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# Activated Sludge and Moving Bed Biofilm Reactor technologies: an experimental comparison for dairy wastewater treatment

February 2017

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E TECNOLOGIA
UNIVERSIDADE DE COIMBRA

**Departamento de Engenharia Mecânica**

# Activated Sludge and Moving Bed Biofilm Reactor technologies: an experimental comparison for dairy wastewater treatment

A Dissertation for Graduate Study in MSc Program
Master of Science in Environmental Engineering

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February 2017

# Acknowledgements

This thesis is in memory of my grandparent, who will be utterly missed.

I would like to state my acknowledgements to Prof. Rosa, PhD. and Prof. Luís Castro, PhD., my supervisors, who guided me, and provided invaluable help, throughout the thesis work.

Special thanks to my girlfriend, Raquel, who has been accompanying me, not only during this journey, and gives me the strength to go on and push further.

My family, for understanding that I couldn't always be available for their needs. Special dedication to my grandmother, who I'm sure is proud of me. Sorry for not paying you the attention you deserve.

To the GERST group, who helped me with everything that I would need, and for all the good moments and fun.

Lastly, I would like to thank all my other friends, and to address to everyone that somewhat contributed to the completion of this work, you have my gratitude.

# Abstract

The Moving Bed Biofilm Reactor (MBBR) is an emerging technology that has been proving quite effective in the removal of high organic strength wastewaters as well as for having diminished operational technicalities. On the other hand, dairy industry effluents are an environmental burden due to their high organic load and large volume of discharge, that, pose a threat to the environment when discarded with inadequated or incomplete treatment.

The present work aims to compare two types of biological treatment. On one side, the Activated Sludge process, which is the most common biological treatment, employed in several applications, from municipal to industrial wastewater treatment plants. On the other side, the MBBR, developed in Norway due to external pressures to develop a good technology for Nitrogen removal, results from the fusion between suspended-growth and attached growth (biofilm) processes, retaining the best of both.

An experimental arrangement was set, comprised of three single staged, laboraty scale reactors: one Activated Sludge reactor (AS) and one Moving Bed Biofilm Reactor (MBBR), both operating in continuous flow mode, and one Moving Bed Biofilm Reactor operated in sequencial batch mode (MB-SBR). The reactors were feeded with synthethical wastewater, simulating a dairy industry effluent, by diluting market low fat milk in water. The organic load was regulated by adjusting the milk ratio in the dilution, being 1 part of milk to 200 of water the lowest dilution tested, corresponding to a chemical oxygen demand of roughly 600 mg/L, Total Carbon of 256 mg/L, and Total Nitrogen of 52 mg/L. The dilutions tested were 1/200, 2/200, and 4/200, which resulted in period A,B, and C, respectively.

The conducted experiments tested the removal capabilities in respect to carbonaceous and nitrogenous matter as well as the quantification of the biomass produced and the excess sludge wasted.

The experimental results obtained conferred very high removal capabilities to all reactors, with special regard to the batch reactor (MB-SBR). However, it was the most sensible to the increase in the organic load. The continuous reactor had a very similar behaviour in respect to carbonaceous matter removal, in which 89.6 % and 92.1 % was the Chemical Oxygen Demand removal, for the AS and the MBBR, respectively. Regarding Total carbon, the global removal efficiencies obtained were 90.6 %, 92.17 %, and 95.6 %, for the AS, MBBR, and the MB-SBR, respectively. Concerning the quantification of excess sludge, the MBBR came with an impressive advantage, producing roughly 50 % of the amount produced in the AS reactor, and 23 % of the amount produced in the MB-SBR. Total Nitrogen results revealed an advantage to the reactor operating in batch mode due to the existence of both anoxic and aerobic periods in the MB-SBR cycle of operation.

**Key words:** Biological treatment, dairy industry, MBBR, Activated Sludge, carbonaceous matter, biomass production, total nitrogen.

# Resumo

O processo designado por Reator de Biofilme com Leito Móvel (MBBR - "Moving Bed Biofilm Reactor) é uma tecnologia emergente que tem dado frutos, nomeadamente, ao nível da facilidade de operação e altas taxas de remoção de carga orgânica. Por outro lado, os efluentes de uma indústria de laticínios caracterizam-se pelo seu grande volume e, mais precisamente, pelo seu elevado teor orgânico que, quando submetidos a tratamento inadequado, geram impactes ambientais pronunciados no meio onde são descarregados.

O presente trabalho teve como finalidade a comparação de dois tipos de tratamento biológico. De um lado, o tratamento por Lamas Ativadas, que consiste no método mais difundido e empregado na maioria dos casos onde o tratamento biológico é eficaz, desde ETAR municipais a ETAR de águas indústriais. Do outro lado, a tecnologia MBBR, desenvolvida na Noruega nos anos 90, tem mostrado um elevadíssimo potencial ao combinar o melhor dos processos de biomassa suspensa e fixa, sem reter o pior de ambos.

Foi montado um sistema experimental constituído por três reatores de escala laboratorial: um reator de lamas ativadas (AS), um reator MBBR, ambos a operar em modo contínuo, e um reator MBBR a operar em modo sequencial (MB-SBR). O efluente utilizado é constituído através de uma diluição de leite e água, regulando-se a diluição conforme o teor orgânico que se queira obter, sendo que a diluição de 1/200 corresponde a uma carência química de oxigénio de 600 mg/L, carbono total de 256 mg/L e azoto total de 52 mg/L. A carga orgânica duplicou-se duas vezes de 1/200 para 2/200 e para 4/200 constituíndo os períodos A,B e C, respectivamente. As experiências incidiram essencialmente em avaliar a capacidade dos processos para a remoção de matéria orgânica e azoto, ao mesmo tempo que se avalia a produção de lamas.

Os resultados obtidos conferiram uma elevada capacidade de redução de carga orgânica aos três reatores, com especial proeminência para o reator MB-SBR, no entanto foi o que mais sentiu o aumento da carga orgânica. Os reatores contínuos tiveram eficiências bastante semelhantes,

sendo 89.6% e 92.1%, para o reator AS e MBBR, respetivamente e em termos da carência química de oxigénio. Referindo-se ao carbono total, tem-se 90.6 %, 92.1 %, e 95.6 %, para o AS, MBBR, e MB-SBR, respetivamente. De salientar que o reator MBBR produziu cerca de 50 % menos lamas que o reator AS e menos 23 % que o seu congénere a operar em modo sequencial (MB-SBR). Em termos da remoção de azoto, a existência de períodos anóxicos e aeróbios no reator MB-SBR determinaram maiores eficiências relativamente aos reatores contínuos.

**Palavras-chave:** Tratamento biológico, indústria láctea, MBBR, Lamas Ativadas, matéria carbonatada, produção de lamas, azoto total.

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# Chapter 1

# Introduction

## 1.1 Motivation

The increase of water deterioration has been one the most important environmental concerns over the past decades together with the increase of water scarcity. Water quality is being gradually diminished thus limiting the sources for water supply. Such conditions can be linked to the explosive demographic and industrial growth over the past century which lead, naturally, to an increase of water demand and disposal of liquid wastes, such as untreated sewage or industrial waste, which are the major sources of pollutants in developing countries (Ali et al., 2011). An environmentally sound management is thus imperative in order to minimize the harmful outcomes from an incorrect waste disposal on the receiving environment, and ultimately on the human being wellness.

Globally, industrial wastewater represents the main source of water pollution (Ali et al., 2011). Amongst all industrial activities, despite having a vital economic importance on developed countries (FAO, 1997), the food sector has one of the highest consumptions of water and is one of the biggest sources of effluents per unit of production, in addition to generating a large volume of sludge in biological treatments (Tikariha and Sahu, 2014). With that in mind and, in order to fulfill regulatory requirements, environmental sustainability is a key aspect that contributes to a proper management of this sector, namely the dairy industry.

Dairy operations can consume large volumes of water to grow feed, cows, as well as to manage manure and process products. Additionally, manure and fertilizer run-off from dairy farms can significantly pollute water resources. The increased nutrients in local waterways contributes

to eutrophication processes which threats the aquatic ecosystem. Also, with the exponential increase in human population, the agroalimentar sector needs to step-up in order to meet the global demand for food. More production may ultimately lead to more waste and pollution.

The increasing awareness about the environmental impact of discharges is leading many investors into building new or upgrading existing wastewater treatment plants. However, the increase of urbanization diminishes the available area for building these new plants. In addition, the requested space for the conventional activated sludge treatment (AS) would be excessive in case of very high pollutant removal efficiency requirements. In order to improve the quality of treated wastewater and meet the demands of environmental regulations, implementation of advanced technologies for treatment is required (Di Trapani et al., 2010).

Moreover, with the rising costs of sludge disposal, the minimization of sludge production has become increasingly important. According to Egemen et al. (2001), the expense of excess sludge treatment has been estimated to be 50–60% of the total cost of municipal wastewater treatment. Therefore, modifications to existing aerobic treatment processes capable of reducing biosolids production are promising and of highly interest (Kulikowska et al., 2007; Ødegaard, 2004).

For this reason, the moving bed biofilm technology is an interesting approach that can override the flaws exhibited by both suspended-growth and attached-growth technologies and potential contribute to an overall better polished effluent and reduced sludge production. It is already being considered as an upgrade option for an increasing number of wastewater treatment facilities due to its small footprint and ease of operation (Forrest et al., 2016). However, in Portugal, MBBR application is still incipient whereas most of the wastewater treatment plants are based in activated sludge processes.

## 1.2 Aim

The aim of this thesis is to provide an experimental comparison between the conventional Activated Sludge and the Moving Bed Biofilm Reactor for treating dairy wastewater. In order to fulfill this, three independent labscale reactors, operated simultaneously, were implemented, a continuous Activated Sludge, and both continuous and batch Moving Bed Biofilm Reactor. Experiments will evaluate:

- The capabilities for removing carbonaceous organic matter and total nitrogen while subject

to different organic loading rates;

- The biomass growth and its quantification in terms of sludge wasted;
- Preliminary determination of kinetics in batch mode utilizing a simplified Monod approach.

## 1.3 Outline of the thesis

- Chapter 1: Introduction. An overall view of the environmental problematic associated to liquid effluents disposal as well as the motivation for developing new technologies.
- Chapter 2: Theory and Literature Review. It presents the fundaments behind biological treatments, refering the most common processes and biochemistry, and explaining the basis of biological reactors such as Activated Sludge and Moving Bed Biofilm Reactor.
- Chapter 3: Dairy Industry Characterization and Environmental Issues. Goes over the production processes of a typical dairy industry as well as shows the common characteristics of dairy wastewaters and the treatment methods employed. In addition, the environmental burden of the wastewaters is exposed as well as the portuguese regulations behind their discharge to the environment.
- Chapter 4: Materials and Methods. Provides description for the experimental arrangement, the conducted experiments, as well as the utilized analytical procedures.
- Chapter 5: Results and Discussion. Presents the development of the experiments as well as the discussion of the obtained experimental results.
- Chapter 6: Conclusions and Future Work. Presents the final conclusions and suggestions for future work.

# Chapter 2

# Theory and Literature Review

## 2.1 Biological wastewater treatment principles

Wastewater treatment processes may be grouped into two general categories, the first being physical/chemical. This category includes screening, sedimentation, filtration, precipitation, and chemical destruct systems. The second category, biological, includes processes which rely on living organisms to remove pollutants from the wastewater. The microbial consortia includes bacteria, protozoa, fungi, and rotifiers (Metcalf and Eddy, 2003). Biotreatment is considered advantageous both in terms of capital investment and operational costs over other processes such as chemical oxidation (Sofiyanti et al., 2015).

Wastewaters are characterized in terms of their physical, chemical, and biological composition. The principal contituents of concern in wastewaters are:

- **Suspended Solids** - Can lead to the generation of sludge deposits in water bodies and further development of anaerobic conditions.
- **Biodegradable organics** - Mostly proteins, carbohydrates, and fats that exerts an oxygen demand in the aquatic environment. Commonly measured in terms of its chemical oxygen demand (COD) or biochemical oxygen demand (BOD). The latter typically after a 5 day degradation period ($BOD_5$).
- **Pathogens** - Infectious Organisms. Appearance by the excretion to wastewater drainage systems by sickened individuals.
- **Nutrients** - Inorganic compounds that are essential for growth. Wastewater discharge of

high concentrations leads to over development of undesired aquatic life, or groundwater pollution if discharged on land.

- **Priority pollutants** - Organic and inorganic compounds suspected of carcinogenicity, mutagenicity, teratogenicity, or toxicity.
- **Refractory organics** - Compounds that resist to conventional treatment methods or are originated from incomplete treatment reactions.
- **Heavy metals** - Compounds that cannot be degraded or destroyed. Appearance on water bodies mainly from industrial wastes and surface drainage. Toxic and poisonous at low concentrations and leads to bioaccumulation.

Biological treatment units are designed for carbon matter removal and nutrient removal such as nitrogen (N) and phosphorous (P).

Biological wastewater treatment employs microorganisms and their metabolic pathways in order to transform dissolved and particulate biodegradable constituents into acceptable end products while setting up appropriate conditions for microoganisms to floculate and form a sludge (Metcalf and Eddy, 2003). The organic matter content is usually expressed in terms of COD and BOD, as stated before. The basic aim in the operation of a biological treatment plant is to create conditions that favor the desired reactions and to attain maximum dissolved oxygen demand reduction of the effluent (Sharma and Ahlert, 1977).

This type of treatment is widely present in wastewater treatment plants and is preceded by a preliminary and/or a primary treatment which uses physical screening and/or sedimentation to remove coarse material, grit and mostly all suspended pollutants, and with it a significant share of contaminants that contribute to oxygen depletion on water bodies. Finally, wastewater treatment plants may also include disinfection systems such as ozonation or exposure to ultra violet radiation prior to discharge (Hoang, 2013). Figure 2.1 represents a common configuration of a wastewater treatment plant.

Biological processes can occur in aerobic, anoxic or anaerobic conditions or even under their combination. Each one has specific conditions for microbial growth and operational procedures. Two major categories are found in biological treatment, one being suspended-growth and the other being attached-growth processes (Metcalf and Eddy, 2003). In suspended-growth the microorganisms are maintained in liquid suspension by adequate mixing methods. The most common suspended-growth process is the Activated Sludge process (AS). Other examples are aerated la-

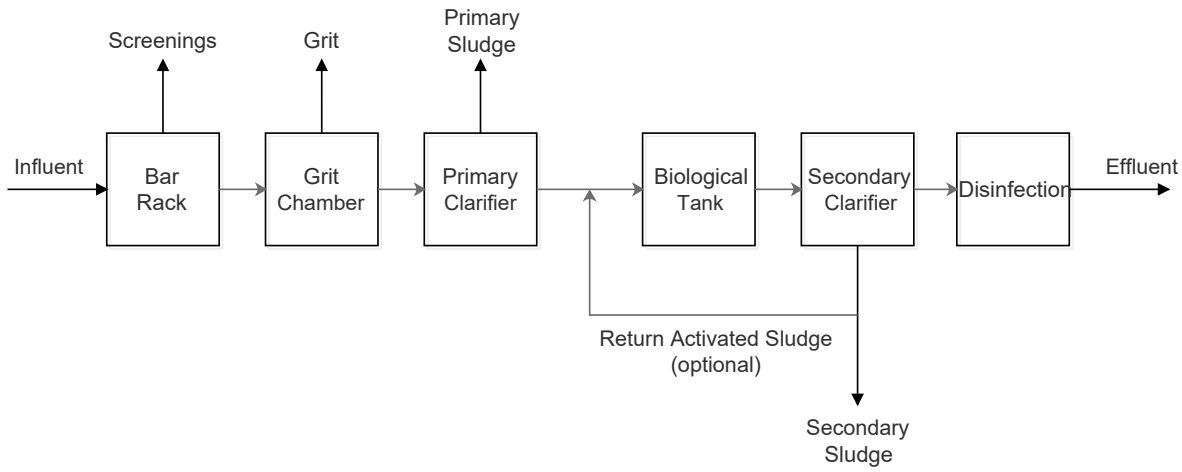



**Fig. 2.1:** Process flow diagram of a wastewater treatment plant employing biological treatment and a final disinfection (Adapted from Metcalf and Eddy (2003))

goons or upflow anaerobic sludge blanket digesters. While in suspension, microorganisms form flocs which aggregate the microbial consortia, and both organic and inorganic particles. On the other hand, in attached-growth processes, the microbian communities naturally attach to inert surfaces that could be either fixed, such as in Trickling Filter's which use packing materials such as rock, slag, sand, or plastic, or suspended, as in the Moving Bed Biofilm Reactor process, forming a biofilm in solid supports. Other example of a biofilm process is the Rotated Biological Contactor in which the biofilm, adhered to the surface of partially submerged rotating discs, is allowed to alternate between exposure to atmospheric air or the wastewater. Biofilm reactors are especially useful when slow growing organisms like nitrifiers have to be kept in a wastewater treatment process (Kermani et al., 2009). Figure 2.2 represents the different types of biomass growth.

Microbial growth is a response to the physio-chemical environment, in which the microbial cells replicate under the influence of four main components: a carbon source, an energy source, a terminal electron acceptor and nutrients. The metabolism comprises a series of redox (reduction-oxidation) reactions that regulate the energy required for cell synthesis, maintenance and endogenous decay; can be either anabolic, in which cells build molecules from smaller ones, or catabolic, in which bigger molecules are broken down into smaller ones. As with all redox equations it envolves an electron donor and an electron acceptor (oxidizing agent) (Hoang, 2013). The microorganism release enzymes which act as a biocatalyst for the naturally slow reactions.

Organism classification based on electron acceptor and common reaction names is shown in Table 2.1.

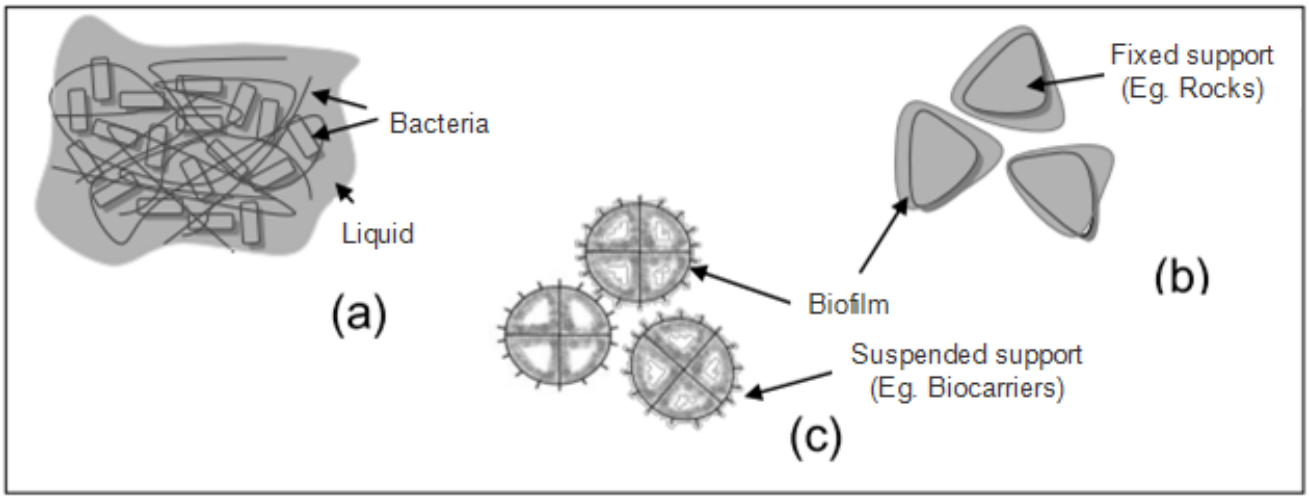



**Fig. 2.2:** Schematic representation of the different types of biomass growth and sustain (Adapted from Von Sperling (2007)): a) suspended-growth; b) fixed attached-growth; c) suspended attached-growth

**Table 2.1:** Organism classification based on carbon source, electron donor, electron acceptor and end products (Adapted from Metcalf and Eddy (2003))

| **Classification** | **Common reaction names** | **Carbon source** | **Electron donor (substrate oxidized)** | **Electron acceptor** | **End products** |
|---|---|---|---|---|---|
| Aerobic heterotrophic | Aerobic oxidation | Organic compounds | Organic compounds | $O_2$ | $CO_2$, $H_2O$ |
| Aerobic autotrophic | Nitrification | $CO_2$ | $NH_3^-$, $NO_2^-$ | $O_2$ | $NO_2$, $NO_3$ |
| | Iron oxidation | $CO_2$ | $Fe^{II}$ | $O_2$ | $Fe^{III}$ |
| | Sulfur oxidation | $CO_2$ | $H_2S$, $S_2O_3{}^2$ | $O_2$ | $SO_4{}^2$ |
| Facultative heterotrophic | Denitrification | Organic compounds | Organic compounds | $NO_2$, $NO_3^-$ | $N_2$, $CO_2$, $H_2O$ |
| Anaerobic heterotrophic | Acid fermentation | Organic compounds | Organic compounds | Organic compounds | VFA's |
| | Sulfate reduction | Organic compounds | Organic compounds | $Fe^{III}$ | $Fe^{II}$, $CO_2$, $H_2O$ |
| | Methanogenesis | Organic compounds | Volatile fatty acids | $CO_2$ | $CH_4$ |

The process of aerobic biodegradation can be described by the following equation:

$$\text{Organic Material} + O_2 \xrightarrow{\text{Bacterial Activity}} CO_2 + H_2O + \text{New Bacteria} \tag{2.1}$$

The particulate organic material needs to be firstly adsorbed by the microbial flocs and broken down under enzimatic activity to the size where it's possible its absorption into the cell, while solube organic matter can be readily absorved and metabolized by the cell.

On the other hand, the anaerobic biodegradation,

$$2\,CH_2O \xrightarrow{\text{Bacterial Activity}} CO_2 + CH_4 \tag{2.2}$$

When utilizing pure microbial cultures, bacterial growth can be characterized by four distinct phases: lag, exponencial growth, stationary, and death phase, which can be represented by the Monod growth curve (Monod, 1949). Description of these phases are presented below aswell as a typical representation of this behaviour in Figure 2.3.

- Lag phase is characterized by the culture inoculation and posterior acclimation to the new environment before biomass production begins to occur. Acclimation consists on enzyme induction and adaptation to pH, salinity, or temperature (Metcalf and Eddy, 2003). Its extension on time depends upon the inoculum age.
- Exponential growth phase is characterized by a maximum growth of bacteria due to high substrate availability and proper acclimation.
- Stationary phase represents a net biomass growth of zero where substrate availability has become limited or inhibition might be occuring.
- Death phase represents the decline of biomass concentration due to substrate depletion.

