UNIVERSITAT DE BARCELONA

# Implementation of a selector for sludge settling enhancement in an activated sludge system treating petrochemical wastewater

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# IMPLEMENTATION OF A SELECTOR FOR SLUDGE SETTLING ENHANCEMENT IN AN ACTIVATED SLUDGE SYSTEM TREATING PETROCHEMICAL WASTEWATER

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The secret of wisdom, power and knowledge is humility

*Ernest Hemingway*

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## SUMMARY

In agreement with the current tendencies, the recently approved version of the *Best Available Techniques Reference Document for Wastewater Treatment in the Chemical Sector (UE 2016/902)* recommends increasingly stringent allowed emission levels to water. Consequently, the existing wastewater treatment processes in Europe need to be upgraded, in order to meet future regulations. Particularly, petrochemical activated sludge systems focused on organic matter removal must face two frequent issues, which difficult sludge settling: i) Filamentous bulking caused by a low food-to-microorganism ratio (F/M) ii) Unintended nitrification in the biological reactor, which results in denitrification with rising sludge in the clarifier.

The literature refers the implementation of selectors in several industrial sectors as a solution to the low F/M filamentous bulking. The present research satisfies the need for additional experimental work on selectors dealing with petrochemical effluents. Conventional solutions to limit nitrification are based on the adjustment of operational parameters, which may not be convenient in this system because of process constraints. This fact justifies the need to develop an alternative strategy, which has been addressed in this thesis through folic acid addition to the biological reactor.

Pilot-scale assays with petrochemical substrate and inoculum have been conducted to assess about the effectiveness of aerobic and anoxic selectors to enhance sludge settling. Eventually, the design parameters of the aerobic selector have been optimized, as well as the operational parameters of the activated sludge system including the selector. The control of unintended nitrification by folic acid addition has been bench-scale tested. Folic acid cost-effective concentrations of 0.4 and 0.9 mg $g^{-1}$VSS $d^{-1}$ have been supplemented to respective petrochemical bioreactors, in comparison to a control. The effect of folic acid on nitrification patterns was assessed during and after the vitamin addition. Also, aiming to evaluate its full-scale implementation, the effect of folic acid on the heterotrophic operational parameters was evaluated.

The experimentation began operating the pilot plant as a continuous stirred tank reactor (CSTR) to confirm the tendency of petrochemical systems to develop low F/M filamentous bulking. Sludge volumetric index (SVI) values higher than 350 mL $g^{-1}$ were registered, corresponding to abundant, cross-linked filaments inside and outside the floc. The SVI in the petrochemical CSTR could only be slightly improved by increasing the F/M (up to 0.4 g COD $g^{-1}$ VSS $d^{-1}$). The filamentous bulking was not definitively solved until a selector was implemented in the pilot activated sludge system. Aerobic

and anoxic selectors improved the biomass quality, reducing the amount of filaments and providing a more compact floc. Nevertheless, when comparing both, the aerobic selector obtained the most reliable performance and the lowest SVI results (range of 30 to 75 in front of 69 to 270 mL $g^{-1}$).

Optimum values for the design parameters of the aerobic selector were identified, though its performance was robust for a wide range. Hence, the optimum F/M and hydraulic retention time (HRT) for the petrochemical aerobic selector were characterized at 35 g sCOD $g^{-1}$ VSS $d^{-1}$ and 30 min, respectively, to obtain average COD and BOD removal efficiencies in the selector of 35% and 95%, respectively. As a result, the optimum substrate equilibrium concentration in the selector was determined at 2.5 g $L^{-1}$, which promoted adequately the mechanism to establish a feed-starve cycle with the reactor. The best substrate for the selector to enhance sludge settling was a high biodegradable (minimum tested 10.4 g BOD $g^{-1}$ VSS $d^{-1}$) and low particulate content one (maximum allowable 65 g tCOD $g^{-1}$ VSS $d^{-1}$).

While sludge settling ability in the CSTR was statistically ($p<0.05$) affected by dissolved oxygen (DO), F/M, sludge retention time (SRT) and ammonia nitrogen concentration, the effect of these variables was no longer significant with the inclusion of a selector, but for DO. Otherwise, other mixed liquor parameters, such as suspended solids, pH, temperature and conductivity continued to have a key role on sludge settling even with the implementation of a selector. Hence, whereas an increase in mixed liquor suspended solid concentration (from 2.3 to 16 mg $L^{-1}$) or in conductivity (from 20 to 60 mS $cm^{-1}$) provided a poorer sludge settling, increasing pH (from 8.0 to 9.0) or temperature (from 30 to 38 ºC) enhanced sludge settling. While pH and temperature determined the ability of bacteria for bio-flocculation, conductivity and temperature affected water properties with an effect on sludge settling, such as density and viscosity. As a drawback, the inclusion of a selector in the activated sludge system resulted in an older sludge, which enhanced unintended nitrification in the biological reactor in a 48% compared to the CSTR. Therefore, the control of biological nitrification acquired more relevance with the implementation of a selector.

The supply of folic acid to the CSTR allowed to limit nitrification, but also had an effect on the operational parameters of the activated sludge system. Hence, the lower vitamin concentration provided a 93.6% reduction in nitrification rates and also improved the sludge volumetric index compared to control (17.4 in front of 67.3 mL $g^{-1}$). However, its feasibility to be full-scale implemented is conditioned to the availability of spare aeration capacity, since oxygen demand increased in a 85.7%, probably due to an older

sludge age (71.4% reduction in observed sludge yield). Reductions up to 97.1% in nitrification rates were obtained during and 60 days after the dosage of the higher vitamin concentration. Despite other advantages, such as increasing the organic matter removal efficiency (60.0%) and reducing oxygen demand (14.7%) relative to control, the high dosed reactor exhibited a worse sludge settling (93.1 mL $g^{-1}$) and more sludge production (57.1% increase in observed sludge yield).

This research provides the petrochemical industry with guidelines to upgrade the activated sludge systems, in order to improve the sludge settling ability. Also, the methodology and conclusions could be extrapolated to other industrial sectors with a similar wastewater's characterization. Particularly, folic acid studies could also be of interest for the agricultural sector, where nitrification is the main responsible for nitrogen losses and nitrogen oxides emissions.

The findings reported in this thesis inspire future research work to test alternative dosing strategies for folic acid and to develop a mechanism of action for nitrogen heterocyclic compounds.

## RESUMEN

De acuerdo con las tendencias actuales, la recientemente aprobada versión de *Best Available Techniques Reference Document for Wastewater Treatment in the Chemical Sector (UE 2016/902)* recomienda límites de vertido de aguas más restrictivos que los actuales. En consecuencia, los procesos de tratamiento europeos deben ser modificados para cumplir las futuras exigencias legislativas. Particularmente, los sistemas petroquímicos de fangos activos focalizados en eliminación de materia orgánica deben afrontar dos problemáticas frecuentes que dificultan la decantación del lodo: i) Proliferación de biomasa filamentosa (o *bulking* filamentoso) causada por una baja razón alimento-biomasa (F/M, del inglés *Food-to-microorganism*), y ii) Nitrificación no deseada en el reactor biológico, con desnitrificación y arrastre superficial de fangos en el clarificador.

La literatura especializada refiere el uso de selectores en varios sectores industriales como solución al *bulking* filamentoso por baja razón F/M. La presente investigación satisface la necesidad de experimentación adicional con selectores tratando aguas petroquímicas. Por otra parte, las soluciones convencionales para limitar la nitrificación consisten en ajustar parámetros operativos, lo cual puede no ser adecuado en este tipo de sistema por limitaciones de proceso. Este hecho justifica la necesidad de desarrollar una estrategia alternativa, la cual en esta tesis se ha centrado en la dosificación de ácido fólico al reactor biológico.

Se han realizado ensayos a escala piloto con inóculo y efluentes petroquímicos para comprobar la eficacia de selectores aerobios y anóxicos en la mejora de la decantación del fango. También se han optimizado los parámetros de diseño del selector aerobio, así como los parámetros operacionales del sistema de fangos activos incluyendo el selector. El control de la nitrificación no deseada por adición de ácido fólico se ha probado a escala laboratorio. Se han aportado concentraciones de ácido fólico de 0,4 y 0,9 mg $g^{-1}$ VSS $d^{-1}$ a reactores biológicos petroquímicos respectivos, en comparación con un reactor de control. Se ha caracterizado el efecto de la dosificación de ácido fólico en las tendencias de generación de nitritos y nitratos, durante y tras la dosificación de vitamina. Adicionalmente, con el objetivo de analizar la viabilidad de su implementación a escala industrial, también se ha evaluado el efecto del ácido fólico en los parámetros operacionales heterótrofos.

La experimentación se inició operando la planta piloto como un reactor tanque mezcla perfecta (RTMP) para confirmar la tendencia de dichos sistemas a desarrollar *bulking*

filamentoso por baja razón F/M. Se obtuvieron valores de índice volumétrico de fangos (IVF) superiores a 350 mL $g^{-1}$, correspondientes a una presencia abundante de filamentos entrecruzados, dentro y fuera del flóculo. El IVF del RTMP petroquímico sólo pudo ser ligeramente mejorado con el aumento de la razón F/M (hasta 0,4 g DQO $g^{-1}$ VSS $d^{-1}$). El *bulking* filamentoso no remitió definitivamente hasta que se implementó un selector en el sistema de fangos activos. La experimentación demostró que selectores anóxicos y aerobios podían mejorar la calidad de la biomasa, reduciendo la cantidad de filamentos y proporcionando un flóculo más compacto. No obstante, al comparar ambos, el selector aerobio mostró una operación más robusta y valores inferiores de IVF (rango de 30 hasta 75 frente a 69 hasta 270 mL $g^{-1}$).

A pesar de que la operación del selector se mostró robusta en un amplio rango de valores para sus parámetros de diseño, se evidenció un óptimo para los mismos. Así, se caracterizaron como razón F/M y tiempo de retención hidráulico (TRH) óptimos para el selector aerobio los valores de 35 g sDQO $g^{-1}$VSS $d^{-1}$ y 30 minutos, respectivamente. Con estos parámetros, se obtuvieron valores promedio de eficiencia en eliminación de DQO y DBO en el selector del 35% y 95%, respectivamente. En consecuencia, la concentración de sustrato óptima fue determinada en 2,5 g $L^{-1}$, la cual evidenció un funcionamiento adecuado del mecanismo del selector, estableciéndose el ciclo alimento-ayuno con el reactor. La calidad de sustrato más adecuada para el selector con el objetivo de mejorar la decantación resultó ser aquella de mayor biodegradabilidad (mínimo ensayado 10,4 g DBO $g^{-1}$VSS $d^{-1}$) y menor contenido en materia particulada (máximo permisible 65 g tDQO $g^{-1}$VSS $d^{-1}$).

Mientras que la decantación del fango en el RTMP estaba estadísticamente ($p<0,05$) afectada por el oxígeno disuelto, la razón F/M, el tiempo de retención celular y la concentración de amonio, el efecto de dichas variables dejó de ser significativo con la inclusión del selector, excepto para el oxígeno disuelto. Por el contrario, otros parámetros del licor mezcla, tales como la concentración de sólidos en suspensión, pH, temperatura y conductividad mantuvieron su efecto significativo en la decantación del fango a pesar de incluir el selector. De esta forma, un incremento en la concentración de sólidos en suspensión (de 2,3 a 16 mg $L^{-1}$) o en la conductividad (de 20 a 60 mS $cm^{-1}$) empeoraron la decantación, mientras que un incremento en el pH (de 8,0 a 9,0) o en la temperatura (de 30 a 38 ºC) mejoraron la decantación del lodo. El pH y la temperatura determinaron la habilidad de las bacterias para la bioflocculación, a la vez que la conductividad y la temperatura afectaron a propiedades del agua con impacto en la decantación del fango, tales como la densidad y la viscosidad. Como inconveniente, la inclusión del selector en el sistema de fangos activos provocó el envejecimiento del

fango, lo cual potenció la nitrificación en el reactor biológico (incremento de un 48% comparado con el RTMP). Por tanto, el control de la nitrificación biológica adquirió mayor relevancia con la inclusión del selector en el sistema de fangos activos.

El suministro de ácido fólico en el RTMP permitió limitar la nitrificación, pero también tuvo un efecto en los parámetros operacionales del sistema de fangos activos. Así, la baja concentración de vitamina aportó resultados satisfactorios en cuanto a reducción de nitrificación (93,6%) y mejoró el IVF comparado con el control (17,4 frente a 67,3 mL $g^{-1}$). Sin embargo, la viabilidad de implementación a escala industrial demostró estar condicionada a la disponibilidad de capacidad sobrante de aireación, puesto que la demanda de oxígeno del reactor se incrementó en un 85,7%, probablemente debido a un incremento en la edad del fango (71,4% de reducción en la producción de fango observada). Se obtuvieron reducciones de hasta el 97,1% en las tasas de nitrificación, durante y 60 días tras la dosificación de la alta concentración de vitamina. A pesar de otras ventajas, tales como un incremento en la eficiencia de eliminación de materia orgánica (60%) y una reducción en la demanda de oxígeno (14,7%) relativo al control, el reactor suministrado con la alta concentración de ácido fólico exhibió una peor decantación (93,1 mL $g^{-1}$) y mayor producción de fango (57,1 % aumento en la tasa de crecimiento bacteriano observada).

Esta investigación proporciona pautas para mejorar los sistemas de fangos activos de la industria petroquímica, potenciando la decantación del fango. Adicionalmente, la metodología y conclusiones pueden ser extrapoladas a otros sectores industriales con una caracterización similar de las aguas residuales. Particularmente, el estudio del ácido fólico podría ser también de interés para el sector agrícola, en el cual la nitrificación es el principal responsable de pérdidas de nitrógeno y de emisiones de óxidos de nitrógeno.

Los resultados obtenidos en esta tesis inspiran futuras investigaciones para probar nuevas alternativas de dosificación del ácido fólico y para desarrollar un mecanismo de acción de los compuestos nitrogenados heterocíclicos.

# 1. Background

***ABSTRACT***

*A new version of the BREF document for Common Wastewater and Waste Gas Treatment/Management Systems in the Chemical Sector, which introduces future more stringent emission levels to water in Europe, has been recently approved by Decision (EU) 2016/902 of 30$^{th}$ May 2016. Consequently, the existing industrial wastewater treatment processes need to be upgraded, in order to improve the effluent quality. Particularly, the present research concentrates on sludge settling enhancement in petrochemical activated sludge systems focused on organic matter removal. Such installations are bound to two frequent issues, which hinder sludge settling and difficult the accomplishment of regulatory waste specifications: i) Filamentous bulking due to a low food-to-microorganism ratio (F/M) in the continuous stirred tank reactor (CSTR) ii) Unintended nitrification in the CSTR and consequently denitrification in the clarifier.*

*The bibliography refers the use of selectors in several industrial sectors to solve the low F/M excessive proliferation of filamentous bacteria. Nevertheless, no references have been found about selectors dealing with petrochemical effluents. Therefore, this thesis covers the need for additional experimental work on selectors in petrochemical activated sludge systems.*

*Traditional approaches to solve denitrification issues have been to limit biological nitrification in the CSTR by the setting of operational parameters. However, since conventional alternatives do not fit in such systems because of process and site constraints, the present manuscript suggests a novel strategy by supplying folic acid to the biological reactor. The vitamin presents nitrogen heterocyclic structure, which reminds of a group of nitrification inhibitors studied in agriculture. Nevertheless, folic acid had not yet been investigated as a nitrification inhibitor. Therefore, the present research assesses on the effect of the vitamin on nitrifying rates.*

*The manuscript aims to provide industrial engineers with a methodology to upgrade their existing activated sludge processes, in order to accomplish future more stringent emission levels. Petrochemical engineers can also find guidelines to optimize their wastewater treatment facilities.*

# 1. Background

Wastewater management has become a great challenge for the industry progress, as the allowed emission levels evolve towards more stringent values. Following this general tendency, a new version of the *BREF document for Common Wastewater and Waste Gas Treatment/Management systems in the Chemical Sector* has been recently approved by *Decision (EU) 2016/902 of 30$^{th}$ May 2016*. Although the Best Available Techniques (BAT) conclusions state just recommendations on the associated emission levels (AEL) to water, this document is the reference for local regulations in Europe. Hence, the *Directive on Industrial Emissions (2010/75/UE)* concludes that local regulations should be equal or more stringent than BAT-AELs recommendations. Consequently, the European industry is urged to improve the effluent water quality not only as an environmental good practice, but also to comply with future regulations. Therefore, the existing wastewater treatment processes should be upgraded in order to improve their efficiency and the quality of the effluent wastewater.

Following this line of research, the present project is focused on the optimization of existing full-scale activated sludge processes. Although this technology is already recognized as being BAT in the *BREF document*, improvement of its efficiency in solid separation is needed in order to adapt it to the new emission levels. Particularly, the thesis concentrates on sludge settling enhancement in petrochemical activated sludge systems focused on organic matter removal.

Such biological treatments often include a continuous stirred tank reactor (CSTR). This configuration is advantageous because of its large dilution capacity for the potential toxics, which may come with the industrial effluents (Metcalf and Eddy, 2003). However, it operates at a low food-to-microorganism ratio (F/M), which enhances the proliferation of filamentous bacteria. Additionally, since some petrochemical substrates present toxicity and a low biodegradability, they are often digested, oxidized or cracked before entering the CSTR (Derakhshan and Fazeli, 2018). These units generate large amounts of carbon dioxide in the oxidation of the organic matter, which is supplied to the biological reactor as carbonates. Therefore, the CSTR presents favourable conditions for the autotrophic nitrifying bacteria to proliferate because of the large inorganic carbon concentrations together with large sludge retention time (SRT) to biodegrade recalcitrant organic matter (Metcalf and Eddy, 2003).

Consequently, two frequent issues, which hinder sludge settling, are bound to happen in such systems: i) An excessive proliferation of filamentous bacteria due to low F/M

ratio, ii) Unintended biological nitrification, which results in denitrification in the clarifiers. The approach to overcome the excessive proliferation of filamentous bacteria has been to implement a selector in the petrochemical activated sludge system, whereas denitrification control in the clarifier has been addressed by a novel strategy to limit nitrification in the CSTR through the addition of folic acid.

References for the use of selectors with paper mill effluents (Durocher *et al.*, 2002), slaughterhouse (Al-Mutairi, 2009) or sugar mill wastewater (Prendl and Kroiss, 1998) have been found in the bibliography. However, no previous works on the use of selectors with petrochemical effluents have been identified. On the other hand, it is recognized in the literature, that the effectiveness and the optimum design of a selector to enhance sludge settling is dependent on the quality of the wastewater (Martins *et al*., 2004; Seviour and Blackall, 2012). Therefore, this thesis covers a need for additional experimental work to assess the effectiveness of a selector to enhance sludge settling in petrochemical systems (Cardete *et al.*, 2017 a, b; Cardete *et al*., 2018).

Traditional strategies based on nitrification kinetics state that the biological reaction can be controlled through the setting of operational parameters, such as SRT (Flores-Alsina *et al*., 2010) or pH (EPA, 2002). However, this possibility may not be feasible in the biological systems studied. Since it is required to biodegrade recalcitrant organic matter, the system must be operated at large SRT conditions, which enhance biological nitrification. Also, the biological reactors in such systems exhibit a strong carbonate-buffer, due to a high carbonate concentration in the feed streams, which come from advanced oxidation processes. Consequently, large amounts of acid should be added to lower the pH in such carbonate-buffered systems from values of 8.0 to 6.0, in order to limit nitrification. An alternative could be to implement a denitrification step before the clarifier. However, apart from requiring huge investment, site constraints such as not enough free space to lay-out or the impossibility to shut down the running process to implement modifications advise to develop a new strategy.

Folic acid offers a promising background for the purpose of limiting biological nitrification without preventing the activity of the heterotrophic bacteria. The vitamin is known in the wastewater treatment field as a resource to reduce biomass production (Stoppa *et al*., 2013; Alishiri and Fataei, 2015; Alexandre *et al*., 2016) without adding toxicity to the effluent (Velho *et al*., 2016). Senorer and Barlas (2004) also demonstrated that the addition of folic acid improved the organic matter removal in biological treatments. On the other hand, its nitrogen heterocyclic structure reminds of a group of nitrification inhibitors studied in agricultural research (Mc Carty, 1999).

Therefore, joining the knowledge developed in both areas, the present manuscript investigates a new functionality of the vitamin to limit biological nitrification, while improving the heterotrophic activity as well (Cardete *et al*., 2019).

This manuscript may be a reference for readers devoted to the industrial sector, who seek for an improvement in their existing wastewater treatment processes, in order to enhance the effluent quality. Particularly, petrochemical engineers can find guidelines to upgrade their activated sludge processes.

# 2. Introduction

***ABSTRACT***

*Among the different biological treatments, the aerobic suspended growth process, known as activated sludge system, could be highlighted in industrial wastewater treatment. The complete mix reactor configuration is often chosen, since it offers a large dilution capacity to withstand potential toxics and load shocks.*

*The understanding of the design and operation of such systems requires considering microbiological, engineering and water chemistry principles, which are interrelated. Two operations must be accomplished in the complete mix activated sludge system: i) The biodegradation of the organic matter in the reactor, and ii) Sludge settling in the clarifier. While the substrate is biologically degraded, also biomass synthesis occurs. Both processes require the supply of oxygen and nutrients to the reactor. In turn, the clarifier must provide thickened sludge and clarified water. For this purpose, it is a key factor the sludge settling ability, which is determined by the shape of the microorganisms and by the presence of exocellular biopolymers that enable bioflocculation. In turn, these factors are selected and given by the operational and design parameters of the system. Hence, associated to scarcity in necessary resources, such as oxygen, organic matter or nutrients, filamentous bacteria tend to dominate. An excessive proliferation of filaments hinders sludge settling. It is useful to characterize them, since the identification gives information on the condition to be corrected in order to improve the biomass quality. Particularly, complete mix reactors are bound to the proliferation of filamentous bacteria due a low food-to-microorganism ratio (F/M). A low F/M filamentous bulking can be definitively overcome by the implementation of aerobic or anoxic selectors, depending on the nature of the wastewater treated. Selectors force a high F/M in a compartmentalized mixing chamber prior to the reactor. This procedure generates a feed-starve cycle in the activated sludge system, which promotes the kinetic selection of floc-forming bacteria. Additionally, anoxic selectors are based on metabolic selection, since most filaments are aerobes.*

*Sludge settling in systems focused on organic matter removal can also be enhanced by limiting nitrification rates, either adjusting the convenient operational parameters or inhibiting the biological reaction. The option chosen should not hinder the heterotrophic bacteria activity, which coexists with the autotrophic microorganisms.*

# 2. Introduction

## 2.1. TYPES OF BIOLOGICAL PROCESSES FOR WASTEWATER TREATMENT

Biological wastewater treatments are based on the natural functionality of bacteria to close the elemental cycles of carbon, nitrogen and phosphorus on earth. The bacteria used in the wastewater treatment plants are the ones that exist in the nature. The design of biological processes is based on providing the resources, which select the microorganisms best adapted. Various media can act as selective: the availability of electron donor (organic matter or ammonium, among others), electron acceptor (oxygen, nitrate or organic matter, among others) and nutrients. Also pH, temperature, hydraulics and other conditions are selective (Mogens *et al*., 2008).

Two main categories of biological processes are used for wastewater treatment: i) Suspended growth processes and ii) Attached growth (or bio-film) processes. The common name and typical applications for suspended and attached growth biological treatment processes are given in Table 2.1.

The suspended growth processes are biological treatment processes, in which the microorganisms responsible for the organic matter biodegradation are kept in liquid suspension by convenient mixing methods. The aeration tank or biological reactor provides contact time for the mixing and aeration of the influent wastewater and the microbial suspension, known as mixed liquor. The processes can operate with a positive dissolved oxygen concentration (aerobic processes), in the presence of nitrates to be biologically converted into nitrogen gas (anoxic processes, also known as denitrification) or in the absence of oxygen (anaerobic processes). The most common type of suspended growth process is the activated sludge process, in which the carbonaceous matter is biodegraded under aerobic conditions. Often, mechanical equipment is used to provide mixing and oxygen transfer into the process. The mixed liquor from the reactor flows into a clarifier, where the microbial suspension settles and thickens. The settled biomass is known as activated sludge, because of the presence of active microorganisms. A portion is returned to the aeration tank and the other part is removed to avoid biomass accumulation. An important objective of the activated sludge process is the formation of floc particles, from 50 to 200 μm, to be removed by gravity settling.

Table 2.1 Biological treatment processes used for wastewater treatment *(after Metcalf and Eddy, 2003)*

| Type | Common name | Objective |
|---|---|---|
| **Suspended growth processes** | | |
| Aerobic | Activated sludge process | Carbonaceous BOD removal, nitrification |
| | Aerobic digestion | Stabilization, carbonaceous BOD removal |
| Anoxic | Suspended growth denitrification | Denitrification |
| Anaerobic | Anaerobic contact process | Carbonaceous BOD removal |
| | Anaerobic digestion | Stabilization, solids destruction, pathogen kill |
| Combined aerobic, anoxic and anaerobic | Single or multistage, various proprietary processes | Carbonaceous BOD removal, nitrification, denitrification, phosphorus removal |
| **Attached growth processes** | | |
| Aerobic | Trickling filters | Carbonaceous BOD removal, nitrification |
| | Rotating biological contactors | Carbonaceous BOD removal, nitrification |
| | Packed-bed reactors | Carbonaceous BOD removal, nitrification |
| Anoxic | Attached growth denitrification | Denitrification |
| Anaerobic | Anaerobic packed and fluidized bed | Carbonaceous BOD removal, waste stabilization, denitrification |
| **Hybrid suspended and attached growth** | | |
| Aerobic | Trickling filter/ Activated sludge | Carbonaceous BOD removal, nitrification |
| Anaerobic | Upflow sludge blanket/Activated sludge | Carbonaceous BOD removal |
| Combined aerobic, anoxic and anaerobic | Single or multistage processes with packing for attached growth | Carbonaceous BOD removal, nitrification, denitrification, phosphorus removal |
| **Lagoon processes** | | |
| Aerobic | Aerobic lagoons | Carbonaceous BOD removal |
| Anaerobic | Anaerobic lagoons | Carbonaceous BOD removal, waste stabilization |
| Facultative | Facultative lagoons | Carbonaceous BOD removal |
| Maturation | Maturation lagoons | Carbonaceous BOD removal, nitrification |

BOD: Biological Oxygen Demand

The attached growth processes are also known as fixed film processes. In attached growth processes, the microorganisms responsible for biodegrading the organic matter are attached to an inert packing material, forming a biofilm. Examples for packing materials are rock, gravel, slag, sand, redwood, plastic and other synthetic materials. Attached growth processes can also be operated as aerobic, anaerobic or anoxic. The packing can be completely submerged in the liquid or not, with air or gas space above the biofilm liquid layer. The trickling filter is the most common aerobic attached growth process. Wastewater is distributed over the top area of a vessel, which contains the non-submerged packing material. Excess biomass sloughs from the attached growth

periodically, so that clarification is required for the effluent, to come to acceptable suspended solids concentration. The solids are collected at the bottom of the clarifier, and removed for waste-sludge processing.

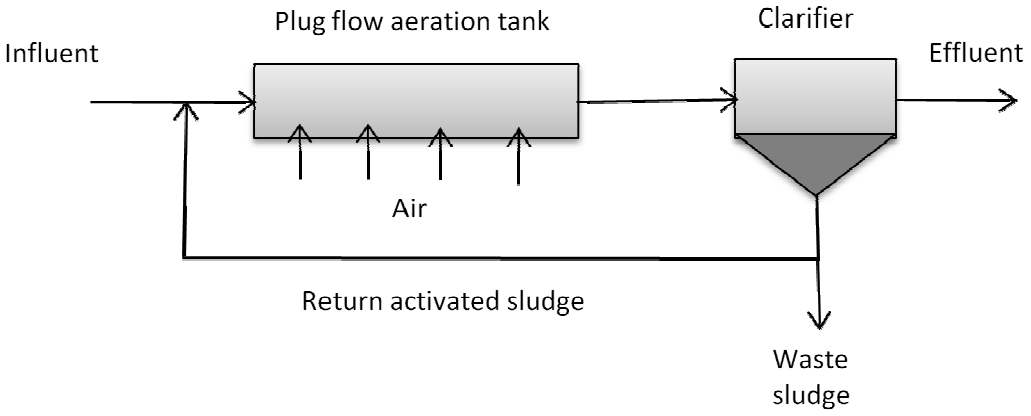
Also, combined suspended and attached growth processes are possible. Additional treatment processes are the Lagoon processes, in which the biological treatment process takes place in ponds or lagoons (Metcalf and Eddy, 2003).

## 2.2. THE ACTIVATED SLUDGE PROCESS

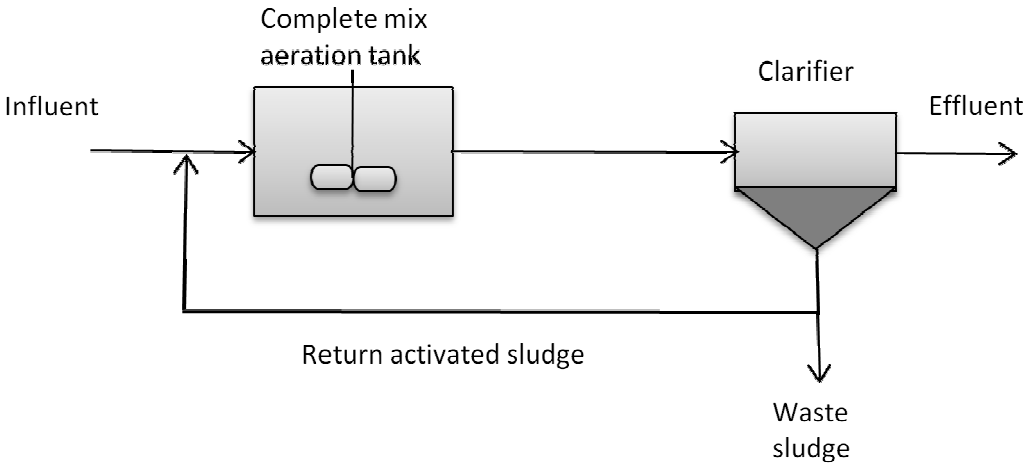
The activated sludge process was developed in England by Ardern and Lockett in 1914 and the process has been through many improvements along the years (Bonadda *et al.*, 2011).

Nowadays, different activated sludge processes are available depending on the reactor configuration, which can be plug flow, complete mix or sequentially operated. Some examples for the plug flow configurations are the conventional plug flow, step feed, contact stabilization, Kraus process, conventional extended aeration, oxidation ditch, Orbal, countercurrent aeration system and Biolac process. Also, sequentially operated systems such as the sequencing batch reactor, the batch decant reactor, intermittent cycle extended aeration system and the cyclic activated sludge system are used. The main differences between the processes rely on their aeration configuration, operating mode, the ability to remove nitrogen and solids retention time (Metcalf and Eddy, 2003). Figure 2.1 illustrates the scheme for the two main configurations of activated sludge process, which are the complete mix and the plug flow reactor.

The selection of an activated sludge process for organic matter removal, as well as its optimum operational parameters, depends mainly on the characterization of the wastewater streams. Also, issues such as specific site constraints, compatibility with existing processes and equipment, present and future needs, level of capacity of the operating staff, operative and investment costs should be taken into account (Hendricks, 2016). Hence, for industrial wastewater treatment, the complete mix activated sludge system is often chosen as a biological treatment. This configuration is advantageous to deal with industrial effluents, since it offers a large dilution capacity for potential toxics that may come with the feed streams (Metcalf and Eddy, 2003).




(a)




(b)

Figure 2.1 Scheme of basic activated sludge processes (a) Plug flow (b) Complete mix activated sludge (CMAS) or continuous stirred tank reactor (CSTR)

### 2.2.1. Historical development and evolution

Nowadays, the activated sludge process is often used for the biological treatment of urban and industrial effluents. The concept of the activated sludge process was initiated by Dr. Angus Smith in the early 1880s, investigating the effect of aeration in wastewater tanks and in the faster oxidation of the organic matter. The aeration of wastewater continued to be further studied and Black and Phelps (1910) attained a considerable reduction in the wastewater to become putrid by forcing air into its containment tanks. Furthermore, Clark and Gage (1912-1913) managed to cultivate growths of organisms in bottles and tanks, partially filled with roofing slate, with a

consequent increase in the degree of the water's purification (Clark and Adams, 1914). Andern and Locket (1914) continued experiencing with aeration tanks, and announced that the sludge played an important role in the results obtained. They named the process as activated sludge because it involved the production of an activated mass of microorganisms capable of aerobic stabilization of the organic matter in the wastewater (Metcalf and Eddy, 2003).

Since its early conception, a number of activated sludge processes and design configurations have been developed. The drivers for this evolution have been i) Engineering development looking forward to obtaining improved quality in the effluent, ii) Technological advances in equipment and process control, iii) A better understanding of the microbiology principles involved in the wastewater treatment and iv) A need to reduce investment and operational costs.

Within the period of the early 1920s, when the process became usual, to the late 1970s, the type of activated sludge process most often used was a plug flow reactor. However, an important feature in the 1960s was the discharge of industrial wastes to domestic wastewater collection systems. Because of the toxic effect of industrial discharges, the plug flow process became problematic. As a solution, the complete mix reactor was developed. Its larger volume allowed for greater dilution, and consequently mitigated the effect of toxic discharges. An additional feature, which promoted the implementation of complete mix reactors, was the limitation of most plug flow systems to feed the necessary oxygen in order to meet the reactor's demand. Hence, the most common type of activated sludge process in the 1970s and 1980s was a single-stage, complete mix activated sludge process (CMAS). However, in Europe the CMAS process has faced a handicap, due to increasingly stringent ammonia standards. In some applications, two-stage systems have been implemented (each stage consisting of an aeration tank and a clarifier). The first stage has been designed for BOD removal and the second stage for nitrification. Alternative activated sludge processes have been also applied, such as, the oxidation ditch (1950), contact stabilization (1950), Krause process (1960), pure oxygen activated sludge (1970), Orbal process (1970), deep shaft aeration (1970) and sequencing batch reactor process (1980).

In the last years, numerous modifications of the activated sludge process have evolved with the objective of accomplishing an efficient removal of nitrogen and phosphorus. Also, membrane technology has found increasing applications to enhance solids separation for water reuse, in the Membrane Biological Reactors.

### 2.2.2. Description of the process

The conventional activated sludge process focused on organic matter removal, as illustrated in Figure 2.1, consists of three basic components: i) The **biological reactor**, in which the microorganisms responsible for COD removal are kept in suspension and aerated, ii) A solid-liquid separation, usually in a **sedimentation tank,** and iii) A **recycle stream** to return part of the separated solids back to the reactor.

Numerous process configurations have evolved using these components. Complementary physical and chemical processes may be used for the preliminary and primary treatment of wastewater, as well as for the post-treatment.

Two main objectives of the activated sludge process are the biological conversion of pollutants in a biological reactor, and solids separation, usually in a gravity clarifier (Jenkins *et al*., 2003).

Nowadays, both functions could also be combined in a single unit, the so-called sequencing batch reactor (SBR). Also, the secondary clarifier may be replaced by a flotation separator or a membrane, in order to remove solids from clarified water.

## 2.3. FUNDAMENTALS FOR THE DESIGN AND OPERATION OF COMPLETE MIX ACTIVATED SLUDGE PROCESSES

To facilitate the understanding of the next chapters, the basic principles of microbiology, engineering and chemistry involved in the operation and design of complete mix activated sludge systems are introduced.

The efficiency of the biodegradation reaction relies on biological considerations, as well as on the design and operational parameters of the reactor and the recycle stream. On the other hand, the efficiency of the settling process is dependent on physical (design and operation of the clarifiers, previous deaeration), chemical (wastewater characteristics) and biological factors (microbiology, degree of bioflocculation) (Wilén, 1995).

### 2.3.1. Microbiology principles

Biological wastewater treatment includes a big variety of microorganisms, such as bacteria, protozoa, fungi, metazoan and possibly algae. Bacteria (0,5-5 µm) are usually the specie responsible for depuration, while protozoa (10-50 µm) are often just known as bioindicators of the wastewater's quality, though they can also biodegrade bacteria, other protozoa and/or particulate organic matter (Madoni, 2009).

Sometimes, achieving a biological wastewater treatment goal can only be accomplished by the presence of specific microbial species. In fact, microorganisms tend to adapt to the media provided for them to grow. Therefore, there is a natural selection with the conditions of the wastewater supplied to the microorganism (Moo-Young *et al*., 1996).

#### 2.3.1.1. The role of microorganisms in wastewater treatment

Microorganisms (mainly bacteria) are responsible for the biological removal of dissolved and particulate carbonaceous BOD in the wastewater. The microorganisms convert the carbonaceous organic matter, by oxidation, into simple end products (carbon dioxide and water) and additional biomass. To carry out the oxidation process, the microorganisms use oxygen and macronutrients, such as ammonia nitrogen and orthophosphate phosphorus.

The term biomass is often referred to, through the simplified molecular formula $C_5H_7NO_2$ or the complete formula $C_5H_7NO_2P_{1/2}$ (Metcalf and Eddy, 2003). The biomass has a specific gravity slightly greater than that of water, which enables its separation from the treated water by gravity settling. In fact, to accomplish the BOD specification in the effluent, the biomass must be removed since it would also be measured as BOD.

#### 2.3.1.2. Microorganism identification

The identification of the microorganisms present in the wastewater informs about the state of bacterial sludge and the operational parameters of the biological system (Jenkins *et al*., 2003). Two common methods are used to identify bacteria: conventional taxonomic methods and phylogenetic classification methods.

Conventional taxonomic methods rely on physical properties of the bacteria (morphologic characteristics) and metabolic characteristics. The tests used to

characterize the bacteria include: i) Microscopic observations to determine morphology (size and shape) ii) Gram staining to determine if the bacteria cell wall absorbs crystal violet dye iii) The type of electron acceptor used in the oxidation-reduction reactions iv) The type of carbon source used for cell growth v) The ability to use various nitrogen and sulphur sources vi) Nutritional needs vii) Cell wall chemistry viii) Cell characteristics, including pigments, segments, cellular inclusions and storage products ix) Resistance to antibiotics x) Environmental effects of temperature and pH (Jenkins *et al*., 2003).

An alternative method is the phylogenetic classification, which is based on the genetic information of the microorganisms. The genetic code for ribosomal ribonucleic acid (rRNA) was chosen for cell identification. rRNA can be separated into two components, 30 S (Svedberg units) and 50 S, based on different centrifugal forces in ultracentrifugation. The 30 S units consist of 16S rRNA which can be extracted from cells for nucleotide sequencing, using molecular techniques. More recent developments use the section of deoxyribonucleic acid (DNA) that encodes the 16S rRNA. After DNA extraction from the cell material, a polymerase chain reaction procedure (PCR) uses DNA primers and a DNA polymerase enzyme to reproduce and amplify artificially the DNA material. The amplified 16S rRNA is then studied for sequencing, to determine its nucleotide sequence. The result is compared to the ribosome sequences available in data base to determine the identity of the organism and its phylogenetic relationship to known organisms (Spiegelman *et al*., 2005).

#### 2.3.1.3. Classification of microorganisms

Microscopic, single-cell microorganisms, such as bacteria, fungi, algae, protozoa and viruses are responsible for the activity in biological wastewater treatment. The basic functional and structural unit of living matter is the cell.

Microbiology principles begin with the classification of the microorganisms in two types, prokaryotes and eukaryotes, depending on their genetic information and cell complexity. Prokaryotes are unicellular organisms which include bacteria, cyanobacteria (blue-green algae) and archaea. On the other hand, eukaryotes include also unicellular organisms, such as protozoa, algae and fungi, and multi-cellular microorganisms (fungi, plants, animals). Table 2.2 includes a comparison of the eukaryote and prokaryote cell characteristics and Figure 2.2 reflects the structure of the prokaryotic and eukaryotic cell.

Table 2.2 Comparison of the prokaryotic and the eukaryotic cell characteristics

| **Cell characteristic** | **Prokaryotic** | **Eukaryotic** |
|---|---|---|
| Phylogenetic group | Bacteria, blue-green algae (cyanobacter), archaea | Single cell: algae, fungi, protozoa; multi-cell: plants, animals |
| Size of cell | 0.2-3.0 μm | 2-100 μm for single cell |
| Glycocalyx | Present as a capsule or slime layer | Present in some cells that lack a cell wall |
| Cell wall | Usually present; chemically complex (typically includes peptidoglycan) | When present, chemically simple (includes cellulose and chitin) |
| Plasma membrane | No carbohydrates and generally lacks sterols | Sterols and carbohydrates serve as receptors |
| Nucleus | No nuclear membrane or nucleoli | True nucleus with nuclear membrane and nucleoli |
| Chromosome (DNA) | Usually single circular chromosome; typically lacks histones | Multiple linear chromosomes with histones |
| Ribosomes | Smaller size (70S) | Larger size (80S) Smaller size (70S) in organelles) |
| Membrane-enclosed organelles | Absent | Present (examples: lyposomes, golgi complex, endoplasmatic reticulum, mitochondria and chloroplasts) |
| Cytoplasm | No cytoskeleton or cytoplasmatic streaming | cytoskeleton and cytoplasmatic streaming |
| Flagella | Consist of two protein building blocks | Complex; consist of multiple microtubules |
| Cell division | Binary fission | Involves mitosis |
| Sexual recombination | Transfer of DNA only | Involves meiosis |

The microorganisms found in wastewater treatment processes can be classified as bacteria, archaea, fungi/yeast, protozoa, metazoan, algae and viruses (Saxena and Awasthi, 2003).

***Bacteria***- Bacteria are single-cell prokaryotic organisms. Inside the cell, the cytoplasm contains a colloidal suspension of proteins, carbohydrates and other complex organic compounds. There is also the ribonucleic acid (RNA), whose major role is the synthesis of proteins, and the DNA, which contains all the necessary information for the reproduction of the cell. Their usual mode of reproduction is by binary fission, although some species reproduce sexually or by budding. Bacteria can adopt several shapes: cocci are spherical-shaped, bacillus is rod-shaped, espirilla have long rod helix shape and vibrio look like a curved rod. Filamentous bacteria are usually row groupings of cocci and/or bacillus. Actually, they grow naturally in little polluted waters, like rivers

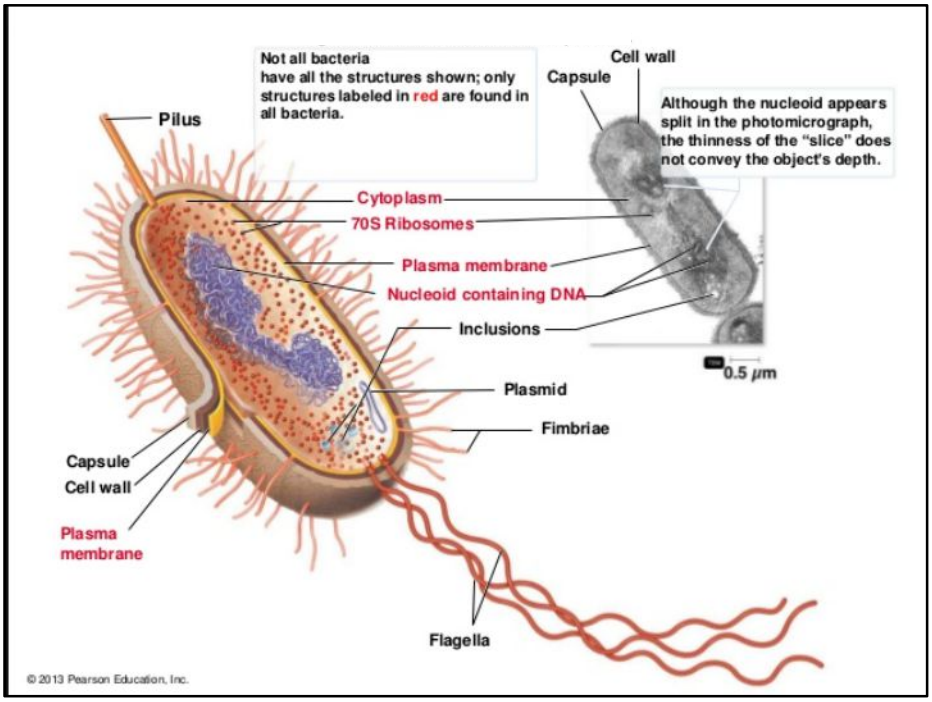
and lakes. Of course, they are also present in the wastewater treatment and form part of the floc. They proliferate especially with nutrient scarcity, causing sludge settling problems. In a wastewater treatment, disperse bacteria associate, together with organic and inorganic particles, in such a way that they create a microstructure or polymer matrix, which is a small floc (30-150 µm). Getting a bigger floc requires the presence of filamentous bacteria. Then a macrostructure (150-1500 µm) is formed, which enhances sludge settling (Schaechter, 2009).

***Archaea-*** Archaea is similar to bacteria in size and basic cell components. However, their cell wall, cell material and RNA composition are different. They are found in anaerobic processes and under extreme conditions of temperature and chemical composition (Schaechter, 2009).

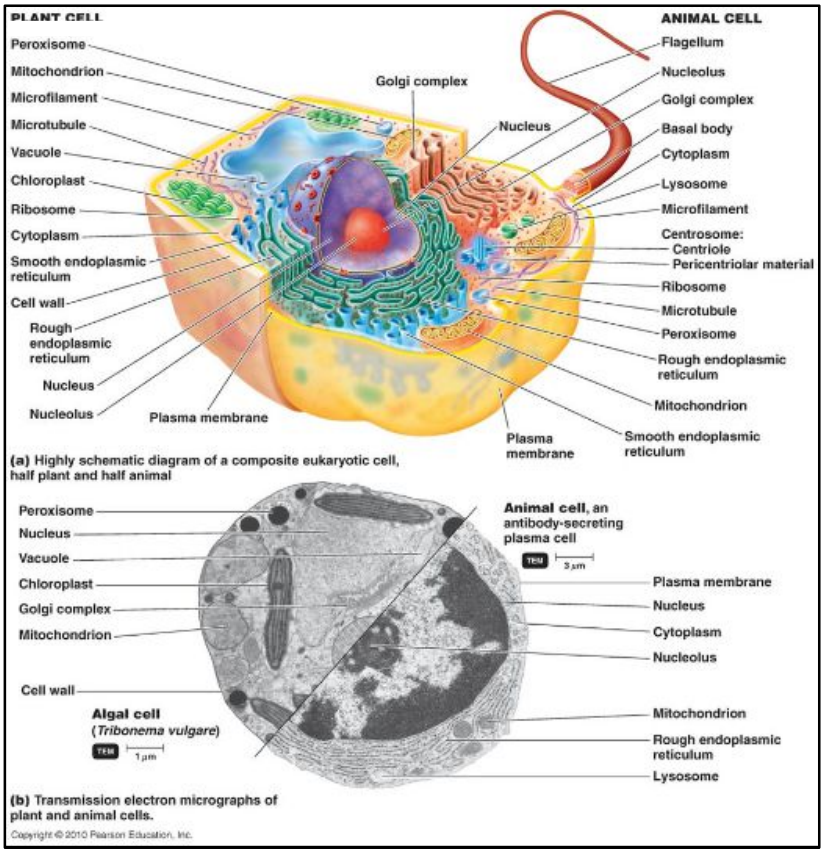
***Fungi and yeast-*** Fungi and yeast are classified as multi-cellular, non-photosynthetic, heterotrophic eukaryotes. Most fungi are strict or facultative aerobes, and reproduce sexually or asexually, by fission, budding or spore formation. Fungi grow under low-moisture, low-nitrogen conditions and can tolerate a relatively low pH. These abilities coupled with the capacity to degrade cellulose determine the use of fungi and yeast in the composting of sludge (Tortora *et al*., 2013).

***Protozoa-*** Protozoa are single-cell, motile, microscopic eukaryotes. Most protozoa are aerobic heterotrophic, some are aero-tolerant anaerobes and a few are anaerobic. Protozoa are an order of magnitude larger than bacteria and often consume bacteria as an energy source, as well as particulate organic matter (Tortora *et al*., 2013). Therefore, they produce a polishing effect in the wastewater treatment effluent (Metcalf and Eddy, 2003).

The protozoa used as bioindicators can be classified as flagellates, amoebas and ciliates. Flagellates (5-20 µm) appear with a high soluble BOD concentration. They are also indicators of low oxygen concentrations. Amoebas (10-200 µm) grow especially with the presence of organic particulate matter. They also tolerate low oxygen concentrations. Particularly, amoebas are usually related to a high concentrated and/or toxic organic effluent. Teak-amoebas may appear with nitrification and low organic loads, which are bound to happen in old-aged sludge. Also, different kind of ciliates can be identified in a wastewater. Borrowed ciliates (20-400 µm) usually indicate unstable but good conditions. On the other hand, attached ones appear with stable sludge conditions. Protozoa are sensitive to toxic materials.




(a)




(b)

Figure 2.2 Structure of the prokaryotic (a) and the eukaryotic cell (b). *Sources: Pearson Education*

***Metazoans-*** Metazoans are pluricellular organisms, such as rotifers, nematodes and mites. Rotifers are aerobic heterotrophic animal eukaryotes. They have two sets of rotating cilia on their head, which serve for motility and food capture. Rotifers are effective in consuming free, dispersed bacteria, and small particles of organic matter. Rotifers (50-500 μm) proliferate in stable, well-oxygenated systems. They can metabolize particulate matter and also, protozoa and bacteria (Tortora *et al*., 2013). All three, rotifers, nematodes and mites, indicate high SRT systems. Their presence in a wastewater indicates a highly efficient aerobic biological purification process.

***Algae-*** Algae are unicellular or multi-cellular, autotrophic, photosynthetic eukaryotes. Their capacity to produce oxygen plays an important role in wastewater treatment lagoons. The blue-green alga cyanobacter is a prokaryotic organism (Tortora *et al*., 2013).

***Viruses***- Viruses consist of a nucleic acid core (DNA or RNA) surrounded by an outer shell of protein (capsid). Viruses can only multiply within a host cell. Bacteriophages are viruses that infect bacteria as the host (Schaechter, 2009).

#### 2.3.1.4. Cell components and cell composition

Bacteria, which are the main responsible for the organic matter biodegradation in the wastewater treatment, are prokaryotic cells (Falkow *et al.,* 2006). Figure 2.2 presents the main components of the prokaryotic cell, which determine its characteristics (Favor, 2005).

***The cell wall-*** The cell wall provides strength to maintain the cell shape and protects the membrane. Some bacteria produce a sticky polysaccharide layer outside the cell wall, called a capsule or slime layer. The components of the cell are just inside the cell wall, without having differentiated organelles. On the contrary, eukaryotic cell does not have a cellular wall, but their inside material is organized in true organelles.

***The cell membrane-*** The cell membrane controls the income of dissolved organics and nutrients into the cell, as well as the waste materials and metabolic by-products out of the cell.

***The cytoplasm-*** The cytoplasm contains the material responsible for the cell functions, which includes water, nutrients, enzymes, ribosomes and small organic molecules.

***Cytoplasmatic inclusions-*** Cytoplasmatic inclusions can contain storage material that provides carbon, nutrients or energy to the cell. These may be carbohydrate deposits, such as polyhydroxybutyrate (PHB) or glycogen, polyphosphates, lipids and sulphur granules.

***DNA, plasmid DNA and Ribosomes***- DNA is a double-stranded helix-shaped molecule that contains genetic information, which determines the nature of the cell protein and enzymes that are produced. Plasmid DNA consists of small circular DNA molecules that can also provide genetic characteristics for the bacteria. Ribosomes are particles in the cytoplasm that are composed of ribonucleic acid (RNA) and protein. They are the sites where proteins are produced. In both types of cells, deoxyribose nucleic acid (DNA) and ribosomes are key components that relate to the cell's genetic information and specific enzymes produced, which determine the capability of the microorganism in wastewater treatment. Ribosomes are the sites of protein synthesis, which are necessary for enzyme production. The DNA provides the genetic information for protein synthesis.

***Flagella-*** Some bacteria have flagella, which are protein hairlike structures, which extend from the cytoplasm membrane, several bacteria lengths out, and provide mobility by rotating at a high speed. Some others have fimbriae and pili, which are short protein hairlike structures that enable bacteria to stick to surfaces. Pili, which are longer, also enable bacteria to attach to each other.

Bacteria found in wastewater treatment plants are typically composed of 75-80% water and 20-25% dry matter. The empirical simplified formula for cells (active biomass) is $C_5H_7NO_2$ and $C_5H_7NO_2P_{1/12}$ (Metcalf and Eddy, 2003), when phosphorus is also considered. Nitrogen and phosphorus are considered as macronutrients because they are needed in relatively large amounts. Prokaryotes also require minority components, such as iron, chlorine, magnesium, calcium, sodium, sulphur, and some trace elements, like Zinc, Manganese, Molybdenum, Selenium, Cobalt, Copper and Nickel. They are known as micronutrients.

#### 2.3.1.5. Bacterial metabolism

As presented in the previous section, bacterial macromolecules include proteins, nucleic acids (DNA and RNA), polysaccharides and lipids. Also, bacterial polymer compounds of significance in wastewater treatment include poly-β-hydroxyalkanoates (PHAs),

glycogen and polyphosphates, which behave as energy reserves. In order to grow, the bacteria must perform some chemical transformations of various precursors to allow the replication of their genetic material, the synthesis of all their constituents and the production of energy (Metcalf and Eddy, 2003).

To reproduce and function properly, the microorganisms need sources of energy and carbon (substrates), inorganic elements (nutrients) and organic nutrients (growth factors).

Metabolism is the sum of all the chemical processes that take place in living cells. The reactions involved can be divided into catabolism and anabolism. Catabolic reactions supply the energy to the cell, by transporting the electrons from a donor to an acceptor through oxidation-reduction reactions. Anabolic reactions use this energy for the synthesis of cellular components, from carbon sources and nutrients. The anabolic process is similar for all the bacteria, but catabolic ones can be different (Sharma, 2007).

Bacterial metabolism relies on the cell carbon and the energy source used for the synthesis of new cell material. As presented in Table 2.3, the microorganisms can be classified based on their metabolism, which is related to the sources of cell carbon, electron donor, electron acceptor and end products produced.

As carbon sources, the microorganisms can obtain the carbon for cell growth from organic matter or carbon dioxide. The organisms that use organic carbon are called heterotrophic, while the organisms consuming carbon dioxide are known as autotrophic. The autotrophic carbon assimilation needs from a reduction process, which requires a net input of energy. Therefore, the autotrophic metabolism demands more energy that the heterotrophic for the synthesis of new cells. As a result, lower yields of cell mass and growth rates are obtained (Sharma, 2007).

Bacteria can oxidize organic or inorganic compounds to gain the energy needed for the cell synthesis, either by using the light or by producing a chemical oxidation reaction. The organisms that have the ability to use the light are called phototrophic, and can be either heterotrophic (sulphur-reducing bacteria) or autotrophic (algae and photosynthetic bacteria). Organisms that take their energy from chemical reactions are called chemotrophs. They can be, as well, autotrophic (nitrifying bacteria) or heterotrophic (protozoa, fungi and most bacteria). Chemoautotrophic obtain their energy from the oxidation of reduced inorganic compounds, such as ammonia, nitrite, ferrous ion and

sulphide. Chemoheterotrophs derive their energy from the oxidation of organic compounds.

Table 2.3 Classification of microorganisms by electron donor, electron acceptor, sources of cell carbon and end products *(after Metcalf and Eddy, 2003)*

| **Type of bacteria** | **Common reaction name** | **Carbon source** | **Electron donor** | **Electron acceptor** | **Products** |
|---|---|---|---|---|---|
| Aerobic heterotrophic | Aerobic oxidation | Organic compounds | Organic compounds | Oxygen | carbon dioxide, water |
| Aerobic autotrophic | Nitrification | Carbon dioxide | Ammonia, nitrite | Oxygen | Nitrite, nitrate |
| | Iron oxidation | Carbon dioxide | Ferrous ion | Oxygen | Ferric ion |
| | Sulphur oxidation | Carbon dioxide | Sulphur, hydrogen sulphide, thiosulphate | Oxygen | Sulphate ion |
| Facultative heterotrophic | Denitrification | Organic compounds | Organic compounds | Nitrite, nitrate | Carbon dioxide, water, nitrogen |
| Anaerobic heterotrophic | Acid fermentation | Organic compounds | Organic compounds | Organic compounds | Volatile fatty acids |
| | Iron reduction | Organic compounds | Organic compounds | Ferric iron | Ferrous iron, dioxide carbon, water |
| | Sulphate reduction | Organic compounds | Organic compounds | Sulphate | Hydrogen sulphide, dioxide carbon, water |
| | Methanogenesis | Organic compounds | Volatile fatty acids | Carbon dioxide | Methane |

The chemical reactions produced by chemotrophs are oxidation-reduction reactions that involve the transfer of electron from an electron donor, which is oxidized, to an electron acceptor, which is reduced. The electron donors and acceptors can be either organic or inorganic compounds. The electron acceptor may be available within the cell during metabolism (endogenous) or it may be obtained from outside the cell, such as dissolved oxygen (exogenous). The production of energy due to the enzymatic transport of electrons to an external electron acceptor is called respiratory metabolism, whereas the use of an internal acceptor is called fermentative metabolism, which yields less energy than respiration. For this reason, heterotrophic organisms that are fermentative present lower growth rates and cell yields than respiratory heterotrophic. If the oxygen is used as electron acceptor, the reaction is said to be aerobic, whereas the reactions involving other electron acceptors are anaerobic. Particularly, when nitrite and nitrate are used as

electron acceptors, the process is considered to be anoxic. Under anoxic conditions, the nitrite and nitrate are reduced to nitrogen gas, in a process known as denitrification. The organisms, which can only use oxygen are called obligate aerobic. The bacteria that can use either oxygen or nitrite/nitrate as electron acceptors are called facultative aerobic. The organisms that can only generate energy by fermentation are said to be obligate anaerobes. Facultative anaerobes have the possibility to perform either aerobic or fermentative respiration, depending on the presence or absence of molecular oxygen. Aerotolerant anaerobes present a strictly fermentative metabolism, but can tolerate the presence of oxygen (Metcalf and Eddy, 2003).

#### 2.3.1.6. Stoichiometry of biological reactions

The microorganisms present in wastewater treatment are both heterotrophic and autotrophic. They can obtain the energy from the oxidation of electron donating molecules, organic and inorganic, respectively. The stoichiometry of the main reactions involved is presented in equations 2.1 to 2.5.

The removal of the organic matter can be accomplished by an aerobic heterotrophic process, in which the organic matter serves as the electron donor and the oxygen as the electron acceptor. The stoichiometry of this reaction (equation 2.1) depends on many factors, especially on the type of substrate and the biomass produced. In this process, new cells are formed (termed as $C_5H_7NO_2$), but as well, existing cells undergo endogenous respiration (equation 2.2). The oxidation of the organic matter into end products produces energy for cell maintenance, whereas the simultaneous biomass synthesis consumes energy. Endogenous respiration is explained in the bibliography through two concepts. The initial linear decay approach is the one represented in equation 2.2 and assumes that in this process, the biomass is completely oxidized into end products (dioxide carbon, ammonia and water) (Metcalf and Eddy, 2003). An alternative approach for modelling decay of heterotrophic biomass is the death-regeneration concept announced by Dold *et al.* (1980). They stated that the biomass is converted into a combination of particulate products and slowly biodegradable substrate, which is then hydrolysed, releasing an equivalent amount of readily biodegradable organic matter. In aerobic conditions, this substrate will be used to form new cells, with the consequent oxygen uptake. In anoxic conditions, cell growth will happen by consuming nitrates. If there is no oxygen or nitrates, no conversion will occur and the slowly biodegradable substrate will accumulate (Henze *et al.*, 2000).

***Heterotrophic oxidation of organic matter and biomass synthesis***

$$\eta_{11}\,(\text{organic material}) + \eta_{21}\,O_2 + \eta_{31}\,NH_3 + \eta_{41}\,PO_4^{3-} \longrightarrow \eta_{51}\,(C_5H_7NO_2) + \eta_{61}CO_2 + \eta_{71}\,H_2O \quad (2.1)$$

Where $\eta_{i\,j}$ is the stoichiometric coefficient of the component i for the reaction j.

***Endogenous respiration (as linear decay)***

$$C_5H_7NO_2 + 5O_2 \longrightarrow 5CO_2 + NH_3 + 2H_2O + Energy \quad (2.2)$$

***Autotrophic oxidation and synthesis-oxidation of ammonia by nitrification***

*Ammonia oxidation and synthesis*

$$55\,NH_4^+ + 76O_2 + 109HCO_3^- \rightarrow 54NO_2^- + C_5H_7NO_2 + 104\,H_2CO_3 + 57H_2O \quad (AOB) \quad (2.3)$$

*Nitrite oxidation and synthesis*

$$400NO_2^- + NH_4^+ + 4H_2CO_3 + HCO_3^- + 195O_2 \rightarrow 400NO_3^- + C_5H_7NO_2 + 3H_2O \quad (NOB) \quad (2.4)$$

*Overall synthesis and oxidation reaction*

$$NH_4^+ + 1.98HCO_3^- + 1.83O_2 \rightarrow 0.98NO_3^- + 0.021C_5H_7NO_2 + 1.041H_2O + 1.88\,H_2CO_3 \quad (2.5)$$

Biological nitrification is an aerobic autotrophic process, in which the energy for bacterial growth is obtained from the oxidation of ammonia nitrogen. As a source of carbon for cell synthesis, carbon dioxide is used. The two main bacteria genera responsible for nitrification are Nitrosomonas and Nitrobacter. However, a variety of

nitrifying bacteria exist in the nature and even, some heterotrophic nitrification could occur (e.g. fungi). The expected growth rates for nitrifying bacteria are much less than for heterotrophic bacteria, since they have a longer generation time of at least 10 to 30 hours. Nitrifying bacteria are also more sensitive to environmental conditions, as well as to growth inhibitors. The growth rate for nitrite oxidizing bacteria (NOB) is much greater than for the ammonia oxidizing bacteria (AOB) at temperatures under 25 ºC. On the contrary, at higher temperatures, AOB exhibit higher growth rates than NOB (Lee and Lin, 2007).

In systems where nitrification takes place, potential denitrification should also be considered. The stoichiometry for denitrification is presented in equations 2.6 to 2.8.

***Heterotrophic denitrification***

*First step for heterotrophic denitrification:*

$$2\,CH_3OH + 6NO_3^- \rightarrow 2CO_2 + 4H_2O + 6NO_2^- \quad (2.6)$$

*Second step for heterotrophic denitrification*

$$3\,CH_3OH + 6NO_2^- \rightarrow 3CO_2 + \,3N_2 + 3H_2O + 6OH^- \quad (2.7)$$

*Overall heterotrophic denitrification reaction*

$$5\,CH_3OH + 6NO_3^- \rightarrow 5CO_2 + \,3N_2 + 7H_2O + 6OH^- \quad (2.8)$$

Denitrification usually happens in anoxic conditions, though it could also take place in aerobic media. Biological denitrification, known as dissimilatory denitrification, is the conversion of nitrates to gaseous nitrogen species and to cell material by heterotrophic facultative aerobic bacteria and some fungi. Denitrifying include bacteria, such as Pseudomonas, Micrococcus, and Bacillus. They can use either nitrate or oxygen as electron acceptor for the conversion of nitrate to nitrogen gas. Denitrification is a two-step process: the first step is a conversion of nitrate to nitrite and the second step converts the nitrite to nitrogen gas. These reactions need an organic carbon source being available. Considering methanol ($CH_3OH$) as the organic carbon source, equations 2.6

to 2.8 represent dissimilatory denitrification reactions (Lee and Lin, 2007), without considering cell synthesis.

#### 2.3.1.7. Bacterial growth

As described in the biological reactions, while the microorganisms consume the substrate and carry out oxidation-reduction reactions, more biomass is produced. Consequently, in wastewater treatment applications, there is a continuous production of biomass. Also, microbial metabolism requires energy for cell synthesis. Therefore, a portion of the electron-donor substrate is used for cell synthesis, while the rest is just used for energy production. Hence, depending on the electron acceptor and donor couple, there is an associated energy production, which determines the biomass yield of the reaction. The biomass true yield or biomass synthesis yield (Y) is typically defined as the ratio of the amount of biomass produced to the amount of substrate consumed (g biomass $g^{-1}$ substrate).

As reflected in the endogenous respiration equation 2.2, the active bacterial cells generated by growth, undergo decay due to maintenance, predation and cell lysis. In fact, during decay, a portion of the active cells become the electron donor to generate more energy and more reaction end products. Consequently, it is useful to report an observed yield ($Y_{obs}$), which should be obtained experimentally, based on the organic matter degraded. For this purpose, nitrogen and phosphorus requirements can be evaluated, taking into account the biomass molecular formula (Mogens *et al*., 2008).

A distinction should be made between $Y_{obs}$ and Y. The biomass observed yield is based on actual measurements of biomass production and substrate consumption. The biomass synthesis yield is the amount of biomass produced from the growth substrate. It cannot be usually directly measured. The synthesis yield for bacterial growth is affected by the energy produced in the oxidation-reduction reactions, by the growth characteristics of the carbon source, by the nitrogen source and by environmental factors, such as temperature, pH and osmotic pressure. The synthesis yield is usually higher than the observed yield due to cell loss by decay (Metcalf and Eddy, 2003).

#### 2.3.1.7.1. Growth phases

Bacterial growth in a batch reactor is characterized by different phases, as illustrated in Figure 2.3. At time zero very small biomass population exists and substrate and nutrients are present in excess. As substrate is consumed, four different growth phases develop sequentially: lag phase, log phase or exponential phase, stationary phase and death phase (Fankhauser, 2004).

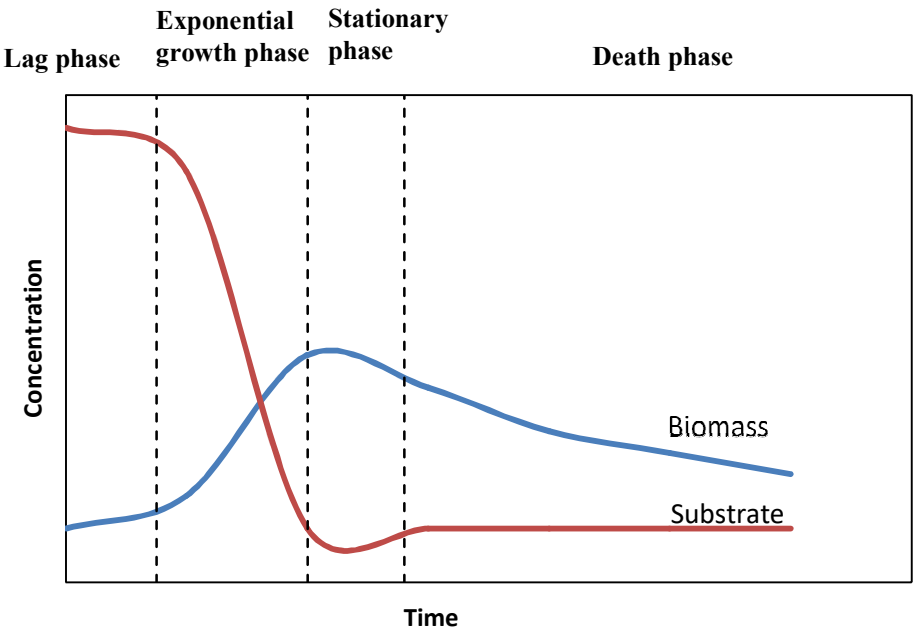



Figure 2.3 Sludge growth stages with the sludge age *(after Metcalf and Eddy, 2003)*

The ***lag phase*** represents the time required for the microorganism to adapt to a new environment before significant biomass production occurs. During this phase, synthesis of RNA, enzymes and other molecules may be occurring, and microorganism should be adapting to pH, temperature, salinity or quality effluent conditions. During this period little or no cell division happens and it can last for 1 hour to several days (Metcalf and Eddy, 2003; Tortora *et al.*, 2013).

The ***exponential growth-phase*** takes place. Here, cells multiply at their maximum rate, as there is no limit due to substrate or nutrients. The biomass growth curve increases exponentially, being temperature the only factor that affects the rate of growth (Panikov, 1995; Metcalf and Eddy, 2003).

Depuration efficiency when operating at this phase is unstable. Also, growing too young microorganisms creates a quality of sludge short in exocellular biopolymer, which helps floc aggregation. Taking to microbiology, flagellates are the dominant protozoa and no filamentous grow at this stage.

Biomass concentration keeps nearly constant with time in the ***stationary phase***. Here the number of cells created is limited by the growth factor and at a time, the growth is offset by the death of cells. At this stage microorganism are mature enough to generate exocellular biopolymer and stand a stable depuration. Microbiology is dominated by ciliates. In case of toxicity, amoebae could also be found. Filaments can also grow, as well as nitrifying bacteria. As far as sludge age increases at this stage, oxygen consumption is increased by endogenous respiration (Metcalf and Eddy, 2003).

In the ***death phase or decline phase***, the substrate has already been depleted, so that no growth occurs, and there's only death cell. In this phase a lot of oxygen is consumed for endogenous respiration. Microorganisms, such as burrowing ciliates, nematodes, mites and rotifers can be observed at this stage (Panikov, 1995).

#### 2.3.1.7.2. Growth kinetics

The performance of the biological processes used for wastewater treatment relies on the dynamics of substrate utilization and microbial growth. In fact, the kinetics of microbial growth governs the oxidation of substrate and the production of biomass.

One of the main concerns in wastewater treatment is the removal of the substrate, which is organic substances for heterotrophic bacteria and ammonia for autotrophic nitrifying bacteria. The rate of substrate utilisation by bacteria depends on factors characteristic of the given microbial group. The most important parameters are the maximum substrate utilisation rate, the half saturation and inhibition constants (Mogens *et al*., 2008).

The equation for the substrate utilisation rate is presented as follows[1]:

$$r_s = K\, M_s\, X \qquad (2.9)$$

[1] The biomass concentration (X) is often identified as the volatile suspended solid concentration (VSS) in the mixed liquor. However, VSS may also include organic matter adsorbed onto the floc. Therefore, if the substrate supplied to the reactor contains important amounts of particulate organic matter, X and VSS may not correspond.

Where

| | |
|---|---|
| $r_s$ | Substrate utilisation rate (g COD $m^{-3}d^{-1}$) |
| $K$ | Maximum specific substrate utilisation rate (g COD $g^{-1}$ VSS $d^{-1}$) |
| $M_s$ | Saturation function for soluble substrate, S (g COD $g^{-1}$ COD) |
| $X$ | Biomass concentration in the reactor (g $m^{-3}$) |

The saturation function ($M_s$) considers the effect of substrate concentration on the rate of reaction. It varies from 0 to 1 as a function of the concentration of substrate available in solution near the biomass.

$$M_s = \frac{S}{K_s+S} \quad (2.10)$$

Where

| | |
|---|---|
| $S$ | Soluble substrate concentration in the reactor (g COD $m^{-3}$) |
| $K_s$ | Substrate half saturation constant (g COD $m^{-3}$) |

The effect of other limiting nutrients (oxygen, ammonia, phosphate) can also be considered in this substrate utilisation rate formula (Mogens *et al*., 2008):

$$r_s = KM_s X \left(M_{O_2}\right)\left(M_{NH_3}\right)\left(M_{PO_4^{3-}}\right) \quad (2.11)$$

Where $M_{O_2}$ , $M_{NH_3}$ and $M_{PO_4^{3-}}$ are the saturation functions for oxygen, ammonia and orthophosphate, respectively.

According to Liebig's law of minimum, however, growth is considered to be limited by just one nutrient. Therefore, it can be just considered the minimum *M*:

$$r_s = KM_s X\ min[\left(M_{O_2}\right)\left(M_{NH_3}\right)\left(M_{PO_4^{3-}}\right)] \quad (2.12)$$

The presence of inhibitory compounds can also be considered, as reflected in equation 2.13, where $I_I$ is the inhibition function for the inhibitory compound expressed in g $g^{-1}$.

$$r_s = K\ I_I\ X \quad (2.13)$$

$$I_I = \frac{S_i}{K_I+S_i} \quad (2.14)$$

Where

$K_I$ Half saturation constant of the inhibitory compound (g $m^{-3}$)

$S_i$ Concentration of the inhibitory compound (g $m^{-3}$)

When the rate of substrate utilisation is at its maximum, the growth rate is also at its maximum and their ratio is, theoretically, that of the true yield. This statement is represented through equation 2.15.

$$\mu_{max} = K\,Y \tag{2.15}$$

Where

$\mu_{max}$ Maximum specific biomass growth rate (g VSS $g^{-1}$VSS $d^{-1}$)

Y Biomass true yield (g VSS $g^{-1}$ COD)

The growth rate of a biomass depends on its rate of substrate utilisation for cell synthesis and on its decay rate, which is proportional to the concentration of biomass present.

$$r_g = Y\,r_s - k_d X \tag{2.16}$$

Where

$r_g$ Biomass growth rate (g VSS $m^{-3}$ $d^{-1}$)

$k_d$ Endogenous decay coefficient (g VSS $g^{-1}$ VSS $d^{-1}$)

Substituting with previous equations:

$$r_g = YK\,M_s\,X - k_d X \tag{2.17}$$

$$r_g = \mu_{max}\,M_s\,X - k_d X \tag{2.18}$$

The specific growth rate is obtained by dividing the growth rate by the biomass concentration.

$$\mu = \frac{r_g}{X} \tag{2.19}$$

Where

$\mu$ Specific biomass growth rate (g VSS $g^{-1}$ VSS $d^{-1}$)

Then,

$$\mu = \mu_{max}\, M_s - k_d \tag{2.20}$$

$$\mu = YK\, M_s - k_d \tag{2.21}$$

From the representation of the last equation, several parameters can be obtained:

- The maximum specific growth rate is obtained at an infinite substrate concentration, at which

$$M_s = \frac{S}{K_s+S} = 1 \quad \text{and} \tag{2.22}$$

$$\mu_{max} = K\, Y - k_d \tag{2.23}$$

- The minimum substrate concentration required, at which the rate of cell synthesis just equals its rate of decay, is when the specific growth rate is zero. It gives:

$$S_{min} = \frac{k_d K_s}{YK - k_d} \tag{2.24}$$

Where

$S_{min}$ Minimum soluble substrate concentration required to achieve a null growth rate (g COD $m^{-3}$)

- At a null substrate concentration (S= 0 g COD $m^{-3}$), the specific growth rate becomes negative and equals the rate of decay.

### 2.3.2. Engineering principles

The activated sludge process relies on five interrelated components: the reactor, the activated sludge, the aeration/mixing system, the clarifier and the returned sludge.

In such a system, the removal of organic matter is accomplished by three mechanisms:

i) Adsorption and agglomeration onto microbial flocs
ii) Assimilation (conversion into new microbial cells)
iii) Mineralization (complete oxidation)

The predominant removal mechanism can be chosen by specific operating conditions (Gray, 2004).

The activated sludge process design requires definition of the desired effluent quality, and a proper characterization of the wastewater to be treated, which is the most critical step of the design. These data determine the volume of the biological reactor, by selecting the required sludge age, in order to deal with the biodegradability of the effluents and accomplish the expected COD removal efficiency. Also, depending on the quality of the effluents and on the reactor operational parameters, such as sludge age, F/M ratio, pH and temperature, the amount of sludge production can be determined. Eventually, as a result of operational parameters, a settling quality of the sludge is obtained. Depending on the clarifier design, the settling sludge is submitted to an upflow velocity, which enhances settling, if it is low enough. The upflow velocity in the clarifier, together with the sludge settling quality and the draw-off flow rate, determine the sludge concentration at the bottom of the clarifier, as well as the quality of the clarified water (Lee and Lin, 2007).

Figure 2.4 shows a typical arrangement for a complete mix activated sludge system with a CSTR as the main equipment. The main operational and design parameters required to control the biological system are reflected.

