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OPERATION AND CONTROL OF SBR PROCESSES FOR ENHANCED BIOLOGICAL NUTRIENT REMOVAL FROM WASTEWATER

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Operation and control of SBR processes for enhanced biological nutrient removal from wastewater

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PhD Thesis

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CERTIFIQUEN

Que el llicenciat **Sebastià Puig Broch** ha dut a terme, sota la seva direcció, el treball que, amb el títol: ***Operation and control of SBR processes for enhanced biological nutrient removal from wastewater***, presenta en aquesta memòria, la qual constitueix la seva Tesi per optar al Grau de Doctor per la Universitat de Girona.

I perquè en prengueu coneixement i tingui els efectes que correspongui, presentem davant la Facultat de Ciències de la Universitat de Girona l'esmentada Tesi, signant aquesta certificació a

Girona, 20 de juliol del 2007

Maria Dolors Balaguer Condom

Jesús Colprim Galceran

Pels meus.

Els que hi han estat, hi són o hi seran.

"If you can dream it, you can do it"

"Si ho pots somiar, ho pots fer"

Walt Disney

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"La felicitat humana generalment no s'aconsegueix a cops de sort, que pot passar algunes vegades, sinó en les coses petites que passen tots els dies"

Benjamin Franklin

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"Investigar és veure el que tot el món ha vist i pensar el que ningú més ha pensat".

Albert Szent-Györgi

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Summary

Water is essential for all socio-economic development and for maintaining healthy ecosystems. As population increases and development calls for increased allocations of groundwater and surface water for domestic, agriculture and industrial sectors; the pressure on water resources intensifies, leading to tensions, conflicts among users, and excessive pressure on the environment.

In the last decades, the awareness of environmental issues has increased in society considerably. A New Water Culture is appearing. The European Water Framework Directive (2000/60/EC) represents these tendencies. Regarding the wastewater treatment field, a decrease in nutrients being discharged into surface waters is required as pointed by the Urban Water Directive (91/271/EC). There is an increasing need to improve the effluent quality of domestic wastewater treatment processes.

In order to deal with these issues, research is focussed on finding and improving technologies for the suitable wastewater treatment where the instrumentation, control and automation of the process is a key factor when the process must be operated to achieve restricted discharge levels and minimize the environmental impacts.

The Sequencing Batch Reactor (SBR) is considered an alternative technology to conventional processes for removing nutrients from wastewater. The SBR is a fill-and-draw activated sludge system for wastewater treatment. While in continuous systems the reaction and settling occur in different reactors, in SBR all the processes are conducted in a single reactor following a sequence of fill, reaction, settling and draw phases. The SBRs have a higher flexibility and controllability, allowing for more rapid adjustment to changing wastewater characteristics. Lower investment and recurrent cost are necessary because secondary settling tanks and sludge return systems are not required. Furthermore, it is especially appropriate for places where there is significant flow and/or load variability or where space problems become a restriction.

Normally SBR technology works with a fixed cycle configuration, which has been developed from the operators' experience and which is repeated over time. These predetermined cycles are sometimes unable to adapt to dynamic changes in the influent characteristics, which leads to excess resources being used. This thesis describes the development, implementation and improvement of an SBR real-time control system treating urban wastewater for organic matter and nitrogen removal purposes. The control system uses the calculated online Oxygen Uptake Rate (OUR) and the Oxidation Reduction Potential (ORP) probe to estimate the status of the biological processes and control the length of the aerobic and/or anoxic phases of the SBR operational cycle.

On the other hand, for Biological Nutrient Removal (BNR) from wastewater, special attention has to be given to the availability and use of the easily biodegradable substrate. The main problem of biological nitrogen removal, when treating low Carbon:Nitrogen (C:N) ratios from wastewater, is the specific use of organic matter for denitrification purposes avoiding the use of complementary carbon sources since easily biodegradable organic matter is also rapidly consumed under aerobic conditions. This thesis describes the

application of the SBR technology for biological nitrogen removal from the wastewater. The step-feed strategy with anoxic filling events and a sequence of anoxic-aerobic phases were applied reaching, in spite of influent variability when treating real urban wastewater, effluent ammonium and nitrate concentrations of 0.1 ± 0.4 mg $N\text{-}NH_4^+ \cdot L^{-1}$ and 1.5 ± 1.1 mg $N\text{-}NO_3^- \cdot L^{-1}$, respectively.

When the wastewater treatment is focussed, at the same time, on biological nitrogen and phosphorus removal, both processes compete for the organic matter available in the raw wastewater. In spite of using some feeding strategies, the organic matter concentration can be insufficient, provoking the destabilization of the system performance. For this reason, the influence of Carbon:Phosphorus (C:P) and Carbon:Nitrogen (C:N) feeding ratios on the efficiency of BNR from wastewater using a lab-scale SBR must be investigated.

The results obtained demonstrate that if the carbon concentration is not enough for BNR from wastewater (when the C:P ratio was below 36 g $COD \cdot g^{-1}$ $P\text{-}PO_4^{3-}$), the Enhanced Biological Phosphorus Removal (EBPR) is affected when evolving under carbon source limitations. However, after a period with low available organic matter, when the influent C:P ratio increased from 36 to 67 g $COD \cdot g^{-1}$ $P\text{-}PO_4^{3-}$, the Polyphosphate Accumulating Organisms (PAOs) responded quickly (15 days) to recover the nutrient removal efficiencies. On the other hand, when the carbon concentration was not enough also for denitrification purposes (the C:N ratio was below 4 g $COD \cdot g^{-1}$ N-TKN), the nitrate accumulated in the SBR, as did phosphorus, causing the breakdown of the system performance. Afterwards, when the influent characteristics changed (the C:N ratio was 9 g $COD \cdot g^{-1}$ N-TKN), the system returned to the normal conditions, recovering quickly the denitrification efficiencies and, after 15 days, the phosphorus removal (i.e BNR performance).

When the influent wastewater composition has not enough organic carbon for BNR, low C:N and C:P ratios, the addition of external carbon source must be considered. The choice of the substrate is important in terms of i) the economic cost for the carbon source and ii) the selective use of the carbon source by PAOs. In general, different substrates have been used for denitrification (i.e. methanol) and phosphate removal (i.e. acetate) purposes. To find out a unique suitable carbon source for both processes, ethanol is proposed in this thesis as an alternative to conventional carbon sources (i.e. acetate and propionate). Ethanol synthetic wastewater is treated in the lab-SBR for BNR purposes achieving high nutrient removal efficiencies. Complementary studies have permitted to compare the behaviour of ethanol as an alternative of conventional Volatile Fatty Acids (VFAs) (i.e. acetate and propionate) as an external carbon source for EBPR from wastewater in ethanol acclimated and unacclimated biomass. In this sense, ethanol has proved to be an useful external carbon source for EBPR. Nevertheless, a period of adaptation is required by PAOs when ethanol is used in unacclimated EBPR processes. After a period of biomass adaptation of 30 days, the population dynamics of the activated sludge evolved to an efficient phosphorus removal process producing Poly-β-HydroxyValerate (PHV) as the main Poly-β-HydroxyAlkaonate (PHA). On the other hand, after a period of biomass adaptation of 140 days and comparing *versus* an unacclimated sludge, the P release rate increased from 1.5 to 6.2 mg $P\text{-}PO_4^{3-} \cdot g^{-1}$ $VSS \cdot h^{-1}$, as well as, phosphate uptake rate to 7.0 mg $P\text{-}PO_4^{3-} \cdot g^{-1}$ $VSS \cdot h^{-1}$. Therefore, if there is a need to support the PAO bacteria with energy over a longer time, a period of adaptation can be accepted and ethanol is suitable taking into account the carbon source addition cost. If EBPR needs to be incidentally supported by substrate addition, VFAs are preferred.

Resum

L'aigua és indispensable pel desenvolupament socioeconòmic i per mantenir en un bon estat els ecosistemes. A mesura que s'incrementa la població i la demanda d'aigua per usos domèstics, agrícoles i industrials, s'intensifica la pressió sobre les fonts d'aigua, creant tensions, conflictes entre usuaris i una excessiva pressió sobre el medi ambient.

En les últimes dècades, la conscienciació respecte els problemes ambientals s'ha incrementat en la societat considerablement. La Nova Cultura de l'Aigua està apareixent. La Directiva Europea Marc de l'Aigua (2000/60/CE) representa aquesta tendència. Respecte al tractament de les aigües residuals, representada en la Directiva d'Aigües Residuals Urbanes (91/271/CE), hi ha una necessitat creixent per millorar la qualitat dels efluents de les estacions depuradores d'aigües residuals.

Per tal de fer front a aquestes necessitats, la recerca es centra en trobar i millorar les tecnologies existents pel tractament adequat de les aigües residuals. En aquest sentit, la instrumentació, el control i l'automatització del procés són factors claus quan el procés ha de ser operat per aconseguir nivells restrictius d'abocament i minimitzar els impactes ambientals.

El reactor seqüencial per càrregues (SBR, acrònim en anglès de *Sequencing Batch Reactor*) és considerat com a una tecnologia alternativa als processos convencionals pel tractament dels nutrients de les aigües residuals. L'SBR és un sistema de fangs activats d'omplerta - buidat utilitzat pel tractament de les aigües residuals. En els sistemes continus, els processos de reacció i sedimentació es realitzen en diferents reactors. En canvi, en els SBRs tots els processos es realitzen en un únic reactor seguint una seqüència de fases d'omplerta, reacció, sedimentació i buidat. Els SBRs tenen una gran flexibilitat i controlabilitat, permeten un ajust més ràpid a canvis en les característiques de l'influent. A més, els decantadors secundaris i els sistemes de recirculació de fangs no són necessaris, dismuint els costos d'inversió. Els SBRs són especialment apropiats per zones on hi ha una variabilitat en el cabal i/o la càrrega o en llocs que presenten problemes d'espai.

Generalment, la tecnologia SBR funciona a partir de la configuració de cicles fixes, els quals han estat desenvolupats a partir de l'experiència dels operaris i es repeteixen al llarg del temps. Aquests cicles predefinits són algunes vegades incapaços d'adaptar-se a canvis dinàmics de l'influent, produint un excessiu consum dels recursos. Aquesta tesi descriu el desenvolupament, implementació i millora d'un sistema de control a temps real pels SBRs per tractar 600L·d^{-1} d'aigua residual urbana per l'eliminació de matèria orgànica i nitrogen. El sistema de control utilitza la velocitat de consum d'oxigen en línia (OUR, acrònim en anglès de *Oxygen Uptake Rate*) i la sonda de potencial RedOx (ORP, acrònim en anglès de *Oxidation Reduction Potential*) per conèixer l'estat dels processos biològics i controlar la durada de les fases aeròbies i/o anòxiques del cicle d'operació de l'SBR.

Per altra banda, per l'eliminació biològica de nutrients (EBN) de les aigües residuals, s'ha de donar una especial atenció a la disponibilitat i la utilització de la matèria orgànica ràpidament biodegradable. El principal problema de l'eliminació biològica de nitrogen, quan es tracten aigües amb baixes relacions

Carboni:Nitrogen (C:N), és l'ús específic de la matèria orgànica per desnitrificar per tal d'evitar la utilització de fonts de carboni complementaries. Aquesta tesi descriu l'aplicació de la tecnologia SBR per l'eliminació de nitrogen de les aigües residuals. Una estratègia d'alimentació esglaonada en condicions anòxiques i una seqüència de fases anòxiques i aeròbies van ser aplicades obtenint, tot i la variabilitat de l'influent quan es tracten aigües urbanes reals, concentracions d'amoni i nitrats en l'efluent de 0.1 ± 0.4 mg $N\text{-}NH_4^+ \cdot L^{-1}$ i 1.5 ± 1.1 mg $N\text{-}NO_3^- \cdot L^{-1}$.

Quan el tractament de les aigües residuals s'enfoca alhora en l'eliminació biològica de nitrogen i fòsfor, els dos processos competeixen per la matèria orgànica disponible en l'aigua residual. Tot i utilitzar una estratègies d'optimització de la matèria orgànica, la concentració d'ella pot ser insuficient, provocant una desestabilització del sistema. Per aquesta raó, la influència de les relacions en l'influent de Carboni:Fòsfor (C:P) i Carboni:Nitrogen (C:N) en els rendiments d'eliminació de nutrients de les aigües residuals utilitzant un SBR a escala de laboratori va ser investigat.

Els resultats obtinguts demostren que si la concentració de carboni no és suficient per l'EBN de les aigües residuals (quan la relació C:P és menor a 36 g $DQO \cdot g^{-1}$ $P\text{-}PO_4^{3-}$), l'eliminació biològica de fòsfor (EBP) es veu afectada. Però, després d'un període de baixa disponibilitat de matèria orgànica, quan la relació C:P en l'influent s'augmenta de 36 a 67 g $DQO \cdot g^{-1}$ $P\text{-}PO_4^{3-}$, els organismes acumuladors de fòsfor (PAOs, acrònim en anglès de *Polyphosphate Accumulating Organisms*) responen ràpidament (15 dies) per recuperar l'eficiència d'eliminació de nutrients. Per altra banda, quan la concentració de carboni també és insuficient per desnitrificar (relació C:N menor de 4 g $DQO \cdot g^{-1}$ TKN), el nitrat s'acumula en l'SBR, a l'igual que el fòsfor, provocant la fallada del procés. Després, quan les característiques de l'influent varien (relació C:N 9 g $DQO \cdot g^{-1}$ TKN), el sistema torna a les condicions normals, recuperant ràpidament les eficiències de desnitrificació i posteriorment, les d'eliminació de fòsfor.

Quan la composició de l'aigua residual no té suficient matèria orgànica per l'eliminació biològica de nutrients, baixes relacions C:N i C:P, s'ha de considerar l'addició d'una font externa de carboni. L'elecció dels substrat és important en termes de: i) cost econòmic de la font de carboni i ii) l'ús selectiu de la font de carboni per part dels PAOs. En general, diferents substrats han estat utilitzats per desnitrificar (ex. metanol) i eliminar fòsfor (ex. acetat). En aquesta tesi es proposa la utilització de l'etanol, com a alternativa a les fonts de carboni convencionals, com a única font de carboni adient pels dos processos. Amb aquest objectiu, s'ha tractat aigua residual rica en etanol en el lab-SBR per eliminar nutrients aconseguint alts rendiments d'eliminació. A més, estudis complementaris han premés comparar el comportament de l'etanol com alternativa als àcids grassos volàtils (VFAs, acrònim en anglès de *Volatile Fatty Acids*) (ex. acetat i propionat) com a font externa de carboni per l'EBP de les aigües residuals en biomassa aclimatada i no aclimatada a l'etanol. En aquest sentit, s'ha provat que l'etanol és una font externa de carboni útil per EBP. Encara que es requereix un període d'adaptació pels PAOs quan l'etanol és utilitzat en processos EBP no aclimatats. Després d'un temps d'adaptació de 30 dies, la dinàmica poblacional dels fangs activats evoluciona cap a un procés eficient d'eliminació de fòsfor produint Poly-β-HidroxiValerat (PHV) com a Poli-β-HidroxiAlcanoat (PHA) majoritari.

Per altra banda, després d'un període d'adaptació de 140 dies i comparant els resultats amb els d'un fang no aclimatat, la velocitat d'alliberament de fòsfor s'incrementa des de 1.5 a 6.2 mg $P\text{-}PO_4^{3-}\cdot g^{-1}$ VSS$\cdot h^{-1}$, a l'igual que augmenta la velocitat de captació de fòsfor a 7.0 mg $P\text{-}PO_4^{3-}\cdot g^{-1}$ VSS$\cdot h^{-1}$. Així doncs, si es necessita un suport de la bactèria PAO amb energia a llarg termini, un període d'adaptació pot ser acceptat tenint en compte el cost de l'addició per l'etanol d'una font de carboni. En cas que l'addició de substrat sigui puntual, els VFAs seran elegits.

Resumen

El agua es indispensable para el desarrollo socioeconómico y para mantener en un buen estado los ecosistemas. A medida que se incrementa la población y la demanda de agua para usos domésticos, agrícolas e industriales, se intensifica la presión sobre las fuentes de aguas, creando tensiones, conflictos entre usuarios y una excesiva presión en el medio ambiente.

En las últimas décadas, la concienciación sobre los problemas ambientales se ha incrementado en nuestra sociedad considerablemente. La Nueva Cultura del Agua está surgiendo. La Directiva Europea Marco del Agua (2000/60/CE) representa esta tendencia. En el tratamiento de las aguas residuales, representada en la Directiva de Aguas Residuales Urbanas (91/271/CE), hay una necesidad creciente para alcanzar la calidad de los efluentes de las estaciones depuradoras.

Para hacer frente a estas necesitades, la investigación se basa en encontrar y mejorar las tecnologías existentes para el tratamiento adecuado de las aguas residuales. En este sentido, la instrumentación, el control y la automatización del proceso son factores claves cuando el proceso tiene que ser operado para conseguir niveles restrictivos de vertido y minimizar el impacto ambiental.

El reactor secuencial por cargas (SBR, acrónimo en inglés de *Sequencing Batch Reactor*) es considerado como una tecnología alternativa a los procesos convencionales para el tratamiento de nutrientes de las aguas residuales. El SBR es un sistema de lodos activados de llenado-vaciado utilizado para el tratamiento de las aguas residuales. En los sistemas continuos, los procesos de reacción y sedimentación se realizan en distintos reactores. En cambio, en los SBRs todos los procesos se realizan en un solo reactor según una secuencia de fases de llenado, reacción, sedimentación y vaciado. Los SBRs tienen una gran flexibilidad y controlabilidad, permitiendo un ajuste más rápido a cambios en las características del agua residual. Con esta tecnología, los decantadores secundarios y los sistemas de recirculación de lodos no son necesarios, disminuyendo los costes de inversión. Además, los SBRs son especialmente apropiados en zonas donde hay una variabilidad en el caudal y/o la carga o en zonas donde existen problemas de espacio.

Generalmente, la tecnología SBR funciona a partir de la configuración de ciclos fijos, los cuales han estado creados a partir de la experiencia de los operarios y se repiten a lo largo del tiempo. Estos ciclos predefinidos son algunas veces incapaces de adaptarse a los cambios dinámicos del agua, produciendo un consumo excesivo de los recursos. Esta tesis describe el desarrollo, implementación y mejora de un sistema de control a tiempo real para los SBRs para tratar 600$L \cdot d^{-1}$ de agua residual urbana para la eliminación de materia orgánica y nitrógeno. El sistema de control utiliza la velocidad de consumo de oxígeno en línea (OUR, acrónimo en inglés de *Oxygen Uptake Rate*) y la sonda de potencial RedOx (ORP, acrónimo en inglés de *Oxidation Reduction Potential*) para conocer el estado de los procesos biológicos y controlar la duración de las fases aerobias y/o anoxias del ciclo de operación del SBR.

Por otro lado, para la eliminación biológica de nutrientes (EBN) de las aguas residuales, debe darse una especial atención a la disponibilidad y la utilización de la materia orgánica rápidamente biodegradable.

El principal problema de la eliminación biológica de nitrógeno, cuando se trata aguas con bajas relaciones Carbono:Nitrógeno (C:N), es el uso específico de la materia orgánica para desnitrificar, con el fin de evitar la utilización de fuentes de carbono complementarias. En esta tesis se describe la aplicación de la tecnología SBR a la eliminación de nitrógeno de las aguas residuales. Una estrategia de alimentación en condiciones anoxias y una secuencia de fases aerobias y anoxias fueron aplicadas obteniendo, a pesar de la variabilidad del agua cuando se tratan aguas urbanas reales, concentraciones de amonio y nitratos en el efluente de 0.1 ± 0.4 mg $N\text{-}NH_4^+ \cdot L^{-1}$ y 1.5 ± 1.1 mg $N\text{-}NO_3^- \cdot L^{-1}$.

Cuando el tratamiento de las aguas residuales se centra a la vez en la eliminación de nitrógeno y fósforo, los dos procesos compiten por la materia orgánica disponible en el agua residual. A pesar de utilizar estrategias de optimización de la materia orgánica, su concentración puede ser insuficiente, causando una desestabilización del sistema. Por este motivo, la influencia de las relaciones en el agua de Carbono:Fósforo (C:P) y C:N en los rendimientos de eliminación de nutrientes de las aguas residuales utilizando un SBR a escala de laboratorio ha sido investigada.

Los resultados obtenidos demuestran que si la concentración de carbono no es suficiente para la EBN de las aguas residuales (cuando la relación C:P es menor a 36 g $DQO \cdot g^{-1}$ $P\text{-}PO_4^{3-}$), la eliminación biológica de fósforo (EBP) se ve afectada. Después de un período de baja disponibilidad de materia orgánica, cuando la relación C:P aumenta de 36 a 67 g $DQO \cdot g^{-1}$ $P\text{-}PO_4^{3-}$, los organismos acumuladores de fósforo (PAOs, acrónimo en inglés de *Polyphosphate Accumulating Organisms*) responden rápidamente (15 días) para recuperar la eficiencia de eliminación de nutrientes. Por otro lado, cuando la concentración de carbono no es tampoco suficiente para desnitrificar (relación C:N menor a 4 g $DQO \cdot g^{-1}$ TKN), los nitratos se acumulan en el SBR, al igual que el fósforo, causando el fallo del proceso. Después, cuando las características del agua varían (relación C:N 9 g $DQO \cdot g^{-1}$ TKN), el sistema vuelve a las condiciones normales, recuperando rápidamente las eficiencias de desnitrificación y después, las de eliminación de fósforo.

Cuando la composición del agua residual no tiene suficiente materia orgánica para la eliminación biológica de nutrientes, bajas relaciones C:N y C:P, se debe considerar la adición de una fuente externa de carbono. La elección del substrato es importante en términos de: i) coste económico de la fuente de carbono i ii) la utilización selectiva de la fuente de carbono de los PAOs. En general, diferentes substratos se han utilizado para desnitrificar (ej. metanol) y eliminar fósforo (ej. acetato y propionato). En esta tesi se propone la utilización del etanol, como alternativa a las fuentes de carbono convencionales, como única fuente de carbono para los dos procesos. Con este fin, se ha tratado agua residual rica en etanol en el lab-SBR eliminando nutrientes con altos rendimientos. Además, estudios complementarios han permitido comparar el comportamiento del etanol como alternativa a los ácidos grasos volátiles (VFAs, acrónimo en inglés de *Volatile Fatty Acids*) (ej. acetato y propionato) como fuente externa de carbono para EBPR de las aguas residuales en biomasa aclimatada y no aclimatada al etanol. En este sentido, se ha probado que el etanol es una fuente externa de carbono útil para EBP. No obstante, se requiere un período de adaptación para los PAOs cuando el etanol es utilizado en procesos EBP no aclimatados. Después de un tiempo de adaptación de 30 días, la dinámica poblacional de los lodos activados evoluciona hacia un proceso eficiente de eliminación de fósforo produciendo Poly-β-HidroxiValerato (PHV) como a Poli-β-HidroxiAlcanoato (PHA) mayoritario.

Por otro lado, después de un período de adaptación de 140 días y comparando los resultados con los de un lodo no aclimatado, la velocidad de liberación del fósforo se incrementa de 1.5 a 6.2 mg $P\text{-}PO_4^{3-}\cdot g^{-1}$ $VSS\cdot h^{-1}$, al igual que la velocidad de captación de fósforo a 7.0 mg $P\text{-}PO_4^{3-}\cdot g^{-1}$ $VSS\cdot h^{-1}$. Así pues, si se necesita un soporte para la bacteria PAO con energía a largo plazo, se puede aceptar un período de adaptación al etanol y teniendo en cuenta el coste de la adición de una fuente de carbono. Si la adición es puntual, los VFAs serán elegidos.

Table of contents

List of Figures

List of Tables

List of Symbols and Abbreviations

AOB	Ammonium Oxidizing Bacteria
ATP	Adenosine 5'-triphosphate
BCFS®	Biological–chemical phosphorus and nitrogen removal process
BNR	Biological Nutrient Removal
C	Carbon
$Ca(OH)_2$	Lime
C:N	Carbon:Nitrogen ratio [g COD·g^{-1} N-TKN]
C:P	Carbon:Phosphorus ratio [g COD·g^{-1} P-PO_4^{3-}]
COD	Total Chemical Oxygen Demand [mg COD·L^{-1}]
COHNS	Organic matter
DME	Dehydrated Meat Extract
DO	Dissolved Oxygen [mg O_2·L^{-1}]
DO_{sat}	Saturation or maximum dissolved oxygen [mg O_2·L^{-1}]
DPAOs	Denitrifying Phosphate Accumulating Organisms
EBPR	Enhanced Biological Phosphorus Removal
EC	European Community
EWFD	European Water Framework Directive
FISH	Fluorescent *In Situ* Hybridisation
FLC	Fuzzy logic-based control
GAOs	Glycogen-Accumulating Organisms
$Gly/C_{upt.}$	Glycogen consumption - carbon uptake ratio [mmols C·$mmols^{-1}$ C]
HNO_2	Un-ionized nitrous acid
HRT	Hydraulic Retention Time [days]
ICA	Instrumentation, Control and Automation
IC	Inorganic Carbon [mg C·L^{-1}]
k^T	Reaction rate constant as a function of temperature
k_{20}	Reaction rate constant at 20ºC
K_La	Oxygen mass transfer coefficient [h^{-1}]

LEQUIA	Laboratory of Chemical and Environmental Engineering
N	Nitrogen
$N\text{-}NH_4^+$	Ammonium [mg $N\text{-}NH_4^+ \cdot L^{-1}$]
$N\text{-}NO_2^-$	Nitrites [mg $N\text{-}NO_2^- \cdot L^{-1}$]
$N\text{-}NO_3^-$	Nitrates [mg $N\text{-}NO_3^- \cdot L^{-1}$]
NOB	Nitrite Oxidizing Bacteria
N-TKN	Total Kjeldalh Nitrogen [mg $N\text{-}TKN \cdot L^{-1}$]
N-TN	Total Nitrogen [mg $N\text{-}TN \cdot L^{-1}$]
OGAR®	Optimized manaGement of Aeration by Redox
ORP	Oxidation Reduction Potential [mV]
ORP_{min}	Minimum Oxidation Reduction Potential [mV]
OUR	Oxygen Uptake Rate [mg $O_2 \cdot L^{-1} \cdot h^{-1}$]
OUR_{END}	Endogenous Oxygen Uptake Rate [mg $O_2 \cdot L^{-1} \cdot h^{-1}$]
P	Phosphorus
PAOs	Polyphosphate Accumulating Organisms
PE	Population Equivalents
PHA	Poly-β-HydroxyAlkaonate
PHB	Poly-β-HydroxyButirate [mg $C \cdot L^{-1}$]
PHV	Poly-β-HydroxyValerate [mg $C \cdot L^{-1}$]
PH2MV	Poly- β -Hydroxy-2-MethylValerate [mg $C \cdot L^{-1}$]
PP	Poly-Phosphates
$P_{rel.}/C_{upt.}$	Phosphate release – Carbon uptake ratio [mmols $P\text{-}PO_4^{3-} \cdot mmols^{-1}$ C]
$P_{upt.}/PHA_{oxid.}$	Phosphate uptake-PHA oxidation ratio [mmols $P\text{-}PO_4^{3-} \cdot mmols^{-1}$ C]
$P\text{-}PO_4^{3-}$	Phosphate [mg $P\text{-}PO_4^{3-} \cdot L^{-1}$]
P_{atm}	Atmospheric pressure [atm]
Q_{air}	Air flow rate [$m^3 \cdot d^{-1}$]
RAS	Return Activated Sludge
RCMP	Residual Carbon Manipulation Point
SBR	Sequencing Batch Reactor
SOUR	Specific Oxygen Uptake Rate [mg $O_2 \cdot g^{-1}$ VSS$\cdot h^{-1}$]
$SOUR_{END}$	Specific Endogenous Oxygen Uptake Rate [mg $O_2 \cdot g^{-1}$ VSS$\cdot h^{-1}$]

SRT	Sludge Retention Time [days]
SVI	Sludge Volume Index [$mL \cdot mg^{-1}$]
T	Temperature [ºC]
t_{min}	Transition time [minutes]
t_{wait}	Waiting time [minutes]
t_{max}	Maximum time length [minutes]
TC	Total Carbon [$mg\ C \cdot L^{-1}$]
TN	Total Nitrogen [$mg\ N\text{-}TN \cdot L^{-1}$]
TOC	Total Organic Carbon [$mg\ C \cdot L^{-1}$]
TSS	Total Suspended Solids [$mg \cdot L^{-1}$]
UCT	University of Cape Town
UdG	University of Girona
V	Reactor volume [m^3]
VFAs	Volatile Fatty Acids
VIP™	Virginia Initiative Plant
VSS	Volatile Suspended Solids [$mg \cdot L^{-1}$]
V_{30}	Settled Sludge Volume in 30 minutes [$mL \cdot L^{-1}$]
WAS	Sludge wastage
WWTPs	WasteWater Treatment Plants

α	Standard deviation
σ_{OUR}	Average viability
θ	Temperature coefficient yield (1.024)
y_{O2}	Fraction of oxygen in air (= 21%)
η	Standard oxygen transfer efficiency (0.21)

Chapter 1.
General introduction

This chapter describes a general introduction to this research including the water and wastewater, biological processes for nutrient removal and the SBR technology.

1.1 Water and wastewater

Water is essential for all socio-economic development and for maintaining healthy ecosystems. As population increases and development calls for increased allocations of groundwater and surface water for domestic, agriculture and industrial sectors; the pressure on water resources intensifies, leading to tensions, conflicts among users and excessive pressure on the environment. The increasing stress on freshwater resources brought about by ever rising demand and profligate use, as well as by growing pollution worldwide, is of serious concern (UN-Water, 2006).

Water use has been growing at more than twice the rate of population increase in the last century, and, although there is no global water scarcity as such, an increasing number of regions are chronically short of water. By 2025, 1800 million people will be living in countries or regions with absolute water scarcity, and two-thirds of the world population could be under stress conditions. The situation will be exacerbated as rapidly growing urban areas place heavy pressure on neighbouring water resources (UN-Water, 2006).

Every community produces both liquid and solid wastes and air emissions. The liquid waste – wastewater – is essentially the water supply of the community after it has been used in a variety of applications. Wastewater contains nutrients, which can stimulate the growth of aquatic plants, and may contain toxic compounds or compounds that potentially may be mutagenic or carcinogenic. For these reasons, the immediate and nuisance-free removal of wastewater from its source of generation, followed by treatment, reuse, or dispersal into the environment is necessary to protect public health and the environment (Metcalf and Eddy, 2003).

1.2 Biological processes in wastewater treatment

The treatment of contaminated wastewater by means of biological and chemical processes has been widely implemented from classical urban to industrial wastewater. From economical and operational points of view, biological treatment has proved to be a robust and more energy efficient way of treating biodegradable wastewater if good process control could be ensured (Grady *et al.,* 1999). A wide variety of contaminants, such as organic matter and nutrients (mainly nitrogen and phosphorus), can be biologically degraded to reduce the discharge levels in water bodies (rivers, lakes and seas).

1.2.1 Aerobic biological organic matter oxidation

Dating back to the early 1900s, the primary purpose of biological wastewater treatment was to remove organic compounds, colloidals and suspended solids and reduce the concentration of pathogenic organisms released to receiving waters (Metcalf and Eddy, 2003).

Organic compounds are normally composed of a combination of Carbon, Oxygen, and Hydrogen, together with Nitrogen and Sulphur in some cases (COHNS). The organic matter in wastewater typically

consists of proteins (40 to 60%), carbohydrates (25 to 50 %), oils and fats (8 to 12 %). Urea, the major constituent of urine, is another important organic compound contributing to fresh wastewater.

The biological **organic matter removal** requires sufficient contact time between wastewater and heterotrophic microorganisms, sufficient oxygen and nutrients (Figure 1.1). In Equation 1.1, the COHNS is used to represent the organic matter in wastewater, which serves as the electron donor while the oxygen serves as the electron acceptor. During the initial biological uptake of organic material, more than half of it is oxidized and the remainder is assimilated as new biomass, which may be further oxidized by endogenous respiration. A wide variety of microorganisms are found for the removal of organic material (Metcalf and Eddy, 2003).

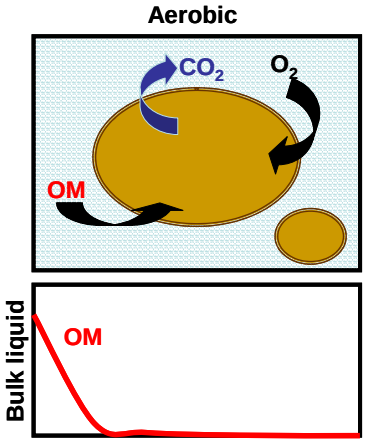

Figure 1.1. Biochemical oxidation of biodegradable organic matter. The picture represents the biomass in brown and the wastewater in blue. The graph gives a schematic representation of the change in concentrations in a plug-type of process.

Oxidation and synthesis:

$$Organic\_matter(COHNS) + O_2 + nutrients \downarrow CO_2 + NH_3 + New\_cells + end\_products$$

Eq.1.1.- Aerobic biological oxidation (Metcalf and Eddy, 2003).

For organic matter removal, pH in the range of 6.0 to 9.0 is tolerable, while optimal performance occurs near a neutral pH. A reactor Dissolved Oxygen (DO) concentration of 2 mg $O_2 \cdot L^{-1}$ is commonly used, and at concentrations above 0.5 mg $O_2 \cdot L^{-1}$ there is little effect on the degradation rate. In some wastewater care must be taken to assure that sufficient nutrients (N and P; Equation 1.1) are available for the amount of organic matter to be treated (Metcalf and Eddy, 2003).

1.2.2 Biological nitrogen removal

Biological nitrogen removal requires a two-step process: **Nitrification and denitrification** (Figure 1.2).

Since the original discovery of the nitrification reaction (Figure 1.2 Left), approximately 100 years ago, there has been considerable attention given to the application of nitrifying bacteria to wastewater treatment. Nitrification is a two-step process: ammonium is firstly oxidized to nitrite (*nitritation process*; Equation 1.2 Up) by Ammonium Oxidizing Bacteria (AOB) and then, nitrite is oxidized to nitrate (*nitratation process*; Equation 1.2 Down) by Nitrite Oxidizing Bacteria (NOB), e.g members of the genera *Nitrobacter*,

Nitrococcus and *Nitrospira* (Schmidt *et al.,* 2003). Autotrophic nitrifying bacteria are the only organisms capable of converting the most reduced form of nitrogen (ammonium) to the most oxidised form (nitrate).

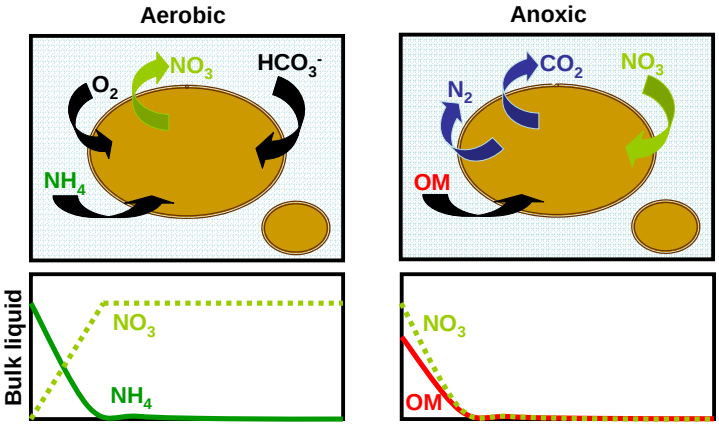

Figure 1.2. Biological nitrogen removal. *Left:* Nitrification process; *Right:* Denitrification process. The pictures represent the biomass in brown and the wastewater in blue.The graph gives a schematic representation of the change in concentrations in a plug-type of process.

$$NH_4^+ + \tfrac{3}{2}O_2 \rightarrow NO_2^- + H_2O + 2H^+$$
$$NO_2^- + \tfrac{1}{2}O_2 \rightarrow NO_3^-$$

Eq. 1.2. Nitrification process reactions. *Up:* Nitritation process. *Down:* Nitratation process.