Biomass is often refered to the volatile suspended solids (VSS) concentration or in lesser extent, to the total suspended solids, or particulate COD concentration in a biological reactor.

Kinetics expressions are equations used to represent the growth rate of cells and the removal of constituents within a system. The reaction rate is the term used to describe the change of a reactive substance and can be expressed by the following equation, under constant volume:

$$r = k[C]^n \tag{2.3}$$

where $k$ is the kinetic coefficient, $C$ the substrate concentration in mg/l, and $n$ is the order of

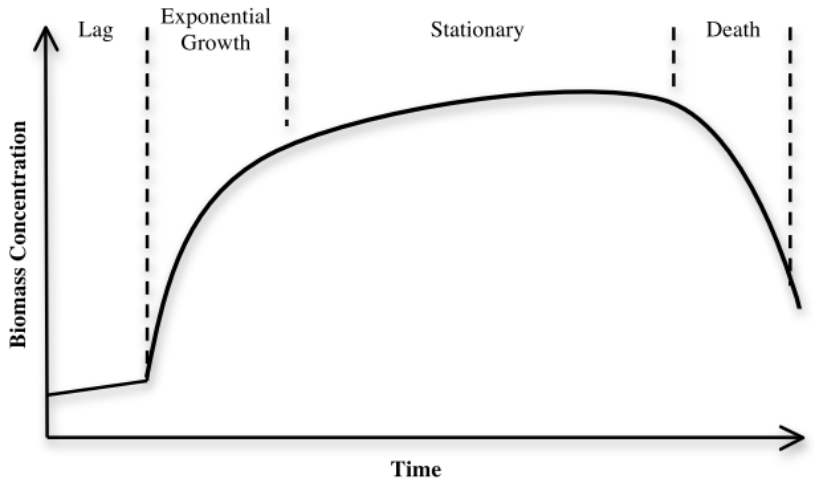



**Fig. 2.3:** Monod growth curve (Copyright from Hoang (2013))

reaction. Common rates are represented by zero, first, or second order.

## 2.2 Biological Nutrients Removal

The accumulation of nitrogen and phosphorus compounds by discharge of wastewater is one of the main causes for eutrophication in water bodies such as lakes and rivers. It is, therefore, necessary to remove these substances from wastewaters for reducing their harm to the environment (Wang et al., 2006). For this reason, the biological processes of nitrification and denitrification are commonly employed. In wastewaters, nitrogen may be found in four forms: organic nitrogen, ammonia nitrogen, nitrite nitrogen, and nitrate nitrogen. In fresh wastewater the nitrogen present is primarily combined in proteinaceous matter and urea as organic nitrogen. Decomposition by heterotrophic bacteria, known as ammonification, readily converts organic nitrogen to ammonia (Cheremisinoff, 1996). The Equation 2.4 shows the ammonia equilibrium in water.

$$NH_3 + H_2O \longleftrightarrow NH_4{}^+ + OH^- \tag{2.4}$$

The presence in the ionized ($NH_4{}^+$) or unionized form ($NH_3$) is controlled by either an acidic or alkaline pH, respectively. In addition, ammonia has been reported toxic to freshwater organisms at concentrations above 0.2 mg/L. The toxicity is primarily attributable to the un-ionized form. Plus, wastewater containing ammonia exerts a nitrate oxygen demand contributing to the total biochemical oxygen demand of the water and ultimately to oxygen depletion on receiving water bodies. Efforts are being made to improve exhisting wastewater treatment plants in order to

accomodate to even more restricting regulations on nitrogen discharge.

On the other hand, anthropogenic phosphorous addition to water bodies is also contributing to the excessive growth of algal organisms leading to low dissolved oxygen concentration. Over-nutrient enrichment is more dangerous towards lakes or stagnant waters as they have a lower capacity for restoring oxygen due to lower transfer coefficient. Likewise for nitrogen, progressively stringent phosphorous discharge limits invariable leads to the upgrade of existing facilities or the implementation beyond the traditional methods.

There are three main categories for phosphorous removal:

- **Physical methods** such as electro-dialysis and membrane technologies, generally expensive and with removal efficiencies as low as 10% (Wang et al., 2006).
- **Chemical methods** comprehend the most well-established processes by means of coagulation but involves the addition of metal salts such as ferric chloride and aluminium sulfate for effective phosphorous precipitation. In addition, produced sludge containing heavy metals is also an environmental concern.
- **Biological methods** have been recently gaining interest as they can remove up to 97% of the total phosphorous (Wang et al., 2006) at relatively lower costs and with minimal additional sludge production through Enhanced Biological Phosphorous Removal (EBPR). However this process can be highly variable due to operational difficulties (Özacar and Şengil, 2003) and overall of difficult implementation. Nonetheless EBPR poses an interesting alternative to chemical precipitation and will be presented further on subsection 2.2.2.

### 2.2.1 Nitrogen

#### 2.2.1.1 Nitrification

Nitrification consists in the first part for nitrogen treatment in wastewater and is a two step reaction in which ammonia is oxidized to nitrate. Performed by autotrophic bacteria, under aerobic conditions, ammonia is firstly oxidized to nitrite according to Equation 2.5.

$$NH_4{}^+ + 1.5\,O_2 \xrightarrow{\text{Nitrosomonas}} 2\,H^+ + H_2O + NO_2{}^- \tag{2.5}$$

Followed by a nitrite oxidation to nitrate according to Equation 2.6.

$$NO_2{}^- + 0.5\,O_2 \xrightarrow{\text{Nitrobacter}} NO_3{}^- \tag{2.6}$$

Ammonia oxidizing bacteria (AOB) are responsible for the first step oxidation and in this group *Nitrosomonas* is the dominant bacteria. The second step is accomplished by Nitrite oxidizing bacteria (NOB), commonly known as *Nitrobacter*. Other autotrophic bacteria genera such as *Nitrococcus, Nitrospira, Nitrospina*, and *Nitroeystis* (prefix with *Nitro-*) can also oxidize nitrite-N to nitrate-N (Metcalf and Eddy, 2003). Nitrite oxidation is happening simultaneously with ammonia oxidation. As soon as nitrite and oxygen is available the reaction can occur. The complete reaction is therefore:

$$NH_4{}^+ + 2\,O_2 \xrightarrow{\text{Nitrification}} 2\,H^+ + H_2O + NO_3{}^- \tag{2.7}$$

The quantity of dissolved oxygen (DO) necessary for full nitrification is $4.57\,\text{g-}O_2\,/\,\text{g-}NH_4{}^+$ oxidized which can be obtained directly from the stoichiometry relationship in Equation 2.7. Moreover, from the stoichiometric relationships of Equation 2.5 and Equation 2.6, it is observed that more oxygen is required for ammonia oxidation than for nitrite oxidation, respectively $3.43\,\text{g-}O_2\,/\,\text{g-}NH_4{}^+$ and $1.14\,\text{g-}O_2\,/\,\text{g-}NH_4{}^+$. The necessary alkalinity required for nitrification is 7.14 $\text{g-}CaCO_3\,/\,\text{g-}NH_4{}^+$ which is determined from the stoichiometric relationship shown in Equation 2.8 (Metcalf and Eddy, 2003).

$$NH_4{}^+ + 2\,HCO_3{}^- + 2\,O_2 \longrightarrow NO_3{}^- + 2\,CO_2 + 2\,H_2O \tag{2.8}$$

The release of Hydrogen ions together with the alkanility consumpton can be enough to bring pH to undesirable levels if the reactor is not sufficiently buffered and may compromise the effectiveness of the process. Rusten et al. (2006) tests at a marine fish farm showed that the nitrification rate in the MBBR at pH 6.7 was only 50% of the nitrification rate observed at pH 7.3.

There are other variables which may interfere significantly. The presence of organic matter will slow down or stop the nitrification process. This is because heterotrophs and nitrifiers will compete for available oxygen and the rapidly growing heterotrophs will dilute or wash-out the

nitrifiers (Gönenç and Harremoës, 1990). Grady et al. (2011) states that the maximum growth rate of heterotrophic bacteria is roughly five times that of nitrifiers. Therefore, maximum nitrification rates are achieved with a minimum COD on the effluent and high oxygen concentration on the reactor as reported by Ødegaard (1999). Nitrification rate has a nearly linearly dependency on the oxygen concentration as seen in Figure 2.4.

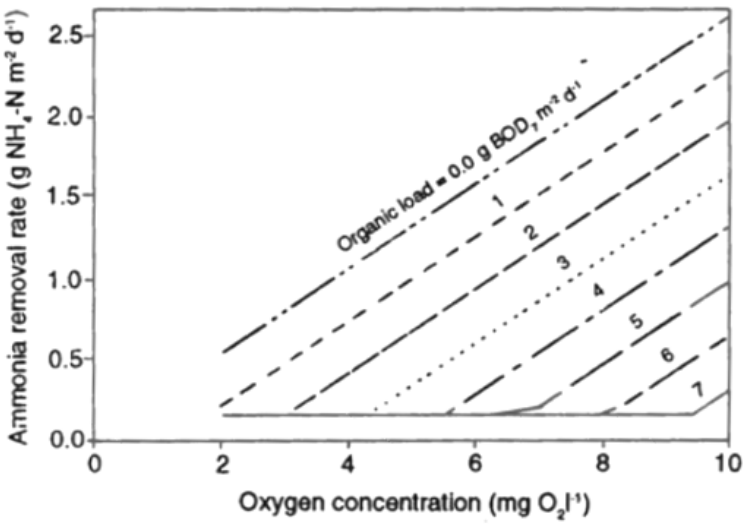



**Fig. 2.4:** Curves for the ammonium removal rate at $15^{o}C$ taking both the oxygen concentration and the organic load into consideration. The lines in the figure represent different organic loads up to $7gBOD_7/m^2.d$ (Copyright from Ødegaard (1999)).

Also, nitrifying bacteria have more specific environmental conditions for growth and are less sensible to temperature variations, oxygen concentration, pH, and to the presence of inhibitory compounds. Temperature effects on the nitrification process were also studied.

According to Rusten et al. (1994), although lower temperatures have an inhibition effect on the nitrifying organisms, the increasing effect on the oxygen solubility may mask this effect and compensate for the lower temperature. However, appropriate air supply rate must be delivered and oxygen cannot be the limiting substrate. Normally oxygen will be the rate limiting substrate at high total ammonium nitrogen (TAN) concentrations, and TAN will be the rate limiting substrate at low TAN concentrations or, alternatively, when the ratio of oxygen to ammonia is lower than 2g O2 /g NH4-N and higher than 5g O2 / gNH4-N, respectively (Salvetti et al., 2006).

#### 2.2.1.2 Denitrification

As stated before, nitrogenous compounds (ammonia, nitrite and nitrate) can cause a significant depletion of dissolved oxygen in receiving waters, exhibit toxicity towards fish, and therefore, decrease the productivity of streams and lakes, and present a public health hazard. Nitrification fails to completely remove nitrogen from wastewater, therefore a subsequent treatment is required. Total nitrogen removal is achieved by a following denitrification process consisting in reducing the nitrate to gaseous nitrogen. Under anoxic conditions, nitrate goes through the following transformation.

$$NO_3{}^-(aq) \longrightarrow NO_2{}^- \longrightarrow NO \longrightarrow N_2O \longrightarrow N_2(g) \tag{2.9}$$

The complete denitrification mechanism is exhibited in Equation 2.10.

$$NO_3{}^- + \text{carbon source} \xrightarrow{\text{denitrification}} N_2 + CO_2 + H_2O + OH^- + \text{new bacterial cell} \tag{2.10}$$

It is performed by facultative heterotrophic bacteria that, under anoxic conditions, replace oxygen for nitrate as electron acceptor. The necessary conditions for denitrification to occur are resumed below: (Cervantes et al., 2006)

- **Presence of nitrate and absence of dissolved oxygen in the mixed liquor:** In order to occur denitrification, anoxic conditions must be accomplished in the reactor. Dissolved oxygen inhibits denitrification and microorganisms will quickly change to an oxygen pathway as it is the preferred oxidant of most bacteria for organic material. The nitrate substrate required for denitrification is often supplied by a previous nitrification process.
- **Presence of suitable bacterial sludge mass:** Most bacteria present in aerobic treatment systems are facultative, therefore, in the absence of oxygen, they can use nitrate to oxidize organic material.
- **Adequate environmental conditions for the microorganisms:** Likewise to all biological processes, abiotic factors such as temperature and pH will have an influence in denitrification rates. Temperature will have a positive effect on denitrification up to 40°C whereas pH should be maintained neutral. Presence of inhibitory compounds can greatly reduce denitrification rates.
- **Presence of organic substrate:** Nitrate reduction to nitrogen gas must be accomplished in the presence of biodegradable organic substrate as electron donor. Could be the organic material present in the influent (pre-denitrification) or introduced from an external source

(post-denitrification), typically methanol or acetic acid.

The Pre-denitrification basic schematic is shown below (Figure 2.5). The system is comprised by two reactors in series, in which the anoxic precedes the aerobic reactor. This way, the organic matter present in the influent is used by the denitrifying organisms. On the next stage, oxygen provides the necessary conditions for nitrification to occur. The nitrate formed in the aerobic reactor is recirculated back to the anoxic for a complete effluent denitrification. For this reason, The nitrate recirculation control is one of the major aspects that contributes to the efficiency of these systems (Von Sperling, 2007). Because most of the organic matter is biodegraded on the anoxic reactor, this system permits the reduction of the aeration tank volume and thus the aeration costs. However, the high nitrate recirculation is considered a trade-off because it increases operational costs.

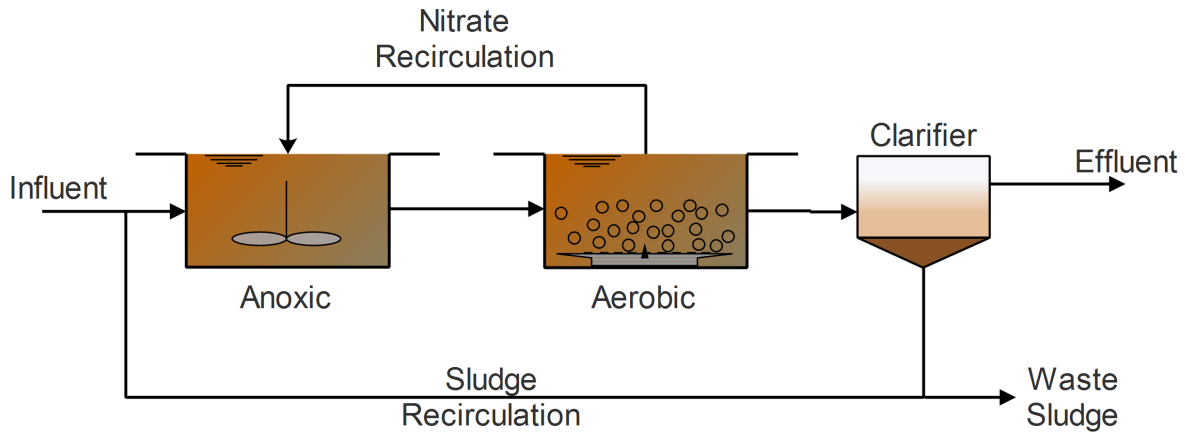



**Fig. 2.5:** Common pre-denitrification schematics

Post-denitrification (Figure 2.6) differs from the previous configuration by having the aerobic, in which organic matter removal and nitrifcation are occurring simultaneously, preceding the anoxic tank, in which the nitrate produced previously is reduced to nitrogen gas. Because the heterotrophs are directly competing with the nitrifiers in the first reactor, a subsequent aerobic reactor could be used to further improve nitrification since organic matter will not be available for heterotrophs to disrupt the nitrifiers. For maximum denitrifications rates on the anoxic reactor, most of the times an external carbon source is used, increasing operational costs.

When choosing predenitrification, biochemical oxygen demand removal should be kept at a minimum. On the other hand, post-denitrification alternative should be accomplished with maximum

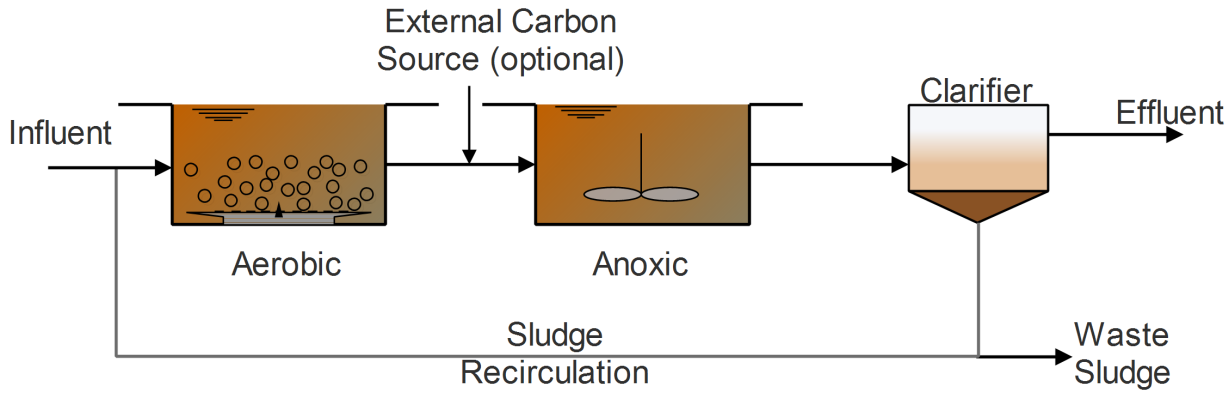



**Fig. 2.6:** Common post-denitrification schematics

BOD removal beforehand in order to reduce size of the reactor (Rusten et al., 1995). The ratio of COD added as external source to the nitrate entering the denitrification reactor is the key parameter when designing post-denitrification units (Rusten et al., 1994).

Four-Stage Bardenpho process comprises the combination of both pre and post-denitrification processes ending in a final reaeration tank. Corresponds to the process with higher nitrogen removal capabilities but also with the larger required total reactor volume (Von Sperling, 2007).

Immobilization of biomass in the form of biofilms is an efficient method to retain slow growing microorganisms, such as nitrifiers, in continuous flow reactors (Wang et al., 2005).

### 2.2.2 Phosphorous

Biological phosphorus removal is performed by phosphate accumulating micro-organisms (PAO) that have the ability to accumulate phosphate over and above what is required for growth. It is performed by microorganisms with specialized metabolic pathways. Although being heterotrophic, *Acinetobacter*, these microorganisms, under anaerobic conditions, can absorb fermentation products such as volatile fatty acids (VFA) and store them until oxygen is available for organic matter oxidation. Thereby, to provide suitable conditions for Enhanced Biological Phosphorous Removal (EBPR) in a treatment plant, the biomass must be exposed to alternating anaerobic and aerobic or anoxic conditions. This can be done in a sequencing batch reactor (SBR), or by moving the biomass from one reactor to another in a continuous process (Helness, 2007).

For the BOD absorption to happen, phosphorous must be released to provide the necessary energy. When oxygen becomes available, *Acinetobacter* grows new biomass and take up, typically, more phosphorous than the one released. This phosphorous release and uptake mechanism is shown on the next figure.

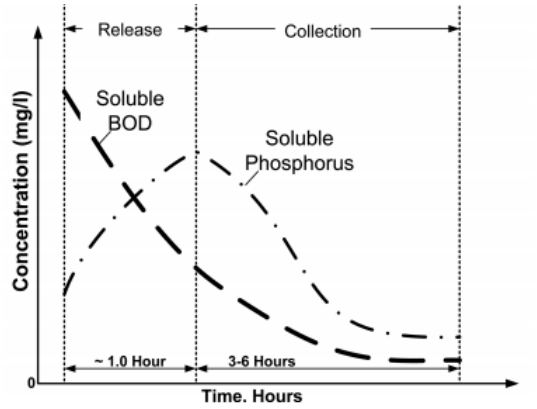



**Fig. 2.7:** Relationship between soluble BOD and Phosphorous on the different stages of EBPR (Adapted from Water Environment Federation (2007))

Biological phosphorus removal is dependent upon the uptake of phosphorus in excess of normal bacterial metabolic requirements and is proposed as an alternative to chemical treatment (Yeoman et al., 1988) In addition, some wastewater facilities have reported that operating in the EBPR mode provides superior sludge settling (Water Environment Federation, 2007).

## 2.3 Activated Sludge Process

Developed in 1917 by Ardnet & locke, the activated sludge process consists on having suspended microorganisms on a tank or reactor, feeding on wastewater pollutants and thereby consuming the required substrates for several metabolic reactions, leading to the creation of new cells and bacterial growth. It is the most known biological wastewater treatment process around the world and it has proved to be both effective and reliable for the majority of domestic or industrial applications. However, big reactor volumes are required when dealing with high strength wastewater and, in general, when high efficiencies are required. Moreover high energy costs due to aeration and pumping equipments, and high biomass production leads to relatively high operation costs and problems with the disposal of large amounts of sludge (Bazari, 2004). In addition, high biomass loadings onto the clarifiers demands the construction of bigger clarifying tanks or higher retention times to provide full settling and good effluent quality.

Figure 2.8 represents the basic schematic of an Activated Sludge (AS) unit, in which a reaction tank, a settling tank (clarifier), and mechanisms for sludge recirculation and excess sludge disposal have to be accounted for.

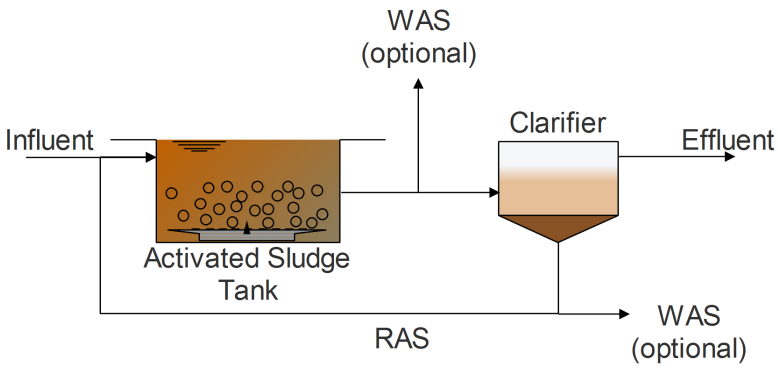



**Fig. 2.8:** Schematic of a Conventional Activated Sludge unit

The biochemical degradation reactions take place in the reaction tank where the micro-organisms reproduce continuously, forming an active sludge. This sludge is removed from the water by settling in post-sedimentation basins due to its proprieties of flocculation The settled sludge forms a blanket in the sedimentation unit and is pumped back to the reactor as recirculated activated sludge (RAS) in order to keep high concentrations of microorganisms and the biological breakdown process going. Clarified effluent is discharged to the recipient or directed to further treatment. Excess sludge coming from bacterial growth has to be wasted and redirected to additional treatment as it may contain several compounds that were present on the wastewater and also because it contains high quantities of living microorganisms that, if discarded without any treatment, pose a threat to the environment. Therefore, sludge treatment or stabilization is a must when dealing with sludge generating processes, however, its treatment goes outside the scope of this thesis. Sludge is wasted either from the activated sludge tank effluent, or from the concentrated sludge in the settling tank and before entering the return sludge line. The latter is usually the common and the best approach when dealing with high amounts of sludge and big AS units.