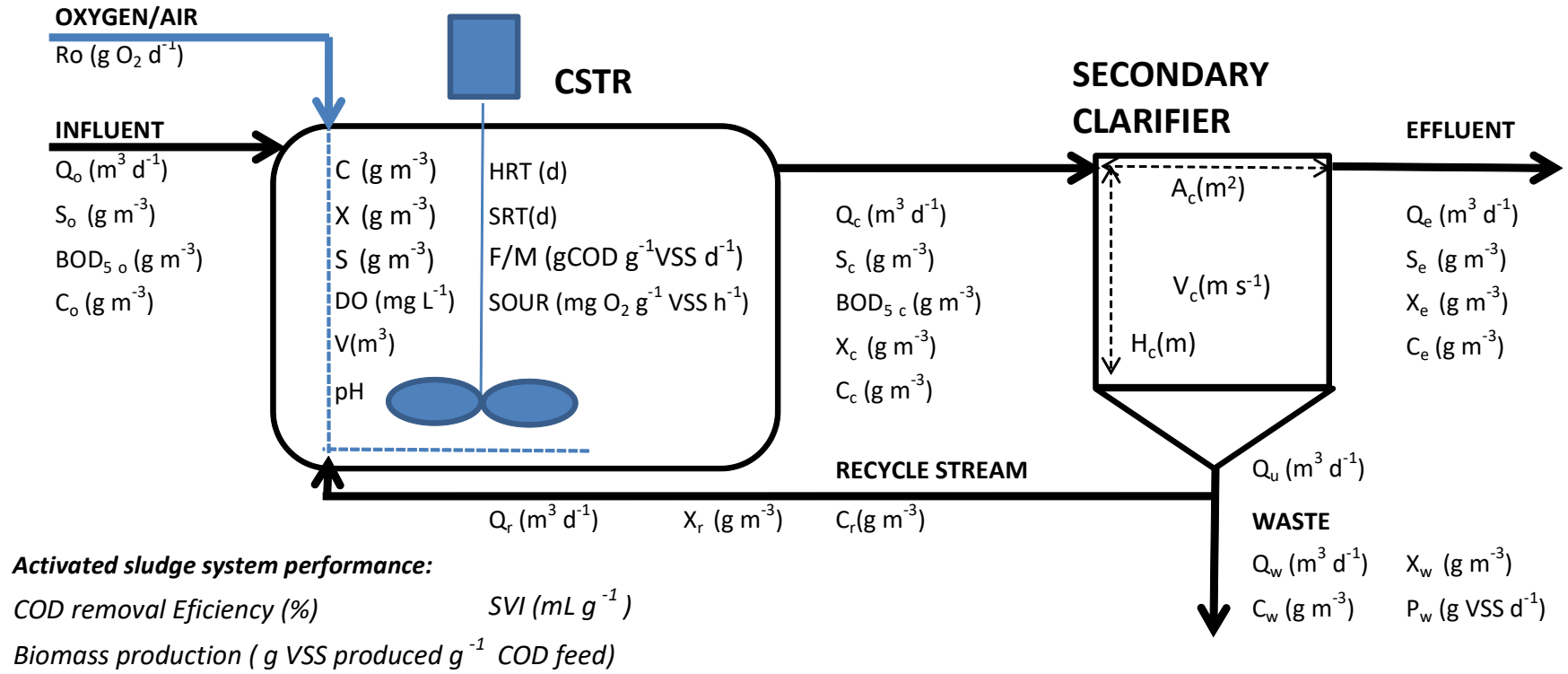



Figure 2.4 General arrangement for a typical activated sludge system. The main operational and design parameters have been included for each equipment and stream.

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Notation | Q | Flow rate | S | Soluble substrate concentration | $BOD_5$ | Five-day biological oxygen demand | C | Suspended solids concentration |
| | T | Temperature | V | Volume | DO | Dissolved oxygen concentration | F/M | Food-to-microorganism ratio |
| | V | Overflow rate | P | Mass flow rate of cells | X | Biomass concentration | R | Oxygen flow rate |
| | A | Cross-flow area | | | | | | |
| Subscripts | o | Influent | - | Reactor | c | Clarifier inlet | e | Effluent |
| | w | Waste | r | Recycle stream | | | | |

### 2.3.2.1. The activated sludge process elements

The activated sludge process elements are described below: biological reactor, activated sludge, air supply, secondary clarifier and recycle stream. The biological reactor, together with the clarifier should be seen as an interactive system (Wilén, 1995).

***Biological reactor*** – The complete mix activated sludge process includes a CSTR, as the main equipment. This configuration is characterized by a homogeneous content of the reactor. Therefore, parameters such as dissolved oxygen or biomass concentration exhibit the same value in every point of the biological reactor including its outlet stream. Consequently, to accomplish a good performance of such reactors, it is necessary to guarantee a good mixing of the mixed liquor, which can be accomplished either by aeration or mechanical agitation. This feature is an important issue, especially in big reactors. At a time, this characteristic allows to withstand shock loads or toxic discharges because of its large dilution capacity. Nevertheless, the organic matter supplied is also dispersed in the total reactor volume, which results in a low food-to-microorganism (F/M) ratio.

The reactor volume must be enough to allocate the necessary aeration capacity and to guarantee the organic matter biodegradation.

***Activated sludge*** – The activated sludge is a mixture of different kinds of microorganisms, mainly bacteria, dead cells and particulate organic and inorganic material. The population of microorganisms present in the mixed liquor depends on the quality of the wastewater being fed into it (Wilén, 1995).

According to their nature, bacteria can basically grow in three different modes: as free or dispersed, as floc-forming and as filaments. Most bacteria in the activated sludge are rod-shaped and gram-negative. Also, as detailed in section 2.3.1.3, other microorganisms, such as fungi, algae, protozoa, and rotifers are present in the media, depending on factors, such as the DO concentration, pH, temperature, sludge's age and wastewater composition. The population of microorganisms in the activated sludge is constantly changing, in order to adapt to the quality of wastewater received (Vallero, 2003).

Bacteria are the microorganisms mainly responsible for degrading the organic matter. Other microorganisms, like protozoa and rotifers degrade small biological flocs and dispersed bacteria. They are, as well, good indicators of the activity and performance of the biological sludge, as described in section 2.3.1.7.1.

Two activities of the microorganisms are necessary to accomplish a good performance of the activated sludge process: the biodegradation and the excretion of exocellular polymers, which promote the flocculation process (Wilén *et al*., 2003). To promote the last objective, section 2.3.2.2 mentions the required operational parameters.

Taking to the organic matter, the dissolved part can be directly uptaken by the microorganisms, while the particulate organic material is as a first step adsorbed onto the sludge flocs. How well the sludge adsorbs the organic material depends on the structure of the activated sludge flocs. Anoxic periods are said to affect negatively the biosorption (Pujol and Canler, 1992).

***Air supply -*** Aeration in the biological reactor should satisfy several objectives:

i) Provide enough agitation to keep the solids in suspension
ii) Supply dissolved oxygen for the biomass activity
iii) Mix the incoming wastewater with the biomass
iv) Strip from solution the excess of carbon dioxide produced in the oxidation of the organic matter.

Therefore, aeration requirements depend on the design of the installation and on the biomass consumption. In turn, the last term can be either estimated experimentally with respirometry tests or calculated with equation 2.25, which considers nitrification and denitrification.

The first term of equation 2.25 accounts for the total mass of organic matter used, whereas the second considers the amount of organic matter as COD incorporated to the new cells produced, based on equation 2.2. The oxygen used for nitrification is included in the third term. The theoretical constant of 4.6 kg $O_2$ ($kg^{-1}NH_4^+$-N oxidized) has been considered, although the observed amount of oxygen consumed is 4.2 or slightly less, due to the fact that some ammonia nitrogen is not oxidized, but assimilated as cellular material ($C_5H_7NO_2$), according to equation 2.5 (Gerardi, 2003). Eventually, the last term accounts for the COD removed in denitrification process (see equation 2.8).

$$R_{or} = Q_o(So - Sc) - 1.42P_w + 4.6\,Q_o\,(NH_4^+ - N)ox - 2.86\,Q_o\,(NO_3^- - N)u \quad (2.25)$$

Where

| | |
|---|---|
| $R_{or}$ | Total oxygen consumed (g $d^{-1}$) |
| $Q_o$ | Influent flow rate to the biological reactor ($m^3$ $d^{-1}$) |
| $S_o$ | Influent COD concentration to the biological reactor (g $m^{-3}$) |
| $S_c$ | Effluent COD concentration from the biological reactor (g $m^{-3}$) |
| 1.42 | COD of the cell tissue (g cell COD $g^{-1}$VSS) |
| $P_w$ | Wasted cells (g $d^{-1}$) |
| 4.6 | Conversion factor for oxygen requirement to oxidize completely ammonia nitrogen (g $O_2$ $g^{-1}NH_4^+$-N) |
| $(NH_4^+ - N)ox$ | Ammonia nitrogen oxidized to nitrate (g $m^{-3}$) |
| 2.86 | Conversion factor for the equivalent oxygen demand to remove the COD consumed in the anoxic process (g $O_2$ $g^{-1}NO_3^-$-N) |
| $(NO_3^- - N)u$ | Nitrogen as nitrate removed (g $m^{-3}$) |

The aeration system is responsible for the gas transfer into the mixed liquor. There are different types of commercialized aeration systems, as reflected in Table 2.4. For air diffusion systems, the air flow rate transferred into the water is known as the Standard oxygen transfer efficiency (SOTE) or Standard oxygen transfer rate (SOTR). On the other hand, mechanical aeration systems are characterized either by SOTR or by Standard aeration efficiency (SAE). These characteristic parameters are provided by the supplier of the aeration system and determined in a performance tests based on ASCE Oxygen Transfer Testing Standard at standard conditions.

Equation 2.26 presents the corrective factors to use, in order to consider the real nature of the wastewater (Metcalf and Eddy, 2003).

$$AOTR = SOTR[\,\beta\,\frac{(DO_{av,S,T,H} - DO)}{DO_{s,20}}1.024^{T-20}\alpha F] \quad (2.26)$$

Where:

| | |
|---|---|
| $AOTR$ | Actual oxygen transfer rate under field conditions (kg $O_2$ $h^{-1}$) |
| $SOTR$ | Standard oxygen transfer rate in clean water (kg $O_2$ $h^{-1}$). |
| β | Correction factor for salinity and surface tension<br>$\beta = \frac{C_s(\text{wastewater})}{C_s(\text{clean water})}$<br>Typically 0.7 to 0.98, being 0.95 the usual value for wastewater. |
| $DO$ | Dissolved oxygen concentration in the biological reactor |

| | |
|---|---|
| $DO_{s,20}$ | Saturation concentration for dissolved oxygen in clean water at 20 ºC y 1 atm (mg $L^{-1}$) |
| $T$ | Operational temperature (ºC) |
| F | Fouling factor (usually between 0.65 and 0.9). |
| α | Oxygen transfer correction factor for wastewater. Correction factor, that considers the effect of mixing intensity and tank's geometry. $\alpha = \frac{K_L a \text{ (wastewater)}}{K_L a \text{ (clean water)}}$ Typical values for diffusion and mechanical aeration systems are 0.4 to 0.8 and 0.6 to 1.2, respectively. |
| $DO_{av.s,T,H}$ | Average dissolved oxygen saturation concentration in clean water in aeration tank at temperature T and altitude H (mg $L^{-1}$) Equation 2.27 reflects the calculation. |

$$DO_{av.s,T,H} = DO_{s,T,H}\ \frac{1}{2}\left(\frac{P_d}{P_{atm,H}} + \frac{O_t}{21}\right) \tag{2.27}$$

Where:

| | |
|---|---|
| $DO_{s,T,H}$ | Oxygen saturation concentration in clean water, at temperature T and altitude H (mg $L^{-1}$) |
| $P_d$ | Pressure at the depth of air release (kPa) |
| $P_{atm,H}$ | Atmospheric pressure at altitude H (kPa) |
| $O_t$ | Percent oxygen concentration leaving tank, usually 18 to 20 % and 1 atm (mg $L^{-1}$) |

The design of the aeration system determines the organic matter removal capacity of the activated sludge process. A discussion arises when selecting aeration equipment on whether to prioritize an easy maintenance or flexibility in capacity. Whereas porous diffusers exhibit a good flexibility, they are also bound to water side clogging. As a solution, removable systems are being designed, in order to avoid stopping the process for cleaning purposes. On the other hand, non-porous diffusers do not usually clog, but they are designed for a particular air flow rate. Therefore, if a biological reactor is being upgraded, it should be taken into account the aeration system configuration, since it may determine the feasibility to implement a solution.

Table 2.4 Characteristics of aeration equipment *(after Water Environmental Federation, 2005)*

| **Equipment type** | **Characteristics** | **Advantages** | **Disadvantages** | **Reported clean water performance[1]** |
|---|---|---|---|---|
| Porous diffusers | Fine bubble ;Materials for diffusers (ceramic, plastic, flexible membranes) ;Configurations for diffusers (dome, disc, plate) | High efficiency; Good flexibility (turn-down about 5:1); Often removable | Air or water-side possible clogging; Usually require air filtration; High initial cost | SOTE: 13-40% |
| Nonporous diffusers | Coarse bubble; Types (fixed orifice, perforated pipe, sparger, slotted tube, valved orifice, static tube) | Not usual clogging; Easy maintenance | Low oxygen transfer efficiency; High initial cost | SOTE: 9-13% |
| Jets | Fine bubble; Compressed air and pumped liquid mixed in nozzle and discharged | Good mixing; High oxygen transfer efficiency | Limited geometry; Requires blowers, pumps and primary treatment | SOTE: 15-24% |
| Low speed mechanical surface equipment (20-60 rpm) | Large diameter turbine with gear reducer; Radial flow; Floating, fixed-bridge or platform mounted | Operational flexibility; High pumping capacity | Higher initial costs than high speed mechanical aerators; Maintenance with gear reducer | SAE:1.2-2.7 kg $kw^{-1}$ $h^{-1}$ |
| High speed mechanical surface equipment (300-1200 rpm) | Direct, motor-driven units mounted on floating structure. Small diameter propeller; Axial flow | Flexible operation; Low initial cost | Poor maintenance accessibility; Mixing capacity may be inadequate | SAE:1.2-1.5 kg $kw^{-1}$ $h^{-1}$ |
| Submerged turbine | Low-speed turbine and compressed air to diffuser rings, open pipe or air draft | Operational flexibility; Valid for deep tank; Good mixing; High capacity input per unit value | Require gear reducer and blower; High total power requirements; High cost | SAE:1.0-1.8 kg $kw^{-1}$ $h^{-1}$ |

[1]Manufacturers data in clean water and standard conditions (20ºC, 0.0 mg $L^{-1}$ dissolved oxygen and 760 mmHg)

***Secondary clarifier -*** In the activated sludge process, sludge settling occurs via bioflocculation and gravity settling. Therefore, the effectiveness of the separation process is dependent on the physical characteristics of the sludge floc, the degree of bioflocculation and the physical characteristics of the mixed liquor. The performance of the clarifier is evaluated by the quality of the clarified water obtained, as well as by the thickness of the sludge separated at the bottom.

Depending on the degree of interaction between the particles, the settling process can be divided into four zones (Metcalf and Eddy, 2003): I) Discrete particle zone II) Flocculation zone III) Hindered zone IV) Compression zone.

In the discrete zone the particles settle independently of each other, according to Newton's law, which is based on the assumption that particles are spherical and with homogeneous diameters. When the particle settles, it accelerates until its effective weight equals the resistance offered by the liquid, because of friction. This point determines the settling velocity ($v_c$). Hazen (1904) developed an ideal horizontal flow model to describe the clarification process in the discrete particle zone. In the design of clarifiers, the procedure is to select a particle with a settling velocity ($v_c$), and design the clarifier so that all the particles that have a terminal velocity equal or greater than $v_c$ will be removed. Therefore, the minimum design area for the clarifier is calculated as stated in equation 2.28.

$$A_c = \frac{Q_c}{v_c} \tag{2.28}$$

Where

| | |
|---|---|
| $Q_c$ | Inlet flow rate to the clarifier ($m^3\ s^{-1}$) |
| $A_c$ | Cross-section area of the clarifier ($m^2$) |
| $v_c$ | Upflow velocity or overflow rate ($m\ s^{-1}$) |

For continuous-flow sedimentation, the depth of the clarifier and the detention time should be such that all particles with the design velocity $v_c$ will settle. The three parameters are related as follows.

$$v_c = \frac{depth}{detention-time} \tag{2.29}$$

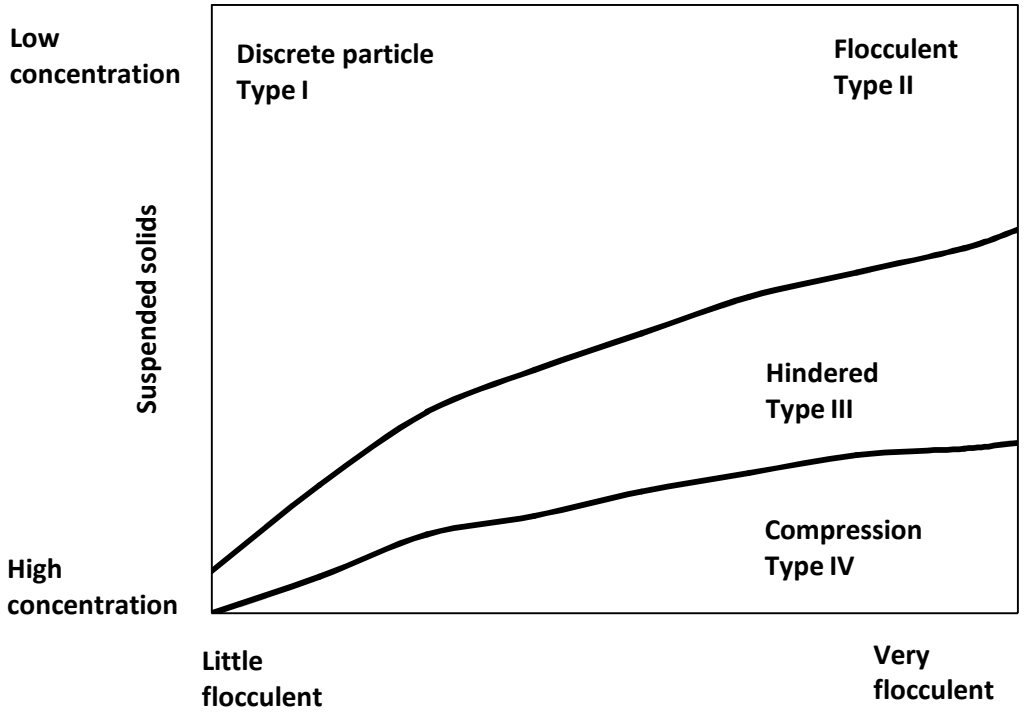



Figure 2.5 Classification of the different types of settling depending on the suspended solids concentration and the degree of flocculation *(after Ekama et al., 1997)*

Fitch (1979) proposed a model, which explains the flocculation zone settling. In this theory, particles in relatively dilute solution coalesce during sedimentation. As coalescence or flocculation occurs, the mass of the particle increases and it settles faster. The extent to which flocculation occurs is dependent on the opportunity for contact, which varies with overflow rate, depth of the basin, velocity gradients in the system, concentration of particles and range of particle sizes. The extent to which flocculation occurs can only be determined by sedimentation tests.

As the concentration of particles increases, the solids begin to interact within each other and this also hinders sludge settling. In these cases, hindered or zone settling, as well as compression settling occurs. Because of the high concentration of particles, the liquid tends to move up through the interstices between the close particles. As a result, the contacting particles tend to settle as a zone or a blanket, keeping always the same position with respect to each other. As the particles settle, a clear layer of water is obtained above the particles in the settling region. The velocity of settling in the hindered region depends on the concentration of solids and their characteristics. When hindered and compression settling happen, experimental tests on a batch settling column are usually required to determine the settling characteristics (Metcalf and Eddy, 2003).

On the basis of experimental observations, two approaches can be considered to obtain the design area of the clarifier: i) *The Solids Flux Theory* developed by Kynch (1952), and ii) *The graphical method of Talmadge and Fitch* (1955).

In the *Solids Flux Theory*, it is considered that when the clarifier is operated at a steady state, a constant flux of solids is transported downwards by two mechanisms, the flow due to gravity and the flow due to underflow of return sludge. The flux of solids may vary depending on the characteristics of the sludge. For this reason, column settling experimental tests are necessary to determine the relationship between the sludge concentration and the settling rate. Therefore, for each suspended solid concentration, the initial settling velocity is determined experimentally. This data allows to calculate the total flux of solids ($SF_t$) through the clarifier, as the sum of the fluxes caused by gravity ($SF_g$) and sludge waste at the bottom of the clarifier ($SF_u$). The parameters $SF_t$, $SF_g$ and $SF_u$ are calculated with equations 2.30 to 2.33.

$$SF_g = C_i V_i \tag{2.30}$$

Where

| | |
|---|---|
| $SF_g$ | Solids flux in the clarifier due to gravity (kg $m^{-2}$ $h^{-1}$) |
| $C_i$ | Concentration of solids in the clarifier at point i (g $m^{-3}$) |
| $V_i$ | Settling velocity in the clarifier at point i (m $h^{-1}$) |

$$SF_u = C_i U_b = \frac{C_i Q_u}{A_c} \tag{2.31}$$

Where

| | |
|---|---|
| $SF_u$ | Solid flux in the clarifier due to underflow (kg $m^{-2}$ $h^{-1}$) |
| $C_i$ | Concentration of solids in the clarifier at point i (g $m^{-3}$) |
| $A_c$ | Cross-section area of the clarifier ($m^2$) |
| $Q_u$ | Clarifier's underflow volumetric rate ($m^3$ $h^{-1}$) |
| $U_b$ | Bulk downward velocity (m $h^{-1}$) |

$$SF_t = SF_g + SF_u \tag{2.32}$$

Figure 2.6 shows the graphical representation of $SF_t$, $SF_g$ and $SF_u$ for the different suspended solid concentrations tested.

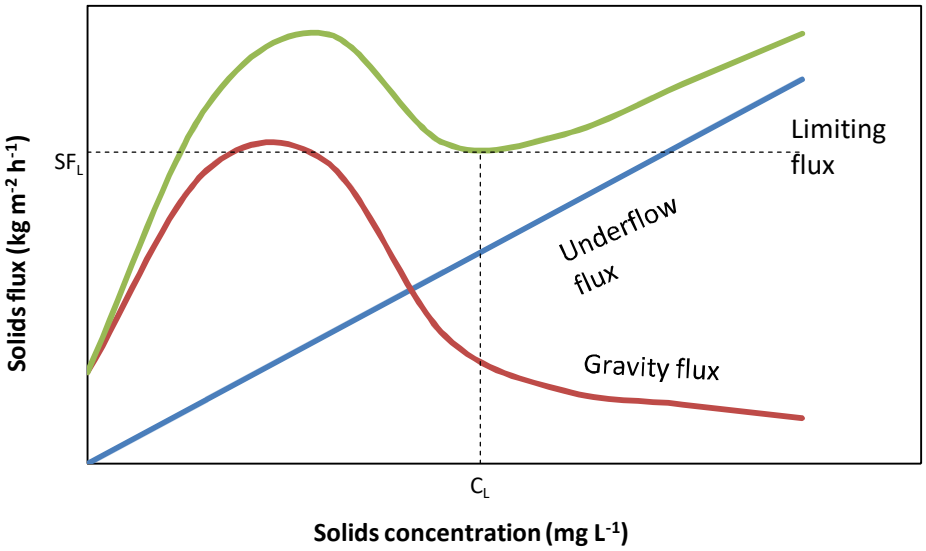



Figure 2.6 Graphical analysis of settling data for the solids flux method *(after Metcalf and Eddy, 2003)*

The flux of solids due to gravity settling depends on the concentration of solids and their settling characteristics. At low solid concentrations, the movement of solids due to the gravity is small, because the settling velocity of the solids is independent of the concentration. As the solid concentration increases, the gravity settling velocity increases also, until high solids concentration where the velocity approaches zero. The solids flux due to bulk transport is a linear function on the solids concentration, with a slope coincident with the underflow velocity ($U_b$). The underflow velocity can be varied, and for this reason is used for control. When the two solid curves are added, a local minimum is identified. It is the limiting flux, so that, if more solids are fed to the clarifier, the solids will eventually fill up the clarifier *(*Metcalf and Eddy, 2003). The limiting solids flux ($SF_L$) is used for the calculation of the required cross-sectional area for thickening, as given by equation 2.33.

$$A_c = \frac{(Q_e+Q_u)C_c}{SF_L} \tag{2.33}$$

Where

| | |
|---|---|
| $A_c$ | Cross-sectional area of the clarifier ($m^2$) |
| $(Q_e + Q_u)$ | Total volumetric flow rate out of the settling basin (overflow and underflow) ($m^3\ d^{-1}$) |
| $C_c$ | Influent solids concentration to the clarifier ($g\ m^{-3}$) |
| $SF_L$ | Limiting solids flux ($kg\ m^{-2}\ d^{-1}$) |

To conclude, the Solid Flux Theory allows to calculate the necessary area of the clarifier to obtain a good clarification for a certain suspended solid concentration. Nevertheless, this model assumes several hypotheses: i) The settling velocity of a particle depends only on the local concentration of solids. Hence, the forces acting on each particle are equilibrated, ii) All the particles have the same shape, size and density, and iii) The concentration of particles is constant in each horizontal section of the clarifier.

On the other hand, *the method of Talmadge and Fitch* allows to calculate the area of the clarifier required to obtain a thickening of the sludge. The methodology is based on an experimental settling curve, as represented in Figure 2.7, for a suspended solid concentration at the entrance of the clarifier ($C_c$). The graphical procedure represented is applied to the settling curve, in order to obtain the solids concentration, $C_2$, which is the critical concentration to determine the solid's handling capacity of the clarifier. The desired concentration of solids at the bottom of the clarifier is chosen ($C_u$). A mass balance as detailed in equation 2.34, is performed to determine the corresponding interface height ($H_u$) .

$$H_u = \frac{H_o C_o}{C_u} \tag{2.34}$$

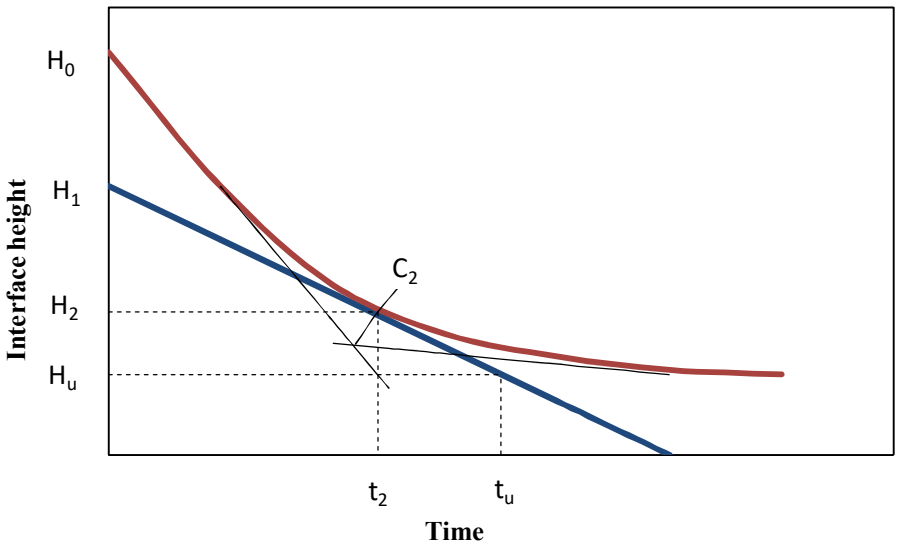



Figure 2.7 Graphical analysis of the hindered interface settling curves *(after Metcalf and Eddy, 2003)*

Known $H_u$, a tangent to the settling curve at this point determines $t_u$, which is the time necessary to obtain the desired solid underflow concentration. The area required to thicken is expressed by equation 2.35.

$$A_t = \frac{Q_c t_u}{H_o} \tag{2.35}$$

Where

| | |
|---|---|
| $A_t$ | Area required to thicken the sludge ($m^2$) |
| $Q_c$ | Inlet flow rate to the clarifier ($m^3\ s^{-1}$) |
| $H_o$ | Initial height of interface in column (m) |
| $t_u$ | Time to reach desired underflow concentration (s) |

To conclude, this methodology is assuming the hypothesis that the settling velocity at the critical point is coincident to that at the bottom outlet of the clarifier, since the same tangent slope is considered. This is only truth if both points are coincident.

Once both methodologies have been applied, the biggest area of the clarifier should be considered for design.

***The recycle stream-*** The recycle stream provides the reactor with biomass coming from the bottom of the clarifier. The recycle stream allows to control the biomass balance in the reactor, in order to ensure the necessary biomass concentration to biodegrade the organic matter fed into the reactor. The required recycle flow rate depends on the flow rate feed.

#### 2.3.2.2. Key operational and design parameters

Key operational and design parameters in the biological system should be tuned upon the effluent's quality, so that all specification requirements are met in the final effluent (Jenkins *et al*., 2003). The main variables to be considered in a complete mix activated sludge system are: sludge retention time, hydraulic retention time, recycle ratio, volatile suspended solid concentration in the mixed liquor, food-to-microorganism ratio and oxygen and nutrients concentration.

***Sludge retention time (SRT) -*** The SRT represents the average period of time during which the sludge remains in the system, understood as the reactor and the clarifier. However, the calculation is often simplified by considering only the biomass in the reactor, since it may have a much bigger volume than the clarifier (Metcalf and Eddy, 2003). Equation 2.36 shows the SRT calculation, which relates the biomass in the reactor to the wasted biomass *(*Lee *et al*., 2007).

The SRT determines the age of the sludge and consequently, sludge production and oxygen requirements.

The SRT selection mainly depends on the biodegradability of the effluents and operational temperature.

$$SRT = \frac{V\,X}{Qw\,Xw + Qe\,Xe} \tag{2.36}$$

Where

| | |
|---|---|
| $SRT$ | Sludge retention time (d) |
| V | Reactor's volume ($m^3$) |
| $X$ | Biomass concentration in the reactor (g $m^{-3}$) |
| $Q_w$ | Waste flow rate ($m^3$ $d^{-1}$) |
| $X_w$ | Biomass concentration in the waste (g $m^{-3}$) |
| $Q_e$ | Effluent flow rate ($m^3$ $d^{-1}$) |
| $X_e$ | Biomass concentration in the effluent (g $m^{-3}$) |

SRT is the operational factor giving control over sludge activity, and it is varied by changing the sludge waste rate. Hence, the sludge age determines the dominant growth stage in the biological system. A short SRT produces sludge with a high growth rate and unstable depuration. For this reason, this condition is often associated to pre-treatment or partial treatment units. On the contrary, a high SRT produces low growth rate sludge and high organic matter removal efficiency. For this reason, this condition is often used in extended aeration systems. However, at high SRT values, filamentous and nitrifying bacteria are bound to appear and difficult sludge settling. Figure 2.8 presents the predominant microorganisms with the sludge age of the system, consistently with the description of each species included in section 2.3.1.3.

Based on the description of the growth phases presented in section 2.3.1.7.1, the operational SRT should be chosen shortly after the start of the stationary phase, aiming for a stable organic matter removal and a good sludge settling helped by exocellular

biopolymer aggregation. Also, oxygen consumption is optimized at this point. In turn, the curve of the growth phases depends on the biodegradability of the effluents. Consequently, the optimum operational SRT is dependent on the effluent's BOD content.

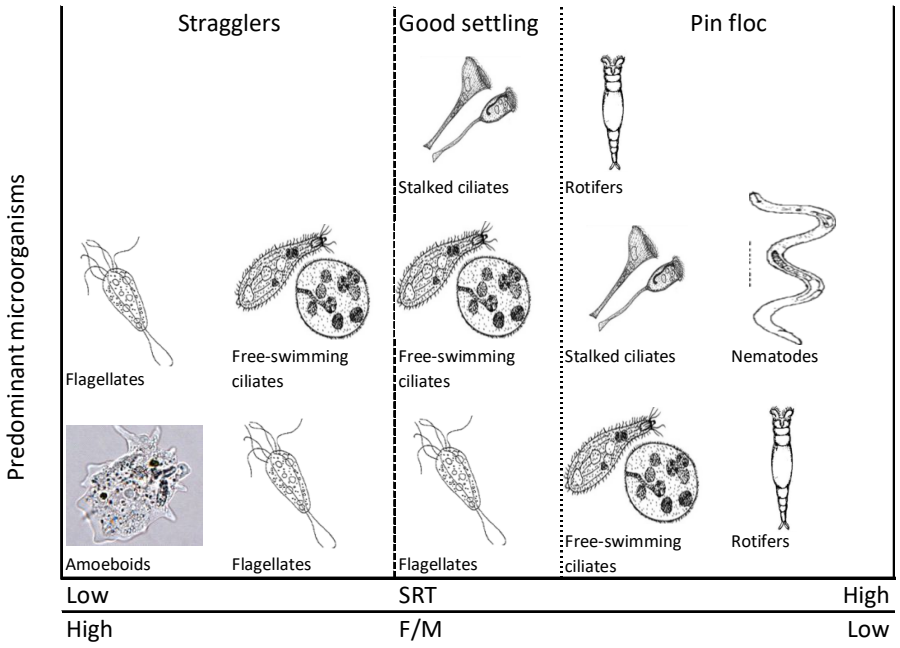



Figure 2.8 Predominant microorganisms based on sludge retention time (SRT) and food-to- microorganism ratio (F/M) *(adapted from Woodard and Curran, 2011)*

Conventional activated sludge processes focused on organic matter removal are referred to exhibit good depuration and settling at SRT values of 3 to 4 days. Long sludge ages, over 6 days, are said to result in poor sludge settling properties (Gray, 2004).

***Hydraulic retention time (HRT) -*** HRT determines how long the water keeps staying in the system, available to be depurated by microorganisms. Less biodegradable streams require longer HRT to be degraded. Or, in other words, if the system is operated at a shorter SRT, higher biomass concentration in the reactor is needed to degrade the same amount of organic matter.

The HRT, also called volumetric loading, is calculated as the relationship between the influent flow rate and the volume of the aeration tank (see equation 2.37).

Consequently, HRT does not take into account the recycled activated sludge flow to the aeration tank, which can be about 25-50% of the total flow (Gray, 2004). Therefore, the real HRT is much less than calculated. For this reason, this calculation is referred to as nominal retention time.

$$HRT = \frac{V}{Q_o} \tag{2.37}$$

Where

| | |
|---|---|
| $V$ | Reactor volume ($m^3$) |
| $Q_o$ | Influent flow rate to the reactor ($m^3\ d^{-1}$) |
| $HRT$ | Hydraulic retention time (d) |

The HRT must be long enough to allow the required degree of adsorption, flocculation and mineralization to occur. As a reference, in conventional plants, the HRT is about 5 h at dry weather flow (DWF), while during storm, when the loading increases to 3 DWF, with maximum sludge recycle, the actual HRT may be as short as 1 h (Gray, 2017).

***Recycle ratio (r) -*** The recycle ratio corresponds to the returned activated sludge flow rate ($Q_r$) divided by the influent flow rate ($Q_o$) to the reactor, as presented in equation 2.38.

$$r = \frac{Q_r}{Q_o} \tag{2.38}$$

Typical ratios are 0.25 to 0.5 for conventional systems. Also ratios between 0.75 and 1.5 are considered for extended aeration systems (Gray, 2004).

Among other parameters, the necessary recycle ratio depends on the growth rates in the biological reactor, which is determined by the sludge age and the nature of the feed streams.

***Volatile suspended solids in the mixed liquor (VSS) -*** Often the term VSS is identified with the biomass concentration in the mixed liquor, although in fact, it also includes any volatile organic solids in the floc. Consequently, it is worth evaluating if the feed streams supply any volatile suspended solids, which could interfere positively with the conventional analytical method to characterize biomass (described in section 5.1.3.1).

The biomass concentration should be enough to degrade the quantity and quality of the organic matter supplied to the biological reactor. When raising the biomass concentration in the reactor, the oxygen requirements increase. Also, upon described for secondary clarifier's design in section 2.3.2.1, a bigger clarifier is needed.

The biomass balance in the reactor is controlled by the flow of a high concentration recycle stream from the bottom of the clarifier. The necessary flow rate to keep the balance depends on the reactor growth yield and the hydraulic load. The mass balance equation 2.39 explains the relationship between VSS, SRT and HRT (Lee *et al.,* 2007).

$$VSS = \left(\frac{SRT}{HRT}\right)\frac{Y\,(So-S)}{1+Kd\;SRT} \qquad (2.39)$$

Where

| | |
|---|---|
| $VSS$ | Mixed liquor volatile suspended solid concentration (g $m^{-3}$) |
| $SRT$ | Sludge retention time (d) |
| $HRT$ | Hydraulic retention time (d) |
| $Y$ | Biomass true yield (mass of cell formed per mass of substrate consumed) |
| $So$ | Influent COD concentration to the reactor (g $m^{-3}$) |
| $S$ | Effluent COD concentration from the reactor (g $m^{-3}$) |
| $K_d$ | Endogenous decay coefficient (typical value is 0.06 $days^{-1}$) |

Therefore, if the quantity of substrate to be degraded is increased, either VSS and/or SRT and/or HRT should also be increased. If the nature of the feed effluents changes to a lower biodegradability, also larger SRT and/or HRT and/or VSS will be necessary to accomplish the same depuration efficiency.

The term suspended solids (SS), or total suspended solids (TSS), includes VSS and also other inorganic molecules. The ratio VSS-to-TSS is process specific, although a common value of 0.75 is referred in the bibliography (Wentzel *et al*., 2002). References indicate that a normal SS range is 1.5 to 3.5 g $L^{-1}$ for conventional activated sludge systems, rising to 8 g $L^{-1}$ for high-rate systems (Gray, 2004).

***Food-to-microorganism ratio (F/M)*** **-** The F/M ratio in the biological reactor is calculated as the relationship between the mass of daily organic matter supplied and the total mass of biomass in the reactor. Equation 2.40 expresses this calculation by

introducing the term *Organic Loading*, which is defined in equation 2.41 as the biochemical oxygen demand load in relation to the tank capacity (Gray, 2017).

$$\frac{F}{M} = \frac{OL}{X} \tag{2.40}$$

Where

$\frac{F}{M}$ Food-to-microorganism ratio (kg BOD $kg^{-1}$VSS $d^{-1}$)

$OL$ Organic loading (kg BOD $m^{-3}$ $d^{-1}$)

$X$ Biomass concentration in the reactor (g $m^{-3}$)

$$OL = \frac{BOD_L}{V} \tag{2.41}$$

Where

$BOD_L$ Biological Oxygen Demand load (kg BOD $d^{-1}$)

$V$ Reactor's volume ($m^3$)

References announce usual values of OL within the range of 0.4 to 1.2 kg BOD $m^{-3}$ $d^{-1}$ for conventional units, while high rate systems exhibit ratios over 2.5 kg BOD $m^{-3}$ $d^{-1}$ and extended aeration systems present figures below 0.3 kg BOD $m^{-3}$ $d^{-1}$ (Gray, 2017).

An alternative approach is to consider the F/M ratio related to the COD supplied instead of the BOD (Spellman, 2013). Current values for this parameter range from 0.1 to 1.0 kg COD $kg^{-1}$ VSS $d^{-1}$.

As it was presented in Figure 2.8, the F/M ratio is linked to the SRT. Therefore, the operational F/M ratio determines the microbiology to be found in the biological system. When the F/M ratio is high, the microorganisms are in the exponential growth phase, with maximum rate of metabolism and large removal of BOD. However, under these conditions, microorganisms do not form flocs and are dispersed, making it difficult for sludge settling. Since food is in excess, not all the substrate is used, so that the final effluent may be high in BOD content. Also, too high F/M ratios can cause toxicity and inhibition. On the contrary, with low F/M ratios, there are food-limited conditions, which rapidly make the rate of metabolism to decline until the microorganisms are in the endogenous respiration phase with cell lysis and eventually, re-synthesis taking place. Almost complete oxidation of substrate occurs, producing a low-biodegradable effluent. If F/M happens to be too low, filamentous bacteria can proliferate, causing sludge settling problems. Consequently, the F/M ratio is another critical variable to

determine the biological system, and therefore, the quality of depuration and sludge settling.

***Oxygen and nutrient concentration -*** To ensure the proper operation of the biological treatment, enough oxygen and nutrients should be supplied to the biological reactor. A way to ensure sufficiency is to check the mixed liquor for a positive residual of 2 mg $L^{-1}$ in these parameters. The main nutrients to be considered are nitrogen and phosphorus, which should be fed as ammonia and orthophosphate, respectively.

Nutrients consumption is related to the quantity of organic load fed into the biological reactor, as well as to its biodegradability, since both factors determine the biomass growth rate (equation 2.1).

The biomass oxygen requirements can be estimated through equation 2.25.

### 2.3.3. Chemistry principles: The water chemistry

Several properties of wastewater, derived from those of water, should be considered in order to get to know its behaviour.

Water is capable of dissolving many substances due to its dipolar nature (having opposite charges at opposite ends of the molecule), ranging from inorganic salts to organic compounds. This fact is responsible for its conductivity and the osmotic pressure phenomenon.

Additionally, some types of matter can be dispersed in water, though they are not truly solubilized. The dispersion of this matter into colloidal systems is accomplished by breaking down the material into extremely small particles, slightly larger than ions and molecules (Flynn, 2009).

Taking to other properties, high surface tension, due to hydrogen bonding and viscosity are to be considered in wastewater treatment, since both have an effect on sludge settling ability.

Also to be considered is the temperature effect, thanks to which dissolved salts and gases can diffuse more rapidly through warmer water, so that chemical treatment is hastened and the physical properties of sedimentation and degasification are faster.

Increasing temperature has also a linear and exponential effect, respectively, on surface tension and viscosity, decreasing both parameters (Flynn, 2009).

#### 2.3.3.1. Solubility of gases in water

The solubility of a gas in a water solution depends on several factors: i) The solubility of the gas as defined by Henry's law, ii) The partial pressure of the gas in the atmosphere, iii) The temperature of water and iv) The concentration of impurities in water (salinity, suspended solids).

Henry's law states that the amount of gas that can be dissolved in water at a given temperature depends on the type of gas and is directly proportional to the partial pressure of the gas above the water surface (Flynn, 2009).

$$P_g = H' X_g \tag{2.42}$$

Where

| | |
|---|---|
| $P_g$ | Partial pressure of gas in air (atm) |
| $H'$ | Henry's law constant (atm (mol gas mol$^{-1}$ air) (mol gas mol$^{-1}$ water)$^{-1}$) |
| $X_g$ | Mole fraction of gas in water (mol gas (mol gas + mol water)$^{-1}$) |

The Henry law's constant is a function of the type of gas, temperature and nature of the liquid.

The solubility of air, oxygen, ammonia and carbon dioxide, as gases, is especially important in wastewater treatment. As reflected in equation 2.1, aerobic bacteria need oxygen and assimilate ammonia to biodegrade the organic matter, whereas $CO_2$ is produced in the biological reaction.

The oxygen to the biological reaction can be provided either with air or with pure oxygen supply. Figure 2.9 shows that oxygen exhibits a higher solubility in water than air. Moreover, the gas solubility tends to decrease when increasing the temperature. Therefore, the availability of oxygen in a biological treatment tends to be more critical in the summer, due to higher ambient temperatures and greater biological activity of the biomass.

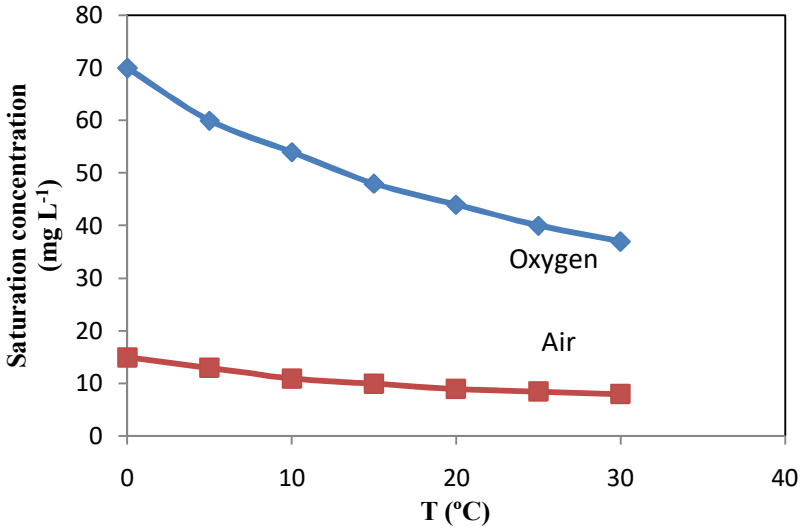



Figure 2.9 Oxygen and air saturation concentration in water as a function of temperature, at 1013 mbar

Ammonia nitrogen exists in aqueous solutions as ammonium ion ($NH_4^+$) or ammonia gas ($NH_3$), depending on the pH of the solution. Equation 2.43 shows the equilibrium reaction.

$$NH_4^+ \leftrightarrow NH_3 + H^+ \tag{2.43}$$

Considering the acid ionization constant (Ka), which holds a value of $10^{-9.25}$, Figure 2.10 presents the distribution of ammonia species as a function of pH. As the pH increases from 7 to 13, the ammonia gas concentration also increases. Consequently, if the biological treatment is operated at a pH over 8.0, undesired ammonia gas stripping by aeration may occur.

Carbon dioxide ($CO_2$) is supplied to wastewater through the biological reaction and the feed wastewater streams coming from advanced oxidation processes (AOP). Dioxide carbon reacts with water when it is in solution, to form carbonic acid ($H_2CO_3$), which ionizes to produce protons ($H^+$), bicarbonate ($HCO_3^-$) and carbonate ($CO_3^{2-}$) ions, depending on the pH. Equation 2.44 presents the equilibrium reactions established between these species. As a consequence, the carbonate system equilibrium provides a buffer effect on the water solution where it is present.

$$CO_2 + H_2O \overset{K_{a1}}{\leftrightarrow} H_2CO_3 \overset{K_{a2}}{\leftrightarrow} H^+ + HCO_3^- \overset{K_{a3}}{\leftrightarrow} H^+ + CO_3^{2-} \tag{2.44}$$

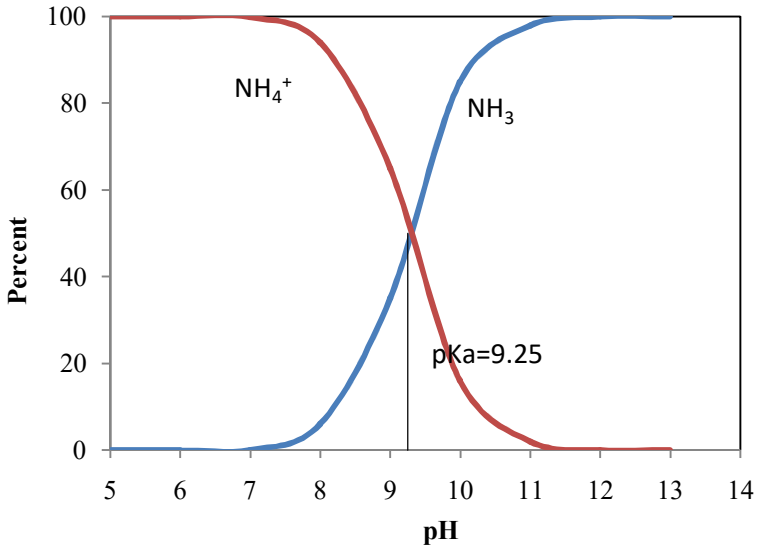



Figure 2.10 Distribution of ammonia ($NH_3$) and ammonium ion ($NH_4^+$) as a function of pH *(after Metcalf and Eddy, 2003)*

The corresponding equilibrium constants are $K_{a1}$= $10^{-2,8}$ , $K_{a2}$= $10^{-6,3}$ , $K_{a3}$= $10^{-10,3}$

The amount of dissolved $CO_2$ in water depends on the pH value and the alkalinity of water. The balance pH, alkalinity and $CO_2$ in water are related to conditions, such as temperature, partial pressure of $CO_2$ and total alkalinity. The total alkalinity is expressed as T and is calculated as indicated in equation 2.45.

$$T = OH^- + HCO_3^- + 2CO_3^{2-} - H^+ \quad (2.45)$$

The partial alkalinity at the phenolphthalein end point (about 8.3) is expressed as P and is calculated with equation 2.46.

$$P = OH^- + ½\, CO_3^{2-} \quad (2.46)$$

The M alkalinity refers to the pH indicator methyl orange, having as end point a pH about 4.3. It includes the ions included in equation 2.47.

$$M = OH^- + HCO_3^- + CO_3^{2-} \quad (2.47)$$

Eventually, the hydrate or caustic alkalinity is expressed as O and corresponds to the hydroxyl ions ($OH^-$), as reflected in equation 2.48.

$$O = OH^- \quad (2.48)$$

M and O equations are only valid when alkalinity is due to carbonate and hydroxide, so that other species like phosphate or ammonia are negligible.

The presence of the different carbonate species ($CO_2$, $HCO_3^-$, $CO_3^{2-}$) is related to the pH and a simplified approximation is represented in the Figure 2.11. The following pH values are the transitions points: i) At 8.3 the carbonate concentration is near 0, ii) At 4.3 all alkalinity is near 0, iii) There are also two transitions around pH 10, so that above 10, $HCO_3^-$ is considered negligible and below 10, $OH^-$ is considered negligible. This approximation may be subject to interferences, especially in contaminated waters with a high ammonia concentration.

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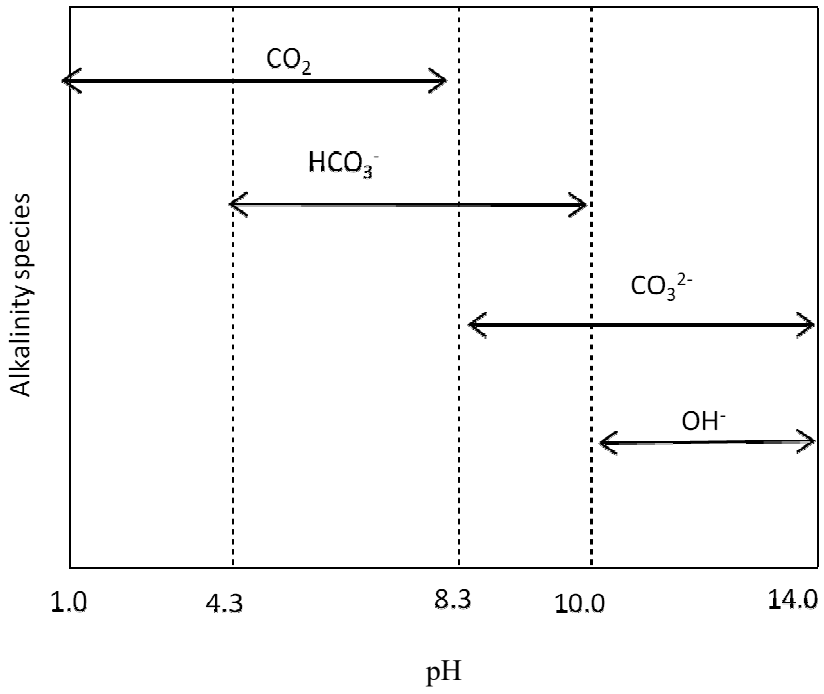



Figure 2.11 Relationship between alkalinity species and *pH (after Flynn, 2009)*

## 2.4. SOLID SEPARATION PROBLEMS IN THE ACTIVATED SLUDGE PROCESS

Section 2.3.2.1 presented two experimental models for the design of secondary clarifiers, whose objective is to ensure a proper clarification and thickening. The design parameters provided by these approaches were based on the quality of the sludge tested. Nevertheless, several operational issues, which can be process, equipment or microbiologically related, may lead to a poor sludge quality for settling, so that they could hinder the proper operation of the clarifier.

### 2.4.1. Process and equipment related factors

Process and equipment related factors have an effect on the clarification in the activated sludge system. Hydraulic load, total suspended solid concentration in the mixed liquor and sludge volumetric index (SVI, see definition in 5.1.3.2) are the main process parameters that affect the clarifier's performance. In turn, the clarifier diameter and height determine its capacity (WEF, 2005). Also, the clarifier's configuration is responsible for a good sludge settling, affecting phenomena such as density currents or short-circuiting (WEF, 2005). The combination of all these factors determines the quality of the clarified water, with regard to the total suspended solid content and turbidity.

Process and equipment factors are interrelated as follows: the sludge settling quality, characterized as SVI, determines the allowed upflow velocity ($v_c$) in the clarifier. The parameter $v_c$ together with the influent hydraulic load results in the diameter of the clarifier. Therefore, an increase in the process hydraulic load into the clarifier causes the upflow velocity to increase and consequently, depending on the settling quality of the sludge, it may cause solid entrainment with the clarified water. Also, the mixed liquor's total suspended solid concentration is a key factor for sludge settling. It has an effect not only on the expected SVI, which determines the clarifier's diameter, but also on the height of the clarifier and the design of the scrapper mechanism. Therefore, an increase in the expected total suspended solid concentration may result in an increase in turbidity and also in overfilling the clarifier with solids.

Moreover, it is especially important to have a good removal of gases dissolved in the mixed liquor in the degasser camera, as well as a good operation of the clarifier's scrapper mechanism, in order to let the bottom sludge out properly.

Also, an elevated concentration of total suspended solids in the effluent can occur because of a poorly flocculated sludge (Das *et al*., 1993). The reason for it can be a lack of time for flocculation or the break-up of already formed flocs. Flocs containing few filaments are specially bound to suffer this issue, resulting in pin floc (see definition in 2.4.2). Sludge flocculation can be accomplished by reducing the mixed liquor input energy, from a level that causes floc shear ($G > 125\ s^{-1}$; Das *et al.,* 1993), to a level that allows floc aggregation ($25 < G < 100\ s^{-1}$; Jenkins *et al*., 2003).

### 2.4.2. Floc structure factors

The basis of the activated sludge floc formation relies on the capacity of the microorganisms to stick to each other and to non-biological particles. There are two accepted models, which explain the mechanism of floc formation: the polymer bridging model (Pavoni *et al*., 1972) and the filamentous backbone model (Sezgin *et al*., 1978). Both models state that the exocellular polymer provides a surface charge to the floc. However, the forces that bind the cells together are referred differently. The polymer bridging model suggests that polymers bind microorganisms together because of electrostatic forces, while the filamentous backbone model suggests that filamentous bacteria create a backbone onto which the microorganisms can attach thanks to the segregation of the exopolymer.

Therefore, there are two key factors to enhance floc compactness: i) The shape of the microorganisms ii) The presence of some exocellular biopolymers which form bridges between them.

Table 2.5 introduces the main solid separation problems due to deficient floc structure, which can be interpreted in terms of exocellular polymer bridging and filamentous organism network existence. The floc appearance in each case is illustrated through microscopic observations in Figure 2.12.

The microorganisms can be relatively spherical or filamentous. The last are necessary in a limited amount to form the floc. If they are in excess, they create extensive networks that difficult a good floc formation and consequently, hinder sludge settling (Jenkins *et al.*, 2003). Several process conditions in the wastewater treatment are bound to cause an excessive proliferation of filamentous microorganisms. In turn, the absence of filaments prevents also from forming a good, compact floc, producing the pin floc phenomena.

Table 2.5 Solid separation problems related with floc structure in activated sludge systems focused on organic matter removal

| **Causes and effects of solid separation problems related with microbiology in activated sludge systems focused on organic matter removal** | | |
|---|---|---|
| **Problem** | **Cause** | **Effect** |
| Dispersed growth | Microorganisms do not attach to each other due to a lack or disruption of exopolymer bridging. Causes: high growth rates (Parker, 1983), high ratio monovalent-to-divalent cations (Novak *et al*., 1998) , poorly biodegradable surfactants and toxics (Bott and Love, 2002) | Turbid effluent |
| Slime, viscous or zoogleal bulking | Caused by excessive amount of exocellular biopolymer. Causes: nutrient deficiency (Jobbagy *et al*., 2002) | Poor settling and compaction; viscous foam |
| Pin floc | Small, compact, weak, spherical flocs, due to having only presence of floc-forming bacteria. Causes: very low F/M, high SRT, toxicity (Richard, 2003) | Low sludge volumetric index, but turbid effluent |
| Filamentous bulking | Large amounts of filamentous bacteria create bridges between the flocs, interfering with compaction, settling and thickening (Eikelboom and Van Buijsen, 1981). Causes: low dissolved oxygen, low F/M, high F/M and nutrient defficiency, among others | High sludge volumetric index with very clear supernatant. Low suspended solid concentration at the bottom of the clarifier |
| Denitrification | Formation of nitrates in the biological reactor. The bacteria remove the oxygen from nitrates in the clarifier, generating nitrogen gas bubbles that cause sludge rising (Henze *et al*., 1993) | Rising sludge and scum of activated sludge in the clarifier surface |
| Foam/Scum | Can be either caused by Nocardioforms, M.Parvicella and Type 1863, or by nutrient deficiency and denitrification. Nocardioforms and M.Parvicella foams are characterized by large, strong bubbles, greasy-looking surface and higher levels of filamentous organisms in the scum compared to the mixed liquor. Type 1863 foam is white-grey and collapses easily. The filamentous organisms are present in the foam at much higher levels than in the mixed liquor. It usually occurs at low SRT. Nutrient deficiency is characterized by sticky, high suspended solid scum caused by exocellular polymers (Novak *et al*., 1994). This foam dewaters poorly compared to the others. Denitrification scum consists of small gas bubbles and there is no difference in the abundance of filamentous organisms between the scum and the mixed liquor | Foams can rise a lot of suspended solids to the surface. Nocardioforms and M.Parvicella foams are persistent and difficult to break mechanically. They can accumulate and putrefy |

Taking to the biopolymer generation, it contributes typically 15-20% to the weight of mixed liquor suspended solids (Urban *et al.,* 1993*).* Different process causes have been identified to produce a lack of exocellular material or to prevent its bridging role. At approximately neutral pH values, the biopolymers carry net negative charges. This is the reason why divalent cations, such as $Ca^{2+}$ and $Mg^{2+}$ enhance bridging by interacting with the polymer (Jenkins *et al*., 2003). On the contrary, an excess of slime on the floc is also bound to cause sludge settling problems.

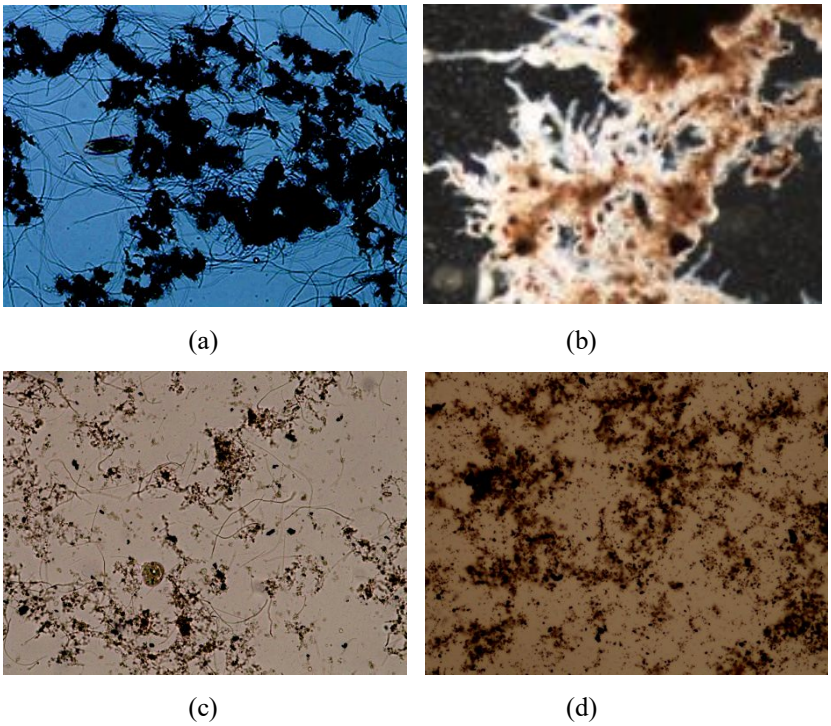



Figure 2.12 Microscopic observations of activated sludge flocs (Original magnification 100X): (a) Filamentous bulking sludge (b) Viscous or zoogleal bulking (c) Pin floc (d) Dispersed growth.

Among the sludge settling issues referred, the discussion will be focused on the filamentous bulking.

There are several process conditions which can be responsible for a filamentous bulking. Each different cause is bound to produce a specific type of filamentous microorganism. Therefore, the identification of the filament type dominant in the mixed liquor can provide information on the process condition, which is causing the sludge settling issue. Section 5.1.3.2 details the methodology followed in this work for filament identification.

As a reference to evaluate the filaments encountered in the mixed liquor, Table 2.6 presents the main causes of filamentous bulking and the usually associated species.

Table 2.6 Process conditions associated to filamentous microorganism growth in activated sludge systems

| **Conditions associated with filamentous organism growth in activated sludge systems** | |
|---|---|
| **Cause** | **Filamentous organism** |
| Low DO concentration | |
| Low to moderate SRT | Type 01701, S.Natans, H.Hydrossis |
| High SRT | M.Parvicella |
| Low F/M | M.Parvicella, Type 0041, Type 0675, Type 0092 Type 0914, Type 0803, Type 0581, Type 1851 |
| Unaerated zones | M. Parvicella, Type 0092, Type 0041, Type 0675 |
| High F/M | |
| Low molecular weight fatty acids | S.Natans, Type 1701, Type 021N,Thiothrix spp., H.Hydrossis,N.Limicola II, N.Limicola III, Type 1851 |
| Lipids | M.Parvicella, Type 1863 |
| Particulate substrates | Type 0041, Type 0675, Type 0092 |
| Hydrogen sulfide | Thiothrix spp., Type 021N, beggiatoa spp., Type 0914 |
| Nutrient deficiency | |
| Nitrogen | Type 021N, Thiothrix spp |
| Phosphorus | S.Natans, H. Hydrossis, N. Limicola III |
| Low pH | Fungi |

## 2.5. FILAMENTOUS BULKING CONTROL

Filamentous bacteria are always present and necessary in a biological system. However, it is important to control its relative weight to floc-forming ones, in order to avoid sludge settling difficulties (Jenkins *et al*., 2003). If filaments dominate the bacterial population, sludge settling issues are bound to generate a poor effluent quality in the clarifier (Jin *et al*., 2003). Figure 2.13 presents the appearance of the clarifier's surface for an activated sludge system suffering a filamentous bulking. The unsettled sludge on the surface is dragged out with the clarified water. Consequently, the effluent may not meet the final specifications on suspended solids and chemical oxygen demand.

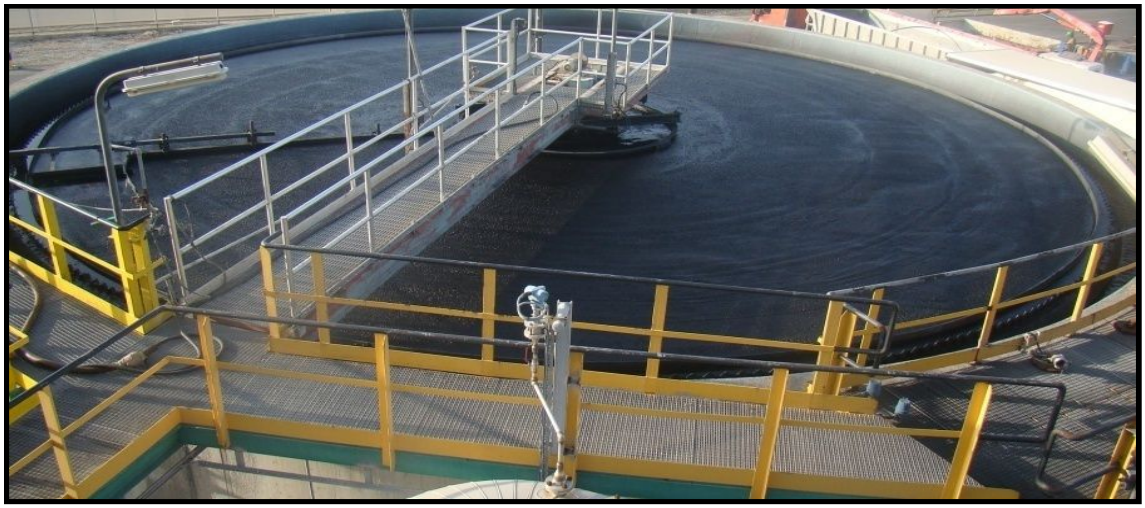


Figure 2.13 Surface of a clarifier belonging to an activated sludge system which experiences a filamentous bulking

If a settling test with filamentous bulking sludge is performed in a crystal glass, it can be observed that the sludge is unsettled and distributed all over the clarifier with an extremely clear supernatant (see Figure 2.14).

There are several ways of dealing with filamentous bacteria. Whenever a filamentous bulking has developed, several quick, non-specific methods may help to keep the effluent within specifications. Usual methodologies are the addition of chemicals and inert solids, or addition of disinfectants to selectively kill filamentous organisms, such as chlorination, peroxide oxygen or ozone (Jenkins *et al*., 2003). However, these are only not-specific, corrective methods. To overcome definitively the filamentous bulking, conditions should be changed in the biological reactor, as a preventive method. As already stated, filamentous bacteria are more competitive when there is scarcity of

resources in the media (Young, 2006). Therefore, the effective way to enhance floc-forming bacteria ahead of filamentous microorganisms is to guarantee sufficiency and well-distribution of oxygen, nutrients and organic matter. Since the type of filamentous microorganism corresponds specifically to the conditions present in the media, the characterization is needed in order to determine the parameter to adjust in order to improve sludge settling.

Figure 2.14 Settling test of a filamentous bulking sludge

Particularly talking about low F/M filamentous bulking, the option of including a selector in the activated sludge system is said to enhance the growth of floc-forming bacteria by different mechanisms (Richard, 2003). Both, anoxic and aerobic selectors have been reported to be successful in filamentous organism suppression at industrial wastewater treatment (Di Marcio *et al.,* 2000).

### 2.5.1. Addition of disinfectants

Addition of disinfectants is often used to selectively kill filamentous organisms and consequently, reduce the bulking effects (Metcalf and Eddy, 2003). The widely used disinfectant is chlorine. Chlorination is usually performed using chlorine or sodium hypochlorite solution. Usually chlorine is dosed at the recirculation stream, where a greater biomass concentration is encountered. It is also dosed directly into the biological reactor, if the HRT is long, in order to provide sufficient frequency of exposure. Chlorine should be added when the target value of SVI is significantly exceeded. Additions should be done until the target value is attained. Chlorine solution should be

added in known and controlled doses. To guarantee the efficiency of chlorine, secondary reactions of this reagent should be minimized. The reactions of chlorine with ammonia and reduced inorganic molecules, such as nitrite and sulfide are important. If chlorine is added to a solution containing an excess of ammonia ($Cl_2$ /N ratio <5), it reacts to form mono-chloramine ($NH_2Cl$). It is not as potent disinfectant as free chlorine, but it remains available longer. Chlorine activity will only be reduced if the ratio $Cl_2/NH_3>10$, where breakpoint reaction will be caused. Then, the chlorine will be reduced to chloride, and its activity will be missed. The parameters that determine chlorine dose are: concentration, overall mass dose rate, and frequency of exposure of solids inventory (Metcalf and Eddy, 2003).

Even if chlorine is preferred because of being more economical and easier to dose, also, Hydrogen Peroxide ($H_2O_2$) is used for bulking control. Usually, the necessary dose is higher than that for chlorine (Jenkins *et al.,* 2003). The addition, could be batch and continuous. Dose points could be located at the aeration basin and/or the recirculation stream. Like chlorine, good initial mixing of the reagent with the activated sludge is basic for efficient filamentous bacteria destruction. The effect of the $H_2O_2$ is similar to that of the chlorine. Both break up filaments, so that they become shorter. Also, cells within the filaments show signs of lysis. In addition to killing filamentous organisms, $H_2O_2$ produces oxygen, which may supplement dissolved oxygen.

Limited use has been made of other disinfectants, such as ozone (Van Leuwen and Pretorius, 1988), or ultraviolet disinfection. However, several limitations to the use of chlorine should be taken into account. Chlorine is not always available on site, and it may be difficult to get immediately the necessary quantities to overcome a severe bulking. There is also a concern over small amounts of chlorinated organic molecules formed from chlorine. Because of the fear of producing halogenated organic compounds, chlorination for bulking control is just recommended as an emergency measure in Germany and central Europe (Jenkins *et al*., 2003).

Chlorination is reported to be successful to control bulking caused by every type of filamentous organism in both, domestic and industrial wastewater treatment. However, most of the filamentous organisms, such as M. Parvicella, grow inside the floc, producing diffuse floc structure. Then, their control by recirculation chlorination can require high and prolonged chlorine doses, which can cause an increase in total suspended solids in the final effluent.

### 2.5.2. Addition of chemicals and inert solids

Addition of chemicals and inert solids may help to settle the sludge, though they do not eliminate the root cause of the problem (Jenkins *et al*., 2003).

Synthetic polymers can be added to the sludge, either to overcome bridging or diffused floc structures, associated to excessive growth of filaments. Polymers can also help to settle activated sludge, containing large amounts of water-retentive extracellular material. A huge range of polymers and doses could be added. Usually, for bulking control, it is recommended a high molecular weight, high cationic charge polymer alone, or in combination with an anionic polymer. The sludge performance in the jar-tests will report about the best polymer for each case and the right dose.

Inert materials, such as carbon or a wide range of zeolites, could also be added to the mixed liquor, in order to assist sludge settling. Density of these materials usually makes the floc denser, so that gravity settling is enhanced. Moreover, these materials are adsorbents. So, having their porous full of, either water or organics, makes them increase their weight, and their efficiency to assist sludge settling.

### 2.5.3. Reactor condition control

The methods described in sections 2.5.1 and 2.5.2 help to manage the filamentous bulking and its consequences. However, to definitively overcome the excess of filamentous bacteria, it is necessary to change the conditions in the biological reactor, so that floc-forming bacteria growth is enhanced. Physical and nutritional factors determine the type of microorganisms and their growth rates (Gaudy and Gaudy, 1988). Hence, physical characteristics of the environment, such as temperature and pH, are selective for a type of microorganism. Also, it is recognized in the literature, that a way to control the proliferation of filaments is to ensure the availability of nutrients, oxygen and organic matter (McKinney, 2004).

Industrial wastewater is often deficient in nutrients (Eckenfelder and Cleary, 2013). Therefore, nutrients, considered as ammonia nitrogen and phosphorus orthophosphate, must be added to the biological system. The usual necessary weight ratio $BOD_5$: N: P is 100:5:1 (Metcalf and Eddy, 2003). Recommendations say that concentrations of total soluble inorganic nitrogen (nitrites, nitrates and ammonia) should not be less than 0.5 to 1.0 mg $L^{-1}$, and soluble phosphorus concentrations should not be less than 0.1 to 0.3 mg

$L^{-1}$. Much higher residuals may be needed, especially when high concentration of readily biodegradable organic matter is treated in aerobic mixed biological reactors. Here, minimum 1 to 3 mg $L^{-1}$ nitrogen and phosphorus residuals are required to guarantee sufficient nutrients inside the floc, in order to meet the nutrient demand corresponding to a high concentration of readily biodegradable substrate (Jenkins *et al*., 2003).

With regard to dissolved oxygen, some experiments show that the level required depends on the F/M ratio. As the F/M increases, the DO required is higher. Therefore, the filamentous bulking could be solved, either by manipulating F/M ratio or DO concentration (Palm *et al*., 1980). Jenkins *et al.* (2003) present several case histories, where settling was improved by providing the sludge a higher DO concentration.

Eventually, scarcity of the organic matter supplied to the biological treatment may result in a low F/M filamentous bulking (Jenkins *et al*., 2003). To solve this issue, the ratio F/M should be increased, either by supplying a higher amount of organic matter or by reducing the biomass concentration in the reactor. However, this solution may not be feasible, especially in complete mix biological reactors, where the carbonaceous substrate concentration is dispersed throughout the entire volume of the biological reactor (Chudoba *et al*., 1973). Therefore, to overcome a low F/M filamentous bulking, additional tools may be required. For this purpose, section 2.5.4 introduces the use of selectors in the activated sludge system.

### 2.5.4. Selectors

The low F/M filamentous bulking has been reported as a frequent issue in complete mix activated sludge systems, whereas plug flow reactors do not show this tendency (Jenkins *et al*., 2003). Therefore, a solution to the excessive proliferation of filamentous bacteria in complete mix biological reactors could be to approach the plug flow regime through the compartmentalization of the aeration basin (Chudoba *et al*., 1973; Rensik *et al.,* 1982). This measure intends to produce a gradient in the carbonaceous substrate concentration, in the aeration basin. In other words, this system aims for a high substrate concentration at the mixing point of the activated sludge recycle and the influent wastewater, followed by a small soluble substrate concentration zone. This way, the activated sludge is first fed and then starved, so that the biomass must develop the ability to rapidly take up the soluble substrate and store it internally for use during the

starvation periods (Van Loosdrecht *et al*., 1997). This procedure allows to select floc-forming bacteria.
Based on this principle, several alternative strategies are referred in the literature to solve a filamentous bulking. For example, some options considered are intermittent feeding of wastewaters (Verachtert *et al*., 1980; Van den Eynde *et al.,* 1982), the use of selectors (Lee *et al*., 1982; Grau *et al*., 1982) or fed-batch operation (Chiesa and Irvine, 1985). The discussion in this manuscript will focus particularly on selectors.

The term selector was introduced by Chudoba *et al*. (1973). A selector consists of one or several mixing tanks, where the recirculation of activated sludge and influent wastewater mix prior to reaching the aeration basin (Lee *et al*., 1982; Grau *et al*., 1982).

The bibliography reports successful results of selectors to solve filamentous bulking issues (Melcer *et al*., 2003; Al-Mutairi, 2009; Ferreira *et al*., 2014), but also some cases are reported, where selectors could not overcome the excessive proliferation of filaments (Jenkins *et al*., 2003). As an example, M. Parvicella growth has not been controlled, since this filamentous microorganism is thought to have a high substrate uptake and storage capacity. Also, Types 0675, 0041 and 0092 can grow with particulate matter, whose concentration is not influenced in a selector (Jenkins *et al*., 2003). Therefore, the success of a selector is conditioned to the quality of the substrate supplied, as announced by Martins *et al.* (2004).

#### 2.5.4.1. Selection mechanisms in a selector

Filamentous bacteria growth can be limited either by kinetic or metabolic control. Also, both mechanisms could apply at a time.

Floc-forming bacteria growth is enhanced through kinetic control by using high soluble substrate concentrations (Chudoba, 1985). At this condition, the growth constant (μ) is much higher for floc-forming than for filamentous bacteria. It is just the contrary for low substrate concentrations in the biological reactor ($S$), as it is shown in Figure 2.15.

On the other hand, metabolic control selects where the energy for the COD biodegradation comes from. In this sense, most filaments are known to be strictly aerobic (Mangrum, 1998), showing much lower denitrification rates than floc-formers. Consequently, anoxic conditions can enhance floc-formers over filaments.

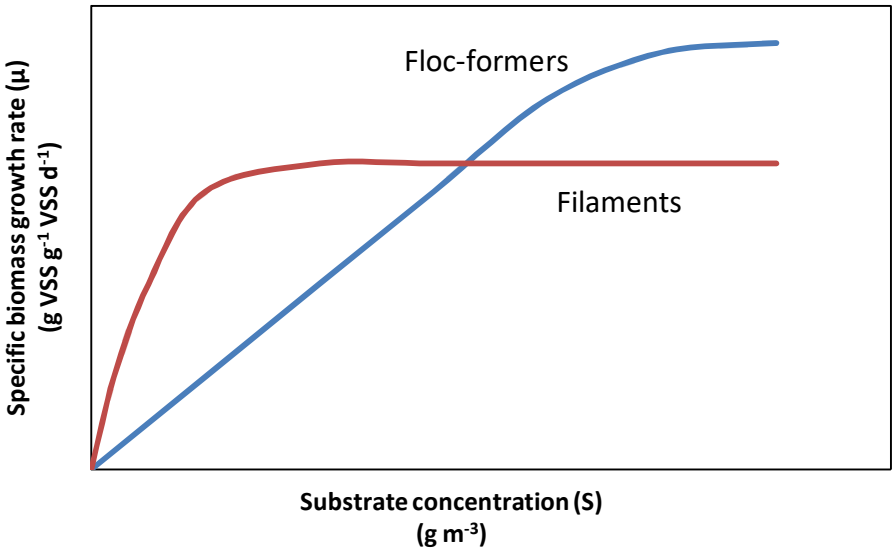



Figure 2.15 Comparative floc-forming and filamentous bacteria growth constant with substrate concentration *(after Metcalf and Eddy, 2003)*

### 2.5.4.2. Selector types and process arrangement

Selectors are usually implemented ahead of the biological reactor, as part of the activated sludge system. The recycle stream, rich in biomass, is mixed together with soluble biodegradable substrate in the selector (Metcalf and Eddy, 2003). There, the readily metabolizable organic substrates are removed.

Depending on the electron acceptor in the biodegradation reaction, there are three types of selectors: aerobic, anoxic and anaerobic.

Aerobic selectors must be supplied with air, since oxygen is the electron acceptor (Jenkins *et al*., 2003). Such systems present the configuration illustrated in Figure 2.16. In this case applies the kinetic control mechanism, forcing a high F/M ratio in the selector and therefore, enhancing the formation of floc-forming bacteria.

Anoxic selector should not be supplied with oxygen, since the electron acceptors are the nitrates formed in the biological reactor (Jenkins *et al*., 2003). Figure 2.17 shows the process configuration for an activated sludge system including an anoxic selector.