Nitrification is affected by a number of environmental factors including: pH (Metcalf and Eddy, 2003), toxicity (Metcalf and Eddy, 2003), metals (Juliastuti *et al.,* 2003), un-ionized ammonia ($N\text{-}NH_3$; Anthonisen *et al.,* 1976; Ganigué *et al.,* 2007), un-ionized nitrous acid (HNO_2; Anthonisen *et al.,* 1976; Ganigué *et al.,* 2007) and reduced sulphur components (Sears *et al.,* 2004). The alkalinity of the wastewater must be enough to maintain the pH in the optimum interval for nitrification, because 7.13 g of alkalinity as $CaCO_3$ is consumed per 1 g of ammonium ($N\text{-}NH_4^+$) oxidized to nitrate ($N\text{-}NO_3^-$) (Metcalf and Eddy, 2003).

Two modes of nitrate reduction can occur in biological systems: **assimilating** and dissimilating or **denitrification** (Figure 1.2 Right). Assimilating nitrate reduction involves the reduction of nitrate to ammonia for use in cell synthesis. Dissimilating nitrate reduction or denitrification is coupled to the respirating electron chain and involves the reduction of nitrate (the highly oxidized forms of nitrogen available for consumption by many groups of organisms) to nitrite to nitric oxide to nitrous oxide to gaseous nitrogen, which is far less accessible to life forms but makes up the bulk of our atmosphere. Denitrification is considered to be an anoxic process, occurring in the absence of oxygen, and requires an organic electron donor (Randall *et al.,* 1992). In the denitrification process, the electron donor is typically one of these three following sources:

1. The organic mater of the influent (Equation 1.3).

$$C_{10}H_{19}O_3N + 10NO_3^- \rightarrow 5N_2 + 10CO_2 + 3H_2O + NH_3 + 10OH^- \qquad \text{Eq. 1.3}$$

2. The organic matter produced during the endogenous decay.

3. An exogenous source such as methanol or acetate (Equations 1.4 and 1.5, respectively).

$$5CH_3OH + 6NO_3^- \rightarrow 3N_2 + 5CO_2 + 7H_2O + 6OH^- \quad \text{Eq. 1.4}$$

$$5CH_3COOH + 8NO_3^- \rightarrow 4N_2 + 10CO_2 + 6H_2O + 8OH^- \quad \text{Eq. 1.5}$$

Denitrification can be described as a kind of anoxic respiration. Electrons originated from e.g. organic matter, reduced sulphur compounds or molecular hydrogen are transferred to oxidized nitrogen compounds instead of oxygen in order to build up a proton motive force usable for Adenosine 5'-triphosphate (ATP) generation (Schmidt *et al.,* 2003). Dissolved oxygen can inhibit nitrate reduction by repressing the nitrate reduction enzyme (Metcalf and Eddy, 2003). Denitrification is performed by various chemoorganotrophics, lithoautotrophic, phototrophic bacteria and some fungi, especially under oxygen-reduced or anoxic conditions (Focht and Chang, 1975; Shoun and Tanimoto, 1991; Zumft, 1997).

Denitrification process originates an increase in the medium alkalinity. One equivalent of alkalinity is produced per equivalent of $N\text{-}NO_3^-$ reduced, which equates to 3.57 g of alkalinity as $CaCO_3$ production per 1 g of $N\text{-}NO_3^-$ reduced (Metcalf and Eddy, 2003).

1.2.3 Biological phosphorus removal

Phosphorous is one of the major nutrients contributing in the increased eutrophication of lakes and natural waters. Its presence causes many water quality problems including increased purification costs, decreased recreational and conservation value of an impoundments, loss of livestock and the possible lethal effect of algal toxins on drinking water.

The traditional method for removing phosphate from wastewater is adding precipitating chemicals (iron or aluminium salts or lime ($Ca(OH)_2$)) to the wastewater. In general, with chemical precipitation 70% to 95% of phosphorus removal can be obtained and effluent total phosphorus concentrations below 0.3 mg $TP \cdot L^{-1}$ can be achieved, depending on the operational conditions (Baetens, 2000). The major disadvantages of chemical phosphorus removal are that it generates large amounts of sludge (an increase of sludge volume by up to 40%), the cost of the precipitants and the negative ecological effect of the concentration of aluminium and iron salts in the effluent (Johansson, 1994). Therefore, adding chemicals in waste treatment should be minimized (Van Loosdrecht *et al.,* 1997).

An alternative is the Enhanced Biological Phosphate Removal (EBPR). Literature reviews on EBPR have been presented by Jenkings and Tandoi (1991), van Loosdrecht *et al.* (1997), Mino *et al.* (1998) and Oehmen *et al.* (2007). In EBPR, the phosphorous in the influent wastewater is incorporated into cell biomass, which is subsequently removed from the process as a result of sludge wasting.

EBPR requires the combination of anaerobic and aerobic/anoxic conditions. During the anaerobic conditions (Figure 1.3 Left), Polyphosphate Accumulating Organisms (PAOs) assimilate fermentation products (i.e. Volatile Fatty Acids; VFAs) into storage products (Poly-β-HydroxyAlkaonate (PHA) within the cells with the concomitant release of phosphorous from stored Poly-Phosphates (PP). VFAs are produced by fermentation of biodegradable organic matter, which is dissolved degradable organic material that can

be easily assimilated by the biomass. Using energy available from stored polyphosphates, PAOs assimilate VFAs and produce intracellular PHA storage products while intracellular glycogen is converted to PHA (Mino *et al.,* 1998). Concurrent with the VFAs uptake is the release of orthophosphates, as well as magnesium, potassium and calcium cations. The PHA content in the PAO increases and the polyphosphate and glycogen decrease.

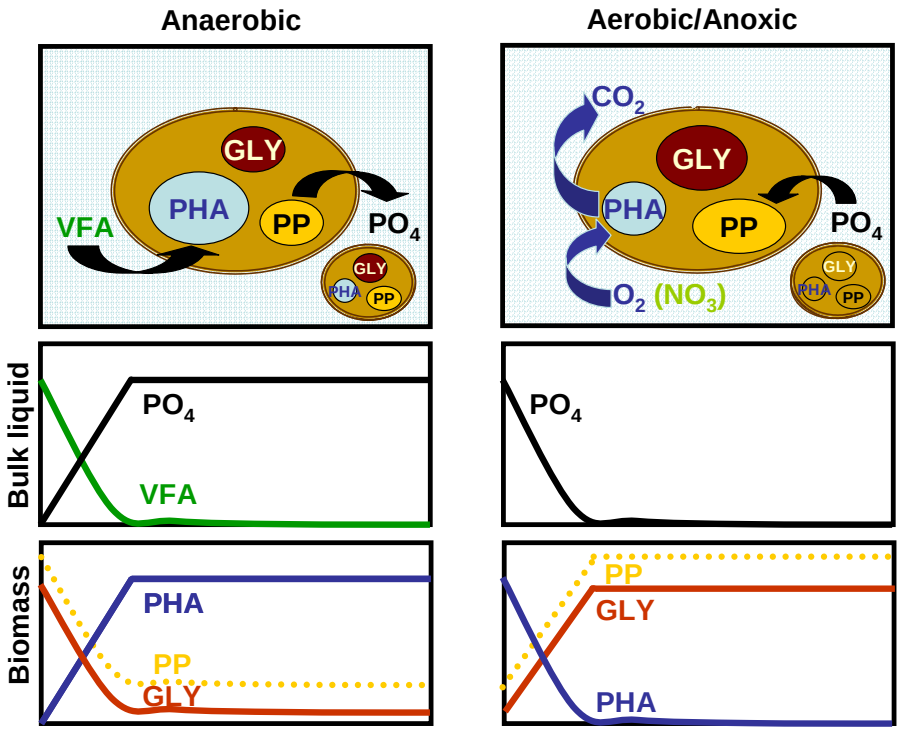

Figure 1.3. Biological phosphorus removal by PAOs. *Left:* Anaerobic conditions; *Right:* Aerobic/Anoxic conditions. The pictures represent the biomass in brown and the wastewater in blue. The graph gives a schematic representation of the change in concentrations in a plug-type of process.

In the anoxic and aerobic conditions (Figure 1.3 Right), energy is produced by the oxidation of storage products and PP storage within the cell increases. Stored PHA is metabolized, providing energy from oxidation and carbon for new cell growth. Some glycogen is produced from PHA metabolism. The energy released from PHA oxidation is used to form PP bonds in cell storage. The soluble orthophosphate is removed from solution and incorporated into PPs within the bacterial cell. PHA utilisation also enhances cell growth and this new biomass with high PP storage accounts for phosphorous removal. As a portion of the biomass is wasted, the stored phosphorous is removed from the biotreatment reactor for ultimate disposal with the waste sludge (Metcalf and Eddy, 2003).

EBPR is affected by a number of environmental factors including pH (Smolders *et al.,* 1994; Liu *et al.,* 1996; Filipe *et al.,* 2001a), excessive aeration (Brdjanovic *et al.,* 1998a), Sludge Retention Time (SRT; Brdjanovic *et al.,* 1998b), nitrate (Puig *et al.,* 2007), nitrite (Saito *et al.,* 2004; Oehmen *et al.,* 2007) and temperature (Brdjanovic *et al.,* 1998c; Panswad *et al.,* 2003). The carbon source used by PAOs is also a factor that influences the EBPR performance. The most prevalent VFA in EBPR plants is acetate, though in plants where prefermentation is employed, propionate is present in substantial quantities. Butyrate, valerate and other VFAs may also be present, but typically in small quantities (Oehmen *et al.,* 2007). Non-

VFA organic substrates (e.g. amino acids, glucose, lactate, starch) are also metabolised by PAOs (Cech and Hartman, 1990; Randall *et al.,* 1997).

PAOs include those organisms with the anaerobic–aerobic phosphorus removal phenotype, and the organisms with the anaerobic–anoxic phosphorus removal phenotype are referred to as Denitrifying Phosphate Accumulating Organisms (DPAOs). DPAOs has similar capacities and characteristics as phosphorus removal in anaerobic-aerobic processes (Kuba *et al.,* 1993). The difference between the metabolism of PAOs and DPAOs is that DPAOs use nitrate instead of oxygen as electron acceptor for phosphorus uptake. The main advantages of applying DPAOs are the savings of energy (for aeration) and organic matter and a lower sludge production in the overall phosphorus and nitrogen removal process (Kuba *et al.,* 1996). The occurrence of DPAOs has been clearly demonstrated in studies using lab-scale reactors (Ahn *et al.,* 2002; Zeng *et al.,* 2003) and in WasteWater Treatment Plants (WWTPs) (Kuba *et al.,* 1997).

Like PAOs, Glycogen-Accumulating Organisms (GAOs) take up VFAs anaerobically and convert them into storage compounds (PHA) (Figure 1.4). They do this via hydrolysis of glycogen which is their sole source of energy. However, they do not contribute to P removal (Mino *et al.,* 1998). The presence of GAOs has important consequences for the phosphorus removal capability of EBPR systems. The competitive advantage of PAOs in EBPR systems is compromised because GAOs use the same substrate (VFAs) under the same conditions. The supply of VFAs to EBPR systems is limited. If a significant amount of GAOs accumulate, the percentage of VFAs available to PAOs is reduced, thereby decreasing the phosphorus removal capability of the system. It is extremely important, therefore, to identify operational conditions that maintain the competitive advantage necessary for the accumulation of PAOs (Filipe *et al.,* 2001a).

Special attention is given in the EBPR research on the competition between PAOs and GAOs studying the factors that influence the microbial competition in EBPR system such as the carbon source, pH and temperature.

Different carbon source, VFAs and non-VFAs have been shown to have an impact on the PAO-GAO competition. While the use of acetate as a carbon source in EBPR systems has been often documented to yield robust and stable phosphorus removal performance, there are also many reported occasions where the phosphorus removal deteriorated due to what is believed to be microbial competition of GAOs with PAOs (Oehmen *et al.,* 2007). Recent studies have suggested that propionate may be a more favourable substrate than acetate for successful EBPR performance in long-term operation (Pijuan *et al.,* 2004; Oehmen *et al.,* 2005).

The effects of pH on the anaerobic metabolism of PAOs and GAOs were studied by Filipe *et al.* (2001a). Those results showed that pH control is a promising strategy for minimizing the accumulation of GAOs and increasing the reliability of biological excess phosphorus removal systems. Filipe *et al.* (2001b and 2001c) studied the stoichiometry and kinetics of acetate uptake by enriched cultures of PAOs and GAOs as a function of pH. They observed that the rate of acetate uptake by GAOs was significantly decreased when the pH of the medium was increased, but that the uptake rate for PAOs was essentially independent of the pH for the range studied (6.5 to 8.0). This strongly suggests that the control of pH in the anaerobic zone of an EBPR system provides a strategy for minimizing the growth of GAOs.

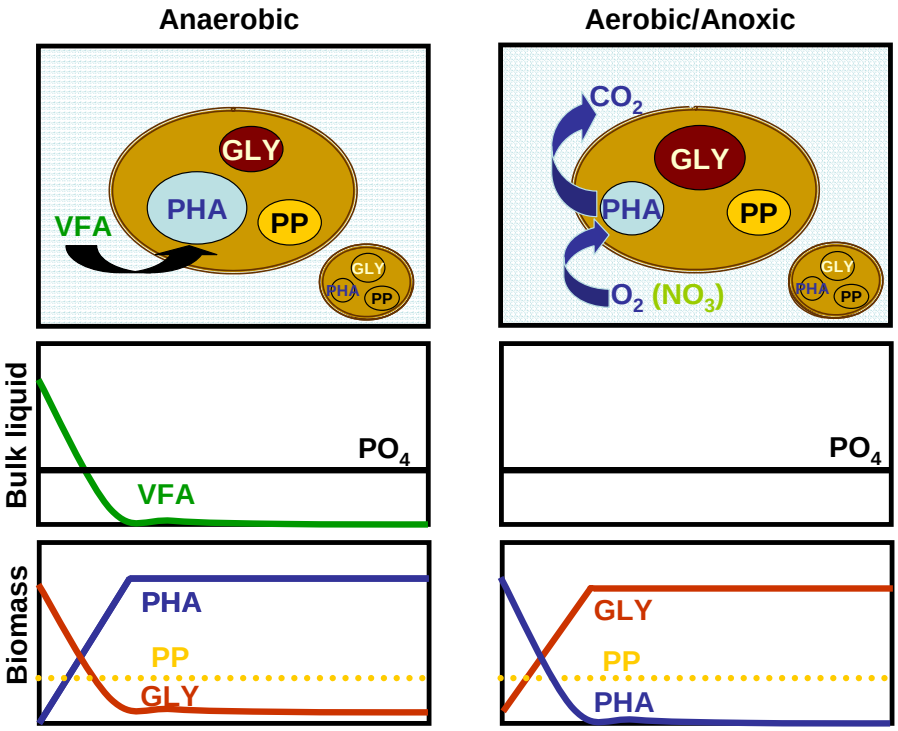

Figure 1.4. Metabolic processes of GAOs. Left: Anaerobic conditions; Right: Aerobic/Anoxic conditions. The pictures represent the biomass in brown and the wastewater in blue.

Temperature variation between 20.0ºC and 35.0ºC affects the microbial community of the EBPR system (Panswad *et al.,* 2003). This study concludes that the PAOs are lower-range mesophiles or perhaps psychrophiles and will predominate only at 20.0ºC or possibly lower. The GAOs are somewhat mid-range mesophilic organisms with optimum temperature between 25.0ºC and 32.5ºC. Lopez-Vazquez *et al.* (2007) proved that GAOs have clear advantages over PAOs for substrate uptake at temperature higher than 20ºC. Below 20ºC, maximum acetate uptake rates of both microorganisms were similar. However, lower maintenance requirements at temperature lower than 30ºC give PAOs metabolic advantages in the PAOs-GAOs competition.

1.3 Activated sludge systems. Process configuration for Biological Nutrient Removal (BNR)

Treatment of wastewater by means of biological processes has been widely implemented for classical urban and industrial wastewater. Moreover, there are many biological nutrient removal processes to deal with nutrient removal such as: Phoredox (A/O) (Figure 1.5), A^2/O^{TM} (Figure 1.6), Five-stage Bardenpho^{TM} (Figure 1.7), UCT (University of Cape Town) (Figure 1.8), modified UCT (Figure 1.9), VIP^{TM} (Virginia Initiative Plant) (Figure 1.10) and Phostrip processes (Figure 1.11). A complete review can be found in Baetens (2000) and Metcalf and Eddy (2003).

The A/O process described in Figure 1.5 is a patented version of Phoredox by *Air Products and Chemicals, Inc.*, and the main difference is the use of multiple-staged anaerobic and aerobic reactors. In

this process there is no nitrification, and the anaerobic retention time is 30 min to 1 h to provide the selective conditions for the biological phosphorus removal.

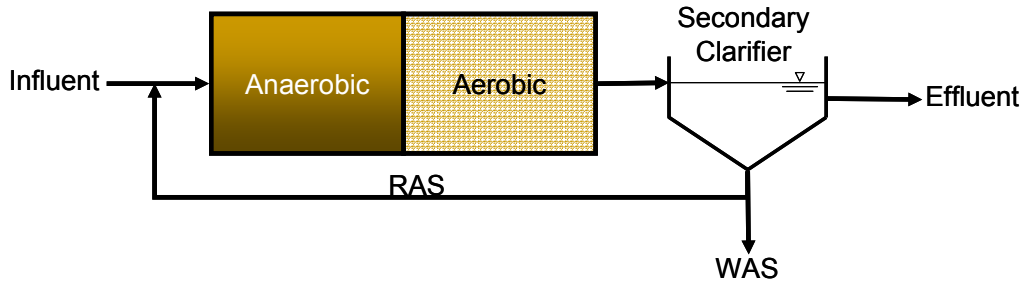

Figure 1.5. Phoredox or A/O process.

The A^2/O process (Figure 1.6), patented by *Air Products and Chemicals, Inc.*,, is a modification of the A/O process and provides an anoxic zone for denitrification with an internal recycle from the aerobic to the anoxic stage.

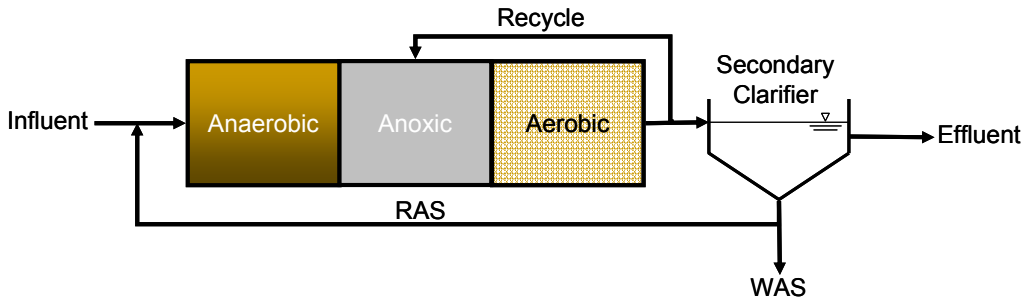

Figure 1.6. A^2/O process.

On the other hand, Barnard (1975) first achieved phosphorus removal in a mainstream process later called the Bardenpho™ process. A four phase anoxic-aerobic-anoxic-aerobic configuration, originally designed for nitrogen removal, was used. Sludge from the secondary clarifier and the mixed liquor from the first aerobic basin are recirculated to the first anoxic reactor. Including an anaerobic reactor ahead of the first anoxic reactor in the Bardenpho™ configuration, fed with sludge from this first anoxic reactor, created favourable conditions for phosphorus removal. This configuration described in Figure 1.7 is known as the five stage Bardenpho™ configuration, or the modified Bardenpho™ process. The 5-stage process uses a longer SRT (10 to 20 days) than an A^2/O process (Figure 1.6), and thus increases the carbon oxidation capacibility.

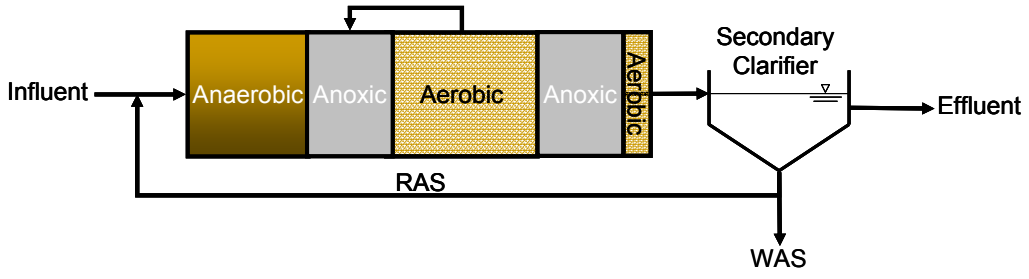

Figure 1.7. Modified Bardenpho (5-stage) process.

The UCT process (Figure 1.8) stands for University of Cape Town in South Africa. The UCT process is similar to the A^2/O process (Figure 1.6) with two exceptions. The Return Activated Sludge (RAS) is recycled to the anoxic stage instead of the aeration stage, and the internal recycle is from the anoxic stage to the anaerobic stage to avoid any negative effects on the initial phosphorus removal efficiency by nitrate present in the return sludge.

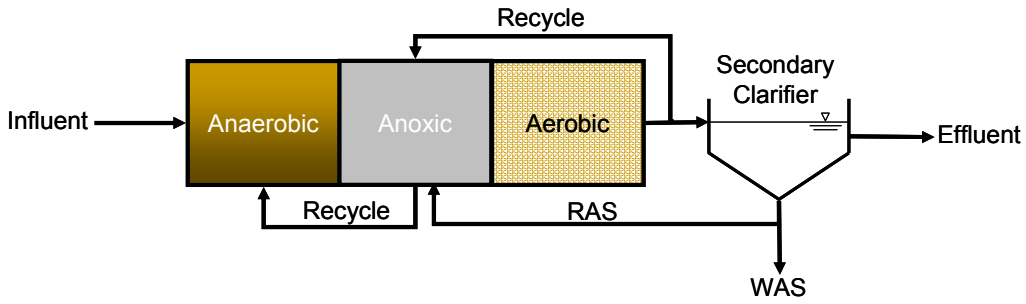

Figure 1.8. University of Cape Town (UCT) process.

In the modified UCT configuration (Figure 1.9), the anoxic reactor from the UCT process is divided in two compartments. The RAS is directed to an anoxic reactor that does not receive internal nitrate recycle flow. The mixed liquor is recycled to the anaerobic phase. The second anoxic reactor allows denitrification of the recycled mixed liquor from the aerobic phase.

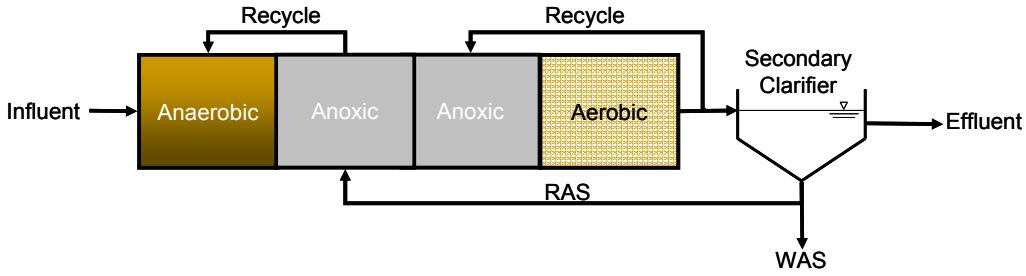

Figure 1.9. Modified UCT process.

The VIP process (Figure 1.10) stands for Virginia Initiative Plant (Daigger *et al.,* 1987). The VIP process is similar to the A^2/O (Figure 1.6) and UCT (Figure 1.9) processes except for the methods used for recycle systems.

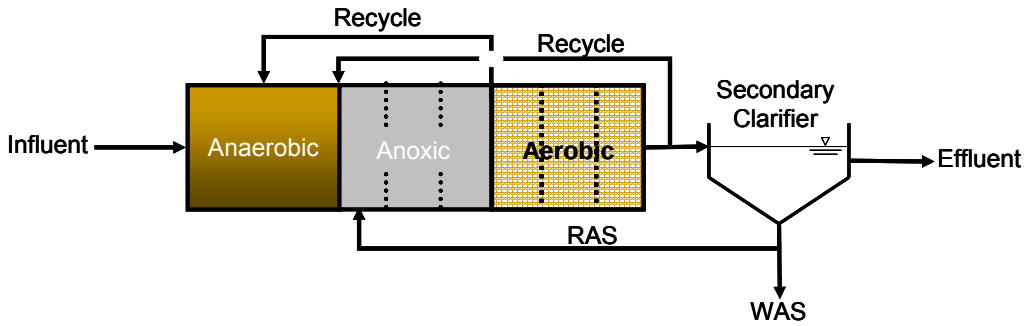

Figure 1.10. Virginia Initiative Plant (VIP) process.

Finally, the PhoStrip process (Figure 1.11) is in essence an anaerobic/aerobic process. Even though chemical treatment is used for phosphorus removal adding lime or alum and ferric salts, there is some enhanced phosphorus removal in the waste sludge due to the development of PAOs.

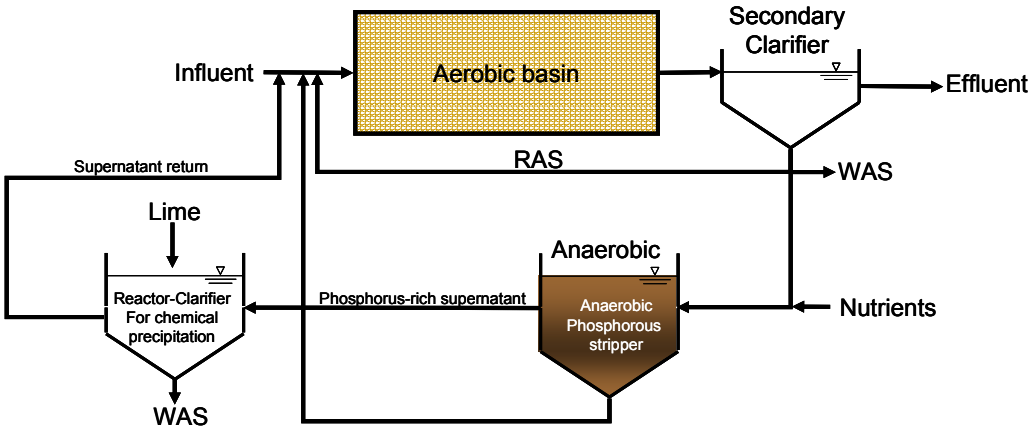

Figure 1.11. PhoStrip process.

Nevertheless, there are some drawbacks of the different EBPR process configurations which must be considered during the process selection, such as:

- Moderate level of nitrogen removal (i.e. A^2/O^{TM} (Figure 1.6), UCT (Figure 1.8) and VIP^{TM} (Figure 1.10) processes).
- Moderate to poor phosphorus removal (i.e. Five-stage Bardenpho^{TM} process) (Figure 1.7).
- Large internal cycle increases pumping energy and maintenance requirements (i.e. Bardenpho^{TM} (Figure 1.7), UCT (Figure 1.8) and VIP^{TM} (Figure 1.10) processes (Baetens, 2000).
- Large reactor volumes required (i.e. A^2/O^{TM} (Figure 1.6) (Grady *et al.,* 1999)).
- Large investments in extra infrastructure (i.e. Phostrip process (Figure 1.11) (Van Loosdrecht *et al.,* 1997)).
- The PhoStrip (Figure 1.11) process requires lime addition for phosphorus precipitation and higher mixed-liquor dissolved oxygen to prevent phosphorus release in final clarifier (Metcalf and Eddy, 2003).

1.4 Sequencing Batch Reactor (SBR) technology

The SBR technology is considered as an alternative to these conventional processes (section 1.3) for removing nutrients from wastewater. This configuration has a higher flexibility and controllability, allowing more rapid adjustment to changing influent characteristics (Baetens, 2000). Lower investment and recurrent cost is necessary because secondary settling tanks and sludge return systems are not required

(Nowak and Lindtner, 2003). Furthermore, it is especially appropriate for places where there is significant flow and load variability (Metcalf and Eddy, 2003) or where space problems become a restriction.

SBRs are widely and commonly used in biological wastewater treatment (Mace and Mata-Alvarez, 2002). SBR technology has been successfully applied in WWTPs treating urban (Lee *et al.,* 2004; Puig *et al.,* 2005) and industrial (Keller *et al.,* 1997; Torrijos *et al.,* 2001; Vives *et al.,* 2003; Cassidy and Belia, 2005) wastewater.

The application of SBR technology is a more apropiate alternative to treat wastewater than conventional continuous systems, but it requires a higher level of control and automation. The Instrumentation, Control and Automation (ICA) of the process is a key factor when the process must be operated to achieve restricted discharge levels. The recent advances in the ICA field have come a long way and it is now an established and recognised area of technology in the profession (Olsson *et al.,* 1998; Olsson, 2002).

1.4.1 SBR history and SBR full-scale applications: a brief review

The SBR technology was first used in 1914 (Ardern and Lockett, 1914). During the 20th century this technology has gained popularity mainly due to its operating advantages and flexibility. In addition, the automation of SBR operations has made their implementation much easier and has definitely contributed to the development of this technology. Research in SBRs began in the 1970's (Irvine and Davis, 1971). Irvine and Busch (1979) described SBRs, suggested an uniform terminology and united batch concepts with modern control strategies. Alleman and Irvine (1980) demonstrated the potential of the SBR to maintain combined organic carbon oxidation and nitrification. Irvine *et al.* (1983) resulted that the SBR technology is a viable alternative to conventional continuous flow activated sludge treatment of domestic wastewater, nitrification, denitrification and chemical precipitation of phosphorus in the first SBR demonstration site. Irvine and Ketchum (1988) demonstrated that the use of static and mixed fill periods could help to achieve nitrogen and phosphorus removal. Norcross (1992) in his overview considered the mechanical, process and control aspects for design of an SBR. Ketchum Jr. (1997) described the SBR physical system and explained approaches used to develop the bases of design needed to meet many different treatment objectives especially for the feeding and reaction periods.

Arora *et al.* (1985) evaluated the SBR technology at several plants in the USA suggesting that equalization, ideal settling, simple operation, compact layout and perhaps cost saving are the major advantages of SBR systems versus the continuous-flow systems. Okada and Sudo (1985) studied the simultaneous removal of phosphorus and nitrogen in a lab-SBR. Rim *et al.* (1997) carried out a successfully full-scale tests for BNR purposes using the SBR technology treating between 47.3 and 52.8 $m^3 \cdot d^{-1}$ of sewage. Helmreich *et al.* (2000) investigated the performance of SBR plants in operation in the State of Bavaria, Germany. The sizes of that SBR plants in Bavaria range from 400 to 25000 Population Equivalents (PE). Teichgräber *et al.* (2001) reported that SBR technology is applied in about 1.3% of the WWTPs in Germany. Keller *et al.* (2001) achieved complete BNR at full-scale in a single tank SBR treating 850 $m^3 \cdot d^{-1}$ of domestic wastewater. Torrijos *et al.* (2001) concluded that the SBR is, from a technical point of view, perfectly adapted to treating cheese production wastewater. Steinmetz *et al.* (2002) evaluated in

view of their effluent quality, treatment efficiency and energy demand four SBR WWTPs designed for approximately 5000, 8000, 15000 and 25000 PE. The study proved that the SBR technology is a good alternative for municipal sewage plants and can help to save investment costs. Peters *et al.* (2004) showed the results of a demonstration of enhanced nutrient removal at two full-scale SBR plants in Australia processing an average of between 2000 and 2500 $m^3 \cdot d^{-1}$ of wastewater. Poo *et al.* (2004) treated strong nitrogen swine wastewater in a full-scale SBR. Finally, Demoulin (2006) reported on the world's largest non-conventional SBR plant, currently under construction in Malaysia for 1.2 million PE.

1.4.2 Characteristics of the SBR technology

The SBR is a fill-and-draw activated sludge system for wastewater treatment. While in continuous systems the reaction and settling occur in different reactors, in SBR all the processes are conducted in a single reactor following a sequence of fill, reaction, settling and draw phases (Figure 1.12). The cycle configuration depends on the wastewater characteristics and legal requirements.

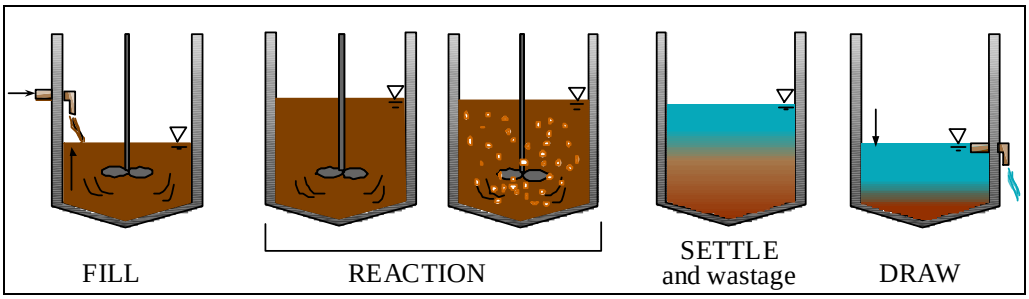

Figure 1.12. Sequence of phases in the SBR operation.

The fill phase (Figure 1.12) may be static, mixed or aerated, depending on treatment objectives. A *static fill* results in minimum energy input and high substrate concentration at the end of the fill phase. A *mixed fill* results in denitrification, if nitrates are present, and provides anaerobic conditions required for biological phosphorus removal. *Aerated fills* result in the beginning of aerobic reactions holds substrate concentrations low, which may be of importance if biodegradable constituents exist that are toxic at high concentrations (Ketchum Jr., 1997). Moreover, the feeding phase could be single or multiple feeding depending on the treatment objective (Chang and Hao, 1996; Lin and Jing, 2001; Vives, 2004; Corominas, 2006). Artan *et al.* (2006) defined the rational framework for understanding the mechanism of nitrogen removal in SBRs with multiple anoxic fill phases.

During the react phase (Figure 1.12), usually under mixing conditions, the biomass consumes the substrate under controlled environmental conditions (aerobic, anoxic or anaerobic) depending on wastewater treatment. In the aerobic react phases, the organic matter oxidation and nitrification take place. Classical heterotrophic denitrification process and the phosphorus uptake required anoxic conditions. During the anaerobic phase, phosphate is released into the liquid phase.

The sludge wasting (Figure 1.12) is another important step in the SBR operation that greatly affects performance (Metcalf and Eddy, 2003). Its target is the regulation of the sludge solids concentration in the

reactor. Sludge wasting could be done at the end of the reaction phase or during the settling phase (Irvine and Busch, 1979).

Solids are allowed to separate from the liquid under quiescent conditions, resulting in a clarifier supernatant that can be discharged as effluent (Figure 1.12). The settling time is based on subsidence of the sludge blanket layer and the concentration of the settled biomass (Ketchum Jr., 1997).

1.5 References

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Chapter 2.
Objectives

The problem definition and research objectives of the thesis are defined in this chapter.

2.1 Problem definition

In the last decades, the awareness of environmental issues has increased in society considerably. A New Water Culture is appearing. The European Water Framework Directive (EWFD, 2000/60/EC) represents these tendencies. Within the scope of the New Water Culture there is pressure to accomplish with environmental requirements. Hence, regarding the field of wastewater treatment a decrease in nutrients being discharged into surface waters is required as pointed by the Urban Water Directive (91/271/EC). There is an increasing need to improve the effluent quality of domestic wastewater treatment processes.

In order to deal with these issues, research is focussed on finding and improving technologies such as Sequencing Batch Reactor (SBR) for the suitable wastewater treatment where the operation, instrumentation, control and automation of the process are a key factor when the process must be operated to achieve restricted discharge levels and minimize the environmental impacts.

On the other hand, for Biological Nutrient Removal (BNR) from wastewater, special attention has to be given to the availability and use of the easily biodegradable substrate. If the available carbon in the raw wastewater is not enough to achieve complete nutrient removal, an additional suitable external carbon source must be required increasing the treatment costs.

2.2 Research objectives

The main motivation of this thesis is to study the **biological nutrient removal from wastewater by applying the SBR technology**. In this side, it pretends to step forward on the SBR operation and control for nutrient removal purposes. These main objectives are divided into more specific goals:

- To study the BNR performance using the SBR technology to treat wastewater applying the step-feed as the strategy to optimize the organic matter available in the raw wastewater. Two situations will be considered:
    - To treat urban wastewater for carbon and nitrogen removal.
    - To treat synthetic wastewater for nutrient removal purposes.
- To define, develop and implement a SBR real-time control system for the optimal treatment of urban wastewater for carbon and nitrogen removal. This control system has to adjust to the duration of the aerobic and anoxic reaction phases based on conventional and economical probe (pH, Dissolved Oxygen (DO) and Oxidation-Reduction Potential (ORP) signals. Thus, more concrete objectives can be defined:
    - To identify the SBR control variables with a key role when removing organic matter and nitrogen.