The successful operation of an activated sludge plant is highly dependant on the efficiency of the solid-liquid separation, which in turn, depends on the quality of the sludge. The quality is, therefore, a function of the sludge settling and thickening capabilities. The microbial consortia presented in the sludge is an indicative of the sludge quality. Sludge bulking is the term com-

monly used to represent the deterioration of sludge quality. Figure 2.8 represents a schematic representation of a healthy sludge floc. The presence of exaggerated amounts of filamentous bacteria is one of the deteriorating factors, which contributes to poor sludge settling.

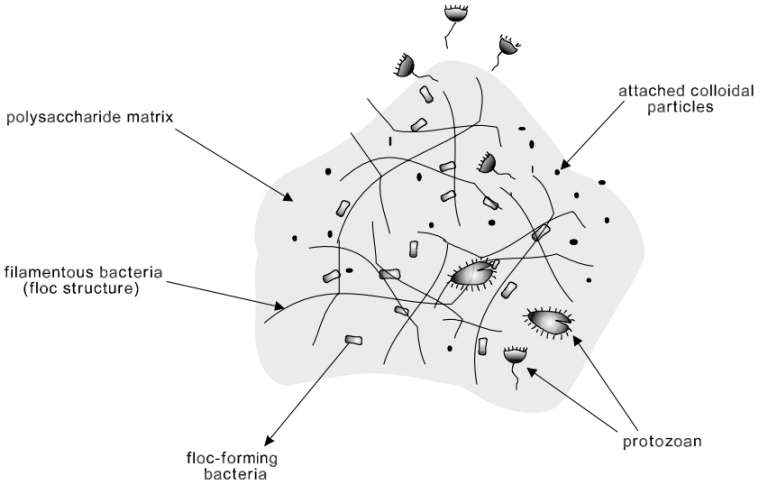



**Fig. 2.9:** Schematic representation of an activated sludge floc (Copyright from Von Sperling (2007)).

### 2.3.1 Operational control parameters

#### 2.3.1.1 Organic Loading Rate

The Organic Loading Rate refers to the amount of substrate entering the reactor, usually in terms of chemical oxygen demand (COD) or biochemical oxygen demand ($BOD_5$), related to the reactor volume. The equation 2.11 is used to calculate this parameter.

$$Organic\ Loading\ Rate = \frac{Q \times S}{V} \tag{2.11}$$

where Q is the influent flow rate, S is the influent substrate concentration, and V is the reactor volume.

#### 2.3.1.2 Food to Microorganism Ratio

The Food to Microorganism ratio (F/M) is a parameter used to maintain a balance between the quantity of substrate available, with the quantity of microorganisms presented in the reactor. Equation 2.12 represents this balance.

$$Food\ to\ Microorganism = \frac{Q \times S}{V \times X} \tag{2.12}$$

where Q, S, and V have the same meaning from the previous equation, and X refers to the concentration of microorganisms in the reactor, usually in terms of the VSS or, in lesser extent, in terms of the TSS concentration.

## 2.4 The Moving Bed Biofilm Reactor

The moving bed biofilm process has been used for many different applications despite being originally developed for upgrading norwegian wastewater treatment plants to complete nitrogen removal (Hem et al., 1994), thus most of the scientific data has been gathered from this application. Later, however, organic matter removal has been more investigated, including high-rate pre-treatment for upgrading of activated sludge plants (Ødegaard, 1999). Although MBBR is an attached growth treatment it also incorporates benefits from suspended growth systems. Presently, there are more than 400 large-scale wastewater treatment plants operating in 22 different countries of the world based on this process (Aygun et al., 2008).

The main idea behind is to have a biofilm reactor with low head-loss and high specific biofilm surface. This is achieved by having the the biofilm grow on small carrier elements that move along with the water in the reactor (Ødegaard, 1999). The source of movement needs to come either from the aeration mechanism in an aerobic reactor or a mechanical stirrer in an anaerobic reactor. Proper design of aeration grids and sieves is very important for optimum performance of the MBBR process (Rusten et al., 2006). Adequated turbulence is essential in order to promote full movement of carriers and good oxygen and substrate diffusion into the biofilm. Secondly, as a biofilm system there is no need for returning settled sludge to the reactor even at short hydraulic retention times, which simplifies the design and control of effluent clarification (Ødegaard, 2006).

The idea behind the development of the Kaldnes MBBR process was to adopt the best features of the activated sludge process as well as those of the biofilter processes, without including the worst (Rusten et al., 2006), such as need for sludge and effluent recirculations, and bigger volume tanks.

For optimal performance the carrier filling fraction (FF), which is the ratio between the bulk

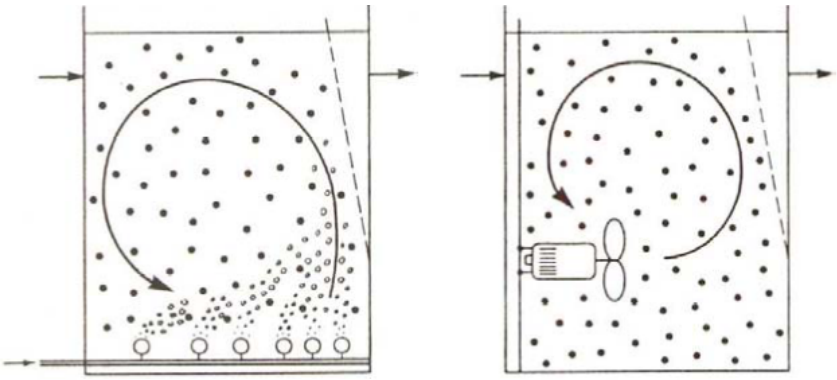


**Fig. 2.10:** Principles behind the moving bed biofilm technology (Copyright from Ødegaard (1999)).

volume occupied by the carriers and the useful volume of the reactor, has to be limited to 70% in order to be able to move the carrier freely (Ødegaard et al., 2000), and Azizi et al. (2013) studies show that anything less than 40% results in a significant performance loss in COD reduction due to lower area available for biofilm growth. However, if operating under low organic loads, 40% or less may be enough to attend a desired efficiency and thus saving on energy costs because less power is required from the aeration systems to keep a smaller number of cariers with adequated turbulence.

Different fill fractions (FF) were also investigated by Di Trapani et al. (2008). Conclusions were that reactor removal efficiency decreases after an optimal filling fraction. This was attributed to competition between suspended and attached biomass and the importance of suspended solids in the MBBR. Indeed, with an increasing fill-fraction the suspended growth concentration decreases. However, low suspended biomass can decrease the MBBR removal efficiency since they have a major role in enzymatic hydrolysis and bio-flocculation in the reactor. It was observed that a fill fraction of 35 % had higher COD removal efficiency than a 66 % fill-fraction. Whereas, a 66% fill fraction had slightly better nitrification efficiency due to higher concentrations of slow growing nitrifies which could be retained in the reactor. These results conclude that the fill fraction is an important parameter in MBBR design and performance and must be chosen based on the treatment objectives.

The constant collision of carrier media and shear in the process prevents substantial biofilm growth on the outside of the supports, making the inner effective specific surface an important design factor.

Contrary to the activated sludge process, the MBBR should not be dimensioned having in mind the volumetric loading rate (OLR) (Equation 2.11), but the surface loading rate (SOLR), which

is the organic loading rate per effective surface area of the carriers contained in the reactor, instead of referring to the reactor volume.

Hem et al. (1994) studied the nitrification process occurring in a moving bed biofilm reactor, in particular the effect of bulk oxygen concentrations, temperature and ammonia concentration and organic load on the nitrification rate. Conclusions were that the liquid film diffusion of substrate into the biofilm is the most rate limiting mechanism. More recently, the ability of the MBBR system to maintain nitrification during extensive exposure to very cold temperatures has promoted these systems as upgrade options to existing plants in the northern and colder regions (Hoang et al., 2014).

In a biofilm process, which has the biomass attached to a physical support, the concentration of suspended solids on the reactor and onto the solid-liquid separator will be much lower than in an activated sludge process, which is an advantage with respect to sludge separation (Helness, 2007). The suspended solids on the reactor is made up of biomass detached from the carrier elements and growth of suspended biomass (on a smaller extent compared to true suspended growth processes). To conclude, a smaller clarifier (or other solid-liquid separator) will be enough to provide the same efficiency because of the reduced solids loading or, alternatively, higher solid loadings can be effectively applied (Rusten et al., 1992).

Andreottola et al. (2000) stressed the following advantages of the MBBR systems: Independent biomass retention time and hydraulic retention time (HRT) due to the fixed biomass, which leads to the formation and possible selection of specialized communities for C and N removal on multi-reactor configurations; non existence of sludge recycling simplifies the whole process; better sludge settling properties and absence of bulking problems.

MBBRs have become an interesting alternative for wastewater treatment as they are a reliable and compact system due to development in their designs and operation which has resulted in decreased footprints, significantly lower suspended solid production, consistent production of high quality and reusable water and minimal waste disposal (Barwal and Chaudhary, 2014).

### 2.4.1 Biofilm mechanics

We've already understand that biofilm processes can be quite advantageous, but what are biofilms ? What kind of interactions exist in a biofilm and how are they structured ? Biofilm can be defined as a structured community of bacterial cells enclosed in a self-produced polymeric matrix and

adherent to an inert or living surface (Costerton, 1999). These structurised communities establish specific locations and develop symbiotic relationships to exploit their environment.

The inclination of bacterial adhesion to surfaces suggests a strong survival instinct and advantage over suspended bacteria (Dunne, 2002). The complex biofilm matrix formed by extracellular polymeric substances (EPS) contains polysaccharides, proteins, glycoproteins, phospholipids, nucleic acid, and humic acid (McSwain et al., 2005).

Biofilms consist of heterogeneous species that form symbiotic relationships with one another; byproducts produced by one organism can act as a substrate for another organism. At the outside will be the conversion with the highest redox potential (general aerobic oxidation), while in the inside the conditions get more reduced (anoxic, sulfate reducing and methanogenic). Within a redox zone a further biomass distribution can occur, where faster growing bacteria are generally found at the outside (e.g. aerobic heterotrophs or acidifyers) while slower growing bacteria (nitrifyers or methanogens) are more inside the biofilm.

This can be seen as an advantage due to the instalment of anaerobic communities onto an aerobic reactor and thus enabling simultaneous anaerobic and aerobic compound removal that otherwise would be impossible in a conventional activated sludge reactor. Simultaneous nitrification and denitrification can be achieved under aerobic conditions in the bulk water phase, in a process with thick biofilm. The deeper layers will be anoxic with denitrifying bacteria utilizing the nitrate produced by the nitrifiers in the outer layer (Helness and Ødegaard, 2001).

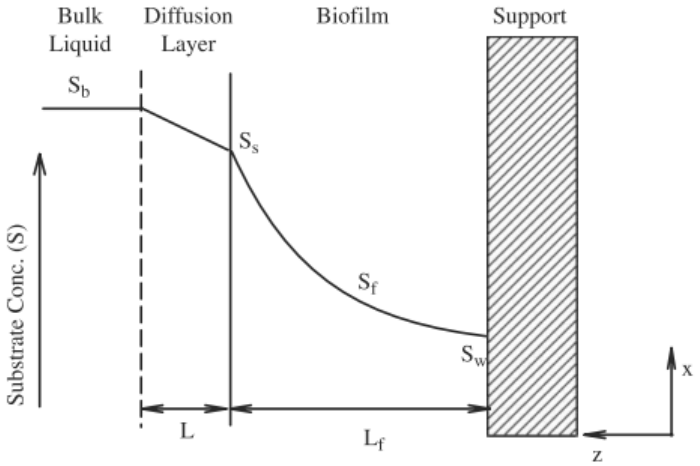



**Fig. 2.11:** Difference of substrate concentration levels in a biofilm process

The substrate and oxygen concentration within the biofilm are lower than the bulk liquid concentration (Figure 2.11). As a result the process is said to be diffusion limited.

Biofilm detachment is one of the most difficult aspects to model in a MBBR. A large variety of factors contribute to the inter-phase transport of biomass from an attached microbial film to the bulk liquid phase. This, has generally been attributed to four different processes (Characklis, 1990), including grazing (the consuming of bacteria from the outer surface of the biofilm by protozoa), sloughing (the periodic loss of large patches of biofilm), erosion (the continuous removal of small particles from the surface of the biofilm, primarily caused by liquid shear stress), and abrasion (analogous to erosion, but caused by collisions of particles).

### 2.4.2 Biocarriers

The original KMT biofilm carrier elements are made of polyethylene (density 0.92–0.96 g/cm3) and shaped like small cylinders with a cross inside the cylinder and longitudinal fins on the outside. Microscopy of the biofilm media from several pilot and full scale moving bed biofilm plants has shown no sign of biofilm growth on the outside of the smooth plastic elements. The reason is believed to be the erosion caused by the frequent collisions between the pieces. Therefore, the biofilm surface area should be calculated based on the internal (protected) surface of the plastic elements (Rusten et al., 1992) as opposed to the total surface area. It is known that the applied loading and carrier type has an influence on the morphology and thickness of the biofilm and subsequently affecting the rate of mass transfer of nutrients and substrates to the microbial community embedded in the biofilm (Young et al., 2016).

Common carrier characteristics are presented in Table 2.2, followed by visual representation Figure 2.12.

**Table 2.2:** Characteristics of common carriers used in MBBR (Adapted from (Rusten et al., 1996))

| Property | K1 | K2 | K3 | Natrix C2 | Natrix F3 | BiofilmChip M | BiofilmChip P | Bioflow 9 |
|---|---|---|---|---|---|---|---|---|
| Nominal diameter (mm) | 9.1 | 15 | 25 | 36 | 64 | 48 | 45 | 9 |
| Nominal length (mm) | 7.2 | 15 | 12 | 30 | 50 | 2.2 | 3.0 | 7 |
| Bulk density ($kg/m^3$) | 150 | 95 | 100 | - | - | - | - | 145 |

**Table 2.2:** Characteristics of common carriers used in MBBR (Adapted from (Rusten et al., 1996))

| Property | K1 | K2 | K3 | Natrix C2 | Natrix F3 | BiofilmChip M | BiofilmChip P | Bioflow 9 |
|---|---|---|---|---|---|---|---|---|
| Total specific surface area ($m^2/m^3$) | 700 | - | - | - | - | - | - | 800 |
| Specific biofilm surface area ($m^2/m^3$) | 500 | 350 | 500 | 220 | 200 | 1200 | 900 | - |
| Specific surface area at 60% filling | 300 | 210 | 300 | 132 | 120 | 720 | 540 | - |

**(a)** **(b)** **(c)**

**Fig. 2.12:** Photo of common biofilm carriers (from left to right): (a) Kaldnes *K1*, *K2* and *K3*; (b) *Natrix F3*, *BiofilmChip M*, *BiofilmChip P*; (c) *Bioflow 9*

## 2.5 Sequencing Batch Reactors

Sequencing Batch Reactors (SBRs), unlike continuous-flow operating systems, operates in a time-orientated system, in which wastewater degradation and sludge settling are carried out in a single tank and in a well-defined repeated time sequence (Morgenroth and Wilderer, 1998). Contrary to conventional systems, SBRs offer various advantages, including minimal space requirements and ease of management (Irvine et al., 1997).

Historically, AS plants first started operating in sequencing batch mode but technical difficulties in the process control at the time made the operation naturally switch to continuous-flow mode. Nowadays, these problems are overcome, with SBR systems being very popular and having significant potentional for biological nutrient removal, particularly because of the existence of anoxic

and aerobic periods which are extremely important for fully nitrogen and phosphorous removal. Irvine and Busch (1979), in the 70's, reintroduced the idea of fully operating activated sludge systems in batch mode. Irvine research team actively investigated the potential of unsteady-state processes, thereby contributing to the development of today's SBR technologies (Morgenroth and Wilderer, 1998).

There are five periods that characterize SBR operation, each lasting a defined period of time. These periods are: fill, react, settle, draw and an iddle phase. These phases are progressively repeated, making up the process cycle of SBR operation. Brief description of each phase is presented below (Patil et al., 2013).

- **Fill** - The wastewater is added to the reactor in a set time period or volume. At this stage high substrate (organic matter) concentration is available. Different modifications of this phase include the use of aeration or mixing. Static filling is characterized by simple filling with no mixing or aeration whereas mixed fill and aerated fill use, respectively, mechanical stirrers and aerators for homogenizing.
- **React** - The biological reactions are fully initiated either by aeration or continously stirring. Modifications include alternating aerobic and anoxic periods.
- **Settle** - Sludge is allowed to settle under quiescent conditions to provide a clarified supernatant. No interferences must happen, susceptible to compromise the process quality.
- **Draw** - The clarified supernatant is decanted within a predetermined cycle time. Figure 2.13 shows the type of decanters used in this phase.
- **Idle** - This is the rest phase, where equalization and sludge wasting may take place and also preparations for the next cycle. Its length depends upon influent flow rate and operating conditions. It is particularly useful for industrial applications and when having various SBRs operating in paralel.

SBR operation can be quite advantageous for combined organic matter and nutrient removal in a single reactor. Combining different aerobic, anoxic, or anaerobic periods, unleashes the possibility of performing each reaction, in different time periods, with good performances. It's a matter of adjusting the length of the periods, or, for high strength wastewater, increasing total cycle time to enable complete degradation.

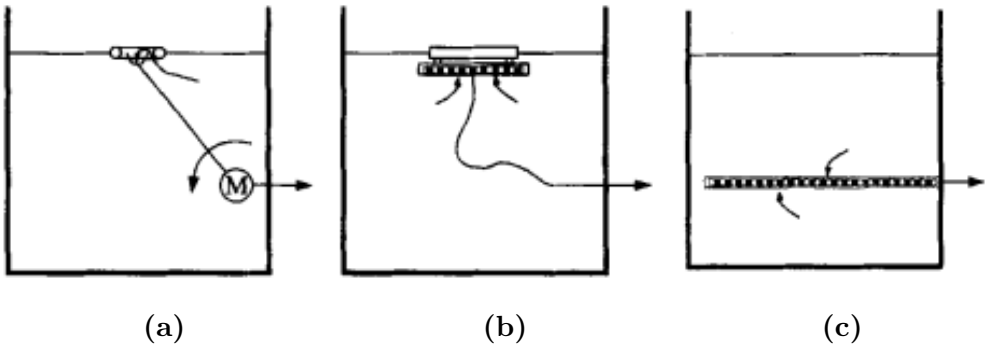



**Fig. 2.13:** Typical decanter mechanisms: (a) Motorized unit rotates header pipe; (b) floating header; (c) fixed-depth decanter (Adapted from Ketchum (1997))

## 2.6 State of the Art

In this section, a summary of experiments related in the literature with the Moving Bed Biofilm Reactor is presented in Table 2.3, followed by a brief explanation and the main ideas to retain about each experiment.

**Table 2.3:** Table overviewing experiments done with Moving Bed Biofilm Reactors

| Carrier | Wastewater | Experimental Conditions | Main Conclusions | Reference |
|---|---|---|---|---|
| *Flocor-RMP* | Municipal wastewater | AS and MBBR pilot-plant comparison; Two 337L reaction tanks for each process; HRT between 3.3-7h for each reactor; 70% FF with very low surface area (160 $m^2/m^3$). | Higher performances for the AS process in respect to total COD and ammonium removal, attributabble to higher biomass concentration; Solube COD removal efficiency was the same for both processes. | Andreottola et al. (2000) |
| *Anox Kaldnes K1* | Synthethic phenolic wastewater | Two 22$L$ MBBR reactors with 70% filling rate; Continuous flow. | COD removal efficiency gradually drecreased due to increase in hydraulic loading rate; Stable against hydraulic and toxic shocks. | Borghei and Hosseini (2004) |

Table overviewing experiments done with Moving Bed Biofilm Reactor (continuation)

| Carrier | Wastewater | Experimental Conditions | Main Conclusions | Reference |
|---|---|---|---|---|
| Alloy of HDPE and nano-sized inorganics | Pesticide wastewater | Pre-treatment by fenton-coagulation processes; 5L Tested with different filling fractions in the MBBR; OLR of 3 $kgCOD/m^3.day$ . | Fenton-coagulation improved wastewater biodegradability; MBBR could tolerate inlet COD loading higher than 37.5 $gCOD/m^2$.carrier.day; High biomass of about 7000 $mg/L$ ensured more than 85% of COD removal efficiency. | Chen et al. (2007) |
| *Anox Kaldnes K1* | Pulp and paper Mill wastewater | 8,55L working volume mbbr reactor with 58% filling fraction; Operated in batch mode; Termophilic conditions; inoculated from a mesophilic activated sludge plant | Removal rates of 1,5-2,4kg SCOD/m3.d at OLR of 2.3-3.8kg SCOD/m3.d giving the fact that 25% of wastewaters soluble COD was not biodegradable; Sludge yields were very similar to those from mesophilic activated sludge treatment. | Jahren et al. (2002) |
| Alloy of HDPE and nano-sized inorganics | landfill leachate | anaerobic-aerobic staged MBBR with a working volume of 4.2 and 2.1L respectively; Ranging HRT from 4 days to 0.5 days and COD concentration from 3965 to 17,500 mg/L | Anaerobic reactor was responsable for an effective 90% COD removal while the aerobic reactor acted as the main mechanism for ammonium removal ; up to 15.70kg COD/m3.d OLR; Overall a good tolerance to loading shocks. | Chen et al. (2008) |
| *Biofilm Chip P* | Pulp and paper Mill wastewater | MBBR tank with 20 $m^3$ of usable volume filled with 10% carriers; Average flow of 6.2 $m^3/h$ giving an average HRT of 3.3 hours; Temperature was in termophilic conditions and pH ranging from 6.5 to 8.5 | Attached biomass of 14,6 $gVSS/m^2$ with $VSS/TSS$ ratio of 0.69; Average removal efficiency of 56 % for an average OLR of 4.4 $kgBOD_{Sol}m^3/d$ and average SOLR of 43.8g $BOD/m^2$; Excellent system stability against hydraulic shocks | de Oliveira (2014) |

Table overviewing experiments done with Moving Bed Biofilm Reactor (continuation)

| Carrier | Wastewater | Experimental Conditions | Main Conclusions | Reference |
|---|---|---|---|---|
| *Flocor-RMP* | Synthethic wastewater - glucose plus macro and micro-nutrients | Four MBBR reactors in series implemented for full phosphorous and nitrogen removal. Filling fraction was 50% with the exception of $R_4$ which was 70%; System HRT ranging from 8 to 48 hours | Maximum phosphorous removal occurs in the aerobic reactor (R4)with 1.047 $gPO4-P$ removed/$kgVSS.h$; TSS biofilm concentration was found to be 0.595 $kgTSS/m^3$ with VSS/TSS equal to 79%; Average nitrification rate was 1.92 g NOx-N produced/kg VSS.h while maximum denitrification rate was 1.3298 g NOx-N removed/m2.day. | Kermani et al. (2009) |

**Andreottola et al. (2000)** performed an experimental comparison between the AS and the MBBR processes treating municipal wastewater. The main aim of the study was to assess the lower limit of MBBR performance using a lower cost carrier with low specific surface (160 $m^2/m^3$), the *Flocor-RMP*. A pilot plant was built, comprised of 2 parallel lines, one for each process. Each process line had two reaction tank of 337L and a final settler. Sludge recirculation was exclusively used for the AS line and the MBBR reaction tanks were filled with 70% of carriers (FF). HRT was common for the process lines, being 3.3-7h for each reactor. Organic matter removal revealed 76% efficiency for the MBBR and 84% for the AS, in respect to total COD, with the influent $COD_{Total}$ concentration averaging 231 and reaching a maximum of 570 mg/L. However, for soluble COD, performance was the same in both systems (71%). These interesting results were explained by the higher biomass concentration in the AS reactor, 1.3 to 3.4 kgTSS/$m^3$, as opposed to 0.8 to 1.5 kgTSS/$m^3$ in the MBBR, and thus higher hydrolization rates in the AS reactor, which resulted in a higher accessable substrate by the AS flocs. Ammonium removal efficiencies averaged 92% for the MBBR and 98% for the AS. To conclude, the MBBR lower performances were explained by the low surface area available for biomass growth, and thus low biomass concentration. Advice is given to atleast have a carrier with 250 $m^2/m^3$, in order to have reasonable performances.

