Universitat Autònoma de Barcelona
Escola Tècnica Superior d'Enginyeria
Departament d'Enginyeria Química

MODELLING BIOLOGICAL ORGANIC MATTER AND NUTRIENT REMOVAL PROCESSES FROM WASTEWATER USING RESPIROMETRIC AND TITRIMETRIC TECHNIQUES

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CERTIFIQUEM:

Que l'enginyer químic Albert Guisasola i Canudas ha realitzat sota la nostra direcció, el treball que amb títol "Modelling biological organic matter and nutrient removal processes from wastewater using respirometric and titrimetric techniques", es presenta en aquesta memòria, i que constitueix la seva Tesi per optar al Grau de Doctor per la Universitat Autònoma de Barcelona.

I perquè en prengueu coneixement i consti als efectes oportuns, presentem a l'Escola Tècnica Superior d'Enginyeria de la Universitat Autònoma de Barcelona l'esmentada Tesi, signant el present certificat a

Bellaterra, juny 2005

Dr. Juan Antonio Baeza Labat   Dr. Julián Carrera Muyo

"I was just guessing at numbers and figures
Pulling the puzzles apart.
Questions of science, science and progress
Don't speak as loud as my heart.
Tell me you love me,
and come back and haunt me,
Oh, when I rush to the start
Running in circles, chasing tails
coming back as we are"

The scientist. COLDPLAY.

Agraïments / Acknowledgments

Sens dubte, aquesta és una de les pàgines més importants de la tesi. No ho és pel fet que sigui la més llegida, sinó perquè un cop he enllestit la tesi, sóc més conscient que mai que aquest treball no s'hagués pogut dur a terme sense l'ajuda de molta gent. I és per això que en aquests moments tinc una gran necessitat d'agrair-los-ho. Com que una imatge val més que unes quantes paraules, la millor manera de descriure el que sento és mitjançant aquest castell on aquesta és la <u>tesi</u> i jo sóc l'anxaneta.

En un castell s'aplaudeix quan l'anxaneta fa l'aleta (és a dir, la defensa la tesi) però per arribar tan amunt cal molta gent acotxadors, primers, dosos, ... fins a arribar a la pinya i al folre.

Voldria mencionar en primer lloc als meus dos acotxadors, en Juan i en Julián, per l'esforç, la il·lusió i la paciència que han tingut amb mi. M'han donat la llibertat necessària per a desenvolupar la meva feina i ha aportat una gran quantitat d'idees a la meva recerca. Heu estat un gran estímul i, per a mi, sou més que els meus directors de tesi.

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I would sincerely like to express my gratitude to Peter Vanrolleghem who had the "courage" to accept me in BIOMATH for almost half a year. You've been an inestimable influence in my work. Dank u wel. In this sense, I am very proud of the fruitful collaboration I had with Gürkan Sin, from who I really learnt a lot. Hocam, tesekkür ederim.

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Finalment, no em vull oblidar de tota la meva gent de Mataró i comarca, familiars i amics, que han estat un complement ideal en aquesta breu però intensa recerca.

Mataró, 27 de juny del 2005

STATEMENT INDICATING THE PARTS OF THE THESIS THAT ARE NOT MY SOLE ORIGINAL WORK

This thesis contains some important contributions that have been made by those with whom I have worked throughout the course of this PhD. These are acknowledged as follows:

- Maite Pijuan of the Department of Chemical Engineering of the Universitat Autònoma de Barcelona complemented part of the work presented in Chapter VII. Her main contribution was the experimental performance of most of the experiments presented in this chapter. The interpretation and discussion of the results of Chapters VII.A and VII.C was a joint effort with equal contribution. This collaboration resulted in two publications and we are first author in one of them.

- Gürkan Sin of the BIOMATH group of Ghent University complemented part of the work presented in Chapter V. We contributed equally in the biological COD removal model development, the interpretation and discussion of the results from Chapters V.A, V.B and V.C and the experimental design and performance of the experiments developed in the hybrid respirometer in Belgium. This collaboration resulted in two publications and we are first author in one of them.

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CHAPTER I

Motivations and research questions

I.1 Water and environment

The natural environment of our planet, and all the biodiversity it contains, are threatened by both water withdrawals and water pollution. The human water requirements must be fulfilled without misusing the natural resources of our planet. Hence, any utilisation of these water resources must be analysed considering the implicit value of the environment, rather than its immediate economic value (UNESCO, 2005). In addition to this, our society must achieve that unpolluted water becomes accessible to every habitant of this planet. In 1992, 20 per cent of the world's population did not have a safe supply of water, and about 50 per cent of the population lacked adequate sanitation. A recent United Nations report states that more than 5 million people die annually from diseases caused by unsafe drinking water, and lack of sanitation and water for hygiene. According to the World Health Organisation, billions of people are at risk due to water-borne diseases.

In the past, human water rejection was naturally treated with the intrinsic capacity of our environment. The natural biological treatment and the dilution effect of rivers and seas was more than enough to avoid water pollution. However, the water demand increased extremely when compared to the population growth due to the growing urbanisation and to the industrial and agricultural systems. This increase in water demand causes that the amount of water demand and the amount of water resources may intersect in this century (UNESCO, 2005), unless this tendency is not changed by human actuation. The restoration of water to the environment after being used should not put at risk its posterior utilisation. This was the idea of the first artificial wastewater treatment systems developed in the 20th century. The increasing amount of wastewater treated and the stringent effluent criteria required cause a continuous research on wastewater treatment processes. These processes have to be the most efficient the better as well as cheap and low-energy consuming.

The activated sludge process represents nowadays the most widespread technology for wastewater purification. Activated sludge plants can be found in different climate conditions - from the tropics to the Polar Regions, from sea level (wastewater treatment plants in ships) to extreme elevations (mountainous hotels). The scale of activated sludge plants ranges from package plants for one family to huge plants serving big metropolises. Wastewater treatment plants (WWTPs) equipped with the activated sludge process are able to fulfil the most stringent effluent criteria. The invention of the activated sludge process is connected with the efforts of British and American engineers at the start of the 20th to intensify biological purification in fixed-film systems. The experiments with wastewater aeration did not provide the expected results until May, 1914 when Ardern and Lockett introduced a recycle of suspension formed during the aeration period. The suspension, known as activated sludge was in fact an active biomass responsible for the improvement of treatment efficiency and process intensity.

In general, the activated sludge process is a continuous or semicontinuous (fill and draw) aerobic method for biological wastewater treatment, including organic matter oxidation and nitrification. This process is based on the aeration of wastewater with flocculating biological growth, followed by separation of treated wastewater from this growth. Part of this growth is then wasted, and the remainder is returned to the system. Usually, the separation of the growth from the treated wastewater is performed by settling (gravity separation) but it may also be done by flotation and other methods. The addition of non-aerobic parts to the system has improved and extended the applicability of these systems. An anoxic zone enables denitrification, and an anaerobic zone enables biological phosphorus removal. In any case, the reader is referred to general books such as Metcalf and Eddy (1991), Randall *et al.* (1992) or Olsson and Newell (1999) for a detailed description of the fundamentals of the activated sludge processes and their implementation in real wastewater treatment plants.

I.2 Framework of the thesis

I.2.1 BACKGROUND ON THE RESEARCH GROUP

Nowadays, the Spanish PhD programme is divided in two different stages. The first stage (approximately the first two years) is proposed so that the student shows its aptitudes to be a potential PhD student. This process involves the qualification of some theoretical courses and the defence of an experimental research work. If the student passes, he/she receives a Master of Sciences (MSc) degree and he/she starts the PhD research. The PhD topic is generally chosen at the start of the whole procedure. Hence, the MSc research is often the starting point of the PhD research, as occurs in this thesis.

This thesis is framed in the research line of the Environmental Engineering Group of Universitat Autònoma de Barcelona (EEG-UAB). This group was born in the 1990s with the aim to conduct research on improving the current biological wastewater treatment systems. Three theses were defended in this group around year 2000 which are the antecedent of this thesis. Firstly, Juan Baeza developed a supervisor control based system to improve the operation of a biological nutrient removal pilot plant (Baeza, 1999). Secondly, David Gabriel examined and modified the Activated Sludge Model n°2d (ASM2d) to monitor the activated sludge process on a pilot plant (Gabriel, 2000). Finally, Julian Carrera investigated the biological treatment of high N-strength wastewaters (Carrera, 2001).

These three theses were conducted in large continuous pilot plants (around 100 L). At this scale, several operating conditions (i.e., plant configuration, control strategies, feeding pattern) can be successfully studied and the evolution of a certain biomass fraction or population can be monitored. Nevertheless, the next logical step was to study the activated sludge process in lab-scale reactors with short-term batch experiments. At this scale, the behaviour of the biomass can be easily monitored and sampled since it is easier to maintain a high controlled and stable environment. Batch experiments with low loaded pulses are a different approach to gain knowledge about the process.

I.2.2 INTRODUCTION TO THE TOPIC: THE MSc DEGREE

The research from the M.Sc. degree started on February 2001 and it aimed to provide a new approach to the activated sludge processes using respirometric short-term batch experiments in a lab-scale reactor (1 L). Respirometry was chosen since it is a technique which provides a lot of information about the biological nutrient removal processes with simple common equipment. "Common equipment" stands for equipment which is frequently used in activated sludge systems such as Dissolved Oxygen (DO) and pH probes or aeration equipment.

The nutrient removal capacity and the biomass growth are two of the most important parameters when monitoring the activated sludge process. Both items are directly linked to oxygen consumption. Hence the oxygen consumption rate of the biomass is an indirect indicator of both the substrate removal and the growth processes. Nevertheless, the direct measurement of the biomass respiration is not possible and the oxygen concentration can only be measured in the gas or the liquid phase. Oxygen balances are required to quantify the amount of oxygen transferred from the gas phase to the liquid phase and the part of this oxygen biologically consumed.

Figure I.1 depicts the basis of the respirometry indicating the link of the available measurements to the desired ones.
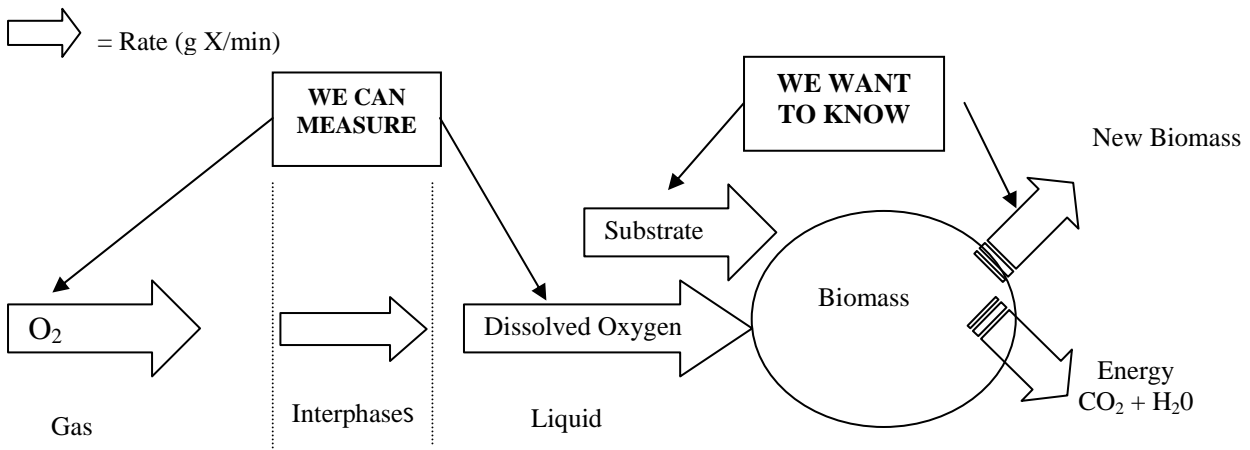
Figure I.1 General diagram about respirometry (adapted from Spanjers *et al.*, 1997)

Respirometric techniques are well-known and used for the monitoring of any aerobic process, which are the most common processes in a typical WWTP (heterotrophic growth and nitrification). Spanjers *et al.* (1997) summarised the applications of respirometry in many fields from model and monitoring to control. The notion of a respirometer involves different equipments with a common characteristic: aerated biological reactors aiming to measure the Oxygen Uptake Rate (OUR) of a certain biomass. The actual classification of respirometers according to the Respirometry group task of the International Water Association (IWA) is depicted on Figure I.2. This classification is based on three characteristics of the respirometer.
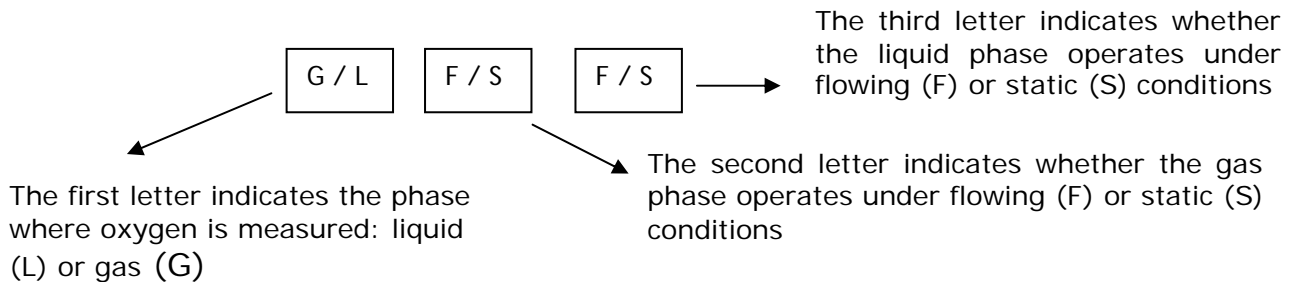
Figure I.2 Classification of respirometers

The EEG-UAB group had some experience in OUR measurement in pilot plants: Baeza *et al.* (2002b) developed a control strategy with OUR as the output variable to optimise a nitrification pilot plant operation. OUR was measured using LSS respirometry, i.e. the aeration was stopped and the OUR value was calculated as the slope of the DO drop. This methodology is still successfully utilised in the pilot plant in view of control and monitoring purposes (Jubany *et al.*, 2004). This technique was also utilised with batch experiments to compare OUR values at different conditions (Torrijos *et al.,* 2004 or Carrera *et al.*, 2002b).

