Simultaneous sludge minimization, biological phosphorous removal and membrane fouling mitigation in a novel plant layout for MBR

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Abstract

The integration of one anaerobic reactor in the mainstream (AMSR) of a pre-denitritication-MBR was evaluated with the aim to achieve simultaneous sludge minimization and phosphorous removal. The excess sludge production was reduced by 64% when the AMSR was operated under 8h of hydraulic retention time (HRT). The highest nutrients removal performances referred to organic carbon (98%), nitrogen (90%) and phosphorous (97%) were obtained under 8 h of HRT. In contrast, prolonged anaerobic-endogenous conditions were found to be detrimental for all nutrients removal performances. Similarly, the lowest membrane fouling tendency (FR=$0.65 \cdot 10^{11}$ m^{-1} d^{-1}) was achieved under 8 h of HRT, whereas it significantly increased under higher HRT. The highest polyphosphate accumulating organisms kinetics were achieved under HRT of 8 h, showing very high exogenous P-release (46.67 $mgPO_4$-P $gVSS^{-1}$ h^{-1}) and P-uptake rates (48.6 $mgPO_4$-P $gVSS^{-1}$ h^{-1}), as well as a not negligible P-release rate under endogenous conditions at low COD/P ratio (≈ 1).

Keywords: Biological nutrients removal; Endogenous P-release; Membrane BioReactor; Membrane fouling; Sludge minimization.

List of abbreviations and symbols

AMSR – Anaerobic Main Stream Reactor

MBR – Membrane BioReactor

HRT – Hydraulic Retention Time

FR – Fouling Rate

PAO – Polyphosphate Accumulating Organisms

SRT – Sludge Retention Time

BNR – Biological Nutrient Removal

CAS – Conventional Activated Sludge

OSA – Oxic Settling Anaerobic

SRR – Sludge Retention Reactor

RAS - Return Activated Sludge

EPS – Extracellular Polymeric Substances

SMP – Soluble Microbial Products

ASSR – Anaerobic Side-Stream Reactor

OHO – Ordinary Heterotrophic Organisms

TSS –Total Suspended Solid

VSS – Volatile Suspended Solid

SCOD – Soluble Chemical Oxygen Demand

COD – Chemical Oxygen Demand

CIP – Clean In Place

DO - Dissolved Oxygen (DO)

ORP – Oxidation Reduction Potential

UCT – University of Cape Town

F/M – Food/Microorganisms

EBPR – Enhanced Biological Nutrient Removal

TN – Total Nitrogen

TP – Total Phosphorous

Rt – Total Resistance

PN – Proteins

PS – Polysaccharides

PDVF – Polyvinylidene fluoride

RIS – Resistance In Series

Y_{sto} – Storage yield coefficient

Y_H – Maximum heterotrophic yield coefficient

Y_{obs} – Observed yield coefficient

f_{XH} – Active fraction of the ordinary heterotrophic biomass

b_H – Endogenous decay coefficient of the ordinary heterotrophic biomass

μ_H – Net growth coefficient of the ordinary heterotrophic biomass

Introduction

The advanced technologies for wastewater treatment based on biological processes are aimed to increase the biological nutrient removal (BNR) performance, while saving energy and minimizing the excess sludge production (Moreira et al., 2015; Ioannou-Ttofa et al., 2016; Semblante et al., 2016a).

Among the new and advanced technologies that have been developed with this aim, the membrane bioreactor (MBR), the moving bed biofilm reactor (MBBR), the aerobic granular sludge (alias Nereda®) are considered the most promising to attain this goal (Pronk et al., 2015). In these systems, nutrients removal significantly improve due to the increase of biomass retention, thus enhancing the plants' loading capacity. Moreover, due to the higher value of the sludge retention time (SRT), low waste-sludge production could be achieved. Indeed, the selection of slow growing microorganisms promotes low sludge yields (Troiani et al., 2011; Devlin et al., 2016).

Nevertheless, similar results could be achieved by retrofitting existing plants based on conventional biological technologies, i.e., conventional activated sludge (CAS) systems. For instance, the oxic-settling-anaerobic (OSA) process, featuring the modification of a CAS plant by placing an anaerobic sludge retention reactor (SRR) in the return activated sludge (RAS) flow, represents one of the most potentially cost-effective and low impact solution to achieve excess sludge minimization (Foladori et al., 2010).