Although phenolic wastewater has a high toxicity and limited application in biological treatment, **Borghei and Hosseini (2004)** studied the treatment of phenolic wastewater in a continuous MBBR. Two 22L reactors were used with the original *Anox Kaldnes K1* carriers with 70% FF. Experiments were conducted at room temperature with DO concentration above 4.5 mg $O_2$/L.

The phenolic wastewater contained sugar beet molasses as the main organic constituent plus added nutrients, resulting in a phenolic COD ($COD_{Ph}$) to total COD concentration ratio of 0.2 to 1.0 ($COD_{Ph}/COD_{Tot}$), with the total COD fixed at 800 mg/l. For start-up, reactors were inoculated with acclimated biomass in batch mode, and the experiments were carried out with HRT ranging from 24 to 8h. COD removal efficiency increased up to an a ratio of 0.6 ($COD_{Ph}/COD_{Tot}$), corresponding to 96% removal efficiency. Beyond this point, inhibitory effects were observed and with that a significant decrease in COD removal efficiency. Photomicrography of the biofilm revealed filamentous bacteria on the biofilm but not in the mixed liquor or the effluent.

Integration of different processes can increase the overall removal efficiency of contaminants. **Chen et al. (2007)** applied fenton-coagulation to a low biodegradable effluent and enabled the application of a subsequent biological treatment that otherwise would be ineffective. Particularly, hazardous and toxic organophosphorous pesticide wastewater with a pH of 2 and a $BOD_5$ to COD ratio of less than 0.2 was submitted to advanced oxidation processes, which allowed a COD reduction from 33700 to 12000 mg/L and enhanced the biodegradability to 0.5. A subsequent $Ca(OH)_2$ addition was made to adjust to pH 7.5 and to further coagulate the pollutants, therefore decreasing COD from 12000 to 9300 mg/L. The MBBR process was then applied. Different FF were tested, ranging from 50 to 10% and hydraulic retention time was kept high (1 day). Removing carriers from the reactor effectively decreased the efficiency of the system, as predicted. The biomass reached 7200 mg/L at FF of 50%. OLR was 3 $kgCOD/m^3.d$ corresponding to an influent COD of 3000 mg/L. Effluent COD was less than 500 mg/L, corresponding to more than 85% removal efficiency.

**Jahren et al. (2002)** studied the treatment of termomechanical pulping whitewater in a lab scale 8.55L batch MBBR, filled with 58% of *Anox Kaldnes K1* carriers. The start-up was made with 36g VSS of already acclimated activated sludge inoculum. The reactor worked in termophilic conditions, with a pH of 7.0. HRT was gradually decreased from 30h to 14h. OLR reached 3.8kgSCOD/$m^3.d$ after 70 days of operation, in which 60 to 65% where sucessfuly degraded. Biomass values in the reactor ranged from 1400 to 1900 mg/L, out of which 80-85% was found as attached biomass. These low values are possibly explained by the low loading rates or nutrient limitation. VSS/TSS ratios were 0.78 in the effluent and 0.91 in the carriers. Average sludge yield based on suspended biomass determinations on the effluent was found to be 0.19 $gVSS/gSCOD_{removed}$. The batch experiments also gave the possibility to determine a degradation

rate of 15.9 gSCOD/gVSS.d in the first hour and 8.6g SCOD/g VSS.d over a 5 hour test.

**Chen et al. (2008)** presented an interesting study about simultaneous COD and ammonium removal utilizing the MBBR process. Activated sludge treatment has been revealed particularly uneffective in leachate treatment due to low rates of removal, sensivity to low temperatures, toxic shocks, flow rate fluctuactions, and loss of active biomass as well. For this reason, an anaerobic-aerobic MBBR system was used with a working volume of 4.2L and a FF of 40% for the anaerobic reactor, and a working volume of 2.1 with a FF of 60% in the aerobic reactor. HRT in the anaerobic was double the one in the aerobic. A 900 $m^2/m^3$ of surface area composite carrier was used. The OLR ranged from 4.08 to 15.70 kgCOD/$m^3$.d and it lead to a slight decrease in the removal efficiency from 94 % to 92%. As for the ammonium removal, the authors reported that for an influent of 350-400 mg$NH_4-N/L$, the removal capabilities were highly dependant on the HRT of the system. In fact, the system removal was consistently above 97% when the HRT was 2.5 days, but only 20% when the HRT was 1.25 days. This was explained by the influence of the HRT in the anaerobic reactor COD removal capabilities, which lead to competition between heterotrophic bacteria and the nitrifiers in the aerobic reactor, which greatly compromised the latter activity.

A pilot scale study was conducted by **de Oliveira (2014)**, which consisted in a single MBBR unit with a useful volume of 20 $m^3$ with 10% FF of the *BiofilmChip P* carriers. The main of the study was to characterize the adhered biomass to the carriers as well as the performance of the pilot-scale MBBR unit. Organic load was in the range of 4.3 kg $BOD_{SOL}/m^3.d$ and performance wise, 56% of removal efficiency was achieved. Very high biomass was contained in the studied carriers.

**Kermani et al. (2009)** studied the removal of nitrogen and phosphorous using the MBBR. It consisted in lab-scale reactors in series with anaerobic, anoxic and aerobic units in four separate reactors that were operated continuously at different loading rates of phosphorus and nitrogen and different hydraulic retention times. Nitrification was nearly complete, with 99.72 % efficiency in the aerobic reactor and an average nitrification rate of 1.92 $g\,NOx-N$ produced / kg volatile suspended solids. Increasing the NOx−N loading resulted in an increase denitrification rate. Average phosphorous removal efficiency was 95.8%. This revealed to be a very effective system for nutrient removal.

# Chapter 3

# Dairy Industry Characterization and Environmental Issues

## 3.1 General Overview

Gathering milk from animals take us back nearly 6 000 years ago. Since early age, man started to domesticate animals to satisfy needs for meat, clothing, milk, etc. Moreover, milk is the only food of a young mammal during the first period of its life, the substances in milk provide both energy and the building materials necessary for growth. Milk also contains antibodies which protect against infection. Man soon realized the importance of milk and it's present in a great varieties of diets, being a relatively cheap source of proteins, vitamins, and minerals (Table 3.1).

**Table 3.1:** Quantitative composition of cow milk

| Main constituent | Range | Mean value |
|---|---|---|
| Water | 85.5 - 89.5 | 87.5 |
| Total Solids | 10.5 - 14.5 | 13.0 |
| Fat | 2.5 - 6.0 | 3.9 |
| Protein | 2.9 - 5.0 | 3.4 |
| Lactose | 3.6 - 5.5 | 4.8 |
| Minerals | 0.6 - 0.9 | 0.8 |

The dairy industry is responsible for gathering raw milk and processing it in a multitude of products such as pasteurized milk, cheese, butter, yoghurt, ice cream, powder products such as milk and whey, etc.

Dairy production is one of the most inefficient processes with respect to water usage in the food industry (Cristian, 2010). Three to four litres of wastewater are generated for producing 1 litre of milk (Gulyás et al., 2015), and may reach up to 15 litres of wastewater (Figueiredo et al., 2001).

Therefore, environmental sustainability of dairy industries constitutes one of the major concerns of a proper integrated management system, bearing in mind the environmental impacts associated with the activity and quality demands. According to environmental guidelines for the dairy processing industry, the main objectives for good environmental performance in a dairy processing plant should begin with:

- Maximum recovery of products;
- Minimisation of losses or emissions to the environment;
- Recycling and/or reuse of wastes;
- Prevention of further environmental degradation.

The existence of a multitude of products derived from milk, is responsible for a very large variety of process lines. Each dairy plant can produce a bit of everything, or be specialized in one or two products. For this reason, a general process schematic is shown in Figure 3.1 (below), representing the main operations of dairy production plants.

Since milk is an highly perishable consumable, appropriate conditions must be given in order to preserve its quality. Within 2 hours of milking, the milk should be chilled to below 4°C. Milk treatment begins with clarification and pasteurization procedures, in which clarification consists in the separation of solid impurities, usually done with centrifugal equipment, and pasteurization consists in submitting milk to high temperatures, 65°C to 140°C depending on the time elapsed, in order to achieve sterilization and kill pathogenic micro-organisms which would otherwise destroy the products. This is the standard for any dairy processing industry.

A variety of products are now ready to be manufactured. For market milk, an homogenization procedure is required, consisting in forcing milk through a small passage at high velocity, which causes disruption of fat globules into much smaller ones, diminishing the tendency of milk to form cream at the surface (Bylund, 1995). For the manufacture of the remaining variety of products, bacteria cultures are added and allowed to grow under controlled conditions. In the course of the resulting fermentation, the bacteria produce substances which give the cultured

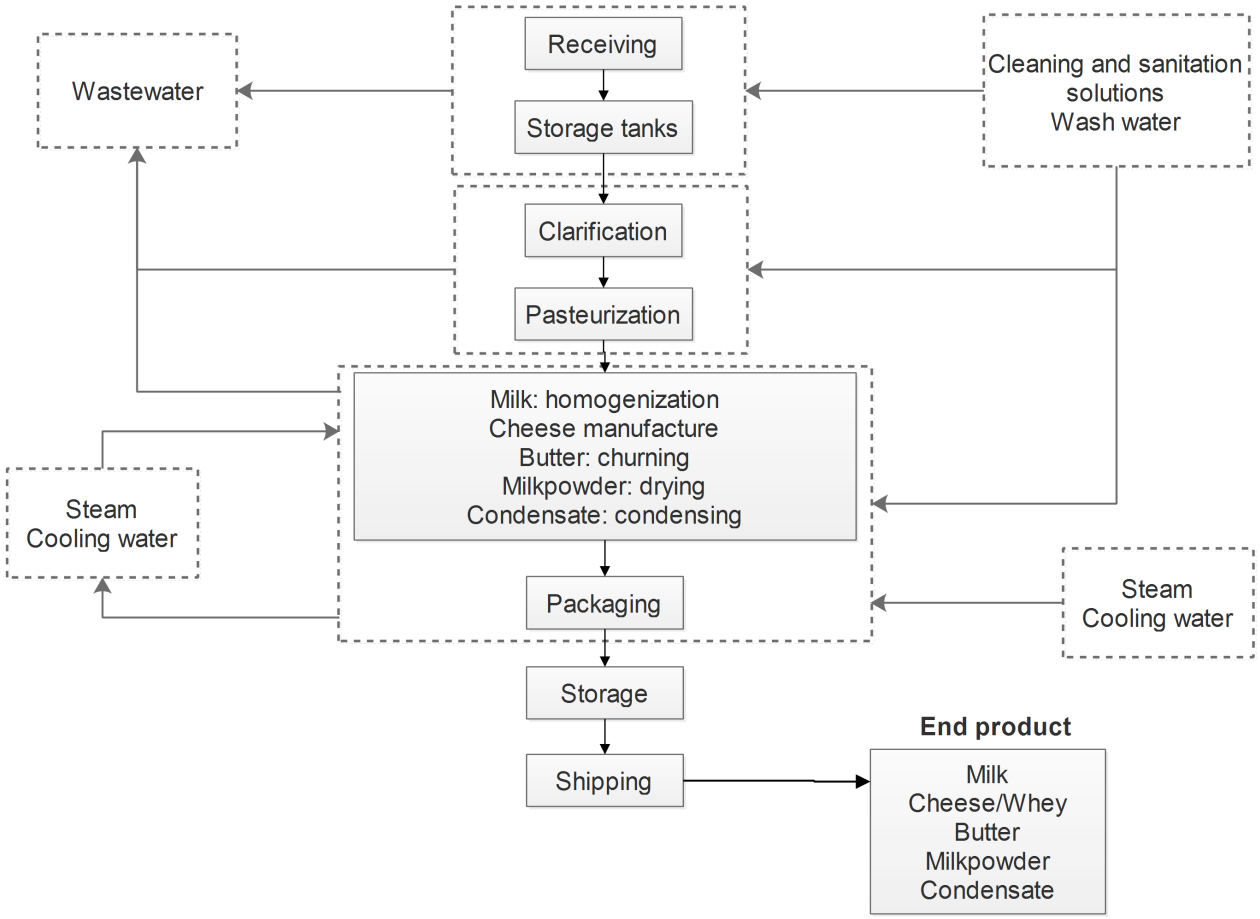



**Fig. 3.1:** Schematic flow sheet of the main dairy products (Adapted from FAO (1996)).

product its characteristic properties such as acidity, flavour, aroma and consistency. Different bacteria cultures produce products with different characteristics.

## 3.2 Dairy Industry in Portugal

In Portugal, the Dairy Industry, the portuguese classification of economic activity (CAE - "Classificação Portuguesa das Actividades Económicas"), aggregates the industry with the manufacturing industry, just like other food and beverages producing industries. Two big groups can be distinguished in the dairy industry, comprised of:

- Larger manufacturing units, processes milk and other dairy products. Represent 5% of all dairy units, of which 69% are licensed units by the DGADR ("Direção-Geral de Agricultura e Desenvolvimento Rural");
- Smaller decentralized units, mainly producing traditional cheese.

The smaller decentralized units are often referred to the ones exerting more environmental pressure, mainly because they often neglect good effluent discharge practices, derived from the less economical and technological means to do so. On the other hand, the larger manufacturing units, are often very specialized, technologically more advanced, and already integrating management systems that contribute to better effluent discharge practices.

Overall the dairy industry in Portugal can be characterized by: (Figueiredo et al., 2001)

- Being predominantly comprised by small and medium enterprises;
- Labors with low qualifications;
- Large technological gaps between the larger manufacturer units, and the smaller decentralized units;
- Very distinct productivity levels.

To understand how the industry is placed in Portugal, the following table is presented (Table 3.2), which shows the latest data available in respect to the number of enterprises and total milk production for the dairy and cheese making industries (CAE 15510), and how its distributed throughout the Portuguese territory.

**Table 3.2:** Dairy industry in Portugal - Number of enterprises and milk production in 2014 (Instituto Nacional de Estatística (2017).

| Geographic localization (NUTS - 2013) | Enterprises | Milk production ($10^3$ litres) |
|---|---|---|
| Portugal | 324 | 2 049 808 |
| Continente | 299 | 1 437 102 |
| Norte | 33 | 778 650 |
| Centro | 128 | 277 060 |
| Lisboa | 32 | 81 314 |
| Alentejo | 105 | 297 232 |
| Algarve | 1 | 2 846 |
| Região Autónoma dos Açores | 42 | 610 585 |
| Região Autónoma da Madeira | 11 | 2 121 |

## 3.3 Dairy wastewater characteristics

The dairy industry is generally considered to be the largest source of food processing wastewater in many countries (Wang et al, 2004). A great amount of water is used in numerous operations. On a milk gathering house, the walls, floors and milking equipment have to be cleaned daily in order to maintain proper sanitation for safely handling the milk (Janni et al., 2009). The cleaning operation will obviously produce a large volume of wastewater, which can account for up to 30% of the total wastewater volume (Wright and Graves, 1998). Apart from the washing procedures the wastewater also results from leaks of raw material and products which can be estimated as 2% of total milk processed (Figueiredo et al., 2001).

The major contaminants in dairy processing wastewater are milk solids that contain milk fat, protein, lactose and lactic acid. Other minor constituents include sodium, potassium, calcium and chloride. Table 3.3 represents a common composition of a dairy industry wastewater.

**Table 3.3:** Common dairy industry effluent composition. Micro-nutrients included (Adapted from Environment Protection Authority State Government of Victoria (1997)).

| Component | Range (mg/L) | Average (mg/L) |
|---|---|---|
| Suspended Solids | 24-5700 | - |
| $BOD_5$ | 450-4790 | 1885 |
| Nitrogen | 15-180 | 76 |
| Phosphorous | 11-160 | 50 |
| Sodium | 60-807 | - |
| Chloride | 48-469 | 276 |
| Calcium | 57-112 | - |
| Magnesium | 25-49 | - |
| Potassium | 11-160 | 67 |
| pH | 4-12 | 7.1 |

For these reasons the wastewater is characterized by high organic loads, resulting in high values of biochemical oxygen demand, and chemical oxygen demand (Table 3.5). There is also a concern about high utilization of detergents and cleaning solutions.

The dairy industry can be divided into several production sectors. Each division produces wastewater of a characteristic composition, depending on the kind of product that is produced

(milk, cheese, butter, milk powder, condensate). The Table 3.4 provides an exemplification of these differences.

**Table 3.4:** Reference wastewater values of different dairy industry sectors

| Sector | TSS | COD | $BOD_5$ | Fat | Total Nitrogen | Total Phosphorous |
|---|---|---|---|---|---|---|
| Milk | 480 | 1 700 | 1 500 | 130 | 50 | 15 |
| Cheese | 1 100 | 12 000 | 5 400 | 380 | 160 | 110 |
| Yoghurt/others | 420 | 2 900 | 1 400 | 230 | 75 | 10 |

Composition of dairy wastewaters presented in some literature experiments are presented in Table 3.5.

**Table 3.5:** Characteristic of different dairy wastewater

| Wastewater type | COD | BOD | pH | TSS | TS | References |
|---|---|---|---|---|---|---|
| Milk & Dairy products factory | 10251.2 | 4840.6 | 8.34 | 5802.6 | | Cristian (2010) |
| Dairy effluent | 1900-2700 | 1200-1800 | 7.2-8.8 | 500-740 | 900-1350 | Deshannavar et al. (2012) |
| Dairy effluent (CPCB 1993) | 1120-3360 | 320-1750 | 5.6-8 | 28-1900 | | Lata et al. (2002) |
| Whey | 71526 | 20000 | 4.1 | 22050 | 56782 | Deshpande et al. (2012) |

## 3.4 Treatment methods

Liquid wastes constitutes constitutes the main environmental problem in dairy industries, especially when discharged directly in water bodies or when submitted to inadequate or low performance treatment. Therefore, effluent management is essential to ensure good environmental sustainability. Proper effluent treatment, water reutilization or even waste valorization techniques are attained to achieve good management practices, taking into account the economic viability for implementing and sustainning these techniques.

A large dairy factory discharging two megalitres of wastewater at a $BOD_5$ of 2,000 mg/L each day means the additional load onto a municipal wastewater treatment plant is equivalent to an

extra 16 000 habitants. Moreover, directly discharge onto municipal sewer may interfere with treatment process, because of the presence of chlorides, oils and fat, and wide variations of pH. Therefore, there is a need for the industries to have their own wastewater treatment plant, be it either for pre-treatment in order to permit discharge onto municipal sewer's, or for complete treatment followed by a directly discharge onto the environment.

Dairy industry wastewaters are generally produced in an intermittent way, resulting in different flows and characteristics of effluents between factories. In fact, this depends on the kind of products produced and the methods of operation, therefore influencing the choice of the wastewater treatment to employ, as specific biological systems have difficulties dealing with wastewater of varying organic loads (Wang et al, 2004). Despite that, given the high amount of biodegradable matter present in dairy wastewater, biological treatment methods are commonly employed. Both aerobic and anaerobic processes have been extensively used.

Conventional treatment of dairy wastewater by aerobic processes includes processes such as activated sludge, trickling filters, aerated lagoons, or a combination of these (Kushwaha et al., 2011). Also, Li and Zhang (2002), studied the SBR technology with success. A single-stage SBR system was tested with 10 000 mg/l COD influent and at an HRT of 1 day while achieving a removal efficiency of 80.2% COD, 75% total kjeldahl nitrogen, and 38.3 % total nitrogen.

However, aerobic biological systems are faced with the problem of sludge generation, and may consequently lead to serious and costly sludge disposal problems. Data from 1998 reveals that the portuguese dairy industries produced 237 764 tonnes of sludge deriving from biological treatment, (Figueiredo et al., 2001) which constitutes one of the most produced wastes for these food industries. The MBBR process comprises an interesting alternative due to its high organic load removal potential, ease of operation, and reduced sludge generation.

The allowed direct discharge limits to the environment according to the Portuguese Decree-Law nº236/98 and the discharge limits into municipal network (Coimbra municipality) is presented below (Table 3.6).

