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Biological minimization of excess sludge in a membrane bioreactor: Effect of plant configuration on sludge production, nutrient removal efficiency and membrane fouling tendency

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Contents

Chapter 1 .... **1**

Introduction *1*

Chapter 2 .... **4**

The excess sludge issue in wastewater biological treatment *4*
2.1 The sludge costs 7
2.2 Normative 11
2.3 Sludge treatment 12
2.4 Final destination of sludge 13

Chapter 3 .... **14**

The excess sludge: from its origin to final destination *14*
3.1 Bacterial growth 14
3.1.1 Requirements for microbial growth 16
3.1.1.1 Electron acceptors 17
3.1.1.2 Temperature 18
3.1.1.3 Nutrients 19
3.1.1.4 pH 20
3.2 Excess sludge production and its main parameters 20
3.2.1 Sludge age 21
3.2.2 Organic load 22
3.2.3 Hydraulic retention time (HRT) 22
3.2.4 Food to microorganism (F/M ratio) 23
3.2.5 Sludge settleability 24
3.2.6 The oxygen demand 25

Chapter 4 .... **26**

Main techniques for excess sludge reduction *26*
4.1 Cryptic growth / Cellular lysis technologies 26
4.1.1 Enzymatic hydrolysis 28
4.1.2 Mechanical disintegration 28
4.1.3 Ultrasound treatment 30
4.1.4 Thermal treatment 30
4.1.5 Chemical and thermo-chemical hydrolysis 32
4.1.6 Chemical oxidation 33
4.1.7 Electrical treatment 34
4.2 Uncoupled metabolism 35
4.2.1 Addition of chemical metabolic uncouplers 38

4.2.2 Side-stream reactors 39
4.2.3 OSA process 40
4.2.4 Microbial community in AS systems with side-stream reactors 43
4.3 Endogenous metabolism 45
4.3.1 MBR 46
4.4 Predation on bacteria 48
4.4.1 Two-stage predation system 48
4.4.2 Oligochaeta 49

Chapter 5 50

The side-stream reactor combined to MBRs 50
5.1 Probable mechanisms of excess sludge reduction 50
5.1.1 Energetic uncoupling 50
5.1.2 Endogenous metabolism / decay 51
5.1.3 Extracellular Polymeric Substances (EPS) destruction 52
5.2 Main process parameters 52
5.2.1 Effect of the Solid Retention Time 52
5.2.2 Effect of the Redox Potential 55
5.2.3 Effect of temperature 56
5.2.4 Effect of HRT 57
5.3 Characteristics of combined process with SSR and MBR 57

Chapter 6 59

Experimental application of MBR Process integrated to an SSR: Materials and methods 59
6.1 Materials and Methods – Phase 1 60
6.1.1 Pilot plant configuration 60
6.1.2 Experimental campaign 62
6.2 Materials and Methods – Phase 2 64
6.2.1 Pilot plant configuration 64
6.2.2 Experimental campaign 65
6.3 Analytical methods 66
6.3.1 Evaluation of biomass growth and heterotrophic kinetic parameters. 67
6.3.2 Activated sludge characterization 68
6.3.3 Membrane fouling analysis 69
6.3.4 SST and SSV analysis 70
6.3.5 COD analysis 71
6.3.6 Nitrogen compounds (NH_4-N, NO_3-N) 71
6.3.7 BOD analysis 71
6.3.8 Hydrophobicity analysis 72
6.3.9 Granulometry and viscosity 72

Chapter 7 73

7.1 Results analysis (Phase 1): Experimental application of a side-stream/main-stream reactor with MBR 73

7.1.1 Heterotrophic biomass growth and waste sludge production 73

7.1.2 Nutrients removal performance 75

7.1.3 Biomass kinetic behavior 79

7.1.4 Activated sludge physical characteristics: flocs size, EPS content and composition 80

7.1.5 Membrane fouling analysis 83

7.1.6 Impact of reactor configuration on sludge minimization 87

7.2 Results analysis (Phase 2) – Integration of an AMSR for sludge reduction and biological phosphorus removal: Effect of HRT 91

7.2.1 Biomass growth and excess sludge production 91

7.2.2 Nutrient removal performances 96

7.2.3 Behavior of OHO (Ordinary Heterotrophic Organisms) and PAOs kinetics 104

7.2.4 EPS content and composition 110

7.2.5 Membrane fouling analysis 112

7.2.6 Mechanism of sludge minimization in the AMSR and implications on the system performances 117

Chapter 8 120

Conclusions *120*

References 122

Chapter 1

Introduction

The achievement of high performances in biological nutrient removal (BNR) processes, while saving energy, footprint and minimizing the excess sludge production, is the basis of the most modern biological technologies for wastewater treatment (Ioannou-Ttofa et al., 2016; Moreira et al., 2015; Semblante et al., 2016a).

In the last two decades, several innovative biotechnologies have been developed with this aim, *inter alia* the membrane bioreactor (MBR), the moving bed biofilm reactor (MBBR), the aerobic granular sludge (AGS, *alias* Nereda®), etc. (Pronk et al., 2015; Safwat, 2018; Sarioglu et al., 2017). In these systems, significant improvements in nutrients removal performance could be easily achieved, mainly due to the increase in the biomass retention ability that enable to operate with higher total suspended solids (TSS) concentration, which in turns enhanced the plants' loading capacity. Moreover, low levels of waste-sludge production could be achieved, because of the higher sludge retention time (SRT) applied and the lower sludge yields due to the selection and proliferation of slow growing microorganisms (Devlin et al., 2016; Troiani et al., 2011).

Nevertheless, similar results could be achieved by retrofitting already existing systems based on conventional biological technologies, i.e., conventional activated sludge (CAS) systems. For instance, the oxic-settling-anaerobic (OSA) process, involving the modification of a CAS plant by placing a sludge retention reactor (SRR) in the return activated sludge (RAS) loop, was suggested as one of the most potentially cost-effective and low impact solution to achieve excess sludge minimization and improvements in nutrient removal efficiencies (Foladori et al., 2010; Sun et al., 2010).

The excess sludge reduction is driven by several biological mechanisms acting separately or simultaneously, like the biological maintenance metabolism, the uncoupling metabolism, the EPS destruction, the bacteria predation, etc. (Wang et al., 2013). Moreover, the sludge cycling between feasting and fasting conditions, as well as anaerobic and aerobic environments, was found to be favorable to the development of polyphosphate accumulating organisms (PAO) and in acceleration of biomass kinetics (Datta et al., 2009; Goel and Noguera, 2006).

The excess sludge minimization in MBR systems was thoroughly studied in the anaerobic side-stream reactor (ASSR) configuration, consisting in the placement of an anaerobic reactor in the RAS line of an MBR (Kim et al., 2012; Semblante et al., 2016a, 2014). In respect of a significant improvement in sludge minimization, the membrane fouling resulted deteriorated because of the increase in the soluble microbial products (SMP) concentration in the liquid bulk (Wang et al., 2013).

Recently, a novel layout for MBR system was proposed with the aim to achieve excess sludge minimization in an anaerobic mainstream reactor (AMSR) configuration, while preserving the membrane permeability (de Oliveira et al., 2018). More precisely, a modification of a conventional pre-denitrification scheme was proposed, consisting in the placement of an anaerobic reactor in the mainstream between the anoxic and the aerobic reactor. In this system, a portion of activated sludge from the anoxic reactor, with a rate approximately equal to the influent flow, before of going to the aerobic reactor passed through an anaerobic reactor, where strictly anaerobic conditions were imposed. In this reactor, because of the lack in substrate availability and the anaerobic starvation, uncoupling metabolism occurred, thereby favoring the achievement of low biomass yield. Thus, it was possible to compare the ASSR configuration with the AMSR and demonstrated that, although the waste sludge reduction was higher in the ASSR (72%), approximately 30% of excess sludge minimization could be achieved in the AMSR configuration, with 6 hours of hydraulic retention time (HRT) in the anaerobic reactor. Nonetheless, was possible to speculate that an increase in the HRT could be beneficial to enable higher levels of sludge reduction. Moreover, in the AMSR configuration a significant increase in nutrients removal performances was obtained, also suggesting the possibility to achieve biological phosphorous removal. In conventional BNR systems, where the anaerobic tank is placed as the more upstream reactor, PAO microorganisms can use low fatty acids that are present in the sewage, releasing phosphate to the surrounding liquid (Zuthi et al., 2013). In contrast, in the AMSR configuration the anaerobic tank is placed downward the anoxic reactor, in which the rapidly biodegradable carbon was already depleted for denitrification (de Oliveira et al., 2018). Therefore, phosphorous release by PAO microorganisms would occur under endogenous conditions because of the low availability of residual carbon source. In this respect, the mechanism of phosphorous release and kinetics in the AMSR configuration should be better investigated.

Lastly, design parameters aimed at assessing the proper operating criteria that maximize the simultaneous effect of sludge minimization and improvement of nutrient removal performances, without compromising the effluent quality, are necessary.

In this light, this study was aimed at evaluating the effects of different configurations and HRTs in the anaerobic reactor on the simultaneous achievement of sludge minimization and biological phosphorous removal in an AMSR-MBR plant. Moreover, insights into the ordinary heterotrophic organisms (OHO) and PAO kinetics, as well as the membrane-fouling tendency were provided.

Chapter 2

The excess sludge issue in wastewater biological treatment

Biological treatment has been widely employed to treat wastewater worldwide, due to its high efficiency and viable costs, when compared to conventional activated sludge (CAS) plants. According to Wang et al. (2015), the major role in biological wastewater treatment is played by activated sludge processes, performed through the conversion of nutrient pollutants and biodegradable organic into bio-solids and gaseous, while the unbiodegradable matter ends in excess sludge. However, activated sludge plants have, among other disadvantages, the high costs with sludge management that represents an important fraction of the entire costs of wastewater treatment plants (WWTPs) management. This technique uses the microorganisms to degrade the organic matter, using it for its metabolism and transforming the organic matter into inert material and new bacterial cells.

The bacteria are, between all the microorganisms present in wastewater, the main protagonists in the wastewater treatment process, absorbing for the cellular membrane the dissolved nutritive substances. Besides the bacteria's importance in the biological treatment, the protozoa have also an important role that consists in reducing the bacteria concentration and organic matter, producing a clear high-quality effluent. The presence of many protozoa in a biological plant means that the plant has a good depurating efficiency (Masotti, 2011).

The sludge (or waste activated sludge – WAS) can be classified in primary or secondary sludge (Metcalf and Eddy, 2003). Primary sludge comes from the primary settler and is composed of settleable solids removed from the raw wastewater. WAS is composed by bacterial products (extracellular polymeric substances - EPS), inorganics from wastewater, recalcitrant organics formed during bacterial decay or originating from wastewater and finally the bacteria that grows on inorganic and organic substances (Wang et al., 2017).

The excess sludge production is a phenomenon that happens due to the decay and growth of heterotrophic biomass, accumulation of inert solids entering the wastewater and accumulation of endogenous residue (Foladori et al., 2010). Many methods have been employed to control the excess sludge production, *e.g.* the manipulation of parameters as sludge retention time (SRT) and dissolved oxygen (DO) in the aerobic reactor or even the addition of chemicals to reduce the biomass growth (Semblante et al., 2016b). The known methods have either positive or negative characteristics resulting in the need of research on the most efficient technologies to reduce the excess sludge production.

The costs of excess sludge treatment accounts for 40-60% of the total costs of an activated sludge treatment plant, which represents an important economic fraction of a CAS plant. The average annual production of excess sludge is 240 million wet tons in Europe, China and USA combined, as well as 3 million wet tons in Australia (Pritchard et al., 2010).

The main methods for sludge disposal are landfill, agricultural use and incineration (that costs e.g. $30-100 per wet ton in Europe and $30-70 per wet ton in Australia) (Batstone et al., 2011). This amount, when related to the dry solids, can reach approximately 6-11 million tons of dry sludge per year in Europe Union (EU), USA and China; and approximately 0,3 million tons of dry sludge in Australia each year (Fytili and Zabaniotou, 2008; Semblante et al., 2014; Yang et al., 2015).

Innovative strategies for reducing excess sludge production have been researched, as the use of advanced oxidation processes (AOPs) that aims to destroy biomass (Wang et al., 2015); chemical addition to disrupt the metabolic processes (Fang et al., 2015; Feng et al., 2014); the sludge cycling in alternating redox conditions (Oxic Settling Anaerobic - OSA process) (Semblante et al., 2014). Most of these alternatives are expensive (Foladori et al., 2010) and/or introduce in the water undesired products (Mahmood and Elliott, 2006).

The side-stream reactor (SRR), also known as sludge holding tank (SHT), can be a potentially cost-efficient and low impact alternative that consists in the insertion of an anaerobic reactor that modifies the conventional activated sludge (CAS) plant by placing an anaerobic (or anoxic) reactor in the return activated sludge (RAS) loop.

The insertion of an SRR is a promising alternative that allows the sludge to be partially biodegraded, before it is returned to the aeration tank, due to low DO and substrate concentration. The interchange of sludge between these tanks with different conditions: rich (the aeration tank) and deficient (the external anoxic reactor/s) in oxygen and substrate results in effective excess sludge minimization (Semblante et al., 2016b). The addition of an SRR also reduces the sludge production when the sludge passes through a substrate-deficient reactor (anaerobic environment) and then recirculates to the main bioreactor, in the same mechanisms of the OSA process (Semblante et al., 2016a). The environmental changes that the microorganisms are exposed cause a stressful situation that obligates the bacteria to use the energy in form of ATP for its vital maintenance instead of producing new biomass.

Many mechanisms have been hypothesized to explain the reactions in the SRR that may reduce the sludge production. They include the enhanced cell lysis, selection of slow-growing bacteria and extracellular polymeric substance degradation (Navaratna et al., 2014; Semblante et al., 2014). The extracellular polymeric substances are necessary to the floc formation, but when founded in high quantity inside the activated sludge (AS) plant, can cause bulking and other functional problems. Furthermore, in the literature some other hypotheses propose that the possible mechanisms of sludge reduction in the SRR reactor are uncoupling metabolism (Troiani et al., 2011) or cell-lysis cryptic growth (Wei et al., 2003), but these processes have not been clearly demonstrated to date (Foladori et al., 2015).

In this context, the hydraulic retention time can be also an important parameter to be evaluated, considering the importance of a balance between the best conditions to perform nitrogen removal and the achievement of satisfactory values of Y_{obs} (bacterial growth field - observed).

Sun et al. (2010) achieved a sludge reduction from 53% to 77% increasing the frequency of return from once a day to four times a day while maintaining the sludge interchange rate (SIR) between a sequence batch reactor (SBR) and an external anaerobic reactor at 10%. On the other hand, Saby et al. (2003) observed the impact of SRT in the external anoxic reactor of OSA over a range of 11-17 days and observed a reduction of 23-58% under smaller SIRs and longer SRTs.

2.1 The sludge costs

Reducing the sludge production is important for many reasons, first of all, the excess sludge production is related to high costs in a WWTP. These costs may vary depending on the plant's location, but the treatment and disposal of excess sludge are quite expensive, and frequently costs about 30 - 60% of the total operation cost in a conventional activated sludge treatment plant (Saby et al., 2003; Wei et al., 2003). The sludge disposal can be performed in several ways and each alternative represents a specific cost. Actually, in Europe, the most chosen option is the disposal for agriculture and landfill (Andreottola G., Foladori P., 2008; Frost & Sullivan, 2003; Ginestet, 2006), the costs for wet weight comprising all the alternatives are shown in Table 1.

Table 1 – Disposal costs of wet sludge in Europe (Andreottola G., Foladori P., 2008; Frost & Sullivan, 2003; Ginestet, 2006).

Alternative	*Percentage of option chosen*	*Cost of the wet weight (€/t wet weight)*	
		Average price	*Min – max*
Discharge	21.0%	71.7	35-120
Agriculture	47.0%	27.8	0-50
Composting	6.5%	41.2	35-70
Incineration	19.9%	74.5	38-98
Others	5.6%	26.0	15-50
Transportation	100%	16.3	0-50
Average price (including transportation)	-	63.4	0-120

The costs shown in Table 1 represent the prices in the European sector and can be converted for unit or dry weight considering three percentage values of dry in sludge: 10%, 20% and 30%, as shown in Table 2.

Table 2 – Disposal costs of dry sludge in Europe (Andreottola G., Foladori P., 2008; Frost & Sullivan, 2003; Ginestet, 2006).

Alternative	*Sludge cost by unit of dry weight (€/t wet weight)*		
Dry percentage values ►	*10%*	*20%*	*30%*
Discharge	717	359	239
Agriculture	278	139	93
Composting	412	206	137
Incineration	745	373	248
Others	260	130	87
Transportation	163	82	54
Average price (including transportation)	634	317	211

One of the drawbacks of biological treatment is the high excess sludge production that represents at least 380-750 € in Europe (Ginestet, 2006). Thus, Italy is one of the nations in Europe where predominates as first option the discharge (on landfill) corresponding to 40% of the total amount; though another interesting data is the cost of sludge disposal in agriculture in Lombardy, that reaches values about 50 – 70 euro/ton (Canziani, 2016). However, is important to have more than one alternative to perform the sludge disposal, considering the possibility of emergency situations that could make unfeasible some of the main methods.

The Energy and Environment Laboratory Piacenza - Italy (LEAP) has estimated that the incineration costs are about 80-90 euro/ton (including depreciation of the plant and transportation) for plants with potential of 20-25000 ton/year of 25% wet sludge, making the incineration an additional option, also for the recovery of phosphate; from the ashes is possible to extract phosphates (not volatile). In Swiss it obligatory the phosphorus recovery and the ashes can be used to produce ceramic material (Canziani, 2016).

Troiani et al. (2011) demonstrated that in Europe, due to the stringent environmental regulations, the production of excess sludge represents a problem. The Directive 2008/98/EC asserts the management of the waste preventing, performing preparation for reuse, recycling, reusing and disposing, besides both quality and quantity were more strictly regulated (Rodriguez-Rodriguez et al., 2011).

The Directive 99/31/EC imposed the reduction of sludge disposal, which is reaching until 2020 a total amount of waste equal to 35% of the quantity disposed of in 1995. All these events led an increase of sludge disposal that in Italy is between 100 and 130 €/ton in 2011 (Troiani et al., 2011).

Ozdemir & Yeningun (2013) have estimated that the excess sludge represents 32% of the total operation costs and 23% of the electricity costs in a plant (Fig.1).

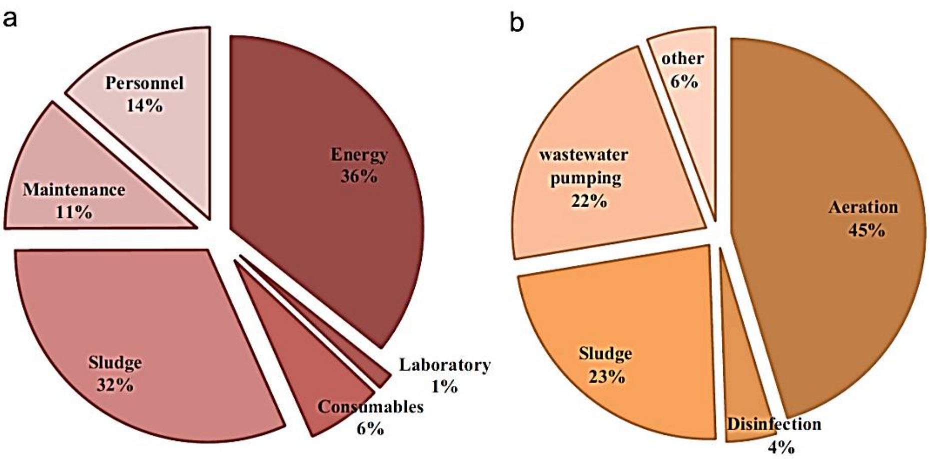


Figure 1- (a) Distribution of important items in total operation costs and (b) distribution of electricity costs (Ozdemir and Yenigun, 2013).

Kroiss and Zessner (2007) have developed researches showing the relevance and costs of sludge disposal for treatment plants in Austria (Table 3).

Table 3: Economic relevance of sludge disposal for treatment plants (in Austria). Adapted from Kroiss and Zessner) (2007)

Operating costs for sludge treatment and disposal	45% of total operating costs
Larger plants (> 50.000)	≥ 5 €/PE/year
Smaller plants (< 10.000)	≥ 10 €/PE/year
Share of disposal only.	≥ 85%
Capital costs for treatment and disposal facilities	4-8 €/p/year
Total yearly costs for sludge disposal including treatment	8-15 €/p/year = 20% of the total yearly costs for WWTP’s

Abbreviations: “PE” - One population equivalent; “p” – one person.

Excess sludge disposal represents an important cost in a WWTP and is necessary to reduce its production using one of the many existing alternatives to reach this goal. All these alternatives have positives and negatives factors, mainly because this management requires a considerable quantity of energy (that also strongly affect the costs) and because controlling the sludge production is a particular operation, aiming to reach a balance between the sludge that must be produced (to treat the wastewater) avoiding the excess sludge production.

2.2 Normative

According to Troiani et al. (2011), in Europe the Directive 2008/98/EC (European Parliament and Council, 2008) focus on a precise hierarchy in the management of waste that is respectively prevention, reuse, recycle and disposal. The 99/31/EC (European Parliament and Council, 1999) established the progressive reduction of sludge landfill disposal, presenting goals for 2020 to dispose a total amount of waste equal to 35% of the quantity disposed of in 1995.

Due to obligations set by the Urban Waste Water Treatment (UWWT) Directive 91/271/EC, a temporary increment of sludge quantities that are disposed in landfills is expected during the following years in EU-12 countries (Milieu Ltd and WRc and (RPA), 2010). During the last 20 years, the implementation of the UWWT Directive 91/271/EC (CEC, 1991) forced the EU-15 countries to improve their wastewater collecting and treatment systems, resulting in an increase of almost 50% of annual sewage sludge production in EU-15 from 6.5 million tons dry solids (DS) in 1992 to 9.8 million tons DS in 2005 (BIOPROS, 2006; EC, 2006, 2004, 1999; EEA, 2002; Hall, 1995).

The UWWT Directive (CEC, 1991) encourages the sludge reuse and forbids the final disposal to surface waters since 31/12/1998. It has established 2005 as deadline for the older EU-15 Member States and 2015 or 2018 for the countries that joined EU after 2004 (EC, 2009).

Besides, according to the Decision 2001/118/EC (CEC, 2001) that context sludge in non-hazardous wastes, the was hierarchy shall apply as a priority order in waste management: prevention, preparing for re-use, recycling, other recovery and disposal. Based on these principles, the Directive 99/31/EC (CEU, 1999) prohibited landfilling of untreated or liquid wastes. This way would be possible to reach a reduction of 65% of produced solid waste in 2020 (Kelessidis and Stasinakis, 2012).

The main legislative texts regarding the sludge management is Sewage sludge Directive 86/278/EEC (CEC, 1986), that describes the benefits of sludge use on solids and encourages safe use of sewage sludge in agriculture, regulating its use aiming to prevent harmful effects on vegetation, soils, humans and animals, specifying rules for the sampling and analysis of sludge and soils. Specifically, 16 countries of 27 EU countries (63%) have set more stringent national normative for heavy metal concentrations in sludge comparing to the European normative (Kelessidis and Stasinakis, 2012).

2.3 Sludge treatment

The most common methods in the Europe Union countries for sewage sludge treatment are sludge stabilization, anaerobic and aerobic digestion. Anaerobic digestion is most used in UK, Italy, Spain, Slovakia and Finland, while aerobic digestion is most commonly chosen in Poland and Czech Republic. It is worth to remind that the aerobic digestion is usually applied in small WWTPs. Besides, in Czech Republic approximately 97% of the sludge is treated by anaerobic stabilization (EC, 2006). In the other hand, chemical solutions are used mainly in 15 countries of EU even if are generally of minor importance, however composting is used in 25 of 27 countries from EU (93%) (Kelessidis and Stasinakis, 2012).

2.4 Final destination of sludge

There are some conditions that may influence the choice of final disposal of sludge, such as land resources, climatic conditions, cost of land, need for fertilizers, transport costs, local regulations and distance to the final disposal location. In general, land use and landfilling are the cheaper alternatives and most used in many countries, or even composting, that is also adopted in many countries as an alternative to sludge disposal (Foladori et al., 2010). The options for sludge treatment and/or disposal depend on many factors as technical and economic factors, ethical factors (values and priorities) related to the acceptability of specific technologies or practices and physic-geographical factors (Bauerfeld et al., 2008; Dentel, 2004).

Regarding the final disposal of sludge, the reuse (including composting and direct agricultural application) seems to be the prevalent choice for sludge management in EU-15 countries (old Member states), reaching approximately 53% of produced sludge), following by incineration (21% of produced sludge). However, the most common final disposal method in EU-12 countries (new Member States that joined the EU after 2004) is still landfilling (Kelessidis and Stasinakis, 2012).

In Eastern Europe, agricultural use and landfill are the most chosen disposal options (38% and 39%, respectively) (Fytili and Zabaniotou, 2008; Jenicek, 2007), while in western European countries the percentage of sludge used in agriculture varies widely in the range 10-70% (Ginestet, 2007). In Japan, incinerated sludge reaches 72% and the final destination of the ashes include industrial reuse and landfill, while in Latin America, agriculture is the most chosen option due to the good effects of nutrients and organic matter in the soil (Barrios, 2007). The disposal of untreated sludge on soil has been prohibited in most of EU countries , in some cases, the biological or chemical stabilizations have been set as obligation for treatment, while some countries allow the use of untreated sludge in specific authorizes circumstances, *e.g.*, Sweden, France and Estonia (Kelessidis and Stasinakis, 2012).

Chapter 3

The excess sludge: from its origin to final destination

The microorganisms are found nearly everywhere and have an important role in the water depuration, performing the macronutrients reduction. Biological treatment has a good efficiency and convenient costs. The production of excess sludge has increased due to more rigorous effluent regulatory requirements and higher number of WWTP in operation (U.S.EPA, 1999).

3.1 Bacterial growth

Biological wastewater treatment aims at adsorbing or degrading dissolved, colloidal, particulate and settleable matter into biofilms or biological flocs. Soluble compounds include non-biodegradable or biodegradable organic matter that in some cases can be toxic and have a considerable quantity of nutrients, including nitrogen and phosphorus. Besides that, the biological treatment is based on the natural role of bacteria to close natural cycles (C, N, P) on the planet (Henze et al., 2008).

In biological treatments, the organic matter is oxidized by heterotrophic microorganisms producing CO_2 and H_2O, this process is called catabolism and requires an electron acceptor (oxygen or nitrate), leading to production of energy as adenosine triphosphate (ATP). This energy is used by the microorganisms to grow forming new cells and to maintenance functions (maintenance of osmotic pressure, motility, nutrient transport, renewal of cellular constituents etc.), this process is called anabolism (Fig. 2). The ratio between the organic matter forming new cells and the organic matter that was oxidized in the process is known as observed growth yield (Y_{obs}). In aerobic conditions, the growth yield can reach approximately 0.60-0.70 gTSS/gCOD, which means an amount of 60-70% of organic biodegradable matter removed is converted into new cellular biomass. (Foladori et al., 2010b).

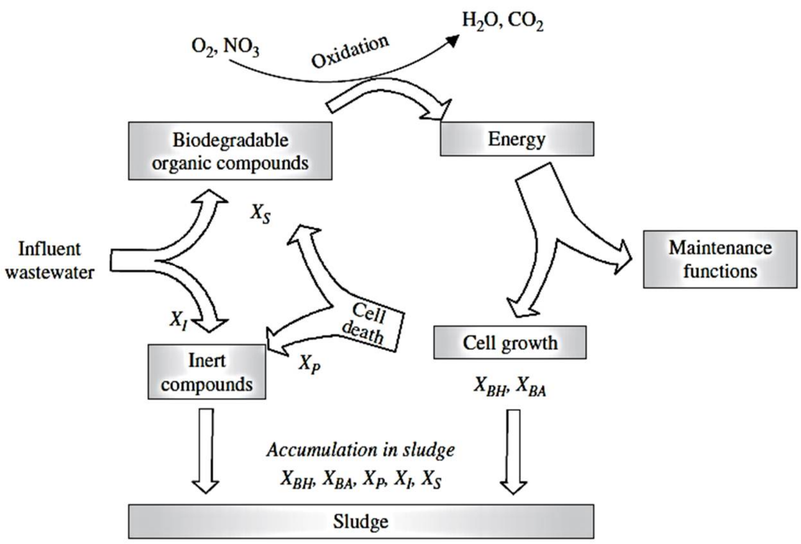


Figure 2 - Simplified scheme of the processes leading to sludge production in the biological treatment of influent wastewater (Foladori et al., 2010a).

Metcalf and Eddy (1991) demonstrated that the ideal bacterial growth for wastewater treatment requires a proper environment condition that involves pH and temperature regulation, nutrients addition, oxygen addition or exclusion and proper mixing. The microorganism growth is necessary to leave them in the system long enough to reproduce and the duration of this period will depend on the growth rate. In biological treatment plants, the rate of growth of bacterial cells can be defined by the following Eq. 1:

$$r_g = \mu X \tag{1}$$

Where:
r_g: rate of bacterial growth, mass/unit volume · time;
μ: specific growth rate, time $^{-1}$;
X: concentration of microorganism, mass/unit volume.

3.1.1 Requirements for microbial growth

Biological plants must maintain enough biomass to metabolize soluble and colloidal organic substances, to perform these processes; many conditions must be fulfilled, such as temperature, necessary nutrients, type and concentration of organic waste (as electron donor). These requirements are essential to design and manage biological treatment plants (Wang et al., 2009).