- To define a control strategy to adjust the length of the reaction phases (aerobic and anoxic phases) based on historical data.
- To develop and implement the real-time control system on a SBR pilot plant to evaluate its effectiveness treating urban wastewater.
- To improve the SBR real-time control system based on experience acquired in the previous implementation.

- To investigate the influence of Carbon:Phosphorus (C:P) and Carbon:Nitrogen (C:N) feeding ratios on the efficiency of BNR from wastewater because the denitrification process and biological phosphorus removal compete both for the organic matter available in the raw wastewater. In this sense, two possible situations will be considered:
  - A modification of the influent phosphorus load.
  - A modification of the influent carbon load.

- To evaluate the behaviour of ethanol as an alternative of conventional Volatile Fatty Acids (VFAs) (i.e. acetate and propionate) as an external carbon source for Enhanced Biological Phosphate Removal (EBPR) from wastewater in ethanol acclimated and unacclimated biomass if the available carbon in the raw wastewater is not enough to achieve complete nutrient removal (low C:N and C:P ratios), an additional suitable external carbon source must be required. More specific goals are:
  - To study the ethanol adaptation time effect on the bio-P biomass.
  - To study the effect of the carbon source on the Poly-β-HydroxyAlkaonate (PHA) produced by Polyphosphate Accumulating Organisms (PAOs) during the anaerobic phase in an EBPR system.
  - To characterise the anaerobic and aerobic stoichiometry of ethanol adapted sludge.

Chapter 3.
Materials and methods

This chapter presents the summary of chemical and microbial analyses, as well as, different experimental set ups have been used along this thesis.

3.1 Experimental set ups

3.1.1 Lab-scale SBR

The lab-scale Sequencing Batch Reactor (SBR) (Figure 3.1) was located at the Science Faculty of the University of Girona. The lab-scale SBR plant was composed of three elements: the storage tank, the reactor and the control panel as described in Figure 3.1.

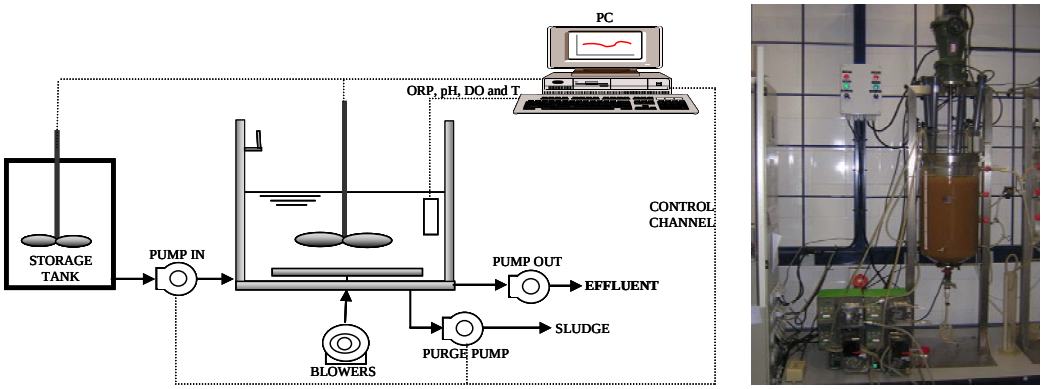

Figure 3.1. Lab-scale SBR. *Left*: Scheme; *Righ*: Picture

The influent wastewater storage tank of 150L is made of stainless steel and is completely mixed. It is equipped with a cooler that allows the temperature of the tank to be controlled. In this case, the influent wastewater is kept at 4ºC in order to minimize the microbiological activity.

The lab-scale SBR (Figure 3.1 Right) is composed of a cylindrical glass reactor working with a maximum volume of 30 L and adjusted to operate at a minimum volume of 20L (which is the residual volume at the end of each SBR cycle). The reactor operates at a predefined SBR cycle, repeated over time, at 20°C in a thermoregulated room. During all filling and reaction phases a mixing device (a marine helix turning at 400 rpm) kept the reactor content under homogenous conditions. Aerobic conditions were achieved by compressed air injections, with on/off Dissolved Oxygen (DO) control. Filling, wastage and draw events were conducted by three different peristaltic pumps (Watson Marlow®). At the end of the reaction time and before the settling phase of each cycle, excess biomass is removed from the reactor under aerobic conditions and while being mixed, to maintain the desired Sludge Retention Time (SRT). During the extraction periods, treated wastewater was discharged from the reactor until a predefined 20 L minimum reactor water level is reached. The reactor is equipped with a floating-probe system for on-line monitoring of the pH (EPH-M10), Oxidation-Reduction Potential (ORP-M10), temperature (PT-100) and DO (WTW OXI 340).

The control panel is equipped with the transmitters, the cards and the computer. The probe signals are filtered in the transmitters and connected to the data acquisition hardware composed of different interface cards (PCL-711B, PCL-728 and PCLD-885 from Advantech). These cards are in charge of

controlling the on/off switch for the process of filling, wastage, drawing of peristaltic pumps, the mixing device, and air supply. Self-developed software programmed in LabWindows® (from National Instruments) was installed in a computer to control the process (Figure 3.2). It is based on user-friendly interfaces and is able to create operating cycles and make them run.

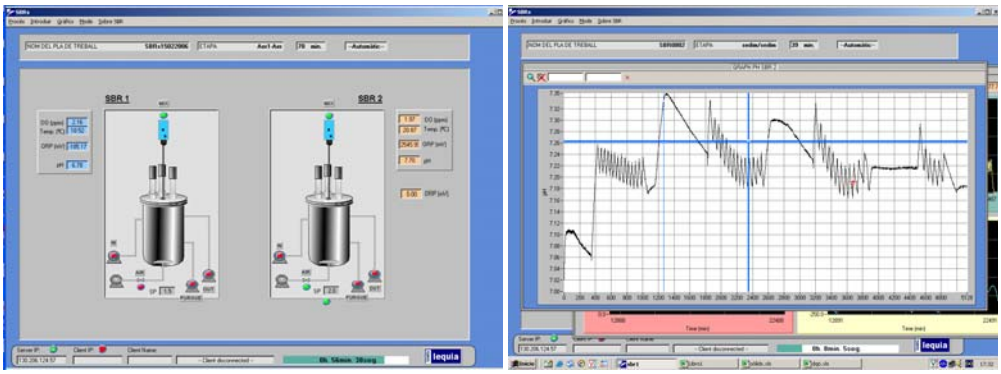

Figure 3.2. Print screen of the main user-friendly interfaces.

3.1.2 SBR pilot plant

The SBR pilot plant (Figure 3.3) was designed specially to be located at WasteWater Treatment Plant (WWTPs) to treat real wastewater. Thus, a compact structure was needed to be able to move the plant (Figure 3.3). The SBR pilot plant is composed of three elements: the storage tank, the reactor and the control panel (Figure 3.4).

Figure 3.3. Picture from outside and inside of the SBR pilot plant.

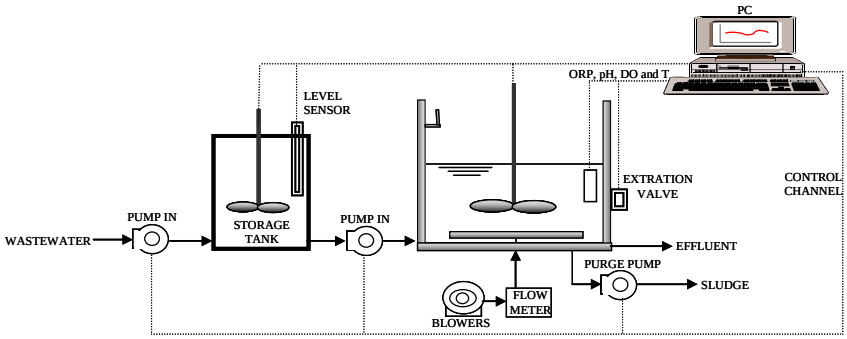

Figure 3.4. Scheme of the SBR pilot plant.

The 300 L storage tank (Figure 3.3 and Figure 3.4) is made of plastic and is completely mixed. Any wastewater temperature control is applied in the storage tank. The wastewater coming from the influent of the WWTP (Figure 3.5) is sequentially introduced into the storage tank using the pretreatment pump (Watson Marlow® 621 F/R 77 RPM).

Figure 3.5. Picture from the WWTP where the wastewater was taken as an influent for the SBR pilot plant.

The SBR pilot plant is composed of a 1000L stainless steel square reactor (Figure 3.3). The wastage in the SBR is performed under mixing and aeration conditions in the last aerobic phase for controlling the sludge or solids retention time of the system, assuming equal concentrations of solids in the wastage and in the reactor.

Furthermore, the plant is equipped with a monitoring and control system consisting of three parts: probes (from Endress-Hauser®), data acquisition and switch on/off cards (from National Instruments), and interfaces developed in LabWindows® (from National Instruments). The SBR is equipped with DO-temperature (OXIMAX- W COS 41), pH (CPF 81) and ORP (CPF 82) probes. Their signals, filtered in the transmitter, are captured by a data acquisition card (PCI-6025E) and control is conducted using a power relay output board (SC-2062), which allowed optimal functioning of the equipment.

The software (Figure 3.6) is able to repeat over time a previously defined operation cycle by controlling the switch on/off process of filling, wastaging, and drawing of peristaltic pumps, the mixing device and the air supply. Online mean values of pH, ORP, DO and temperature are obtained every 5 s (software sampling time, 0.05 s) and stored in a simple ASCII file for further processing.

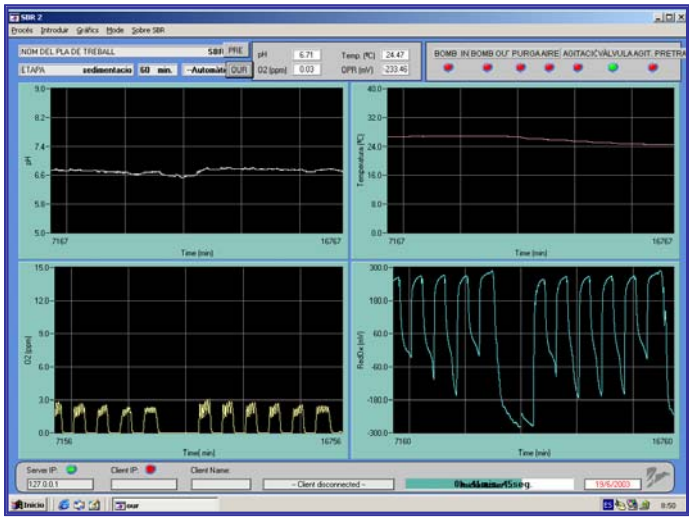

Figure 3.6. Print screen of the main user-friendly interfaces of the software applied in the SBR pilot plant.

3.1.3 Batch reactor

The batch reactor (BIOSTAT B PLUS-SARTORIUS AG) (Figure 3.7) is a cylindrical glass reactor with a maximum capacity of 5L with a cap at the top that permits hermetic conditions to be obtained. Mixing is conducted by a stirrer. The gassing system consists of a rotameter, solenoid valves and mass flow controller. The system is thermostated at 20ºC with a circulation pump or dry heating with a controlled cooling water valve. pH is controlled with two peristaltic pumps adding acid or base solutions.

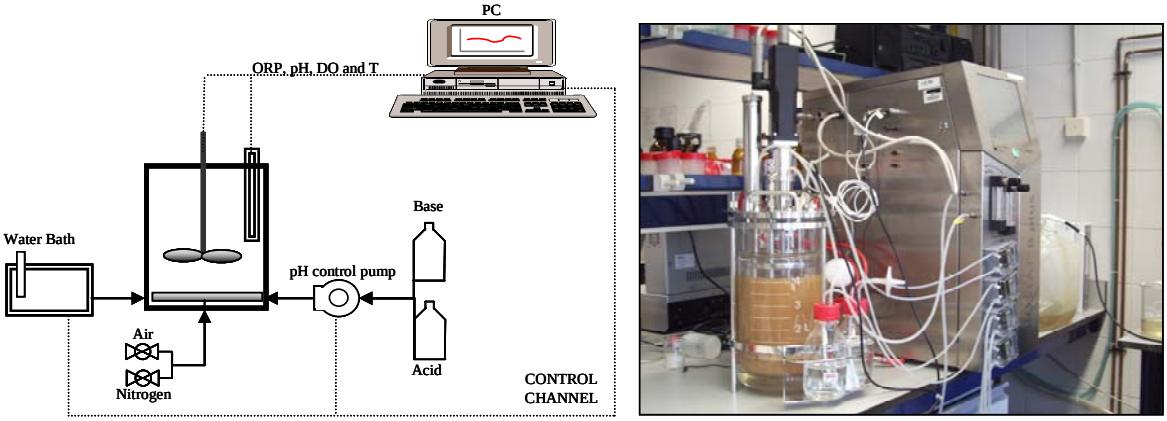

Figure 3.7. Batch reactor.

It is equipped with pH, ORP, conductivity, DO and temperature probes. A supervisory software MFCS/DA for extended visualization, data acquisition and trend display is included. Main features include on-line data acquisition and storage, sample data management for off-line measured values, process evaluation and visualization, as well as event dependent process control, process documentation via data export and batch reporting.

3.2 Chemical analyses

Different analytical methods were used for the period analysis. They were measured in accordance with standard methods (APHA, 2005). More information about the analytical measurements used in this thesis can be found in Vives (2004).

- Total Chemical Oxygen Demand (COD). APHA method number: 5220B.
- Total Kjeldalh Nitrogen (N-TKN). APHA method number: 4500-Norg.B.
- Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS). APHA method numbers: 2540D and 2540E.
- Settled Sludge Volume in 30 minutes (V_{30}). Plave 1.0L sample in settling column and distribute solids by covering the top and inverting cyclinder three times. Insert stirring rods, activate strirring mechanism, start the stopwatch, and let suspension settle. Determine volume occupied by suspension at desired measured time. APHA method number: 2710C.

- Sludge Volume Index (SVI). SVI is the volume in milliliters occupied by 1g of a suspension after 30 minutes settling. The procedure consists on determine the TSS of a well-mixed sample of the suspension and the V_{30}. APHA method number: 2710D.

- Ammonium ($N\text{-}NH_4^+$) concentration of the supernatant was determined distilling (Büchi B324) a sample into a solution in boric acid. The ammonia in the distillate could be determined either tritimetrically (Titrino 719S Metrohm) with a standard H_2SO_4 and pHmeter. APHA method number: 4500-NH_3.B-C.

- Nitrites ($N\text{-}NO_2^-$), nitrates ($N\text{-}NO_3^-$) and phosphate ($P\text{-}PO_4^{3-}$) were analyzed using ionic cromatrography (Metrohm® 761-Compact). APHA method number: 4110B.

- Volatile Fatty Acids (VFAs) were measured using a ThermoInstruments® Trace 2000 gas chromatograph, with an FFAP capillary column (0.25 mm ID and 30 m long) and a flame ionization detector. It was used as the carrier gas with a flow rate of 1 $mL \cdot min^{-1}$. More details about the method in Campos (2001).

- Inorganic Carbon (IC), Total Organic Carbon (TOC) and Total Carbon (TC) were measured by Shimadzu TOC-VC SH analyzer. APHA method number: S310.

- Total Nitrogen (TN) was calculated as the sum of N-TKN, nitrite and nitrate concentrations as mg $N\text{-}TN \cdot L^{-1}$.

- Poly-β-HydroxyValerate (PHV) and Poly-β-HydroxyButyrate (PHB) as the main Poly-β-HydroxyAlkaonates (PHAs) and the glycogen content in the sludge were performed as reported in Smolders *et al.* (1994). Poly-β-Hydroxy-2-MethylValerate (PH2MV) was calculated from the C balance taking into account the carbon dosed, the PHA produced and glycogen consumed.

3.3 FISH analysis

A Fluorescent *In Situ* Hybridisation (FISH) analysis was performed as specified by Amann (1995) using the Cy5-labelled EUBMIX probe to target the entire bacterial community (Daims *et al.,* 1999), the Cy3-labelled PAOMIX (consisting of PAO462, PAO651 and PAO846 probes) was used for Accumulibacter, a known PAO, Fluos-labelled GAOMIX (consisting of GAO431 and GAO989 probes) to target Competibacter, a known GAO (Crocetti *et al.,* 2002) and a group of alphaproteobacterial GAOs Fluos-labelled (ALF969 for some Alphaproteobacteria and to be used with c969a and c969b (Oehmen *et al.,* 2006), SBR9-1a probe (Beer *et al.,* 2004), TFO-DF218 and TFO-DF618 probes (Wong *et al.,* 2004), DF988 and DF1020 be used in conjunction with helper probes H966 and H1038 (Meyer *et al.,* 2006)). The probed sludge was examined using an Epifluorescence Zeiss® microscope or Leica confocal laser scanning microscope. The area containing specific labelled probe cells (Cy3 and Fluos for PAOMIX and GAOMIX or alphaproteobacterial GAOs, respectively) was quantified as a percentage of the area of entire bacterial population (EUBMIX). The probes used are summarized in Table 3.1.

Table 3.1. Oligonucleotide probes used in this thesis.

	Probe name	Probe sequence (5′-3′)	Specificity	% Formamide	Reference
EUBMIX	EUB338	GCT GCC TCC CGT AGG AGT	Many but not all Bacteria	0-70	Amann *et al.* (1999)
	EUB338-II	GCA GCC ACC CGT AGG TGT	Planctomyces branch	0-50	Daims *et al.* (1999)
	EUB338-III	GCT GCC ACC CGT AGG TGT	Verrucomicrobia	0-50	Daims *et al.* (1999)
PAOMIX	PAO462	CCG TCA TCT ACW CAG GGT ATT AAC	Candidatus Accumulibacter posphatis	35	Crocetti *et al.* (2000)
	PAO651	CCC TCT GCC AAA CTC CAG	Candidatus Accumulibacter posphatis	35	Crocetti *et al.* (2000)
	PAO846	GTT AGC TAC GGC ACT AAA AGG	Candidatus Accumulibacter posphatis	35	Crocetti *et al.* (2000)
GAOMIX	GAOQ431	TCC CCG CCT AAA GGG CTT	Candidatus Competibacter posphatis	35	Crocetti *et al.* (2002)
	GAOQ989	CAC CTC CCG ACC ACA TTT	Candidatus Competibacter posphatis	35	Crocetti *et al.* (2002)
	SBR9-1a	AAG CGC AAG TTC CCA GGT TG	Sphingomonas-related organisms	30	Beer *et al.* (2004)
DF1MIX	TFO-DF218	GAA GCC TTT GCC CCT CAG	Defluvicoccus - related TFO in Alphaproteobacteria	35	Wong *et al.* (2004)
	TFO-DF618	GCC TCA CTT GTC TAA CCG	Defluvicoccus - related TFO in Alphaproteobacteria	35	Wong *et al.* (2004)
	ALF-1B	CGT TCG YTC TGA GCC AG	Some alphaproteobacteria	20	Manz *et al.* (1992)
	ALF-969	TGG TAA GGT TCT GCG CGT	Some alphaproteobacteria	35	Lemaire and other unpublished results. More details in Oehmen *et al.* (2006)
	c969A	AGG TAA GGT TCT GCG CGT	To be used with ALF-969	0	
	c969B	GGG TAA GGT TCT GCG CGT	To be used with ALF-969	0	
DF2MIX	DF988	GAT ACG ACG CCC ATG TCA AGG G	Cluster 1 D. Vanus	35	Meyer *et al.* (2006)
	DF1020	CCG GCC GAA CCG ACT CCC	Cluster 2 D. Vanus	35	Meyer *et al.* (2006)
	H966	CTG GTA AGG TTC TGC GCG TTG C	To be used with DF2MIX	0	Meyer *et al.* (2006)
	H1038	AGC AGC CAT GCA GCA CCT GTG TGG CGT	To be used with DF2MIX	0	Meyer *et al.* (2006)

3.4 References

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Chapter 4. Nitrogen removal from urban wastewater using the SBR technology

The main problem of biological nitrogen removal, when treating low Carbon:Nitrogen (C:N) ratios, from wastewater is the specific use of organic matter for denitrification purposes in order to avoid the use of complementary carbon sources. Since easily biodegradable organic matter is rapidly consumed under aerobic or anoxic conditions (i.e. aerobic oxidation or anoxic denitrification, respectively), it is an important factor to consider in Sequencing Batch Reactors (SBRs) for nitrogen removal purposes.

The objective of this chapter was to study the biological nitrogen removal performance in a SBR pilot plant treating 600L·d^{-1} of urban wastewater applying the step-feed as the strategy to optimize the use of the organic matter available in the raw wastewater.

This chapter has been the basis of the following publication:

Puig, S., Vives, M.T., Corominas, Ll., Balaguer, M.D. and Colprim, J. 2004. Wastewater nitrogen removal in SBRs, applying a step-feed strategy: from lab-scale to pilot-plant operation. *Water Sci. Technol.,* **50**(10), 89-96.

4.1 Introduction

The use of Sequencing Batch Reactors (SBRs) in the biological treatment of wastewater has been widely extended from lab-scale studies to real WasteWater Treatment Plants (WWTPs) (Brenner *et al.,* 2000; Artan *et al.,* 2001; Keller *et al.,* 2001; Mace and Mata-Alvarez, 2002; Steinmetz *et al.,* 2002; among others). While lab-scale SBRs have been used for basic and applied research on nutrient removal and the development of urban/industrial wastewater biodegradability assays (Spanjers and Vanrolleghem, 1995; Zeng *et al.,* 2003; Vives, 2004), real plant applications, unless in Catalonia or Spain, are still mainly focused on carbon and ammonium removal (ammonium oxidation). Nevertheless, when operating real plant SBRs with aerobic and anoxic phases, the efficiency of nitrogen removal turns out to be better than the legally required effluent standards (Keller *et al.,* 2001; Teichgräber *et al.,* 2001).

When operating SBRs for nitrogen removal (i.e. biological nitrification/denitrification), special attention has to be given to the availability and use of the easily biodegradable substrate for denitrification purposes. If the easily biodegradable substrate is not focused on the denitrification process, high effluent nitrate concentrations may be found due to partial denitrification. Table 4.1 summarizes the strategies in literature applied to increase denitrification efficiency.

Table 4.1. Strategies in the bibliography applied to increase the organic matter available for denitrification purposes.

Feeding strategy	References
Supplying an external carbon source	Acetate: Bernardes and Klapwijk, 1996; Hallin *et al.,* 1996; Ghyoot *et al.,* 1999. Methanol: Hallin *et al.,* 1996; Hallin and Pell, 1998; Ghyoot *et al.,* 1999; Cheng and Liu, 2001; Louzerio *et al.,* 2002. Ethanol: Hallin and Pell, 1998.
Adding sludge during the anoxic phases	Bernet *et al.,* 2000; Ra *et al.,* 2000.
Controlling the nitrate concentration at the end of the anoxic zone	Van Loosdrecht *et al.,* 1998; Yuan *et al.,* 2002.
Optimizing the feeding phases during the anoxic phases	Ayesa *et al.,* 1998; Andreottola *et al.,* 2001; Keller *et al.,* 2001; Sin *et al.,* 2004; Vives, 2004; Artan *et al.,* 2006.

When the influent characteristics are shortened of available biodegradable carbon source, the addition of an external carbon source (i.e. acetate, methanol or ethanol) is a strategy which is widely applied (Table 4.1). Nevertheless, denitrification with some complementary carbon source like methanol or ethanol requires an adaptation period (Hallin and Pell, 1998; Ghyoot *et al.,* 1999). Thus, to avoid the higher treatment costs, the application of different operational strategies should be considered to increase the use of easily biodegradable substrate for denitrification purposes.

Adding sludge from an aerobic reactor during anoxic periods has also been used (Brenet *et al.,* 2000; Ra *et al.,* 2000), but this strategy is not applied in SBR technology unless the plant employs two SBRs working in parallel.

Controlling the nitrate recirculation flow has also attracted considerable interest (Van Loosdrecht *et al.,* 1998; Yuan *et al.,* 2002). A common strategy is to control the nitrate concentration at the end of the anoxic zone at a level of about 1–2 mg $N\text{-}NO_3^-\cdot L^{-1}$. This strategy maximizes the use of influent organic matter for denitrification, but has limited effectiveness in maintaining the effluent nitrate level, as the amount of nitrate that can be removed is predominantly determined by the influent to Carbon:Nitrogen (C:N) ratio when external Chemical Oxygen Demand (COD) is not used (Yuan and Keller, 2003). The nitrate recirculation flow strategy like the addition of sludge is only applicable in facilities where at least two SBRs are working in parallel.

The uniform introduction of influent into the bottom of the tank during settling or compression can help to optimize the supply and utilization of the easily biodegradable substrate (i.e. the UniFed® process) (Keller *et al.,* 2001). Moreover, the introduction of the feed distribution system allows the use of the installed hydraulic capacity for virtually 100% of the time, eliminating the "non-productive" periods of settling and decant since during this time, the lower part of the reactor is used for the anoxic and anaerobic processes to take place.

Another option for enhancing denitrification is the step-feed strategy. Step-feed strategy for nitrogen removal means that the influent filling phases must be carried out under anoxic conditions to increase the denitrification efficiencies (Andreottola *et al.,* 2001; Lin and Jing, 2001; Vives, 2004; Corominas *et al.,* 2006a). In a single-feed strategy, substantial amounts of nitrate might remain in the effluent because endogenous denitrification cannot reduce nitrate efficiently in the anoxic reaction and settle period as there is no organic electron donor (Chang and Hao, 1996). However, in the step-feed strategy, with anoxic filling events between aerobic phases, there is an increase in the availability of organic matter in wastewater for exogenous denitrification in the anoxic phases, and subsequently this allows nitrification to occur under lower organic loading in the oxic periods (Lin and Jing, 2001). The step-feed strategy has been applied successfully in SBRs plants at Laboratory of Chemical and Environmental Engineering (LEQUIA) group (University of Girona (UdG)) treating synthetic (Corominas, 2006b), urban (Vives, 2004) and industrial (Vives *et al.,* 2003) wastewater.

4.2 Objectives

The objective of this chapter was to study the biological nitrogen removal performance in a SBR pilot plant treating $600L\cdot d^{-1}$ of urban wastewater applying the step-feed as the strategy to optimize the organic matter available in the raw wastewater.

4.3 Materials and methods

4.3.1 Methodology

Two experimental set ups were used for this study: 30L lab-scale SBR (Figure 3.1) and a 1000 L SBR pilot plant (Figure 3.3). The lab-SBR was located at the Science Faculty of the University of Girona (Girona, N.E. Spain) and the SBR pilot plant at the Cassà WWTP (Girona, N.E. Spain). Figure 4.1

summarizes the two operational periods conducted with the lab scale and the pilot-plant SBRs. These operational cycles were adapted from previous studies with synthetic wastewater and carried out on a lab scale (Vives, 2004). Both reactors had an 8 h cycle time divided into reaction (390 min), settling (60 min) and discharge (30 min) phases. Thus, 53.8% of the entire reaction time took place under anoxic conditions and 46.2% under aerobic conditions with influent filling during the anoxic phases (step-feed strategy). The last reaction phase was an aerobic phase without feeding to oxidize the organic matter and remove the N_2 bubbles kept inside the sludge in the previous anoxic phase. Thus, the sludge settling characteristics improved and this prevented the sludge from rising in the settler (Larrea *et al.,* 2001).

Since different aeration methods were applied to each reactor (constant air flow during aeration phases for the lab scale SBR whereas the SBR pilot plant was Dissolved Oxygen (DO) controlled at 2.0 mg $DO \cdot L^{-1}$ set point by an air on/off strategy), different filling sequences were considered. Thus, in the lab scale SBR a small filling event (1 min) was carried out at the start of each anoxic phase to reduce the high initial DO levels (around 6–8 mg $DO \cdot L^{-1}$) which were achieved at the end of each aerobic phase because of the depletion of biodegradable organic matter and ammonium. After reaching anoxic conditions (i.e. 5 minutes later), there was a second filling event to obtain the desired wastewater volume (indicated with two arrows in Figure 4.1). This filling strategy was adopted to enhance denitrification by using the easily biodegradable organic matter from the wastewater (Vives, 2004). The number of filling events was determined based on a simple nitrogen mass balance (during the last filling event) and, considering a complete nitrification/denitrification (under aerobic or anoxic conditions, respectively).

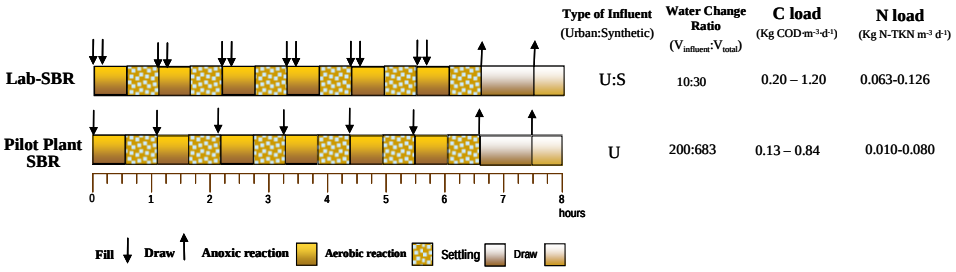

Figure 4.1. Operational periods of the two SBRs with filling strategy. On the right, the treated wastewater origin, water change ratio per cycle and minimum-maximum carbon and nitrogen loads applied for each operational period.

The operational conditions during the experimental period of the 1000L SBR pilot plant are presented in Table 4.2.

Table 4.2. Operational SBR conditions during the experimental period in the Cassà WWTP.

Parameter	Mean ±σ	Range	Units
HRT	1.1 ± 0.1	0.9 – 1.3	*days*
SRT	22.9 ± 10.0	10.2 – 49.0	*days*
SVI_{30}	155 ± 71	41 – 280	-
V_{30}	456 ± 223	167 – 970	$mL \cdot L^{-1}$
$TSS_{REACTOR}$	3043 ± 586	1790 – 4105	$mg \cdot L^{-1}$

where Hydraulic Retention Time (*HRT*), Sludge Retention Time(*SRT*), Sludge Volume Index after 30 minutes of settling (*SVI*), Settled Sludge Volume in 30 minutes (V_{30}) and Total Suspended Solids ($TSS_{REACTOR}$)

To achieve nitrogen removal in the SBR, the hydraulic retention time (HRT) and solids retention time (SRT) were maintained at 1.11 day and 22.9 days on average, respectively. The average SRT applied was considered according to the reference values to achieve complete organic matter and nitrogen removal (Mace and Mata-Alvarez, 2002; Metcalf and Eddy, 2003).

4.3.2 Wastewater

The 30 L lab scale SBR (Figure 3.1) was fed with real urban wastewater from the Girona WWTP (Girona, N.E. Spain). Twice a week 150L of fresh wastewater were transported (a journey of about 60 min) to our laboratory and stored at 4°C in a stainless steel mixing tank to minimize microbiological activity. The COD and nitrogen content of the fresh wastewater were low. Therefore, in order to maintain the desired carbon and nitrogen loads, synthetic sources of carbon (200 mg $COD \cdot L^{-1}$) and nitrogen (30 mg $N\text{-}NH_4 \cdot L^{-1}$) were added to the fresh wastewater. The wastewater characteristics (considering the addition of synthetic sources) are presented in Table 4.3.

Table 4.3. Characterization of the wastewater during the experimental study.

	Lab-SBR		**SBR Pilot Plant**		
Parameter	**Mean**	**Range**	**Mean ±σ**	**Range**	**Units**
COD	584	200-1200	495 ± 197	140 - 935	*mg $COD \cdot L^{-1}$*
N-TN	-	-	46.8 ± 17.1	8.2 – 88.0	*mg $N\text{-}TN \cdot L^{-1}$*
N-TKN	86	63-126	45.7 ± 16.9	8.2 – 87.3	*mg $N\text{-}TKN \cdot L^{-1}$*
$N\text{-}NH_4^+$	74	56-125	24.2 ± 9.7	2.5 – 47.3	*mg $N\text{-}NH_4^+ \cdot L^{-1}$*
$N\text{-}NO_2^-$	-	-	0.69 ± 0.80	0.00 – 4.17	*mg $N\text{-}NO_2^- \cdot L^{-1}$*
$N\text{-}NO_3^-$	-	-	1.44 ± 2.15	0.0 – 8.59	*mg $N\text{-}NO_3^- \cdot L^{-1}$*

The 1000L SBR pilot plant (Figure 3.2) was located at the Cassà WWTP and treated the same wastewater from the real WWTP (Table 4.3). In contrast to the lab-scale SBR, the wastewater pumped to the storage tank (15 min pumping every 40 min) was stored at ambient temperature without refrigeration, under mixing conditions. It is important to note that influent wastewater from the Cassà WWTP showed unusually high organic nitrogen content (about 50% of the total nitrogen) which might be caused by industrial activity connected to the sewer network (i.e. cork, textile and furniture factories).

It is also important to note the high variability of the COD concentration, which ranged from 200 to 1200 g $COD \cdot L^{-1}$ in the lab-scale SBR, and from 140 to 935 g $COD \cdot L^{-1}$ in the SBR pilot plant. This variability can be explained by the size and characteristics of the WWTPs selected as wastewater sources. In both cases, they are small to medium sized WWTPs (the Girona and the Cassà WWTPs serve around 40000 and 10000 Population Equivalents (PE), respectively).

4.4 Results and discussion

4.4.1 Background

Figure 4.2 shows the evolution of the COD concentrations (A) and nitrogen (B) components during the experimental study in the lab-scale SBR treating urban wastewater.

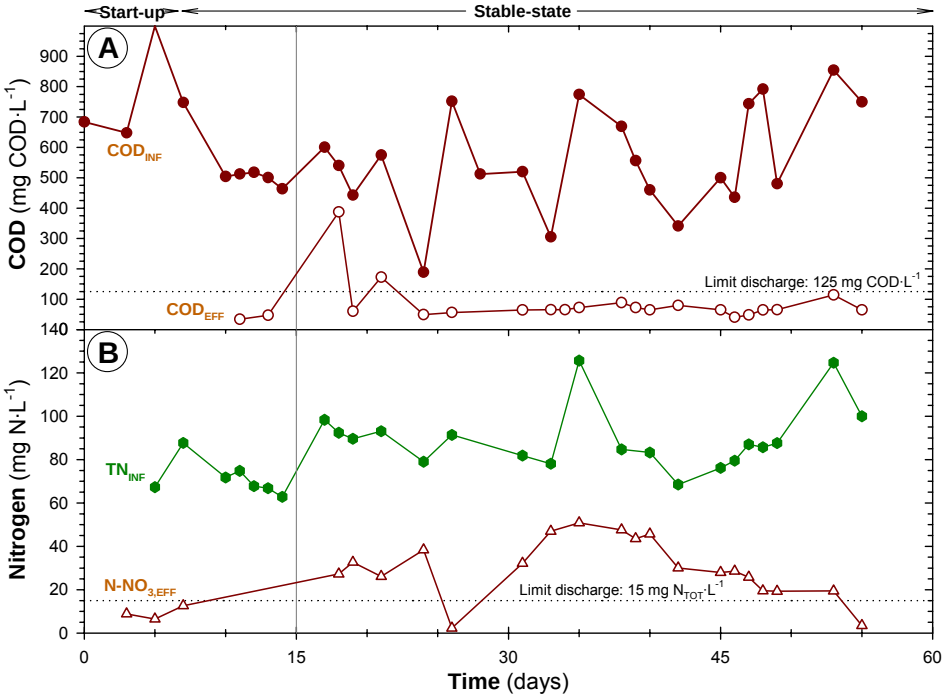

Figure 4.2. Evolution of influent (coloured dots), effluent (white dots) for: (A) total COD and (B) nitrogen during the lab-SBR operation (Vives, 2004).