LSS respirometry was the first attempt to measure OUR in lab-scale batch experiments. Figure I.3 is an example of one of these first experiments with ammonium as substrate. This methodology was found not to be suitable to achieve our goals (i.e., the monitoring of the aerobic consumption of a low loaded pulse). The main disadvantage of LSS respirometry is the low measuring frequency. Then, processes occurring in a short time cannot be measured (e.g. the start-up process described below) and the parameter estimation values from experimental OUR data may not be reliable enough. In addition, it had some technical problems since the non-aerated periods required the reactor to be hermetic for an accurate OUR calculation and the aerated periods needed an open-air reactor.
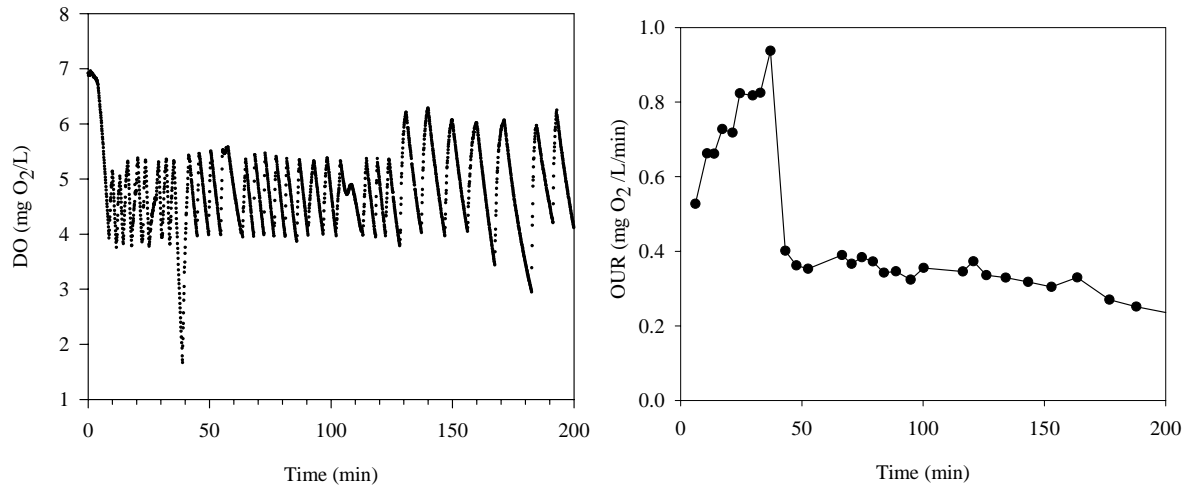
Figure I.3 Example of one of the first respirometric experiments

An alternative OUR measurement technique was searched in the literature and the LFS respirometry developed in Ghent University (Vanrolleghem *et al.*, 1994) was found. This technique was observed to be suitable to meet our goals and a LFS respirometer was built. This equipment is detailed in the equipment section (Chapter III.1.1). The main advantage of the LFS respirometer is that a continuous OUR profile can be obtained (OUR frequency equals DO frequency). Moreover, its implementation is simple since it only requires an accurate mass flow meter and a fast DO probe. The main disadvantage of this setup is that the k_La_{O2} of the system is required to measure OUR (Vanrolleghem *et al.,* 1998) and there is not an agreed and simple method for its estimation. The most common methodology is based on a perturbation on the aeration of the system (i.e. turning the aeration off for a period and then reaerate the system).

The main objective of the MSc degree was to develop and validate a LFS respirometer. In addition to the physical build-up, a new software for OUR calculation was required, which was programmed in MATLAB and Visual Basic. Several experiments were conducted with different biodegradable substrates with different loads. Figure I.4 shows an example of a set of experiments with different ammonium loads.
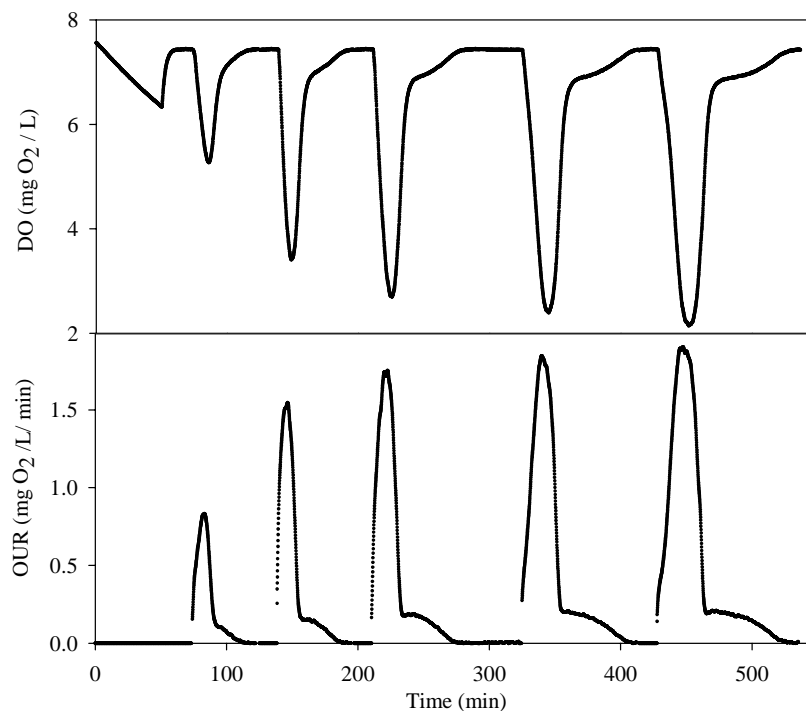
Figure I.4 Respirometric profiles for different loaded $N-NH_4^+$ pulses (5 to 12.5 mg $N-NH_4^+$/L)

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The modelling of the experimental results was also examined in this research. Nowadays, (i.e. three years after), one realises that this was a rough approach to the modelling since some important issues such as parameter identifiability were not specifically considered. Activated Sludge Model n°1 (ASM1) was the model chosen to describe the experimental profiles. The major achievement of this modelling investigation was the consideration of the start-up process. This process describes the fast transient period in reaching the maximum OUR observed in respirometric batch profiles. A high frequency OUR measurement is needed to observe this phenomenon. Figure I.5 compares the theoretical behaviour (LEFT) with an example of an OUR profile obtained where the start-up phase can be clearly seen (RIGHT).
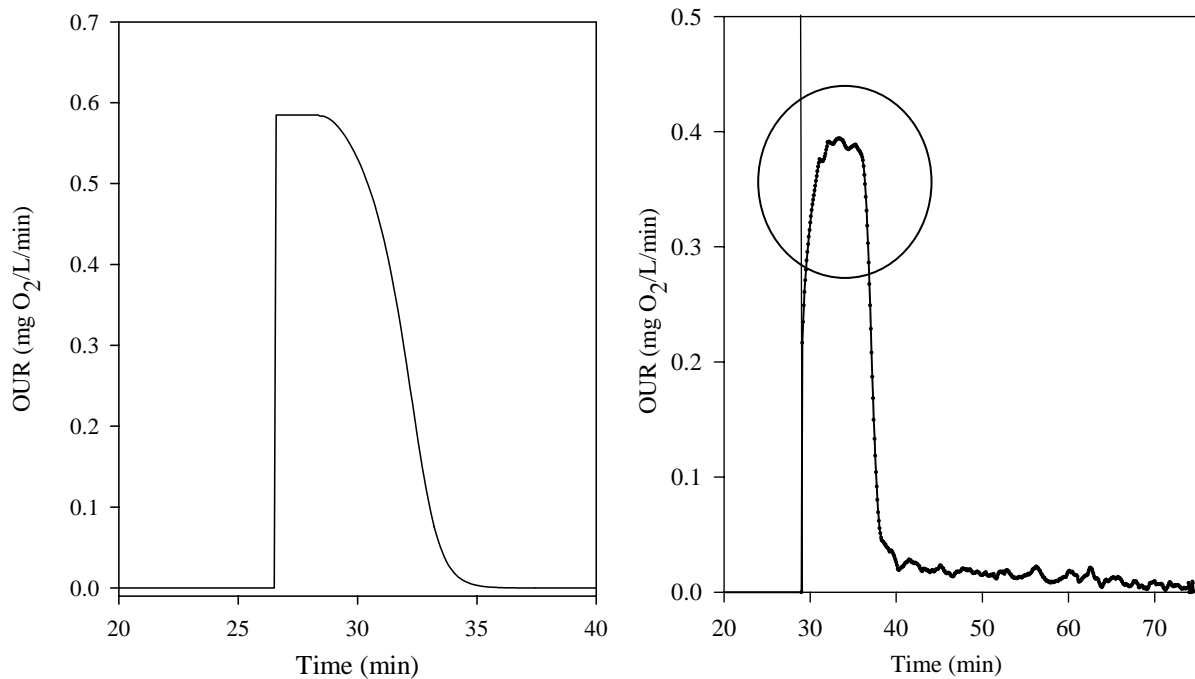
Figure I.5 Simulated OUR (LEFT) profile versus experimental OUR profile (RIGHT).

The start-up process was simply modelled as a first order delay. This certainly resulted to be a very accurate approach since a deeper study of this process conducted in parallel by Vanrolleghem *et al.*, (1998b, 2004) also used this mathematical approach. Vanrolleghem *et al.*, (2004) demonstrated that a detailed analysis of the experimental set-up dynamics (i.e. substrate diffusion, DO probe time response…) could partially explain this phenomenon, yet could not describe it completely. Hence, they stated that the start-up phenomenon is more likely due to the substrate metabolism. In addition, they demonstrated that if this period is not taken into account, the error is propagated (and amplified) into the estimates of the kinetic parameters of the biomass.

Finally, a toxicity study using respirometry was conducted for a particular wastewater. A chemical company observed a nitrification failure in their WWTP due to the presence of one compound: *p*-phenylenediamine. This compound was biodegradable for the acclimated heterotrophic biomass of their WWTP although it was toxic for nitrifiers. Hence, a typical toxicity test comparing the OUR profile of a reference compound (ammonium or acetate) and the OUR profile of the reference compound plus the toxic could not be used: the oxygen consumption due to the toxic may mask or hide the oxygen consumption due to the reference compound.

A new procedure was developed using four different profiles. This procedure aimed to distinguish between the oxygen consumption of the possible toxic and the oxygen consumption due to the reference compound. This procedure is published as Guisasola *et al.*, (2003).

This last section I.2 has summarised the main content of the MSc degree research defended on February 2003. This work is not explicitly included in this thesis, though it was essential for the thesis development.

I.3 Research questions

After finishing my MSc degree, I stayed for five months with the BIOMATH group of the Ghent University (spring-summer of 2003). This is one of the leader groups in the field on activated sludge processes modelling and they had a lot of experience on titrimetric techniques. In this staying, we collaborated in developing a new model for a correct description of the biological Chemical Oxygen Demand (COD) removal process and I could gain knowledge of titrimetry and mathematical tools for modelling processes. This knowledge together with the previous experience in respirometry was the starting point of this thesis.