Besides energy, microorganisms need source of inorganic compounds and carbon to synthesize cellular components. Bacteria found in WWTP are composed typically by 20-25% dry matter and 75-80% water (Henze et al., 2008). The Table 4 presents the typical composition of dry matter (TSS) of bacteria.

Table 4: Typical composition of bacteria (adapted from *Metcalf & Eddy* (2003) in Henze et al., 2008).

Constituent or elements	%TSS	Empirical formula for cells $C_5H_7O_2N$
Major cellular constituents		
Protein	55.0	
Polysaccharides	5.0	
Lipid	9.1	
DNA	3.1	
RNA	20.5	
Other (sugars, aminoacids)	6.3	
Inorganic ions	1.0	
As cell elements		%VSS
Organic (VSS)	93.0	
Carbon	50.0	53.1
Oxygen	22.0	28.3
Nitrogen	12.0	12.4
Hydrogen	9.0	6.2
Inorganics (FSS)	7.0	
Phosphorus	2.0	

Sulfur	1.0
Potassium	1.0
Sodium	1.0
Calcium	0.5
Magnesium	0.5
Chlorine	0.5
Iron	0.2
Other trace elements	0.3

3.1.1.1 Electron acceptors

The wastewater treatment can be performed either by aerobic or anaerobic processes. For aerobic biodegradation, dissolved oxygen (DO) serves as electron acceptor; under anaerobic conditions, many compounds can act as electron acceptor, for example SO_4, CO_2, etc., as well as in anoxic conditions, where the electron acceptor is the nitrate (NO_3).

The need, sensitivity or tolerance to molecular oxygen (O_2) varies among microorganisms. Aerobes use oxygen as electron acceptors and may need it, function in its absence (facultative microorganisms) or require oxygen in low levels, as the microaerophilic. Anaerobes microorganisms do not use oxygen as electron acceptor but may tolerate it (aerotolerant) or not (obligate). While the absence of oxygen is related to anoxic (without O_2) or anaerobic (without air) by microbiologists, some engineers make a distinction between these conditions. Besides, in the absence of O_2, the presence or absence of oxidized nitrogen (nitrate or nitrite) is referred to as anoxic and anaerobic conditions, respectively (Henze et al., 2008).

In aerobic environments, the oxygen demand can be calculated by laboratory tests (BOD, COD) or by stoichiometry. The theoretical oxygen (ThOD) demand is the quantity of oxygen required to oxidize completely the organic carbon to water and carbon dioxide (Wang, Shammas and Hung, 2009) based on stoichiometry calculation. The Chemical Oxygen Demand (COD) is defined as an indicative measure of the amount of oxygen that can be consumed by chemical reactions;

lastly, Biochemical Oxygen Demand (BOD) is the amount of dissolved oxygen needed by aerobic biological organisms to break down organic material present in each water sample at certain temperature over a specific period. The BOD value is most commonly expressed in milligrams of oxygen consumed per liter of sample during 5 days of incubation at 20 °C.

3.1.1.2 Temperature

The response of a biological treatment plant depends strongly of the temperature variations, even if different groups of bacteria have various temperature optimums. The higher is the temperature, the faster are the reactions inside the biological reactors. The values of α for most biological treatment systems are between 1.0 – 1.14 (Wang, Shammas and Hung, 2009).

The effect of the temperature variation can be described by Eq. 2:

$$r_T = r_{20}\alpha^{(T-20)} \tag{2}$$

Where:

r_T = biodegradation rate at temperature T;
r_{20} = biodegradation rate at 20°C;
α = temperature-activity coefficient;
T = Temperature, °C.

Temperature is an important parameter in the microorganism's growth (Fig. 3). With an increasing temperature, a continuous increase in growth rate is observed until a brusque drop is observed due to the denaturation of proteins that occurs at a higher temperature. The most used terms to describe these microorganisms are psychrophilic (below 15°C), mesophilic for 15-40°C, thermophile at 40-70°C and hyperthermophile (above 77°C up to approximately 110°C) (Henze et al., 2008).

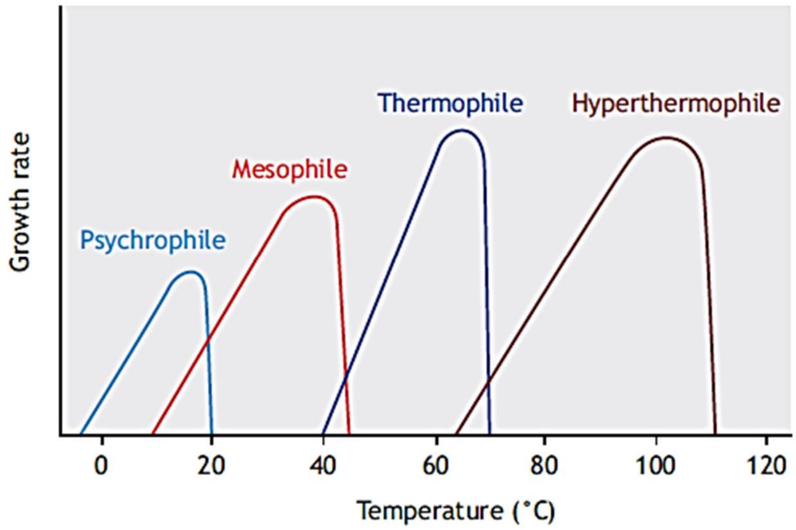


Figure 3 - Effect of temperature on microbial growth rate (adapted from Rittmann and McCarty, 2001).

3.1.1.3 Nutrients

The bacterial nutrition happens through the cellular membrane and because of this phenomenon; the bacteria - for nutritional requirement - absorb only dissolved particles. According to Henze et al. (2008), in addition to energy, microorganisms require sources of inorganic compounds and carbon to synthesize cellular components. Bacteria in WWTP are typically composed of 75-80% water, while the other fraction is composed by dry matter.

The microorganisms of AS plants require nutrients to function, *e.g.* nitrogen, iron, phosphorus and other trace metals. The accepted minimum ratio of carbon to nitrogen, phosphorus and iron is 100 parts carbon to 5 parts nitrogen, 0.5 parts iron and 1 part phosphorus (Spellman, 2003). The main macro nutrients for cell growth are nitrogen and phosphorus (Grady et al., 1999), even if other elements are also necessary for cell growth as the ones showed previously in Table 4.

3.1.1.4 pH

In biological treatments, most bacteria can function in the range of 6 to 8. When the values of pH in the reactor goes out the optimal range is expected to the microbial population drop significantly resulting in failure of the process (Wang, Shammas and Hung., 2009).

The pH is a parameter that can vary due to the alkalis and acids produced by the metabolism of microorganisms, even if it can be artificially adjusted, it has a significant effect on the physical properties of sludge. The levels of H^+ or OH^- in the supernatant can change the interaction between sludge particles by the change of surface properties of sludge particles (Tang and Zhang, 2014).

Tixier et al. (2003) investigated the effect of pH on the interparticle interactions of flocs in activated sludge systems and concluded that viscosity increased with increased pH for all tested samples. The increment in viscosity is higher in high and low levels of pH, considering that the viscosity is a parameter that reflects the interactions between particles and flocs of sludge, which are influenced by the surface charge of sludge particles. A decrease in pH levels facilitates a decrease in the thickness of electrostatic double layers, reducing the electrostatic repulsion and decreasing the viscosity.

3.2 Excess sludge production and its main parameters

In biological treatment plants, the microorganisms are not able to degrade completely the organic compounds; furthermore, there is an accumulation of inorganic substances, present in the wastewater, these factors contribute to the excess sludge production. One of the main units that is useful to understand the velocity of bacterial growth is the sludge growth rate (KF), that means the daily growth in terms of weight inside the plant compared to the quantity of sludge taken off the reactor (to maintain constant the sludge concentration in the aerobic reactor) and the sludge quantity inside the plant (Masotti, 2011).

Specific sludge production in WWTP varies from 35-85g dry solids (total solids – TS) per population equivalent (PE) per day (gTS PE^{-1} d^{-1}) (Foladori et al., 2010). In Germany, a typical daily sludge production pro capita is nearly 60-80 gTS PR^{-1} d^{-1} (Ginestet, 2007); in Italy, the values or sludge production can reach 65 gTS PE^{-1} d^{-1} or 250g of wet sludge PE^{-1} d^{-1} (Battistoni et al., 2002).

3.2.1 Sludge age

The sludge residence time (SRT), also known as sludge retention time, or sludge age, affects the condition of the AS flocs in the aeration basin and it is calculated as the amount of sludge solids in the system divided by the rate of loss of sludge from the system (Grady et al., 1999). In this case, the Eq. 3 is used:

$$t_s = V\,X \,/\, [(Q_w X_w) + (Q_e X_e)] \qquad (3)$$

Where:

V is the volume of liquid in the aeration tank (m^3);
X the MLSS (mg l^{-1});
Qw the sludge wastage rate (m^3 d^{-1}), Xw the MLSS (mg l^{-1}) in the waste sludge stream;
Qe the effluent discharge rate (m^3 d^{-1});
Xe the effluent suspended solids concentration (mgl^{-1});
ts the SRT in days.

If it is assumed constant, the proportion of microbial cells in the MLSS the SRT can be referred also as the mean cell residence time (MCRT) or the sludge age. SRT is an important operational factor over sludge activity because it is the reciprocal of the net specific growth rate of the sludge. A low SRT (<0.5 d) indicates a sludge with a high growth rate as used in high-rate units for pretreatment or partial treatment, a high SRT (>5d) indicates a low growth rate sludge such as extended aeration systems. Conventional AS systems have an

SRT of between 3 and 4 d presenting good settling properties (Grady et al., 1999). Besides, increasing sludge retention time (SRT) or decreasing sludge rate in aerobic processes can reduce the sludge production (Van Loosdrecht and Henze, 1999).

3.2.2 Organic load

Organic loading is defined by the amount of organic matter entering the WWTP and it is usually measured as BOD. An organic overload happens when the quantity of BOD entering the plant exceeds the designed capacity of the WWTP and results in increased demand for oxygen. Besides that, the organic underload occurs when the amount of BPD that enters the system is significantly less than the designed capacity of the plant (Spellman, 2003).

3.2.3 Hydraulic retention time (HRT)

The hydraulic retention time, also known as hydraulic detention time refers to the theoretical time (average length of time) a drop of water/wastewater or suspended particles remain in a reactor/tank or channel. The division of the wastewater in the tank by the flow rate through the tank results in the HRT. The units of flow rate depend on whether the HRT is calculated: in days, hours, minutes or seconds. Generally it is associated with how many time is required for a tank to empty, it is usually measured in minutes (Spellman, 2003). The ways to calculate HRT are expressed in Eq. 4 (for days), Eq. 5 (for hours) and Eq. 6 (for minutes).

$$HDT = \frac{Tank\ volume}{Flow\ rate} \tag{4}$$

$$HDT\ (h) = \frac{Tank\ volume\ (ft^3) x\ 7.48 gal/ft^3 x\ 24h/d}{flow\ (gal/d)} \tag{5}$$

$$HDT\ (\mathrm{min}) = \frac{Tank\ volume\ (ft^3) x\ 7.48 gal/ft^3 x\ 1440\ min/d}{flow\ (gal/d)} \tag{6}$$

3.2.4 Food to microorganism (F/M ratio)

The food to microorganism ratio (F/M ratio), also known as sludge loading, is a process control method and calculation based upon keeping a balance between available food materials, *i.e.* BOD or COD in the aeration tank mixed liquor volatile suspended solids (MLVSS) concentration and in the aeration tank influent. To calculate the F/M ratio, is necessary to obtain information as: aeration tank influent flow rate (MGD), aeration tank MLVSS (mg/L), aeration tank volume (MG) and aeration tank influent BOD or COD (mg/L) (Spellman, 2003). Then it is calculated according to Eq. 7, while typical F/M ratios for activated sludge processes are the ones shown in the Table 5:

$$F/M\ ratio = \frac{primary\ effluent\ COD/BOD\ (mg/L)\ \times flow\ (MGD) \times\ 8{,}34\ lb/mg/L/MG}{MLVSS\ (mg/L)\ \times aerator\ volume\ (MG) \times 8{,}34\ lb/mg/L/MG} \tag{7}$$

Liu et al. (2012) classified the F/M ratio as the balance between the food supply and the mass of microorganism inside the system. Besides, it is a relevant operational variable parameter that affects directly the organic removal efficiency, microbial composition and sludge properties (Li et al., 2011; Tay and Yan, 1996), influencing also the process and membrane fouling in MBRs.

A high F/M ratio affords a high driving force for microbial growth and metabolic activity, and high overall rates of waste converse to methane (Lobos et al., 2008; Sung and Dague, 1995). Though, a too high value of F/M may disturb the balance that exists between the methanation and hydrolyzing, resulting in sludge deflocculation by affecting the microbial ecology and the process efficiency (Liu et al., 2012).

Table 5: Typical F/M ratios for activated sludge processes (Spellman, 2003).

Process	**BOD (lb)/MLVSS (lb)**	**COD (lb)/MLVSS (lb)**
Conventional	0.2-0.4	0.5-1.0
Contact stabilization	0.2-0.6	0.5-1.0
Extended aeration	0.05-0.15	0.2-0.5
Pure oxygen	0.25-1.0	0.5-2.0

Besides, a low F/M ratio enhances organic removal capability and sludge deflocculation, increasing the settleability of biomass in the tank; though, if this parameter is too low, sludge deflocculation may also occur due to the limited cell growth (Lobos et al., 2005). There is no consensus of an optimal F/M ratio in the literature (Ghangrekar et al., 2005; Prashanth et al., 2006). Considering the industrial WWTP, a high F/M ratio potentially applicable, can be, for example, 3.75 g COD/g MLSS · d (He et al., 2005).

3.2.5 Sludge settleability

The sludge volume index (SVI) is useful to understand the flocculation and settling characteristics of the activated sludge present in the plant. The SVI is obtained by a physical analysis performed in a cylinder glass after 30 minutes settling as shown in the following Eq. 8. The lower the SVI is, the better settling and flocculation characteristics, actually, values nearly 100 ml/g are considered satisfactory (Henze et al., 1997).

$$SVI = \frac{1}{x_{0.5}} \text{ (unit normally ml/g SS)} \tag{8}$$

Where:

$x_{0.5}$ = Sludge concentration in sludge phase after 30 minutes.

3.2.6 The oxygen demand

The sensitivity or need to molecular oxygen (O_2) varies between microorganisms. Indeed, aerobes may need it (obligate), while facultative can function in its absence or require it in low levels, *i.e.* microaerophilic. On the other hand, anaerobes do not use O_2, but may tolerate it (aerotolerant). In aerobes, enzymes for oxygen reduction are regularly induced, while denitrifiers (facultative aerobes), also have constitutive enzymes for oxygen reduction, but enzymes for nitrate or nitrite reduction need to be induced and this situation requires the absence of oxygen. Microbiologists use to refer to the absence of anoxic (without O_2) or anaerobic (without air), while engineers make a distinction between these conditions, *i.e.,* in the absence of oxygen, the presence of absence of oxidizes nitrogen (nitrate or nitrite) is referred to as anoxic and anaerobic environments, respectively. (Henze et al., 2008) .

Chapter 4

Main techniques for excess sludge reduction

The excess sludge is one of the main problems in WWTP and due to this fact, the researches over this argument has grown exponentially. Many techniques can be used to reduce the excess sludge production; each one has its own advantages and disadvantages. These methods can be applied in the water line or in the sludge line, and can be based on many mechanisms, as the cryptic growth/cellular lysis, uncoupled metabolism, endogenous metabolism or predation on bacteria.

4.1 Cryptic growth / Cellular lysis technologies

The term "cryptic growth", also referred as endogenous activity or autodigestion, was introduced to indicate the reutilization of intracellular compounds (both nutrients and carbonaceous compounds released from cell lysis), for the growth of viable cells of the same community) (Foladori et al., 2010b). In other words, the cryptic growth (Fig. 4) consists in the disintegration of microbial cells, releasing in the liquid the microorganism cell contents that are reused by the new cells for metabolism (Chu et al., 2009).

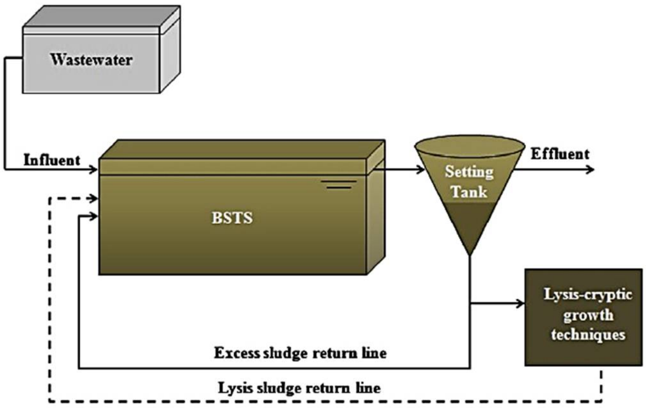


Figure 4 – Schematic diagram of lysis-cryptic growth process (Guo et al., 2013).

Guo et al. (2013) performed an analysis of the pros and cons of the sludge reduction by lysis-cryptic growth (Table 6), concluding the predominance of disadvantages in this process.

Table 6: Evaluation of advantages and disadvantages on in-situ activated sludge reduction processes (Guo et al., 2013).

Sludge reduction by lysis-cryptic growth	
Advantages	Disadvantages
1. Improve the sludge settling properties and dewaterability	1. Disintegrate the microorganism and organic materials without selection
2. Enhance the biodegradability	2. Corrode the reactor and system
3. Shorten the retention time	3. Produce undesirable hazardous by-products
4. Reduce the bulking and scumming	4. High energy and operational cost
	5. Complicated operation/control process
	6. Difficult to optimize the chemical oxidation reagent dosage
	7. Deteriorate the effluent quality, especially TP or TN concentrations

4.1.1 Enzymatic hydrolysis

A large number of enzymes were identified and have been reported to play an important function for sludge treatment purposes, acting on specific recalcitrant pollutants to remove them by precipitation or transformation to other innocuous products, changing the characteristics of the waste to render it more susceptible to treatment (Karam and Nicell, 1997).

Parmar et al. (2001) found interesting results with enzyme preparations, obtaining solid reductions of 21.4%, 25% and 32.1% for lipase, cellulase and alkaline protease, respectively. Besides, slight increases in solid reduction were observed with increasing enzyme concentrations, obtaining a significant improvement of settleability with proteases. The authors concluded that the use of enzymes can reach a reduction of 50% of excess sludge production, also improving the sludge settleability properties. Considering that organic matter of sewage sludge solids amounts nearly 60%, the enzyme treatment has the potential to reduce about 80% of the organic fraction of biosolids.

Foladori et al. (2010) highlighted that the main mechanisms which occur during enzymatic treatment are sludge solubilization, cryptic growth and cell lysis and that sludge solubilization by enzymes is more intense the nearer the temperature is to the 50°C, considered excellent for hydrolytic enzyme activities. Therefore, the enzymatic treatment is best applied with anaerobic digestion in thermophilic or mesophilic conditions.

4.1.2 Mechanical disintegration

The mechanical disintegration (Fig. 5) is a process known for obtaining intracellular products such as enzymes or proteins in biotechnological applications (Schwedes and Bunge, 1992). This process has the aim to disrupt sludge flocs, improve sludge biodegradability, disintegrate bacteria cells and reduce sludge production.

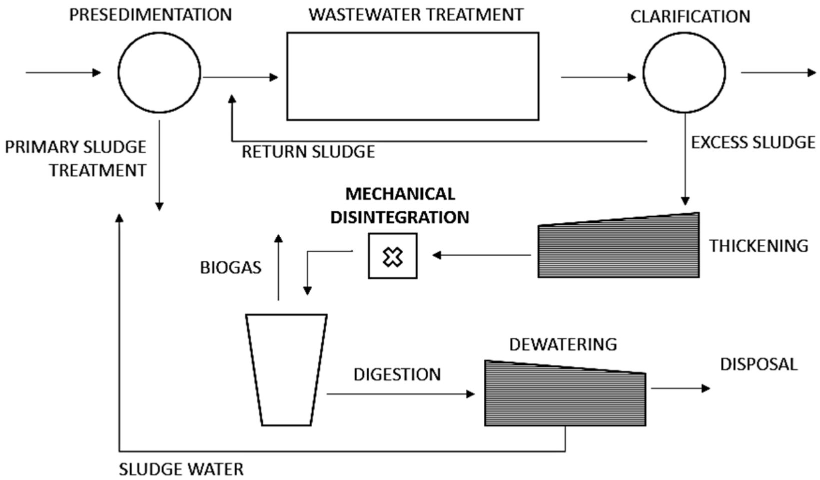


Figure 5- Flow sheet of sludge treatment with mechanical disintegration of excess sludge. Adapted from Kopp et al. (1997).

According to Muller et al. (2004) the changes in the sludge can be influenced for microorganisms, sludge solubilization, floc size reduction, foaming reduction, viscosity reduction and improvement/worsening of settling and dewatering. Mechanical disintegration can be achieved using many equipment as lysis-thickening centrifuge, stirred ball mills, high pressure homogenizers, high pressure jet and collision, rotor-stator disintegration system and ultrasonic disintegration (Foladori et al., 2010b).

According to Lehne (2001), digestion is a typical method to enhance the dewaterability of excess sludge and reduce the amount of sludge in a WWTP. Besides, excess sludge contains approximately 70% of bacteria, the hydrolysis of these microorganisms limits the speed of the entire anaerobic process, reaching nearly 40% of degradation of the organic material, reducing the amount of digested sludge that must be disposed and enhancing the production of biogas.

A high degree of disintegration in combination with an economical energy consumption and a high release of organic material are indispensable for the beneficial use of mechanical disintegration to improve the anaerobic digestion (Lehne et al., 2001).

4.1.3 Ultrasound treatment

Ultrasound treatment (also known as ultrasonic treatment) has been reported as a technology with many benefits, avoiding the disadvantages of chemical oxidation and improving the sludge dewaterability, biodegrability and biosolids quality (Khanal et al., 2007; Weemaes and Verstraete, 1998).

This methodology is generally performed putting the sludge for several seconds to 2.5h (usually < 1h) at 9-41 kHz (mostly at 20kHz) with an enhanced VS removal of 9-36% improving CH_4/biogas production of 24-138% (Wang et al., 2017).

4.1.4 Thermal treatment

The thermal treatment of sludge, *i.e.* the use of high temperatures, is one of the most common techniques to reduce the excess sludge production and can be also used to increase biogas production in anaerobic digestion, improving dewaterability, pathogen inactivation and reduction of sludge produced. This method (Fig. 6) produces some effects as the breakdown of the sludge structure (disaggregating the biological flocs), lysis of bacterial cells (releasing intracellular constituents and bound water) and high level of sludge solubilization (Foladori et al., 2010b).

Thermal hydrolysis, also known as thermal hydrolytic pre-treatment (THP), is recognized as a method to enhance degradability of long-age AS and involves heat treatment of the process fluid (150-160°C saturated steam) and pressure. Therefore, in a full-scale system, the use of this technique produces recalcitrant, coloured compounds that may have downstream impacts, *i.e.,* increased effluent nitrogen or failure of UV disinfection (Dwyer et al., 2008).

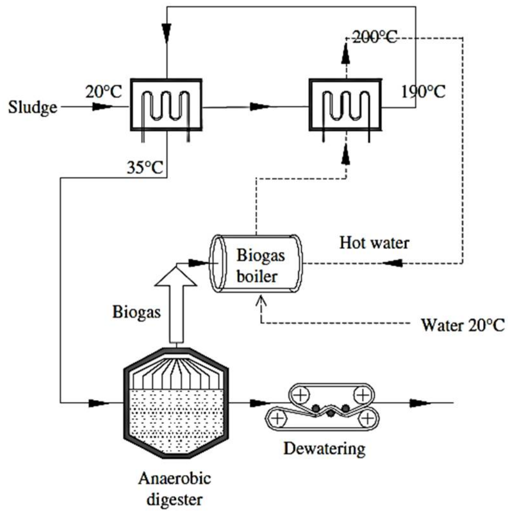


Figure 6 - Scheme of a thermal treatment + anaerobic digestion (Bougrier et al., 2007).

In cases where the thermal treatment is used, the viscosity of sludge changes as cell water is freed under hydrolysis, these cells are rich in dissolved organic compounds. Some experiments performed with municipal sewage sludge showed that the highest yield of hydrolysis could be achieved at 165 and 180°C, while for industrial waste activated sludge (WAS) - mainly from the paper and pulp industry - had as optimal hydrolysis temperature from 150 to 165°C. A reason for this can be that the industrial wastewater sludge is more pure biomass than municipal WAS (Kepp et al., 2000).

Some researchers concluded that temperatures below 100°C are already able to contribute to partial sludge reduction, by increasing the biodegradability. Besides, temperatures above 150°C (combined with pressure of 600-2500 kPa) must be reached to liquefy sludge, contributing to sludge reduction, therefore this process is costly (Foladori et al., 2010b).

Dohányos et al. (2004) proposed extremely short contact times (lasting 1 minute) in the thermal treatment (at 170°C), to improve anaerobic digestion, while moderate temperatures (under 100 °C) require a longer contact time that can last from few hours to one day).

4.1.5 Chemical and thermo-chemical hydrolysis

According to Foladori et al. (2010), these techniques are based on alkaline, acid reagents or combination. Coupling an increase in temperature with an energetic change in pH, away from the best values for microorganisms, occurs the cell breakage and besides these types of treatment enhance the course of cell lysis-cryptic growth. When compared to the thermal treatment, the thermo-chemical treatment is more efficient in sludge solubilization (at the same temperature). Acid or alkaline agents can be considered as "catalysts" in the thermal hydrolysis of organic macromolecules. Therefore, the application of a thermo-chemical treatment of sludge is aimed at improving biodegradability, promoting hydrolysis and solubilization of complex polymeric substances and improving dewatering and settling properties. The thermo-chemical treatment of sludge can be performed by using:

- Alkaline reagents, as NaOH, KOH, CaO, $Mg(OH)_2$ or $Ca(OH)_2$;

- Acidic reagents, as HCl or H_2SO_4.

Neyens et al. (2003) demonstrated that alkaline thermal hydrolysis using $Ca(OH)_2$ is efficient in reducing the residual sludge and improve dewaterability. The goals were fully met at 100°C, pH approximately 10 and 60-min reaction time, where all pathogens are moreover eliminated.

Rocher et al. (1999) highlighted that sludge treatment by thermal, alkaline, acid or its combination can perform some cell treatment to reach the cryptic growth of microorganisms (microbial growth on its lysates) and found that in thermal-chemical hydrolysis, sodium hydroxide was the most efficient for inducing cell lysis. Besides, further researches performed by Rocher et al. (2001) showed that biodegradation of the soluble fraction of lysates by fresh sludge was able to reach 75% and 90% after 48 and 350 hours of incubation, respectively, obtaining a reduction of 37% of the excess sludge without changing the purification yield of the process.

Thus, Liu (2003) performed experiments using sludge alkaline-thermal treatment, activated sludge-ozonation process, chlorination-combined AS process, sludge reduction by metabolic uncouplers and high DO activated sludge process to reduce the excess sludge production. Based on the achieved results, he concluded that compared to microbiological methods (*e.g.* endogenous respiration, lysis, predation and decay), the chemical-combined AS processes would be more efficient for excess sludge reduction, besides the chemical-assisted sludge reduction processes could have some advantages as stable performance, high operation flexibility and easy control.

4.1.6 Chemical oxidation

In chemical oxidation cell techniques, the use of ozone (strong cell lysis ability) is reported as the most efficient and powerful oxidant (Cui and Jahng, 2004). It was well reported that the ozonation pretreatment could improve the sludge settleability, reducing bulking and scumming and facilitating the biodegradability (Chu et al., 2009).

Another chlorine compounds, as chlorine dioxide (ClO_2), or hydrogen peroxide (H_2O_2), can be used instead of ozone (Guo et al., 2013), but these oxidants even if get to lower the costs of the plant, are much weaker and can produce undesirable chlorinated by-products such as trihalomethanes (THMs), which are harmful to human beings (Gallard and Von Gunten, 2002; Park, 2011).

A chlorine dose of 0.066 gCl_2/g MLSS reduced the excess sludge production by 65%, but between the chlorine disadvantages we can highlight the bad sludge settleability, formation of trihalomethanes (THMs) and significant increase of soluble chemical oxygen demand (sCOD) (Wei et al., 2003).

Yasui and Shibata (1994) developed a process for reducing the excess sludge production in AS systems based in ozonation stage and biodegradation stage, in which a fraction of recycled sludge passes through the ozonation unit and then the sludge is decomposed in the subsequent phases of the process. In the ozonation unit happens the disintegration of suspended solids (solubilization) and the mineralization (that happens due to oxidation of sludge reduction). The recycling of solubilized sludge in the aerobic reactor induces the cryptic growth mechanism.

4.1.7 Electrical treatment

The electrical treatment for treating sludge, also known as pulsed electric field (PEF), has been widely used in medicine, biology and food applications for years, and recently has been proposed in the wastewater treatment scenario die to the breakage effect on microbial cells. It works sending high-voltage (>20kV) electrical pulses thousands of times per second across the sludge, attacking this way the peptidoglycan and phospholipids, that are the main constituents of cell walls and membranes), consequently causing the opening of the cell pores resulting in cell ruptures and lysis (Foladori et al., 2010b).

Although the cell destruction can optimize the anaerobic stabilization of sewage sludge, depending on the sludge composition and the disintegration process, it leads to an increase of the gas yield, degradation degree and partially to a minimization of foaming in the digester (Kopplow et al., 2004).