When treating real wastewater from the Girona WWTP, low denitrification efficiencies were detected in the lab-SBR (39% of the nitrate was denitrified), reaching nitrate effluent concentrations of 50.0 mg $N\text{-}NO_3 \cdot L^{-1}$. First of all, the operational cycle was modified to increase the anoxic reaction percentage time from 23.1% to 53.8% and decrease the aerobic reaction percentage time from 76.9% to 46.2%. However, these changes were not enough, because the real wastewater that was renewed twice a week constantly degraded inside the storage tank at 4ºC. As a consequence, the organic matter that remained after a few days was not rapidly biodegradable (Vives, 2004). Thus, the influent was dosed with around 200 mg $COD \cdot L^{-1}$ of extra organic matter. Moreover, an analysis of the process efficiency and the DO profile in lab-scale experiences showed that an 8 mg $DO \cdot L^{-1}$ at the end of the aerobic phases was normal when working without DO control. Such high DO values delayed the start of the next anoxic phase, and the organic matter recently added from the wastewater was mainly degraded aerobically. Both the high DO concentration at the beginning of anoxic periods and the ageing of the wastewater lowered the level of biodegradability of the wastewater, which led to low denitrification in the system. In order to reduce these effects, the filling during anoxic phases in the lab scale SBR was divided into two events: an initial short filling period followed by an idle time (under mixing conditions) to enhance DO reduction up to values near 0.0 mg $DO \cdot L^{-1}$, and a second filling in which the rest of the wastewater volume was added for

denitrification purposes. After these changes, the lab-scale SBR reached close to complete nitrogen removal efficiency (94%) and the effluent total nitrogen concentration was lower than 4.5 mg N-TN·L^{-1} (Figure 4.2).

4.4.2 From lab- to pilot-scale SBR plant

From the results presented in the lab-scale operation (Figure 4.2) and the previous experience with lab-scale SBRs (Vives *et al.,* 2003; Vives, 2004; Corominas, 2006) for organic matter and nitrogen removal of urban and industrial wastewater, some key points were considered in order to scale up the process from lab-scale (30 L) to a 1000 L maximum capacity SBR pilot plant.

Optimization of the organic matter available

The use of the biodegradable organic matter of the raw wastewater is a key parameter for the wastewater treatment. The step-feed could be used as the strategy to optimize the organic matter available in the raw wastewater. On the other hand, from the lab-scale SBR studies in the LEQUIA's group, the biodegradable organic matter was degradated inside the storage tank. The SBR pilot plant had to be set up *in situ* in an urban WWTP. In this way, the influent was renewed daily and the denitrification efficiencies should have increased.

DO Control

High DO concentration at the beginning of each anoxic phase of the lab-scale SBR was reached, when system worked without a DO control, it provoked a decrease of the denitrification efficiencies. From these experiences, DO control was applied in the SBR pilot plant. The DO control system implemented was based on a simple on/off switch strategy with a 2.0 mg DO·L^{-1} set-point to avoid high DO levels at the end of the aerobic phases, as previously mentioned. This strategy also reduces operational costs (Corominas *et al.,* 2006).

Temperature control

The temperature dependence of the biological reaction rate constants is very important in assessing the overall efficiency of a biological treatment process (Metcalf and Eddy, 2003). The effect of the temperature on the reaction rate of biological process (Equation 4.1) can be estimated using the van't Hoff-Arrhenius relationship.

$$k_T = k_{20}\theta^{(T-20)} \qquad \textbf{(Eq. 4.1)}$$

where k_T is reaction rate constant as a function of *temperature* (T), k_{20} is reaction rate constant at 20ºC and θ is the temperature coefficient yield.

The lab-scale SBR operated at 20°C in a thermo regulated room during all the experiences (Vives, 2004; Corominas, 2006). In this way, the removal efficiencies were not affected by the temperature. However, in full scale WWTPs the temperature in the reactor is not controlled. For this reason, the SBR pilot plant worked without temperature control.

4.4.3 SBR pilot plant performance

The SBR pilot plant was set up *in situ* at the Cassà WWTP, treating only real, fresh wastewater from the sewers arriving at the facility. This avoided problems concerning the biodegradation of the wastewater duing the storage time. The SBR pilot plant was inoculated with mixed liquor from the non nitrifying oxidation ditch for organic matter removal of the same WWTP. The process was operated for more than four months.

Figure 4.3 shows (a) the organic matter, (b) nitrogen compounds and (c) influent C:N ratio evolution during the SBR operation.

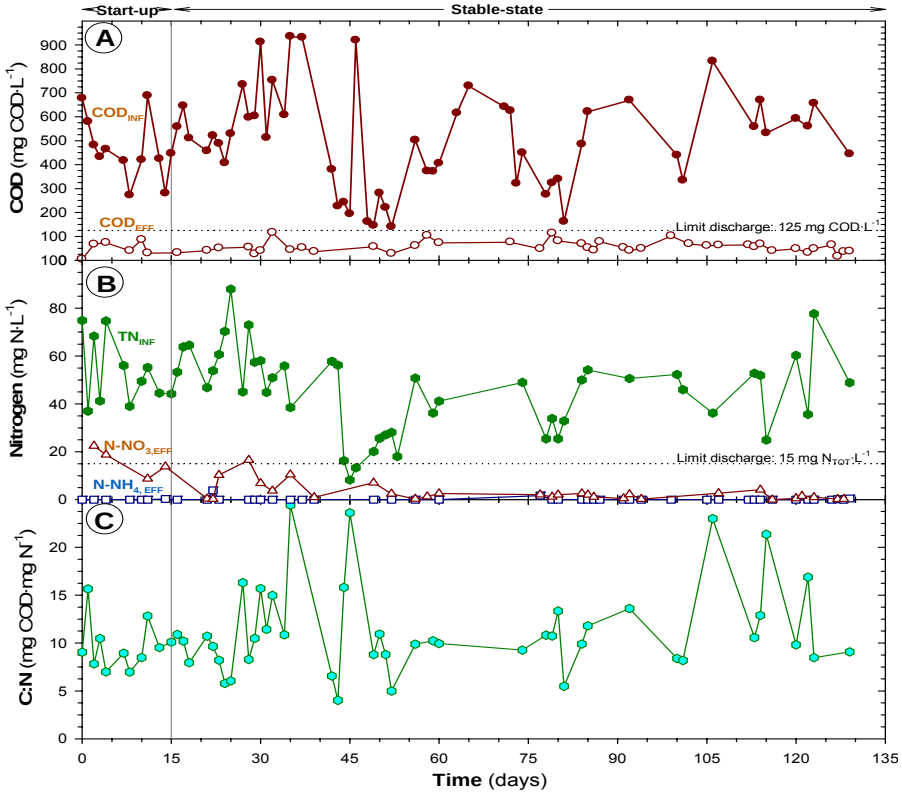

Figure 4.3. Evolution of influent (coloured dots), effluent (white dots) for: (A) total COD, (B) nitrogen and (C) C:N ratio during the SBR pilot plant operation at the Cassà WWTP.

During the first month the SBR pilot plant quickly achieved COD removal (from the first day) and nitrogen removal (i.e. nitrification and denitrification). When observing COD evolution in Figure 4.3A, the variability of influent COD (from 140 to 935 mg COD·L^{-1}; Table 4.4) is noticeably high. The high intraday variation was mainly because of the size of the WWTP, the influence of rainy weather periods (from days 42 to 52) and some contributions from industrial zones. Nevertheless, COD removal efficiencies of around 90% were easily achieved and always with effluent concentrations (mean effluent of 57 mg COD·L^{-1}) lower than those of the European Directive 91/217/CEE (125 mg of COD·L^{-1}).

It is important to note that, even with high influent COD and TN variations (Table 4.3), after the start up period (around 15 days), in the stable state the SBR efficiency was always able to keep the effluent's total nitrogen levels lower than 3.0 mg N-TN·L^{-1} (Table 4.4) with ammonium and nitrate levels of 0.4 and

2.61 mg $N \cdot L^{-1}$ on average (Figure 4.3B). The nitrification process, when run properly with an ammonium effluent concentration, was close to zero during the stable operation. The C:N ratio was 11 ± 4 mg $COD \cdot mg^{-1}$ N during the experimental period (Figure 4.3C) helped that the denitrification process also worked properly during the experimental period. However, on days 28, 34 and 49, the effluent nitrate concentration presented high variability, 0.00 - 16.24 mg $N\text{-}NO_3^- \cdot L^{-1}$, due to failure of the equipment and rainy events. In spite of this, on 92 % of the days the total nitrogen concentration in the effluent had the standard requirements with high average nitrification and denitrification efficiencies (99% and 92%, respectively).

Table 4.4. Organic matter and nitrogen concentrations in the effluent of the SBR pilot plant treating urban wastewater from the Cassà WWTP during the stable state.

Parameter	Mean	Range	Units
Daily flow	624 ± 31	531 - 741	$L \cdot d^{-1}$
COD	57 ± 23	16 - 116	$mg\ COD \cdot L^{-1}$
N-TN	2.9 ± 4.0	0.0 - 16.5	$mg\ N\text{-}TN \cdot L^{-1}$
$N\text{-}NH_4^+$	0.4 ± 1.7	0.0 - 9.8	$mg\ N\text{-}NH_4^+ \cdot L^{-1}$
$N\text{-}NO_2^-$	0.27 ± 0.35	0.00 - 1.28	$mg\ N\text{-}NO_2^- \cdot L^{-1}$
$N\text{-}NO_3^-$	2.61 ± 3.77	0.00 – 16.24	$mg\ N\text{-}NO_3^- \cdot L^{-1}$

Figure 4.4 presents the nitrogen removal efficiencies and the temperature in the SBR pilot plant (expressed as the daily mean value and its standard deviation) during the experimental period treating urban wastewater from the Cassà WWTP.

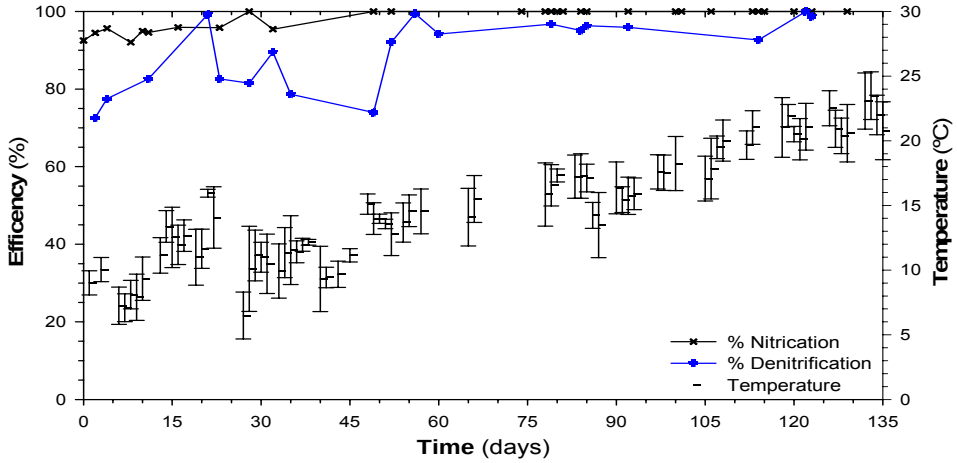

Figure 4.4. Nitrogen removal efficiencies and reactor temperature during the experimental period in the SBR treating urban wastewater.

In spite of the high variability of the temperature inside the reactor (average temperatures from 24.8ºC to 5.2 ºC) (Figure 4.4), high average nitrification and denitrification efficiencies (99%±1% and 92%±8%, respectively) were achieved in the SBR pilot plant. The nitrification process remained stable during the experimental period without taking into account the low temperatures achieved inside the

reactor. However, the denitrification process dropped from days 20 to 50 not only was affected by the temperature and the influence of rainy/dry weather (Figure 4.3), also for the failure of the equipment and rainy events.

4.4.4 Optimization of the SBR cycle length

After achieving the stable state operation, an analysis of the SBR's cycle profiles using pH, DO and Oxidation-Reduction Potential (ORP) probes was carried out. The analysis provide information about the state of the process (i.e. nitrification and denitrification). Figure 4.5 shows pH, DO and ORP profiles of a typical cycle (8 hour total cycle length) of the SBR pilot plant when nitrification/denitrification efficiencies were higher than 95%.

In the aerobic phase when the ammonium is depleted a minimum point called *ammonia valley* appears in the pH profile (Figure 4.5, point A) as a consequence of the equilibrium between proton production during the nitrification process (decreasing pH) and stripping of CO_2 (increasing pH). Before the end of nitrification the pH decreases because proton production in the nitrification process is higher than the stripping effect. However, once nitrification is finished no more protons are produced and the stripping effect is considerably larger, which represents an increase in the pH (Al-Ghusain and Hao, 1995). The *ammonia valley* could not be observed easily because of the combination of aeration on/off events which kept the DO concentration around 2.0 mg $DO \cdot L^{-1}$. Nevertheless, in Figure 4.5 a decreasing and finally an increasing pH tendency (point A) can be seen during the aerobic phases.

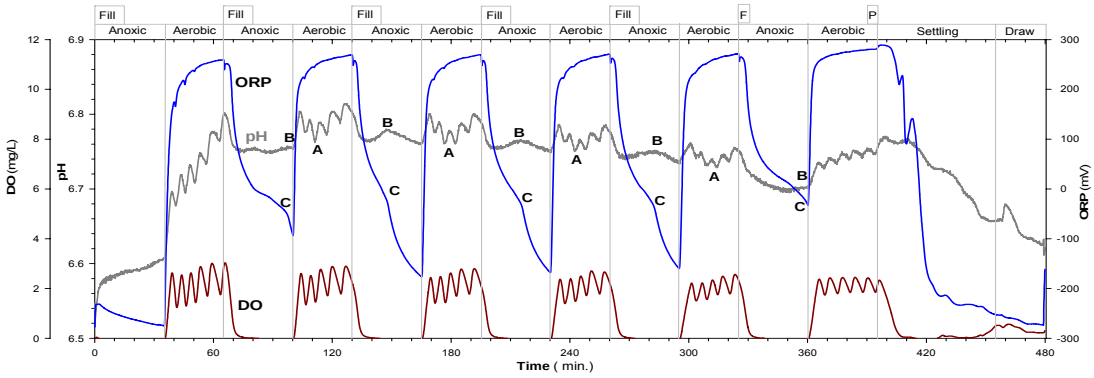

Figure 4.5. Typical pH, DO and ORP profiles obtained during an 8 hour cycle of the SBR pilot plant located at the Cassà WWTP treating urban wastewater for carbon and nitrogen removal.

Complete nitrite/nitrate removal is achieved by the end of each anoxic phase (at a cycle time of about 95 min, 150 min, 215 min, 280 min and 350 min), when the *nitrate knee* (Figure 4.5, point C) was clearly detected from the ORP profile. The flex point is related to the electron acceptor changing from nitrate to sulphates or other components (Al-Ghusain and Hao, 1995; Plisson-Saune *et al.,* 1996). At the same time that the *nitrate knee* appeared in the pH profile, the *nitrate apex* (pH maximum) was also detected in some anoxic phases (Figure 4.5, point B). This maximum in the pH is caused by the equilibrium between OH^- production during denitrification and the alkalinity of the system (Al-Ghusain and Hao, 1995). From the viewing of these points before the end of the anoxic and aerobic reaction phases, it is possible to optimize their length and the overall cycle length.

4.5 Conclusions

When conducting lab-scale studies for biodegradability or nitrogen removal efficiencies, the process of scaling it up to real plant size must be carried out knowing that there may be increased denitrification efficiency due to the presence of easily biodegradable carbon sources. This chapter describes a successful implementation in the SBRs using 6 filling events with a sequence of anoxic-aerobic phases to treat urban wastewater for nitrogen removal purposes with low ammonium and nitrate levels (0.1 ± 0.4 and 1.5 ± 1.1 mg $N \cdot L^{-1}$, respectively) in the discharge phase in spite of influent variability when treating real urban wastewater (dairy mean flow 624 ± 31 $L \cdot d^{-1}$).

Normally SBR technology works with a fixed cycle configuration, which has been developed from the operators' experience and which is repeated over time. Special attention is given to security factors since the responsibility is higher when treating real wastewater. Thus, the predetermined cycle is sometimes unable to adapt to dynamic changes in the influent, which leads to excess resources being used. Therefore, it appears to be necessary to adjust the length of the different process stages according to effluent quality and possible changes in the quality of the wastewater to be treated. This can be achieved by managing the SBR with online control of the duration of the reaction phases. To overcome analytical difficulties for online control, interpreting the progression of simple physical–chemical parameters (i.e. pH, DO and ORP probes) in the SBR can help to define phase durations. Thus, some characteristic points (i.e. *ammonia valley, nitrate knee* and *nitrate apex*) which appear in the reaction phases allow us to learn the state of the system and therefore determine the end of the nitrification and denitrification reactions.

4.6 References

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Chapter 5.
Development, implementation and improvement of an SBR real-time control system for carbon and nitrogen removal from urban wastewater

This chapter presents the development, implementation and improvement of a Sequencing Batch Reactor (SBR) real-time control system for carbon and nitrogen removal from urban wastewater. From the results obtained in Chapter 4, a real-time control was designed, constructed and implemented in the 1000L SBR pilot plant treating 600 $L \cdot d^{-1}$ of urban wastewater. The control system was an algorithm that automatically adjusted the cycle length to the influent wastewater characteristics according to the Oxidation Reduction Potential (ORP) and the Oxygen Uptake Rate (OUR) values as the anoxic and aerobic phase end points respectively.

On the other hand, an improvement on the previously described real-time control system was made after improving the Dissolved Oxygen (DO) control. Thus, the objectives of the control system were different according to the aeration phase during the whole cycle. The targets applied were the detection of the ammonia removal (*ammonia valley*) for the first phases or carbon and ammonia removal (double checking condition: *ammonia valley* and OUR_v) for the last aerobic phase prior to settling and discharge.

This chapter has been the basis of the following publications:

Puig, S., Corominas, Ll., Vives, M.T., Colomer, J., Balaguer, M.D. and Colprim, J. 2005a. Development and implementation of a real-time control system for nitrogen removal using OUR and ORP as endpoints. *Ind. Eng. Chem. Res.* **44**(9), 3367–3373.

Puig, S., Corominas, Ll., Colomer, J., Balaguer, M.D. and Colprim J. 2005b. On-line oxygen uptake rate as a new tool for monitoring and controlling the SBR process. *European Symposium on Computer Aided Process Engineering - 15, Vol.Computer-Aided Chemical Engineering* **20 A/B** , 1291-1296, Ed: Luis Puigjaner (Barcelona). ISBN:0-444-51987-4

Puig, S., Corominas, Ll., Traore, A., Colomer, J., Balaguer, M.D. and Colprim, J. 2006. An on-line optimization of a SBR cycle for carbon and nitrogen removal based on on-line pH and OUR: the role of dissolved oxygen control. *Water Sci. Technol.* **53**(4-5), 171-178.

5.1 Introduction

The biological processes in Sequencing Batch Reactor (SBR) technology are conducted in a complete mix reactor following a sequence of different operational conditions. The common practice used in SBRs is based on executing a predefined cycle over time (Keller *et al.,* 2001; Teichgräber *et al.,* 2001; Steinmetz *et al.,* 2002). In SBRs operating with a fixed cycle scheme, the daily variations in the influent composition are considered by assuming a reaction phase that is able to deal with the worst operational conditions. Otherwise, in some cases different predefined cycles could be used under different influent flow or concentration conditions. However, it is possible to use the flexibility of SBR technology to greater advantage by finding the correct duration of the aerobic and anoxic phases to achieve complete nitrification and suitable denitrification respectively (Andreottola *et al.,* 2001).

Instead of using a fixed SBR cycle, information gained from simple online probes (Dissolved Oxygen (DO), pH and Oxidation Reduction Potential (ORP)) can be used as an indicator of the SBR cycle phase status. Moreover, the Oxygen Uptake Rate (OUR; Section 5.3.4) was calculated from the DO probe values.

The biological nitrogen removal could be characterized by observing and detecting the aerobic/anoxic end points from the profiles gathered by the different signals probes. When focussing on nitrogen removal, differents end points for the anoxic and aerobic phases could be observed during a cycle as presented in Figure 5.1. The observed end points are related to the achievement of nitrogen removal and different end points could be used for the detection of complete nitrification and denitrification during aerobic and anoxic phases, respectively.

End points of the nitrification process in the aerobic phase:

- *Ammonia valley* (Figure 5.1A). *Ammonia valley* appears as a consequence of the equilibrium between proton production during the nitrification process (decreases pH) and stripping of dissolved inorganic carbon as CO_2 (increases pH). During the nitrification, the pH decreases because proton production in the nitrification process is higher than the stripping effect, but once nitrification is finished no more protons are produced and the stripping effect is considerably large, representing an increase in the pH (Al-Ghusain and Hao, 1995). Nevertheless, such pH increase in also related to the net charge balance of the wastewater and should be more noticeable for the system that lacks strong buffer capacity (Chang and Hao, 1996).

- *Residual Carbon Manipulation Point* (*RCMP*; Figure 5.1B). At the point of complete nitrification of ammonium, an abrupt change is observed in the ORP curve. Following the nitrogen break point, the only reaction which occurs is organic carbon oxidation. At this point (the RCMP according to Ra *et al.* (1998)) a relatively constant slope change begins in the ORP curve.

- The σ_{O2} *point* (Figure 5.1C). When the ammonium is depleated, a flex point (the σ_{O2} *point*) in the DO profile is observed (Mauret *et al.,* 2001; Battistoni *et al.,* 2003) and caused by a suddenly reduction of the DO comsuption. The σ_{O2} *point* only appears under constant aeration conditions.

- σ_{OUR} or average viability (Watts and Garber, 1995; Figure 5.1D) represents the average respiration rate for the period in which nitrification and oxidation of the less readily biodegradable materials occur and it has been identified by a sharp decrease in the OUR profile (Demuynck *et al.,* 1994; Vanrolleghem and Coen, 1995).

End points of the denitrification process in the anoxic phase:

- *Nitrate knee* (Figure 5.1E). This flex point in the ORP profile corresponds to the elimination of accumulated nitrates and more directly to the sulphate-reductase activity (production of sulphide) as a new electron acceptor. A very low sulphide concentration (0.07 mg Hg) induces a strong ORP decrease (100 mV) (Plisson-Saune *et al.,* 1996; Paul *et al.,* 1998).

- *Nitrate apex* (Figure 5.1F). During the anoxic cycle, denitrification results in a rise in the alkalinity of the system, with a corresponding increase in pH. A small quantity of N_2 purging does not affect the initial pH value. When the nitrate depletion is complete, a sharp change in the slope of the pH profile can be seen. The *nitrate apex* corresponds to the depletion of nitrates and the onset of anaerobiosis (Al-Ghusain and Hao, 1995).

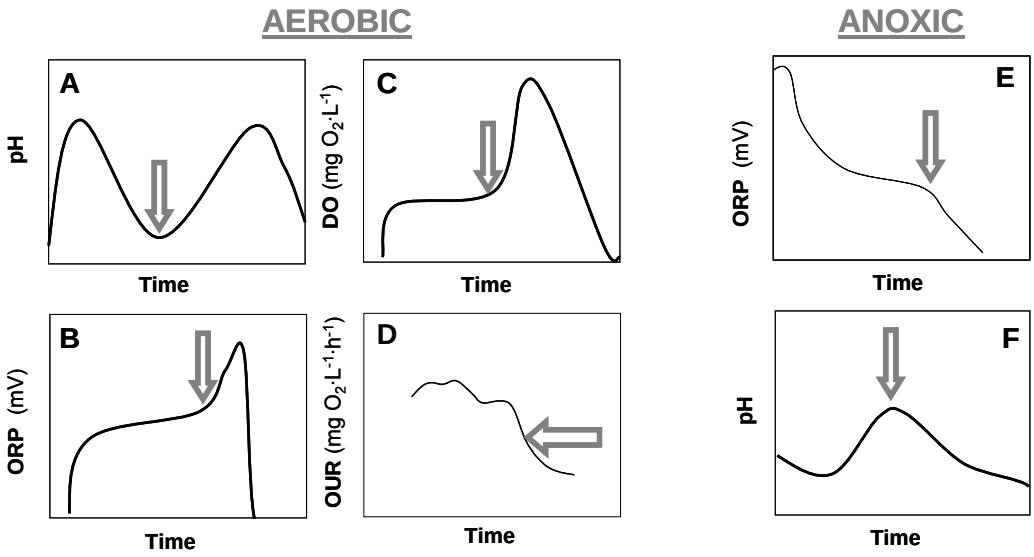

Figure 5.1. End points of biological nitrogen removal during the aerobic (A: *Ammonia valley*; B: Residual Carbon Manipulation Point (*RCMP)*; C: σO_2 and D: σOUR) and anoxic (E: *Nitrate knee* and F: *Nitrate apex*) phases.

Many researchers have used these end points to control the nitrogen removal efficiencies in biological wastewater treatment. Table 5.1 gives a detailed list of the end points used in previous studies to control

the end of the nitrification and denitrification processes of the wastewater in the aerobic and anoxic phases, respectively.

Ammonium depletion in the aerobic phase is mainly controlled using the pH, ORP and/or DO probes (Table 5.1). Controlling the end of the aerobic phases can only be achieved using pH or ORP sensors, but OUR provides information on incoming load and effluent toxicity that pH and ORP cannot give. After an initial high OUR value due to the biomass, an important decrease in its value is observed which corresponds to the end of carbon oxidation and nitrification (Figure 5.1D). It is important to note that the respiration rate is directly linked to two important aerobic biochemical processes that must be controlled in a WasteWater Treatment Plant (WWTP): biomass growth (endogenous OUR) and substrate consumption (exogenous OUR) as reported by Spanjers *et al.* (1998). Many respirometry-based control strategies have been proposed in the literature, but very few real implementations based on OUR measurements or calculations have been reported.

During the anoxic phases, the ORP signal is commonly used to establish the end of the denitrification process (Chang and Hao, 1996; Tomlins *et al.,* 2002; Kishida *et al.,* 2003). Casellas *et al.* (2006) demonstrated that during exogenous denitrification ORP was the more reliable sensor. This result was in agreement with Akin and Ugurlu (2005) who showed that ORP gave more information during anoxic phases (exogenous denitrification; organic substrate available) than pH. However, during endogenous denitrification, pH is a more reliable sensor because is able to identify the final pH removery caused by the denitrification metabolism (Casellas *et al.,* 2006).

As well as the just described this scientific works, three patented industrial systems for nitrogen removal based on the detection of different end points have been developed:

1. A specific control strategy, called "***INFLEX***" control was described by Ferrand *et al.* (1998). It is based on detecting bending points in the ORP and DO profiles and is backed up by timetables and threshold values when any DO control is applied. The "*INFLEX*" control showed good efficiency for fitting aeration time to the demand (Mauret *et al.,* 2001).

2. ***OGAR®*** (Optimized manaGement of Aeration by Redox) is an industrial process control system, developed by Ondeo Services, that uses online redox (ORP) measurements to control the aeration sequence in an activated sludge process (Caulet *et al.,* 1998; Tomlins *et al.,* 2002).

The efficient combination of chemical and biological phosphorus removal was one of the reasons for the development of the ***BCFS®*** process (Biological–chemical phosphorus and nitrogen removal; Van Loosdrecht *et al.* (1998)). In this technology, in combination with optimal operating conditions for biological nitrogen removal, chemical precipitation of phosphorus is used to ensure compliance with effluent standards using online redox (ORP) measurements to control the recirculation flows.

Table 5.1. Summary of the end points used in works from the bibliography to control the end of the nitrification and denitrification processes of wastewater.

Reactor	Aim	Aerobic phase	Anoxic phase	Wastewater	Reference
A/A Digestion	C, N and P	A. Valley or pH= 6	N. Knee or pH= 8	Waste activated sludge	Al-Ghusain and Hao, (1995)
Alternate oxic-anoxic process	C and N	α_{O2} or OD= 4	N. Knee or ORP= - 220mv	Urban wastewater	Battistoni *et al.* (2003)
Alternate oxic-anoxic process	C and N	OUR decrease and $[N\text{-}NH_4^+]$= 0	OUR increase and $[N\text{-}NO_3^-]$= 0	Urban wastewater + external carbon source	Klapwijk *et al.* (1998)
Alternate oxic-anoxic process	C and N	α_{O2}	-	Slaughterhouse wastewater	Mauret *et al.* (2001)
Alternate oxic-anoxic process	C and N	ORP= 20 mV	ORP= - 75 mV	Urban wastewater	Zipper *et al.* (1998)
SBR	C and $N\text{-}NH_4^+$	α_{O2}	-	Synthetic wastewater	Cohen *et al.* (2003)
SBR	C, N and P	A. Valley	N. Knee and N. Apex	Urban + industrial wastewater	Casellas *et al.* (2006)
SBR	C and N	A.Valley, α_{O2} and RCMP	N. Knee and N. Apex	Swine wastewater+ external carbon source	Kishida *et al.* (2003)
SBR	C and N	ORP= 400 mV	ORP= 150 mV	Urban wastewater	Tomlins *et al.* (2002)
SBR	C, N and P	A. Valley	N. Knee	Urban wastewater + external carbon source	Chang and Hao, (1996)
SBR	C, N and P	A. Valley and $[N\text{-}NH_4^+]$= 0	N. Knee, N. Apex and $[N\text{-}NO_3^-]$= 0	Synthetic wastewater	Cho *et al.* (2001)
SBR	C, N and P	RCMP	-	Swine wastewater	Ra *et al.* (1998)
SBR	C, N and P	RCMP	-	Swine wastewater	Ra *et al.* (1999)
SBR	C, N and P	A. Valley and RCMP	N. Knee and N. Apex	Synthetic wastewater	Yu *et al.* (1997)
SBR	C, N and P	A. Valley and RCMP	N. Knee and N. Apex	Synthetic wastewater	Yu *et al.* (1998; 2000; 2001)

5.2 Objectives

Many researchers have used end points based on the conventional probes (Table 5.1) but any combination of *in situ* respirometry measurements, an on-line OUR, and the ORP probe to detect the end

of the organic matter removal, nitrification and denitrification processes, respectively. Therefore, the aims of the research presented in this chapter were:

- To develop, implement and optimize a real-time control system for the optimal treatment of real urban wastewater for carbon and nitrogen removal in the 1000 L SBR pilot plant. This control system has to adjust to the optimal duration of the aerobic and anoxic reaction phases based on conventional and economical probe (pH, DO and ORP) signals.

- Study the role of DO control in the probes' profiles and its effect on controlling the length of the aerobic phase.

5.3 Materials and methods

5.3.1 Methodology

The SBR pilot plant was set up at two different WWTPs: Cassà and Celrà WWTPs (N.E. Spain). Both treated fresh wastewater from the sewers arriving at the facility. The cycle characteristics for achieving complete nitrification and denitrification were defined by previous laboratory studies (Vives, 2004 and Chapter 4). Figure 5.2 presents the fixed cycle used during the experimental period. An 8 h cycle with six feeding steps was implemented, introducing the wastewater in the anoxic phases and then alternating the anoxic and aerobic phases in order to improve carbon and nitrogen removal in the WWTP (Puig *et al.*, 2004). The cycle was divided into a reaction phase (395 min; 46.2% under aerobic conditions), a settling phase (60 min) and a draw phase (25 min). The wastage in the SBR was performed under mixing and aeration conditions in the last aerobic phase to control the sludge or Solid Retention Time (SRT) of the system at 20 days on average, assuming equal concentrations of solids in the wastage and in the reactor.

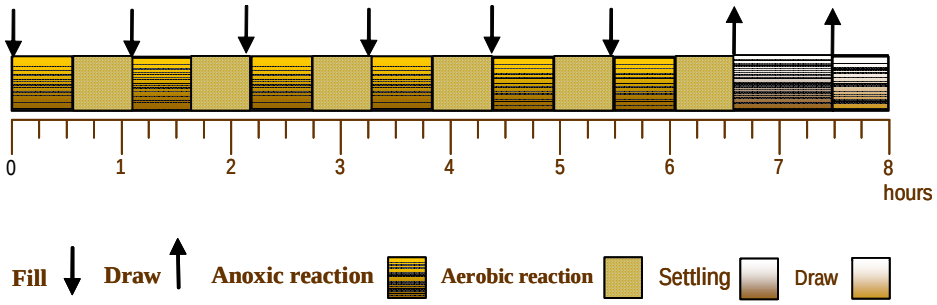

Figure 5.2. Operational periods of the SBR pilot plant with the step-feed strategy used to treat urban wastewater.

5.3.2 Wastewater characteristics

Table 5.2 presents the average characteristics of the Cassà and Celrà WWTPs wastewater treated in the SBR pilot plant during the experimental study. The main difference between both wastewater was the organic matter content. The low COD values (259 ± 58 mg COD·L^{-1}), the percentage of biodegradability

(28 ± 14 %) and the Carbon:Nitrogen (C:N) feeding ratio (6 ± 2 mg COD·mg^{-1} N) of the Celrà wastewater could *a priori* affect the denitrification process.

Table 5.2. Characteristics of the Cassà and Celrà WWTP wastewater during the experimental study.

Parameter	Cassà WWTP	Celrà WWTP	Units
	Mean ±σ	Mean ±σ	
COD	527 ± 220	259 ± 58	*mg COD·L^{-1}*
N-TKN	51.1 ± 17.1	42.9 ± 10.5	*mg N-TKN·L^{-1}*
N-NH_4^+	30.7 ± 12.3	25.2 ± 5.7	*mg N-NH_4^+·L^{-1}*
N-NO_3^-	0.73 ± 1.13	2.05 ± 2.82	*mg N-NO_3^-·L^{-1}*
C:N	13 ± 11	6 ± 2	*mg COD·mg^{-1} N*

5.3.3 DO control

DO control in the SBR pilot plant is necessary to avoid final aerobic phases at high DO levels. Owing to the repeated anoxic–aerobic pairs, the beginning of anoxic phases at high DO levels concluded with a reduction in the time and carbon source, which was suitable for denitrification purposes. In this chapter, two DO control strategies have been applied with a fixed DO set-point of 2.0 mg DO·L^{-1}:

1. **On/Off DO controller**. On/off DO controller was implemented by using an on/off valve working with a fixed air flow. As long as the DO value was lower than the fixed set point, the command variable was set to 100% (on). As soon as the DO values reach or exceed the set point, the command variable was set to 0% (off).

2. **Fuzzy Logic-based DO Controller (FLC)**. The FLC was implemented after installing a variable frequency engine coupled with an air flow meter. The developed FLC, a Mamdani FIS (Mamdani and Assilian, 1975), was based on the error between the DO measured, the DO set point and the cycle phase number running. The output from the FLC is the air quantity injected into the reactor expressed in terms of the voltage supplied to the engine. This structure (only two inputs and one output) allows a simple representation of the various fuzzy rules, with easy integration and interpretation of human knowledge (Traoré *et al.,* 2005).

5.3.4 Online OUR calculation using an on/off DO controller

During the aerobic phases of the cycle, in which no influent or effluent flow occurs in the SBR and assuming a complete mix reactor achieved with the aeration plus the mechanical agitation, a DO mass balance in the liquid phase of the reactor could be represented by Equation 5.1

$$OUR(t) = K_L a^{(T)} \cdot \left(DO_{sat}^{(T)} - DO^T(t)\right) - \frac{dDO}{dt} \quad \text{(Eq. 5.1)}$$

where *OUR* is the calculated oxygen uptake rate (mg $L^{-1} \cdot h^{-1}$), *DO* is the dissolved oxygen in the SBR (mg·L^{-1}), DO_{sat} is the saturation or maximum dissolved oxygen as a function of *temperature* (T) (mg·L^{-1}), and K_La is the oxygen mass transfer coefficient (h^{-1}) as a function of compressed air flow, air diffuser efficiency and the reactor volume.

Thus, during air off periods resulting from the on/off control strategy and according to Equation 5.1, the OUR can be calculated by adjusting the DO measured over time to a linear regression as the calculated slope (Equation 5.2).

$$OUR(t) = -\frac{dDO}{dt} \quad \text{(Eq. 5.2)}$$

This calculated OUR does not account for the surface aeration. Nevertheless, for control purposes the evolution in the OUR measurements is the key point, not the absolute values themselves. Thus, the effect of the surface aeration is considered to be small and constant during the operation and therefore does not affect the signal trends.

