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EXPERIMENTAL STUDY IN CONTINUOUS FLOW FOR DAIRY WASTEWATER WITH AS, IFAS AND MBBR SYSTEMS: TREATMENT AND MICROBIOLOGY

Dissertation in the ambit of the Integrated Master's in Environmental Engineering, supervised by Professor Luís Miguel Moura Neves de Castro and Professor Rosa Maria de Oliveira Quinta-Ferreira, presented for Dissertation in Technology and Environmental / Mechanical Engineering Department

February 2020

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FACULDADE DE CIÊNCIAS E TECNOLOGIA
UNIVERSIDADE D COIMBRA

Experimental study in continuous flow for dairy wastewater with AS, IFAS and MBBR systems: treatment and microbiology

A dissertation for graduated study in MSC program for Master of Science in environmental engineering

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Coimbra, February, 2020

When the last tree is cut down, the last river poisoned, the last fish caught. Then only will man discover that he cannot eat money

-Native American proverbe

Acknowledgements

First of all, I would to acknowledge Professor Rosa Quinta-Ferreira for the opportunity to investigate such an interesting subject and for all the words of support and motivations.

A special thanks to Professor Luis Miguel Castro for providing both installations and all the equipment necessary for the experience and for all their patience and support.

I would also want to demonstrate my gratitude to Professor António Amaral for his availability and attention regarding the microbiology area.

To my parents, a huge thanks, there will never be enough words to thank them for everything they have done for me throughout my life, and for the encouraging words in the most difficult times that were my support during the entire journey, without them I would never have been able to proceed.

To my companion, Samuel Costa, a gigantic thanks for all the unconditional support, for always getting me to feel that I was capable to achieve all my dreams and for making me believe that I would be able to continue and conclude my thesis.

To my lab colleague, João Silva, who was always there to help through the whole experience, which was not always easy, a big thank you not only for all your help but also for your support and persistence that was fundamental throughout the entire experience.

Last but not least, to all my friends for all the motivations and jokes in the most difficult times that helped a lot during this journey

Abstract

The Moving Bed Biofilm Reactor, MBBR, and the Integrated Fixed-Film Activated Sludge, IFAS, are advanced biological systems for wastewater treatment. The advantages associated with their applications, contributed to carry out many investigations on these systems, as they provide an easier operation, with high organic load removal efficiencies in addition to producing less amount of sludge, being a great economical advantage as the sludge disposal corresponds to the majority of the operations costs, when compared with the Activated Sludge, AS. IFAS system can be easily implemented in water treatment plants, that already use the AS system, without the need of expansion of the system. The greatest advantages of the MBBR system is that it does not need a sludge recycling system.

This study aimed to compare three systems, AS, IFAS and MBBR in terms of treatment efficiency, biomass characterization and microbial identification. The experimental system consisted in three independent reactors that operated in a continuous mode throughout the experiment. The effluent used was composed by a dilution of milk with water, in order to resemble a real dairy effluent. The experiment was divided in two periods, A and B. In both periods the same filling fractions were used, 44.5% for MBBR and IFAS reactors. Period A, lasted 37 days and a dilution of 1:200 was used, whereas period B lasted 25 days and the dilution was 1:100. With the variation in the amount of organic load entering each reactor, it was possible to obtain the performances and relate each one, compared to the organic load increase as well as the variation in the microbial consortium.

IFAS showed better performance, in the analysed parameters for both periods compared to MBBR and AS. Chemical Oxygen Demand (COD) removal efficiencies in period A were 63%, 73% e 71% for AS IFAS and MBBR, respectively. Period B obtained higher efficiencies for the analysed parameters, with the exception of MBBR, which suffered a washout in the second day after the begging of the experiment, that led the sludge out of the reactor, to the treated effluent, and subsequently the loss of biofilm provoked by the detachment phenomena. The increase in the organic load and air diffusers problems may justify the results. The CQO removal efficiencies were 82%, 84% e 79% for AS, IFAS and

MBBR, respectively. During period B, IFAS produced less 36.5 % of sludge than AS, whereas MBBR produced less 85.5%.

For biomass characterization, the suspended biomass was used as a sample. It is concluded during the analysed periods, that there was no evidence of *zoogleal bulking* or filamentous bulking, but, nevertheless, there was signs of pinpoint in the MBBR reactor, which was justified through the biomass characterization and microbiological observation itself, since the quantity of microorganism and biomass present in the mixed liquor decreased dramatically.

The mesoflocs predominated in the three reactors for both periods, this type of flocs is associated with good settling conditions. The analysis of the morphological parameters, in both periods, allowed to conclude that smaller flocs have a less elongated structure, are denser and have smoother edges, while larger flocs have a more elongated shape, are less dense and have rougher edges. It is also concluded that ciliates were the dominant protozoa group, during the experiment, mainly at IFAS. On the other hand, it was possible to observe that the AS reactor had a higher concentration of metazoa compared to MBBR and IFAS. MBBR was the system with the lowest microorganism concentration, the ciliates and smaller metazoa were observed. The tardigrade specie was detected at the end of period A which is associated with an advanced sludge age. The most common species of ciliate were, *Colpidium sp, Zoothamnium sp, Vorticella micróstoma sp, Aspidisca cicada* and *Vorticella convallaria sp*, and the main group of metazoa were the rotifers in which it was possible to visualize mainly the *Digononta sp* and *Monogononta sp*.

Keywords: biological treatment, MBBR, IFAS, AS, biomass, COD, *zoogleal bulking*, *filamentous bulking*, microorganisms, protozoa, metazoa

Resumo

O Reator de Biofilme com Leito Móvel, mais conhecido como MBBR (Moving Bed Biofim Reator em inglês) e o Reator Integrado de Lamas Ativadas com Biofilme em Leito Móvel, ou IFAS (Integraded Fixed-Film Activated Sludge em inglês) são sistemas biológicos avançados para o tratamento de águas residuais. As vantagens associadas às suas aplicações contribuíram para que muitos cientistas realizassem estudos sobre esses sistemas, uma vez que proporcionam uma operação mais fácil, com elevadas eficiências de remoção de carga orgânica para além de produzirem menos quantidade de lama, sendo uma vantagem a nível económico muito grande pois o despejo das lamas corresponde à maioria dos custos de operação, quando comparados com o sistema de Lamas Ativadas. O IFAS consegue ser facilmente implementado numa estação de tratamento de água que já utilize o sistema LA, sem que sejam necessários custos associados à ampliação do sistema. O MBBR tem como grande vantagem o facto de não necessitar de um sistema de recirculação de lamas.

O presente trabalho teve como objetivo a comparação de três reatores, LA, IFAS e o MBBR em termos de eficiência de tratamento, caracterização da biomassa e identificação microbiológica. O sistema experimental consistiu em três reatores independentes que operaram em modo continuo ao longo da experiência. O efluente utilizado foi composto por uma diluição de leite com água, de modo a assemelhar-se o mais possível com um efluente proveniente de uma indústria de lacticínios real.

A experiência foi dividida em dois períodos, A e B. Em ambos os períodos foram utilizados as mesmas frações de enchimento, 44.5% no reator MBBR e no reator IFAS. O período A, durou 37 dias e utilizou-se uma diluição de 1:200, já o período B teve uma duração de 25 dias e a diluição passou a ser de 1:100. Com a variação da quantidade de carga orgânica a entrar em cada um dos reatores, foi possível obter os desempenhos e relacionar cada um, face ao aumento da carga orgânica, bem como a variação do consórcio de microrganismos.

O IFAS mostrou melhor desempenho, nos parâmetros analisados, em ambos os períodos face ao MBBR e ao LA. As eficiências de remoção de Carência Química de Oxigénio (CQO) no período A foram de 63%, 73% e 71% para LA, IFAS e MBBR respetivamente. O período B

obteve melhores eficiências de remoção para os parâmetros analisados, com a exceção do MBBR, que no segundo dia, após o início da experiência, sofreu um *washout* que levou à saída das lamas de dentro do reator para o tanque do efluente tratado, e subsequentemente o biofilme sofreu o fenómeno de *dettachement*, que fez com que grande parte do biofilme fosse perdido. O aumento da carga orgânica e problemas associados aos difusores de ar podem justificar o problema. As eficiências de remoção do CQO foram 82%, 84% e 79% para o LA, IFAS e MBBR, respetivamente. Durante o período B, o IFAS produziu menos 36.5% de lamas que o LA, enquanto que o MBBR produziu menos 85.5%. Para a caracterização microbiológica, foi utilizada como amostra, a biomassa suspensa. Conclui-se, que durante os períodos analisados não houve indícios de *zoogleal bulking* ou de *filamentous bulking*, mas, no entanto, surgiram indícios de *pinpoint* no reator MBBR, que foram justificados através da caracterização da biomassa e da própria observação microbiológica, uma vez que tanto a quantidade de microrganismos e de biomassa presentes no licor misto diminuíram drasticamente.

Os mesoflocos predominaram nos três reatores em ambos os períodos, sendo esses associados a boas condições de sedimentação. As análises dos parâmetros morfológicos da biomassa, em ambos os períodos, permitiu concluir que os flocos de menores dimensões têm uma estrutura menos alongada, são mais densos e têm fronteiras mais suaves, enquanto que os flocos de maiores dimensões têm uma forma mais alongada, são menos densos e as suas fronteiras são mais ásperas. Conclui-se também que os ciliados foram o grupo de protozoários dominante, durante o decorrer da experiência, principalmente no IFAS. Por outro lado, foi possível observar que o reator LA tinha maior concentração de metazoários quando comparado com o MBBR e o IFAS. O MBBR foi o sistema com menor concentração de microrganismos, tendo sido observado ciliados e alguns metazoários de menores dimensões, foi detetado no final do período A, a espécie *tardígrados*, que está associada a idades de lamas avançadas. As espécies mais comuns de ciliados foram *Colpidium sp, Zoothamnium sp, Vorticella micróstoma sp, Aspidisca cicada* e *Vorticella convallaria sp*, e o principal grupo de metazoários foram os rotíferos em que foi possível principalmente as espécies *Digononta* e *Monogononta*.

Palavras-chave: tratamento biológico, MBBR, IFAS, LA, biomassa, CQO, *zoogleal bulking, filamentous bulking*, microrganismos, protozoários, metazoários.

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ACRONYMS

AS – Activated Sludge

AOB – Ammonia-Oxidizing Bacteria

BOD – Biochemical Oxygen Demand

BNR – Biological Nutrient Removal

CAS – Conventional Activated Sludge

COD – Chemical Oxygen Demand

CODs – Soluble Chemical Oxygen Demand

DO – Dissolved Oxygen

EBPR – Enhanced Biological Phosphorous Removal

EPS – Extracellular Polymeric Substances

F/M – Food to microorganism Ratio

FF – Filling Fraction

HDPE – High Density Polyethylene

HRT – Hydraulic Retention Time

IFAS – Integrated Fixed-Film Activated Sludge

MBBR – Moving Bed Biofilm Reactor

NDIR – Non-dispersive Infrared

NOB – Nitrite-Oxidizing Bacteria

PAOs – Phosphorus Accumulating Organisms

RAS – Recirculated Activated Sludge

SRT – Solids Retention Time

SVI – Sludge Volume Index

TN – Total Nitrogen

TS – Total Solids

TSS – Total Suspended Solids

VSS – Volatile Suspended Solids

WWTP – Wastewater Treatment Plant

1. INTRODUCTION

1.1. Motivation

Water is an indispensable asset for any ecosystem. Population overrun, an industrial exponential growth and climate change, are the mainly responsible for water deterioration and scarcity, as larger volumes of water are increasingly needed to meet the need (Sousa *et al.,* 2018 ; Kubota and Cantorski, 2013). The water deterioration and scarcity are among the main environmental problems nowadays, so in order to raise awareness many convention, directives and protocol have been adopted in the past two decades (Cvetnić *et al.,* 2019). Several environmental measures have been taken to preserve this good; the European Union for example established a framework for Community action in regards to water policy, called EU Water Framework Directive (EU WFD), whose objective is to safeguard water quality by identifying certain pollutants previously established by the European Commission (Kaika, 2003). As the legislation concerning wastewater treatment is becoming stricter on effluent discharge limits, more advanced technologies are demanded to maintain the water quality since the present treatment plants are overloaded and do not comply with the legislation imposed (Leyva-Díaz *et al.,* 2017).

Industrial wastewaters are one of the major sources of environmental pollution (Monib et al. 2010). Among all industries, the dairy industry besides being one of the major industries economically important in the agricultural sector (Chokshi *et al.,* 2016) is also one of the most polluting industries in terms of water consumption and disposal (Willers *et al.,* 2014), producing a large volume of sludge in biological treatment (Porwal, Mane, and Velhal 2015)

The quantity and quality of wastewater from the dairy industry varies greatly according to the type of products that are produced (Goli *et al.,* 2019), as dried milk, butter, cheese, yogurt, condensed milk ant others (Kavitha *et al.,* 2013). The maintenance of the dairy industry requires high volumes of water and uses many acid and alkaline detergents in order to clean all the equipment used, making the pH levels of these wastewater vary greatly (Hung, Britz, and van Schalkwyk, 2010; Andreottola *et al.,* 2002). The most commmon environmental problems associated with this kind of wastewater are eutrophication, toxicity and excess of oxygen consumption in the receiving environments (Kasmi, 2018), because of the high

content of organic matter, total solids (TS), total nitrogen (TN), total phosphorus (TP), biochemical and chemical oxygen demand (BOD and COD), and pathogenic (Yirgu, 2018).

The treatment of dairy wastewater typically includes mechanical, physicochemical, chemical and biological methods. The preliminary treatment, where the suspended solids are removed by mechanical processes such as screening, sedimentation and flotation, uses physical and chemical methods. This method tries to eliminate milk fat and protein colloids through equalisation tanks with the help of flocculants and coagulants, also including pH correction. Within the secondary treatment, which aims to remove the dissolved organic matter by biological processes, the most common biological process used is the conventional activated sludge (CAS) (Slavov, 2017; Gonçalves, Pires and Simões 2017).

The conventional activated sludge (CAS) is an effective system in terms of organic matter and nutrients removal, being nitrogen also easily degraded. It is seen as an eco-friendly method, but it presents some disadvantages such as the need for large reactors and settling tanks, bulking, foam production that diminishes sludge settling, precipitation of iron and carbonates. Moreover, the excessive sludge production requires its treatment with a cost that is about 50% of the total operating costs (Leonard *et al.*, 2011). Also, there is a decrease in efficiency during winter periods (Leyva-Díaz *et al.*, 2017; Slavov, 2017) and during aeration it is required a large oxygen supply making it a major energy-consumption operation (Hung *et al.*, 2010).

In this regard, advanced technologies for wastewaters are been developed in order to control stricter effluent limits, to upgrade the already existing CAS plants and to overcome the problems associated with CAS. Among the new emergent technologies, moving bed biofilm reactors (MBBR) and integrated fixed film activated sludge reactors (IFAS) have been attracting attention in the past two decades since they have incorporated several advantages over CAS. The MBBR uses carriers that are constantly in movement inside the reactor to promote the biofilm growth and IFAS or MBBR-based IFAS process combine the benefits of MBBR and CAS (Leyva-Díaz *et al.*, 2014; Mannina *et al.*, 2017).

1.2. Aim and Objectives

The aim of this thesis is to provide an experimental comparison in a continuous flow between three biological treatments: the conventional Activated Sludge (CAS), the Moving Bed Biofilm Reactor (MBBR) and the Integrated Fixed-Film Activated Film (IFAS) or Hybrid MBBR. For the comparison to be possible it was necessary to assemble 3 independent laboratory size reactors, that work simultaneously and with a continuous flow rate.

The objectives were to asses:

- Efficiency of the treatment and sludge production with two different organic loads rate;
- Microbial consortium characterization;
- Suspended biomass characterization

1.3. Structures of the thesis

This thesis is organized in five chapters:

- Chapter 1: Introduction. It describes the current state of water and indicates which are the main responsible for its deterioration and scarcity. Brief description of the role of Europe in preventing water quality. Overview of the dairy industry, dairy wastewater, most common methods for dairy wastewaters and the development of new technologies dairy wastewater.
- Chapter 2: Theory and Literature Review. Presents the fundamentals behind biological treatment, microbial growth and characterization, biofilm growth and explains the concept and metabolism of AS, IFAS and MBBR
- Chapter 3: Materials and Methods. All the parameters used are described as well as the experimental arrangements in order to evaluate the 3 reactors
- Chapter 4: Results and Discussion. The development of the experiments and the data that were obtained during the experiment are discussed.
- Chapter 5: Conclusions and Future Work. Presents the overall important conclusions of the experiments and suggestion for future work.

2. THEORY AND LITERATURE REVIEW

2.1. Biological wastewater basis

Wastewater treatment generally involves the combination of physical, chemical and biological processes and operations aimed at the removal of solids, organic load and nutrients from wastewater. The Table 2.1 shows the processes and main associated operations.

These processes and operations can be grouped into four steps of treatment: preliminary, primary, secondary and tertiary. The latter treatment is not so usual as the previous ones, and is usually applied under conditions where the final effluent has very strict discharges limits or when the effluent has a high toxicity

Table 2.1: Main processes and operation for the wastewater treatment.

Processes	Operations
Physical	Screening, mixing, flocculation, sedimentation, flotation, filtration.
Chemical	Precipitation, gas transfer, adsorption, disinfection.
Biological	Living organisms are responsible to remove pollutants from the wastewater

The preliminary treatment removes larger suspended solids such as rags, sticks, floatables, grift and grease. The purpose of using this process is to prevent damage to mechanical equipment. Primary treatments use physical processes to remove suspended solids and organic matter from the wastewater since sludge settlement is provided. Secondary treatment refers to chemical and biological unit processes. This treatment aims to remove the biodegradable organic load through bacterial growth and remove nutrients as nitrogen and phosphorous. Finally, tertiary treatment is applied after the secondary treatment and consists in the removal of residual suspended solids through cloth filters and medium filters, as it happens in prior treatment, and disinfection is included in order to remove nutrients (Metcalf and Eddy *et al.,* 2014).

Nowadays biological treatment has proven to be efficient in treating both industrial and domestic wastewater or even mixing of the two types of wastewater sources, over other treatment processes like chemical oxidation or thermal oxidation and others. Besides it is economically advantageous, both in terms of investment and operational costs (Mittal, 2011). Figure 2.1 represents the typical flow of wastewater treatment plants (WWTP) with tertiary treatment incorporated.

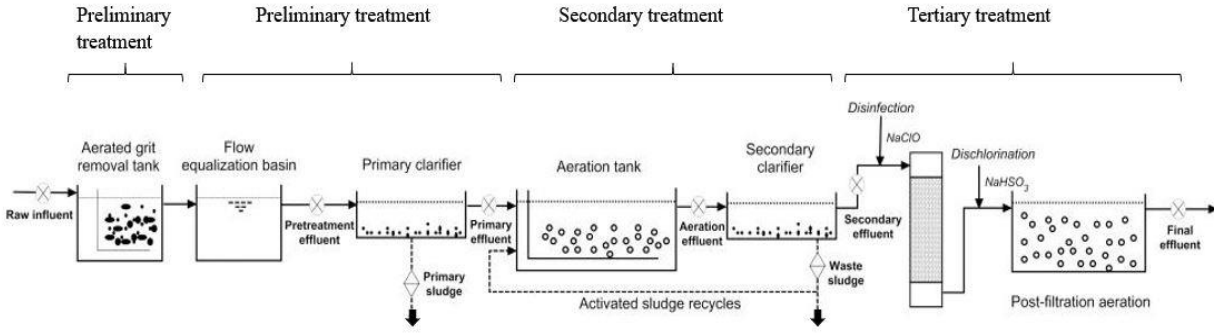

Figure 2.1: Diagram flow for wastewater treatment (Gao *et al.*, 2012).

Wastewaters have a wide variety of constituents and usually tend to be characterized by physical, chemical and biological properties. According to (Metcalf and Eddy *et al.*, 2014) the principal constituents are:

- **Biodegradable organics:** Composed mainly of carbohydrates, proteins and fats. They are determined by chemical oxygen demand (COD) and biochemical oxygen demand (BOD). When discharged without any treatment may lead to septic conditions;
- **Suspended solids:** When discharged without treatment can lead to anaerobic conditions and increment of sludge deposits;
- **Nutrients:** Phosphorous and nitrogen are important inorganic compounds for growth. Excessive amounts of these compounds can then lead to eutrophication of receiving waters. Detergents affect the aquatic life;
- **Pathogens:** Provide communicable diseases when present in the wastewater;
- **Dissolved inorganics:** Inorganic compounds such as calcium, sodium and sulphate are added for domestic supply;

- **Priority pollutants:** Organic and inorganic compounds likely carcinogenic, mutagenic or highly toxic;
- **Refractory organics:** Compounds that generally resist to conventional treatment;
- **Heavy metals:** They are typically added via industrial and commercial wastewaters.