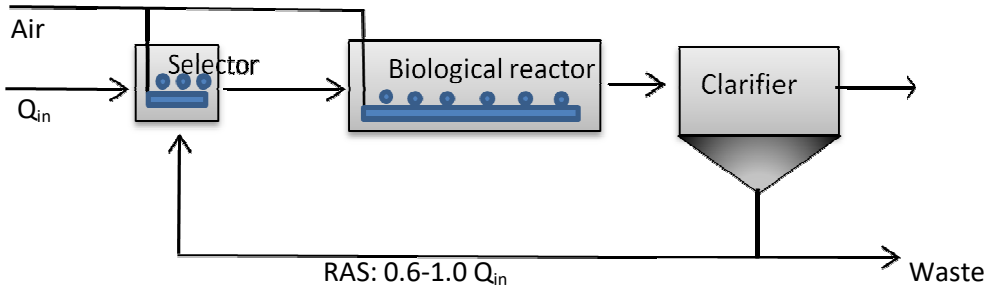



Figure 2.16 Conventional configuration of a biological system with an aerobic selector. $Q_{in}$ stands for influent flow rate to the selector and RAS for recirculation of activated sludge

Nitrates are formed in the biological reactor and transferred through an internal recycle stream to the selector. Nitrate production in the biological reactor requires oxygen, ammonia nitrogen and alkalinity (4.57 kg $O_2$ $kg^{-1}$ $NH_4^+$-N and 7.14 kg alk $kg^{-1}$ $NH_4^+$-N). On the other hand, the denitrification produced in the selector tends to buffer by producing 5.57 kg alk.$CaCO_3$ $kg^{-1}$ $NO_3^-$-N denitrified. Anoxic selectors are said to require a lower F/M ratio than aerobic selectors. References remark the need of enough, but not superficial agitation, in order to guarantee homogeneity without superficial aeration. Dissolved oxygen should be kept at less than 1 mg $L^{-1}$ in the selector and the nitrate supply should be within the range of 6 to 8 kg $NO_3^-$-N $kg^{-1}$ COD or 3 to 5 kg $NO_3^-$-N $kg^{-1}$ $BOD_5$. The HRT in the selector is recommended to be around 60 to 90 min (Jenkins *et al.*, 2003; Metcalf and Eddy, 2003). For anoxic selectors apply the kinetic and the metabolic selection mechanisms.

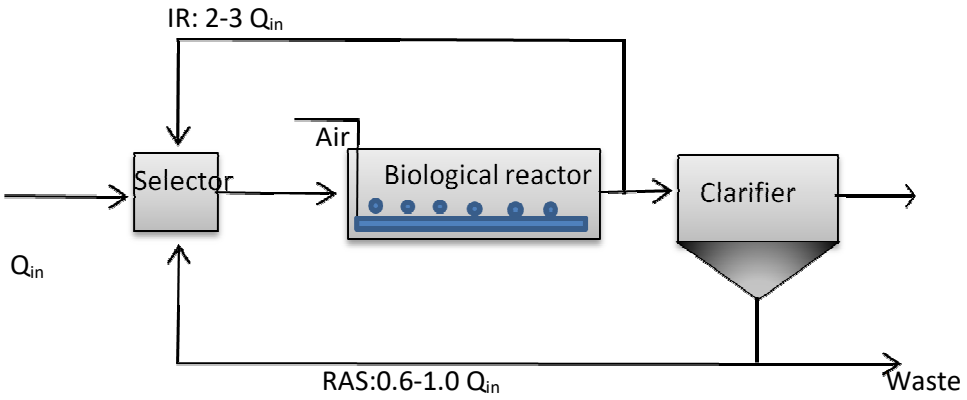



Figure 2.17 Conventional configuration of a biological system with an anoxic selector. $Q_{in}$ stands for influent flow rate, IR for the internal recycle and RAS for recirculation of activated sludge

In anaerobic selectors, oxygen and nitrates must be absent. Here, polyhydroxyalkanoate (PHA) storage, hydrolysis of stored inorganic polyphosphate and fermentation of stored glycogen are the major metabolic activities removing soluble substrate (Jenkins *et al.*, 2003). The configuration of anaerobic selectors is shown in Figure 2.18. Anaerobic selectors can be used for Enhanced Biological Phosphorus Removal (EBPR). In this process, inorganic polyphosphate hydrolysis takes place in the aeration basin, so that the growth of microorganisms capable of polyphosphate storage should be promoted. The overall sizing is determined by the rate at which soluble organic matter is taken up and orthophosphate is released under anaerobic conditions. As an example, for domestic wastewater, the total anaerobic selector HRT is usually in the range of 0.75 to 2.0 h. The recommendation is to divide the selector in at least three compartments with the same F/M ratio. Anoxic selectors use both, kinetic and metabolic selection processes.

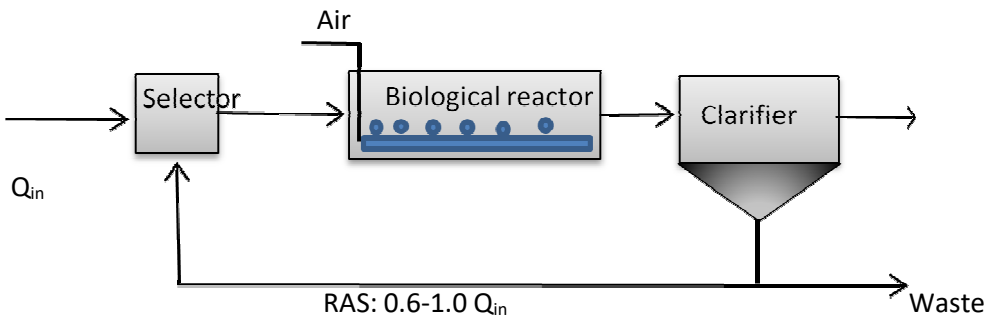



Figure 2.18 Conventional configuration of a biological system with an anaerobic selector. $Q_{in}$ stands for influent flow rate and RAS for recirculation of activated sludge

The mechanisms described above are mutually exclusive, because of the high competitive environment that exists in the activated sludge. The energy released by aerobic oxidation of an organic substrate is greater than for denitrification, and also, this is greater than for polyphosphate hydrolysis. Therefore, in the presence of oxygen, aerobic oxidation will dominate, even if nitrates are present. Likewise, the presence of dissolved oxygen or nitrates will suppress polyphosphate hydrolysis mechanism.

The choice of a type of selector may be based on general rules stated in the bibliography (Martins *et al.*, 2004). Aerobic and anoxic selectors have been described to be effective to overcome a low F/M filamentous bulking (Wanner *et al.*, 1987; Scruggs and Randall, 1998; Di Marzio *et al.*, 2000; Richard, 2003). As a reference, aiming to solve a type 021N filamentous bulking, Prendl and Kroiss (1998) suggested an aerobic selector for a

belt sugar mill treatment plant, whereas other authors (Wanner *et al*., 1987; Di Marzio *et al*., 2000) suggested an anoxic selector or even the combination of aerobic and anoxic selectors to solve a Thiotrix I filamentous bulking. However, it is still widely recognized that the effectiveness and design of selectors is dependent on the particular wastewater characteristics (Chambers and Tomlinson, 1982; Tandoi *et al.*, 2006; Al-Mutairi, 2009). Therefore, due to the variability of industrial wastewater, the design of selectors relies on experimentation (Martins *et al*., 2004; Seviour and Blackall, 2012).

From the review of this section, it can be outlined that the ratio F/M in the selector is a critical variable to determine its effectiveness to enhance sludge settling. Some experimental works show a relationship between SVI and initial F/M ratio, suggesting that the F/M should be greater than 3 kg $BOD_5$ $kg^{-1}$ VSS $d^{-1}$ in order to produce a SVI of 100 to 150 mL $g^{-1}$ SS (Tomlinson, 1976). Taking to selector sizing, it is reported that its value should be a certain fractional value of the aeration basin (Lee *et al*., 1982), so that the selector provides enough time to remove all soluble readily metabolizable organic matter. Selector's design practice in Czechoslovakia and Austria suggest criteria for COD removal of 80% of the removable organic matter (Wanner *et al*., 1987). Although these general criteria can guide a selector's design, lots of questions arise when it comes to the detailed design of the process. Answers to these questions should be provided by pilot tests using real-like wastewater streams.

## 2.6. CONTROL OF UNINTENDED BIOLOGICAL NITRIFICATION

Despite being focused on organic matter removal, some activated sludge systems accomplish the necessary conditions to enable biological nitrification. As an example, in petrochemical wastewater treatments, advanced oxidation processes often precede the biological reactor (Derakhshan and Fazeli, 2018), in order to improve the biodegradability of the effluents. As a consequence, the CSTR is provided with feed streams rich in inorganic carbon, which enhances the proliferation of nitrifying bacteria (Metcalf and Eddy, 2003). In such systems, temperature, pH and long SRT exhibit also favourable values for nitrification. Once nitrites and nitrates are formed in the biological reactor, they get to the clarifier, where the bacterial activity keeps on, as long as there is a residual organic load. Since oxygen is needed for the biodegradation process, the bacteria obtain it from the nitrates, converting them into nitrogen gas. The gas bubbles rise in the clarifier dragging sludge particles up to the clarifier's surface (Eckenfelder, 1998), as shown in Figure 2.19. Therefore, to obtain a good sludge settling in such

systems, it is necessary to limit either biological nitrification in the reactor or biological denitrification in the clarifier.

(a)

(b)

Figure 2.19 Surface of a secondary clarifier with rising sludge due to denitrification (a) Settling test of an activated sludge with a high concentration of nitrates (b)

If nitrates are formed in the reactor and they are not used in a denitrification step, the nitrogen concentration as nitrates in the effluent is expected to increase. Authorized discharge emission levels usually limit the amount of nitrogen, as it will be discussed in chapter 3. Therefore, the option to limit nitrification in the biological reactor prevails over avoiding denitrification in the secondary clarifier.

### 2.6.1. Biological nitrification

Equations 2.3 to 2.5 presented the stoichiometry for the autotrophic oxidation and synthesis oxidation of ammonia by nitrification. Nitrification is a two step biological process, in which ammonia ($NH_4^+$) is first oxidized into nitrite ($NO_2^-$), and in turn, nitrite is oxidized into nitrate ($NO_3^-$). The autotrophic bacteria responsible for this process are Nitrosomonas (also known as Ammonia Oxidizing Bacteria, AOB) and Nitrobacter (also known as Nitrite Oxidizing Bacteria, NOB), respectively.

Equation 2.5 indicates that the nitrification reaction requires from the presence of oxygen and alkalinity to occur. On the other hand, the nitrification reaction produces few new biomass cells, nitrates and acidity. Consequently, the nitrification reaction lowers the pH in the biological reactor.

In order to limit unintended nitrification in activated sludge systems focused on organic matter removal, the factors that enhance the reaction need to be identified and controlled. Nitrification rates are a function of nitrogen and oxygen concentration in the biological reactor, as reflected in equation 2.48 for the specific growth rate of nitrifying bacteria.

$$\mu_n = \left(\frac{\mu_{nm}\, N}{K_n + N}\right)\left(\frac{DO}{K_o + DO}\right) - k_{dn} \qquad (2.48)$$

Where

| | |
|---|---|
| $\mu_n$ | Specific growth rate of nitrifying bacteria (g new cells $g^{-1}$ cells $day^{-1}$) |
| $\mu_{nm}$ | Maximum specific growth rate of nitrifying bacteria (g new cells $g^{-1}$ cells $day^{-1}$) |
| N | Nitrogen concentration (g $m^{-3}$) |
| $K_n$ | Half-velocity constant, substrate concentration at one-half the maximum specific substrate utilization rate (g $m^{-3}$) |
| $k_{dn}$ | Endogenous decay coefficient for nitrifying organisms (g VSS $g^{-1}$ VSS $d^{-1}$) |
| $DO$ | Dissolved oxygen concentration (g $m^{-3}$) |
| $K_o$ | Half-saturation coefficient for DO (g $m^{-3}$) |

Low dissolved oxygen and nitrogen concentrations in the mixed liquor limit the nitrifying activity, as reflected in equation 2.48. Particularly, at low DO concentrations (below 0.5 mg $L^{-1}$) nitrification rates are inhibited, more for NOB than for AOB. As a

consequence, incomplete nitrification happens, raising the concentration of nitrite in the effluent.

Also, nitrification rates are affected by other factors, such as the pH, temperature, SRT and inorganic carbon concentration in the biological reactor. The rate of nitrification declines significantly at pH values below 6.8. As shown in Figure 2.20, optimal nitrification rates occur at pH values in the range of 7.5 and 8.0 (Metcalf and Eddy, 2003). At lower and higher pH values, the nitrifying activity is significantly reduced.

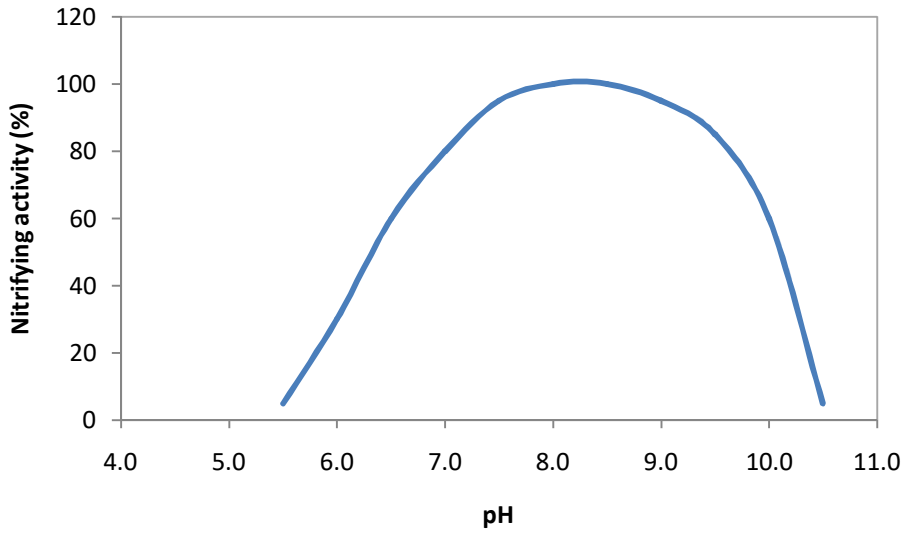



Figure 2.20 Variation of the nitrifying activity with the pH in the mixed liquor *(data from Shammas, 1986)*

The optimal nitrification temperature has been identified at 30 to 36 ºC, as represented in Figure 2.21. At low temperatures, the ammonia oxidation kinetics is limiting versus the nitrite-oxidation kinetic. At elevated temperatures, the relative kinetics of ammonia and nitrite oxidation change, so that operating at lower SRT values nitrite accumulates.

Maximum specific growth rates of nitrifying bacteria are lower than for heterotrophic bacteria. For this reason, longer SRT are required for nitrification activities. Whenever nitrification is a purpose, typical design SRT values range from 10 to 20 days at 10ºC to 4 to 7 days at 20ºC (Metcalf and Eddy, 2003). Additionally, as represented in equation 2.5, the nitrification reaction requires from the presence of inorganic carbon to occur, since it is the source of carbon for autotrophic bacteria.

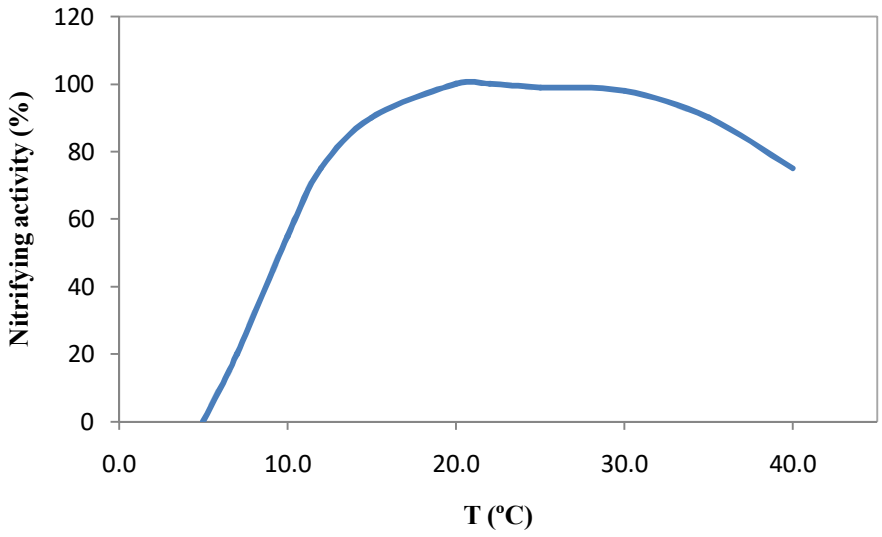



Figure 2.21 Variation of the nitrifying activity with the temperature in the mixed liquor *(data from Shammas, 1986)*

This review of parameters suggests that keeping any of them at unfavourable values for nitrification could prevent the biological reaction. However, it must be taken into account that in activated sludge systems focused on organic matter removal, the heterotrophic biomass coexists with the autotrophic and its performance must be enhanced in order to accomplish the treatment objectives. Therefore, dissolved oxygen and ammonia nitrogen should not be lowered below 2 mg $L^{-1}$ and SRT may be required at high values to degrade recalcitrant organic matter. The heterotrophic biodegradation produces carbon dioxide, which results in inorganic carbon in the mixed liquor. The pH range for heterotrophic activity is often within the range of 6 to 9. The temperature affects the extent of the heterotrophic biodegradation reaction, providing higher removal efficiencies on organic matter for higher temperatures. Consequently, it may be not possible in such systems to limit biological nitrification by adjusting the mentioned operational parameters.

An alternative way to limit nitrification could be explored, based on the nitrifying bacteria sensitiveness to the presence of certain chemical compounds, which act as inhibitors. Along the last years, a vast research has been performed on nitrification inhibition in soils, since nitrification is the main cause for nitrogen losses (Subbarao *et al.*, 2009) and nitrogen oxides emissions (Torralbo *et al.*, 2017). Ammonia oxidizing bacteria inhibitors can act in different ways:

i) Binding to the active site of Ammonia Monoxygenase (AMO), which is the enzyme that catalyzes the conversion of ammonia to hydroxylamine, as a first step of nitrification. The substrates of AMO have shown competitive and non-competitive kinetics.

ii) Mechanism based inhibition consists in the inactivation of AMO enzyme by modification through covalent binding of its proteins to the inhibitors. There are a wide range of products showing this mechanism, such as sulphurs or acetylenic compounds.

iii) Nitrogen heterocyclic compounds are also highly effective nitrification inhibitors. Compounds that contain two or three adjacent ring (pyridazine, pyrazole, indazole) produce a significantly higher inhibitory effect than compounds containing non-adjacent nitrogen atoms or singular nitrogen ring atoms (pyridine, pyrrole) (Mc Carty, 1999). Mc Carty and Bremmer (1989) demonstrated that un-substituted nitrogen heterocyclic compounds containing two adjacent ring nitrogen atoms inhibit nitrification in soils and that two of these compounds, pyrazole and 1,2,4-triazole are potent inhibitors. They also showed that several substituted pyrazoles and triazoles are good inhibitors in soils (3- methylpyrazole and 3,4-dichloro-1,2,5-thiodiazole. Although the action mechanism of nitrogen heterocyclic compounds has been related to the presence of the nitrogen ring (Mc Carty, 1999), its mode of action is not yet well understood (Torralbo *et al*., 2017).

As a summary of all the research performed, Blum and Speece (1991) published a list of recognized nitrification inhibitors. In order to select a nitrification inhibitor for the mixed autotrophic-heterotrophic system, it is a condition to choose a compound, which does not hinder the heterotrophic activity.

### 2.6.2. Biological denitrification

Assuming that nitrification takes place, an additional strategy to prevent the rising sludge in the secondary clarifier of the activated sludge system focused on organic matter removal is to force denitrification in a prior step to the clarifier. The understanding of this possibility takes to the analysis of biological denitrification.

There are two possible reactions for nitrate removal in the biological processes: i) The assimilating nitrate reduction, which consists in the reduction of the nitrate to ammonia

for use in cell synthesis. This reaction occurs in absence of ammonia, and is independent of DO concentration ii) The dissimilating nitrate reduction or biological denitrification, which involves the reduction of nitrate to nitric oxide, nitrous oxide and nitrogen gas. In this reaction, nitrate and nitrite are used as electron acceptors for the oxidation of organic matter.

A wide range of autotrophic and heterotrophic bacteria are capable of denitrification. Among them, Pseudomonas is the most common specie of denitrifying bacteria. For denitrification, they can use organic compounds such as methanol, organic acids, alcohols, benzoates or other aromatic compounds (Payne, 1981). Most of these bacteria are facultative. The electron donor in these reactions can be either the biodegradable organic matter contained in wastewater or an exogenous source, such as methanol or acetate, as reflected in equations 2.6 to 2.8. From the stoichiometry presented in the overall denitrification reaction, one equivalent of alkalinity is produced per equivalent of nitrate reduced.

As a rule of thumb, Barth *et al.* (1968) estimated that 4 g of BOD is needed per 1 g of nitrate reduced. However, the actual necessary amount of BOD will depend on the operating conditions and the type of electron donor used for denitrification. An expression can be developed to determine the necessary biodegradable matter to consume nitrates (Randall *et al.,* 1992), as reflected in equation 2.52.

The nitrifying bacteria use the biodegradable soluble COD removed ($bsCOD_{r)}$ for oxidation ($bsCOD_o$ ) or for cell synthesis ($bsCOD_{syn}$).

$$bsCOD_r = bsCOD_{syn} + bsCOD_o \tag{2.49}$$

The nitrifying cell synthesis component is calculated as indicated in equation 2.50.

$$bsCOD_{syn} = 1.42\, Y_n\, bsCOD_r \tag{2.50}$$

Where

$Y_n$ Net biomass yield (g VSS $g^{-1} bsCOD_r$)

The biodegradable substrate oxidized ($bsCOD_o$) is equal to the oxygen equivalent of the nitrate used for the oxidation of the biodegradable substrate, as reflected in equation 2.51.

$$bsCOD_o = 2.86\ NO_x \tag{2.51}$$

Where

| | |
|---|---|
| 2.86 | Oxygen equivalent of nitrate ($g\ O_2\ g^{-1} NO_3^-$) |
| $NO_x$ | Nitrate and nitrite reduced ($g\ d^{-1}$) |

Rearranging equations 2.49 to 2.51 yield equation 2.52.

$$g\ bsCOD_r \Big/ NO_3^- = \frac{2.86}{1-1.42\ Y_n} \tag{2.52}$$

and

$$Y_n = \frac{Y}{1+\ k_d\ SRT} \tag{2.53}$$

Where

| | |
|---|---|
| $Y$ | Synthesis yield coefficient ($g\ VSS\ g^{-1} bsCOD$) |
| $k_d$ | Endogenous decay coefficient ($g\ VSS\ g^{-1} VSS\ d^{-1}$) |

Therefore, denitrification requires from the absence of oxygen and the presence of biodegradable organic matter. As long as conditions in the biological reactor hasten organic matter removal efficiency, denitrification in the later steps will be less intense. In order to avoid denitrification in the secondary clarifier, a prior denitrification step could be implemented in the activated sludge system, using the residual organic matter from the biological reactor. However, the feasibility of this solution is site-specific, since the inclusion of this step needs to stop the running process for modifications and also needs available space to be layed-out.

# 3. Legislative framework

***ABSTRACT***

*The European Union Authorities regulate pollutant emissions from industrial installations through Directive 2010/75/EU on Industrial Emissions, which came into force on the 6th January 2011. Its main objective was to protect the human health and the environment as a whole. To accomplish this goal, the Directive requests to operate the installations in accordance with an integrated permit, granted by the competent Authorities in the Member States. The permit must include the allowed emission levels, which should be based on the Best Available Technologies (BAT) conclusions. Therefore, the European Union must define BAT and BAT associated environmental performance, which is reflected in the BAT Reference Documents, known as BREFs. The conclusions, as part of this document where the allowed emission levels are included, are adopted as a Decision. Hence, for common wastewater in the chemical sector, the BAT conclusions are nowadays implemented through Decision (EU) 2016/902 the 30th May 2016. This regulation announces future more stringent allowed emission levels to water than in previous versions of the BREF document. For example, total suspended solids emission is limited to 35 mg $L^{-1}$. The BREF document also presents references on emission levels of currently running wastewater treatment plants. Focussing on activated sludge systems with sedimentation as the main solid removal step, the document reports yearly average values within 5 to 2900 mg $L^{-1}$. Obviously, to accomplish the future legislation, it is necessary to upgrade activated sludge processes, in order to enhance solid separation.*

# 3. Legislative framework

*Directive 2010/75/EU of the European Parliament and the Council on Industrial Emissions* (*IED*) is the main tool of the European Union Authorities to regulate pollutant emissions from industrial installations (European Commission, 2018). The *IED* recasts seven existing Directives related to industrial emissions: the IPPC Directive, the large combustion plants Directive, the waste incineration Directive, the solvents emissions Directive and three Directives on Titanium Dioxide (European Commission, 2016). This *Directive* covers several industrial activities (chemicals, waste management, energy, metal production and processing, minerals and other sectors, such as slaughterhouses, pulp and paper and intensive rearing of poultry and pigs), among which highlights the production of chemicals (Eur-lex, 2015). Therefore, this Directive is addressed to the petrochemical activity, which is the focus of this thesis.

The *IED* was adopted on the 24$^{th}$ November 2010, entered into force on the 6$^{th}$ January 2011 and was to be transposed by Member States by 7$^{th}$ January 2013 (European Commission, 2018). The main objective of this legislation was to protect the human health and the environment, considered as a whole, by reducing the industrial emissions, thanks to a better application of the Best Available Technologies (BAT). All the installations covered by the *Directive* are urged to prevent and reduce pollution by implementing the BAT. It is also requested an efficient use of energy, waste prevention and management and the prevention of accidents (Eur-lex, 2015). The way to reach these standards is through the requirement to operate the installations in possession of an integrated permit, granted by the competent Authorities in the Member States (European Commission, 2018). The *IED* is based on several concepts:

i. An integrated approach, which is materialized in a permit that considers the whole environmental performance of the installation (European Commission, 2018), to avoid pollution being shifted from one medium to another. Prevention of pollution at source is a priority (Eur-lex, 2015).

ii. The permit should include the allowed emission levels, which must be based on the BAT conclusions (European Commission, 2018).

iii. Flexibility for the competent Authorities to set less strict emission levels when the BAT conclusions assess that reaching the BREF limits would lead to too higher costs compared to the environmental benefits (European Commission, 2018).

iv. Environmental inspections to installations become mandatory for competent authorities (European Commission, 2018).

v. The public can participate in the decision process, by acceding to permits, permit application and the results of monitoring (European Commission, 2018). Additionally, the European Pollutant Release and Transfer Register (E-PRTR) stands as a public register for emission data of the major industrial activities.

Since the operation permit requested by *IED* must be based on recognized best available technologies, BAT and environmental performance principles according to BAT must be defined for the European Union. For this purpose, the Commission has organized a multidisciplinary team, at which experts from the Member States, industry and environmental organizations take part. Their work, being coordinated by the European IPPC Bureau, ends up in the BAT Reference Documents, known as BREFs. They contain the BAT conclusions, where allowed emission levels are included, as the base for the *IED*'s permit condition. The commission adopts this chapter as Implementing Decisions. Hence, *Decision (EU) 2013/84 of the 11th February 2013* established the BAT conclusions for common wastewater in the chemical sector. In 2016, it was revised by *Decision (EU) 2016/902 of 30$^{th}$ May 2016,* which suggested more stringent allowed emission levels (BAT-AEL) to water. Table 5.1 reflects the BAT-AEL for direct discharges to a receiving water body proposed by this Decision.

Also, the BREF document reports reference data from existing installations. Hence, the current emission levels of some wastewater treatment plants are included in its chapter 2. Despite no specific values for petrochemical activated sludge systems have been identified, data from similar wastewater treatment installations are provided. Particularly, emission levels for activated sludge systems with sedimentation as the main solid removal step could be taken as reference in this work, with regard to the total suspended solids (TSS). The BREF document reports a wide range of 5 to 2900 mg TSS $L^{-1}$ in the effluent for current activated sludge systems in operation. This statement is supported by data on particular installations with such configuration. For example, the installations identified as code # 28 and #25, dedicated to organic fine chemicals production, have reported a TSS emission within the range of 500 to 3000 mg $L^{-1}$ and around 250 mg $L^{-1}$, respectively. The wastewater treatment facility code #48, dedicated to large volume organic chemicals, reports TSS emission concentrations within the range of 15 to 240 mg TSS $L^{-1}$.

The wide range of values provided as yearly average evidence variable process situations. By the comparison of the emission levels reported for running installations with the limit of 35 mg TSS $L^{-1}$ stated in the BREF document, it is obvious that activated sludge systems need to be upgraded in order to improve their solid separation performance.

Table 3.1 BAT-AELs for direct discharges to a receiving water body (*source: Best Available Techniques Reference Document for Common Waste Water and Waste Gas Treatment/Management Systems in the Chemical Sector, chapter 4, Table 4.1*)

| Parameter | BAT-AEL (yearly average) | Conditions |
|---|---|---|
| Total organic carbon (TOC) | < 10-33 mg $L^{-1}$ (1) (2) | The BAT-AEL applies if the emission exceeds 3.3 t/year |
| Chemical oxygen demand (COD) | < 30-100 mg $L^{-1}$ (1) (2) | The BAT-AEL applies if the emission exceeds 10 t/year |
| Total suspended solids (TSS) | 5.0-35 mg $L^{-1}$ | The BAT-AEL applies if the emission exceeds 3.5 t/year |
| Total nitrogen (TN) | 5.0-25 mg $L^{-1}$ | The BAT-AEL applies if the emission exceeds 2.5 t/year |
| Total inorganic nitrogen ($N_{inorg}$) | 5.0-20 mg $L^{-1}$ | The BAT-AEL applies if the emission exceeds 2.0 t/year |
| Total phosphorous (TP) | 0.50-3.0 mg $L^{-1}$ | The BAT-AEL applies if the emission exceeds 300 kg/year |
| Adsorbable organically bound halogens (AOX) | 0.20-1.0 mg $L^{-1}$ | The BAT-AEL applies if the emission exceeds 100 kg/year |
| Chromium (expressed as Cr) | 5.0-25 µg $L^{-1}$ | The BAT-AEL applies if the emission exceeds 2.5 kg/year |
| Copper (expressed as Cu) | 5.0-50 µg $L^{-1}$ | The BAT-AEL applies if the emission exceeds 5.0 kg/year |
| Nickel (expressed as Ni) | 5.0-50 µg $L^{-1}$ | The BAT-AEL applies if the emission exceeds 5.0 kg/year |
| Zinc (expressed as Zn) | 20-300 µg $L^{-1}$ | The BAT-AEL applies if the emission exceeds 30 kg/year |

(1)The upper end of the range may be up to 100 mg $L^{-1}$ for TOC and 300 mg $L^{-1}$ for COD as yearly average if the following two conditions are fulfilled: a) abatement efficiency of the global treatment as a yearly average ≥90%. b) A low-loaded biological treatment is used (≤0.25 kg COD $kg^{-1}$ organic dry matter of sludge).
(2)The upper end of the range may not apply if the following conditions are fulfilled: a) abatement efficiency of the global treatment as a yearly average ≥95%. b) The same as condition b) in note (1) c) The influent to the final wastewater treatments exhibits these values: TOC> 2 g $L^{-1}$ (or COD>6 g $L^{-1}$) as a yearly average and a high proportion of refractory compounds.

# 4. Objectives and thesis structure

***ABSTRACT***

*The main goal of this thesis is the optimization of existing full-scale petrochemical activated sludge systems focused on organic matter removal, in order to enhance sludge settling. Two frequent issues, which difficult sludge settling in such systems, must be faced: i) Low food-to-microorganism (F/M) filamentous bulking and ii) Unintended nitrification in the biological reactor and consequent denitrification in the clarifier. To overcome the excessive proliferation of filamentous bacteria, the implementation of a selector in the activated sludge system has been considered. To limit unintended biological nitrification, the supply of folic acid to the biological reactor is investigated.*

*The scope of the thesis includes the following objectives, to accomplish the main goal stated above: i) To assess the effectiveness of aerobic and anoxic selectors to improve the biomass quality in order to enhance sludge settling, ii) To optimize design and operational parameters of the selector, iii) To optimize the mixed liquor parameters in the activated sludge system including a selector to enhance sludge settling and iv) To assess the effect of folic addition on nitrification rates.*

*The thesis begins with a background section, where the need for this work is justified. An introduction chapter sets the theoretical basis to understand the content of the manuscript. The core of the document is the experimental methodology used to accomplish the objectives stated, as well as the results and conclusions obtained. Eventually, conclusions and recommendations are provided.*

# 4. Objectives and thesis structure

## 4.1. OBJECTIVES

The main goal of this thesis is to enhance sludge settling in existing full-scale petrochemical activated sludge systems focused on organic matter removal. For this purpose, two main issues must be addressed: i) Strong tendency of such systems to develop low food-to-microorganism ratio (F/M) filamentous bulking, and ii) Unintended biological nitrification in the continuous stirred tank reactor (CSTR).

Considering bibliographic recommendations, the approach to overcome the low F/M filamentous bulking has been to improve the biomass quality through the implementation of a selector in the activated sludge system. Hence, the literature presents the use of selectors on different wastewater sources, such as urban (Ferreira *et al*., 2014), slaughterhouse (Al-Mutairi, 2009), sugar mill (Prendl and Kroiss, 1998), fiber mill (Melcer *et al.,* 2003) and paper mill (Durocher *et al*., 2002). However, no references have been found on the use of selectors for petrochemical biological systems. As well, it is widely recognized that the design of a selector depends on the wastewater characteristics (Chambers and Tomlinson, 1982). Moreover, as stated by Seviour and Blackall (2012), although the literature reports general rules, the design of a selector relies still on experimental work. Therefore, the present research involves experimental objectives to assess the effectiveness of selectors in order to enhance sludge settling in the petrochemical system.

With regard to limiting biological nitrification, despite folic acid holds promising nitrogen heterocyclic structure (McCarty, 1999) the vitamin has not been referenced as a nitrification inhibitor. Additionally, the mode of action of such inhibitors is not yet clearly known (Torralbo *et al*., 2017). Consequently, to initiate this research line, it is necessary to evaluate the effect of folic acid supply on nitrification rates. Positive results at this initial step could lead to further studies in order to confirm the inhibitory effect of folic and determine its action mechanism.

Therefore, to achieve the stated overall goal, the following objectives are proposed:

i. To assess the effect of the selector's electron acceptor ($O_2$, $NO_3^-$) on sludge settling.

This objective allows to conclude about the effectiveness of a selector to enhance sludge settling in the petrochemical system. It is also an objective to identify the best type of selector for the petrochemical activated sludge system.

ii. To optimize the selector's design variables (hydraulic retention time and F/M) in order to enhance sludge settling.
Since petrochemical effluents often exhibit a low biodegradability and high particulate matter content, it is also an objective to determine the effect of the selector's influent biodegradability and particulate matter content on sludge settling. This objective aims to provide the parameters in order to carry out the design of the full-scale petrochemical selector.

iii. To optimize the mixed liquor operational parameters to enhance sludge settling for the activated sludge system including a selector.
Since sludge settling not only depends on the biomass quality, but also on the water characteristics, the effect of the mixed liquor parameters is evaluated for the new configuration of the activated sludge system including the selector.

iv. To assess the effect of folic acid on biological nitrification and on the operational parameters of the activated sludge system.
This objective intends to conclude about the effectiveness of folic acid to limit nitrification rates. Aiming to determine the feasibility of its full-scale implementation, it must also be evaluated the effect of folic acid on the operational parameters of the activated sludge system, such as organic matter removal efficiency, biomass growth rates, sludge volumetric index and oxygen requirements.

## 4.2. THESIS STRUCTURE

In order to reach the objectives stated in the previous section, pilot-scale tests have been conducted to assess the effectiveness and optimum design of the selector. Complementarily, bench-scale assays have been performed to test folic acid effect on the nitrification reaction and the performance parameters of the heterotrophic biological system. The description of the experimental work, the results obtained and the conclusions have been structured in the following chapters.

**CHAPTER 1: Background**

The Background section justifies the need for this research work. The challenge of the current European industrial wastewater treatment plants to adapt to increasingly stringent regulations is presented. On the other hand, two main issues related with sludge settling in petrochemical activated sludge systems, which difficult the accomplishment of such regulations, are stated. Taking as a base the existing bibliography, it is justified why additional experimental research is required to solve these issues. Chapter 1 also indicates the readers to whom the manuscript is addressed.

**CHAPTER 2: Introduction**

Chapter 2 introduces the biological treatment principles for the design and operation of industrial installations, in order to facilitate an understanding of the thesis content. Especial attention is paid to the activated sludge process, since it is the type of biological treatment used in this experimental research. Particularly, this chapter focuses on sludge settling issues, such as the filamentous bulking and unintended nitrification in systems based on organic matter removal, which are the main subjects to deal with in the present manuscript.

**CHAPTER 3: Legislative framework**

The legal framework introduces the principles to follow in the wastewater treatment and the objectives to reach in the final effluent. The main legislation that applies to industrial installations is presented. The content of this section drives to the conclusion that regulatory compliance after the 2016 version of the BREF reference document makes compulsory to enhance sludge settling in the activated sludge system.

**CHAPTER 4: Objectives and thesis structure**

This chapter states the objectives of the research. As well, it summarizes the content introduced in each chapter of the thesis document.

**CHAPTER 5: Materials and methods**

This chapter summarizes the properties of the petrochemical wastewater streams and the inoculum used in the experimental tests. Features of the pilot plant and bench-scale set up used for the experimentation are described. Considerations about folic acid manipulation have been included, since they have been key to conduct a successful experimentation. For the evaluation of results, a review and brief description of the analytical methods that have been used is also included. Eventually, the methodology for the statistical evaluation of the results obtained in the experimentations has been referred.

**CHAPTER 6: Effect of the selector's electron acceptor on sludge settling**

The operation of a pilot petrochemical CSTR has demonstrated the tendency of such systems to low F/M filamentous bulking. Tools to enhance sludge settling were tested in the pilot-scale installation, such as increasing the reactor's F/M as well as introducing aerobic and anoxic selectors in the activated sludge system. Sludge settling has been evaluated for each configuration, through microscopic observations and sludge volumetric index. As well, aiming to the full-scale implementation of the selector, the operational parameters of the activated sludge system, including or not the selector have been compared. The introduction of both selectors was effective to overcome the low F/M filamentous bulking, although the aerobic selector presented better sludge settling results and a more reliable operation. However, the inclusion of the selector in the activated sludge system resulted in a more intense unintended biological nitrification.

**CHAPTER 7: Optimization of the operational and design parameters of an aerobic selector**

The operational and design variables of the aerobic selector included in the activated sludge system were optimized, in order to enhance its effectiveness to improve sludge settling. The effect of selector's variables such as the hydraulic retention time, F/M ratio, influent biodegradability and influent particulate matter on sludge settling was experimentally tested in the pilot activated sludge system. This work allows to design a full-scale selector and to make a choice on the most convenient petrochemical effluents to be supplied to the selector.

**CHAPTER 8: Effect of the mixed liquor parameters on settling for activated sludge with selector**

The experimental work presented in chapters 6 and 7 has aimed to enhance sludge settling through the improvement of the biomass quality. However, sludge settling is not only affected by the sludge quality, but also by the mixed liquor's parameters. Bibliographic references report about the effect of the mixed liquor's variables on sludge settling for CSTR systems. The present research complements the existing bibliography by experiencing the effect of the mixed liquor's parameters on sludge settling for a system being operated with a selector. Pilot plant and laboratory-scale tests are presented and statistically evaluated to conclude about the effect of the mixed liquor's parameters on sludge settling.

**CHAPTER 9: Biological nitrification control by addition of folic acid**

The implementation of a selector allowed to overcome the frequent low F/M filamentous bulking. However, it enhanced nitrification by producing an older sludge. Therefore, a strategy to limit unintended nitrification had to be implemented, complementarily to the inclusion of a selector, in order to enhance sludge settling in the petrochemical activated sludge system. This chapter presents an alternative to conventional methods to limit biological nitrification, through the addition of folic acid. Bench-scale tests have been conducted in order to assess the effect of folic acid on nitrification patterns. Also, to verify whether folic acid addition is a full-scale feasible alternative, the effect of the vitamin on the heterotrophic operational parameters has been presented.

**CHAPTER 10: General conclusions and recommendations**

General conclusions from the experimentation carried out are presented. Recommendations for future work are proposed.

# 5. Materials and methods

***ABSTRACT***

*A pilot-scale activated sludge system was used in order to assess the effectiveness of a selector to overcome a low F/M filamentous bulking in the petrochemical system. The pilot plant could be configured as a CSTR or including a one-compartment selector, which could be operated as anoxic or aerobic. Three real petrochemical wastewater streams (A, B and C) were fed into the petrochemical system. Their flow rates could be adjusted upon convenience. Streams A and B came from oxidation processes and consequently, they were the most biodegradable ones. For this reason, they were fed to the selector when it was operative. Stream B provided the highest content in organic particulate matter. Stream C was a non-pre-treated one and was fed to the main reactor. The inoculum, as well, came from an industrial petrochemical CSTR.*

*With regard to the control of unintended nitrification in the petrochemical treatment focused on organic matter removal, bench-scale assays were organized to assess the effect of folic acid on the biological reaction and also on the operational parameters of the biological system. Two bioreactors supplied with vitamin doses of 0.4 and 0.9 mg* $g^{-1}$ *VSS* $d^{-1}$ *were run in parallel, in comparison to a control. The bench bioreactors were supplied with a mixture of two petrochemical feed streams (A and D). Stream D had already been pre-treated in a biological system. Folic acid came from commercial tablets ACFOL, which were grounded and dissolved in a carbonate-buffered solution at a pH of 9.0. The short-term toxicity supplied by folic acid to the effluent water was determined by respirometry and the chronic toxicity was analyzed based on the bioluminescence inhibition of Vibrio Fischeri.*

*Both systems were determined using analytical methods based on ASTM standards and sludge settling was characterized through sludge volumetric index and microscopic examinations.*

# 5. Materials and methods

## 5.1. FILAMENTOUS BULKING CONTROL BY THE IMPLEMENTATION OF A SELECTOR

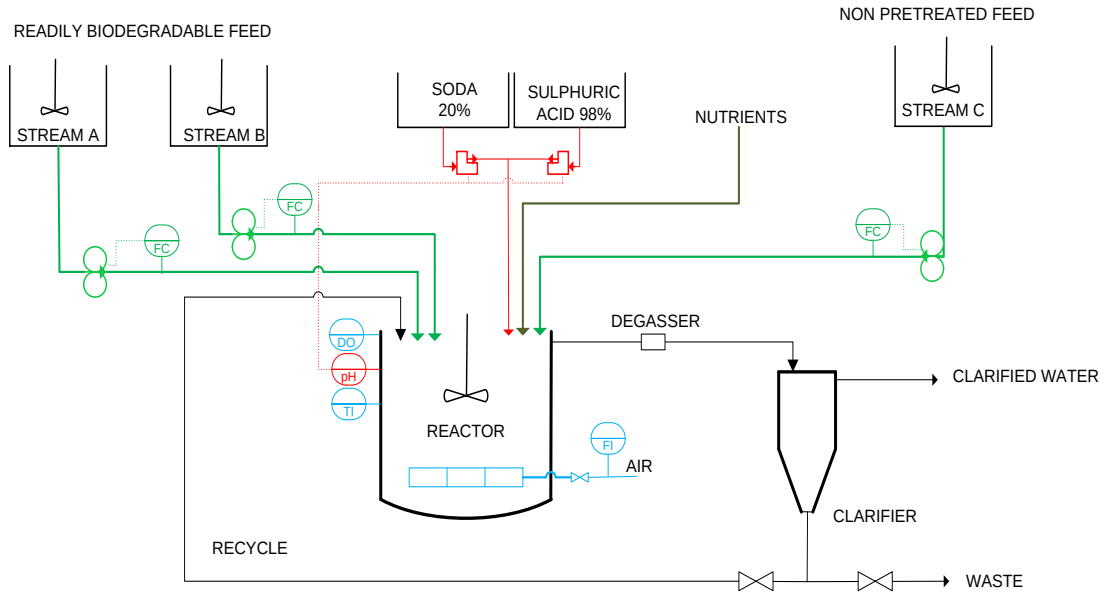
Pilot-scale tests with an activated sludge system were conducted to assess the effectiveness of a selector to overcome the low F/M filamentous bulking. The pilot biological system was supplied with petrochemical substrate and inoculum. The operational parameters were determined analytically by ASTM methods. The quality of the sludge for settling was evaluated macroscopically by the sludge volumetric index and also through microscopic observations. The comparison of the results obtained with and without the inclusion of the selector was based on statistical methods.

### 5.1.1. Pilot plant set up and operation

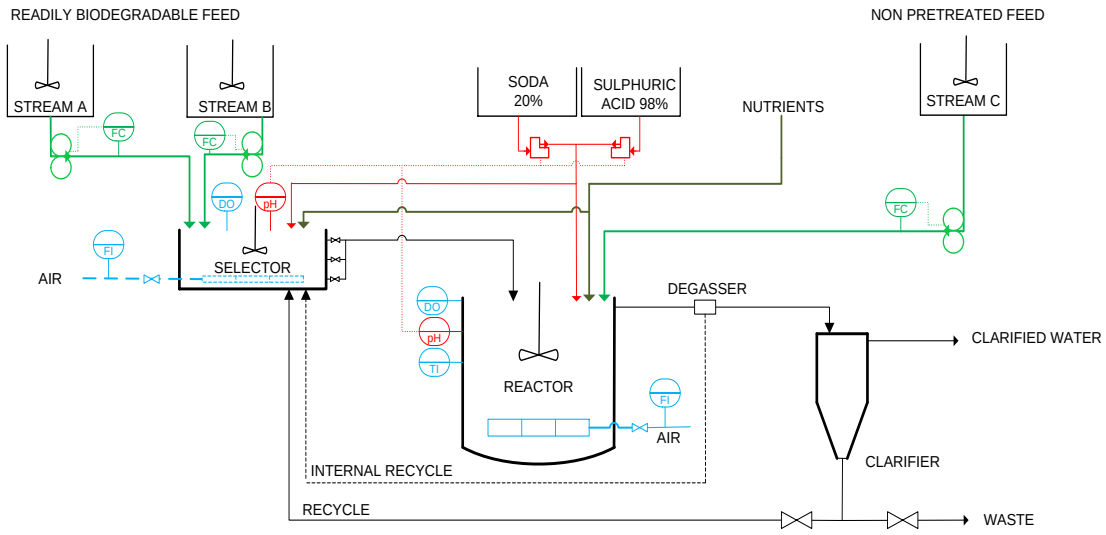
Figure 5.1 presents the pilot plant used in this experimental study, as well as the different configurations available to operate the installation. The pilot activated sludge system could be configured as a CSTR (Figure 5.1 a) or including a one-compartment selector (Figure 5.1 b), which could be operated as anoxic or aerobic. Optional air supply and internal recycle were available for the selector. When the aerobic selector configuration was tested, air was provided to the selector and the reactor. When the anoxic selector configuration was to be implemented, the air supply to the selector was turned off and the internal recycle was set operative, in order to provide nitrates to the selector's bacteria. The reactor volume was 500 L and the selector could work at three different volumes (namely 3.3, 6.7 or 10 L) in order to accomplish the required HRT.

The SRT was also established by wasting part of the concentrated sludge accumulated at the bottom of the clarifier. So as to help a better control of the quantity of biomass wasted, the waste was done discontinuously, into a leveled vessel. A sample of the daily total waste was analyzed for suspended solid content, and eventually for biomass. This allowed to know the wasted volume and the biomass concentration in it.

As reflected in Figure 5.1, nutrients could be provided to both the selector and the reactor. As nutrients, a sulphate ammonia solution 20%w and phosphoric acid 75%w could be dosed.




(a)




(b)

Figure 5.1 Scheme of the pilot-scale activated sludge process working as a CSTR configuration (a) and including an aerobic or an anoxic selector (b) *(Source: Cardete et al., 2017 a)*

Three feed streams (A, B and C) and an inoculum belonging to the petrochemical industry were fed into the pilot biological system. Each of the three streams was stored in each of the three available batch vessels, in order to be fed into the system. The pilot plant operation required batch charges for every feedstock vessel with the feed streams A, B and C. Analytical characterization of the content was made after replenishing.

Stirrers were always on, to ensure homogeneity of the fluid. The high and low level of the vessels was alarmed by switches for every vessel. To start operation, feed streams had to be aligned to the desired destination. The total desired flow for the feed streams was fixed as a set point on the flow controller that moved the speed of the respective pump. If the flow needed to be split in two ways, the respective needle valves for both destinations should be adjusted to get the desired measure in the flow indicator installed in one of the streams. To confirm proper adjustment, flow capacity was tested.

The main variables such as flow rate, pH, dissolved oxygen (DO) and temperature (T), could be monitored by a programmable logic controller (PLC). Particular emphasis was made on the control of DO and pH in the reactor and the selector. Air was introduced through membrane diffusers, and its flow was continuously monitored and manually regulated. Air was continuously supplied to the selector (18 NL $min^{-1}$) and the reactor (33 NL $min^{-1}$), through a one-point injection tube and a porous plate, respectively, located at the bottom of the equipment. The total air flow rate consumed in the pilot plant was measured by a mass flow controller (Tylan Mod.2920) and split afterwards into the reactor and the selector. The air introduced into the reactor was registered by a mass flow indicator (Tylan Mod.2910), whereas the supply to the selector was calculated by the difference of the two mass flow indicators. DO in the reactor and the selector was measured by galvanic membrane probes (Desin, Mod.TM-6679/TCO and HACH, Model 5740sc, respectively) located at the top of the equipment, which included temperature indication and compensation.

The pH was adjusted at 8.2 ± 0.2 in the reactor with sulphuric acid 98%w addition, taking as a reference a pH indicator (HACH, Model si792x P). The pH in the selector was monitored also by a HACH, Model si792xP probe at the mentioned range, without the need for acid (sulphuric acid 98%w) or base (soda 20%w) addition, though they were available.

Contrast probes were daily available for pH (Metrohm 826pH Mobile) and DO (Orion 810-3756) to confirm the measurements obtained and identify the need to recalibrate.

The reactor had the possibility to be heated up with an electrical trace, but not to be cooled. During the period of tests, the temperature in the reactor was 32 ± 2 ºC. The selector was partially heat-insulated and kept temperatures around 20ºC.

The experimental device was equipped with several sampling points. The selector could be sampled at the draw-off line into the reactor, whereas the samples of the reactor and

each of the feed vessels could be obtained either from the bottom or the top. Any stream, including the sludge recycle, could be sampled at its final destination point.

### 5.1.2. Substrate and inoculum

Three wastewater feed streams (A, B and C), as well as an inoculum belonging to the petrochemical industry were fed into the pilot biological system. Their main characteristics are summarized in Table 5.1.

Feed streams A and B came from full-scale advanced oxidation processes (AOP). Consequently, they showed a high total inorganic carbon content (TIC), due to the carbon dioxide generated in the oxidation reaction. Stream A provided the main quantity of carbonaceous matter, while stream B supplied the highest portion of particulate organic matter. Streams A and B presented a high biodegradability. For this reason, they were fed into the selector when it was operative. Stream B also contained ammonia and orthophosphate, as nutrients for the biomass. Therefore, stream B combined all the elements needed for the biomass growth. As a consequence, a significant biomass growth was detected in the vessel, in which stream B was stored. Hence, when planning vessel renewal time, it had to be taken into account the lack of stability of this stream.

On the other hand, stream C, was a non-pretreated wastewater, which explained its low biodegradability. Consequently, even with the selector configuration, this stream was fed to the reactor.

Short oxidized molecules (mainly alcohols and organic acids) were dominant in streams A and B, whereas stream C contained a variety of organic compounds, including those of streams A and B, and also traces of aliphatic and aromatic molecules. The composition of streams A and B was constant, while stream C experienced certain variability.

The inoculum came from an industrial petrochemical CSTR and exhibited average diluted sludge volumetric index (DSVI) values of 500 mL $g^{-1}$.

Table 5.1 Characterization of the inoculum and substrates employed in the pilot plant *(adapted from Cardete et al., 2017 a, b)*

| | | FEED STREAMS | | | MIXED LIQUOR |
|---|---|---|---|---|---|
| | | A | B | C | INOCULUM |
| | UNITS | AVERAGE (RANGE) | | | |
| **PARAMETERS** | | | | | |
| pH | | 8.1 (8.0-8.2) | 8.5 (8.2-8.5) | 4.0 (2.3- 6.5) | 8.2 (8.0-8.5) |
| TIC | g $L^{-1}$ | 3.2 (2.7-3.5) | 1.5 (1.3-1.6) | 0.10 (0.05- 0.20) | 2.7 (2.6-2.9) |
| Conductivity | mS $cm^{-1}$ | 33 (30-35) | 18 (16-20) | 22 (20-24) | 21 (18-23) |
| TOC | g $L^{-1}$ | 17.2 (16.0-19.5) | 2.5 (1.5-2.7) | 3.2 (2.2- 4.5) | 0.10 (0.07-0.15) |
| sCOD | g $L^{-1}$ | 50.9 (40.0-59.6) | 6.2 (4.5-7.5) | 8.8 (5.5-12.1) | 0.25 (0.20-0.30) |
| tCOD | g $L^{-1}$ | 50.6 (47.3-60.2) | 31.2 (19.5-62.5) | 12.3 (6.5-15.6) | 0.3 (0.2-0.4) |
| sCOD/TOC | | 2.9 (2.9-3.1) | 2.8 (2.5-3.0) | 2.9 (2.5-3.1) | 2.7 (2.5-3.0) |
| $BOD_5$/tCOD | | 0.35 (0.10-0.45) | 0.18 (0.11-0.25) | 0.10 (0.01-0.15) | 0.05 (0.01-0.08) |
| $NH_4^+$-N | mg $L^{-1}$ | <1 | 1200 (750-1500) | <1 | 2 |
| Norganic | mg $L^{-1}$ | <1 | <1 | <1 | <1 |
| $NO_3^-$-N | mg $L^{-1}$ | <1 | <1 | <1 | <1 |
| $PO_4^{3-}$-P | mg $L^{-1}$ | <1 | 75 (55-85) | <1 | 2 |
| TSS | g $L^{-1}$ | 0.4 (0.2-0.6) | 21.3 (20.1-25.6) | 3.2 (3.0-4.3) | 12.0 (10.1-17.2) |
| VSS | g $L^{-1}$ | 0.03 (0.02-0.04) | 0.1 (0.0-0.2) | - | 3.0 (2.5-3.5) |
| Inert | g $L^{-1}$ | <0.01 | 11.0 (10.1-12.8) | <0.01 | 3.3 (2.5-3.5) |
| **COMPOSITION** | | | | | |
| Short-chain alcohols | % | 0.10 (0.09-0.12) | | 0.04(0.01-0.08) | |
| Phenol compounds | % | 0.10 (0.09-0.12) | | 0.01 (0.01-0.02) | |
| Short-chain fatty acids | % | 3.02 (3.0-3.1) | 0.25(0.23-0.26) | | |
| Aromatic acids | % | | 0.23(0.20-0.25) | | |
| Aromatic compounds | % | | | 0.03(0.01-0.05) | |
| Ketones | % | | | 0.06(0.03-0.07) | |

| Acronyms | | | | |
|---|---|---|---|---|
| | TIC | Total inorganic carbon | TOC | Total organic carbon |
| | tCOD | Total chemical oxygen demand | sCOD | Soluble chemical oxygen demand |
| | $BOD_5$ | Five-day biological oxygen demand | $NH_4^+$-N | Ammonia nitrogen |
| | NTK | Kjeldhal nitrogen | $NO_3^-$N | Nitrite nitrogen |
| | $PO_4^{3-}$-P | Orthophosphate phosphorus | TSS | Total suspended solid |
| | VSS | Volatile suspended solid | | |

### 5.1.3. Analytical methods

The characterization of the feed streams, as well as the operational parameters of the mixed liquor, was carried out according to the ASTM standards (Rice *et al*., 2012).

Soluble and total chemical oxygen demand (sCOD and tCOD, respectively) were analyzed by digestion in a closed tube and spectrophotometry, with a HACH DR/2010 spectrophotometer at 600 nm wavelength. Total organic carbon (TOC) and the total inorganic carbon (TIC) were obtained from a total carbon analyzer Shimadzu TOC 5050A. The five-day biological oxygen demand ($BOD_5$) was measured with BOD Aqualytico material. The analysis of Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS) were performed using WHATMAN GF/A fiberglass filters, a muffle furnace working at 550 ± 50 ºC and a melting pot Haldenwanger Ò 82 A/3. The pH was determined by an Orion pH-meter model 710 A. Ammonia nitrogen was determined with a specific-ion electrode (Orion 95-12), while the quantification of orthophosphate was performed by a Shimadzu UV-VIS spectrophotometer (Compact UV-2600), at 880 nm wavelength. Nitrites ($NO_2^-$) and nitrates ($NO_3^-$) were analyzed with an ion-chromatograph 761 Compact IC Metrohm, provided with a chemical suppressor module. Kjeldhal nitrogen (NTK) characterization used a Büchidigestor model 425, with a Büchi distillation unit model 323.

Periodic respirometry tests were performed on the selector and reactor sludge with a Surcis BM-T respirometer equipped with a thermostatic unit MD40 in order to get information on the oxygen uptake rate (OUR) of the sludge.

The sludge settling ability was monitored through diluted sludge volumetric index (DSVI) calculated on suspended solids basis and microscopic examination. This technique was used to compare the floc characteristics and the overall abundance of filaments obtained in each test (Jenkins *et al.*, 2003). The wet mount examination, at 100X, rendered information on floc size and characteristics, filamentous organism's abundance and effect of the filamentous organisms on the floc structure. Next, a 400X observation yielded information on the filamentous organisms, such as characterization, branching, shape and location. Gram and Neisser stains were complementarily used to characterize the filaments. Moreover biological indicators could be observed, as representative of the biological quality of the sludge. Polymerase chain reaction (PCR) methodology could not be applied to characterize the filaments, due to matrix interference.

#### 5.1.3.1. Analytical methods for process control

Some complementary information on the analytical methods used to control the pilot activated sludge process is specified in this section.

***pH*** **-** It is a measure of the hydrogen-ion concentration, as reflected in equation 5.1, where $[H^+]$ is the concentration of protons expressed in mol $L^{-1}$.

$$pH = -log[H^+] \quad (5.1)$$

The pH range to allow for biological activity is quite narrow and critical, typically within 6 to 9 (Metcalf and Eddy, 2003). Therefore, the pH must be controlled in biological systems. In this study, the pH in aqueous systems has been measured with a pH-meter (Orion, model 710A). The pH of the sample has been determined electrometrically using either a glass electrode in combination with a reference potential or a combination electrode. The measurement device had been previously calibrated using standard solutions of a known pH. The samples analyzed contained colour, turbidity, colloidal matter, oxidants, reducers and moderate salinity (< 0.1 M), which does not usually interfere in the measurement. Temperature affects on the electrometric determination of pH, as the electrode output is different at various temperatures. This was considered by having a temperature compensated instrument. Also pH papers and indicator solutions that change color at a definite pH value were occasionally used. The pH was determined by comparing the color of the paper or solution to a standard. Reference standards are ASTM D1293 and ASTM E70.

***Temperature*** **-** The temperature of water is an important parameter, since it determines reaction rates, the solubility of gases, the type of microbiology and the suitability of water for microorganism's life. Optimum temperatures for bacterial activity are in the range from 25 to 35 ºC. Both, aerobic digestion and nitrification stop at temperatures higher than 50 ºC. When temperature drops down to 5 ºC the autotrophic bacteria (nitrifying bacteria) stop their function. At 2 ºC, even the chemoheterotrophic bacteria that feed from carbonaceous organic matter become inactive (Metcalf and Eddy, 2003). Therefore, in this study, temperature was continuously measured in the mixed liquor with normalized ASTM E2251-07 instruments.

***Conductivity*** **-** The electrical conductivity of water is an indirect measure of the number of ions contained in it (Standard Methods 2510), similar to the total dissolved solids. The conductivity measurement is referred in the literature as a quick estimation of the total dissolved solids (Standard Methods, 1998), as reflected in equation 5.2.

$$TDS = EC\ (0.55 - 0.70) \quad (5.2)$$

Where

$TDS$ Total dissolved solids (mg $L^{-1}$)

$EC$ Electrical conductivity (dS $m^{-1}$)

However, it was proofed that this relationship does not necessarily apply to industrial wastewater. Therefore, the estimation could only be occasionally used for the biological system's effluent water. To measure the conductivity, a conductivity meter (METROHM mod. 644 or 660) was used, as well as a cell for conductivity determination, METROHM model 6.0901.110.

***Alkalinity*** **-** Alkalinity in water is a measure of the concentration of hydroxides ($OH^-$), carbonates ($CO_3^{2-}$) and bicarbonates ($HCO_3^-$) of a variety of elements, such as calcium, magnesium, sodium, potassium and ammonia. Borates, silicates, phosphates can also contribute to the alkalinity (Metcalf and Eddy, 2003). As the alkalinity in wastewater increases, more acid is needed to change the pH. Alkalinity plays an important role in biological treatments, especially in biological nutrient removal and ammonia stripping by air.

The methodology used to evaluate alkalinity was based on Standard Methods 2320. The alkalinity was determined by titrating against a standard acid (0.02 N sulphuric acid). Alkalinities are classified according to the endpoint of titration with a strong acid, as it was explained in section 2.3.3.1.

***Total organic carbon and total inorganic carbon*** **-** The total organic carbon (TOC) and the total inorganic carbon (TIC) have been analyzed with a Shimadzu TOC-5050. As a pretreatment, to protect the equipment's injection system from obstructions, the sample was filtered at 20 µc. Also, considering the measuring range of the analyzer (from 50 µg $L^{-1}$ to 4000 mg $L^{-1}$), some samples needed to be diluted.

The equipment provided results for the total carbon and for the inorganic carbon. The organic carbon was obtained by difference between both. To determine the total carbon, the sample was automatically injected in a high temperature combustion tube filled with a platinum catalyst, where the totality of the carbon is oxidized with high purity air. The carbon dioxide resulting from the oxidation process flows into a non-dispersive infrared analyzer (NDIR), where it is quantified. To determine the inorganic carbon, the sample is introduced in a reaction chamber with a phosphoric acid solution, where only the

inorganic carbon compounds are decomposed into dioxide carbon, which is quantified in the NDIR.

However, the literature refers that in such analyzers, large TIC concentrations can induce overestimation of the total carbon and consequently of the total organic carbon (Findlay *et al*., 2010). Therefore, in the samples of mixed liquor, where the TIC concentration was much higher than the TOC, to conduct the TOC characterization, the sample was previously acidified with sulphuric acid 98%w under agitation. This preliminary procedure allowed to eliminate the inorganic carbon and its interference to determine the TOC. Eventually, upon the analytical results, there was a typical ratio chemical oxygen demand-to-total organic carbon (COD/TOC) between 2.5 and 3.0 for all the samples analyzed.

***Total and soluble chemical oxygen demand*** - The methodology used to analyze chemical oxygen demand (COD) was based on Standard Methods 5220 and ASTM D1252 (Standard test methods for Chemical Oxygen Demand of water).

The COD test is used to measure the oxygen equivalent to the organic material contained in wastewater. The organic matter is oxidized with potassium dichromate (electron acceptor) in very strong oxidizing conditions (elevated temperature, 70% sulfuric acid and silver catalyst, among others). Therefore, it is expected to achieve a complete oxidation of most organic compounds into carbon dioxide. In turn, dichromate potassium ($K_2Cr_2O_7$) undergoes the reduction reaction stated in equation 5.3.

$$Cr_2O_7^{2-} + 14\,H^+ + 6\,é \xrightarrow{Ag_2SO_4} 2\,Cr^{3+} + 7\,H_2O \qquad (5.3)$$

Since dichromate potassium is added in excess, the fraction that has not been reduced by the organic or inorganic matter in the wastewater is titrated with Mohr's salt, which is an inorganic compound with the formula $(NH_4)_2\ Fe(SO_4)_2.6H_2O$. Equation 5.4 represents the redox reaction to determine the excess of dichromate.

$$Cr_2O_7^{2-} + 14\,H^+ + 6\,Fe^{2+} \rightarrow 2\,Cr^{3+} + 7\,H_2O + 6\,Fe^{3+} \qquad (5.4)$$

The two main fractions of COD are particulate and soluble. Since total chemical oxygen demand (tCOD) includes both, the sample was not filtered to determine it. When the soluble fraction (sCOD) was to be characterized, the sample was filtered at 20 µc.

***Biological Oxygen Demand*** **-** The methodology used for biological oxygen demand (BOD) analytical was based on Standard Methods 5210. This test provides an indirect measure of the organic material content, by measuring the oxygen consumed as electron acceptor during the biological oxidation process. Three aerobic reactions occur in the biological oxidation process of the organic matter contained in the wastewater, in which the oxygen is consumed. The global generalized equation 2.1, presented in chapter 2, accounts for two of these processes, which are the oxidation of part of the organic matter into end products and the simultaneous biomass synthesis reaction. The third process refers to the endogenous respiration, which was represented in equation 2.2.

The oxygen required to complete the three reactions is known as the ultimate carbonaceous or first stage BOD (UBOD). In most readily degradable substrates, phase one is completed in 24 to 36 hours (Eckenfelder, 1998).

Usually UBOD and COD are not coincident. There are several reasons that explain the differences:

- Many organic substances are difficult to oxidize biologically, but can be oxidized chemically.
- Inorganic substances can be oxidized by dichromate, and consequently increase the apparent organic content.
- Certain organic substances may be toxic to the microorganisms in the BOD test

The BOD test is a batch analysis. The assay was conducted in closed, dark glass bottles to prevent oxygen transfer from the atmosphere and exposure to light to exclude photosynthesis, respectively. The volume of the BOD bottles was 300 mL. A small sample of the wastewater to be tested was placed in the BOD bottle, which was then filled with dilution water saturated in oxygen and containing the nutrients required for the biological growth. Since industrial wastewaters are not expected to provide microorganisms, a seeded test was conducted by adding a small quantity of mixed liquor from the pilot biological reactor. Several dilutions of the problem wastewater were prepared to cover the complete range of possible BOD values. Also, 2-chloro-6-(trichloromethyl)pyridine, prepared at a concentration of 1.24 g $L^{-1}$ and diluted with water at 1 mL $L^{-1}$, as nitrification inhibitor was added, in order to avoid consuming oxygen in nitrification. Before the bottle was stoppered, its oxygen concentration was measured. After 5 days of incubation at 20 ºC, the oxygen was measured again in the bottle. Also, in parallel with the test bottles, a control test was run using a standard substrate of known BOD. As well, a BOD bottle supplemented with seed but without

problem wastewater was used as a control. The BOD of the problem wastewater was calculated as indicated in equation 5.5.

$$BOD = (D_1 - D_2) - (B_1 - B_2)\left(\frac{f}{p}\right) \quad (5.5)$$

Where

| | |
|---|---|
| $BOD$ | Biological oxygen demand (mg $L^{-1}$) |
| $D_1$ | Dissolved oxygen of diluted sample just after preparation (mg $L^{-1}$) |
| $D_2$ | Dissolved oxygen of diluted sample after 5-day incubation at 20 ºC (mg $L^{-1}$) |
| $B_1$ | Dissolved oxygen of seed control before incubation (mg $L^{-1}$) |
| $B_2$ | Dissolved oxygen of seed control after incubation (mg $L^{-1}$) |
| $f$ | Volumetric fraction seeded dilution water in sample to seed-control (mL $mL^{-1}$) |
| $p$ | Volumetric fraction of wastewater sample to total combined volume (mL $mL^{-1}$) |

The BOD test has some limitations:

- Simultaneous oxidation of inorganic compounds causes an overestimation of the oxygen demand.
- Usually, the oxidation of the organic matter is not completed in 5 days. Therefore, a constant is needed to correlate the five-day biological oxygen demand ($BOD_5$) with UBOD.
- The test period is long (5 days).
- The presence of toxic materials may interfere with the biological activity during the test, so that the measured BOD will underestimate the true organic content in the sample. This issue is bound to happen in industrial wastewaters.
- Characteristics of the seed organisms may influence the rate of oxidation of organics.

The incubation period used in the BOD tests performed was 5 days, though longer tests could have been planned. Biochemical oxidation, theoretically takes an infinite time to go to completion, as the rate of oxidation is proportional to the amount of organic matter remaining. Within a period of 20 days, it is expected to accomplish a 95 to 99 % oxidation of the carbonaceous substrate, while in a five-day period just a 60 or 70 % is completed (Metcalf and Eddy, 2003).

The rate of BOD oxidation is model based on the assumption that the amount of organic material remaining at any time is governed by a first-order function (Metcalf and Eddy, 2003).

$$\frac{dBOD_r}{dt} = -K_1 BOD_r \quad (5.6)$$

Integrating between the limits of UBOD and $BOD_r$ at t=0 and t=t

$$BOD_r = UBOD\ e^{-K_1 t} \quad (5.7)$$

Where

| | |
|---|---|
| $BOD_r$ | Amount of waste remaining at time t (mg $L^{-1}$) |
| $k_1$ | First-order reaction rate constant (1 $d^{-1}$) |
| $UBOD$ | Total or ultimate BOD (mg $L^{-1}$) |
| $t$ | Time (days) |

Then, the BOD degraded at time $t$ is:

$$BOD_t = UBOD - BOD_r = UBOD - UBOD\ e^{-K_1 t} = UBOD(1 - e^{-K_1 t}) \quad (5.8)$$

The bibliography suggests values for the constant $k_1$ . Hence, for untreated wastewater it is usually about 0.12 to 0.46 $d^{-1}$, with a typical value of about 0.23 $d^{-1}$. The value of $k_1$ for effluents, which have been treated in a biological process is within the values 0.12 to 0.23 $d^{-1}$ (Metcalf and Eddy, 2003).

***Solids content*** **-** The wastewater and the mixed liquor contain a variety of solid materials, ranging from small to colloidal particles. The method used for analyzing the different fractions of solids was based on Standard Methods 2540. Solids could be identified into different fractions.

- *Total solids (TS)*: It was characterized as the residue remaining after the wastewater sample had been evaporated and dried at a temperature of 105 ºC.

- *Total suspended solids (TSS)*: It was identified as the portion of the TS retained on a filter with a nominal pore size of 2 µc, measured after being dried at a temperature of 105 ºC.

- *Total dissolved solids (TDS)*: This fraction identifies colloidal and dissolved solids that pass through the filter and are then evaporated and dried up at a

specific temperature of 105 ºC. It was calculated as the difference between TS and TSS.

- *Volatile suspended solids (VSS)*: They were characterized as the solids that could be volatilized and burned off when the TSS fraction was ignited (500 ± 50 ºC).

- *Fixed suspended solids (FSS)*: It was considered as the residue that remained after ignition of the TSS at 500 ± 50 ºC.

- *Volatile dissolved solids (VDS)*: It was determined as the fraction of solids that could be volatilized and burned off when the TDS were ignited at 500 ± 50 ºC

- *Fixed dissolved solids (FDS)*: It was calculated as the residue that remained after TDS were ignited at 500 ± 50 ºC.

- *Total volatile solids (TVS)*: Those solids that could be volatilized and burned off when the TS were ignited at 500 ± 50 ºC.

- *Total fixed solids (TFS)*: The residue that remained after TS were ignited at 500 ± 50 ºC.

The fraction of VSS in the mixed liquor is often identified with the content in biomass. However, it was proofed that when feed streams supplied organic particulate matter to the mixed liquor, it interfered causing overestimation in the biomass content. Therefore, in the system studied, the VSS analysis was complemented with nitrogen Kjeldhal (NTK) characterization, in order to identify the biomass content.

***Phosphorus*** - Phosphorus is found in aqueous solutions in different chemical forms, such as orthophosphate, polyphosphate and organic phosphate. The orthophosphates $PO_4^{3-}$, $HPO_4^{2-}$, $H_2PO_4^-$ and $H_3PO_4$ are the ready source of phosphorus for the biomass. The polyphosphates include molecules with two or more phosphorus atoms, combined with oxygen and hydrogen. Polyphosphates can undergo a slow hydrolysis in aqueous solution, and revert to the orthophosphate forms.

The analytical procedure used to characterize orthophosphates was based on ASTM D-515-82 and Standard Methods 425. Orthophosphate was determined by directly adding a mixture of ammonium molybdate solution with ascorbic acid 0.01 M, which

forms a coloured complex with the phosphate. The measurement was performed with a spectrophotometer UV/VIS at 880 nm.

To determine the total phosphorus, the polyphosphate and organic phosphate must be converted into orthophosphates using a previous digestion step with concentrated sulphuric acid and nitric acid.

***Nitrogen*** **-** Different nitrogen species were characterized in the wastewater feed streams and the mixed liquor**.**

- *Ammonia nitrogen*: Ammonia nitrogen was analyzed based on ASTM D-1426 by an Orion 95-12 selective ammonia electrode. This electrode uses a hydrophobic gas permeable membrane to separate the sample to be measured from an ammonium chloride internal solution. The ammonia contained in the sample as $NH_4^+$ is converted into $NH_3$ (aq) by adding a soda solution until pH between 11 and 14 is attained. The $NH_3$ (aq) in the sample diffuses through the membrane of the electrode, changing the pH of the internal solution.

- *Kjeldhal nitrogen (NTK)*: The parameter NTK determines the organic nitrogen plus de ammonia nitrogen. NTK determination was conducted based on ASTM D3590. The sample was heated in the presence of concentrated sulphuric acid. A neutral salt ($K_2SO_4$) was added to increase the boiling point of the acid solution. This way increases the digestion temperature and favours decomposition. Also, a reducer catalyst agent was added($HgSO_4$). An issue taken into consideration was nitrite and nitrate concentration, which causes NTK underestimation when the nitrite and nitrate are about 10 times the NTK concentration (EPA-600/7-77-017, 1977). Whenever this happened, the mixed liquor was kept without aeration for a period of time, until the nitrates had been consumed by the biomass. The NTK was characterized afterwards.

- *Nitrites and nitrates*: The characterization was performed by ionic chromatography, based on ASTM D4327. Nitrites and nitrates were determined in the mixed liquor immediately after sampling, since their concentration decreases with the time because of their use as electron acceptors by the bacteria. An alternative methodology was to acidify the sample just at the sampling moment, in order to inactivate the bacteria. This alternative was used

occasionally when the residual TOC in the mixed liquor was high, which enhanced the bacteria's use of nitrates as electron acceptor.

Also, test strips for nitrite and nitrate measurement from HACH were occasionally used, as a contrast to the ionic chromatography results.

### 5.1.3.2. Analytical methods for sludge quality evaluation

***Sludge Volumetric Index -*** A macroscopic evaluation of sludge settling was performed through the sludge volumetric index (SVI). A one-litre sample was taken from the pilot mixed liquor and settled for 30 minutes in a graduated cylinder. The settled volume of the sludge, known as V30, was registered. Also, the TSS concentration in the sample was analyzed. The SVI was calculated as follows, in order to account for the effect of TSS concentration on sludge settling.

$$SVI = \frac{(\mathrm{V30}) * (1000)}{(\mathrm{TSS})} \tag{5.9}$$

V30 was expressed in mL $L^{-1}$ and TSS in mg $L^{-1}$. SVI was calculated in mL $g^{-1}$.

Criteria have been published to evaluate the sludge settling ability upon the SVI results. Hence, Parker *et al*. (1998) stated that a good sludge settling corresponds to a SVI below 100 mL $g^{-1}$ 90% of the time. Later, Gray (2004) postulated that a good sludge settling should exhibit a *SVI* value below 80 mL $g^{-1}$, and a very good settling should be below 50 mL $g^{-1}$. Values higher than 120 mL $g^{-1}$ were said to indicate poor settling properties.

For bulking sludge, characterized by SVI values over 150 mL $g^{-1}$ (Mangrum, 1998; Parker *et al*., 2001), the SVI resulted to provide poor sensitivity to determine a small improvement on sludge settling. Moreover, it is well known that the SVI test is severely affected by total suspended solid concentrations in the mixed liquor higher than 4 g $L^{-1}$ (Gray, 2004). Also, due to quiescent conditions in the SVI test, settling occurs past the hindered zone and more near the compression zone, making the test less representative. For these reasons, in these assays the diluted sludge volumetric index (DSVI) was used. A 25% dilution of the mixed liquor with clarified water from the biological reactor was performed. The conventional V30 test and the determination of TSS were conducted on

the diluted mixed liquor. The DSVI was calculated from these results, as expressed in equation 5.9.

***Microscopic examination*** - Microscopic examinations performed with a Nikon Eclipse 80i microscope allowed to identify sludge settling characteristics through the floc appearance, types and abundance of filamentous organisms (Jenkins *et al*., 2003). As well, monitoring biological indicators reported the quality of the influent wastewater and the resulting mixed liquor in the biological reactor.

When examining the wet mount under direct and phase contrast illumination at 100X, the following characteristics were observed (Jenkins *et al*., 2003):

- *Floc size*. A usual methodology is to measure around 10 to 20 approximately spherical flocs and place them in the following categories based on their diameters:

  Small ≤150µm Medium 150-500µm Large ≥500µm

  Nevertheless, the flocs observed were not usually spherical. Therefore, the size was qualitatively evaluated by comparing the results obtained in the different tests.

- *Structure of activated sludge flocs*. The flocs observed were categorized as round or irregular, compact or diffuse, firm or weak.

- The *presence* of protozoa and other macro-organisms as *bio-indicators*.

- *Non-biological organic and inorganic particles*. Much of the biological material was not visible, while non biological material was readily observed under direct illumination.