The main objective of the thesis was to examine the existing models for the biological nutrient removal processes and, if necessary, extend them for a correct description of the experimental observations. These models are thought to be calibrated using short-term batch experiments with low-loaded pulses. It was emphasized that calibration was performed using common on-line measurements as OUR. However, OUR as the sole measurement does not contain enough information for a reliable calibration of such a complex models. A new technique which was simpler as OUR and provided complementary information about the process was required: titrimetry. This technique is based on pH as the measured variable. Hence, the models proposed in this thesis were extended to be calibrated with OUR and H^+ Production Rate (HPR) as output variables.

Each of the models is based on an exhaustive review of the previous models proposed in the literature. Each modification, improvement or extension is discussed in detail and justified in the corresponding chapter. Next, the main research questions in each of the proposed topics are shown together with a short review/introduction of each case.

Nevertheless, why would one focus his/her thesis on modelling?

In order to model a process from a mechanistic way, a good knowledge of the process is required. Hence, the effort dedicated for the model development is also dedicated to understanding the process. The main reason why this thesis is focused in modelling is that modelling is a good approach to get a deeper insight of the process. In addition, several applications of the model can be found once the model is developed. Olsson and Newell (1999) summarised them:

- Research and design: explore the consequences of new knowledge, to test new control strategies on the process, explore the influence of different input disturbances and parameter changes in order to meet the required goals (i.e. plant upgrading and retrofitting). In summary, models are used to predict the system behaviour under different circumstances.

- Operation and control: Bring the process to the desired performance with the use of on-line instrumentation and laboratory measurements to develop new control actions.

- Diagnosis: to interpret patterns in plant data, detect abnormalities and suggest causes.

I.3.1 TITRIMETRIC TECHNIQUES

Titrimetric measurements are very useful for the monitoring of biological processes since they provide high quality information about the process with simple equipment (a pH control loop). The basis of the titrimetry is that the proton production (or consumption) rate can be indirectly measured with the amount of base (or acid) dosage necessary to maintain the pH at a certain setpoint value.

For example, nitrification is a proton producing process. Hence, the system is acidified whereas ammonium is oxidised. Theoretically, the amount of base dosage necessary to maintain the pH at a certain setpoint should equal to the amount of protons produced in the process. The base dosage rate is an indirect measurement of the proton-producing rate, i.e. the nitrification rate. However, in practice this is not that easy since the existing chemical equilibriums of the rest of the species of the medium have to be considered. In particular, the acid carbonic equilibrium is very influencing: carbon dioxide is continuously stripped from the medium because of the aeration and this process has a noticeable effect on the pH. Hence, this effect has to be correctly accounted to be distinguished from the biological processes. Figure I.6 shows an example of a nitrification experiment, where the amount of acid and base added can be observed.
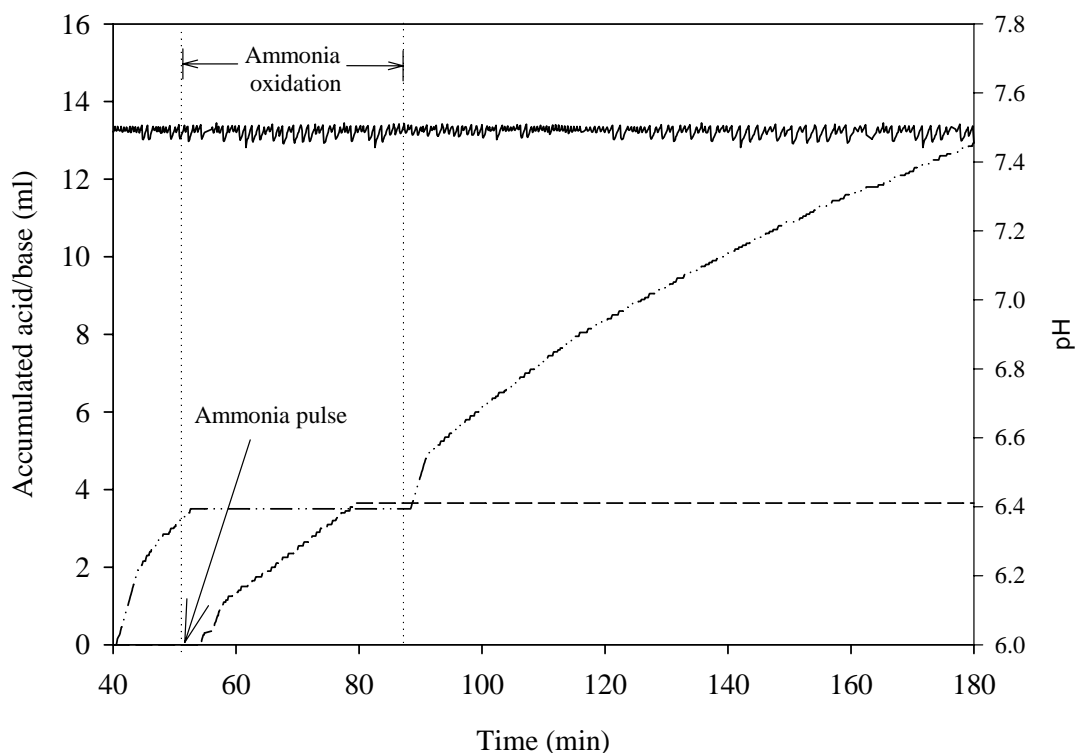
Figure I.6 Titrimetric measurements. Accumulated acid (dash-dotted), base (dashed) and pH (solid).

As can be observed, acid dosage was necessary to maintain the pH constant at 7.5 when there was no ammonia in the medium. This is because carbon dioxide was stripped from the medium (the carbon dioxide stripping process is deeply examined in the thesis). On the other hand, base dosage is required whereas ammonia is oxidised since protons are produced. The base dosage rate is an indirect indicator of the ammonium oxidation rate. Hence, titrimetry techniques can be used to monitor any process which has an effect on pH. For instance, the substrate uptake process basifies the medium in the biological COD removal. Both the COD removal and nitrification models are extended to be calibrated with titrimetric measurements. This implies the modelling of other processes such as the carbon dioxide stripping and the chemical equilibriums of the carbonic acid.

Proton production (HP) can be calculated from the acid/base dosage profiles according to equation I.1.

$$HP= \frac{V_{BASE} \cdot C_{BASE} - V_{ACID} \cdot C_{ACID}}{V_R} \quad [mol\ eq/L] \qquad (I.1)$$

where V_{ACID}/V_{BASE} = Accumulated volume of acid/base (ml)
C_{ACID}/C_{BASE} = Acid/base concentration (mol/ml)
V_R = reactor volume (L)

As can be deduced, for a proton consuming process (i.e. CO_2 stripping) HP will be negative and acid dosage would be required and for a proton-producing process as nitritation HP will be positive. HPR is calculated as the first derivative of HP in time and provides interesting information about the process rates.

Titrimetric measurements have already been used to monitor biological aerobic and anoxic carbon removal processes (Gernaey *et al.*, 2002a,b; Pratt *et al.*, 2004; Sin, 2004). Successful applications of titrimetric techniques for nitrification monitoring are Ramadori *et al.*, (1980); Massone *et al.*, (1996), Gernaey *et al.*, (1997); Gernaey *et al.*, (1998); Ficcara *et al.*, (2003) and Gapes *et al.*, (2003) among others.

I.3.2 PARAMETER ESTIMATION ERROR METHODOLOGY

As described before, the utilization of modeling tools in view of process design and process characterization has become very widespread. Model parameters are estimated through minimization algorithms with respect to experimental data and, afterwards, the calibrated model can be used for the process improvement (e.g. in process design or process control). However, for a reliable posterior utilization of the estimated parameters, the assessment of their confidence intervals should be as important as the estimation of the parameter value itself (Dochain and Vanrolleghem, 2001 or Brun *et al.*, 2002, among many others).

When calibrating a model, the quantity and quality of the output measurements available define which parameters can be identifiable or not. An identifiability analysis of the model with the experimental data used has to be performed before model calibration in order to decide which parameters can be estimated and which should be previously calculated or assumed form literature. For example, it is illogical to measure the volume of the vessel to model the dependence of the solubility of a certain compound on temperature. This reasoning can be done in simple models; however in complex models a mathematical approach is necessary because some factors, such as parameter correlations, cannot be detected *a priori*.

A lot of research is being conducted in assessing the parameter estimation accuracy from experimental data, though confidence intervals assessment is not a straightforward issue. There are many different factors involved which have to be considered to decide whether a parameter estimation value is reliable or not (i.e., the quality and quantity of experimental data, the inherent structure of the model or the minimization approach used (Beck, 1987)). In this sense, the utilization of the Fisher Information Matrix (FIM) is gaining importance in view of examining the model identifiability and for parameter estimation error assessment. This matrix integrates the sensitivity of the measured outputs with the estimated parameters with the quantity and quality of the experimental data.

Model identifiability is the ability to obtain a unique parameter set able to describe accurately the behaviour of the system.

Based on the information obtained with the FIM, Dochain and Vanrolleghem (2001) distinguished two different kind of identifiability:

- Structural identifiability: assuming a certain number of outputs (without experimental error), can we obtain a unique set of parameter values that describe our system? This part deals with the model structure itself.

- Practical identifiability: assuming a model structurally identifiable, is the information contained in our experimental data enough for a reliable estimation of our parameters? This part deals with the quantity and quality of our experimental measurements (Holmberg, 1982)

Hence, before model calibration, one should decide which of the model parameters can be identifiable with the available experimental data. As described above, this thesis aims to calibrate all the models with "common on-line measurements" such as DO or pH. This will restrict the amount of parameters identifiable. This thesis is aware of this fact and a previous identifiability analyses is performed in each model.

In addition, Chapter IV shows an applied exercise to understand that parameter estimation assessment is fundamental in process modeling. In addition to this, the parameter estimation procedure is extensively reviewed. The importance of data quantity and quality is discussed using the well-known Andrews model for substrate inhibition.

I.3.3 BIOLOGICAL COD REMOVAL

At first glance, the biological COD removal process was considered a simple process where part of the substrate was directly used for growth and the rest was oxidised for energy obtainment. This idea is the basis of ASM1, the model introduced by the IWA for the description of the biological COD and nitrogen removal processes. (Henze *et al.*, 2000). This model was based in the death-regeneration concept that Dold introduced in the 1980s. According to this concept, the oxygen consumption observed in absence of external substrate is due to the hydrolysis of the slowly degradable substrate, which is partly produced in the biomass decay. Figure I.7 depicts a scheme of ASM1. Biomass is considered to grow solely on the readily biodegradable substrate (S_S).
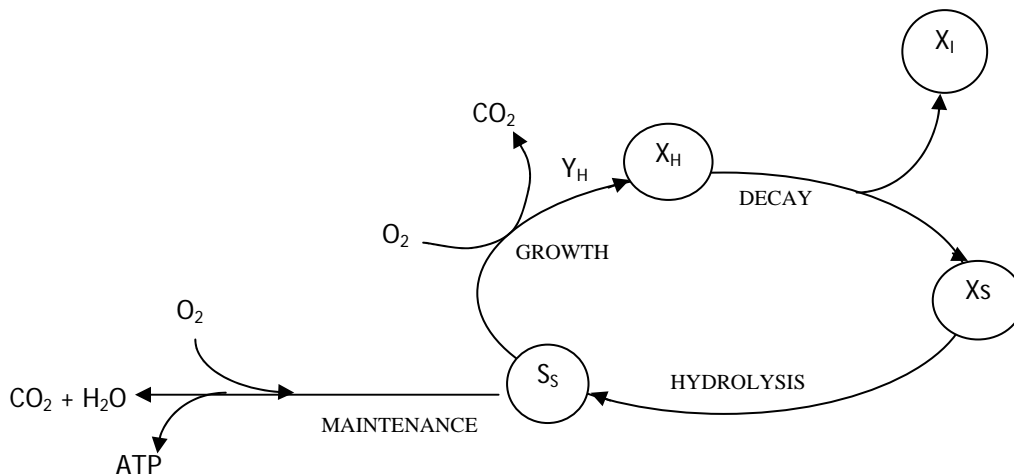
Figure I.7 Scheme of ASM1

However, storage polymers were reckoned to play a very important role in the biological COD removal, because part of the substrate was not directly used for growth but stored (van Loosdrecht et al., 1997). In 1999, a new model for the biological COD removal process (ASM3) was developed mainly to take this storage phenomenon into account

(Gujer *et al.*, 1999). The main innovation of this model is the assumption that all the readily biodegradable organic substrates taken up under feast conditions are directly converted into stored material. Figure I.8 depicts a scheme of ASM3. These stored compounds become the carbon and energy source for growth purposes in the subsequent famine period. In ASM3, the decay processes are replaced with the endogenous processes. Hence, the oxygen consumption experimentally observed in absence of external substrate is explained with the biomass growth on the storage product and because of the lyses processes.
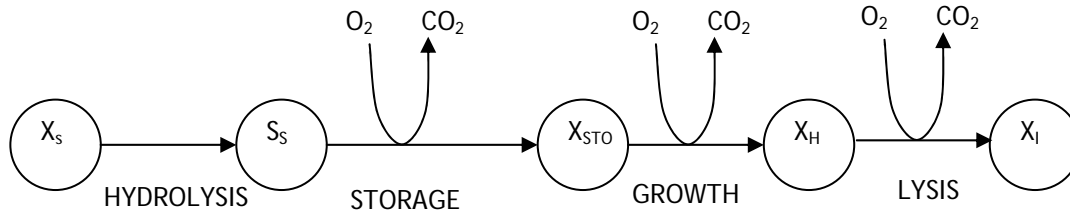
Figure I.8 Scheme of ASM3

The conceptual basis of both ASM1 and ASM3 has been largely criticized. On the one hand, it has been experimentally demonstrated that biomass stores part of the substrate and uses the rest for growth. Hence, reality is a mixture of both models. Alternative models to ASM1 and ASM3 taking into account simultaneous storage and growth processes have been proposed (e.g. van Aalst-van Leeuwen *et al.,* 1997;, Krishna and Van Loosdrecht, 1999; Beccari *et al.,* 2002; van Loosdrecht and Heijnen, 2002 or Karahan Gül *et al.,* 2003).