The secondary treatments are mostly based on biological treatments followed by a secondary sedimentation. These treatments may be performed by microorganisms such as algae, fungi, bacteria and some plants under aerobic and anaerobic conditions, that guarantee oxidation or the incorporation of most of the biodegradable organic matter which is subsequently removed by secondary sedimentation (Samer, 2015). This process can be called as bioremediation, once pollution control is done through biological systems that catalyse the degradation or transformation of various toxic chemicals to less harmful forms (Nadu and Blair, 2011). Potential bacterial strains are used for biodegradation of industrial effluents (Monib *et al*., 2010).

Anaerobic treatment is often followed by aerobic treatment in order to remove as much as soluble organic matter, for phosphorous and nitrogen reduction, biological nutrient removal (BNR) is applied. Water is often reused, so that it is necessary to carry out processes of disinfection such as the case of chlorination oxygenation and ultra violet radiation exposure (Kushwaha *et al,.* 2011; Hoang, 2013).

Biological processes can be divided into two categories: suspended biomass (free cell), where microorganisms are maintained in liquid suspension whit mixing techniques and attached biomass (biofilm). Suspended biomass processes can be effective for the elimination of organic matter and nutrients (Sonwani *et al.*, 2019), since microorganism tend to form flocs that aggregate the microbial consortium while in suspension. The most common is the activated sludge process and other examples are aerated lagoons, membrane bioreactors, aerobic digestion and nitritation processes, with some drawbacks when exposed to high hydraulic and organic loads (Leyva-Díaz *et al.,* 2013). The biofilm is simply a consortium of microorganisms that are attached to various types of surfaces, where a biofilm can be created, that can be either fixed, like trickling biofilters (TBF) that use packing materials such as rocks, plastic slag, or suspended supports such as the MBBR process. Other types of biofilm examples used for the treatment of organic pollutants are: rotating biological contactors

(RBC), fluidized bed bioreactors (FBBR), packed bed bioreactors (PBBR), granular media biofilters (GBF) (Sonwani *et al.,* 2019).

Attached biomass has more advantages when compared with suspended biomass, such as large surface area, possible reuse of biomass, prevention of minor reactor clogging, simpler liquid-solid separation and easy reuse of biomass (Banerjee and Ghoshal, 2017). Moreover, greater resistance to negative environmental factors, high viability, increased catalytic activities, increased solid residence time (SRT) with minimal clogging in continuous flow systems (Ismail and Khudhair, 2018) are also advantages of these methodologies that can occur in anaerobic, aerobic and anoxic processes or in combinations of them.

Inorganic compounds as nitrogen and phosphorous can be a serious threat to the receiving water bodies since the presence of this type of compounds can cause eutrophication demising water quality. The use of BNR processes are being increasingly exploited as they avoid the use of chemicals and therefore the problem of chemical sludge disposal is overtaken (Mannina *et al.,* 2017).

2.1.1. Microbial metabolism

To select the type of biological process it is very important to understand the biochemical activities of the microorganisms. Microbial metabolism includes several series of reduction-oxidation reactions that regulate the amount of energy required for cell synthesis, maintenance and endogenous decay. In order to speed up reactions, microorganism release enzymes that act as catalysts. The reactions may be anabolic, when cells build molecules from smaller ones, or catholic when bigger molecules are broken into smaller subunits. Both of them involve an electron donor which is the reducing agent and give electrons to an electron acceptor which is the oxidizing agent and they can be either organic or inorganic (Hoang 2013; Metcalf and Eddy *et al.,* 2014). According to Metcalf and Eddy *et al,(*2014), the classification of microorganism can be done through those that are electron donors, electron receivers, source of cell carbon and end products, the Table 0.1 presented in Annex A represent the classification.

Depending of the carbon source, microorganism can be heterotrophs or autotrophs. The difference between them is that, heterotrophs use organic carbon, while autotrophs use carbon dioxide, to form new cells (Metcalf and Eddy *et al.,* 2014).

The aerobic biodegradation of organic matter and following removal of nutrients is demonstrated in the Equation 2.1.

$$Organic\ matter + O_2 \xrightarrow{\text{MICROORGANISM}} \text{new cells} + CO_2 + H_2O \quad (2.1)$$

Particulate organic material is firstly adsorbed by microbial flakes and broken under enzymatic activity and is later absorbed in the cell. Soluble organic compounds in turn can be easily absorbed and metabolized by the cell.

2.1.1.1. Microorganisms

The microorganisms present in the suspended and attached biomass can be very different. In activated sludge systems, AS, where biomass is suspended, the most common is the existence of *aerobic or facultative anaerobic heterotrophic bacteria*, as the main responsible for organic matter removal (Abtahi *et al.*, 2018).

Protozoa are very common both in suspended and attached biomass and they are associated to longer solid retention times (STR) than *the aerobic or facultative anaerobic heterotrophic bacteria*. STR is the average time solids remain inside the reactor, so longer sludge age is associated to such microorganisms. Metazoa such as *rotifers* and *nematodes* are also associated with longer STR.

During the last two decades several studies have shown the importance of biodiversity in biological systems and microbial communities with richer microbial diversity since they have higher functionality and stability than microbial communities with lower richness (Torresi *et al., 2016)*. Biofilms have a highly diverse microbial community and this trait would possibly enable the biofilm to outperform the suspended biomass for removal of bio-refractory (Abtahi *et al.*, 2018).

The most common microorganisms found in wastewater treatment are bacteria, archaea, fungi/yeast, protozoa, helminths, rotifers, algae and viruses (Metcalf and Eddy *et al.,* 2014).

2.1.1.2. Protozoa and Metazoa

Protozoa and metazoa monitoring can be considered an advantageous tool to evaluate the operation of the biological WWTP (A. Luís Amaral *et al.*, 2018). Protozoa are important in wastewater purification and work as bioindicators since the present of some types of protozoa

is associated which a good effluent and plant performance. Metazoa can also work, mainly rotifers, as bioindicators since they are associated to longer sludge age (Amanatidou *et al.*, 2016). There are about 200 species of these microorganisms.

Protozoa can be classified in three main groups (António L. Amaral *et al.*, 2013):

- **Flagellates:** Appear mostly in the early stages of untreated effluents; they indicate a low sludge age, increased organic load or lack of oxygen. They move by flagella and are resistant to toxic and anoxic conditions;
- **Sarcodina (amoeboid):** Small amoebas are related to transient phenomena, high organic load and to a mediocre final effluent, while big amoebas are related to high quality final effluent. They move by pseudopods.
- **Ciliates:** They are the biggest fraction of the protozoa community in aeration tanks in WWTP and have a crucial role in the purification of wastewaters. This implies that the more of these microorganisms, the better the final effluent quality will be. They move through a small locomotive designated as eyelashes which are dispersed throughout the body. There are basically four groups of ciliates: free-swimmers, crawlers, stalked and suctorial.

Metazoa are multicellular that feed on bacteria. They have higher gestation times than protozoa, which implies a more advanced age of sludge. Contribute to flocculation/ deflocculating processes through a mucus, that causes filamentous bacteria to adhere and through fractionation break the oversize flocks as a result of mixed liquor mobility (Amaral and Leal, 2013). According to the same authors there are three types of metazoan more common in WWTP:

- **Rotifers:** They are the most representative, indicating a high age of sludge associated with good aeration. Correlated with a high-quality final effluent;
- **Nematodes:** Present in all types of organic loads, existing in all of them in a small number. Resistant to under-aeration conditions and do not correlate with a specific final effluent quality;
- **Annelids:** They are only present in high age sludge and low organic loads. Associated with a high quality of the final effluent with nitrification occurrence.

The major role of protozoa and metazoa is the elimination of coliforms and pathogenic bacteria through their predation since the majority is bacterivorous. Furthermore, they contribute to flocculation/ deflocculating processes as previously mentioned. Therefore, it can

be stated that protozoa and metazoa are intrinsically related to hydraulic time (HT), the age of the sludge and the organic load of the effluent (Amaral and Leal, 2013).

2.1.2. Microbial growth kinetics

Microbial growth is dependent on some conditions such as pH, dissolved oxygen (DO) and nutrients, hence there is the need to control all these parameters so that the treatment can be as efficient as possible (Metcalf and Eddy *et al.*, 2014).

The principals of microbial growth kinetics are based on the Monod's model. The biological growth is associated to the synthesis of cellular constituents (biomass) and the multiplication of individual cells to produce offspring (Egli, 2009). The Monod growth curve has four distinct phases (Metcalf and Eddy *et al.,* 2014) described below. In the Figure 2.2 the typical curve with the four phases is represented:

- **Lag phase:** Represents the time required for organism to adapt to the new environment before cell division and biomass production. The time required depends on the inoculum age;
- **Exponential growth:** In this stage the bacteria multiply at a maximum rate and there is no other limiting factor besides temperature;
- **Stationary phase:** Biomass concentration is practically constant; substrate and nutrients are limiting factors. The amount of growth is offset by death of cells;
- **Death phase:** The substrate is depleted so there is no growth; the variation in biomass occurs exclusively because of cell death.

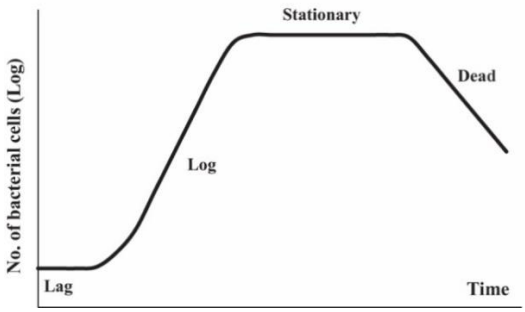

Figure 2.2: Typical Monod growth curve characterized by four phases (S. Wang *et al.*, 2009).

2.1.3. Biological nutrients removal

The continuous discharge of wastewater without suitable treatment into water bodies can pose serious pollution problems, such as the eutrophication phenomenon which consists of enriching the waters resources with nutrients mainly nitrogen and phosphorous. This type of phenomenon is responsible for the degradation of freshwater ecosystems as it provides oxygen depletion through the development of algae blooms and the spread of aquatic plants (Ismail and Khudhair, 2018). The blooms are also linked to a higher risk for public health. Hence there is the need to apply an effective treatment to diminish the concentration of nitrogen and phosphorus in wastewater before the discharge into the environment.

2.1.3.1. Nitrogen removal

Nitrogen removal is an important part of WWTP since the presence of pollutants such as ammonia (NH_4-N) and nitrite (NO_2-N) in the hydric resources are responsible for depletion of oxygen, fish toxicity and eutrophication. Nitrogen removal is done biologically, and it is achieved in two processes: **Nitrification** and **Denitrification**.

In the past, the removal of the pollutants above was performed by physical, biochemical and the combination of both processes. These methods have as disadvantages the facts that the heterotrophic denitrification process may be inhibited by carbon source limitation and that they are expensive methods (Wang *et al.*, 2017; Xia *et al.*, 2005).

2.1.3.1.1. Nitrification

Nitrification occurs practically in aerobic suspended growth and fixed film biological processes in order to remove nitrogen or ammonia. This process is accomplished in two steps through autotrophic bacteria; first ammonium is oxidized to nitrate (NO_2-N), the reaction is demonstrated in Equation 2.5, and in the second and final step, the nitrate is oxidized to nitrate (NO_3-N), Equation 2.6 (Ma *et al.*, 2016). In the first step, the conversion of ammonium into nitrite is carried out by *Ammonia-Oxidizing bacteria (AOB)* that are responsible for oxidation, *Nitrosomonas* is the most representative bacteria in this group and in the second step the conversion of nitrite into nitrate is carried out by *Nitrate-Oxidizing Bacteria (NOB)* that are responsible for the oxidation and it is known as *Nitrobacter*. Both

AOB and *NOB* are aerobic chemoautotrophs as they us CO_2 for their carbon source and need dissolved oxygen to oxidize NH_4-N and NO_2-N to gain cell energy (Metcalf and Eddy *et al.*, 2014; Leyva-Díaz *et al.*, 2015).

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O \quad (2.5)$$

$$2NO_4^+ + O_2 \rightarrow 2NO_3^- \quad (2.6)$$

This process is influenced by the same environmental factors as other aerobic processes: NH_4-N and NO_2-N concentration, DO concentration, pH, salinity in the bioreactor. These factors cause nitrifying bacteria to dominate both suspended growth and fixed film growth. Bacteria can be divided in r-strategists and k-strategists, regarding the concentration of nitrogen and DO. The k-strategists grow faster than r-strategist at a low substrate concentration and the nitrifying bacteria most common in this case are: *Nitrosospira* and *Nitrospira* for *AOB* and *NOB* respectively. Nevertheless, in a higher substrate concentration the r-strategists grow faster than k-strategists and in this case the most usually bacteria are: *Nitrosomonas Europea* for *AOB* and *Nitrobacter spp.* (Metcalf and Eddy *et al.*, 2014). The total oxidation reaction is showed in Equation 2.7.

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O \quad (2.7)$$

2.1.3.1.1. Denitrification

Nitrification cannot completely remove nitrogen from wastewater, hence the need for subsequent treatment is required. Denitrification involves reducing nitrate or nitrite to nitrogen gas and the Equation 2.8 shows the process. Nitrate removal is possible by two methods: assimilatory or dissimilatory nitrate reduction. The first is independent of DO concentrations and involves the reduction of nitrate to NH_4-N for cell synthesis processes when NH_4-N is not present. The second method is responsible for the biological denitrification for greater nitrogen removal and involves nitrate/nitrite as the largest electron acceptor rather than oxygen for oxidation of a variety of organic and inorganic substrate. In dissimilatory biological denitrification

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \quad (2.8)$$

As the equation above demonstrates, nitrate reduction occurs through several intermediate products, and one of them, N_2O creates some concern as it is one of the most powerful greenhouse gases.

In dissimilatory biological denitrification there are two processes which are usually used: preanoxic denitrification and postanoxic denitrification. The first one is designated this way since before the aeration tank (nitrification) the anoxic tank (denitrification) comes and it is needed a recirculation flow for the nitrate to enter in the anoxic tank. The second process happens on the contrary, i.e. BOD removal has occurred in the nitrification tank and is not available to lead the nitrate reduction reaction, so it is necessary to add an external carbon source, such as acetate or methanol, so that there is enough BOD to reduce nitrate and rise the denitrification level.

The bacteria responsible for the denitrification can be either heterotrophic or autotrophic, being that heterotrophic bacteria are the more common. These bacteria are all facultative aerobes and can use either oxygen or nitrate/nitrite and when none of these compounds are present, some of these bacteria can carry out fermentation (Metcalf and Eddy *et al.*, 2014).

2.1.3.2. Phosphorous

Phosphorus removal can be done through chemical treatment, biological phosphorus removal or a combination of both. In chemical treatment, alum or iron salts are usually used followed by tertiary filtration or by passing through a membrane separation. The second involves the incorporation of phosphorus into the cell biomass removed with sludge wasting. This process can only remove 10% to 30%, therefore it was necessary to create other processes that are more efficient, such as the enhanced biological phosphorous (EBPR) also designated as phosphorus accumulating organism (PAOs). EBPR has a removal efficiency above 80% and has the main advantages of reducing costs associated with chemicals and lower sludge production when compared to chemical precipitation (Metcalf and Eddy *et al.*, 2014; Rossetto, 2012).

2.2. Activated Sludge (AS) system

The Activated Sludge (AS) was the first mechanically equipped wastewater treatment in history; it was developed in 1914 by Ardern and Lockett in England (Ali *et al.,* 2015). The principle of this system is based on large masses of aerobic microorganism that are held in suspension by mixing and aeration systems, inside of an aeration tank/reactor, that converts organic matter or other constituents present in the wastewater, into cell tissue and/or gases (Metcalf and Eddy *et al.,* 2014). This system is the most common worldwide and the oldest biological treatment, being largely used to treat municipal and industrial wastewater (Mittal, 2011). Although AS system has been associated with high COD removal efficiencies, it consumes a huge amount of energy and produces high amounts of sludge which are associated with high treatment costs before disposal (Daverey *et al.,* 2019). Other setbacks are the sludge settleability, the need of large reactors and settling tanks and the need for having biomass recycling.

The AS system is generally composed of aeration tank (reactor), settling tank (secondary sedimentation tank), sludge recirculation and excess sludge removal. In Figure 2.3 a typical activated sludge system is shown. Inside the aeration tank (or biological reactor) where biochemical degradation reactions take place microorganisms are responsible for the treatment and are maintained in suspension and aerated. Microorganisms are continuously reproducing using the substrate in the reactor, forming an activated sludge. In the secondary sedimentation tank, the settling of the solids occurs, which subsequently leads to a clarified effluent. Some of the settled solids will be recirculated back into the reactor, to keep the biomass concentration high which is responsible for the high efficiency of the system. The other part of the settled solids, excess sludge, is discarded from the system and forwarded to the sludge treatment station (Von Sperling, 2015; Metcalf and Eddy *et al.,* 2014). The biomass is separated in the secondary sedimentation tank due to its flocculation and settlement properties. These properties are derived from a gelatinous matrix that enables agglutination of bacteria, protozoa and metazoa into macroscopics flocs and, since the flocs are much larger than the microorganisms, it facilitates the sedimentation (Von Sperling, 2015).

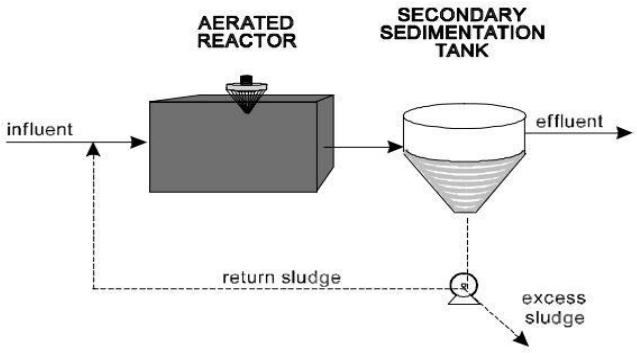

Figure 2.3: Representation of the main components of an activated sludge system (AS) (Von Sperling, 2015).

The most common problems associated to the Activated Sludge (AS) systems are sludge bulking and foaming. Bulking refers to the phenomenon of poor sedimentation, the suspended solids are visible in most of the settlement tank and in more extreme cases they are lost over the outlet dams to pollute the effluent discharge. Foaming is mainly linked to the presence of three types of filamentous microorganism: *Microthrix parvicella*, *Nocardia* and *Nostocoidia limilocola* (Mara and Horan, 2003). There are mostly three types of bulking, they are presented below (Amaral and Ferreira, 2005; Metcalf and Eddy *et al.*, 2014). To know if the system is having any of these problems it is necessary to use the indicator Sludge Volume Index (SVI).

- **Filamentous bulking:** It is the biggest problem in AS and happens because of the excess of filamentous bacteria, which leads to large, paint-covered flocs. When AS is operating with DO or nutrients at low concentrations, conditions are favourable for filamentous bacteria linked to a low Food to Microorganism ratio (F/M);
- **Zoogloeal bulking or viscous bulking:** It happens when there is a large of amount of extracellular biopolymer that results in a sludge with a gelatinous consistency, low density, low settling velocities and poor compaction. Is linked to a high Food to Microorganism (F/M);
- **Pinpoint flocs bulking:** Is linked to the absence of filamentous bacteria, leading to hardly settleable small flocs.

Figure 2.4 depicts the initial state of a normal floc (in equilibrium) that later undergoes changes leading to pin-point floc and bulking sludge.

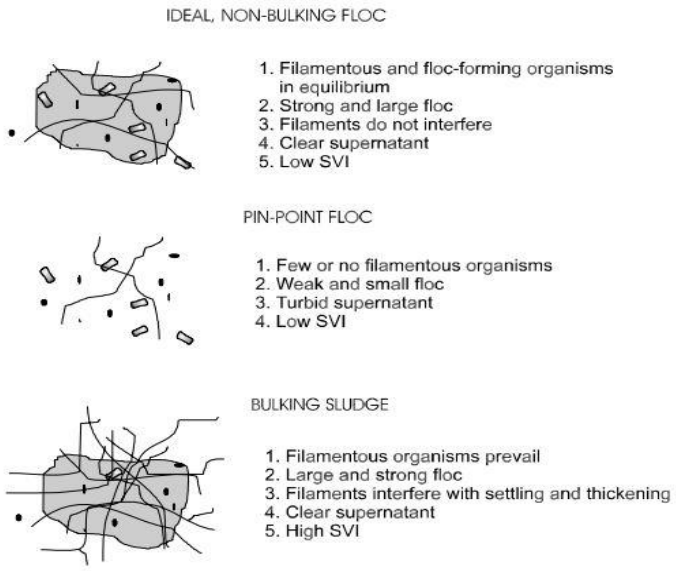

Figure 2.4: Effect on flocs structure in AS system, due to filamentous organisms.(Von Sperling (2015)**).**

2.3. Moving Bed Biofilm Reactor (MBBR) system

The Moving Bed Biofilm Reactor (MBBR) was developed in Norway by Leiknes and Ødegaard between 1980 and 1990 to achieve nitrogen removal at cold temperatures. In Norway most of WWP are built in enclosed spaces hence there was the need to build a compact system as an alternative to AS system (Dale and Water, 2015), in order to reduce the amount of nitrogen that was discharged into the North Sea (Metcalf and Eddy *et al.,* 2014). This type of system was implemented over 700 WWTP worldwide (Zhang *et al.*,2017).