- *Bacterial Colonies*. The presence of bacterial colonies, that were composed by specific types of microorganisms (fingered zooglea, nitrifying bacteria) were identified.

- *Effects of filamentous organisms on floc structure*, such as bridging and open floc. Bridging was characterized by filaments extending from the floc surface

into the bulk solution and bridging between the flocs. Open floc structure was determined when floc population grew around the filaments, leading to large, irregularly shaped flocs with substantial internal voids.

- *Filamentous organism abundance*: Filaments were observed at 100X and 400X. Its abundance was qualitatively determined, by comparison between the samples.

To identify the filamentous microorganisms, Gram and Neisser stains were used previous to phase contrast microscopic observation (Jenkins *et al*., 2003). Stains were performed in an external laboratory.

***Respirometry*** **-** In the respirometry test the biological oxygen consumption rate is measured under well-defined experimental conditions.

The respirometry test is useful to monitor the behaviour of the activated sludge process, since the respiration rate of the bacteria is directly related to substrate removal and growth. Hence, the respirometers are based on a technique which allows to determine the rate at which the bacteria take up the DO from the mixed liquor. This can be performed by measuring directly DO or indirectly by measuring gaseous oxygen.

Determining DO usually involves electrochemical measurements based on the Clark-cell, whereas gaseous oxygen concentration can be measured by physical techniques, such as the paramagnetic method. In this test, the bacterial respiration is characterized through two parameters: OUR (oxygen uptake rate) measured in mg $O_2$ $L^{-1}h^{-1}$ and SOUR (specific oxygen uptake rate) measured in mg $O_2$ $g^{-1}$VSS $h^{-1}$. OUR is calculated as the slope of a straight line which represents bacteria's oxygen consumption in front of time. To account for the dependence of OUR on the biomass concentration, OUR can be divided by the VSS concentration, so that the specific term SOUR is obtained.

The BMT respirometer used for the tests consisted of a one-litre-volume reactor vessel, where the mixed liquor should be allocated (see Figure 5.2). The vessel was provided with a thermostatic jacket, which was connected to a thermostatic bath in order to keep a constant temperature. To maintain a homogeneous mixture of the mixed liquor, the system was equipped with a recirculation peristaltic pump. Air was introduced in the reactor vessel through a porous diffuser connected to an air compressor. An oxygen probe submerged in the liquid indicated DO in the mixed liquor.

Figure 5.2 Image of the BMT respirometer model used for the respirometry tests (*Source: Surcis.com*)

The procedure followed to perform the respirometry aimed to measure the decrease in DO due to respiration as function of the time. Because of limitations in the air supply of the respirometer, the mixed liquor of the pilot plant had to be diluted with effluent water from the clarifier to a total suspended solid concentration of 1 g $L^{-1}$. A three-litre-volume of this preparation was aerated to saturation and stabilized at 30ºC in a parallel thermostatic bath. Next, one-litre batch volumes of the diluted mixed liquor were used to perform each respirometry assay. To conduct a respirometry assay, the mixed liquor introduced in the reactor vessel was saturated with air supply until the DO probe lecture stabilized at values around 6.5 mg $O_2$ $L^{-1}$ (saturation). At this point, the air supply was stopped.

The first respirometry assay was to evaluate the endogenous respiration of the sludge, without supplying any feed stream. The DO tendency was evaluated along the time in such conditions. As presented in Figure 5.3, DO experienced a slight reduction along the time. The OUR obtained is conditioned by several factors: the amount of biomass, the sludge age and the residual organic matter concentration in the mixed liquor.

A second respirometry assay was performed, while supplying a small volume of feed mix (usually 0.15 mL). Consequently, as shown in the example of Figure 5.3, DO decreased faster due to a higher activity of the biomass. Eventually, a third respirometry assay was usually conducted, while a higher volume of feed stream was provided to the mixed liquor (2.4 mL). The expected tendency was an even faster reduction of DO than

in the second respirometry. The tendencies obtained during the pilot and bench-scale assays corresponded to the patterns described. Otherwise, it should have been interpreted as a sign of toxicity in the effluents supplied.

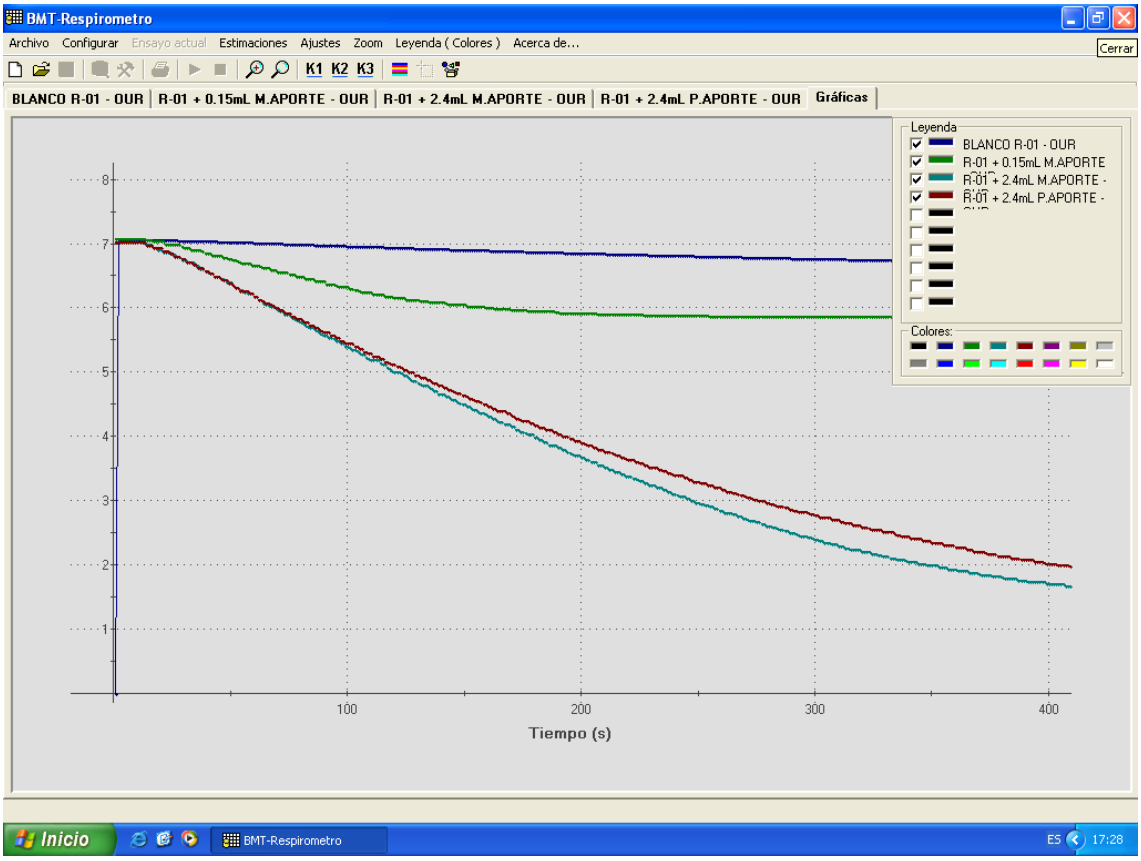



Figure 5.3 Typical tendencies obtained in a respirometry test. The oxygen consumption in the mixed liquor (mg $L^{-1}$) is represented in front of the elapsed time (s), for different volumes of feed supplied.

### 5.1.4. Statistical analysis tools

To conclude about the significance of the differences between the results obtained, statistical techniques were applied. A t-test and an F-test were run to compare, respectively, the means and the variances of the studied variables. Also, experimental design arrangements $2^k$ were run, in order to assess the effect of process variables on sludge settling. The ANOVA analysis was used to conclude about significant effects. The statistical significance of the differences was tested for a 95% confidence level. As support software, *Statgraphics Plus for Windows 3.3 (1994-1998)* was used.

## 5.2. BIOLOGICAL NITRIFICATION CONTROL BY ADDITION OF FOLIC ACID

Bench-scale tests were conducted to assess about the effectiveness of folic acid to limit biological nitrification. Bench bioreactors, supplied with petrochemical substrate and inoculum, were supplemented with folic acid. To guarantee the vitamin stability, a solid compound was chosen. Nevertheless, dosing strategies needed to be adopted in order to ensure its supply to the bioreactors. Also, the analytical determination of folic acid required the development of a methodology, based on Matias *et al.* (2014) premises. Eventually, the short-term and chronic toxicity caused by folic acid addition was evaluated.

### 5.2.1. Bench-scale set up and operation

The experimental arrangement consisted of two, aerobic, five-litre bioreactors supplied with folic acid, which were run in parallel, in comparison to a control one.

The content of the experimental bioreactors was agitated and oxygenated with air supply, through a multiple-orifice distributor, up to an oxygen residual above 2 mg $L^{-1}$. Before being introduced in the bioreactor, the air was forced through a water trap to be humidified and hence, to avoid evaporation. A petrochemical feed mix, an acid solution and a carbonate-buffered solution were continuously fed to the bioreactors with a multichannel peristaltic pump Watson Marlow 205C, working at a constant flow rate of 7 mL $h^{-1}$ each. The three bioreactors were inoculated with the same quality of mixed liquor and were also provided with the same feed mix. The acid solution dosage allowed to control the pH in the mixed liquor at values of 8.0 ± 0.3. For the two bioreactors supplied with folic acid, the carbonate-buffered solution was supplemented with the corresponding amount of vitamin. The concentration of the vitamin in the buffered solutions provided to the bioreactors was analytically confirmed after preparation. The two test bioreactors were operated at folic acid concentrations in the mixed liquor of 0.4 and 0.9 mg $g^{-1}$ VSS $d^{-1}$, each. The bioreactors were operated on a semi-continuous basis. Therefore, initially, the bioreactors were filled with a one-litre working volume of mixed liquor. As the supply streams were fed, the volume of the bioreactors increased. Once a day, the working volume was restored to one litre by centrifuging the extra-volume (3000 rpm, 10 minutes) and returning the sludge contained in it to the bioreactors. The surplus liquid was used to perform routine analysis.

Dissolved oxygen in the bioreactors was monitored with a portable Orion 810-3756 probe and registered always concentrations over 2 mg $L^{-1}$. The pH in the bioreactors was determined twice a day with an Orion pH-meter model 710 A. Temperature was monitored with a thermometer ASTM 91C.

The experimentation was conducted in two stages. Firstly, two of the three bioreactors were supplied with constant folic acid concentrations of 0.4 and 0.9 mg $g^{-1}$VSS $d^{-1}$, each. After this experimental period, the folic acid dose was interrupted. In the second stage, habituation to the vitamin was evaluated.

The mixed liquor SOUR was daily tested in the BMT-respirometer by addition of 2.5 mL of mix feed stream to 1 L of mixed liquor.

### 5.2.2. Substrate and inoculum

The substrate and inoculum used in the bench bioreactors were collected from a full-scale petrochemical activated sludge system. Two effluents, A and D, keeping a volumetric proportion of 7.5: 92.5, respectively, were mixed to produce the feed stream to the bioreactors. Stream A had already been used in the selector's assays and was defined in section 5.1.2. Stream D had already been pre-treated in a biological system. The mixture was supplemented with sulphate ammonia solution 20%w and phosphoric acid 75%w to keep a residual higher than 2 mg $L^{-1}$ of ammonia nitrogen and orthophosphate phosphorus in the mixed liquor, respectively. As the resulting feed stream supplied an amount of particulate organic matter, it was filtered at 20 µc before being fed into the bench reactors to avoid variability in the organic matter content and interference in the volatile suspended solid analysis. Table 5.2 reflects the main parameters for the filtered feed stream and the inoculum. Additionally, an acid solution was prepared by addition of sulphuric acid 98%w to demineralized water and was added to the bioreactors, in order to keep a pH in the mixed liquor of 8.0 ± 0.3.

### 5.2.3. Commercial drug: vehicle and stability

Stability problems arise more frequently with liquid pharmaceutical preparations than with solid dosage forms (Vignesh *et al*., 2012). For this reason, commercial tablets (ACFOL) with a content of 5 mg folic acid, manufactured by ITALFARMACO (Batch number: EKA 7399 Expired: 03 2022; Batch number: EKA 7405 Expired: 06 2022;

Batch number: EKA 7415 Expired: 08 2022) were used for the compounding of the folic acid suspension.

Folic acid is a water soluble vitamin, unstable in the presence of water due to the change in pH (Vignesh *et al*., 2012). To solve this issue, upon the proposal of Gazzali *et al.* (2016) and Matias *et al.* (2014), folic acid was dissolved in a carbonate-buffered solution at a pH of 9.0 to favour the stability and solubility of the solid and also to allow the determination of the concentration in the solution to be dosed. Following the methodology used by Gunasekaran *et al*. (2015), the folic acid tablets were grounded using a mortar and pestled to form fine powder. The powder was added gradually into the carbonate-buffer solution under mixing conditions. The suspension was stirred with a glass rod until a uniform solution was formed. Buffered solutions at different folic acid concentrations, within the range of 1.0 to 15.0 mg $L^{-1}$ were prepared to be fed to the bioreactors, according to the folic acid concentration required in the mixed liquor.

Table 5.2 Characterization of the petrochemical wastewater feed stream and inoculum used in the bench-scale experimentation *(Source: Cardete et al., 2019)*

| *Parameter* | *Description* | *Units* | *Feed stream* | *Inoculum* |
|---|---|---|---|---|
| COD | Chemical Oxygen Demand | mg $L^{-1}$ | 4490.7 ± 816.3 | 177.0 ± 1.4 |
| TOC | Total Organic Carbon | mg $L^{-1}$ | 1750.2 ± 296.2 | 100.0 ± 28.2 |
| $BOD_5$ | Biological Oxygen Demand | mg $L^{-1}$ | 808.3± 343.5 | - |
| TIC | Total Inorganic Carbon | mg $L^{-1}$ | 1335.3 ± 588.9 | 992.5 ± 95.4 |
| $NH_4^+$-N | Ammonia Nitrogen | mg $L^{-1}$ | 416.1 ± 66.6 | 1.9 ± 0.1 |
| TSS | Total Suspended Solids | g $L^{-1}$ | - | 1.67 ± 0.05 |
| VSS | Volatile Suspended Solids | g $L^{-1}$ | - | 1.24 ± 0.04 |
| pH | pH | | 8.12 ± 0.04 | 8.14 ± 0.02 |
| SVI | Sludge Volumetric Index | mL $g^{-1}$ | - | 87.7 ± 24.7 |

The soluble COD (sCOD) and five-day biological oxygen demand ($BOD_5$) of the folic acid buffered solution were tested for two different concentrations of the buffered solution (see Table 5.3).

Stability considerations determined the manipulation premises for the folic acid solution. The degradation of folic acid is said to be enhanced by factors such as light, temperature, oxygen and pH (Gazzali *et al*., 2016). To avoid the light effect, the daily folic acid buffered solution was stored in a beaker covered with a silver paper. The

solution was kept at ambient temperature, which according to Tripet and Kesselring (1975) did not produce significant folic acid decomposition. Day and Gregory (1983) recommendation to operate folic acid solution under nitrogen atmosphere could not be implemented. Instead, Liang *et al*. (2013) suggestion to add an antioxidant (vitamin C) was occasionally used at long weekends to stabilize and minimize oxidation of the folic acid solution. Also, in this study, special emphasis was made on the daily preparation of the folic acid solution. Folic acid has a low solubility in water at 25ºC (0.01 mg $mL^{-1}$). Its solubility is said to improve in alkaline or acidic medium, although it is reported to be more stable in alkaline medium (Araújo *et al*. 2011). Hence, the buffered solution was prepared at a pH of 9.0.

Table 5.3.Folic acid buffered solution characterization *(Source: adapted from Cardete et al., 2019)*

| Buffered solution | Folic acid concentration mg $L^{-1}$ | COD mg $L^{-1}$ | $BOD_5$ mg $L^{-1}$ | $COD/BOD_5$ - |
|---|---|---|---|---|
| 1 | 7.0 | 218 | 34.65 | 0.16 |
| 2 | 15.0 | 524 | 68.75 | 0.13 |

### 5.2.4. Analytical methods

All the analysis performed to characterize the bench-scale petrochemical activated sludge system were conducted according to Standard Methods (APHA, 2005).

The wastewater feed streams were analyzed to determine the TOC with a total carbon analyzer Shimadzu TOC 5050A. The sCOD was analyzed by digestion with a HACH DR/2010 spectrophotometer at 600 nm wavelength. In the mixed liquor, the pH was controlled with an Orion pH-meter model 710 A, and the DO was registered with an Orion 810-3756 portable probe. Residual ammonia nitrogen and orthophosphate phosphorus were determined with a specific-ion electrode (Orion 95-12), and with a Shimadzu UV-VIS spectrophotometer (Compact UV-2600) at 880 nm wavelength, respectively. The analysis of TSS and VSS were performed using WHATMAN GF/A fiberglass filters, a muffle furnace working at 550 ± 50 ºC and a melting pot Haldenwanger Ò 82 A/3. Nitrates were analyzed with an ion-chromatograph (Metrohm Compact 761 IC) provided with a chemical suppressor module and a column Metrohm 6.1006.100.

The UV spectrophotometry method presented by Matias *et al*. (2014) for the determination of folic acid in commercial tablets was used to quantify the vitamin. The concentration of folic acid in the pH 9.0 buffered solution was determined at $\lambda_{max}$ of 282.5 nm in a linear range of 1.0-17.5 mg $L^{-1}$ with a $R^2$>0.999. To quantify the folic acid concentration in the bioreactor's mixed liquor, UV spectrophotometry was also used after centrifuging the sample and adjusting the pH at 9.0. However, in this case, to avoid matrix interference, the standard addition method was carried out. The determination was performed by addition of known amounts of folic acid (0.5, 1.0, 2.0 and 4.0 mg $L^{-1}$) to the problem mixed liquor, through pestled solid ACFOL tablet.

The $BOD_5$ of the folic acid buffered solution was measured with BOD Aqualytico material, using a buffer-nutrient solution HACHÓ, ref. 14160-66 and a nitrification inhibitor ATH Aqualytico ref. 418642.

Folic acid and its metabolites were identified by high-performance liquid chromatography (HPLC) with a diode array detector (DAD) and an Agilent macro-porous reversed-phase column C18.

Sludge settling of the mixed liquor was characterized by SVI. The volume of settleable solids used to calculate the SVI was obtained by sedimentation in a 25 mL graduated cylinder (Alexandre *et al*., 2016) and the settling time was 30 minutes. Consequently, the SVI values are to be considered only for comparison between the control and the test bioreactors.

Microscopic examinations performed with a Nikon Eclipse 80i microscope at 100X and 400X assessed about the floc appearance, the dominance of filaments and biological indicators in the mixed liquor. Observations on dark-field and phase-contrast microscopy assessed about the morphology of the sludge flocs (Mesquita *et al*., 2013) and the proliferation of Zooglea Ramigera (Vázquez *et al*., 2010).

### 5.2.5. Toxicity assays

As an initial test, to assess the potential inhibition of folic acid on heterotrophic bacteria activity, short-term toxicity assays were performed through respirometry. The SOUR of a one-litre volume mixed liquor, supplied with feed stream, was tested for different folic acid dosage. The tests were organized as summarized in Table 5.4.

Each test was repeated five times with different mixed liquor samples, in order to account for variability in the inoculum. Also, chronic toxicity supplied by folic acid addition to the effluent was evaluated for each operational period. The method used was Microtox (ISO 11348-3:2007), based on bioluminescence inhibition of Vibrio Fischeri (formerly known as Photobacterium Phosphorum, NRRL B-11177). The toxicity data provided by this methodology is expressed as the effective concentration of the inhibitory compound that produces a 50% reduction of the initial bacterial luminescence ($EC_{50}$) (Onorati and Mecozzi, 2004). The toxicity units (TU) were calculated from $EC_{50}$ as follows in equation 5.10.

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$$TU = \frac{100}{EC_{50}} \tag{5.10}$$

The samples were obtained from the bioreactor's mixed liquor. They were centrifuged at 3000 rpm during 10 minutes, in order to remove turbidity. As the toxicity can change with the time, the samples were frozen from collection until the analysis was performed. The methodology also required pH adjustment in some samples to neutral values.

Table 5.4 Conditions for short-term toxicity tests of folic acid on heterotrophic bacteria through respirometry *(Source: Cardete et al., 2019)*

| Respirometry test | Supply | | Mixed liquor [(2)] |
|---|---|---|---|
| | Feed | FA [(1)] | |
| | mL | mL | mg FA $L^{-1}$ |
| 1 | 12 | 0 | 0.0 |
| 2 | 12 | 8 | 0.5 |
| 3 | 12 | 17 | 1.0 |
| 4 | 12 | 33 | 2.0 |
| 5 | 12 | 50 | 3.0 |
| 6 | 12 | 67 | 4.0 |

[(1)] 60 mg $L^{-1}$ folic acid buffered solution

[(2)] Folic acid concentration in the mixed liquor

### 5.2.6. Statistical analysis tools

To conclude about significant differences between the control and the test bioreactors, the results obtained were statistically compared by the t-test and the F-test with 95% confidence using *Statgraphics Plus for Windows 3.3 (1994-1998)* as a support software.

# 6. Effect of the selector's electron acceptor on sludge settling

***ABSTRACT***

*Industrial continuous stirred tank aerobic biological reactors (CSTR) often suffer from low food-to-microorganism (F/M) filamentous bulking, which results in deficient sludge settling. In order to find solutions to this issue, a pilot-scale activated sludge system treating petrochemical wastewater was operated under three different conditions: i) Increasing the F/M supplied to the CSTR ii) Including an anoxic selector iii) Including an aerobic selector. The initial sludge quality, characterized by diluted sludge volumetric index values (DSVI) of 500 mL $g^{-1}$, open floc structure and bridging filaments was slightly improved by increasing the F/M from 0.10 up to 0.40 g COD $g^{-1}$VSS $d^{-1}$. However, DSVI did not reach values lower than 100 mL $g^{-1}$ until a selector was implemented. Anoxic and aerobic selectors were effective to improve sludge settling, reducing DSVI to average values of 80 and 45 mL $g^{-1}$, respectively. However, whereas the anoxic selector produced lots of short filaments, the aerobic selector reduced significantly the number of filaments, obtained a more compact floc structure and leaded to a more reliable operation. Biomass and nitrogen balances suggested that production of new biomass cells and substrate uptake for storage occurred in both selectors. The aerobic selector system, compared to the CSTR, also leaded to a slightly higher soluble COD removal efficiency, as well as lower observed ammonia assimilation and lower observed volatile suspended solid production per unit of COD, in the main reactor. The last factor indicated that including a selector resulted in an older sludge, susceptible of experiencing nitrification issues.*

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# 6. Effect of the selector's electron acceptor on sludge settling

## 6.1. INTRODUCTION

Industrial wastewater treatment with biodegradable organic matter as the main pollutant frequently involves the operation of a continuous stirred tank aerobic biological reactor (CSTR), as a cost-effective operation to remove carbonaceous matter. Because of their large dilution capacity, these reactors may be a good option when potential toxic compounds, ranging from metals, sulphites or hazardous organics, may inhibit the biomass activity. However, CSTR may suffer from an excessive presence of filamentous bacteria, characterized by diluted sludge volumetric index (DSVI) greater than 150 mL $g^{-1}$ (Mangrum, 1998), mainly due to its low operational food-to-microorganism ratio (F/M) (Chambers and Tomlinson, 1982; Chiesa and Irvine, 1985; Scruggs and Randall, 1998; Richard, 2003). One possibility to overcome the low F/M filamentous bulking is to introduce a selector (Richard, 2003).

A selector is defined as a small separated initial zone of a biological reactor, which is fed with readily biodegradable organic matter and biomass from a recycle stream. In the selector a high readily biodegradable COD uptake rate takes place, with virtually complete readily biodegradable organic matter removal (Jenkins *et al.,* 2003).

Selectors can enhance the production of floc-forming bacteria over filamentous bacteria either by kinetic or metabolic selection (Jenkins *et al*., 2003; Metcalf and Eddy, 2003). The kinetic selection theory establishes that floc-forming bacteria grow faster than filaments at higher BOD loading rates. Consequently, small reactors with high organic loading rates, such as selectors, create an enabling environment for floc-formers to predominate over filamentous bacteria. The kinetic selection mechanism involves processes of substrate storage in the selector and subsequent metabolism in the reactor. Therefore, the biomass is expected to increase without forming new cells in the selector, whereas afterwards, in the reactor takes place the replicative growth, which is associated to protein synthesis using the accumulated or stored substrate (Gujer and Jenkins, 1975). The reactor, as aerobic stage, must provide a sufficiently long period of starvation to re-establish the storage capacity of the cells (Van Loosdrecht *et al*., 1997; Beun *et al*., 1999). Taking to metabolic selection, most filaments are known to be strictly aerobic (Mangrum, 1998), so that anoxic and anaerobic conditions can enhance floc-formers over filaments.

Depending on the convenient selection mechanism for a filamentous bulking issue, different types of selectors can be introduced (Albertson, 1987; Albertson and Hendricks, 1992) in the activated sludge system. Among them, aerobic and anoxic selectors have been described to be effective to overcome a low F/M filamentous bulking (Wanner *et al*., 1987; Scruggs and Randall, 1998; Di Marzio *et al*., 2000; Richard, 2003), conditioned to the availability of nutrients and oxygen in the biological reactor (Prendl and Kroiss, 1998).

Aerobic selectors are based on the kinetic selection mechanism, whereas anoxic selectors rely on both, the kinetic and the metabolic selection mechanisms. Also, the biochemical metabolic processes of heterotrophic bacteria are different in both selectors: the biodegradation of the organic matter is produced through energy-yielding oxidation-reduction reactions, in which reduced organic compounds behave as hydrogen donor and oxidized organic or inorganic compounds act as hydrogen acceptors. The energy stored in the organic matter is released by dehydrogenation of the substrate, followed by the transfer of hydrogen or electrons to an acceptor. While aerobic selectors use oxygen as electron acceptor, the anoxic selectors use nitrates. Aerobic metabolism, using oxygen, yields a greater amount of energy than the facultative metabolism, using oxygen bound in nitrates.

The choice of an aerobic or an anoxic selector to overcome a filamentous bulking may be based on general rules stated in the bibliography (Martins *et al.,* 2004). As a reference, aiming to solve a type 021 N filamentous bulking, Prendl and Kroiss (1998) suggested an aerobic selector for a belt sugar mill treatment plant, whereas other authors (Wanner *et al*., 1987; Di Marzio *et al*., 2000) suggested an anoxic selector, or even the combination of aerobic and anoxic selectors to solve a Thiotrix I filamentous bulking. However, it is still widely recognized the need for further empirical observations in order to accomplish the proper selection and design of a selector (Martins *et al.,* 2004; Seviour and Blackall, 2012).

The present research introduces an experimental work which seeks for tools to overcome a low F/M filamentous bulking in a pilot-scale CSTR. Particularly, the study focuses on confirming the efficiency of a selector to improve sludge settling ability and on the selection of the most convenient type of selector to deal successfully with real petrochemical wastewater. Although the literature reports successful performance of selectors to overcome filamentous bulking with urban wastewater (Ferreira *et al*., 2014), slaughterhouse activated sludge (Al-Mutairi, 2009), sugar mill wastewater (Prendl and Kroiss, 1998), fiber mill (Melcer *et al.,* 2003) and paper mill effluents (Durocher *et al*.,

2002), there are also references which confirm that the optimum design of a selector is dependent on the wastewater characteristics (Al-Mutairi, 2009). The present research intends to complement the existing bibliography considering the particular behaviour of petrochemical wastewater and give a response to the needs for further experimental work on selectors.

## 6.2. METHODOLOGY

In order to assess the effectiveness of a selector to enhance sludge settling, the pilot plant described in chapter 5.1.1 was operated with three different configurations: i) As a CSTR, ii) Including an aerobic selector and iii) Including an anoxic selector. When the aerobic selector configuration was tested, air was provided to the selector and the reactor. When the anoxic selector configuration was to be implemented, the air supply to the selector was turned off and the internal recycle was set operative, in order to provide nitrates to the selector's bacteria.

Three effluents (A, B and C) and an inoculums coming from the petrochemical industry were supplied to the pilot system, as referred in chapter 5.1.2.

Taking to the duration of the tests, it was considered to be required about 2 times the mean cell residence time of the system, in order to take a sludge bulking to a non bulking condition (Palm *et al*., 1980) and also to improve settling (Wheeler *et al*., 1984; Boe *et al.*, 1996). Therefore, as the average sludge retention time (SRT) of the pilot-scale system was 17 days, the duration of the tests was around 30 days. Additionally, to guarantee the reproducibility of the results, for each condition evaluated, two thirty-day tests were performed, using in each of them fresh mixed liquor proceeding from a different operational condition in the industrial biological reactor.

To prepare the experimentation, Eckenfelder and Cleary's (2013) concern about selectors having an optimum operational F/M was considered. Some bibliography provides recommendations for the selector's F/M. As an example, Jenkins *et al*. (2003) suggest an F/M ratio of 12 g COD $g^{-1}$VSS $d^{-1}$. However, it is also recognized that the optimum operational parameters of a selector depend on the influent wastewater (Al-Mutairi, 2009). Taking into consideration this statement, previous screening tests were performed in order to identify the optimum selector's F/M for the system studied. Different selector's F/M ratios were tested, to compare the sludge settling quality

obtained and the efficiency of the selector. As a result, an optimum F/M range was identified for the selector at 30 to 40 g COD $g^{-1}$VSS $d^{-1}$.

To conclude about the significance of the difference between the DSVI values obtained with each configuration of the pilot plant, statistical techniques were applied as described in chapter 5.1.4.

For the analysis of experimental data, soluble COD (sCOD) and $BOD_5$ balances were performed over the selector and reactor, in order to evaluate soluble COD and $BOD_5$ removal efficiencies. Soluble COD balances were considered rather than total COD because of the low biodegradability of the particulate matter and the better reproducibility of soluble COD analysis.

The assimilated ammonia per unit of consumed COD (g $NH_4^+$-N assimilated $g^{-1}$ COD removed), represented as $N_{ASSIM}$, was calculated through a nitrogen and COD balance. As the average pH of the mixed liquor was 8.2, ammonia stripping was evaluated in the system to conclude whether it should be taken into account. Considering the average selector (12 to 20 ºC and up to 70 mg $NH_4^+$-N $L^{-1}$) and reactor (33 ºC and up to 2 mg $NH_4^+$-N $L^{-1}$) conditions, the free ammonia susceptible of being stripped by aeration was calculated at 5.5% and 11.1% of the $NH_4^+$-N residual, respectively. The introduction of these figures into the calculation of the ratio $N_{ASSIM}$ indicated a maximum overestimation of 0.001 g $NH_4^+$-N assimilated $g^{-1}$ COD removed for the selector and 0.00001 g $NH_4^+$-N assimilated $g^{-1}$ COD removed for the reactor, if stripping was not considered. Therefore, it was concluded that stripping had no significant effect on the calculated ratio $N_{ASSIM}$. Furthermore, considering the presence of a low quantity of volatile suspended solids (VSS) in the raw wastewaters, the VSS balance was validated with the kjeldhal nitrogen analysis in the mixed liquor, as the feed streams did not supply any organic nitrogen. The VSS balance provided results on the observed VSS production per unit of consumed COD (g VSS produced $g^{-1}$ COD removed), represented as $P_{VSS}$. A calculated ratio $N_{ASSIM}$/ $P_{VSS}$ (g $NH_4^+$-N assimilated $g^{-1}$ VSS produced) was compared to its theoretical limit value (0.12 g $NH_4^+$-N assimilated $g^{-1}$ VSS produced), based on the biomass molecular stoichiometry as $C_5H_7NO_2$ (0.086 mg $NH_4^+$-N $mg^{-1}$ cell COD), and the COD/VSS ratio (1.42 mg cell COD $mg^{-1}$ VSS). The difference between both ratios suggested the amount of carbonaceous substrate uptaken as biomass storage in front of new cell production.

## 6.3. RESULTS AND DISCUSSION

### 6.3.1. Pilot tests of an activated sludge system without a selector

During this stage, the pilot plant was set to a configuration where the selector was not operative, since the objective was to test the settling quality of sludge from the aerobic CSTR.

Five different conditions were implemented in the pilot CSTR, by increasing progressively the F/M (see Table 6.1). The mixture of streams A, B and C in the feed was kept constant for all the tests, so that the ratio $BOD_5$/sCOD was always 0.5, except for period 2, where incidences in the flow control caused the ratio to be only 0.2. Dissolved oxygen concentration (DO) in the biological reactor was kept at an average value of 1.0 mg $L^{-1}$ due to air supply poor control, which was solved in period 5, so that a DO concentration of 2.0 mg $L^{-1}$ was attained. Hydraulic retention time (HRT) and SRT were set at 10.5 and 17 days, respectively. Nearly constant values of pH (8.6), temperature (25.5 ºC) and conductivity (18.3 mS $cm^{-1}$) were recorded in this stage. Ammonia nitrogen and orthophosphate, as nutrients, were also available at concentrations over 2 mg $L^{-1}$. Eventually, to avoid interference, the ratio inert-to-VSS in the mixed liquor was always kept constant to a value of 1.0 since the presence of inert may enhance settling.

Sludge settling quality was evaluated by means of DSVI and microscopic observations.

High DSVI values, around 500 mL $g^{-1}$, were obtained for every period, which is considered to be a bulking sludge. As it is reflected in Figure 6.1, a correlation could be established, in general terms, between DSVI values and operational F/M: the general tendency showed that as F/M increased, within the values of 0.10 to 0.25 g COD $g^{-1}$VSS $d^{-1}$, DSVI got lower (from 650 to 380 mL $g^{-1}$). However, when the operating F/M increased from 0.25 to 0.40 g COD $g^{-1}$VSS $d^{-1}$, an increase in DSVI (from 380 to 500 mL $g^{-1}$) was registered. This result could be explained by the presence of a larger quantity of filaments, probably due to an insufficient increase of the oxygen supply, according to the increase in the F/M ratio. Sezgin *et al.* (1978) presented a similar correlation and stated that bulk substrate concentration together with bulk DO concentration determine the DO gradient inside the floc, which determines the proportion of filament's growth.

Table 6.1 Operational parameters of the pilot aerobic CSTR for different F/M ratios: dissolved oxygen (DO), COD removal efficiency (COD RE), nitrogen assimilation ($N_{ASSIM}$), VSS production ($P_{VSS}$), OUR, nitrates and sludge volumetric index (SVI)

| Parameter | Units | PERIOD 1 | PERIOD 2 | PERIOD 3 | PERIOD 4 | PERIOD 5 |
|---|---|---|---|---|---|---|
| sCOD feed | g $h^{-1}$ | 11.0 (10.5-11.5) | 15.1 (13.8-17.8) | 26.3(25.9-26.6) | 27.1(26.7-27.2) | 33.0 (30.1-33.0) |
| F/M | g COD $g^{-1}$ VSS $d^{-1}$ | 0.10 (0.07-0.12) | 0.16 (0.10-0.17) | 0.18 (0.17-0.18) | 0.25 (0.24-0.27) | 0.40 (0.30-0.40) |
| $BOD_5$/sCOD | g $g^{-1}$ | 0.5 (0.5-0.6) | 0.2 (0.1-0.3) | 0.5 (0.5-0.6) | 0.6 (0.5-0.6) | 0.5 (0.5-0.6) |
| DO | mg $L^{-1}$ | 1.0 (0-2.0) | 1.0 (0-2.0) | 1.0 (0-2.0) | 1.0 (0-2.0) | 2.0 (1.5-2.5) |
| Nitrates | mg $NO_3^-$-N $L^{-1}$ | 25.4 (0.00-40.0) | 15.6 (0.0-23.6) | 11.4 (0.0-15.9) | 6.5 (0.0-10.1) | 17.9 (0.0-28.6) |
| COD RE | % | 99.1 (98.5-99.3) | 99.2 (98.9-99.5) | 99.1(99.0-99.3) | 99.1 (99.0-99.3) | 99.1 (99.0-99.3) |
| $N_{ASSIM}$ | g $NH_4^+$-N assimilated $g^{-1}$ COD assimilated | 0.012 (0.008-0.014) | 0.020 (0.019-0.022) | 0.030 (0.028-0.031) | 0.030 (0.027-0.031) | 0.028 (0.027-0.041) |
| $P_{VSS}$ | g VSS produced $g^{-1}$ COD assimilated | 0.12 (0.07-0.19) | 0.27 (0.23-0.26) | 0.29 (0.26-0.30) | 0.32 (0.31-0.37) | 0.29 (0.23-0.33) |
| $N_{ASSIM}$ /$P_{VSS}$ | g $NH_4^+$-N assimilated $g^{-1}$ VSS produced | 0.10 | 0.07 | 0.10 | 0.09 | 0.10 |
| OUR | mg $O_2$ $L^{-1}$ $h^{-1}$ | 12.8 | | 28.4 | 51.0 | 49.0 |
| SVI | mL $g^{-1}$ | 650 (580-700) | 580 (550-720) | 600 (550-650) | 380 (300-450) | 500 (380-890) |

Period 2 worked under a similar F/M, but with a lower $BOD_5$/sCOD ratio than period 3 (see Table 6.1). The average DSVI value obtained for both tests was very similar (namely, 580 and 600 mL $g^{-1}$, respectively).

Denitrification was not observed during the DSVI tests though there was nitrification in the biological reactor, due to the high SRT and average inorganic carbon concentration (0.9 g $L^{-1}$), as well as the favourable pH and temperature conditions. As shown in Figure 6.2 a, nitrification tended to increase when decreasing the feed of carbonaceous matter. Therefore, period 1, in which the minimum COD flow rate was supplied (F/M of 0.10 g COD $g^{-1}$VSS $d^{-1}$), produced the highest concentration of nitrates.

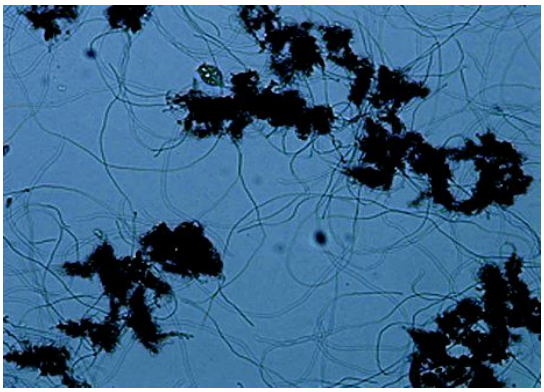
However, period 5 presented also one of the highest nitrification rates although supplying the highest F/M (0.40 g COD $g^{-1}$VSS $d^{-1}$), because of a greater DO availability.

In agreement with the high DSVI values, microscopic observations (see Figure 6.1) showed for each period, a significant presence of long, bridging filaments. The lower the F/M ratio, the more dominant was the presence of long filaments, corresponding to higher DSVI values (Sezgin *et al*., 1978; Richard, 2003). Martins *et al*. (2003) hypothesized that the development of filamentous bulking is caused by substrate gradients in the sludge. At low bulk liquid substrate concentration, filamentous bacteria are favoured, as they can access more easily to the feed outside the floc.

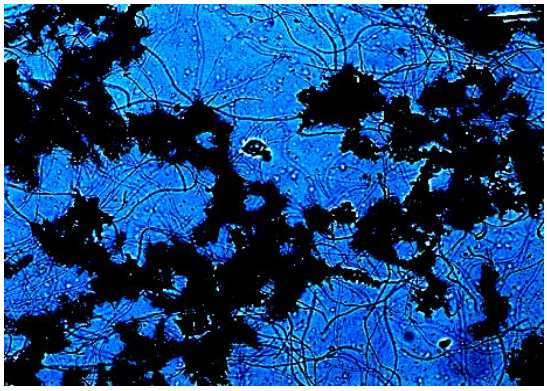
The presence of a specific filament is likely due to the presence of a specific limiting substrate. Low F/M filaments are expected to proliferate in full-scale plants with unaerated fractions (Ekama *et al*., 1996). Periods 5 and 4 showed a more compact floc, corresponding to more oxygen availability (Sezgin *et al*., 1978) and a higher F/M ratio (Palm *et al*., 1980). The filamentous population obtained could not be characterized by PCR methodology due to matrix interferences. Instead, microscopic examinations based on morphology and staining techniques were performed. The observations showed a dominant presence of Nocardia spp., Type 021N and Thiothrix I and II. Occasionally, Type 0092, H. Hydrossis and M. Parvicella were also found. Most of these species are reported to correspond to low F/M conditions (Scruggs and Randall, 1998), associated to complete mix aeration basins at low substrate concentration. As biological indicators, flagellates dominated in the media, when enough oxygen supply could not be guaranteed. Different kinds of sessile ciliates were also observed, such as Vorticella, which showed a tendency to detach when F/M was increased.

Performance parameters, such as the soluble COD removal efficiency (COD RE), observed ammonia assimilation per unit of COD ($N_{ASSIM}$), observed VSS production per unit of COD ($P_{VSS}$) and Oxygen Uptake Rate (OUR) were also assessed (see Table 6.1).

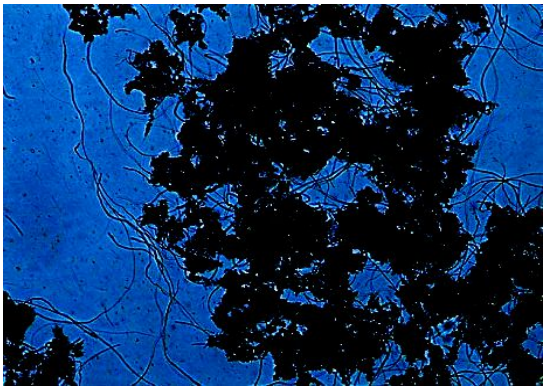
(a) DSVI: 650 mL $g^{-1}$



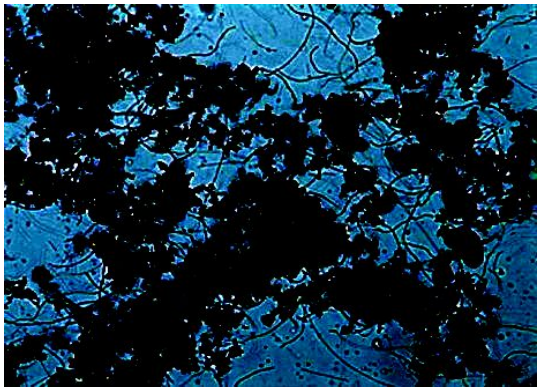
(b) DSVI: 580 mL $g^{-1}$



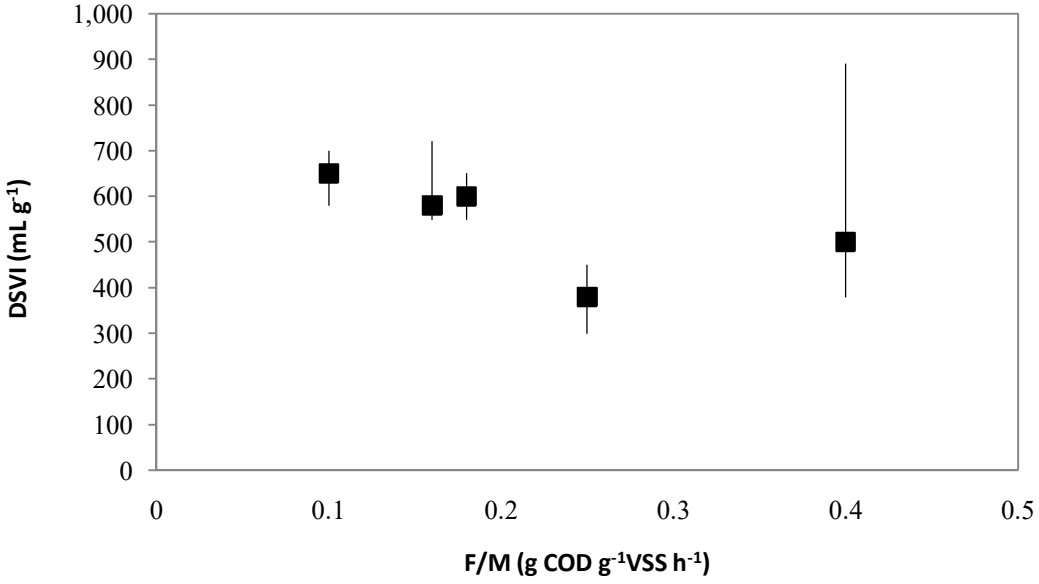
(c) DSVI: 600 mL $g^{-1}$



(d) DSVI: 380 mL $g^{-1}$



(e) DSVI: 500 mL $g^{-1}$

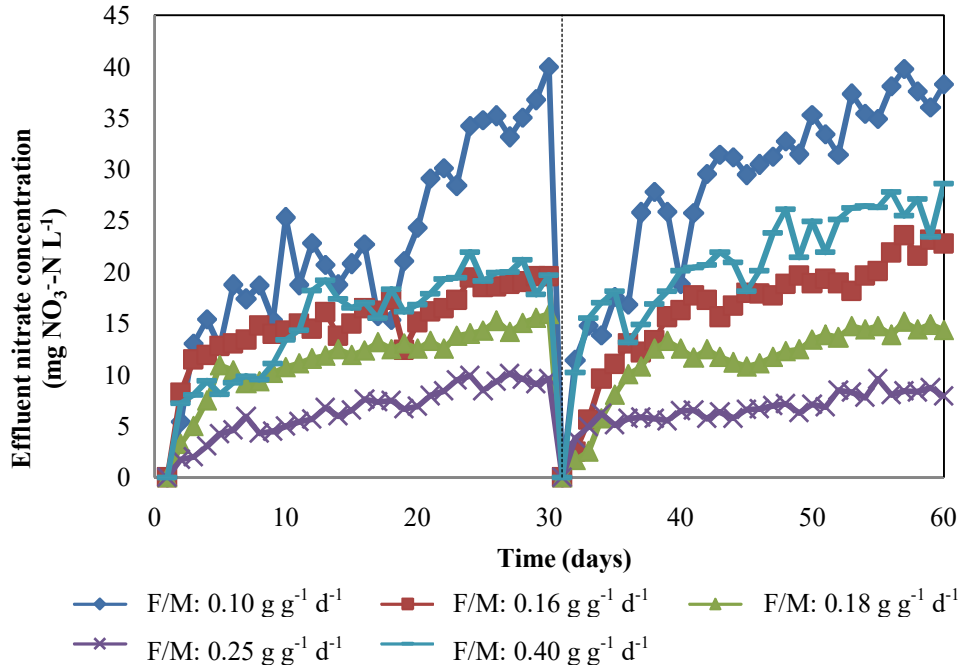



(f)

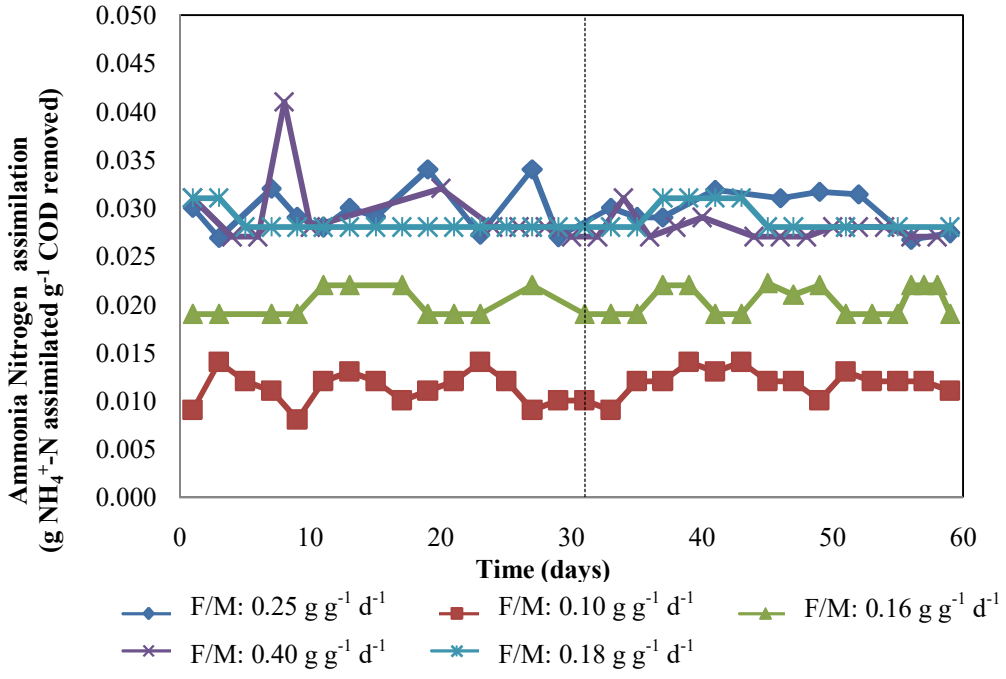
Figure 6.1 Microscopic observations of the pilot CSTR for different F/M ratios (100X): (a) Period 1 at 0.10 g COD $g^{-1}$VSS $d^{-1}$ (b) Period 2 at 0.16 g COD $g^{-1}$VSS $d^{-1}$ (c) Period 3 at 0.18 g COD $g^{-1}$VSS $d^{-1}$ (d) Period 4 at 0.25 g COD $g^{-1}$VSS $d^{-1}$ and (e) Period 5 at 0.40 g COD $g^{-1}$ VSS $d^{-1}$.(f) Representation of the diluted sludge volumetric index (DSVI) obtained for different F/M ratio values in the pilot aerobic biological CSTR.

For every period, COD removal efficiencies above 99.1 % were obtained, and $N_{ASSIM}$ and $P_{VSS}$ values increased as F/M was increased in the different periods (see Figure 6.2 b). Ammonia nitrogen assimilation values ranging from 0.012 to 0.030 g $NH_4^+$-N assimilated $g^{-1}$ COD removed, and VSS production with values in the interval of 0.12 to 0.32 g VSS produced $g^{-1}$ COD removed were obtained. As a result, the observed nitrogen assimilation per unit of VSS produced ($N_{ASSIM}$ / $P_{VSS}$) for every period was around 0.10 g $NH_4^+$-N assimilated $g^{-1}$ VSS produced, near the stoichiometric limit of nitrogen conversion into VSS (0.12 g $NH_4^+$-N assimilated $g^{-1}$ VSS produced). The comparison of both values suggested that little substrate storage had taken place in the CSTR, as most carbonaceous matter had been converted into new cells.

Eventually, respirometry tests also indicated an increasing OUR value from 12.8 to 51.0 mg $O_2$ $L^{-1}$ $h^{-1}$ in periods 1 and 4, respectively, as the F/M ratio increased, while keeping for each period a COD removal efficiency of 99.1%. Whereas the OUR increased, more oxygen was supplied to the reactor, registering a positive residual (see Table 6.1).




(a)




(b)

Figure 6.2 Monitoring of $NO_3^-$-N (a) and $NH_4^+$-N assimilation (b) in the pilot aerobic CSTR for the five operational periods. The dotted vertical line indicates re-inoculation.

As the best settling results were obtained for period 4, it was considered as a reference to program the comparative selector's tests.

Tables 6.4 and 6.5 show, respectively, a summary of the operational and performance parameters of the CSTR in period 4, compared to the activated sludge system operated with an anoxic and an aerobic selector.

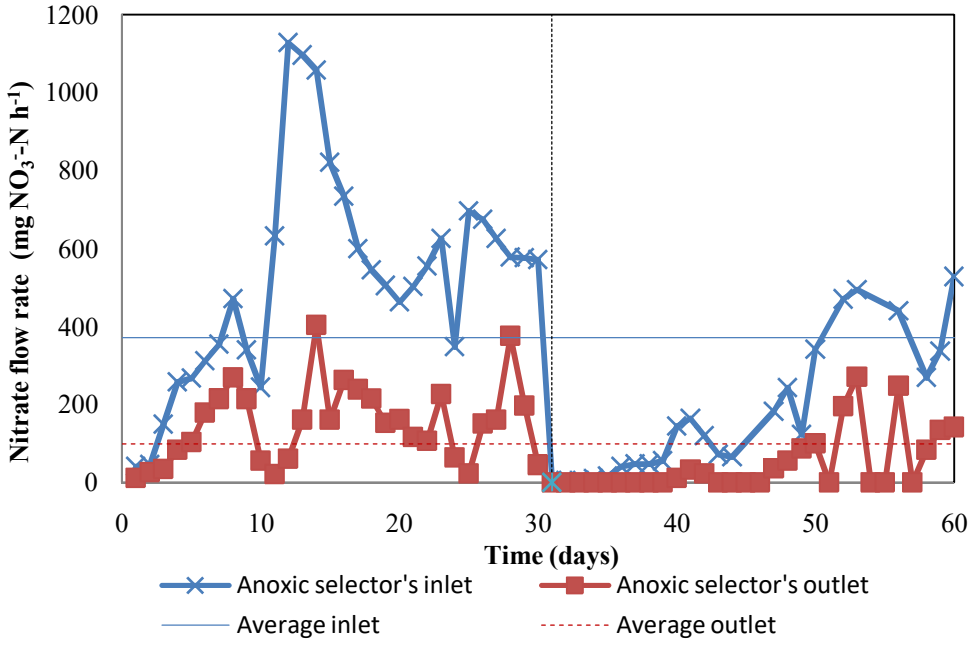
### 6.3.2. Pilot tests of an activated sludge system including an anoxic selector

In order to test the impact of including an anoxic selector on sludge settling, the pilot plant configuration introduced the selector and an additional internal recycle from the degasser, so as to provide the selector with nitrates, as electron acceptor. No air was supplied to the selector, in order to force the oxygen uptake from the nitrates. The biodegradable streams, A and B, as well as the conventional recycle stream from the bottom of the clarifier were fed into the anoxic selector. The feed flow rates to the activated sludge system were established similar to that used in the CSTR, in order to compare results (see Table 6.2).

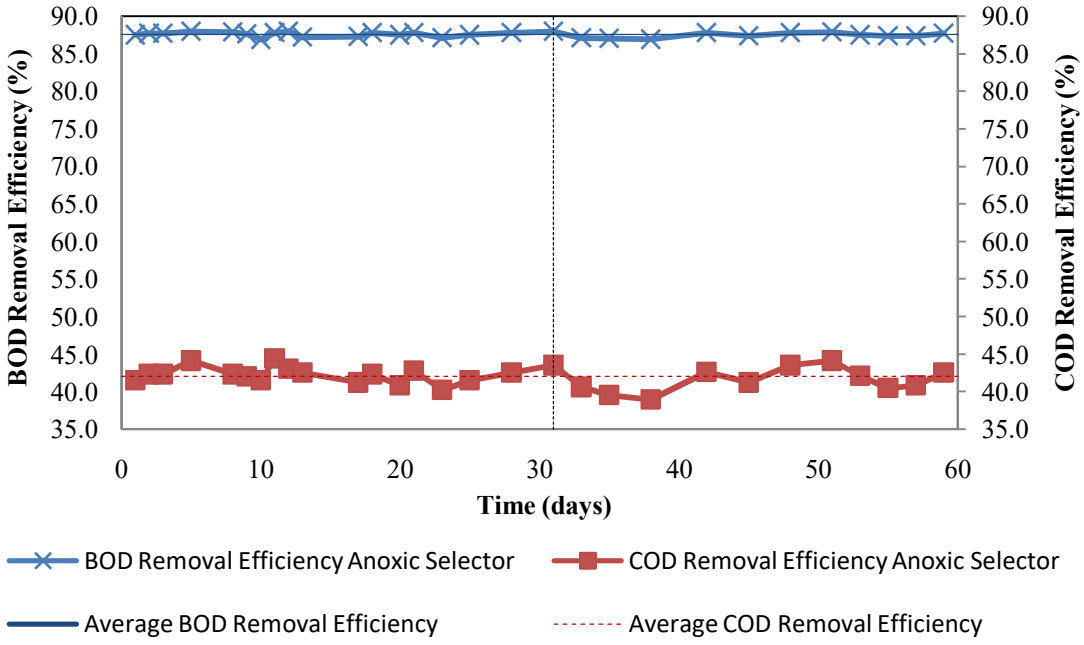
The selector's hydraulic retention time was set at 45 minutes (Jenkins *et al*., 2003) and the pH was controlled at 8.3±0.3. Stream C and the effluent of the selector were fed into the main reactor, whose parameters were controlled at values that favoured the nitrification reaction.

The sludge waste was established to keep a SRT of 20 days, and the reactor's HRT registered values of 10.1 days. Nitrification rate was also determined by the main reactor's parameters, such as pH, temperature, ammonia, DO and the total inorganic carbon concentration, as the carbon source for autotrophic nitrifying bacteria (Metcalf and Eddy, 2003; He *et al*., 2012; Liu and Jianmin, 2015). Control of nitrification in the biological reactor was difficult, due to the variability in the ammonia and dissolved oxygen concentration. As a consequence, the nitrate concentration in the selector showed unsteady tendencies during the test (see Figure 6.3 a). However, there was sustained nitrate consumption in the selector and consistently, the DSVI values and microscopic observations showed an improvement on sludge settling quality.

The average DSVI value obtained was 80 mL $g^{-1}$, although the maximum value was 270 mL $g^{-1}$, higher than 150 mL $g^{-1}$.




(a)




(b)

Figure 6.3 Operational parameters of the activated sludge system including an anoxic selector. Variation of (a) $NO_3^-$-N flow rate in and out of the selector (b) BOD and COD removal efficiency in the selector. The dotted vertical line indicates re-inoculation of the pilot biological system.

Microscopic observations showed an open, moderately compact floc, with lots of short filaments, both inside and outside the floc, as shown in Figure 6.5. In agreement with the results obtained, the bibliography reports successful performance of anoxic selectors to enhance settling, dealing with domestic effluents (Cha *et al.,* 1992; Mangrum, 1998; Guo *et al*., 2014), as well as with chemical and paper mill wastewater (De Lorme *et al.,* 1990; Di Marzio *et al*., 2000). In this study, even if sludge settling quality was improved, the unsteady control of nitrification in the main reactor could cause settling problems in the clarifier.

The performance parameters of the activated sludge system including the anoxic selector were also evaluated and presented in Table 6.2. The selector produced a nearly constant, average sCOD removal efficiency of 42.0 % and a high BOD removal efficiency of 87.6 %, as requested in the bibliography (Jenkins *et al.,* 2003) (see tendencies in Figure 6.3 b). A ratio $N_{ASSIM}$ / $P_{VSS}$ was calculated (0.05 g $NH_4^+$-N assimilated $g^{-1}$ VSS produced) and compared to the stoichiometric nitrogen assimilation value for biomass growth (0.12 g $NH_4^+$-N assimilated $g^{-1}$ VSS produced). The result obtained suggests that both mechanisms have taken place in the selector: new biomass growth, because of the ammonia nitrogen assimilation and storage because of the difference between the limit and the calculated value of the ratio $N_{ASSIM}$ / $P_{VSS}$.

Expressing the observed ammonia nitrogen assimilation (0.016 g $NH_4^+$-N assimilated $g^{-1}$ COD removed) as new biomass production (0.129 g new cells produced $g^{-1}$ COD removed) and comparing it with the observed biomass production (0.30 g VSS produced $g^{-1}$ COD removed), the results indicate that 56.7% of the observed biomass production was dedicated to storage, whereas 43.3% resulted in new biomass cells.

The sCOD removal efficiency of the reactor and the activated sludge system was 99.2%. The specific observed ammonia assimilation in the reactor was similar to that in the selector, whereas the observed production of VSS was lower in the reactor than in the selector. Therefore, the calculated ratio $N_{ASSIM}$ / $P_{VSS}$ (0.08 g $NH_4^+$-N assimilated $g^{-1}$ VSS produced) in the reactor was higher than in the anoxic selector, which suggested having, comparatively, less storage in the main reactor than in the selector. The Oxygen uptake rate (OUR) value for the reactor was quantified at 62.7 mg $O_2$ $L^{-1}$ $h^{-1}$, and was related to both, heterotrophic and autotrophic bacteria.

Table 6.2 Operational and performance parameters of the activated sludge system including an anoxic selector

| Parameter | Units | SELECTOR | REACTOR | GLOBAL |
|---|---|---|---|---|
| sCOD feed | g $h^{-1}$ | 22.3 (18.1-23.8) | 18.3 (8.3-19.4) | 27.7(23.2-29.4) |
| F/M | g COD $g^{-1}$ VSS $d^{-1}$ | 42.0 (40.5-44.3) | 0.2 (0.1-0.2) | |
| $BOD_5$/sCOD | g $g^{-1}$ | 0.7 (0.6-0.7) | 0.2 (0.1-0.2) | |
| DO | mg $L^{-1}$ | <1 | 2.0 (1.5-2.5) | |
| COD Equilibrium Concentration | g $L^{-1}$ | 2.8 (2.5-3.0) | | |
| $BOD_5$ Removal Efficiency | % | 87.6 (86.9-88.0) | | |
| sCOD Removal Efficiency | % | 42.0 (38.9-44.4) | 99.2 (99.1-99.6) | 99.2 (98.5-99.2) |
| $N_{ASSIM}$ | g $NH_4^+$-N assimilated $g^{-1}$ COD assimilated | 0.016 (0.002-0.049) | 0.015 (0.014-0.020) | 0.015 (0.013-0.034) |
| $P_{VSS}$ | g VSS produced $g^{-1}$ COD assimilated | 0.30 (0.19-0.38) | 0.20 (0.09-0.22) | 0.23 (0.20-0.25) |
| $N_{ASSIM}$ /$P_{VSS}$ | g $NH_4^+$-N assimilated $g^{-1}$ VSS produced | 0.05 | 0.08 | 0.07 |
| OUR | mg $O_2$ $L^{-1}$ $h^{-1}$ | | 62.7 | |
| NUR | mg $N\text{-}NO_3^-$ $L^{-1}$ $h^{-1}$ | 35.0 | | |
| SVI | mL $g^{-1}$ | | | 80 (69-270) |

| | | | |
|---|---|---|---|
| sCOD | Soluble chemical oxygen demand | NUR | Nitrogen uptake rate |
| F/M | Food-to-microorganism ratio | SVI | Sludge volumetric index |
| $BOD_5$ | Five-day biological oxygen demand | | |
| DO | Dissolved oxygen in the mixed liquor | | |
| $N_{ASSIM}$ | Assimilated ammonia per unit of consumed COD | | |
| $P_{VSS}$ | Observed volatile suspended solid production per unit of consumed COD | | |
| OUR | Oxygen uptake rate | | |

### 6.3.3. Pilot tests of an activated sludge system including an aerobic selector

During this period, the aerobic selector activated sludge system configuration was set operative in the pilot plant. Air was fed into the selector, to keep a DO concentration of 2.5 ± 0.5 mg $L^{-1}$. Table 6.3 presents the operational parameters of the system. A short HRT of 30 minutes was set to the selector, in order to guarantee its effectiveness (Metcalf and Eddy, 2003). Microscopic observations showed similar biological indicators as in the previous tests, which was in agreement with a similar sludge age and operational conditions.

Table 6.3 Operational and performance parameters of the activated sludge system including an aerobic selector

| Parameter | Units | SELECTOR | REACTOR | GLOBAL |
|---|---|---|---|---|
| sCOD feed | g $h^{-1}$ | 23.3 (21.3-25.1) | 19.7(15.7-53.0) | 25.1(18.1-30.4) |
| F/M | g COD $g^{-1}$ VSS $d^{-1}$ | 35.0 (16.5-45.5) | 0.3 (0.1-0.4) | |
| $BOD_5$/sCOD | g $g^{-1}$ | 0.5 (0.5-0.6) | 0.2 (0.1-0.2) | |
| DO | mg $L^{-1}$ | 2.5 (1.2-4) | 3.7 (2.4-4.9) | |
| COD Equilibrium Concentration | g $L^{-1}$ | 2.8 (2.5-3.0) | | |
| BOD Removal Efficiency | % | 80.7(0-82.7) | | |
| sCOD Removal Efficiency | % | 35.5(0-42.3) | 99.2(99.1-99.6) | 99.2(98.5-99.2) |
| $N_{ASSIM}$ | g $NH_4^+$-N assimilated $g^{-1}$ COD assimilated | 0.013 (0.007-0.039) | 0.019(0.010-0.025) | 0.019 (0.018-0.036) |
| $P_{VSS}$ | g VSS produced $g^{-1}$ COD assimilated | 0.57 (0.09-0.69) | 0.28 (0.11-0.48) | 0.41(0.36-0.56) |
| $N_{ASSIM}$ /$P_{VSS}$ | g $NH_4^+$-N assimilated $g^{-1}$ VSS produced | 0.02 | 0.07 | 0.05 |
| OUR | mg $O_2$ $L^{-1}$ $h^{-1}$ | 52.1 | 30.0 | |
| SVI | mL $g^{-1}$ | | | 45 (30-75) |

sCOD Soluble chemical oxygen demand
F/M Food-to-microorganism ratio
$BOD_5$ Five-day biological oxygen demand
DO Dissolved oxygen in the mixed liquor
$N_{ASSIM}$ Assimilated ammonia per unit of consumed COD
$P_{VSS}$ Observed volatile suspended solid production per unit of consumed COD
OUR Oxygen uptake rate
SVI Sludge volumetric index

The average DSVI obtained with the inclusion of the aerobic selector was 45 mL $g^{-1}$, in a range of 30 to 75 mL $g^{-1}$. Consequently, the aerobic selector succeeded to overcome the filamentous bulking, in agreement with other experiences reported in the bibliography, dealing with sugar mill wastewater (Prendl and Kroiss, 1998), fiber mill effluents (Melcer *et al.,* 2003) and slaughterhouse residues (Al-Mutairi, 2009). However, the results reported were obtained with a one compartment aerobic selector, different to Duine and Kunst (2002) findings with industrial wastewater, in which a sectionalized selector was needed to control filaments type 021N to achieve a stable DSVI below 150 mL $g^{-1}$. Taking into account that the reactor's SRT was 20 days, these results differ from that obtained by Cha *et al.* (1992), on bench-scale activated sludge experiments in California primary effluent, who found that an aerobic selector was

effective to control Nocardia at SRT of 5 days but not at longer SRT values (10 days). For such long SRT, they obtained better results with an anoxic selector. Although an activated sludge that settles very quickly (DSVI below 70 mL $g^{-1}$) may produce a turbid supernatant, condition known as pin-floc (Palm *et al.*, 1980), the microscopic observations shown in Figure 6.5 indicate that the aerobic selector offered the most compact floc and the less interflocular material sludge.

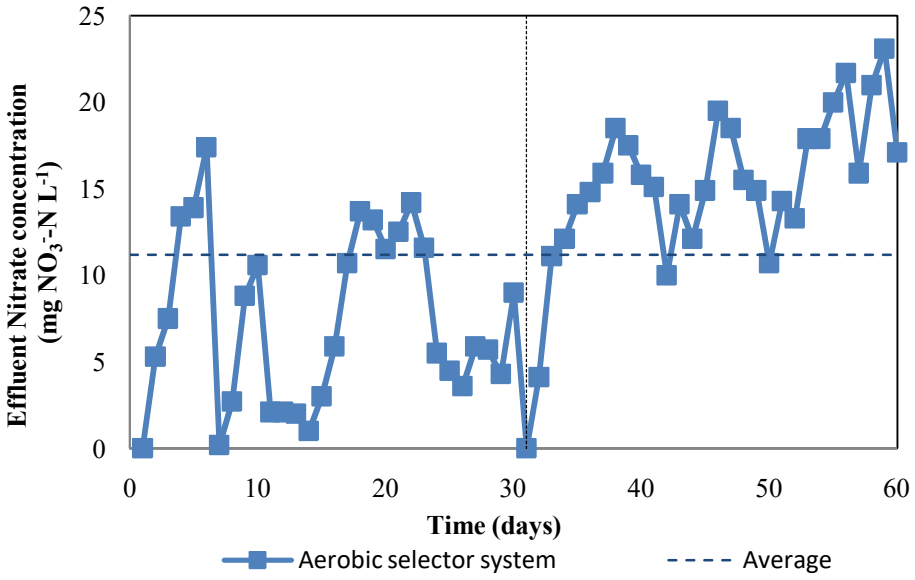
As reflected in Table 6.3, the selector's average sCOD removal efficiency was 35.5 %, whereas the BOD removal efficiency was 80.7 % (see tendencies in Figure 6.4 b). As a reference, Daigger *et al.* (1990) reported a sCOD removal efficiency of 45 % and BOD removal efficiency of 60 % for an aerobic selector. Taking to the biomass production in the selector, the observed ammonia assimilation ($N_{ASSIM}$) was assessed by the nitrogen balance. This data combined with the observed VSS production ($P_{VSS}$) calculated from the biomass balance in the selector produced a ratio $N_{ASSIM}$ / $P_{VSS}$ of 0.02 g $NH_4^+$-N assimilated $g^{-1}$ VSS produced (see Table 6.3). This value, compared to the stoichiometric limit of 0.12 g $NH_4^+$-N assimilated $g^{-1}$ VSS produced suggested the accumulation of carbonaceous matter as biomass storage material. Using the biomass molecular stoichiometry ($C_5H_7NO_2$), the data of observed ammonia assimilation (0.013 g $NH_4^+$-N assimilated $g^{-1}$ COD removed) can be converted into the production of new cells (0.105g new cells $g^{-1}$ COD removed). The comparison of this value with the observed VSS production (0.57 g VSS produced $g^{-1}$ COD removed) indicates that 18.4% of the biomass production was in the form of new cells and 81.6% was dedicated to storage.

Table 6.3 presents the performance parameters obtained for the reactor and for the activated sludge system including the aerobic selector.

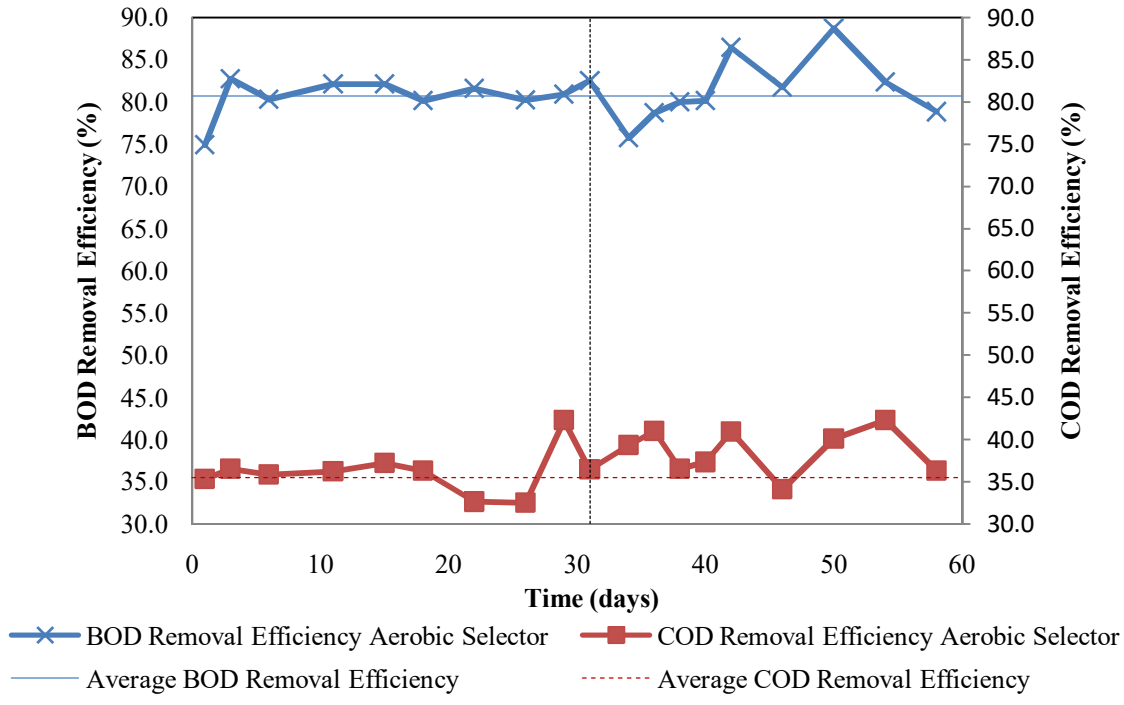
In the main reactor, an average sCOD removal efficiency of 99.2% was observed.

The biomass growth pointed out to be lower than in the selector, with lower specific nitrogen assimilation ($N_{ASSIM}$) and lower observed VSS production ($P_{VSS}$). Both figures resulted in a $N_{ASSIM}$ / $P_{VSS}$ ratio of 0.07 g $NH_4^+$-N assimilated $g^{-1}$ VSS produced, higher than in the selector. The required SRT for the system was 20 days. Hence, due to the favourable conditions for the proliferation of nitrifying bacteria and for nitrification, an average concentration of 11.2 mg $NO_3^+$-N $L^{-1}$ was registered in the reactor (see nitrate tendencies in Figure 6.4 a).

The whole process showed an overall sCOD removal efficiency of 99.2 %, slightly higher than the system without a selector, similar to the results obtained by Munirathinam (2003) for the treatment of pulp and paper industry wastewater.




(a)




(b)

Figure 6.4 Operational parameters of the activated sludge system including an aerobic selector. Variation of (a) The effluent's $NO_3^-$-N concentration (b) BOD and COD removal efficiency in the selector. The dotted vertical line indicates re-inoculation of the pilot biological system.

### 6.3.4. Comparison of the sludge settleability in the three systems

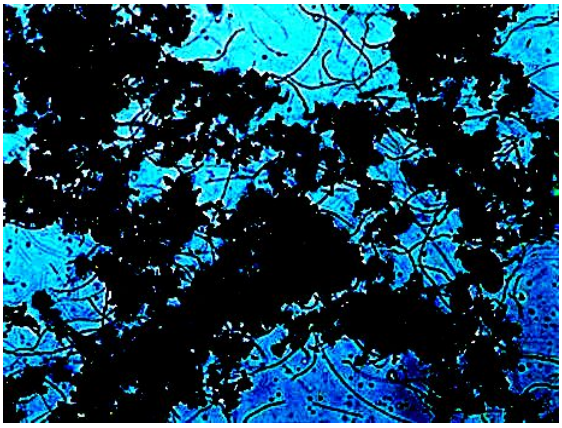
A comparison of the operational parameters registered for the three configurations is shown in Table 6.4.

Table 6.4 Operational parameters of the pilot activated sludge system without a selector (CSTR), with an anoxic selector and with an aerobic selector.

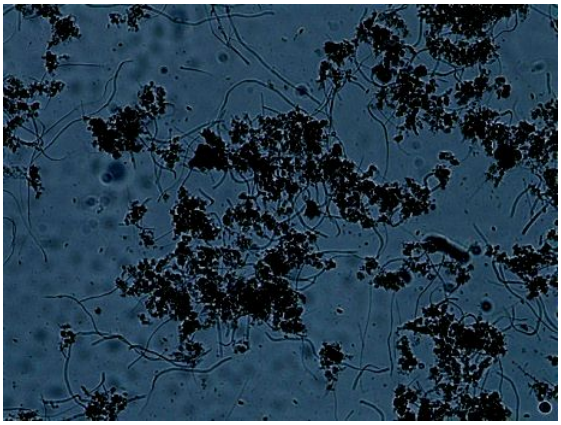
| PARAMETER | UNITS | CSTR | ANOXIC SELECTOR +CSTR | AEROBIC SELECTOR +CSTR |
|---|---|---|---|---|
| **SELECTOR** | | | | |
| sCOD feed | g $h^{-1}$ | | 23.1 (18.1-23.8) | 23.3 (21.3-25.1) |
| F/M | g COD $g^{-1}$ VSS $d^{-1}$ | | 29.4 (22.5-44.3) | 30.5 (16.5-45.5) |
| $BOD_5$/sCOD feed | g $g^{-1}$ | | 0.30 (0.11-0.45) | 0.30 (0.11-0.45) |
| DO | mg $L^{-1}$ | | 0.1(0.0-1.0) | 2.5 (2.0-3.0) |
| HRT | min | | 45 (40-50) | 30 (25-35) |
| VSS | g VSS $L^{-1}$ | | 2.8 (1.3-4.3) | 2.7 (1.2-3.8) |
| VSS/TSS | - | | 0.3 (0.1-0.4) | 0.2 (0.1-0.4) |
| **REACTOR** | | | | |
| sCOD feed | g $h^{-1}$ | 21.7 (19.7-27.2) | 15.4 (8.3-19.4) | 16.9 (15.7-53.0) |
| F/M | g COD $g^{-1}$ VSS $d^{-1}$ | 0.25 (0.24-0.27) | 0.20 (0.10-0.20) | 0.30 (0.10-0.40) |
| $BOD_5$/tCOD | g $g^{-1}$ | 0.25 (0.20-0.40) | 0.15 (0.10-0.20) | 0.15 (0.10-0.20) |
| DO | mg $L^{-1}$ | 1.0 (0.0-2.0) | 2.0 (0.0-3.0) | 3.7 (2.4-4.9) |
| VSS | g VSS $L^{-1}$ | 4.0 (2.5-4.5) | 3.0 (2.7-3.5) | 2.7 (2.0-3.3) |
| VSS/TSS | - | 0.2 (0.2-0.4) | 0.2 (0.2-0.3) | 0.2 (0.2-0.3) |
| Nitrates | mg $NO_3^-$-N/L | 6.5 (0.0-10.1) | 38.7 (0.4-117.5) | 11.2 (0.0-23.1) |
| SRT | days | 17 (16-18) | 20 (19-21) | 20 (19-22) |
| HRT | days | 10.5 (10.0-11.0) | 10.1 (10.0-11.0) | 10.2 (10.0-11.0) |
| pH | - | 8.6 (8.3-8.6) | 8.3 (8.0-8.6) | 8.3 (8.0-8.6) |
| T | ºC | 25.5 (23.5-27.5) | 33.3 (31.3-35.3) | 33.3 (31.3-35.3) |
| Ammonia | mg $L^{-1}$ | 2.5 (2.0-3.0) | 1.0 (0.0-5.6) | 2.5 (2.0-3.0) |
| Orthophosphate | mg $L^{-1}$ | 2.5 (2.0-3.0) | 2.5 (2.0-3.0) | 2.5 (2.0-3.0) |
| Inert-to-VSS | - | 1.0 | 0.9 | 0.9 |
| **GLOBAL ACTIVATED SLUDGE SYSTEM** | | | | |
| sCOD feed | g $h^{-1}$ | 21.7(19.7-27.2) | 25.1(23.2-29.4) | 25.1(18.1-30.4) |

sCOD Soluble chemical oxygen demand
F/M Food-to-microorganism ratio
$BOD_5$ Five-day biological oxygen demand
DO Dissolved oxygen in the mixed liquor
HRT Hydraulic retention time
VSS Volatile suspended solids
T Temperature

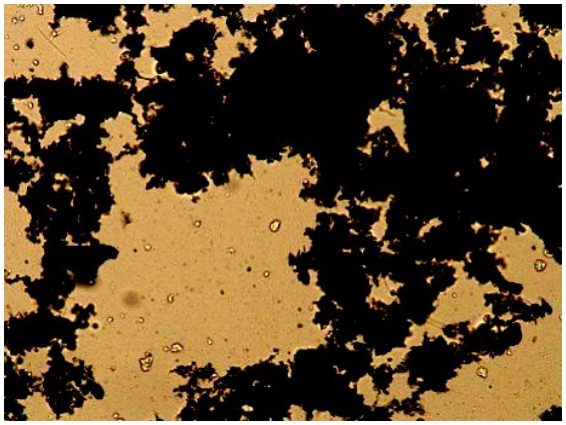
The experimental tests with petrochemical wastewater demonstrated that the implementation of properly dimensioned aerobic and anoxic selectors could deal successfully with a filamentous bulking caused by a low F/M ratio (Richard, 2003), and consequently, improve sludge settling by a reduction in the number of filaments (Azimi and Zamanzadeh, 2006). Whereas the pilot CSTR produced an average DSVI of 380 mL $g^{-1}$, the implementation of either an aerobic or an anoxic selector allowed to control DSVI below 100 mL $g^{-1}$. In agreement with the DSVI comparative, the microscopic observations in Figure 6.5, show a reduction in the number of filaments and an increase in the floc compactness when the selector was implemented.