Hence, to obtain the oxygen consumption only the DO derivative has to be determined during air off periods. This can be done by measuring the decrease in DO as a function of time due to biological activity, which is equivalent to approximating the differential terms with a finite difference term. The dynamic of the sensor (i.e. transmitter plus probe delay signal) must be taken into account and therefore the first measurements (50 seconds) after deactivating the aeration system are not used. Next, DO values are taken until the airflow is switched on again, and the linear regression can finally be obtained.

5.3.5 Calculating OUR using a fuzzy logic DO controller

When the FLC was applied, the air flow to the reactor was modified over time. Under these conditions, the oxygen mass transfer coefficient (K_La) must be calculated from the measured air flow data in order to calculate the OUR (Equation 5.1). The different terms of Equation 5.1 can be determined as follows.

$K_La^{(T)}$ calculation: K_La depends on the airflow rate, the partial pressure of oxygen, the dissolved oxygen saturation concentration, the volume, the type of diffusers and the temperature. $K_La^{(T)}$ is calculated in days using Equation 5.3:

$$K_L a^{(T)} = \left(\frac{\alpha \cdot Q_{air} \cdot \eta \cdot \gamma_{O_2} \cdot 1333.3}{V \cdot DO_{sat}^{(T)}} \right) \cdot \theta^{(T-20)} \quad \text{(Eq. 5.3)}$$

where Q_{air} is the air flow rate ($m^3 \cdot d^{-1}$); y_{O2} is the fraction of oxygen in air (= 21%); *V* is the reactor volume (m^3); $DO^{(T)}_{sat}$ is the saturation or maximum dissolved oxygen as a function of temperature (T) (mg·L^{-1}); and the correction factors: *1333.3* = g $O_2 \cdot m^{-3}$ unit conversion; θ is the temperature correction factor (1.024); α is the factor to correct the device parameters which are normally determined for a range of operating conditions using tap water; and η is the standard oxygen transfer efficiency (0.21).

$DO_{sat}^{(T)}$ calculation: This parameter depends on the temperature and the atmospheric pressure. Equation 5.4 has been obtained by adjusting a third order polynomial to Henry's constant values at different temperatures obtained from Foust *et al.* (1960).

$$DO_{sat}^{(T)} = P_{atm} \cdot \left[14.515 - 0.3286T + 4.173 \cdot 10^{-3} T^2 - 1.815 \cdot 10^{-5} T^3\right] \quad \text{(Eq. 5.4)}$$

where $DO^{(T)}_{sat}$ is the saturation or maximum dissolved oxygen as a function of *temperature* (T) (mg·L^{-1}) and P_{atm} is the atmospheric pressure (atm).

Derivative of oxygen concentration: The derivative is approximated to small increments and the difference in the DO concentration is calculated at each time interval of the monitoring software (Equation 5.5).

$$\frac{dDO}{dt} = \frac{\Delta DO}{\Delta t} = \frac{DO_t - DO_{t-1}}{\Delta t} \quad \text{(Eq. 5.5)}$$

The Specific Oxygen Uptake Rate (SOUR), also known as the oxygen consumption or respiration rate, is defined as the milligrams of oxygen consumed per gram of Volatile Suspended Solids (VSS) per hour (Equation 5.6).

$$SOUR(t) = \frac{OUR(t)}{VSS} \quad \text{(Eq. 5.6)}$$

5.4 Results and discussion

5.4.1 Background

The SBR pilot plant located at Cassà WWTP (Girona, NE Spain) ran for 200 days using the fixed cycle described in Figure 5.2. The results obtained in Chapter 4 demonstrated that under those conditions the SBR presented a successful performance for carbon and nitrogen removal from a real wastewater (532 ± 220 mg of COD·L^{-1} and 53.6 ± 25.0 mg of N·L^{-1} on average), keeping effluent concentrations (54 ± 25 mg of COD·L^{-1} and 4.7 ± 5.6 mg of N·L^{-1} on average) lower than those required by the European Directive 91/217/CEE (125 mg of COD·L^{-1} and 15 mg of N·L^{-1}).

Figure 5.3 presents pH, ORP and OUR profiles of a typical 8h cycle of the SBR pilot plant when nitrification and denitrification were completed (0.0 mg of N-NH_4^+·L^{-1} and 0.13 mg of N-NO_3^-·L^{-1} in the effluent).

The *ammonia valley* (Figure 5.3, points A) cannot be observed easily in the aerobic phases because of the CO_2 stripping effect of the on/off aeration control. However, the σ_{OUR} is clearly identifyed (Figure 5.3, points D). During anoxic phases, the appearance of the *nitrate knee* (in the ORP profile, Figure 5.3, points C) and of the *nitrate apex* (in the pH profile, Figure 5.3, points B) indicates the end of the

denitrification process. Thus, considering those clearly identified end points, it may be possible to optimize the aerobic and anoxic phases length using these end points to adapt these phases length to the influent wastewater characteristics without affecting the effluent quality.

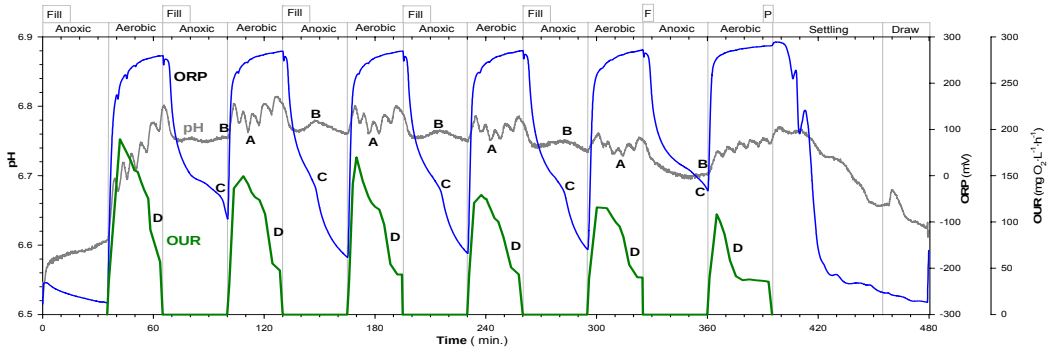

Figure 5.3. pH, ORP and OUR profiles during the 8h total cycle length employed in the SBR pilot plant treating urban wastewater.

Thus, a module to control the alternation of aerobic and anoxic phases was developed based on monitoring and control systems, the background knowledge acquired and the end points detected. Due to the fluctuations in the pH profile during the aerobic phase (Figure 5.3), which made it complicated to detect the *ammonia valley*, the OUR was proposed as the parameter to control the end of the aerobic phase. The *nitrate knee,* which is a robust parameter, was used as the end point for the anoxic phase. In Figure 5.3, for example, using the control system at least 160 minutes (33% time reduction) of the total cycle length would be optimized by using both parameters as end points for biological carbon and nitrogen removal from urban wastewater.

5.4.2 Control system module

Thus, in order to improve and optimise the reaction phases of the SBR, a control system able to adapt the reaction phases (aerobic and anoxic phases) of the SBR cycle was developed and implemented. The aerobic phases length was adjusted by the online OUR calculations, while the ORP profile was used to adapt the anoxic phases.

Aerobic phase cycle length control

In recent years, OUR analysis has demonstrated its usefulness as an off-line tool for characterizing wastewater or biomass in terms of biodegradable fractions, biological nitrification tests and modelling purposes (Spanjers and Vanrolleghem, 1995; Brouwer *et al.,* 1998; Gutierrez, 2003). To improve the SBR's performance, a tool based on the online calculated OUR (Equation 5.2) was implemented to know the state of the plant and control the length of the aerobic phases in each cycle. Figure 5.4 presents the calculated OUR, pH and DO during a typical aerobic phase of an SBR cycle treating urban wastewater.

Different stages related to the biological reactions involved in wastewater treatment could be identified in the evolution of the OUR:

- *Stage A:* The OUR values decrease, from 110 to 95 mg $O_2 \cdot L^{-1} \cdot h^{-1}$, due to the degradation of rapidly biodegradable organic matter in the SBR.

- *Stage B:* The calculated OUR values stabilize due to the oxidation of ammonium and biodegradable organic matter.

- *Stage C:* After the 13th minute, a huge decrease in the OUR values, to around 50% of the initial OUR value (σ_{OUR} point), can be observed due to ammonium depletion. At the same time (at minute 18), the *ammonia valley* can be observed in the pH profile and the frequency of oscillation of the DO profile becomes slower.

- *Stage D:* The OUR decreases slowly ("the tail shape") when the slowly biodegradable organic matter is removed until it stabilizes at the 24th minute when endogenous respiration is achieved. In this moment, the oxygen consumption is only used to maintain the biomass, and the biodegradable organic matter and ammonium are completely removed.

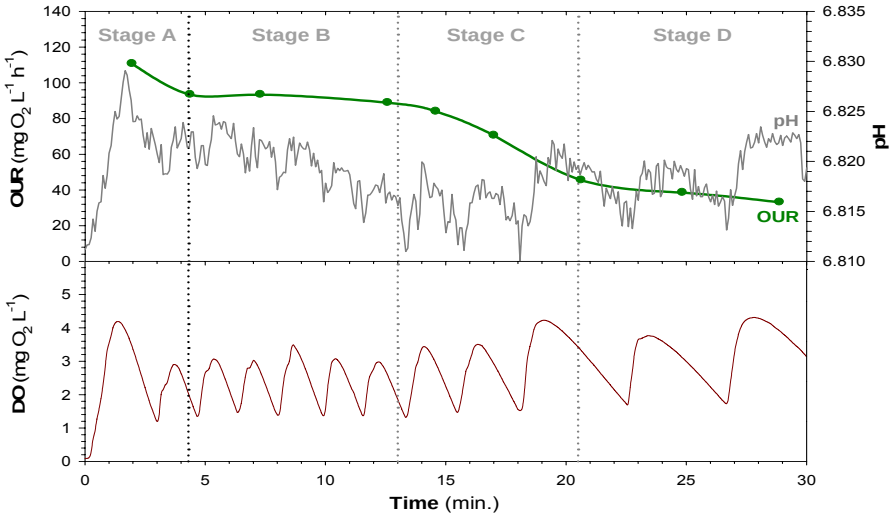

Figure 5.4. Identifying the state of the pilot plant SBR using the calculated OUR, pH and DO during a typical aerobic phase of the 8 h cycle.

To identify the end of the aerobic phase, different cycles were analyzed using OUR data from previous cycles with high nitrification efficiencies treating urban wastewater. Considering the six anoxic-aerobic pairs cycle presented in Figure 5.2, in Figure 5.5 is showed the evolution over time of online OUR measurements in the last two aerobic phases of different cycles.

At the beginning of the aerobic phase there is an increasing trend for OUR values, which is caused by the changing conditions (from the previous anoxic phase to the aerobic phase). This transient response of the activated sludge is most probably due to the sequence of intracellular reactions involved in substrate degradation of the activated sludge (Vanrolleghem *et al.,* 2004). For this reason, a minimum time, t_{min}, of 5 min will be considered in the control system designed in order to avoid misinterpretations of an increasing OUR. After this initial transient response of the OUR (t_{min}), the OUR profile presented a decreased until minute 15. At this moment, the OUR values kept constant around a minimum OUR value,

OUR_V, near 35 mg of $O_2 \cdot L^{-1} \cdot h^{-1}$ or 15.85 mg $O_2 \cdot g^{-1}$ VSS$\cdot h^{-1}$. Therefore, the control of the aerobic length was defined according to an end point reference based on reaching the OUR value lower than 35 mg of $O_2 \cdot L^{-1} \cdot h^{-1}$. When reaching the OUR_V value, referred to by Watts and Garber (1995) as the *time to endogenous*, the system is assumed to be under endogenous conditions when at least 95% of the organic materials in the waste have been treated. Thus, the OUR_V value could be considered as the endogenous OUR value (OUR_{END}).

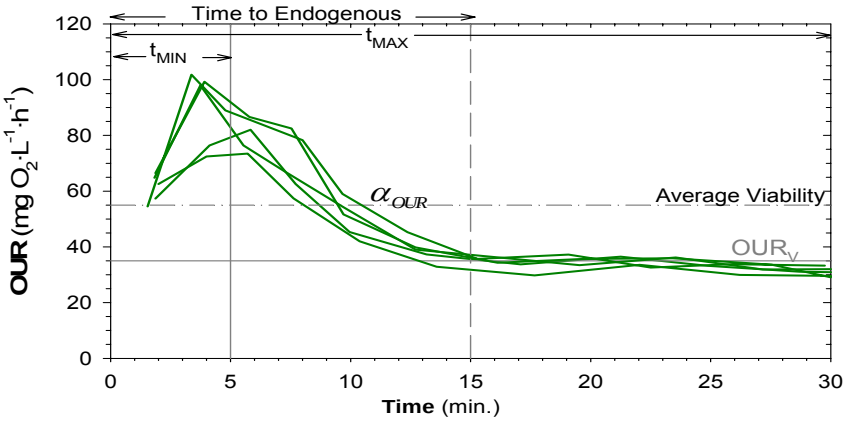

Figure 5.5. Analysis of previous OUR data corresponding to aerobic phases of different cycles.

Table 5.3 compares the Specific Endogenous Oxygen Uptake Rate ($SOUR_{END}$) found in this study with the bibliography values. The experimental value (15.85 mg $O_2 \cdot g^{-1}$ VSS$\cdot h^{-1}$; Table 5.3) was close to that of Madoni *et al.* (1999). Both experiments were done with an activated sludge sample treating urban wastewater with some industrial components. However, it is important to note that the operation and design of the WWTP, including the composition of incoming wastewater and the sludge age of the activated sludge sample taken, plays an important role in the experimental results obtained (Dircks *et al.*, 1999).

Table 5.3. Data from the literature concerning the endogenous SOUR in wastewater treatment systems.

References	Wastewater	$SOUR_{END.}$ ($mgO_2 \cdot g^{-1}$ VSS$\cdot h^{-1}$)
This study	Urban wastewater	*15.85*
Dirck *et al.* (1999)	Urban wastewater	*3.5* *9.0*
Madoni *et al.* (1999)	Urban and industrial wastewater	18.5 – 31.0

Anoxic phase cycle length control

The ORP parameter was used to control the duration of the anoxic phase. The *nitrate knee* was observed in all the anoxic phases. Thus, to determine a relationship between the end of the anoxic phase and the ORP, data from all the anoxic phases of various profiles were analyzed (Figure 5.6).

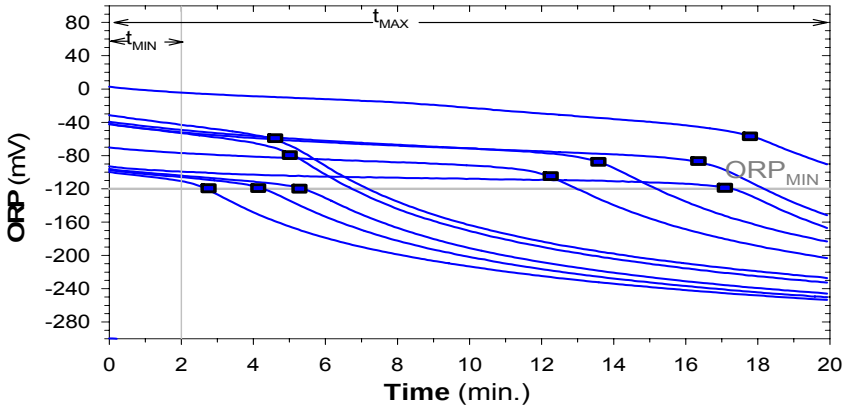

Figure 5.6. Analysis of previous ORP data corresponding to the anoxic phases of various cycles.

Occurrence of the *nitrate knee* depends on the characteristics of the influent wastewater and on the performance of the SBR. When the ORP profiles from the various anoxic phases were compared (Figure 5.6), the *nitrate knee* always took place between -40 and -120 mV, with a minimum value of -120 mV, ORP_{min}. Thus, in this case an ORP minimum value was selected as the indicator of the anoxic phase end point. Such criteria has also been reported by other authors as a end point in a control system as described in Table 5.4.

Table 5.4. Data from the literature concerning the ORP mimimum values to identify the end point of the anoxic phases or to control the aeration devices in different wastewater treatment systems.

References	Wastewater	ORP	Reactor
This study	Urban wastewater	-120 mV	SBR
Battistoni *et al.* (2003)	Urban wastewater	-220 mV	Alternate oxic-anoxic process
Fiter (2006)	Urban wastewater	20 mV	Oxidation Ditch
Van Loosdrecht *et al.* (1998)	Urban wastewater	-100 mV	BCFS® process
Zipper *et al.* (1998)	Urban wastewater	-75 mV	Alternate oxic-anoxic process

However, it is important to note that the ORP minimum value depends on the influent characteristics and the aim of the study. The ORP_{min} found in this chapter was similar to the values found by Battistoni *et al.* (2003) and Van Loosdrecht *et al.* (1998) with high nitrogen efficiencies. Zipper *et al.* (1998) reached an average nitrogen removal of 89% using -75 mV as the anoxic end point. Fiter (2006) used a fixed ORP value to control the anoxic phases in the Taradell WWTP and achieved high nitrogen removal efficiencies.

5.4.3 Development of the control system

The software control program developed using LabWindows® was based on three synchronized modules that communicate with each other:

- Signal acquisition and monitoring device.

- Online OUR calculation module.
- The core of the control system module. The core of the control system module was responsible for:
    - To detect of which kind of reaction phase is running according to a predefined SBR cycle and the functioning of the aeration devices.
    - To remain with no action from the start time until reaching the transition time (t_{min}) to start to identify if the end point has been reached.
    - To detect the end points of the aerobic and anoxic phases using minimum OUR and ORP values (OUR_V and ORP_{min}, respectively).
    - To wait for a fixed time (t_{wait}) prior to force the change to the next phase after the detection of the end point.

The diagram of the control strategy is shown in Figure 5.7, and Table 5.5 presents the selected values of the control system parameters. It is important to note that all the parameter values defined in Table 5.5 were selected according to the previous experimental analysis of the gathered OUR and ORP profiles and thus, they are highly depended on the influent characteristics and the operational cycle defined. Thus, their values need to be periodically adjusted.

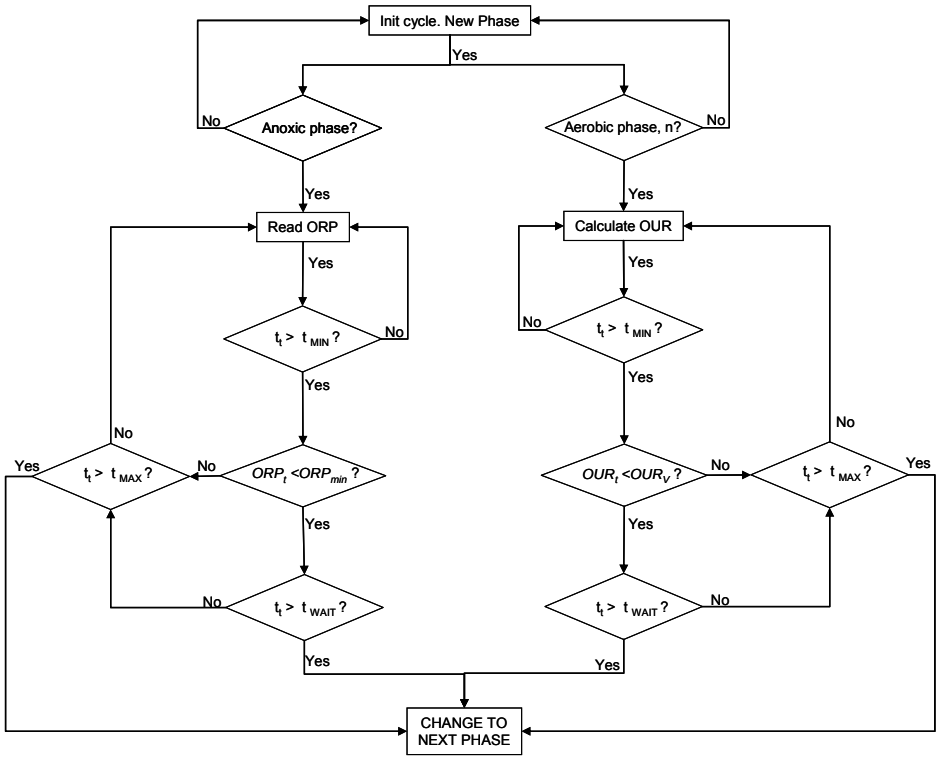

Figure 5.7. Real-time control strategy flow diagram applied in the SBR pilot plant for carbon and nitrogen removal from urban wastewater.

Table 5.5. Parameter values for the control strategy applied in the SBR pilot plant treating urban wastewater from the Cassà WWTP.

Phase	OUR_V ($mgO_2 \cdot L^{-1} \cdot h^{-1}$)	ORP_{min} (mV)	t_{min} (min)	t_{wait} (min)	t_{max} (min)	Control Target
Aerobic	35	-	2	5	30	Ammonium removal
Anoxic	-	- 120	2	5	35	Nitrate removal

First, the control system detected whether the phase was a reaction phase (aerobic or anoxic) or another phase (i.e. filling, settling, wastage or extraction). If the phase was anoxic or aerobic, after a minimum time (t_{min}), it started to check the measured values with the fixed minimum values to find the end point (OUR_V or ORP_{min}, in the aerobic or anoxic phase, respectively). When the measured value was lower than the minimum, a waiting time (t_{wait}) for stabilization was added. However, if the minimum value is not reached, the device functioned to guarantee the process performance by adopting a maximum time length (t_{max}) for each phase. If the minimum value was not achieved before t_{max}, the control system changed to the next phase.

After the minimum OUR or ORP was achieved, a stabilization time, t_{wait}, of 2 minutes in the control strategy was applied to ensure that the system was under suitable conditions. The device also functioned to guarantee the process performance by adopting a maximum time length (t_{max}) of 30 or 35 minutes for the aerobic and anoxic phase, which is equal to the fixed cycle.

5.4.4 Implementation of the control system

After developing the real-time control system, the next step was to test it. The control system was encoded on the software responsible for the operation and monitoring of the SBR pilot plant and tested for 3 months treating real urban wastewater from the Cassà WWTP. Figure 5.8 shows ORP (A) and OUR (B) profiles of a typical cycle from the SBR pilot plant applying the developed control system.

In Figure 5.8A, the largest reductions always took place during the first anoxic step of the cycle because of the rapid decrease in the ORP profile due to the nitrate concentration in the reactor being the same as the effluent concentration of the previous cycle. Then, at the end of each anoxic phase, the *nitrate knee* was clearly observed at around 73 min, 124 min, 175 min, 224 min and 287 min. After the *nitrate knee* appeared, the control system detected that the ORP values were lower than the ORP_{min} value, and it changed to the following phase. Thus, the anoxic periods were reduced by around 56%.

Figure 5.8B shows the evolution of the OUR profile during the aerobic phases of the cycle applying the control system. The aerobic time reduction was around 12%. The largest reduction always occurred in the last step of the cycle, when the substrate was depleted and OUR values reached the aerobic end point value (OUR_V; Table 5.5).

The length of the fixed cycle was 480 min, while the cycle length when applying the control system was around 390 min, which is a 12% total cycle reduction. Despite the high cycle length reduction, which depends on the cycle used and the influent characteristics, the aim of the control system was to adapt the cycle length to the influent conditions. If a high effluent quality could be achieved, this would demonstrate that the control system was operating correctly.

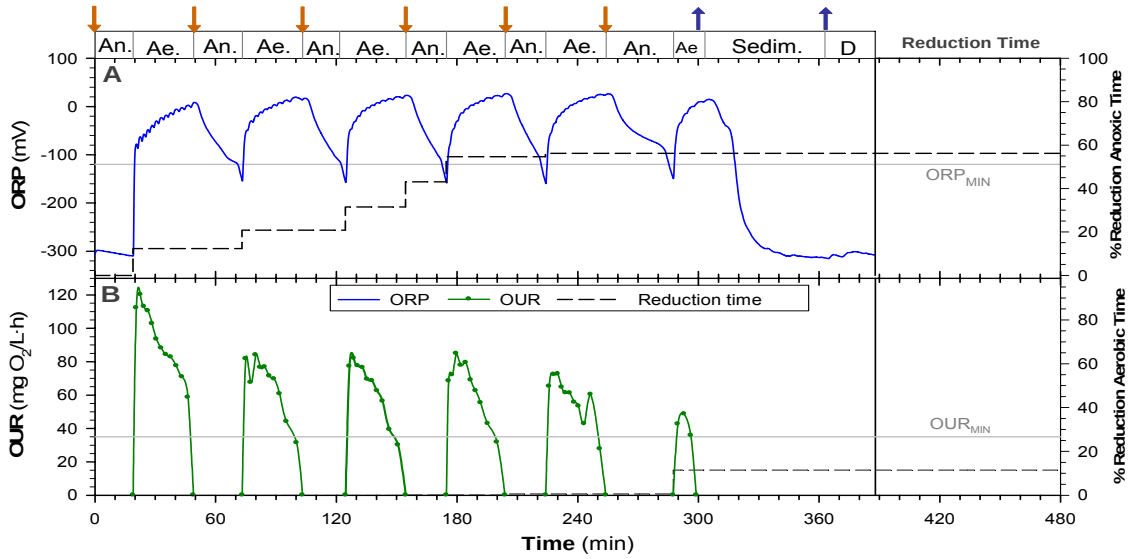

Figure 5.8. ORP (A) and OUR (B) profiles of an optimized cycle using the control system treating urban wastewater. Dotted lines represent the percentatge of reduction time for the anoxic or aerobic phases.

Figure 5.9 shows the evolution of the organic matter, both influent and effluent, during the two experimental periods: operation with the fixed cycle and with the control system (after day 220).

In spite of the variability of the organic matter influent (527 ± 220 mg of COD·L^{-1} on average; Table 5.2), the organic matter effluent (57 mg of COD·L^{-1} on average) was always lower than the standard requirements when the SBR real-time control system was applied to treat urban wastewater. There were no major differences between the two periods in the effluent profile.

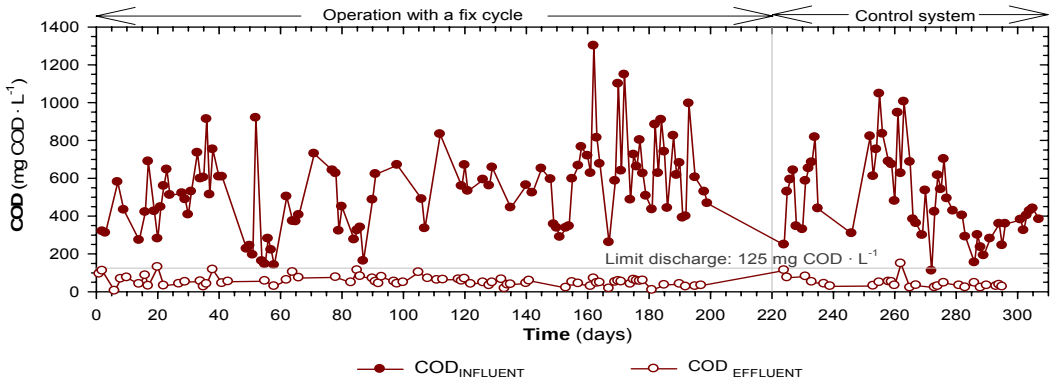

Figure 5.9. Evolution of the influent and effluent for the total COD during the SBR pilot plant operation.

Figure 5.10 presents the evolution of the nitrogen compounds, influent and effluent, during the experimental period. On day 220, the real-time control system was applied.

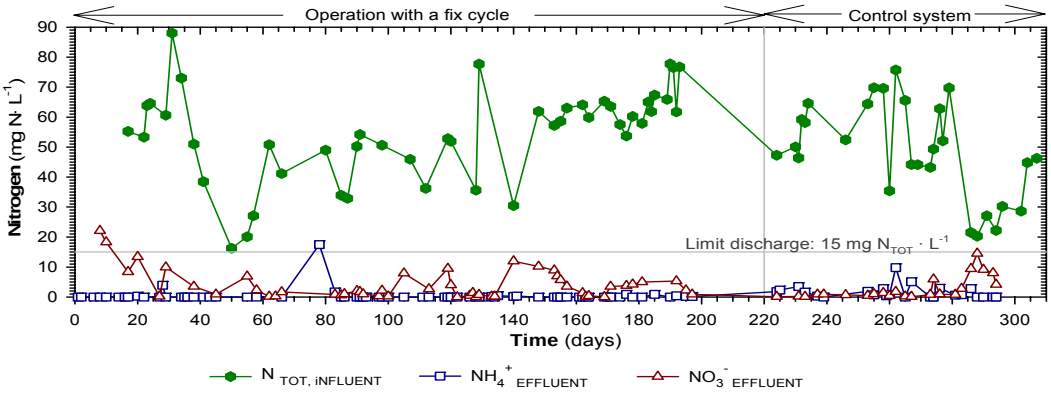

Figure 5.10. Evolution of the influent and effluent for nitrogen removal during the SBR pilot plant operation at the Cassà WWTP.

Like the organic matter (Figure 5.9), the high nitrogen influent concentration variability (51.1 ± 17.1 mg $N \cdot L^{-1}$ on average; Table 5.2) did not affect the effluent quality, 4.6 mg of $N_{TOT} \cdot L^{-1}$ (1.6 mg of $N\text{-}NH_4^+ \cdot L^{-1}$ and 3.0 mg of $N\text{-}NO_3^- \cdot L^{-1}$ on average), and it was always lower than the European Directive values. However, on days 260 and 290, the SBR pilot plant had some incidents that led to DO control system malfunction (i.e. air flow to the reactor was kept active for a whole cycle, leading to high nitrification and lower denitrification) and a change in the settling properties of the sludge (i.e. some colloidal organic matter was observed in the effluent) due to strong variation in the influent wastewater quality (i.e. influent COD and nitrogen greatly decreased after a continuous rainy period). Nevertheless, after these incidents, the performance of the SBR rapidly recovered its normal efficiency without any need to apply extra actions.

5.4.5 Effect of DO control on the real-time control system

Once the control system was successfully developed, implemented and evaluated to treat urban wastewater from the Cassà WWTP, the SBR pilot plant was set up at the Celrà WWTP (Girona, N.E. Spain). The SBR treated 233 $L \cdot d^{-1}$ of fresh wastewater arriving at the facility from the sewers (259 ± 58 mg of $COD \cdot L^{-1}$ and 42.9 ± 10.5 mg of $N \cdot L^{-1}$ on average; Table 5.2). The SBR operated for 4 months according to the fixed cycle described in Figure 5.2, with high organic matter and ammonium removal efficiencies. The effluent concentration was 54 ± 25 mg of $COD \cdot L^{-1}$ and 4.7 ± 5.6 mg of $N \cdot L^{-1}$, on average. The change in the WWTP facility was made to study the effect of the DO controller on the real-time control system and to optimize its treatment of urban wastewater with different characteristics (mainly a lower C/N ratio; Table 5.2).

The aerobic phases evolution was also monitored with an online calculated OUR to extract process behaviour knowledge based on a comparison of the monitored (i.e. pH and DO) and calculated (OUR) data. Figure 5.11 shows a normal pattern for measured and calculated data results obtained when applying an on/off DO controller in the SBR pilot plant treating urban wastewater from the Celrà WWTP.

In Figure 5.11 significant DO set point overtakings (between 1.4 and 3.0 mg $O_2 \cdot L^{-1}$), due to the DO probe delay (30 s), are observed. The on/off DO controller is also responsible for an observed pH fluctuation. Even though the pH minimum could be established from a full cycle profile during aerobic phases, online detection of the *ammonia valley* was difficult because of this fluctuation (Figure 5.11, point A). At the same time, the plotted calculated OUR data in Figure 5.11 shows a large reduction in the OUR profile (i.e. *σOUR*; point B). Thus, the calculated OUR data remains in a flat pattern until the end of the aerobic phase (Figure 5.11, point C). Such a stable minimum OUR value (OUR_v), clearly observed during the last aerobic phase, indicates that the system was under endogenous conditions and the organic matter and ammonium were completely oxidized (Watts and Garber, 1995).

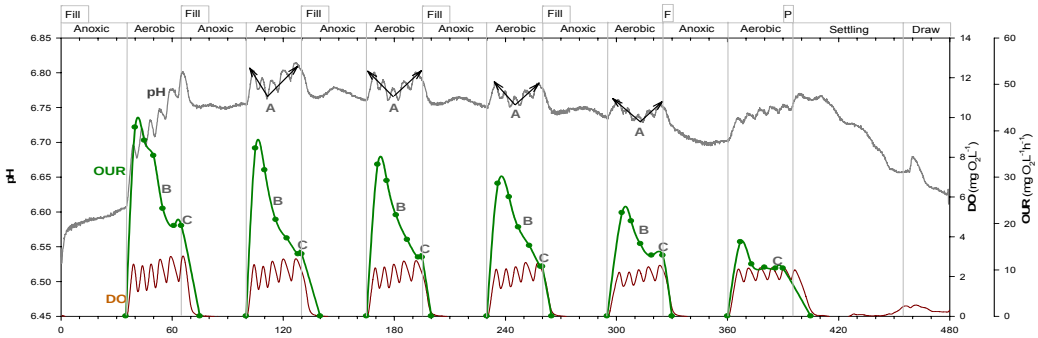

Figure 5.11. Monitored and calculated data for an 8h cycle with a DO on/off control in the SBR pilot plant treating urban wastewater from the Celrà WWTP.

To avoid the oscillations in the DO profile and to get a more continuous OUR profile, we considered applying a control system for the DO control. Nevertheless, because of the changing volume during the the aerobic phases, applying a unique PID control to all the stages was not possible and the DO control presented some lacks of stability. To solve this problem, the possibility of developing local controllers for each stage was considered. However, it was noticed that the same controller applied to the same stage was greatly affected by daily changes in water pollution level (Traoré *et al.,* 2005).

On the other hand, a Fuzzy Logic Control (FLC) for the DO was tested in order to reduce the troubles of stability presented by the PID controller. The basis of the FLC are presented by Traoré *et al.* (2005) and Figure 5.12 presents the evolution of a full cycle with such DO control system (pH, DO and calculated OUR). One of the advantages obtained was a continuous OUR profile with more available OUR values (section 5.3.4). Also, as a consequence of lower air flow variations to the reactor, pH and DO fluctuations observed with the previous on/off control strategy (Figure 5.11) were considerably reduced. Measured DO values were always between 1.9 and 2.1 mg $O_2 \cdot L^{-1}$ and, in spite of the pH trend displayed, the data obtained with coupled noise were enough to detect the *ammonia valley* (Figure 5.12, points A) when using an episode representation of the pH evolution (Rubio *et al.,* 2004).

Comparing on/off control and FLC, presented in Figure 5.11 and Figure 5.12, FLC identifies different bending points. Furthermore, no severe signal fluctuations are observed using FLC and, with the monitoring of the air flow, it is possible to obtain an online OUR profile. The pH profile analysis is characterized by the manifestation of a minimum value (i.e. *ammonia valley*, point A, Figure 5.11 and

Figure 5.12). This bending point appears at the same time as another bending point related to OUR evolution (i.e. *σOUR*, point B, Figure 5.11 and Figure 5.12). Finally, when analyzing the OUR evolution, a two plateau pattern can be observed when using FLC (Figure 5.12). Thus, all aerobic phases reached a final OUR constant value (OUR_v; Figure 5.12, point C).

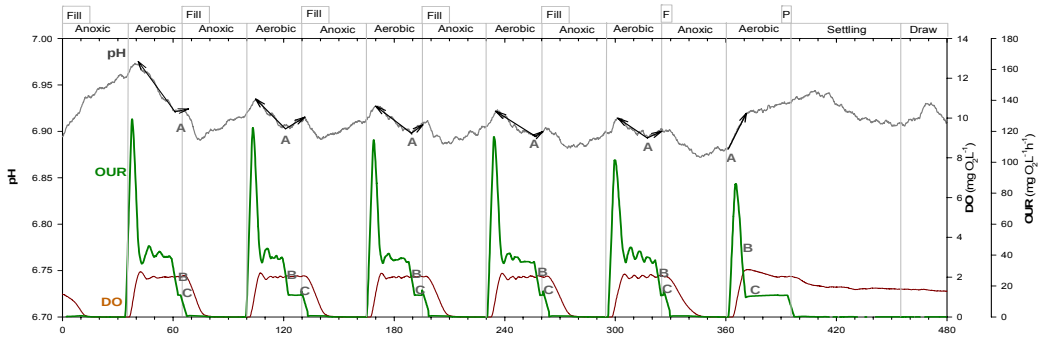

Figure 5.12. pH, ORP and DO profiles during the 8 hour total cycle length applied in the SBR pilot plant using fuzzy DO control.