From the existing background on the modelling of COD removal process, the next research questions arise:

1. ARE THESE MODELS ACCURATE ENOUGH TO DESCRIBE BATCH EXPERIMENTS?
2. IF NOT, HOW CAN WE IMPROVE/EXTEND THEM?
3. CAN TITRIMETRIC MEASUREMENTS IMPROVE MODEL CALIBRATION WHEN ADDED TO RESPIROMETRY?
4. CAN THIS MODEL DESCRIBE THE EXPERIMENTAL PROFILES OBTAINED WITH BIOMASS FROM DIFFERENT WWTPS OPERATED UNDER DIFFERENT CONDITIONS?

Chapter V deals with the biological COD removal topic. The applicability of ASM1 and ASM3 is discussed when calibrated with respirometric batch experiments. It is demonstrated that these models should be extended to take into account that storage and growth on external substrate occur simultaneously as opposed to the assumption of ASM3 that only storage occurs during the feast phase. Then, a new model is developed which includes the simultaneous growth and storage approach for biological COD removal under aerobic conditions. This model is calibrated with several respirometric and titrimetric experiments with biomass withdrawn from different WWTPs (operated under different conditions).

I.3.4 NITRIFICATION

Nitrification is the biological oxidation of ammonia to nitrate. There are two phylogenetically different groups of bacteria that collectively perform nitrification. Thus, this process can be considered a two-step process. Ammonia oxidising biomass (AOB) performs nitritation (i.e. oxidation of ammonia to nitrite) and nitrite oxidising biomass (NOB) performs nitratation (i.e. oxidation of nitrite to nitrate).

$$\text{Nitritation:} \quad NH_4^+ + 3/2\ O_2 \rightarrow NO_2^- + 2\ H^+ + H_2O$$
$$\text{Nitratation:} \quad NO_2^- + 1/2\ O_2 \rightarrow NO_3^-$$
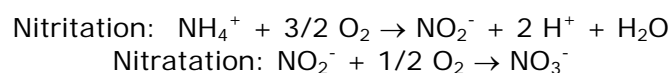
However, the first nitrification models described the process as a single step system where ammonia was oxidised to nitrate based on the assumption that the limiting step was the oxidation of ammonia to nitrite. This single step modelling was used in the ASM models, the reference models in the biological nutrient removal field (Henze *et al.*, 2000)

Hence, in the last years the single-step simplification has been avoided and, nowadays, two-step nitrification models are the most common in the literature (Gee *et al.*, 1990a; Sheintuch *et al.*, 1995; Picioreanu *et al.*, 1997; Ossenbruggen *et al.*, 1996; Nowak *et al.*, 1995;, Chandran and Smets, 2000; Petersen, 2000; Carrera, 2001; Hao *et al.*, 2002; Gapes *et al.*, 2003 or Pratt *et al.*, 2004 among many others).

5. CAN WE EXTEND THE PREVIOUS TWO-STEP NITRIFICATION MODELS TO BE EASILY CALIBRATED WITH OUR AND HPR?
6. WHICH PARAMETERS OF THIS MODEL CAN BE RELIABLY ESTIMATED FROM BATCH EXPERIMENTS?
7. CAN EXTRA SUBSTRATE MEASUREMENTS (AMMONIA, NITRITE, CO_2) IMPROVE THE PARAMETER IDENTIFIABILITY?

Chapter VI deals with the biological nitrogen oxidation. A two-step nitrification model is developed to be calibrated using both respirometric and titrimetric data, hence, the prediction of the proton production/consumption is also developed for each of the involved processes. From an identifiability point of view, not all the parameters could be reliably estimated using only short-term batch respirometric and titrimetric profiles and particular experiments would be required. The parameters of the model are examined to decide which of them can be either calculated or assumed from the literature and which of them should be estimated.

Nowadays, biological nitrogen removal systems have evolved from the classical nitrification/denitrification systems in view of treating more efficiently different N-loaded wastewaters. One of the recent advances in the biological nitrogen removal field is the combination between the SHARON® (Hellinga *et al.*, 1998, 1999) and the Anammox® processes (van Loosdrecht and Jetten, 1998). The combination of these processes represents an upgrading of the classical nitrification/denitrification process since no organic matter is required for biological nitrogen removal. These processes sometimes operate under limiting substrate conditions.

8. HOW DO SUBSTRATE LIMITATIONS INFLUENCE THE NITRIFICATION PROCESS?

Hence, for a deeper knowledge of the process, a special emphasis was put in the modelling of substrate limitations of the process. As the nitrification is an aerobic and autotrophic process, its kinetics is highly dependent on the DO and inorganic carbon concentration in the medium. Hence, the lack of any of them should result in a decrease of the process rate. Both the oxygen and the inorganic carbon limitations are examined for both the AOB and the NOB populations in Chapter VI.

I.3.5 ENHANCED BIOLOGICAL PHOSPHORUS REMOVAL (EBPR)

In parallel to this thesis, research on EBPR was conducted on EEG-UAB by the formed PhD student Maite Pijuan. The EBPR process is based on the enrichment of activated sludge with Polyphosphate Accumulating Organisms (PAO). She examined the optimal conditions to favour this PAO enrichment from non-EBPR sludge.

Figure I.9 depicts the PAO mechanisms. Under anaerobic conditions, PAO take up Volatile Fatty Acids (VFA) and store them as polyhydroxyalkanoates (PHA), while the degradation of intracellular glycogen provides reducing equivalents (Mino *et al.* 1987, Smolders *et al.*, 1995). The energy for this process is obtained partly from the glycogen degradation but

mostly from the hydrolysis of the intracellular stored polyP, resulting in an orthophosphate release into solution. In the subsequent aerobic phase, PAO take up excessive amounts of orthophosphate to recover the intracellular polyP levels by oxidising the stored PHA. Meanwhile they grow and replenish the glycogen pools using PHA as both carbon and energy sources (Smolders *et al.*, 1995). Net phosphorus removal is achieved by wasting sludge after the aerobic period when the biomass contains high levels of polyP.
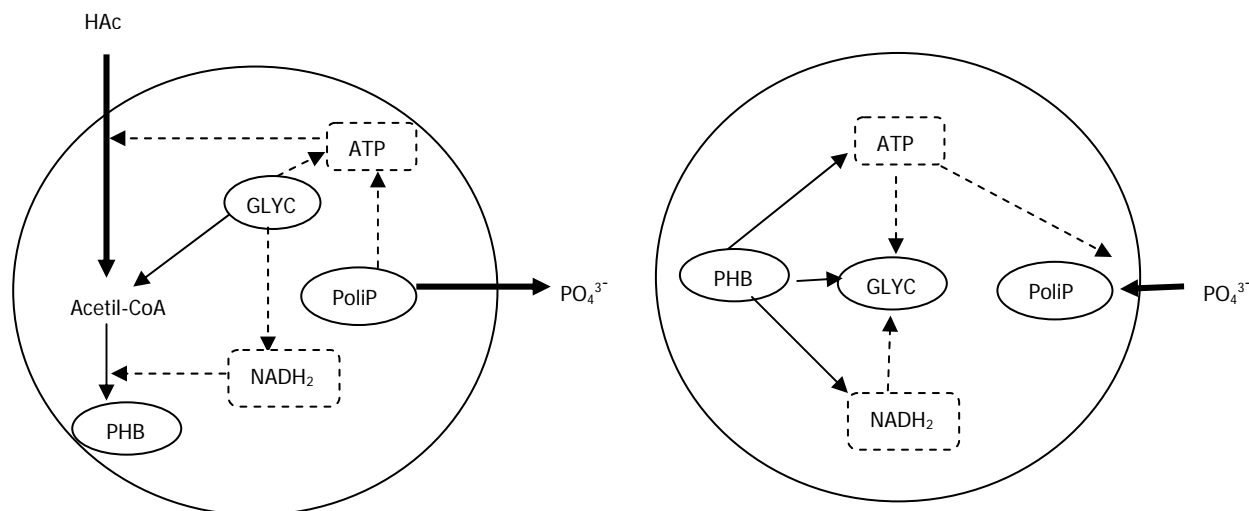
Figure I.9 PAO mechanisms. Anaerobic (LEFT), aerobic (RIGHT)

Maite Pijuan investigated the PAO behaviour under different conditions. The modelling of the experimental data was required for a better understanding of the process. Hence, one of the objectives of this thesis was to develop a model which was not only capable to describe the conventional anaerobic-aerobic EBPR processes, but a model capable to describe the experimental observation under different conditions.

From the existing background on EBPR, the next research questions arise:

9. ARE THE EXISITNG MODELS OF THE LITERATURE ACCURATE ENOUGH TO DESCRIBE EBPR BATCH EXPERIMENTS?
10. HOW DOES A CHANGE IN THE CARBON SOURCE (PROPIONATE FOR ACETATE) AFFECTS TO THE EBPR PARAMETER VALUES?

Two different modelling approaches to the EPBR process can be found in the literature. The first approach is a detailed metabolic model such as Smolders *et al.* (1995) or Oehmen *et al.* (2005). On the other hand, the IWA developed a different approach to the EBPR modelling by using "grey models" for a macroscopic description of the evolution of the main compounds involved, but without deepening in the metabolic details of these transformations. ASM2 (and ASM2d) are examples of these models.

After a literature review on EBPR modelling and a new model was developed which derived from a modification of ASM2. The development of this model is detailed in Chapter VII. The most important modification was the inclusion of glycogen economy which has a very important role, particularly when describing batch experiments. Hence, its inclusion has become indispensable in view of process modelling. This model was used to examine the differences between using either acetate or propionate as the sole carbon source. This study was conducted in collaboration with the Advanced Wastewater Management Centre (AWMC) of the University of Brisbane (UB), Australia. The experimental work was conducted in Australia during a research staying of Maite Pijuan at AWMC in 2003.

11. CAN THE EBPR MODELS DESCRIBE THE BEHAVIOUR OF PAO UNDER CONDITIONS THAT DIFFER FROM THE CLASSICAL ANAEROBIC-AEROBIC CONDITIONS?

Once the classical anaerobic-aerobic PAO behaviour is established, one step beyond in PAO knowledge is to investigate PAO behaviour under different conditions. As described n Figure I.9, EBPR is based on the physical separation between the electron donor (COD) and the final electron acceptor (oxygen or nitrate). A very interesting topic is the analysis of the effect of the simultaneous presence of both the electron donor and the final electron acceptor. Some research can be found in the literature about the effects of the coexistence of COD as electron donor and nitrate as electron acceptor (as for example in Kuba *et al.*, 1996; Pereira *et al.*, 1996 or Filipe and Daigger, 1999). These works are mainly focused in studying the importance of the Denitrifying Polyphosphate Accumulating Organisms (DPAO) in the bio-P sludge.