The MBBR is one of the most efficient biological treatment systems being considered as an advanced wastewater treatment process (Rodriguez-Sanchez *et al.,* 2018; Leyva-Díaz *et al.,* 2014) in which the biomass grows as attached biomass in the surface of the carriers (Sonwani *et al.,* 2019). The attached biomass moves freely throughout the reactor volume inside the carrier forming the biofilm. The carriers have a density similar to the water, in order to be held in suspension with the lowest mixing energy possible provided by aeration or mechanical mixing (Lariyah *et al.,* 2016).

The growth of attached biomass inside the reactor will lead to an increment of the solids concentration without increasing the suspended solids concentration, leading to easier biomass separation procedures and, thus, to a more specialized biomass development enabled by growth in fixed biofilms (Sonwani *et al.,* 2019). The fixed biofilm on the carriers is kept in continuous movement through agitation caused by diffusers in aerobic bioreactors or by

mechanical stirrers in anaerobic/anoxic bioreactors without requiring a sludge recycling system (Leyva-Díaz *et al.*, 2017). Turbulence in the reactor is needed in order to transport the substrates to the biofilm and to maintain a low thickness of the biofilm by shearing forces (Rusten *et al.,* 2006). The Figure 2.5 shows the different configurations possibilities of the MBBR for aerobic and anaerobic reaction.

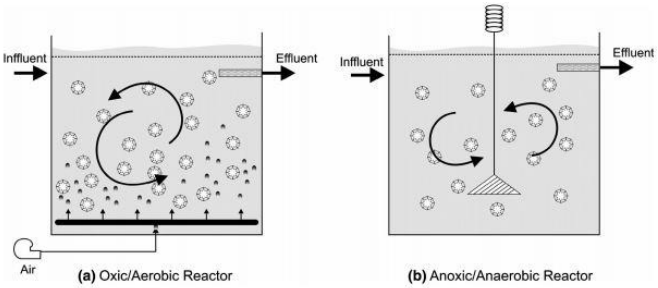

Figure 2.5: MBBR scheme of possible configurations for aerobic and anaerobic environments (Barwal and Chaudhary, 2014).

The MBBR undergoes shear forces that will directly influence biofilm thickness and substrate diffusion. Due to diffusion limitations, the biofilm activity depends not merely on the protection of the carrier's surface but also on the biofilm thickness (Piculell *et al.*, 2014). As result of diffusion limitations in a tighter substrate penetration, the ideal MBBR biofilm should be thinner and comprehend the entire surface of the carrier. When the biofilm thickness on the carrier surface is lower than 100 μm it can be assumed that the biofilm is fully penetrated (Levstek and Plazl, 2009).

Carriers require space to stay in suspension, so several investigators propose a filling fraction, (FF) percentage of reactor volume occupied with carriers in empty tank, ranging from 30% to 70% maximus (Rodriguez-Sanchez *et al.*, 2018; Safwat, 2018; Leyva-Díaz *et al.*, 2017). Higher FF is known to have associated lower efficiencies. The various studies about FF in MBBR system indicate that FF is a key parameter for the design, performance and is intrinsically dependent on the type of treatment purposes.

The MBBR system can be used/adopted for different purposes, such as organic matter removal (BOD and COD), nitrification, denitrification and removal of phosphorous (Safwat, 2018), in order to be able to comply with all prepositus, implying different configurations.

The MBBR systems have many advantages such as (Morgan-Sagastume, 2018; Metcalf and Eddy *et al.*, 2014; Ali *et al.*, 2015; Xia *et al.*, 2005):

- Higher volumetric reaction rates and capacity;

- Increased biofilm-related process stability;
- Lower solids effluent output compared to CAS;
- Simplicity of operation;
- This system can operate in continuous mode without backwashing or sludge return;
- Better oxygen transfer;
- Can achieve nitrification due to the growth of nitrifiers;
- Possible to treat wastewater under extreme low temperatures;
- Has higher concentration of active biomass when compared to CAS;
- The treatment efficiency is not actively influenced by final sedimentation;
- No bulking formation and the inherent problems;
- Higher resistance to organic and hydraulic load shocks;
- When compared to other attached biomass systems like trickling filters, fixed media submerged biofilters, granular media biofilters etc, the MBBR system is more versatile and highly adaptable for biological nitrogen removal. Furthermore, in continuous mode it does not need special operation attention or flushing out excess solids.

Beside all these advantages, the MBBR process has some disadvantages when compared to the CAS process such as (Metcalf and Eddy *et al.*, 2014):

- Higher consumption of energy when compared with CAS since the MBBR process needs an elevated DO concentration;
- Needs to use adequate media;
- The limitation of phosphorous removal that can only be done by chemical addition;
- The need for improved influent wastewater screening.

2.4. Integrated Fixed-Film Activated Sludge (IFAS) system

The Integrated Fixed-Film Activated Sludge (IFAS) or Hybrid-MBBR is a biological treatment that encompasses both the advantages of AS as those of the MBBR and was developed to overcome the drawbacks of the conventional AS process. WWTP that use CAS processes are often at or beyond their capacity, and therefore a need for an expansion is required. In this case, the IFAS process is applied since it offers a compact treatment plant design to overcome the drawbacks of the traditional treatment and on the other hand, it produces a higher quality effluent even with a smaller foot print (Lariyah *et al.*, 2016).

This type of process has the particularity of working with both suspended and attached biomass (biofilm) (Mannina *et al.*, 2017). The addition of the attached biomass inside of the reactor, also happenings in the MBBR system, will increment the solids concentration without increasing the suspended solids concentration. This action can result in increased SRT which increases both nitrification capacity and efficiency (Metcalf and Eddy *et al.*, 2014). Like MBBR, this system requires high levels of DO (saturation levels) in order to avoid problems in the efficiency of the treatment as well in the microbial communities (Singh *et al.*, 2016).
Figure 2.6 describes the IFAS operations and treatment units with a coupled settling tank and as can be seen, in this type of treatment there is a sludge recirculation unlike in the MBBR. The filling fraction (FF) is lower than MBBR, ranging 30% a 60% (Johnson *et al.*, 2012).

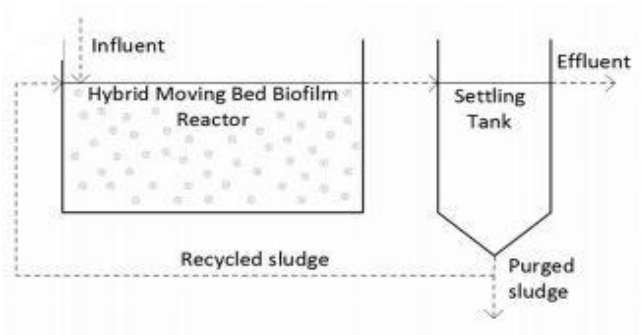

Figure 2.6: Operating scheme of the IFAS or Hybrid-MBBR treatment procedure (Leyva-Díaz *et al.*, 2017).

The IFAS process has advantages when compared with CAS such as (Metcalf and Eddy *et al.*, 2014; Mittal, 2011):

- It can be easily incorporated in the existing CAS;
- Smaller footprint;
- Increase treatment capacity in smaller space;
- Can reach simultaneously, nitrification/denitrification, controlling the DO levels;
- Produces less sludge, leading to lower sludge treatment costs.

This process has some disadvantages such as (Johnson *et al.*, 2012; Metcalf & Eddy *et al.*, 2014) :

- Elevated energy consumption due to high DO concentrations;
- Needs to use proprietary media;
- Free-floating media are retained in the aeration basin by sieves contributing to in head losses.

2.5. Biocarriers

Most biocarriers or biofilm carriers for attached growth-based systems can differ from each other in material composition, specific surface area, shape and treatment capabilities (Levstek and Plazl, 2009). The effective surface area of the biocarriers is a very important parameter as the biofilm only grows in the protected interior (Barwal and Chaudhary, 2014) allowing the biofilm growth shielded from abrasion by carrier collision (Morgan-Sagastume, 2018).

Biocarriers are made of various materials such as: high density polyethylene (HDPE), polypropylene (PP) or polyethylene (PE) (Barwal and Chaudhary, 2014). The densities of the carriers must be similar to the water density; carrier density usually vary between 0.94 to 0.98 g/mL (Morgan-Sagastume, 2018). They are shaped as small cylinders with cross on the inside, in the outside they have longitudinal "fins" (Barwal and Chaudhary, 2014). Table 2.2 shows the most commons biocarriers available on the marked and their characteristics including the carrier used in this study, *Bioflow 9*.

Table 2.2: Settings of some biocarriers used in attached biomass systems (Barwal and Chaudhary, 2014**;** Cortés-Lorenzo *et al.*, 2012)**.**

Carrier	**K1**	**K2**	**K3**	**BiofilmChip M**	**BiofilmChip P**	**Bioflow 9**
Material	HDPE	HDPE	HDPE	HDPE	HDPE	HDPE
Length (mm)	7	15	12	2.2	3	7
Diameter (mm)	9	15	25	48	45	9
Total Specific surface (m^2/m^3)	700	-	-	-	-	800
Bulk density (kg/m^3)	150	95	100	-	-	145
Specific biofilm surface area (m^2/m^3)	500	350	500	1200	900	-

Nowadays the K1 carrier from AnoxKaldnes® still dominates the market, even though it was the first to be developed (Morgan-Sagastume, 2018). In Figure 2.7, some biocarriers made by AnoxKaldnes® are demonstrated.

Some new developments are being made in the shape of the biocarrier, as the Z-MBB R or Z-carrier. The saddle formed Z-carrier is different from other carriers, as the biofilm grows in the outside of the carrier. However, the biofilm is protected due to a grid covering the surface of the carrier, avoiding the scouring action of other carriers (Piculell *et al.*, 2016). In the present work the carrier used was *Bioflow 9* and is represented in the Figure 2.8.

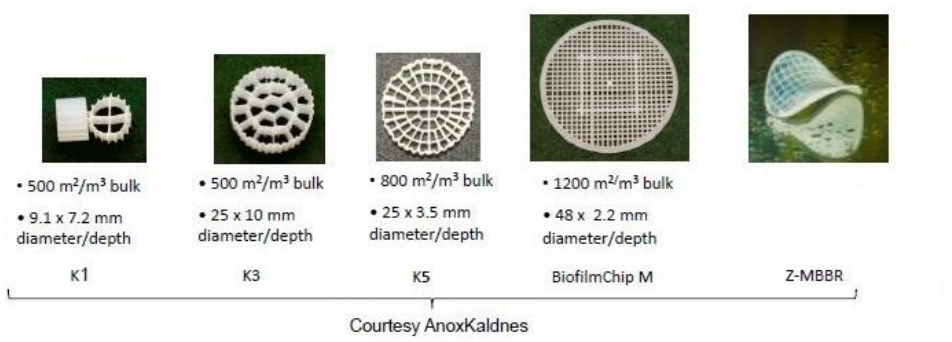

Figure 2.7: Representation of the most commonly used carriers in MBBR and IFAS systems (Hallvard Ødegaard, 2014).

Figure 2.8: Carriers used in the experiment, *Bioflow9*.

2.6. Biofilm mechanics

Biofilms are complex, heterogeneous and hydrated microbial structures composed of a community of microorganisms aggregated and incorporated into an extracellular polymeric substances (EPS) matrix self-produced with associated non-cellular material and interstitial voids, all bound and developed on a solid surface (Morgan-Sagastume, 2018). The matrix has three dimensional architecture and enables mechanical stability for the biofilm and also intensifies the resistance of the microbial community when coming in contact with the outside (Zhu *et al.*, 2015). The biofilm consists of heterogeneous species that form symbiotic relationships with each other's and the by-products produced can act as substrate for an organism (Hoang, 2013). The biofilm is practically composed of polysaccharides,

phospholipids, nucleic acid and proteins such as glycoproteins and glycolipids (Cydzik-Kwiatkowska and Zielińska, 2016).

Biofilms can grow in many types of surface such as plastic, metal, wood, tissues, soil particle and glass (Ali *et al.,* 2015). The goal of the biofilm is to be a resistant enough structure to deal with stress factors such as antibiosis, UV radiation, desiccation and predation (Ahmad and Husain, 2017). The biofilm system when compared to the CAS system has demonstrated to have higher biomass stabilization (Ali *et al.,* 2015).

The biofilm can contain an aerobic layer with a higher redox potential and anoxic/anaerobic inner layer where reduced reactions are more common, so biofilm-based systems can either be used for organic carbonaceous matter removal, nitrification and nitrogen removal (Safwat, 2018). In the redox zone is typically found faster growing bacteria as heterotrophs or acidifiers and further into the biofilm, anoxic micro-zone is common to have slower growing bacteria as is the case of nitrifying bacteria (Leyva-Díaz *et al.,* 2015).

Biofilms attach to solids surface basically by three reasons (Mara and Horan, 2003):

- Substrate availability;
- Protection from hostile environment;
- Interaction of physical forces such as attachment, adsorption and adhesion.

In Figure 2.9 the five stages of the biofilm development are described. Stages 1 and 2 is where the microorganism started to adhere to the carrier, stages 3 and 4 represent the steps of the cells growth and where the EPS are produced and finally the stage 5, when the biofilm starts to detach, releasing extracellular materials to continue the cycle of the development of biofilm (Morgan-Sagastume, 2018).

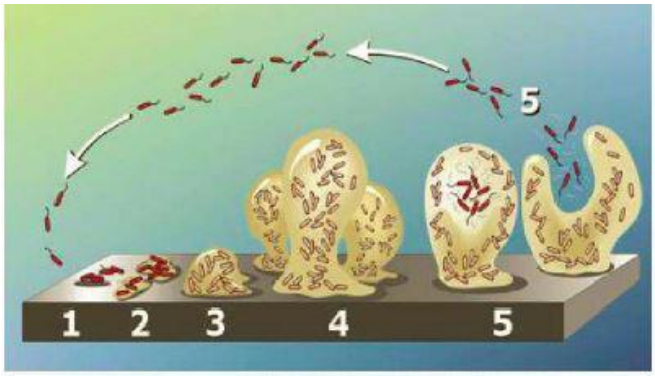

Figure 2.9: Scheme of biofilm formation: 1-Initial attachment of cells; 2-Irreversiaonal attachment; 3-EPS production, initial maturation; 4- Biofilm development, maturation; 5- Dispersion of biofilms cells. (Shahot *et al., 2014)***.**

2.7. State of Art

In this section, the state of art of the MBBR system and IFAS reactor will be briefly approached. The data collected are shown in Table 2.3, in which emphasis is placed on the experimental conditions and on the main conclusions associated to each experiment.

Table 2.3: Bibliographic review on MBBR and IFAS reactor.

Carrier	Effluent	Experimental Conditions	Major Conclusions	Reference
Cosmo Ball	Synthetic river water	Parallel MBBR and CAS pilot scale comparison; One influent tank with 2000L; Two 5L reactor; Influent flow was maintained at 2.5 L/h and COD values between 100 and 200 mg/L;50% of FF; HRT of 4h.	The experiment reveals that DO range between 2.0-7.0 mg/L and pH values range from 7.0 to 8.4, indicating satisfactory biological processes in both reactors. MBBR shown in overall higher removals efficiencies compared with AS; MBBR produced less sludge than AS system.	Ali *et al.*, (2015)
Ceramics granules unmodified and modified with sepiolite	Oilfield produced water (OPW)	AS and MBBR pilot scale comparison; Two 5L MBBR reactor with an FF=50% and one with unmodified ceramic and the other with modified; one 5L AS rector; HRT between 36-10h.	Higher COD removal efficiencies for sepiolite-modified carries, then for the MBBR reactor with the unmodified carriers and finally for the AS reactor at an HRT of 10h.	Dong *et al.*, (2011)
FLOCOR-RMP	Dairy wastewater	A 905 L single-stage MBBR reactor and a 94 L settler were placed in a full-scale treatment plant; FF=60% of MBBR reactor; Influent flow was set at 29-78 $L.h^{-1.}$ HRT of 11.5 to 31h for the MBBR rector and 1.2 to 3.8h.	COD efficiencies range between 80% to 90%, but the total COD concentration in effluent was 251 mg L^{-1}, so it was not possible to remove all the nutrients, due to high organic loads and their fluctuations. TN removal efficiency varied widely (13.3-96%).	Andreottola *et al.*, (2002)

Biotextil Cleartec (Fixed Curtains)	Municipal wastewater	A pilot scale fixed media IFAS reactor was assembled in a sewage pumping station, under different DO (0.5-4.5 mg. L^{-1}) concentrations in order to evaluate the effects on the biological treatment potential and in the bacterial. community; FF=0.5%; HRT of 11.1h.	It was observed that different DO affect the performance of the system; in fact, the COD removal efficiencies were 81, 90 an 94% DO levels of 0.5, 2.5 and 4.5 respectively. It was also observed that at lower DO, the sludge tends to have a higher SVI; DO concentrations have direct impact on the microbial.	Singh, Kazmi, and Starkl (2016)
Bioflow 9	Oil sands process-affected water (OSPW)	Three reactors were installed in a pilot scale (MBBR, IFAS and MBR) in order to compare microbial characteristics and biological treatment performance of five types of microbial biomass (MBBR-biofilm, IFAS-biofilm, IFAS-floc, MBR-aerobic-floc, and MBR-anoxic-floc) compared in batch tests; FF=60% for MBBR and IFAS; HRT of 96h for IFAS and MBBR and 12 h for MBR; The influent flow were 2.0 L/d for IFAS and AS and 3.2L/d for MBR ; Biomass applied was 500mg TSS/L.	MBR demonstrated COD removal efficiencies higher than MBBR and IFAS, but for AEF the MBBR and IFAS had higher removal efficiencies. In the microbial analysis, the bacterial 16S rRNA gene abundance was significantly higher in the batch bioreactors with suspended flocs than in those with biofilm but the denitrifiers bacterial were more abundant in the suspended phase of the activated sludge floc (IFAS and MBR).	Huang *et al.,* (2017)
Z-MBBR by *AnoxKaldnes*	Secondary-treated municipal wastewater	Two lab-scale MBBR reactors with 3.1L were evaluated in terms of micropollutants (MPs) removal; HRT and COD influent ranges between 20-4h and 500 to 100 mg. L^{-1}, respectively. FF=40%.	COD removal efficiencies were higher (80%) in both reactor at HRT= 4h. The MPRs reduction was ascribed to the sportion onto the biomass, since it was not observed any MPs reduction through photodegradation and volatilization. Comparing the abiotic and biotic aspects,	Abtahi *et al.,* (2018)

	it is concluded that the biotic removal was greater for the MPs analysed. The MPs overall removal were higher for the HRT=4h.

2.8. Dairy Wastewater

2.8.1. Dairy wastewater characteristics

Dairy wastewater properties can vary widely, as they are intrinsically associated with the intended lactate products and production technologies available (Melchiors *et al.*, 2016). Nevertheless, it is common to find the following components in dairy wastewater: lactose, milk carbohydrates and soluble proteins, lipids, mineral salts, detergents and small concentrations of heavy metals from equipment cleaning can also being found (Karadag, Köro, *et al.*, 2015). These compounds generate wastewaters with a high organic load, with a COD concentration above 800 mg/L (Chokshi *et al.*, 2016; Melchiors *et al.*, 2016). In Table 0.2 presented in the Annex A the main characteristics of raw dairy wastewater are represented.

Discarding untreated or improperly treated dairy wastewater can lead to multiple environmental problems and consequently to human and animal health problems (Bortoluzzi *et al.*, 2017; Porwal *et al*,. 2015).

Biological processes are widely used as they are more economic and lead to a cleaner wastewater, than physicochemical treatment processes (Kasmi, 2018).

The dairy wastewater can be divided into three major categories (Slavov, 2017):

- **Processing water:** Is formed in the cooling of milk in coolers and condensers, as well as condensates from the evaporation of milk or whey. This waters lack pollutants, and after pre-treatment, they can be reused or discharged (reuse is only possible when the installations are not in direct contact with derived products);
- **Cleaning wastewater:** Comes from washing equipment which is in direct contact with milk or dairy product, including milk and product spillage, whey, CIP ("clean in place") effluents or equipment malfunction and even operational errors. These

effluents are in high volumes and very polluted, since over 90 % of organic solids in dairy effluents come from milk and manufacturing residues;

- **Sanitary wastewater:** Is from the lavatories, shower room, etc, an is very similar in terms of composition to municipal wastewater. This type of wastewater is generally piped directly to sewage or it can be used as nitrogen source for unbalanced dairy effluents before a secondary aerobic treatment.

The cleaning operations generates most of the large volumes of wastewater associated with this type of industry, due to high water use (Goli *et al.*, 2019).

2.8.2. Dairy wastewater treatment

The dairy wastewaters are essentially composed by different dilutions of milk, and washing water from the cleaning of all the equipment's and maintenance of the industry, containing alkaline and acidic chemicals (Carvalho *et al.*, 2013). These compounds are then part of the wastewater composition, since almost all the milk processing unit uses CIP systems which pumps cleaning solutions through all equipment such as caustic solution (sodium hydroxide), phosphoric or nitric acid (acid solutions), sodium hypo-chlorite disinfectant, which are subsequently washed with water (Tikariha and Sahu, 2014).