**Table 3.6:** Allowed effluent discharge limits

| Parameter | Maximum allowed direct discharge[1] | Maximum allowed municipal discharge[2] |
|---|---|---|
| pH | 6.0-9.0 | 5.5-9.5 |
| $BOD_5$ | 40 | 800 |
| COD | 150 | 1000 |
| TSS | 60 | 1000 |
| Phosphorous | 10 | 25 |
| Ammonium | 10 | 100 |
| Total Nitrogen | 15 | 125 |
| Nitrate | 50 | 100 |

[1]According to Portuguese Decree-Law nº236/98 ("Decreto lei nº236/98, 1 de agosto")

[2]In "Águas de Coimbra - Valores Limite de Emissão para águas residuais industriais"

# Chapter 4

# Materials and Methods

## 4.1 Analytical Methods

For this investigation, influent and effluent, mixed liquors, and carriers samples were analysed with respect to organic matter content, nitrogen, and suspended solids. The influent, effluent and mixed liquors were measured in terms of the chemical oxygen demand (COD), total carbon (TC), and total nitrogen (TN). Solid assessment was carried out in respect to mixed liquors (suspended biomass) and carriers (attached biomass). In order to evaluate any change in sludge settleability, the sludge volume index (SVI) in the AS tank was periodically analysed. pH and temperature were also monitorized.

For these tests, samples were analysed in duplicate to reduce the effect of experimental errors. Effluent samples are composite from 36 to 48 hours time period.

Samples were measured according to Standard Methods for Water and Wastewater Examination (SMWWE), (APHA et al., 1998), namely: COD - *5220 D. Closed reflux, Colorimetric Method*; TSS - *2540 D. Total Suspended Solids dried at 103-105°C*; VSS - *2540 E. Fixed and Volatile Solids Ignited at 550°C*; SVI - *2710 D. Sludge Volume Index.*

Biomass in the carriers were measured according to the methodology described by de Oliveira (2008).

### 4.1.1 Chemical Oxygen Demand

This methods allows to indirectly quantify the organic matter present in a sample by measuring the amount of oxygen needed to degrade the sample. It was performed based on the *5220D closed reflux colorimetric method of the SMWWE*.

The basis for this method is that, under the presence of a boiling mixture of chromic and sulfuric acids, the organic matter present in a sample is completely oxidized. The acidic conditions are provided by an acid solution, containing silver sulfate ($Ag_2SO_4$) dissolved on sulfuric acid ($H_2SO_4$) concentrate. The digestion is made using a solution of potassium dichromate. Dichromate ions ($Cr_2O_7{}^{2-}$) oxidize the organic matter and will be reduced to chromic ion ($Cr^{3+}$). Both of these chromium species are colored and absorb in the visible region of the spectrum. Two digestion solutions were prepared, a low range for measuring COD between 0-100 mg/L and a high range for the 100-1000 mg/L interval.

Before technique appliance, a calibration is required. A solution of Potassium Hydrogen Phtalate (KHP) with a known concentration was prepared and giving the fact that 1 g of KHP corresponds to $0.9\,\mathrm{mg\,O_2/L}$ several dillutions were made in order to calibrate the photometer with different concentrations. A linear dependency between the absorvance measured and the KHP concentrations was obtained and with that, the calibration curve.

The analysis was performed by pipetting 1.5mL of digestion solutions, 3.5 mL of acid solution and 2.5 mL of sample to a borosilicat vial which was placed for 2hours at 150°C on a VELP SCIENTIFICA ECO25 thermoreactor. The absorvances were read at 445 nm for the low range and 605 nm for the high range.

### 4.1.2 Total Carbon and Total Nitrogen

Total carbon and total nitrogen concentration in the effluent samples were measured by high-temperature catalytic oxidation with non-dispersive infrared detection, and by chemiluminescence, respectively, in TOC-V CPN and Total N TNM-1, Shimadzu. Prior to analysis, the samples were centrifuged and refrigerated to 4 °C.

### 4.1.3 Solids determination

Solids refer to matter suspended or dissolved in water or wastewater. The importance of solids analysis is due to the fact that it may harmfully affect water or effluent quality, reason why it is a regulatory requirement for effluent discharge, and also because of its importance in the control of biological and physical wastewater treatment processes.

#### 4.1.3.1 Total Suspended Solids and Volatile Suspended Solids

For the suspended solids assessment a few preparatory steps are needed: Firstly, the glass fibre disk is rinsed with distilled water through the vacuum filtrating system. The filter paper is then placed in a watch glass, dried at 103-105 °C for 1 hour, cooled to ambient temperature in a desiccator, and weighed. This procedure is repeated until successive weighs offer no change in the glass fibre weigh ($m_0$).

After these preparatory steps, the **total suspended solids** are obtained by submitting a well-mixed sample to the vacuum filtration system, through the previously weighed glass fibre disk, cooled, dried, and weighed ($m_1$). The TSS are determined by applying Equation 4.1:

$$Total\ Suspended\ Solids\ (mg/l)\ = \frac{m_1\ -\ m_0}{sample\ volume} \tag{4.1}$$

For the **volatile suspended solids** analysis, the previous sample is further submitted to a muffle furnace, in a previously weighed ceramic dish ($m_2$), for ignition at 550°C for roughly 1 hour. The ignite residue is cooled to ambient temperature and weighed ($m_3$). The VSS are determined by applying Equation 4.2.

$$Volatile\ Suspended\ Solids\ (mg/l)\ = \frac{(m_2\ +\ m_1)\ -\ m_3}{sample\ volume} \tag{4.2}$$

#### 4.1.3.2 Attached biomass

Analysis of the biomass adhered to the carriers was based on the methodologies described by de Oliveira (2008). The assessment of TSS and VSS on the fixed biomass contained on the carrier elements was determined by detaching the biofilm from a known quantity of carriers and after

vaccuum filtration and drying (in accordance with the TSS and VSS methodology), the weight was related to a single carrier element.

Bulk carrier (number/litre) was determinated by manually counting the number of carriers occupying a volume of 400 mL in a graduated cylinder and then extrapolating to the volume occupied by the carriers in the reactor.

### 4.1.4 Sludge Volume Index

The sludge volume index (SVI) is the volume in milliliters occupied by 1 g of a suspension after 30 min settling. SVI typically is used to monitor settling characteristics of activated sludge and other biological suspensions. To assess the SVI, the sludge is allowed to settle in a beaker glass for a period of 30 minutes. After the TSS determinations, the following equation is used:

$$Sludge\ Volume\ Index\ (ml/mg) = \frac{settled\ sludge\ volume\ (ml/l)}{total\ suspended\ solids\ (mg/l)} \tag{4.3}$$

The SVI was measured periodically in the AS reactor because of its importance in the process operationality. For the Moving Bed systems, SVI was measured once.

### 4.1.5 pH and temperature

For the pH measurement, a Crimson pH analyser was used, while a portable Testo 925 thermometer was used for temperature. On the start-up phase, pH was measured on a daily basis. Periodically measurements were performed atleast once a week on the subsequent experience phases.

## 4.2 Experimental Unit Description

In order to evaluate the performance of the proposed technologies, a system with three independent reactors was implemented. One activated sludge reactor (AS), and one Moving Bed Biofilm Reactor (MBBR) operated in a continuous flow mode. To further test the Moving Bed capabilities, a third MBBR reactor was operated in sequencing batch mode (MB-SBR). The laboratory scale reactors are made of plexiglass with a total liquid volume of 3.5 L.

Both continuous reactors had a sedimentation unit with a liquid volume estimated of 3 liters. Initially the continuous MBBR did not possess a proper sedimentation unit but that approach was quickly dropped. A clarifier was later installed into the MBBR system which allowed for adequate sludge quantification and better visual representation and comparison of the excess sludge in both systems. The period in which the MBBR reactor was operating without the sedimentation unit was considered part of the start-up period (section 4.5). The full process flow diagram of the experimental set-up is showed on Figure 4.1.

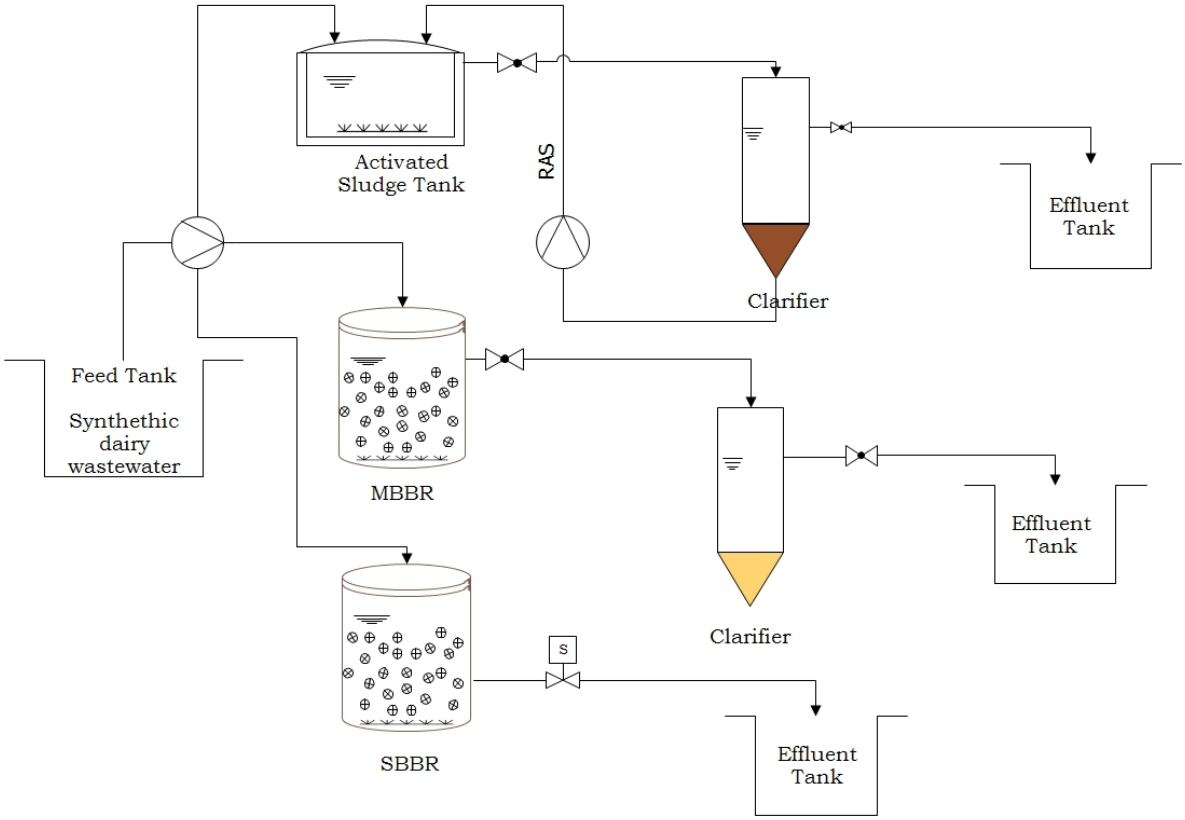
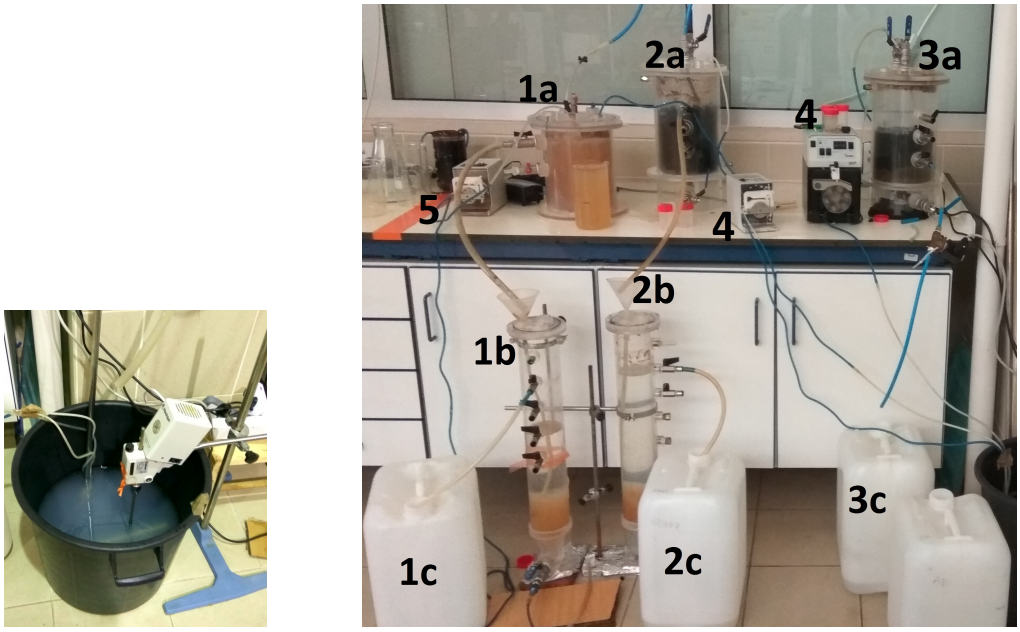



**Fig. 4.1:** Scheme of the experimental arrangement

Sludge recycling was applied only in the AS system. Feeding flow rates were controlled by peristaltic pumps, previously calibrated using a stopwatch and a graduated cylinder. Calibration was performed at least once every two weeks to ensure constant flow rates.

Sufficient DO was supplied to each reactor in order to maintain its concentration at saturation levels, hence preventing oxygen from becoming a limiting factor throughout the study.

The SBR system was operated in automatic mode, meaning wastewater admission and discharge was controlled by a peristaltic pump and a solenoid valve, respectively, both attached to electrical timers. Discharge was made by a fixed-depth decanting (Figure 2.13c) The aeration time was also controlled using an electrical timer. The incorporation of the different operations comprised in




(a) (b)

**Fig. 4.2:** View of the experimental arrangement: (a) Wastewater feeding tank and mixer; (b) AS reactor (1), MBBR (2), MB-SBR (3), feeding pump (4), recirculation pump (5). Letter *a* for reactor, letter *b* for clarifiers, and *c* for effluent tanks

the MB-SBR cycle is represented in Figure 4.3. SBR operation stopped during weekends.

| ID | Operation | Duration | Hours 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Static Fill | 0.5 h. | | | | | | | | | | | | |
| 2 | Aerate | 8 h. | | | | | | | | | | | | |
| 3 | Settle | 2.5 h. | | | | | | | | | | | | |
| 4 | Draw and | 1 h. | | | | | | | | | | | | |

**Fig. 4.3:** Representation of the MB-SBR cycle

For both systems (continuous and batch) the oxygen was firstly introduced via the available compressed air grid attached to a bubble diffuser at the bottom of the reactor and later via a VWR air bomb.

## 4.3 Biocarriers

The carrier used in this work was the *bioflow 9* which dimensions are 9 mm × 7 mm made from High Density Polyethilene (HDPE) and a bulk density of 145 kg/m$^3$. The total superficial area available for biofilm growth specified by the manufacturer is 800 $m^2/m^3$. However, under optimum hydraulic conditions, only the interior surface area should be considered for biofilm growth, this was not covered in the manufacturer's specification brochure.

Filling fraction (FF) was set to be roughly 50% of the reactor liquid volume, which is in accordance to literature values, setting up the specific surface area to 0.875 m$^2$. The Figure 2.12c (subsection 2.4.2) provides visual representation of the biocarrier.

## 4.4 Wastewater

Synthethic dairy wastewater was used because of its simplicity, since it can be approximated to a real milk processing industry and it provides stable loading rates within limited variations. Moreover, it also prevents the introduction of solids to the reactors, hence enabling an investigation of the biologically produced solids without interactive effects of influent solids.

Wastewater was prepared roughly three times per week by diluting low fat milk in tap water without added nutrients.

## 4.5 Seeding and Start-up

The Activated sludge reactor (AS) was inoculated with activated sludge from the wastewater treatment plant of Ribeira de Frades, in Coimbra, which operates as a traditional activated slude WWTP. The moving bed systems (MBBR, and MB-SBR) were seeded with already inoculated carriers from the wastewater treatment plant of Arzila, Coimbra. Start-up period lasted roughly two weeks, and it allowed the microbian communities to adapt to their new environment and to acclimate to the milked influent.

## 4.6 Experiments

The experiments were divided in two phases. The **continuous phase** is characterized by a continuous operation of the three single staged reactors, two in continuous flow, and one in sequencing batch mode. The **batch phase** is the second phase of the experiments, characterized by the realization of various batch tests in which the removal of organic and nitrogenous matter is assessed in an hourly base.

### 4.6.1 Continuous Phase

The main objective is to compare the three reactors under identical conditions, and assess the removal rate in respect to the organic matter and nitrogen content. To achieve this, different organic loading rates were tested, gradually increasing the milk content in the wastewater dilution. Flow rate was set to 7 L/d, equivalent to an hydraulic retention time of 12 hours.

The first part of this experiment, Period A, had a duration of 17 days, corresponds to a low fat milk dilution of 1/200. Followed by Period B, which lasted 14 days, milk concentration has been doubled in the dilution, so 2/200. Period C, is characterized by the highest organic loading which resulted yet from another duplication of the milk concentration to 4/200, in which sampling occurred for 9 days. The total length of this experience was then, 40 days, starting from November fourth and ending on December fourteenth.

Influent COD was measured every time the influent was prepared, while TC and TN influent concentrations was assessed through characterizing the 1/200 dilution and the mathematical relationship between the remaining dilutions (2/200 and 4/200).

### 4.6.2 Batch Phase

The batch phase consisted in preliminary batch tests with the MB-SBR in order to assess the kinetics involved. Prior to the experiments, the mixed liquor suspended solids (MLSS) in excess was removed.

For the first test, the main objective was to understand how the Mixed Liquor Suspended Solids (MLSS) and the chemical oxygen demand content evolved during the 8 hour degradation period. To achieve this, samples were taken every hour and measured in terms of the soluble COD

content, and the suspended biomass concentration. The initial COD concentration was set with a 3/200 milk dilution.

For the second test, the initial organic concentration was reduced to the 2/200 dilution, and an attempt to determine the substrate removal kinetics in respect to both carbon and nitrogen removal was made.

# Chapter 5

# Results and Discussion

## 5.1 Continuous Phase

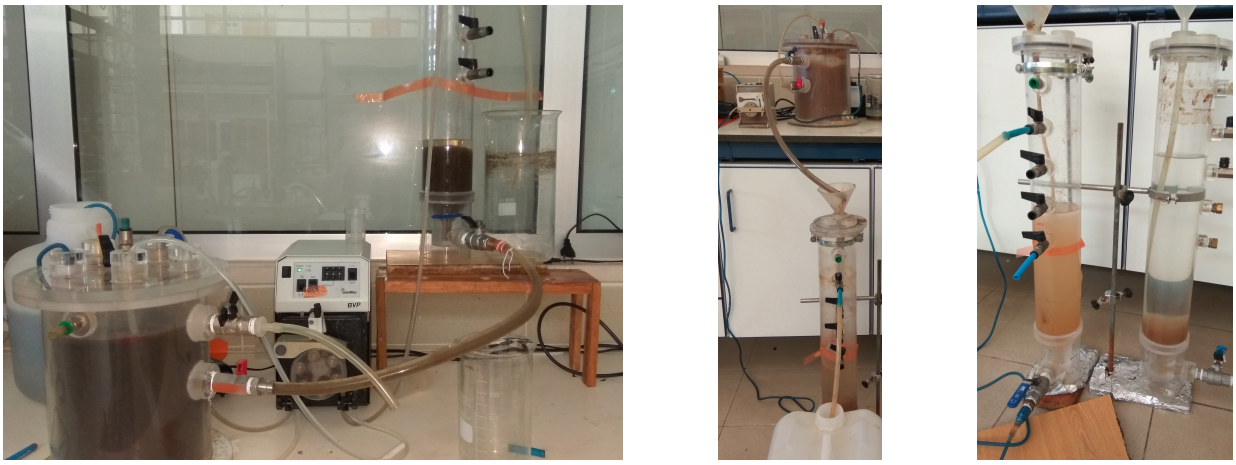
### 5.1.1 Initial considerations

Before presenting the results, some aspects that will contribute to a better understanding of this section will be addressed. In the start of the experiments there were some difficulties for retaining the biomass in the activated sludge (AS) reactor. This was due to a less adequate initial configuration and mid-process changes in the experimental set-up. In the beginning, the AS reactor was operating with the mixed liquor being pumped to the clarifier, and sludge recirculation being done by gravity and controlled by manually opening the entrance valve on the reactor (Figure 5.1a). Although the period in which this configuration was running, was considered as part of the start-up period, it had repercussions in the first part of the continuous phase, namely in period A. This starting configuration was proven not to be very effective for a few reasons, namely:

- The peristaltic pump made the tube leading to the clarifier be in constant shaking, thus interfering with the sludge settling and ultimately to the quality of the effluent;
- The sludge recirculation was not functioning properly. During the weekends or, whenever nobody was present, there was no sludge being recirculated. The sludge would then build-up excessively in the clarifier, leading to lesser biomass concentration in the reactor.

Sludge settleability was also very poor, with sludge volume index ranging from 400 to 700 mL/mg.

These reasons contributed to low biomass retention levels. A new and definite set-up was then configured (Figure 5.1b). The mixed liquor was discharged to the clarifier by gravity and sludge recirculating was made by a peristaltic pump, controlled by an electrical timer that made the pump operate for fifteen minutes every half an hour. However, this did not fix the biomass concentration issues because of the poor settleability allied with low solids concentration, resulting in too much biomass being gradually lost with the effluent. Figure 5.1c shows the poor settleability of the AS effluent on the left clarifier. On November sixteenth new biomass was added to the reactor, upping its concentration to roughly 2500 mg/L. The AS reactor was then, finally, operating flawlessly.



**(a)** **(b)** **(c)**

**Fig. 5.1:** (a) Initial AS system configuration; (b) Final AS system configuration; (c) Left: AS clarifier; Right: MBBR clarifier

Another aspect to bear in mind, was the existence of two unprogrammed air compressor failures, estimated to be due to a power failure. The **continuous-flow** reactors were cut from oxygen and the exact lasted period is unknown. While the air compressor did not have the capacity to reboot itself, the peristaltic bombs responsible for the wastewater feeding did, which simultaneously overloaded the already oxygen-short reactors. The MB-SBR was not affected since it was not connected to the compressed air grid. The Table 5.1 summarizes the dates for the unprogrammed occurrences. For the last 10 days of this experience, both the AS and the MBBR had its oxygen supply changed to VWR air bombs.