However, the coexistence of oxygen and VFA with bio-P sludge is a less studied scenario, because the anaerobic phase has always been considered essential to favour PAO against other aerobic species. However, some experiments have shown that P-release may occur under strictly aerobic conditions linked to external substrate uptake (Ahn *et al.*, 2002 or Serafim *et al.*, 2004). Chapter VII examines this phenomenon and analyses PAO behaviour under strictly aerobic questions. Under these conditions (i.e. aerobic conditions with VFA presence), Ordinary Heterotrophic Biomass (OHO) should also be considered in the process. Moreover, as it is an aerobic process, the OUR measurement can also provide a lot of information about the process rate and the process yields.

12. CAN WE USE THESE MODELS TO PROPOSE A NEW CONTROL STRATEGY TO REDUCE THE START-UP OF AN EBPR SYSTEM FROM A NON-EBPR WWTP SLUDGE?

EBPR process is based on PAO enrichment from non-EBPR WWTP sludge. The experience accumulated in EEG-UAB says that this process can be very slow and not always successful since PAO are scarce in conventional WWTP sludge. Hence, one motivation of this thesis was to use the previously developed model to test new control strategies to improve the EBPR start-up process. These control strategies aim to improve the EBPR start-up process by increasing its efficiency skipping periods where PAO growth is not favoured.

CHAPTER II

Objectives

This thesis aims to develop, calibrate and validate a model for each of the main biological nutrient removal processes:

- Biological COD removal
- Nitrification
- Enhanced Biological Phosphorus Removal

These models should be developed so that they can be calibrated with respirometric and titrimetric techniques and using the mathematical tools necessary to ensure a reliable parameter estimation.

This objective can be divided into:

1. Examine a mathematical tool for the assessment of the parameter estimation error. This tool should be easily and generally applicable and should consider the quality and quantity of experimental data used for parameter estimation.

2. Particularly, in the case of biological COD removal, this thesis aims to improve the classical Activated Sludge Models (ASM), with a new model that includes the simultaneous growth and storage hypothesis. This new model aims to be successfully calibrated and validated using respirometric and titrimetric techniques with biomass withdrawn from different WWTP.

3. Develop and calibrate a new model for the two-step nitrification process.

    a. Decide which of the model parameters can be estimated with respirometric-titrimetric measurements and which should be assumed form the literature.
    b. Experimentally assess the existing oxygen and inorganic carbon limitations on the nitrification process.

4. Improve EBPR modelling by modifying the existing ASM2d model with the inclusion of glycogen for a proper description of EBPR batch tests.

    a. Extend the model to describe EBPR behaviour under conditions that differ from the classical anaerobic-aerobic.
    b. Calibrate the model with respirometric-titrimetric batch tests.
    c. Develop new control strategies to reduce the start-up length in an EBPR system in a SBR.

CHAPTER III

Materials and methods

III.1 Description of the equipments

This section describes the experimental setups used in this thesis. Despite there are different equipments, most of them operated as a LFS respirometer (i.e. an aerated bioreactor with a pH control loop and DO monitoring). The choice of the equipment for each experiment was done according to the sort of measurement and the sample volume required. There is also a description of the pilot plants from which the biomass was withdrawn. These pilot plants were operated to enrich the biomass with either nitrifying biomass or Phosphate Accumulating Organisms (PAO). These plants were respectively maintained by two PhD students of our group: Irene Jubany and Maite Pijuan.

III.1.1 LFS RESPIROMETER

A. EXPERIMENTAL SETUP

The LFS respirometer was the most used equipment in this thesis because it was very flexible and only required 1 L of mixed liquor. In addition, the data acquisition and the monitoring was adapted to our needs through an original software programmed in Visual Basic 6.0 (see below). A respirometer is essentially an aerated bioreactor utilised to measure the biological oxygen consumption rate (i.e. OUR).

According to the classification described in Chapter I (Figure I.2), a LFS respirometer consists of a continuously aerated bioreactor without continuous liquid inputs or outputs where the oxygen is measured in the liquid phase. The substrate is introduced to the respirometer by pulses. The LFS respirometer used in this thesis is schematically depicted on Figure III.1.
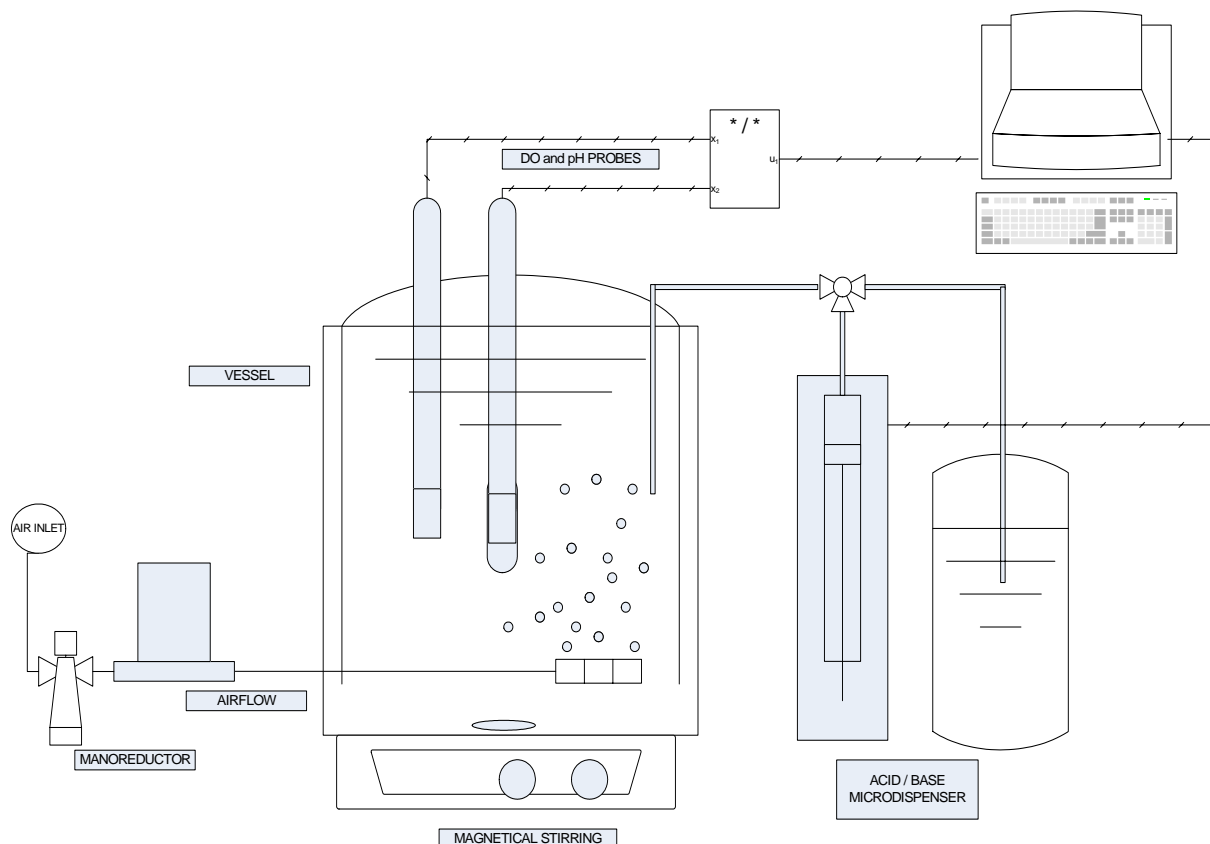

Figure III.1 Schematic representation of the LFS respirometer

As can be observed, the air enters to the respirometer through a manoreductor and a massflow controller. This massflow controller was necessary for an accurate OUR calculation. A rotameter is cheaper but it was not chosen because it is not a controller and it could not ensure as constant airflow as a massflow controller does, particularly in low values. It was essential that the flow value was kept constant during the whole experiment, despite the fact that the absolute value of the airflow was not needed for calculations. Otherwise, the oxygen transfer through the interphase (i.e. k_La) could fluctuate during the experiment. The massflow controller used in this equipment was the model F201C from Bronkhorst HiTec, and had a range of 0-1 L/min. The airflow was normally set to values in the range of 0.1-0.3 L/min which means 0.1-0.4 vvm.

The airflow was bubbled to the liquid phase through a microdiffuser which ensured that the air bubbles were small enough to:

- Obtain good mass transfer from the gas to the liquid
- Avoid biomass overflow
- Prevent the bubbles from sticking to the membrane of the DO probe, which would add noise to the signal

The respirometer vessel was thermally controlled by submerging it into a bath. Figure III.2 shows the respirometric vessel of 1L. As can be observed, it has 4 different outlets. One outlet was set at the bottom for air inlet. Another outlet was set at one side of the vessel for the DO probe, which entered diagonally to the respirometer. This inclination prevent the bubbles from sticking to the DO probe membrane and causing disturbances in the OUR measurement. The system was magnetically stirred at high speed to ensure homogenisation and also to avoid any biomass flocs or bubbles sticking into the membrane. Two more outlets were set on the top for the pH probe and a possible extra probe (i.e. reduction-oxidation potential) and the acid/base dosage. The pH probe model chosen was WTW Sentix 81 and the DO probe was WTW CellOx 325. This last probe was chosen due to its short time response which allowed reliable DO measurements despite sudden changes in the system. The DO probe time response should not be neglected as will be shown along this thesis and as described in Spanjers and Olson, 1992 or Baeza *et al.*, 2002b.

Figure III.2 LFS respirometric vessel

These pH and DO probes were connected to a multiparametric reception equipment (INOLAB 3 of WTW). This multiparametric equipment was connected via RS232 to a PC that monitored the data and stored it in a Microsoft Excel sheet through the Visual Basic 6.0 software. The software was also capable to manipulate a microdispenser equipment

in order to keep the pH constant with acid/base dosage. Figure III.3 shows an overview photograph of the whole equipment.

Figure III.3 Overview of the whole LFS respirometer equipment

B. OUR CALCULATION

Most of the equipments used in this thesis were operated as LFS respirometer for OUR calculation. The methodology of OUR calculation from a typical LFS respirogram (i.e. DO profile) is briefly described next as it was regularly used in this thesis. The fundamentals of this methodology can also be found in Vanrolleghem *et al.*, (1994) or Spanjers *et al.*, (1997).

Equation III.1 shows the DO balance in the liquid phase of an aerated bioreactor:

$$\frac{d(V_L \cdot S_O)}{dt} = V_L \cdot k_L a \left[S_O * - S_O(t) \right] - OUR \cdot V_L \tag{III.1}$$

where $k_L a$ = global oxygen transfer coefficient (1/min)
$\quad S_O$ = dissolved oxygen concentration (mg O_2/L)
$\quad S_O*$ = dissolved oxygen saturation (mg O_2/L)
$\quad V_L$ = Volume of the liquid phase (L)

If the volume of the liquid phase is constant and OUR is divided into endogenous OUR (OUR_{END}) and exogenous OUR (OUR_{EX}), the DO balance becomes:

$$\frac{dS_O}{dt} = k_L a \left[S_O * - S_O(t) \right] - OUR_{END} - OUR_{EX} \tag{III.2}$$