In Europe, dairy industries typically produce on the daily basis 50 m^3 of wastewater with high amounts of organic matter (fat, protein and carbohydrates) and nutrients (nitrogen and phosphorous) originating from the milk and the milk products (Tikariha and Sahu, 2014).

In addition to producing high volumes of wastewater, by-products that can be valorised instead of discharging them within the wastewaters are also produced (Ganju and Gogate, 2017).

Whey is one of the sub products when cheese or casein are produced and is the principal pollutant in milk processing due to its high organic and volumetric load (Slavov, 2017). Whey has a high nutritional added value and, in terms of an economic point a view, is a valuable asset since it contains lactose, soluble proteins and/or whey proteins, enzymes, organic acids, water-soluble vitamins and minerals (Goli *et al.,* 2019). However, in most of the dairy industries, whey is often considered as waste so it is discharged with the dairy wastewater (Carvalho *et al.*, 2013). When whey is mixed along with the remaining wastewaters from the dairy industries, the COD levels increase up to seven times higher (Karadag *et al.*, 2015).

In recent years, dairy industries have been steadily increasing recovery of proteins, lactose and minerals from the treatment of the whey using various technologies. This new approach, the transformation of whey into a valuable product, helps reducing environmental pollution and it also provides an economic incentive as it is possible to recover these processed products (Ganju and Gogate, 2017).

The recovery and reutilization of water is another manner to reduce the volume of dairy wastewater and many studies are been conducted in this regard. The objective of several studies covers the improvement of certain technologies and techniques through cleaner production (CP), that facilitate the reuse and recycling of resources and waste management (Kubota and Cantorski, 2013; Kasmi, 2018).

As mentioned, dairy wastewater is characterized by having high COD and great amount of dissolved or suspend solids, hence the need for proper treatment before disposal *(Mehrdadi et al.,* 2012) in order to diminish the environmental problems. In dairy wastewater treatments exist a lot of strategies that essentially depend on the amount of COD or BOD present in the wastewater, Figure 0.1 presented in Annex A demonstrates these strategic options. Almost all the treatments for the dairy wastewater consist in conventional coagulation and flotation. Conventional treatments, or physicochemical treatments fail due to the high levels of proteins and lipids, causing many problems such as alkaline pH, high levels of COD and BOD, besides that it also generates secondary pollution as result of the use of chemical reagents (Melchiors *et al.,* 2016). The cost associates with this type of treatment is extremely elevated and additionally physicochemical methods only treat the wastewater partially, i.e. can only eliminate a very low percentage of soluble chemical demand, COD (Slavov, 2017). Therefore biological methods are more suitable alternatives, since they achieve a better removal of the pollutants, are less expensive and the application is straightforward (Porwal *et al.,* 2015).

3. MATERIALS AND METHODS

3.1. Analytical Methods

In this section, the analytical techniques used in the laboratory during the experiments, to evaluate the AS, IFAS and MBBR performances are described. All the samples were placed in closed recipients and stored at a temperature of about 4ºC.

3.1.1. Chemical Oxygen Demand

Chemical Oxygen Demand is a very important parameter because it quantifies, indirectly, the contend of organic matter present in the sample trough measurement of the amount of oxygen needed to degrade the sample. The method used was the *Closed Reflux, Colorimetric Method 5220D of the Standard Methods for the Examination of Water and Wastewater*. The quantity of oxidant consumed is expressed in terms of its oxygen equivalence.

The basis of this method consists in a complete oxidation of the organic matter in the sample into carbon dioxide through a boiling mixture of chromic and sulfuric acids. The acidic conditions are ensured since the acid solution contains silver sulphate dissolved in sulfuric acid. The digestion solution is obtained adding potassium dichromate ($K_2Cr_2O_7$), sulfuric acid and mercury sulphate; in this investigation the mercury sulphate was not added since there was no need because there were no chlorides.

The specific chemical properties of the dichromate iron ($Cr_2O_7^{2-}$) give the conditions to be a stout oxidant under the acidic conditions; it is reduced to chromic iron (Cr^{3+}) after the oxidation is finished. The chromic ion absorbs very well in the 600 nm region, unlike the dichromate which has almost zero absorption in this range. Using ultraviolet-visible spectrophotometry it is possible to indirectly quantify the amount of organic matter in the respective sample, since the amount of chromic iron can be measured. The method is used to determine values of COD between 100 and 900 mg/L, while higher concentrations need to be properly diluted.

For the application of the method, in each digestion reagent tube 2.5 mL of sample, 1.5 mL of digestion solution and 3.5 mL of acid solution are pipetted and then the tube is placed in the thermoreactor for 2 hours at 150ºC. After the 2 hours the tubes were placed in the dark for 1hour to cool down, then the absorvances were read at 600 nm in the Thermo Spectronic Helios Delta Visible Spectrophotometer.

3.1.2. Solids

The concentration of solids is referred as the amount of suspend or dissolved matter present in water or in the effluent and it can be organic or inorganic. The presence of these solids in water environments can come from natural origin (natural erosion) or anthropical origin (domestic and industrial effluents).

Solids analyses are important in the control of biological and physical wastewater treatment processes and, depending on their concentration, solids may negatively affect the water quality and for that reason these analyses are a regulatory requirement for effluent discharge.

All the samples were performed based on *2540 Method from Standard Methods for the Examination of Water and Wastewater* respectably, TSS- *2540 D. Total Suspend Solids Dried at 103-105ºC,* VSS- *2540 E. Fixed and Volatile Solids Ignited at 550ºC,* TS and VS- *2540 g. Total, Fixed, and Volatile Solids in Solid and Semisolid Samples*.

For the determination of suspended solids some preliminary steps for sample analysis are required, for valid results to be obtained, and all the samples were made in duplicated. Firstly, the porcelain crucible with the glass microfiber filter inside it is put at 105ºC for 1 hour than it goes to the desiccator until it reaches ambient temperature; after that it is weighted. In the next step the glass microfiber filter is moistened with distilled water through the vacuum filtrating system. After that the glass microfiber filter is placed again in the porcelain crucible, dried for 1 hour at 103-105ºC, cooled down until reaching the ambient temperature in a desiccator and finally weighed (m_0). The procedure of drying, cooling, desiccating and weighing is repeated until a constant weight or the weighted change is less than 4% of the previous weight.

3.1.2.1. Total Suspended Solids and Volatile Suspended Solids

After the previous steps, **Total Suspend Solids, TSS**, are determined with a well-mixed sample that is filtered, by the vacuum filtration system, using the previous weighed porcelain crucible with the microfiber glass filter and repeating the previous procedure of the cooled, dried and weighed sample (m_1). The determination of TSS is obtained by applying the Equation 3.1.

$$TSS\ (mg/l) = \frac{m_1 - m_0}{sample\ volume} \tag{3.1}$$

To evaluate the **Volatile Suspend Solids, VSS**, the preceding sample (m_1) is introduced into the muffle for 1 hour at 550ºC to ignite. After it was cooled down it is weighed (m_2). The VSS is obtained by applying the Equation 3.2.

$$VSS\ (mg/l) = \frac{m_2 - m_1}{sample\ volume} \tag{3.2}$$

3.1.2.2. Total Solids and Volatile Solids

For the determination of TS and VS some previous steps were needed. Firstly, the porcelain crucible is put on the muffle for 1 hour and after it is cooled, dried and weighted (m_0).

To determinate the **Total Solids**, **TS**, it is placed a known volume of a well-mixed sample in the previously weighted porcelain crucible and it is submitted to the oven at 103-105ºC for 24 hours, then it is cooled, dried and weighted (m_1) until a constant weight or the weighted change is less than 4% of the previous weight . The TS is obtained through Equation 3.3.

$$TS\ (mg/l) = \frac{m_1 - m_0}{sample\ volume} \quad (3.3)$$

To evaluate the **Volatile Solids**, **VS**, the proceeding sample (m_1) is placed in the muffle at 550ºC for 1 hour maximum and after that it is cooled, dried and weighted (m_2). To obtain the value it was used the Equation 3.4.

$$VS\ (mg/l) = \frac{m_2 - m_1}{sample\ volume} \quad (3.4)$$

3.1.3. Sludge Volume Index

The Sludge Volume Index, SVI, was determinate based on the *2710 D. Sludge Volume Index of the Standard Methods for the Examination of Water and Wastewater*. SVI is usually used to monitor the sludge settlement of biological suspensions. The method is based on the volume occupied by 1 g of sludge after 30 minutes settling in a beaker glass. The SVI its determinate by the immediate Equation 3.5.

$$SVI\ (ml/g) = \frac{\text{settled sludge volume } \left(\frac{\text{ml}}{\text{l}}\right) * 1000}{\text{total suspended solids } \left(\frac{\text{mg}}{\text{l}}\right)} \quad (3.5)$$

3.1.4. Temperature and pH

During the entire time, temperature and pH were measured almost every weekday. A Hanna Instruments HI98106 champ pH tester was used to measure the pH in the 3 reactors and in the alimentation tank in order to assure that the feed is not too acid because of the heat. For the determination of the temperature a glass thermometer was used, and measurements were made in the 3 reactors and in the feed tank as well.

3.1.5. Total Carbon and Total Nitrogen

Total Carbon, TC, is a method where it is possible to measure the amount of organic matter present in the sample. There are two types of carbon present in water: organic and inorganic carbon. The difference between the two lies in the chemical structure; while organic carbon (TOC) is bound to oxygen or hydrogen molecules, inorganic carbon (IC), is bound to gas carbonate and carbonate ions. The two forms of carbon are referred as Total Carbon (TC) and it is expressed in Equation 3.6.

$$TC = TOC + IC \tag{3.6}$$

In order to be possible to measure TC in a sample, the *680ºC Combustion Catalytic Oxidation with non-dispersive infrared detection method* was applied. The sample is burned in the combustion tube transforming the TC components in carbon dioxide; after that the combustion products are then transported by a carrier gas, which leads them through the combustion tube to the dehumidifier where it is cooled and subsequently dehydrated. The carrier gas then conducts the combustion products through a halogen scrubber to remove chlorine and other halogenates. Finally, combustion products arrive at the cell of a non-dispersive infrared (NDIR) gas analyser where the carbon dioxide is detected. A calibration curve is used to obtain the TC concentrations. The equipment used was TOC-V CPN, incorporated with ASI-V, in order to be automatic and with TMN-1 to be able to measure TN; all the equipment was from Shimadzu.

Just like Total Carbon (TC), Total Nitrogen (TN) is also present in water, organically and inorganically, and analogously to TC, TN is the sum of organic and inorganic nitrogen.

To measure the TN, the *chemiluminescence method* was used. The sample containing TN when introduced into the combustion tube is decomposed into nitrogen monoxide. The carrier gas containing the nitrogen monoxide is cooled and dehumidified and then transported to the chemiluminescence gas analyser, where the nitrogen monoxide is detected. The equipment used was TNM-1, from Shimadzu and the procedure is represented in Figure 0.2 presented in Annex A.

3.1.6. Microbial characterization

The objective of the microbiological analyses in the experiments, was the identification, quantification and monitoring of protozoa and metazoa, and the determination of morphological parameters of microbial aggregates (flocs and filaments). To obtain all the above parameters it was necessary to use a laboratory optical microscope, *Leica DM 2000*, in order to acquire the necessary images to later use the Matlab program established by Amaral and Ferreira (2005).

3.2. Experimental Unit Description

To determine the performance of each biological process studied in this work, it was necessary to assemble 3 independent laboratory size reactors: one Integrated Fixed-Film Activated Sludge (IFAS), one Moving Bed Biofilm Reactor (MBBR) and one Activated Sludge (AS). All reactors consisted of the same material, plexiglass, and had the same volume, 5 L.

The experiments were set up in the Department of Chemical and Biological Engineering (DEQB) of the Coimbra Institute of Engineering (ISEC).

Since the reactors were operating on a continuous-flow rate it was necessary to use in IFAS and AS system, one decanter and a sludge recycling system in each of them. For the MBBR system it was only used an Erlenmeyer flask for a more efficient way to observe the excess sludge and to avoid effluent contamination. A known portion of excess sludge was removed from time to time in AS and IFAS, to reduce the amount of suspend matter and so that the sludge does not get too old.

The oxygen was administered via a circular bubble diffuser placed at the bottom of all reactors, connected by a tube to an air pump; in this way the Dissolved Oxygen was not a limiting factor in the treatment, and in the case of IFAS and MBBR systems all the carriers were assured to be suspended and fill the entire reactor.

Initially the feeding flows were controlled by three peristaltic pumps, each one serves one reactor with the same flow rate, but due to the flow control the pumps were replaced a week and half later, since they did not obey the desired hydraulic retention time. The Figure 3.1 shows the initial assembly with the three pumps (in the left). This period was

considered as the incubation time of the biofilm in the carriers and the preliminary trials of the study will be explained in section 2.6.

In order to meet the hydraulic retention time, it was necessary to replace the three pumps with another two peristaltic pumps that managed the feed rate according to the desired hydraulic retention time. In Figure 3.1 it is showed the configuration of the experimental set-up with the new pumps (on the right). Almost a month later, both pumps started to have problems and one of them had a malfunction due to one of the bearings being broken; as an alternative a solenoid valve was assembled, and it worked for two weeks and fed two reactors, IFAS and AS, until another peristaltic pump arrived that fed both reactors at the same time and with the same flow. During this period there were some flow fluctuations in IFAS and AS, as it was necessary to branch the tube that was connected to the solenoid valve. Another peristaltic pump was used to feed the MBBR reactor as the previous one was already starting to cause problems.

Figure 3.1: View of the initial experiment arrangement; 1: Initial arrangement with the firsts pumps, incubation period; 2: Second arrangement with new pumps, beginning of the experiment.

The last and final arrangement of the full flow process is described in Figure 3.2. All the peristaltic pumps were set automatically by an electric timer, to work all the days of the experiments. Air pumps were set up to go off only when the recirculation system was turned on. The feed peristaltic pump in MBBR system worked 24 hours a day since the type of pump used in this system allowed to deliver a very low flow rate.

The effluent discharge was made using tube connected to the decanter, in the case off the IFAS and AS system, or to the Erlenmeyer in the MBBR system, and it worked only using gravity.

Feeding flow rates were delivered by two peristaltic pumps, one served the IFAS and AS systems and the other one served the MBBR system. Calibration was performed every two weeks to certify the flow did not change. The feeding-rate flow was equal in all the reactors controlled by peristaltic pumps.

In Figure 3.3 it is possible to observe the display of the experimental set-up in the laboratory.

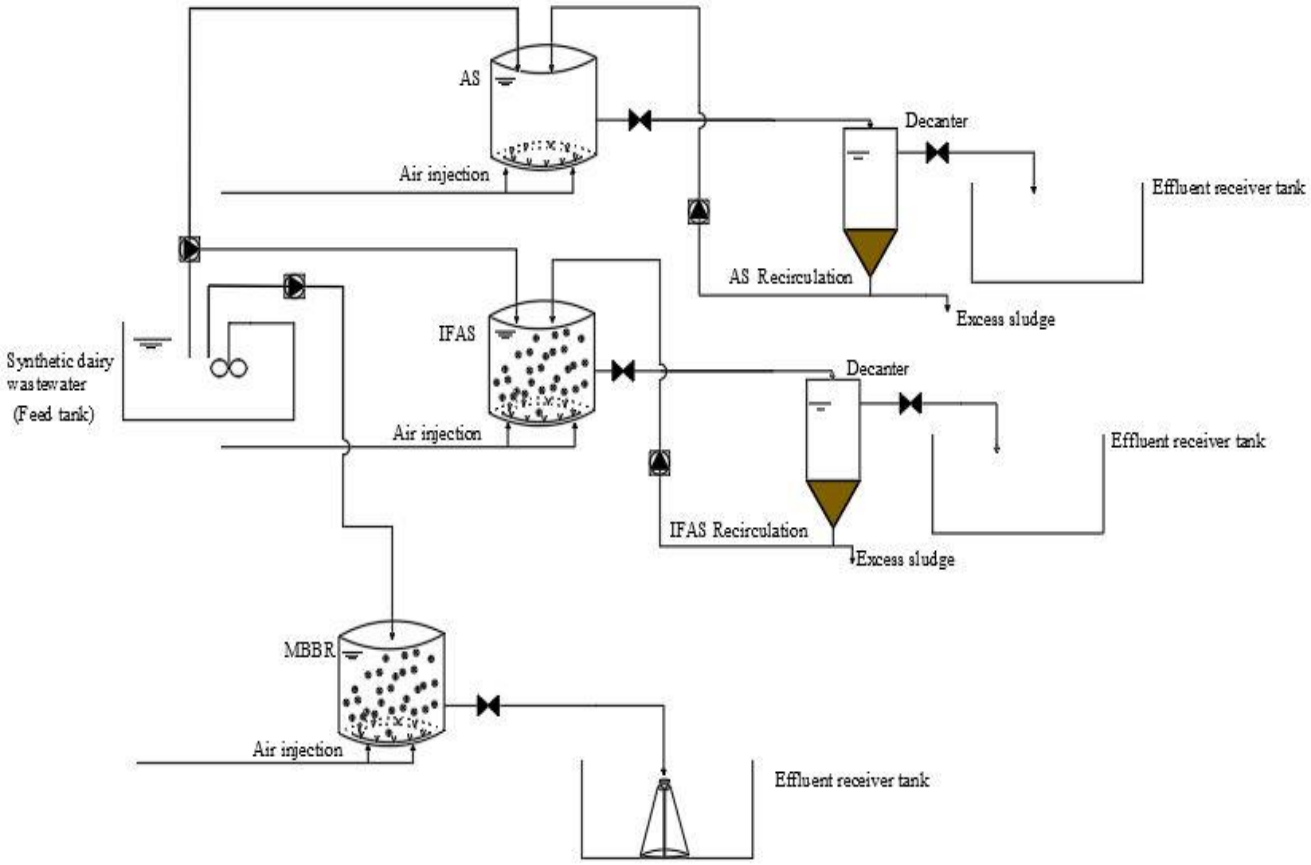

Figure 3.2: Experimental arrangement diagram.

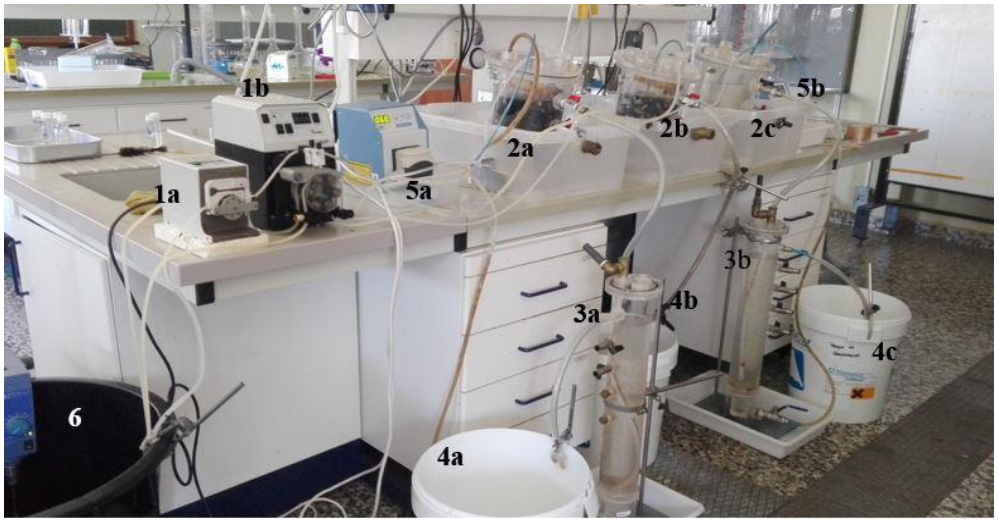

Figure 3.3: View of the experiment assembled and operating in the laboratory; 1a & 1b: peristaltic feeding pumps, 1a feeding MBBR and 1b feeding IFAS and AS; 2a: IFAS reactor; 2b: MBBR reactor; 2c: AS reactor; 3a: decanter for the IFAS reactor; 3b: decanter for AS reactor; 4a & 4b & 4c: receiver tanks for effluent from MBBR, IFAS, AS respectively; 5a & 5b: Sludge recirculation peristaltic pumps from IFAS and AS respectively; 6: feeding tank.

3.2.1. Biocarriers

The carriers used in the experiment were *Bioflow 9* both in IFAS and MBBR systems. These carriers are made made from High Density Polyethilene (HDPE) and have the folowing characteristics: dimensions are 9mm x 7mm , bulk density of 145 kg/m^3 and the total superficial area available for biomass growth is 800 m^2/m^3. More informacion is given in section 2.5.

3.2.2. Wastewater

During the present study a synthetic dairy wastewater, composed of skimmed milk and water, was used as this would ensure a very low variability in terms of organic load. This simplification also helped to prevent solids from entering the reactors, which in turn facilitated the study of solids produced by biomass.

Every day, except weekend, a new dairy wastewater was used, since the tank was exposed to air which causes a faster acidification of the milk, which was further aggravated due to the high temperatures experienced during the study. This acidification could damage the growth of microorganism within reactors.