(a) DSVI: 380 mL $g^{-1}$



(b) DSVI: 80 mL $g^{-1}$



(c) DSVI: 45 mL $g^{-1}$

Figure 6.5 Images (100X) of (a) the cross-linked filament net obtained in the floc with the CSTR (b) the anoxic selector configuration sludge, with short filaments and (c) the compact sludge floc obtained with the aerobic selector

The aerobic selector presented the best sludge settling results, compared with the non selector and the anoxic selector system. Whereas the SVI of the anoxic selector system ranged from 69 to 270 mL $g^{-1}$, with an average of 80 mL $g^{-1}$, the aerobic selector produced lower SVI values (average 45 mL $g^{-1}$), always below 100 mL $g^{-1}$ (range of 30 to 75 mL $g^{-1}$). A statistical comparison of both figures indicated that the SVI obtained with the aerobic selector was significantly lower. In agreement with these results, the microscopic examinations presented in Figure 6.5, show that the bridging filaments in the CSTR turned into short, isolated filaments in the system operated with the anoxic selector. The aerobic selector improved further the sludge appearance by suppressing completely the interfloc filaments.

Several references also report similar results with other types of wastewater. As an example, an aerobic selector was successful in solving a filamentous bulking at a pulp and mill wastewater system, which could not be remedied by an anoxic selector (Durocher *et al*., 2002) or by an anaerobic selector (Munirathinam, 2003). Additionally, although filaments type 021N, Thiotrix spp and H. Hydrossis could be removed by anoxic selectors (Wanner *et al*., 1987; Di Marzio *et al.,* 2000; Azimi and Zamanzadeh, 2006), some literature suggests to implement an aerobic selector ahead of the aerobic biological reactor to ensure degradation of intracellular nitric oxide, which had been shown to inhibit the growth of floc forming bacteria (Casey *et al*., 1994).

Taking to sludge age, Gray *et al.* (2006) also demonstrated the conditions in which selectors are efficient to improve sludge settling quality. Aerobic selectors were reported to be useful for biological systems with an SRT below 30 days, as the conditions tested in this study.

With regard to performance parameters (see Table 6.5), the anoxic selector presented higher COD and BOD removal efficiency than the aerobic selector (42.0 and 87.6 % in front of 35.5 and 80.7 %, respectively), probably due to a higher HRT (45 in front of 30 min, respectively) (see Table 6.4).

Consequently, the reactor of the aerobic selector system assimilated more carbonaceous matter than the one of the anoxic selector system, considering that the COD removal efficiency in both reactors was the same (99.2%). In agreement with the results obtained for the CSTR tests, the reactor of the aerobic selector system provided higher observed ammonia assimilation ($N_{ASSIM}$) and VSS production ($P_{VSS}$) than the reactor of the anoxic selector system. Consistently, the CSTR produced the highest $N_{ASSIM}$ and $P_{VSS}$, as it was the reactor dealing with a higher COD feed. Therefore, the inclusion of a

selector reduced the production of VSS per unit of COD in the main reactor. Consequently, higher SRT values were required for the systems operated with a selector (20 days, in front of 17 days for the CSTR). As a result, nitrification was enhanced in the selector's systems (average 6.5 mg $NO_3^-$-N $L^{-1}$ for the effluent of the CSTR, in front of 11.2 mg $NO_3^-$-N $L^{-1}$ for the effluent of the aerobic selector system).

Table 6.5 Performance of the pilot activated sludge system without a selector (CSTR), with an anoxic selector and with an aerobic selector.

| PARAMETER | UNITS | CSTR | ANOXIC SELECTOR +CSTR | AEROBIC SELECTOR +CSTR |
|---|---|---|---|---|
| SVI | mL g-1 | 380 (300-450) | 80 (69-270) | 45 (30-75) |
| **SELECTOR** | | | | |
| $BOD_5$ Removal Efficiency | % | | 87.6 (86.9-88.0) | 80.7(0-82.7) |
| sCOD Removal Efficiency | % | | 42.0 (38.9-44.4) | 35.5(0-42.3) |
| $N_{ASSIM}$ | g $NH_4^+$-N assimilated $g^{-1}$ COD removed | | 0.016 (0.002-0.049) | 0.013 (0.007-0.039) |
| $P_{VSS}$ | g VSS produced $g^{-1}$ COD removed | | 0.30 (0.19-0.38) | 0.57 (0.09-0.69) |
| $N_{ASSIM}/P_{VSS}$ | g $NH_4^+$-N assimilated $g^{-1}$ VSS produced | | 0.05 | 0.02 |
| **REACTOR** | | | | |
| OUR | mg $O_2$ $L^{-1}$ $h^{-1}$ | 51.0 | 62.7 | 30.0 |
| sCOD Removal Efficiency | % | 99.1 (99.0-99.3) | 99.2 (99.1-99.6) | 99.2(99.1-99.6) |
| $N_{ASSIM}$ | g $NH_4^+$-N assimilated $g^{-1}$ COD removed | 0.030 (0.027-0.031) | 0.015 (0.014-0.020) | 0.019(0.010-0.025) |
| $P_{VSS}$ | g VSS produced $g^{-1}$ COD removed | 0.32 (0.31-0.37) | 0.20 (0.09-0.22) | 0.28 (0.11-0.48) |
| $N_{ASSIM}/P_{VSS}$ | g $NH_4^+$-N assimilated $g^{-1}$ VSS produced | 0.09 | 0.08 | 0.07 |
| **GLOBAL ACTIVATED SLUDGE SYSTEM** | | | | |
| sCOD Removal Efficiency | % | 99.1 (99.0-99.3) | 99.2 (98.5-99.2) | 99.2(98.5-99.2) |
| $N_{ASSIM}$ | g $NH_4^+$-N assimilated $g^{-1}$ COD removed | 0.030 (0.027-0.031) | 0.015 (0.013-0.034) | 0.019 (0.018-0.036) |
| $P_{VSS}$ | g VSS produced $g^{-1}$ COD removed | 0.32 (0.31-0.37) | 0.23 (0.20-0.25) | 0.41(0.36-0.56) |
| $N_{ASSIM}/P_{VSS}$ | g $NH_4^+$-N assimilated $g^{-1}$ VSS produced | 0.09 | 0.07 | 0.05 |

COD Chemical oxygen demand

$BOD_5$ Five-day biological oxygen demand

$N_{ASSIM}$ Assimilated ammonia per unit of consumed COD

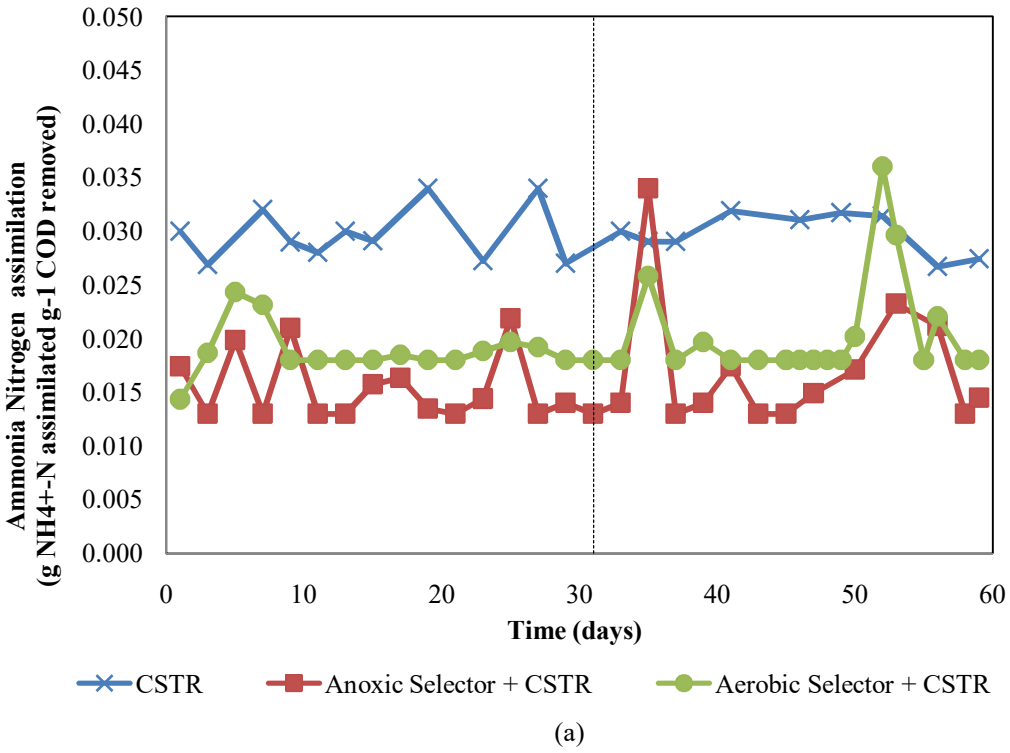
$P_{VSS}$ Observed volatile suspended solid production per unit of consumed COD

OUR Oxygen uptake rate

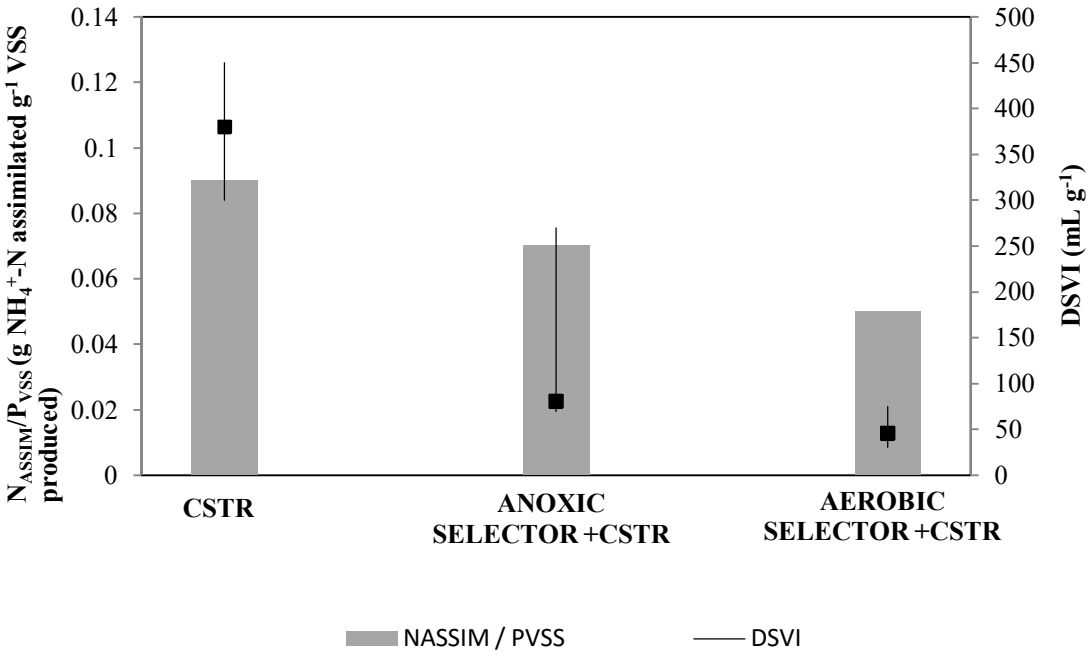
Table 6.5 presents the OUR values registered for the CSTR (51.0 mg $O_2$ $L^{-1}$ $h^{-1}$) and for the reactors of the aerobic and anoxic activated sludge systems (30.0 and 62.7 mg $O_2$ $L^{-1}$ $h^{-1}$, respectively). The reactor of the anoxic selector system provided the highest OUR, as the oxygen was not only dedicated to the biomass respiration but also to nitrate formation. However, when comparing the CSTR with the reactor of the aerobic selector system, the second exhibited the lowest oxygen consumption, consistently with degrading less COD, as part of it had already been consumed in the selector.

The comparison of the global operational parameters obtained with and without a selector (Table 6.5), also showed the effect of including a selector in the activated sludge system. The operation of the biological system with a selector, allowed to improve slightly the global sCOD removal efficiency from 99.1 % to 99.2 %, in agreement with the results obtained by Durocher *et al.* (2002). As it can be observed in Figure 6.6 a, the global observed ammonia assimilation ($N_{ASSIM}$) was lower for the systems including a selector, whereas the observed VSS production ($P_{VSS}$) was higher for the aerobic selector activated sludge system. These results suggest a lower new cell growth in the systems including a selector, according to the results announced by Ferreira *et al*. (2014), working with an aerobic selector in an urban wastewater treatment system, and ~~a~~ higher storage of carbonaceous matter. Figure 6.6 b shows a comparative representation of the calculated ratio $N_{ASSIM}/P_{VSS}$ (g $NH_4^+$-N assimilated $g^{-1}$ VSS produced) for the system operating without a selector (CSTR), and with an anoxic and aerobic selector. The CSTR provided the highest ratio $N_{ASSIM}$ / $P_{VSS}$ (0.09 g $NH_4^+$-N assimilated $g^{-1}$ VSS produced), whereas the selector's systems showed the lower values (0.07 and 0.05 for the anoxic selector and aerobic selector activated sludge system, respectively), suggesting a higher proportion of storage material.

Also the reactors belonging to the configurations which included an anoxic and an aerobic selector, showed a lower ratio $N_{ASSIM}$ / $P_{VSS}$ than the CSTR (0.08 and 0.07, respectively, in front of 0.09 g $NH_4^+$-N assimilated $g^{-1}$ VSS produced for the CSTR). In agreement with a lower $N_{ASSIM}$ / $P_{VSS}$ ratio, the selector systems produced a lower DSVI results, as stated in Figure 6.6 b. Particularly, the aerobic selector activated sludge system presented the lowest DSVI and suggested the highest accumulation of storage material.




(a)




(b)

Figure 6.6 Comparison of operational parameters for the CSTR and for the systems including an anoxic and an aerobic selector. (a) Variation of the global ammonia nitrogen assimilation. The dotted vertical lines indicates re-inoculation of the systems (b) Diluted sludge volumetric index (DSVI) values and calculated $N_{ASSIM}/P_{VSS}$ ratio

## 6.4. CONCLUSIONS

A pilot-scale aerobic biological CSTR, treating petrochemical effluents, leaded to a filamentous bulking, with diluted sludge volumetric index (DSVI) values of 500 mL $g^{-1}$ when working with a low F/M ratio of 0.10 g COD $g^{-1}$VSS $d^{-1}$. Although increasing the F/M ratio to 0.40 g COD $g^{-1}$VSS $d^{-1}$ and guaranteeing the availability of dissolved oxygen (> 2 mg $L^{-1}$) improved the floc structure, it was not enough to overcome the filamentous bulking. The DSVI could not be significantly reduced below 100 mL $g^{-1}$ until a selector was implemented.

Anoxic and aerobic selectors worked properly in the pilot petrochemical activated sludge system. Therefore, a high portion of the readily biodegradable matter was biodegraded in the selectors and they represented an effective solution to the filamentous bulking. However, the aerobic selector was the best option, as it attained a significantly lower SVI than the anoxic selector, a more significant reduction of filaments and a more reliable operation. An additional issue to be considered was the sensitivity of nitrifying bacteria to potential toxics coming from the industrial processes, which may prevent the system effectiveness. Biomass and nitrogen balances suggested having storage and new biomass cells production, as mechanisms of carbonaceous matter uptake in the selector. The activated sludge system including the aerobic selector was the one showing more proportion of storage, coincident with producing the lowest SVI.

The inclusion of a selector in the pilot activated sludge system had an effect on its performance parameters. The system operated with a selector provided slightly higher soluble COD removal efficiency as well as lower specific ammonia consumption and biomass production, per unit of COD, in the main reactor. Also, the reactor of the aerobic selector system presented lower oxygen requirements than the CSTR. Consequently, the implementation of a selector allowed to improve the soluble COD removal efficiency with less oxygen supply to the main reactor. However, less biomass was produced in the main reactor which may result in an older aged sludge system, susceptible of experiencing nitrification issues due to the high amount of inorganic carbon present in the petrochemical wastewaters treated.

# 7. Optimization of the operational and design parameters of an aerobic selector

***ABSTRACT***

*Selectors have been widely recognized as a solution to control filamentous bulking and consequently enhance sludge settling. However, to guarantee the selector's effectiveness (sludge volumetric index< 100 mL $g^{-1}$ during 90% of the time) in a full-scale installation, it is compulsory to find the proper design and operational parameters, according to the quality of the wastewater treated. In order to identify the key parameters to optimize the selector's performance, petrochemical wastewater was tested in a pilot-scale activated sludge system including an aerobic selector. The optimum conditions in the selector were a hydraulic retention time (HRT) of 30 minutes and a food-to-microorganism ratio (F/M) from 30 to 35 g COD $g^{-1}$ VSS $d^{-1}$. They corresponded to the selector's maximum COD (37.4%) and $BOD_5$ (95.1%) removal efficiency and the dominance of the storage mechanism in front of replicative growth (ratio Nitrogen assimilation-to-Volatile Suspended Solid production of 0.07 g $NH_4^+$-N assimilated $g^{-1}$ VSS produced). Feeding a more biodegradable influent to the selector (up to 45 g $BOD_5$ $g^{-1}$ VSS $d^{-1}$) enhanced its effectiveness, whereas increasing the supply of particulate matter (up to 139.6 g tCOD $g^{-1}$ VSS $d^{-1}$) showed a negative effect on sludge settling. The inclusion of the aerobic selector in the activated sludge system, operated at the optimum parameters, resulted in an older aged sludge. Increasing the selector's F/M above the optimum value or reducing the influent $BOD_5$ produced a progressive loss of efficiency of the activated sludge system and higher oxygen requirements.*

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# 7. Optimization of the operational and design parameters of an aerobic selector

## 7.1. INTRODUCTION

Selectors were introduced by Chudoba *et al*. (1973), as a way to suppress filamentous microorganisms in mixed cultures and improve sludge volumetric index (SVI). They defined a selector as the initial part of a continuous biological reactor, characterized by a low dispersion number and by an adequate concentration gradient.

Nowadays, different types of selectors are available, depending on the electron acceptor used (Jenkins *et al*., 2003; Metcalf and Eddy, 2003). Particularly, aerobic selectors have been widely implemented as a cost-effective operation to deal with filamentous bulking (Melcer *et al*., 2003; Al-Mutairi, 2009; Ferreira *et al*., 2014). They use oxygen as the electron acceptor and rely on kinetic selection (Metcalf and Eddy, 2003). This selection mechanism uses differential growth kinetics to promote the development of floc-forming microorganisms over filamentous bacteria. According to the saturation kinetic's model, the high substrate concentration under aerobic conditions, results in favourable conditions for the dominance of floc-formers over filamentous bacteria (Al-Mutairi, 2009; Ferreira *et al*., 2014; Eckenfelder and Cleary, 2013). The kinetic selection of floc-forming microorganisms relies on initial substrate storage followed by its metabolization (Grady *et al*., 2011; Eckenfelder and Cleary, 2013). Hence, in the selector, the substrate is mainly taken up by the microorganisms and converted into internal stores, through a non-replicative process called oxidative assimilation or bio-sorption. As a result, the biomass is increased as storage, without increasing the number of cells. In this process, a small quantity of substrate is oxidized to provide the energy for storage (Gaudy and Gaudy, 1988). Later, in the aeration basin, replicative growth or cell division happens, which is associated with protein synthesis, from supplied nitrogen and the material stored in the cells (Jenkins and Wanner, 2014). This procedure establishes a feed-starve cycle, so that the storage capacity of the microorganisms is regenerated (Henze *et al*., 2008). Replicative growth can also happen in the selector if most of the substrate has been removed and the bacteria auto-digest the internal stores (Gaudy and Gaudy, 1988).

A target for the proper design of a selector, is to obtain a good settling sludge with a SVI <100 mL $g^{-1}$ for 90% of the time (Parker *et al*., 1998). To achieve the optimum selector's performance two opposite conditions have to be met at the same time. The first principle is to obtain a high substrate equilibrium concentration in the selector, in

order to generate a high growth rate, which allows rapid substrate uptake. This way enables the selection of floc-forming microorganisms. The second statement is to achieve high substrate removal efficiency in the selector so that the feed starve cycle is established in the activated sludge system (Patoczka and Eckenfelder, 1990; Mangrum, 1998; Eckenfelder and Cleary, 2013). Also, the selector must remove almost all of the readily biodegradable substrate in order to create the feed starve cycle (Chambers and Tomlinson; 1982; Jenkins *et al*., 2003; Tandoi *et al.*, 2006; Grady *et al*., 2011).

References can be found on the optimization of the design and operational parameters of selectors, in order to obtain a good sludge settling. As an example, Gabb *et al*. (2010) presented data from full-scale wastewater treatment plants which identified key parameters, such as the influent five-day biological oxygen demand ($BOD_5$) concentration, the selector hydraulic retention time (HRT) and the food-to-microorganism ratio (F/M) of the initial selector's compartment. However, it is widely recognized in the bibliography that the optimum parameters of a selector depend on the influent wastewater characteristics and upstream unit processes (Chambers and Tomlinson, 1982; Tandoi *et al*., 2006; Al-Mutairi, 2009). Therefore, as stated by Seviour and Blackall (2012) the design of a selector relies still on empirical results, considering the particular behaviour of the wastewater treated.

Referring particularly to petrochemical wastewater, the previous chapter reported successful results of an aerobic selector in order to solve a low F/M filamentous bulking. However, to come to an industrial design of the aerobic selector, it is still necessary to optimize the main design and operational parameters, according to the quality of the petrochemical wastewater. Moreover, it is required further knowledge about the effect of implementing a selector on the activated sludge system performance.

The present chapter introduces an experimental study with petrochemical effluents, in which a pilot activated sludge system including an aerobic selector was run in order to optimize the selector's design and operational parameters. The effect of selector's variables such as F/M, HRT, influent biodegradability and particulate matter has been tested on sludge settling and on the activated sludge system output. The methodology and the findings presented in this chapter could not only provide guidelines for the design of a selector, but also inspire future work on the optimization of an activated sludge system operated with a selector.

## 7.2. METHODOLOGY

As summarized in Table 7.1, eighteen operational periods were planned in order to assess the effect of the F/M ratio, HRT, influent biodegradability and influent particulate matter on the selector's performance. Comparative F/M ratios were established in the selector for every period, based on soluble COD (ratio F/M), total COD which included the particulate matter (ratio F/M (tCOD)) and $BOD_5$ (ratio F/M (BOD)).

Table 7.1 General information on test periods conducted to assess the effect of HRT, F/M, influent particulate matter and influent biodegradability on the selector's performance

| | Selector parameters (Units) | | | |
|---|---|---|---|---|
| Period Nº | HRT (min) | F/M (g sCOD $g^{-1}$ VSS $d^{-1}$) | F/M (t COD) (g tCOD $g^{-1}$ VSS $d^{-1}$) | F/M (BOD) (g $BOD_5$ $g^{-1}$ VSS $d^{-1}$) |
| 1 | 15 | 37.5 | 60.5 | 29.3 |
| 2 | 20 | 37.7 | 60.8 | 29.4 |
| 3 | 30 | 32.0 | 50.6 | 22.4 |
| 4 | 60 | 35.4 | 57.1 | 27.6 |
| 5 | 20 | 16.4 | 46.9 | 8.2 |
| 6 | 30 | 10.9 | 35.0 | 8.3 |
| 7 | 60 | 11.9 | 34.0 | 7.1 |
| 8 | 30 | 30.0 | 139.6 | 19.5 |
| 9 | 30 | 31.4 | 56.1 | 10.4 |
| 10 | 30 | 33.0 | 53.6 | 23.1 |
| 11 | 30 | 33.7 | 64.6 | 19.5 |
| 12 | 30 | 35.2 | 54.3 | 46.9 |
| 13 | 30 | 39.8 | 233.6 | 21.9 |
| 14 | 30 | 40.0 | 55.7 | 34.0 |
| 15 | 30 | 43.0 | 64.2 | 20.2 |
| 16 | 30 | 70.0 | 83.0 | 46.9 |
| 17 | 30 | 74.2 | 157.1 | 48.2 |
| 18 | 30 | 88.0 | 103.8 | 61.6 |

Different HRT were tested in the selector at a constant F/M. In order to adjust the HRT, the selector was operated at different volumes and additionally raw water dilution of the feed streams was used. To keep constant the selector's F/M, the flow of recycled biomass was changed according to the total selector's flow rate, to get the desired biomass concentration in the selector.

To vary the F/M into the selector, the feed flow rates were adjusted. Also, by varying the relative flow rates of the feed streams A and B, the influent $BOD_5$ and particulate COD into the selector varied.

According to Henze *et al*. (2008) recommendation, dissolved oxygen concentration (DO) in the aerobic selector was always guaranteed (higher than 2 mg $L^{-1}$).

The reactor and the selector were filled before every period began with fresh inoculum. The duration of each test period was calculated as about 2 times the sludge retention time (SRT) of the activated sludge system, which was characterized at 12 days.

To conclude about the selector's performance, soluble chemical oxygen demand removal efficiency and biological oxygen demand removal efficiency were calculated based on COD and $BOD_5$ mass balances to the selector, respectively.

Soluble chemical oxygen demand was preferred to total chemical oxygen demand, as the particulate matter fed showed low biodegradability. Also the COD analysis was more reproducible than the total COD analysis.

The COD equilibrium concentration in the selector was also considered as relevant data, whereas the biomass production was calculated from the biomass and the nitrogen balance to the selector.

The observed nitrogen assimilation in the selector (g $NH_4^+$-N assimilated $g^{-1}$ COD removed) provided information about the replicative growth process, expressed with the parameter $N_{ASSIM}$. As the average pH in the selector was 8.2, ammonia stripping in the selector was evaluated in order to quantify the maximum overestimation of the parameter $N_{ASSIM}$. Considering the average selector's conditions (20 ºC and up to 47 mg $NH_4^+$-N $L^{-1}$) the maximum potential stripping was evaluated at 5.95% of the ammonia concentration. As a result, the maximum overestimation for the parameter $N_{ASSIM}$ was 0.001 g $NH_4^+$-N assimilated $g^{-1}$ COD removed. The volatile suspended solid (VSS) mass balance to the selector yielded the observed VSS production (g VSS produced $g^{-1}$

COD removed), reported through the parameter $P_{VSS}$. The biomass balance was validated with NTK analysis in the mixed liquor to discard interferences with the low VSS concentration in the feed streams. Eventually, a ratio $N_{ASSIM}$ / $P_{VSS}$ was calculated in order to quantify the dominance of the storage mechanism over the new cell production, by comparison with the stoichiometric value (0.12 g $NH_4^+$-N assimilated $g^{-1}$ VSS produced), considering the biomass formula as $C_5H_7NO_2$.

The results obtained were statistically evaluated by comparison of the means and the variances using, respectively, the Student and the Fisher tests. The statistical significance of the differences was tested for a 95% confidence level. *Statgraphics Plus for Windows 3.3 (1994-1998)* was used as support software.

## 7.3. RESULTS AND DISCUSSION

### 7.3.1. Effect of F/M and HRT on the selector's performance

During the seven initial operational periods, the pilot-scale activated sludge system was operated at different selector's F/M and HRT, in order to assess the effect of these variables on the selector's performance. The optimum F/M determines the biomass recycle to the selector, depending on the organic load supplied. Identifying the optimum HRT for the selector provides information on its design volume.

As reflected in Table 7.2, the first four periods of operation used an average F/M ratio of 35.7 g COD $g^{-1}$ VSS $d^{-1}$, and increased progressively the HRT from 15 minutes (Period 1) to 60 minutes (Period 4). A similar comparative was also studied with a lower average F/M ratio of 13.1 g COD $g^{-1}$ VSS $d^{-1}$, increasing the HRT from 20 minutes (Period 5) to 60 minutes (Period 7).

As performance variables in the selector, COD equilibrium concentration, COD and BOD removal efficiency, SVI, observed ammonia nitrogen assimilation and observed VSS production have been quantified and statistically evaluated. The results belonging to periods operated at the same F/M but different HRT were compared in order to test the statistical effect of the HRT. Also, the impact of varying the F/M was statistically tested by comparing the periods operated at the same HRT and different F/M.

Table 7.2 Performance data of an aerobic selector included in a pilot activated sludge system. Different selector's F/M ratio and HRT were tested in seven operational periods

| | | PERIOD 1 | PERIOD 2 | PERIOD 3 | PERIOD 4 | PERIOD 5 | PERIOD 6 | PERIOD 7 |
|---|---|---|---|---|---|---|---|---|
| Parameters | Units | | | | | | | |
| HRT[1] | min | 15 | 20 | 30 | 60 | 20 | 30 | 60 |
| F/M[2] | g COD $g^{-1}$ VSS $d^{-1}$ | 37.5 ± 3.5 | 37.6 ± 5.2 | 34.1 ± 5.1 | 35.4 ± 4.3 | 16.4 ± 1.1 | 10.9 ± 0.7 | 12.0 ± 1.7 |
| COD EC[3] | g $L^{-1}$ | 3.3 ± 0.9 | 3.1 ± 0.3 | 2.6 ± 0.1 | 1.6 ± 0.5 | 2.2 ± 0.1 | 1.5 ± 0.1 | 0.8 ± 0.1 |
| COD RE[4] | % | 26.2 ± 4.9 | 29.2 ± 4.5 | 37.4 ± 7.8 | 33.1 ± 1.6 | 13.3 ± 6.4 | 17.6 ± 0.7 | 17.2 ± 2.7 |
| BOD RE[5] | % | 88.7 ± 3.5 | 91.6 ± 0.7 | 95.1 ± 1.1 | 88.5 ± 3.3 | 86.7 ± 1.4 | 90.3 ± 0.6 | 87.4 ± 1.4 |
| $N_{ASSIM}$[6] | g $NH_4^+$-N assimilated $g^{-1}$ COD removed | 0.0447 ± 0.0015 | 0.0349 ± 0.0010 | 0.0238 ± 0.0015 | 0.0193 ± 0.0009 | 0.0143 ± 0.0010 | 0.0103 ± 0.0012 | 0.0059 ± 0.0007 |
| $P_{VSS}$[7] | g VSS produced $g^{-1}$ COD removed | 0.56 ± 0.02 | 0.44 ± 0.01 | 0.34 ± 0.02 | 0.32 ± 0.02 | 0.29 ± 0.02 | 0.26 ± 0.05 | 0.20 ± 0.02 |
| $N_{ASSIM}/P_{VSS}$ | g $NH_4^+$-N assimilated $g^{-1}$ VSS produced | 0.08 | 0.08 | 0.07 | 0.06 | 0.05 | 0.04 | 0.03 |
| SVI[8] | mL $g^{-1}$ | 76.8 ± 4.3 | 56.7 ± 6.1 | 22.0 ± 3.0 | 65.1 ± 6.3 | 65.2 ± 3.4 | 44.8 ± 6.8 | 62.3 ± 3.7 |

[1]Hydraulic Retention Time

[2]Food-to-microorganism ratio

[3]COD Equilibrium Concentration

[4]COD Removal Efficiency

[5]BOD Removal Efficiency

[6]Observed Ammonia nitrogen assimilation per substrate removed

[7]Observed production of Volatile Suspended Solids per substrate removed

[8]Sludge Volumetric Index

The implementation of a selector was effective to improve sludge settling for every period, as the SVI values obtained (see Table 7.2) were always below 100 mL $g^{-1}$, meeting the criteria of bibliographic references (Henze *et al.*, 2008). However, Figure 7.1 shows a minimum SVI value for period 3, which indicates that optimum conditions can be established in the selector to improve sludge settling. This result is in agreement with the literature, which states that there is an optimum F/M (Eckenfelder and Cleary, 2013) and HRT (Henze *et al.*, 2008) for the selector's performance. To confirm this statement, a statistical comparison was performed between the SVI values of correlative periods being operated at the same F/M but different HRT. It was concluded that the SVI result obtained for each HRT was significantly different with a probability of 95% (see Table 7.3).

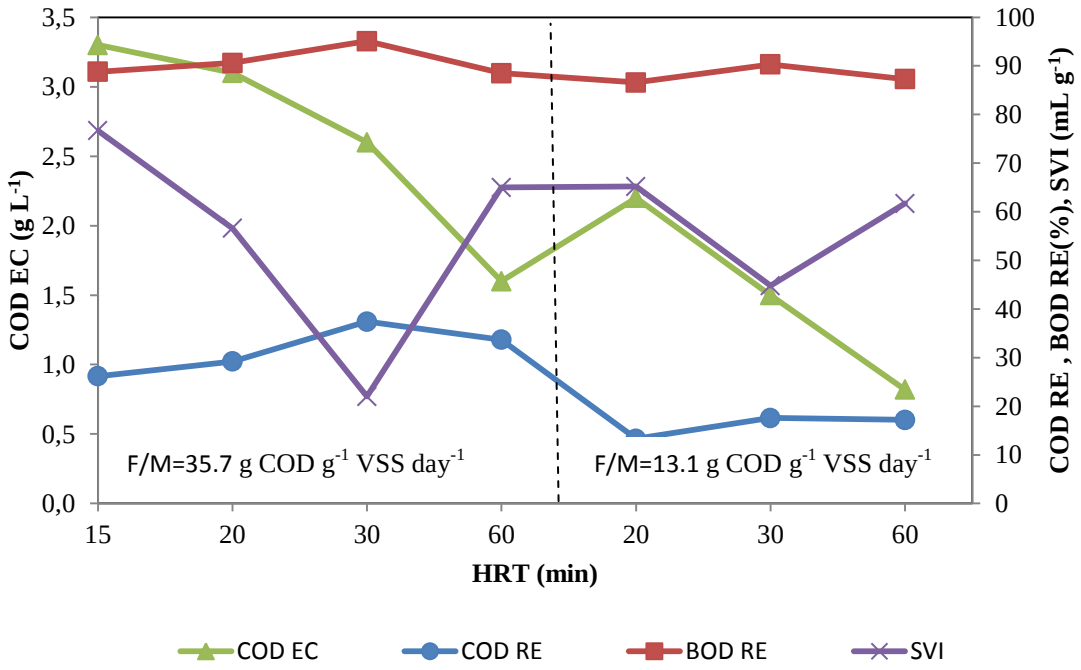



Figure 7.1 Operational variables in the aerobic selector for different F/M and HRT: COD equilibrium concentration (COD EC), COD removal efficiency (COD RE), BOD removal efficiency (BOD RE), Sludge Volumetric Index (SVI)

Only the difference between the SVI of Period 1(HRT of 15 min) and Period 2 (HRT of 20 min) could not be concluded as significantly different. Also, the SVI between periods run at the same HRT and different F/M were statistically compared. As a conclusion, the differences between the SVI values obtained for each HRT at low and high F/M were statistically significant with a 95% probability. Therefore, the selector's

HRT and F/M were confirmed as variables affecting the sludge settling ability. Period 3 presented a statistically significant minimum SVI value, related to optimum operational conditions in the selector.

Table 7.3 Statistical analysis of the selector's operational parameters. Fisher and Student tests for a 95% confidence level were considered for the statistical comparison of the values obtained.

| | | Selector's operational parameters | | | | | |
|---|---|---|---|---|---|---|---|
| | Compared periods | COD EC[1] | COD RE[2] | BOD RE[3] | $N_{ASSIM}$[4] | $P_{VSS}$[5] | SVI[6] |
| Different HRT, the same F/M | Period 1 and 2 | NS | NS | S | S | S | NS |
| | Period 2 and 3 | S | S | S | S | S | S |
| | Period 3 and 4 | S | S | S | S | S | S |
| | Period 5 and 6 | S | S | S | S | S | S |
| | Period 6 and 7 | S | NS | S | S | S | S |
| Different F/M, the same HRT | Period 2 and 5 | S | S | S | NS | S | S |
| | Period 3 and 6 | S | S | S | NS | S | S |
| | Period 4 and 7 | S | S | S | NS | S | S |

[1]COD Equilibrium Concentration

[2]COD Removal Efficiency

[3]BOD Removal Efficiency

[4]Observed Ammonia nitrogen assimilation per substrate removed

[5]Observed production of Volatile Suspended Solids per su removed

[6] Sludge Volumetric Index

S= Statistically significant difference between results

NS= Statistically non-significant difference between results

Reaching an optimum COD equilibrium concentration in the selector (not too low and not too high) is recognized as a key issue to accomplish its maximum efficiency to improve sludge settling (Mangrum, 1998; Albertson, 2005). Figure 7.1 shows the tendency of COD equilibrium concentration to decrease from period 1 (3.3 g $L^{-1}$) to period 7 (0.82 g $L^{-1}$), as the F/M fed to the selector decreases, and the HRT of the selector increases. The difference between the COD equilibrium concentrations obtained for the different periods was confirmed to be statistically significant, but for periods 1 (HRT of 15 min) and 2 (HRT of 20 min). Therefore, it could be concluded that each operational condition drove to a different COD equilibrium concentration in the selector, which determined sludge settling. The comparison of the COD equilibrium concentration and the SVI trends indicates that there is an optimum COD equilibrium concentration in the selector (Period 3), where the SVI is minimum (22.0 mL $g^{-1}$). When increasing the COD equilibrium concentration (Periods 1 and 2) by high F/M feed and

short HRT, the SVI shows a tendency to increase. On the contrary, when decreasing the COD equilibrium concentration by long HRT and low F/M feed, the SVI also tends to increase. Therefore, there was an optimum COD equilibrium concentration in the selector to enable rapid substrate uptake, which was characterized at 2.6 g $L^{-1}$, when operating at an F/M of 32.0 g COD $g^{-1}$ VSS $d^{-1}$and a HRT of 30 minutes.

An additional requirement for the selector to improve settling is to accomplish enough COD removal efficiency in order to establish the feed-starve cycle in the activated sludge system (Patoczka and Eckenfelder, 1990; Mangrum, 1998; Grady *et al*., 2011). The figures presented in Table 7.2 for COD removal efficiency confirm this statement, by showing the maximum COD removal efficiency (37.4%) in Period 3, coincident with the minimum SVI obtained (22.0 mL $g^{-1}$). Lower COD removal efficiency was obtained for periods 1 (26.2%) and 2 (29.2%), being operated at a shorter HRT. Periods 5 to 7, exhibited the lowest COD removal efficiency, with values around 15 %. This last result showed that, according to Mangrum (1998) finding, as the organic loading in the selector increased, the substrate removal rate also increased. The conclusions obtained were confirmed statistically by comparing the COD removal efficiency produced by periods operated at the same F/M but different HRT and by periods operated at the same HRT but different F/M. Hence, the COD removal efficiency obtained for each period was significantly different with a 95% confidence level, except for periods 1 and 2, in agreement with the statistical comparison for the SVI and the COD equilibrium concentration. Also the comparison of the COD removal efficiency for periods 6 and 7 showed no significant difference, due to similar average values (17.6% for period 6 and 17.2% for period 7).

With regard to $BOD_5$ removal efficiency in the selector, the bibliography reports that it is required a nearly complete removal of the biodegradable substrate to guarantee the effectiveness of the selector (Jenkins *et al.*, 2003; Chambers and Tomlinson, 1982; Tandoi *et al*., 2006). The $BOD_5$ removal efficiency results obtained for every period comply with this statement. However, the maximum $BOD_5$ removal efficiency was, as well, accomplished in period 3 with a value of 95.1 %. The other three periods performed at an average F/M ratio of 35.7 g COD $g^{-1}$ VSS $d^{-1}$ presented a lower $BOD_5$ removal efficiency, around 90%. The periods tested at the lowest F/M ratio (13.1 g COD $g^{-1}$ VSS $d^{-1}$) provided the lowest $BOD_5$ removal efficiency with values around 87%, except for period 6, performed at a HRT of 30 minutes, which attained a higher value of 90.3%. The significant difference between the $BOD_5$ removal efficiency values was confirmed statistically. Even periods 1 and 2, which provided no significant

difference in the SVI, COD equilibrium concentration and COD removal efficiency values, presented significantly different values for the BOD removal efficiency.

Performance parameters, such as observed ammonia assimilation ($N_{ASSIM}$) and observed VSS production ($P_{VSS}$) were also assessed.

In every period, there was an assimilation of ammonia nitrogen in the selector, so that $N_{ASSIM}$ showed values ranging from 0.006 to 0.045 g $NH_4^+$-N assimilated $g^{-1}$ COD removed. Therefore, it was evident, that replicative growth had taken place in the selector, as well as oxidative assimilation. As presented in Table 3, higher values of $N_{ASSIM}$ were obtained when decreasing HRT and increasing F/M ratio, as the COD equilibrium concentration was higher. This tendency supports previous findings, which reported that the higher the selector's substrate concentration, the faster the microorganisms growth (Chambers and Tomlinson, 1982; Gaudy and Gaudy, 1988; Jenkins *et al*., 2003). However, a remark must be made in this statement: when $N_{ASSIM}$ results were statistically evaluated, only the differences between $N_{ASSIM}$ of periods performed under different HRT were significant. On the contrary, the $N_{ASSIM}$ of the periods performed at the same HRT and different F/M were not significantly different in any case. Therefore, increasing the HRT decreased significantly the observed nitrogen assimilation per unit of substrate removed in the selector, whereas increasing the F/M provided a non significant increase of the nitrogen assimilation, with a 95% confidence level.

$P_{VSS}$ accounted for the observed VSS production which tended to increase as the F/M ratio increased and the HRT decreased, with values ranging from 0.20 to 0.58 g VSS produced $g^{-1}$ COD removed (see Table 7.1). The statistical evaluation of the results showed that these tendencies were significant, as every $P_{VSS}$ value was significantly different from others with a 95% confidence level.

As a result, the calculated ratio $N_{ASSIM}$ / $P_{VSS}$ (see Table 7.2) reports lower values than the stoichiometric limit of nitrogen conversion into VSS (0.12 g $NH_4^+$-N assimilated $g^{-1}$ VSS produced), which suggests the dominance of oxidative assimilation in front of new cell production, as growth mechanism. The periods performed at lower F/M (Periods 5 to 7) and higher HRT (Periods 3 and 4) exhibited a lower ratio $N_{ASSIM}$ / $P_{VSS}$, corresponding to a higher amount of carbonaceous matter dedicated to storage. The results confirm the hypothesis of Mangrum (1998), who reported that F/M limits how fast oxidative assimilation takes place, but not the capacity of the microorganism to do it. Therefore, as the bibliography states (Gaudy and Gaudy, 1988; Mangrum, 1998;

Jenkins *et al*., 2003), the dominant mechanism responsible for rapid substrate removal in the selector was determined by floc loading: low floc loadings favoured substrate storage while high rate metabolism was dominant at higher floc loadings.

The bibliography reports significant advantages of substrate storage in the selector, taking to sludge settling. It is stated that oxidative assimilation allows the selection of floc-forming bacteria over filamentous microorganisms by producing high substrate removal rates and so, an effective feed starve cycle. Also, unbalanced growth would improve bio-flocculation due to the production of the polymer poly-hydroxybutyrate (Mangrum, 1998). In agreement with this statement, the selector's objective stated by Henze *et al*. (2008) was accomplished by obtaining SVI values below 100 mL $g^{-1}$, for every test, 100% of the time. However, not always the lower SVI results corresponded to the minimum $N_{ASSIM}$ / $P_{VSS}$ ratio. Periods operated at a low F/M showed the lowest $N_{ASSIM}$ / $P_{VSS}$ ratio, but the highest SVI results, probably due to a low COD equilibrium concentration.

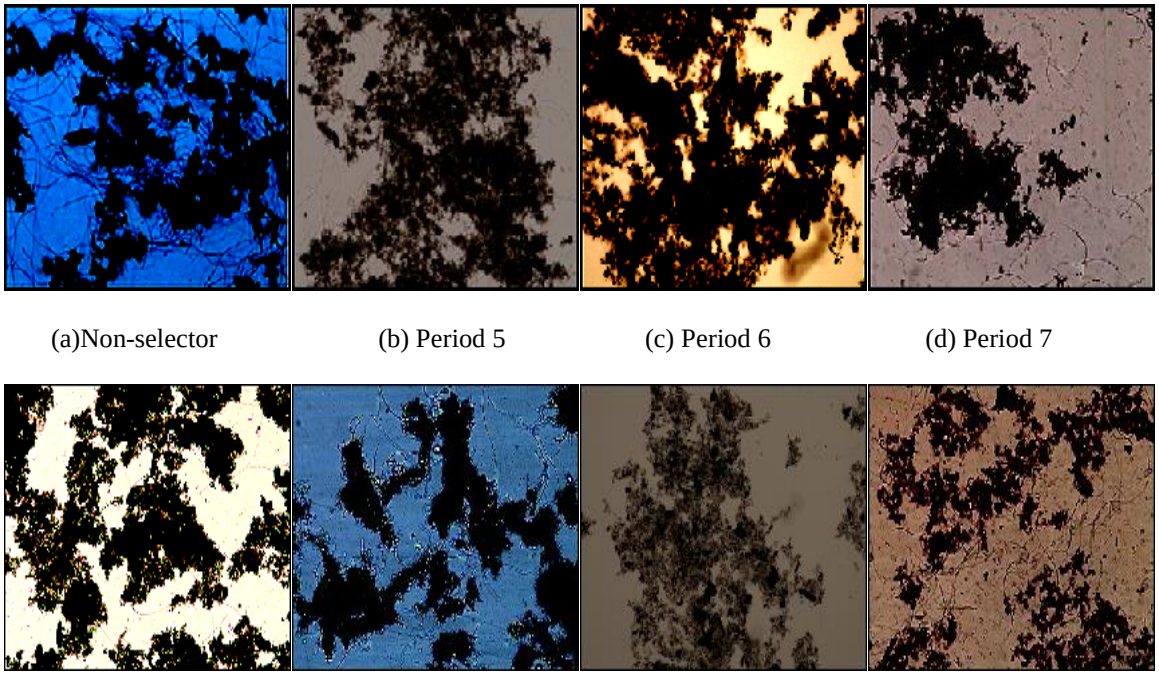
Comparative microscopic observations of the sludge at the beginning and during the tests, showed a quick response of the implementation of a selector. Even if the SRT was 12 days for every period, within a week of operation, a significant reduction in the number of filaments was registered, as well as SVI improvement.

The microscopic observations showed the filamentous appearance on the initial mixed liquor used for the different tests (Figure 7.2 a), in comparison to the floc obtained operating the activated sludge system with a selector at low F/M periods (Figures 7.2 b-d) and high F/M periods (Figures 7.2 e-h). For every period, the selector was effective to control the filamentous bulking, by creating a BOD concentration gradient, as stated by Gabb *et al*. (2010).

For the lower F/M periods, the less filamentous and most compact flocs were obtained at HRT 30 min (Period 6, Figure 7.2 c), corresponding to the lowest SVI value (44.8 mL $g^{-1}$) and the highest $BOD_5$ removal efficiency (90.3%).

For the higher F/M periods, the best floc was also obtained at HRT of 30 min (Period 3, Figure 7.2 g), which corresponded, as well, to the minimum SVI (22.0 mL $g^{-1}$) and the highest COD (37.4%) and $BOD_5$ removal efficiency (95.1%). This result confirms the theory introduced by Van Niekerk *et al*. (1988), and confirmed by Tandoi *et al*. (2006), who stated that there was an optimum selector's size, dependent on the influent substrate concentration and flow rate. They also reported that if the selector is sized too small

(short HRT), its efficiency decreases (as reflected in periods 1, 2 and 5) and a large portion of soluble substrate enters the aerobic reactor, enhancing the growth of filamentous bacteria (as reflected in Figures 7.2 e, f and b). On the other hand, if the selector is oversized (long HRT), the substrate gradient is not high enough to induce rapid soluble uptake, which also leads to large amounts of soluble substrate coming into the aerobic reactor (as reflected in periods 4 and 7), and enabling the filaments to proliferate (as reflected in Figures 7.2 h and d).



(a)Non-selector (b) Period 5 (c) Period 6 (d) Period 7

(e) Period 1 (f) Period 2 (g) Period 3 (h) Period 4

Figure 7.2 Microscopic observation at 100X of the sludge produced by a pilot activated sludge system including an aerobic selector. (a) Seed sludge from a non-selector system. Low F/M periods at different HRT (b) 20 min (c) 30 min (d) 60 min. High F/M periods at different HRT (e) 15 min (f) 20 min (g) 30 min (h) 60 min

The review of the selector's performance variables together with the observation of the microscopic appearance of the sludge for each operational condition provides the petrochemical industry with references to evaluate the performance of a selector. Additionally, the optimum conditions obtained can be regarded as guidelines for the design and operation of a petrochemical selector, complementing the existing bibliography.

### 7.3.2. Effect of influent $BOD_5$ and particulate COD on the selector's performance

In order to determine the impact of influent $BOD_5$ and particulate COD on the selector effectiveness to improve sludge settling, twelve operational periods from Table 7.1 (Periods 3, 6 and 8 to 17) were considered and organized (Test 1 to 12) as reflected in Table 7.4. The aerobic selector's HRT was kept constant at a value of 30 minutes.

As presented in Table 7.4, the ratio F/M provided to the selector increased progressively from test 1 (10.9 g COD $g^{-1}$ VSS $d^{-1}$) to test 12 (74.2 g COD $g^{-1}$ VSS $d^{-1}$), in four steps. Periods with a similar F/M, were used to test the effect of supplying different F/M (tCOD) and F/M (BOD) ratios to the selector. Hence, the average F/M (tCOD) was within the range of 50.6 to 64.6 g COD $g^{-1}$ VSS $d^{-1}$. However, in tests 2, 8 and 12 the ratio F/M (tCOD) increased up to 139.6, 233.6 and 157.1 g COD $g^{-1}$ VSS $d^{-1}$, respectively, in order to assess the effect of high particulate COD concentrations. Also, the effect of influent $BOD_5$ was analyzed by increasing progressively the ratio F/M (BOD) from 10.4 g BOD $g^{-1}$ VSS $d^{-1}$ in test 3 to 46.9 g BOD $g^{-1}$ VSS $d^{-1}$ in test 7.

Table 7.4 Calculated ratios food-to-microorganism for the aerobic selector: F/M (based on soluble COD), F/M (tCOD) (based on total COD) and F/M (BOD) (based on $BOD_5$).

| Parameter | F/M | F/M (tCOD) | F/M (BOD) |
|---|---|---|---|
| Units | g COD $g^{-1}$ VSS $d^{-1}$ | g COD $g^{-1}$ VSS $d^{-1}$ | g BOD $g^{-1}$ VSS $d^{-1}$ |
| Period 6 | 10.9±0.7 | 35±1.2 | 8.3±0.2 |
| Period 8 | 32.9±3.3 | 139.6±7.9 | 19.5±0.5 |
| Period 9 | | 55.8±5.6 | 10.4±0.4 |
| Period 3 | | | 22.4±1.0 |
| Period 10 | | | 23.1±0.8 |
| Period 11 | | | 19.5±0.5 |
| Period 12 | | | 46.9±1.2 |
| Period 13 | 40.9±3.1 | 233.6±13.4 | 21.9±0.6 |
| Period 14 | | 60±5.2 | 34.0±1.1 |
| Period 15 | | | 20.21±2.2 |
| Period 16 | 72.1±7.1 | 83±2.9 | 46.9±6.1 |
| Period 17 | | 157.1±6.9 | 48.23±1.1 |

Figure 7.3 shows the resulting performance parameters of the selector for every period. The selector's COD equilibrium concentration (see Figure 7.3 a) increased either by increasing F/M or F/M (tCOD), as reported in the bibliography (Mangrum, 1998). As an

example, tests 2 and 8, in which a higher F/M (tCOD) than average was supplied, produced average COD equilibrium concentration values of 4.15 and 4.35 g $L^{-1}$. Tests 3 and 9, which fed a similar F/M ratio, presented significantly lower values of 2.9 and 3.7 g $L^{-1}$, respectively. A similar comparative can be established between tests 12 and 11, with COD equilibrium concentrations of 5.76 and 4.71g $L^{-1}$, respectively.

The highest COD removal efficiency in the selector was obtained for tests 3 to 7 (around 35%), which fed an F/M within the range of 30.0 to 35.2 g COD $g^{-1}$ VSS $d^{-1}$. In agreement with Mangrum (1998) findings, test 1 (feeding a lower F/M) and tests 8 to 12 (feeding a higher F/M) obtained significantly lower COD removal efficiency. Periods performed at high F/M (tCOD) ratios (tests 2, 8 and 12) assessed poor results for the selector, providing practically no degradation of organic matter. Consequently, the requirement stated in the literature (Patoczka and Eckenfelder, 1990; Mangrum, 1998; Eckenfelder and Cleary, 2013) to establish the feed starve cycle in the activated sludge system was not accomplished. When comparing periods with a different influent $BOD_5$, the period using the highest F/M (BOD) obtained the highest COD removal efficiency. As an example, test 9, feeding an average F/M(BOD) of 34.0 g BOD $g^{-1}$ VSS $d^{-1}$ obtained a significantly higher COD removal efficiency (26.0%) than test 10 (21.5%), which fed an average F/M (BOD) of 20.2 g BOD $g^{-1}$ VSS $d^{-1}$. Also, tests 3 to 7, which increased progressively F/M (BOD) from 10.4 to 46.9 g BOD $g^{-1}$ VSS $d^{-1}$, obtained progressively higher COD removal efficiency (from 25.8 to 40.1%, respectively).

As reflected in Figure 7.3 b, the SVI trend followed an inverse tendency to the COD removal efficiency. Therefore, when no COD removal efficiency was registered in the selector (tests 2, 8, 11 and 12), the SVI got significantly higher than 100 mL $g^{-1}$ (163, 177, 129 and 154 mL $g^{-1}$, respectively). On the contrary, tests 7 and 5, which exhibited the highest COD removal efficiency (40.1% and 36.0%), presented one of the lowest SVI result (19 mL $g^{-1}$). Test 9, feeding a moderate F/M and F/M (tCOD) (40.0 and 55.7 g COD $g^{-1}$ VSS $d^{-1}$, respectively) and a high F/M (BOD) (34.0 g BOD $g^{-1}$ VSS $d^{-1}$), produced the lowest average SVI value (15 mL $g^{-1}$). Tests 1, 3 and 10, which presented intermediate COD removal efficiencies (17.2, 25.8 and 21.5%, respectively) also obtained intermediate SVI values (44.8, 68.0 and 62.0 mL $g^{-1}$, respectively). As Mangrum (1998) stated, feeding a low soluble COD fraction, together with a high particulate COD charge, is not a proper condition for a selector to be effective. This result outlines the importance of controlling the particulate matter content in the selector's feed streams, to guarantee its proper performance.

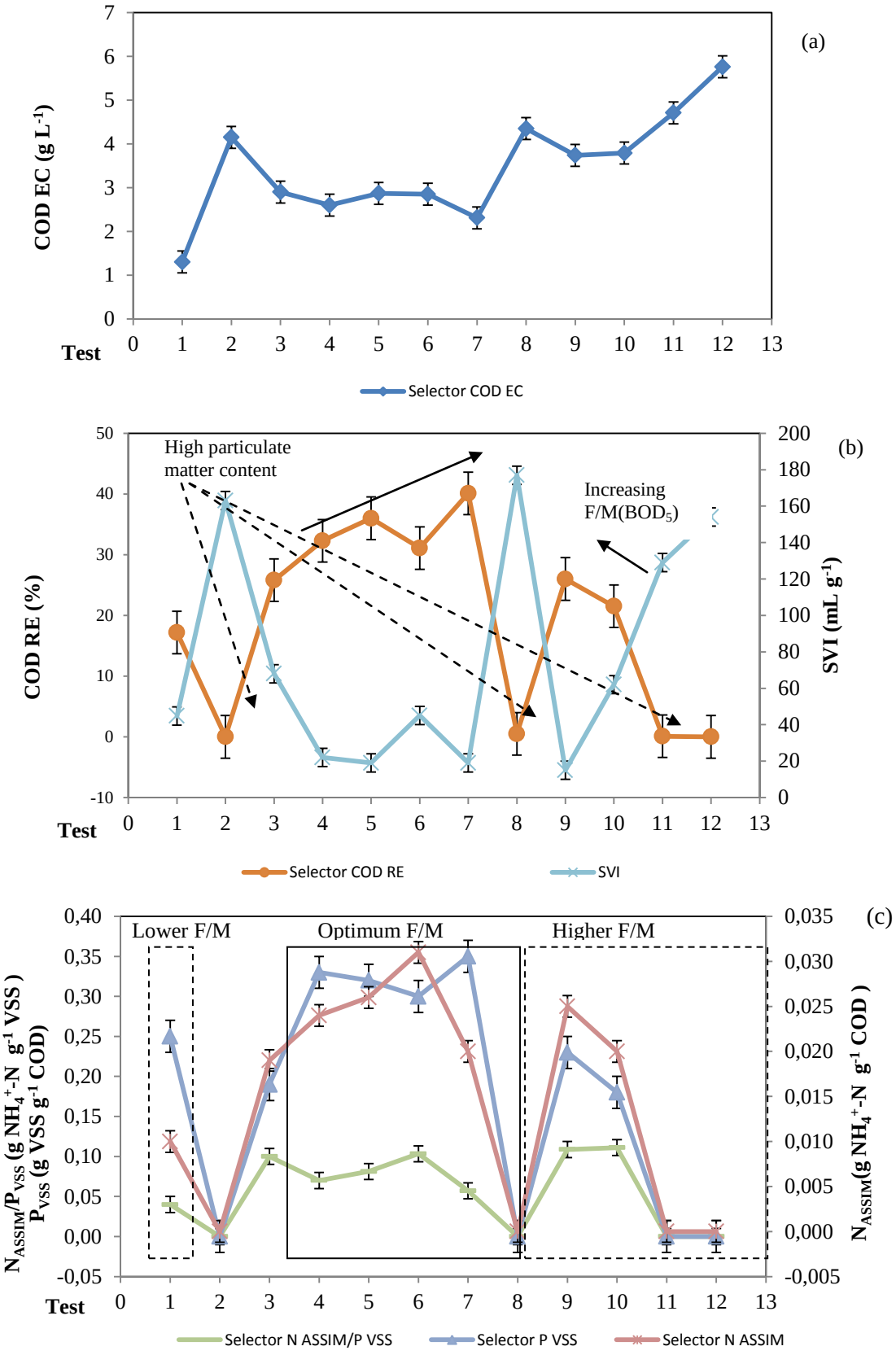



Figure 7.3 Effect of influent $BOD_5$ (F/M(BOD)) and particulate matter (F/M(tCOD)) on the aerobic selector performance (a) COD equilibrium concentration (COD EC), (b) COD removal efficiency (COD RE) and Sludge volumetric index (SVI), (c) Observed ammonia Assimilation ($N_{ASSIM}$) and VSS production ($P_{VSS}$). Error bars represent the standard deviation for each test.

The comparison of periods feeding a different BOD showed that increasing the F/M(BOD) improved settling: tests 3 (10.4 g BOD $g^{-1}$ VSS $d^{-1}$), 4 to 6 (average 21.7 g BOD $g^{-1}$ VSS $d^{-1}$) and 7 (46.9 g BOD $g^{-1}$ VSS $d^{-1}$) obtained progressively significantly lower SVI values (68.0, average 28.7 and 19.0 mL $g^{-1}$, respectively). Also comparing tests 9 and 10 (34.0 and 20.2 g BOD $g^{-1}$ VSS $d^{-1}$) indicated that test 9 obtained significantly lower SVI (15 in front of 62 mL $g^{-1}$). This result states a question about the necessity of a minimum influent $BOD_5$ for the selector to be effective, In fact, the bibliography recommends a minimum $BOD_5$ feed of 2.0 g $BOD_5$ $g^{-1}$ VSS $d^{-1}$ (Albertson, 1991).

Biomass production, as well as ammonia nitrogen assimilation, occurred only in the periods which showed COD removal efficiency values higher than zero. Hence, the periods feeding a higher particulate matter concentration than average (tests 2, 8 and 12), where no COD degradation was accounted, showed neither biomass production nor nitrogen assimilation. On the other hand, the periods that presented the highest COD removal efficiency (coincident with feeding a F/M ratio of 30.0 to 35.2 g COD $g^{-1}$ VSS $d^{-1}$), also provided the highest average biomass production (0.35 g VSS produced $g^{-1}$ COD removed in test 4) and ammonia nitrogen assimilation (0.031 g $NH_4^+$-N assimilated $g^{-1}$ COD removed in test 6). Comparing periods feeding similar F/M and F/M (tCOD), when increasing the ratio F/M (BOD), the results indicate an increase in biomass production, but not necessarily in the ammonia nitrogen assimilation.

Therefore, the ratio $N_{ASSIM}$ / $P_{VSS}$ tends to decrease as the influent biodegradability increases, suggesting a more relevant presence of the storage mechanism. As an example, test 5, with a F/M (BOD) of 23.1 g BOD $g^{-1}$ VSS $d^{-1}$ produced a nitrogen assimilation of 0.026 g $NH_4^+$-N assimilated $g^{-1}$ COD removed and a biomass production of 0.32 g VSS produced $g^{-1}$ COD removed, whereas test 7, with a F/M (BOD) of 46.9 g BOD $g^{-1}$ VSS $d^{-1}$, presented values of 0.020 g $NH_4^+$-N assimilated $g^{-1}$ COD removed and 0.35 g VSS produced $g^{-1}$ COD removed. Consequently, in agreement with Dalentoft and Thulin (1997) statement for lab-scale tests performed with waste paper mill effluents, the results showed that for every period in which a fraction of COD was degraded in the selector, both biomass growth mechanisms (storage and new cell production) were present in the selector. The results also confirmed Gaudy and Gaudy (1988) theory, that oxidative assimilation (storage) happens even if a nitrogen source is fed into the selector. The calculation of the ratio $N_{ASSIM}$/ $P_{VSS}$, in comparison with the stoichiometric value of 0.12 g $NH_4^+$-N assimilated $g^{-1}$ VSS produced, was related to occurring substrate uptake by the storage mechanism. Each period with COD degradation showed a ratio $N_{ASSIM}$ / $P_{VSS}$ below 0.12 g $NH_4^+$-N assimilated $g^{-1}$ VSS

produced, which suggested that unbalanced growth mechanism happened. This finding supports the theory of rapid substrate uptake happening in the selector presented by Grau *et al.* (1982) and confirmed by other authors (Mangrum, 1998; Henze *et al*., 2008).

The evaluation of different influent particulate matter and BOD conditions to the selector has stated limits for the selector's effectiveness to enhance settling. The figures obtained become references to predict if a selector can be successful to improve sludge settling, by knowing the quality of the wastewater fed. Hence, this knowledge can also be extended to industrial sectors other than the petrochemical industry.

### 7.3.3. Effect of selector's parameters on the reactor performance

Six periods from Table 7.1 (periods 3, 9, 11, 15, 16 and 18) using different F/M ratios and influent $BOD_5$ conditions (F/M(BOD)) to the selector were considered (Tests 1 to 6) in order to assess the performance of the main aerobic reactor in the activated sludge system.

As stated in Table 7.5, tests 1 to 3 fed a similar F/M to the selector of around 32.0 g COD $g^{-1}$ VSS $d^{-1}$, which had been identified in the previous tests within the optimum F/M range. In the next periods, the F/M increased progressively to a maximum average ratio of 88.0 g COD $g^{-1}$ VSS $d^{-1}$ (test 6). To analyze the effect of the selector's influent $BOD_5$, the average F/M (BOD) increased progressively from 10.4 g BOD $g^{-1}$ VSS $d^{-1}$ in test 1 to 22.4 g BOD $g^{-1}$ VSS $d^{-1}$ in test 3.

According to the results obtained in the previous tests, as the selector's F/M increased above the optimum value, its COD and BOD removal efficiency decreased (see Figure 7.4 a). Consequently the F/M of the main aerobic reactor increased, which resulted in reducing its capacity to regenerate the storage (Gaudy and Gaudy, 1988; Mangrum, 1998; Chambers and Tomlinson, 1982; Goel and Gaudy, 1968; Eickelboom, 1982) and in a progressive reduction of its COD removal efficiency (see Table 7.5). Also, because of a higher amount of carbonaceous matter being degraded in the reactor, more oxygen was required. Therefore, as the air flow rate supplied was constant, the dissolved oxygen available in the reactor dropped so that tests 5 and 6 showed average DO values below 1 mg $L^{-1}$ (see Table 6). The lack of DO in the reactor, joined to the progressive loss of the selector's efficiency in the last two periods, resulted in a SVI significantly higher than 100 mL $g^{-1}$ (see Table 7.5). Consequently, sludge settling worsened when

the selector's F/M was increased up from the optimum value (Eckenfelder and Cleary, 2013).

Table 7.5 Effect of the selector's F/M and influent $BOD_5$ on the main reactor's performance parameters

| Parameter | Units | PERIOD 9 | PERIOD 11 | PERIOD 3 | PERIOD 15 | PERIOD 16 | PERIOD 18 |
|---|---|---|---|---|---|---|---|
| **Selector** | | | | | | | |
| F/M[1] | g COD $g^{-1}$VSS $d^{-1}$ | 31.4 ± 2.7 | 33.7 ± 1.9 | 32.0 ± 2.6 | 43.0 ± 4.7 | 70.0 ± 9.1 | 88.0 ± 9.3 |
| F/M (BOD)[2] | g BOD $g^{-1}$VSS $d^{-1}$ | 10.4 ± 0.4 | 19.5 ± 0.5 | 22.4 ± 1.0 | 20.2 ± 2.2 | 46.9 ± 6.1 | 61.6 ± 8.4 |
| **Reactor** | | | | | | | |
| F/M[1] | g COD $g^{-1}$VSS $d^{-1}$ | 0.30 ± 0.05 | 0.25 ± 0.07 | 0.20 ± 0.06 | 0.50 ± 0.09 | 0.63 ± 0.05 | 0.92 ± 0.04 |
| F/M (BOD)[2] | g BOD $g^{-1}$VSS $d^{-1}$ | 0.03 ± 0.01 | 0.02 ± 0.002 | 0.01 ± 0.004 | 0.04 ± 0.002 | 0.40 ±0.02 | 0.64 ± 0.03 |
| COD RE[3] | % | 99.1 ± 2.1 | 99.2 ± 0.2 | 99.5 ± 0.08 | 98.7 ± 1.0 | 97.0 ± 0.5 | 97.0 ± 0.4 |
| $N_{ASSIM}$ /$P_{VSS}$[4] | g $NH_4^+$-N assimilated $g^{-1}$ VSS produced | 0.07 | 0.06 | 0.06 | 0.06 | 0.12 | 0.12 |
| DO[5] | mg $L^{-1}$ | 1.5 ± 0.2 | 3.0 ± 0.4 | 3.7 ± 0.4 | 0.5 ± 0.2 | 0.3 ± 0.1 | 0.1 ± 0.1 |
| SVI[6] | mL $g^{-1}$ | 68 ± 8 | 45 ± 3 | 22 ± 5 | 62 ± 6 | 129 ± 7 | 205 ± 10 |

[1]Food-to-microorganism ratio (based on soluble COD)

[2]Food-to-microorganism ratio (based on $BOD_5$)

[3]COD removal efficiency

[4]Assimilated ammonia nitrogen per unit of biomass produced

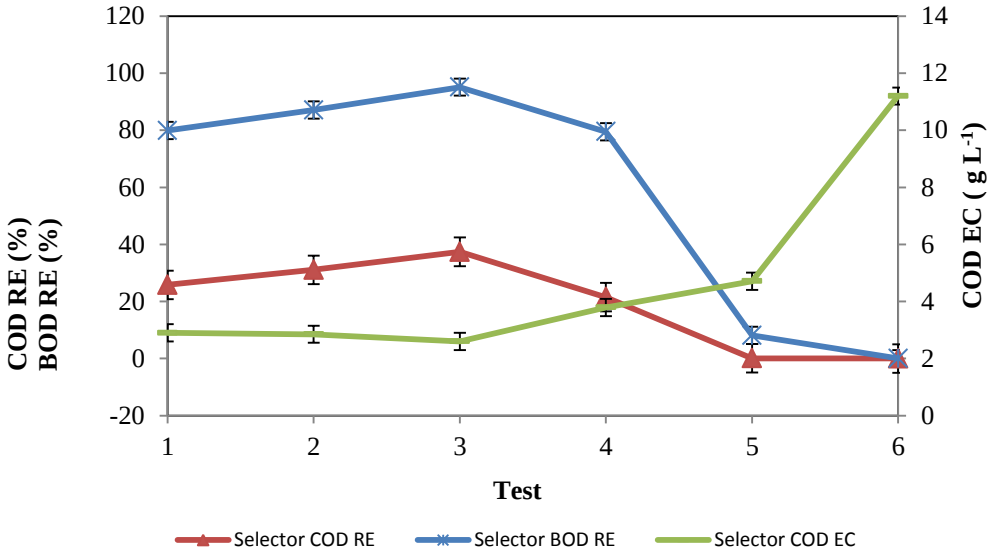
[5]Dissolved oxygen

[6]Sludge volumetric index

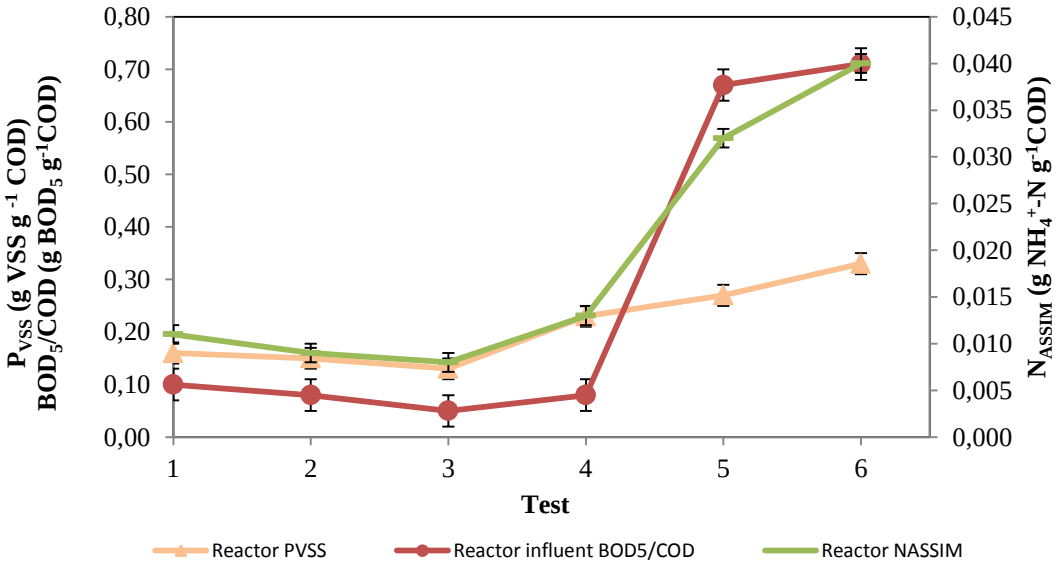
Figure 7.4 b reflects that the observed nitrogen assimilation per unit of COD degraded ($N_{ASSIM}$) increased in the main reactor while increasing the influent ratio $BOD_5$/COD. Therefore, the periods that showed the lowest BOD removal efficiency in the selector provided the highest $N_{ASSIM}$ values in the reactor, in correspondence with a higher influent $BOD_5$/COD ratio.

The observed production of VSS per unit of COD degraded ($P_{VSS}$) in the main reactor increased with the F/M, so that the lowest $P_{VSS}$ values corresponded to the optimum selector's performance conditions (see Figure 4 b). Consequently, the implementation of a selector in the activated sludge system resulted in an older aged sludge. The calculated

ratio $N_{ASSIM}/P_{VSS}$ presented values around 0.12 g $NH_4^+$-N assimilated $g^{-1}$VSS produced, similar to the stoichiometric ratio, for the periods in which the selector was not effective. Tests 1 to 4, where the operational conditions allowed the selector to be effective, produced a lower $N_{ASSIM}/P_{VSS}$ ratio in the reactor (0.06 g $NH_4^+$-N assimilated $g^{-1}$VSS produced).




(a)




(b)

Figure 7.4 Effect of the F/M and influent $BOD_5$ (F/M (BOD)) on (a) the selector's performance: COD equilibrium concentration (COD EC), COD and BOD removal efficiency (COD RE and BOD RE, respectively), and on (b) the reactor's performance: influent $BOD_5$/COD, observed nitrogen assimilation ($N_{ASSIM}$) and observed VSS production ($P_{VSS}$). Error bars represent the standard deviation for each test.

Figure 7.4a shows that an increase in the selector's influent biodegradability, while keeping constant the COD feed, resulted in a lower COD equilibrium concentration. Consistently, the COD and $BOD_5$ removal efficiency in the selector increased progressively from test 1 to test 3. Consequently, when increasing the influent's biodegradability to the selector, the reactor's F/M (BOD) and F/M decreased, so that its efficiency increased (see Table 6). Also, feeding a more biodegradable effluent to the selector produced a better sludge settling. Hence, tests 1 to 3 obtained progressively a significantly lower SVI, in agreement with Albertson's (1991) theory.

The observed nitrogen assimilation in the reactor decreased, as the influent ratio $BOD_5$/COD decreased, while the observed VSS production also decreased when decreasing the F/M. Therefore, increasing the biodegradability of the selector's influent resulted in lower observed nitrogen assimilation, and observed VSS production in the reactor, which could lead to an older aged sludge.

As a result, the operational parameters set to the selector determine not only the selector's effectiveness to improve sludge settling, but also the activated sludge system performance. The optimum selector's parameters to enhance sludge settling provide also a high COD removal efficiency in the main reactor. However, the activated sludge system is bound to operate at higher sludge ages, due a lower biomass production. This finding can either be advantageous or an inconvenience, depending on the wastewater system treated. In any case, it starts the way for future research, either on taking advantage of a lower biomass production or on solving undesired nitrification issues due to an older sludge.

## 7.4. CONCLUSIONS

For the petrochemical wastewater studied, the design of an industrial aerobic selector should consider the optimization of variables such as F/M, HRT, influent biodegradability and particulate matter. They have been demonstrated to be key parameters, not only to optimize the selector to enhance sludge settling, but also to determine the performance of the activated sludge system.

To minimize the SVI, the design volume and biomass recycle to the selector should meet the optimum HRT (30 minutes) and F/M (32.0 g sCOD $g^{-1}$ VSS $d^{-1}$) identified. Also, the best selector's influent to enhance sludge settling should provide a high biodegradability and a low content in particulate matter. Operating at these conditions,

the selector is characterized by the dominance of the storage mechanism and by attaining a maximum COD (37.4%) and $BOD_5$ (95.1%) removal efficiency, which reduces the F/M into the reactor. As a result, an older aged sludge is produced in the activated sludge system, compared to the operation without a selector. If the selector is operated over its optimum F/M, either due to soluble or particulate matter, its COD removal efficiency is reduced and the main reactor is expected to deal with a higher amount of carbonaceous matter. Consequently, higher volatile suspended solid concentration and oxygen consumption is required.