Hence, the OUR profile can be calculated from the S_O profile if k_La, S_O* and OUR_{END} are known. The experimental calculation of S_O* is somehow tedious, however as it is shown below, it can be avoided. Figure III.4 shows a typical example of a LFS respirogram.
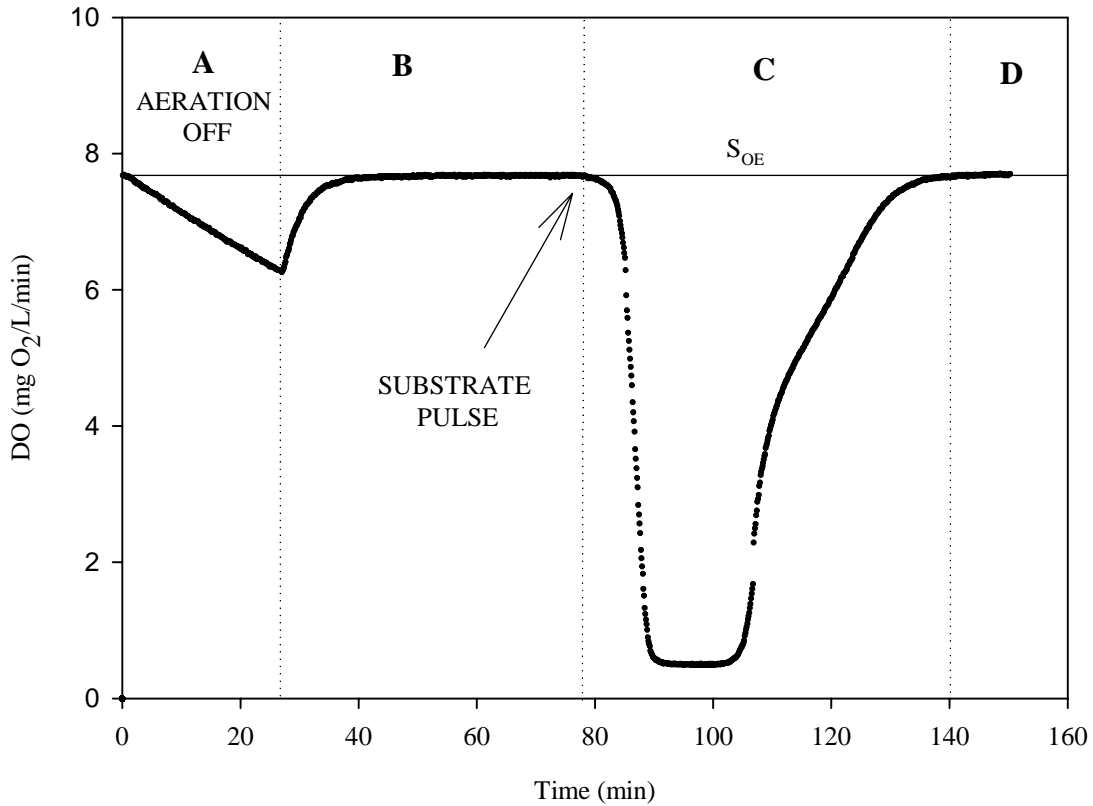

Figure III.4 Typical LFS respirogram

Before region A, biomass was left aerated overnight under endogenous conditions to ensure that any readily biodegradable substrate is present when the pulse is added. Equation III.3 describes the oxygen evolution under these conditions:

$$\frac{dS_O}{dt} = k_La\left[S_O * -S_O(t)\right] - OUR_{END} \qquad (III.3)$$

The S_O tends to a constant value where the oxygen transfer is balanced with the endogenous OUR. This S_O value, named S_{OE}, is constant as long as endogenous OUR is constant.

$$\frac{dS_O}{dt} = 0 \ \ (S_O = S_{OE}) \ \ ; \ \ k_La\left[S_O * -S_{OE}\right] = OUR_{END} \qquad (III.4a,b)$$

In region A, aeration is stopped and endogenous OUR is calculated as:

$$\frac{dS_O}{dt} = -OUR_{END} \qquad (III.5)$$

Afterwards, the system is reaerated (in region B) and the DO balance corresponds again to equation III.3. As S_O is lower than S_{OE}, the S_O increases to reach S_{OE}. The curvature of this increase depends on the oxygen transfer efficiency from the gas phase to the liquid. Hence, this reaeration profile is used for k_La estimation as described in Bandyopadhyay *et al.*, (1976). Equation III.3 can be transformed to equation III.6:

$$\frac{1}{k_La}\cdot\frac{dS_O}{dt} + S_O(t) = S_O{}^* - \frac{OUR_{END}}{k_La} \qquad (III.6)$$

The description of the reaeration profile is obtained by combining this last equation with equation III.4b:

$$\frac{1}{k_La}\cdot\frac{dS_O}{dt} + S_O(t) = S_{OE} \qquad (III.7)$$

The theoretical DO [eq. III.8] profile is obtained solving this ordinary differential equation. Mathematically, the k_La is the inverse of the time constant of a first order system. Hence, high k_La values imply low response time and then short reaeration profiles.

$$S_O(t) = (S_O(0) - S_{OE})\cdot e^{(-k_La\cdot t)} + S_{OE} \qquad (III.8)$$

Once the reaeration profile reaches S_{OE}, a pulse of substrate is added to the system (region C) and S_O decreases because of the oxygen consumption linked to substrate uptake [eq. III.2]. Equation III.9 comes from the combination of this equation with III.4b.

$$OUR_{EX}(t) = k_La\cdot[S_O{}^* - S_O(t)] - k_La\cdot[S_O{}^* - S_{OE}] - \frac{dS_O}{dt} \qquad (III.8)$$

Finally, and simplifying the equation, the OUR due to an external substrate can be calculated with equation III.10:

$$OUR_{EX} = k_La\cdot[S_{OE} - S_O(t)] - \frac{dS_O}{dt} \qquad (III.10)$$

Once the biodegradable fraction of the substrate pulse is depleted, the biomass returns to endogenous conditions and the S_O returns again to the S_{OE} level (region D).

C. LFS RESPIROMETER SOFTWARE

In the LFS respirometer, the PC was connected to the multiparametric equipment and received, monitored and stored all the data. It also controlled the microdispenser equipment for the acid/base dosage to control the pH.

Figure III.5a, b show typical screenshots of the program. As can be observed the software provides the on-line experimental profiles. This software has MATLAB as a calculation engine and the OUR is calculated from the respirogram by using equation III.10 in each oxygen sample measurement.
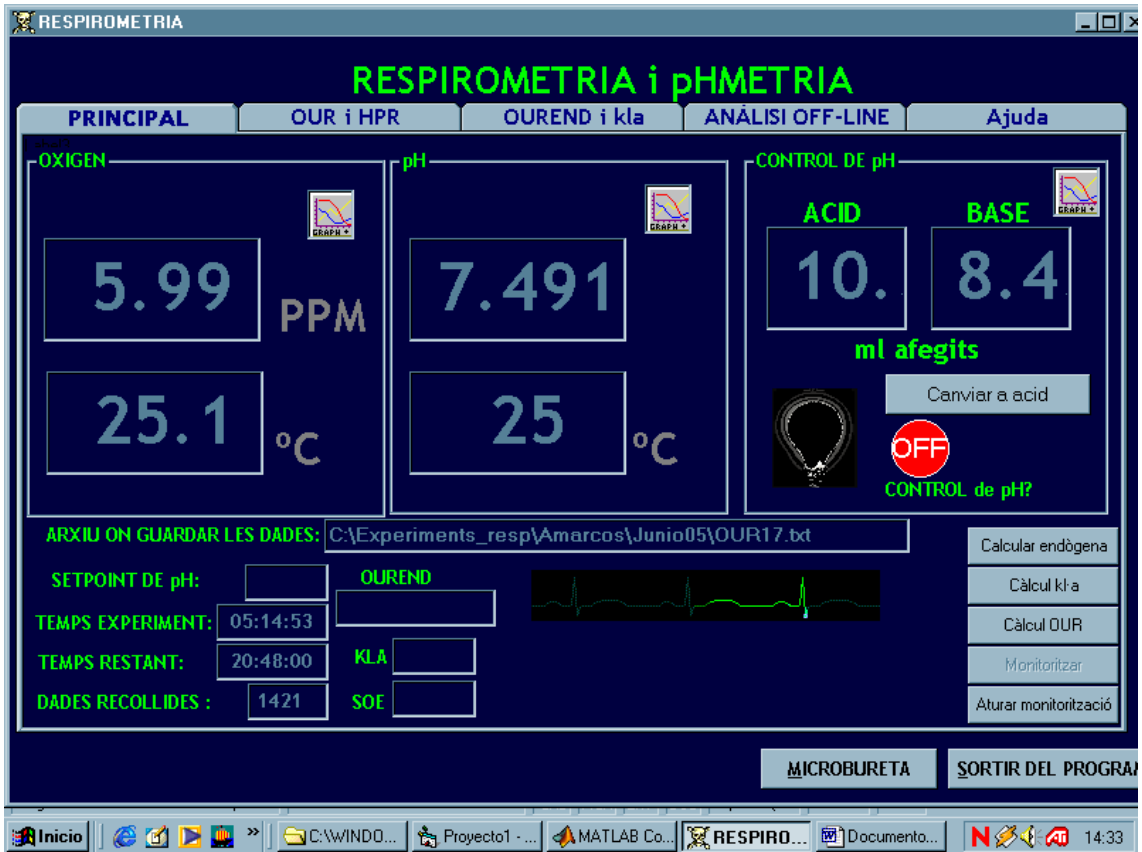
Figure III.5a Typical LFS respirometer screenshot, with where the oxygen, pH and acid/base dosage are displayed together with other data.
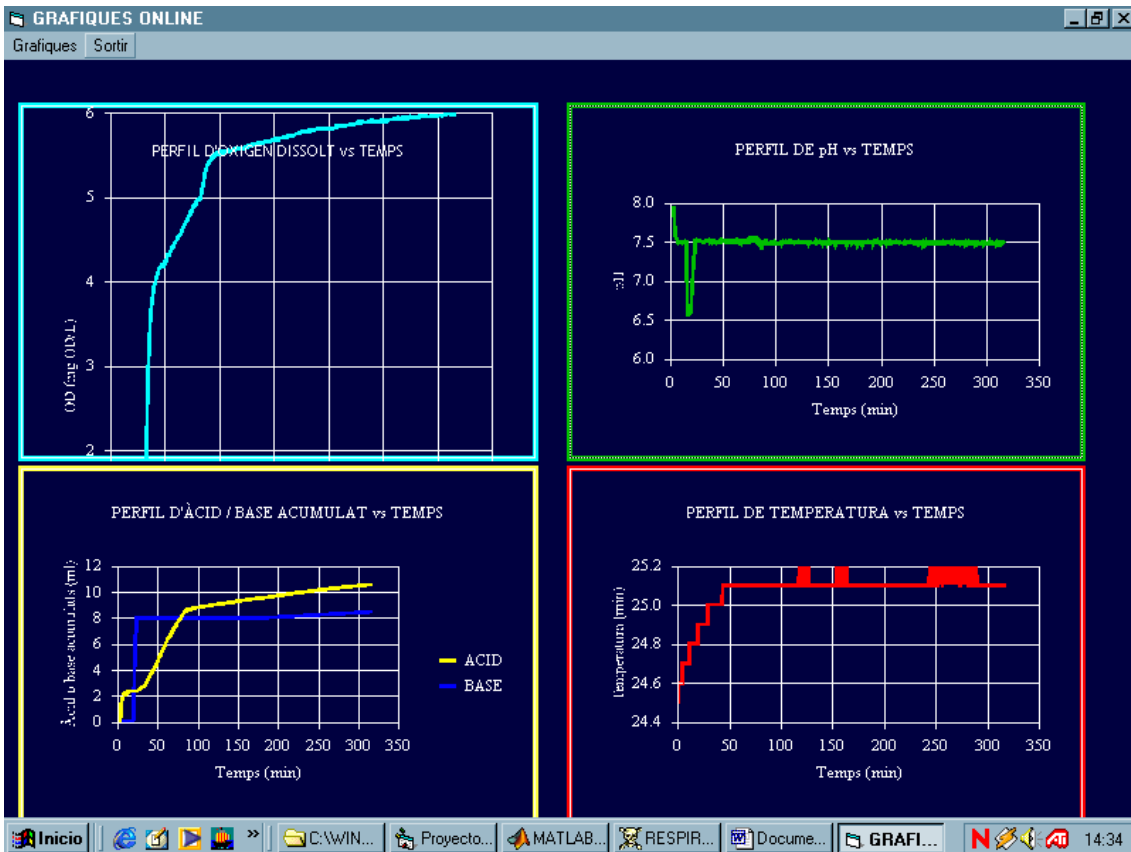

Figure III.5a Typical LFS respirometer screenshot, with where the oxygen, pH, acid/base dosage and temperature are plotted.

III.1.2 BIOSTAT B FERMENTER

The BIOSTAT B fermenter (Braun Biotech Int.) consists of an aerated vessel of 6 L with DO, pH and temperature monitoring. The scheme of this equipment is the same as the one depicted in figure III.1, except for the gas outlet, which is connected to mass spectrophotometer for off-line gas analysis. This system was used only when CO_2 measurements were required.

Figure III.6 BIOSTAT B Fermenter

This equipment consists of a jacketed vessel with DO, T and pH probes and a mechanical stirrer, which are connected to a Digital Control Unit (DCU). The DCU is capable to control the process variables with manual tuning PID loops. The pH was controlled with two peristaltic pumps. The DCU is connected to a PC where the variables are monitored and stored for a posterior utilisation. Pinsach (2003) previously programmed this software also in Visual Basic 6.0 code. The inlet flow was controlled with the same massflow controller as in the LFS respirometer. The massflow controller (Figure III.7-left) is also connected to the PC to register its measurements.

Figure III.7 LEFT- Massflow controller and RIGHT – Mass spectrophotometer

The gas outlet went through a silica-dewatering column before entering to the mass spectrophotometer (Balzers Quadstar 422) depicted in Figure III.7-right. This equipment estimated the molar fraction of the gas main components and was also connected to the PC to monitor and register its measurements.