**Table 5.1:** Dates of the unprogrammed occurences

| Failure | Date |
|---|---|
| Power failure A | 18/11 |
| Power failure B | 1/12 |

### 5.1.2 Wastewater

As described in the Materials and Methods chapter, the organic loading rate has been increased stepwise, through increasing the milk ratio in the wastewater dilution by successive doubling the concentration, from 1/200 to 4/200. The COD of the wastewater was determined everytime the feed tank was filled, while the TC and TN of the influent wastewater was determined based on the fact that the 1/200 dilution corresponds to an average TC of 256 mg/L and TN of 52 mg/L. So the theoretic values expected should be double when using the 2/200 dilution, and quadrupled on the 4/200 dilution. The following table provides a comprehension of the measured influent COD values for each dilution, which also characterizes each period.

**Table 5.2:** Influent wastewater composition in the continuous phase of experiments

| Dilution | Period | Date | n | Average (ST.D.) | Min | Max |
|---|---|---|---|---|---|---|
| 1/200 | A | 4/11 to 21/11 | 8 | 582 (± 65) | 462 | 647 |
| 2/200 | B | 21/11 to 5/12 | 6 | 1397 (± 131) | 1277 | 1572 |
| 4/200 | C | 5/12 to 14/12 | 4 | 2646 (± 276) | 2303 | 2874 |

### 5.1.3 Carrier characterization

Although Rusten et al. (2006) observed that after 15 years of uninterrupted operation no wear and tear was found on the *Kaldnes K1* carriers, it was possible to observe physical degradation in the carriers used in the present work. The *bioflow 9*, with roughly 10 years of use, presented wear-off marks, with the external saliences being greatly shredded. Figure 5.2 compares a new bioflow 9 carrier with another being used for several years. In this regard, this indicates that not only the carrier composition is important, since both *K1* and bioflow 9 are made from HDPE, but also its configuration, or even the hydrodynamic conditions that the carriers are subject to. A more violent agitation of the carriers puts more stress on the outside walls and the shear forces

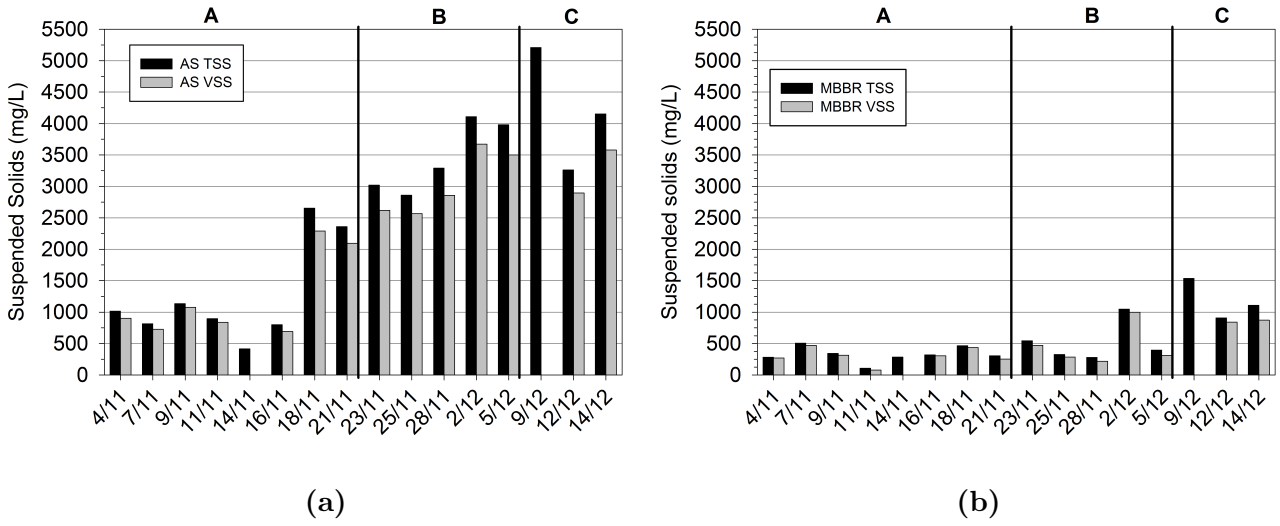
will be superior, contributing to a greater wear off.

The *bioflow 9* carrier put more emphasis on the outside area compared to the *K1* for instance, and this abrasion effect diminished the superfical area, but since biofilm will not effectively grow in the outer area, it won't have any significant effect in diminishing the surface available for biofilm growth. However, it might indicate the need for carrier replacement somewhere in the future, contrary to what is said in the cited literature.

**Fig. 5.2:** Carrier comparison; new (left) vs one with about 10 years of utilization (right)

### 5.1.4 Biomass assessment

The results from the total suspended and volatile suspended biomass determination in the continuous reactors are shown below (Figure 5.3).




**(a)** **(b)**

**Fig. 5.3:** Total Suspended and Volatile Suspended Solids in the continuous reactors. (a) concerns the Activated Sludge reactor while (b) belongs to the MBBR.

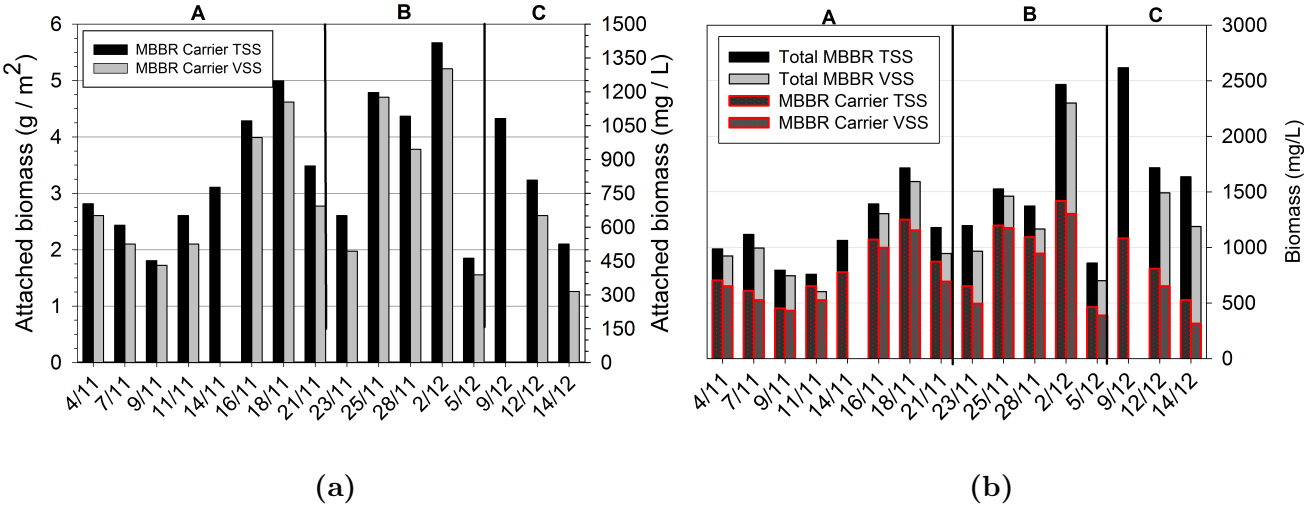
In the AS reactor, the period A (feeding with the 1/200 dilution) was characterized by low biomass retention until the addition of new biomass, as stated before. During period B (feeding with 2/200

dilution), the biomass concentration continuously increased until it reached a maximum of 5 210 mg TSS/L on 9/12. At this point the reactor was already operating at the maximum organic loading rate (period C).

On the other hand, the MBBR operated with fairly low TSS concentrations and no correlation was found between the amount of COD entering the system and the suspended biomass concentration. Bear in mind this is not taking into account the biomass contained in the carriers (attached biomass). In the first of December, power failure B happened and with it a shutdown in the air compressor system. The reactors were without oxygen provisioning for a long period and destabilized the entire system. In the MBBR , a period of biomass detachment from the carriers was observed and it lead to an increase in the MLSS concentration. However, this was only noted by December 5 when withdrawing carriers for sampling. To speed-up the biofilm replenishment, the MLSS concentration was increased, hence the relatively high values for the subsequent days (9/12 and beyond).

For a complete analysis of the MBBR solids, one must take into account the biomass contained in the carriers. The number of carriers in the reactor was achieved by manually counting the volume occupied by the carriers in the graduated cylinder test, as described in subsubsection 4.1.3.2. A value of 1 050 carriers/L was obtained for the *bioflow 9*, which was extrapolated to the bulk volume occupied by the carriers in the reactors. According to the work of Ødegaard et al. (2000), a value of 1 030 carriers/L for the original *Anox Kaldnes K1* was found. To sum up, the number of carriers contained in each reactor was approximately 1838, which allowed to convert the attached biomass concentration into mg/L values, and thus enabling the direct comparison with the AS reactor values. The attached biomass concentration is also presented in ($g/m^2$) which is the total mass of attached biomass per total surface area for biofilm growth (0.875 $m^2$). Figure 5.4a shows the results for the attached TSS and VSS concentration in the MBBR reactor.

In the first period of the continuous experiments, the mean value was 3.192 ($\pm$1.037) $g/m^2$ corresponding to 798 ($\pm$260) $mg/L$. For the second period, the mean value was 3.860 ($\pm$1.583) corresponding to 964 ($\pm$396) $mg/L$. From the 17/11 to the 18/11 power failure A happened and it's clear its impact in the biofilm biomass. In fact, in the subsequent days, a period of pronounced biofilm detachment was observed and it lasted for 4 days. Despite that, a slight increase in the biofilm biomass from period A to B can be seen, as it is expected as result of the increase in the substrate concentration entering the reactor.

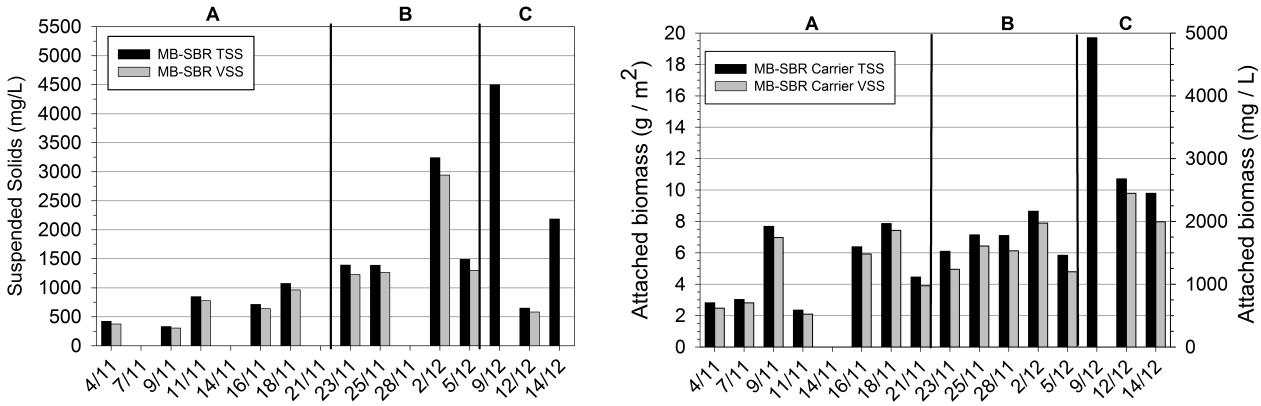



**(a)** **(b)**

**Fig. 5.4:** MBBR total biomass concentration. (a) Attached biomass concentration; (b) Attached plus suspended biomass concentration

Results from period C were seriously affected by the air compressor failure, harmfully influencing the biomass concentration on the MBBR carriers. Experiments ended before any sign of biofilm recovery could be observed, and not only that, but also a subsequent gradually decrease can be seen in the biomass concentration of the carriers. By January, the biofilm had been completely reestablished without any added substrate during the fifteen days of holiday period, only by keeping high MLSS concentration in the reactor. The total biomass concentration in the MBBR is the combination of both attached and suspended biomass, which is presented in 5.4b. The MB-SBR biomass results are presented in Figure 5.5.

The MBBR and MB-SBR started with the same attached biomass concentration. Comparing the remaining values (Figure 5.4a, andFigure 5.5b, respectively), it's clear that the MB-SBR operated with bigger attached biomass concentration. Although the organic load over a period of 12 hours was the same for both reactors, operating in batch mode implies that the biomass has access to bigger substrate concentration, promoting therefore, a higher growth of the microorganisms. Also, it is presumed that promoting different conditions in the reactor, lead to the possibility for a wider variety of microorganisms to develop. These reasons contributed to a much thicker and dense biofilm, compared to the continuous flow MBBR.




**(a)** **(b)**

**Fig. 5.5:** Biomass concentration in the MB-SBR: (a) refers to both attached and suspended concentration (b) attached only

#### 5.1.4.1 Volatile to Total Suspended Solids Ratio

In this subsection, a brief discussion on the ratio of volatile (VSS) to total suspended solids (TSS) will be made. In this regard, Figure 5.6 represents the VSS/TSS ratio along the tested periods.

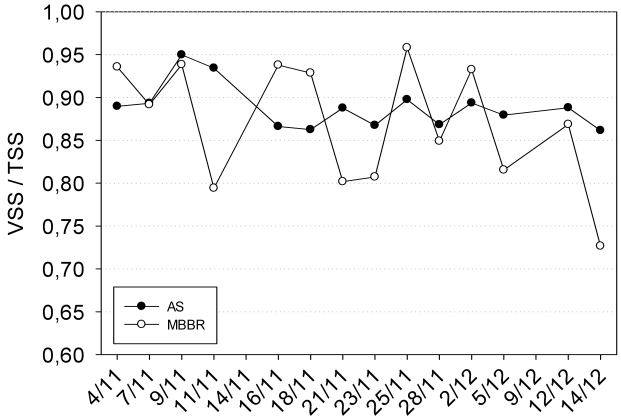


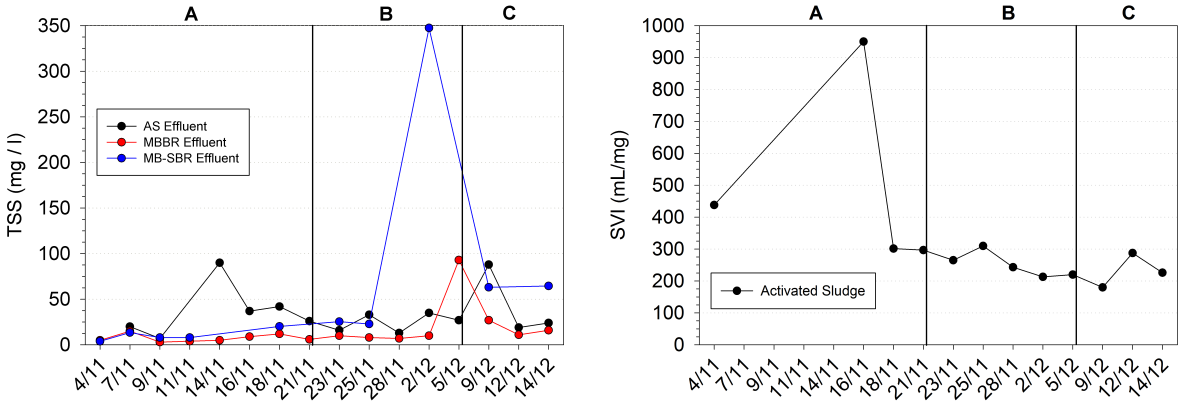

**Fig. 5.6:** Ratio of volatile to total suspended solids for the continuous reactors

Both continuous reactors operated with fairly high VSS/TSS ratios, with the AS reactor providing more stable values whereas the MBBR produced more oscillating ratios. Nonetheless, and for the majority of the continuous experience, the VSS/TSS ratio stayed in a range between 0.8

and 0.95. In the last days of the MBBR, the VSS concentration in the reactor was dropping immensely, again, due to power failure B.

#### 5.1.4.2 Total Suspended Solids in the Effluent

Observing the TSS concentration of the effluents (Figure 5.7a) it is clear the superiority of the MBBR. By operating with lower MLSS concentrations, the solids loading into the clarifier is much lesser in comparison to the AS reactor, leading to a better polished effluent with lower TSS. Note that the AS and MBBR had a dedicated clarifier while the MB-SBR effluent was discharged trough the discharge port on the reactor after a period of sedimentation.




**(a)** **(b)**

**Fig. 5.7:** Effluent TSS concentration

However, sludge settleability also played a major role here. The MLSS of the AS reactor had poor settleability with a mean SVI of 694 mL/mg in the inital period (before biomass addition) and a mean SVI of 254 ($\pm$44) mL/mg after the biomass addition (16/11) (Figure 5.7). Missing measurements are due to the sludge being unable to settle at all, during the 30 minutes of the test. On the other hand, SVI from the MBBR reactor had much greater settleability (SVI $\leqslant$ 90 mL/mg). Although the SVI of the MBBR was only measured once, daily observations concluded that the sludge derived from both Movind Bed systems (MBBR and MB-SBR) had greater settling capacities, which is in accordance to what is described in the literature. Unfortunately, no comparisons were made between the sludge derived from the continuous MBBR and the MB-SBR. Concluding, if the sludge from AS reactor had better settling proprieties, it is our belief that the results would be different, in which the MBBR would be only slightly ahead in terms of

effluent quality.

As for the reactor operating in batch mode (MB-SBR), very similar amounts of solids in the effluent during Period A. However, a gradual increase between the periods can be observed, worsening during period B which coincided with the power failure. The power failure had disrupted the SBR operation and compromised the cycle, however that did not have any repercussions in the operation of subsequent cycles.

It was also noted that although the sludge from biofilms have better sludge settleability, having carriers in a reactor is a factor that influences the amount of sludge that is settled (when operating in batch), which directly interferes with the decanting process. Moreover, when the reactor was operating with high concentrations of suspended solids, during the settling phase, considerably amounts of sludge have been observed near the floating carriers, capably of interfering with the decanting operation, specially when using fixed-type decanting (Figure 2.13c). However, this should not be a problem when operating with lower organic loads or doing more frequent sludge wastings, or even by raising the depth at which the effluent is drained (which causes a decrease in the useful volume of the reactor). These are important aspects to have into account when designing Moving Bed Biofilm Reactors having in mind the batch operation.

### 5.1.5 Organic matter removal performance

In this section the results from the continuous experiment will be presented. The graphs that represent the data from the COD (Figure 5.8) and TC (Figure 5.9) analysis per sampling day are presented below. The graphs correspond to the full experiment duration divided in the three periods that characterized this experience: Period A, B, and C.

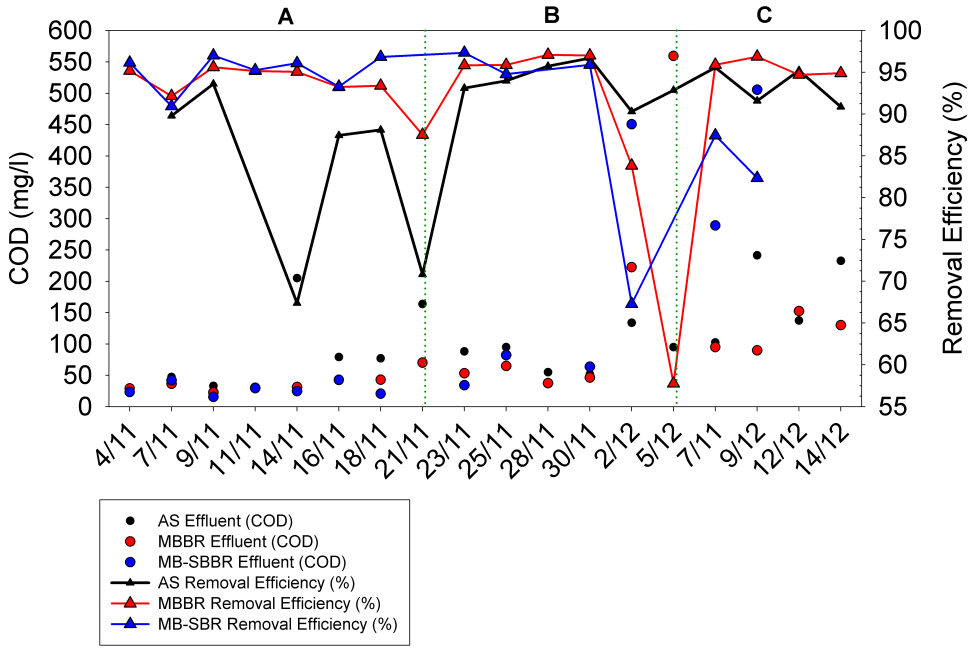



**Fig. 5.8:** Comparison COD values in the effluent per sampling day

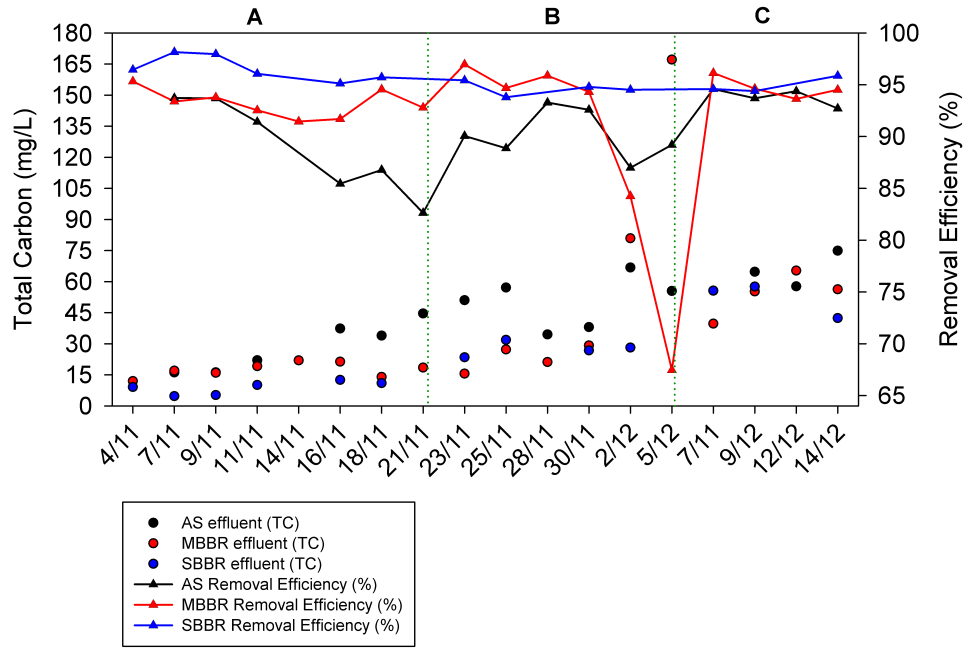



**Fig. 5.9:** Comparison of TC values in the effluent per sampling day

#### 5.1.5.1 Period A

Period A is characterized by the lower COD concentrations in the influent (Table 5.2), thus high removal efficiencies, which means the organic load entering the systems was very far from the

true removal potential.