Reviewing the performance parameters of the selector and of the activated sludge system has provided references and guidelines for the design and operation of aerobic selectors. Though the tests were performed with petrochemical wastewater, some of the conclusions could be extended to other industrial sectors.

# 8. Effect of the mixed liquor parameters on settling for activated sludge with selector

***ABSTRACT***

*Sludge settling is determined by the biomass quality, which in turn is dependent on the mixed liquor parameters. In order to assess their effect on sludge volumetric index (SVI) in an activated sludge system including an aerobic selector, experimental design arrangements were organized in a pilot-scale installation fed with petrochemical wastewater. Experiments on a CSTR revealed that while increasing dissolved oxygen (DO) (from <1 to 2-3 mg $L^{-1}$), food-to-microorganism ratio (from 0.2 to 0.5 g COD $g^{-1}$ VSS $d^{-1}$) and ammonia nitrogen concentration (from <1 to >2 mg $L^{-1}$) and while reducing sludge retention time (from >22 to <19 days), sludge settling presented a statistically significant improvement. When the aerobic selector was included in the activated sludge system, DO above 2 mg $L^{-1}$ was the only requirement in the reactor to obtain SVI below 100 mL $g^{-1}$. The critical parameters became those of the selector, where the flocculent biomass was formed, while degrading almost completely the $BOD_5$. Despite the better quality of the biomass obtained with the selector, bench-scale, short-term experiments demonstrated that gravity settling principles, bio-flocculation and water properties still played a significant role on sludge settling. With an increase in suspended solid concentration in the mixed liquor (from 2.3 to 16 mg $L^{-1}$) or in conductivity (from 20 to 60 mS $cm^{-1}$), sludge settling was significantly worse. Increasing pH (from 8.0 to 9.0) or temperature (from 30 to 38 ºC) enhanced sludge settling. Measurements of the sludge electrical particle charge with a Mütek system indicated an optimum value to obtain the minimum SVI, which could be attained by the addition of coagulants.*

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# 8. Effect of the mixed liquor parameters on settling for activated sludge with selector

## 8.1. INTRODUCTION

Sludge quality determines sludge settling ability (Jenkins *et al*., 2003). As evidence, sludge settling in petrochemical continuous stirred tank reactors (CSTR) is often hindered by the deficient quality of the biomass (Cardete *et al*., 2017a), as stated in chapter 6. In turn, sludge microbiological and morphological characteristics are said to be due to mixed liquor parameters, such as dissolved oxygen (DO) (Scruggs and Randall, 1998; Amanatidou *et al*., 2015), food-to-microorganism ratio (F/M) (Gabb *et al.,* 1991; Barbusinski and Koscielniak, 1995; Amanatidou *et al*., 2015), ammonia concentration ($NH_4^+$-N) (Amanatidou *et al*., 2015) and sludge retention time (SRT) (Tuntoolavest and Grady, 1983; Chapman, 1983; Lovette *et al*., 1983; Amanatidou *et al*, 2015). Hence, as presented in chapter 6, frequent conditions in petrochemical CSTR are low F/M and large SRT, as well as poor control of DO and ammonia concentration, due to unintended nitrification. Consequently, petrochemical CSTR are bound to suffer from an excess of different types of filamentous bacteria, which difficult sludge settling.

In the previous chapters, the implementation of aerobic and anoxic selectors succeeded to overcome a low F/M filamentous bulking in a pilot CSTR treating petrochemical wastewater (Cardete *et al*., 2017a, b). The assays conducted demonstrated that the aerobic selector was effective to enhance sludge settling, provided it was operated at the optimum parameters (Cardete *et al*., 2017b). Upon the research presented, DO, F/M, nutrients and HRT in the selector are key elements to guarantee its efficiency to improve sludge settling (Cardete *et al*., 2017b). The results assessed the mechanism of action in the selector: The aerobic selector works as a small aerobic pre-reactor with recommended HRT of 30 minutes (Cardete *et al*., 2017b), where a high F/M of 30 to 35 g COD $g^{-1}$ VSS $d^{-1}$ is forced (Cardete *et al*., 2017b) to enable the selection of flocculating bacteria in front of filaments through a kinetic selection mechanism (Cardete *et al*., 2017a).

As expressed by the results in chapters 6 and 7, the biomass is formed in the selector according to its conditions and then finishes growth in the main reactor (Cardete *et al*., 2017a, b). From this statement, when implementing a selector the question arises, whether the reactor parameters still determine sludge settling or otherwise, the selector's variables referred in chapter 7 (Cardete *et al*., 2017b) have become the only critical ones in the activated sludge system.

Therefore, to complement the previous research, the objective of the present chapter is to explore the potential of the aerobic selector to deal with other adverse conditions in the reactor, such as large SRT, low DO and/or low ammonia concentration.

Additionally, the bibliography reports other parameters related to the mixed liquor quality in a CSTR with an effect on sludge settling. Hence, according to the physical principles of particle settling, the mixed liquor suspended solid concentration (MLSS) is a key factor to determine sludge gravity settling (Tuntoolavest and Grady, 1983; Chapman, 1983; Luque, 2005). Moreover, wastewater characteristics, such as pH (Ghanizadeh and Sarrafpour, 2001), temperature (T) (Cetin and Sürükü, 1989; Ghanizadeh and Sarrafpour, 2001; Winkler *et al*., 2012; Yahya *et al*., 2012) and conductivity (Bayo *et al*., 2006; Winkler *et al*., 2012; Yahya *et al*., 2012) can not only enhance the biomass ability for bio-flocculation, but also determine water properties with an effect on sludge settling, as density and viscosity.

The pH is said to affect the enzymatic activity of bacteria. An increase of pH above the isoelectric point makes the polymeric chain to be longer, and hence, contributes to form bridges between bacterial cells, improving biological flocculation (Ghanizadeh and Sarrafpour, 2001).

Temperature is reported to cause changes in the structure of extracellular polymer and the bacterial cell wall, which in turn, affect the surface charge of bacteria. Also, at high temperature, the viscosity of extracellular polymer decreases, which results in a reduction of bio-flocculation (Ghanizadeh and Sarrafpour, 2001). On the other hand, when increasing temperature, the viscosity of water decreases, which helps sludge settling (Winkler *et al*., 2012).

The effect of the conductivity on sludge settling is explained through an increase in the density of water because of salt solution as ions. Since the density of sludge is only slightly different to that of water, changes in the density of water can have a significant impact on sludge settling (Winkler *et al*., 2012).

Eventually, sludge settling is as well determined by the ability of bacteria to induce a stable floc formation through their surface charge, caused by the ionization of carboxyl and amino groups (Zita and Hermansson, 1994). In this sense, the addition of coagulants is a widely recognized solution to improve sludge settling (Al-Jasser, 2009; Lema and Suarez, 2017).

Furthermore, when implementing a selector, it should be elucidated if the better quality of the biomass produced can make all these mixed liquor parameters not to be significant for sludge settling.

To conclude, if the operational parameters of the selector were the only significant ones in the activated sludge system to enhance sludge settling, a wider operational range could be afforded for the variables in the reactor. Consequently, sludge settling issues due to large SRT and poor control of DO and ammonia residual could be solved. Also, the possibility to adjust pH and temperature in the main reactor to avoid calcium carbonate scaling on piping and dynamic equipment would improve significantly the petrochemical wastewater treatment, provided these variables do not affect sludge settling.

Therefore, to complement the previous research about sludge settling enhancement in petrochemical activated sludge processes, the pilot system, with and without the aerobic selector, has been experimentally tested with the objective of determining the effect of the main aerobic reactor's DO, F/M, $NH_4^+$-N and SRT on sludge settling ability. As in the previous assays, typical petrochemical wastewater streams have been continuously fed into the system. The study has been completed with additional bench-scale tests to assess the effect of MLSS and mixed liquor's pH, temperature, conductivity and particle charge on sludge settling. The different possible combinations of the parameters have suggested a statistical approach through an experimental design arrangement. The findings from this research provide operational guidelines for an activated sludge system including a selector, in order to enhance sludge settling. The results could be extrapolated to other wastewater treatment plants with a similar process scheme.

## 8.2. METHODOLOGY

### 8.2.1. Pilot-scale tests

The pilot plant and petrochemical feed streams presented in chapter 5 were used to carry out this experimentation.

For the tests performed with the CSTR, variations were introduced in DO, F/M, $NH_4^+$-N and SRT. To attain the desired DO concentration in the CSTR, the air flow rate supplied was manually adjusted. To set the objective F/M to the CSTR, variable flow rates of the three feed streams could be supplied, keeping always the same volumetric proportion

(1:1:1.3 for A: B : C, respectively). Ammonia supplement to the CSTR with the sulphate ammonia solution 20%w was calculated to obtain the desired ammonia concentration. Eventually, the SRT was regulated by adjusting the sludge waste from the bottom of the clarifier.

When the aerobic selector was included in the pilot plant configuration, variation was also introduced in the main reactor's DO, F/M, $NH_4^+$-N and SRT. While changing the reactor's conditions, the selector's parameters were always kept at its optimum values in order to enhance sludge settling, according to the previous chapter: DO was always over 2 mg $L^{-1}$, the F/M ratio was established at 30 to 35 g COD $g^{-1}$ VSS $d^{-1}$ and the HRT of the selector was 30 min (Cardete *et al,.* 2017b).

Also, the most biodegradable feed streams (A, B) were fed to the selector. Additionally, nutrients were supplied with stream B, which could be supplemented with sulphate ammonia 20%w.

To adjust DO in the reactor, the air inflow to the pilot plant could be manually regulated through a throttle valve. The F/M ratio selected for the reactor was fixed by regulating the flow rate of streams A, B and C, keeping always the same volumetric proportion. The volume of the selector had to be adjusted to A and B flow rates, to keep the HRT, so that the COD equilibrium concentration in the selector was constant. Consequently, the COD mass flow rate into the reactor changed with the supply of stream C and with the volumetric flow rate of the selector's effluent. To control the $NH_4^+$-N concentration in the reactor, the addition of the sulphate ammonia solution 20%w to the reactor was adjusted. Eventually, the sludge wasted from the bottom of the clarifier was calculated to get the desired SRT.

### 8.2.2. Bench-scale tests

Laboratory tests, performed with mixed liquor from the pilot plant including a selector, were performed in order to assess the effect of MLSS, pH, temperature, conductivity and particle charge on sludge settling. When the samples were taken, the activated sludge system was fed with streams A and B to the selector, and stream C to the reactor. The same volumetric proportion as in the CSTR was used for the feed mix. As a result, the selector was operated at its optimum parameters to enhance sludge settling upon the previous chapter (Cardete *et al.,* 2017b), and the reactor was operated at F/M of 0.3 g

COD $g^{-1}$ VSS $d^{-1}$. DO and ammonia concentration over 2 mg $L^{-1}$ were registered in the CSTR, as well as a SRT of 17 days. The pH was measured at 8.0.

To test the effect of the MLSS concentration, 16.0 g $L^{-1}$ mixed liquor from the pilot reactor was diluted with clarified water to different suspended solid concentrations, ranging from 16.0 to 3.2 g $L^{-1}$. The initial settling velocity was evaluated for each dilution in a V-cone, by accounting the reduction in the volume of sludge accomplished in the first five minutes of settling.

The effect of pH, temperature, conductivity and particle charge on sludge settling was also assessed by splitting a sample of mixed liquor from the reactor into smaller fractions so that pH, temperature and conductivity could be adjusted to the desired values.

The initial pH of the mixed liquor was 8.0 and soda 20% was added to attain higher values of 8.5 and 9.0. The conductivity was adjusted to the desired range by addition of chloride sodium salt. The initial value was around 20.0 mS $cm^{-1}$ and salt was added, until values of 40.0 mS $cm^{-1}$ and 60.0 mS $cm^{-1}$ were obtained. Whereas the addition of divalent cations, such as calcium and magnesium, are said to strengthen the floc and improve settling, the dosing of a monovalent cation, such as sodium, has been reported to deteriorate settling, when the monovalent to divalent cation ratio exceeds approximately 2 to 1, expressed on an equivalent basis (Higgins and Novak, 1997; Novak *et al*., 1998). Also, Tandoi *et al*. (2006) findings reported that an overabundance of monovalent cations causes the flocs to lose their resistance to shear, resulting in dispersed particles, which are difficult to settle. Because of these statements, it was ensured that the quantity of sodium added in the pH and conductivity adjustment did not change significantly the ratio monovalent-to-divalent cation in the mixed liquor.

Taking to the mixed liquor's temperature control, a thermostatic bath was regulated at values of 28, 30 and 38 ºC. The selected ranges for pH, temperature and conductivity correspond to usual values accomplished by large petrochemical bioreactors in the southern Mediterranean coast, considering capacity, seasonal variability and cooling water supply. Eventually, the charge of the colloidal particles was determined in each of the previous mixed liquor samples, completing them with the addition of 50 mg $L^{-1}$ of an organic coagulant (N-8130). Practically all substances dissolved colloidally and solid particles in water carry electric charges. This leads to a concentration of oppositely charged ions (counter-ions) on the colloids' surfaces, which stabilizes them in solution. In order to characterize the particle charge, a streaming current can be measured in mV,

if these counter-ions are separated from the dissociated macromolecule or particle. If positive (cationic) or negative (anionic) streaming current is denoted, the system may need charge compensation to neutralize the particle charge and allow the particle's flocculation and settling.

### 8.2.3. Statistical methodology

The effect of the main reactor's variables, such as DO, F/M, $NH_4^+$-N, SRT, pH, temperature, conductivity and MLSS on sludge settling was assessed by evaluation of the SVI. In order to identify cross-effects between the variables, two-level factorial designs were applied to program the pilot tests. The organization of the tests and the treatment of the results followed the procedures shown in Box *et al.* (2008). This methodology allowed to determine the impact of the main parameters (DO, F/M, $NH_4^+$-N, SRT, pH, temperature, conductivity and MLSS) and their interactions on the analyzed variable (SVI). Additionally, the significance of each effect could be determined with an ANOVA variance analysis. As a support software, *Statgraphics Plus for Windows 3.3 (1994-1998)* was also used.

## 8.3. RESULTS AND DISCUSSION

### 8.3.1. Effect of DO, F/M, $NH_4^+$-N and SRT on sludge settling for a CSTR

A total of sixteen operational periods, based on an experimental design arrangement with the parameters DO, F/M, $NH_4^+$-N and SRT, were set in the pilot CSTR, in order to assess the influence on sludge settling (see the experimental design matrix with codified variables in Table 8.1). The variables were adjusted to the desired values with the methodology described in section 8.2.

Adjusting one parameter caused unavoidable changes in other variables. Hence, whereas pH and temperature were controlled at fixed values in the pilot CSTR, conductivity was a consequence of the air and feed streams supplied. To reduce DO in the CSTR, the air flow rate was limited. Consequently, less inorganic carbon was introduced in the system, so that the conductivity decreased from 20.0 to 18.5 mS $cm^{-1}$. Also, less air stripping happened, so that the feed degraded may have been higher. As the feed mix of streams A, B and C always kept the same proportion, when increasing the F/M, the flow rate of the three streams increased. Consequently, a higher

conductivity was registered in the mixed liquor, due to a higher supply of streams A and B. Conditions with high air supply and large SRT caused undesired nitrification issues, which hindered the control of the ammonia residual. Eventually, the operation at low F/M and low SRT presented difficulties in keeping constant the biomass concentration, which obliged to adjust the biomass recycle to keep constant the F/M ratio.

Table 8.1 Experimental design arrangement to test the effect of the aerobic reactor's DO, F/M, $NH_4^+$-N and SRT on diluted sludge volumetric index, for an activated sludge system without selector.

| TEST | **DO** | **F/M** | **$NH_4^+$-N** | **SRT** | **DSVI** |
|---|---|---|---|---|---|
| | mg $L^{-1}$ | g COD $g^{-1}$ VSS $d^{-1}$ | mg $L^{-1}$ | days | mL $g^{-1}$ |
| | | *Codified effect units* | | | |
| 1 | - | - | - | - | 223 |
| 2 | - | - | - | + | 243 |
| 3 | - | - | + | - | 218 |
| 4 | - | - | + | + | 234 |
| 5 | - | + | - | - | 230 |
| 6 | - | + | - | + | 237 |
| 7 | - | + | + | - | 228 |
| 8 | - | + | + | + | 210 |
| 9 | + | - | - | - | 202 |
| 10 | + | - | - | + | 193 |
| 11 | + | - | + | - | 192 |
| 12 | + | - | + | + | 201 |
| 13 | + | + | - | - | 135 |
| 14 | + | + | - | + | 187 |
| 15 | + | + | + | - | 95 |
| 16 | + | + | + | + | 166 |
| | DO | F/M | $NH_4^+$-N | SRT | |
| | mg $L^{-1}$ | g COD $g^{-1}$ VSS $d^{-1}$ | mg $L^{-1}$ | days | |
| | - + | - + | - + | - + | |
| | <1 2-3 | 0.2 0.5 | <1 >2 | <19 >22 | |

To focus the study on sludge settling, the main effect of each parameter on DSVI was calculated. The statistical analysis showed a negative effect of the variables DO (-56.5 ± 10.6), F/M (-27.25 ± 10.6) and $NH_4^+$-N (-13.25 ± 10.6), so that DSVI decreased and consequently, sludge settling improved, when these variables increased. On the contrary, the parameter SRT reported a positive effect (18.5 ± 10.6) in the range tested, so that DSVI increased when SRT increased, as stated by Moreno (2004), who found long sludge ages to be susceptible of creating pin point floc and of suffering from

filamentous bulking. The variable DO showed the greater absolute effect value, followed by the F/M. Also, the impact of the two-variable interaction on SVI was calculated. Every double interaction showed a negative effect on DSVI, except for the combinations where the SRT took part, that provided a positive effect.

To complete the analysis, the impact of the variables on SVI was tested for significance with an ANOVA table analysis. The results showed that the only significant variable was DO concentration (p-value < 0.05).

However, when the complete statistical analysis was repeated, considering only the eight tests that had been performed at the high range of DO concentration, the effect of the reactor's F/M, $NH_4^+$-N, SRT and their dual interactions presented a significant effect on sludge settling (p-value < 0.05) (see Table 8.2) .

Table 8.2 Pilot plant tests of an activated sludge system without selector, with the aerobic reactor's DO > 2 mg $L^{-1}$. Effect of the aerobic reactor's F/M, $NH_4^+$-N and SRT on diluted sludge volumetric index (DSVI) and analysis of variance with ANOVA table.

| Effect | Estimated effect ± typical deviation |
|---|---|
| Average | 171.375 ± 0.125 |
| F/M | -51.25 ±- 0.25 |
| $NH_4^+$-N | -15.75 ± 0.25 |
| SRT | 30.75 ± 0.25 |
| F/M x $NH_4^+$-N | -14.75 ± 0.25 |
| F/M x SRT | 30.75 ± 0.25 |
| $NH_4^+$-N x SRT | 9.25 ± 0.25 |

| Source | Sum of squares | DF | Mean Square | F-ratio | P-Value |
|---|---|---|---|---|---|
| F/M | 5253.13 | 1 | 5253.13 | 42025.00 | 0.0031 |
| $NH_4^+$-N | 496.125 | 1 | 496.125 | 3969.00 | 0.0101 |
| SRT | 1891.13 | 1 | 1891.13 | 15129.00 | 0.0052 |
| F/M x $NH_4^+$-N | 435.125 | 1 | 435.125 | 3481.00 | 0.0108 |
| F/M x SRT | 1891.13 | 1 | 1891.13 | 15129.00 | 0.0052 |
| $NH_4^+$-N x SRT | 171.125 | 1 | 171.125 | 1369.00 | 0.0172 |
| Total error | 0.125 | 1 | 0.125 | | |
| Total (corr.) | 10137.9 | 7 | | | |

This result agrees with Adonadaga (2015) findings, which stated that DO is not the only plant operating condition to affect sludge settling. The significance of the dual interaction effects, as presented in Table 8.2, states important information on the basic behaviour of the biological system: the ammonia requirements are dependent on the operational F/M ratio and SRT, whereas F/M ratio is related to SRT. Because of the methodology followed to increase F/M, supplying more COD resulted also in providing significantly more $BOD_5$. This could explain a higher growth rate (Cardete *et al.*, 2017a), linked to shorter sludge ages and higher $NH_4^+$-N requirements.

Complementarily, microscopic observations were used to characterize the effect of each variable on the sludge appearance. Microscopic examination, based on floc appearance and biological indicators, turned out to be sensitive to changes in conditions, as well as DSVI, according to the bibliography (Jenkins *et al.*, 2003). The observations support Amanatidou *et al.* (2015) statement that filamentous bacteria proliferate under low DO, high SRT, low F/M, and low nutrient conditions. Also, as Gabb *et al.* (1991) stated, the DSVI results and the microscopic observations showed that low F/M conditions are linked to long sludge ages, where filamentous bacteria proliferate.

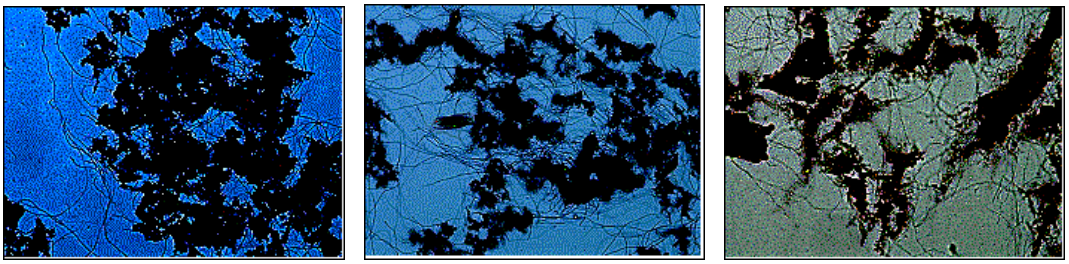
Figure 8.1a shows a typical floc structure for the CSTR working at the conditions that provided the lowest DSVI (high DO, high F/M, high nutrient concentration and low SRT). The general appearance of the sludge produced without a selector was dominated by an excess of filamentous bacteria and an opened floc configuration.

When working with low concentrations of the required resources, such as DO, carbonaceous matter (F/M) and ammonia nitrogen ($NH_4^+$-N), the floc became less compact, more opened and unbundled, in order to make it easier for the biomass to get the scarce resource. Also, in these circumstances, bacterial filamentous shape became more competitive than flocculent, probably due to the higher surface area to volume ratio (Young, 2006). As an example, Figure 8.1b shows how floc disintegration and filamentous presence has been enhanced by low DO conditions. These observations agree with Scruggs and Randall (1998), who experienced at laboratory and full-scale and concluded that DO and F/M ratios were key factors affecting filamentous growth in the activated sludge.

Wilén and Balmér (1999) stated that lower DO resulted in poorer sludge settling and higher effluent turbidity, pointing to filamentous bacteria proliferation as the main reason.

Adonadaga (2015), as well, verified the effect of mixed liquor DO concentrations in wastewater treatment plants dealing with municipal and industrial wastewater and found that the size of the flocs were greater when increasing DO concentration, in agreement with the results obtained.

Also, increasing the loading was said to increase the size of the floc (Barbusinski and Koscielniak, 1995). According to Wilén (2010) when lowering DO concentration, poorer effluent quality and poorer sludge properties were obtained. To explain these facts, Starkey and Karr (1984) had postulated inhibition of exopolymer production and inhibition of eukaryotic population (they keep the effluent clear by consuming free bacteria) as two possible mechanisms related to DO scarcity.



(a) (b) (c)

Figure 8.1 Sludge floc appearance at 100X for a pilot CSTR operated with typical petrochemical wastewater. The reactor conditions were (a) high DO, F/M and $NH_4^+$-N (b) low DO (c) low $NH_4^+$-N

Figure 8.1c presents a viscous, sticky sludge probably caused by an excess of polysaccharide, due to low ammonia concentrations (Peng *et al.,* 2003). Poor settling was obtained in these conditions. Taking to biological indicators, stable sludge was characterized by the presence of sessile ciliates, such as Vorticella. Also, flagellates have appeared to be the most resistant microorganisms to adverse situations, such as anoxic conditions. On the other hand, long-aged sludge showed borrowing ciliates.

The statistical analysis and the microscopic observations have revealed the effect of the mixed liquor parameters on sludge settling. The findings reported allow to establish operational rules to enhance sludge settling in a petrochemical CSTR, although the results show that only limited improvement can be accomplished without the implementation of a selector.

### 8.3.2. Effect of the reactor's DO, F/M, $NH_4^+$-N and SRT on sludge settling for an aerobic selector system

Sixteen operational periods based on an experimental design arrangement with the main reactor's DO, F/M, $NH_4^+$-N and SRT as studied variables were set in the pilot activated sludge system including an aerobic selector, in order to assess the effect on SVI (see the experimental design matrix with codified variables in Table 8.3). The methodology to vary the reactor's parameters is detailed in section 8.2, as well as the aerobic selector variables, which were always set at its optimum to enhance sludge settling.

As reflected in the previous chapters, the inclusion of a selector enhanced nitrification (Cardete *et al*., 2017 a, b), which hindered the control of DO and $NH_4^+$-N concentration in the CSTR.

From the SVI results obtained, the reactor DO concentration appeared to be the most important variable to determine sludge settling: high DSVI (285 to 135 $mL^{-1}$ g) results were obtained for low DO condition in the biological reactor (< 1 mg $L^{-1}$), while low SVI values (12 to 54 $mL^{-1}$ g) were found for the highest DO concentration tested (2 to 3 mg $L^{-1}$). These figures remind of Prendl and Kroiss (1998), who stated the importance of DO in the reactor, for the selector's effectiveness to enhance sludge settling.

To verify statistically this observation, the estimated effect of each variable on SVI was calculated. The results repeated the same tendencies obtained for the study without selector. Therefore, DO (-174.0 ± 19.5), F/M (-27.5 ± 19.5) and $NH_4^+$-N (-24.0 ± 19.5) presented a negative effect on SVI, whereas SRT (16.0 ± 19.5) showed a positive effect.

As well, the ANOVA table indicated that with the inclusion of a selector in the activated sludge system, the only significant effect was DO (p-value<0.05). Following the same methodology as in the study with the CSTR, the analysis was repeated considering only the tests performed with a high oxygen level. In this case, the main effect calculation and the ANOVA table (Table 8.4) confirmed that $NH_4^+$-N, F/M , SRT in the reactor and their dual interactions had no longer a significant effect (p-value>0.05) on SVI, when including a selector.

Due to the strategy followed to vary F/M in the reactor, a significant change in the COD feed resulted only in a small variation in the $BOD_5$ supply to the reactor. This is explained because the selector biodegrades almost completely the $BOD_5$ received, so that its effluent presents very low biodegradability ($BOD_5$/COD ratio lower than 0.1)

(Cardete *et al*., 2017 b). Consequently, the small $BOD_5$ feed change could be a reason for F/M and SRT in the reactor not to have a significant effect on sludge settling. Also, less biomass growth is expected to happen (Cardete *et al*., 2017 a, b), which could explain the non-significant effect of $NH_4^+$-N concentration.

Table 8.3 Experimental design arrangement to test the effect of the aerobic reactor's DO, F/M, $NH_4^+$-N and SRT on diluted sludge volumetric index, for an activated sludge system including an aerobic selector.

| TEST | **DO** | **F/M** | **$NH_4^+$-N** | **SRT** | **SVI** |
|---|---|---|---|---|---|
| | mg $L^{-1}$ | g COD $g^{-1}$ VSS $d^{-1}$ | mg $L^{-1}$ | days | mL $g^{-1}$ |
| | | *Codified effect units* | | | |
| 1 | - | - | - | - | 285 |
| 2 | - | - | - | + | 184 |
| 3 | - | - | + | - | 192 |
| 4 | - | - | + | + | 270 |
| 5 | - | + | - | - | 185 |
| 6 | - | + | - | + | 231 |
| 7 | - | + | + | - | 135 |
| 8 | - | + | + | + | 175 |
| 9 | + | - | - | - | 38 |
| 10 | + | - | - | + | 54 |
| 11 | + | - | + | - | 20 |
| 12 | + | - | + | + | 28 |
| 13 | + | + | - | - | 30 |
| 14 | + | + | - | + | 50 |
| 15 | + | + | + | - | 12 |
| 16 | + | + | + | + | 33 |

| | DO mg $L^{-1}$ | | F/M g COD $g^{-1}$ VSS.$d^{-1}$ | | $NH_4^+$-N mg $L^{-1}$ | | SRT days | |
|---|---|---|---|---|---|---|---|---|
| | - | + | - | + | - | + | - | + |
| | <1 | 2-3 | 0.1 | 0.5 | <1 | >2 | <16 | >26 |

This finding introduces additional knowledge about the effect of a selector on the activated sludge system performance, completing the results presented in the previous chapter (Cardete *et al.,* 2017b).

In such systems, the biomass is formed in the selector, whose parameters determine the sludge quality and settling ability (Cardete *et al.,* 2017 b). Therefore, introducing a

selector in the activated sludge system has been demonstrated as a tool to widen the operational interval for parameters in the reactor such as the F/M, $NH_4^+$-N and SRT, keeping still a good sludge settling. In this sense, including a selector could solve sludge settling issues in the petrochemical activated sludge system caused by low F/M, large SRT and poor control of ammonia concentration in the main reactor.

Consistently, based on the previous chapter (Cardete *et al.,* 2017b) research, when including a selector, the critical F/M, residence time and ammonia concentration are those of the selector, where the biomass is formed. On the other hand, DO in the reactor still keeps to have a significant effect on the biomass quality, even if an aerobic selector is included in the activated sludge system.

Table 8.4 Pilot plant tests of an activated sludge system including a selector, for the aerobic reactor's DO concentration > 2 mg $L^{-1}$. Effect of the aerobic reactor's F/M, $NH_4^+$-N and SRT on Sludge Volumetric Index and analysis of variance

| Effect | Estimated effect ± typical deviation |
|---|---|
| Average | 33.125 ± 1.125 |
| F/M | -3.75 ± 2.,25 |
| $NH_4^+$-N | -19.75 ± 2.25 |
| SRT | 16.25 ± 2.25 |
| F/M x $NH_4^+$-N | 2.25 ± 2.25 |
| F/M x SRT | 4.25 ± 2.25 |
| $NH_4^+$-N x SRT | -1.75 ± 2.25 |

| Source | Sum of squares | DF | Mean Square | F-ratio | P-Value |
|---|---|---|---|---|---|
| F/M | 28.125 | 1 | 28.125 | 2.78 | 0.3440 |
| $NH_4^+$-N | 780.125 | 1 | 780.125 | 77.05 | 0.0722 |
| SRT | 528.125 | 1 | 528.13 | 52.16 | 0.0876 |
| F/M x $NH_4^+$-N | 10.125 | 1 | 10.125 | 1.00 | 0.5000 |
| F/M x SRT | 36.125 | 1 | 36.125 | 3.57 | 0.3100 |
| $NH_4^+$-N x SRT | 6.125 | 1 | 6.125 | 0.60 | 0.5792 |
| Total error | 10.125 | 1 | 10.125 | | |
| Total (corr.) | 1398.88 | 7 | | | |

### 8.3.3. Comparison of the SVI in the CSTR and the aerobic selector system

Since the DO concentration in the main reactor always had a significant effect, the comparison of the SVI results obtained for the activated sludge system with and without a selector has been analyzed in two separate stages, for the periods performed at the low DO range (< 1 mg $L^{-1}$), and for the periods performed at high DO range (> 2 mg $L^{-1}$).

To determine whether the average SVI values obtained with and without a selector were significantly different, a comparison of the variances was performed with the *F-distribution test*, followed by a comparison of the average values with the *t-distribution test* (see Table 8.5).

When operating at low DO concentrations in the main reactor, the comparison with the Fisher test concludes no significant difference between the variances obtained for the results with and without a selector, for a 95% probability. As a consequence, it cannot be concluded that the SVI results obtained with and without a selector were different.

Table 8.5 Comparison of SVI results obtained for the tests without and with a selector, at the low DO range concentrations in the main aerobic reactor (DO< 1 mg $L^{-1}$) and at the high DO range concentrations in the main aerobic reactor (DO>2 mg $L^{-1}$)

| | Reactor's DO < 1 mg $L^{-1}$ | | Reactor's DO > 2 mg $L^{-1}$ | |
|---|---|---|---|---|
| | No Selector | Selector | No Selector | Selector |
| Average | 227.89 | 207.13 | 171.34 | 33.13 |
| Min | 210.10 | 135.00 | 95.40 | 12.00 |
| Max | 243.40 | 285.00 | 201.80 | 54.00 |
| Desvest (S) | 10.63 | 50.81 | 37.85 | 14.14 |
| Variance($S^2$) | 112.93 | 2582.13 | 1432.90 | 199.84 |
| Observations(n) | 8 | 8 | 8 | 8 |
| Av. Variance ($S^2$ $\dot{X}_1\dot{X}_2$) | 36.71 | | 28.57 | |
| $F_{calc.}$ (7, 7) | 0.32 | | 23.40 | |
| $F_{0,05}$ (7, 7) | >3.79 | | >3.79 | |
| Degrees of freedom (DF) | 14 | | 14 | |
| $T_{calc.}$ (14) | 1.13 | | 9.67 | |
| $t_{0.05}$ (14) | >1.761 | | >1.761 | |

The same comparison was analyzed with tests performed with a high DO level in the main aerobic reactor. The comparison of variances with the Fisher test and the comparison of averages with Student test show that the difference between the SVI results obtained with and without a selector are significant when operating the main reactor at high DO concentration (Prendl and Kroiss, 1998). This result complements the findings presented in the previous chapters (Cardete *et al.,* 2017 a b), by introducing DO in the main reactor, as a condition for the selector's effectiveness to enhance sludge settling. Hence, even if flocculating bacteria are formed in the selector by the kinetic selection mechanism, the results suggest that not providing enough DO in the reactor would cause filament's proliferation.

### 8.3.4. Effect of the mixed liquor suspended solid concentration on sludge settling

Sludge from the pilot petrochemical activated sludge system including an aerobic selector, produced with the conditions stated in section 8.2, was tested for settling at different MLSS concentration. The initial settling velocity was characterized for the different MLSS concentrations. The results presented in Figure 8.2 indicate that initial settling velocity decreases sharply when increasing MLSS concentration from 4 to 6 g $L^{-1}$. For values higher than 6 g $L^{-1}$ the initial settling velocity was low, with values around 0.1 m $h^{-1}$.

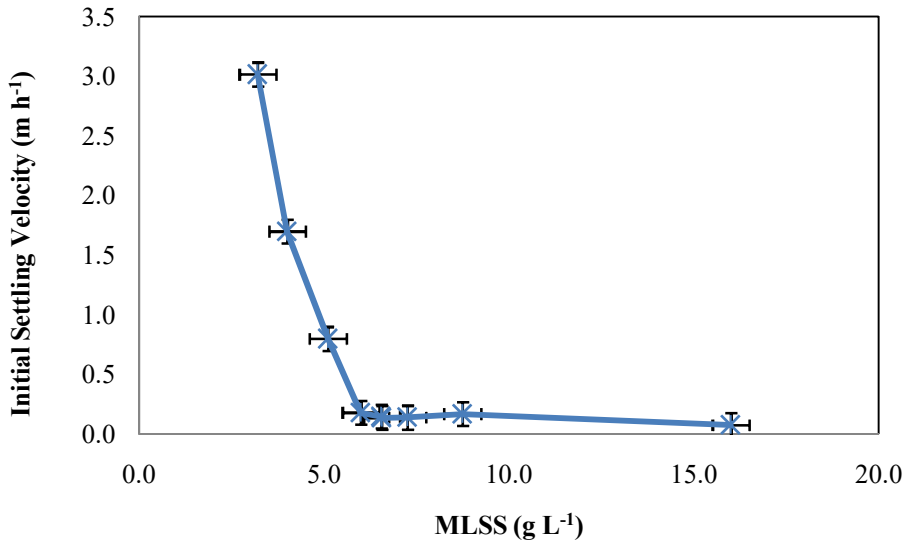



Figure 8.2 Effect of the MLSS concentration on initial settling velocity for sludge from a pilot petrochemical activated sludge system, including an aerobic selector. Error bars represent the standard deviation for each test.

As a consequence, the results show an effect of the MLSS concentration on sludge settling ability, even if a selector is included in the activated sludge system. Therefore, the better quality of the biomass obtained with the inclusion of a selector could not suppress the physical aspects related to gravity particle settling. This result supports previous research works performed on CSTR sludge, which also remarked the importance of MLSS concentration on sludge settling. As an example, Tuntoolavest and Grady (1983) identified the mixed liquor suspended solid concentration as the most important parameter to affect the clarifier's water quality among variables such as the solids retention time, sludge recycle rate or turbulence level in the aeration tank.

Chapman (1983) identified as well, the MLSS concentration as the most important variable within others, such as the settler feed flow rate and underflow rate, air flow rate in the biological reactor or depth of the inlet feedwell. As a consequence of this result, it becomes a key issue to optimize the MLSS concentration in the activated sludge system in order to enhance sludge settling. Therefore, finding the optimum F/M is not only necessary to guarantee the effectiveness of the selector to promote flocculating bacteria (Cardete *et al*., 2017b) but it is also a tool to increase sludge settling velocity.

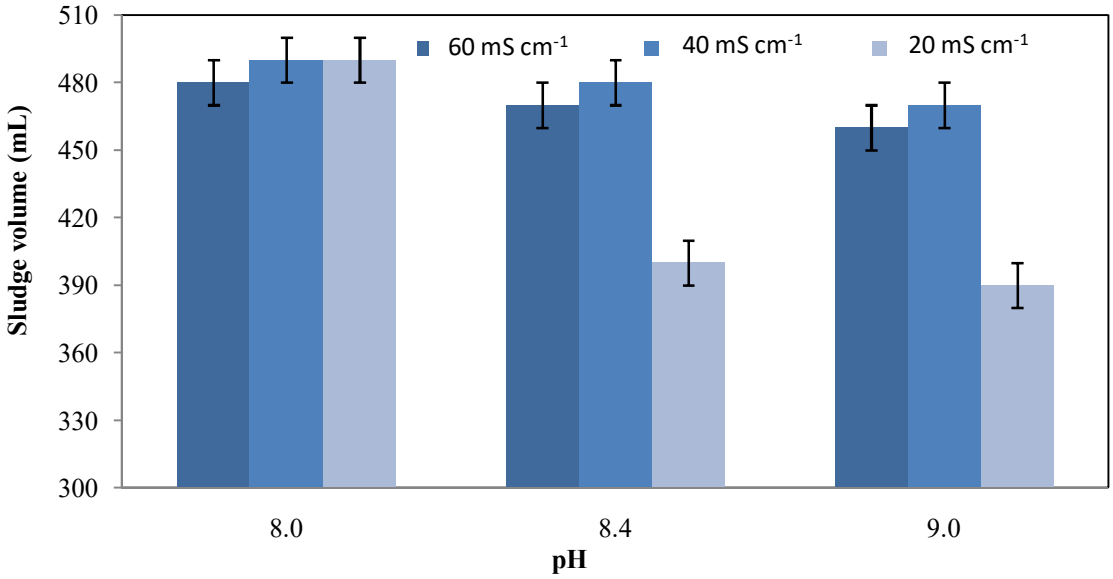
### 8.3.5. Effect of the mixed liquor pH, temperature and conductivity on sludge settling

The sludge settling ability of mixed liquor from the pilot petrochemical activated sludge system including an aerobic selector, produced as presented in section 8.2, was evaluated in batch tests at different pH, temperature and conductivity conditions. Sludge settling comparison was based on the sludge settled volume, as the suspended solid concentration was the same for all the tests.

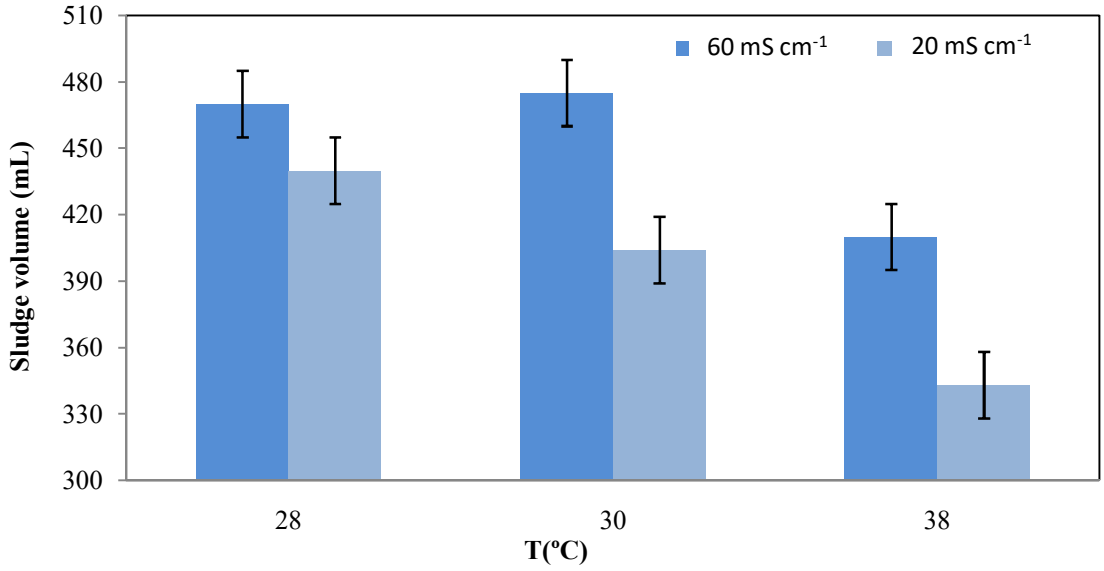
The pH, temperature and conductivity of the initial mixed liquor was adjusted in the laboratory to the different values at which settling was to be checked. The pH was tested within the range of 8.0 to 9.0, whereas the temperature range considered was from 28 to 38 ºC. The conductivity varied from 20.0 mS $cm^{-1}$ to 60.0 mS $cm^{-1}$.

Figure 8.3a reflects the sludge settled volume for each pH and conductivity value, at a temperature of 28 ºC. In agreement with Ghanizadeh and Sarrafpour (2001) theory about bioflocculation, for the three conductivity levels tested, the highest pH (9.0) was the one to obtain a better settling sludge (lower sludge volume) at short term. Also, when comparing the results for a different conductivity, it was the lower conductivity

(20.0 mS $cm^{-1}$) to obtain the lower volume of settled sludge, according to Winkler *et al.* (2012). Giokas *et al*. (2003) postulated that temperature has an influence on sludge settling. In this study, the effect of temperature at short term conditions is shown in Figure 8.3b, where the sludge settled volume is reduced as the temperature increases in the range studied, for the two levels of conductivity, 20 and 60 mS $cm^{-1}$.




(a)




(b)

Figure 8.3 Effect of the mixed liquor's (a) pH and conductivity at 28ºC (b) temperature and conductivity at a pH of 8.5 on sludge settling. Error bars represent the standard deviation for each test

An experimental design was applied to the sludge volume data available, in order to conclude whether the observed tendencies were significant (see Table 8.6).

In agreement with Ghanizadeh and Sarrafpour (2001), who reported temperature and pH as important factors affecting efficiency of flocculation and settling properties, the results obtained show that the three analyzed parameters (pH, T, conductivity) have a significant effect (p-value<0.05) on sludge settling, whereas their dual interactions show no statistically significant effect (see Table 8.7).

Temperature (-47.5 ± 2.0) and pH (-43.0 ± 2.0) reflect a negative effect, whereas conductivity (48.5 ± 2.0) shows a positive effect on sludge settling volume. This finding supports Gray (2004) statement, that low pH, together with other factors such as low DO and toxic shock loads, worsen settling by producing pin-point flocs.

Table 8.6 Experimental design matrix to test the effect of the mixed liquor's pH, temperature and conductivity on the sludge settled volume. The mixed liquor proceeded from a pilot activated sludge system with an aerobic selector.

| TEST | pH | T | Conductivity | V30 |
|---|---|---|---|---|
| | | ºC | mS $cm^{-1}$ | mL |
| | | *Codified effect units* | | |
| 1 | + | + | + | 410 |
| 2 | + | + | - | 343 |
| 3 | + | - | + | 475 |
| 4 | + | - | - | 404 |
| 5 | - | + | + | 450 |
| 6 | - | + | - | 420 |
| 7 | - | - | + | 480 |
| 8 | - | - | - | 454 |

| | pH | | T (ºC) | | Conductivity (mS $cm^{-1}$) | |
|---|---|---|---|---|---|---|
| | - | + | - | + | - | + |
| | 8.0 | 8.5 | 30 | 38 | 20.0 | 60.0 |

The results are also in agreement with Ghanizadeh and Sarrafpour (2001), who stated that an increase in pH (from 5.7 to 9) resulted in an improvement of SVI, and with Cetin and Sürükü (1989), who said that an increase of pH in the reactor caused the SVI to decrease.

References upon the temperature effect on SVI report alternate tendencies, probably due to its opposite effect on bioflocculation and the water properties (density and viscosity) (Winkler *et al*., 2012). For example, Ghanizadeh and Sarrafpour (2001) noted that an increase in temperature (from 15 to 35 ºC) resulted in SVI to increase, whereas Cetin and Sürükü (1989) observed that up to 25 ºC sludge showed low compressibility, and they became highly compressible by the increase in temperature after 25 ºC, in accordance with the results obtained. Other experimental works report a linear regression between SVI and temperature, and a cubic curve regression between SVI and pH (Yahya *et al*., 2012).

Taking to conductivity, the results obtained correspond with Bayo *et al.* (2006) findings, which indicated that the SVI increased with conductivity, probably due to a higher density of the mixed liquor (Winkler *et al*., 2012). Winkler *et al.* (2012) also did further work on conductivity, concluding that temperature and salt concentration had an effect on settling velocity in granular sludge. In agreement with the results obtained, they observed slower settling velocity when the temperature of water decreased from 40ºC to 5 ºC and when salt concentration increased.

Table 8.7 Analysis of variance for the sludge settled volume on a V30 test. Effect variables: mixed liquor's pH, temperature and conductivity. Mixed liquor from a petrochemical pilot activated sludge system, including an aerobic selector, was used.

| Source | Sum of squares | DF | Mean Square | F-ratio | P-Value |
|---|---|---|---|---|---|
| pH | 3698.0 | 1 | 3698.0 | 462.25 | 0.0296 |
| T | 4512.5 | 1 | 4512.5 | 564.06 | 0.0268 |
| Conductivity | 4704.5 | 1 | 4704.5 | 588.06 | 0.0268 |
| pH x T | 480.5 | 1 | 480.5 | 60.06 | 0.0817 |
| pH x Conductivity | 840.5 | 1 | 840.5 | 105.06 | 0.0619 |
| T x Conductivity | 0 | 1 | 0.0 | 0.00 | 1.0000 |
| Total error | 8.0 | 1 | 8.0 | | |
| Total (corr.) | 14244.0 | 7 | | | |

Consequently, despite the better quality of the biomass obtained with a selector, the reactor's pH, temperature and conductivity kept a significant effect on sludge settling.

The ability for bioflocculation, determined by pH and temperature, has been evidenced as a key factor to enhance sludge settling, regardless of the type of biomass. As well, the mixed liquor density and viscosity, determined by the temperature and conductivity,

have been shown to play a significant role on sludge settling, even with flocculating biomass. Therefore, even if a selector is included in the activated sludge system, pH, temperature and conductivity in the main reactor need to be adjusted to the optimum values, in order to enhance sludge settling.

Unfortunately, optimum sludge settling values within the range tested (pH of 8.5, temperature of 38 ºC) result in other operational issues in the petrochemical system, such as calcium carbonate scaling.

### 8.3.6. Effect of the particle's surface charge on sludge settling

The samples from the activated sludge system including a selector, which had been produced to assess the effect of pH, temperature and conductivity were completed with 50 mg $L^{-1}$ of an organic coagulant (N-8130) and were also tested for their particle's surface charge with a Mütek equipment.

At the pH range studied, the sludge flocs become negatively charged, so that the mixed liquor ions of the contrary charge (counter-ions) can attach to their surface, and stabilize them in solution. Consequently, a streaming current is established between the sludge flocs and the mixed liquor counter-ions when both are separated. The streaming current is indicative of the charge that needs compensation to allow the particle flocculation and settling.

A correlation was obtained between the sludge settled volume and the streaming current, which was characterized by the Mütek equipment.

As Figure 8.4 shows, for the system studied, there was an optimum particle charge, corresponding to a streaming current of -10 mV, where the volume occupied by the settled sludge was minimal. The results support bibliographic references (Garikipati, 2005), which state that SVI correlates with the surface charge of colloidal solids. Goodwin and Forster (1985) found a linear relationship between the SVI and the surface charge.

Consequently, the results suggest that the addition of coagulants to compensate particle's charge may be an alternative for systems including a selector in order to attain their optimum streaming current and enhance sludge settling.

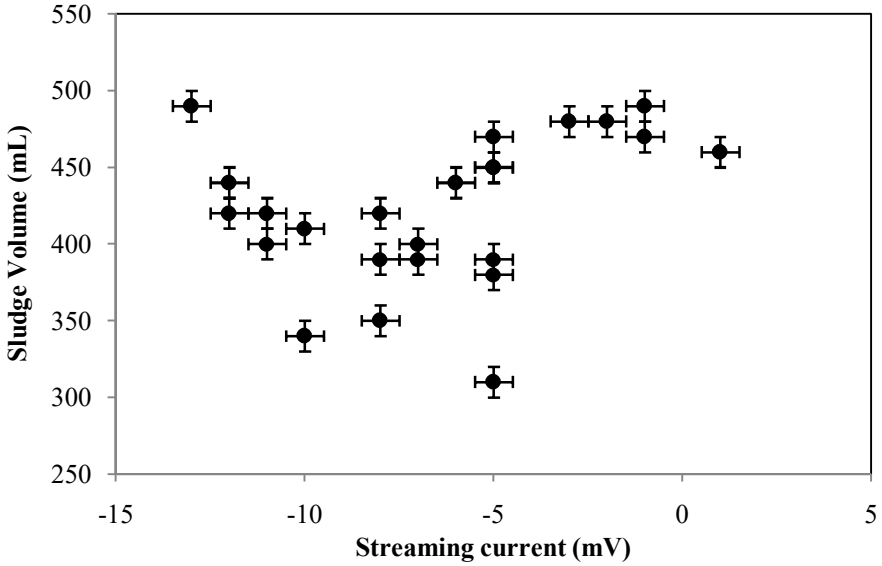



Figure 8.4 Effect of the particle's surface charge, measured through the streaming current with a Mütek equipment, in front of the sludge settling volume. Error bars represent the standard deviation between samples for both measurements.

## 4. CONCLUSIONS

This chapter provides operational guidelines and complements previous research about sludge setting enhancement by the implementation of selectors in petrochemical activated sludge systems. Whereas DO, $NH_4^+$-N, F/M and SRT affect significantly sludge settling in a petrochemical CSTR, when a selector is included, only DO values in the main reactor above 2 mg $L^{-1}$ need to be guaranteed. This result could be explained through the selector mechanism, which suggests almost complete degradation of the $BOD_5$ before entering the reactor. Hence, the critical parameters become those of the selector, where the biomass is formed. Therefore, the inclusion of a selector can provide a solution to typical sludge settling issues due to large SRT values and poor control of ammonia concentration in the reactor, caused by undesired nitrification. Despite the better quality of the biomass formed in the selector, bioflocculation determined by pH and temperature, played a key role on sludge settling. As well, the water density and viscosity, consequence of the temperature and conductivity, demonstrated to have still a significant effect on sludge settling. The addition of coagulants in the selector system resulted as a feasible alternative to enhance sludge settling, by providing the sludge with an optimum electrical particle charge.

# 9. Biological nitrification control by addition of folic acid

***ABSTRACT***

*The operational conditions of petrochemical, aerobic activated sludge systems focused on organic matter removal often lead to the unintended proliferation of nitrifying bacteria. Consequently, undesired denitrification occurs in the clarifier, hindering the accomplishment of each time more stringent emission levels. The addition of folic acid to limit nitrification is presented as an advantageous alternative, due to its low investment cost and easy implementation. To assess the effectiveness of folic acid for this purpose, cost-effective concentrations of 0.4 and 0.9 mg $g^{-1}$ VSS $d^{-1}$ have been supplemented to respective bench-scale, petrochemical bioreactors in comparison to a control. Eventually, the addition was interrupted to observe lasting effects. Based on Vibrio Fischeri assay, the folic acid concentrations tested did not add significant toxicity to the effluent. The supply of the lower vitamin concentration provided satisfactory results regarding to nitrification reduction (93.6%) and improvement of the sludge volumetric index compared to control (17.4 in front of 67.3 mL $g^{-1}$). However, its feasibility is conditioned to the availability of spare aeration capacity, since oxygen demand increased in 85.7%, probably due to an older sludge age (71.4% reduction in observed sludge yield). Reductions up to 97.1% in nitrification rates were obtained during and 60 days after the dosage of the higher vitamin concentration. Despite other advantages, such as increasing the organic matter removal efficiency (60.0%) and reducing oxygen demand (14.7%) relative to control, the high dosed reactor exhibited a worse sludge settling (93.1 mL $g^{-1}$) and more sludge production (57.1% increase in observed sludge yield).*

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*The most relevant parts of this chapter are published in:*

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# 9. Biological nitrification control by addition of folic acid

## 9.1. INTRODUCTION

Since petrochemical effluents exhibit a low biodegradability, the CSTR in such systems is often preceded by advanced oxidation processes (Derakhshan and Fazeli, 2018), which generate rich-carbonate feed effluents for the biological treatment. The presence of high inorganic carbon concentrations, together with large sludge retention times (SRT) and favourable conditions on temperature and pH, result in the proliferation of autotrophic nitrifying bacteria, which convert into nitrites and nitrates the ammonia nitrogen supplied as a nutrient to the heterotrophic system.

The introduction of a selector ahead of the CSTR was proposed in the previous chapters (Cardete *et al.*, 2017 a, b) as a solution to overcome the low F/M filamentous bulking. However, the results presented in chapters 6 and 7 indicated that the implementation of a selector enhances nitrification rates in the biological system (Cardete *et al.*, 2017 a, b). As drawbacks, nitrification increases oxygen demand and hinders sludge settling because of uncontrolled denitrification in the clarifier (Eckenfelder, 1998). Therefore, to improve sludge settling in petrochemical activated sludge systems, nitrification control must be approached complementarily to the inclusion of a selector.

Considering additional investment, a denitrification step could be included previous to the clarifier. However, this option may not be convenient because of the cost, site constraints and need to stop the process to implement modifications.

Otherwise, nitrification could be limited by several operational strategies, such as lowering the mixed liquor pH (EPA, 2002). However, this possibility presents two disadvantages: i) As concluded in chapter 8, sludge settling is enhanced in such systems at pH values between 8.0 and 8.5 (Cardete *et al*., 2018) and ii) The addition of large acid doses is required in such carbonate-buffered systems.

Another possibility is to operate the biological reactor at a shorter sludge retention time (SRT) (Flores-Alsina *et al*., 2010). However, insufficient bacterial growth and the requirement to degrade low biodegradable organic matter limit the minimum feasible sludge age in the biological system.

As an alternative, this chapter presents a novel strategy to limit biological nitrification in wastewater treatment through the control of ammonia oxidizing activity by folic acid (FA) addition.

Folic acid highlights among the different vitamin supplements because of its capacity to improve biological systems (Burgess *et al*., 2000) and its affordable cost (Barnett, 2013). The literature reports the use of folic acid in wastewater treatments to reduce sludge production (Stoppa *et al*., 2013; Alexandre *et al*., 2016). Nevertheless, its effect on the operational parameters of the biological system is not clear, since the bibliography concludes changing tendencies. Moreover, they have only been evaluated during folic acid dosage but not afterwards, to account for habituation. Folic acid is a water-soluble vitamin, known as Vitamin $B_9$ or $B_c$, which stands for N- [4-(2-amino-3,4-dihydro-4-oxo-6-pteridinylmethylamino)-benzoyl]-L-glutamic acid. This molecule is formed by a pteroic acid, which consists of two blocks (pteridine and p-aminobenzoic acid, known as PABA) and a L-glutamic acid. The nitrogen heterocyclic structure of folic acid reminds of nitrification inhibition studies in soils: Mc Carty and Bremmer (1989) postulated the efficiency of heterocyclic nitrogen compounds to inhibit nitrification. McCarty (1999) confirmed their inhibitory effect, related to the presence of the nitrogen ring. Later studies were conducted on particular compounds, such as dimethylpirazole phosphate (Quiñones *et al*., 2009) or dicyandiamide (Gong *et al*., 2013) to assess their efficiency as nitrification inhibitors. However, the mode of action of nitrogen heterocyclic compounds is not yet clearly known (Torralbo *et al*., 2017). Despite its structure, folic acid has neither been identified as a nitrification inhibitor. Therefore, the present research introduces a new functionality for folic acid.

To assess the effectiveness of folic acid to limit nitrification, bench-scale tests performed with wastewater and inoculum from the petrochemical industry were conducted in an activated sludge system. The research aims to conclude whether folic acid addition could be a feasible alternative for existing industrial processes focused on COD removal, in order to enhance sludge settling by preventing unintended nitrification. To serve this purpose, the effect of folic acid during and after dosage on the operational parameters of the biological system must be clarified. Additionally, this study reports the behaviour patterns of the ammonia and nitrite oxidizing bacteria (Nitrosomonas and Nitrobacter, respectively) during and after folic acid addition, which could serve as a background to develop a mechanism of action for nitrogen heterocyclic compounds.

## 9.2. METHODOLOGY

### 9.2.1. Experimental methodology

The effectiveness of folic acid to inhibit nitrification was tested in the bench-scale set-up described in chapter 5. Two bioreactors were run in parallel and supplied with folic acid concentrations of 0.4 and 0.9 mg $g^{-1}$ VSS $d^{-1}$, in comparison to a control. The three were fed with the same quality of industrial petrochemical substrate, whose characterization was described in chapter 5.

The experimental period was differentiated into two stages. In stage 1, to test the effect of folic acid, the control reactor was run without vitamin supply, while the other two bioreactors were provided with concentrations of 0.8 and 1.6 mg $L^{-1}$ of folic acid in the total feed. In stage 2, the folic acid dosage was stopped at the last two bioreactors, in order to assess lasting effects. The duration of each experiment, as minimum, doubled the calculated SRT, which was dependent on the biomass growth rate. Hence, in stage 1, the higher folic acid concentration was supplied to the bioreactor for 50 days, whereas the lower folic acid concentration test lasted 80 days. The control bioreactor was operated for 80 days to compare with the test bioreactors. After the folic addition was stopped (stage 2), the test bioreactors were operated during 103 and 41 days, respectively, until their performance parameters moved back to values similar to the control reactor.

Since the bench bioreactors were continuously supplied with feed mix, acid solution and carbonate buffered solution, their volume increased daily from 1.0 L to 1.5 L (at weekends, up to 2.5 L). To restore the initial volume, the excess of mixed liquor was daily extracted and centrifuged (3000 rpm, 10 minutes). The solids separated were returned back into the bench bioreactor and the clarified water was used for routine analysis to characterize the mixed liquor. Afterwards, an additional 20 mL sample was daily extracted from the bioreactors to determine total suspended solids and volatile suspended solids. This volume was accounted as sludge waste, which was daily calculated and performed in order to keep constant the biomass concentration in the bioreactors. This allowed to compare results between bioreactors at a constant F/M ratio, since it is a factor that affects nitrification potential, according to the results presented in chapter 6 (Cardete *et al.*, 2017a). When additional sludge waste was necessary, the calculated volume of mixed liquor was extracted from the bioreactor and centrifuged. The clean supernatant was returned back into the reactor and the sludge

was purged. As a result of this procedure, the allowable SRT was a consequence of the biomass growth yield.

### 9.2.2. Observed sludge yield and sludge retention time calculation

The observed sludge yield ($Y_{obs}$) quantifies the true growth yield minus the endogenous decay of biomass. The $Y_{obs}$ was determined for each experimental test by using a regression method between the masses of the daily VSS produced and organic matter removed (Velho *et al*., 2016).

The mass of VSS produced ($\Delta VSS_{produced}$) was calculated as expressed in equation 9.1.

$$\Delta VSS_{produced} = (X_{tf} - X_{to}) \, . \, V_{ML\,t} + V_{ML\,w} . X_{w} \quad (9.1)$$

Where X is the biomass concentration and $V_{ML}$ is the mixed liquor volume. The subscripts "t", "tf" and "to" indicate, respectively, daily, final and initial sampling time, whereas "w" stands for waste.

The mass of soluble COD removed ($\Delta sCOD_{removed}$) was calculated with equation 9.2.

$$\Delta sCOD_{removed} = (sCOD_{in} . V_{in} + sCOD_{ML\,to} . V_{MLto}) - (sCOD_{MLtf} . V_{MLtf}) \quad (9.2)$$

Where the subscripts "in" and "ML" represent, respectively, influent and mixed liquor.

The mean value of $Y_{obs}$ for each experimental test was calculated as the slope of the linear regression between both terms, as it is represented in equation 9.3.

$$Y_{obs} = \frac{\Delta VSS_{produced}}{\Delta sCOD_{removed}} \quad (9.3)$$

The COD removal efficiency ($RE_{COD}$) was calculated from equation 9.4.

$$RE_{COD} = \frac{\Delta sCOD_{removed}}{(sCOD_{in} . V_{in} + sCOD_{ML\,to} . V_{MLto})} . 100 \quad (9.4)$$

Sludge retention time (SRT) was calculated according to Kutz (2009) as the ratio between the mass of VSS in the reactor and the mass of VSS wasted daily from the

system. It was proven that the quantity of biomass lost with the supernatant water after centrifugation was not significant (data not shown). Therefore, the SRT was calculated as stated in equation 9.5.

$$SRT = \frac{X_t . V_{MLt}}{X_w . V_{MLw}} \quad (9.5)$$

## 9.3. RESULTS AND DISCUSSION

### 9.3.1. Short-term toxicity of folic acid on heterotrophic bacteria

To assess the short-term toxicity of folic acid on the inoculum treating petrochemical wastewater, SOUR was characterized by respirometry, while adding different concentrations of the vitamin (0.0, 0.5, 1.0, 2.0, 3.0 and 4.0 mg $L^{-1}$). The SOUR of the control bioreactor was characterized at 208 ± 66 mg $O_2$ $g^{-1}$ $h^{-1}$ and was statistically compared with the results for any of the folic acid concentrations tested. No significant differences were observed ($p>0.05$) by comparing the averages and standard deviations for a 95% confidence level. Therefore, folic acid fulfilled the condition not to inhibit the heterotrophic biodegradation.

### 9.3.2. Effect of folic acid addition to the bioreactors (Stage 1)

A control and two test bioreactors were run in parallel, in order to assess the effect of folic acid on nitrification. The test bioreactors were supplemented with vitamin concentrations of 0.8 and 1.6 mg $L^{-1}$ in the total feed supply. Apart from nitrite and nitrate biological production, the mixed liquor parameters considered to characterize the bioreactors performance were COD RE, SVI, $Y_{obs}$ and SOUR. Considering the research of Stoppa *et al.* (2013), the effect of folic acid on these performance parameters is dependent on the dose of vitamin supplied.

To allow the comparison of results, the three bioreactors were run under similar conditions (see Table 9.1). Since biological nitrification is affected by parameters such as F/M (Cardete *et al*., 2017a), pH, temperature, ammonia nitrogen residual, inorganic carbon concentration, oxygen availability and SRT (Metcalf and Eddy, 2003), the tests were conducted aiming to the same values for these variables in each bioreactor. According to this premise, the statistical evaluation of the operational parameters indicated that they registered non significant differences between the three bioreactors

($p>0.05$) but for ammonia nitrogen concentration and SRT. The sludge age was controlled by the volume of wasted sludge from the bioreactors, which in turn was determined to keep a constant biomass concentration in the mixed liquor. Consequently the three bioreactors were operated at constant and similar F/M ratio. As a difference to other studies (Stoppa *et al*., 2013; Alexandre *et al*., 2016; Velho *et al*., 2016), this procedure allowed to run the test bioreactors at a constant ratio folic acid-to-VSS (FA/VSS) of 0.44 ±0.09 and 0.9 ±0.3 mg FA $g^{-1}$ VSS $d^{-1}$. Both figures have been statistically confirmed as significantly different ($p<0.05$). Also, the same total feed flow rate (21 mL $h^{-1}$) was supplied to each one-litre bioreactor, so that the hydraulic retention time was calculated at 2 days for all of them.

Folic acid addition was effective to limit nitrite ($NO_2^-$) and nitrate ($NO_3^-$) concentration in the effluent for any of the two concentrations tested, as reflected in Figure 9.1. The control bioreactor showed steady nitrification rates, achieving average values of 51 ± 6 mg $NO_2^-$-N $L^{-1}$ and 233 ± 13 mg $NO_3^-$-N $L^{-1}$. Within days 30 to 60, nitrification increased up to average values of 68 ± 4 mg $NO_2^-$ -N $L^{-1}$ and 276 ± 3 mg $NO_3^-$ -N $L^{-1}$, probably due to a less biodegradable feed stream. Since heterotrophic cell growth is faster than nitrifying, nitrification rates increased with diminishing biodegradable organic loading (Celenza, 2000).

In the higher vitamin dosed bioreactor (0.9 mg FA $g^{-1}$ VSS $d^{-1}$), the nitrite concentration fell down to zero in three days and afterwards kept always at the null value, except for days 21 to 23, where concentrations up to 8 mg $NO_2^-$-N $L^{-1}$ were registered. Incidences in the supply of folic acid could explain this isolated peak of nitrites. The nitrate concentration decreased progressively and attained values of zero after fifteen days, regardless of the feed stream quality. On the contrary, the bioreactor complemented with the lower folic acid concentration (0.4 mg FA $g^{-1}$ VSS $d^{-1}$) kept the sensitivity to the feed stream biodegradability, obtaining a constant reduction in nitrification rates of 93.6 ± 4.1 % compared to control. Nitrites descended to values below 10 mg $NO_2^-$ -N $L^{-1}$ in two days, and registered an increase to average values of 30 ± 8 mg $NO_2^-$ -N $L^{-1}$ during days 30 to 60.

Table 9.1 Operational conditions of the three bioreactors at each stage (average values ± standard deviation)

| Parameters [1] | Units | Bioreactor | | | | | |
|---|---|---|---|---|---|---|---|
| | | Stage 1: Folic acid addition | | | Stage 2: After folic acid addition[3] | | |
| | | Control 1 | Test 1 A | Test 1 B | Control 2 | Test 2 A | Test 2 B |
| Time of operation | days | 80 | 80 | 50 | 103 | 41 | 103 |
| Folic Acid feed | mg $L^{-1}$ | - | 0.80 ± 0.05* | 1.60 ± 0.20* | - | - | - |
| VSS | g $L^{-1}$ | 1.2 ± 0.4 | 1.2 ± 0.2 | 1.2 ± 0.4 | 1.4 ± 0.4 | 0.8 ± 0.2 | 0.9 ± 0.2 |
| Folic Acid/VSS [2] | mg FA (g VSS d)$^{-1}$ | - | 0.44 ± 0.09 * | 0.9 ± 0.3 * | - | - | - |
| F/M | g COD (g VSS d)$^{-1}$ | 0.4 ± 0.2 | 0.6 ± 0.1 | 0.4 ± 0.1 | 0.5 ± 0.1 | 0.5 ± 0.1 | 0.5 ± 0.1 |
| pH | | 8.4 ± 0.3 | 8.2 ± 0.3 | 8.5 ± 0.3 | 8.2 ± 0.3 | 8.5 ± 0.3 | 8.5 ± 0.3 |
| Temperature | ºC | 28± 1 | 28± 1 | 28± 1 | 30 ± 1 | 30± 1 | 30± 1 |
| SRT | days | 24 ± 3 | 40 ± 5 * | 14 ± 8 * | 17 ± 6 | 16 ± 6 | 19 ± 6 |
| Ammonia nitrogen | mg $L^{-1}$ | 2 ± 1 | 18 ± 8* | 11 ± 5* | 2 ± 1 | 11 ± 5* | 14 ± 5* |
| Dissolved oxygen | mg $L^{-1}$ | 6.3 ± 0.1 | 6.3 ± 0.1 | 6.3 ± 0.1 | 6.3 ± 0.1 | 6.3 ± 0.1 | 6.3 ± 0.1 |
| TIC | mg $L^{-1}$ | 372 ± 289 | 337 ± 195 | 383 ± 290 | 518 ± 249 | 370 ± 172* | 401 ± 232 |

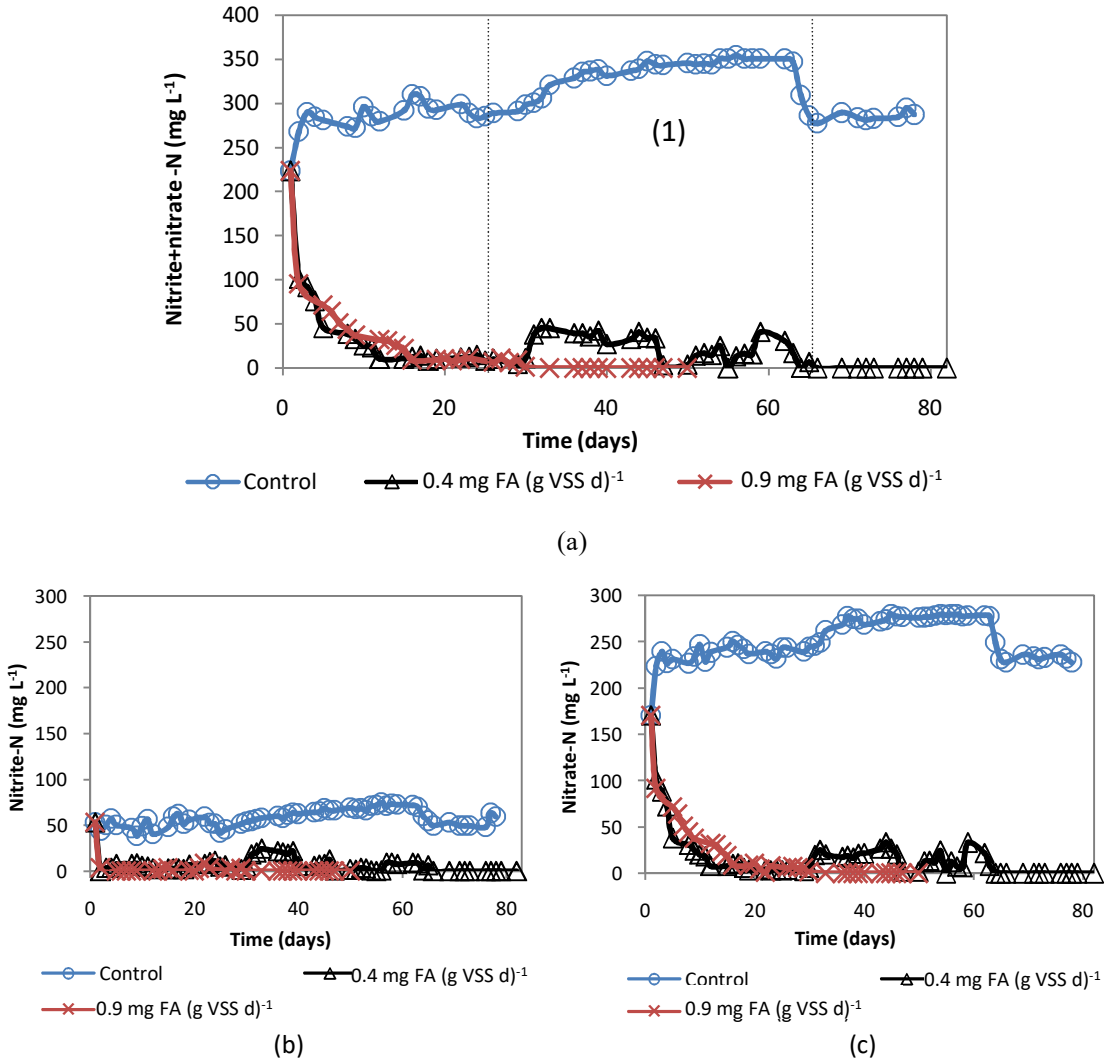
[1] Daily averaged values

[2] Daily Folic acid supplied-to-Volatile Suspended Solids in the mixed liquor

[3]Folic acid addition was interrupted the day before beginning this stage

* statistically significant difference from control ($p < 0.05$)

In turn, the reduction in the nitrate concentration was progressive to values below 15 mg $NO_3^-$-N $L^{-1}$ in the first twenty days, although the low biodegradability period exhibited values of 50 ± 8 mg $NO_3^-$-N $L^{-1}$.




(1) Period with a low biodegradability feed stream, within pointed lines.

Figure 9.1 Monitoring of (a) nitrogen as nitrite+nitrate (b) nitrogen as nitrite ($NO_2^-$-N) and (c) nitrogen as nitrate ($NO_3^-$-N) concentration in the effluent for the three bench bioreactors during folic acid (FA) addition (stage 1).

Therefore, despite providing different nitrification rates, both bioreactors supplied with folic acid experienced a statistically significant reduction in the concentrations of nitrites and nitrates. Also, both folic acid concentrations produced the same pattern of nitrification: an immediate, sharp reduction in nitrite concentration and a progressive

decrease of nitrates. These tendencies suggest that folic acid may be acting on ammonia oxidizing bacteria, limiting the nitrite production. However, since folic acid is an organic molecule, it is expected to be degraded by the heterotrophic bacteria in the system (Rappold and Bacher, 1974). HPLC was used to confirm the presence of folic acid and pteroic acid ($C_{14}H_{12}N_6O_3$), as a metabolite, in the mixed liquor. Apart from registering higher concentrations of these compounds in the mixed liquor, an additional factor which could explain a more effective nitrification reduction in the 0.9 mg FA $g^{-1}$ VSS $d^{-1}$ bioreactor is a lower SRT, as a consequence of higher growth yields induced by folic acid dosage.