The PEF treatment (Fig.7) in wastewater plants was applied by Heinz (2007), that addicted a heat exchanger prior to the PEF treatment allowing the sludge temperature to be increased up to 35°C, enhancing the effect of the electrical pulses and consequently causing a further temperature increase about 20°C in the treated sludge. When applied a specific energy of 100.000 kJ/m^3, the TSS reduction reached 27-45%. Besides, the advantages of this technology are: short contact time, compact system, no odor production, reduction of filamentous microorganisms; while the drawbacks are erosion of electrodes, high-energy consumption and that this process is not yet fully investigated.

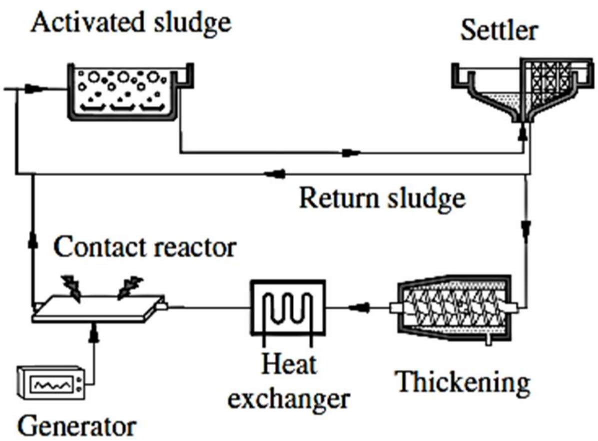


Figure 7 - Electrical treatment integrated in wastewater handling unit. Adapted from Foladori et al. (2010)

4.2 Uncoupled metabolism

Uncoupled (or uncoupling) metabolism is based on the disassociation with the energy coupling between anabolism and catabolism (Guo et al., 2013) and can be induced in different ways, as the use of chemical uncouplers (Qiao et al., 2011), oxic-settling-anaerobic (OSA) process (Chen et al., 2003) and repeatedly coupling of aerobes/anaerobes environment (Xing et al., 2008).

The metabolism of microorganisms is based in biochemical transformations that are related to the catabolic and anabolic reactions. The microorganisms during the catabolism produces energy in form of adenosine triphosphate (ATP) that is used for the microorganisms for metabolism to produce new cells. In the uncoupling methods, this phenomenon is interrupted and the anabolism (production of new cells) is interrupted. In most of cases the anabolism and catabolism are coupled through the transfer of generated energy (Aragón et al., 2009).

The possibility of enhancing the conventional aerobic digestion process (in which the tank is continuously aerated) including alternating aerobic/anoxic (and/or anaerobic) phases, can favor nitrogen removal through the alternation of the nitrification/denitrification process. In theory, the sludge reduction occurs since the heterotrophic growth yield assumes lower values under anoxic conditions, when compared to aerobic ones. Generally, the anoxic growth yield (0.30 - 0.36gVSS/gCOD) is 67-80% of the aerobic yield (0.45 gVSS/gCOD) (Foladori et al., 2010b).

Considering the huge number of bacteria in the sludge, even if it represents only 15-30% of TSS (Foladori et al., 2010b), many of these microorganisms have a particular behavior when exposed to different environments. The presence or the absence of oxygen inside the different reactors in the plant put the bacteria through a stressful condition that reduces their growth.

The resulting reduction that occurs when the biomass is exposed to these different environments is attributed mainly to the adenosine

triphosphate (ATP), that plays an important role between the substrate oxidation process (produced during catabolism) and the biomass synthesis reactions (anabolism) (Coma et al., 2013). This reaction is called uncoupled metabolism and it occurs when microorganisms are subjected to a physiological shock created by the absence of O_2 and substrate, using ATP as a source of energy. When they are returned to the aerobic reactor they rebuild they energy reserves at the expense of growth (Chudoba et al., 1992a).

For most of aerobic bacteria the energy in ATP form is generated by electrons donations between the substrate (donor) to the final electron acceptor (O_2), this process is also called oxidative phosphorylation. The uncoupling approach means to increase the discrepancy of energy level between anabolism and catabolism, resulting in absence of energy to the anabolism process, and consequently declining the observed growth yield of biomass (Wei et al., 2003). The advantages and disadvantages of the uncoupled metabolism are shown in Table 7.

Table 7: Sludge reduction by uncoupled metabolism (Guo et al., 2013).

OSA system and novel systems based on repeatedly coupling of aerobic/anaerobic process	
Advantages	Disadvantages
1. No extra-chemical or physical addition	1. Lack of practical application experience
2. Improving the settling ability	
3. Capable of treating complex components or high strength organic pollutants	
4. Flexible to operate and easy to be meliorated	
5. Economical efficiency and environmental friendliness	
Uncouplers-induced uncoupling metabolism	
Advantages	Disadvantages
1. No significant change in the configuration of the CAS processes	1. Little is known about the fates and the potential hazards of the metabolic uncouplers for eco-environment, sludge ecosystem, and even the human
	2. The application of single metabolic uncouplers might result in microbial acclimatization during a long-term running period
	3. The selection and optimization of appropriate metabolic uncouplers need a mass of fussy and reduplicative experimental inputs that always reach inconsistent results
	4. Deteriorate the effluent nutrient removal efficiency

4.2.1 Addition of chemical metabolic uncouplers

The addition of chemical metabolic uncouplers can reduce excess sludge in many biological treatment systems, as membrane bioreactor (MBR), sequencing batch reactor (SBR), continuous stirred-tank reactor (CSTR) and full-scale AS systems (Chen et al., 2008, 2006; Chong et al., 2011; Henriques et al., 2005).

During the oxidative phosphorylation with chemical uncouplers, the substrate oxidation process creates a proton motive force across the intracellular cytoplasm membrane providing the force necessary to perform the oxidative phosphorylation (Wei et al., 2003).

The main chemical uncouplers such as chlorophenol, nitrophenol, para-nitrophenol (pNP), 2,4-dinitrophenol (dNP), pentachlorophenol, 2,4,5-trichlorophenol (TCP), 3,3',4'5-tetrachlorosalicylanilide (TCS), cresol and aminophenol can be effectively lower the sludge yield during the biological treatment (Aragón et al., 2009; Low, 2000; Qiao JL, Wang L, 2011; Yang et al., 2003).

Although its effectiveness, many reports conclude that many of chemical uncouplers have residual toxicity and may be even toxic to microorganism and environment. Chen et al. (2002) and Chong et al. (2011) reported the toxicity of metabolism uncouplers including, 3.3',4'5-tetrachlorosalicylanilide, 2.4-dinitro-phenol and 2.4-dichlorophenol p-nitrophenol.

Guo et al. (2013) reported many disadvantages of the use of chemical uncouplers, some of them are: (1) the long-term application of a single chemical uncoupler can result in biological acclimation phenomenon, that might lead to lower sludge reduction capability; (2) the use of metabolic uncouplers can deteriorate the effluent nutrient removal efficiency; (3) most of metabolic uncouplers are potentially harmful to environment.

4.2.2 Side-stream reactors

The uncoupling metabolism could be also driven through biological methods. The side-stream reactors (SSR) – also called sludge retention reactors (SRR) – are an additional reactor (anoxic or anaerobic) that can be merged in the water or in the sludge line and aims to put the microorganisms under a stressful condition, that helps to reduce the excess sludge production. Some hypotheses have been proposed in the literature to explain the possible mechanisms of sludge reduction in these techniques, such as cell-lysis-cryptic growth (Quan et al., 2012; Wei et al., 2003) and uncoupling metabolism (P. Chudoba et al., 1992b; Troiani et al., 2011).

The research performed by Foladori et al. (2015) demonstrates that viable bacteria does not decrease during the anaerobic phase, indicating that at ambient temperature, the anaerobiosis does not produce a significant cell lysis. They also proposed two mechanisms for sludge reduction: in aerobic conditions occurs bacterial cell lysis and oxidation of released biodegradable compounds and under anaerobic conditions occurs the sludge hydrolysis of non-bacterial material. In addition, they concluded that in the transition from anaerobiosis to aerobiosis, only the aerobic phase contributes to bacteria reduction due to a significant aerobic decay rate.

Kim et al. (2012) observed in an anaerobic side-stream reactor (ASSR) with a 10% interchange rate (equivalent to HRT of 10d), the presence of different bacterial communities, even if these stages were connected via continuous sludge recirculation, resulting in modifications in the structure and composition of sludge. The insertion of a side-stream reactor in a CAS plant can result in many benefits, such as good process stability, easy management and low operational costs (J. Wang et al., 2008). Chon et al. (2011) and Coma et al. (2013) have found in these systems good results for sludge reduction such as Y_{obs} of 0.350 ± 0.004 kgTSS/kgCOD in the presence of SSR system, compared to 0.042 ± 0.002 kgTSS/kgCOD before the introduction of the SSR system.

The Cannibal® process, is an anaerobic SSR tank (Foladori et al., 2015) and is an alternative for reducing sludge production that consists in: part of the return activated sludge initially passes through and intermediate tank, drum screen and hydrocyclone, removing grit and other inert particles (solids), then sludge goes to an interchange reactor (anaerobic/anoxic) (Wang et al., 2017). Novak et al. (2007) achieved a 60% sludge reduction in the Cannibal process.

Among many techniques to reduce the sludge production, the SSR process shows significant sludge reduction without causing negative effects on sludge settling and effluent properties (Novak et al., 2007). In SSR systems, a portion of the return activated sludge - RAS is recycled through the anaerobic reactor with intentionally minimized sludge wasting (Chon et al., 2011). The OSA process shares some system configuration with SSR, except for that all of the settled (return) sludge undergoes short anaerobic treatment before reaching the main aeration basin (Saby et al., 2003).

4.2.3 OSA process

The OSA process (Fig. 8) reduces the excess sludge production due to the low oxidation-reduction potential (ORP) level that is the main factor to the sludge decay and lysis in the SSR of the OSA process. This process consists in a modification of the conventional activated sludge system (CAS) with anoxic/anaerobic treatment of the return sludge where it is inserted a sludge holding tank in the sludge return line (Chen et al., 2001). This system can represent almost 30-50% of excess sludge, offering a cost-efficient solution because neither metabolic inhibitor nor chemical/physical pretreatment of excess sludge is needed (Wang et al., 2015).

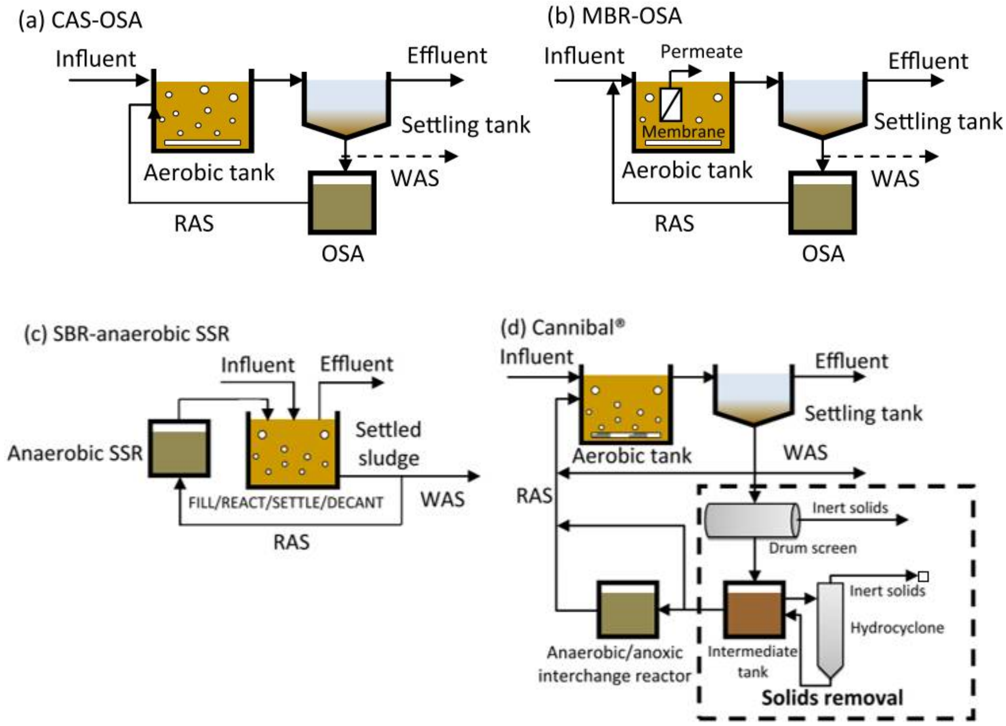


Figure 8 - Various configurations: CAS–OSA that routes 80-100% of the thickened sludge from the settling tank to the anaerobic or anoxic holding tank (a); MBR–OSA (b); SBR-anaerobic SSR that treats thickened sludge in the SSR R prior to its return to the SBR during the FILL stage (c); full-scale Cannibal with solids removal module (d) (Semblante et al., 2014).

Wei et al. (2003) have observed that in CAS system and OSA system, the sludge yields were found in the range from 0.13 to 0.29 kg SS/kg COD removed and from 0.28 to 0.47 kg SS/kg COD removed, respectively.

Chudoba et al. (1992a) and Chudoba et al. (1992b) suggested a theory that ATP content in the sludge depletes when the sludge is retained inside the anaerobic reactor. Indeed, when the sludge returns to the aerobic tank, ATP in the sludge would proliferate under the food-sufficient and aerobic conditions, this cyclic change in ATP leads to an energy uncoupling between anabolism and catabolism, resulting in sludge reduction.

The main limitations of OSA process is mainly attributed to a low oxidation-reduction potential (ORP) required in the SRR. Wang et al. (2015) suggested that sludge decay with the aid of low ORP in the sludge hold tank could be the major cause of the reduction in excess sludge in the OSA process (Chen et al., 2003). When the ORP level reaches - 250 mV, disintegration, sludge decay and solubilization could be accelerated effectively (Saby et al., 2003); though, this requirement could induce extra expense considering that the ORP control in an OSA process is usually associated with dosing with reductant or injection of pure nitrogen gas (Chen et al., 2003, 2001).

There are two theories about the mechanism involved in OSA process. The first one is related to the uncoupling mechanism, when occurs a depletion of cell energy in the form of ATP or food storage and the microorganisms stay in an oxygen-deficient and starved condition (Dawes and Sutherland, 1992) returning after that to aerobic conditions with nutrition supplied and re-synthesizing ATP storage (energy reserves) prior to biosynthesis (Chudoba et al., 1991). The cycle of alternative environments dissociates the anabolism from catabolism reducing the sludge production. The second theory is related to the sludge decay, that is accelerated effectively under a low ORP inside the anaerobic reactor, the increase in the sludge decay coefficient when induced by a low ORP is related to the low production rate of excess sludge in the OSA reactor (Chen et al., 2003).

Wang et al (2008) demonstrated that sludge decay is the main cause of sludge production reduction in OSA process accounting for 66.7% and also that sludge decay includes hydrolysis and acidogenesis of dead microorganisms and also particle organic carbon that is adsorbed in sludge floc and endogenous metabolism; they also concluded that there is energetic uncoupling in the OSA system since microorganisms were exposed to alternative anaerobic and aerobic environment, accounting for about 7.5% of sludge production reduction. .

Soluble chemical oxygen demand (sCOD) released from the anaerobic sludge tank in the OSA process is also used as substrate for cryptic growth, then the substrate is used for anoxic denitrifying, anaerobic phosphorus release, methane production and sulfate reduction. These anaerobic reactions inside the anaerobic reactor may lead to approximately 23% of sludge reduction in the OSA process. This way, an OSA process plant can reduce excess sludge production by 23% to 58% (J. Wang et al., 2008).

4.2.4 Microbial community in AS systems with side-stream reactors

Researches on microbial community structure in OSA process have been conducted generally with molecular biology methods (Ye and Li, 2010; Ye et al., 2008) and indirect methods, as the determination of the yield coefficient (Chen et al., 2003). On the other hand, these methods enable the detection of the abundant microbial species in function, but lack sufficient sequences to obtain comprehensive and also systematic information when compared with the vast genetic diversity that is present in the major part of AS systems (Hu et al., 2012; Ma et al., 2013).

Chudoba et al. (1992) found that 50 - 60% of microbial species present in OSA process was the phosphate accumulating organisms (PAOs) with low yield coefficient, and found that in the same way the existence of slow growers including denitrifying biomass and PAOs. Thereafter, Chen et al. (2003) reported that the sludge reduction reached in the OSA systems could not be associated to the domination of slow growers. However, Ye et al. (2008) found that no distinct shift in the diversity of the predominant species was found in microbial populations of the OSA systems.

Zhou et al. (2015) reported that *Anaerolineae* and *Actinobacteria* classes are responsible for fermentation and hydrolysis, while organic matter were enriched in SRR and played a significant role in the sludge reduction in the OSA process. In this research, the authors also used the Pyrosequencing developed by Roche 454 Life Science (Branford, CT, USA), that is a high-throughput analytical method that can generate huge amount of DNA reads in a thickly parallel sequencing-by-synthesis approach (Hu et al., 2012; Zhang et al., 2012). This technology has been widely used to analyze the microbial community in many WWTP, including MBR (Hu et al., 2012).

However, in the aforementioned experimentation, Zhou et al. (2015) observed an abundance of the phylum level, in total 15 identified phyla with predominance of *Proteobacteria* and *bacteroidetes*, accounting for 46.6-64.4% and 11.3-20.7%& of total effective bacterial sequences. Other dominant phyla (abundance >1%) were *Chloroflexi* (4.3-9.3%), *Firmicutes* (3.4-2.5%), *Planctomycetes* (1.3-3.8%) and *Actinobacteria* (1.5-4.3%). With low abundance it was also detected the presence of *Cyanobacteria*, *Elusimicrobia* e *Fibrobacteres*; concluding that one of the causes for sludge reduction in the OSA process is related to a change in the microbial species from fast growers to slow growers species. For example, *Trichococcus* (slow grower of fermentation bacteria) was founded in the anerobic fermentation system (Yasir et al., 2009), was unique in the SRR. Thus, the 454-pyrosequencing results reported the unique existence of predatory specie in the SRR, such as *Bacteriovorax* (Chen et al., 2012).

The different phyla in samples of anoxic OSA and anoxic-OSA systems performed by Zhou et al. (2015) are described in Fig. 9.

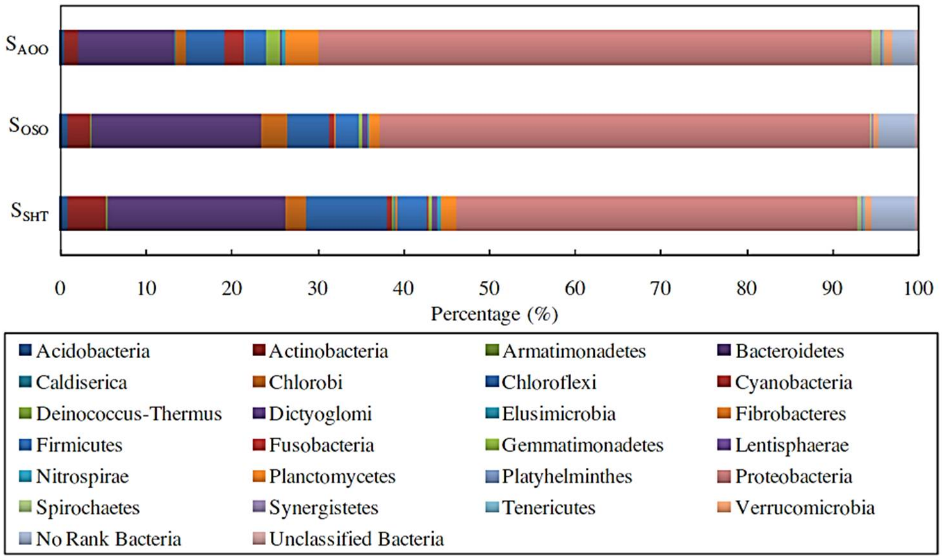


Figure 9- Abundances of different phyla in samples from anoxic OSA systems and anoxic-oxic systems, Where S_{OSO} (oxic tank of the anoxic OSA system), S_{SHT} (of the anoxic OSA system) and S_{AOO} (oxic tank of the Anoxic-oxic system) (Zhou et al., 2015).

4.3 Endogenous metabolism

Most of microorganisms can survive in the absence of nutrients, even for longer periods. The suspension of facultative/aerobic organisms in water consume oxygen and liberate CO_2, indicating that reserve materials within the cell are being oxidized to provide the energy necessary for life functions. The endogenous metabolism of a microorganism (Fig.10) is defined as the metabolic reactions that occur within the living cell when it is in absence of compounds that may serve as exogenous substrates (Dawes and Ribbons, 1961).

Besides, the concept of endogenous metabolism can be described as the observation that storage compounds are used by the cells for maintenance purposes when the external substrate is effectively depleted, *i.e.* a state when no net growth is possible, but cells consume energy to remain viable (Foladori et al., 2010b). In other words, by increasing energy requirements for non-growth activities (e.g., maintenance functions), the quantity of energy available for the growth of biomass decreases, meaning that an effective reduction of sludge production is reached by maximizing the energy used for maintenance requirements rather than for cellular synthesis (Low and Chase, 1999).

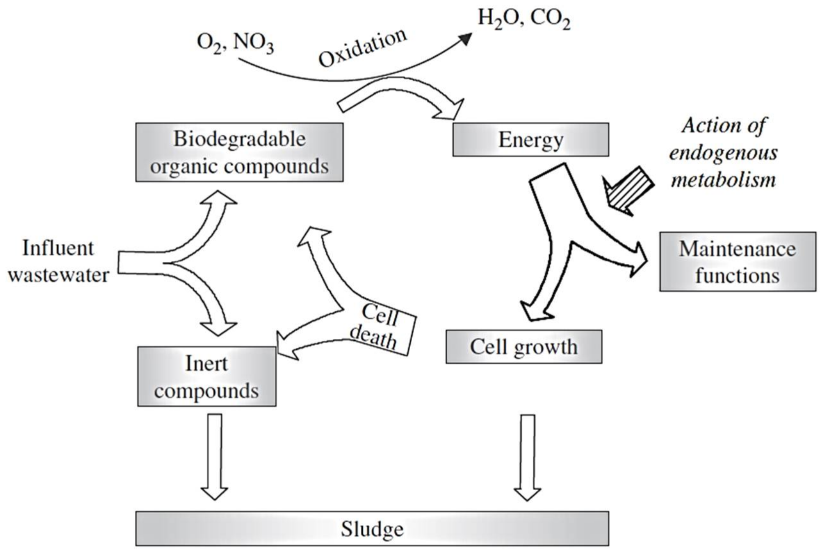


Figure 10 - Effect on endogenous metabolism in the scheme of sludge production (Foladori et al., 2010b).

The maintenance functions in the absence of substrates performed during the endogenous metabolisms are: (a) serving as a source of energy for the cells; (b) performing special functions, *e.g.* furnishing a source of reducing power in some phototrophic and chemoautotrophic bacteria, or a source of sulfur or phosphorus in organisms that store sulfur or volutin granules; (c) providing carbon substrates for resynthesis of degraded cellular constituents (Dawes and Ribbons, 1961).

4.3.1 MBR

A membrane is a material that performs the separation between the liquid phase and other components, that depending on their sizes, pass through the membrane pores or are retained within the system. The most used membranes in wastewater treatment applications, are the microfiltration (MF) and ultrafiltration (UF) membranes. The UF membranes have smaller pores and retain some viruses, while the MF membranes reject particulate matter and retain some bacteria.

Membrane bioreactors (MBR) are the combination between the biological treatment and the membrane separation. According to Judd (2008), the main benefits of an MBR process are:

- Production of a high quality, clarified and disinfected permeate production;

- Absolute control of solids retention time (SRT) and hydraulic retention time (HRT) parameters;

- Operation at a higher mixed liquor suspended solids (MLSS) concentrations, which reduces the required reactor size and enhances ammonia removal developing specific nitrifying bacteria;

- Reduced excess sludge production;

- Operation at a longer sludge retention time (SRT) selecting slow-growing bacterial with possible enhanced treatment.

MBR process has many advantages over CAS processes, *e.g.* less sludge production, flexibility of operation, small footprint and excellent effluent quality (Visvanathan et al., 2000). However, MBR process has also problems encountered under high SRT operation, as poor oxygenation leading to increased aeration costs, high viscosity, weak and small sludge flocs, and membrane fouling that requires frequent maintenance and cleaning (Wei et al., 2003).

4.4 Predation on bacteria

The principle of this method is using predation to reduce the excess sludge production, according to the food chain theory, this mechanism happens due to the metabolic maintained needs and higher living organisms' formation (Guo et al., 2013). Biological wastewater treatment is considered an artificial ecosystem and there are many other microorganisms in addition to bacteria, where the bacteria are predated by protozoa e metazoa, resulting in the excess sludge reduction.

Protozoa represents <1% of the total dry-weight of a wastewater biomass, and can be divided into four groups: ciliates (represent 70% of all protozoa), flagellates, heliozoa and amoeba (Eikelboom, 2000, 1988; Ratsak et al., 1996). Metazoa are normally nematoda and rotifera types and both protozoa and metazoa in the past were usually used as indicators of efficiency and process performance in wastewater treatment (Wei et al., 2003).

The most commonly used protozoa/metazoa are *T. tubifex, Lumbriculus variegates* and *Tubificidae*. These microorganisms may reduce the sludge production of 12-75%, this mechanism happens under a long sludge retention time, considering that the protozoa and metazoa need sufficient time to grow (Wang et al., 2017).

4.4.1 Two-stage predation system

Even if the use of predators in wastewater treatment systems could have good results, researchers found that the distribution and application of predators are uncontrollable (Khursheed and Kazmi, 2011). To solve this issue, a two-stage system was proposed by Ratsak (1994), where a pattern (first stage) is referred to the bacterial stage in a mixed reactor without biomass retention (to stimulate the growth of dispersed bacterial). The second one is designed as a predator stage with a long SRT for the growth of protozoa and metazoa, in these cases about 60-80 % sludge yield was reduced in the second stage when compared to the first stage (Lee and Welander, 1996).

Ratsak et al. (1996) studied the predation on bacteria induced by the ciliate *T. pyriformis* grazing on *P. fluorescens* in a two-stage pure culture chemostat-system, and observed at least 12-43% of biomass reduction.

4.4.2 Oligochaeta

Recently, many researchers are focusing on the use of oligochaetes on sludge reduction. The main types of worms present in trickling filters and AS systems are *Aeolosomatidae, Naididae* and *Tubificidae* (Wei and Liu, 2005). They also concluded that the presence of worms in aerobic wastewater treatment may lead to a significant sludge reduction, however this technique is still uncontrollable in WWTP because of the instability of worm growth. In their study, they developed a new bioreactor for worm growth consisting of three sections, one for free swimming worm growth, one for sessile worm growth and one for sludge settling.

Tubifex worms (a genus from the family *Tubificidae*) are specific in the fact that they build tubes, where they attach themselves within the tube and wave their posterior end in the water to make more oxygen available to their body surfaces and circulate the water. Most of these worms are red (presence of hemoglobin in their blood) and may be very abundant in environments where other macroinvertebrates are absent. They can subsist in very low oxygen levels and live with no oxygen at all for short periods. *Tubifex* is frequently found in polluted streams, and feed on sewage (Spellman, 2003).

Chapter 5

The side-stream reactor combined to MBRs

Considering the need to reduce the excess sludge production, Westgarth (1963) demonstrated that when an adequate exposure time inside the anoxic reactor and sufficient food in the aerobic reactor were maintained, more than 50% of excess sludge could be reduced by a full-scale OSA process. In this context, the combination of an SSR with the MBR can provide an interesting result regarding to the excess sludge reduction.

5.1 Probable mechanisms of excess sludge reduction

5.1.1 Energetic uncoupling

The energetic uncoupling is one of the main mechanisms of the reduction of excess sludge in a side-stream reactor. The cycling between aerobic conditions, rich of substrate, and anaerobic conditions (complete absence of new substrate), results in the uncoupling of the catabolic phase from the anabolic phase, reaching an efficient reduction of the cell growth yield.

Chudoba and Morel (1992) specified this separation phenomenon describing the role of an intermediate between catabolism and anabolism: adenosine triphosphate (ATP) that is a compound possessing three phosphate bounds well provided in free energy. They highlighted that the reactions of catabolism are someway controlled by energy consumption (anabolism), concluding that the growth yield may be reduced by conditions of energetic uncoupling between catabolic and anabolic processes, what is expressed in Fig. 11.

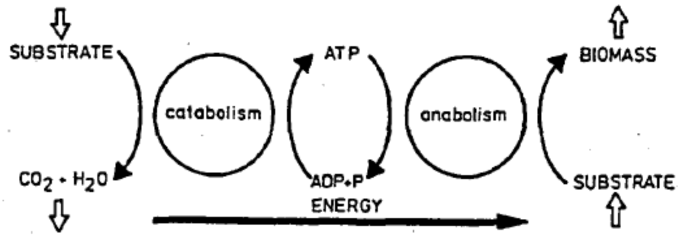


Figure 11 - Simplified relationship between catabolism and anabolism (Chudoba, 1991).

Chen and Liu (1999) proposed a sludge “fasting/feasting" explanation to understand the sludge phenomenon in the OSA process. Fasting refers to the insufficiency of food in an exposure of the settled sludge to an anoxic environment, causing an stressful condition that starve the microorganisms (Maruyama et al., 1971), leading to a depletion of ATP (cell energy) or food storage (Dawes and Sutherland, 1992). Thus, Feasting means that when the fasted microorganisms return to an oxic environment where they have available food, they may achieve a harvest of cell energy, storing energy in form of ATP (Chen et al., 2001). This cycle is able to yield a disassociation of energy level between anabolism and catabolism, resulting in energy uncoupling to regulate cell metabolism (Forrest, 1969).