In order to check the end of the nitrification process with the detected bending points, an offline analysis of soluble ammonium in the reactor was conducted during one aerobic phase of a cycle (Figure 5.13). Figure 5.13A shows the evolution of pH and calculated OUR taken from online measurements from the pilot plant treating urban wastewater. The conversion of the initial aerobic ammonium concentration is shown in Figure 5.13B in order to compare the nitrification process with the measured pH and the calculated OUR. When ammonium conversion levels reach values over 90% (minute 26), the pH profile is characterized by an *ammonia valley*, while the OUR profile is identified by a sudden decrease (*σOUR* point). Therefore, both the *ammonia valley* and *σOUR* point could be used to detect the ammonium depletion. However, at the end of the aerobic phase and after the depletion of ammonium (from minute 27), OUR values decreased until they remained at a constant value (OUR_v). At that moment the ammonium and the biodegradable organic matter were removed.

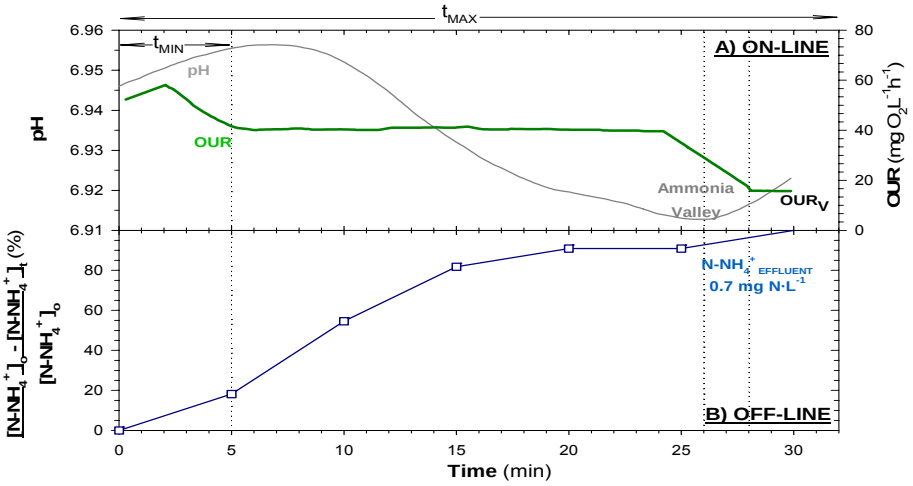

Figure 5.13. Relation between online values (pH and calculated OUR) and the ammonium conversion to nitrate during an aerobic cycle phase.

5.4.6 The proposed online optimization control

Using a step-feed strategy and previously observed profiles with a FLC for the DO, aerobic phases of the SBR cycle can be adjusted according to the evolution of the monitored pH and calculated OUR. All observed cycles (Figure 5.11 with on/off DO control and Figure 5.12 with FLC) are characterized by longer aeration phases than are needed. All of them easily reached the endogenous OUR values (OUR_V). Nevertheless, not all the aerobic phases could have the same goal. While the first aerobic phases are followed by successive fillings and anoxic–aerobic pairs, the last one is responsible for the final effluent ammonium and organic matter discharge. Therefore, to optimize operational costs (especially the aeration ones), different control strategies to each aerobic phase must be implemented:

- During the first five aerobic phases, reaching the *ammonia valley* is enough to define a phase change to the next anoxic phase because the end of nitrification has been achieved and if available organic matter still remains into the bulk liquid, it will serve as a starting basis for the next anoxic denitrification phase.
- The last aerobic phase, as the responsible of the final effluent quality, must be controlled by achieving a lower OUR constant value (OUR_V), which indicates the complete depletion of ammonium and organic matter (i.e. endogenous conditions).

Figure 5.14 shows the proposed algorithm developed from the knowledge acquired when observing the pH and OUR profiles as the optimized version of the real-time control system implemented in the SBR pilot plant treating urban wastewater from the Cassà WWTP. This control algorithm should be applied during aerobic and anoxic phases of the fixed cycle used (Figure 5.2). All the control system parameter values are presented in Table 5.6.

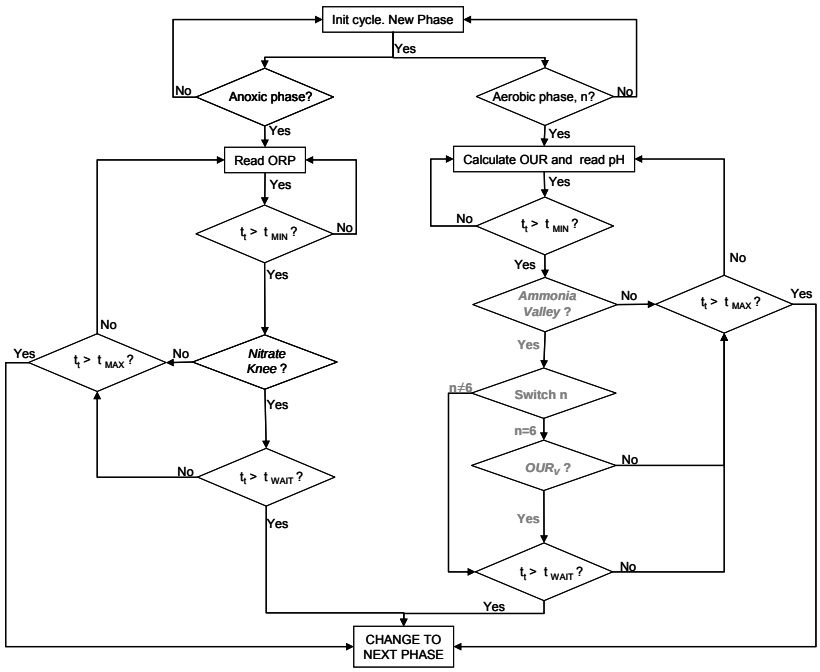

Figure 5.14. A proposed real-time control scheme for adjusting aerobic reaction phases in an SBR operating with a step-feed strategy.

Table 5.6. Parameter values for the control strategy of the proposed algorithm.

Phase		pH	OUR ($mgO_2 \cdot L^{-1} \cdot h^{-1}$)	ORP (mV)	t_{min} (min)	t_{wait} (min)	t_{max} (min)	Control Target
Aerobic	n≠ 6	*Ammonia valley*	-	-	2	5	30	Ammonium removal
	n= 6	*Ammonia valley*	OUR_v	-				Ammonium + carbon removal
Anoxic	All	-	-	*Nitrate Knee*	2	5	35	Denitrification

During the aerobic phases, the control system proposed firstly identifies the aerobic running phase (n) and reads the pH and calculated OUR values from the monitoring module. A minimum aerobic time (5 min, t_{min}) is considered to avoid a premature aerobic phase ending caused by the transient response of the activated sludge. After the minimum aerobic time is reached, the system starts to check for a possible *ammonia valley* by using episode representation of pH evolution (Rubio *et al.,* 2004) at each time step. If no *ammonia valley* is detected, the algorithm checks whether the maximum aerobic time has passed (t_{max}). This maximum aerobic time must be considered to avoid an excessive aerobic time that could result in a longer SBR cycle. For aerobic phases one to five, detecting the *ammonia valley* is enough to cause a phase change. However, for the sixth aerobic phase, a second termination criterion must be reached. To ensure a final effluent with low levels of ammonium and organic matter, the last aerobic phase is finished when, after achieving the *ammonia valley*, the calculated OUR values reach values lower than OUR_v. Finally, for all the aerobic phases, after identifying the end of the phase, another security timer was used, i.e. the waiting time (2 min, t_{wait}).

In the anoxic phase the control system controls nitrate depletion by detecting the *nitrate knee*. First, the system waits a minimum time (t_{min}). After that, the system starts to check for a possible *nitrate knee* in the ORP profile by using episode representation (Rubio *et al.,* 2004) at each control step. If no *nitrate knee* is detected, the algorithm checks whether the maximum anoxic time has passed (t_{max}). This maximum anoxic time must be considered to avoid an excessive anoxic time that could result in a longer SBR cycle. If the *nitrate knee* is reached, the system waits a security time (t_{wait}) and then changes to the next phase.

5.5 Conclusions

This chapter describes the successful development, implementation and improvement of an SBR real-time control system treating urban wastewater for organic matter and nitrogen removal purposes. More specific conclusions are:

1. Using the online OUR and the ORP, it is possible to estimate the status of the biological processes and control the length of the aerobic and/or anoxic phases of the SBR operational

cycle in real time. Some security factors related to minimum and maximum aerobic phase duration must be considered to avoid mistakes or persistent aerobic phases.

2. The SBR real-time control system worked treating 0.6L·d^{-1} of urban wastewater for more than 4 months and reached effluent levels lower than legally required by the European Directive 91/ 217/CEE (i.e. 57 mg of COD·L^{-1} and 4.7 mg of N·L^{-1}).

3. This study demonstrates the importance of the aeration control strategy applied in a real-time control system. Clearer profiles were obtained which can be used for control strategies when on/off DO control is changed to a control scheme based on FLC.

4. When DO control had been improved, identifying the bending points for the pH (*ammonia valley*) and the online calculated OUR (*OURv*) can optimize the aerobic phase of the SBR cycle for removing organic matter and ammonium.

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Chapter 6.
Biological nutrient removal in SBR technology: C:N:P influent ratio effects

Biological Nutrient Removal (BNR) in Sequencing Batch Reactor (SBR) technology requires a sequence of anaerobic–anoxic–aerobic phases with multiple feeding events over one cycle. This filling strategy was adapted to enhance denitrification and phosphate release, using the easily biodegradable organic matter from the wastewater. In this chapter, the successful BNR of wastewater using SBR technology in a single reactor is presented.

On the other hand, in some wastewater the available organic matter concentration can be insufficient, provoking the destabilization of the system performance. For that reason, the influence of Carbon:Phosphorus (C:P) and Carbon:Nitrogen (C:N) feeding ratios on the efficiency of BNR from wastewater using a lab-scale SBR is also investigated.

This chapter has been the basis of the following publications:

Puig, S., Corominas, Ll., Balaguer, M.D. and Colprim, J. 2007a. Biological nutrient removal by applying SBR technology in small wastewater treatment plants: carbon source and C/N/P ratio effects. *Water Sci. Technol.* **55**(7), 135-141.

Puig, S., Coma, M., van Loosdrecht, M.C.M., Colprim, J. and Balaguer M.D. 2007b. Biological nutrient removal in a sequencing batch reactor using ethanol as the carbon source. *J. Chem. Technol. Biotechnol.* **82**(10), 898-904.

6.1 Introduction

Biological Nutrient Removal (BNR) in Sequencing Batch Reactors (SBRs) requires a combination of anaerobic, anoxic and aerobic phases. During the anaerobic phase, the carbon source, generally in the form of Volatile Fatty Acids (VFAs), is taken up by Polyphosphate Accumulating Organisms (PAOs) releasing phosphate into the liquid phase. Within the cells, VFAs are stored basically as PolyHydroxyAlkanoates (PHAs) while intracellular glycogen is converted to PHA. In the anoxic phases, if a soluble and biodegradable carbon source is present, a classical heterotrophic denitrification process takes place. Finally, under aerobic conditions, PAOs grow and accumulate phosphate in the cells, while autotrophic biomass is responsible for the nitrification process. Phosphorus removal is achieved through the wastage of excess sludge with high poly-P content.

Nevertheless, Glycogen Accumulating Organisms (GAOs) are a group of bacteria capable of competing with PAOs during anaerobic VFA uptake. Like PAOs, GAOs take up VFAs anaerobically and convert them into PHAs via hydrolysis of glycogen as their sole source of energy for this process (Mino *et al.,* 1998).

When operating SBRs for BNR, special attention has to given to the availability and use of the easily biodegradable substrate. Classical heterotrophic denitrification process and phosphate release process by PAOs both compete for the organic matter. From here, a first analysis about the influent composition must be conducted in order to identify the suitability of biological nitrogen removal and biological phosphorous removal. Table 6.1 provides general guidelines about the amenability of various wastewater to biological nitrogen removal. The values given can be used to screen candidate wastewater to determine how difficult it may be to achieve good nitrogen removal (Grady *et al.,* 1999).

Table 6.1. Relationship between expected biological nitrogen removal efficiency and influent organic matter to nitrogen ratios (Grady *et al.,* 1999).

Nitrogen removal efficiency	Poor	Moderate	Good	Excellent
C:N	< 5	5 - 7	7 - 9	> 9

In reference to biological phosphorous removal, the amount of organic matter required to remove phosphorus is highly depend on the organic matter composition and there is a lack of reference values in the literature. Nevertheless, Table 6.2 shows a review of some minimum C:P ratios presented by different authors as a minimum value for a suitable biological phosphorous removal.

Table 6.2. Amount of organic matter required to remove phosphorus in previous studies.

Reference	Randall *et al.* (1992)	Henze *et al.* (2002)	Metcalf and Eddy, (2003)
C:P	> 33	> 20	> 10

Randall *et al.* (1992) clearly noted that an effluent total phosphorus below 1 mg $P\text{-}PO_4^{3-}\cdot L^{-1}$ is achieved at a C:P ratio of 33 or higher. Henze *et al.* (2002) proposed 20 g of Chemical Oxygen Demand (COD) (mainly acetate) per each gram of phosphate removed. Metcalf and Eddy (2003) suggested that about 10 g of COD (mainly acetate) will be required to remove 1g of phosphorus by the biological storage mechanism.

In spite of using the feeding strategies (Chapter 4), the influent organic matter concentration can be insufficient affecting the normal performance of the system for nutrient removal purposes. A decrease of the C:P ratio causes the depletion of the polyphosphate content in PAOs, eventually leading to their replacement by GAOs as the majority (Liu *et al.* 1997).

6.2 Objectives

The aim of this chapter was to study the cycle characteristics of wastewater treatment for BNR purposes using SBR technology in a single reactor. The chapter focuses on the influence of Carbon:Phosphorus (C:P) and Carbon:Nitrogen (C:N) feeding ratios on the efficiency of BNR from wastewater using a lab-scale SBR.

6.3 Materials and methods

6.3.1 Experimental set-up

The lab-scale SBR was composed of a cylindrical reactor with maximum and minimum working volumes of 30L and 20L respectively. A full description of the SBR is presented in Section 3.1.1. To achieve nutrient removal in the SBR, the Hydraulic Retention Time (HRT) and Solids Retention Time (SRT) were maintained, respectively, at 1 day and 20 days on average. The SBR, which treated synthetic wastewater for nutrient removal purposes, was seeded with sludge from the Sils-Vidreres WasteWater Treatment Plant (WWTP) (Girona, Spain).

6.3.2 Synthetic feed

The synthetic wastewater (Table 6.3) was basically composed of a mixed carbon source (ethanol, Dehydrated Meat Extract (DME), milk and landfill leachate), an ammonium solution, a phosphate source, alkalinity control ($NaHCO_3$) and a microelement solution (adapted from Dangcong *et al.,* 2000). DME is a commercial mixture of starch, yeast, monosodium glutamate and chicken extract. It is considered a soluble fermentation product (Boeije *et al.,* 1999). Raw leachate was supplied from the CORSA landfill located near the city of Reus (41° 6' 28"N, 1° 7' 4" E, Catalonia, Spain) as the fraction of slowly biodegradable organic matter in the synthetic influent (average BOD_5:COD ratio between 0.10 to 0.18) (Ganigué *et al.,* 2007).

The synthetic wastewater was prepared twice a week with an average concentration of 502±175 mg·L^{-1} COD, 61.5±10.1 mg·L^{-1} N-TKN, 47.9±7.3 mg·L^{-1} N-NH_4^+ and 9.1±3.8 mg P- PO_4^{3-}·L^{-1} during stable state operation, giving C:N and C:P ratios of 8±2 mg COD·mg^{-1} N-TKN and 52±16 mg COD·mg^{-1} P-PO_4 respectively.

Table 6.3. Synthetic wastewater composition.

Name	Formula	Concentration	Solution
Ethanol	CH_3CH_2OH	0.27 mL·L^{-1}	
Dehydrated meat extract (DME)	-	560 mg·L^{-1}	**Carbon Source**
Milk	-	0.4 mL·L^{-1}	
Sodium bicarbonate	$NaHCO_3$	280 mg ·L^{-1}	**Alkalinity source**
Ammonium chloride	NH_4Cl	183 mg ·L^{-1}	**Ammonium source**
Manganese (II) chloride tetrahydrate	$MnCl_2·4H_2O$	0.19 mg ·L^{-1}	
Zinc chloride dihydrate	$ZnCl_2·2H_2O$	0.0018 mg ·L^{-1}	
Copper (II) chloride dehydrate	$CuCl_2·2H_2O$	0.022 mg ·L^{-1}	**Microelements solution**
Magnesium sulphate heptahydrate	$MgSO_4·7H_2O$	5.6 mg ·L^{-1}	
Iron (III) chloride hexahydrate	$FeCl_3·6H_2O$	0.88 mg·L^{-1}	
Calcium chloride dehydrate	$CaCl_2·2H_2O$	1.3 mg·L^{-1}	
Potassium dihydrogen phosphate	KH_2PO_4	7.0 mg·L^{-1}	
Dipotassium hydrogen phosphate	K_2HPO_4	18 mg ·L^{-1}	**Phosphorus source**
Disodium hydrogen phosphate heptahydrate	$Na_2HPO_4·7H_2O$	14 mg ·L^{-1}	
Landfill leachate	-	13.3 mL·L^{-1}	**Complex mixture**

6.4 Results

6.4.1 From nitrogen to nutrient removal SBR

Chapter 4 described a successful implementation in the SBRs using 6 filling events with a sequence of anoxic-aerobic phases treating urban wastewater for nitrogen removal purposes. Figure 6.1A presents the 8h SBR cycle implemented for nitrogen removal purposes in the SBR pilot plant. 53.8% of the entire reaction time was under anoxic conditions and 46.2% was under aerobic conditions. The last influent

feeding flow was 3 times lower than the others to assure low values of final nitrogen concentration in the effluent.

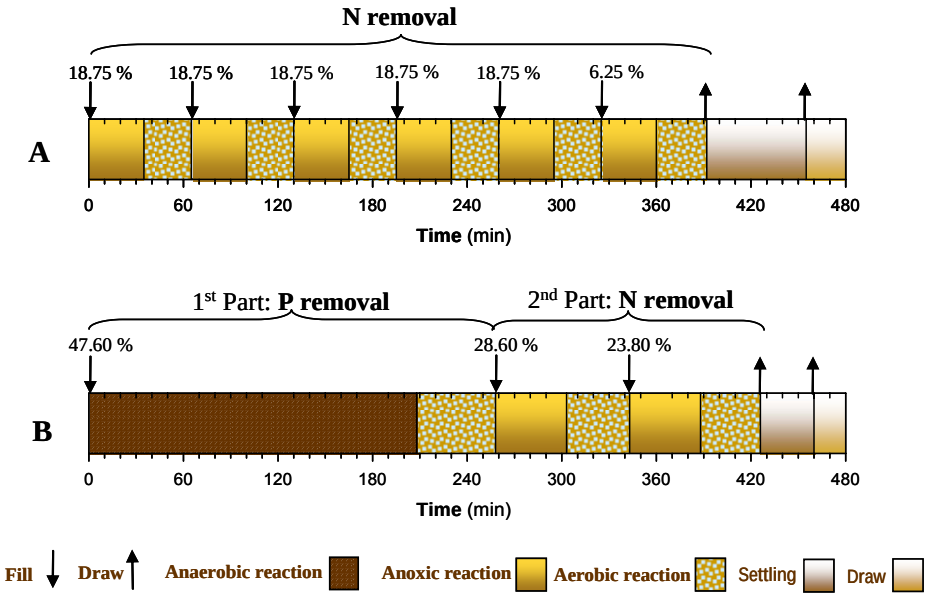

Figure 6.1. Definition of the SBR cycles used during the experimental study for nitrogen removal (a) and BNR (b) purposes.

However, to achieve biological phosphorous removal, anaerobic conditions are required for VFAs uptake and storage into PHA. Thus, the option was to add an anaerobic phase to improve the uptake of readily biodegradable organic matter by PAOs, followed by an aerobic phase for the phosphate uptake with the consumption of stored products. For this reason, the nitrogen removal cycle (Figure 6.1A) was modified to provide the different environmental conditions of at least three separate periods. Figure 6.1B presents the 8 hour SBR cycle implemented for BNR with three feeding steps (the first one doubling the volume of the others). The 8h cycles were designed with different reaction phases conducted under: 3.5 h (43.33 %) anaerobic, 2.2 h (27.08 %) aerobic, 1.5 h (19.17 %) anoxic, 0.5 h (6.25 %) settle and 0.3 h (4.17 %) decant. The organization of the reaction phase was divided into two parts focussed on firstly phosphorus removal and afterwards on the nitrogen removal. In such sense, the first anaerobic-aerobic pair of phases was related to phosphorus removal lasting 4.3 h (80% under anaerobic conditions); and the second part (2.8 h) consisted of a sequence of anoxic-aerobic phases for anoxic denitrification (52.9 % of the reaction time) and aerobic autotrophic nitrification (47.1 % of the reaction time). The wastewater was always introduced under anaerobic or anoxic conditions to enhance phosphate release (first feeding) and denitrification (second and third feeding).

6.4.2 SBR operation and performance

The lab-scale SBR operated for five months using an 8h cycle (Figure 6.1B) under nutrient removal conditions. The dynamics of nitrogen, phosphorous and carbon compounds when the system achieved stable operation are shown in Figure 6.2.

Figure 6.2A presents the ammonium and nitrate profiles during an SBR cycle. The nitrification process took place in the first aerobic phase, and the ammonium concentration decreased from 9.6 to 0.7 mg N-NH_4^+·L^{-1}, while nitrate concentration peaked at 6.2 mg N-NO_3^-·L^{-1}. In the second and third aerobic phases, the ammonium concentration decreased to 0.4 and 0.9 mg N-NH_4^+·L^{-1}, respectively. During the anoxic phase, nitrate was depleted because of the biological denitrification process. At the end of the cycle, the total nitrogen effluent concentration was below 3 mg N-TN·L^{-1} (influent nitrogen concentration was 70.5 mg N-TN·L^{-1}).

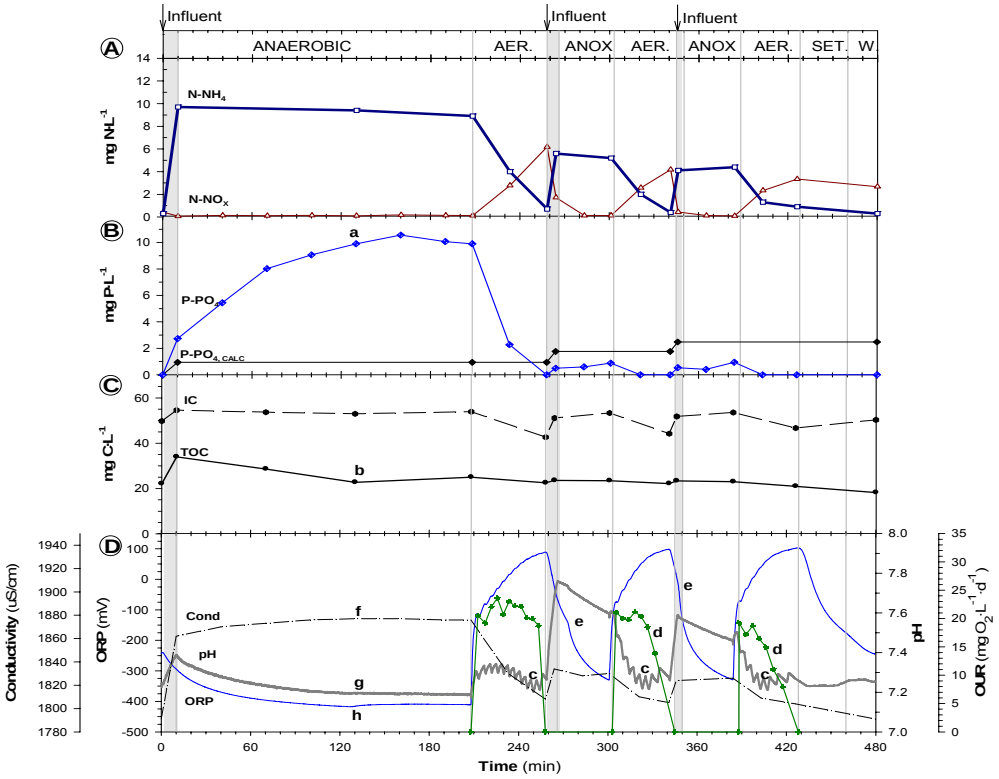

Figure 6.2. Evolution of nitrogen (A, as ammonia and oxidised nitrogen, mg N·L^{-1}), phosphorus (B, soluble and calculated orthophosphate assuming no chemical/biological reactions, mg P·L^{-1}), carbon (C, as organic and inorganic total carbon, mg C·L^{-1}) compounds and on-line probes (D, ORP, pH, OUR and conductivity) profiles during the 8h total cycle length employed in the lab-scale SBR. Grey areas refer to the filling phases of the cycle.

Related to phosphorous profile, Figure 6.2B compares the observed profile of experimental phosphate and the expected phosphate if no biological reactions occur (P-calculated) during the SBR cycle. The influent phosphate concentration was 6.93 mg P-PO_4^{3-}·L^{-1}. During the anaerobic phase, phosphorus was released up to 10.56 mg P-PO_4^{3-}·L^{-1}. In the aerobic phase the phosphate was quickly taken up by PAOs so that there was less than 0.05 mg P-PO_4^{3-}·L^{-1} at the end of the cycle. If no chemical/biological reactions had taken place, the calculated orthophosphate concentration would have been 2.50 mg P-PO_4^{3-}·L^{-1}.

Because of the effect of storage during the anaerobic phase, Figure 6.2C shows the bulk liquid Total Organic Carbon (TOC) and the Inorganic Carbon (IC) profiles evolution during an 8 h cycle. The TOC concentration in the influent was 191 mg C·L^{-1} (510 mg COD·L^{-1}). During the anaerobic phase, the TOC was consumed from 34 to 22 mg C·L^{-1} for the organic substrate uptake by PAOs. In spite of feeding twice the reactor, The TOC concentration remained more or less constant due to the denitrification process took place during the feeding phases. Thus, at the end of the cycle, the TOC was 18 mg C·L^{-1} (COD of 61 mg

$O_2 \cdot L^{-1}$). This value could be due to the influent non-biodegradable organic matter. The inorganic carbon profile (Figure 6.2C) during the cycle followed a profile of a classical nitrification-denitrification process. In the aerobic phases, the IC decreased because, during the nitrification process, 7.14 g of alkalinity (as $CaCO_3$) was consumed per g of $N\text{-}NH_4$ oxidized. Some of the alkalinity was restored by the denitrification process. This process produces 3.57 g of alkalinity (as $CaCO_3$) per g of $N\text{-}NO_3^-$ reduced (Metcalf and Eddy, 2003).

Finally, the evolution of online probes data (i.e. pH, Oxidation Reduction Potential (ORP), Oxygen Uptake Rate (OUR) and conductivity) profiles are presented in Figure 6.2D for a typical 8 h cycle of the lab scale SBR when nitrification, denitrification and biological phosphorous removal were achieved. As stated before in Chapter 5, some end points of these processes could be observed. The end of the nitrification process could be identified by detecting the *ammonium valley* and α_{OUR} (Figure 6.2D; points c and d respectively), while the appearance of a *nitrate knee* (Figure 6.2D; point e) demonstrated the nitrate depletion. Under anaerobic conditions, when PAOs released all the phosphate (Figure 6.2B; point a) to get energy to take up the carbon source (Figure 6.2C; point b), three characteristic points appeared in the conventional online probes, while the phosphate profile remained stable (Figure 6.2B; point a): *i)* the conductivity (Figure 6.2D; point f) and the pH (Figure 6.2D; point g) profiles levelled off and *ii)* the ORP profile presented a slight increase (Figure 6.2D; point h)

6.4.3 Influence of C:N:P influent ratios on the BNR efficiency

Once the 8h cycle was successfully evaluated for BNR purposes, the next research task was to study the influence on the BNR efficiency of C:N:P influent ratios. For this reason, two possible situations were considered: a modification of the influent phosphorus load (Case 1) and a modification of the influent carbon load (Case 2).

Case 1: A modification of the influent phosphorus load

The purpose of this study was to know the effect on the BNR performance after a sudden increase in the influent phosphate load. Thus, the plant was inoculated again and operated for three months using the 8h cycle (Figure 6.1B) for nutrient removal and treating $15.7 L \cdot d^{-1}$ of wastewater. The HRT and SRT were maintained at 1.3 day and 16.7 days respectively.

Period 1: Start-up of the SBR

The SBR was seeded with sludge from an urban WWTP (Sils-Vidreres WWTP, 41º 47' 58"N, 2º 45' 7" E; Girona, Spain). The WWTP treated 3100 m3·day-1 of urban wastewater for organic matter and nitrogen removal purposes. The lab scale SBR was fed with synthetic wastewater as described in Table 6.3. Figure 6.3 presents the evolution of carbon, nitrogen and phosphorous components during the experimental period applying an 8h cycle in the SBR. The stable state was reached after 2 weeks of operation. The COD, TN and phosphate average effluent concentrations of 44, 2.2 and 0.15 mg·L-1, respectively, leaded to removal efficiencies of 94%, 95% and 98% for carbon, nitrogen and phosphorous respectively.

Table 6.4 describes the different load working conditions (according to the C:N and C:P ratios) applied in the lab scale SBR. Figure 6.3 shows the evolution of the C:N and C:P feeding ratios (A), and carbon (B), nitrogen (C) and phosphorous (D) components during the experimental study.

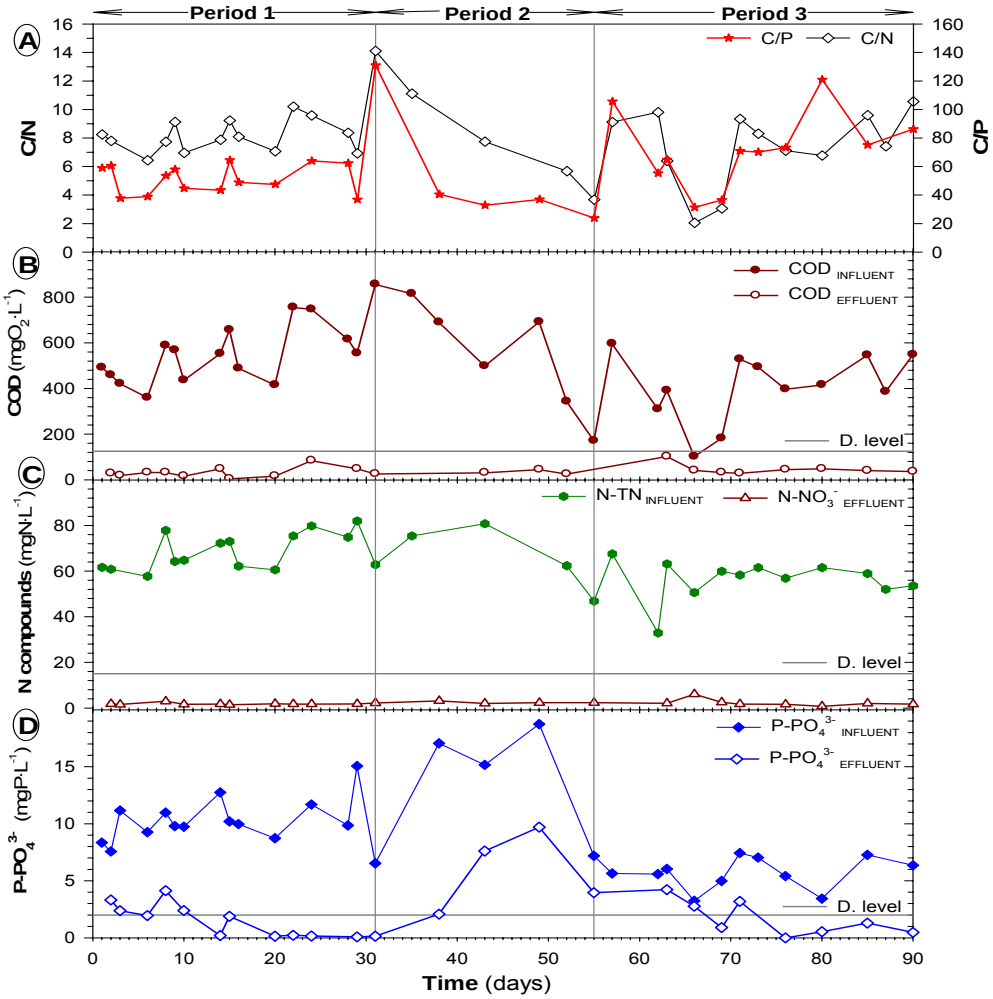

Figure 6.3. Evolution of carbon, nitrogen and phosphorous components during the experimental period applying an 8 h cycle in the SBR pilot plant. (D.level: Maximum discharge concentration admissible).

Period 1: Start-up of the SBR

The SBR was seeded with sludge from an urban WWTP (Sils-Vidreres WWTP, 41º 47' 58"N, 2º 45' 7" E; Girona, Spain). The WWTP treated 3100 $m^3 \cdot day^{-1}$ of urban wastewater for organic matter and nitrogen removal purposes. The lab scale SBR was fed with synthetic wastewater as described in Table 6.3. Figure 6.3 presents the evolution of carbon, nitrogen and phosphorous components during the experimental period applying an 8h cycle in the SBR. The stable state was reached after 2 weeks of operation. The COD, TN and phosphate average effluent concentrations of 44, 2.2 and 0.15 $mg \cdot L^{-1}$, respectively, leaded to removal efficiencies of 94%, 95% and 98% for carbon, nitrogen and phosphorous respectively.

Period 2: Increase of the phosphorus loading rate

After achieving complete nutrient removal in Period 1, an increase in the influent phosphate concentration (17 mg $P\text{-}PO_4^{3-} \cdot L^{-1}$, on average; Figure 6.3D) was applied during Period 2. The phosphate

load increased from 13.6 to 22.7 mg $P\text{-}PO_4^{3-}\cdot L^{-1}\cdot d^{-1}$, representing an increment of 69%. At the same time, the carbon and nitrogen concentrations were maintained similar to Period 1 (Table 6.4).

Despite the limited amount of carbon source available for nutrient removal (Figure 6.3B), the nitrogen effluent concentrations (0.3 mg $N\text{-}NH_4\cdot L^{-1}$ and 2.5 mg $N\text{-}NO_3\cdot L^{-1}$) were not affected and their concentrations were always lower than the European Directive values (D. level, Figure 6.3C). However, the lower amount of influent available organic matter (C:P ratio of 36 g $COD\cdot g^{-1}$ $P\text{-}PO_4^{3-}$ *versus* 55 g $COD\cdot g^{-1}$ $P\text{-}PO_4^{3-}$ in Period 1) clearly affected the phosphorous removal performance (Figure 6.3D) since the phosphate concentration rose steadily from 0.1 (day 29) to 9.71 mg $P\text{-}PO_4^{3-}\cdot L^{-1}$ (day 49).

Table 6.4. Definition of the different periods of study according to the C:N and C:P ratios to treat nutrients from wastewater.

	Period 1	**Period 2**	**Period 3**	**Units**
Tag	Start-up	P load increase	Recover	-
Length	31	20	37	*days*
C:P ratio	55 ± 16	36 ± 4	67 ± 21	*g $COD\cdot g^{-1}$ $P\text{-}PO_4^{3-}$*
C:N ratio	8 ± 2	9 ± 3	7 ± 3	*g $COD\cdot g^{-1}$ N-TKN*
C load	754	811	526	*mg $C\cdot L^{-1}\cdot d^{-1}$*
N load	90.2	88.0	72.9	*mg $N\cdot L^{-1}\cdot d^{-1}$*
P load	**13.6**	**22.7**	**7.8**	***mg $P\cdot L^{-1}\cdot d^{-1}$***

Period 3: Recuperation time for complete nutrient removal

The aim of this period was to find out the time needed to recover complete nutrient removal after a period of phosphate accumulation (9.71 mg P- $PO_4^{3-}\cdot L^{-1}$ in the SBR effluent; Figure 6.3D). For this reason, the C:P was increased from 36 to 67 g $COD\cdot g^{-1}$ $P\text{-}PO_4^{3-}$ as described in Table 6.4. Although the PAOs' responded slowly to recover the phosphorous removal, after 15 days of operation, complete phosphorus removal was achieved again. The effluent concentrations reached were lower than 1.0 mg $P\text{-}PO_4^{3-}\cdot L^{-1}$ (Figure 6.3D). The COD and nitrogen effluent concentration were always lower than the legal standards.