The operational conditions of the fermenter were set so that it could operate as a LFS respirometer. The k_La of the system was set to a low value (lowest airflow and stirring feasible), so that a significant decrease on the DO level linked to substrate consumption could be observed. The airflow was maintained strictly constant and the k_La and the endogenous OUR value were previously estimated. Hence, the OUR could be calculated as described for an LFS respirometer (see above). Enabling the fermenter to work as an LFS respirometer was a major contribution of this thesis, despite the OUR could be directly measured with the mass spectrophotometer connected to the BIOSTAT B.

The mass spectrophotometer registered the inlet and outlet oxygen fraction and OUR could be calculated with a simple mass balance in the gas phase. However, the amount of oxygen consumed in these experiments was very low when compared to the oxygen amount in the gas phase. Hence, the differences between the inlet and outlet were so small that the OUR value obtained with the mass spectrophotometer was too noisy.

Figure III.8 compares a typical experimental OUR profile estimated through LFS respirometry (black solid line) and through the mass spectrophotometer (grey line). The latter had a lot of noise and it became almost useless for parameter estimation since it would bring too much uncertainty to the parameter estimation process. These noisy OUR profiles are characteristic of these systems as can be observed in Gapes (2004) or Pratt (2004). However, an OUR profile valid for parameter estimation (i.e. without noise) could be obtained with a proper filtering. For example, the black dashed line was calculated using a 15th level mean mobile filtering. This filtering visually improved a lot the OUR profile, however it introduced in certain delay.
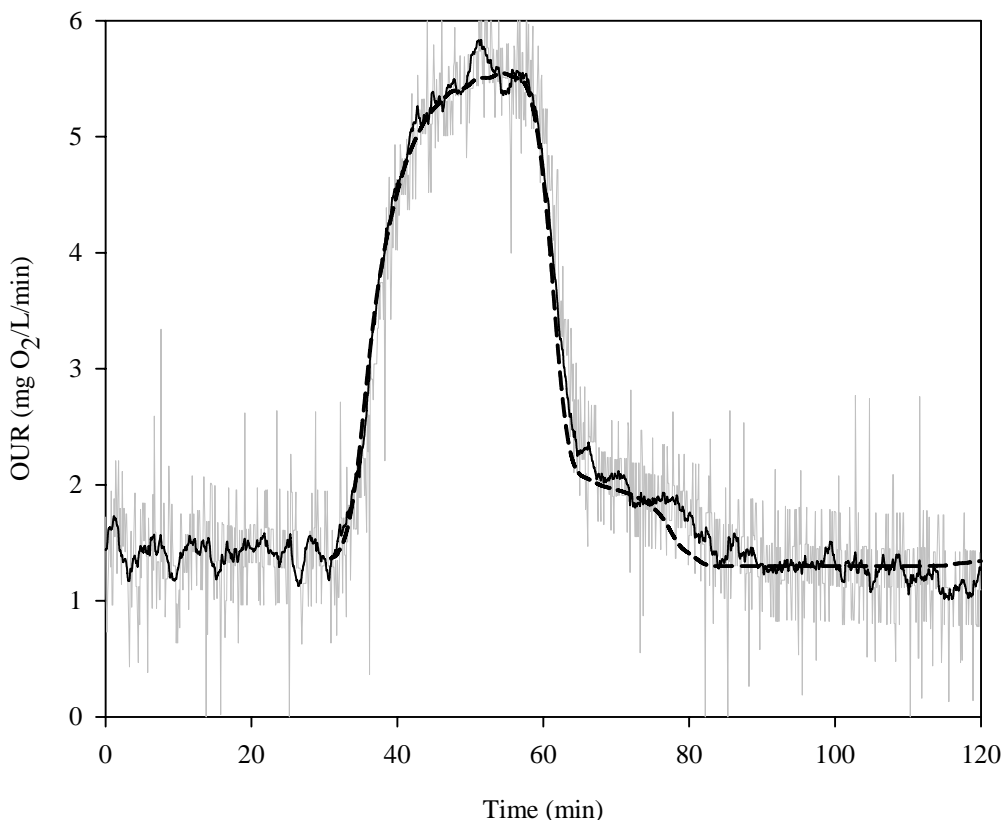

Figure III.8 Comparison of OUR profiles form a typical batch experiment: LFS estimated OUR (dashed), measured OUR (solid grey) and filtered OUR (solid line)

III.1.3 EBPR-SBR PILOT PLANT

A. EXPERIMENTAL SET-UP

The biomass necessary for the EBPR experiments conducted in UAB was withdrawn from a pilot plant developed during this thesis. This pilot plant consisted in two identical fully monitored SBRs. Having two different SBRs allowed the comparison of different strategies of operation. Figure III.9 depicts and scheme of one of the SBRs with all of its instrumentation. Both SBRs were controlled by the same PLC (Siemens SIMATIC S7-226), which was on top of the control system. It controlled the inlet and outlet pumps, the air and nitrogen valves, the mechanical stirring and received all the analogic measurement signals. The PLC was connected via RS-232 to a PC, which monitored and stored the data received through an original software programmed with Visual Basic 6.0 (see below)
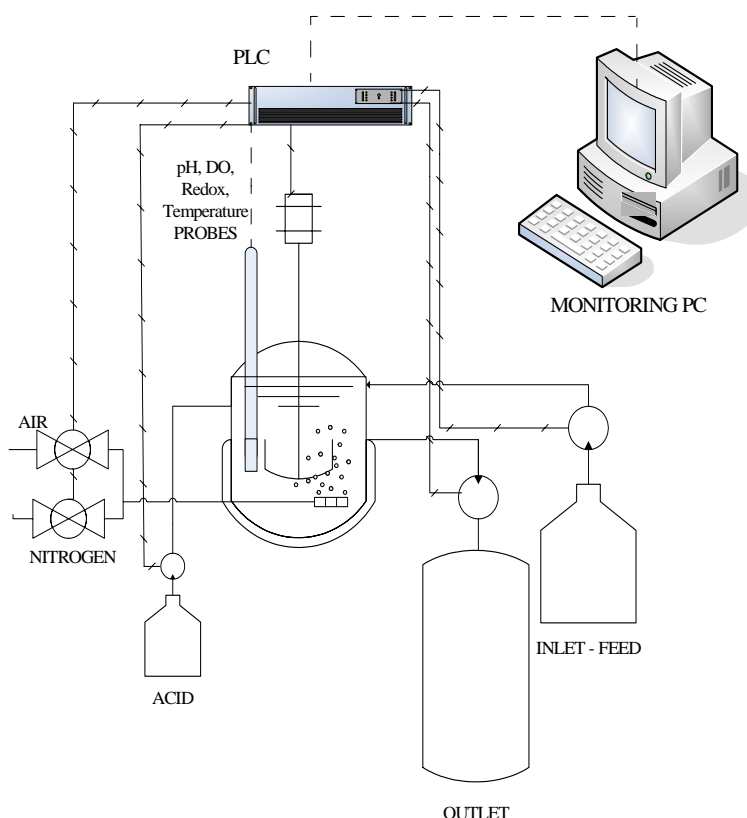

Figure III.9 Schematic representation of the EBPR-SBR

Each SBR was operated with 4 cycles per day with a controlled temperature of 25°C. Each cycle consisted of 2 h anaerobic react, 3.5 h aerobic react, 25 min of settling and, in the last 5 min, extraction of 5 L of the supernatant. A volume of 5 L of synthetic wastewater was added during the first 5 min of the subsequent cycle, producing a hydraulic residence time (HRT) of 12 h. The sludge residence time (SRT) was kept at 9 d by periodic sludge wastage during the end of the aerobic period but before the mixing was stopped. The pH was controlled during the aerobic period at 7.0 ± 0.1 with 1M HCl.

The feed for normal SBR operation was prepared daily from a stock solution and distilled water. It had the following composition (mg·L^{-1}): 594 $NaCH_3COO$, 270 KH_2PO_4, 101 NH_4Cl, 8.5 $CO(NH_2)_2$, 9 yeast extract, 38 $NaCl$, 126 $MgCl·6H_2O$, 45 $CaCl_2$, 3.1 $FeSO_4·7 H_2O$, 1.8 $MnSO_4·H_2O$, 3.1 $ZnSO_4·7H_2O$, 1.5 $CuSO_4·5H_2O$, 0.015 H_3BO_3, 0.050 KI, 0.046 $CoCl_2·7H_2O$, 0.015 $Na_2MoO_4·2H_2O$ and 3.1 EDTA.

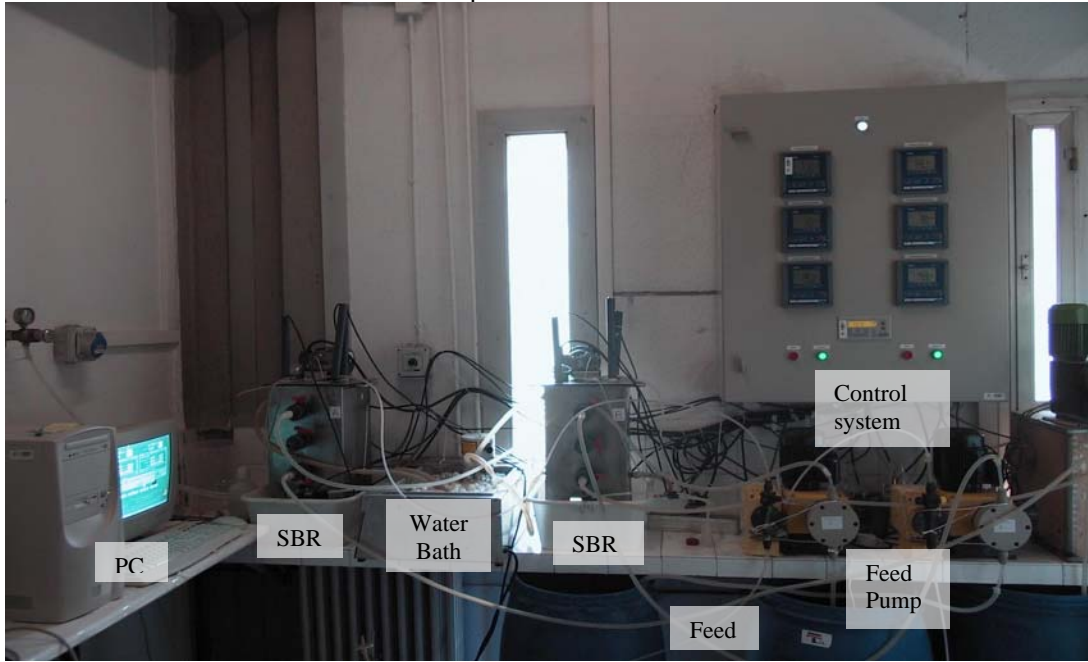Figure III.10 is an overview of the complete EBPR-SBR set.