3.2.3. Biofilm inoculation and preliminary trial

The reactors had a volume of 5 L and were filled with 2 L of the carriers and 4 L of sludge brought, from a Coimbra Wastewater Treatment Plant (WWTP), in order to achieve a proper biofilm inoculation. The aeration tank was turned on into the IFAS and MBBR reactors to achieve good mixing conditions, and for a proper distribution of substrates to the biofilm, 50 ml of semi-skimmed milk was also added every weekday.

For the biofilm growth to occur, the air system was also turned on. After a few days, the AS reactor was filled with 4 L of the same sludge and 50 ml of semi-skimmed milk were introduced.

After almost two weeks, the preliminary trial started and in this period all the experiments were assembled and lasted twelve days allowing the microbial communities to grow and adapt to the new environment. The bulk carriers (kg/L) in IFAS and MBBR were obtained by multiplying the volume of carriers used by the bulk density of the carriers (Equation 3.7 shows how it was obtained). The amount of carrier matches to a volume fraction of 44.4% for both reactors.

$$Bulk\ carriers\ \left(\frac{kg}{l}\right) = Bulk\ dens\left(\frac{kg}{l}\right) * Vl.of\ carreirs\ used(l) * Vl.ocuppied\ in\ reactor(l) \quad (3.7)$$

In Figure 3.4 a) it is showed the inoculation period for the biofilm to fix in the carriers and in Figure 3.4 b) it is represented the initial setup associated with the preliminary trials.

Figure 3.4: a) Inoculation of the carriers in the reactor. IFAS reactor in the left and MBBR reactor at the right. b) Initial experimental set up, corresponding to the AS sludge inoculation period and preliminary trial.

3.3. Experiments

3.3.1. Continuous experiments

The three reactors were opperated under the same conditions in order to appraise the performance in terms of carbon nitrogen and organic matter removal efficiency and to evaluate the kinetic growth of microorgannism. The experimental work was devided in two periods**, Period A** and **Period B**, were the difference resides on the amount of milk that was diluted in water on the feedig tank. The flow rate was established to give 7 L/day for each reactor in order to keep the hydraulic retention time of 12 hours. The experiments endured for 62 days.

The tests were all automatically programmed, with the help of electrical timers, in order to maintain the feeding flow rate, air suply and the recirculation system. Figure 3.5 shows how this equipment was programmed, each quadrant conrresponds to a gap of 15 minutes.

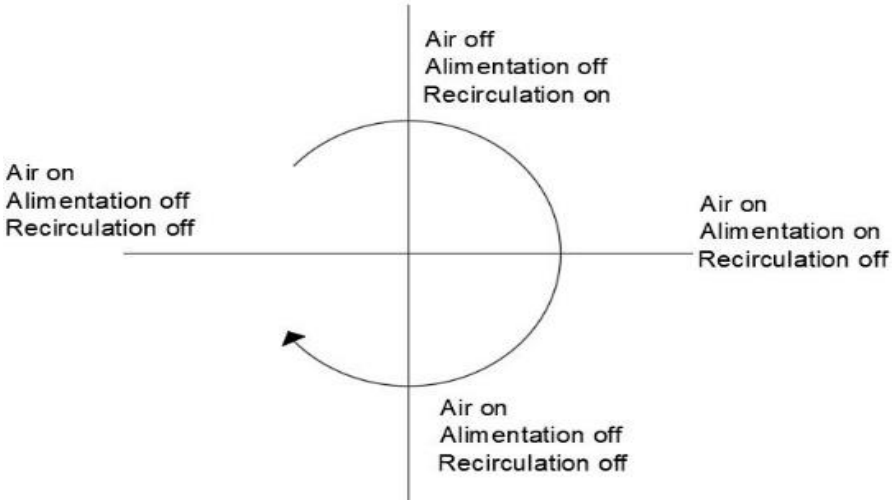

Figure 3.5: Schedule of the operating time of air, feeding and recirculation pumps. Schedule represented for 1h and this process repeated every hour for 24 hours.

In both periods, the same parameters were evaluated with the same frequency for a proper comparison between them. Table 3.1 represents the parameters and their analytical frequency, The COD, TC and TN were analysed in terms of raw and filtered (soluble) effluent.

Table 3.1: Parameters analysed together with the analytical frequency.

Parameters	Analytical Frequency
Chemical Oxygen Demand (COD and CODs)	2-3 times a week
Total Solids (TS) and Volatile Solids (VS)	Every weekday
Total Suspend Solids (TSS) and Volatile Suspend Solids (VSS)	2-3 times a week
Total Carbon (TC and TCs) and Total Nitrogen (TNs)	Every weekday
Temperature and pH	Every weekday
Sludge Volume Index (SVI)	1 time in each period
Microbial Characterization	2-3 times a week

3.3.1.1. Period A

The experiment lasted 37 days, with a synthetic wastewater made of 1/200 skimmed milk dilution entering daily in each reactor. The Filling Fraction (FF) in the IFAS and MBBR reactors was around 44.5%, that is 2 L were occupied by the carriers and 2.5 L were filled with liquid.

During this time the peristaltic feed pumps broke and until new pumps arrive experiments were conducted by a solenoid valve. Moreover, during the first 16 days the TN reading machine had a technical problem, which made readings impossible during that time; for this reason, all other parameters, only started to be counted from that time forward.

3.3.1.2. Period B

The second and finally set of experiments lasted 25 days, the synthetic wastewater was made from a dilution of 1/100 skimmed milk and it was introduced in all reactors. In this study all the reactors were initially emptied and cleaned. The sludge was all removed and filtered through a sieve, and then distributed among the 3 reactors to be in the same conditions. The decanters were also cleaned. In AS an IFAS reactors 1 L of sludge was introduced and in the MBBR only 650 mL. The Filling Fraction was the same as in the

previous period, but the carriers used were pre-inoculated in a separated reactor, under the same conditions as the other carries.

3.3.2. Microbiological analysis

3.3.2.1. Protozoa and Metazoa

In order to achieve a characterization of the microorganisms, a representative sample of 250 mL from each reactor had to be collected. From each sample was pipetted 10 µL, onto a microscope slide and covered with a cover for viewing on the LEICA DM 200 optical microscope. They were visualized in a 100x magnification, where it was possible to count and identify all the protozoa and metazoan (Amaral and Ferreira, 2005). For proper results this was made in triplicate, so around 90 images per sample were acquired (30 for each microscope slide).

3.3.2.2. Image acquisition, processing and analysis

The software used to acquire the images was Leica Application Suite V.3.3.0 with a DFC391 FX camera coupled to the microscope LEICA DM 2000. The image was acquired in 8 bits greyscale with an image size of 1392x1040 pixies. Using a Matlab program developed by Amaral and Ferreira (2005), the images were processed and analysed in order to obtain binary images. With binary images the morphological parameters of the flocs and filaments were obtained.

4. Results and Discussion

4.1. Sludge characteristics

During the experiments, the sludge that was used was the same in both periods.

In period A, the sludge came from the aeration system of the 'Estação de Tratamento de Águas Residuais do Choupal, Coimbra'. The results from Table 4.1 correspond to the initial state of the sludge used in period A.

Table 4.1: Parameters used to determine the sludge characteristics in period A.

Parameter	Value
pH	7.1
COD (mgO_2/L)	1022.547
CODs (mgO_2/L)	937.576
TCs (mgO_2/L)	112.123
TNs (mgO_2/L)	11.780
TSS (mg/ L)	20.000
VSS (mg/L)	12.000
TS (mg/L)	508.750
VS (mg/L)	441.250

The sludge used in period B was obtained by emptying and mixing the sludge from the three reactor and the two decanters (IFAS and AS), in order to obtain a sludge as similar as possible to ensuring the same conditions in the three reactors. The results from the sludge are represented in Table 4.2.

Table 4.2: Parameters used to determine the sludge characteristics in period B.

Parameter	Value
pH	7.4
COD (mgO_2/L)	1186.360
CODs (mgO_2/L)	1084.627

TCs (mgO_2/L)	172.576
TNs (mgO_2/L)	17.992
TSS (mg/ L)	24.000
VSS (mg/L)	20.000
TS (mg/L)	831.250
VS (mg/L)	735.000

4.2. Wastewater

The wastewater that was used in the present work was synthetic in order to guarantee constant parameters throughout the entire study.

In the period A the wastewater was composed by water and skim milk with a concentration dilution of 1:200. Table 4.3 shows the analysed parameters for the characterization of the wastewater. The results of each parameter corresponded to the average values of the values obtained over the period A.

Table 4.3: Parameters used for the characterization of the wastewater for the period A.

Parameter	**Value**
pH	5.0
COD (mg O_2/L)	1154.658
CODs (mg O_2/L)	978.569
TC (mg/L)	231.450
TN (mg/L)	39.210
TSS (mg/L)	20.000
VSS (mg/L)	12.000
TS (mg/L)	508.750
VS (mg/L)	441.250

Period B was operated also with the same synthetic wastewaters as Period A but with a different diluent factor, 1:100, origination a higher organic load since the feeding flow duplicated in the amount of skimmed milk entering. Table 4.4 shows the analysed parameters for the characterization of the wastewater.

Table 4.4: Parameters used for the characterization of the wastewater for the period B.

Parameter	Value
pH	5.3
COD (mg O_2/L)	1176.449
CODs (mg O_2/L)	1019.004
TC (mg/L)	1581.500
TN (mg/L)	181.475
TSS (mg/L)	4468.000
VSS (mg/L)	106.000
TS (mg/L)	831.250
VS (mg/L)	735.000

During period B there were some fluctuations in the values due to the high temperature in the laboratory, increasing wastewater degradation, very dull with cream particles from the milk, and another factor was the obstruction inside the feeding tubes.

During the weekends the experiments were running, and therefore the feeding tank needed to have more wastewater to ensure supply during the days on which the laboratory was closed, but when it opened on Monday the wastewater present in the tanks was dull and with a darker colour containing a lot of cream particles from the milk associated with a bad smell.

4.3. Biomass assessment

The attached biomass was not measured in this study, so the biomass was not fully assessed, due to lack of time and technical problems that were previously described.

During the experiments there was a need to clean manually the air diffusers, as part of the orifices became clogged, and some solids began to settle in the bottom of the reactor. Even with this effort some solids, despite being much less, continued to sediment at the bottom. Therefore, sampling of suspended solids inside the reactor was not entirely homogeneous, contributing in part to creating some fluctuations in the concentration suspended biomass, and this is demonstrated in Figure 4.1 and 4.2 for period A and B, respectively.

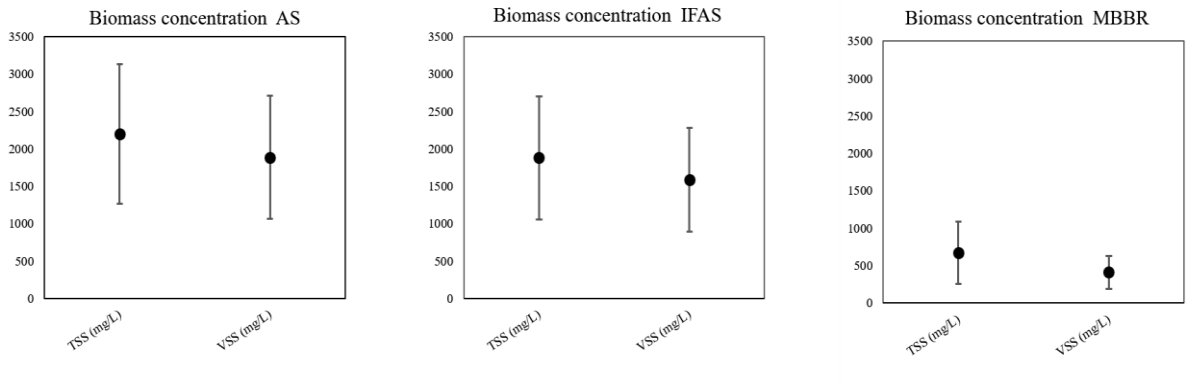

Figure 4.1: Total Suspended Solids and Volatile Suspended Solids inside of the three reactors working in continuous flow in period A.

The intended retention time was 12h, so for each reactor should have been provided 7 L/d of wastewater from the feeding pumps, which did not happen for AS, MBBR and IFAS systems during period A. In fact, it only provided about 4.5 to 6 L/d. The inability to control the feeding pumps during the period A, since the pumps breakdown between 15th to 30th of May, which were then replaced by a solenoid valve during that period, caused some oscillations in AS and IFAS suspended biomass values. After that the solenoid valve was replaced for peristaltic pumps and the values started to settle. Period A is characterized by a low suspended biomass concentration due to the previously described factors.

The AS system worked with the highest biomass concentration as expected, around 2197.439 mg TSS/ L and 1887.597 mg VSS/ L. Notwithstanding the MBBR system operated with an average of 669.796 mg TSS/ L and 408.432 mg VSS/ L, being the system that operated at a lower biomass concentration. No correlation was found between the suspended biomass amount and the COD entering in the reactor. During the breakdown of the feeding pumps the MBBR system was maintained with the same pump, even when it started showing some mechanical problems until its replacement, on 05/31 (month/day).

The IFAS system had a suspended biomass concentration higher than the MBBR system, contributing for a greater substrate consumption and diminishing the substrate availability for the biofilm. The average values were 1881.015 mg TSS/ L and 1584.516 mg VSS/ L.

The suspended biomass showed more significant oscillation values in the IFAS and AS system as already mentioned, but this oscillations can also be related to problems associated with the sludge recirculation system, since the tube sometimes was clogged, preventing the normal passage of recirculated sludge from the decanter to the reactor.

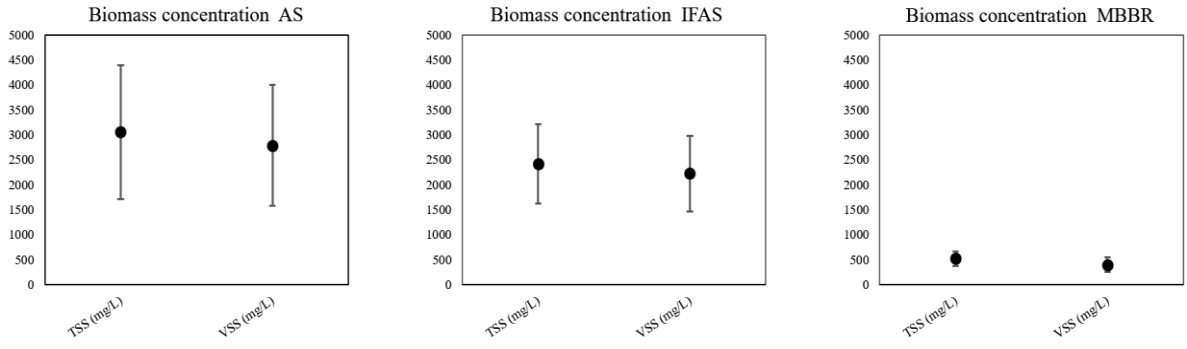

Figure 4.2: Total Suspended Solids and Volatile Suspended Solids inside of the three reactors working in continuous flow in period B.

Period B was characterized by a higher concentration of suspended biomass, except for the MBBR system which for unknow reasons suffered a washout the day after period B started, displacing most of the sludge that was inside of the reactor to the treated effluent tank.

The feeding pumps functioned without mechanical problems throughout the entire period, unlike air differs that were quickly obstructed, and their cleaning became more frequent in order to ensure homogeneity in all reactors volume. The problems with air diffusers became more problematic in the AS system for perhaps operating with only suspended biomass and having a recirculating system.

AS system operated with higher suspended biomass concentration, with an average amount of 2829.733 mg TSS/ L and 2578.257 mg VSS/ L, in 6/26 the sludge, in some parts of the reactor, turned reddish which may be link to the air diffusers. In Figure 0.1 presented in Appendix A, it is shown the reddish spots in the AS reactor and in the effluent.

In this period, IFAS system operated with an average amount of 2256.476 mg TSS/ L and 2060.314 mg VSS/ L, and in this period the suspended biomass remained much more stable.

During the entire period, the MBBR system operated alongside some problems in terms of retained the sludge inside of the reactor, polluting the clarified effluent making it darker and duller. The darker colour was manifested in the second day, when the washout phenomenon occurred, and it was also observed that most carriers suffered biomass detachment leading to the loss of biofilm. The effects of the phenomenon went throughout the period B, since the biofilm never fully recovery, even when the sludge was put back into the reactor, with the purpose of recovering /maintaining the biofilm and controlling the

sludge concentration in the reactor. These problems led to higher TC and CODs levels and lower concentration of biomass in the reactor, harming the biodegradations reactions. The average values were 556.448 mg TSS/ L and 428.971 mg VSS/ L.

4.4. Characterization of the treated effluent

Several parameters, such as COD, TC, TN and Suspended Solids were determined in order to obtain the characterization of the treated effluent from both periods. TCs and TNs values were multiplied by 10 to facilitate representation on the figures below The suspended solids in the output stream are negligible since their values are extremely low, so they not appear in the graphics below.

Figure 4.3 represents the average values of CODs, TCs x10 and TNs x 10 and the evaluation throughout period A for the AS system. The average values of CODs, TCs and TNs were 347.027 mgO_2/ L, 23.634 mgO_2/ L and 12.279 mgO_2/ L respectively. During the 15^{th} to the 30^{th} of May the overall values are slightly higher, this is since the peristaltic pump, responsible for the distribution of the wastewater, was replaced for a solenoid pump that contributed to an abnormal supply to the AS reactor. In 4/6 the CDOs and TCs values increased while TNs decreased, this anomaly is likely to be associated with the washout that was observed when taking the samples for analysis. After taking the samples, the sludge was placed inside the reactor with the assistance of a decanter.

The results of the three reactors in terms of substrate and nitrogen removal are presented below. The SVI was calculated for the AS and IFAS in periods A and B, and in both cases, they presented good settleability since the values were <150 mL/g, which is in accordance to what is recommended in the literature. The temperature and pH were also measured in the reactors for both periods, Tables 0.1 and 0.2 presented in Appendix A, show the average values of these parameters and it is possible to observe the increase in temperature, practically 1ºC more, and in period B also pH increases the pH.

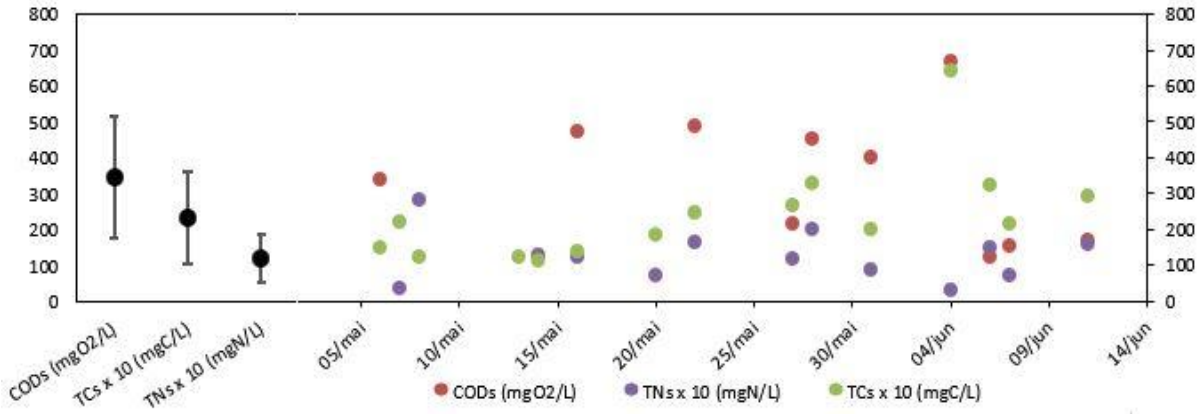

Figure 4.3: AS output concentration evolution in period A.

The TCs and TNs values for IFAS and MBBR systems are represented in the same way that was described for the AS system. The Figure 4.4 shows the average values and the standard deviation for CODs, TCs x10 and TNs x10 and furthermore, demonstrates how these values varied over period A. The averages values for CODs, TCs and TNs in IFAS systems are 257.156 mgO_2/ L, 12.265 mgO_2/ L and 7.134 mgO_2/ L. During the 15th to 30th of May the overall values increased, as with AS system as they shared the same peristaltic pump that breakdown and was replaced with a solenoid pump. Nevertheless, the rise was not as accentuated as in the AS system, especially in the values of the TCs and TNs, which did not change very much from the other values obtained outside of that malfunction period. Through the Figure 4.4 it is possible to observe that the values remained stable.