Case 2: Modification of the influent carbon load

Once the effect on the BNR performance after a sudden increase of the influent phosphate load was investigated, the next study focused on a sudden drop in the influent carbon load and its effect on the behaviour of the BNR system.

The lab-scale SBR was seeded again and operated for five months using the same 8h cycle (Figure 6.1B) for nutrient removal purposes and treating 17.9 $L\cdot d^{-1}$ of wastewater. The HRT and SRT were maintained at 1.3 and 20.5 days respectively. Table 6.5 presents the different load conditions divided into three periods according to the C:N and C:P ratios applied in the lab-scale SBR. Figure 6.4 shows the evolution of the C:N and C:P feeding ratios (A), and carbon (B), nitrogen (C) and phosphorous (D) components during the experimental study.

Period 4: Start-up of the SBR

The reactor was seeded again with sludge from an urban WWTP (Sils-Vidreres WWTP) and fed with synthetic wastewater (section 6.3.1) as in Case 1. Initially (from days 0 to 30), the low influent C:N applied (5 mg COD·mg^{-1} N; Figure 6.4A) resulted in high nitrate concentrations in the effluent (7.6 mg N-NO_3^-·L^{-1}; Figure 6.4C). At the same time, the phosphorus removal performance was affected by the nitrate presence at the beginning of the anaerobic phase reaching 2.0 mg P-PO_4^{3-}·L^{-1} in the effluent (Figure 6.4D). However, when the C:N ratio was increased from 5 to 10 (Figure 6.4A), from day 36 the process achieved complete BNR (3.3 mg N-NO_3^-·L^{-1} and 0.05 mg P-PO_4^{3-}·L^{-1}, at the end of the period).

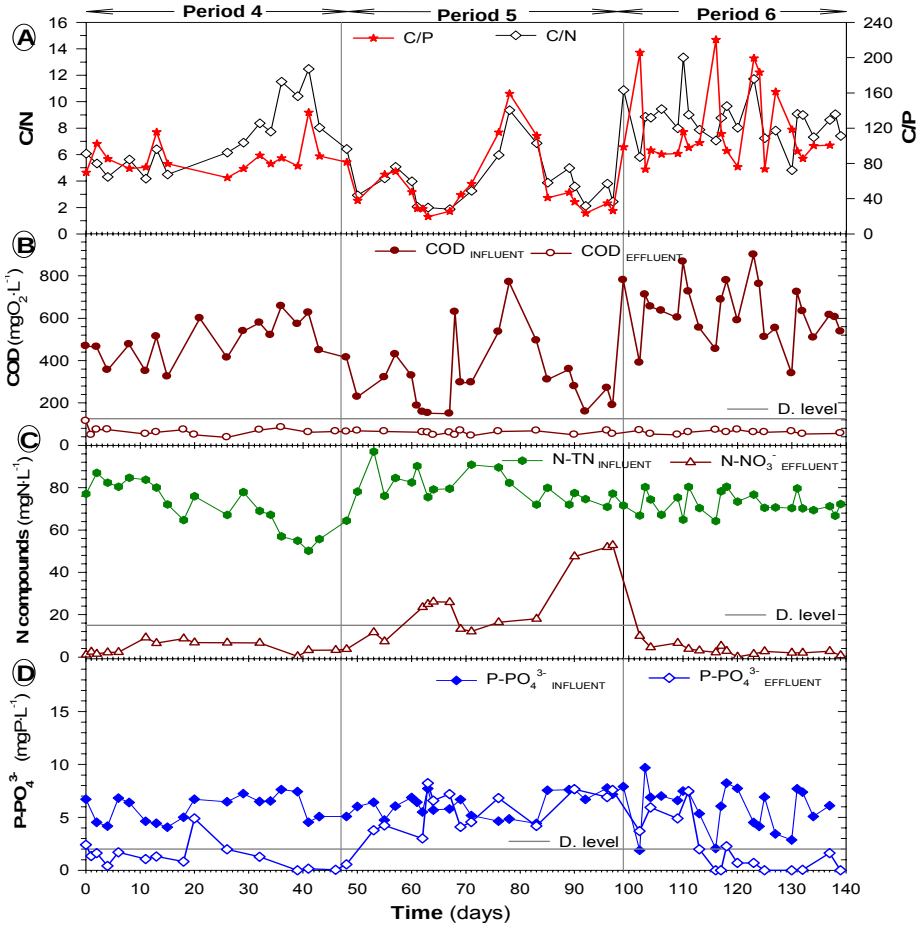

Figure 6.4. Evolution of carbon, nitrogen and phosphorous components during Case 2. (D.level: Maximum discharge concentration admissible).

Period 5: Decrease of the influent carbon load

After achieving the complete nutrient removal in the previous period, the carbon load fell from 630 to 425 mg C·L^{-1}·d^{-1} (Table 6.5). This reduction of the carbon load (around 33%) provoked the breakdown of the process. The nitrogen and phosphorus removal efficiencies decreased from 94% and 85% to 69% and 26% respectively. First, the system lost the biological phosphorus removal, and some days after the nitrate started to accumulate in the reactor (Figure 6.4). At the end of this period, the low influent C:N ratio (4 g COD·g^{-1} N-TKN) affected the performance of the process, and the effluent nitrate and phosphorus concentrations were 52.88 mg N-NO_3·L^{-1} and 7.18 mg P-PO_4^{3-}·L^{-1} respectively.

Table 6.5. Average influent characteristics and operation conditions during Case 2.

	Period 4	**Period 5**	**Period 6**	**Units**
Tag	Start-up	C load decrease	Recover	-
Length	47	50	41	*days*
C:N ratio	7 ± 3	4 ± 2	9 ± 2	*g COD·g^{-1} N-TKN*
C:P ratio	81 ± 28	55 ± 37	118 ± 45	*g COD·g^{-1} P-PO$_4^{3-}$*
C load	**630**	**425**	**786**	***mg C·L^{-1}·d^{-1}***
N load	89.7	100.2	89.1	*mg N·L^{-1}·d^{-1}*
P load	7.4	8.0	7.5	*mg P·L^{-1}·d^{-1}*

Period 6: Recuperation time for the complete nutrient removal

In this period the C:N and C:P were established to normal levels (from 2 and 25 (day 97) to 11 g COD·g^{-1} N-TKN and 99 g COD·g^{-1} P-PO$_4^{3-}$, respectively). The organic matter concentration shot up from 189 to 779 mg COD·L^{-1} on day 99th (Figure 6.4B). The effluent nitrate concentration decreased gradually from 52.88 (day 97) to 9.96 (day 102) and 4.61 mg N-NO$_3$·L^{-1} (day 104; Figure 6.4C). Once the system achieved complete denitrification efficiencies, the competition for organic matter available between the denitrification process and phosphorus removal disappeared. The phosphate removal took 16 days to recover the complete phosphate removal efficiencies (Figure 6.4D). The effluent phosphorus concentration was 0.00 mg P-PO$_4^{3-}$·L^{-1} on day 116.

6.5 Discussion

6.5.1 SBR operation and control

The design of the appropriate SBR cycle configuration is one of the bottlenecks of the operation process. The cycle configuration depends on influent characteristics, daily flow and admissible effluent discharge concentration. Thus, the 8h cycle used for BNR purposes was successfully applied in the lab scale SBR treating synthetic wastewater (influent loads: 401 mg COD ·L^{-1}·d^{-1}, 55.9 mg N·L^{-1}·d^{-1} and 5.5 mg P·L^{-1}·d^{-1}) and resulted in excellent nutrient removal efficiencies (96% and 99.9% for nitrogen and phosphorus respectively).

Some characteristic points were seen in the cycle profiles obtained from the conventional probes related to biologic reactions involved in the wastewater treatment such us ammonium and nitrate depletion, and the end of phosphate release. The presence of these points confirms that an optimization of the SBR cycle is possible. Thus, to develop a control system for nutrient removal, these points will be useful, especially to determine the end of the anaerobic phase. Marsili-Libelli (2006) found that, when all the available nitrate is denitrified and all the phosphorus released, the pH and ORP level off. Both were

observed experimentally during the anaerobic phase, but also the conductivity profile levelled off when all the phosphate was released.

On the other hand, during the aerobic phase, nitrification and phosphorus uptake occur at the same time. For this reason, pH may exhibit differing patterns depending on which ends first (Marsili-Libelli, 2006). This complex behaviour was thoroughly examined by Spagni *et al.* (2001) who observed differing pH patterns as a consequence of the relative duration of two concurrent processes, ammonium oxidation and phosphorus uptake, in addition to CO_2 stripping, responsible for a rapid increase of pH at the beginning of the aerobic phase. Although, ammonium depletion patterns (*ammonia valley* and α_{OUR}) were observed.

6.5.2 Influence of the C:N:P influent ratios on the BNR performance

The efficiency of the BNR process is clearly dependent on the configuration of the system and the influent characteristics. Once the effect of the carbon source on the BNR performance is known, a comparison between the experimental results and the results reported by other researchers in terms of the variation of C:N, C:P influent ratios, nitrogen and phosphorus removal efficiencies were carried out and summarized in Table 6.6.

In spite of the competition for the carbon sources between PAOs and denitrifiers in Period 1, C:P (55 g COD·g^{-1} P-PO_4^{3-}) and C:N (8 g COD·g^{-1} N-TKN) ratios were enough to achieve complete nutrient removal of wastewater. However, when the C:P ratio was decreased to 36 g COD·g^{-1} P-PO_4^{3-} in Period 2, there was not enough available organic matter to be taken up by PAOs and therefore phosphate accumulated in the reactor (Figure 6.3D), decreasing the removal efficiency from 99% to 77% (Table 6.6).

On the other hand, in Period 5 the carbon load decreased resulting in low C:N (4 g COD·g^{-1} N-TKN) ratio. The low organic matter available in the influent, which affected both processes (denitrification and phosphate removal), caused their breakdown (69% and 26% nitrogen and phosphorus removal efficiencies respectively). The nitrate remained in the system (52.88 mg N-NO_3^-·L^{-1}; Figure 6.4C) and there was competition for the carbon source, which led to the final reduction of phosphorus removal efficiency (Table 6.6). The current interpretation of this phenomenon is that nitrate entering the anaerobic period will be utilized as an electron acceptor in the growth of non-polyP heterotrophs. This reduces the amount of substrate available for sequestration by the polyP organisms, and hence reduces the amount of P removal that can be achieved (Barker and Dold, 1996; Zou *et al.,* 2006).

Moreover, comparing the data shown in Table 6.6, some variability can be observed between the C:N and C:P feeding ratios and nitrogen and phosphorus removal efficiencies in the different studies. The nitrogen efficiencies presented (periods 1, 2 and 3) are higher than those obtained by other researchers working with the same or higher C:N ratio (C:N of 7-10 g COD·g^{-1} N-TKN) and applying the step-feed strategy. In single-feed or continuous feeding strategies (Yu *et al.,* 1997; Akin and Ugurlu, 2004; Ma *et al.,* 2005) or in the case that the influent C:N ratio was low (Period 5), substantial amounts of nitrate might remain in the effluent because endogenous denitrification cannot reduce nitrate efficiently in the

anoxic reaction and settle period due to the lack of an organic electron donor (Chang and Hao, 1996). Moreover, phosphate removal efficiency is affected by competition for the organic substrate between denitrifiers and PAOs leading to high phosphate concentrations in the effluent.

Table 6.6. Comparison of C:N and C:P influent ratios, nitrogen and phosphorus removal efficiencies in different studies of nutrient removal.

Reference	Kind of wastewater	Carbon source	Reactor Configuration	C:N	C:P	C:N:P	% N removal	% P removal
This study (Period 1)	Synthetic	Ethanol + DME	SBR	8	55	100:12:1.8	95	99
This study (Period 2)	Synthetic	Ethanol + DME	SBR	9	36	100:11:2.8	97	77
This study (Period 3)	Synthetic	Ethanol + DME	SBR	7	67	100:14:1.5	96	90
This study (Period 5)	Synthetic	Ethanol + DME	SBR	4	55	100:24:1.9	69	26
Kargi and Uygur, (2003)	Synthetic	Glucose + Acetate	SBR	10 - 50	40 - 250	100:2:0.54	94	99
Keller *et al.* (2001)	Urban	-	SBR	10	58	100:10:1.7	96	84
Ma et al. (2005)	Synthetic	Synthetic brewage*	A^2/O	6	18 to 61	100:17: (1.6 to 5.6)	75 - 84	90 - 98
Yu *et al.* (1997)	Synthetic	Lactose + Glucose	SBR	7	55	100:14:1.8	81	61

* The main element of the synthetic wastewater: alcohol (31–47%); acetate acid (29–33%); propionate acid (3–6%); butyric acid (3.8–5%); slow biodegradable COD (5.9–20.9%); inert COD(3.1–12.3%), the proportion was that each component accounts for the Total COD (TCOD).

The step-feed strategy also improved phosphorus removal in the experimental study working with the same C:P ratios (55-58 g COD·g^{-1} P-PO_4^{3-}) as other researchers that used continuous feeding (Yu *et al.,* 1997) and feeding during the settle phase strategies (Keller *et al.,* 2001) have also reported.

6.6 Conclusions

BNR has been successfully achieved by using only one reactor and working with a low organic matter ratio in the influent (C:N:P ratio of 100:12:1.8). The high nutrient efficiencies achieved (94%, 95% and 98% for carbon, nitrogen and phosphorous respectively) proved that the sequence of anaerobic-anoxic-aerobic phases with multiple feeding events over one cycle is a promising strategy for removing nutrients from wastewater.

The BNR performance is strongly influenced by the C:N:P influent ratios. If the carbon concentration is not enough for BNR from wastewater, the first process affected is the Enhanced Biological Phosphorus Removal (EBPR). Thus, when the C:P ratio was below 36 g COD·g^{-1} P-PO_4^{3-}, this affected the phosphorus

performance and the accumulating in the reactor. After a period of phosphate accumulation and applying a higher C:P ratio of 67 g COD/g $P\text{-}PO_4^{3-}$, the PAOs responded quickly (15 days) to recover the nutrient removal efficiencies. In cases in which the carbon concentration is also not enough for denitrification purposes (the C:N ratio was below 4 g $COD \cdot g^{-1}$ N-TKN), the nitrate accumulated (52.88 mg $N\text{-}NO_3^- \cdot L^{-1}$), as did phosphorus, causing system performance destabilization. Afterwards, when the influent characteristics recovered (the C:N ratio was 9 g $COD \cdot g^{-1}$ N-TKN), the system returned to the normal conditions, first, recovering the denitrification efficiencies and after 15 days the phosphorus removal.

6.7 References

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Chapter 7.
Ethanol as a carbon source for biological nutrient removal from wastewater

When operating Sequencing Batch Reactors (SBRs) for Biological Nutrient Removal (BNR) from wastewater, special attention has to be given to the availability and use of the easily biodegradable substrate. If the available carbon in the raw wastewater is not enough to achieve complete nutrient removal, an additional suitable external carbon source must be required.

The choice of the substrate is important in terms of i) the economic cost for the carbon source and ii) the selective use of the carbon source by Polyphosphate Accumulating Organisms (PAOs). In this chapter we studied the usage of ethanol as the external carbon source for Enhanced Biological Phosphorus Removal (EBPR) from wastewater in adapted or unadapted activated sludge.

This chapter has been the basis of the following publications:

Puig S., Coma M., van Loosdrecht M.C.M., Colprim J. and Balaguer M.D. 2007b. Biological nutrient removal in a sequencing batch reactor using ethanol as the carbon source. *J. Chem. Technol. Biotechnol.* **82**(10), 898-904.

Puig S., Coma M., Monclús, H., van Loosdrecht M.C.M., Colprim J. and Balaguer M.D. 2007c. Selection between alcohols and volatile fatty acids as external carbon sources for EBPR. *Water Res.* (*in press*). doi:10.1016/j.watres.2007.07.050.

7.1 Introduction

In recent years Enhanced Biological Phosphorus Removal (EBPR) from wastewater has been successfully applied to achieve low phosphorus concentrations in effluents (Van Loosdrecht *et al.,* 1998; Tykesson *et al.,* 2005; Kishida *et al.,* 2006; Tsuneda *et al.,* 2006). In this process, special attention has to be given to the availability and the use of organic substrate because the phosphate release by Polyphosphate Accumulating Organisms (PAOs) and classical heterotrophic denitrification process compete both for the organic matter.

If the available carbon in the raw wastewater is not enough to achieve complete nutrient removal, an additional suitable external carbon source must be required. In this way, acetate has been successfully tested to improve the bio-P removal process performance (Issacs and Henze, 1995; Pastorelli *et al.,* 1999). Other short chain Volatile Fatty Acids (VFAs), such as propionate, butyrate, valerate, isovalerate and lactate have been used for EBPR purposes (Oehmen *et al.,* 2004; Pijuan *et al.,* 2004). However, the addition of VFAs considerably increases the overall treatment costs. Therefore, the choice of the substrate is critical in terms of i) the economic cost for the carbon source and ii) the selective use of the carbon source by PAOs against GAOs.

In general different substrates have been used for denitrification (i.e. methanol) and phosphate removal (i.e. acetate) purposes. However, Cho and Molof (2004) suggested that the use of acetic acid can cause nitrate to inhibit phosphorus removal since acetic acid reacts preferentially with nitrate over phosphorus. Methanol is most commonly used at various WasteWater Treatment Plants (WWTPs) for the enhancement of denitrification, however, has insignificant or negligible effects on biological phosphorus removal (Randall *et al.,* 1995).

In order to find other suitable substrates, Chapter 5 achieved high nutrient removal efficiencies using ethanol as the carbon source for Biological Nutrient Removal (BNR) in a lab-scale Sequencing Batch Reactor (SBR). Hallin *et al.* (1998) demonstrated that the adaptation of heterotrophic denitrifying bacteria to ethanol was rapid, and the denitrification capacities with several compounds, including primary alcohols and VFAs, were higher in adapted than in unadapted sludge. However, some drawbacks remain which must be considered, such as if ethanol is also used by PAOs, the storage polymer produced by PAOs using ethanol, the response of unacclimated activated sludge and a comparison between ethanol acclimated or unacclimated biomass. This comparison is important because certain studies of Chen *et al.* (2005) found that propionic acid enriched wastewater was less efficient for EBPR than acetic acid in short-term tests, but much more efficient in the long-term cultured experiments. According to Chen *et al.* (2002), this implies that population dynamics is so significant in EBPR that extrapolating batch experiments can lead to erroneous conclusions.

7.2 Objectives

The purpose of this work was to study the behaviour of ethanol as alternative to conventional VFAs (i.e. acetate and propionate) as an external carbon source for EBPR from wastewater.

A comparison between the different carbon sources (i.e. acetate, propionate, methanol and ethanol) was performed based on biological phosphorus removal activities and stoichiometry parameters in ethanol acclimated and unacclimated biomass.

7.3 Materials and methods

7.3.1 Experimental set-up

Two experimental set-ups were used during this study: 30L lab-SBR and the 5L batch reactor. The lab-SBR was composed of a cylindrical reactor with maximum and minimum working volumes of 30L and 20L, respectively. A full description of the SBR is presented in section 3.1.1. To achieve nutrient removal in the SBR, the Hydraulic Retention Time (HRT) and Solids Retention Time (SRT) were maintained at 1.3 day and 19.0 days on average, respectively. The SBR was seeded with sludge from the Sils-Vidreres wastewater treatment plant (Girona, Spain) and it treated synthetic wastewater for nutrient removal purposes (described in Chapter 6).

The batch reactor consisted of a cylindrical glass reactor with a maximum capacity of 5L and a cap at the top to permit fully closed conditions. Dissolved oxygen concentration was fixed at 30% oxygen saturation during aerobic periods (2.7 mg $O_2 \cdot L^{-1}$ at 20ºC). The system was thermostated at 20.0 ± 0.5ºC with a circulation pump or dry heating with a controlled cooling water valve. pH was controlled at 6.8±0.1 to avoid calcium phosphate precipitation (the raw wastewater calcium concentration was 47.9 mg $Ca \cdot L^{-1}$, on average). Moreover, Smolders *et al.* (1994) found that the anaerobic P-release rate depending on the pH value. An optimum pH of 6.8 ± 0.7 was recommended by Liu *et al.* (1996) for anaerobic acetate metabolism and biological phosphorus removal. Filipe *et al.* (2001a) suggested optimal pH value for the dominance of PAO over GAO which was around 7.25. Filipe *et al.* (2001b and 2001c) found that the phosphorus uptake rate for PAOs was essentially independent of the pH for the range from 6.5 to 8.0. A full description of the batch reactor is presented in section 3.1.3.

7.3.2 Experimental procedure

Activated sludge samples were collected from two plants:

- A 1000 L SBR pilot plant located *in situ* at the Quart WWTP (Catalonia, Spain). The SBR pilot plant treated urban sewage for nutrient removal purposes without any external carbon source addition. The sludge from this plant was labelled as unadapted sludge.

- The 30 L lab scale SBR was run with ethanol added to the synthetic wastewater for BNR (Puig *et al.,* 2007a). In this case, samples after 30 and 140 days of adaptation were obtained for the analysis of the ethanol performance as the external carbon source for EBPR.

A series of anaerobic-aerobic batch cycle experiments were performed in the batch reactor with unacclimated ethanol biomass from the SBR pilot plant (Tests 0 to 2), with 30-day ethanol acclimated sludge (Tests 3 to 8) from the lab scale SBR and with 140-day ethanol acclimated sludge (Tests 9 and 10) from the lab scale SBR.

The carbon sources tested in these experiments were: two VFAs (i.e. acetate and propionate) and two alcohols (i.e. methanol and ethanol). Each batch test lasted for 5.7 hours (3.5 h under anaerobic conditions plus 2.2 h under aerobic conditions). The batch reactor was filled with 4L of mixed liquor (previously washed twice with tap water to achieve endogenous conditions) taken from the SBR pilot plant (Tests 0 to 2) or from the lab-SBR (Tests 3 to 10). After the sludge was washed, it was then mixed with a synthetic feed solution to get the initial carbon, nitrogen and phosphorous concentrations presented in Table 7.1.

Table 7.1. Initial nutrient concentrations after feeding in the batch reactor.

	Carbon Source	Biomass	$[C]_o$ (mg C·L^{-1})	$[N]_o$ (mg N·L^{-1})	$[P\text{-}PO_4^{3-}]_o$ (mg P·L^{-1})
Test 0	Control	Unadapted urban sludge	1.0	20.1	19.7
Test 1	Acetate	Unadapted urban sludge	53.2	20.5	18.7
Test 2	Ethanol	Unadapted urban sludge	59.5	20.5	18.5
Test 3	Acetate	30-day ethanol adapted sludge	44.4	5.4	0.6
Test 4	Propionate	30-day ethanol adapted sludge	47.5	6.1	0.5
Test 5	Methanol	30-day ethanol adapted sludge	29.6	5.4	0.5
Test 6	Ethanol	30-day ethanol adapted sludge	27.8	5.1	0.8
Test 7	Ethanol	30-day ethanol adapted sludge	45.5	5.2	0.4
Test 8	Ethanol	30-day ethanol adapted sludge	64.2	5.3	8.5
Test 9	Ethanol	140-day ethanol adapted sludge	25.3	9.1	1.0
Test 10	Acetate	140-day ethanol adapted sludge	27.9	3.5	0.9

7.4 Results and discussion

7.4.1 Ethanol adaptation time effect on the bio-P biomass

The activity of PAOs when using acetate and ethanol was initially verified over non acclimated activated sludge from the urban SBR pilot plant. Afterwards, batch experiments were also conducted over 30- and 140 day ethanol acclimated sludge.

Figure 7.1 presents the specific phosphate profiles during anaerobic-aerobic conditions in an ethanol unadapted sludge from the SBR pilot plant using no organic substrate (control; Test 0), acetate (Test 1) and ethanol (Test 2) as the external carbon sources for EBPR. The initial carbon, ammonium and phosphate concentrations for each test are presented in Table 7.1.

The results showed that the PAO biomass from the SBR pilot plant, when acetate was dosed (Figure 7.1; Test 1), released up to 25.2 mg $P\text{-}PO_4^{3-}\cdot g^{-1}$ VSS at the end of the anaerobic phase. In the aerobic phase, phosphate was taken up by PAOs. The net phosphate removal or *P luxury* was 5.6 mg $P\text{-}PO_4^{3-}$ g^{-1} VSS when the experiment ended. On the other hand, when ethanol was dosed to the unadapted ethanol sludge (Figure 7.1; Test 2), the phosphate profile followed the same trend as for the control test reaching net phosphate release concentrations of 3.4 and 3.8 mg $P\text{-}PO_4^{3-}\cdot$ g^{-1} VSS; Test 0 and 2, respectively) with low phosphate variations in the release and uptake periods. The net phosphate removals were 2.8 and 2.4 mg $P\text{-}PO_4^{3-}\cdot$ g^{-1} VSS, respectively. Therefore, an unacclimated biomass was not able to perform a suitable EBPR when ethanol was dosed.

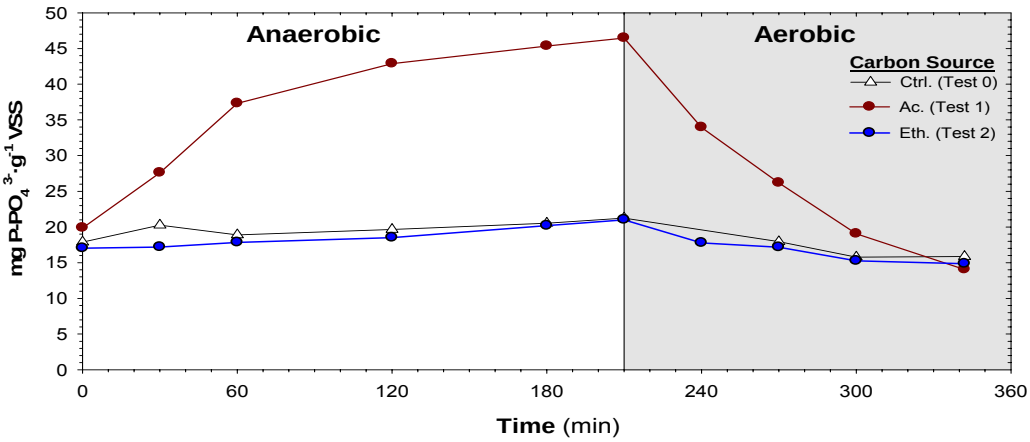

Figure 7.1. Specific phosphate release and uptake using no organic substrate (Test 0), acetate (Test 1) and ethanol (Test 2) as the carbon sources for EBPR purposes using unacclimated sludge.

In a second series of tests we used sludge which was acclimatized to ethanol during 30 days in the lab scale SBR for EBPR. Substrates tested were: acetate (Test 3), propionate (Test 4), methanol (Test 5) and ethanol (Test 6). Figure 7.2 presents the evolution of soluble phosphate, VFA, glycogen and PHA contents during the whole series of batch experiments. Moreover, in Figure 7.2 Tests 5 and 6, when methanol and ethanol are used as the carbon source, Total Organic Carbon (TOC) is also presented to verify the reduction of soluble organic carbon.

When acetate or propionate (Figure 7.2, Tests 3 and 4, respectively) were used as carbon sources for EBPR, phosphate was released during the anaerobic phase at levels of 17.0 and 18.2 mg $P\text{-}PO_4^{3-}\cdot L^{-1}$, respectively. Meanwhile, the VFAs were depleted, glycogen was partially consumed and PHA produced. Then, in the following aerobic phase the soluble phosphate was taken up by PAOs using the PHAs accumulated to restore the storage pools of poly-phosphate and glycogen. In the aerobic phase phosphate was quickly taken up by PAOs (4.4 and 4.8 mg $P\text{-}PO_4^{3-}\cdot g^{-1}$ VSS$\cdot h^{-1}$ for acetate and propionate, respectively). At the end of the tests, the effluent phosphorus concentrations obtained were lower than 0.2 mg $P\text{-}PO_4^{3-}\cdot L^{-1}$ for both cases.

Methanol (Figure 7.2; Test 5) as the most simple and economical alcohol on the market, was also tested for EBPR purposes in a 30-day ethanol acclimated sludge. In the anaerobic period, phosphate was poorly released (7.2 mg $P\text{-}PO_4^{3-}\cdot L^{-1}$) compared to acetate and propionate (Figure 7.2; Tests 3 and 4). The phosphate was taken up in the following aerobic phase; however, the TOC and glycogen concentration remained constant. Therefore, PAOs might not use methanol, and the slight P release observed could also be related to the use of their intracellular storage compounds. This fact and the low P uptake rate would confirm that methanol was not a suitable carbon source for EBPR in ethanol enriched sludge in short-term tests. Similarly, Louziero *et al.* (2002) suggested that methanol was not utilized as a carbon source for the EBPR process.

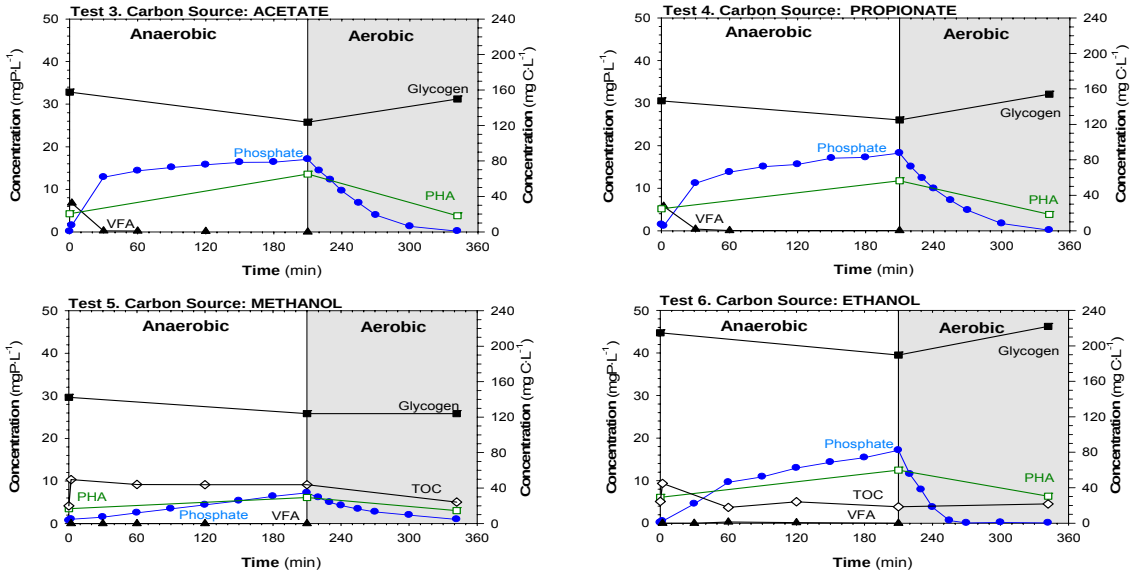

Figure 7.2. Batch experiment profiles with acetate (Test 3), propionate (Test 4), methanol (Test 5) and ethanol (Test 6) as the carbon sources for a 30-day ethanol acclimated biomass. Phosphate(●), VFAs(▲), TOC(◇), glycogen(■) and PHA(□) were measured during the cycle.

The last alcohol tested was ethanol (Figure 7.2; Test 6). During the anaerobic phase, phosphate was released until 17.1 mg $P\text{-}PO_4^{3-}\cdot L^{-1}$, consuming glycogen and accumulating PHAs. No VFAs were present in the reactor, even though the TOC profile showed that the only soluble biodegradable organic substrate (ethanol) was consumed from 44.9 to 18.6 mg $C\cdot L^{-1}$. Therefore, ethanol is used directly by PAOs during the first 60 minutes, and then no biodegradable organic matter remained inside the reactor. In the aerobic period the phosphate was taken up, restoring the glycogen pool and the PHA concentration decreased. At the end of the cycle, the effluent phosphate concentration was 0.1 mg $P\text{-}PO_4^{3-}\cdot L^{-1}$.

In the last series of tests we used sludge which was acclimated to ethanol during 140 days. Figure 7.3 presents the results of the anaerobic-aerobic batch experiments carried out using ethanol (Test 9) and acetate (Test 10; as a reference) as carbon sources for EBPR purposes. Their initial carbon, nitrogen and phosphorous concentrations are presented in Table 7.1.

Figure 7.3 shows that for the carbon sources used, the phosphate concentration at the end of each experiment was close to zero. The specific phosphate release amounts, shown in Figure 7.3, were highest

for the acetate test (8.7 mg $P\text{-}PO_4^{3-}\cdot g^{-1}$ VSS$\cdot h^{-1}$; Test 10) than for ethanol test (6.2 mg $P\text{-}PO_4^{3-}\cdot g^{-1}$ VSS$\cdot h^{-1}$; Test 9). The phosphate released was quickly taken up in the aerobic period.

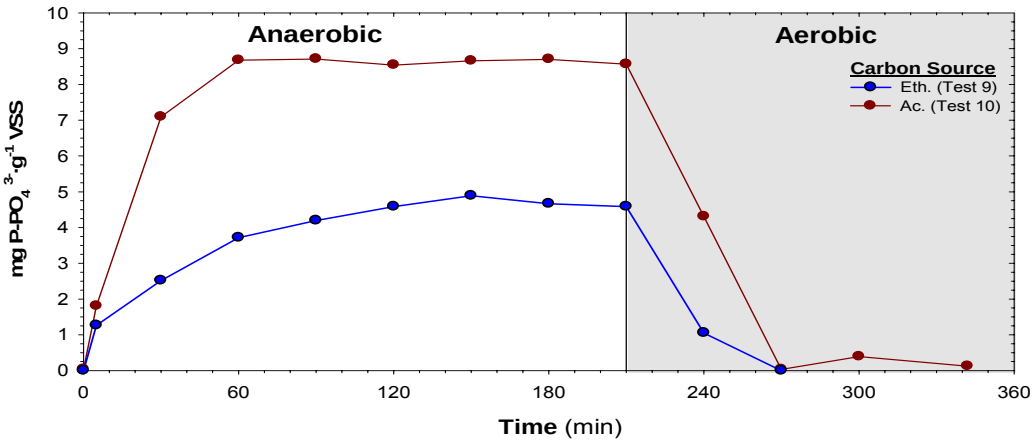

Figure 7.3. Specific phosphate release and uptake in batch tests using acetate and ethanol as carbon sources used by PAOs for biological phosphorus removal in a 140-day ethanol adapted sludge.

Table 7.2 compares the response of ethanol addition on ethanol unadapted activated sludge and in 30- and 140- day ethanol adapted activated sludge.

Table 7.2. Comparison between ethanol unadapted activated sludge and 30- and 140-day ethanol enriched sludge.

	Unadapted sludge	**30-day adapted sludge**	**140-day adapted sludge**	**Units**
Carbon source	Ethanol	Ethanol	Ethanol	-
References	Test 2	Test 6	Test 9	-
1st $P_{release}$ rate	1.5	1.8	6.2	*mg $P\text{-}PO_4^{3-}\cdot g^{-1}$ VSS$\cdot h^{-1}$*
2nd $P_{release}$ rate	-	0.6	0.7	*mg $P\text{-}PO_4^{3-}\cdot g^{-1}$ VSS$\cdot h^{-1}$*
P_{uptake} rate	-	1.5	7.0	*mg $P\text{-}PO_4^{3-}\cdot g^{-1}$ VSS$\cdot h^{-1}$*
$P_{rel.}/C_{upt.}$	0.1	0.2	0.4	*mmols P·mmols^{-1} C*
PAOs	9.4	11.5 ± 5.1	38.0	*%*
Total GAOs	3.1	1.2 ± 1.7	13.1	*%*

The results showed that the P release rate rose (Table 7.2), as the adaptation time by PAOs to ethanol increased (from 0 to 140 days). Moreover, the P release – carbon uptake ratio increased (from 0.1 to 0.4 mmols P·mmols^{-1} C) from the unadapted to adapted ethanol activated sludge. Although an unacclimated biomass was not able to perform a suitable EBPR, after a period of biomass adaptation (30-140 days), the population dynamics of the activated sludge evolved to an efficient phosphorus removal process as well as the PAOs population grew.