Figure III.10 EBPR-SBR Plant

B. EBPR-SBR SOFTWARE

In the EBPR-SBR equipment, the PC was connected to the PLC and received all analogical and digital inputs. It was in charge of monitoring and storing all the data received. Figure III.11a, b show a typical screenshots of the program.
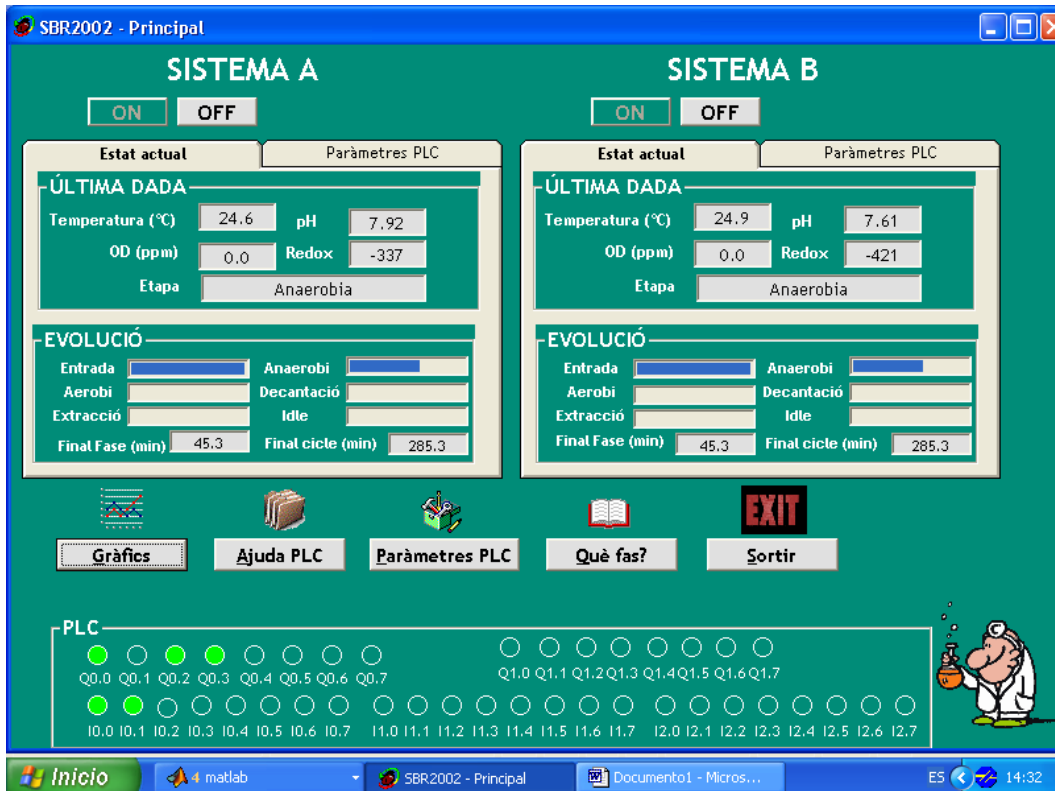

Figure III.11a Typical EBPR-SBR screenshot where the current state of both SBRs can be easily observed, together with the last value of the on-line data (oxygen, pH, ORP and temperature).
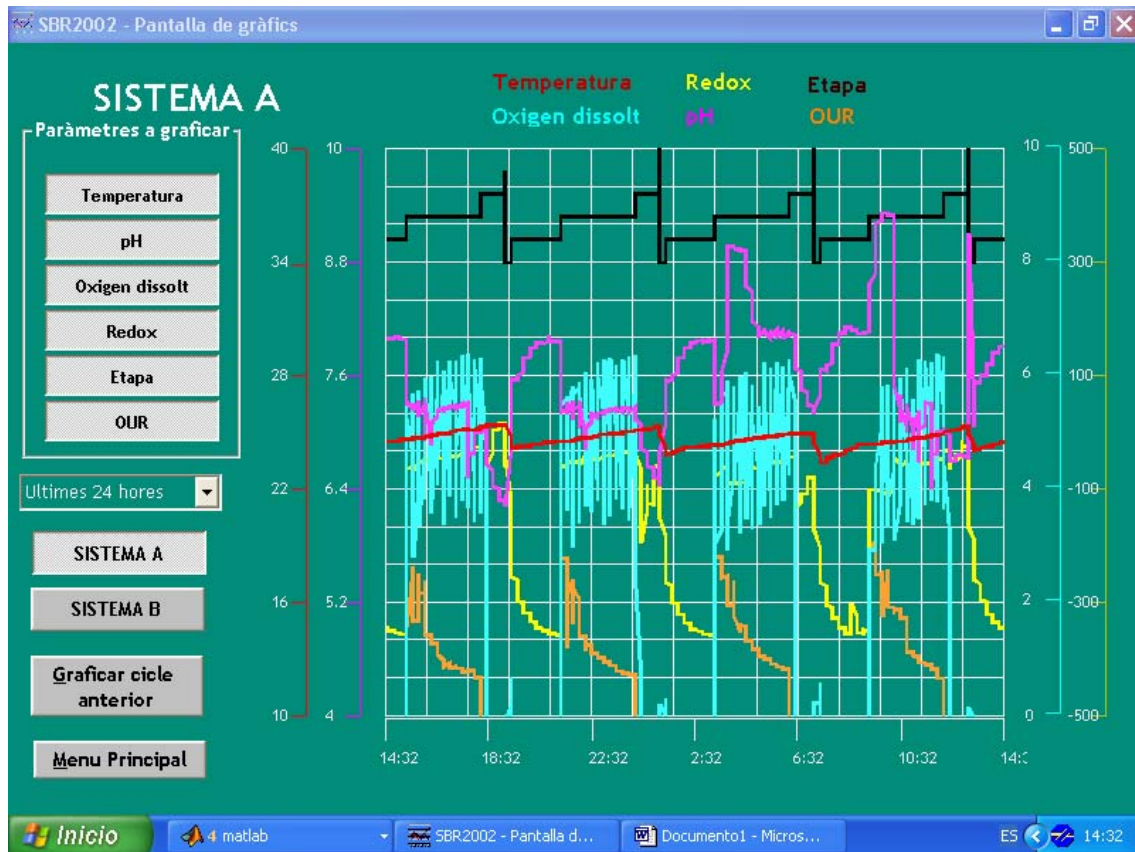
Figure III.11b Typical EBPR-SBR screenshot

III.1.4 AEROBIC-SBR

There were some experiments in the EBPR section that required the measurement of internal polymers (such as polyhydroxyalkanoates or glycogen) as well as OUR measurements. The sampling volume necessary for the measurement of these polymers was too high to use the LFS respirometer (around 50 ml). Hence, a larger SBR was operated as an LFS respirometer with a continuous and controlled airflow and previous k_La estimation. This experimental set-up is conceptually the same as the LFS respirometer (Figure III.1) but with a higher volume. Even the same massflow controller and the same multiparametric equipment were used for these experiments. The SBR was a 10 L jacketed batch reactor mechanically stirred with DO, pH, ORP and temperature monitoring. Figure III.12 shows an overview of this equipment.

III.1.5 HYBRID RESPIROMETER

The hybrid respirometer was the equipment used in the experiments performed in collaboration with BIOMATH group in Ghent University (Belgium). These experiments are only used in the biological COD removal section in the experiments conducted in the BIOMATH laboratory. The conceptual basis of this equipment is fully detailed in Vanrolleghem and Spanjers, 1998, and one example of its utilisation can be seen in Sin *et al.* (2003). Essentially, it consists of two reactors (one aerated and one non-aerated) connected so that the mixed liquor is continuously circulating from one vessel to another. OUR is calculated from a mass balance between the S_O values in both vessels. According to their authors, the precision of the LSS respirometry and the frequency of the LFS respirometry are combined in a new and more robust respirometer.

Figure III.12 Overview of the aerobic-SBR equipment.

III.1.6 NITRIFICATION PILOT PLANT

This was the pilot plant where all the enriched nitrifying biomass was withdrawn from. This activated sludge system consisted of three 26 L aerobic reactors and a 25 L settler. It was inoculated with municipal WWTP sludge and it was enriched for three moths with synthetic wastewater with 11.5 g/L NH_4Cl (3 g/L $N-NH_4^+$) and the following compounds (in mg/L): 145.0 CH_3COOH, 20.0 KH_2PO_4, 16.0 NaCl, 18.0 $MgCl_2 \cdot 7H_2O$, 0.4 $FeSO_4 \cdot 7H_2O$, 0.3 $MnSO_4 \cdot H_2O$, 0.4 $ZnSO_4 \cdot 7H_2O$, 0.2 $CuSO_4 \cdot 5H_2O$, 0.02 H_3BO_3, and 8.0 $CaCl_2 \cdot 2H_2O$. The nitrogen loading rate (NLR) was 0.6 g $N-NH_4^+$ g/VSS/d.

The hydraulic (HRT) and sludge retention times (SRT) were maintained at 4 and 20 days, respectively, and the biomass concentration in each reactor was around 1000 mg/VSS under steady-state conditions.

Figure III.13 shows an schematic overview of the plant and Figure III.14 shows a photograph of the plant. The reader is referred to Baeza (2000) for further information about the development of this pilot plant and to Baeza *et al.*, (2002a) or Jubany *et al.*, (2004) for a successful application of it.
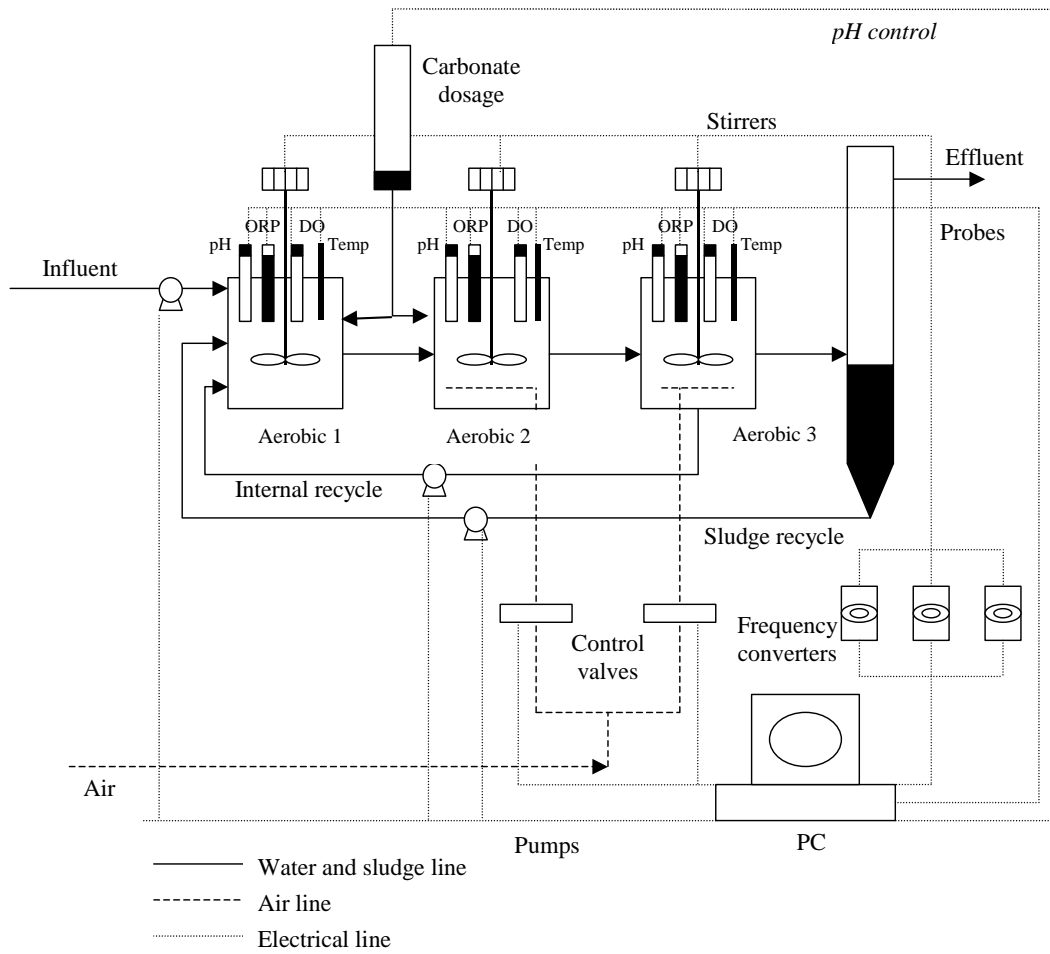
Figure III.13 Schematic overview of the nitrogen pilot plant

Figure III.14 Nitrogen pilot plant photograph

III.2 Chemical analyses

Analyses of phosphate, nitrite and nitrate filtered samples were performed with an Electrophoresis Capillar System (Quanta 4000E CE - WATERS). The electrolite used was a commercial solution (Ionselect High Mobility Anion Electrolite). In some experiments, these measurements were done with the kits of Dr. Lange for the analyses of ammonia and nitrite (Dr Lange, mod LCK301, LCK304)

Glycogen was determined by a modification of the method of Smolders *et al.* (1994a) as described in Pijuan *et al.*, 2004a. In short, 50 mg of lyophilised sludge samples were digested in a water bath with 0.6 N HCl aqueous solution at 100 °C during 5 hours. After cooling and filtering through 0.22 μm filter (Millipore), the concentration of glucose was measured using a Yellow Spring Instrument (2700 Select). Triplicates of each sample were done.

Polyhydroxyalkanoates were measured according to a modification of the method of Comeau *et al.* (1988) as describe in Pijuan *et al.* (2004a), 40 mg of lyophilised sludge samples were digested and methylated with 4 ml of acidulated methanol (3% H_2SO_4) and 4 ml of chloroform during 3.5 hours at 100 °C. Benzoic acid was used as internal standard. The analyses were performed in a GC system (Hewlett Packard 5890). Triplicates of each sample were done.

Total Inorganic Carbon (TIC) was analysed using the 1020A O-I-Analytical TOC analyser.

Finally, total suspended solids (TSS) and volatile suspended solids (VSS) were done according to the Standard Methods (APHA, 1995).