AS poor efficiencies in some cases are explained by the low biomass concentration in the reactor and poor settleability resulting in too much biomass being lost with the effluent and thus contributing to relatively high COD values (Figure5.7a). That's why TC values are important, they show us the soluble carbon matter, without being compromised by the quality of the separation. Figure 5.9 presents the effluent TC concentration per sampling day and it confirms that the high COD values were in part due to the high concentration of solids in the effluent, and due to low biomass concentration in the reactor, which effectively decreased the performance in this period. On the other hand, very high effluent quality was obtained from the MBBR and MB-SBR systems, the latter with slightly better efficiency, 93.4% and 95.1% (Table 5.3), respectively.

The effects of Power failure A were only felt in the effluent of day 21, and produced an inverted peak for both MBBR and AS, meaning a sudden decrease in the removal efficiency, in which the MBBR showed better stability than the AS reactor. The effects of the power failure were more pronounced in the AS reactor along period B but that could be due to the recent addition of sludge to the reactor and thus, not being yet in completely stable conditions. However, recovery from the power failure was quick for both MBBR and AS reactor. Full period A results are summarized in Table 5.3.

**Table 5.3:** Results summary from period A in respect to effluent COD, TC, and TSS analysis in the continuous phase of experiments

| **Effluent** | **Parameter (unit)** | **N** | **Average (STD.)** | **Min** | **Max** | **Removal Efficiency (%)** |
|---|---|---|---|---|---|---|
| AS | COD (mg/L) | 6 | 100.7 (± 68.3) | 32.9 | 204.9 | 82.9 |
| | TC (mg/L) | 6 | 28.4 (± 11.9) | 16.1 | 44.6 | 89.0 |
| | SST (mg/L) | 6 | 37 (± 29) | 7 | 90 | - |
| MBBR | COD (mg/L) | 8 | 38.0 (± 14.6) | 22.6 | 69.9 | 93.4 |
| | TC (mg/L) | 8 | 17.5(± 3.5) | 12.0 | 22.0 | 93.2 |
| | SST (mg/L) | 8 | 7 (± 4 ) | 3 | 15 | - |
| MB-SBR | COD (mg/L) | 7 | 28.1 (± 10.4) | 15.4 | 42.3 | 95.1 |
| | TC (mg/L) | 6 | 8.8 (± 3.2 ) | 4.7 | 12.5 | 96.6 |
| | SST (mg/L) | 7 | 11 (± 6) | 4 | 20 | - |

#### 5.1.5.2 Period B

Period B demonstrates even greater performance. COD of the wastewater entering the system was averaging roughly 1 397 $mgO_2/L$ (Table 5.2) and a very high removal efficiency period that ranged from day 23 of November to 1 of December, which marked the beginning of power failure B, was found. Until this failure, very stable conditions were met, with an average efficiency of 94.8 %, 96.4 % and 96.0 % for the AS reactor, MBBR and MB-SBR, respectively.

The effects of the power failure B were very pronounced in the effluent quality of the MBBR, mainly because of the subsequent biomass detachment from the carriers that was clearly observed. It contributed to very high concentration of solids in the effluent (TSS of 90mg/L), thus high COD values, and to low concentration of biomass in the reactor, hence limiting the biodegradation reactions. This can be seen in 5/12 where both COD (Figure 5.8) and TC (Figure 5.9) values are very high, confirming the previous statement. Reasons that lead to this detachment are not completely known but it was probably due to the extended period without aeration that the biofilm was subjected to.

On the other hand, the AS reactor showed great stability, in contrary to what was experienced in power failure A. The MB-SBR performance drop in 2/12 is explained by a high concentration of suspended solids in the effluent. The reactor was operating with very high concentration of TSS and it harmfully influenced the settling capabilities. This is corroborated by observing the TC (Figure 5.9) values for day 2/12, which clearly demonstrates the infuence of the effluent TSS concentration in the COD values. The summary of period B results are presented in Table 5.4.

**Table 5.4:** Results summary from period B in respect to effluent COD, TC, and TSS analysis in the continuous phase of experiments

| Effluent | Parameter (unit) | N | Average (STD.) | Min | Max | Removal Efficiency (%) |
|---|---|---|---|---|---|---|
| AS | COD (mg/L) | 6 | 86.1 (± 30.3) | 51.3 | 133.5 | 93.8 |
| | TC (mg/L) | 6 | 50.5 (± 12.2) | 34.5 | 66.8 | 90.2 |
| | SST (mg/L) | 5 | 25 (± 10) | 13 | 35 | - |
| MBBR | COD (mg/L) | 6 | 163.9 (± 205.8) | 37.3 | 559.4 | 87.9 |
| | TC (mg/L) | 6 | 56.9 (± 58.9 ) | 15.6 | 167.1 | 88.9 |

| Effluent | Parameter (unit) | N | Average (STD.) | Min | Max | Removal Efficiency (%) |
|---|---|---|---|---|---|---|
| | SST (mg/L) | 5 | 26 (± 38 ) | 7 | 93 | - |
| MB-SBR | COD (mg/L) | 4 | 157.5 (± 196.3) | 33.9 | 450.4 | 88.8 |
| | TC (mg/L) | 4 | 27.6 (± 3.5) | 23.5 | 31.9 | 94.6 |
| | SST (mg/L) | 3 | 131.9 (± 6) | 22.9 | 347.5 | - |

#### 5.1.5.3 Period C

Day 5/12 marks the beginning of period C. COD concentration entering the reactors was increased to roughly 2646 mg$O_2$/L (Table 5.2). The MB-SBR high TSS concentrations (Figure 5.5b) kept influencing the COD results, but now the effect was even more pronounced. More frequent sludge wasting should have been accounted for, in order to accommodate to the higher biomass growth due to higher organic load. Indeed, biomass growth was tremendous, with sludge wasting having to be removed almost every 3 to 4 cycles of operation (36 to 48 hours).

For the MBBR, despite having relatively low biomass concentration in the carriers(Figure 2.10), a performance drop was not seen, maybe because the relatively high MLSS concentration in the reactor was compensating for biofilm loss. However, even when operating with fewer total biomass, the MBBR was slightly better than the AS reactor.

COD removal efficiencies for this period were 93.3 %, 95.6 %, and 84.9 % for the ASR, MBBR, and MB-SBR, respectively. TC values, once again, demonstrate the influence of TSS concentration, mainly in the MB-SBR. Removal efficiencies were 93.8 %, 94.7 %, and 95,0 % for the ASR, MBBR, and MB-SBR.

**Table 5.5:** Results summary from period C in respect to effluent COD, TC, and TSS analysis in the continuous phase of experiments

| Effluent | Parameter (unit) | n | Average (ST.D.) | Min | Max | Removal Efficiency (%) |
|---|---|---|---|---|---|---|
| AS | COD (mg/L) | 4 | 178.3 (± 69.2 ) | 102.2 | 241.2 | 93.3 |
| | TC (mg/L) | 4 | 63.2 (± 8.7 ) | 55.6 | 74.9 | 93.8 |
| | SST (mg/L) | 3 | 44 (± 38) | 19 | 88 | - |
| | COD (mg/L) | 4 | 116.6 (± 29.7 ) | 89.7 | 152.3 | 95.6 |

| Effluent | Parameter (unit) | n | Average (ST.D.) | Min | Max | Removal Efficiency (%) |
|---|---|---|---|---|---|---|
| MBBR | TC (mg/L) | 4 | 54.1 (± 10.6 ) | 39.7 | 65.3 | 94.7 |
| | SST (mg/L) | 3 | 18 (± 8 ) | 11 | 27 | - |
| | COD (mg/L) | 2 | 397.2 (± 153.2 ) | 288.8 | 505.5 | 84.9 |
| MB-SBR | TC (mg/L) | 3 | 51.9 (± 8.3 ) | 42.4 | 57.6 | 95.0 |
| | SST (mg/L) | 2 | 64 (± 1 ) | 63 | 65 | - |

### 5.1.6 Total Nitrogen removal performance

As we have seen, the total nitrogen content in the influent wastewater increased stepwise from roughly 52 mg/L to 208 mg/L throughout the three periods.

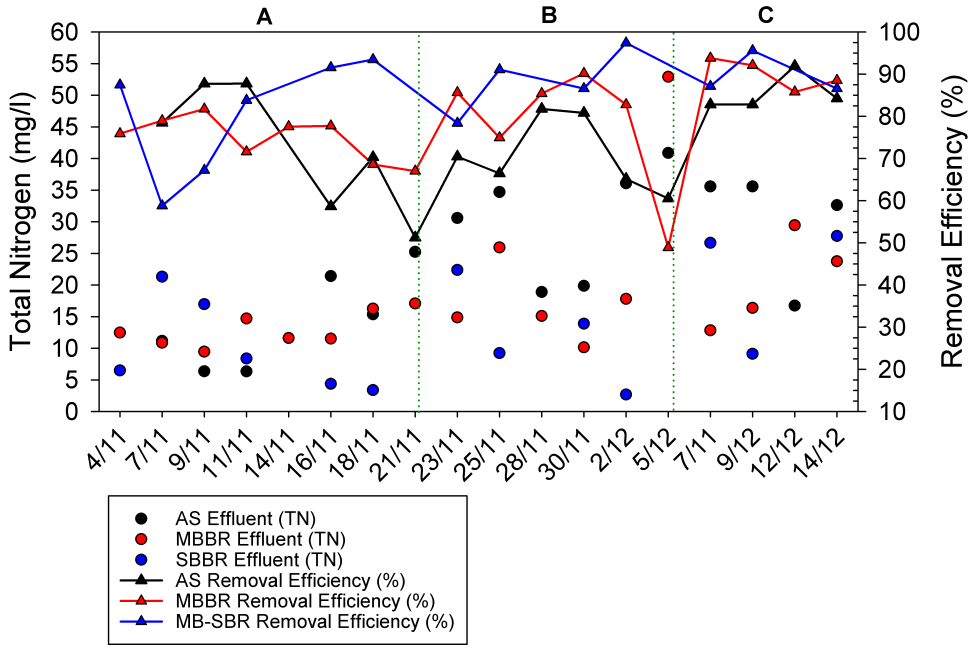



**Fig. 5.10:** Comparison of TN values in the effluent per sampling day

By observation of the data presented in the Figure 5.10 and in the table summary below (Table 5.6), it is noted that the efficiency of each reactor increased slightly with the increase in the organic load. Overall, the MB-SBR had the best nitrogen removal capabilities, which was expected, since it incorporated both aerobic and anoxic periods, which promoted the nitrification and denitrification reactions, respectively. However, from Period B to period C the efficiency remained similar, which could mean the MB-SBR was near or at its removal limit. Overall efficiency for the batch reactor was 85.0 %.

On the other hand, the continuous reactors did manage to have good TN removal efficiencies, despite not being specifically designed for nitrogen removal. In the AS reactor the main mechanism for nitrogen removal was the nitrogen assimilation by heterotrophic bacteria and incorporation into new biomass, and maybe some level of nitrification, which resulted in an overall efficiency of 75.1 %. The MBBR had an efficiency of 79.3 %, slightly better than the AS reactor, which may indicate that not only nitrogen assimilation and some level of nitrification was taking part, but also, the nitrogen reduction reactions from the interior zones of the biofilm, which is believed to be absent of oxygen, thus contributing to the denitrifiers activity.

**Table 5.6:** Results summary from Total Nitrogen analysis in the continuous phase of experiments

| Reactor | Period | N | Average (ST.D.) | Min | Max | Removal Efficiency (%) |
|---|---|---|---|---|---|---|
| AS | Period A | 6 | 14.3 (± 7.8) | 6.3 | 25.2 | 72.3 |
| | Period B | 6 | 30.2 (± 9.0) | 18.9 | 40.9 | 70.9 |
| | Period C | 4 | 30.1 (± 9.0) | 13 | 35.6 | 85.4 |
| MBBR | Period A | 8 | 13.0 (± 5) | 9.5 | 17.1 | 74.9 |
| | Period B | 6 | 22.8 (± 15.6 ) | 10.2 | 52.9 | 78.0 |
| | Period C | 4 | 20.6 (± 7.4 ) | 12.8 | 29.4 | 90.1 |
| MB-SBR | Period A | 6 | 10.1 (± 7.3) | 3.4 | 21.3 | 80.4 |
| | Period B | 4 | 12.0 (± 8.3) | 2.7 | 22.4 | 88.4 |
| | Period C | 3 | 21.2 (± 10.5) | 9.1 | 27.85 | 89.8 |

Comparing these values with the maximum discharge limits in the current legislation (Decree-law nº236/98, Annex XVIII), it is observed that the averages from the three systems in period A are below the discharge threshold (TN $\leqslant$ 15 mg/L), which could possibly indicate that there is no need for a specialized nitrogen removal system when dealing with lower organic loads (nitrogen content of 52 mg/L in period A). However, when the organic load was increased, the total nitrogen levels in the effluent were not fully compatible with direct discharge onto the aquatic environment in the continuous reactors for period B, and C. The batch reactor (MB-SBR) has the best potential for nitrogen removal, and it only falls very short of the discharge limit ($\leqslant$ 15 mg/L) in period C, when the influent TN concentration was estimated to be roughly 208 mg/L (4/200 dilution). This could be fixed by increasing the total cycle time, and is not that uncommon to have batch cycles higher than 12 hours, or by testing different operating conditions, such as increasing the length of the anoxic period (without increasing the total cycle time).

### 5.1.7 Final considerations

#### 5.1.7.1 Total sludge production

In this section the solid production results are presented. The total solids production was calculated in a period in which stable or pseudo-stable conditions were found, which was from 18/11 to 2/12. The figure 5.11 represents the solids production in each reactor, broken down per purged solids, which are the solids removed through sampling and the wasted solids, per difference in the reactor solids within the respective period, and per solids contained in the effluent (total mass of suspended solids that were discharged in the treated effluent).

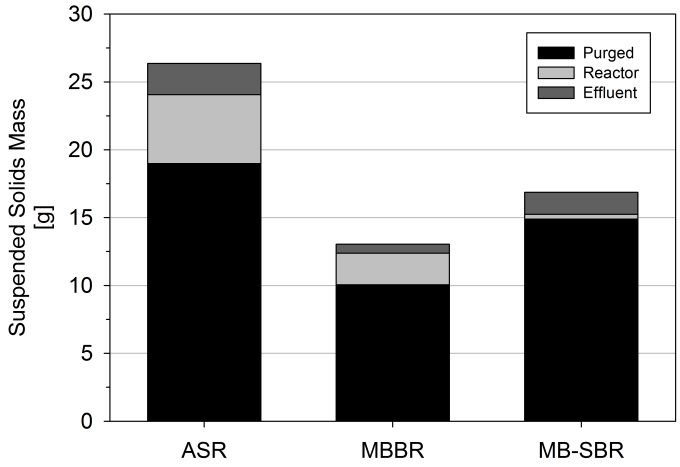



**Fig. 5.11:** Comparison of the produced solids

The difference in the MB-SBR reactor solids was only due to the attached biomass concentration, since the suspended solids difference is already being considered in the purge.

The total produced solids for the AS, MBBR, and MB-SBR was 26.26, 13.03, 16.86 g TSS, which is equivalent to 1.88, 0.93, and 1.20 gTSS/d, respectively. If relating the solid production per amount of substrate removed then we have 0.25, 0.12, and 0.16 g TSS/g removed COD, respectively. The daily amount of COD entering the system was calculated by the average of the COD values in the respective period multiplied by the flow rate (7 L/d), which was 8.26 g COD/d. The removed COD was calculated with the average efficiencies for each system in the referred period.

Aygun et al. (2008) studied the production of solids in a MBBR with an average of 2.85 g COD/d and found that the MBBR produced 0.35 g TSS/d which is equivalent to 0.12 g TSS/g removed

COD, the same value found for the MBBR in this continuous experience, which gives validation to the values that were reached in the present work. The value found for the MBBR is certainly lower than the common values for the activated sludge process.

#### 5.1.7.2 Food to Microorganism ratio

One important aspect to consider is the food to microorganism ratio that the reactors were submitted to. The next figure represents the evolution of the food-to-microorganism ratio in this experiment, related to the TC values of the influent. Note that the F/M for the MBBR was calculated having in mind both attached and suspended biomass.

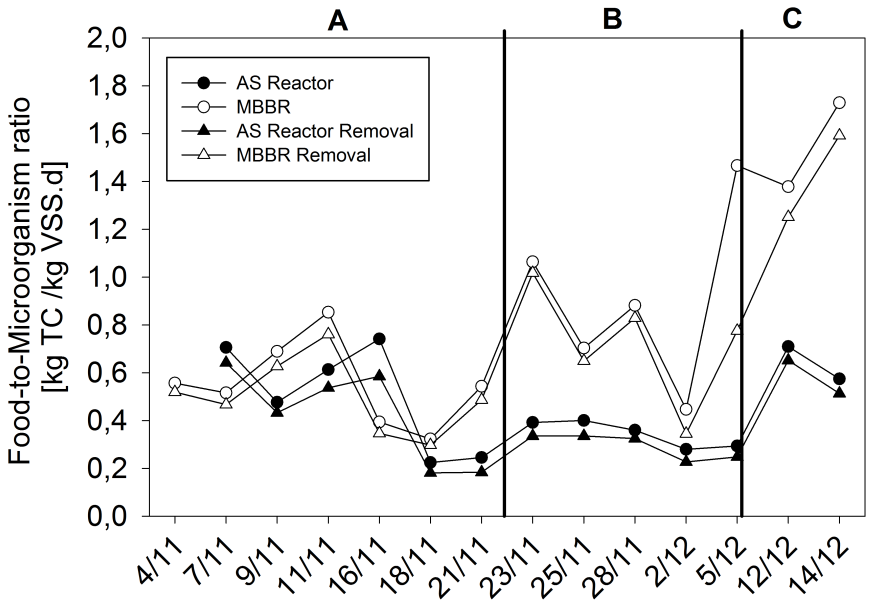



**Fig. 5.12:** Food to Microorganism ratio in the continuous phase

Both F/M loading and F/M removed are presented. The F/M removed, represents the food to microorganism ratio multiplied by the daily efficiencies of each system. The gap between the two (F/M and F/M removed) represents the residual substrate contained in the effluent per biomass, which means the bigger the gap, the lower the removal efficiency.

But what's important here is that both the reactors achieved similarly efficiencies, 85.0 % and 86.3 % for the Activated Sludge and the MBBR respectively, however, the MBBR operated with nearly double the F/M ratio, which is impressive.

An high F/M means the microorganism are submitted to bigger loads of "food" per microor-

ganism. This means that, although the MBBR operated with lower biomass concentration, that didn't have any impact on the removal performance, which is indicative that the biomass contained in the biofilm has higher activity.

Results in terms of COD certainly have the influence of the sludge settling in it, so, in terms of TC (Figure 5.12), the average was 0.47 and 0.82 kgTC/(kgVSS.d), and the removed was 0.40 and 0.711 kg removedTC/(kgVSS.d), equivalent to 85.0 and 86.3 %, respectively, as stated before.

Because literature values are more commonly found in terms of BOD or COD, then for comparison purposes, the average F/M in terms of its chemical oxygen demand obtained in the present work was 1.14 and 2.10 kg COD/(kgVSS.d) for Activated sludge and the MBBR, respectively. If the removal is referred to the biomass in the reactor, then we have 1.00 and 1.84 kg removedCOD/(kgVSS.d) which is equivalent to a removal percentage of 87.6 and 89.4 %.

Jahren et al. (2002) found values of 1.5–2.6 kg sCOD/(kgVSS.d) and removal rates of 1.1-1.8 kg sCODkg/(VSS.d) in a MBBR reactor. For the AS process, literature values of F/M for the conventional AS are situated between 0.2 and 0.5 in terms of the $BOD_5$, which one could say it's equivalent to a F/M between 0.4 and 1.0 in terms of its chemical oxygen demand (assuming a BOD to COD ratio of 0.5 (Table 3.5)). The average for the AS in this study is quite a bit higher than the maximum suggested limit, but it was derived from the initial period in which the biomass concentration in the AS reactor was very low. But, overall, the AS reactor still operated with fairly high F/M ratios.

#### 5.1.7.3 Effluent COD/TC ratio

Since both COD and TC are both measurements to the organic matter present in the wastewater, one could expect a reasonable correlation between these parameters. However, since TC analysis is measured from the soluble fraction only, and the COD analysis is performed in relation to the total sample (soluble and particulate), some differences between the two were observed, which could be explained by the presence of particulate matter (biomass) in the effluent. The correlation (Figure 5.13) is used in order to assess the extent of these differences for each reactor.

It is observed that the MBBR COD values in the effluent could be well correlated to their TC values, and this is due to lesser biomass in the effluent. It also confirms that the AS reactor and the MB-SBR COD values had the influence of particulate matter (biomass) in the effluent.

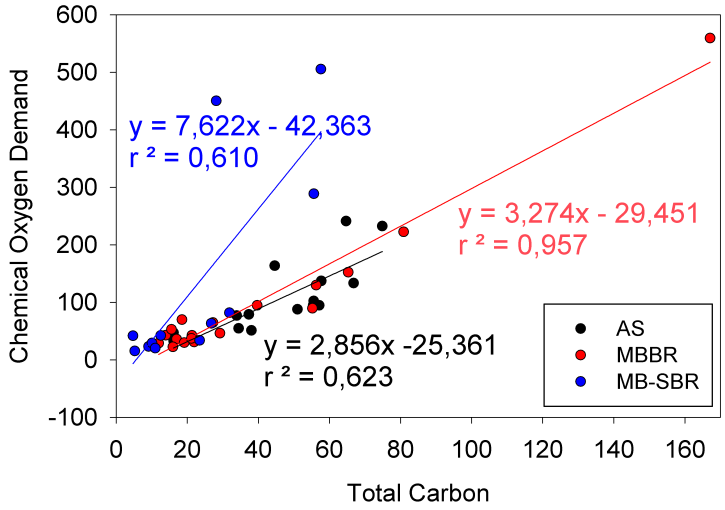



**Fig. 5.13:** Effluent COD/TC ratio

## 5.2 Batch Phase

The following section concerns the second phase of the experiments, the Batch phase. It consisted in batch tests in order to understand the evolution of the conditions inside the reactor as well as the kinetics for each reaction. The reactor operated at 15.8 °C.