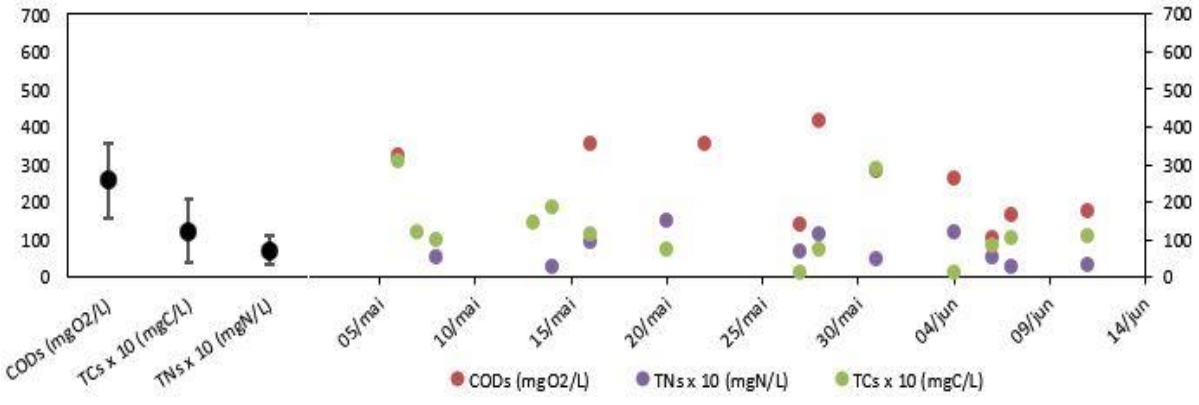

Figure 4.4: IFAS output concentration evolution in period A.

The MBBR system did not have many fluctuations as the AS and IFAS system, since it had no problems with the peristaltic pumps during period A, as it is possible to observe in Figure 4.5. The average values were 274.774 mgO_2/ L 14.556 mgO_2/ L and 11.354 mgO_2/

L for CODs, TC and TN respectively. Even so, the IFAS system was the one that got the lowest values and in turn better organic matter removal performance, as shown in Table 4.5.

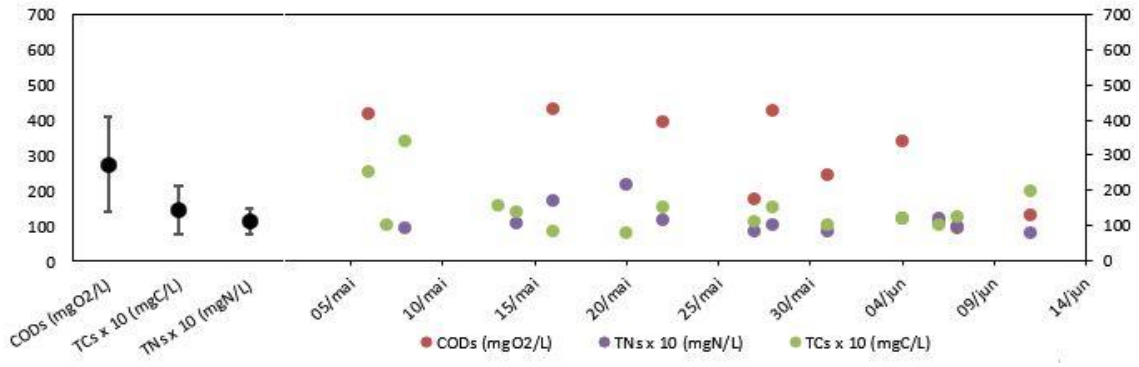

Figure 4.5: MBBR output concentration evolution in period A.

During period B all the pumps were already replaced, ceasing to be a limiting factor on the experience and as it is possible to observe, through the images bellow, the values of the analysed parameters did not suffer so many oscillations in the case of the AS and IFAS system, as it happened in the previous period.

AS system shown a higher effluent quality compared with period A, since the averages values of CODS, TCs and TNs drop to 178.510 mgO_2/ L 23.753 mgO_2/ L and 6.557 mgO_2/ L, respectively, and over the period it is observed through the Figure 4.6, that the values of the analysed parameters are gradually decreasing.

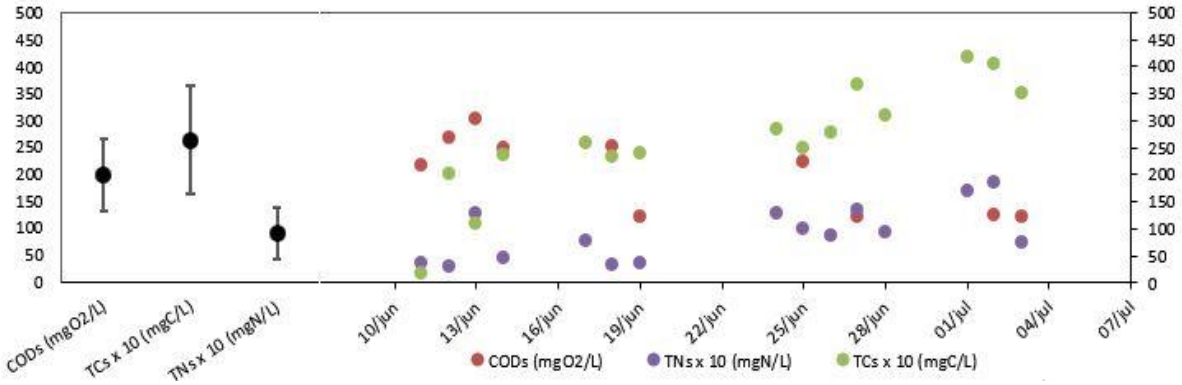

Figure 4.6: AS output concentration evolution in period B.

The effluent quality was more noticeable in IFAS system, once the averages values were the lowest among the other two systems. Through the visualization of the Figure 4.7 it is concluded that the values remained practically stable during period B, except for the first

days of the beginning of the experiment; this can be explained because this system operated with suspended and attached matter.

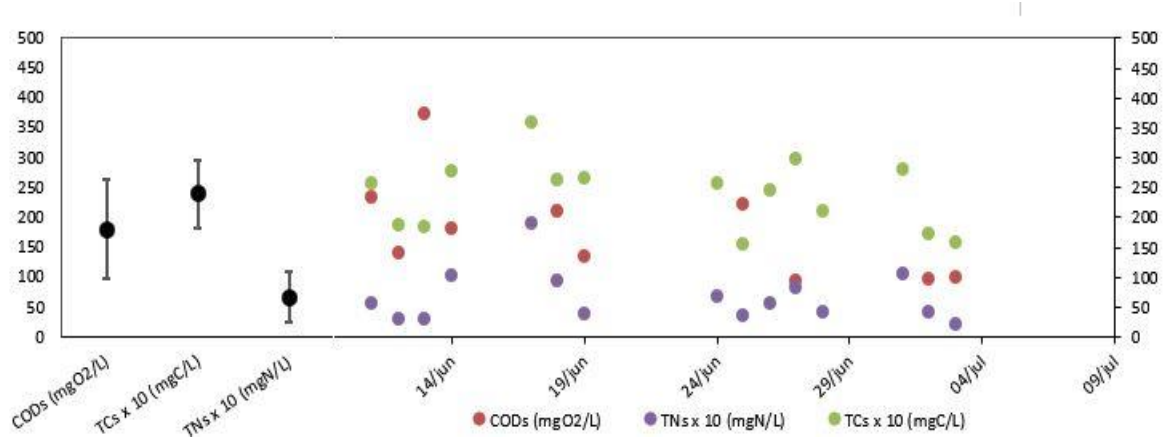

Figure 4.7: IFAS output concentration evolution in period B.

As already mentioned, the MBBR system suffered a washout the day after starting the new experimental period. Greatly damaging the effluent quality. The Figure 4.8 demonstrates primarily the values of TCs and TNs increase throughout the period B. In addition to the washout, there may have been some contamination in the reactor or in the tubes that connects to the feeding pump, since for this period all equipment was properly cleaned and only after the new experience began.

In general, the IFAS system was the one that obtained the best results regarding the quality of the final effluent and this can be recognized in Table 4.5 and 4.6.

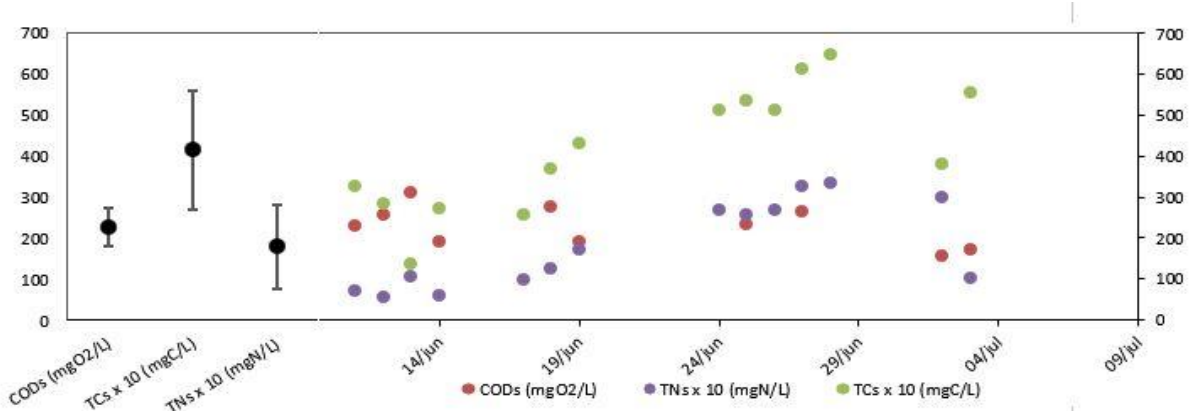

Figure 4.8: MBBR output concentration evolution in period B.

4.4.1. Organic matter removal performance

Period A generally corresponds to a lower removal's efficiencies, whereas period B corresponds to a higher efficiency. The increase of the efficiency can be explained through

the increment of the organic load entering in the reactors, or the fact that the feeding pumps operated without mechanical problems. The increase in removal efficiencies was only visible in the AS and IFAS system, since MBBR had problems in its performance as already mentioned. The Table 4.5 and Table 4.6 correspond to the efficiencies of the parameters analysed in the effluent through period A and Period B, respectively.

In period A, even with the difficulties, IFAS system achieved significant efficiencies in organic removal, which indicates it is a system that manages to keep constant even when it suffers disturbances. The MBBR revealed similar values but slighter removal efficiencies.

The poor CODs efficiencies can be explained by the low biomass concentration inside the reactor and for deficient settleability, so is often replaced by TC since it can determinate the soluble carbon matter without taking in account the quality of the separation.

Table 4.5: Resume of parameters evaluated for each system with the corresponded efficiencies for period A.

Treated effluent	Parameters	Average	Standard Deviation (±)	Removal Efficiency
AS	CODs	347.027	170.065	63%
	TCs	23.634	12.894	79%
	TNs	12.279	6.670	20%
	TSS	9.731	3.862	51%
	VSS	7.308	3.123	39%
	TS	60.578	44.255	88%
	VS	22.500	6.124	95%
IFAS	CODs	257.156	127.089	73%
	TCs	12.265	100.443	89%
	TNs	7.134	8.344	54%
	TSS	6.615	3.901	67%
	VSS	4.785	2.193	60%
	TS	61.259	51.822	88%
	VS	13.125	7.369	97%

	CODs	274.774	133.952	71%
	TCs	14.556	6.865	87%
	TNs	11.354	4.552	26%
MBBR	TSS	23.815	6.047	43%
	VSS	7.255	4.447	29%
	TS	20.423	50.962	88%
	VS	17.500	5.863	96%

Period B has better removal efficiencies, except for MBBR system. The IFAS system was again superior in terms of the removal efficiency when compared with AS and MBBR. The rise of the efficiencies seems to reveal an increased efficiency of this type of wastewater treatment technology. The MBBR system suffered the washout as referred, but the fact that the treated effluent remained contaminated, may be link to the increase in the organic load that caused eutrophication. Significant is also the improved efficiency in the reduction of soluble nitrogen (TNs) that the IFAS system present, which might reveal that the denitrification step during the periods of no aeration is more effective in the IFAS reactor.

Table 4.6: Resume of parameters evaluated for each system with the corresponded efficiencies for period B.

Treated effluent	**Parameters**	**Average**	**Standard Deviation (±)**	**Removal Efficiency**
	CODs	199.711	67.056	82%
	TCs	27.471	8.129	84%
	TNs	9.066	4.844	50%
AS	TSS	8.311	8.073	82%
	VSS	3.622	3.319	87%
	TS	36.167	13.342	96%
	VS	23.500	9.866	97%

IFAS	CODs	178.510	82.367	84%
	TCs	23.753	5.638	86%
	TNs	6.557	4.244	64%
	TSS	7.667	5.808	83%
	VSS	4.422	3.309	90%
	TS	39.167	13.875	96%
	VS	27	13.363	98%
MBBR	CODs	227.509	47.719	79%
	TCs	41.581	14.548	76%
	TNs	16.881	14.878	6%
	TSS	16.489	20.727	59%
	VSS	10.871	18.840	86%
	TS	46.000	23.550	96%
	VS	31.333	19.037	97%

4.5. Sludge Production

The sludge production was only considered in the period B in which conditions were stable except for the MBBR system. The figures 4.9, 4.10 and 4.11 demonstrate how the sludge concentration varied in relation with the amount of VSS present inside the reactor through period B.

From the analysis of the figures beneath it is possible to conclude that the AS process produced greater amounts of sludge. In Figure 4.9 it is observed that the amounts of produced sludge were virtually constant except for the 6/26 and for 7/3. The higher amounts of sludge may relate to the appearance of the red spots in the reactor and the display of worms that were increased over time. The total amount of sludge produced was 38780.19 mg VSS and that quantity represents a total of 1.55 g/d. In the last days of the period B, the AS reactor was already working with sludge excess. This is observable through the SSV values, represented in Figure 4.9.

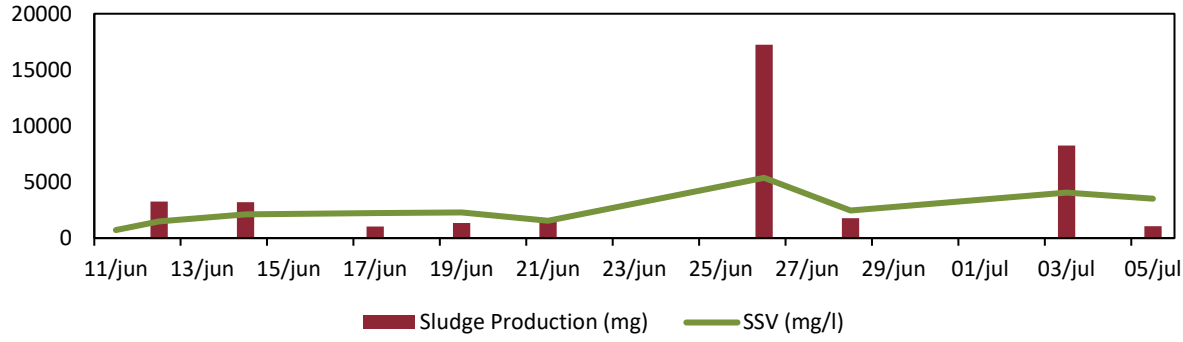

Figure 4.9: Amount of sludge production with the corresponded SVV values along period B in AS system.

IFAS system when compared to AS system, leads to conclude that less sludge is produced during the same period; the VSS concentration was practically always below the amount of sludge produced and it is also noticed that the quantity of sludge was much more constant, except for the 6/17. This can be confirmed with the representation in the Figure 4.10 where 24631.38 mg VSS were produced, representing a total of 0.985 g/d, which represents a reduction of 36% compared to AS. This reveals a significant advantage of the IFAS system moreover when sludge management in the wastewater treatment plants assumes a significant fraction of the total operational costs.

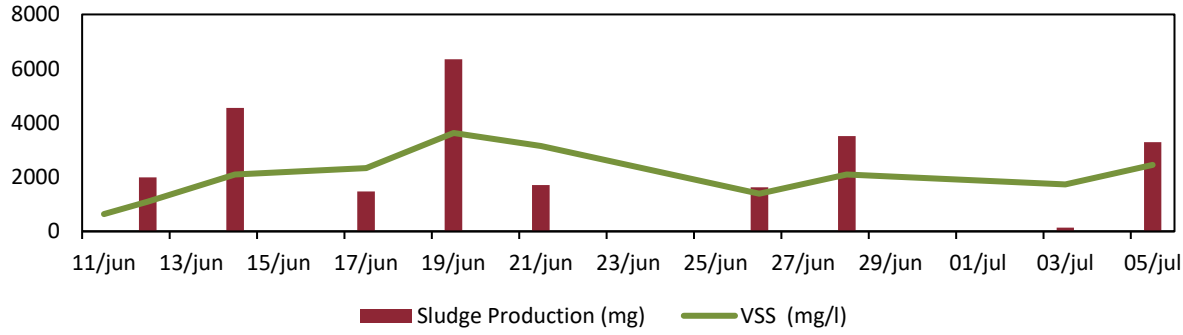

Figure 4.10: Amount of sludge production with the corresponded SVV values along period B in IFAS system.

The values obtained for the MBBR may not be completely correct, since there was the washout phenomenon and all the problems associated with the phenomenon, including the loss of biofilm, but it was speculated, through the literature, that the MBBR values were lower than the other two systems. Figure 4.11 shows how the sludge production and the amount of VSS varied through period B. The total produced amount of sludge was 4040.381 mg VSS, representing a total of 0.162 g/d.

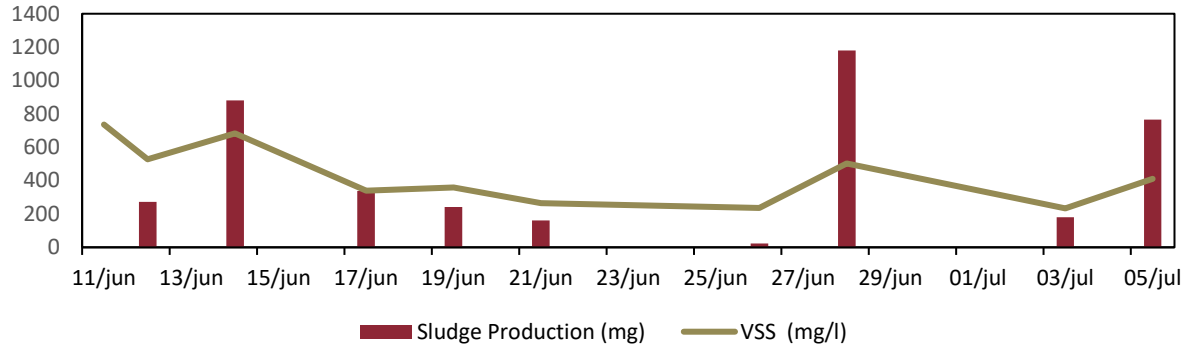

Figure 4.11: Amount of sludge production with the corresponded SVV values along period B in MBBR system.

4.6. Microbiological characterization

In this section the results from the analyses of the microbial aggregates in terms of morphology content and amount of filamentous bacterial were determined. The morphological descriptors were obtained using a Matlab program described in section 3.3.2; in the end of the image treatment binary images were attained, where the flocs and filamentous are represented by value one and background by value zero. The Figure 4.12 shows how the program was applied during the experiments.

Based on the binary images it was possible to determine the morphological parameters of the flocs, such as the total area per volume (TA/Vol), percentage of microflocs (Diamter equivalent (D_{eq}) < 25 µm), mesoflocs (D_{eq} between 25 µm and 250 µm) and macroflocs (D_{eq} > 250 µm). According to Mesquita *et al.* (2009), these parameters correlate with settleability of the sludge and the existence of zoogloeal bulking. For the characterization of the filamentous bacteria other parameters as the total length per volume (TL/Vol) and total length per area (TL/TA) were analysed in order to determine if the sludge has filamentous bulking properties. Through these parameters it was possible to analyse the evolution of the amount of aggregated biomass and filamentous bacteria.

The microscope images from the three reactors for period A and period B are represented in Figure 4.13 and around 30 images were obtained per sample. Both in period A as in period B, seven observations were made and their consequent characterization.

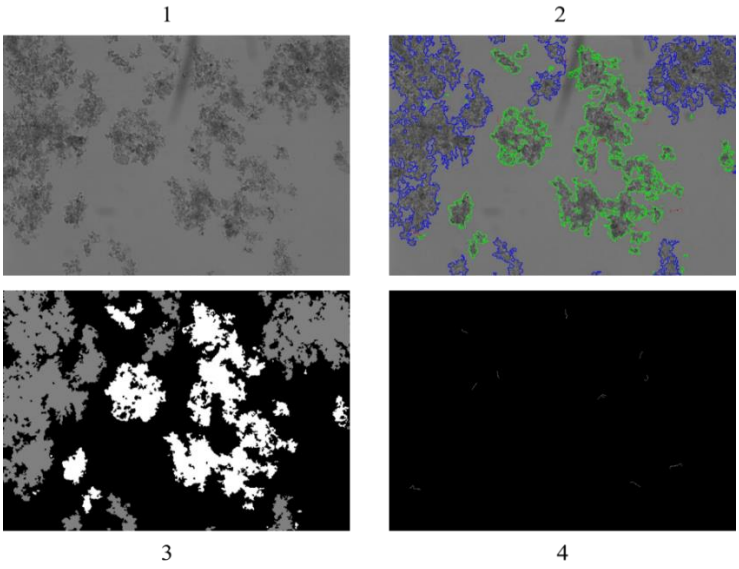

Figure 4.12: Representation of the image treatment: 1- Original images in 8 bits greyscale; 2- Image treated by the program, in which the green flocs represent those who are not in contact with the images border, therefore they are the ones that were counted, the blue flocs are the ones that were not casted, since they are in contact with the image border, and finally in red are the filaments (filamentous bacteria); 3- Treated images from flocs; 4- Binary images of filaments.