7.4.2 FISH analysis of the biomass

Periodic FISH analyses of the PAO and GAO communities were done to assess the population dynamics evolution during the experimental period. Figure 7.4 presents the FISH images of the initial sludge seed from an urban WWTP or unadapted sludge (A), evolved sludge after 30 days (B) and after 140 days (C) of SBR operation with ethanol synthetic wastewater. Figure 7.5 shows the FISH images of the 140-day evolved sludge and the contrast phase picture.

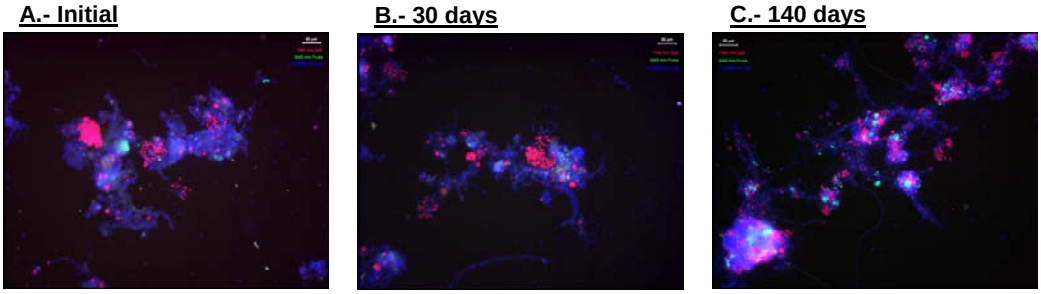

Figure 7.4. In situ hybridization of activated sludge samples with probes EUBmix (Cy5; in blue), PAOmix (Cy3; in red) and GAOmix (Fluos; in green) in initial sludge (A), evolved sludge after 30 days (B) and 140 days (C), respectively. The scale bar on panel (20 μm).

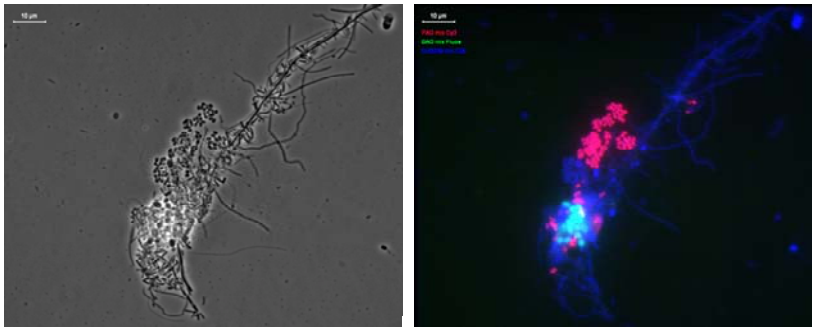

Figure 7.5. In situ hybridization of activated sludge samples with probes EUBmix (Cy5; in blue), PAOmix (Cy3; in red) and GAOmix (Fluos; in green) in evolved sludge after 140 days (right) and contrast phase picture (left). The scale bar on panel (10 μm).

FISH image analysis quantification was performed from the photographs obtained from Figure 7.4. The results obtained are summarized in Table 7.3.

Table 7.3. Summary of FISH quantification of the initial and evolved sludges.

	Initial	30-day	140-day	Units
PAOs	9.4	11.5 ± 5.1	38.5	%
Total GAOs	3.1	1.2 ± 1.7	13.1	%

FISH analysis showed that the seed biomass contained a low population of PAOs (9.4%), while the SBR biomass after five month of operation had grown and enriched with PAOs (38.5%) in spite of the sludge presenting a significant change in wastewater quality (from urban to synthetic wastewater). On the

other hand, the GAO population also increased from 3.1 to 13.1% (Table 7.3) during the experimental study.

Bio-P bacteria (PAOs) usually become dominant in the culture when organic substrate is introduced under anaerobic conditions (Satoh *et al.,* 1996; Seviour *et al.,* 2003). Furthermore, Kong *et al.,* (2005) suggested that the availability of proper organic substrates may be a key factor for selecting the different EBPR species. It is most likely that the low phosphorus concentration in the second and third anoxic/anaerobic periods of the SBR cycle eventually led to a competitive advantage for GAOs (Liu *et al.,* 1997). Moreover, Liu *et al.* (1997) suggested that the polyP accumulation by PAOs inside is prevented only due to the limitation of P in the feed. As a result, the amount of acetate taken up by PAOs under anaerobic conditions decreased. The growth of PAOs is suppressed, and growth of GAOs is promoted.

7.4.3 Carbon source effect on the specific phosphate release and uptake rates

Once it was demonstrated that ethanol and VFAs were used by PAOs for biological phosphorus removal purposes in a 30-day ethanol acclimated sludge, a study of the carbon source effect on the specific phosphate release and uptake rates at the similar concentrations of external carbon addition (Table 7.4) was carried out during anaerobic-aerobic experiments as presented in Figure 7.6.

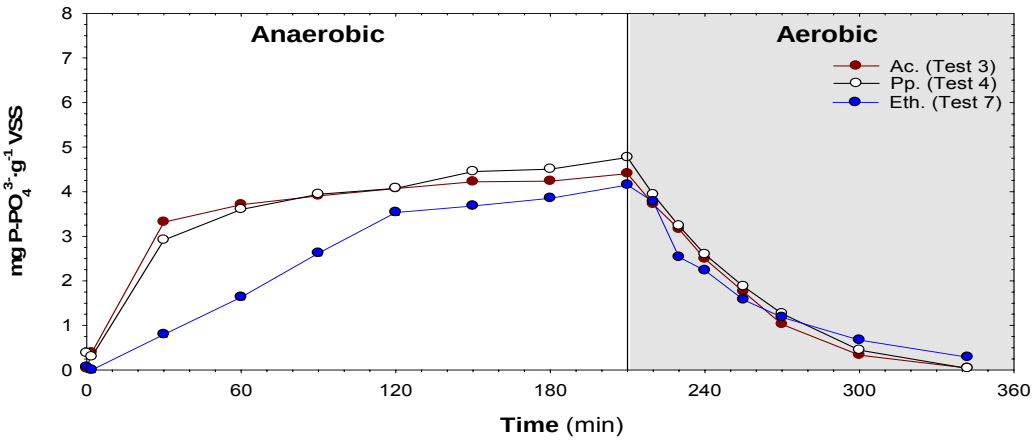

Figure 7.6. Specific phosphate release and uptake using different carbon sources for EBPR purposes at the same carbon concentration: acetate (44.37 mg C·L^{-1}; Test 3), propionate (47.54 mg C·L^{-1}; Test 4) and ethanol (45.46 mg C·L^{-1}; Test 7).

In the anaerobic period, for all the substrates used, phosphate was released dosing similar carbon concentration (between 44.4 – 47.5 mg C·L^{-1}; Table 7.4). Propionate (Test 4), acetate (Test 3) and ethanol (Test 7) tests released 4.8, 4.4 and 4.2 mg P-PO_4^{3-}·g^{-1} VSS, respectively. In each experiment, phosphate was released at two different rates. In the acetate and propionate tests (Figure 7.6), an initial high specific P release rates (1st P release) were seen when the organic substrates were taken up in the first 30 minutes. In the ethanol test (Test 7), the 1st P release took more time, around 120 minutes, with a P-release rate of 1.8 mg P-PO_4^{3-}·g^{-1} VSS·h^{-1} (Table 7.4). This rate was 3 times lower than the P-release rates for acetate (5.9 mg P-PO_4^{3-}·g^{-1} VSS·h^{-1}) and propionate (5.2 mg P-PO_4^{3-}·g^{-1} VSS·h^{-1}).

Randall *et al.* (1997) suggested that the carboxyl function group is biochemically significant in EBPR because PHA polymerization requires an activated carboxylic acid. The second P-release rate was likely due to maintenance energy needed (Smolders *et al.,* 1994). However, When the same test were performed using the 140 days ethanol adapted sludge, the 1st P release was 6.2 mg $P\text{-}PO_4^{3-}\cdot g^{-1}$ VSS$\cdot h^{-1}$ (Table 7.2).

On the other hand, in Table 7.4 the specific P uptake rates for all the carbon sources tested in Figure 7.6 were similar (from 4.4 - 4.7 mg $P\text{-}PO_4^{3-}\cdot g^{-1}$ VSS$\cdot h^{-1}$), suggesting that it was not dependent on carbon source but rather the PHA produced was the same.

Table 7.4. Summary of the P release and uptake rates using different carbon sources with the same carbon concentration.

	Carbon Source	$[C]_o$	1st $P_{release}$ rate	2nd $P_{release}$ rate	P_{uptake} rate
		mg C·L^{-1}	*mg P-$PO_4^{3-}\cdot g^{-1}$ VSS$\cdot h^{-1}$*		
Test 3	Acetate	44.4	5.9	0.4	4.4
Test 4	Propionate	47.5	5.2	0.6	4.7
Test 7	Ethanol	45.5	1.8	0.4	4.4

7.4.4 Ethanol dosing effect on the specific phosphate release and uptake rates

Following Smolders *et al.* (1994), the P release depends on the carbon amount added in the anaerobic phase in an EBPR system. In order to know if ethanol behaved similar, a study of the P release at different ethanol concentrations was carried out. Figure 7.7 presents the specific phosphate release and uptake in a 30-day initial ethanol adapted sludge using ethanol as the carbon source for EBPR purposes at three different carbon concentrations: 27.8 mg C·L^{-1} (Test 6), 45.5 mg C·L^{-1} (Test 7) and 64.2 mg C·L^{-1} (Test 8).

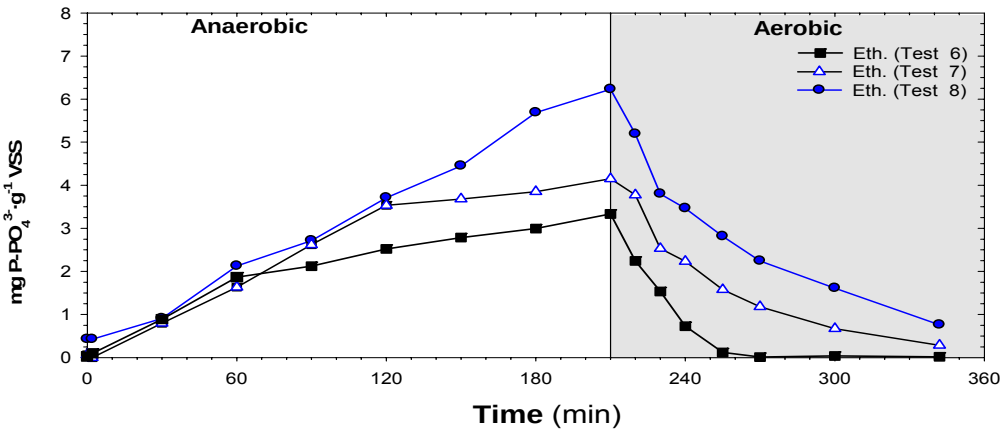

Figure 7.7. Specific phosphate release and uptake using ethanol as the carbon source for EBPR purposes at the different concentrations: ethanol (27.78 mg C·L^{-1}; Test 6), ethanol (45.46 mg C·L^{-1}; Test 7) and ethanol (64.17 mg C·L^{-1}; Test 8).

Figure 7.7 shows that when the ethanol dose was increased (from Test 6 to Test 8), so was the specific maximum P release increased (from 3.3 to 6.2 mg $P\text{-}PO_4^{3-}\cdot g^{-1}$ VSS). In Tests 6 and 7, two P released rates were observed (Figure 7.7). In Test 8, however, because of the higher carbon concentration added, the PAOs did not have enough anaerobic time to take up all the dosed ethanol. The initial P release rates kept constant for all the tests (between 1.8 and 1.7 mg $P\text{-}PO_4^{3-}\cdot g^{-1}$ VSS$\cdot h^{-1}$; Table 7.5).

Table 7.5. Summary of the P release and uptake rates using ethanol as the carbon source at different carbon concentrations.

	Carbon Source	$[C]_o$	1st $P_{release}$ rate	2nd $P_{release}$ rate	P_{uptake} rate
		mg C·L^{-1}	*mg P-PO_4^{3-}·g^{-1} VSS·h^{-1}*		
Test 6	Ethanol	27.8	1.8	0.6	1.5
Test 7	Ethanol	45.5	1.8	0.4	4.4
Test 8	Ethanol	64.2	1.7	-	5.9

7.4.5 Effect of the carbon source on the PHA produced by PAOs during the anaerobic phase in an EBPR system

The anaerobic-aerobic experiments performed showed that acetate, propionate and ethanol were used by PAOs for EBPR purposes in ethanol acclimated sludge. The PHA compounds produced during the anaerobic phase for each carbon source tested (acetate, propionate, methanol and ethanol) in an EBPR system are presented in Figure 7.8.

In the acetate tests, PAOs accumulated PHB (46.6%) and for the propionate tests PAOs accumulated PHV (50.7%) and PH2MV (47.2%). These results followed the literature references shown in Table 7.6. At the same carbon dose (Table 7.1**;** Test 3 and 4), PHA production was higher for acetate (65.0 mg C·L^{-1}) than propionate (59.8 mg C·L^{-1}).

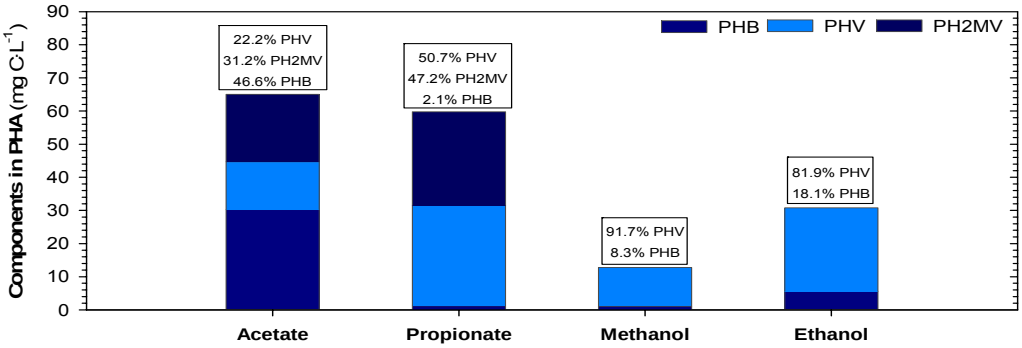

Figure 7.8. The content of accumulated PHA and the percentage of PHB, PHV and PH2MV at the end of the anaerobic period for each carbon source used.

When ethanol was added in the reactor for EBPR purposes, the storage polymer produced by PAOs in a 30-day ethanol acclimated sludge was mainly PHV (81.9%; 71.2 mg C·L^{-1}). Puig *et al.* (2007b) found

that PAOs also produced mainly PHV (73.5%) in 140-day ethanol acclimated biomass. More glycogen is needed than acetate or propionate to produce PHV because ethanol is a more reduced compound. These results are in accordance with Wu and Hickey (1996) that found that the ethanol degradation produces propionate, n-propanol and acetate in their studies about n-propanol production.

Table 7.6. Data from the literature concerning the PHA accumulated and form produced in EBPR systems using acetate, propionate, methanol, ethanol, DME, glucose, valeric acid and isovaleric acid as carbon sources.

Carbon Source	% PHB	% PHV	% PH2MV	% Others	Reference
Acetate	46.6	22.2	31.2	-	Test 3
Propionate	2.1	50.7	47.2	-	Test 4
Methanol	8.3	91.7	-	-	Test 5
Ethanol	18.1	81.9	-	-	Test 6
Ethanol + DME	26.5	73.5	-	-	Puig *et al.* (2007b)
Acetate	90.2	9.8	-	-	Smolders *et al.* (1994)
Propionate	2.0	45.0	53.0	-	Oehmen *et al.* (2005)
Propionate	4.0	46.5	49.0	0.5	Pijuan *et al.* (2004)
Valeric acid	0.0	95.0	-	5.0	Liu *et al.* (2002)
Isovaleric acid	85.0	15.0	-	-	Liu *et al.* (2002)

7.4.6 Characterising the anaerobic and aerobic stoichiometry of ethanol adapted sludge

Table 7.7 summarizes the P release and uptake rates with different carbon sources in ethanol adapted and unadapted sludge. In the 30-day adapted sludge, the higher phosphate release rate was observed when acetate was used as the carbon source, followed by propionate and ethanol. For these three carbon sources, two specific P release rates were observed in the anaerobic phase. The initial high specific P release rates (1st P release) were seen when the organic substrates were taken up (5.9, 5.2 and 1.8 mg P-PO_4^{3-}·g^{-1} VSS·h^{-1}, respectively). In acetate and propionate cases, it corresponds with the VFA depletion (the first 30 minutes; Figure 7.2). In the ethanol test, it corresponds with the organic substrate depletion after 60 minutes (Figure 7.2). The 2nd specific phosphate release rates (0.4 and 0.6 mg P-PO_4^{3-}·g^{-1} VSS·h^{-1}, respectively) could result from the enriched PAOs using their excess intracellular poly-P to generate energy which could be required for maintenance (Smolders *et al.,* 1994). In the methanol test, a low specific P release rate of 0.4 mg P-PO_4^{3-}·g^{-1} VSS·h^{-1} was observed. This rate was similar to the 2nd P release rates obtained using acetate, propionate or ethanol (Table 7.7).

On the other hand, the stoichiometry of an ethanol acclimated sludge for EBPR has not been described previously. Table 7.7 summarizes the biochemical transformations found experimentally in this study using different carbon sources for EBPR purposes and compared with the bibliography values.

High glycogen consumption - carbon uptake ratios ($Gly/C_{upt.}$; from 0.6 to 0.8 mmols C·mmols^{-1} C) and low phosphate release – carbon uptake ratios ($P_{rel.}/C_{upt.}$; from 0.1 to 0.2 mmols P-PO_4^{3-}·mmols^{-1} C) for all the carbon sources tested in the 30-days sludge were in contrast with the reference values (Table 7.7). Two possible hypotheses could explain this difference. The first one could be the GAOs abundance in the 30-days sludge. However, the FISH analysis performed resulted with low GAOs population (Table 7.7). Chen *et al.* (2005) suggested that less poly-P degradation is required when more glycogen is degraded, provided that glycogen is metabolized through the same pathway and ATP production in the tricarboxylic acid (TCA) cycle is not changed, which results in a low $P_{rel.}/C_{upt.}$ ratio. Moreover, Chen *et al.* (2005) demonstrated that with acetic and propionic acid wastewater the acclimated biomass utilised less glycogen and more phosphate was released than in the case of the unacclimated. In test 9 using 140-day ethanol adapted sludge reached a $P_{rel.}/C_{upt}$ of 0.4 mmols P-PO_4^{3-}·mmols^{-1} C in comparison with the 0.2 mmols P-PO_4^{3-}·mmols^{-1} C achieved with 30-day ethanol activated sludge (Test 6; Table 7.2). From the results obtained, it is proved the influence of the acclimatation time on the stoichiometry values when ethanol is used as external carbon source for EBPR.

In the aerobic phase, the parameter studied was the phosphate uptake-PHA oxidation ratio ($P_{upt.}/PHA_{oxid.}$). Low $P_{upt.}/PHA_{oxid}$ experimental ratios were found for all the carbon sources tested (from 0.1 to 0.2 mmols P-PO_4^{3-}·mmols^{-1} C). This low ratio was in accordance with the low $P_{rel.}/C_{upt}$ found in the anaerobic phase and that the PAOs restored the glycogen pool. Chen *et al.* (2005) suggested that the unacclimated biomass had a lower $P_{upt.}/PHA_{oxid}$ degradation ratio than the acclimated one. On the other hand, Randall and Liu (2002) found that the PHAs type was significant in determining the amount of $P_{upt.}/PHA_{oxid}$ because the PHB resulted in over twice the amount as PHV.

Finally, an economical comparison was done as the first insight to know the viability to change the VFAs addition for an ethanol solution. The prices are shown in Table 7.7. The cost of adding ethanol (2.7 € per gram of phosphorus removed) is 2.7 and 4.9 times cheaper than acetate and propionate sodium, respectively (PANREAC®, 2006).

Table 7.7. Comparison between P release and uptake rates with different carbon sources. Summary of the chemical transformations and FISH analysis of PAO and GAO populations.

	Experimental data						Literature references		
	Initial	30-day adapted sludge				140-day			
Carbon source	**Ethanol**	**Acetate**	**Propionate**	**Methanol**	**Ethanol**	**Ethanol**	**Acetate**	**Propionate**	**Units**
References	Test 2	Test 3	Test 4	Test 5	Test 6	Test 9	Smolders *et al.* (1994)	Oehmen *et al.* (2005)	
1st $P_{release}$ rate	1.5	5.9	5.2	0.0	1.8	6.2	-	-	*mg $P\text{-}PO_4^{3-}$·g^{-1} VSS·h^{-1}*
2nd $P_{release}$ rate		0.4	0.6	0.4	0.6	0.7	-	-	*mg $P\text{-}PO_4^{3-}$·g^{-1} VSS·h^{-1}*
P_{uptake} rate	-	4.4	4.7	0.7	1.5	7.0	-	-	*mg $P\text{-}PO_4^{3-}$·g^{-1} VSS·h^{-1}*
$P_{rel.}$ /$C_{upt.}$	-	0.1	0.2	-	0.2	0.4	0.5	0.4	*mmols P·mmols^{-1} C*
Gly /$C_{upt.}$	-	0.7	0.6	-	0.8	-	0.5	0.3	*mmols C·mmols^{-1} C*
PHB /$C_{upt.}$	-	0.7	0.0	-	0.2	0.3	1.3	0.0	*mmols C·mmols^{-1} C*
PHV /$C_{upt.}$	-	0.3	0.8	-	0.8	0.8	-	0.5	*mmols C·mmols^{-1} C*
PH2MV /$C_{upt.}$	-	0.4	0.7	-	-	-	-	0.6	*mmols C·mmols^{-1} C*
PHA /$C_{upt.}$	-	1.4	0.8	-	1.0	1.2	1.3	1.2	*mmols C·mmols^{-1} C*
$P_{upt.}$ /$PHA_{oxid.}$	-	0.1	0.2	0.2	0.2	-	0.3	-	*mmols P· mmols^{-1} C*
PAOs	9.4	11.5 ± 5.1	11.5 ± 5.1	11.5 ± 5.1	11.5 ± 5.1	38.5	-	63.0 ± 1.3	*%*
Total GAOs	3.1	1.2 ± 1.7	1.2 ± 1.7	1.2 ± 1.7	1.2 ± 1.7	13.1	-	< 1.0	*%*
Price	-	7.3	13.2	-	2.7	-	-	-	*€·g^{-1} $P_{REMOVED}$*

7.5 Conclusions

Ethanol has proved to be an useful external carbon source for EBPR. Nevertheless, a period of adaptation is required by PAOs when ethanol is used in unacclimated EBPR processes. After a period of biomass adaptation, the population dynamics of the activated sludge evolved to an efficient phosphorus removal process producing PHV as the main PHA and increasing the P release rate from 1.5 to 6.2 mg P-PO_4^{3-}·g^{-1} VSS·h^{-1}, as well as, phosphate uptake rate to 7.0 mg P-PO_4^{3-}·g^{-1} VSS·h^{-1}.

Therefore, if there is a need to support the PAO bacteria with energy over a longer time, a period of adaptation can be accepted and ethanol is suitable taking into account the carbon source addition cost. If EBPR needs to be incidentally supported by substrate addition, VFAs are preferred.

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Chapter 8. General conclusions and future perspectives

This chapter gives an overview of the main achievements of this work and points out the topics for future research derived from this thesis.

8.1 General conclusions

This thesis describes the application of the Sequencing Batch Reactor (SBR) technology for Biological Nutrient Removal (BNR) from the wastewater. In particular, the work presented evolves from the nitrogen removal to the biological nutrient removal (i.e. nitrogen plus phosphorous removal) with special attention to the operational strategy design, the identification of possible reactor cycle controls or the influent composition related to the process efficiency. In such sense, also the use of ethanol as an external carbon (when low influent Carbon:Phosphorus (C:P) or Carbon:Nitrogen (C:N) ratios are presented) are studied as an alternative to maintain the BNR efficiency. Thus, the conclusions of this work can be classified into the following sections:

SBR performance for biological nitrogen removal purposes

A 1000 Liters SBR pilot plant was operated to treat an average daily flow of 624$L \cdot d^{-1}$ of urban wastewater for carbon and nitrogen removal purposes. The SBR cycle was defined according an step-fed strategy with six anoxic filling events and with a sequence of anoxic-aerobic phases reaching, in spite of influent variability when treating real urban wastewater (daily mean flow), effluent ammonium and nitrate concentrations of 0.1 ± 0.4 mg $N\text{-}NH_4^+ \cdot L^{-1}$ and 1.5 ± 1.1 mg $N\text{-}NO_3^- \cdot L^{-1}$, respectively.

Control of the SBR cycle length for carbon and nitrogen removal

From the analysis and interpretation of the online profiles (i.e. pH, Dissolved Oxygen (DO), Oxidation-Reduction Potential (ORP)) and the online calculated Oxygen Uptake Rate (OUR), the characteristics points related to the end of nitrification and denitrification were identified. From this point, a control strategy for the adjustment of the aerobic and anoxic phases was successfully designed, developed, implemented and verified over real wastewater in the 1000 litres SBR pilot plant treating 600L per day for organic matter and nitrogen removal purposes.

The control system was based on the online calculation of the OUR during the aerobic phases and the analysis of the ORP values during the anoxic phases. In this way, it was possible to estimate the status of the biological processes and define them as the control keys for adjusting the length of the aerobic and/or anoxic phases of the SBR operational cycle in real-time. The control system also considered some security factors related to minimum and maximum aerobic phase duration to avoid misinterpretation of online profiles or persistent aerobic phases.

The SBR real-time control system worked treating 600 $L \cdot d^{-1}$ of urban wastewater for more than 4 months and reached effluent levels lower than legally required by the European Directive 91/ 217/CEE (i.e. 57 mg of $COD \cdot L^{-1}$ and 4.7 mg of $N \cdot L^{-1}$).

Moreover, this study demonstrates the importance of the aeration control strategy applied in a real-time control system. Clearer profiles were obtained which can be used for control strategies when on/off

DO control is changed to a control scheme based on fuzzy logic control (FLC). When the DO FLC was applied, continuous OUR profiles were obtained getting more available OUR values.

An improvement on the previously described real-time control system was made after improving the DO control. Thus, the objectives of the control system were different according to the aeration phase during the whole cycle. For the first aerobic phases, the real-time control system detect *ammonia valley* for ammonia removal purposes and this parameter is used for the adjustment (cut-off) of the aeration phase. For the last aerobic phase, prior to settling and discharge, the aim is the carbon and ammonia removal, therefore, a double checking condition (*ammonia valley* and OUR_v) was considered for the regulation of this last aeration phase.

SBR performance for biological nutrient removal purposes

When moving from carbon and nitrogen to nutrient removal (i.e BNR: carbon, nitrogen and phosphorous), special attention is required in the availability of the organic carbon for denitrification (under anoxic conditions) and phosphorous removal (anaerobic phases, P release). The results obtained (94% of nutrient removal efficiencies, on average) in the lab scale SBR proved that the sequence of anaerobic-anoxic-aerobic phases with multiple feeding events over one cycle is a promising strategy for removing nutrients from wastewater with a low organic matter ratio in the influent (C:N:P ratio of 100:12:1.8).

The role of organic matter in the raw wastewater on the BNR performance

Low influent C:N and C:P ratios influence on the BNR performance. In such sense the influent ratio between C:N:P was analysed. From experimental work with the 30 litres lab scale SBR with C:P ratios lower than 36 g $COD \cdot g^{-1}$ $P\text{-}PO_4^{3-}$, the organic matter available is not enough to perform nutrient removal. In such sense, when evolving under carbon source limitations, the Enhanced Biological Phosphorus Removal (EBPR) is affected. However, after a period with low available organic matter, when the influent C:P ratio increases from 36 to 67 g $COD \cdot g^{-1}$ $P\text{-}PO_4^{3-}$, the PAOs responded quickly (15 days) to recover the nutrient removal efficiencies.

In the case in which the organic carbon also is not enough for denitrification purposes (C:N ratio below 4 g $COD \cdot g^{-1}$ TKN), the nitrate accumulated in the SBR leading to effluent concentrations of 52.88 mg $N\text{-}NO_3^- \cdot L^{-1}$. The same behaviour was observed for the phosphorous, causing the breakdown of the system performance. Afterwards, when recovering from an influent C:N ratio of 4 to 9 g $COD \cdot g^{-1}$ TKN, the system was able to return to the normal BNR conditions. First of all, the denitrification was rapidly achieved and after 15 days the SBR was able to remove also the phosphorus (i.e BNR performance).

Ethanol as an alternative carbon source for biological nutrient removal

When the influent wastewater composition has not enough organic carbon for biological nutrient removal, the addition of external carbon source must be considered to enhance the BNR. We have studied the use of ethanol as a suitable carbon source for BNR and from the presented results we conclude that:

When treating ethanol synthetic wastewater (502 ± 175mg·L^{-1} COD, 61.5 ± 10.1mg·L^{-1} N-TKN and 9.1 ± 3.8mg·L^{-1} P-PO_4^{3-}) in a lab-SBR for BNR purposes, high nutrient removal efficiencies can be achieved (94%, 95% and 98% for carbon, nitrogen and phosphorus, respectively) proving that ethanol can be a useful external carbon source for BNR.

Nevertheless, a period of adaptation is required by PAOs when ethanol is used in unacclimated EBPR processes. After a period of biomass adaptation of 30 days, the population dynamics of the activated sludge evolved to an efficient phosphorus removal process.

FISH analysis showed that the seed biomass contained a low population of PAOs (9.4%), while the SBR biomass after five month of operation had grown and enriched with Polyphosphate Accumulating Organisms (PAOs) (38.5%) in spite of the sludge presenting a significant change in wastewater quality (from urban to synthetic wastewater).

When ethanol is used as a carbon source for EBPR purposes, poly-β-hydroxyvalerate (PHV) as the main Poly-β-HydroxyAlkaonate (PHA) is produced in the anaerobic phase.

The more time the ethanol adaptation increased from 0 to 140 days, the anaerobic P release rate for the ethanol test rose from 1.5 to 6.2 mg P-PO_4^{3-}·g^{-1} VSS·h^{-1}, as well as, phosphate uptake rate to 7.0 mg P-PO_4^{3-}·g^{-1} VSS·h^{-1}.

When using ethanol, the carbon concentration in the influent did not affect the P release rates in the anaerobic phase. For this, the initial specific P release rates kept constant between 1.8 and 1.7 mg P-PO_4^{3-}·g^{-1} VSS·h^{-1} in spite of the different initial carbon concentrations. However, the influent carbon concentration affected the maximum phosphate concentration released in the anaerobic phase.

High glycogen consumption - carbon uptake ratios (Gly/$C_{upt.}$; from 0.6 to 0.8 mmols C·$mmols^{-1}$ C) and low phosphate release – carbon uptake ratios ($P_{rel.}/C_{upt.}$; from 0.1 to 0.2 mmols P-PO_4^{3-}·$mmols^{-1}$ C) were obtained for all the carbon sources tested in the 30-days sludge. From the results obtained, it is proved the influence of the acclimatation time on the stoichiometry values when ethanol is used as carbon source for EBPR.

If there is a need to support the PAO bacteria with energy over a longer time, a period of adaptation can be accepted and ethanol is suitable taking into account the carbon source addition cost. If EBPR needs to be incidentally supported by substrate addition, VFAs are preferred.

8.2 Future perspectives

Control of the SBR cycle length for BNR purposes

SBR real-time control implemented was focussed on removing carbon and nitrogen from wastewater using conventional probes to adjust the cycle length. The next step, it will be the development of a real-time control system (RCTS) for BNR purposes. In this sense, analysing the typical cycle profiles obtained when the SBR operated for BNR purposes, some characteristic points related to the biological phosphorus removal process appeared in the conventional online probes profiles. These points could be seen when PAOs released all the phosphate in the anaerobic phase to get energy to take up the carbon source. The conductivity and the pH profiles levelled off and the ORP profile presented a slight increase in the anaerobic phase while the phosphate profile remained stable. Based on these results and further research an algorithm for BNR could be designed and implemented.

Ethanol as a suitable carbon source for BNR purposes

Further research is required in my point of view of two important topics:

1. Ethanol has been tested in unadapted and adapted activated sludge in lab scale SBR, now it should be proved in long-term operation. This fact, it will permit to know the real potential of this substrate versus the others substrates in an operational (removal efficiencies, sludge production…) and economical points of view.

2. Further research is still necessary to know the metabolic pathway used by PAOs. Is it the acetate or the propionic metabolic pathway? Or maybe a new one?

Curriculum Vitae

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Bachelor in **Chemical sciences** for the University of Girona on July 2002.

Master Thesis in **Environmental Sciences** for the University of Girona on July 2004.

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FELLOWSHIPS

Colaborating Fellowship with the Laboratory of Chemical and Environmental Engineering (LEQUIA), Faculty of Chemistry, University of Girona (October 2002 - December 2003).

Predoctoral Fellowship of the University of Girona within the BR program (BR03/11) with the Laboratory of Chemical and Environmental Engineering (LEQUIA) (January 2004 – December 2007).

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Data evaluation of full-scale wastewater treatment plants by mass balance. Department of Biotechnology. Environmental Biotechnology (EBT) group. Delft University of Technology. Delft (Netherlands). 18 to 21 April 2006.

RESEARCH PROJECTS and R+D CONTRACTS WITH ENTERPRISES

The main research projects, that I have participated, were:

Development of a control and supervision system and its implementation to a Sequencing Batch Reactor (SBR) for organic matter, nitrogen and phosphorus removal.

Financing and duration: Spanish Ministry of Education and Science. DPI2002-04579-C02-02 (2002-2005).

Description: The project focuses on studying the operational conditions for nutrient removal from wastewater with a biological treatment using the SBR technology. The knowledge acquired permits to develop and implement a control system to adjust the individual steps of the treatment cycle adapting them to the influent characteristics, using on line data from pH, ORP, DO and temperature sensors.

Development of an intelligent control system for a Sequencing Batch Reactor (SBR) for organic matter, nitrogen and phosphorus removal.

Financing and duration: Spanish Ministry of Education and Science. DPI2005-08922-C02-01 (2006-2009).

Description: This project is structured in two big parts. Firsts, there is the acquisition of a basic knowledge about the variations, which occur in the process caused by microorganism activity during the different operation conditions. On the other hand, starting from historical data about the performance of an SBR designed for nutrient removal, an intelligent control system will be developed. This intelligent control system is based on statistic control and a case based system, which allows the identification of the process situation and to propose solutions in case of malfunctioning.

Closing the nitrogen cycle from urban landfill leachate by biological nitrogen removal over nitrite and thermal treatment.

Financing and duration: UE Program LIFE-Environment. LIFE 03 ENV/E/000140 (2004-2006).

Description: Ammonium removal from landfill leachate by biological treatment over partial nitrite conversion (SHARON) and autotrophic denitrification to nitrogen (Anammox). This is an adequate treatment to remove high loads of nitrogen in waste streams with low organic content. The goal was to obtain a new technology for nitrogen removal in effluents with high ammonium loads, cheaper that the present treatment technologies.

Partial nitrification and anaerobic oxidation of ammonium present in the landfill leachate using the PANI-SBR and ANAMMOX process (PANAMMOX).

Financing and duration: Spanish Ministry of Education and Science. CIT-310200-2007-90 (2007-2008).

Description: This is a project of knowledge transfer (PROFIT) to the CESPA enterprise to apply the combination of the partial nitrification of ammonium (PANI-SBR) and autotrophic denitrification (ANAMMOX) for nitrogen removal purposes treating landfill leachate.

Consolidated groups: Laboratory of Chemical and Environmental Engineering (LEQUIA).

Financing and duration: Catalan Government. AGAUR (Comission for Universities and Research). 2005SGR 00406 (2005-2008).

Description: Grants for consolidated research groups.