5.1.2 Endogenous metabolism / decay

The endogenous metabolism is usually described as the observation of storage compounds used for maintenance purposes when the external substrate is completely depleted, *i.e.* a state when no net growth is possible, but even so, the cells consume energy to remain viable. This phenomenon occurs, mainly, because when external substrate is available, the energy obtained from the substrate biodegradation is used for maintenance requirement of bacteria (*e.g.* renewal of proteins, membrane potential, motility etc.); though, in the absence of external substrate, only a part of cellular constituents can be oxidized to CO_2 and water to produce the energy needed for cell maintenance requirements (Foladori et al., 2010b).

Van Loosdrecht and Henze (1999) defined the endogenous respiration as the "respiration with oxygen or nitrate using cell internal components". Besides, a great reduction of sludge production can be achieved by increasing the energy used for maintenance requirements rather than for cellular synthesis (Low and Chase, 1999). Foladori et al. (2010) also highlighted that sludge production is lower in AS plants with log SRT and operating at a low applied loads or low food-to-microorganisms (F/M) ratios.

5.1.3 Extracellular Polymeric Substances (EPS) destruction

The extracellular polymeric substances are an important factor to floc formation, they are composed of several kinds of biopolymers, as protein, polysaccharide, glycoprotein and glycolipid; forming a matrix that hold the microorganisms together (flocs). The relative levels of these polymers and their surface functional groups facilitate electrostatic and gel-like interactions between the sludge flocs present in the reactor, increasing the bound water content, that influences directly the physicochemical properties of EPS that is important for the cohesion of sludge flocs (Marinetti et al., 2010). A decrease in the concentration of these polymeric substances reduces the strength of sludge flocs, decreasing also the viscoelastic properties (Tang and Zhang, 2014).

5.2 Main process parameters

5.2.1 Effect of the Solid Retention Time

Also known as sludge retention time, the SRT is an important parameter for excess sludge reduction. Liu and Tay (2001) defined this parameter as the average time a unit of biomass remains inside the treatment system and showed that this is the most operational parameter in AS processes. Besides, they highlighted that SRT is inversely related to the specific growth rate (Y_{obs}).

Lawrence and McCarty (1970) described the SRT by Eq. 9:

$$\frac{1}{Y_{obs}} = \frac{1}{Y_{max}} + \frac{SRT \cdot k_d}{Y_{max}} \quad (9)$$

Where Y_{max} is the true growth yield and k_d the specific endogenous rate. The Eq. 9 provides a theoretical basis, relevant to controlling total sludge production by adjusting the SRT during the biological treatment and showing that the observed growth yield is inversely dependent on the SRT and endogenous rate in steady state AS process.

Besides, Foladori et al. (2010) specified two different ways to calculate the SRT (Eq. 10, 11), defined as the mass of solids in the AS reactors divided by the solids removed daily. Those are:

a) when excess sludge is taken from the secondary settler:

$$SRT\ (d) = \frac{V \cdot x}{Q_s \cdot x_s} \quad (10)$$

b) when excess sludge is taken from the reactor, SRT can be calculated dividing V by Q_s, because, in this case $x = x_{s:}$

$$SRT\ (d) = \frac{V \cdot x}{Q_s \cdot x} = \frac{V}{Q_s} \quad (11)$$

Where:
V = volume of biological reactors (m^3);
X = TSS concentration in biological reactors ($kgTSS/m^3$);
Qs = daily excess sludge flow rate (m^3/d);
x_s = TSS concentration in excess sludge flow ($kgTSS/m^3$).

Pollice et al. (2008) investigated the process conditions with different SRT values and observed that filtration characteristics are minimized in the SRT between 40 and 80 days. Therefore, the viscosity seems to be more sensitive above 60 days, suggesting that MBR can be conveniently operated at SRT higher than 40 days without relevant drawbacks in terms of cleaning needs, filterability and biological activity.

The SRT can change the state of biomass in an AS system (Chaize and Huyard, 1991) and the concentration of MLSS in the bioreactor increased with SRT (Yamamoto and Win, 1991). Some MBR plants were operated with an infinite SRT aiming to maintain large amounts of biomass, however, is expected that the treatment efficiency cannot be linearly proportioned to biomass concentration because the specific bioactivity can be reduced at substrate deficient states (Han et al., 2005).

The SRT value is important for better biological nutrient removal processes (BNR), meaning a good economical choice for nitrogen and phosphorus removal (Randall et al., 1992) and having significant effects on biological activity and membrane performance. Nevertheless, a different approach to achieve a lower sludge production is to enhance the cryptic growth that is in other words, the microbial growth on lysates (Liu and Tay, 2001).

Rosenberger et al. (2000) highlighted the prospect to reduce the cell growth operating in an infinite SRT, possible in MBR systems. Even if, in practical conditions, is not possible to maintain in every cases a high value of SRT, due to negative effects that can change the sludge m characteristics (Wei et al., 2003). Fig. 12 compares Y_{obs} to the SRT in some results obtained from experiments with activated sludge.

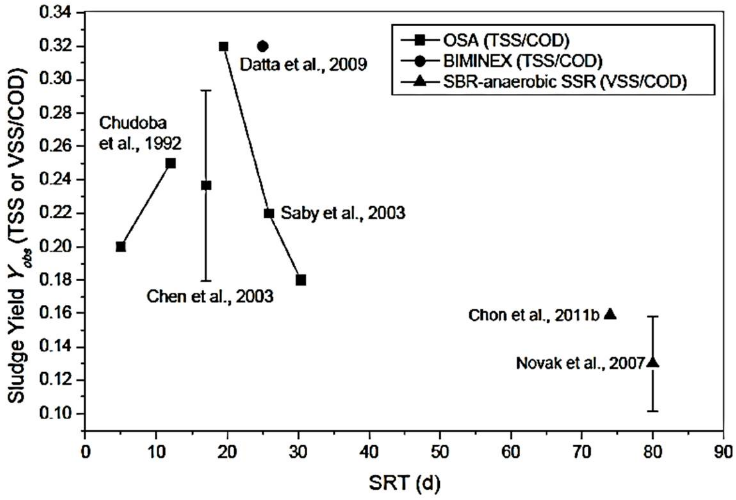


Figure 12 - Y_{obs} compared to SRT by different authors (Semblante et al. 2014).

5.2.2 Effect of the Redox Potential

The ORP is one of the main parameters in an anaerobic reactor, mainly because when sludge is under low ORP values is observed a growth of the cell death speed. Saby et al. (2003) found a better reduction (between 23 and 58%) when inserting an OSA reactor in an MBR plant, reducing the ORP values from +100 to -250. Chen et al. (2003) have indicated the low ORP as the main and only reason of the sludge reduction when fasting/feasting, reaching a result of 36 and 58% of reduction, comparing to a CAS, with ORP -250 and +100 respectively.

Takdastan et al. (2014) explored the influence of ORP in the OSA process for sludge reduction using a sequencing batch reactor (SBR) and a synthetic influent at COD 600 mg/L to control the efficacy of uncoupled metabolism in cell growth yield (Y), keeping the sludge inside the reactor from 1-8 hours varying the ORP values. The results obtained considering ORP from -36 to -246 mV are the ones in the Fig. 13 showing the reduction of Y_{obs} with the increase of SRT values.

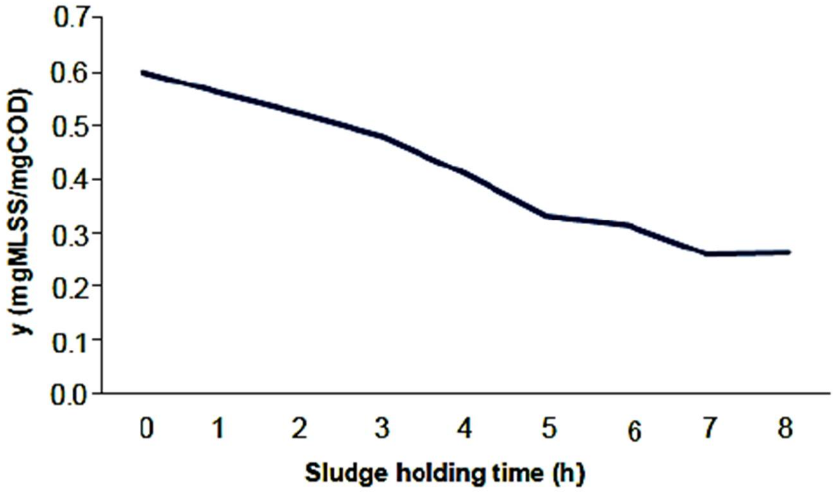


Figure 13- The effect of various ORP on biomass yield at different sludge holding times. Takdastan et al. (2014).

5.2.3 Effect of temperature

High temperatures are one of the factors that provides the uncoupled metabolism; *e.g.*, in an abnormal environment, microorganism cells undergo an uncoupled metabolism, reducing the production of excess sludge (Chen et al., 2002). Based on this statement, a high temperature can induce an uncoupled metabolism. Zhang et al., (2006) reached an optimal condition at 29°C. This can be explained by the fact that a higher temperature could harm microorganism activities, decrease the cell particle diameter to become soluble substances or decrease the viscosity of sludge flocs, resulting in a high percentage of cell death or decomposition. Furthermore, a high temperature might increase the degree of sludge decay or enhance the uncoupling metabolism (Yang et al., 2011).

5.2.4 Effect of HRT

The HRT is an important parameter when considering the sludge reduction effect and can vary according to certain conditions of the plant. The best HRT is the one able to achieve a good effectiveness for sludge reduction without affecting the effluent quality or the performance of the process. Yang et al. (2011) found a suitable HRT around 8 - 9.7h, with optimal condition for sludge reduction at HRT 9.1h; while Wang et al. (2008) found values between 8 - 12 h. Thus, Yang et al. (2011) concluded that around 9h an uncoupled metabolism or sludge decay (or even the combination of both factors) could play a critical role in excess sludge reduction.

5.3 Characteristics of combined process with SSR and MBR

The combination of an SRR and the MBR provides many mechanisms for reduction of excess sludge as the endogenous metabolism from the MBR and the processes in the side-stream reactor as the mechanisms described in the Chapter 4. Some authors, as Chen et al. (2003) and Saby et al. (2003), studied the combination of a side-stream reactor and an MBR and they have concluded that this combination have a sludge production yield about 2.3 - 3.6 g/day, that is 23 - 51% lower than an MBR system. Saby et al. (2003) obtained values of Y_{obs} of 0.18 - 0.32 (SST/COD) for the SSR+MBR system, that were 20 - 55% lower than the only MBR system (0.40 SST/COD). In both studies, the ORP values were between -250 to +100 mV.

Saby et al. (2003) observed a decrease of the Y_{obs} based in many configurations of OSA systems and concluded that the MBR-OSA has the minor cell growth yield with SRT of 30.4 days and ORP -250mV. Observing the reduction of Y_{obs} (0.42. 0.32. 022 SST/COD) and the growth of SRT values (19.5, 25.9, 30.4 days). Furthermore, they compared the sludge production to the ORP values in the MBR plant and the MBR + OSA plant and concluded that with the OSA system the excess sludge production decreases as the ORP also decreases.

Based on the comparison made by Takdastan et al., (2014) in Table 8, is possible to conclude that between the various configurations of OSA process, the one combined with MBR has the lowest value of Y_{obs}, with SRT 30.4 days and ORP -250mV.

Table 8: Literature studies of OSA techniques for reducing excess sludge (Takdastan et al., 2014).

Operating condition	$Y\ \frac{(mgBiomas)}{mg\ \ COD}$	Effluent quality	References
Pilot scale OSA system, COD = 300mg/l			
1. Conventional activated sludge	0.4	Good	Saby et al. (2003)
2. OSA system at ORP = +100mV	0.32	Excellent	
3. OSA system at ORP = -100mV	0.22	Excellent	
4. OSA system at ORP = -250mV	0.18	Excellent	
Pilot scale OSA system, COD = 365mg/l			
1. Conventional activated sludge	0.53	Good	Wang (2008)
2. OSA system ORP = -250mV	0.38	Excellent	
Pilot scale OSA system under anoxic-anaerobic zone, COD = 600mg/l			
1. At ORP = -30mV	0.6	Good	Current research
2. At ORP = -80mV	0.56	Good	
3. At ORP = -190mV	0.41	Good	
4. At ORP = -230mV	0.31	Excellent	
5. At ORP = -246mV	0.26	Excellent	

In these processes, is important to highlight the effect of EPS in the membrane fouling; considering that EPS, excreted by bacteria (membrane walls) or SMP (soluble microbial products), are the main factors in membrane fouling (Wang et al., 2009).

Chapter 6

Experimental application of MBR Process integrated to an SSR: Materials and methods

The feasibility to implement an anaerobic SRR in a submerged MBR (sMBR) with a pre-denitrification scheme was examined in this study. The whole experiment was divided in two phases, named "Phase 1" and "Phase 2". In Phase 1, two plant configurations (different from the original MBR layout) were considered, involving a different placement of the SRR in the pre-denitrification MBR layout. Fig. 14 illustrates the original MBR layout (Fig.14a) as well as the two investigated alternatives, implementing the SRR in the side stream (Fig.14b) or in the mainstream line (Fig.14c). Thus, a comparison between ASSR configuration with the AMSR demonstrated that, although the waste sludge reduction was higher in the ASSR (72%), approximately 30% of excess sludge minimization could be achieved in the AMSR configuration, with 6 hours of hydraulic retention time (HRT) in the anaerobic reactor.

In Phase 2, a novel layout for MBR system was proposed with the aim to achieve excess sludge minimization in an anaerobic mainstream reactor (AMSR) configuration, while preserving the membrane permeability. More precisely, was performed a modification of a conventional pre-denitrification scheme, consisting in the placement of an anaerobic reactor in the mainstream between the anoxic and the aerobic reactor. In this system, a portion of activated sludge from the anoxic reactor, with a rate approximately equal to the influent flow, before of going to the aerobic reactor passed through an anaerobic reactor, where strictly anaerobic conditions were imposed. In this reactor, because of the lack in substrate availability and the anaerobic starvation, uncoupling metabolism occurred, thereby favoring the achievement of low biomass yield. Nonetheless, the speculation that an increase in the HRT could be beneficial to enable higher levels of sludge reduction was valuated. Moreover, in the AMSR configuration a significant increase in nutrients removal performances was obtained, also suggesting the possibility to achieve biological phosphorous removal. In conventional BNR systems, where the anaerobic tank is placed as the more upstream reactor, PAO microorganisms can use low fatty acids that are present in the sewage, releasing phosphate to the surrounding liquid (Zuthi et al., 2013). In contrast, in the AMSR configuration the anaerobic tank is placed downward the anoxic reactor, in which the rapidly biodegradable carbon was already depleted for denitrification, phosphorous release by PAO microorganisms would occur under endogenous conditions because of the low availability of residual carbon source. In this respect, the mechanism of phosphorous release and kinetics in the AMSR configuration should be better investigated.

6.1 Materials and Methods – Phase 1

6.1.1 Pilot plant configuration

The original MBR layout (Fig.14a) was realized according to a pre-denitrification scheme. It consisted of one anoxic (18 L) and one aerobic tank (24 L). The MBR plant was fed in continuous mode with a flow rate of 2.3 L h^{-1}. The mixed liquor was pumped to the anoxic tank via an internal recycling characterized by a flow rate equal to 11.5 L h^{-1} (RAS). The solid-liquid separation phase was achieved by an ultrafiltration hollow-fiber membrane module (PURON® Single bundle Demo, nominal pore size 0.03 µm, membrane area 0.47 m^2) placed within the aerobic tank in a submerged configuration. The membrane flux was maintained to approximately 4.9 L m^{-2} h^{-1}. The filtration cycle had a duration equal to 6 minutes, divided into 5 minutes of permeate extraction and 1 minute of backwashing. The membrane backwashing was carried out by pumping a volume of permeate back through the membrane fibers from the Clean in Place (CIP) tank.

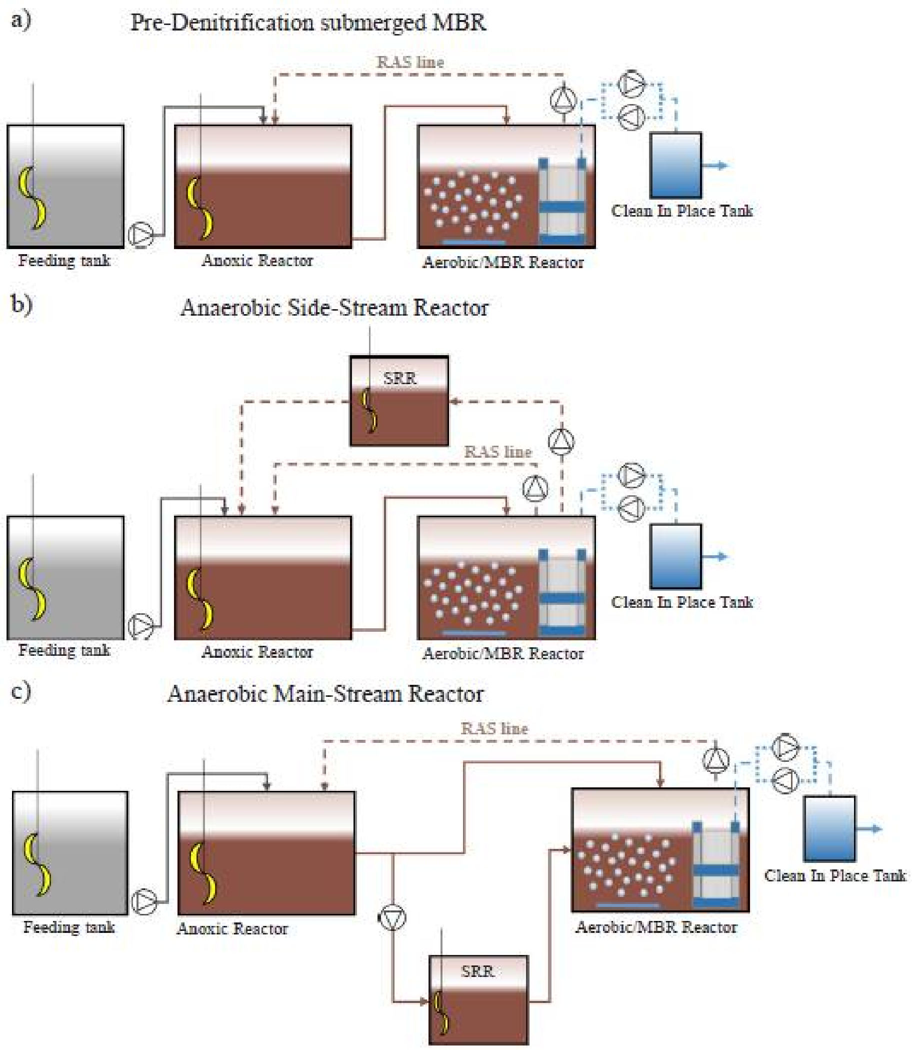


Figure 14 - Pilot plant layout: submerged MBR with pre-denitrification scheme (a); MBR with an anaerobic side-stream reactor (MBR+ASSR) (b); MBR with an anaerobic main-stream reactor (MBR+AMSR) (c).

In the configuration depicted in Fig. 14b, the RAS line from the aerobic to the anoxic tank was pumped first into an SRR (13.8 L volume) with a flow rate equal to 2.3 L h^{-1} and then recycled to the anoxic reactor. As above discussed, this configuration was named anaerobic side-stream reactor (ASSR).

In the configuration depicted in Fig. 14c, the SRR was placed in the main stream, between the anoxic and the aerobic tank. This configuration was named anaerobic main-stream reactor (AMSR), as previously mentioned.

In both configurations, the interchange rate (the rate of solids passed through the SRR) was 100% and the hydraulic retention time (HRT) in the SRR was 6 hours, equal to the 25% of the entire plant HRT. The anoxic and the SRR were continuously mixed by a mechanical stirrer. In the aerobic/membrane reactor, the oxygen was supplied by an air blower connected to a fine bubble diffuser placed at the bottom of the reactor. Furthermore, the same air blower supplied air to the membrane for fibers scouring, in order to mitigate the fouling extent.

6.1.2 Experimental campaign

The MBR was monitored for 153 days. The experimental campaign was divided into three periods, named MBR (I), MBR+ASSR (II) and MBR+AMSR (III), during which the MBR plant was operated according to the configurations above described. Specifically, the MBR operated with the conventional pre-denitrification scheme for 18 days, until steady conditions in terms of nutrient removal performance and excess sludge production were achieved. During this period, the excess sludge production was evaluated in terms of observed heterotrophic growth yield (Y_{obs}) and this latter was assumed as the reference value to evaluate the impact of the other plant configurations in terms of sludge minimization efficiency. Hereafter, the MBR operated in ASSR configuration for 45 days until steady-state excess sludge production was achieved. Lastly, the MBR operated in AMSR configuration for 90 days until the end of the experiment.

The MBR was seeded with activated sludge collected from a municipal WWTP with a conventional activated sludge scheme (inoculum TSS equal to 3.63 gTSS L^{-1}). The sludge retention time (SRT) was not controlled and no dedicated wasting operations of sludge were carried out, excepting the samples withdrawn to perform chemical-physical analyses. Approximately 100 mL of mixed liquor were withdrawn daily, thereby resulting in an SRT more than 500 days. Therefore, it can be assumed that the pilot plant was operated with a complete sludge retention strategy. The achievement of steady state conditions in each phase was evaluated basing on the biological performance, kinetic parameters and sludge production.

The MBR was fed with synthetic wastewater during the entire experiment. The synthetic wastewater composition was (in 100 L of tap water): 4.5 g of peptone, 15 g of sodium acetate (CH_3COONa), 4 g of urea (CH_4N_2O), 14.5 g of ammonium chloride (NH_4Cl) and 6 g of dipotassium phosphate (K_2HPO_4). Table 9 summarizes the average features of the influent wastewater as well as the main operating conditions throughout experiments.

Table 9: Summary of the wastewater characteristics and operating conditions of the MBR pilot plant during the experiment.

Parameter	Unit	MBR	MBR + ASSR	MBR + AMSR
		Value		
Total COD	[mg L^{-1}]	521 ± 13	539 ± 14	523 ± 11
Soluble COD	[mg L^{-1}]	411 ± 21	423 ± 16	419 ± 14
Total nitrogen (TN)	[mg L^{-1}]	47 ± 6	50 ± 4	49 ± 3
Total phosphorous (TP)	[mg L^{-1}]	3.7 ± 1.2	4.0 ± 0.6	3.8 ± 0.3
Flow rate	[L h^{-1}]	2.3	2.3	2.3
SRT	[d]	∞	∞	∞
HRT	[h]	18	24	24
Duration	[d]	18	45	90

6.2 Materials and Methods – Phase 2

6.2.1 Pilot plant configuration

The experiment was carried out in an MBR pilot plant operating at room temperature (20 ± 6 °C). The MBR was realized according to a pre-denitrification scheme, consisting of an anoxic reactor followed by an aerobic one, each of 22.5 L of volume. The influent wastewater was pumped from a continuously stirred storage tank to the anoxic reactor with a flow rate of 2.4 L h^{-1} in continuous mode. Then, the mixed liquor passed from the anoxic reactor to the aerobic by gravity through a bottom opening. From the aerobic reactor, the mixed liquor was returned to the anoxic tank via an internal recycling characterized by a flow rate equal to 13.5 L h^{-1} (RAS). The solid-liquid separation phase was achieved by an ultrafiltration (UF) hollow-fiber membrane module (PURON® Single bundle Demo, nominal pore size 0.03 µm, membrane area 0.47 m^2) placed within the aerobic tank in a submerged configuration. The UF membrane was operated at constant flux of approximately 8.4 L m^{-2} h^{-1}. The extraction of the permeate was periodically stopped for 1 minute every 5 minutes to allow the membrane backwashing. The membrane backwashing was carried out by pumping a volume of permeate back through the membrane fibers from the Clean in Place (CIP) tank, with a flow rate of approximately 4.32 L h^{-1}. In the aerobic/membrane reactor, the aerobic conditions were maintained through oxygen supplying, which was provided by an air blower connected to a fine bubble diffuser placed at the bottom of the reactor. Furthermore, additional air was supplied air to enable the scouring of the membrane fibers, with the aim to mitigate the fouling extent.

Subsequently, the pre-denitrification scheme was modified by placing a sludge retention reactor, maintained under anaerobic conditions, between the anoxic and the aerobic ones. This configuration was named anaerobic mainstream reactor (AMSR). More precisely, a portion of activated sludge flow from the anoxic reactor, with a rate approximately equal to the influent flow (4.32 L h^{-1}), before of going to the aerobic reactor, passed through the anaerobic reactor that was continuously mixed by a mechanical stirrer. Different HRT in the anaerobic reactor were studied during the experiment. In detail, HRT of 6 h, 8 h and 10 h were imposed by increasing the reactor volume, while maintaining the same influent flow coming from the anoxic reactor. The activated sludge from the anaerobic reactor was pumped to the aerobic one with the same flow rate of the inlet.

6.2.2 Experimental campaign

The MBR was seeded with activated sludge collected from a municipal WWTP with a conventional activated sludge scheme (inoculum TSS equal to 6.15 gTSS L^{-1}) and it was fed with synthetic wastewater during the entire experiment (de Oliveira et al., 2018). The MBR was monitored for 198 days, divided into four periods, named Period 1 (56 days), Period 2 (49 days), Period 3 (49 days) and Period 4 (44 days). Specifically, during Period 1 the MBR operated with the conventional pre-denitrification scheme for 56 days, until steady conditions were achieved. In this period, for the first 21 days, the sludge retention time (SRT) was not controlled and no dedicated wasting operations of sludge were carried out, to enable the activated sludge adaptation to the synthetic medium and the new plant configuration. To avoid the activated sludge ageing, during the remaining 35 days, a known amount of sludge was daily withdrawn, including the samples for physical-chemical analysis, with the aim to maintain an SRT of approximately 35-40 days. The same SRT was imposed during the following experimental periods. In this period, the excess sludge production was evaluated in terms of observed heterotrophic growth yield (Y_{obs}) and the latter was assumed as the reference value to evaluate the sludge minimization efficiency achieved in the other experimental periods.

In Period 2 the MBR operated in AMSR configuration for 49 days with an HRT in the anaerobic reactor of 6 h. When steady-state excess sludge production was achieved, the HRT was increased to 8 h and 10 h in Period 3 and Period 4, respectively. Because of the relatively long SRT applied, the achievement of steady state conditions in each period was evaluated based on the biological performance, kinetic parameters and excess sludge production, instead of basing on the conventional time equal to three times the SRT.

Table 10 summarizes the main operating conditions and the average characteristics of the influent wastewater throughout experiments.

Table 10: Summary of the wastewater characteristics and the main operating conditions of the MBR

Parameter	Unit	Period 1	Period 2	Period 3	Period 4
		Value	Value	Value	Value
Soluble COD (SCOD)	[mg L^{-1}]	440±18	477±21	566±13	571±15
Ammonium nitrogen (TN)	[mg L^{-1}]	41±3	40±5	41±4	43±3
Total phosphorous (TP)	[mg L^{-1}]	11.8±1.6	12.4±1.3	11.5±0.8	11.0±0.9
Influent flow rate	[L h^{-1}]	2.4	2.4	2.4	2.4
Food to microorganism (F/M)	[kgCOD $kgTSSd^{-1}$]	0.08±0.02	0.08±0.01	0.09±0.02	0.12±0.01
SRT	[d]	∞ - 35/40	35/40	35/40	35/40
Total plant HRT	[h]	18.75	24.6	24.6	24.6
Volume of AMSR	[L]	-	14.4	19.2	24
AMSR HRT	[h]	-	6	8	10
Period duration	[d]	56	49	49	44

6.3 Analytical methods

All the chemical-physical analyses including total and volatile suspended solid (TSS, VSS) concentrations, total chemical oxygen demand (TCOD), total nitrogen (TN), ammonium nitrogen (NH_4-N), nitrate nitrogen (NO_3-N), nitrite nitrogen (NO_2-N) and total phosphorous (TP) were performed according to standard methods (APHA, 2005). TSS and VSS were measured in the mixed liquor of all the reactors. The COD, TN, NH_4-N, NO_3-N, NO_2-N were measured at the inlet and at the outlet of each reactor as well as in the permeate. Specifically, the TCOD was measured in the supernatant of mixed liquor samples (after centrifugation at 4000 rpm for 30 minutes). Dissolved oxygen (DO) concentration, oxidation-reduction potential (ORP) and pH were measured in all the reactors by means of specific probes (WTW 3310).

6.3.1 Evaluation of biomass growth and heterotrophic kinetic parameters

The effectiveness of the implemented process configurations to sludge minimization was evaluated in terms of reduction in the heterotrophic growth yield.

The observed heterotrophic yield coefficient (Y_{obs}), was calculated through mass balances between sludge withdrawn and sludge production, dividing by the cumulated TCOD removed, according to the literature (Torregrossa et al., 2012) (Eq. 12):

$$Y\text{obs} = \frac{[(X2 - X1)V + X\text{s}V\text{s}]}{(TCOD\text{in} - TCOD\text{out})Q} \qquad [gVSS\ gCOD\text{-}1] \tag{12}$$

Where X_2 and X_1 are the biomass concentrations (g VSS L^{-1}) at day (n) and (n-1), V is the working volume of the reactor, Q is the influent flow, Xs is the concentration of the waste biomass (gVSS L^{-1}), Vs is the volume of waste sludge on a daily base, COD_{in} and COD_{out} are the influent and effluent TCOD concentration (g L^{-1}), respectively. The Y_{obs} was calculated in each reactor (anoxic, aerobic and SRR) and the average value was determined.