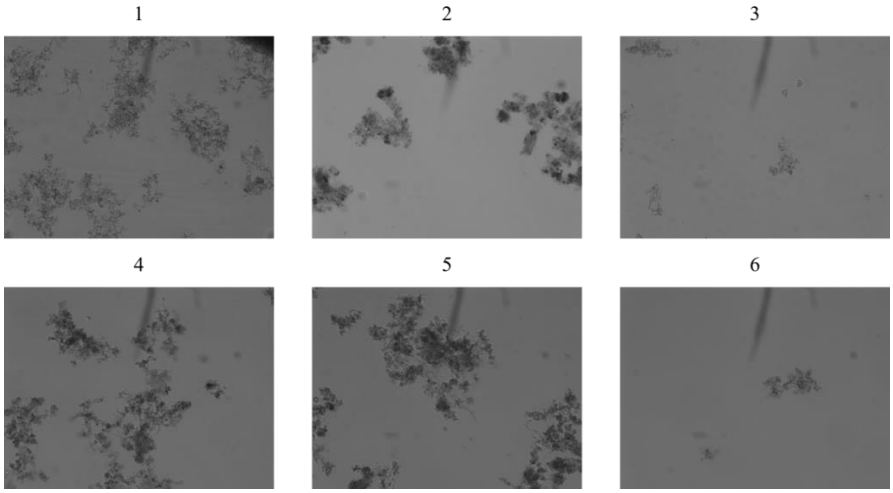

Figure 4.13: Microscope images for the three reactor in period A and period B: 1- IFAS during period A; 2- AS during period A; 3- MBBR during period A; 4- IFAS during period B; 5- AS during period B; 6- MBBR during period B.

Through the observations of the images in Figure 4.13, it is possible to conclude that IFAS both in period A and period B, occupies a larger area compared to the other two reactors, since the flocs are more dispersed. MBBR, as expected, has less flocs since this type of reactor works with less suspended biomass. Regarding the AS reactor has more dense

flocs, compared to the IFAS, making the occupied area smaller. TA/ Vol is an indirect measure of the amount of aggregated biomass.

During the experiment the three reactors were analysed, as described in Section 3.3.2 both for period A and period B, with a sample volume of 10 μm. The Figure 4.14 represents the average and standard deviation for each period in terms of total area of the flocs per volume (TA/Vol).

Through the results below it is possible to conclude that IFAS has a larger total area in terms of sludge aggregate flocs, than AS and finally the MBBR reactor; it will be expected that the reactor with vaster biomass concentration would have the higher TA/ Vol ratio. The average values for the IFAS, AS and MBBR reactors for period A are 932.887, 809.552 and 292.808 mm^2/ml respectively. For period B the averages values increased as expected, once the organic load of the affluent has doubled, the TA/Vol for the IFAS, AS and MBBR were 1037.273, 1006.247 and 344.923 mm^2/ml respectively.

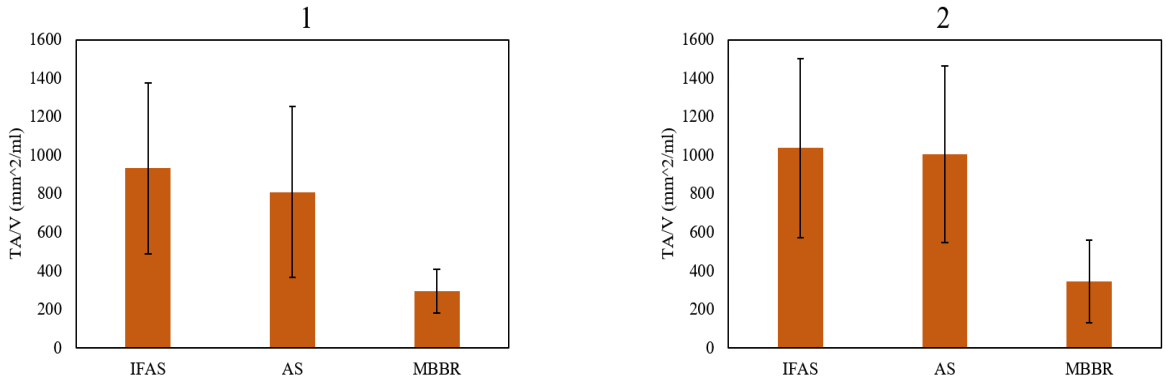

Figure 4.14: Total area of flocs by volume: 1- TA/Vol in the three reactors through period A; 2- TA/Vol in the three reactors through period B.

The TSS has an influence on the area occupied per flocs, as Figures 4.15 and 4.16 demonstrate. Whenever there is a decrease or increase in the TSS values, the TA (total area, mm^2/mL) tend to follow these variations, suggesting a direct relation between TSS and TA. During period A the TSS values fluctuated due to the problems already mentioned, and the TA also oscillated through this period according to TSS values. During the mechanical problems (15^{th} and 30^{th} of May), there was a decrease in TSS values in IFAS and MBBR reactors while in the AS reactor there was an increase; this may be due to the amount of organic load that entered in each reactor.

During the beginning of period A the TA/Vol values are very low, compared to the TSS, since the microorganisms are still developing and adapting to the new environment, increasing over time. On 4th July AS suffered a washout and it is possible to observe in the second images (Figure 4.15) that TSS values decline briskly and consequently TA/Vol also decreases.

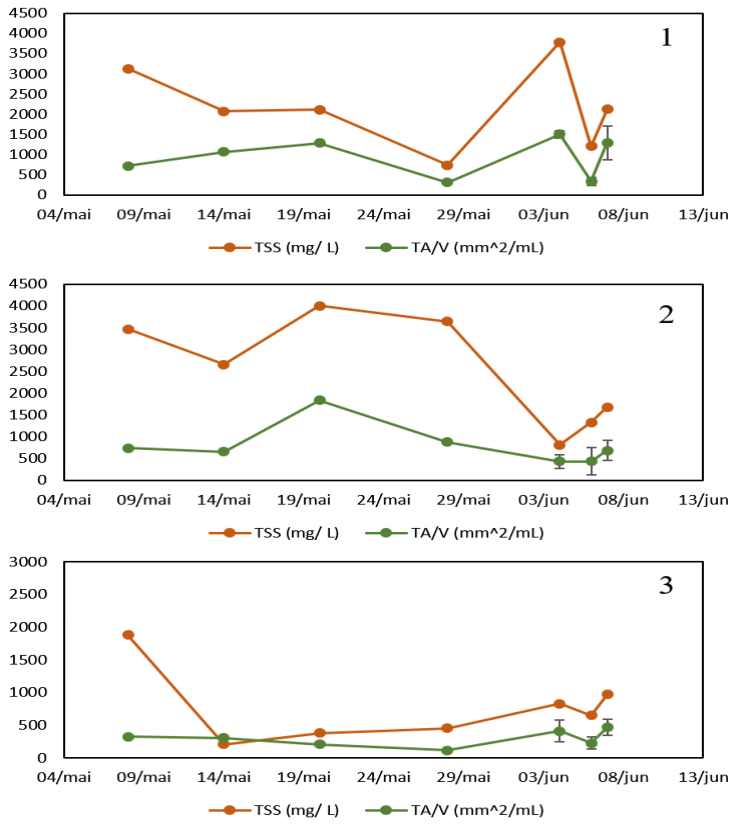

Figure 4.15: TSS (mm^2/ml) and TSS (mg/l) evolution through period A: 1-IFAS reactor; 2-AS reactor: 3-MBBR reactor.

As described in Section 3.3.1.2, the sludge was distributed into the reactors, hence they were initially practically with the same TSS and TA/Vol values, as it is possible to observe through the Figure 4.16. Through the time these parameters increase mainly in IFAS and then in AS reactor. The MBBR reactor, as mentioned, suffered a washout on the second day (6/12). As a consequence, the biofilm as well most of the flocs suffered a sharp decrease, causing lower TA/Vol values practically along the entire period, indicating that the reactor was never able to fully recovery.

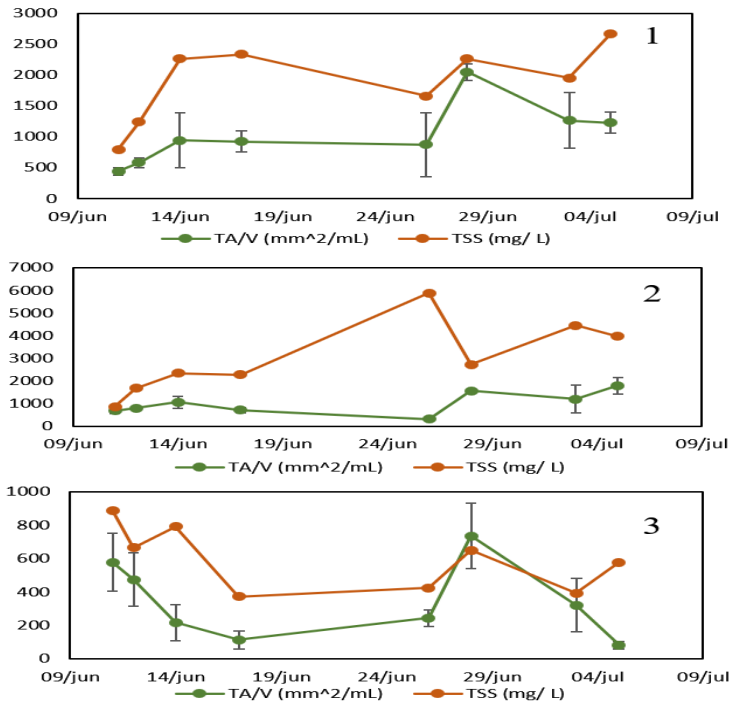

Figure 4.16: TSS (mm2/ml) and TSS (mg/l) evolution through period B: 1-IFAS reactor; 2-AS reactor: 3-MBBR reactor.

Sludge bulking is a problem that is associated with the deterioration of the sludge characteristics and is caused by the imbalance between the populations of microorganisms that form the floc.

The flocs can be represented depending on its diameter. For D_{eq}< 25 μm is Microfloc or pinpoint floc, 25 μm< D_{eq}< 250 μm is mesofloc or normal floc and finally for D_{eq} > 250 μm is macrofloc or zoogleal floc. The conditions for the formation of the Pinpoint floc, zoogleal floc or normal floc are described in Figure 2.4. Zoogleal bulking conditions were studied by Amaral and Leal (2013) and they concluded that when the perimeter and length of the mesofloc is higher than 610 μm and 120 μm, respectively, this phenomenon can occur. In the present work this phenomenon was not verified.

During the experiment, the percentage of micro, meso and macroflocs was considered and the Figure 4.17 and 4.18 represent the percentages of the reactor that are occupied in the three systems during period A and period B, respectively. Through the data provided in the Figure 4.17 it is concluded that mesoflocs are the predominant type of flocs, followed by the microflocs in both periods. Mesoflocs are associated with a good sludge sedimentation. In period A the average area percentages of mesoflocs were 46.0%, 55.1% and 49.2 % for

AS, IFAS and MBBR, respectively. In period B the concentration of meso and microflocs slightly decreased and in contrast the amount of macroflocs increased for IFAS and AS. The average area percentages of mesoflocs for AS, IFAS and MBBR were 44.9, 53.7 and 49.63 %.

Comparing the two periods it is noticed for AS and IFAS, that both micro and mesoflocs suffer a small decrease, while the macroflocs increased significantly. Microflcocs were the ones that had a bigger decrease which can be justified through a washout or aggregation phenomena.

The AS and MBBR reactor had problems related to washouts, and this is noticeable through the observations of the amount of microflocs; these types of flocs are associated with pinpoint phenomenon. The pinpoint phenomenon occurs when the floc has reduced dimensions, making it more fragile and eventually sheared due to the turbulence inside of the reactor. This type of floc tends to have a more spherical and compact form, they settle slowly creating a high amount of solids concentration in the clarifier supernatant, leading to the washout phenomenon (Jenkins *et al.*, 2004; Mesquita, 2010). According to the data, the pinpoint floc maybe happened in the AS and MBBR reactors since the D_{eq} for microflocs was lower than 25 μm during both periods. On the other hand, the zoogleal bulking did not occur during both periods.

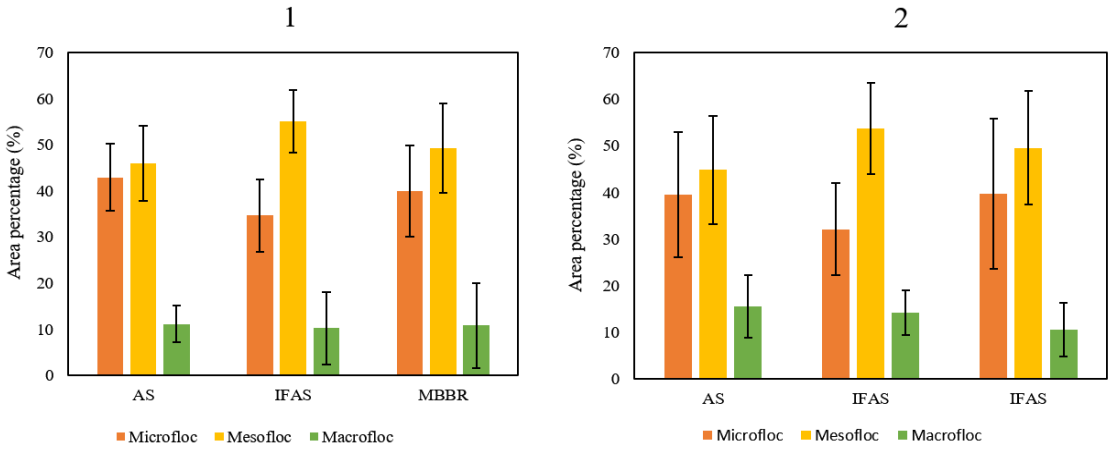

Figure 4.17: Area percentage occupied by microfloc, mesofloc and macrofloc: 1- During period A; 2- During period B.

For the present thesis, the morphological parameters analysed were solidity, that is the aggregate capacity to occupy the smallest possible place; eccentricity, is the aggregate elongation and convexity, is the aggregate edges roughness.

Figure 4.18 represents, the average values for the morphological parameters during period A.

In period A, according with the available data in Figure 4.18 the smaller flocs tend to be more elongated in the three reactors, since the eccentricity values are nearer to one than zero, suggesting a more elongated/elliptical shape, being more dense since the solidity values are closer to one and have smoother edges, since the convexity values are close to 1, making smaller aggregates less prone to erosion effects. Unlike small aggregates, larger ones are looser since they are less dense and have rougher edges, making the effects of erosion more propitious. It is possible to observe that the larger aggregates, the greater their eccentricity values, while smaller aggregates have lower values since they are represented with fewer pixies being subject to higher errors. In general, mesoflocs e microflocs are the predominant flocs.

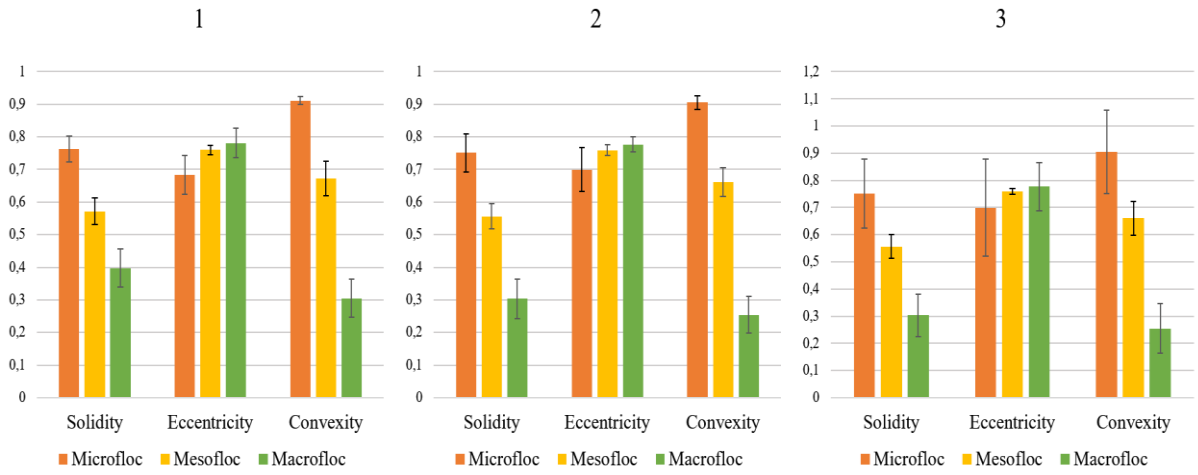

Figure 4.18: Morphological parameters analysed during period A: 1-AS; 2- IFAS: 3-MBBR.

As shown in Figure 4.19, period B also has a larger amount of smaller flocs, with a more elongate shape and presenting smooth edges for the AS and IFAS sludge. The macrofloc values oscillated a lot in all analysed parameters in the MBBR reactor, the larger aggregate is less dense and have rougher edges, that may enhance the effects of erosion and displacement.

Analysing both periods, it is possible to conclude that MBBR operated with the lowest solidity values which could mean that it operated with the less biomass density.

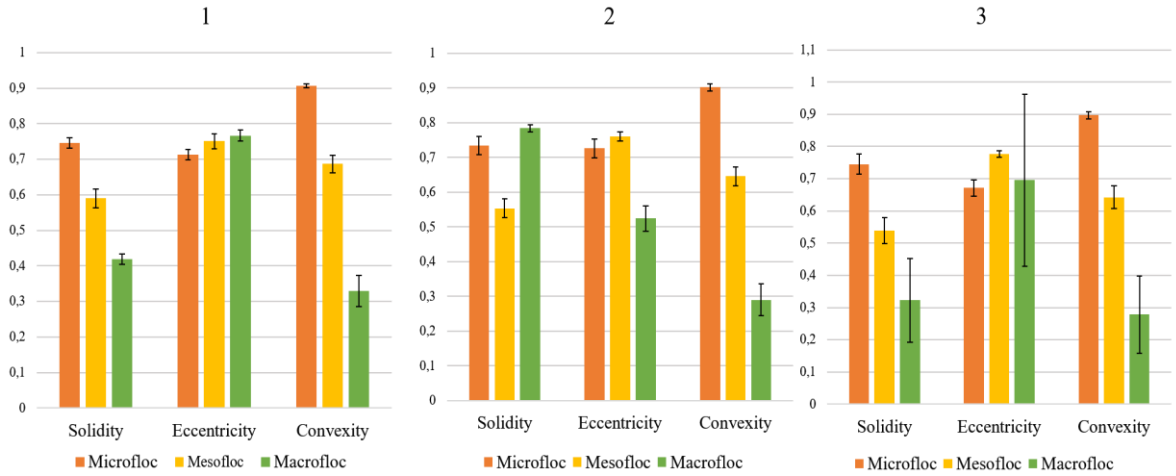

Figure 4.19: Morphological parameters analysed during period B: 1-AS; 2- IFAS: 3-MBBR.

The total length per volume (TL/ Vol) was also determined as the total length per total area (TL/TA). TL/ Vol is an indirect measure of the amount filamentous bacteria. Figure 4.20 presents the TA/Vol (mm/ml) values for each reactor in both periods. Period A generally shows more oscillations, which are caused by mechanical problems that were detected through this period. The averages values in period A for the TL/ Vol were 4934.789, 1967.774 and 1247.653 mm/mL for AS, IFAS and MBBR. Period B compared with period A has fewer amounts of filamentous bacteria, except for AS, since the averages numbers were 3084.268, 2288.821 and 2904.306 mm/mL. In general, the AS system operated with the higher concentration of filamentous bacteria in both periods.

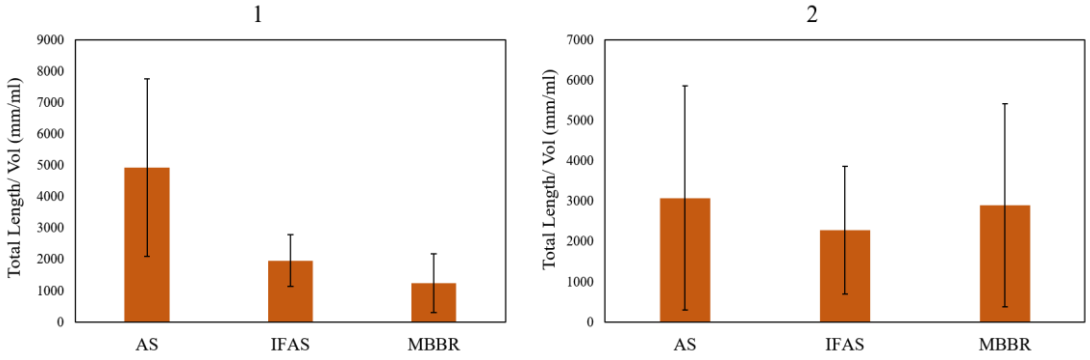

Figure 4.20: Total length per Volume for each reactors: 1-During period A; 2- During period B.