### 5.2.1 First batch test

The aim of this test was to evaluate not only the degree of biomass growth within the reactor, in respect to the MLSS concentration, but also the degree of organic matter removal, and how they are related to each other. The MB-SBR results for the first batch test are presented below (Figure 5.14).

In an overall observation, its clear that the 8 hour reaction period was not enough to degrade all the organic matter. Bear in mind that this is the soluble COD concentration, so no influence of the solids here was taken into account. The bacterial growth is quite visible, in which the first three hours are characteristic of an exponential growth phase of a typical Monod curve when great amounts of substrate are available for growth. After the 3 hour mark, the biomass concentration remained more or less constant. The small variance could be assumed to be from experimental errors, which are very characteristic of a TSS analysis.

COD started at 1792 mg/L and after 8 hours of degradation was at 205 mg/L. The first hour was

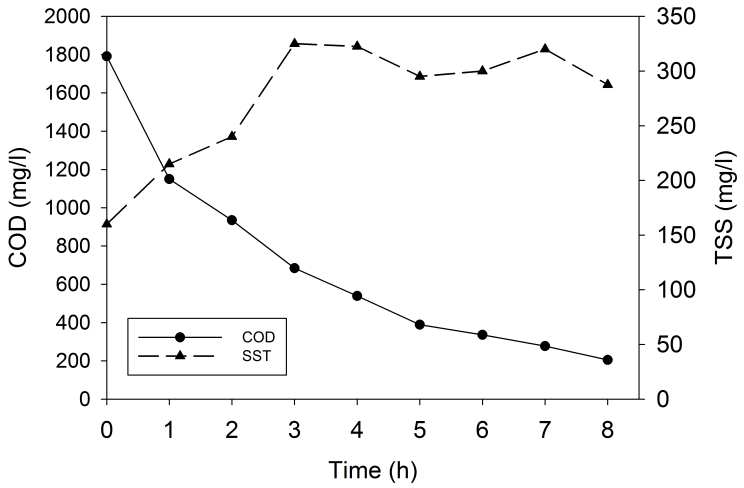



**Fig. 5.14:** sCOD and TSS concentration in the first 8 hour batch test

responsible for 36 % of degradation and after 8 hours, 89 % of the COD was effectively degraded. A gradually decrease in the substrate degradation velocity is seen, with the first 3 hours being responsible for the majority of the degradation. To assess the reaction kinetics, the reaction was assumed to follow Monod kinetics, according to Equation 5.1.

$$\mu = \frac{\mu_{max} \times S}{K_s + S} \tag{5.1}$$

During the so-called exponential growth, characterized by a high availability of substrate, the above equation may be simplified to $\mu = \mu_{max}$. Assuming first-order reaction rate kinetics and that the volume remains constant (reaction in liquid phase), the mass balance to the biomass comes:

$$\frac{dX}{dt} = \mu_{max} X \tag{5.2}$$

and

$$\int_{X_0}^{X} \frac{dX}{X} = \mu_{max} \int_{0}^{t} dt \tag{5.3}$$

where X is the biomass concentration at a given hour, $X_0$ is the initial biomass concentration, both in mgVSS/L, and $\mu_{max}$ is the maximum specific growth rate of the microorganisms ($t^{-1}$). Integrating Equation 5.2 results in:

$$\ln \frac{X}{X_0} = \mu_{max} t \tag{5.4}$$

This equation is plotted and the result is presented in Figure 5.15. The biomass in mgVSS/L was calculated assuming a VSS/TSS ratio of 0.85, based on the data obtained in the previous continuous experiment (subsubsection 5.1.4.1).

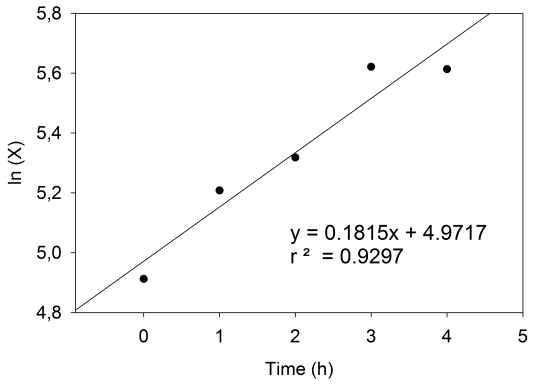


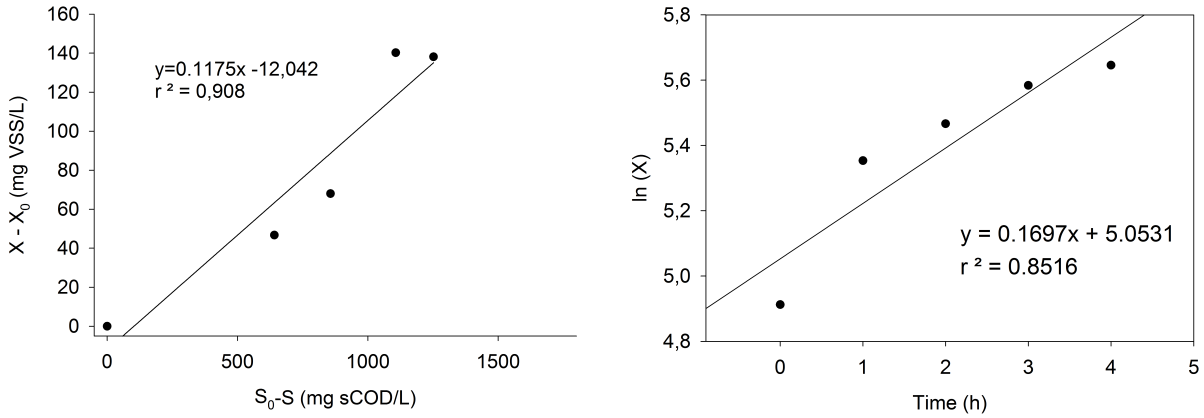

**Fig. 5.15:** First order reaction linearization in the first batch test

The slope from the plot represents the $\mu_{max}$ in Equation 5.4, so $\mu_{max} = 0.182$ h$^{-1}$ or 4.36 day$^{-1}$. It is also possible to correlate the amount of biomass produced per amount of substrate that is consumed, the $Y_{OBS}$. In order to do this the following equation is used:

$$X - X_0 = Y'_{OBS} \times (S_0 - S) \tag{5.5}$$

Where X is the biomass concentration at a given hour and $X_0$ is the initial biomass concentration, both in mg VSS/L, $S_0$ is the initial substrate concentration and S is the substrate concentration at a given hour, both in mg sCOD/L. The data from the experience is plotted according to the equation above and the result is presented in Figure 5.16a (below). The slope of the plot represents the $Y'_{OBS} \approx 0.118$ mg VSS/mg COD. Typical values for the Activated Sludge are $Y'_{OBS} = 0.35$ mgVSS/mg sCOD.

Equation 5.5 could now be used to estimate a theoretical value for the biomass based on the substrate concentration and considering Equation 5.4, a new relationship is plotted to determine the $\mu_{max}$ that is now related with the sCOD measurements instead of the biomass measurements. The slope of the plot represents the $\mu_{max}$=0.1697 h$^{-1}$, or 4.07 d$^{-1}$, which is slightly lower than the previous (Figure 5.15). However, since the determination through the substrate leads to a lower correlation, $r^2$= 0.852 in Figure 5.16b, the $\mu_{max}$ value was referred to the one found in (Figure 5.15) ($r^2 = 0.93$), so $\mu_{max}$=4.36 d$^{-1}$ at 15.8 °C. This parameter is dependant upon the temperature, common values for activated sludge system are $\mu_{max} = 4.1$ day$^{-1}$ at 15 °C, and




**(a)** **(b)**

**Fig. 5.16:** (a) Y'$_{OBS}$ plot results; (b) Estimation of $\mu_{max}$ parameter using the Y'$_{OBS}$

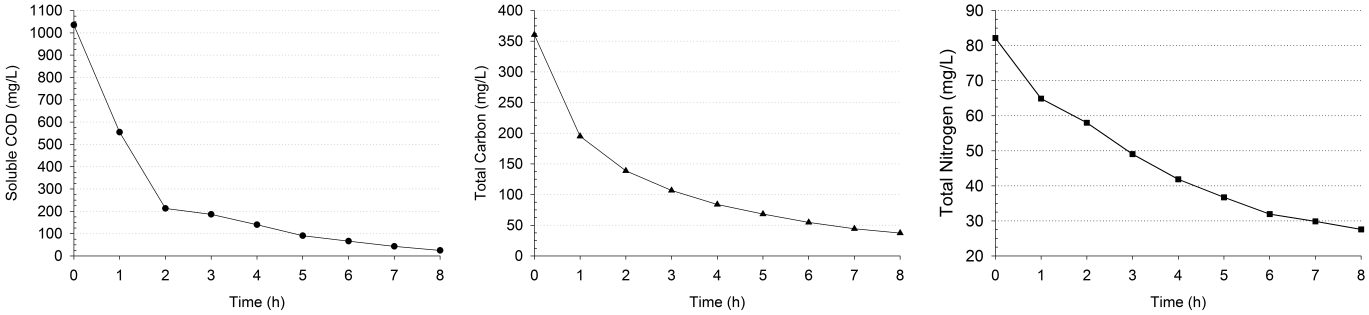
$\mu_{max} = 6.0$ day$^{-1}$ at 20 °C (Grady et al., 2011), which means that the values found in the present are very similar to the ones reported in the literature for the activated sludge.

### 5.2.2 Second batch test

In the second batch test, the COD was purposely lowered, from 1817 to 1060 mg/L, given the previous test which resulted in organic matter present in the influent not being completely degraded and also because the objective of this experiment was also to assess total nitrogen removal in the aerobic period, and how the carbon and nitrogen removal related to each other. The data for this experience is represented in figure 5.17. Observing the COD (Figure 5.17a) and TC (Figure 5.17b) values for carbonaceous matter removal, it is seen that roughly 46 % of the organic matter is degraded in the first hour and after a four hour period of reaction, the effluent could be directly discharged onto the environment.

However, total nitrogen removal (Figure 5.17c) was a little disappointing, in comparison to the results obtained by the MB-SBR in the continuous phase of experiments, and it might show the influence of the anoxic period. The MB-SBR from the previous experience operating with the same organic load, and the same aerobic period length, permitted effluent concentrations of total nitrogen as low as 9.2 mg/L with an average of 12.0 mg/L (n=4), as it was observed in subsection 5.1.6.

A good correlation between the TC and the sCOD values was found (Figure 5.18), as expected,




**(a)** **(b)** **(c)**

**Fig. 5.17:** Second batch test results: (a) Soluble COD concentration; (b) TC concentration; (c) TN concentration

since the COD values are referred to the soluble component only, as the TC is. The Monod

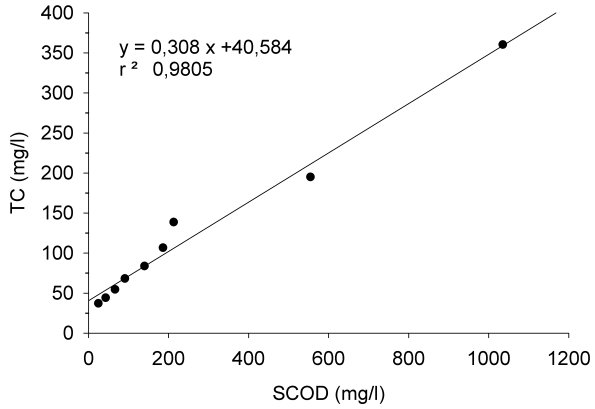



**Fig. 5.18:** Relationship between total carbon and soluble chemical oxygen demand in the second batch test

kinetics for the TC and sCOD were determined, utilizing Equation 5.4, with the biomass (X values) being calculated from the substrate concentration using the Equation 5.5, as in the previous test. TC concentration was converted to sCOD values by utilizing the correlation found in Figure 5.18. So, plotting Equation 5.4 for each COD and TC data results in the Figure 5.19 (below). As before, if the slope of each plot represents the maximum growth $\mu_{max}$, then we have $\mu_{max}$= 0.123 $h^{-1}$ and $\mu_{max}$= 0.1095 $h^{-1}$, or $\mu_{max}$=2.957 $d^{-1}$ and $\mu_{max}$=2.628 $d^{-1}$ for soluble COD and TC, respectively.

As for the total nitrogen, the linearization according to a first order kinetic did not quite describe the behaviour of the TN concentration and, as such, the derivative method was applied, in order to find the order value, $n$.

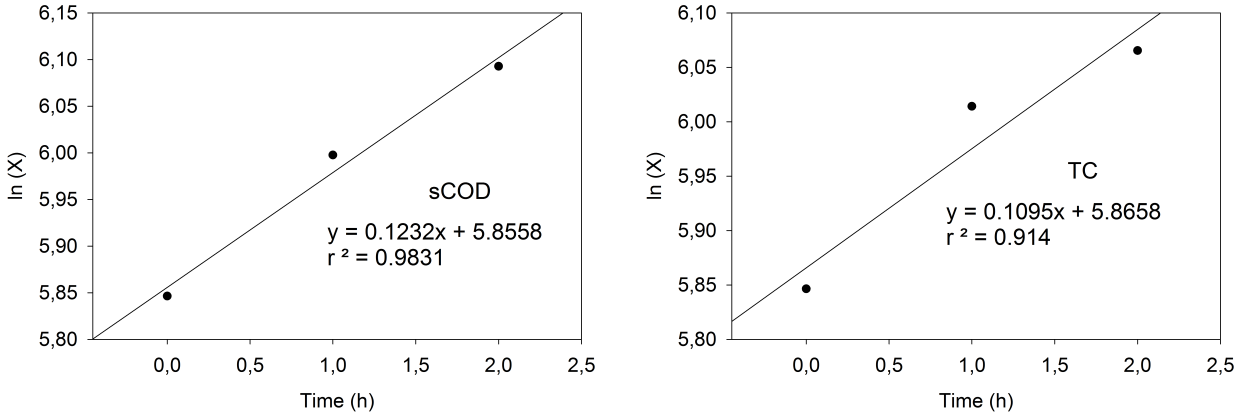



**Fig. 5.19:** Reaction rate kinetics assessment in the second batch test. (a) Soluble COD linearization; (b) Total Carbon linearization

The first step was to interpolate the evolution of TN concentrations in order to get a polynomial function, easy to derivate, and that adjusted adequately to the experimental results. Secondly, the reaction kinetics were assumed to follow as:

$$-\frac{dC}{dt} = k\ C^n \tag{5.6}$$

where C is the TN concentration, and t, the time in hours. Integrating Equation 5.6 gives:

$$\ln(-\frac{dC}{dt}) = \ln k + n \ln C \tag{5.7}$$

So, the order $n$, is the slope of the plot between the first derivate of the polynomial function that describes the TN evolution and the natural logarithm of the TN concentration, which is found in Figure 5.20.

The order that resulted from the linearization was n=1.76, with an $r^2 = 0.98$ and a reaction rate constant, k, of $e^{-4.8994} = 0.0075 = k$. However, because the order is very close to a second order reaction, the evolution of TN concentration (Figure 5.17c) was plotted according to second-order reaction kinetics, by using Equation 5.6, but now admitting an order n = 2. Integration results in Equation 5.8.

$$\frac{1}{C} = kt + \frac{1}{C_0} \tag{5.8}$$

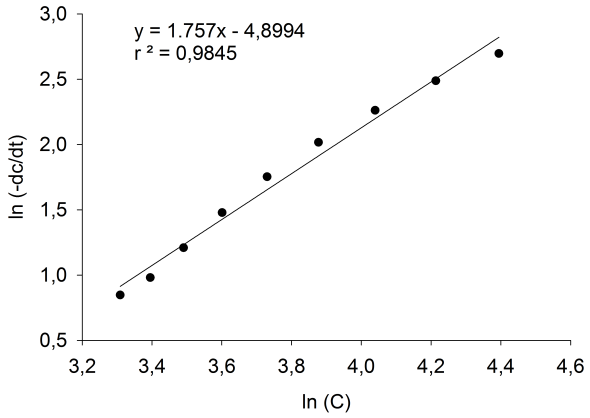



**Fig. 5.20:** Derivative method utilizing a polynomial function for the determination of the order for TN substrate removal

where $C_0$ is the initial TN concentration, C is the TN concentration at a given hour, both in mg/L, t is the time in hours, and k is the reaction constant ($t^{-1}$). So, plotting $\frac{1}{C}$ versus t results in the Figure 5.21 (below).

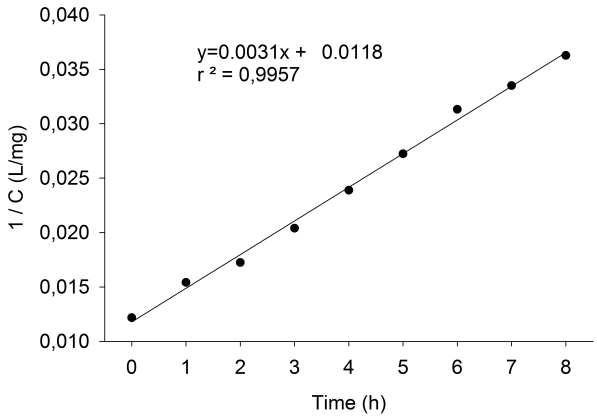



**Fig. 5.21:** Plot of 1/C versus t in respect to Total Nitrogen in batch test 2

The reaction coefficient takes the value of the slope of the graph, in which comes, k = 0.0031 $h^{-1}$.

# Chapter 6

# Conclusions and Future Work

## 6.1 Conclusions

With the development of this work it is possible to conclude that every system offered very good treatment capabilities for a dairy industry effluent. In fact, in terms of the organic removal capabilities the proposed technologies presented similar performances, with a slight advantage to the Moving bed systems.

With a HRT of 12 hours, the three reactors were able to provide the necessary conditions for a direct discharge onto the aquatic environment, in accordance with the current portuguese legislation (Decree-law nº236/98, 1 of August), with an influent COD concentration of up to 2 647 $mgO_2/L$ (average), corresponding to organic loading rates varying from 0.92 to 5.7 $kgCOD/m^3.d$. However, during the period with higher organic load (period C) the effluent COD concentration was approaching the maximum discharge values (150 $mgO_2/L$).

The global COD removal efficiencies were 89.6 %, 92.1 %, and 91.6 % for the AS, MBBR, and the MB-SBR, respectively. Regarding total carbon, the global removal efficiencies obtained were 90.6 %, 92.17 %, and 95.6 %, for the AS, MBBR, and the MB-SBR, respectively. Total nitrogen removal efficiencies were greater in the moving bed system operated in batch mode (MB-SBR), which could constitute an important aspect in the design and treatment selection for the dairy industry. Overall, the MB-SBR presented the higher performances, especially in period A and B, where organic loads where lower. However, with the increase in the organic load, a slight decrease in the efficiency was noted, that would be more prominent if higher loads were tested.

Another aspect that plays in favor of the moving bed biofilm systems is the simplicity of the process. No sludge recirculation was needed in the MBBR and MB-SBR systems. Also, continuous MBBR's operate with lower suspended biomass concentrations, which gives the possibility of having smaller clarifiers, without compromising the treatment quality. Moreover, the simplicity of the process, could offer the opportunity in reducing investment and operational costs.

If one refers to the treatment performance in terms of the quantity of biomass present in the reactor, the MBBR comes with an impressive advantage, capable of dealing with very high loads, while not necessarily meaning higher biomass production. In fact, the MBBR produced 50 % less of excess sludge than the AS and 26 % less than the MB-SBR during stable conditions of the continuous experiment. These reasons could constitute a considerably economic advantage by effectively decreasing the amount of excess sludge produced and therefore the amount of excess sludge disposed, which could be the main expense in a wastewater treatment plant.

The kinetic modelation showed interesting results with a sequencing batch MBBR. Values of $Y_{OBS} = 0.12$ were found, that seem to be lower than the ones found in the literature. However, $\mu_{max}$ determinations revealed results that are similar to the ones found in the literature. To mention that these results were only preliminary, and should serve as basis for future work.

## 6.2 Future Work

The result of any investigation is an unfinished work as many more experiences can be elaborated. Time was brief and aside the answered questions many more arised. To further test the MBBR capabilities, additional work should be continued. Suggestions for future work are presented below.

- Microscopic analysis to the biomass, in order to understand the differences and types of microorganisms involved;
- Experimenting with different biocarriers as there are enumerous models on the market and each one is particularly different, with different configurations and thus different conditions for the microorganisms to attach;
- Testing reduced hydraulic retention times, more proximate to the ones used in real world wastewater treatment plants;
- Further study of the kinetics involved and comparison with the conventional activated

sludge process;

- Further test reactors performance to hydraulic shocks or air supply shortages. The ones studied happened due to malfunctioning or technical issues and were not premeditated. Studying this episodes on a controlled scenario provides much more reliable results;
- Implementation of various continuous reactors in series each one optimized for a different purpose such as nitrification, denitrification or carbon matter removal, instead of accomplishing every process in a single underperforming reactor. Also, testing different predispositions of the reactors and how they affect the overall removal rate;
- Testing configurations that are suitable for biological phosphorous removal;
- Experimenting on real dairy industry effluent. Although synthethic wastewater experiments can be proximate to real effluent studies, testing with real wastewater provides more realistic results;
- Final but not the least, transitioning from lab-scale reactors to pilot scale plants.

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# Acronyms and symbols

| | **Acronyms and terms** |
|---|---|
| AS | Activated Sludge |
| BNR | Biological Nutrient Removal |
| BOD | Biochemical Oxygen Demand |
| COD | Chemical Oxygen Demand |
| DO | Dissolved Oxygen |
| EBPR | Enhanced Biological Phosphorous Removal |
| F/M | Food to microorganism Ratio |
| HDPE | High Density Polyethylene |
| MB-SBR | Moving Bed-Sequencing Batch Reactor |
| MLSS | Mixed Liquor Suspended Solids |
| MLVSS | Mixed Liquor Volatile Suspended Solids |
| NOx | Nitrate-N; Nitrite-N |
| OLR | Organic Loading Rate |
| SBR | Sequencing Batch Reactor |
| sCOD | Soluble Chemical Oxygen Demand |
| SLR | Surface Loading Rate |
| SRT | Solids Retention Time |
| SVI | Sludge Volume Index |
| TC | Total Carbon |
| TN | Total Nitrogen |
| TSS | Total Suspended Solids |
| VSS | Volatile Suspended Solids |
| VFA | Volatile Fatty Acids |
| RAS | Recirculated Activated Sludge |