The heterotrophic kinetic parameters, including the endogenous decay coefficient (b_H), the net growth coefficient (μ_H), the yield coefficient (Y_H) and the active fraction of the heterotrophic biomass (f_{XH}), were evaluated according to the literature (Capodici et al., 2016).

Moreover, specific batch tests aimed at assessing the PAO kinetics in terms of phosphate release and uptake rates were carried out at the end of each experimental period. More precisely, these tests were performed in batch reactors (1.5 L) at controlled temperature (20 ± 0.1 °C). A known volume of mixed liquor was withdrawn from the anoxic reactor and put in the batch reactor where it was diluted with the permeate in order to obtain a TSS concentration of approximately

3 gTSS L^{-1} (2.1 gVSS L^{-1}). The sample was continuously mixed through a magnetic stirrer.

The sample was maintained under endogenous conditions until nitrates were completely depleted. At this point, a known amount of sodium acetate was added, in order to obtain a COD concentration of approximately 200±20 mg L^{-1}. The ORP was continuously monitored in order to ensure the achievement of anaerobic conditions (ORP <-150 mV). Successively, samples were taken at regular time interval (15-20 minutes) and PO_4-P and COD was measured after filtration through 0.45 µm membrane. Sampling was stopped when the phosphate release reached its maximum value. Hereafter, the batch reactor was aerated and the oxygen concentration was maintained close to the saturation value (9 mg L^{-1}). During this phase, phosphate uptake occurred very rapidly, thus the sampling interval was increased (10 minutes) until all the phosphate concentration was close to 1 mg L^{-1}.

The phosphate release rate was calculated in the anaerobic period as the ratio between the variation of the phosphate concentration and the time interval during which the release occurred. More precisely, the P-release was calculated both in the presence of external COD (named exogenous P-release) and in absence of this (named endogenous P-release). The exogenous P-release was calculated as the release occurred until the COD in the batch reactor was completely depleted, whereas the endogenous P-release was measured as the P-release occurred from that time onward.

6.3.2 Activated sludge characterization

The physical properties of the activated sludge were evaluated in terms of extracellular polymeric substance (EPS) content and composition, as well as flocs size and morphology.

The EPS extraction was carried out according to the Heating Method described by (Le-Clech et al., 2006). Briefly, the microbial products (SMPs) were obtained by centrifugation of a 50 mL of mixed liquor sample at 5,000 rpm for 5 min, whereas the bound EPS

(EPSBound) content was extracted by means of the thermal extraction method (10 minutes of thermal bath at 80°C followed by a centrifugation at 7,000 rpm at 4°C for 10 minutes). Both supernatants obtained from the first and the second centrifugation were filtered through a 0.22 µm membrane in order to obtain a free-cells sample. Thereafter, for both SMP and EPS, the polysaccharides and protein concentrations were determined according to the phenol-sulphuric acid method with glucose as the standard (Dubois et al., 1956) and by the Folin method with bovine serum albumin as the standard (Lowry et al., 1951), respectively.

The size and morphology of the activated sludge flocs were examined by means of a high-speed image analyses sensor (Sympatec Qicpic) that provided the particle size distribution and the granulometric curve.

6.3.3 Membrane fouling analysis

The membrane fouling analysis was performed by measuring the total resistance to filtration (R_T) according to the following expression, derived from the general form of the Darcy's law (Eq. 13):

$$R_T = \frac{TMP}{\mu \cdot J} \tag{13}$$

Where, R_T is the total fouling resistance (10^{12} m^{-1}), TMP is the transmembrane pressure (Pa), µ the permeate viscosity (Pa·s), and J the permeation flux (m s^{-1}).

The resistance-in-series (RIS) model was applied with the aim to investigate the specific deposition mechanisms. Specifically, the RIS model allowed the total resistance to filtration (R_T) decomposition according to the following Eq. 14:

$$R_T = R_m + R_{PB} + R_{c,irr} + R_{c,rev} \tag{14}$$

Where R_m represents the intrinsic membrane resistance, R_{PB} the irreversible resistance due to particles deposition into the membrane pore (pore blocking), $R_{C,irr}$ the fouling resistance related to irreversible superficial cake deposition (removable with extraordinary physical cleaning), $R_{C,rev}$ the fouling resistance related to superficial cake deposition which is removed by ordinary backwashing.

Specifically, the R_m was evaluated before the membrane was started-up by placing the new membrane module in a tank with ultrapure water and monitoring the TMP and J data. The resistances, due to membrane fouling were achieved by applying the RIS model after physical cleaning operation. Briefly, the membrane module was removed from the reactor and each membrane fiber was individually washed with tap water to remove the cake layer from its surface. After, the cleaned membrane module was placed in a tank with tap water, and the resistance to filtration (R_{T1}) was calculated by measuring the TMP and J, according to Eq. 14. Since it was hypothesized that all the superficial deposition (cake layer) was removed by the physical cleaning, the total resistance R_{T1} was considered as the sum of R_m and R_{PB} only. Therefore, R_{PB} was calculated as the difference between R_{T1} and R_m. Afterwards, the membrane module was submerged into the mixed liquor and the total resistance after physical cleaning (R_{T2}) was calculated after the first suction-backwashing cycle by monitoring TMP and J data. In this case, R_{T2} was considered as the sum of R_m, R_{PB} and $R_{C,rev}$. Therefore the difference between R_{T2} and R_{T1} gave the $R_{C,rev}$ value. In this way, $R_{C,irr}$ was calculated as the difference between R_T and R_{T2}, where R_T is the total resistance to filtration before physical cleaning. Lastly, the fouling rate (FR) was calculated as the increment in TMP on a daily basis.

6.3.4 SST and SSV analysis

The solid analysis were performed according to the Standard Methods (APHA, 2005). First of all, the sample was filtrated (0.7 um filters) and then, used to perform the COD and nitrogen analysis.

6.3.5 COD analysis

The COD analysis (total and soluble) were performed in the anoxic, aerobic and SRR, using the supernatant obtained from the sample for the SST analysis, in the period I the samples were obtained by centrifugation and in the following phases by filtration (filter with porosity of 70mm). For the total COD in the first period it was performed for influent and aerobic tanks, the soluble COD was performed for all the tanks (feed tank, anoxic, aerobic, permeate, SRR).

In the analysis, kits from Merck were used. After the insertion of the samples inside the cuvettes, the analysis continued with the insertion of the prepared cuvettes in a termoreactor at 148°C for 120 minutes after reaching ambient temperature, to then perform the data reading in the photometer NOVA 60 from Merck.

6.3.6 Nitrogen compounds (NH_4-N, NO_3-N)

The samples were filtered in a 70 mm filter obtaining a clear surnatant. The kits of NH_4 and NO_3 were used to obtain the values, read in the photometer NOVA 60 from Merck.

6.3.7 BOD analysis

A “BOD sensor” from VELP Scientifica, that uses an electronic system with a mixing system (System 10), performed the analysis of BOD. The BOD sensor is screwed in a bottle containing the sample according to the chosen range (Table 11). The intern microprocessor controls the pressure transducer transforming the value in the BOD data, memorizing 5 BOD values, each 24 hours.

Table 11: Scales for the use of the BOD analysis from Velp Scientifica

	Scale	Sample Volume
A	0 ÷ 1,000 mg O_2/l	100 ml
B	0 ÷ 600 mg O_2/l	150 ml
C	0 ÷ 250 mg O_2/l	250 ml
D	0 ÷ 90 mg O_2/l	400 ml

6.3.8 Hydrophobicity analysis

Hydrophobicity is the physical property of sludge to not absorb and not retain water inside or on their surface. The analysis of this parameter was performed with the method proposed by (Rosenberg et al., 1980).

6.3.9 Granulometry and viscosity

Granulometric analysis were carried out with the Sympatec granulator, which yields the granulometric distribution of particles from 0 to 4 mm using an optical principle. In addition, viscosity analysis was carried out using the Brookfield DV-E viscometer that returns the viscosity values in cP and with an accuracy of 1%.

Chapter 7

7.1 Results analysis (Phase 1): Experimental application of a side-stream/main-stream reactor with MBR

7.1.1 Heterotrophic biomass growth and waste sludge production

Among the main parameters monitored during the experiment, the most important were the total and volatile suspended solids concentration. Indeed, the variation of the biomass concentration in the biological tanks and the amount of biomass wasted daily, helped to calculate the net sludge production. The trends of the TSS and VSS concentration, as well as the VSS/TSS ratio during the experiment are reported in Fig.15.

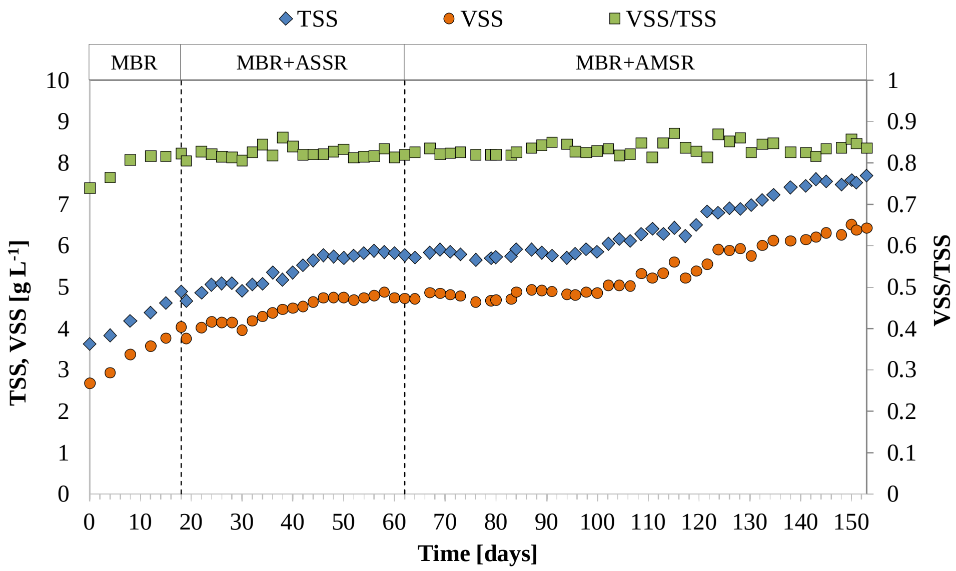


Figure 15 - Trends of total (TSS) and volatile suspended solids (VSS), as well as VSS/TSS ratio during the experiment.

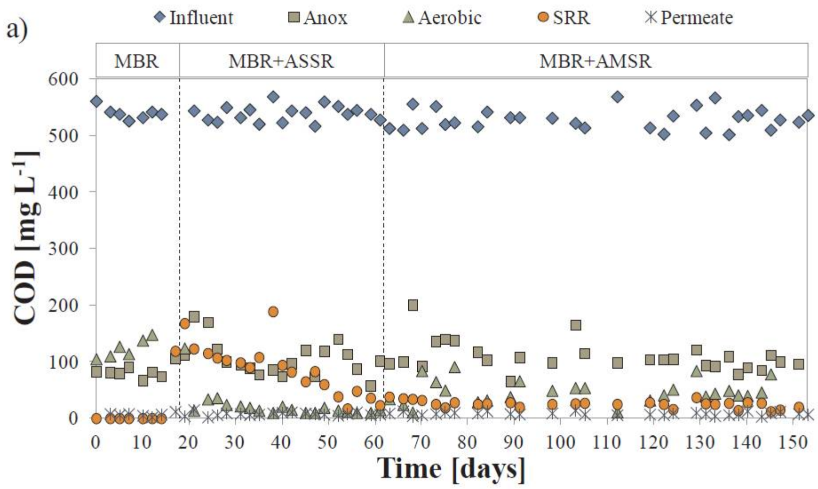
The MBR was started up with a conventional activated sludge inoculum at a biomass concentration close to 3.63 gTSS L^{-1}. The TSS concentration increased up to 5 gTSS L^{-1} until the plant was operated in MBR configuration. When the plant configuration was changed to MBR+ASSR the TSS concentration still increased, although a lower rate was observed, compared to the previous period. At the end of the MBR+ASSR configuration, the TSS concentration reached a steady value close to 5.8 gTSS L^{-1}. When the plant configuration was changed to MBR+AMSR, the TSS concentration began to increase and the TSS concentration reached a value of approximately 7.5 gTSS L^{-1} at the end of the experiments. It is worth mentioning that the TSS increase in the MBR+AMSR configuration occurred with a delay of approximately 35 days after the change of plant configuration. The trend of VSS was similar to the TSS trend. Accordingly, the VSS/TSS ratio was almost constant at a value of approximately 0.82 during the overall experimental campaign.

The heterotrophic biomass growth was assessed by calculating the Y_{obs} and the Y_H. The Y_{obs} is the observed sludge yield (*i.e.,* the fraction of the removed COD that was converted into new biomass) evaluated by means of mass balance equations, whereas the Y_H is the maximum sludge yield, calculated through respirometric batch tests in the absence of limiting growth conditions to exclude decay phenomena. The Y_{obs} and Y_H in the original MBR configuration were on average 0.34 kgVSS $kgCOD^{-1}$ and 0.52 kgVSS $kgCOD^{-1}$, respectively. These values were slightly higher compared with those obtained in other MBR systems (Wang et al., 2013) likely because the organic matter in the synthetic feed was composed of approximately 80% by readily biodegradable substrate (sodium acetate). The Y_{obs} significantly decreased in the ASSR configuration to less than 0.08 kgVSS $kgCOD^{-1}$, and accordingly the Y_H decreased to 0.38 kgVSS kgCOD−1. In contrast, in the AMSR configuration both the Y_{obs} and Y_H increased approximately to 0.22 kgVSS $kgCOD_{-1}$ and 0.41 kgVSS $kgCOD^{-1}$, respectively.

The obtained results demonstrated that the implementation of the SRR in the original MBR scheme enabled to decrease the excess sludge production. Specifically, the ASSR configuration provided a sludge minimization efficiency of approximately 75%, whereas it was lower in the AMSR (35%). The net waste sludge production in the MBR was of approximately 2 gTSS d^{-1}. Thereafter, the sludge production decreased to 0.09 gTSS d^{-1} and 1.17 and gTSS d^{-1} in the ASSM and AMSR configuration, respectively. These findings confirmed that the implementation of the SRR in the original MBR configuration helped to decrease the biomass production. On the other hand, biomass production in the ASSM configuration was lower than AMSR of approximately 35%.

7.1.2 Nutrients removal performance

The MBR was periodically monitored to evaluate COD and nitrogen removal performance. Fig. 16a depicts the COD concentration in the influent, in the supernatant of each reactor and in the permeate. In Fig. 16b the average COD removal efficiency in each MBR section during the three experimental periods are reported.



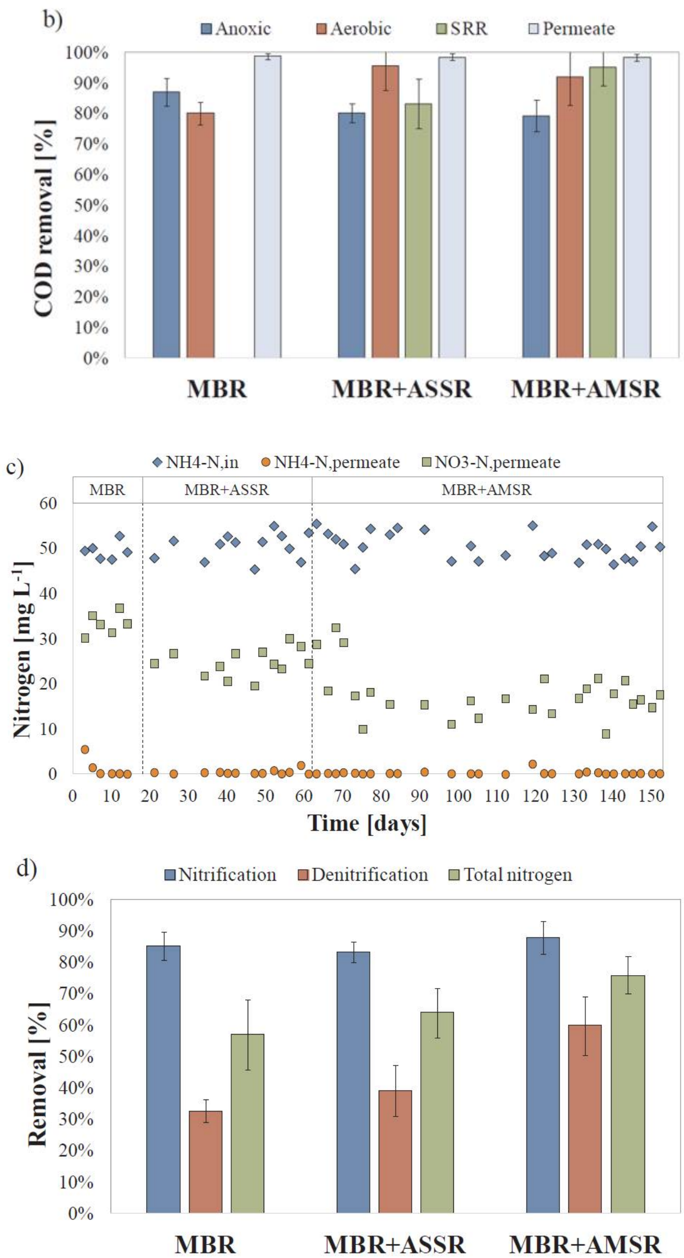


Figure 16 - Time course of COD concentration in the influent, in the supernatant of the anoxic, aerobic and SRR and in the permeate (a); average COD removal efficiency in each MBR section (b); ammonia nitrogen in the influent and ammonia and nitrate in the permeate (c); average values of nitrification, denitrification and total nitrogen removal efficiencies in the three MBR, ASSR + MBR and AMSR + MBR configurations (d).

Moreover, nitrogen forms concentrations in the influent and effluent of the MBR, as well as in the supernatant of each reactor, and the nitrification, denitrification and total nitrogen removal efficiencies (average values) are reported in Fig. 16c and Fig. 16d, respectively.

The TCOD concentration ranged between 520-550 mg L^{-1} in the influent synthetic wastewater. The TCOD in the supernatant of the anoxic and aerobic reactors ranged between 90-160 mg L^{-1} and between 15-80 mg L^{-1} respectively, without showing any significant relationship with the change in the process configuration. Nevertheless, the TCOD concentration in the supernatant of the SRR was higher in the ASSR configuration, when it ranged between 40-170 mg L^{-1}, thereby suggesting the occurrence of lysis phenomena. In contrast, in the AMSR configuration, the average TCOD was approximately of 30 mg L^{-1}, without showing any significant fluctuations. Lastly, the TCOD in the permeate was always lower than 10 mg L^{-1}, thereby indicating that membrane contributed around 15% to the total COD removal (Fig. 16b).

Based on the results above, the implementation of SRR in the MBR scheme did not compromise the effluent quality. Although it was previously highlighted that enhancing biomass decay may result in the deterioration of COD removal efficiency (Wei et al.,2003), in this study no significant modifications were observed after SRR implementing. A not negligible release of COD within the SRR was detected in the ASSR+MBR configuration, likely due to the EPS hydrolysis. Nonetheless, this COD was consumed once the sludge was returned to the anoxic reactor, thereby not worsening the effluent quality. In this respect, implementation of the SRR in the RAS line slightly improved the denitrification process. The release of COD was not detected in the AMSR+MBR configuration, because biomass decay did not occur in the SRR under this configuration.

Complete nitrification was achieved regardless the plant configuration (Fig. 16c). Moreover, no nitrites were observed in the effluent during the entire experiment. However, the nitrate concentration in the permeate was not constant during the experiment, thus highlighting that denitrification efficiency was affected by the plant configuration. Specifically, the nitrate concentration in the permeate ranged between 30 and 40 mg L^{-1} in the original MBR configuration.

Afterwards, the implementation of the SRR reactor provided an improvement of the denitrification efficiency. Indeed, the nitrate concentration in the effluent decreased to 25–30 mg L^{-1} and to 10–20 mg L^{-1} in the ASSM and AMSR configuration respectively. Overall, the results depicted in Fig. 16d indicate that the nitrification efficiency was quite similar in all the three configurations (> 85%).

The denitrification efficiency, instead, was lowest in the MBR configuration (32%), whereas it slightly increased in the ASSR (37%) and significantly increased in the AMSR (58%). Denitrification was significantly improved in the AMSR+MBR configuration because the extension of the not aerated volume in the mainstream. Consequently, the lowest TN removal performance was observed in the original MBR configuration (57%), whereas the highest one in the AMSR configuration (77%). The ASSM configuration did not provide a significant improvement in the TN removal (63%). These results indicated that the AMSR configuration provided the best performance in terms of nutrient removal.

Nitrogen removal performance might be affected by some environmental parameters like pH, DO, ORP and temperature. The average data of these parameters during the experimentation are summarized in Table 12.

The DO was constantly zero in the anoxic and the SRR tank, whereas it was close to 7 mg L^{-1} in the aerobic reactor due to the additional aeration system of the membrane module for fiber scouring, in order to mitigate the fouling development. The pH was in general lower in the aerobic reactor due to development of nitrification reactions, whereas it was slightly higher in the anoxic and SRR, thereby suggesting the establishment of reducing reactions.

Accordingly, the ORP was positive in the aerobic reactor, whereas it was negative in the anoxic and SRR. Interestingly, the ORP in the SRR was approximately of -1.7 mV, thereby suggesting that the anaerobic conditions were not reached in the SRR during the ASSR configuration. This result suggests that 6 h HRT in the SRR was not enough to establish anaerobic conditions in the ASSR configuration. The average ORP in the SRR of AMSR configuration was −224 mV, thereby indicating the achievement of the anaerobic conditions.

Table 12: Average data of the main environmental parameters in the anoxic, aerobic and SRR reactors in the three investigated configurations.

Parameter	Unit	MBR			MBR+ASSR			MBR+AMSR		
		Anox	*Aerobic*	*SRR*	*Anox*	*Aerobic*	*SRR*	*Anox*	*Aerobic*	*SRR*
Dissolved Oxygen	[mg L^{-1}]	0	6.90	-	0	7.21	0	0	6.89	0
pH	[-]	7.66	7.58	-	8.08	8.00	7.93	8.37	8.26	8.40
ORP	[mV]	-59.3	223	-	-110.5	232	-1.7	-137.5	219	-224.7
Temperature	[°C]	16	17	-	18	19	18	18	18	18

7.1.3 Biomass kinetic behavior

To give a more comprehensive insight into the impact of the change in plant configuration on biomass metabolisms, some kinetics parameters were monitored. Specifically, the endogenous decay coefficient (b_H), the heterotrophic active fraction (f_{XH}), the maximum heterotrophic growth rate ($\mu_{max,H}$) and the yield on internal storage products (Y_{sto}) were examined by periodically performing respirometric batch tests.

The highest (1.57 d^{-1}) and lowest (0.99 d^{-1}) values of the endogenous decay coefficient (b_H) were observed when the plant was operated in ASSR and AMSR configuration, respectively. This result indicated that the implementation of the SRR caused the increase of the bacterial decay rate (ASSR) on a side and its decrease (AMSR) on the other, respect to the original MBR configuration.

The maximum heterotrophic growth rate ($\mu_{max,H}$) significantly reduced from 2.368 d^{-1} to 1.392 d^{-1} when the SRR was implemented in ASSR configuration, thereby indicating a decrease in new bacterial cell synthesis. When the plant configuration was changed into AMSR, the $\mu_{max,H}$ slightly increased approximately to 1.59 d^{-1}, thereby suggesting the establishment of growth-enhancing conditions.

The heterotrophic active fraction decreased during the entire experiment from approximately 8% (MBR) to 5% (ASSR) and less than 2% in (AMSR). It is worth to note that, although the heterotrophic growth rate increased when the plant configuration was changed from ASSR to AMSR, the f_{XH} decreased. This finding was likely affected by the complete sludge retention strategy that caused an excessive biomass ageing.

No significant changes were observed in the Y_{sto} when the SRR was implemented in the original MBR in ASSR configuration (0.57 gVSS $gCOD^{-1}$). Interestingly, the Y_{sto} significantly increased approximately to 0.79 gVSS $gCOD^{-1}$ when the SRR was implemented in AMSR configuration. This value was significantly higher than the typical values observed in MBR systems (0.50 gVSS $gCOD^{-1}$) (Capodici et al., 2016).

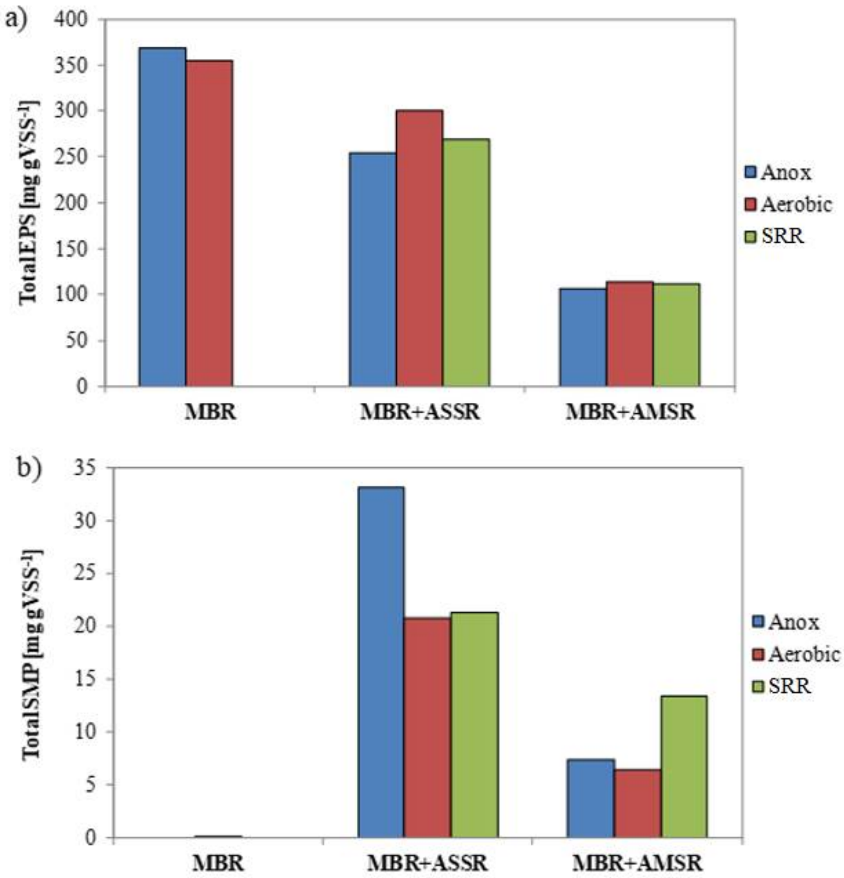
The above findings suggested that the implementation of the SRR significantly affected the biomass kinetic behavior. Specifically, in ASSR configuration, biomass decay prevailed over growth. In contrast, in AMSR configuration the growth of biomass with internal storage capacity was stimulated.

7.1.4 Activated sludge physical characteristics: flocs size, EPS content and composition

The size and morphology of the activated sludge flocs changed during the experiment. The mean size of the inoculum activated sludge was approximately 115 µm. The average size of flocs decreased at the end of the MBR phase reaching a value of approximately 92 µm. When the SRR was implemented in ASSR configuration, the average size of flocs still decreased, reaching the lowest value of 63 µm. In contrast, when the plant configuration was changed into AMSR configuration, the average size of flocs slightly increased at approximately 86 µm. These results can be likely related to the changes occurred in the EPS content and composition.

The EPS are a complex high-molecular-weight mixture of polymers that have a significant influence on the physicochemical properties of microbial aggregates (Sheng et al., 2010). The SMP are organic compounds that are released into solution resulting from the biomass decay and EPS destruction (Barker and Stuckey, 1999).

The specific total EPS and SMP content, as well as the bound EPS composition in terms of protein to carbohydrate ratio are reported in Fig. 17.



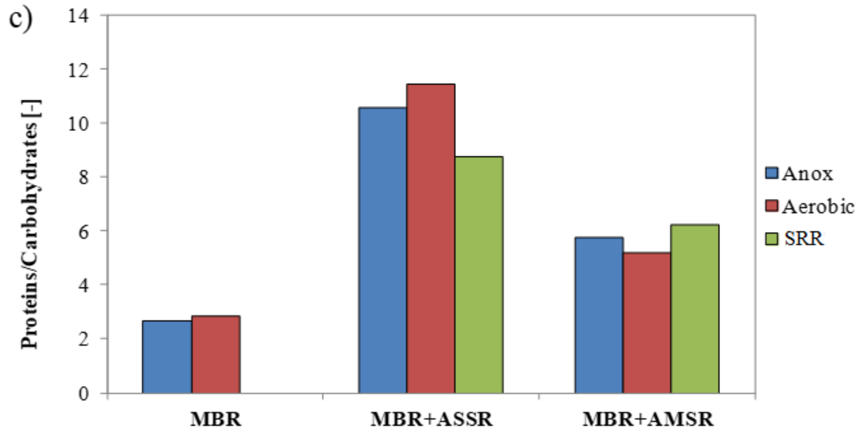


Figure 17 - Average values of the specific total EPS content (a), and SMP (b), as well as proteins to carbohydrate ratio in the bound EPS (c) in different plant configuration.