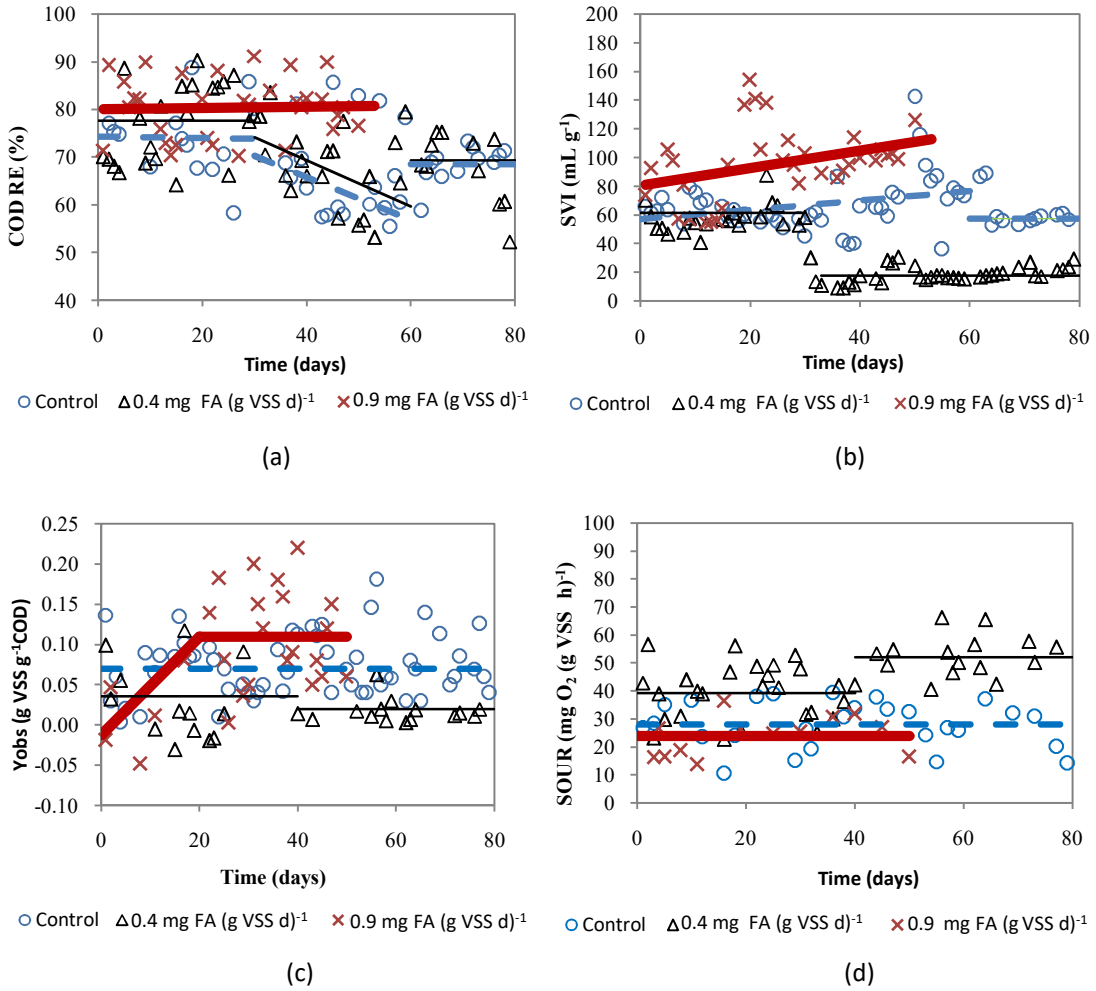
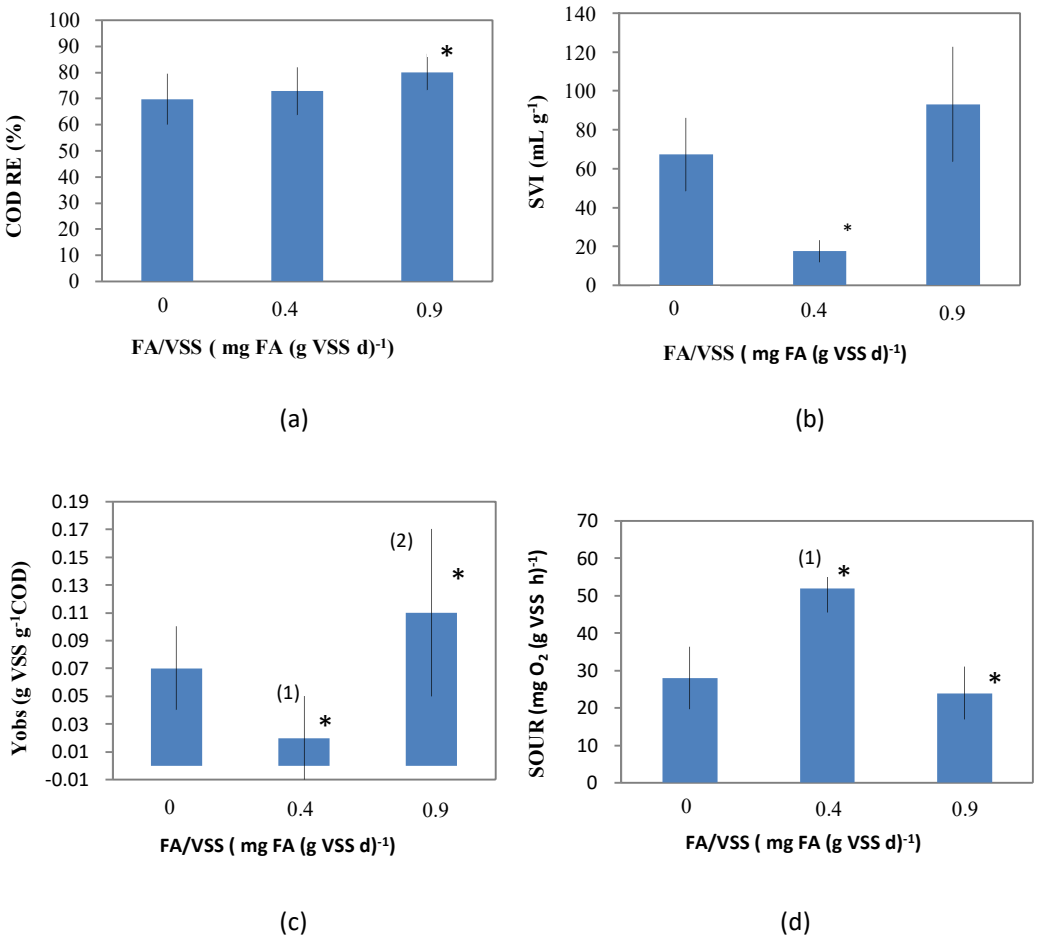



Figure 9.2 Evolution of the (a) organic matter removal efficiency (COD RE) (b) sludge volumetric index (SVI) (c) observed sludge yield ($Y_{obs}$) and (d) specific oxygen uptake rate (SOUR) during folic acid (FA) addition (stage 1). The lines indicate average values for the control ( _ _ ), low ( ____ ) and high folic acid concentration ( ▬ ).

The tendencies observed for COD RE (Figure 9.2 a) were also sensitive to the feed quality. The control bioreactor produced an average COD RE of 69.7 ± 9.7 %, except for the period within days 30 to 60, at which the COD RE showed a decreasing profile. Such low organic matter removal was probably due to the poor biodegradability of the feed stream, since part of it had already been biologically treated. Supplementing 0.4 mg FA $g^{-1}$ VSS $d^{-1}$ provided only a slightly higher COD RE of 72.7 ± 9.1 %, reproducing also the decreasing tendency during the low-biodegradable-feed period. The parallel bioreactor supplied with 0.9 mg FA $g^{-1}$ VSS $d^{-1}$ presented a constant, higher value for COD RE (80.0 ± 6.9 %), with statistical significance at 95 % confidence level, compared with the other two bioreactors (see Figure 9.3 a).

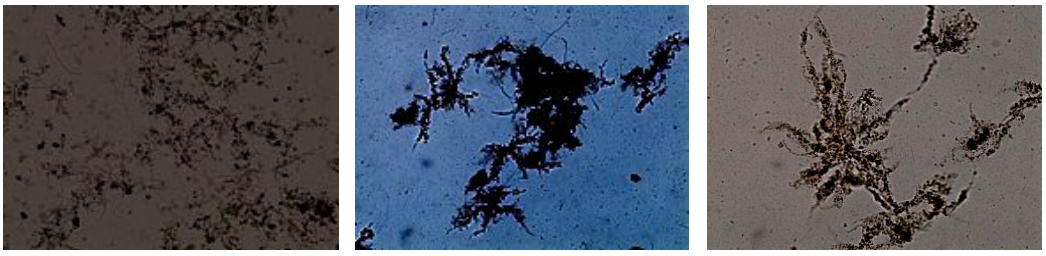



(1) After 40-day transition period
(2) After progressive 20-day acclimation

Figure 9.3 Average values of (a) COD removal efficiency (COD RE) (b) sludge volumetric index (SVI) (c) observed sludge yield ($Y_{obs}$) and (d) specific oxygen uptake rate (SOUR) during folic acid (FA) addition in the three bioreactors. Error bars represent the standard deviation. Asterisks over bars indicate significant difference from the control ($p<0.05$).

Therefore, according to Senorer and Barlas (2004), the addition of folic acid has increased the efficiency of the mixed liquor to degrade organic matter. As an explanation, Strunkheide (2004) postulated the functionality of folic acid to enhance the one-carbon metabolism (breakdown of formic acid with oxygen, into carbon dioxide and water), which is normally limited by the low concentrations of this vitamin in the nature, due to its limited stability in aqueous solutions.

In agreement with Stoppa *et al*. (2013) research, opposite effects were observed at sludge settling with both folic acid concentrations tested (see Figure 9.2 b). Whereas the higher folic acid concentration registered higher SVI values (93.02 ± 29.5 mL $g^{-1}$) compared to the control bioreactor (67.3 ± 18.8 mL $g^{-1}$), the lower folic acid dose caused an improvement in sludge settling (17.4 ± 5.5 mL $g^{-1}$), after an acclimation period. The differences between the three results were statistically significant with a 95 % confidence level (see Figure 9.3 b). To explore the reason for these tendencies, microscopic observations were performed periodically on the mixed liquor of the three bioreactors.



(a) (b) (c)

Figure 9.4 Microscopic observations (100X) of the mixed liquor in (a) the control (b) the 0.4 and (c) the 0.9 mg FA $g^{-1}$ VSS $d^{-1}$ bioreactors

Epiphytic growth appeared in the bioreactors supplied with folic acid (see Figure 9.4), which could indicate that the filaments were unhealthy or decaying (Wanner, 2014). Therefore, according to Dubé *et al*. (2002), folic acid could be considered as an alternative to chlorination for the filamentous bulking control. This fact could explain the lower SVI in the 0.4 mg FA $g^{-1}$ VSS $d^{-1}$ bioreactor. The poorer sludge settling in the higher dosed bioreactor could be understood through a jelly appearance of the sludge. To evaluate this observation, additional examinations on dark-field and phase-contrast microscopy assessed the morphology of the sludge flocs (Mesquita *et al*., 2013) and the proliferation of Zooglea Ramigera (Vázquez *et al*., 2010). While extracellular slime was

organized in fingered colonies in the 0.9 mg FA $g^{-1}$ VSS $d^{-1}$ bioreactor, in the other two tests, it was distributed all over the floc. Wanner (2014) identified toxicity and a high organic matter gradient as possible causes for the increase of viscosity in the floc. While toxicity was discarded by Vibrio Fischeri test in the assays performed, the increase in $RE_{COD}$ resulted in a higher COD gradient. The coincident, sustained increase in $RE_{COD}$ and SVI suggests that the jelly appearance of the floc could be related to a faster assimilation of the organic matter.

It was also evaluated the effect of folic acid addition on $Y_{obs}$, which was characterized at values of 0.07 ± 0.03 g VSS $g^{-1}$ COD for the control bioreactor. As reflected in Figure 9.3c , the tests performed indicated an increase in $Y_{obs}$ when the folic acid was supplied at a concentration of 0.9 mg FA $g^{-1}$ VSS $d^{-1}$ (0.11 ± 0.06 g VSS $g^{-1}$ COD), after a 20-day acclimation period. Otherwise, feeding 0.4 mg FA $g^{-1}$ VSS $d^{-1}$ slowed down the biomass growth to 0.02 ± 0.03 g VSS $g^{-1}$ COD, after a 40-day acclimation period. The statistical comparison of the three figures showed significant differences between the values obtained for a 95 % confidence level (see Figure 9.3 c). These results are in agreement with Stoppa *et al.* (2013) findings, working with kraft pulp mill effluents. They characterized $Y_{obs}$ for folic acid concentrations in the mixed liquor, ranging from 0.5 to 4 mg $L^{-1}$, concluding that supplying 0.5 mg $L^{-1}$ reduced $Y_{obs}$, whereas providing concentrations of 1 to 4 mg $L^{-1}$ increased progressively the growth rates, compared to a control reactor. Since tetrahydrofolic acid is an essential co-enzyme synthesized by microorganisms from folic acid (Senorer and Barlas, 2004), the changing tendency of the two concentrations tested, as well as the results obtained by Stoppa *et al.* (2013), could be explained in kinetic terms by Kompala's (2013) modified Monod rate equation, which considers the effect of varying key enzyme concentration. Combining the equations of Kompala (2013) and Metcalf and Eddy (2003), the specific biomass growth rate (μ) can be defined as indicated in equation 9.6.

$$\mu = \frac{\mu_{max}.S.E_R}{K_S+S} - K_d \qquad (9.6)$$

where $\mu_{max}$ represents the maximum specific bacterial growth rate, $K_s$ the half-velocity constant, S the growth-limiting substrate concentration in solution, $E_R$ the relative amount of key enzyme and $K_d$ the endogenous decay coefficient.

Upon Stoppa *et al.* (2013), the term $K_d$ is expected to increase with folic acid supply, due to the acceleration of metabolic processes. Consequently, a small increase in $E_R$ may not be enough to compensate for a higher $K_d$ and globally growth rates decrease.

When more vitamin is supplied, the first term overcomes the increase in $K_d$, exhibiting higher growth rates.

Eventually, the SOUR is expected to be a consequence of the SRT and the biodegradation activity (Stoppa *et al*., 2013). The control bioreactor hold steady values of 28.0 ± 8.3 mg $O_2$ $g^{-1}$ VSS $d^{-1}$, which were significantly higher than the respiration rates registered for the 0.9 mg FA $g^{-1}$ VSS $d^{-1}$ bioreactor test (23.9 ± 7.0 mg $O_2$ $g^{-1}$ VSS $d^{-1}$). Although the organic matter removal efficiency was higher with the highest folic acid supplement, this reduction in oxygen consumption could be due to a younger sludge, as a consequence of a higher biomass growth yield. On the contrary, the bioreactor provided with 0.4 mg FA $g^{-1}$ VSS $d^{-1}$ experienced an increase in SOUR, after a 40-day acclimation period (52.0 ± 6.5 mg $O_2$ $g^{-1}$ VSS $d^{-1}$) (see figures 9.3 d and 9.4(d)). This behaviour could be explained by a slightly higher COD removal efficiency and especially by an older sludge, more intensive in endogenous respiration (Metcalf and Eddy, 2003).

### 9.3.3. Lasting effects after folic acid addition to the bioreactors (Stage 2)

After stage 1 was completed, folic acid addition to the two test bioreactors was interrupted to assess lasting effects.

The operational conditions in stage 2 were similar to those registered in stage 1 but for SRT in the test bioreactors, due to a different $Y_{obs}$. In turn, during stage 2, the parameters of the control and the test bioreactors did not differ significantly, except for ammonia nitrogen concentration, which was supplemented in excess in the test bioreactors, in order to favour nitrification conditions (see Table 9.1).

In stage 2, the control bioreactor attained average values of 73 ± 30 mg $NO_2$-N $L^{-1}$ and 195 ± 39 mg $NO_3$-N $L^{-1}$, which were slightly higher than in stage 1, probably due to a higher operational temperature. The bioreactor supplemented with 0.4 mg FA $g^{-1}$ VSS $d^{-1}$ initiated nitrification immediately after interrupting the vitamin addition reaching 78 ± 29 mg $NO_2$-N $L^{-1}$ and 136 ± 73 mg $NO_3$-N $L^{-1}$ which are values statistically comparable to the control (see Figure 9.5). However, the initial ratio nitrate-to-nitrite was lower in the test bioreactor and increased to comparable values to control at the end of the test.

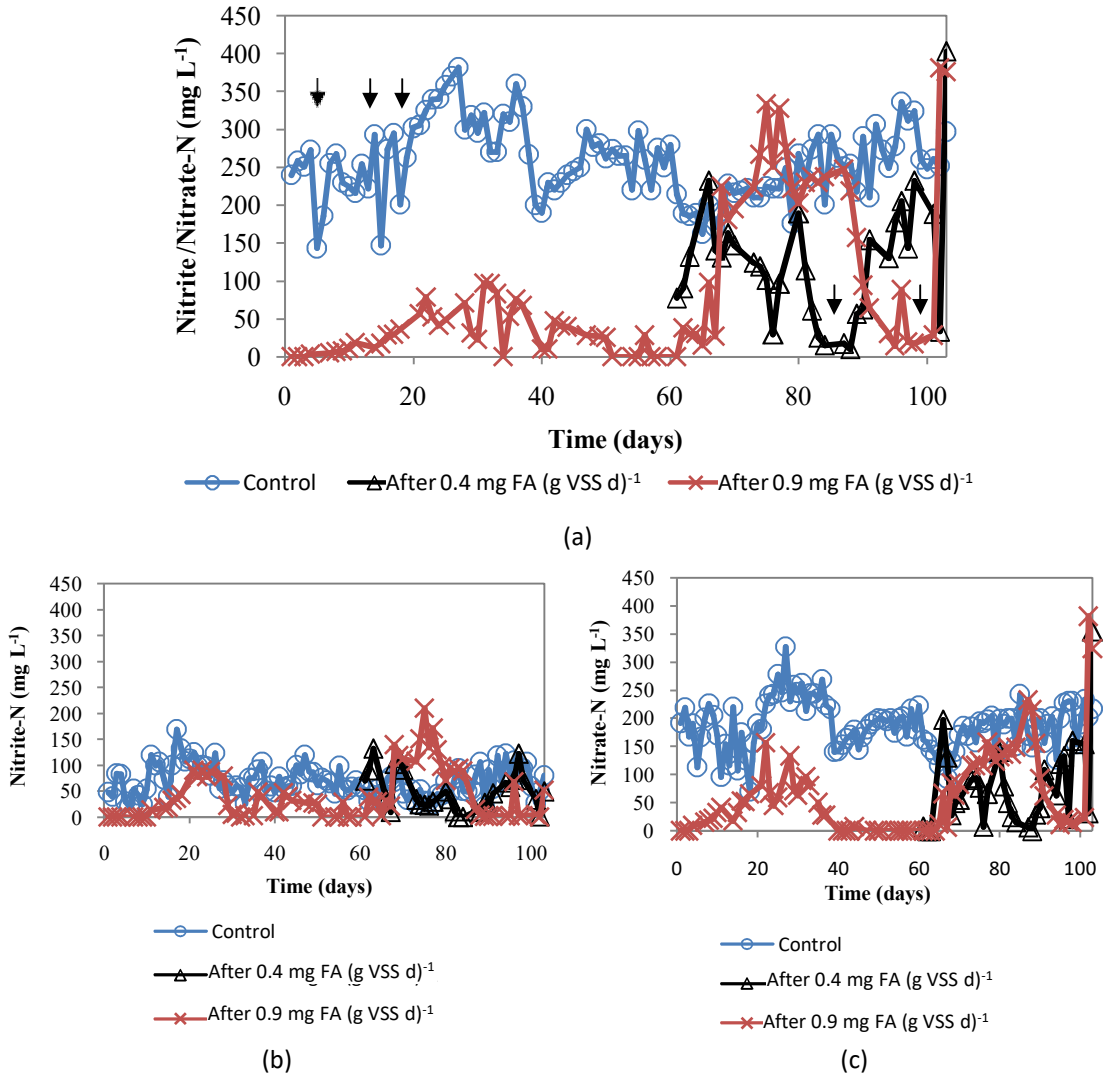



Figure 9.5 Monitoring of (a) nitrogen as nitrite+nitrate (b) nitrogen as nitrite ($NO_2^-$-N) and (c) nitrogen as nitrate ($NO_3^-$-N) concentration in the effluent for the three bench bioreactors after folic acid (FA) addition (stage 2).

Also, nitrifying bacteria appeared to be extremely sensitive to the pH control incidences registered in stage 2. Whereas pH values above 9.0 and below 6.0 only caused a short-term inhibitory effect in the control bioreactor, they resulted in a one-week stop of nitrification rates in the test bioreactor. This fact evidenced a weak recovery of the nitrifying activity. The same pattern taking to pH effect was verified in the test bioreactor, which had been provided with 0.9 mg FA $g^{-1}$ VSS $d^{-1}$.

In the high concentration assay, nitrates initiated a progressive resurgence at the fifth day after interrupting folic acid addition, whereas it took 20 days to obtain a nitrite

concentration different to zero. Therefore, in this initial step, nitrite generation seemed to be limiting. According to the observations in stage 1, these tendencies suggest affection on the ammonia oxidizing bacteria, dependent on the folic acid concentration supplied. From day 20, the 0.9 mg FA $g^{-1}$ VSS $d^{-1}$ bioreactor experienced nitrification rates reduced in 82 ± 9 % compared to the control, with equilibrated concentrations of nitrites and nitrates. It was not until day 60 after interrupting folic acid supply, that the nitrifying activity was completely recovered. However, nitrite and nitrate concentrations were still equilibrated. It was only after 90 days that the ratio nitrate-to-nitrite in the test bioreactor showed comparable values to the control. The relative concentrations of nitrite and nitrate during the recovery period in both tests indicated a decrease in nitrite consumption by Nitrobacter. A similar result was obtained by Odokuma and Akponah (2008) experiencing with drilling fluids in soils. They attributed this effect to the sensitivity of the enzyme mediating the oxidation process of nitrite to nitrate by Nitrobacter. This enzyme is said to be located in the outer membrane, which is of high permeability in Nitrobacter.

Along stage 2, the performance parameters of the test bioreactors moved to similar values to the control. Tendencies during the test period are shown in figure 9.6 and average values after acclimation are presented in Figure 9.7. The average values and the standard deviations obtained after acclimation for the three bioreactors were statistically compared. The results indicated no significant difference between them with a 95% confidence level.

In stage 2, the COD RE in the control bioreactor decreased compared to stage 1, probably due to a less biodegradable feed stream. The test bioreactors, which had increased $RE_{COD}$ with folic acid addition, experienced a reduction in their efficiency after interrupting the vitamin dosage. Whereas the COD RE in the 0.4 mg FA $g^{-1}$ VSS $d^{-1}$ bioreactor stabilized immediately after stopping folic acid dosage in values similar to the control, the 0.9 mg FA $g^{-1}$ VSS $d^{-1}$ test required an acclimation period of 30 days, with a progressive reduction in $RE_{COD}$. Therefore, a period of almost twice the sludge age was necessary for the effect of folic acid on COD RE to disappear completely. The stimulation of the one-carbon metabolism by folic acid addition (Strunkheide, 2004) appeared to dim as the vitamin concentration decreased in the mixed liquor. A consistent pattern applied for sludge settling. Stopping the lower folic acid dosage, which had improved sludge settling, resulted in a progressive increase of the SVI after a 10-day transition period. Therefore, compared to Goldwyn *et al*. (2010) findings about chlorination, folic acid manifested itself as a short-term method to control filamentous

bacteria proliferation, attaining a significant reduction in SVI during and shortly after the vitamin addition.

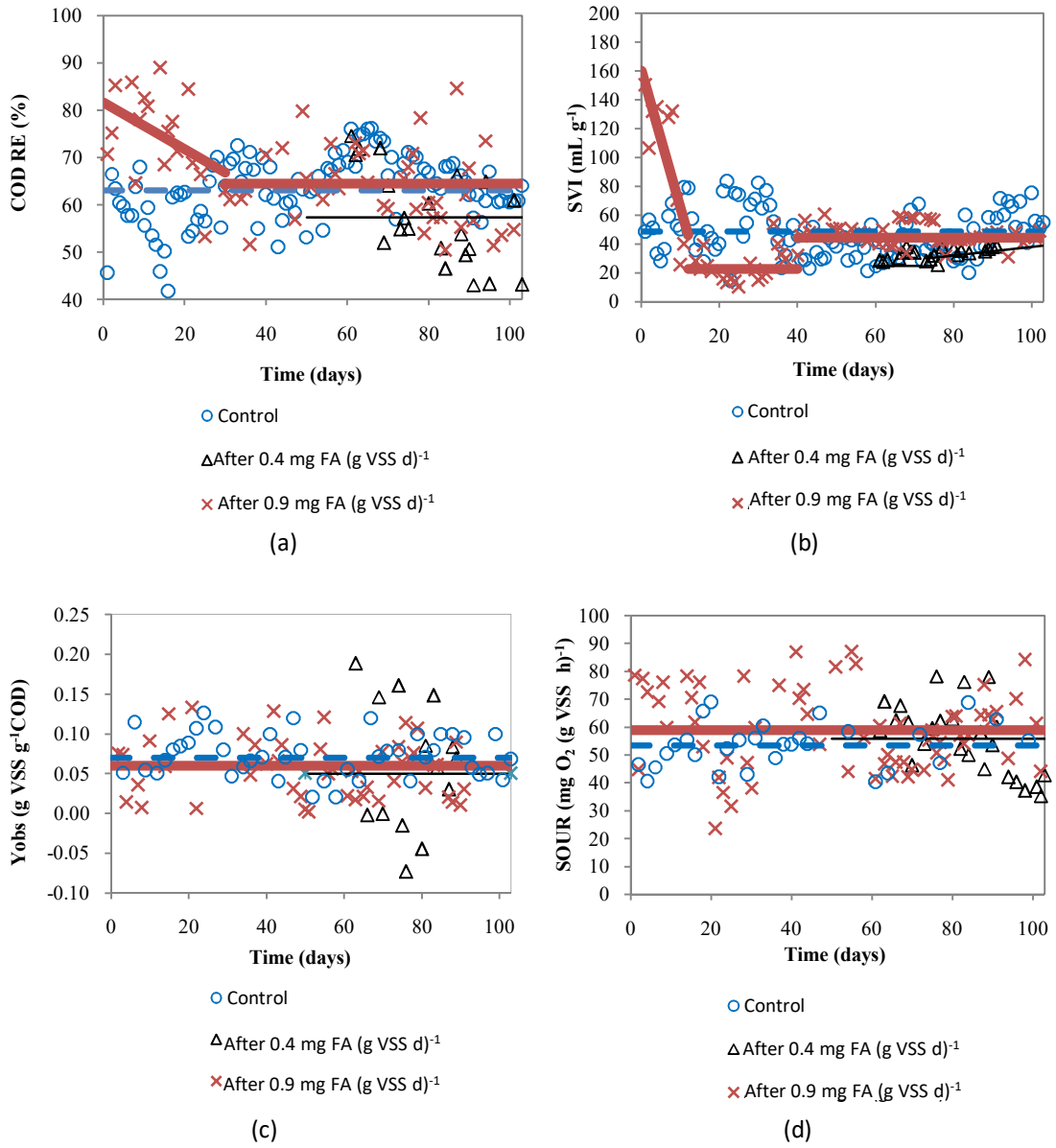
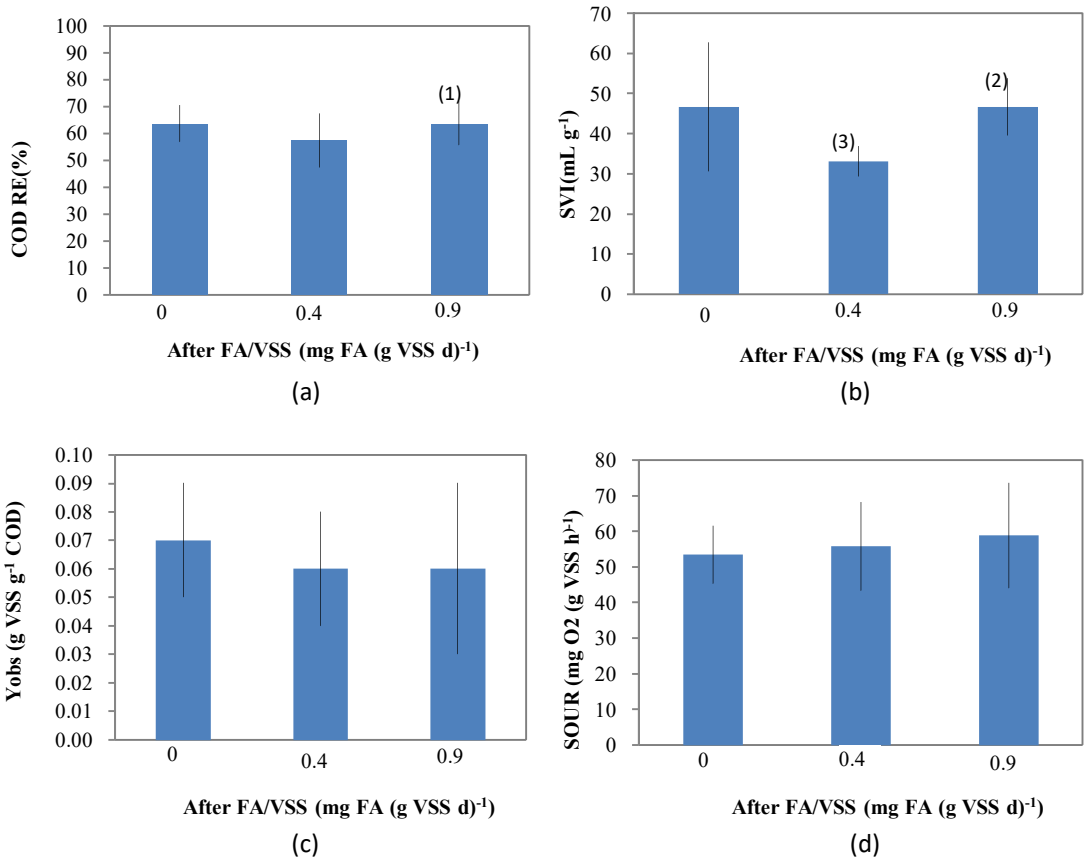



Figure 9.6 Evolution of the (a) organic matter removal efficiency ($RE_{COD}$) (b) sludge volumetric index (SVI) (c) observed sludge yield ($Y_{obs}$) and (d) specific oxygen uptake rate (SOUR) after folic acid (FA) addition (stage 2). The lines indicate average values for the control ( ), low ( ) and high folic acid concentration ( ).

With the interruption of the higher folic acid concentration, lower SVI values than the control were progressively attained during a 12-day acclimation period. In turn,

eventually after 28 days, the SVI increased to the reference values in the control bioreactor.

Microscopic observations provided an explanation to the SVI tendencies. The jelly appearance induced by the 0.9 mg FA $g^{-1}$ VSS $d^{-1}$ folic acid addition, ceased to occur progressively along the initial 10 days after stopping the additive, as the $RE_{COD}$ returned to the usual values. The coincidence of both acclimation periods could confirm the hypothesis that the jelly appearance caused by folic acid is linked to the increase in COD RE. Coincident microbiology was generally observed in the three bioreactors during stage 2, which indicated a similar sludge age (Jenkins *et al.*, 2003).




(1) After 30-day transition period
(2) After 28-day acclimation period
(3) After a progressive 10-day transition

Figure 9.7 Values attained for (a) COD removal efficiency (COD RE) (b) sludge volumetric index (SVI) (c) observed sludge yield ($Y_{obs}$) (d) specific oxygen uptake rate (SOUR) in the three bioreactors after stopping folic acid (FA) addition. Error bars indicate standard deviation.

Consistently, immediately after folic acid interruption $Y_{obs}$ was calculated at similar values to the control in both test bioreactors. This result controverts Strunkheide (2004) hypothesis, that the effect of folic acid on bacterial growth is related to the increase in $RE_{COD}$ and consequently on higher endogenous respiration. Since growth rates have attained the control values beforeCOD RE, this finding suggests independent affection of folic acid on bacterial growth and organic matter removal efficiency.

On the other hand, SOUR confirmed to be dominated by sludge age rather than by COD RE. Therefore, since SRT was similar in stage 2 for the three bioreactors (see Table 9.1), SOUR registered similar values.

### 9.3.4. Long-term toxicity effects of folic acid

The assays conducted to assess the chronic toxicity supplied by folic acid to the effluent indicated similar results for the control and the test bioreactors, in stages 1 and 2 ($EC_{50}$> 45%, TU< 2.2). Therefore, the doses of vitamin supplied did not provide a significant increase in the effluent's toxicity, either during or after addition. This result complements the existing bibliography, which presents alternative assays to evaluate folic acid toxicity. Hence, Velho *et al.* (2016) came to the same conclusion by using a Dafnia Magna test performed on a domestic wastewater system supplemented with folic acid. Otherwise, Stoppa *et al.* (2013) reported an increase in toxicity with the test Pseudokichneriella Subcapitata 72 h algal growth inhibition with typical bleached kraft pulp mill effluents.

## 9.4. CONCLUSIONS

Cost-effective doses of folic acid have allowed to limit undesired biological nitrification in a petrochemical wastewater treatment focused on organic matter removal, without adding significant toxicity to the effluent. Therefore, folic acid addition stands as a new feasible strategy to control undesired nitrification. This procedure is advantageous compared to the traditional ones because (1) there is no need to adjust operational parameters with an effect on the performance of the biological system, (2) requires low investment cost, (3) demands small area to lay-out and (4) can be implemented without interference with the current process. Nevertheless, the full-scale feasibility of the process deserves specific site consideration, since the vitamin dosage has also influenced the operational parameters of the mixed biological system. Hence, to provide

a continuous dose of 0.4 mg FA $g^{-1}$ VSS $d^{-1}$, spare capacity of aeration should be available, as oxygen demand increased. Otherwise, a continuous supply of 0.9 mg FA $g^{-1}$ VSS $d^{-1}$ resulted in higher growth yields, which require more sludge waste. Comparing both doses tested, 0.9 mg FA $g^{-1}$ VSS $d^{-1}$ provided several advantages such as a greater improvement in COD removal efficiency, better and longer nitrification control, even until 60 days after stopping the vitamin addition. However, apart from doubling operative costs, the concentration of 0.9 mg FA $g^{-1}$ VSS $d^{-1}$ worsened sludge settling. This scenario suggests an alternative strategy to explore in future research, to dose discontinuous doses of 0.9 mg FA $g^{-1}$ VSS $d^{-1}$.

# 10. General conclusions and recommendations

## 10.1. GENERAL CONCLUSIONS

Pilot and bench-scale tests have confirmed, respectively, the tendency of petrochemical activated sludge systems focused on organic matter removal to develop low F/M filamentous bulking and unintended biological nitrification. The first issue lead to a poor sludge settling, characterized by sludge volumetric index values above 350 mL $g^{-1}$. The second provided nitrogen as nitrite and nitrate concentrations up to 300 mg $L^{-1}$, susceptible to cause rising sludge in the clarifier because of denitrification.

In order to adapt to future more stringent emission levels, the present research has developed full-scale feasible tools to enhance sludge settling in such systems by improving the biomass quality, optimizing the mixed liquor parameters and limiting unintended nitrification. Therefore, a novel strategy in the petrochemical sector has been tested, to upgrade the existing wastewater treatment processes by including an aerobic selector and supplying cost-effective doses of folic acid to the CSTR.

The selector has allowed to overcome the filamentous bulking, providing SVI values below 100 mL $g^{-1}$, 100% of the time. Aiming to its full-scale implementation, guidelines are provided for the design and operation of the selector and the activated sludge system. Despite the selector evidenced optimum design variables (HRT of 30 min and F/M of 35 g sCOD $g^{-1}$ VSS $d^{-1}$), its performance was robust to improve sludge settling with variations in the key parameters. As a drawback, the inclusion of the aerobic selector in the activated sludge system enhanced unintended nitrification, which highlights folic acid dosage as a complementary solution.

This proposal is advantageous to control nitrification in existing installations, since it can deal with usual site constraints, such as minimizing the necessary space to lay-out and being built while the process is running. Folic acid concentrations of 0.4 and 0.9 mg $g^{-1}$VSS $d^{-1}$ provided nitrogen as nitrite and nitrate concentrations below 10 mg $L^{-1}$ without adding significant toxicity to the effluent and even improving the organic matter removal efficiency. Nevertheless, the vitamin supply modified the operational parameters of the activated sludge system. Therefore, its full-scale implementation deserves specific site consideration. Hence, the dose of 0.4 mg $g^{-1}$ VSS $d^{-1}$ increased oxygen demand in 85.7%, which requires spare aeration capacity. In turn, the concentration of 0.9 mg $g^{-1}$ VSS $d^{-1}$ increased biomass production and worsened sludge settling.

Despite all the experimental assays were conducted with petrochemical substrate, the methodology and the conclusions could be extrapolated to other industrial sectors holding a similar characterization of the effluents.

## 10.2 RECOMMENDATIONS

Full-scale petrochemical activated sludge systems focused on organic matter removal should be upgraded to attain consistently the future, more stringent allowed emission levels.

The implementation of an aerobic selector is suggested to overcome frequent low F/M filamentous bulking. Guidelines for its design and operation should be considered, such as hydraulic retention time of 30 min and F/M ratio of 35 g sCOD $g^{-1}$VSS $d^{-1}$. Among the available feed streams, the most biodegradables ones should be chosen to be supplied to the selector (minimum 10 g BOD $g^{-1}$VSS $d^{-1}$). As well, there is a limit of 65 g tCOD $g^{-1}$ VSS $d^{-1}$ as the maximum amount of particulate matter to be provided. Since the introduction of a selector in the activated sludge system is bound to increase unintended nitrification, it is recommended, as a complimentary solution, to supply cost-effective doses of folic acid to limit the biological reaction. Nevertheless, before the full-scale implementation of this solution, it is recommended to complete the experimentation by assessing the effect of other dosing strategies, such as a discontinuous supply of 0.9 mg $g^{-1}$VSS $d^{-1}$ of folic acid. Testing folic acid addition at pilot-scale on an activated sludge system including a selector is also suggested.

Also, this manuscript can inspire future research lines related to sludge settling enhancement in activated sludge systems. Hence, the methodology presented to assess about selectors could be used to reproduce similar experiences for other industrial sectors, which have not been already explored in the bibliography. As well, folic acid could be investigated as a nitrification inhibitor, not only for wastewater treatments, but also in the agricultural field. The behaviour patterns obtained for ammonia oxidizing bacteria during and after folic acid supply could be the baseline to figure out a mechanism of action for folic acid.

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# 12. Notation

| SYMBOL/ ACRONIM | DESCRIPTION | UNITS |
|---|---|---|
| $A_c$ | Cross-section area of the clarifier | ($m^2$) |
| $A_t$ | Area required for sludge thickening | ($m^2$) |
| $AEL$ | Associated emission levels | (-) |
| $AOB$ | Ammonia oxidizing bacteria | (-) |
| $AOP$ | Advanced oxidation processes | (-) |
| $AOX$ | Adsorbable organically bound halogens | (-) |
| ASCE | Amertican Society of Civil Engineers | (-) |
| ASTM | American Society of Testing Materials | (-) |
| α | Oxygen transfer correction factor for wastewater | (-) |
| β | Correction factor for salinity and surface tension | (-) |
| $BAT$ | Best available techniques | (-) |
| $BOD$ | Biological oxygen demand concentration | (g $m^{-3}$) |
| $BOD_{5\,c}$ | Influent $BOD_5$concentration into the clarifier | (g $BOD_5$ $m^{-3}$) |
| $BOD_L$ | Biological oxygen demand load | (kg BOD $d^{-1}$) |
| $BOD_5$ | Five-day biological oxygen demand concentration | (g $m^{-3}$) |
| $BOD_r$ | Remaining BOD at a time | (g $m^{-3}$) |
| $BOD_t$ | BOD degraded at time t | (g $m^{-3}$) |
| $BOD_{5\,o}$ | Influent $BOD_5$concentration to the reactor | (g $BOD_5$ $m^{-3}$) |
| $BOD\ RE$ | BOD removal efficiency | (%) |
| $BREF$ | BAT reference document | (-) |
| $bsCOD_o$ | Biodegradable soluble COD oxidized in denitrification | (g $d^{-1}$) |
| $bsCOD_r$ | Biodegradable soluble COD removed in denitrification | (g $d^{-1}$) |
| $bsCOD_{syn}$ | Biodegradable soluble COD used for cell synthesis in denitrification | (g $d^{-1}$) |
| $C$ | Suspended solid concentration in the reactor | (g SS $m^{-3}$) |
| $C_2$ | Critical suspended solid concentration in the clarifier | (g SS $m^{-3}$) |
| $C_c$ | Suspended solid concentration into the clarifier | (g SS $m^{-3}$) |
| $C_e$ | Suspended solid concentration in the effluent | (g SS $m^{-3}$) |
| $C_i$ | Concentration of solids in the clarifier at point i | (g $m^{-3}$) |
| $C_o$ | Influent suspended solid concentration to the reactor | (g SS $m^{-3}$) |
| $C_r$ | Suspended solid concentration in the recycle | (g SS $m^{-3}$) |
| $C_u$ | Suspended solid concentration at the bottom of the clarifier | (g SS $m^{-3}$) |

| SYMBOL/ ACRONIM | DESCRIPTION | UNITS |
|---|---|---|
| $C_w$ | Suspended solid concentration in the waste stream | (g SS $m^{-3}$) |
| $CMAS$ | Complete mix activated sludge | (-) |
| $COD$ | Chemical oxygen demand | (g $m^{-3}$) |
| $COD\ EC$ | COD equilibrium concentration | (g $L^{-1)}$ |
| $COD\ RE$ | COD removal efficiency | (%) |
| $CSTR$ | Continuous Stirred Tank Reactor | (-) |
| $DNA$ | Deoxyribonucleic acid | (-) |
| $B_1$ | Dissolved oxygen of seed control before incubation for BOD test | (mg $L^{-1}$) |
| $B_2$ | Dissolved oxygen of seed control after incubation for BOD test | (mg $L^{-1}$) |
| $D_1$ | Dissolved oxygen of diluted sample after preparation for BOD test | (mg $L^{-1}$) |
| $D_2$ | Dissolved oxygen of diluted sample after 5-day incubation at 20 ºC for BOD test | (mg $L^{-1}$) |
| $DAD$ | Diode array detector | (-) |
| $DO$ | Dissolved oxygen concentration in reactor | (mg $L^{-1}$) |
| $DO_{s,20}$ | Dissolved oxygen saturation concentration in clean water (20 ºC ,1 atm) | (mg $L^{-1}$) |
| $DO_{av,S,T,H}$ | Average dissolved oxygen saturation concentration in clean water (T, H) | (mg $L^{-1}$) |
| $DO_{s,T,H}$ | Dissolved oxygen saturation concentration in clean water (T, H) | (mg $L^{-1}$) |
| $DSVI$ | Diluted sludge volumetric index | (mL $g^{-1}$) |
| $\Delta VSS_{produced}$ | Mass of volatile suspended solids produced | (g) |
| $\Delta sCOD_{removed}$ | Mass of soluble COD removed | (g) |
| $DWF$ | Dry weather flow | (-) |
| $EBPR$ | Enhanced biological phosporus removal | (-) |
| $EC$ | Electrical conductivity | (dS $m^{-1}$) |
| $EC_{50}$ | Effective concentration to produce 50% reduction | (%) |
| $E-PRTR$ | European Pollutant Release and transfer Register | (-) |
| $EPA$ | Environmental protection agency | (-) |
| $f$ | Fraction of seeded dilution for the BOD test | (-) |
| $F$ | Fouling factor | (-) |
| $FA$ | Folic acid | (-) |
| $F/M$ | Food-to-microorganism ratio based on soluble COD | (g sCOD $g^{-1}$VSS $d^{-1}$) |

| SYMBOL/ ACRONIM | DESCRIPTION | UNITS |
|---|---|---|
| $F/M\ (tCOD)$ | Food-to-microorganism ratio based on total COD | (g tCOD $g^{-1}$VSS $d^{-1}$) |
| $F/M\ (BOD)$ | Food-to-microorganism ratio based on BOD | (g BOD $g^{-1}$VSS $d^{-1}$) |
| $FDS$ | Fixed dissolved solids | (g $m^{-3}$) |
| $FSS$ | Fixed suspended solids | (g $m^{-3}$) |
| $H'$ | Henry's law constant | (atm(mol gas/mol air) /(mol gas/mol air)) |
| $H$ | Altitude | (m) |
| $H_c$ | Height of the clarifier | (m) |
| $H_o$ | Initial height of interface in column | (m) |
| $H_u$ | Sludge interface height at time $t_u$ | (m) |
| $H_2$ | Sludge height at time $t_2$ | (m) |
| $HPLC$ | High-performance liquid cromatography | (-) |
| $HRT$ | Hydraulic retention time | (d) |
| $i$ | Sludge settling constant for a given suspension | (-) |
| $IED$ | Industrial Emissions Directive | (-) |
| $I_I$ | Inhibition function for the inhibitory compound | (g $g^{-1}$) |
| $K$ | Maximum specific substrate utilisation rate | (g CODg$^{-1}$ VSS $d^{-1}$) |
| $K_d$ | Endogenous decay coefficient | (g VSS $g^{-1}$ VSS $d^{-1}$) |
| $K_{dn}$ | Endogenous decay coefficient for nitrifying bacteria | (g VSS $g^{-1}$ VSS $d^{-1}$) |
| $K_I$ | Half saturation constant of the inhibitory compound | (g $m^{-3}$) |
| $K_n$ | Half-velocity constant for nitrifying bacteria | (g $m^{-3}$) |
| $K_o$ | Half-saturation coefficient for DO | (g $m^{-3}$) |
| $K_S$ | Substrate half saturation constant | (g COD $m^{-3}$) |
| $K_1$ | First-order reaction rate constant | (1/day) |
| $M_{NH_3}$ | Saturation function for ammonia | (g $NH_3$ $g^{-1}NH_3$) |
| $M_{O_2}$ | Saturation function for oxygen | (g $O_2$ $g^{-1}O_2$) |
| $M_{PO_4^{3-}}$ | Saturation function for orthophosphate | (g $PO_4^{3-}$ $g^{-1}PO_4^{3-}$) |
| $M_s$ | Saturation function for soluble substrate | (g COD $g^{-1}$ COD) |
| $MLSS$ | Mixed liquor suspended solid concentration | (g $m^{-3}$) |
| $\mu$ | Specific biomass growth rate | (g VSS $g^{-1}$ VSS $d^{-1}$) |
| $\mu_{max}$ | Maximum specific biomass growth rate | (g VSS $g^{-1}$VSS $d^{-1}$) |
| $\mu_n$ | Specific growth rate of nitrifying bacteria | (g new cells $g^{-1}$ cells $d^{-1}$) |

| SYMBOL/ ACRONIM | DESCRIPTION | UNITS |
|---|---|---|
| $\mu_{nm}$ | Maximum specific growth rate of nitrifying bacteria | (g new cells $g^{-1}$ cells $d^{-1}$) |
| $N$ | Nitrogen concentration | (g $m^{-3}$) |
| $N_{ASSIM}$ | Assimilated ammonia per unit of consumed COD | (g $NH_4^+$-N assim. $g^{-1}$ COD removed) |
| $NDIR$ | Non dispersive infrared analyzer | (-) |
| $NH_4^+ - N$ | Ammonia nitrogen concentration | (mg $L^{-1}$) |
| $(NH_4^+ - N)ox$ | Ammonia nitrogen oxidized to nitrate | (g $m^{-3}$) |
| $(NO_3^- - N)u$ | Nitrate used | (g $m^{-3}$) |
| $NO_x^-$ | Nitrate and nitrite concentration | (g $d^{-1}$) |
| $NO_2^- — N$ | Nitrogen as nitrite concentration | (mg $L^{-1}$) |
| $NO_3^- — N$ | Nitrogen as nitrate concentration | (mg $L^{-1}$) |
| $NOB$ | Nitrite oxidizing bacteria | (-) |
| $NUR$ | Nitrate uptake rate | (mg $NO_3^-$-N $L^{-1}$ $h^{-1}$) |
| $NTK$ | Kjeldhal nitrogen | (-) |
| $\eta_{ij}$ | Stoichiometric coefficient of component i in reaction j | (-) |
| $O_t$ | Oxygen leaving the tank | (mg $L^{-1}$) |
| $OL$ | Organic loading | (kg BOD $m^{-3}$ $d^{-1}$) |
| $OUR$ | Oxygen uptake rate | (mg $O_2$ $L^{-1}$ $h^{-1}$) |
| $p$ | Fraction of wastewater to total volume in BOD test | (mL $mL^{-1}$) |
| $PABA$ | p-aminobenzoic acid | (-) |
| $P_{atm,H}$ | Atmospheric pressure at altitude H | (kPa) |
| $P_d$ | Pressure at the depth of air release | (kPa) |
| $PCR$ | Polymerase chain reaction procedure | (-) |
| $P_g$ | Partial pressure of gas in air | (atm) |
| $PHA$ | Poly-β-hydroxyalkanoates | (-) |
| $PHB$ | Polyhydroxybutyrate | (-) |
| PLC | Programmable logic controller | (-) |
| $(PO_4^{3-} - P)$ | Phosphor as orthophosphate concentration | (mg $L^{-1}$) |
| $P_{VSS}$ | Observed VSS production per unit of consumed COD | (g VSS produced $g^{-1}$ COD removed) |
| $P_w$ | Wasted sludge mass flow rate | (g $VSSd^{-1}$) |
| $Q_c$ | Inlet flow rate to the clarifier | ($m^3d^{-1}$) |
| $Q_e$ | Clarifier's effluent or overflow volumetric flow rate | ($m^3$ $d^{-1}$) |
| $Q_{in}$ | Influent flow rate to the selector | ($m^3$ $d^{-1}$) |

| SYMBOL/ ACRONIM | DESCRIPTION | UNITS |
|---|---|---|
| $Q_o$ | Influent flow rate to the reactor | ($m^3$ $d^{-1}$) |
| $Q_r$ | Returned activated sludge flow rate | ($m^3$ $d^{-1}$) |
| $Q_u$ | Clarifier's underflow volumetric rate | ($m^3 d^{-1}$) |
| $Q_w$ | Volumetric waste flow rate | ($m^3$ $d^{-1}$) |
| $r$ | Recycle ratio | (-) |
| $RAS$ | Recirculation of activated sludge | (-) |
| $r_g$ | Biomass growth rate | (g VSS $m^{-3} d^{-1}$) |
| $RNA$ | Ribonucleic acid | (-) |
| $rRNA$ | Ribosomal ribonucleic acid | (-) |
| $R_o$ | Influent oxygen flow rate to the reactor | (g $O_2$ $d^{-1}$) |
| $R_{or}$ | Total oxygen required in the reactor | (g $O_2$ $d^{-1}$) |
| $r_s$ | Substrate utilisation rate | (g COD $m^{-3} d^{-1}$) |
| $S$ | Soluble substrate concentration in the reactor | (g COD $m^{-3}$) |
| $SAE$ | Standard aeration efficiency | (kg $O_2$ $kW^{-1}$ $h^{-1}$) |
| $S_c$ | Soluble substrate concentration into the clarifier | (g COD $m^{-3}$) |
| $sCOD$ | Soluble chemical oxygen demand | (g $m^{-3}$) |
| $sCOD_{in}$ | Influent soluble chemical oxygen demand | (g $m^{-3}$) |
| $sCOD_{MLto}$ | Soluble chemical oxygen demand in the mixed liquor at initial time | (g $m^{-3}$) |
| $sCOD_{MLtf}$ | Soluble chemical oxygen demand in the mixed liquor at final time | (g $m^{-3}$) |
| $S_e$ | Soluble substrate concentration in the effluent | (g COD $m^{-3}$) |
| $S_i$ | Concentration of the inhibitory compound | (g $m^{-3}$) |
| $S_o$ | Influent substrate concentration to the reactor | (g COD $m^{-3}$) |
| $S_{min}$ | Minimum soluble substrate concentration | (g COD $m^{-3}$) |
| $SBR$ | Sequencing batch reactor | (-) |
| $SF_g$ | Solid flux in the clarifier due to gravity | (kg $m^{-2}$ $h^{-1}$) |
| $SF_L$ | Limiting solid flux | (kg $m^{-2}$ $d^{-1}$) |
| $SF_t$ | Total flux of solids | (kg $m^{-2}$ $h^{-1}$) |
| $SF_u$ | Solid flux in the clarifier due to underflow | (kg $m^{-2}$ $h^{-1}$) |
| $SRT$ | Sludge retention time | (d) |
| $SS$ | Suspended solid | (g $m^{-3}$) |
| $SOTE$ | Standard oxygen transfer efficiency | (%) |
| $SOTR$ | Standard oxygen transfer rate | (kg $O_2$ $h^{-1}$) |
| $SOUR$ | Specific oxygen uptake rate | ($mgO_2 g^{-1}$VSS $h^{-1}$) |
| $SVI$ | Sludge volumetric index | (mL $g^{-1}$) |

| SYMBOL/ ACRONIM | DESCRIPTION | UNITS |
|---|---|---|
| $T$ | Operational temperature | (ºC) |
| $TU$ | Toxicity units | (-) |
| $t$ | Time | (days) |
| $tCOD$ | Total chemical oxygen demand | (g $m^{-3}$) |
| $TDS$ | Total dissolved solids | (mg $L^{-1}$) |
| $TFS$ | Total fixed solids | (g $m^{-3}$) |
| $TIC$ | Total inorganic carbon | (g $m^{-3}$) |
| $TN$ | Total Nitrogen | (mg $L^{-1}$) |
| $TP$ | Total phosphorous | (mg $L^{-1}$) |
| $t_u$ | Time to reach desired underflow concentration | (s) |
| $TOC$ | Total organic carbon | (g $m^{-3}$) |
| $TS$ | Total solids | (g $m^{-3}$) |
| $TSS$ | Total suspended solid | (g $m^{-3}$) |
| $TVS$ | Total volatile solids | (g $m^{-3}$) |
| $U_b$ | Bulk downward velocity | (m $h^{-1}$) |
| UBOD | Total or ultimate biological oxygen demand | (g $m^{-3}$) |
| $\mathrm{UV-VIS}$ | Ultraviolet-visible | (-) |
| V | Reactor's volume | ($m^3$) |
| $V_c$ | Upflow velocity | (m $s^{-1}$) |
| $V_i$ | Settling velocity in the clarifier at point i | (m $h^{-1}$) |
| $V_{ML\,t}$ | Mixed liquor volume at the sampling moment | (L) |
| $V_{ML\,to}$ | Volume of mixed liquor at initial time | (L) |
| $V_{ML\,tf}$ | Volume of mixed liquor at final time | (L) |
| $V_{ML\,w}$ | Wasted mixed liquor volume | (L) |
| $VDS$ | Volatile dissolved solids | (g $m^{-3}$) |
| $VSS$ | Volatile suspended solid | (g $m^{-3}$) |
| $X$ | Biomass concentration in the reactor | (g $m^{-3}$) |
| $X_c$ | Biomass concentration into the clarifier | (g $m^{-3}$) |
| $X_e$ | Biomass concentration in the effluent | (g $m^{-3}$) |
| $X_g$ | Mole fraction of gas in water | (mol gas/(mol gas+mol water)) |
| $X_r$ | Biomass concentration in the recycle stream | (g $m^{-3}$) |
| $X_t$ | Biomass concentration at the sampling moment | (g $m^{-3}$) |
| $X_{tf}$ | Final biomass concentration | (g $m^{-3}$) |
| $X_{to}$ | Initial biomass concentration | (g $m^{-3}$) |
| $X_w$ | Biomass concentration in the waste | (g $m^{-3}$) |

| SYMBOL/ ACRONIM | DESCRIPTION | UNITS |
|---|---|---|
| $Y$ | Biomass true yield or biomass synthesis yield | (g biomass $g^{-1}$ substrate) |
| $Y_n$ | Net biomass yield in denitrification | (g VSS $g^{-1} bsCOD_r$) |
| $Y_{obs}$ | Observed sludge yield | (g biomass $g^{-1}$ substrate) |

## 13. PUBLICATIONS

Cardete, M.A., Mata-Álvarez, J., Dosta, J., Nieto-Sánchez, R. (2017a) Sludge settling enhancement in a pilot scale activated sludge process treating petrochemical wastewater by implementing aerobic or anoxic selectors, J. Environ. Chem. Eng., 5, 3472-3482
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# 14. Resumen en castellano

## 14.1. ANTECEDENTES

En coherencia con las tendencias actuales, por la *Decisión (EU) 2016/902 de 30 Mayo de 2016*, ha sido aprobada una nueva versión del documento BREF de tratamiento de aguas residuales para la industria química. Éste sienta las bases para la imposición de futuros límites de vertido más restrictivos que los actuales. En consecuencia, los procesos industriales existentes de tratamiento de aguas deben ser optimizados con el objetivo de mejorar la calidad de su efluente. La presente investigación se centra concretamente en desarrollar un proceso viable a escala industrial, que permita mejorar la decantación en sistemas de fangos activos petroquímicos focalizados en la degradación de la materia orgánica. Aquí son frecuentes dos problemáticas que dificultan el cumplimiento de las nuevas especificaciones de vertido: i) Fuerte tendencia a desarrollar *bulking* filamentoso, debido a baja razón alimento-biomasa (F/M, del inglés *food-to-microorganism*) en el reactor ii) Nitrificación biológica no deseada en el reactor, que provoca desnitrificación en el clarificador con arrastre de fangos en el efluente (Eckenfelder, 1998).

La resolución del *bulking* filamentoso se aborda con la mejora de la calidad de la biomasa mediante la implementación de un selector. Existen referencias del empleo de selectores en otros sectores industriales (Prendl and Kroiss, 1998; Durocher *et al*., 2002; Al-Mutairi, 2009). Esta tesis responde a la necesidad de trabajo experimental adicional con selectores (Martins *et al*., 2004; Seviour and Blackall, 2012), para validar su uso con efluentes petroquímicos.

Las soluciones tradicionales para el control de la nitrificación biológica pasan por ajustar parámetros operativos como la edad del fango (Flores-Alsina *et al*., 2010) o el pH (EPA, 2002). Sin embargo, éstas no resultan adecuadas en estos sistemas por limitaciones de proceso o de implantación. Por tanto, la necesidad de encontrar una solución alternativa justifica el trabajo experimental desarrollado en esta tesis, el cual se fundamenta en la adición de pequeñas dosis de ácido fólico al reactor biológico para limitar la nitrificación.

Esta tesis está dirigida al sector industrial de aguas residuales, proporcionando una metodología para optimizar los procesos de fangos activos focalizados en degradación de materia orgánica. Particularmente, se presentan pautas de operación y diseño para instalaciones dirigidas al sector petroquímico.

## 14.2. INTRODUCCIÓN

Dentro del amplio abanico de procesos biológicos disponibles en el mercado, esta tesis se centra en el estudio de procesos aerobios de crecimiento suspendido con reactor de mezcla perfecta. Dicha configuración es ampliamente utilizada en tratamientos de aguas industriales debido a su gran capacidad de dilución de tóxicos potenciales.

Este sistema consta de un reactor biológico, donde la biomasa degrada la materia orgánica. Su efluente se dirige a un clarificador, cuyo objetivo es el espesamiento del fango y la obtención de un efluente libre de sólidos. Para mantener la concentración de biomasa en el reactor, se establece un reciclo de fangos desde el fondo del clarificador.

A la vez que se produce la degradación de la materia orgánica (proceso heterótrofo), también se generan nuevas células bacterianas, como se representa en la ecuación 14.1.

$$\eta_{11}\,(\text{materia orgánica}) + \eta_{21}\,O_2 + \eta_{31}\,NH_3 + \eta_{41}\,PO_4^{3-} \longrightarrow \eta_{51}\,(\text{nuevas células}) + \eta_{61}CO_2 + \eta_{71}\,H_2O \qquad (14.1)$$

Donde $\eta_{i\,j}$ es el coeficiente estequiométrico del componente i para la reacción j. Para producirse el proceso de oxidación biológica, los microorganismos necesitan consumir oxígeno ($O_2$), nitrógeno amoniacal ($NH_3$) y fósforo como ortofosfato ($PO_4^{3-}$). En el proceso de oxidación se producen nuevas células de biomasa y productos finales como el dióxido de carbono ($CO_2$) y agua ($H_2O$). La biomasa se suele representar como $C_5H_7NO_2$ (Metcalf and Eddy, 2003). El crecimiento bacteriano se suele caracterizar mediante los términos Y e $Y_{obs}$ para la producción real de biomasa y la producción observada de biomasa, respectivamente. El primer término es mayor que el segundo, puesto que este último se ve afectado por la muerte de células. El término $Y_{obs}$ se obtiene experimentalmente, en función de la materia orgánica degradada. Para este propósito, el consumo de nitrógeno y fósforo pueden ser evaluados, considerando la fórmula molecular de la biomasa (Mogens *et al*., 2008). El término Y no se puede medir directamente. En términos cinéticos, Y puede ser expresado según la ecuación 14.2.

$$\mu_{max} = K\,Y \qquad (14.2)$$

Donde

| | |
|---|---|
| $K$ | Ratio máximo específico de utilización de sustrato (g DQO $g^{-1}$ VSS $d^{-1}$) |
| $\mu_{max}$ | Ratio máximo específico de crecimiento de biomasa (g VSS $g^{-1}$VSS $d^{-1}$) |

En coexistencia con la degradación heterótrofa, en los sistemas estudiados también se producen procesos autótrofos. Las bacterias amonio-oxidantes (AOB) convierten el amonio ($NH_4^+$) en nitritos, y éstos a su vez son transformados en nitratos ($NO_3^-$), en segunda instancia, por las bacterias nitrito-oxidantes (NOB). Como se observa en la ecuación 14.3 que representa el proceso global, para producirse la nitrificación es necesaria la presencia de carbono inorgánico ($HCO_3^-$) y oxígeno (Crites and Tchobanoglous, 1998).

$$NH_4^+ + 1.98HCO_3^- + 1.83O_2 \rightarrow 0.98NO_3^- + 0.021C_5H_7NO_2 + 1.041H_2O + 1.88\,H_2CO_3 \quad (14.3)$$

La degradación de la materia orgánica es realizada principalmente por las bacterias. No obstante, en el licor mezcla también coexisten protozoos y metazoos, cuya caracterización es interesante puesto que es indicativa de la calidad del sistema biológico y de sus parámetros operativos. De entre estos, se mencionan a continuación las variables principales que determinan la calidad del sistema biológico.

***Edad del fango o SRT*** (del inglés *Sludge retention time*) - La edad del fango representa el periodo medio durante el cual el fango permanece en el sistema, bien en el reactor o bien en el decantador (Lee *et al.*, 2007). La edad del fango determina el tipo de biología presente en el sistema (Woodard and Curran, 2011). A su vez, el tipo de microorganismos presentes en el fango determina la calidad de la depuración y de la decantación del lodo.

***Sólidos en suspensión volátiles o VSS*** (del inglés *Volatile suspended solids*) - Generalmente, la mayor parte de los sólidos en suspensión que se encuentran en el reactor son sólidos volátiles. La fracción VSS se suele identificar con la biomasa. No obstante, ambas no siempre coinciden, puesto que en el análisis de VSS se puede determinar adicionalmente materia orgánica particulada adsorbida en el flóculo. La concentración de biomasa en el reactor debe ser suficiente para degradar la materia orgánica influente. No obstante, al incrementar la concentración de biomasa, también aumentan los requerimientos de oxígeno y el tamaño de clarificador requerido para la decantación. Para aumentar la concentración de biomasa en el reactor se debe incrementar el caudal de reciclo de fangos. El caudal de reciclo necesario depende del balance hidráulico en el reactor y del crecimiento bacteriano obtenido en el mismo.

***Tiempo de retención hidráulico o TRH -*** Determina cuánto tiempo permanece el agua en el sistema quedando disponible para ser depurada por los microorganismos. Por tanto, las corrientes menos biodegradables requieren mayor TRH para ser depuradas.

***Ratio de recirculación*** - Se calcula como el cociente entre el caudal de reciclo de fangos y el caudal influente al reactor. El caudal de reciclo necesario queda determinado por el balance hidráulico del reactor biológico y por las tasas de crecimiento bacteriano, las cuales a su vez, dependen de la edad del fango y de la naturaleza de las corrientes de alimentación.

***Relación alimento-biomasa o F/M*** (del inglés *food-to-microorganism*) – Esta relación se calcula como el caudal másico diario de materia orgánica alimentada respecto de la masa de biomasa. Este ratio se puede calcular en base a $DBO_5$ alimentada o a DQO (kg DBO kg $^{-1}$ VSS $d^{-1}$ o kg DQO kg $^{-1}$ VSS $d^{-1}$). Generalmente, las relaciones F/M bajas se asocian con la presencia de bacterias filamentosas. Por el contrario, los altos ratios pueden causar toxicidad e inhibición (Jenkins *et al*., 2003).

El tipo de microorganismos y su capacidad de floculación son dos características que determinan la capacidad de decantación del lodo. Por desequilibrios en las condiciones de proceso, se pueden dar diversas incidencias repercuten en la generación de un sistema biológico, el cual dificulta la decantación del fango. De entre ellas, la discusión se centrará en dos causas que se encuentran habitualmente en los sistemas de fangos activos de mezcla perfecta focalizados en la degradación de materia orgánica: el *bulking* filamentoso causado por baja relación F/M y la desnitrificación biológica.

Para corregir el *bulking* filamentoso, hay métodos no específicos, tales como la dosificación de sólidos inertes para dar más peso al flóculo. También se emplean técnicas para dañar selectivamente a los organismos filamentosos, tales como la cloración. No obstante, para superar definitivamente la situación de *bulking* filamentoso, se deben aplicar técnicas preventivas que modifiquen las condiciones del reactor biológico. El caso particular de *bulking* filamentoso causado por baja relación F/M se puede abordar con la implementación de un selector (Richard, 2003). Existen diferentes tipos de selectores, en función de su aceptor de electrones: aerobios, anóxicos y anaerobios. De entre ellos, los selectores anóxicos y aerobios presentan referencias exitosas para el control de *bulking* filamentoso en tratamientos de aguas residuales industriales (Di Marzio *et al*., 2000). Un selector es un pre-reactor biológico que opera con un corto tiempo de residencia en el cual se fuerza una elevada relación F/M. De esta forma, se establece un ciclo alimentación-ayuno entre el selector y el reactor biológico,

de manera que en el selector se consume y almacena el sustrato, mientras que, posteriormente, en el reactor se metaboliza (Patoczka and Eckenfelder, 1990). Con la implementación de esta estrategia, el sistema de fangos activos de mezcla perfecta se aproxima a un sistema de flujo en pistón, donde ya no se demuestran los problemas de formación de *bulking* filamentoso (Jenkins *et al*., 2003). La Figura 14.1 muestra la configuración del sistema de fangos activos incluyendo selectores aerobios y anóxicos.

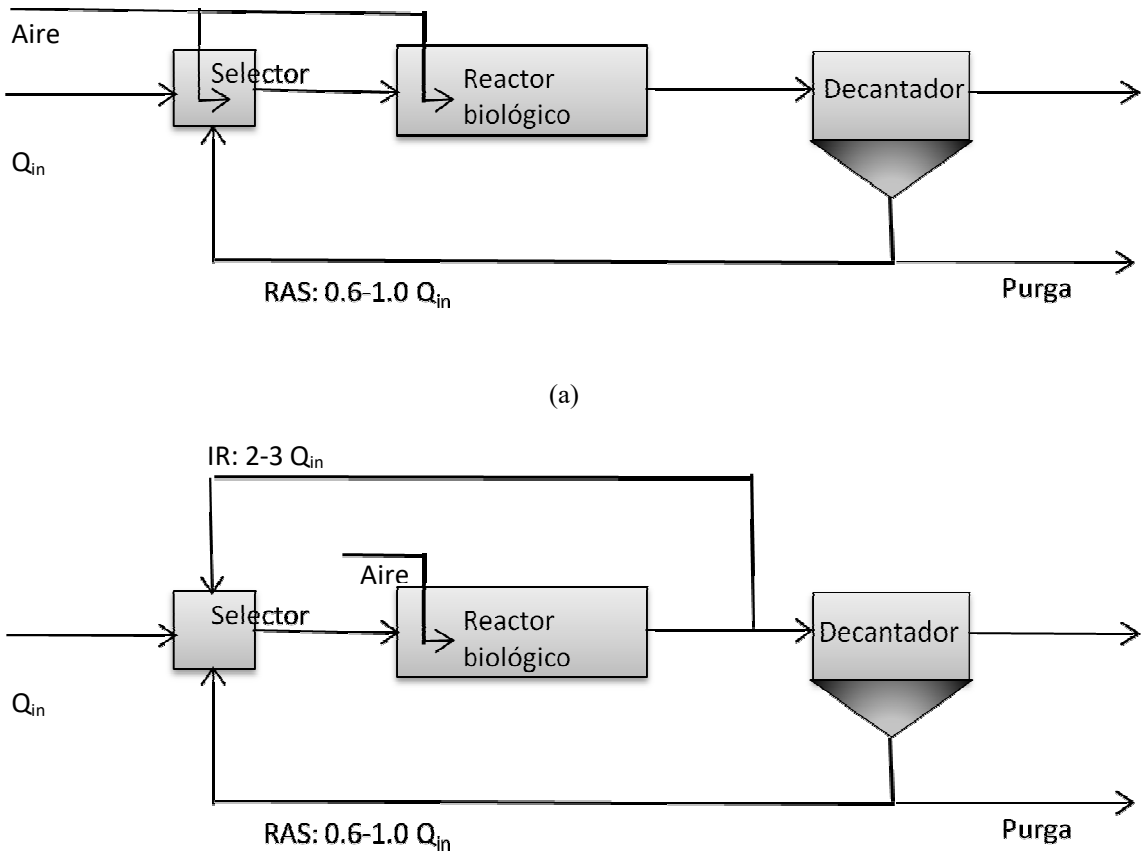



Figura 14.1 Esquema de un proceso de fangos activos con selector aerobio (a) y anóxico (b). El caudal volumétrico de recirculación al selector (RAS) es proporcional al caudal volumétrico de sustrato alimentado al selector (Qin). Adicionalmente, en el selector anóxico se establece un reciclo interno (IR).

El selector aerobio utiliza como aceptor de electrones el oxígeno del aire suministrado, mientras que el selector anóxico utiliza los nitratos formados en el reactor, y aportados al selector mediante un reciclo interno. Por tanto, al selector anóxico no se le suministra aire, y se debe controlar que su residual de oxígeno sea inferior a 0,5 mg $L^{-1}$. La proliferación de bacterias filamentosas se controla por dos mecanismos de selección, el cinético y el metabólico. El mecanismo cinético se aplica en los selectores aerobios y anóxicos y consiste en operarlos con una alta relación F/M. En estas condiciones las

bacterias floculantes están cinéticamente favorecidas frente a las bacterias filamentosas (Chudoba, 1985). El mecanismo metabólico se fundamenta en que la mayoría de filamentos son estrictamente aerobios (Mangrum, 1998). Por tanto, las condiciones anóxicas potencian el crecimiento de bacterias floculantes frente a filamentosas.

Por otra parte, el control de la nitrificación biológica se puede abordar aprovechando la extrema sensibilidad de las bacterias nitrificantes a algunos compuestos, como alternativa a los métodos tradicionales de ajuste de variables operativas. Esta vía se ha investigado especialmente en el sector agrícola, en el ámbito del cual Blum and Speece (1991) publicaron una lista de inhibidores de nitrificación. Estas sustancias pueden actuar por distintos mecanismos para evitar la reacción biológica. Algunas se unen al sitio activo de la enzima amonio monooxigenasa (AMO), la cual cataliza la conversión del amonio a hidroxilamina, como primer paso de la nitrificación. Otro mecanismo de inhibición consiste en la inactivación de AMO, modificándola mediante la formación de un enlace covalente de sus proteínas con el inhibidor. Finalmente, se identificó un tercer grupo de inhibidores, que son los heterociclos nitrogenados (McCarty, 1999). Se ha comprobado que cuantos más heterociclos nitrogenados tiene una molécula, mayor es su capacidad inhibidora. A su vez, se atribuye mayor potencia inhibidora a los heterociclos no sustituidos que a los sustituidos (McCarty and Bremmer, 1989).

El ácido fólico, todo y no haber sido reportado en la bibliografía como un inhibidor de nitrificación, posee estructura de heterociclo nitrogenado. Existen referencias en la literatura de su dosificación en tratamientos biológicos, y de su efecto de mejora de la eficiencia del tratamiento para eliminar la DQO (Burgess *et al*., 2000). La principal aplicación que se reporta para el ácido fólico en tratamientos biológicos es su uso para reducir el crecimiento bacteriano (Alexandre *et al*., 2016). A su vez, el efecto del ácido fólico en el crecimiento bacteriano depende de la concentración en que es aplicado (Stoppa *et al*., 2013).

Por tanto, uniendo la bibliografía desarrollada en el entorno del sector agrícola con la de aguas residuales, el escenario sugiere la posibilidad de limitar la nitrificación biológica por adición de ácido fólico, el cual, además, dosificado en cantidades adecuadas, permite mejorar el tratamiento biológico.

## 14.3. OBJETIVOS

El objetivo principal de esta tesis consiste en mejorar la decantación del lodo en sistemas petroquímicos de fangos activos dedicados a la degradación de materia orgánica. Con este propósito, se abordan dos problemáticas frecuentes en estos sistemas: i) *Bulking* filamentoso por baja relación F/M, ii) Nitrificación no deseada en el reactor y la consiguiente desnitrificación en el clarificador. La resolución del primer aspecto se enfoca a través de la implementación de un selector en el sistema de fangos activos. El segundo aspecto se trata mediante la limitación de la actividad nitrificante gracias a la dosificación de ácido fólico.

Con estas premisas y para la consecución del objetivo principal mencionado, el alcance de la tesis incluye los siguientes objetivos parciales:

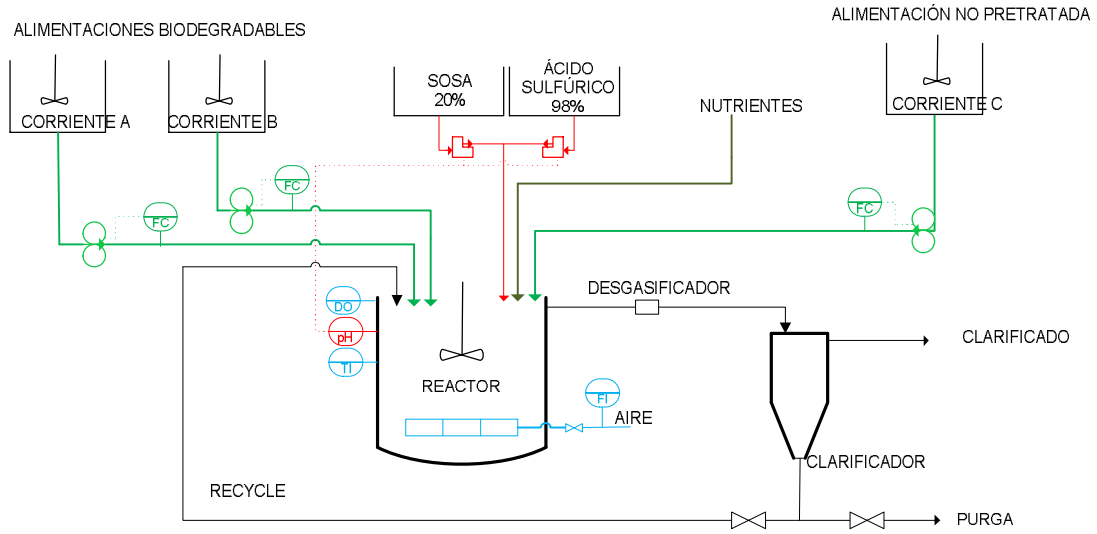
i. Determinar el efecto del aceptor de electrones ($O_2$, $NO_3^-$) en el selector para mejorar la decantación del fango (publicado en Cardete *et al*., 2017 a)
ii. Optimizar los parámetros operacionales y de diseño del selector: determinar el efecto de tiempo de retención hidráulico, F/M e influente de $DBO_5$ y de materia particulada, en la decantación del fango (publicado en Cardete *et al*., 2017 b)
iii. Optimizar los parámetros operacionales del licor mezcla para mejorar la decantación del lodo en un sistema piloto de fangos activos que incluye un selector (publicado en Cardete *et al*., 2018)
iv. Determinar el efecto del ácido fólico en la nitrificación bacteriana y en los parámetros operativos del sistema biológico (publicado en Cardete *et al*., 2019)

## 14.4. MATERIALES Y MÉTODOS

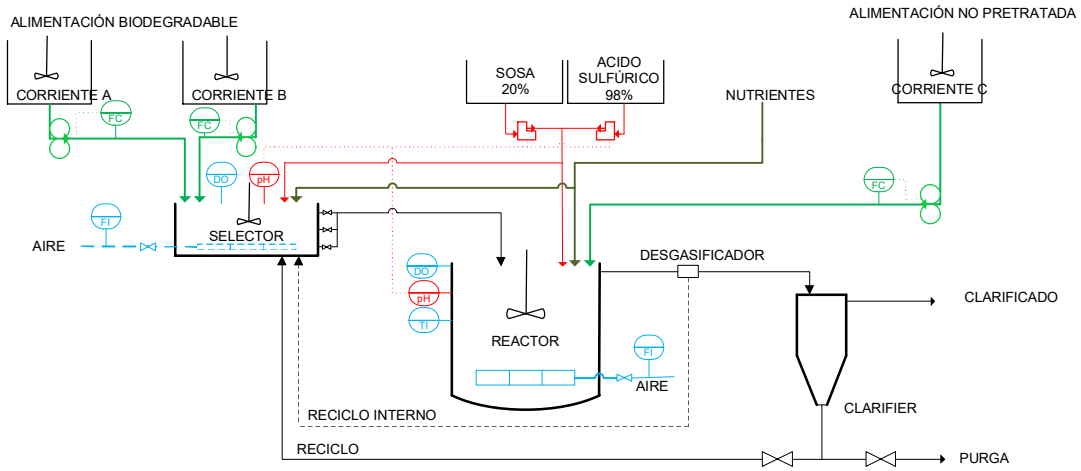
### 14.4.1. Descripción y operación de la planta piloto

En la Figura 14.2 se presenta la planta piloto utilizada para experimentar la inclusión del selector en el sistema de fangos activos. Ésta se podía utilizar como un RTMP (Figura 14.2 a) o incluyendo un selector de un solo compartimento, el cual podía ser operado como aerobio o anóxico (Figura 14.2 b).

El selector podía trabajar con tres volúmenes diferentes (3,3, 6,7 ó 10 L) para alcanzar el tiempo de retención hidráulico deseado. Los nutrientes se dosificaban a través de sendas disoluciones de sulfato amónico 20%p y ácido fosfórico 75%p.




(a)




(b)

Figura 14.2. Esquema del sistema de fangos activos piloto, operando como (a) RTMP e (b) incluyendo un selector aerobio o anóxico

Cada una de las tres corrientes de alimentación (A, B y C) se almacenaba en un depósito diferente. La operación de la planta piloto requería reponer el contenido de estos depósitos por cargas. Tras cada operación de rellenado, se caracterizaba el contenido de los depósitos. Los depósitos estaban equipados con agitadores para mantener homogéneo su contenido y también con sensores de alto y bajo nivel, que indicaban la necesidad de reponer su contenido. Al iniciar la operación, las corrientes de alimentación se debían alinear hacia el destino deseado. El caudal deseado de cada corriente se fijaba en un controlador de caudal que actuaba sobre el variador de su bomba respectiva. En los casos en los que el flujo necesitaba ser repartido entre dos destinos diferentes, válvulas manuales de aguja permitían regular los caudales

dedicados a cada destino. Para comprobar la operación de las bombas y de los ajustes realizados se aforaba periódicamente el caudal en el punto de destino.