Through Figure 4.21 it is possible to conclude that the relation between the total length of filaments and the total area of floc had different behaviours in the two periods. In period A the AS show the highest values 5.785 mm / mm^2 what was not verified in period B, which had an average value of 2.549 mm/ mm^2, which is related to the fact that the area of the

flocs increased in that period, compared to the length of the filamentous. IFAS maintained the averages values that were 2.180 and 2.283 mm / mm^2 for period A and B respectively. In period B the MBBR increased compared to period A, the averages values were 4.562 (Period A) and 6.401 mm / mm^2.

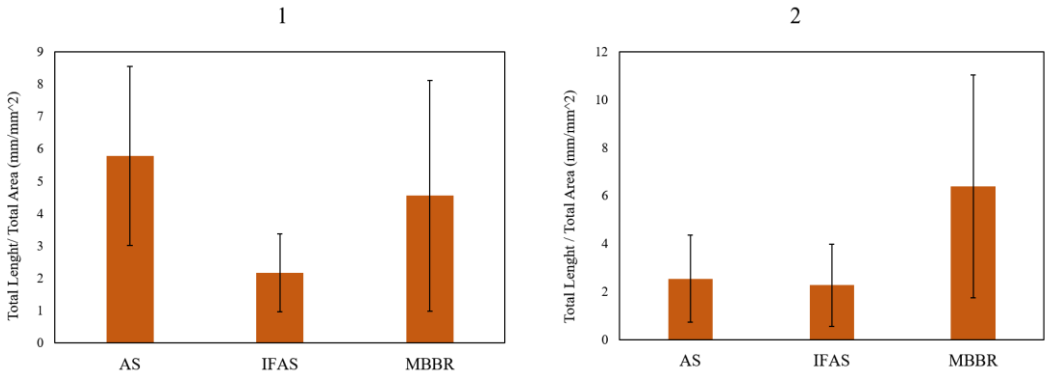

Figure 4.21: Total length per total area for each reactor: 1- During period A; 2- During period B.

During the course of the experiment is possible to conclude that there was no filamentous bulking, since according to Leal *et al*., (2020) the threshold values for filamentous bulking are TL/V > 20000 mm / mL and TL/TA > 15 mm /mm^2 and values obtained were much lower.

4.6.1. Microbial Consortium

The identification of the main protozoa and metazoa was carried out in both periods a total of seven times per reactor in each period.

During the period A, it was possible to observe in the IFAS, AS and MBBR reactors a predominance of protozoa, mainly ciliates, as represented in Table 4.7, but there was also a strong presence of metazoa mainly in AS and IFAS, which was lower compared to AS reactor. These microorganisms are associated with a high age of the sludge, good quality of the final effluent, aeration and nitrification and they are found when the organic load is low.

In MBBR, the *tardigrate* was seen twice on different days, which indicates a high age of the sludge.

In general, the microorganism found in period A indicate an advanced sludge age, an effluent with reasonable to good quality, good aeration and high nitrification.

Table 4.7: Main microorganism observed in the three reactors during period A.

Microorganism	Group	Reactor		
		IFAS	**AS**	**MBBR**
Protozoa	Flagellates	*Peranema*	-	-
	Sacordina	*Arcella*	*Arcella*	-
	Ciliates	*Colpidium*	*Colpidium*	*Colpidium*
		Vorticella aquadulcis	*Vorticella aquadulcis*	*Vorticella aquadulcis*
		Zoothamnium	*Zoothamnium*	*Zoothamnium*
		Vorticella microstoma	*Vorticella microstoma*	*Vorticella microstoma*
		Vorticella convallaria	*Aspidisca cicada*	*Vorticella convallaria*
Metazoa	Rotifers	*Monogononta*	*Monogononta*	*Digononta*
		Digononta	*Digononta*	
	Nematodes	*Nematoda*	*Nematoda*	-
	Annelids	*Aelosoma*	*Aelosoma*	Tardigrate

During period B, there was an increase in the number of ciliates seen mainly in IFAS and AS, as can be seen in Table 4.8. The MBBR during this period suffered a washout phenomenon as mentioned before, thereby decreasing the amount of microorganisms present, and those that were possible to view in the beginning of the period; most of them are associated with a transient phenomenon and washout conditions.

It can be concluded that period B had higher biodiversity of microorganism for AS and IFAS reactors and that the final effluent had better quality.

Figures 0.2 and 0.3, show some of the microorganism found in period A and B, respectively.

Table 4.8: Main microorganism observed in the three reactors during period B.

Microorganism	Group	Reactor		
		IFAS	**AS**	**MBBR**
Protozoa	Flagellates	*Peranema*	*Peranema*	*Peranema*
	Sacordina	*Arcella*	*Arcella*	*Arcella*
	Ciliates	*Colpidium*	*Colpidium*	*Litonotus*
		Vorticella aquadulcis	*Vorticella aquadulcis*	*Vorticella aquadulcis*
		Zoothamnium	*Zoothamnium*	*Zoothamnium*
		Vorticella microstoma	*Vorticella microstoma*	*Vorticella microstoma*

		Vorticella convallaria	*Aspidisca cicada*	*Aspidisca cicada*
		Aspidisca cicada	*Litonotus*	*Depranomonas*
		Euplotes	*Epistylis*	
Metazoa	Rotifers	*Digononta*	*Monogononta*	*Digononta*
	Nematodes	*Nematoda*	*Nematoda*	*Nematoda*
	Annelids	-	-	*Aelosoma*

5. Conclusions and Future Work

5.1. Conclusions

This thesis aimed to compare three biological reactors, activated sludge, Activated Sludge (AS), Integrated Fixed-Film Activated Sludge (IFAS) and Moving Bed Biofilm Reactor (MBBR), in terms of treatment, biomass and microbial characterization through two different periods, namely period A and period B. The IFAS and MBBR systems had a filling fraction of 44.2% in both periods.

Period A lasted 37 days and operated with an average COD of 379 mgO_2 / L. The period was characterized by relatively low COD removal efficiencies, 63%, 73% and 71% for AS, IFAS and MBBR, respectively. The low efficiencies are related to the fact that a larger part of period A was conditioned due to mechanical problems in the equipment.

Period B lasted 25 days and operated with an average CODs of 1019 mg_{O2} / L, CODs removal efficiency was 82%, 84% and 79% for AS, IFAS and MBBR. The removal efficiencies compared to period A increased by 21%, 11% and 8%, respectively. The increase in the case of AS and IFAS may have been the proper function of the equipment or the increase in the organic load, which may have provided more food to the microorganisms favouring the treatment. The small rise in MBBR removal efficiency can be associated with the washout phenomena, that occurred on the second day where much of the sludge was lost, dragging the majority of the microorganism and biofilm that never fully recovered or the increase in the organic load coupled with air distribution problems that caused eutrophication.

In both periods IFAS was the most efficient reactor and it can also be concluded that it was the one that most easily adapt to variations in the organic load, compared to AS and MBBR.

The TN removal efficiency was also evaluated, with an average TNs of 39 mg / L, and it can be concluded that period B have higher removal efficiencies for the AS and IFAS, 50% and 64% compared to period A which had only 20 % and 54 % respectively. This increase

in period B can also be correlated with the microbial consortium, since it was possible to visualize that during this period there was an increase in protozoa ciliate which is indicator of good nitrification conditions. The TN efficiency as decreased in the MBBR reactor that might be explained by the fact that in the MBBR there wasn´t any period of lack of aeration like in the other two reactors, reducing, this way, the denitrification step of the biological treatment.

Sludge production was only measured in period B, since period A was too unstable to have a correct quantification of the sludge production. MBBR was the system that produces less sludge, 0.162 g / d, followed by IFAS with 0.985 g / d and finally AS, which had produced the higher amount of sludge, 1.550 g/d. The sludge that was produced by MBBR could be tampered, because when the washout occurred, a large amount of sludge was removed from the reactor; part of the sludge was not possible to recover thus reducing the amount of sludge in the reactor.

The deposition of sludge accounts for most of the operation costs, so from an economical point of view, MBBR and IFAS will be more economic, since they produce less sludge.

For the characterization of the biomass present in the mixed liquor, the bacteria floc aggregates and filamentous bacterial were analysed for each reactor in both periods, to find out if there were bulking problems (filamentous, zoogleal and pinpoint) during the experiment. The area occupied by flocs was greater in period B, 1037.273, 1006.242 and 344.293 mm^2/ mL, than in period A, 932.887, 809.552 and 292.808 mm^2/ mL for AS, IFAS and MBBR, respectively. The influence of TSS in the area occupied by the flocs was also analysed, and it was concluded that whenever there was a decrease in TSS values, there would also be a decrease in the area occupied by the flocs. As expected, the MBBR reactor had the smallest area occupied by the flocs, since the values of TSS are the lowest of the three reactors.

It was verified over both periods, that mesoflocs predominated, followed by micrflocs and finally macroflocs. In period A, IFAS had the higher percentage of mesoflocs, 55.1%, followed by MBBR with 49.2% and finally the AS with 46.0%. In period B, IFAS has again the predominant percentage of mesoflocs with 53.7%, followed by MBBR, 49.6% and in last the AS with 44.9%, indicating a better sludge settling in period A. During the course of the experiment there was no zoogleal bulking, but the pinpoint floc may have

occurred in MBBR, since it obtained D_{eq} < 25μm and in period B the washout can be link with pinpoint floc.

Both in period A and B there were no filamentous bulking, since the averages values obtained were well below the representative filamentous bulking values. The data for the total length for period A were 4934.8, 1967.8 and 1247.7 mm/ mL and for period B was 3084.3, 228.8 and 2904.3 mm/ mL for AS, IFAS and MBBR respectively. The averages values for the TL/TA for period A were 5.8, 2.2 and 4.6 mm/ mm^2 and for period B were 2.5, 2.3 and 6.4 mm/ mm^2.

Through the analysis of the morphological parameters in both periods and for the three reactors, it was possible to conclude that the flocs with reduced diameters have a less elongate structure, more dense and have smoother edges, while in larger flocs they have a more elongate shape, less dense and have rougher edges.

The ciliates predominated throughout the experiment but mainly in period B, they are associated with a good quality of the final effluent, good aeration conditions and favourable nitrification conditions. In period A, a greater amount of metazoa was visualized in the three system having tended to decrease over the rest of the experiment. The predominant protozoa found belonged to the ciliate group and they were *Colpidium sp, Zoothamnium sp, Vorticella micróstoma sp, Aspidisca cicada* and *Vorticella convallaria sp*. In terms of metazoa the predominant group was the rotifers in which the *Digononta sp* and *Monogononta sp* were observed. At the beginning of the period A, a large number of *Aelosoma* in the AS reactor, indicated a high aging sludge.

5.2. Future work

As virtually happens in all research works, there are always questions / approaches that remain unanswered or ascertained in order to know the full capabilities of the MBBR and IFAS systems. In this regard, some suggestions for future work are described below.

- Use different types of carriers in order to evaluate the attachment conditions, more specifically the Z-MBB R or Z-carrier, since this type of carrier is new, and it is already shown great results in treatment since the biofilm growth occurred outside of the carrier;
- Experimenting with a real effluent from dairy wastewaters for more realistic results;

- Using different hydraulic retention times in continuous mode for the three systems (AS, IFAS and MBBR) to evaluate the behaviour of the systems;
- More detailed study of biofilm development, using different inoculation times, where the microbiology and biofilm thickness are assessed;
- Compare different biological treatment to IFAS and/or MBBR systems, such as microalgae treatment in order to determinate the best method for nitrogenous and phosphorous removal;
- Using the same two systems, IFAS and MBBR, but with different filling fraction, FF, and determinate the most ideal FF, for this experiment;
- Extrapolate the same models to different configurations, such as nitrification and/or denitrification;

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ANNEX A

Table 0.1: Microorganism classification base on donor electron, receiving electron, source of cell carbon and end products. Adapted from Metcalf and Eddy *et al.*, (2014)

Type of bacteria	Reaction name	Carbon source	Electron donor	Electron acceptor	Products
Aerobic heterotrophic	Aerobic oxidation	Organic compound	Organic compound	O_2	CO_2, H_2O
Aerobic autotrophic	Nitrification	CO_2	NH_4^+, NO_2^-	O_2	NO_2^-, NO_3^-
	Iron oxidation	CO_2	FE (II)	O_2	Ferric Iron FE(III)
	Sulphur oxidation	CO_2	H_2S, S^0, $S_2O_3^{2-}$	O_2	SO_4^{2-}
Facultative heterotrophic	Denitrification	Organic compound	Organic compound	NO_2^-, NO_3^-	N_2, CO_2, H_2O

Table 0.2: Characterization of a dairy wastewater (Mehrdadi *et al.*, 2012).

Parameters	Concentration (Average)
Non-Filtered TKN (mg/L)	98.000
Nitrate (mg/L)	1772.000
NH_3 (mg/L)	46.500
Non-Filtrated Phosphate (mg/L)	40.200
COD (mg/L)	1958.000
BOD (mg/L)	836.000
TSS (mg/L)	608.000
pH	7.100
Temperature (ºC)	17.000

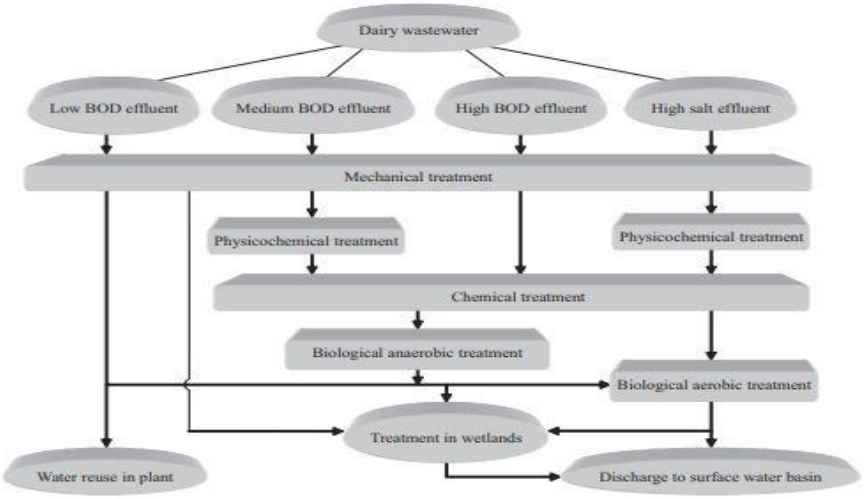

Figure 0.1: Dairy wastewater treatment options depending on the organic load present (Torresi et al., 2016).

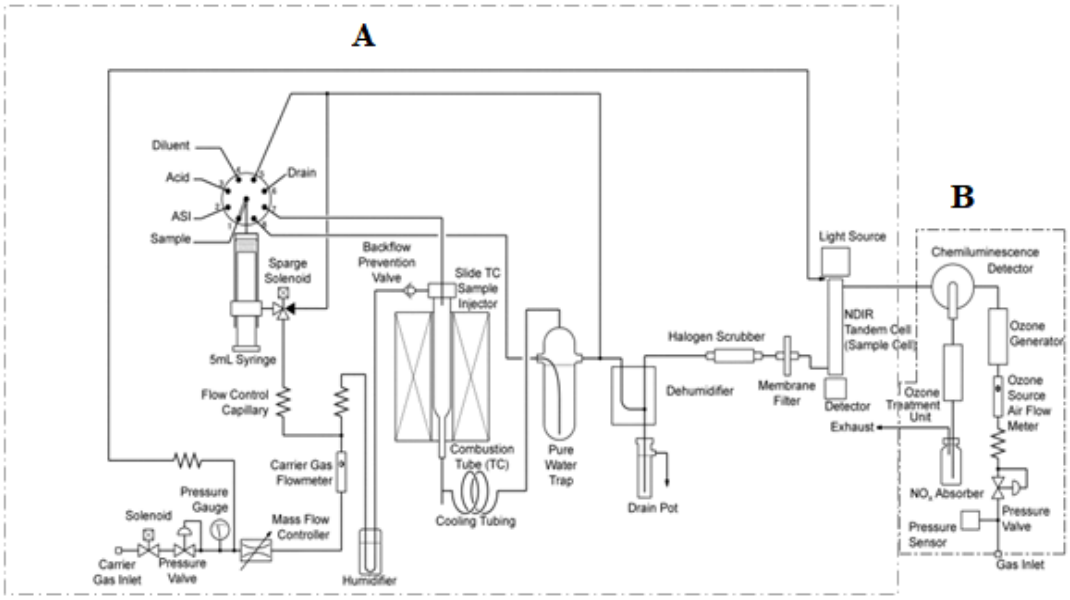

Figure 0.2: Flow diagram of a TOC-Vcph+TNM-1. A) Represents the TOC-Vcph flow and B) Represents the TNM-1 flow (Adapt from SHIMADZU user's manual).

APPENDIX A

1 2

Figure 0.1: Problems observed in AS in period B: 1- Reddish sludge spots observed in the AS system in period B; 2- Reddish spots appearing in the bottom of the treated effluent tank.

Table 0.1: Average values of the pH and temperature for each reactor in period A.

Reactor	pH	Temperature (ºC)
AS	6.9	22.7
IFAS	6.6	22.5
MBBR	6.7	22.3

Table 0.2: Average values of the pH and temperature for each reactor in period B.

Reactor	pH	Temperature (ºC)
AS	7.2	23.7
IFAS	7.1	23.4
MBBR	7.3	23.5

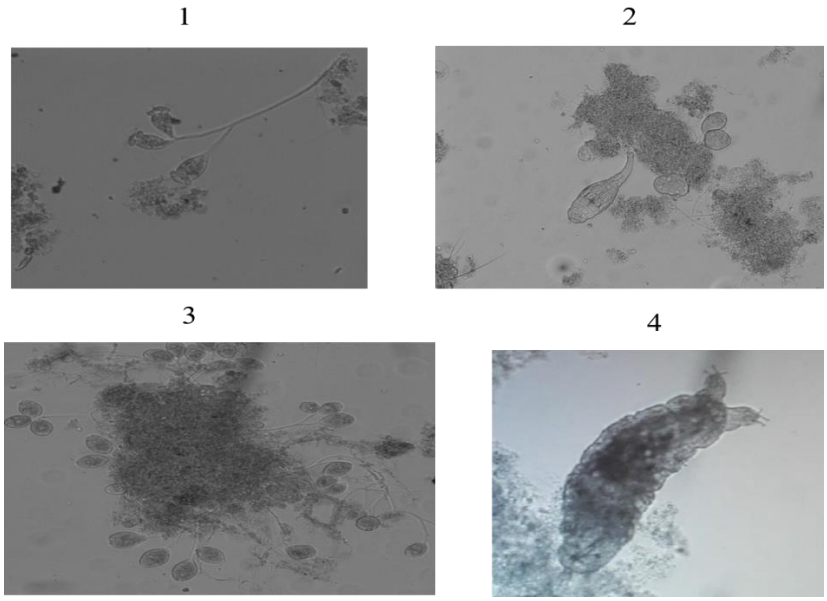

Figure 0.2: Examples of the protozoa and metazoan found during period A: 1- *Vorticella convalária* found in IFAS; 2- *Digononta* and *Vorticella microstoma* found in AS; 3- *Zoothamnium* found in AS; 4- *Tardigrate* found in MBBR.

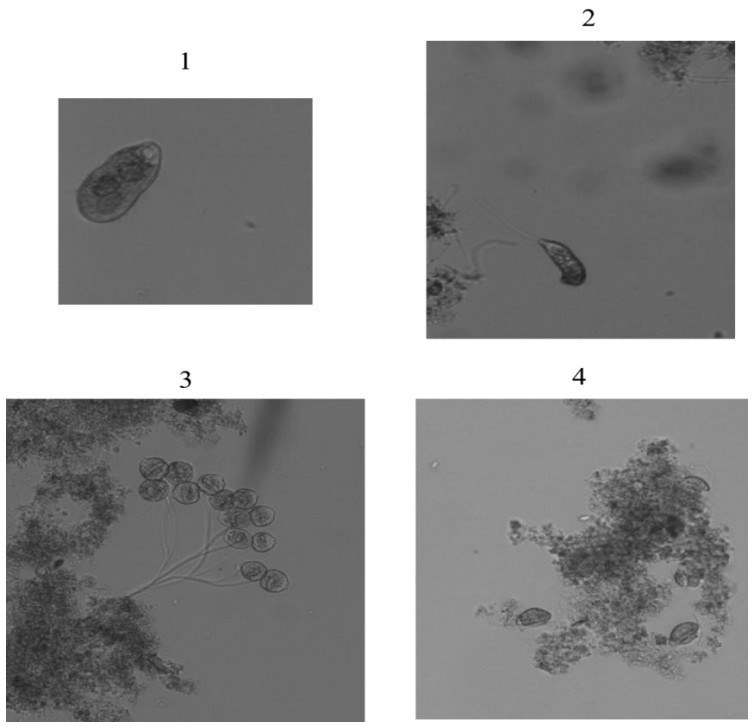

Figure 0.3: Examples of the protozoa and metazoan found during period B: 1- *Depranomonas* found in MBBR; 2- *Peranema* foung in IFAS; 3- *Zoothamnium* found in AS; 4- *Aspidisca cicada* found in IFAS.