The specific total EPS concentration decreased during the experiment. The average EPS content in the MBR configuration was approximately of 350 mgEPS $gVSS^{-1}$. After the change of plant configuration, the EPS contend decreased to approximately 265 mgEPS $gVSS^{-1}$ and 100 mgEPS $gVSS^{-1}$ in the ASSR and AMSR, respectively. Moreover, no statistically significant differences between the total EPS values within the anoxic, aerobic and SRR were observed. The decrease in EPS content likely affected the strength of the activate sludge flocs, thereby causing the sludge deflocculation.

The SMP concentration was negligible in the MBR configuration, whereas it significantly increased in the ASSR (23 mgSMP $gVSS^{-1}$), thereby suggesting that bacterial cell lysis and EPS destruction simultaneously occurred in the ASSR configuration. In contrast, the SMP concentration decreased in the AMSR configuration (7.5 mgSMP $gVSS^{-1}$). Interestingly, the decrease in the EPS content did not result in an increase of the SMP concentration in the AMSR configuration. This result likely indicating that bacterial cell lysis and EPS destruction did not take place or played a marginal role in the sludge minimization process.

The EPS composition was significantly affected by the plant configuration. Indeed, the proteins to carbohydrate ratio significantly increased from approximately 2 (MBR) to 9 and 5.5 in the ASSM and AMSR configuration, respectively. This result clearly indicated that implementation of the SRR in the original MBR configuration caused a significant enrichment in the protein content of the activated sludge.

Because of the implementation of an anaerobic reactor in the main or side stream of the MBR original layout, biomass underwent to metabolic stress conditions given by the lack of organic substrate or low ORP level. Under metabolic stress condition, bacteria likely use EPS as carbon source for its metabolism. Being carbohydrates more simple from a molecular point of view, microorganisms degrade the carbohydrates as a priority than the proteins, thereby resulting in the increase of PN/PS ratio (particular proteins/polysaccharides) (Corsino et al., 2017).

Based on the obtained results, it is worth to note that the change in EPS content and composition changed after the change of plant configuration. Moreover, the extent of these changes was strictly related to the placement of the SRR within the plant scheme.

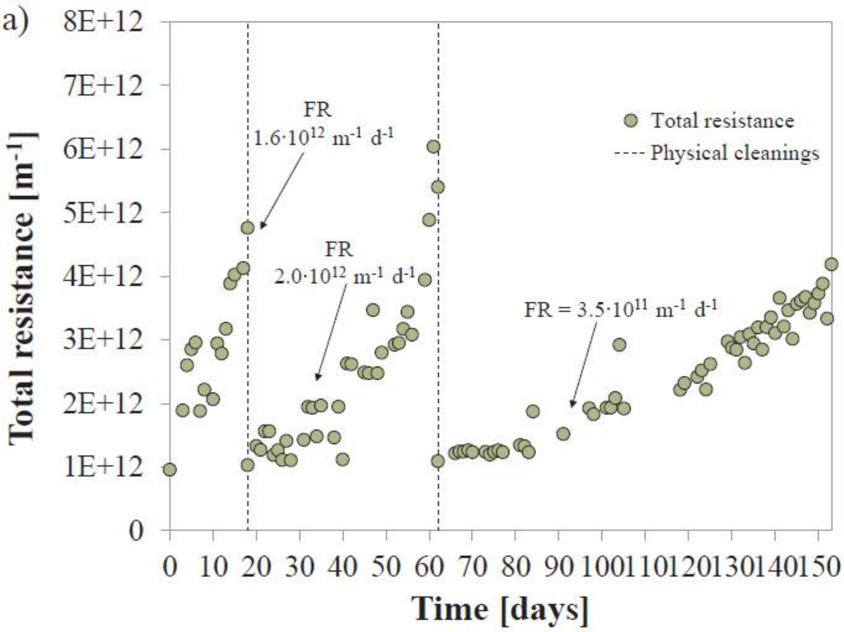
7.1.5 Membrane fouling analysis

Metabolic stress conditions imposed by the implementation of the SRR caused the change of the mixed liquor characteristics and in turns the membrane fouling tendency. Fig. 18 depicts the trend of the total resistance of the membrane (a), the fouling rate (b) and the total resistance decomposition according to Eq. 14.

During the experiment three physical cleanings were performed, when the plant configuration was changed (18th, 62th and 153th day), whereas any chemical cleaning was performed. The total resistance increased during the period in which the plant was operated with the original MBR configuration, reaching a value of approximately $4.8 \cdot 10^{12}$ m^{-1}. The increase in the total resistance was more pronounced when the plant configuration was changed in ASSR, likely due to the stress effect exerted by the implementation of the SRR. The maximum total resistance observed in this phase was $6.1 \cdot 10^{12}$ m^{-1} after 44 days of operation. In contrast, a lower fouling tendency was observed when the plant configuration was changed in AMSR. The total resistance slightly increased during the entire phase, thereby reaching a value of approximately $4.1 \cdot 10^{12}$ m^{-1} after 91 days of operation.

In terms of FR, the implementation of the SRR caused a significant worsening of the membrane fouling in ASSR configuration ($1.98 \cdot 10^{12}$ $m^{-1}d^{-1}$), whereas in AMSR the fouling tendency was significantly mitigated ($3.8 \cdot 10^{11}$ $m^{-1}d^{-1}$). The increase in FR in ASSM configuration compared to the original MBR (+25%) indicated that this configuration was not optimized in terms of membrane fouling. In contrast, the AMSR configuration enabled a significantly lower FR, comparable with that observed in the original MBR.

a)



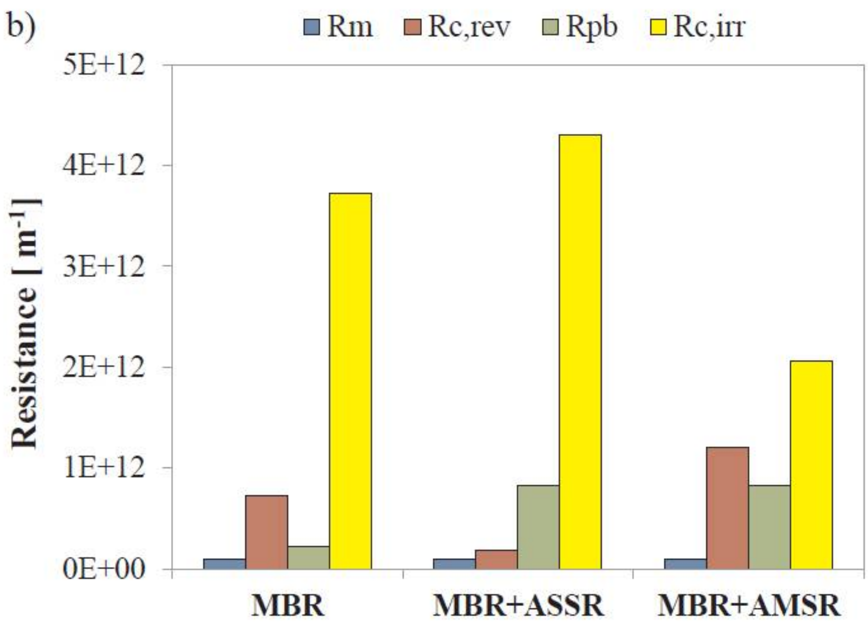


Figure 18 - Trends of the total resistance of the membrane and the fouling rate (a), the total resistance decomposition according to the RIS model (b).

Fig. 18b shows the membrane resistance decomposition in the three plant configurations investigated. As shown in Fig. 18, the irreversible cake deposition was the main membrane fouling mechanism in the MBR and in the MBR+ASSR ($3.85 \cdot 10^{12}$ m^{-1}), contributing by approximately 84% to the total resistance. It is worth to note that the resistance due to pore blocking significantly increased when the SRR was implemented in ASSR configuration (400%), thereby suggesting that this configuration could potentially increase the irreversible loss in membrane permeability. This result was likely due to the increase in the SMP concentration and the decrease in the activated sludge flocs size.

Interestingly, in the AMSR configuration, the contribution of the irreversible cake deposition to the total resistance decreased (40%) and no significant increase in the pore blocking was noted. On the other hand, the reversible cake deposition significantly increased by approximately 7 times.

The results suggested that the placement of the SRR in the MBR plant configuration played a key role in the change of the activated sludge physical characteristics. As above discussed, several mechanisms involved in minimizing sludge production in ASSR+MBR configuration. Among these, destruction of EPS caused on the one hand the increase in the SMP concentration and on the other hand the decrease in the flocs average size. Both occurrences involved a possible occlusion of the membrane pores by dispersed microorganisms and soluble products (pore blocking mechanism), thereby causing a significant worsening in membrane filtration efficiency.

Besides, endogenous decay mechanisms could have affected the EPS composition, resulting in the increase in protein. As reported in the literature, cell lysis, induced by extended endogenous conditions, causes the release of endoenzymes and extracellular products, including proteins and polysaccharides (Sponza, 2002). Since the polysaccharides are more readily biodegradable than protein, bacteria are able to degrade them. In contrast, proteins, that are molecularly more complex, accumulated in the EPS matrix, thereby resulting in their accumulation within the flocs (Campo et al., 2017).

Because of the hydrophobic nature of proteins, their increase contributed to the irreversible cake deposition, thereby resulting in a higher fouling rate. In terms of membrane fouling tendency, the AMSR configuration enabled better performance than the ASSR+MBR. The larger size of flocs on a side and the lower EPS content on the other likely helped to increase the pre-filter effect exerted by the cake layer, thereby preventing fouling due to pore blocking and irreversible cake deposition (di Bella et al., 2010).

The EPS concentration/composition could have affected the features of the membrane fouling: indeed, in Period II (ASSR+MBR), it was observed a higher total EPS and SMP concentration compared to Period III (ASMR+MBR), and a higher protein contribution. This situation could have promoted the development of irreversible fouling in the ASSR+MBR configuration, in agreement with previous studies (Lin et al., 2014).

The found results demonstrated that, if on the one hand endogenous decay and EPS destruction significantly contributed to sludge minimization, on the other hand the worsening in the sludge physical characteristics significantly affected the membrane filtration performance.

7.1.6 Impact of reactor configuration on sludge minimization

Published studies in the literature demonstrated the effectiveness of using OSA process for excess sludge reduction (Foladori et al., 2010b; Velho et al., 2016). Nevertheless, it should be carefully considered that effective sludge minimization must be accomplished without compromising the effluent quality neither the hydraulic functionality of the membrane. The integration of an anaerobic SRR in a sMBR for biological nutrient removal poses a challenge about its correct placement in the process scheme. In this study, two different solutions were examined. The first involved the placement of the SRR in the RAS line from the membrane tank to the anoxic reactor (ASSR+MBR). In the second configuration, the SRR was placed between the anoxic and the aerobic/MBR tank (AMSR+MBR). Obviously, environmental conditions in the SRR were different based on its placement in the wastewater line.

Based on the above results, the inclusion of the SRR in the wastewater treatment line enabled to reduce the excess sludge production in the MBR system. In this study, several mechanisms were found to contribute to decrease the excess sludge production. Metabolism uncoupling, destruction of EPS, endogenous decay and selective enrichment of bacteria population simultaneously occurred in both configurations, thereby contributing to sludge minimization. However, the contribution of each mechanism changed according to the process scheme investigated.

The endogenous decay mechanism was mainly observed in the ASSR+MBR configuration, as highlighted by the higher endogenous decay coefficient, assessed through respirometry. The ORP value (−1.7 mV) indicated that anaerobic conditions were not obtained within the SRR in the ASSR+MBR configuration. However, as the sludge was subjected to extended substrate-limitation within the SRR, biomass was induced to limit cell production while using energy for basic metabolism (Hao et al., 2010).

Moreover, bacterial predation by protozoa or metazoa could also take place in the ASS+MBR configuration; however, such aspect should be verified in future activities. On the other hand, in the AMSR+MBR configuration, the sludge was under anaerobic conditions with a residual availability of organic substrate deriving from the anoxic reactor. Although anaerobic conditions were effectively obtained in the SRR of the AMSR+MBR configuration, the availability of carbon source limited the biomass decay. Therefore, this mechanism contributed to a lower extent to the overall sludge minimization process.

Concerning the destruction of EPS, it was speculated that bacteria used EPS as carbon source in the SRR because of the lack of external carbon source or, in general, under metabolic stress conditions (Chon et al., 2011). This hypothesis was confirmed by the observed increase of the SMP concentration after the implementation of the SRR reactor. Indeed, depending on the system configuration, biomass underwent to metabolic stress conditions given by the lack of organic substrate (ASSR+MBR) or low ORP level (AMSR+MBR). Specifically, under stress conditions, the proteins and carbohydrates of EPS were released into the bulk as SMP.

This mechanism, which is known as destruction of EPS (Semblante et al., 2014), was mainly observed in the ASSR+MBR configuration, whereas it was almost negligible in the AMSR+MBR. The results indicated that the EPS destruction mechanism was mainly driven by the lack of carbon source (ASSR+MBR) instead of low ORP level (AMSR+MBR). This could have two important implications for the operation of the MBR. Indeed, if on the one hand, this helped to enable higher excess sludge reduction, on the other hand it increased the membrane fouling tendency because of the increase in the SMP.

Focusing on the uncoupled metabolism, the investigated process configurations highlighted interesting aspects. In the ASSR+MBR configuration, mixed liquor from the aerobic tank was enriched in oxygen and nitrates from the nitrification process. Furthermore, as the organic carbon was mostly depleted within the upstream reactors (anoxic and aerobic), oxygen and nitrate depletion in the SRR occurred very slowly. Therefore, the results suggested that in ASSR+MBR configuration the SRR worked as an oxygen depletion reactor with extended HRT.

Therefore, because nitrates were not depleted, anaerobic conditions were hardly achievable in this reactor. Nevertheless, the sludge underwent a state of fasting condition within the SRR because of the lack in carbon substrate. Thereafter, when the starved sludge was returned to the anoxic reactor, cells began feasting on available substrate to replenish energy stores. According to the literature, this lead bacteria to uncouple catabolism and anabolism, thereby reducing the biomass growth rate (Troiani et al., 2011; Wang et al., 2017).

In AMSR+MBR configuration, the sludge passed from the anoxic reactor to the SRR with no oxygen and low nitrates. Therefore, anaerobic conditions were easily obtained, as also confirmed by the lowest ORP values (<−210 mV). Although residual organic carbon was still present in the outflow of the anoxic tank, fasting conditions were accomplished because of the low ORP in the SRR (<−210 mV) (Semblante et al., 2014). Subsequently, cells were able to replenish energy stores when the sludge was returned to the aerobic reactor. In summary, in the ASSR+MBR fasting conditions were driven by low substrate availability, whereas feasting condition occurred in the anoxic reactor characterized by high substrate availability. In contrast, in the AMSR+MBR configuration, fasting condition was driven by low ORP and feasting occurred in aerobic environment with low substrate availability. The obtained results indicated that the ASSR+MBR was more effective in terms of sludge minimization, indicating that the strategy of fasting by withholding substrate and feasting under anoxic condition could be more beneficial for sludge minimization. It can be speculated that energy uncoupling prevailed in ASSR rather than AMSR configuration.

Interestingly, selection of bacteria with internal storage capacity and low biomass growth rate likely occurred in AMSR+MBR configuration. The results above indicated that the amount of the specific EPS decreased by almost 70% in AMSR+MBR configuration compared with the ASSR+MBR, without a corresponding increase of SMP concentration. At the same time, the growth rate on internal storage product (Y_{sto}) increased.

Based on these results, is possible to speculate that the mechanism of substrate storage of bacteria changed according to the process configuration. In AMSR+MBR, bacteria underwent anaerobic conditions in the SRR with a not negligible amount of carbon substrate (approximately 160 mg L^{-1}). It could be assumed that the availability of organic matter under anaerobic conditions led to selection of bacteria with internal storage capacity. Alternation of anaerobic and aerobic conditions is known to be beneficial to polyphosphate-accumulating organisms (PAOs) selection. These microorganisms are able to convert organic substrate into storage polyhydroxyalkanoate (PHA) under anaerobic conditions, which are subsequently degraded under aerobic conditions (Chudoba et al., 1992). The significant increase in the Y_{sto} observed under the AMSR + MBR configuration (>50%) supports this assumption. However, biomolecular tests should be performed in future research activities to confirm this assumption. Similar results were also found by others authors (Chudoba et al., 1992), therefore it is realistic that AMSR+MBR configuration enabled a selective enrichment of PAO population.

The results above reported indicated that under the operating conditions of the AMSR+MBR configuration a selective speciation of the biomass rather than an overall bacterial growth reduction likely occurred. Indeed, the lower biomass decay and SMP concentration, as well as the higher observed growth yield, suggested that in the AMSR+MBR configuration the sludge was less subjected to environmental stress factors. If one the one hand the AMSR+MBR configuration provided a lower sludge minimization than the ASSR+MBR, in the former it was speculated that biomass was significantly enriched in PHA. Therefore, the AMSR+MBR explores the possibility of PHA recovery from the waste sludge, thereby balancing the lower sludge minimization capacity.

7.2 Results analysis (Phase 2) – Integration of an AMSR for sludge reduction and biological phosphorus removal: Effect of HRT

7.2.1 Biomass growth and excess sludge production

The trends of the TSS concentration and the ratio between VSS and TSS throughout the experiment are shown in Figure 19.

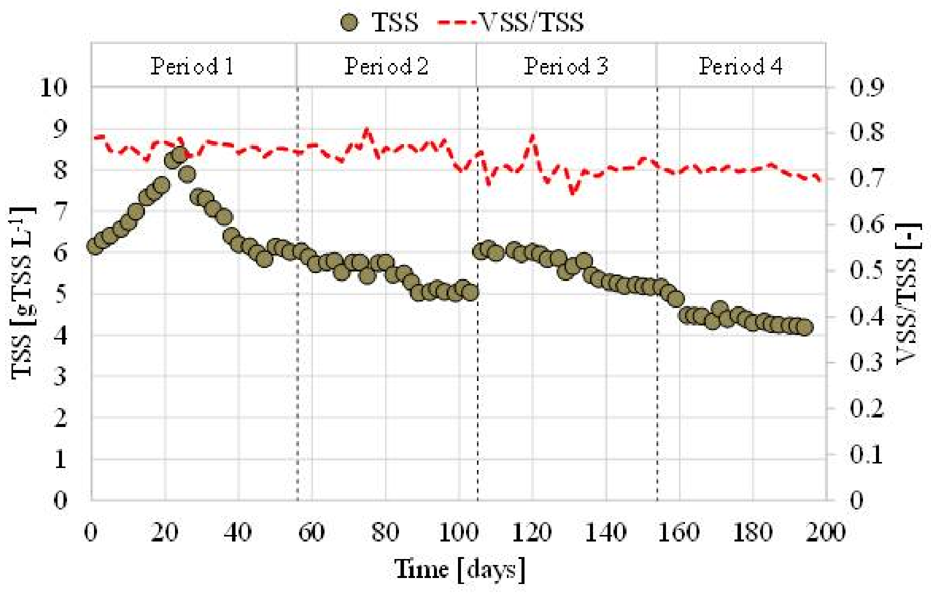


Figure 19 - Trends of TSS concentration and VSS/TSS ratio during the experiment.

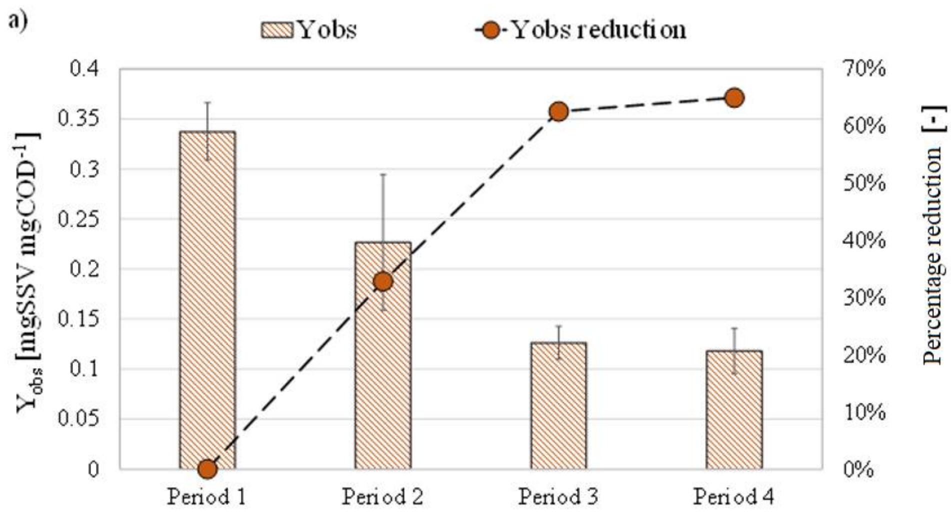
In Period 1, when a complete sludge retention strategy was adopted, the TSS concentration increased from 6.15 gTSS L^{-1} to approximately 8.5 gTSS L^{-1}. In this short period, the high growth rate indicated that the biomass was successfully acclimated to the new operating conditions of the MBR system. Hereafter, when a regular sludge withdrawn was performed, the TSS concentration decreased standing at a constant value of approximately 6 gTSS L^{-1}.

In Period 2, when the system configuration was changed to AMSR, the TSS concentration showed a slightly decreasing trend, reaching a steady value of 5 gTSS L^{-1} at the end of the period. This result indicated that the TSS mass balance was negative, thereby suggesting that the net growth was lower than the wasted activated sludge.

Beginning in Period 3, the TSS concentration was increased to 6 gTSS L^{-1} by adding a part of the sludge wasted in the previous days, in order to have the same initial conditions of the previous period in terms of TSS and F/M. During Period 3, the TSS concentration decreased as observed during Period 2, standing at a value of approximately 5.20 gTSS L^{-1} at the end of the period. Compared to the previous experimental periods, the decreasing trend observed during Period 3 showed a higher slope, indicating that the higher was the HRT in the anaerobic reactor, the lower was the excess sludge production. This confirmed that the integration of the anaerobic reactor in the AMSR scheme involved a decrease in the biomass net growth, thus favoring a lower excess sludge production. Even in Period 4 the TSS concentration decreased from 5.20 gTSS L^{-1} to approximately 4.22 gTSS L^{-1} at the end of the experiment, showing a similar trend with that observed in Period 3.

The VSS/TSS ratio showed a slightly decreased trend during the entire experiment. Indeed, the VSS/TSS of the inoculum was close to 0.77, whereas it decreased to approximately 0.73 at the end of the experiment. This result suggested that by operating at constant SRT ranging between 35-40 days, the accumulation of inert material deriving from the activated sludge mineralization only partially occurred occur. In general, longer SRTs caused low sludge production since the SRT is inversely proportional to sludge yield due to the diversion of energy towards cell maintenance rather than synthesis (Semblante et al., 2016b). Therefore, when a long SRT strategy is imposed, it is difficult to establish if the excess sludge reduction occurred because of the long SRT rather than the change in the plant configuration. The above results indicated that the regular sludge withdrawn avoided the enhancement of the bacterial decay, thereby suggesting that sludge reduction was achieved because of the change in the plant configuration.

The average values of the observed yield coefficient (Y_{obs}) and the maximum yield coefficient (Y_{max}), as well as their respective percentage reductions obtained in each experimental period are depicted in Fig. 20.



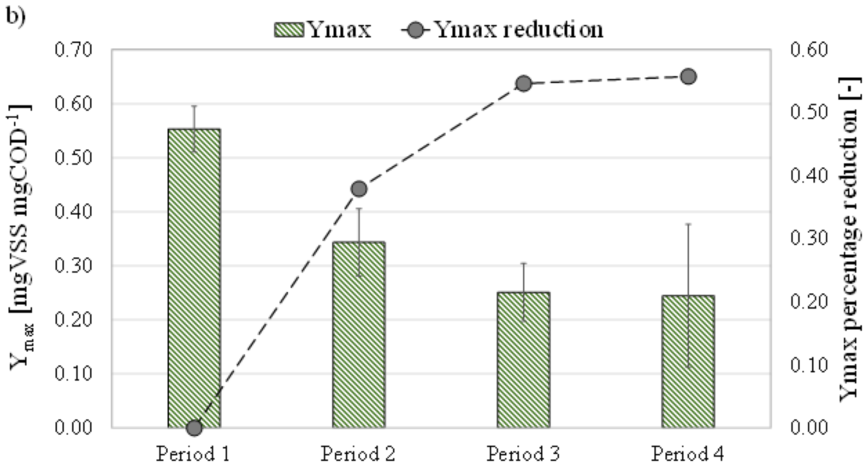


Figure 20 - Average values of the observed yield coefficient (Y_{obs}), the maximum yield coefficient (Y_{max}) and their respective percentage reduction obtained in the four experimental periods.

The Y_{obs} is the observed sludge yield (Fig. 20a), calculated by means of a mass balance equation as the fraction of the removed COD that was converted into new biomass as VSS. The average value of the Y_{obs} in Period 1, referred to the phase in which the MBR operated under controlled SRT, was approximately 0.33 kgVSS kgCOD^{-1} that was slightly higher compared with that obtained in other MBR systems operating under SRT and F/M similar with those applied in this study (Wang et al., 2013). Nevertheless, the Y_{obs} was very similar with that observed by de Oliveira et al. (2018) that operated with acetate based synthetic wastewater under prolonged SRT. After the plant configuration was changed to AMSR in Period 2, the Y_{obs} decreased to 0.22 kgVSS kgCOD^{-1}, thereby reducing of approximately 33% compared to the previous period. This result was in accordance with that reported in a previous study (de Oliveira et al., 2018), thus confirming that by operating under an HRT of 6 h in the anaerobic reactor in the AMSR configuration, approximately 30% of sludge reduction could be obtained. When the HRT of the anaerobic reactor was increased to 8 h (Period 3), the Y_{obs} decreased to 0.12 kgVSS kgCOD^{-1}, thereby showing an overall decrease of 62% in excess sludge production. In Period 4, the increase in the HRT of the anaerobic reactor did not result in a significant decrease in sludge minimization. Indeed, the Y_{obs} was approximately 0.11 kgVSS $kgCOD^{-1}$, which very close to the value achieved in the previous period. This result indicated that the increase in the HRT of the anaerobic reactor from 8 h to 10 h did not provide any significant advantage in terms of sludge minimization, suggesting that 8 h of HRT could be assumed as reference value for designing the anaerobic reactor in an AMSR configuration.

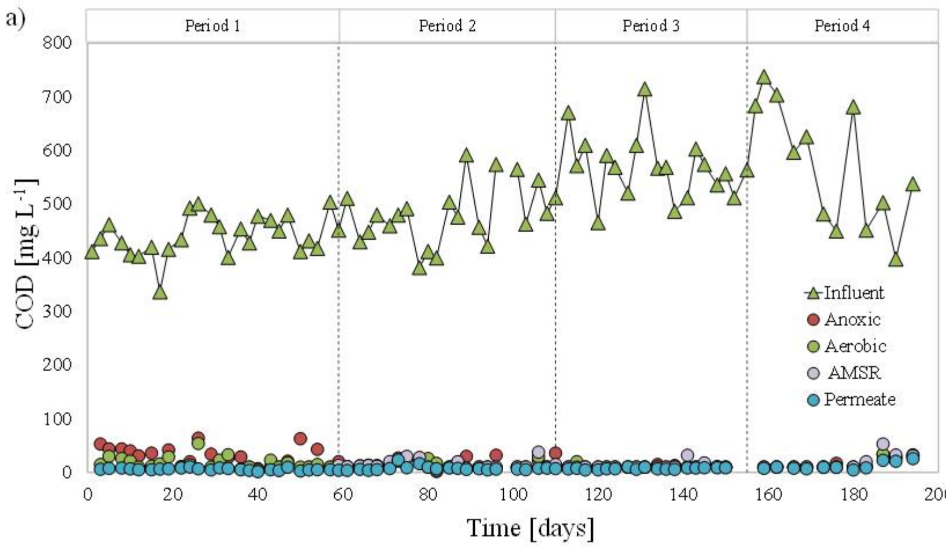
The Y_{max} is the maximum sludge yield evaluated in the absence of limiting growth conditions. The Y_{max} decreased from the initial value of approximately 0.55 kgVSS $kgCOD^{-1}$ (Period 1) to a minimum value of 0.22 kgVSS $kgCOD^{-1}$ obtained in Period 3 and Period 4 (Fig. 20b). As observed for the Y_{obs}, the maximum effect in terms of sludge reduction was obtained under an HRT of 8 h, whereas no significant improvements were achieved under 10 h of HRT.