Las variables principales, como el caudal, pH, oxígeno disuelto y temperatura podían ser monitorizadas en un controlador lógico programable (PLC). El control de oxígeno y pH en reactor y selector era especialmente crítico. El aire se introducía a través de difusores de membrana en el reactor y de un tubo de inyección en el selector. El flujo total suministrado era continuamente monitorizado (Tylan Mod. 2920) y regulado manualmente. Posteriormente, el flujo se bifurcaba entre reactor y selector, siendo el caudal del primero registrado en un medidor Tylan Mod. 2910. El suministro al selector se calculaba por diferencia entre los dos medidores anteriores. El oxígeno disuelto en reactor y selector se medía con sondas galvánicas (Desin, Mod. TM-6679/TCO y HACH, modelo 5740sc, respectivamente) ubicadas en la zona superior de los equipos, las cuales incluían indicación y compensación por temperatura. El pH se ajustaba en valores de 8,2 ±0,2 en el reactor, con ácido sulfúrico 98%, tomando como referencia la lectura de un indicador de pH (HACH, modelo si792xP). Sondas de contraste estaban diariamente disponibles para el pH (Metrohm 826 pH Mobile) y para el oxígeno disuelto (Orion 810-3756), para confirmar las medidas disponibles y detectar la necesidad de recalibración.

El reactor podía ser calentado con una resistencia eléctrica, pero no enfriado. Durante los periodos de test la temperatura en el reactor fue de 32 ± 2 ºC. El selector estaba sólo parcialmente calorifugado y presentó temperaturas de alrededor de 20ºC.

También se establecía una purga del fango concentrado en el fondo del clarificador. Para facilitar un mejor control de la biomasa purgada, esta operación se realizaba en discontinuo sobre un depósito graduado. Se tomaba una muestra del total de purga diaria para analizar el contenido en sólidos en suspensión y biomasa de esta corriente.

El sistema experimental disponía de varios puntos de muestreo. Se podía tomar muestra del selector en su línea de salida hacia el reactor. Las muestras del reactor y de los depósitos de alimentación se tomaban del fondo de los mismos. Todas las corrientes, incluyendo el reciclo de fangos, podían ser muestreadas en su punto de destino final.

Como metodología de trabajo, en primer lugar se operó la planta piloto como RTMP, para confirmar la tendencia de los sistemas petroquímicos al *bulking* filamentoso. A continuación, se incluyeron secuencialmente las configuraciones de selector anóxico y aerobio para seleccionar la más favorable para la decantación del fango. Una vez

identificada, se programaron ensayos para optimizar sus parámetros operativos y de diseño. En base a referencias bibliográficas, los parámetros del selector considerados fueron la relación F/M (Eckenfelder and Cleary, 2013) y TRH (Henze *et al*., 2008). También se evaluó cuáles eran las corrientes residuales óptimas para ser alimentadas al selector y cuál era su impacto en el comportamiento del mismo. Para ello se evaluaron dos variables de las corrientes influentes: la biodegradabilidad y el contenido en materia orgánica particulada. Estos ensayos se realizaron variando los caudales de las corrientes alimentadas al selector, así como su volumen de trabajo. Finalmente, mediante un diseño experimental, se organizaron periodos de prueba para comprobar el efecto de los parámetros del licor mezcla en la decantación del fango, para el sistema de fangos activos incluyendo un selector.

La duración de todos los ensayos fue como mínimo el doble del tiempo de retención celular (Palm *et al*., 1980).

### 14.4.2. Descripción y operación del montaje experimental a escala laboratorio

El montaje experimental consistía en dos biorreactores de cinco litros que operaban en paralelo, a los cuales se suministraban dosis diferentes de ácido fólico, en comparación con otro reactor de control. Inicialmente, los biorreactores se inoculaban con un volumen de un litro de licor mezcla, procedente de un proceso industrial petroquímico. Estos eran a su vez, oxigenados y agitados por un aporte de aire realizado a través de un distribuidor multiorificio, hasta conseguir un residual de oxígeno en el licor mezcla superior a 2 mg $L^{-1}$. Antes de ser introducido en los biorreactores, el aire era forzado a través de un borboteo con agua con objeto de evitar evaporación en los biorreactores. A los tres reactores se les alimentaba la misma corriente residual (E), una solución ácida para ajustar su pH y una solución tamponada de carbonatos a pH de 9,0. En los biorreactores suplementados con ácido fólico, la vitamina se consumía en polvo y se disolvía en esta solución tamponada. La concentración de ácido fólico aportada a los reactores era confirmada analíticamente. La dosificación de todas las corrientes a los tres reactores se realizó mediante una bomba peristáltica multicanal Watson Marlow 205 C, que operaba a un caudal constante de 7 mL $h^{-1}$ por cada canal.

Los ensayos se realizaron en dos fases. Inicialmente, se suplementaron concentraciones de ácido fólico de 0,4 y 0,9 mg $g^{-1}$VSS $d^{-1}$ a respectivos biorreactores, en comparación con el reactor de control, al cual no se le suministró vitamina. A continuación, se interrumpió la dosificación de ácido fólico a los dos biorreactores inicialmente

suplementados. En estos ensayos se caracterizó el efecto del ácido fólico en la nitrificación biológica y también en los parámetros operativos del sistema de fangos activos.

### 14.4.3. Sustrato e inóculo

Tres corrientes residuales petroquímicas (A, B y C) se suministraron a la planta piloto para ensayar la efectividad de un selector para superar el *bulking* filamentoso. Los parámetros principales de estas corrientes se incluyen en la Tabla 14.1. Las corrientes de alimentación A y B provenían de procesos de oxidación avanzada, motivo por el cual mostraban una elevada concentración de carbono inorgánico. Las corrientes A y B eran las más biodegradables, por lo que se alimentaban al selector cuando éste estaba en servicio. La corriente C era una corriente no pre-tratada, lo que explica su baja biodegradabilidad.

Tabla 14.1. Caracterización de las corrientes residuales e inóculo empleados en la planta piloto

| | | CORRIENTES DE ALIMENTACIÓN | | | INÓCULO DE LICOR MEZCLA |
|---|---|---|---|---|---|
| | | A | B | C | |
| PARÁMETRO | UNIDAD | PROMEDIO (RANGO) | | | |
| pH | | 8,1 (8,0-8,2) | 8,5 (8,2-8,5) | 4,0 (2,3- 6,5) | 8,2 (8,0-8,5) |
| TIC | $g\ L^{-1}$ | 3,2 (2,7-3,5) | 1,5 (1,3-1,6) | 0,1 (0,05- 0,2) | 2,7 (2,6-2,9) |
| Conductividad | $mS\ cm^{-1}$ | 33 (30-35) | 18(16-20) | 22 (20-24) | 21 (18-23) |
| TOC | $g\ L^{-1}$ | 17,1 (16,0-19,5) | 2,5 (1,5-2,7) | 3,.3 (2,2- 4,5) | 0,1 (0,07-0,15) |
| sDQO | $g\ L^{-1}$ | 50,0 (41,7-59,6) | 6,2 (4,5-7,5) | 8,8 (5,5-12,1) | 0,25 (0,20-0,30) |
| DQO | $g\ L^{-1}$ | 50,6 (47,3-60,2) | 31,2 (19,5-62,5) | 12,1(6,5-15,6) | 0,3 (0,2-0,4) |
| sDQO/TOC | $g\ g^{-1}$ | 2,9 (2,9-3,1) | 2,8 (2,5-3,0) | 2,9 (2,5-3,1) | 2,7 (2,5-3,0) |
| $DBO_5$/DQO | $g\ g^{-1}$ | 0,35 (0,10-0,45) | 0,18 (0,11-0,25) | 0,10 (0,.01-0,15) | 0,05 (0,01-0,08) |
| $NH_4^+$-N | $mg\ L^{-1}$ | <1 | 1200 (750-1500) | <1 | 2 |
| Norgánico | $mg\ L^{-1}$ | <1 | <1 | <1 | <1 |
| $NO_3^-$-N | $mg\ L^{-1}$ | <1 | <1 | <1 | <1 |
| $PO_4^{3-}$-P | $mg\ L^{-1}$ | <1 | 75 (55-85) | <1 | 2 |
| TSS | $g\ L^{-1}$ | 0,4 (0,2-0,6) | 21,3 (20,1-25,6) | 3,2 (3,0-4,3) | 12,0 (10,1-17,2) |
| VSS | $g\ L^{-1}$ | 0,03 (0,02-0,04) | 0,1 (0,0-0,2) | 0,01 (0,00-0,.02) | 3,3 (2,5-3,5) |
| Inertes | $g\ L^{-1}$ | <0,01 | 11,0 (10,1-12,8) | <0,01 | 3,3 (2,5-3,5) |

Acrónimos:

| | | | |
|---|---|---|---|
| TIC | Carbono total inorgánico | TOC | Carbono orgánico total |
| DQO | Demanda química orgánica total | sDQO | Demanda química orgánica soluble |
| $DBO_5$ | Demanda biológica de oxígeno a 5 días | $NH_4^+$-N | Nitrógeno amoniacal |
| NTK | Nitrógeno Kjeldhal | $NO_3^-$N | Nitrógeno en forma de nitrato |
| $PO_4^{3-}$-P | Fósforo como ortofosfato | TSS | Sólidos en suspensión totales |
| VSS | Sólidos en suspensión volátiles | | |

Los compuestos dominantes en las corrientes A y B eran moléculas cortas oxidadas (principalmente alcoholes en concentración inferior al 1%p y ácidos orgánicos en concentración inferior al 5%p), mientras que la corriente C contenía variedad de compuestos orgánicos, incluyendo los de las corrientes anteriores y también trazas de moléculas alifáticas y aromáticas. La composición de las corrientes A y B fue constante, mientras que la corriente C experimentó cierta variabilidad.

El inóculo utilizado en estos ensayos provenía de un reactor RTMP industrial, el cual exhibía valores medios de índice volumétrico de fangos diluido (IVFD) de 500 mL $g^{-1}$.

En los ensayos de laboratorio, se utilizó como sustrato la corriente residual E, la cual era mezcla de las corrientes A y D, con una proporción volumétrica de 7,5: 92,5, respectivamente. La corriente residual D había sido ya pre-tratada en un tratamiento biológico. La mezcla se suplementaba con disolución de sulfato amónico 20% p y ácido fosfórico 75%p para mantener un residual superior a 2 mg $L^{-1}$ de nitrógeno amoniacal y de fósforo como ortofosfato en el licor mezcla. Dado que la corriente de alimentación contenía materia orgánica particulada, esta fue filtrada a 20 μc previamente a ser introducida en los biorreactores, para evitar variabilidad en su contenido de materia orgánica e interferencia en el análisis de sólidos en suspensión volátiles. La Tabla 14.2 refleja los parámetros principales de la corriente de alimentación filtrada y del inóculo empleado en los biorreactores de laboratorio. Para estos ensayos, adicionalmente se preparó una solución ácida por adición de ácido sulfúrico 98%p a agua desmineralizada, con objeto de ajustar el pH de los reactores biológicos a 8,0 ± 0,3.

Tabla 14.2. Caracterización de la corriente residual petroquímica y del inóculo utilizados en los ensayos de laboratorio con ácido fólico

| *Parámetro* | *Descripción* | *Unidades* | *Alimentación* | *Inóculo* |
|---|---|---|---|---|
| DQO | Demanda química de oxígeno | mg $L^{-1}$ | 4490,7 ± 816,3 | 177,0 ± 1,4 |
| TOC | Carbón orgánico total | mg $L^{-1}$ | 1750,2 ± 296,2 | 100,0 ± 28,2 |
| $DBO_5$ | Demanda biológica de oxígeno | mg $L^{-1}$ | 808,3± 343,5 | - |
| TIC | Carbono inorgánico total | mg $L^{-1}$ | 1335,3 ± 588,9 | 992,5 ± 95,4 |
| $NH_4^+$-N | Nitrógeno amoniacal | mg $L^{-1}$ | 416,1 ± 66,6 | 1,9 ± 0,1 |
| TSS | Sólidos en suspensión totales | g $L^{-1}$ | - | 1,67 ± 0,05 |
| VSS | Sólidos en suspensión volátiles | g $L^{-1}$ | - | 1,24 ± 0,04 |
| pH | pH | | 8,12 ± 0,04 | 8,14 ± 0,02 |
| IVF | Índice volumétrico de fangos | mL $g^{-1}$ | - | 87,7 ± 24,7 |

### 14.4.4. Ácido fólico: manipulación y estabilidad

Para evitar problemas de estabilidad con formas líquidas del ácido fólico, se ha empleado un formato sólido (Vignesh *et al*., 2012). En la experimentación se han usado pastillas comerciales ACFOL con un contenido de 5 mg de ácido fólico, fabricadas por ITALFARMACO (Número batch: EKA 7399 Caducidad: 03 2022; Número batch: EKA 7405 Caducidad: 06 2022; Número batch: EKA 7415 Caducidad: 08 2022). El ácido fólico es una vitamina soluble en agua, pero inestable debido al cambio de pH (Vignesh *et al*., 2012). Para resolver este aspecto, el ácido fólico se disolvió en una disolución tampón de carbonatos a pH de 9,0 con objeto de favorecer su estabilidad y solubilidad (Gazzali *et al*., 2016) y también de permitir su determinación (Matias *et al*., 2014). Se prepararon disoluciones tamponadas a diferentes concentraciones de ácido fólico, dentro del rango 1,0 a 15,0 mg $L^{-1}$, de acuerdo con la concentración requerida en el licor mezcla. La Tabla 14.3 refleja las características analíticas de la solución tampón de ácido fólico.

Tabla 14.3. Caracterización de la solución tamponada de ácido fólico

| Concentración de ácido fólico en la solución tamponada mg $L^{-1}$ | sDQO mg $L^{-1}$ | $DBO_5$ mg $L^{-1}$ | $DBO_5$/sDQO |
|---|---|---|---|
| 7,0 | 218 | 34,65 | 0,16 |
| 15,0 | 524 | 68,75 | 0,13 |

Las premisas para la manipulación del ácido fólico se establecieron a partir de consideraciones de estabilidad del producto. La bibliografía indica que la degradación del ácido fólico se ve acentuada por factores como la luz, oxígeno y pH (Gazzali *et al*., 2016). Para evitar el efecto de la luz, la preparación diaria de ácido fólico se almacenaba en un vaso de precipitados cubierto por papel de plata. La solución se mantenía a temperatura ambiente, lo cual según Tripet and Kesselring (1975) no producía una descomposición significativa del ácido fólico. Para evitar la oxidación del ácido fólico, no se pudo implementar la recomendación de Day and Gregory (1983) de trabajar en atmósfera inerte. Como alternativa, se incidió en la preparación diaria de solución fresca. Ocasionalmente, en fines de semana se empleó la sugerencia de Liang *et al*. (2013) de añadir antioxidante (vitamina C).

### 14.4.5. Métodos analíticos

Todos los métodos analíticos empleados eran ASTM. La demanda química de oxígeno total y soluble fue analizada por digestión y posterior espectrofotometría con un equipo HACH DR/2010 a una longitud de onda de 600 nm. El carbono orgánico total y el carbono inorgánico total fueron analizados con un equipo Shimadzu TOC 5050 A. El pH en el laboratorio, fue determinado con una sonda Orion modelo 710 A. El nitrógeno amoniacal se determinó por electrodo selectivo (Orion 95-12), mientras que el ortofosfato se cuantificó por espectrofotometría UV-VIS (Shimadzu Compact UV-2600) a longitud de onda de 880 nm. Los nitritos y nitratos se analizaron por cromatografía iónica (761 Compact IC metrohm). Las respirometrías se realizaron con un respirómetro BM-T Surcis, equipado con una unidad termostática MD40.

Las analíticas de sólidos en suspensión y sólidos en suspensión volátiles se realizaron por métodos gravimétricos. El objetivo de la segunda era caracterizar la cantidad de biomasa presente en el sistema. No obstante, en esta analítica, para los ensayos de la planta piloto (donde la alimentación no estaba pre-filtrada) se observaron desviaciones positivas debidas a la presencia de materia orgánica particulada. Por tanto, para determinar la biomasa, se utilizó un método alternativo por nitrógeno Kjeldhal, teniendo en cuenta que ninguna de las corrientes residuales aportaba nitrógeno orgánico. Para caracterizar el nitrógeno Kjeldhal se utilizó un Büchidigestor modelo 425, con una unidad de destilación Büchi, modelo 323.

La decantación del fango se caracterizó macroscópicamente mediante el índice volumétrico de fangos y el índice volumétrico de fangos diluido, según procedía. En la investigación realizada en la planta piloto se emplearon volúmenes de 1 litro de licor mezcla para realizar este test, según marcan las normas. No obstante, en los ensayos de laboratorio, por disponibilidad, el volumen utilizado fue únicamente de 25 mL, según la metodología empleada por Alexandre *et al*. (2016). En consecuencia, los resultados obtenidos fueron únicamente válidos para ser comparados entre sí en este ensayo. La calidad del fango también fue evaluada microscópicamente, utilizando un microscopio Nikon Eclipse 80 i a 100X y 400X, por iluminación directa y por contraste de fases.

Para cuantificar el ácido fólico se utilizó la metodología desarrollada por Matias *et al.* (2014) por espectrofotometría UV-VIS a longitud de onda 282,5 nm para concentraciones de entre 1,0 y 17,5 mg $L^{-1}$. En la determinación realizada sobre el licor mezcla, adicionalmente se empleó el método de adiciones estándar para evitar interferencias de la matriz.

### 14.4.6. Evaluación de la toxicidad aportada por el ácido fólico en el agua efluente

Para comprobar la inhibición del ácido fólico en la actividad de las bacterias heterótrofas se realizaron ensayos de respirometría, según las pautas presentadas en la Tabla 14.4. Cada test se repitió cinco veces con diferentes muestras de licor mezcla, para tener en cuenta la variabilidad del inóculo.

Tabla 14.4. Condiciones para evaluar la toxicidad a corto plazo del ácido fólico en las bacterias heterótrofas por respirometría

| Test respirometría | Aportes | | Licor mezcla [2] |
|---|---|---|---|
| | Alimento | FA [1] | |
| | mL | mL | mg FA $L^{-1}$ |
| 1 | 12 | 0 | 0,0 |
| 2 | 12 | 8 | 0,5 |
| 3 | 12 | 17 | 1,0 |
| 4 | 12 | 33 | 2,0 |
| 5 | 12 | 50 | 3,0 |
| 6 | 12 | 67 | 4,0 |

[1] solución tamponada de ácido fólico a 60 mg $L^{-1}$

[2] Concentración de ácido fólico en el licor mezcla

La toxicidad crónica provocada por el ácido fólico en el efluente también fue evaluada en cada periodo de operación. El método utilizado fue Microtox, basado en la inhibición de la bioluminescencia de Vibrio Fischeri (formalmente conocida como Photobacterium Phosphorum, NRRL, B-11177). Las muestras utilizadas para evaluar la toxicidad se obtuvieron del licor mezcla de los reactores y se centrifugaron a 3000 rpm durante 10 minutos para eliminar la turbidez. Puesto que la toxicidad puede cambiar con el tiempo, las muestras se congelaron hasta que el análisis se realizó. En algunas muestras se requirió ajuste de pH a valores neutros.

### 14.4.7. Técnicas estadísticas

Para concluir sobre diferencias significativas entre resultados se han utilizado los tests T y F con un nivel de confianza del 95%, para la comparación de medias y varianzas, respectivamente. Como software de apoyo se utilizó *Statgraphics Plus* para Windows 3.3 (1994-1998).

## 14.5. DISCUSIÓN DE RESULTADOS Y CONCLUSIONES

### 14.5.1. Efecto del aceptor de electrones del selector en la decantación del fango

La experimentación se inició con un ensayo de blanco, configurando la planta piloto como RTMP, con objeto de demostrar la tendencia de los sistemas petroquímicos al *bulking* filamentoso por baja relación F/M. Se llevaron a cabo cinco periodos de operación con relación F/M entre 0,10 y 0,40 g DQO $g^{-1}$ VSS $d^{-1}$. En todos ellos se obtuvieron valores de IVF superiores a 250 mL $g^{-1}$, observándose sólo una ligera mejora al aumentar la relación F/M dentro del rango estudiado. Se caracterizaron los filamentos obtenidos, con una presencia dominante de Tipo 0092 y M. Parvicella. Estas especies corresponden a condiciones de baja relación F/M y la bibliografía reporta que pueden ser eliminadas con la implementación de selectores anóxicos o aerobios (Jenkins *et al.*, 2003). Adicionalmente, en estos ensayos se evidenció que al bajar la relación F/M se incrementaba la nitrificación biológica no deseada (ver Figura 14.3).

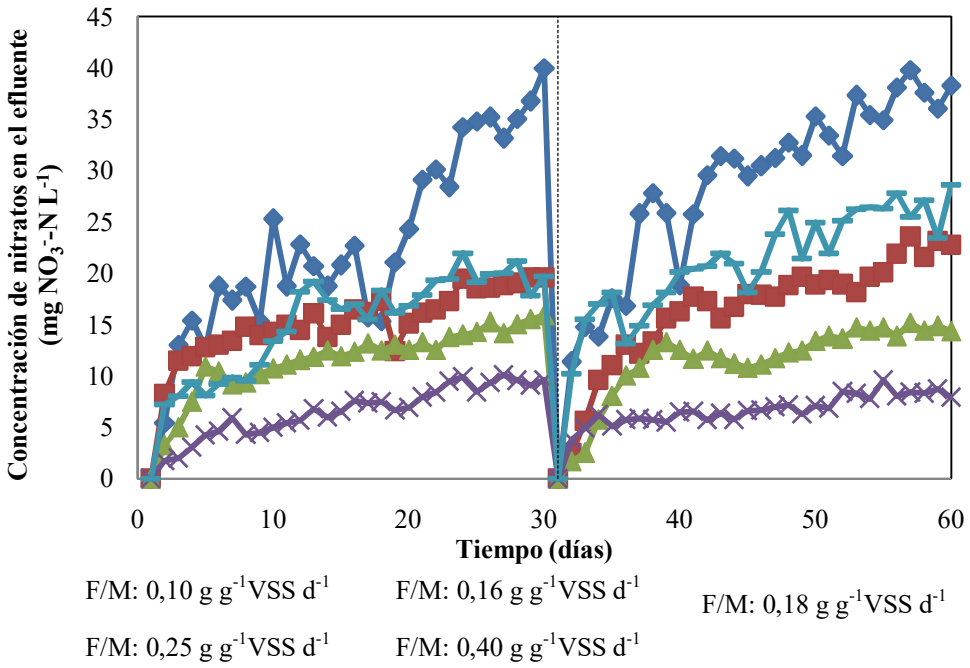
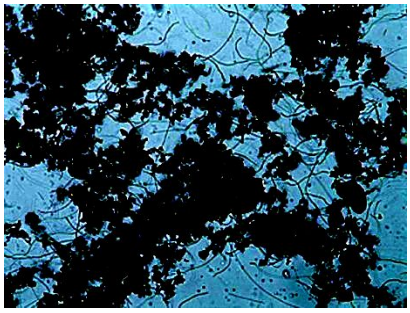



Figura 14.3. Monitorización de la evolución de la concentración de nitratos en el efluente del RTMP piloto para diferentes relaciones F/M. La línea punteado indica re-inoculación

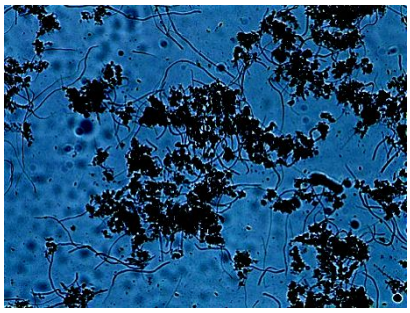
A continuación, se introdujo el selector en la configuración de la planta piloto. Primero fue operado como anóxico y posteriormente como aerobio. Para poder operar el

selector, se empezó por realizar unos ensayos preliminares en los que se concluyó que su F/M adecuada era de 30 g DQO $g^{-1}$VSS $d^{-1}$. En cuanto a tiempo de residencia, se utilizaron valores de 45 minutos para el selector anóxico (Jenkins *et al*., 2003) y 30 minutos para el selector aerobio (Metcalf and Eddy, 2003), siguiendo recomendaciones bibliográficas.

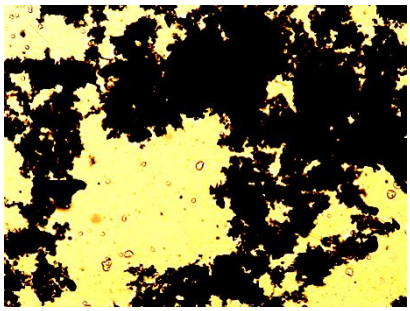
Con estas condiciones, en ambos casos se consiguió reducir considerablemente el valor de IVF, obteniendo valores promedios de 80 y 45 mL $g^{-1}$. No obstante, la operación del selector aerobio fue más robusta que la del anóxico. Con esta última, la nitrificación obtenida en el reactor biológico fue inestable, lo que motivó una concentración variable de nitratos en el selector, que se tradujo en valores de índice volumétrico de fangos variables, con algunos resultados superiores a 100 mL $g^{-1}$. La Figura 14.4 muestra imágenes comparativas de las observaciones microscópicas realizadas sobre el licor mezcla de las tres configuraciones ensayadas. Mientras que con el RTMP se registró la presencia de abundantes filamentos largos entrecruzados, con la inclusión del selector anóxico se redujo la abundancia y la longitud de los filamentos. El selector aerobio proporcionó un fango compacto, con escasos filamentos fuera del flóculo.



(a) 380 (300-450) mL $g^{-1}$



(b) 80 (69-270) mL $g^{-1}$



[1]

(c) 45 (30-75) mL $g^{-1}$

Figura 14.4. Imágenes (100x) del flóculo obtenido con (a) RTMP (b) inclusión de selector anóxico (c) inclusión de selector aerobio. Debajo de cada imagen se indica el valor promedio de IVF obtenido y entre paréntesis el rango de valores

Para confirmar el funcionamiento efectivo del selector, además de la decantación del fango, se evaluaron también los parámetros operacionales de selector, reactor y conjunto de sistema de fangos activos (ver resumen de resultados en Tabla 14.5).

Tabla 14.5. Parámetros operacionales del sistema de fangos activos operado como sin selector (RTMP), con selector anóxico y con selector aerobio

| **PARÁMETRO** | **UNIDADES** | **RTMP** | **SELECTOR ANÓXICO +RTMP** | **SELECTOR AEROBIO +RTMP** |
|---|---|---|---|---|
| IVF | mL $g^{-1}$ | 380 (300-450) | 80 (69-270) | 45 (30-75) |
| **SELECTOR** | | | | |
| Eficiencia eliminación BOD | % | | 87,6 (86,9-88,0) | 80,7(0,0-82,7) |
| Eficiencia eliminación DQO | % | | 42,0 (38,9-44,4) | 35,5(0,0-42,3) |
| $N_{ASSIM}$ | g $NH_4^+$-N asimilado $g^{-1}$ COD eliminado | | 0,016 (0,002-0,049) | 0,013 (0,007-0,039) |
| $P_{VSS}$ | g VSS producido $g^{-1}$ COD eliminado | | 0,30 (0,19-0,38) | 0,57 (0,09-0,69) |
| $N_{ASSIM}/P_{VSS}$ | g $NH_4^+$-N asimilado $g^{-1}$ VSS producido | | 0,05 | 0,02 |
| **REACTOR** | | | | |
| OUR | mg $O_2$ $L^{-1}$ $h^{-1}$ | 51,0 | 62,7 | 30,0 |
| Eficiencia eliminación DQO | % | 99,1 (99,0-99,3) | 99,2 (99,1-99,6) | 99,2(99,1-99,6) |
| $N_{ASSIM}$ | g $NH_4^+$-N asimilado $g^{-1}$ COD eliminado | 0,030 (0,027-0,031) | 0,015 (0,014-0,020) | 0,019(0,010-0,025) |
| $P_{VSS}$ | g VSS producido $g^{-1}$ COD eliminado | 0,32 (0,31-0,37) | 0,20 (0,09-0,22) | 0,28 (0,11-0,48) |
| $N_{ASSIM}/P_{VSS}$ | g $NH_4^+$-N asimilado $g^{-1}$ VSS producido | 0,09 | 0,08 | 0,07 |
| **SISTEMA DE FANGOS ACTIVOS GLOBAL** | | | | |
| Eficiencia en eliminación DQO | % | 99,1 (99,0-99,.3) | 99,2 (98,5-99,2) | 99,2(98,5-99,2) |
| $N_{ASSIM}$ | g $NH_4^+$-N asimilado $g^{-1}$ COD eliminado | 0,030 (0,027-0,031) | 0,015 (0,013-0,034) | 0,019 (0,018-0,036) |
| $P_{VSS}$ | g VSS producido $g^{-1}$ COD eliminado | 0,32 (0,31-0.37) | 0,23 (0,20-0,25) | 0,41(0,36-0,56) |
| $N_{ASSIM}/P_{VSS}$ | g $NH_4^+$-N asimilado $g^{-1}$ VSS producido | 0,09 | 0,07 | 0,05 |

Con ambas configuraciones de selector, se cuantificaron elevadas eficiencias de degradación de DBO en el selector, tal y como requiere la literatura (Jenkins *et al*., 2003), y valores de eficiencia de degradación de DQO en el rango de 35-40%.

En el selector, hubo consumo de nitrógeno amoniacal, caracterizado con el parámetro $N_{ASSIM}$, el cual era indicativo de formación de nuevas células bacterianas. Por otra parte, la producción observada de VSS con respecto a la DQO consumida fue cuantificada mediante el parámetro $P_{VSS}$. Con ello, se calculó en los selectores un ratio $N_{ASSIM}$ / $P_{VSS}$, el cual se comparó con el valor estequiométrico de 0,12 correspondiente a la consideración de la célula de biomasa como $C_5H_7NO_2$. Puesto que los resultados obtenidos fueron inferiores al valor de referencia, se interpreta que en el selector se produjo acumulación de sustrato. Estos resultados son indicativos del buen funcionamiento del selector, según las teorías bibliográficas (Gujer and Jenkins, 1975; Van Loosdrecht *et al*., 1997; Beun *et al*., 1999).

Otro resultado destacado fue un ligero incremento en la eficiencia en degradación de materia orgánica obtenida para el sistema global, de acuerdo con la bibliografía (Munirathinam, 2003). No obstante, el crecimiento bacteriano observado en el reactor se redujo, con un consiguiente incremento en la edad del fango. Ésta pudo ser la causa de un incremento en la nitrificación biológica no deseada (Flores-Alsina *et al*., 2010) con la inclusión del selector aerobio.

De esta primera fase de ensayos se concluyó que la implementación de un selector aerobio en el sistema petroquímico de fangos activos era la opción más efectiva y robusta de superar la fuerte tendencia al *bulking* filamentoso. La inclusión del selector aumentó la eficiencia en eliminación de materia orgánica del sistema de fangos pero provocó un incremento en la nitrificación no deseada.

### 14.5.2. Optimización de parámetros de diseño y operación del selector aerobio

Una vez confirmada la efectividad del selector aerobio para controlar el *bulking* filamentoso se procedió a programar ensayos para la optimización de sus parámetros operativos y de diseño. En base a referencias bibliográficas, los parámetros del selector considerados fueron la relación F/M (Eckenfelder and Cleary, 2013) y TRH (Henze *et al*., 2008). También se evaluó, dentro de las posibles corrientes residuales disponibles (A, B y C), cuáles eran las óptimas para ser alimentadas al selector y cuál era su impacto en el comportamiento del mismo. De esta forma se caracterizó el impacto de

dos variables en la calidad de las corrientes alimentadas al selector: la biodegradabilidad y el contenido en materia orgánica particulada.

Para analizar el efecto del ratio F/M y del TRH del selector en la decantación del fango, se desarrollaron 7 periodos experimentales en la planta piloto, con las características operativas del selector que indica la Tabla 14.6.

En todos los ensayos realizados se obtuvo asimilación de materia orgánica y consumo de nitrógeno amoniacal en el selector. Igual que en el caso anterior se evaluó el ratio $N_{ASSIM}/P_{VSS}$, siendo en todos los casos inferior a 0,12 y sugiriendo así la acumulación de sustrato en el selector, y por tanto, un correcto funcionamiento del mismo.

Los resultados de IVF obtenidos fueron en todos los casos inferiores a 100 mL $g^{-1}$, de acuerdo con las recomendaciones de la literatura (Henze *et al*., 2008), y en coherencia con imágenes microscópicas que mostraban una reducción en la presencia de filamentos con respecto del RTMP. Por tanto, los resultados fueron indicativos de que el selector estaba siendo efectivo para mejorar la decantación del fango en todas las condiciones ensayadas.

No obstante, tal y como indican las referencias (Mangrum, 1998; Albertson, 2005), se caracterizó un valor óptimo para la concentración de equilibrio de sustrato en el selector en 2,6 g $L^{-1}$ (periodo 3), para el cual se obtuvo el valor mínimo de IVF, con diferencia estadísticamente significativa respecto del resto para un nivel de confianza del 95%. Este periodo también consiguió la máxima eficiencia en degradación de DQO y $DBO_5$, lo que es considerado como un requisito para establecer el ciclo alimentación-ayuno en el sistema de fangos activos, en el cual se fundamenta el éxito del selector para mejorar la calidad de la biomasa (Patoczka and Eckenfelder, 1990; Tandoi *et al*., 2006; Grady *et al*., 2011). Este resultado confirma el concepto que introdujeron Van Niekerk *et al*. (1988) y que posteriormente confirmaron Tandoi *et al*. (2006) de que existe un tamaño óptimo de selector, en función de la concentración de materia orgánica en la corriente influente y del caudal. En condiciones correspondientes a mayor concentración de equilibrio de sustrato, la eficiencia del selector se reduce y una mayor porción de sustrato soluble va a parar al reactor, favoreciendo el crecimiento de bacterias filamentosas. Por otra parte, si el selector se sobredimensiona y ocasiona concentraciones de equilibrio de sustrato inferiores al óptimo, no hay suficiente gradiente de sustrato para inducir una asimilación rápida del mismo, lo que conduce a que grandes cantidades de sustrato soluble pasen igualmente al reactor, posibilitando el crecimiento de filamentos (Tandoi *et al*., 2006).

Tabla 14.6 Datos operacionales del selector aerobio, incluido en el sistema piloto de fangos activos, para su configuración con diferentes valores de TRH y F/M

| Parámetros | Unidades | PERIODO 1 | PERIODO 2 | PERIODO 3 | PERIODO 4 | PERIODO 5 | PERIODO 6 | PERIODO 7 |
|---|---|---|---|---|---|---|---|---|
| TRH[1] | min | 15 | 20 | 30 | 60 | 20 | 30 | 60 |
| F/M[2] | g DQO $g^{-1}$ VSS $d^{-1}$ | 37,5 ± 3,5 | 37,6 ± 5,2 | 34,1 ± 5,1 | 35,4 ± 4,3 | 16,4 ± 1,1 | 10,9 ± 0,7 | 12,0 ± 1,7 |
| DQO EC[3] | g $L^{-1}$ | 3,3 ± 0,.9 | 3,1 ± 0,3 | 2,6 ± 0,1 | 1,6 ± 0,5 | 2,2 ± 0,1 | 1,5 ± 0,1 | 0,8 ± 0,1 |
| DQO RE[4] | % | 26,2 ± 4,9 | 29,2 ± 4,5 | 37,4 ± 7,8 | 33,1 ± 1,6 | 13,3 ± 6,4 | 17,6 ± 0,7 | 17,2 ± 2,7 |
| DBO RE[5] | % | 88,7 ± 3,5 | 91,6 ± 0,7 | 95,1 ± 1,1 | 88,5 ± 3,3 | 86,7 ± 1,4 | 90,3 ± 0,6 | 87,4 ± 1,4 |
| $N_{ASSIM}$[6] | g $NH_4^+$-N asimilado $g^{-1}$ DQO consumida | 0,0447 ± 0,0015 | 0,0349 ± 0,0010 | 0,0238 ± 0,0015 | 0,0193 ± 0,0009 | 0,0143 ± 0,0010 | 0,0103 ± 0,0012 | 0,0059 ± 0,0007 |
| $P_{VSS}$[7] | g VSS producido $g^{-1}$ DQO consumida | 0,56 ± 0,02 | 0,44 ± 0,01 | 0,34 ± 0,02 | 0,32 ± 0,02 | 0,29 ± 0,02 | 0,26 ± 0,05 | 0,20 ± 0,02 |
| $N_{ASSIM}/P_{VSS}$ | g $NH_4^+$-N asimilado $g^{-1}$ VSS producido | 0,08 | 0,08 | 0,07 | 0,06 | 0,05 | 0,04 | 0,03 |
| IVF[8] | mL $g^{-1}$ | 76,8 ± 4,3 | 56,7 ± 6,1 | 22,0 ± 3,0 | 65,1 ± 6,3 | 65,2 ± 3,4 | 44,8 ± 6,8 | 62,3 ± 3,7 |

[1]Tiempo de retención hidráulico

[2]Ratio alimento-biomasa

[3]Concentración de equilibrio de DQO

[4]Eficiencia en eliminación de DQO

[5]Eficiencia en eliminación de $DBO_5$

[6]Asimilación observada de nitrógeno amoniacal por unidad de sustrato consumido

[7]Producción observada de sólidos en suspensión volátiles por unidad de sustrato consumido

[8]Índice volumétrico de fangos

Como conclusión, se identificaron como condiciones óptimas de diseño del selector un TRH de 30 minutos y un valor F/M de 35 g DQO $g^{-1}$ VSS $d^{-1}$. Nuevamente, la operación del selector aerobio se manifestó robusta para su implementación a escala industrial, puesto que a pesar de no operar en condiciones óptimas fue efectivo para mejorar el IVF, obteniendo siempre valores inferiores a 100 mL $g^{-1}$.

Para comprobar el efecto de la DBO y la materia orgánica particulada influente en el selector, se diseñaron 12 periodos experimentales en base a tres ratios F/M en el selector: F/M basado en la DQO soluble suministrada, F/M (tDQO) calculado con la DQO total alimentada y F/M (DBO) a partir de la $DBO_5$ introducida. En la Tabla 14.7 se presentan los valores empleados para los tres ratios en cada experimento.

Tabla 14.7 Ratios calculados alimento-biomasa para el selector aerobio, en cada periodo de operación del sistema piloto de fangos activos

| Parámetro | F/M | F/M (tDQO) | F/M (DBO) |
|---|---|---|---|
| Unidades | g DQO $g^{-1}$ VSS $d^{-1}$ | g DQO $g^{-1}$ VSS $d^{-1}$ | g DBO $g^{-1}$ VSS $d^{-1}$ |
| Periodo 1 | 10,9±0,7 | 35,0±1,2 | 8,3±0,2 |
| Periodo 2 | 32,9±3,3 | 139,6±7,9 | 19,5±0,5 |
| Periodo 3 | | | 10,4±0,4 |
| Periodo 4 | | 55,8±5,6 | 22,4±1,0 |
| Periodo 5 | | | 23,1±0,8 |
| Periodo 6 | | | 19,5±0,5 |
| Periodo 7 | | | 46,9±1,.2 |
| Periodo 8 | 40,9±3,1 | 233,6±13,4 | 21,9±0,6 |
| Periodo 9 | | 60,0±5,2 | 34,0±1,1 |
| Periodo 10 | | | 20,2±2,2 |
| Periodo 11 | 72,1±7,1 | 83,0±2,9 | 46,9±6,1 |
| Periodo 12 | | 157,1±6,9 | 48,2±1,1 |

Los resultados confirmaron que al aumentar el contenido del influente en materia orgánica particulada se incrementaba la concentración de equilibrio de materia orgánica en el selector excesivamente (Mangrum, 1998), de manera que empeoraba la decantación del fango. En casos extremos, para valores de F/M (tDQO) superiores a 139 g DQO $g^{-1}$ VSS $d^{-1}$, la eficiencia del selector para degradar la materia orgánica descendió a valores próximos a cero, mostrando una eficiencia nula del selector, y por tanto valores de IVF mayores de 100 mL $g^{-1}$. Por el contrario, un incremento en la biodegradabilidad del efluente alimentado, tuvo un impacto positivo en la efectividad

del selector para mejorar la decantación. De forma coherente, al aumentar biodegradabilidad en el influente del selector, se incrementó su eficiencia en degradación de materia orgánica (ver Figura 14.6).

Como conclusión, para potenciar la capacidad del selector de mejorar la decantación del lodo en el sistema de fangos activos, es conveniente suministrarle las corrientes disponibles de mayor biodegradabilidad. En el sistema estudiado, esta observación corresponde a las corrientes residuales A y B, las cuales son las que presentan mayor biodegradabilidad. No obstante, como la corriente B adicionalmente tiene un alto contenido en materia orgánica particulada, la cantidad alimentada de este efluente se debe limitar en función de su DQO total, para no alcanzar los ratios F/M (tDQO) que se han identificado como excesivos para permitir la acción del selector.

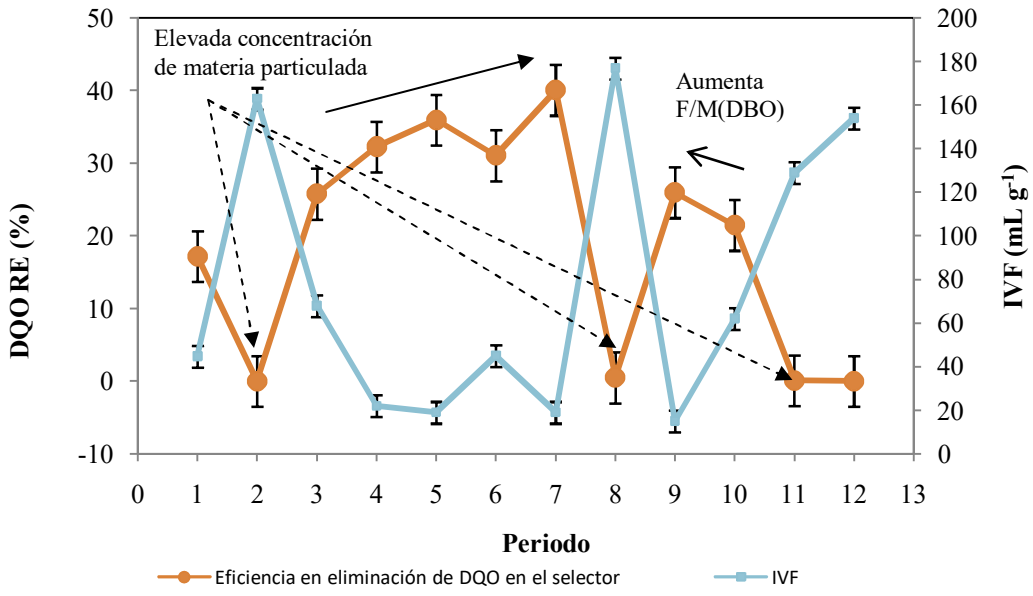



Figura 14.6 Efecto de biodegradabilidad (F/M (BOD)) y materia orgánica particulada (F/M(tCOD)) del influente del selector en su capacidad para mejorar la decantación del lodo. Se indica el índice volumétrico de fangos (IVF) y eficiencia en eliminación de DQO del selector (DQO RE) para cada periodo.

### 14.5.3. Efecto de los parámetros del licor mezcla en la decantación para un sistema de fangos activos con selector

Con objeto de completar la optimización del sistema de fangos activos para mejorar la decantación del lodo, se programan ensayos experimentales para concretar los valores óptimos de oxígeno disuelto (DO), F/M, concentración de nitrógeno amoniacal

($NH_4^+$-N), edad del fango (SRT), concentración de sólidos en suspensión (TSS), pH, temperatura y conductividad en el reactor biológico. Existen referencias bibliográficas que dan pautas sobre valores recomendados para estas variables, con el fin de mejorar la decantación en un RTMP. No obstante, la optimización de estos parámetros para un sistema de fangos activos que incluya un selector no dispone de referentes.

Para empezar, se realizó un diseño experimental, configurando la planta piloto como RTMP, y verificando el efecto de los parámetros DO, F/M, $NH_4^+$-N y SRT en el IVF. Los resultados confirmaron teorías previamente descritas en la bibliografía, para el sistema piloto petroquímico. Todas las variables mencionadas presentaron un efecto estadísticamente significativo con un 95% de nivel de confianza sobre la decantación del fango ($p<0,05$), así como sus interacciones duales. De acuerdo con Adonadaga (2015), todo y que el DO es la variable con mayor impacto en el IVF, no es la única variable de proceso que condiciona la decantación. Bajas concentraciones de DO (< 1 mg $L^{-1}$) resultaron en un excesivo crecimiento de bacterias filamentosas y un flóculo disperso, lo cual empeoró la decantación (Amanatidou *et al*., 2015). El mismo efecto se produjo para bajas relaciones F/M (0,2 g DQO $g^{-1}$VSS $d^{-1}$), las cuales van unidas a altas edades del fango (> 22 días) (Gabb *et al*., 1991). La teoría de Young (2006) explicaba que en condiciones de escasez de recursos la forma filamentosa de la bacteria es más competitiva que la floculante, por disponer de mayor ratio área superficial-volumen. En la misma línea, Barbusinski and Koscielniak (1995) demostraron que al aumentar la carga orgánica, aumentaba el tamaño del flóculo. De acuerdo con los resultados obtenidos, Wilén (2010) postuló que a concentraciones bajas de oxígeno la decantación y la calidad del efluente empeoraban. La teoría de Starkey and Karr (1984) proporciona una explicación a estos hechos, indicando que la escasez de oxígeno provoca la inhibición en la producción de exopolímero y también en el crecimiento de población eucariota, la cual consume bacterias libres, reduciendo la turbidez del efluente. Con concentraciones bajas de nitrógeno amoniacal (< 1 mg $L^{-1}$) se obtuvo un flóculo de aspecto viscoso, observándose un incremento en la producción de exopolímero (Peng *et al*., 2003) que resultó en un empeoramiento de la decantación del fango.

A continuación se practicaron 16 periodos experimentales incluyendo el selector aerobio en el sistema de fangos activos, cuyas condiciones respondieron a un diseño experimental para comprobar el efecto de las variables del reactor DO, F/M, $NH_4^+$-N y SRT sobre el IVF. A diferencia del caso anterior, con la inclusión del selector, la única variable que presentó un efecto estadísticamente significativo ($p<0,05$) en la decantación fue el DO. Por tanto, se concluyó que para que el selector fuera efectivo en

la mejora de la decantación, era condición necesaria disponer de un DO suficiente en el reactor, de acuerdo a la carga orgánica alimentada.

Para completar estos ensayos, se evaluó el efecto de TSS, pH, temperatura y conductividad en el IVF, realizando ensayos de laboratorio con licor mezcla procedente del sistema de fangos activos piloto operado con selector aerobio. En la bibliografía se recoge como estos parámetros afectan a la decantación en un RTMP (Luque, 2005; Bayo *et al*., 2006; Winkler *et al*., 2012; Yahya *et al*., 2012). Los resultados concluyeron que a pesar de la implementación del selector, el efecto de estos parámetros en la decantación del fango seguía siendo estadísticamente significativo.

Se comprobó como la velocidad de decantación del fango disminuye al incrementar la concentración de sólidos en suspensión en el licor mezcla. Por tanto, se concluyó que para favorecer la decantación del fango interesa operar el sistema de lodos activos a la mínima concentración necesaria de sólidos en suspensión.

El efecto del pH se comprobó en un rango de entre 8,0 y 9,0 así como el efecto de la temperatura se ensayó entre 20 y 38 ºC. De acuerdo con la teoría de biofloculación de Ghanizadeh and Sarrafpour (2001), se obtuvo mejor decantación para valores más elevados de pH y de temperatura. Esta teoría indica que el pH afecta a la actividad enzimática de las bacterias. Un incremento de pH por encima del punto isoeléctrico hace que se generen cadenas poliméricas más largas, lo que contribuye a formar puentes entre las células bacterianas, mejorando la floculación biológica. Para la temperatura existen dos efectos contrapuestos. Al incrementarse la temperatura, se reduce la viscosidad del exopolímero, lo que empeora la biofloculación. Sin embargo, se reduce también la viscosidad del agua, lo que ayuda a la decantación (Winkler *et al*., 2012).

En cuanto a la conductividad, la cual fue ensayada en el rango de 20 a 60 mS $cm^{-1}$, los resultados evidenciaron que un incremento en esta variable repercutía en un empeoramiento en la decantación del lodo. Estos resultados se explican con la teoría de Winker *et al.* (2012), quienes consideraron determinante el incremento de densidad provocado en el agua por la mayor concentración de sales.

En conclusión, para favorecer la decantación del lodo interesa operar el sistema a valores de pH elevados, dentro del rango permisible para la biodegradación. Temperaturas de 38 ºC han obtenido los resultados más favorables para decantar el fango, no obstante, se debe considerar en el sistema biológico, la disminución en la solubilidad del gas a estas elevadas temperaturas. Por otra parte, de los resultados se

deduce que la elevada concentración de sales de las corrientes A y B afectará negativamente a la decantación, tanto más, cuanto mayor cantidad de estas corrientes se alimente al licor mezcla.

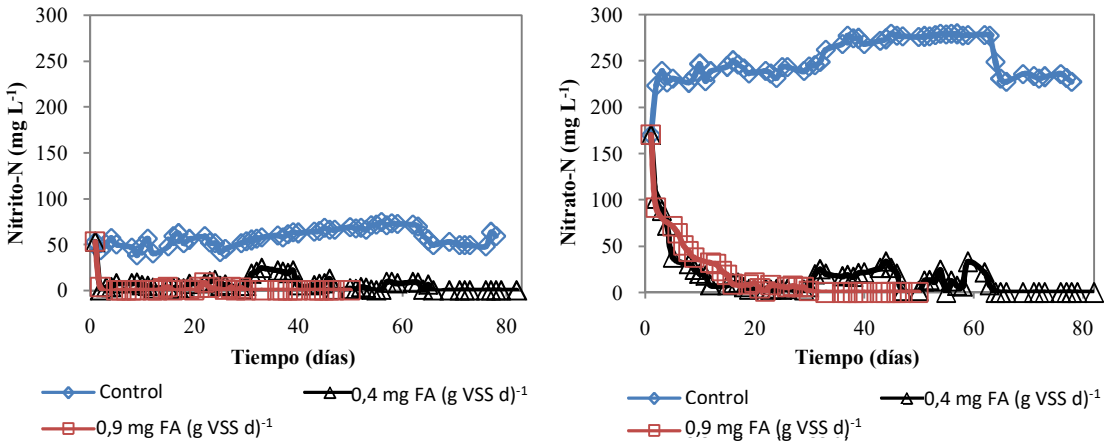
### 14.5.4. Dosificación de ácido fólico al sistema biológico de fangos activos para limitar la nitrificación biológica

Una vez resuelto el *bulking* filamentoso con la implementación de un selector en el sistema de fangos activos, se ha comprobado experimentalmente que la nitrificación biológica no deseada se incrementa. Por tanto, el control de la nitrificación biológica cobra mayor importancia en sistemas de fangos activos que incluyen un selector aerobio. Como solución alternativa a los métodos convencionales, se ha experimentado a escala laboratorio con la adición de ácido fólico al reactor biológico.

En el sistema biológico de estudio coinciden poblaciones bacterianas heterótrofas y autótrofas. Las primeras son responsables de la necesaria biodegradación de la materia orgánica y las segundas provocan la nitrificación no deseada. Por tanto, la vitamina seleccionada debe cumplir la función de limitar la reacción de nitrificación, pero sin afectar negativamente a la capacidad de degradación de materia heterótrofa. Para confirmar en este sentido que la selección de la vitamina era adecuada, se programaron ensayos de respirometría que permitieron verificar que la adición de ácido fólico no inhibía la actividad heterótrofa. En este ensayo, se obtuvieron valores de SOUR similares para el licor mezcla de blanco y para los licores mezcla suplementados con diferentes cantidades de ácido fólico ($208 \pm 66$ mg $O_2$ $g^{-1}$ $h^{-1}$).

Se alimentó ácido fólico en concentraciones de 0,4 y 0,9 mg $g^{-1}$VSS $d^{-1}$ a dos biorreactores de test, en comparación con un reactor de control al que no se suplementaba la vitamina. Durante esta fase, se evidenció la fuerte tendencia a la nitrificación del biorreactor no suplementado, el cual exhibió valores nitrógeno en forma de nitritos y nitratos de hasta 50 y 250 mg $L^{-1}$, respectivamente. Hubo un periodo en el que se experimentó un incremento en la concentración de nitratos, alcanzando valores próximos 300 mg $NO_3^-$-N $L^{-1}$, probablemente debido a una alimentación de menor biodegradabilidad (Celenza, 2000). En los reactores dosificados con ácido fólico, se experimentó un rápido descenso de la concentración de nitritos y nitratos, alcanzando valores inferiores a 10 mg $NO_3^-$N $L^{-1}$ y 10 mg $NO_2^-$N $L^{-1}$. No obstante, mientras que el reactor suplementado con 0,9 mg $g^{-1}$VSS $d^{-1}$ de ácido fólico mantuvo esta tendencia de manera sostenida durante el periodo de duración del ensayo,

el biorreactor suplementado con 0,4 mg $g^{-1}$VSS $d^{-1}$ mantuvo la sensibilidad a la calidad de la alimentación, incrementando ligeramente la nitrificación en el periodo de alimentación de menor biodegradabilidad (ver Figura 14.7).




(a) (b)

Figura 14.7 Monitorización de la concentración de (a) nitrógeno como nitrito y (b) nitrógeno como nitrato en el efluente para los tres biorreactores durante el periodo de adición de ácido fólico

Esta misma tendencia se observó al evaluar la eficiencia en degradación de materia orgánica (Figura 14.8), donde el biorreactor suplementado con 0,9 mg $g^{-1}$VSS $d^{-1}$ de ácido fólico obtuvo valores significativamente superiores ($p<0,05$), de acuerdo con la experiencia de Senorer and Barlas (2004). Como explicación, Strunkheide (2004) postuló la habilidad del ácido fólico para potenciar el metabolismo C-1 ("carbono-uno"), el cual generalmente está limitado por las bajas concentraciones de esta vitamina.

De acuerdo con las observaciones de Stoppa *et al.* (2013), se observaron efectos opuestos con ambas dosificaciones para el IVF. Con la dosis de 0,4 mg $g^{-1}$VSS $d^{-1}$ el IVF se redujo de manera significativa, mientras que con la de 0,9 el IVF se incrementó. En ambos casos, se observó crecimiento epifítico, el cual era indicativo de que los filamentos presentes habían quedado dañados (Wanner, 2014). Por tanto, este resultado confirma la conclusión de Dubé *et al.* (2002) de que el ácido fólico podía ser considerado como una alternativa a la cloración para el control del *bulking* filamentoso. Este hecho podía explicar la mejora en la decantación observada con la menor dosis de ácido fólico. El empeoramiento en la decantación observado en el reactor de mayor dosis de ácido fólico se podría explicar a través de una apariencia viscosa del flóculo. Por microscopía de campo oscuro y de contraste de fases se pudo observar la proliferación de Zooglea Ramígera (Vázquez *et al.*, 2010), en forma de colonias

digitiformes. Wanner (2014) identificó el alto gradiente de carga orgánica como posible causa de incremento de viscosidad en el flóculo. Por tanto, el valor de IVF obtenido pudo venir determinado por una más rápida asimilación de la materia orgánica, dada por la mayor eficiencia en eliminación de materia orgánica.

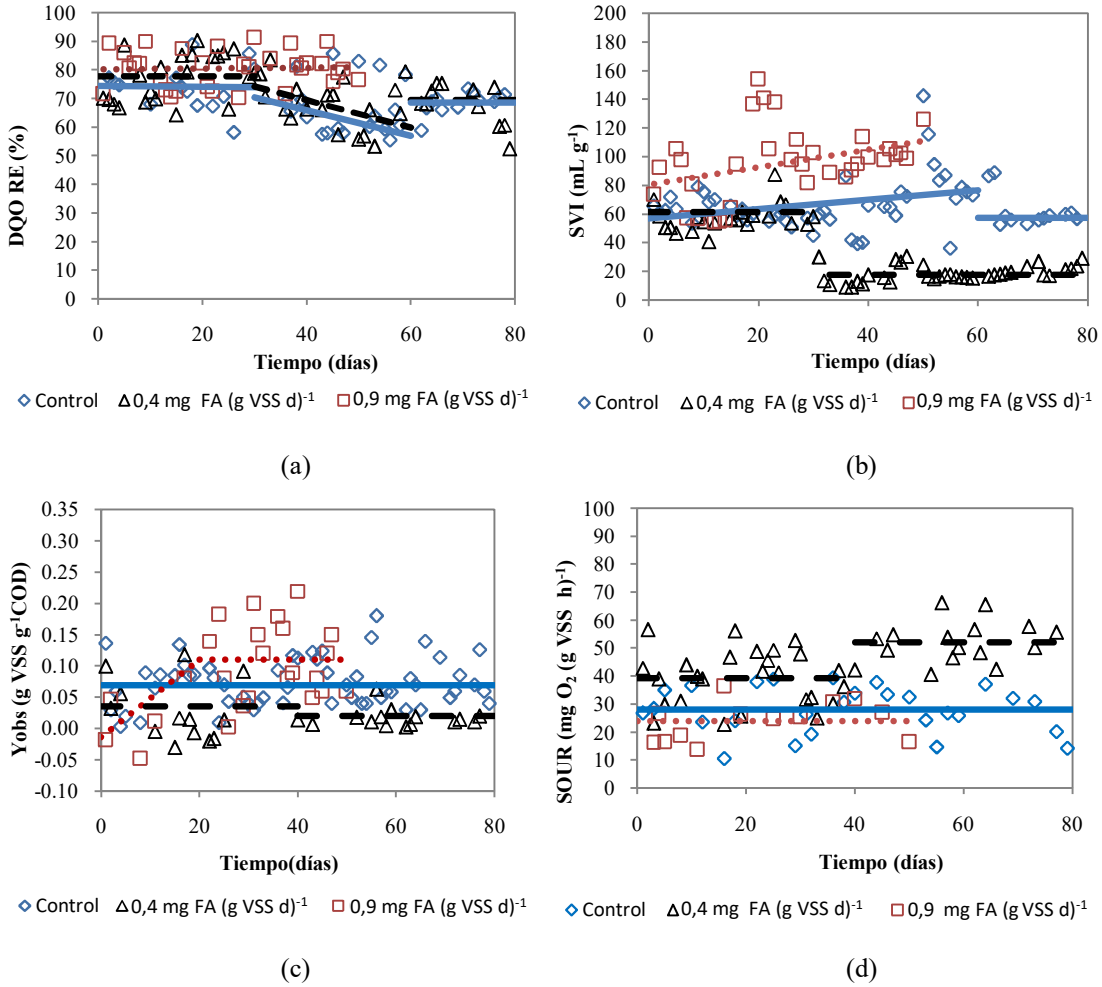



Figura 14.8 Evolución de (a) eficiencia en eliminación de materia orgánica (DQO RE) (b) índice volumétrico de fangos (IVF) (c) tasa de crecimiento bacteriano observado ($Y_{obs}$) y tasa específica de consumo de oxígeno (SOUR) durante la adición de ácido fólico. Las líneas indican valores promedio para el control (——), baja ( - - - -) y alta (……..) concentración de ácido fólico.

El crecimiento bacteriano observado ($Y_{obs}$) también se vio afectado por la dosificación de la vitamina mostrando valores mayores para la dosis superior y menores al control para la dosis inferior. Estos resultados son coherentes con las observaciones de Stoppa *et al* (2013), trabajando con efluentes de la industria papelera, y se pueden explicar en términos cinéticos por la ecuación de Monod modificada por Kompala (2013).

Combinando esta ecuación con el crecimiento específico de biomasa (μ) (Metcalf and Eddy, 2003) se obtiene la ecuación 14.4.

$$\mu = \frac{\mu_{máx}.S.E_R}{K_S+S} - K_d \qquad (14.4)$$

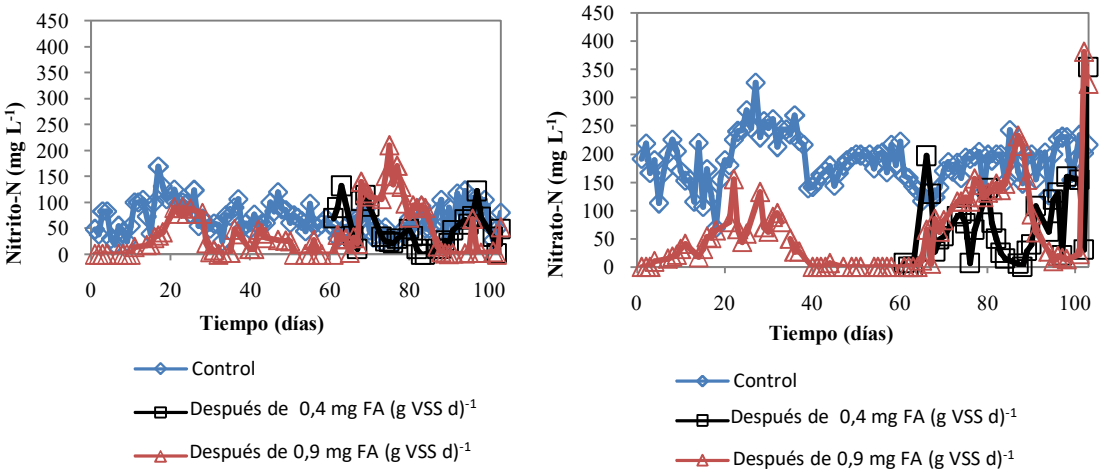
Donde $\mu_{máx}$ representa la velocidad específica de crecimiento bacteriano máxima, $K_s$ la constante de media velocidad, S la concentración en solución del sustrato limitante para el crecimiento, $E_R$ la cantidad relativa de enzima existente en la célula respecto de la máxima y $K_d$ el coeficiente de decaimiento endógeno. Según Stoppa *et al.* (2013), se espera que el término $K_d$ aumente con el suministro de ácido fólico, por una aceleración de los procesos metabólicos. En consecuencia, un pequeño aumento en $E_R$ (suplemento de baja concentración), podría no ser suficiente para compensar una $K_d$ más elevada, por lo que globalmente se reduce el crecimiento. Cuando más cantidad de vitamina es suministrada, el primer término supera el aumento en $K_d$, exhibiendo mayor crecimiento bacteriano.

Finalmente, se espera que el SOUR sea una consecuencia del SRT y de la actividad de biodegradación (Stoppa *et al*., 2013). El biorreactor suplementado con 0,9 mg $g^{-1}$ VSS $d^{-1}$ de ácido fólico presentó valores de respiración bacteriana inferiores al control, a pesar de una mayor eficiencia en degradación de materia orgánica. Esto pudo ser debido a una menor edad del fango, ocasionada por un mayor crecimiento bacteriano. Por el contrario, el biorreactor suministrado con 0,4 mg $g^{-1}$VSS $d^{-1}$ experimentó un mayor consumo de oxígeno, probablemente debido a una mayor edad del fango que conlleva más respiración endógena (Metcalf and Eddy, 2003).

Posteriormente, se interrumpió la dosificación de ácido fólico a los dos biorreactores previamente suplementados. En esta fase se observó como el reactor que había sido alimentado con 0,4 mg $g^{-1}$VSS $d^{-1}$ de ácido fólico recuperó inmediatamente la producción de nitritos y nitratos, alcanzando los valores del control (ver Figura 14.9).

El reactor previamente suplementado con 0,9 mg $g^{-1}$VSS $d^{-1}$ de ácido fólico tardó 60 días en alcanzar los ratios de nitrificación del control. Durante todo este periodo inicial de recuperación, la generación de nitritos fue limitante, pudiendo indicar una afectación de la bacteria amonio-oxidante. Sin embargo, mientras que en el reactor de control la concentración de nitratos era dominante sobre la de nitritos, en el reactor alimentado con ácido fólico ambas concentraciones quedaron igualadas. Hasta el día 90 no se alcanzaron ratios similares al control entre nitratos y nitritos. Por tanto, en este ensayo, las concentraciones relativas de nitritos y nitratos durante el periodo de recuperación

indicaron un descenso en el consumo de nitritos por parte de Nitrobacter. Odokuma y Akponah (2008) obtuvieron un resultado similar experimentando en suelos con fluidos para perforación. Este hecho se atribuyó a la sensibilidad de la enzima que posibilita la oxidación de nitrito a nitrato, la cual está ubicada en la membrana exterior de Nitrobacter, siendo ésta de gran permeabilidad.




(a) (b)

Figura 14.9 Monitorización de la concentración de (a) nitrógeno como nitrito y (b) nitrógeno como nitrato en el efluente de los biorreactores, tras haber interrumpido la dosificación de ácido fólico

Tras interrumpir la adición de ácido fólico, los parámetros operativos de los reactores que habían sido suplementados con la vitamina tendieron a adecuarse a los valores del reactor de control, después de un cierto periodo de aclimatación (ver Figura 14.10).

Finalmente, se comprobó la toxicidad aguda aportada por el ácido fólico en el licor mezcla de los diferentes ensayos realizados, durante y después de la adición de ácido fólico. En todos los casos se obtuvieron valores de $EC_{50}$> 45%, TU< 2,2, por lo que se concluyó que el ácido fólico no aportaba toxicidad significativa, de acuerdo con los resultados obtenidos por Velho *et al.* (2016), experimentando con un test de Dafnia Magnia en aguas domésticas.

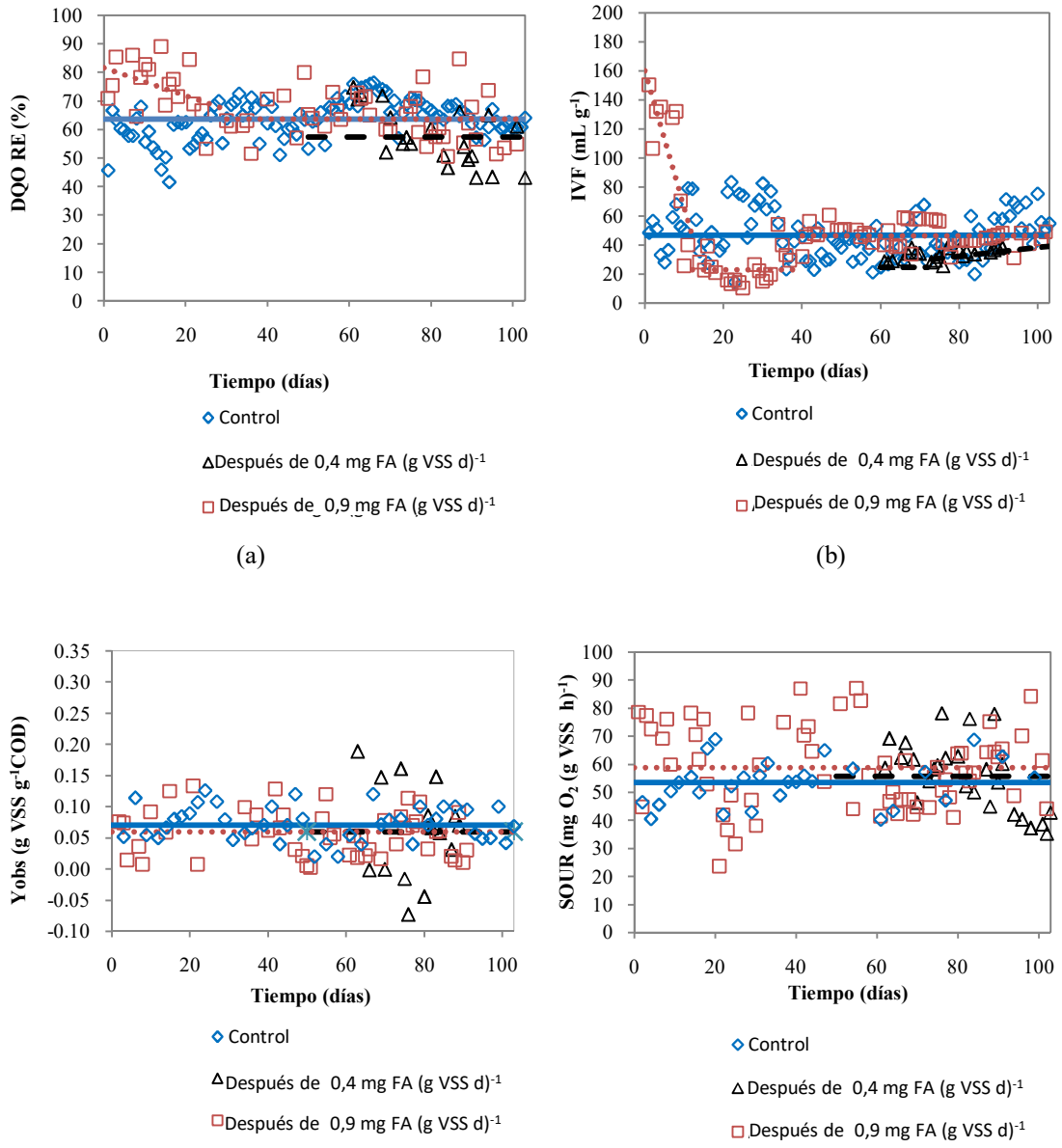



Figura 14.10 Evolución de (a) eficiencia en eliminación de materia orgánica (DQO RE) (b) índice volumétrico de fangos (IVF) (c) crecimiento bacteriano observado ($Y_{obs}$) (d) tasa específica de consumo de oxígeno (SOUR) tras la adición de ácido fólico. Las líneas indican valores promedio para el control ( ——— ), baja ( - - - - ) y alta (……..) concentración de ácido fólico.

Como conclusión de los resultados obtenidos, dosis de ácido fólico efectivas en coste han permitido limitar la nitrificación bacteriana en el sistema petroquímico de lodos activos, sin aportar toxicidad significativa al efluente. Sin embargo, la viabilidad de implementar esta estrategia a nivel industrial debe ser considerada particularmente en cada caso, puesto que la adición de la vitamina ha afectado a las variables operativas del sistema. Si se dosifica una dosis continua de 0,4 mg $g^{-1}$VSS $d^{-1}$ de ácido fólico, es

necesaria capacidad de aireación sobrante, puesto que aumenta la demanda de oxígeno. Por otra parte, un suministro continuo de 0,9 mg $g^{-1}$VSS $d^{-1}$ de ácido fólico provoca mayor crecimiento bacteriano, con lo cual se incrementará el volumen de fangos purgados. Comparando ambas dosis ensayadas, la mayor concentración proporciona varias ventajas, como una mayor eficiencia en degradación de materia orgánica o un mejor y más duradero control de la nitrificación. Sin embargo, además de duplicar los costes operativos, esta concentración empeoró la decantación del lodo. Este escenario sugiere investigar nuevas estrategias de dosificación, como por ejemplo el suplemento de dosis discontinuas de 0,9 mg $g^{-1}$VSS $d^{-1}$ de ácido fólico.

## 14.6. Recomendaciones

Los sistemas petroquímicos de fangos activos existentes, orientados a la degradación de materia orgánica, son susceptibles de ser modificados para alcanzar de forma consistente las especificaciones de vertido impuestas por la legislación próximamente aplicable. Para resolver la problemática habitual de decantación deficiente del fango por *bulking* filamentoso se propone la implementación de un selector aerobio. Como pautas de diseño para el mismo, se sugiere disponer de un tiempo de retención hidráulico de 30 minutos y operar a un ratio alimento-biomasa (F/M) de 35 g DQO $g^{-1}$VSS $d^{-1}$. Se deben conducir al selector las corrientes más biodegradables, limitando el aporte de materia orgánica particulada, considerando valores máximos de 50 g DQO total $g^{-1}$VSS $d^{-1}$. Como se espera que con la implementación del selector se incremente la nitrificación biológica no deseada, se recomienda como solución complementaria el aporte de pequeñas dosis de ácido fólico para limitar dicha reacción. En este último aspecto, previo a su implementación industrial, se recomienda completar la experimentación, ensayando estrategias alternativas de dosificación del ácido fólico, tales como un aporte discontinuo de la vitamina. Una vez identificada la estrategia de dosificación óptima, se recomienda ensayar la dosificación de ácido fólico en el sistema piloto, con inclusión del selector aerobio. Finalmente, de forma paralela, se recomienda profundizar en el mecanismo de acción del ácido fólico, como compuesto heterocíclico nitrogenado, sobre la reacción de nitrificación biológica.

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## 14.8. NOMENCLATURA

| SÍMBOLO | DESCRIPCIÓN | UNIDADES |
|---|---|---|
| $AMO$ | Amonio monooxigenasa | (-) |
| $AOB$ | Bacterias amonio-oxidantes | (-) |
| $ASTM$ | *American Society of Testing Materials* | (-) |
| $BREF$ | Documento de referencia de las mejores tecnologías disponibles | (-) |
| $DBO$ | Demanda biológica de oxígeno | (g $m^{-3}$) |
| $DBO_5$ | Demanda biológica de oxígeno a cinco días | (g $m^{-3}$) |
| $DBO\ RE$ | Eficiencia en eliminación de DBO | (%) |
| $DO$ | Concentración de oxígeno disuelto | (mg $L^{-1}$) |
| $DQO$ | Demanda química de oxígeno | (g $m^{-3}$) |
| $DQO\ EC$ | Concentración de equilibrio de DQO | (g $L^{-1}$) |
| $DQO\ RE$ | Eficiencia en la eliminación de DQO | (%) |
| $EC_{50}$ | Concentración efectiva 50% reducción | (%) |
| $E_R$ | Cantidad relativa de enzima | (g $g^{-1}$ máx) |
| $F/M$ | Razón alimento-biomasa en base a DQO soluble | (g sDQO $g^{-1}$VSS $d^{-1}$) |
| $F/M(DBO)$ | Razón alimento-bioma sa en base a DBO | (g DBO $g^{-1}$VSS $d^{-1}$) |
| $F/M(tDQO)$ | Razón alimento-biomasa en base a DQO total | (g tDQO $g^{-1}$VSS $d^{-1}$) |
| $IR$ | Reciclo interno | (-) |
| $IVF$ | Índice volumétrico de fangos | (-) |
| $IVFD$ | Índice volumétrico de fangos diluido | (-) |
| $K$ | Ratio máximo específico de utilización de sustrato | (g DQO $g^{-1}$ VSS $d^{-1}$) |
| $K_d$ | Coeficiente endógeno | (g VSS $g^{-1}$ VSS $d^{-1}$) |
| $K_s$ | Constante de media velocidad | (g COD $m^{-3}$) |
| μ | Velocidad específica de crecimiento de biomasa | (g VSS $g^{-1}$ VSS $d^{-1}$) |
| $\mu_{max}$ | Máxima velocidad específica de crecimiento de biomasa | (g VSS $g^{-1}$VSS $d^{-1}$) |
| $N_{ASSIM}$ | Nitrógeno amoniacal asimilado por unidad de DQO consumida | (g $NH_4^+$-N asim. $g^{-1}$ COD eliminado) |
| $NH_4^+ - N$ | Concentración de nitrógeno amoniacal | (mg $L^{-1}$) |
| $NO_2^- - N$ | Concentración de nitrógeno como nitrito | (mg $L^{-1}$) |
| $NO_3^- - N$ | Concentración de nitrógeno como nitrato | (mg $L^{-1}$) |
| $NOB$ | Bacterias nitrito-oxidantes | (-) |
| $OUR$ | Tasa de consumo de oxígeno | (mg $O_2$ $L^{-1}$ $h^{-1}$) |
| $Q_{in}$ | Caudal de entrada al selector | ($m^3$ $d^{-1}$) |
| $PLC$ | Controlador lógico programable | (-) |

| SÍMBOLO | DESCRIPCIÓN | UNIDADES |
|---|---|---|
| $PO_4^{3-} - P$ | Concentración de fósforo como ortofosfato | (mg $L^{-1}$) |
| $P_{VSS}$ | Producción observada de VSS por unidad de DQO eliminada | (g VSS producido $g^{-1}$ DQO eliminada) |
| $RAS$ | Recirculación de fangos activos | (-) |
| $RTMP$ | Reactor tanque mezcla perfecta | (-) |
| $S$ | Concentración de sustrato soluble en el reactor | (g DQO $m^{-3}$) |
| $sDQO$ | Demanda química de oxígeno soluble | (g $m^{-3}$) |
| $SOUR$ | Tasa específica de consumo de oxígeno | $(\text{mg } O_2 g^{-1} \text{VSS } h^{-1})$ |
| $SRT$ | Edad del fango ó *Sludge Retention Time* | (d) |
| $TIC$ | Concentración de carbono inorgánico total | (g $m^{-3}$) |
| $TOC$ | Concentración de carbono orgánico total | (g $m^{-3}$) |
| $TRH$ | Tiempo de retención hidráulico | (d) |
| $TSS$ | Sólidos en suspensión totales | (g $m^{-3}$) |
| $TU$ | Unidades de toxicidad | (-) |
| $VSS$ | Sólidos en suspensión volátiles | (g $m^{-3}$) |
| $Y$ | Tasa de crecimiento real de biomasa | (g biomasa $g^{-1}$ sustrato) |
| $Y_{obs}$ | Tasa de crecimiento de biomasa observada | (g biomasa $g^{-1}$ sustrato) |