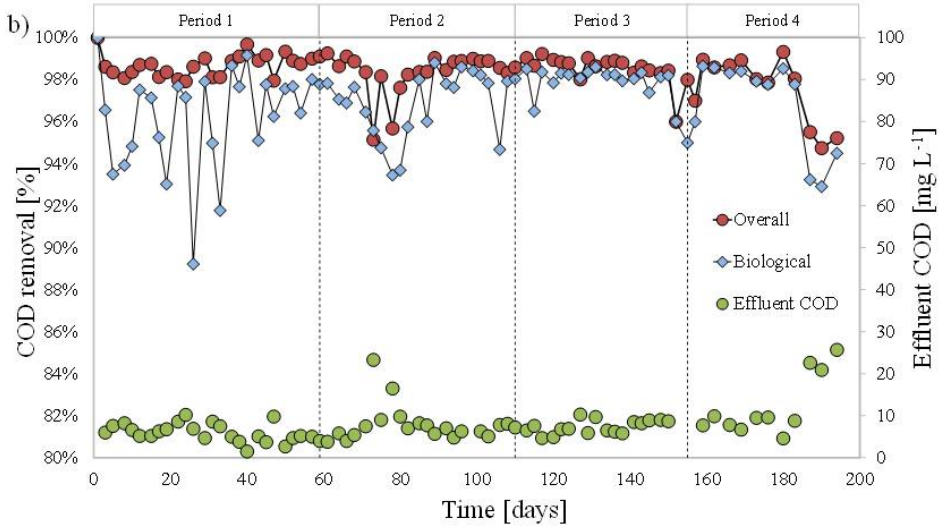
The results above indicated that the integration of an anaerobic reactor in the mainstream of a pre-denitrification scheme enabled approximately the 62% of sludge reduction by operating under HRT of 8 h in the AMSR. Compared with previous literature, the AMSR configuration enabled a slightly lower sludge minimization compared with the ASSR (62 vs 72%) even at higher HRT (8 h vs 6 h) (de Oliveira et al., 2018). Nevertheless, it is worth mentioning that in this study, the SRT was significantly lower (35-40 d *vs* infinite SRT), whereby the contribution of the decay phenomena to sludge minimization was certainly lower in this experiment. While comparing the above results with others obtained under controlled SRT (63 days) in ASSR configuration, it was noted that Y_{obs} was similar with that observed in the AMSR configuration (0.12 vs 0.13 kgVSS $kgCOD^{-1}$) but under lower HRT (8 h vs 10 h) (Kim et al., 2012). Similarly, 35% of sludge reduction was obtained in a SBR attached to an anaerobic side stream reactor operating under 12 h of HRT and 30 days of SRT (Semblante et al., 2016b). In another study carried out in ASSR-MBR systems, the maximum sludge reduction (55-58%) was achieved under 10-11 h of HRT in the anaerobic reactor (Ferrentino et al., 2016; Saby et al., 2003). Similarly, Cheng et al. (2017) observed that the sludge yield decreased of approximately 49.7% in a ASSR-MBR system with HRT of 5 h in the anaerobic reactor, whereas Coma et al. (2015) obtained a sludge reduction of 18% operating in a University of Cape Town (UCT) system coupled with an anaerobic side stream reactor under an HRT of 5.9 h.

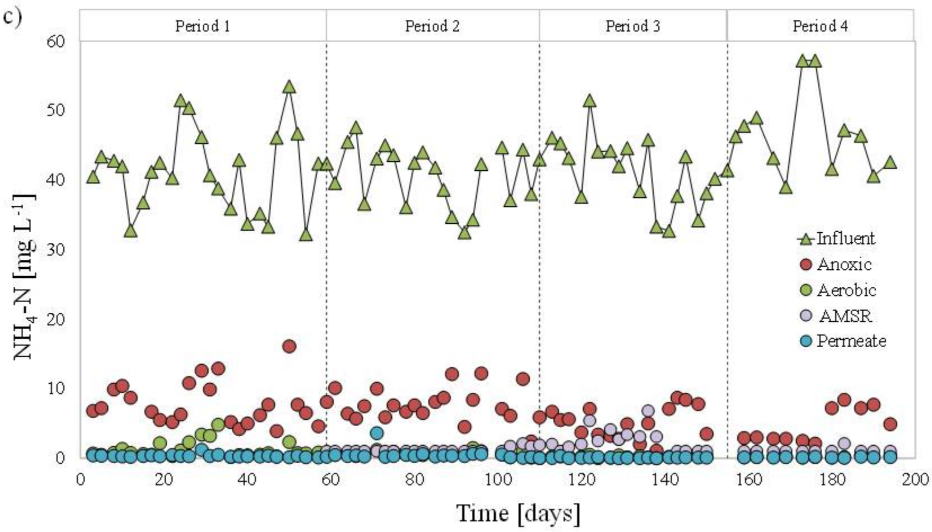
The results obtained in this study suggested that in general the AMSR configuration enabled a higher sludge minimization than the ASSR operating under similar HRT and SRT.

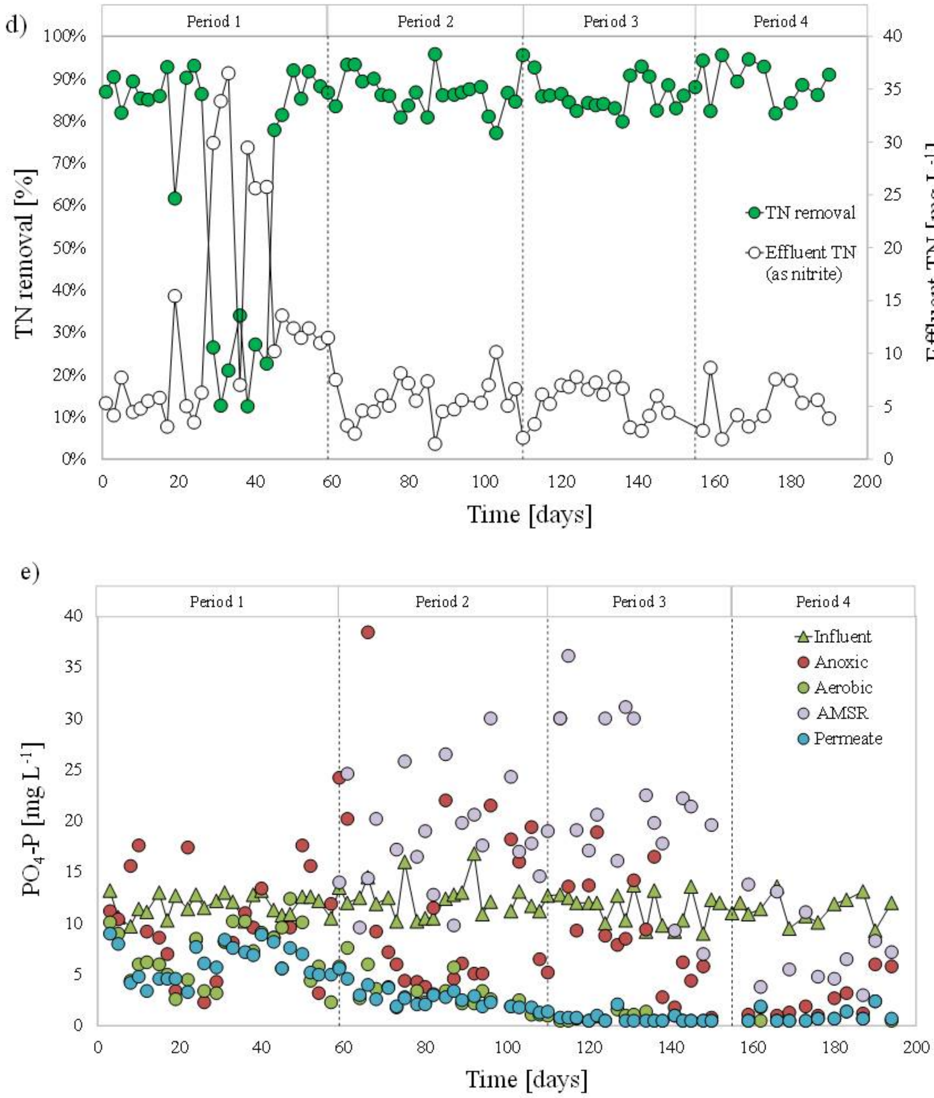
7.2.2 Nutrient removal performances

It is worth mentioning that the implementation of the anaerobic reactor in the AMSR configuration for excess sludge minimization must not compromise the effluent quality. With this aim, the MBR plant was periodically monitored to evaluate COD, nitrogen and phosphorous removal performances (Fig. 21).




b)
Period 1
Period 2
Period 3
Period 4
COD removal [%]
Effluent COD [mg L-1]
Overall
Biological
Effluent COD
Time [days]


c)
Period 1
Period 2
Period 3
Period 4
NH4-N [mg L-1]
Influent
Anoxic
Aerobic
AMSR
Permeate
Time [days]


d)
Period 1
Period 2
Period 3
Period 4
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
TN removal [%]
40
35
30
25
20
15
10
5
0
Effluent TN [mg L-1]
TN removal
Effluent TN (as nitrite)
0
20
40
60
80
100
120
140
160
180
200
Time [days]
e)
Period 1
Period 2
Period 3
Period 4
40
35
30
25
20
15
10
5
0
PO4-P [mg L-1]
Influent
Anoxic
Aerobic
AMSR
Permeate
0
20
40
60
80
100
120
140
160
180
200
Time [days]

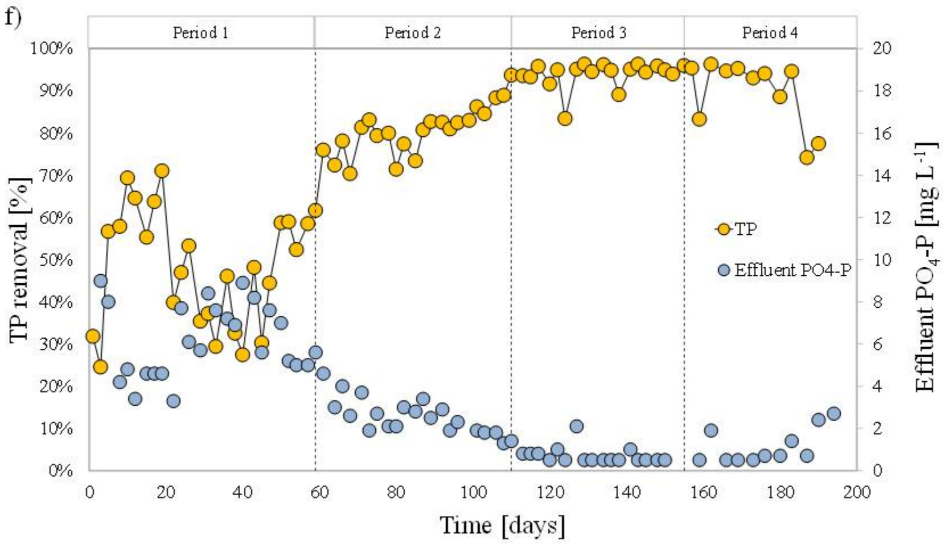


Figure 21 - Trends of the COD concentration in the influent, in the supernatant of the anoxic, aerobic and AMSR and in the permeate (a); biological and overall COD removal efficiency and effluent COD concentration (b); ammonia nitrogen in the influent and in the supernatant of each reactor (c); TN removal efficiency and TN concentration (as nitrate) in the permeate (d); PO_4-P concentration in the influent, in the supernatant of each reactor (e); TP removal efficiency and PO_4-P concentration in the permeate.

The trends of the COD concentration in the influent and in the supernatant of each reactor are shown in Fig. 21a, whereas Fig. 21b depicts the COD removal performances due to the biological process (biological) and the combination of the biological process and membrane retention (overall), as well as the COD concentration in the permeate. The influent COD concentration was ranged between 380–680 mg L^{-1} to maintain a steady F/M ratio according to the TSS concentration variation.

In Period 1, the COD concentration in the supernatant of the anoxic reactor was close to 18 mg L^{-1}, on average, and it slightly decreased after the plant configuration was changed to AMSR, likely due to a more effective use of the organic carbon for denitrification process. In contrast, the COD concentration was constant when the HRT was increased in Periods 2-4. The COD concentration in the supernatant of the aerobic reactor decreased during the experiment from nearly 17 mg L^{-1} (average value in Period 1) to 10 mg L^{-1} (average value in Period 3), whereas it increases to 19 mg L^{-1} on average in Period 4, showing an increasing trend with the HRT of the anaerobic reactor. Similarly, the COD in the supernatant of the anaerobic reactor increased with the HRT, reaching a maximum value of approximately 28.9 mg L^{-1} in Period 4. These results suggested the occurrence of lysis phenomena in the anaerobic reactor under prolonged HRT, thereby resulting in the deterioration of COD removal efficiency. Nevertheless, it is worth mentioning that a significant increase of the COD concentration in the supernatant of the anaerobic reactor was observed under an HRT higher than 8 h, indicating that HRT lower than, or equal to, this threshold value did not compromise the effluent quality in terms of COD. Moreover, the released COD was mainly degraded once the sludge was returned to the MBR, suggesting that the COD generated in the anaerobic reactor was biodegradable and had a minimal impact on overall COD removal efficiency.

Referring to COD removal efficiency, during Period 1 the COD removal due to biological process gradually increased suggesting the acclimation of the biomass to the new operating conditions (Fig. 21b). Beginning in Period 2, after the startup of the AMSR configuration, the biological contribution to COD removal slightly decreased from 98% to approximately 93% but it was gradually recovered, standing at 98% at the end of the period. In Period 3, the biological COD removal was stable at approximately 96%, although showing a decreasing trend at the end of the period. During these three periods, the overall COD removal was close to 97% on average, without showing any significant relationship neither with the change in plant configuration nor with the increase of the HRT in the anaerobic reactor. This result suggested that the membrane retention was able to ensure high COD removal, thus coping with the temporary decreases in the biological contribution to the organic carbon removal performance. In contrast, in Period 4 both the biological and the membrane removal efficiencies decreased in the long-term, suggesting that prolonged HRT (higher than 8 h) in the anaerobic reactor could be detrimental for the effluent quality.

The above results were in good agreement with the literature, indicating that the released COD in the anaerobic reactor increased with the rising in the HRT (Cheng et al., 2017). Although previous studies reported that the implementation of the anaerobic reactor improved the COD removal efficiency (Saby et al., 2003; Semblante et al., 2014), the findings of this study demonstrated that the COD removal efficiency deteriorated under HRT higher than 8 h according to what previously reported by Ye et al. (2008). It is possible to speculate that under prolonged anaerobic condition, the decrease of the biomass activity is so massive that bacteria resulted unable to cope with the COD release that occurred in the anaerobic reactor.

The trends of the influent ammonium concentration and that in the supernatant of each reactor are shown in Fig. 21c. The average ammonium concentration in the anoxic reactor was approximately equal to 10 mg L^{-1} during the entire experiment, indicating that the ammonium removed for heterotrophic synthesis accounted for approximately the 64% of the total nitrogen removal. The ammonium concentration in the supernatant of the aerobic reactor was always lower than 1 mg L^{-1}, indicating that complete nitrification was successfully achieved during the entire experiment. In the anaerobic reactor a slightly increase in the ammonium concentration occurred only during Period 3, whereas no significant variations were observed during the other experimental periods.

The main nitrogen form in the permeate was nitrate (Fig. 21d) that accounted for more than 98% of the effluent total nitrogen, whereas nitrites were not detected during the entire experiment. In Period 1, the nitrate concentration in the effluent increased during the first two weeks of operation, reaching a maximum value of approximately 36 mg L^{-1} on the 38th day, whereas it decreased to approximately 12 mg L^{-1} at the end of the period, indicating the achievement of steady conditions. The TN removal efficiency at steady state was close to 85%. In Period 2, the nitrate concentration in the permeate decreased of approximately 50%, standing at an average value of 5 mg L^{-1} during the rest of the experiment. Accordingly, the TN removal efficiency increased from 86% to approximately 91% because of the implementation of the anaerobic mainstream reactor, but no significant improvements were observed as a result of higher HRTs. The obtained results were in good agreement with previous literature (de Oliveira et al., 2018; Semblante et al., 2014), indicating that the AMSR configuration enabled a not negligible improvement in nitrogen removal performance.

The trends of orthophosphate in the supernatant of each reactor and the removal efficiency are depicted in Figure 21e and Figure 21f, respectively. In Period 1, a slightly increase of the phosphorous concentration in the supernatant of the anoxic reactor was periodically observed, suggesting that anaerobic condition occasionally occurred likely due to the completion of denitrification reaction. Indeed, the P-release in the anoxic reactor occurred when the TN removal efficiency was of approximately 90% during Period 1. Overall, in Period 1 the TP removal efficiency was of approximately 50%. In Period 2, a significant release of phosphorous was observed in the anaerobic reactor. The average concentration of orthophosphate in the supernatant of the anaerobic reactor was of approximately 20 mg L^{-1}, whereas it was significant lower in the aerobic reactor (3.28 mg L^{-1}) indicating that the integration of the anaerobic reactor in the mainstream favored the release and the uptake of orthophosphate by PAO. Similarly, in Period 3 the PO_4-P concentration in the anaerobic reactor slightly increased to 25 mg L^{-1}, whereas that in the aerobic reactor decreased to 0.76 mg L^{-1}, indicating an increase in TP removal. As can be noticed in Fig. 21f, the average TP removal efficiency in Period 2 was close to 78%, although showing an increasing trend during the entire period, whereas it significantly increased in Period 3 where it reached its maximum value of 97% at steady state. In Period 4, a significantly lower release of orthophosphate was observed in the anaerobic reactor, where the average PO_4-P concentration was of approximately 7 mg L^{-1}, which was slightly higher than that measured in the anoxic reactor (5 mg L^{-1}). Overall, the TP removal efficiency was higher than 90%, on average, while showing a decreasing trend at the end of the experiment.

The results above demonstrated that the change in the plant configuration to AMSR enabled the achievement of high TP removal performance. In the anaerobic reactor, similar conditions in terms of ORP (< -350 mV) compared to that of enhanced biological phosphorus removal (EBPR) systems occurred (Chudoba et al., 1992). In previous literature, it was observed that the integration of an anaerobic reactor in the plant layout (*i.e*. OSA process), encouraged the selection of PAO (Semblante et al., 2014). However, many researchers found contradictory results regarding phosphorous removal in these systems. Among these, Ye et al. (2008) observed that TP removal efficiency increased from 48% to 58% in a CAS-OSA system, whereas Saby et al. (2003) reported that TP removal in an MBR-OSA decreased from 55% to 28% when the ORP in the anaerobic reactor was adjusted to -250 mV. Besides, other authors observed that coupling an ASSR with an UCT-scheme had negative effects on the phosphorous removal performance (Velho et al., 2016). The authors emphasized than the main drawback affecting phosphorus removal in ASSR configuration is that release of orthophosphate under anaerobic conditions cannot be prevented especially if prolonged anaerobic conditions (ORP < - 250 mV) are imposed to maximize the excess sludge minimization.

In the AMSR configuration, significant differences compared to conventional EBPR can be found. Indeed, the COD/P ratio in the AMSR, close to 1:1, is significantly lower than that commonly observed in EBPR systems (> 20:1). Moreover, it is worth mentioning that in conventional EBPR systems, phosphorous is separated from wastewater through disposal of the waste sludge enriched in orthophosphate, whereas in the AMSR system the achievement of sludge minimization reduces the amount of sludge to be withdrawn, thus leading to a potential accumulation of PO_4-P within the system.

The results obtained in study demonstrated that very high TP removal efficiency were achieved in the AMSR configuration, although the low COD/P ratio and the low amount of sludge withdrawn. This result suggested that a different mechanism of phosphorous removal occurred in the AMSR system, which favored the simultaneous achievement of P-removal and sludge minimization.

7.2.3 Behavior of OHO (Ordinary Heterotrophic Organisms) and PAOs kinetics

With the aim to give an insight into the impact of the HRT in the AMSR on the biomass metabolism, kinetic tests were carried out to determine the biokinetics parameters of the OHO and investigate on the phosphorous removal mechanism. The main biokinetics parameters referred to the OHO are summarized in Table 13.

Table 13: Summary of the main biokinetics parameters of the OHO.

Parameter	Unit	Period 1	Period 2	Period 3	Period 4
Maximum heterotrophic growth rate ($\mu_{max,H}$)	[d^{-1}]	2.512±0.131	1.426±0.093	1.190±0.078	1.230±0.025
Endogenous decay coefficient (b_H)	[d^{-1}]	0.109±0.246	0.157±0.104	0.287±0.086	0.291±0.046
Heterotrophic active biomass (f_{XH})	[%]	13.80±0.4%	3.74±0.2%	3.40±0.2%	1.14±0.16%
Specific Oxygen Uptake Rate (SOUR)	[$mgO_2\ L^{-1}h^{-1}$]	30.46±11.2	30.82±4.6	31.73±5.2	40.31±3.6
Storage Yield Coefficient (Y_{sto})	[$mgCOD\ mgCOD^{-1}$]	0.57±0.12	0.67±0.15	0.69±0.09	0.63±0.08

The maximum heterotrophic growth rate ($\mu_{max,H}$) significantly reduced from 2.512 d^{-1} to 1.426 d^{-1} from Period 1 to Period 2, thereby indicating a decrease in new bacterial cell synthesis following the startup of the AMSR. When the HRT in the AMSR was increased to 8 h in Period 3, the $\mu_{max,H}$ decreased approximately to 1.19 d^{-1}, whereas it slightly increased in Period 4 to 1.23 d^{-1}.

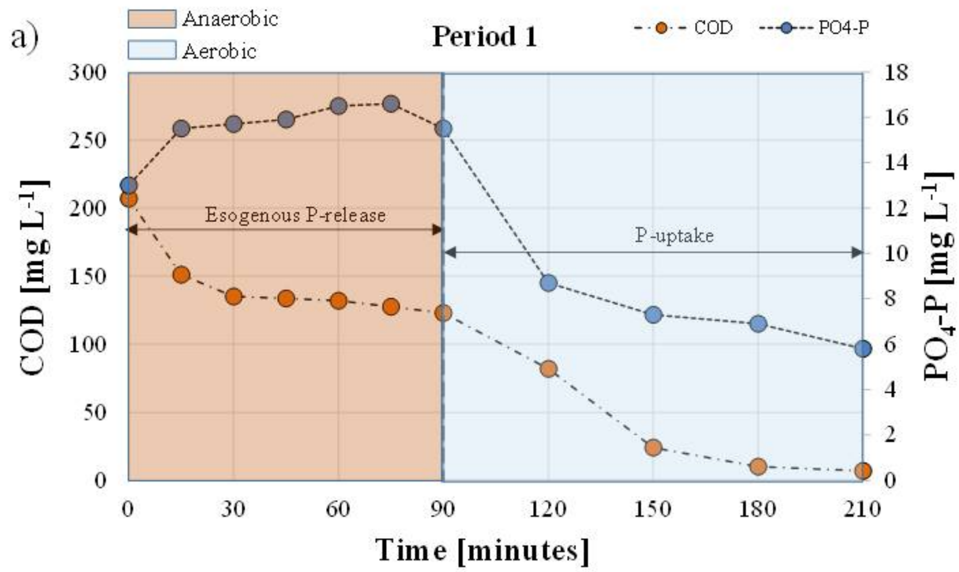
The endogenous decay coefficient (b_H) increased according to the rise of the HRT in the AMSR. The maximum value of the b_H was observed in Period 4 (0.291 d^{-1}), although there was not a significantly increase when the HRT was extended from 8 h to 10 h. Overall, the minimum net growth rate, evaluated as the difference between $\mu_{max,H}$ and b_H, resulted minimum in Period 3, when the AMSR operated under HRT of 8 h. This finding was in good agreement with the previous discussed results, confirming that the maximum efficiency in terms of sludge minimization was observed in Period 3.

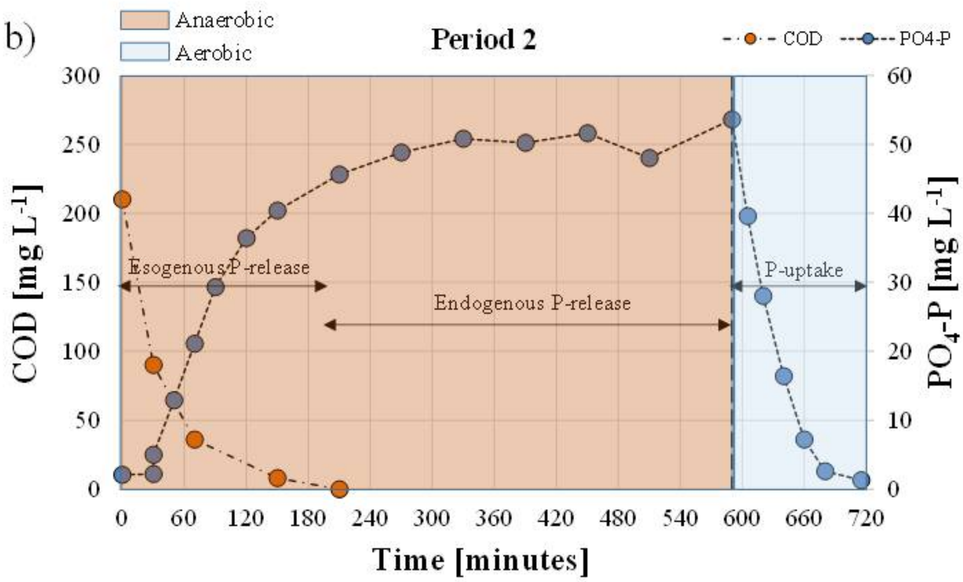
The heterotrophic active fraction (f_{XH}) significantly decreased from Period 1 (13.8%) to Period 2 (3.74%). Hereafter, the f_{XH} slightly decreased with the increase of the HRT, reaching its minimum value of approximately 1.14% in Period 4. Considering that the VSS/TSS was constant during the experiment, the above result indicated that a significant accumulation of endogenous residue occurred likely due to the membrane retention.

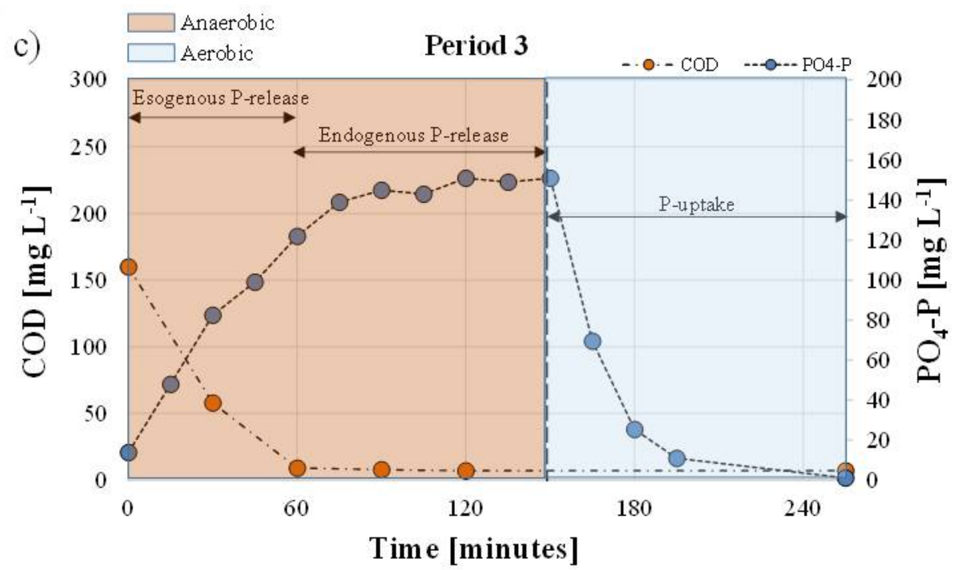
The specific oxygen uptake rate (SOUR) increased during the entire experiment from 30 mg O_2 L^{-1} h^{-1} to approximately 40 mgO_2 L^{-1} h^{-1}, according to the decreasing trend previously observed for the heterotrophic active fraction.

Lastly, the storage yield coefficient (Y_{sto}) that represents the amount of organic substrate converted into internal storage products, increased from 0.57 mgCOD mgCOD^{-1} to 0.67 mgCOD mgCOD^{-1} when the AMSR was started-up in Period 2. The maximum Y_{sto} was obtained in Period 3 (0.69 mgCOD mgCOD^{-1}), whereas it slightly decreased in Period 4 (0.63 mgCOD mgCOD^{-1}) when the HRT in the AMSR was increased from 8h to 10 h. These results indicated that the integration of the AMSR was favorable to the development of bacteria with internal storage capacity (*i.e.,* PAO).

In parallel to the OHO biokinetics evaluation, specific kinetic tests aimed at assessing the PAO kinetic behavior were assessed. Fig. 22 shows the trends of orthophosphate and COD during the batch tests performed from Period 1 to Period 4.


a)
Anaerobic
Aerobic
Period 1
COD
PO4-P
Esogenous P-release
P-uptake
COD [mg L-1]
PO4-P [mg L-1]
Time [minutes]


b)
Anaerobic
Aerobic
Period 2
COD
PO4-P
Esogenous P-release
Endogenous P-release
P-uptake
COD [mg L-1]
PO4-P [mg L-1]
Time [minutes]



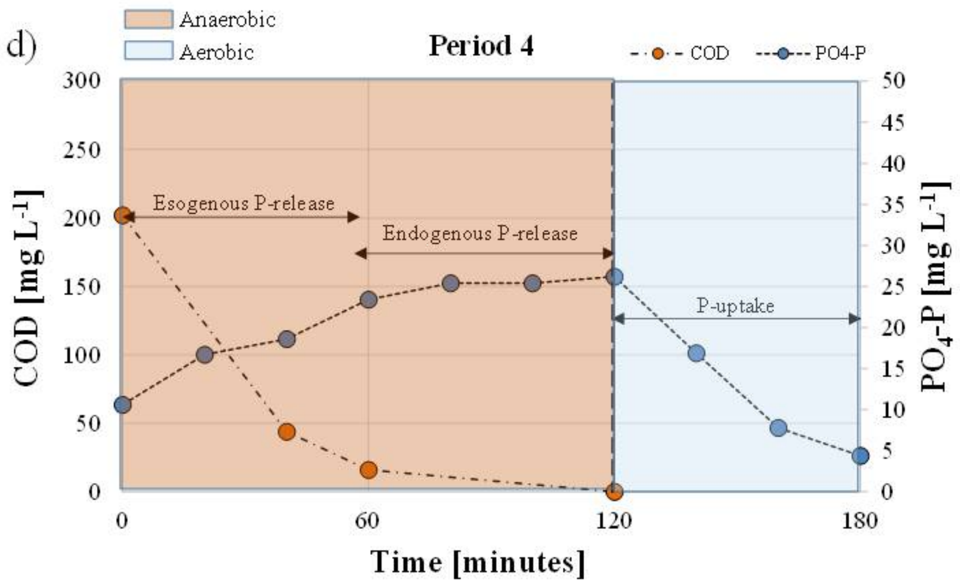


Figure 22 - Results of the kinetic tests carried out in Period 1 (a), Period 2 (b), Period 3 (c) and Period (4).

In Period 1, no significant P-release occurred during the anaerobic phase, suggesting a very poor presence and activity of PAO. In the aerobic phase, a not negligible P-uptake was observed, resulting in a removal of approximately the 50%, according to what previously discussed with reference to TP removal efficiency observed in Period 1. The P-release occurred in presence of the external carbon source (sodium acetate) supplied at the beginning of the batch test, resulting in a P-release rate of approximately 0.76 mgPO_4-P gVSS^{-1} h^{-1}, whereas the P-uptake rate was 2.31 mgPO_4-P gVSS^{-1} h^{-1}. In Period 2, during the anaerobic phase a significant P-release was observed. More precisely, in the presence of the external carbon source the PO_4-P concentration increased from 12 mg L^{-1} to 48 mg L^{-1} with a P-release rate of 5.90 mgPO_4-P gVSS^{-1} h^{-1}. Interestingly, P-release was still observed even in the absence of the external carbon source, thereby suggesting that orthophosphate release occurred under endogenous conditions. The release rate under endogenous conditions was of approximately 0.6 mgPO_4-P gVSS^{-1} h^{-1}, thus resulting lower of approximately an order of magnitude compared to the exogenous P-release rate. The P-uptake during the aerobic phase increased compared to the previous period resulting close to 39.7 mgPO_4-P gVSS^{-1} h^{-1}. In Period 3, the exogenous P-release was of approximately 18.90 mgPO_4-P L^{-1} h^{-1}, indicating the achievement of the maximum activity of PAO. Even in this case, the P-release under endogenous conditions was observed, with a release rate of approximately 3.08 mgPO_4-P gVSS^{-1} h^{-1}. The P-uptake during the aerobic was close to 48.6 mgPO_4-P gVSS^{-1} h^{-1}, thus indicating a significant increase compared with the previous period. In Period 4, both the exogenous and endogenous P-release decreased to approximately 6.10 mgPO_4-P gVSS^{-1} h^{-1} and 1.33 mgPO_4-P gVSS^{-1}h^{-1}, respectively, as well as the P-uptake during the aerobic phase that decreased to 10.38 mgPO_4-P gVSS^{-1} h^{-1}.

The obtained results were in good agreement with the TP removal performances previously discussed. Indeed, considering only the steady values achieved at the end of each experimental periods, the maximum TP removal efficiency was obtained in Period 3, followed by Period 2, Period 4 and Period 1, according to the P-release and P-uptake rates measured during the batch tests. Both the exogenous and the endogenous P-release and P-uptake increased with the HRT of the AMSR reaching their respective maximum values at 8 h of HRT.

Comparing the P-release and P-uptake rates obtained in this study with those reported in the literature, it was found that the exogenous P-release was considered very good in Period 3 (P-release > 7 $mgPO_4$-P gVSS h^{-1}), whereas it was good in Period 2 and Period 4 (P-release = 3÷7 $mgPO_4$-P $gVSS^{-1}$ h^{-1}), according to the classification proposed by Janssen et al. (2002) Similarly, the endogenous P-release was classifiable as good in Period 3 (3.08 $mgPO_4$-P $gVSS^{-1}$ h^{-1}), whereas it was moderate in Period 2 (0.6 $mgPO_4$-P $gVSS^{-1}$ h^{-1}) and Period 4 (1.33 $mgPO_4$-P $gVSS^{-1}$ h^{-1}). In contrast, the P-uptake was classifiable as very good in all the periods (P-uptake > 7 $mgPO_4$-P $gVSS^{-1}$ h^{-1}), suggesting that the anaerobic P-release was the limiting process affecting the phosphorous removal performance.

Our findings demonstrated that phosphorous removal was achieved even under endogenous conditions, thus with C/P ratio significantly lower than that of conventional EPBR systems (Chuang et al., 2011). The mechanism involving phosphorous removal in the AMSR is different compared to that occurring in conventional EBPR systems. A possible explanation for P removal in the AMSR-MBR could be that under extended endogenous-anaerobic conditions, bacterial lysis could result in the release of intracellular substrates that were likely subjected to hydrolysis and fermentation within the anaerobic reactor. This promoted the formation of simple organic molecules, which were stored by PAO in intracellular solids such as polyhydroxybutyrate. As aforementioned, a significant decrease in the heterotrophic active fraction was observed during the experiment because of decay phenomena due to the AMSR implementation. Nevertheless, the VSS/TSS ratio was almost constant during the experiment, thereby suggesting that a significant amount of endogenous residue, deriving from the bacteria decay phenomena, was retained within the system by the membrane. The endogenous residue, being constituted by biodegradable organic substances (Ramdani et al., 2012), could be used as substrate by PAO, thereby proving the carbon source necessary to drive the release of orthophosphate under anaerobic conditions. In this respect, the membrane provided a crucial contribution to enable the endogenous residue retention. This would explain the simultaneous achievement of phosphorous removal and sludge minimization achieved in the AMSR-MBR system.

As previously observed referring to COD and TN removal, also kinetic tests revealed that the HRT of 8 h enabled the highest TP removal efficiency and PAO biokinetics. Based on the above results, it is possible to speculate that HRT within the range of 6-8 h were favorable to the selection of slow growing microorganisms (*i.e.*, PAO) at the expense of others, whereas the prolonged anaerobic conditions in the AMSR in Period 4 improved the decay of the biomass including PAO.

7.2.4 EPS content and composition

Some studies suggested that one possible mechanism leading to sludge reduction in sludge cycling systems is the destruction of EPS that occurs under anaerobic conditions. The average EPS content during the experimental periods in the mixed liquor of each reactor is shown in Fig. 23.

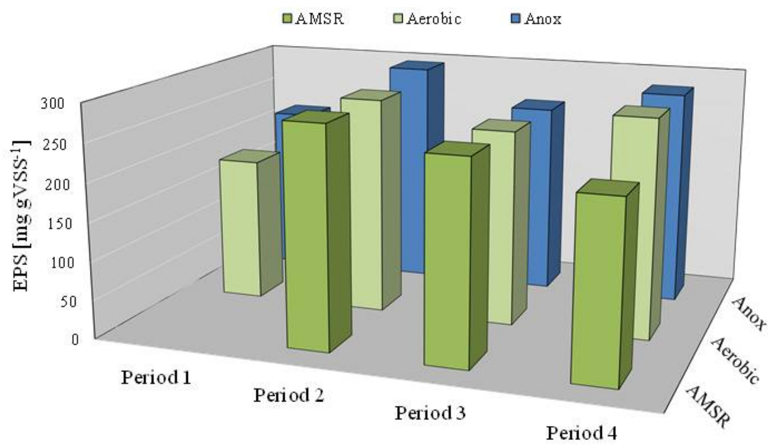


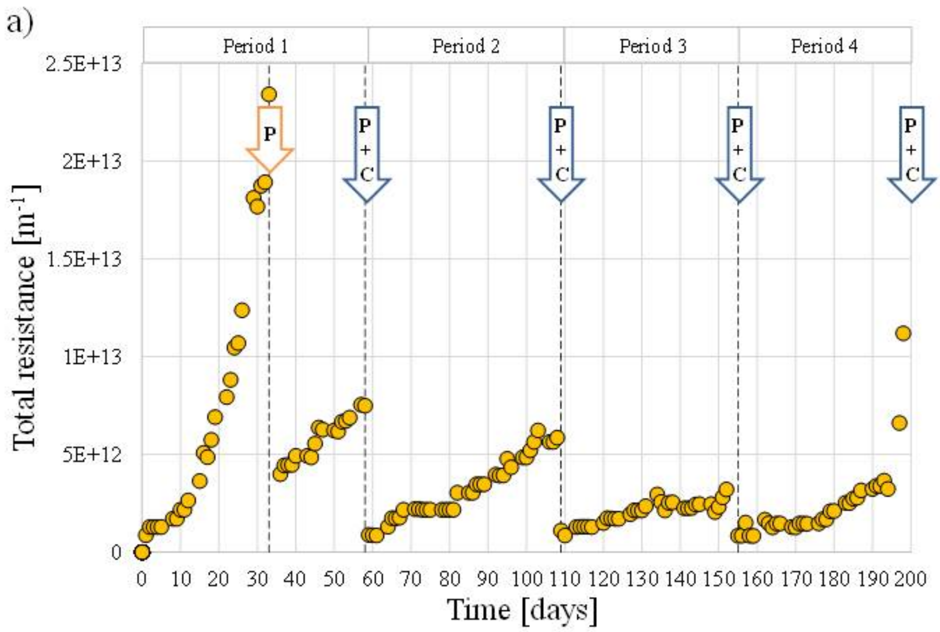
Figure 23 - Average EPS content in the anoxic, aerobic and AMSR during the four experimental periods.

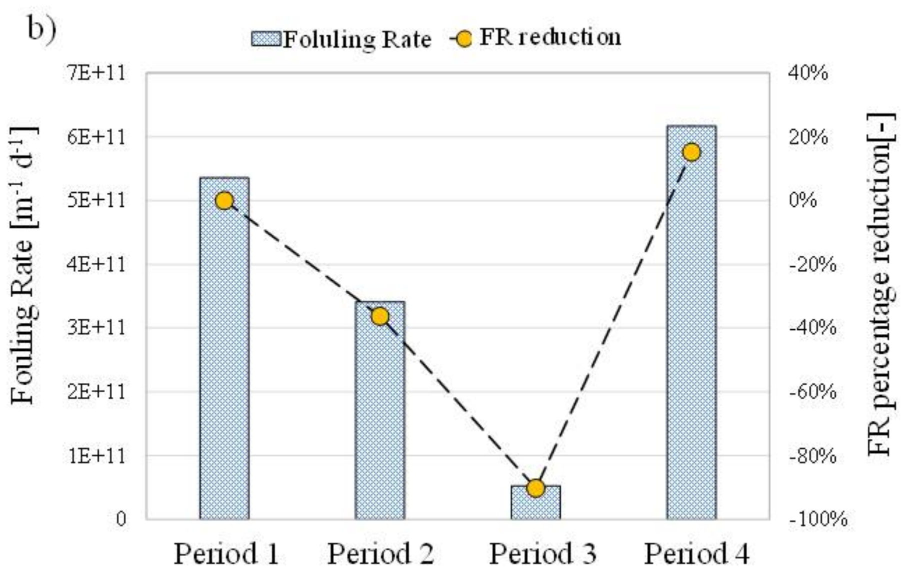
The average EPSs content in Period 1 was of approximately 200 mgEPS $gVSS^{-1}$ and the same were mainly constituted by the bound fraction that accounted for more than 99% of the entire EPS amount. The ratio between protein and carbohydrates (PN/PS) was on average close to 6, indicating the predominance of proteinaceous exopolymers in the EPS matrix. The average EPS content increased to approximately 290 mgEPS $gVSS^{-1}$ in Period 1 and no significant differences were observed among the anoxic, aerobic and the AMSR reactors. The amount of soluble microbial products (SMP) was negligible (< 2 mgSMP $gVSS^{-1}$) indicating that the destruction of EPS did not occur when the AMSR was implemented in the original MBR layout in Period 2. The PN/PS ratio significantly increased to approximately 10.7 because a significant decrease in the carbohydrates (> 40%) amount in the EPS. In Period 3, the EPS content slightly decreased to approximately 235 mgEPS $gVSS^{-1}$ in all the reactors. As observed during the previous period, the amount of SMP was negligible, whereas the PN/PS ratio decreased to 5.8, this time because of the decrease in the protein content (35%). In Period 4, the average EPS content slightly increased to 260 mgEPS $gVSS^{-1}$, but in contrast to what observed in the previous periods, the EPS content was lower in the AMSR for approximately 20%, indicating that the destruction of EPS occurred when the HRT in the anaerobic reactor was higher than 8 h. Nevertheless, no SMP was measured in Period 4 likely because it was degraded by bacteria. The PN/PS ratio was similar compared to the previous period, thereby indicating that no significant changes in the EPS matrix composition occurred.

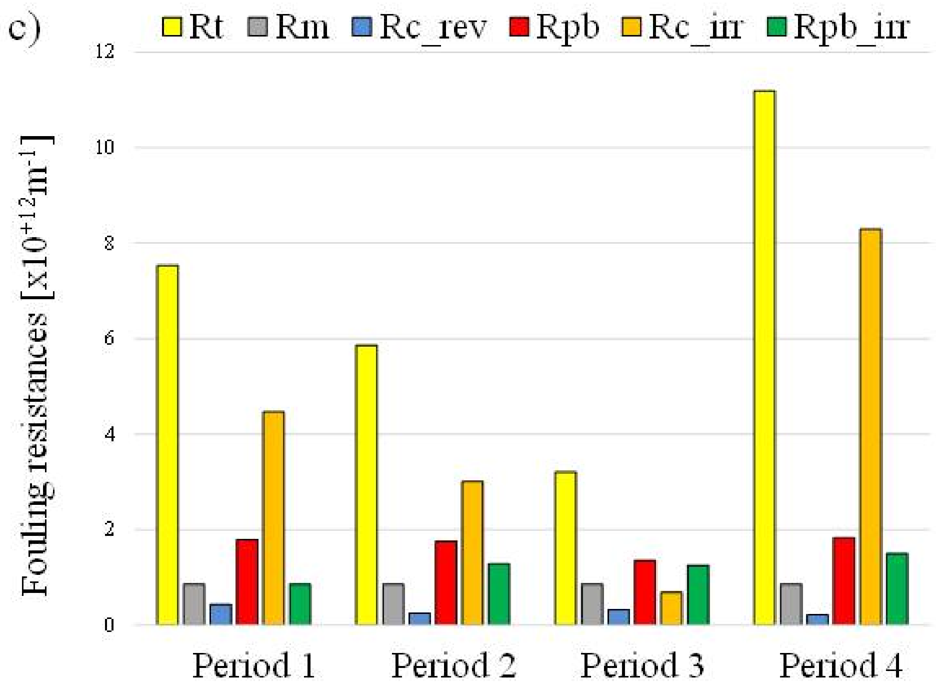
Based on the obtained results, a more extensively destruction on EPS occurred in Period 4, whereas in Period 2 and Period 3 only a partial decrease of certain fractions of EPS was observed. More precisely, the amount of carbohydrates slightly decreased under HRT of 6 h, whereas that of proteins decreased as a result of HRT of 8 h. These results are in contrast with that previously reported by de Oliveira et al. (2018), which observed a significant decrease in the total EPS content when the AMSR (HRT = 6 h) was implemented in the pre-denitrification MBR layout. Because the only difference between this study and the above cited was the SRT (35-40 d vs infinite SRT), it is possible to speculate that under HRT lower than 8 h, the destruction of EPS is mainly driven by the prolonged SRT. On the other hand, under controlled SRT the destruction of EPS occurred because of prolonged HRT in the anaerobic reactor (> 8 h). This finding should be taken into account in the light of the effects that EPS destructuration could exert on the membrane fouling tendency (Campo et al., 2017).

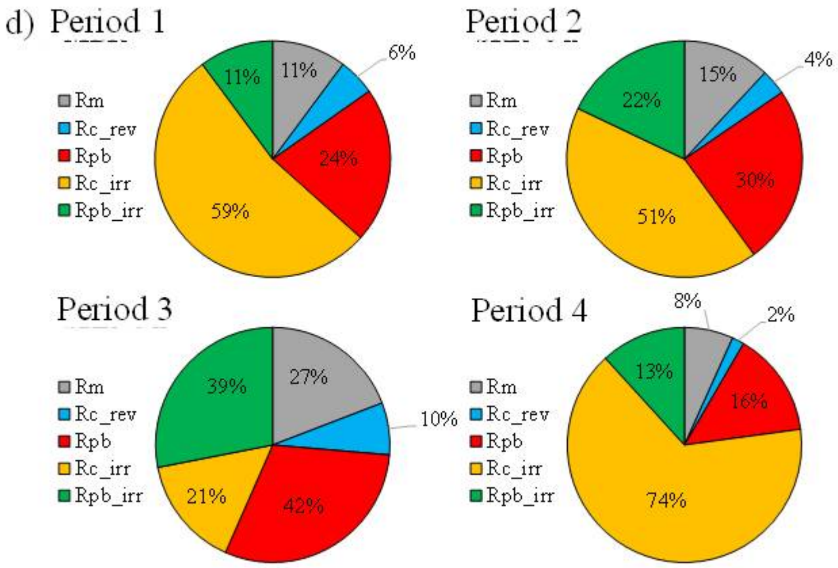
7.2.5 Membrane fouling analysis

It was previously emphasized that the sludge minimization must not compromise the effluent quality. Nevertheless, in an MBR system, also the hydraulic performances of the membrane should be considered with the aim to optimize the entire process. Fig. 24 depicts the trend of the total fouling resistance, the fouling rate, as well as the mechanisms involved in the membrane fouling mechanism.




b)
Foluling Rate
FR reduction
Fouling Rate [m-1 d-1]
FR percentage reduction[-]
7E+11
6E+11
5E+11
4E+11
3E+11
2E+11
1E+11
0
40%
20%
0%
-20%
-40%
-60%
-80%
-100%
Period 1
Period 2
Period 3
Period 4


c)
Rt
Rm
Rc_rev
Rpb
Rc_irr
Rpb_irr
Fouling resistances [x10+12m-1]
12
10
8
6
4
2
0
Period 1
Period 2
Period 3
Period 4



Legend: P: membrane physical cleaning; P+C: membrane physical+chemical cleaning; R_t: total resistance; R_m: membrane resistance; R_{c_rev}: reversible cake resistance; R_{pb}: pore blocking resistance; R_{c_irr}: irreversible cake resistance; R_{pb_irr}: irremovable pore blocking resistance

Figure 24 - The trend of the total resistance of the membrane (a), the average fouling rate during the four experimental periods (b), the total resistance decomposition (c), and the contribution of each fouling mechanism to the overall membrane fouling (d).

In Period 1, the increase of the total resistance (R_t) followed two different trends (Fig. 24a). Indeed, the R_t increased rapidly when the MBR operated under a complete sludge retention strategy, whereas it increased more slowly when a regular sludge withdrawn was performed. The average FR in this period was of approximately $5.3 \cdot 10^{11}$ m^{-1} d^{-1} (Fig. 24b). In Period 2, the R_t increased to approximately $6.3 \cdot 10^{12}$ m^{-1} after 49 days of operation before the membrane was subjected to a physical-chemical cleaning.

The FR decreased compared to the previous period, resulting close to $3.4 \cdot 10^{11}$ m^{-1} d^{-1} on average. In Period 3, the R_t reached a maximum of $4.1 \cdot 10^{12}$ m^{-1} after 48 days of operation, whereas the FR ($0.65 \cdot 10^{11}$ m^{-1} d^{-1}) was significantly lower than that observed in the previous periods. In Period 4, the Rt reached its maximum value of approximately 1.18·1013 m^{-1} after 44 days of operation, thereby showing the highest FR ($6.15 \cdot 10^{11}$ m^{-1} d^{-1}) of the entire experiment. The latter result was in good agreement with the destruction of EPS above discussed, indicating that the destructuration of the extracellular polymeric matrix could be detrimental for the membrane fouling tendency.

The results above confirmed that after the AMSR was implemented, the fouling tendency was significantly mitigated (de Oliveira et al., 2018). In this respect, the optimum HRT of the AMSR was found to be 8 h, which corresponded to the minimum FR achieved.

As noticeable from the Figure 24c and Figure 24d, the irreversible cake deposition in Period 1 was the main fouling mechanism, accounting for approximately 60%, whereas the pore-blocking and the reversible cake deposition contributed for approximately 24% and 11%, respectively. In Period 2, the contribution of the irreversible cake decreased to 51%, while in contrast those of the pore-blocking and the reversible cake increased to 30% and 22%, respectively. In Period 3, the same trend observed during the previous period was observed. Indeed, the irreversible cake significantly decreased to 21%, while in contrast that of the pore-blocking and the reversible cake increased to 41% and 39%, respectively. Lastly, in Period 4 the fouling mechanism was similar to that observed in Period 1, with the irreversible cake deposition, the pore-blocking and the reversible cake deposition accounting for 74%, 16%, 13% respectively.

From a point of view of the membrane service-life preservation, the pore-blocking mechanism should be minimized, or at least, the recovery of the membrane original permeability following chemical cleanings should be maximized. Bearing in mind this, the highest irreversible pore-blocking, means as the residual fouling after chemical cleanings, was observed in Period 4, whereas the lowest in Period 1. This result indicated that the integration of the AMSR caused a potential decrease in the membrane service-life. Nevertheless, the minimum irreversible pore-blocking resistance was observed in Period 3, suggesting that 8 h resulted the proper value of the HRT to achieve sludge minimization, while preserving the membrane fouling.

It is worth mentioning that the irreversible cake deposition could significantly decrease the membrane flux, thereby reducing the plant loading potential (Janus and Ulanicki, 2015). In this respect, the minimum contribution of the irreversible cake deposition to the overall membrane fouling was observed in Period 3 (21%). The resistance due to the irreversible cake was found to be in good agreement with the hydrophobicity of the cake layer (Fig. 25).

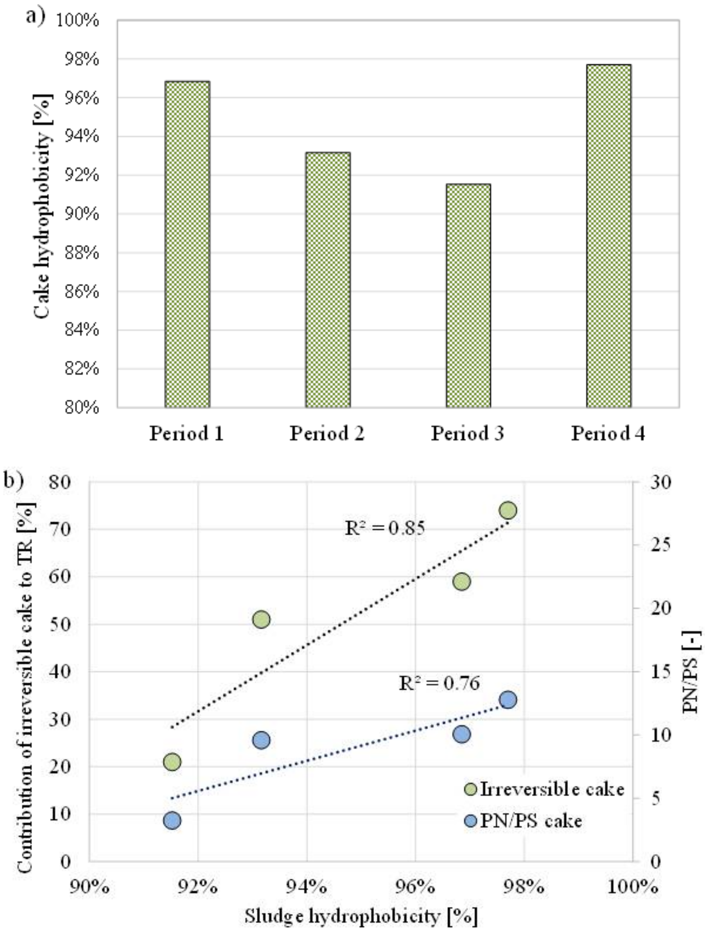


Figure 25 - Hydrophobicity of the cake layer during the four experimental periods (a); relationship between the sludge hydrophobicity with the contribution of the irreversible cake to the total resistance and the PN/PS.

Indeed, the minimum value of the cake hydrophobicity was observed in Period 3 (91.7%) in correspondence with the lowest contribution of the irreversible cake deposition to the membrane fouling. In contrast, the maximum cake hydrophobicity was observed in Period 4 (97.7%), when the contribution of the irreversible cake deposition to the membrane fouling was the highest (74%). Referring to the cake layer composition, it was observed that the amount of proteinaceous EPS was minimum in Period 3, resulting in the lowest PN/PS ratio of both the bound (3.5) and soluble EPS (4.3) observed during the entire experiment. In contrast, the PN/PS ratio of the bound EPS was higher in Period 1 (10) and Period 2 (12), whereas the PN/PS in the soluble EPS was approximately equal to that observed in Period 2. These findings demonstrated that the increase in the irreversible cake resistance was related to the change in the cake composition. More precisely, the higher was the amount of proteins in relation to carbohydrates, the higher resulted the cake hydrophobicity. Indeed, as confirmed by previous studies, proteins are more hydrophobic than carbohydrates, thus their predominance resulted in the increase in sludge hydrophobicity (Niu et al., 2016). The prevalence of proteins was considered as important reason for its abundant existence on membrane surface, because of the establishment of hydrophobic iterations with the fibers membrane (PDVF).

7.2.6 Mechanism of sludge minimization in the AMSR and implications on the system performances

The findings achieved in this study demonstrated the effectiveness of integrating the AMSR for the simultaneous achievement of sludge minimization and phosphorous removal, enabling higher efficiency than that reported in the literature with ASSR configuration (de Oliveira et al., 2018; Ferrentino et al., 2014; Kim et al., 2012). Based on the above results, the AMSR integration enabled a significant decrease in sludge production, whose yield increased with the HRT in the anaerobic reactor. More precisely, the proper HRT was found to be 8 h that compensated the excess sludge minimization with the worsening of the effluent quality and the membrane fouling tendency that occurred under higher HRT (10 h).

The mechanisms involved in sludge minimization were different, which acted simultaneously to each other, making it difficult to identify the prevailing one. Indeed, the bacterial decay increased with the HRT in the AMSR as evidenced by the significant decrease in the heterotrophic active fraction and the increase in the endogenous decay rate. Moreover, another mechanism involved in sludge minimization was the selection of slow growing bacteria (PAO). Indeed, in Period 2 ad Period 3, a very high activity of PAO was observed, indicating that the amount of these microorganisms in the activated sludge significantly increased when the AMSR was integrated in the original MBR layout. This significantly improved the TP removal performances that rose to approximately 97%, resulting in PO_4-P concentration in the effluent close to 0.5 mg L^{-1}. Besides, kinetic tests pointed out that phosphorous release occurred in absence of external carbon source. This was likely because cell lysis released biodegradable low molecular weight compounds that were utilized by bacteria as a secondary substrate, suggesting the existence of the cryptic growth process (Quan et al., 2012). Lastly, it should be considered that the sludge cycling between anaerobic and aerobic conditions provided a basis for the energy uncoupling mechanism and the feasting/fasting conditions that contributed to the excess sludge minimization.

Although the sludge minimization efficiency increased with the HRT in the AMSR, it was noted that prolonged anaerobic-endogenous conditions were detrimental for both the nutrients removal performances, including the phosphorous, and the membrane fouling tendency. Indeed, the excessive decrease of the heterotrophic active fraction, the accumulation of endogenous residue, as well as the destruction of EPS, contributed to worsen the effluent quality. Moreover, under HRT higher than 8 h, the over-accumulation of proteinaceous EPS and SMP in the membrane cake layer caused a drastic increase in the membrane fouling rate and the irreversible cake deposition, thereby limiting the applicable flux.

The results demonstrated that the integration of the AMSR is a valuable management solution to achieve sludge minimization, although prolonged anaerobic-endogenous conditions are not encouraged to ensure that neither the effluent quality nor the hydraulic functionality of the membrane are compromised.

Chapter 8

Conclusions

Minimization of excess sludge was studied in an MBR. Two configurations involving the placement of an anaerobic tank in side-stream (ASSR+MBR) and in main-stream (AMSR+MBR) configurations were investigated. The ASSR+MBR enabled higher sludge reduction (74%), while achieving lower biological nutrient removal (BNR) (TN=63%) and membrane fouling tendency (FR=2.1 · 10^{12} m^{-1} d^{-1}). In contrast, the AMSR+MBR enabled lower sludge reduction (32%), while achieving lower membrane fouling tendency (FR=4.0 · 10^{11} m^{-1} d^{-1}) and higher BNR performance (TN=78%). Metabolism uncoupling, destruction of EPS and endogenous decay simultaneously occurred in the ASSR+MBR, whereas selective enrichment of bacteria with low biomass yield provided excess sludge reduction in the AMSR+MBR.

Therefore, the simultaneous achievement of sludge minimization and phosphorous removal was studied in an MBR system integrated with an anaerobic reactor placed in mainstream configuration. The AMSR operated under different HRT, from 6 h to 10 h. The AMSR enabled a sludge reduction of 64% and the highest removal performance of organic carbon (98%), nitrogen (90%) and phosphorous (97%) when it was operated under 8 h of HRT, as well as the lowest membrane fouling tendency (FR=0.65·10^{11} m^{-1} d^{-1}). In contrast, prolonged anaerobic-endogenous conditions (HRT = 10 h) were found to be detrimental for both the nutrients removal performances, including the phosphorous, and the membrane fouling tendency, because of the excessive decrease in the heterotrophic active fraction, the accumulation of endogenous residue, as well as the destruction of EPS. A significant high metabolic activity by PAO was observed in Period 3, showing very high exogenous P-release (98 mgPO_4-P L^{-1} h^{-1}) and P-uptake rates (100 mgPO_4-P L^{-1} h^{-1}), as well as a not negligible release rate under endogenous conditions (5.85 mgPO_4-P L^{-1} h^{-1}) at low C/P ratio (≈ 1).

The results suggested that the mechanism driving phosphorous removal in the AMSR could involve the use of the biodegradable low molecular weight compounds deriving from the bacterial lysis, as secondary substrate by PAO. This would lead the biomass to the cryptic growth process, thereby explaining the simultaneous achievement of phosphorous removal and sludge minimization achieved in the AMSR-MBR system.

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