Several biological mechanisms contribute to the excess sludge reduction (even simultaneously). Among these, the biological maintenance metabolism, the uncoupling metabolism, the extracellular polymeric substances (EPS) destruction, the bacteria predation, have been highly debated in previous literature. (Wang et al., 2013). Moreover, many studies states that the sludge alternation between feasting and fasting conditions under anaerobic and aerobic environments, could be favorable to the development of polyphosphate accumulating organisms (PAO) (Goel and Noguera, 2006; Datta et al., 2009).

The excess sludge minimization in MBR systems was thoroughly investigated in anaerobic side-stream reactor (ASSR) configuration, consisting in the placement of one anaerobic reactor in the RAS line of the MBR plant (Kim et al., 2012; Semblante et al., 2014, 2016a). Although the majority of the studies reported excellent results towards sludge minimization, a collateral issue that arises might be represented by a long-term worsening of membrane permeability. Indeed, a worsening of the microbial cells features, including the production of EPS and the increase of soluble microbial products (SMP) in the bulk liquid deriving from the cellular lysis, might enhance the membrane fouling (in this case also referred to as "biofouling") (Wang et al., 2013).

Recently, a novel layout for MBR system was proposed with the aim to achieve excess sludge minimization by applying the anaerobic mainstream reactor (AMSR) configuration, while preserving the membrane permeability (de Oliveira et al., 2018). de Oliveira and co-workers proposed a modification of the conventional pre-denitrification scheme, by placing one anaerobic reactor in the mainstream between the anoxic and the aerobic reactor. In this system, a portion of the activated sludge flow from the anoxic reactor was fed to the anaerobic SRR and subsequently to the aerobic reactor. In the SRR, because of the anaerobic starvation, uncoupling metabolism occurred, thereby favoring the achievement of low biomass yield (Semblante et al, 2016a). de Oliveira and co-workers compared the ASSR configuration with the AMSR one and demonstrated that approximately 30% of excess sludge minimization could be achieved operating with 6 hours of hydraulic retention time (HRT) in the anaerobic reactor. Nonetheless, the authors suggested that an increase in the HRT could be beneficial to achieve higher excess sludge reduction. Moreover, the authors observed that in the AMSR configuration a significant increase of nutrients removal was obtained, thus suggesting the feasibility to achieve significant biological phosphorous removal. However, because in the AMSR configuration the anaerobic tank is placed downstream the anoxic reactor, where the rapidly biodegradable carbon was already depleted for denitrification, phosphorous release by PAOs would occur under endogenous conditions because of the low availability of residual carbon source, in contrast with what generally observed in conventional BNR plants (Zuthi et al., 2013).

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

Materials and methods

2.1 Pilot plant configuration

The experimental campaign was carried out on a MBR pilot plant operating at room temperature (20 ± 6 °C). The MBR pilot plant layout was realized according to a pre-denitrification scheme, consisting of one anoxic reactor followed by one aerobic, each characterized by a volume of 22.5 L. Further details about the plant configuration can be found in literature (de Oliveira et al., 2018).

Subsequently, the pre-denitrification scheme was modified by placing a sludge retention reactor, operated under anaerobic conditions, between the anoxic and the aerobic ones. This configuration was referred to as anaerobic mainstream reactor (AMSR). A portion of the activated sludge flow from the anoxic reactor was fed to the anaerobic reactor (continuously mixed by a mechanical stirrer) with a flow rate almost equal to the influent one (4.32 L h^{-1}) and then fed to the aerobic reactor. Different HRTs in the anaerobic reactor were investigated during the experiments. In detail, HRTs 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.

2.2 Experimental set-up

The MBR plant was seeded with activated sludge collected from a municipal WWTP characterized by a conventional activated sludge scheme (inoculum sludge concentration: 6.15 gTSS L^{-1}) and it was fed with synthetic wastewater. The composition of the synthetic wastewater is reported as supplementary information (SI). The experimental campaign had a duration of 198 days and it was divided into four periods, namely: 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, until steady conditions were achieved. During the first 21 days of Period 1 the sludge retention time (SRT) was not controlled and no dedicated sludge wasting operations were carried out. The aim was 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 of Period 1, a known amount of sludge was withdrawn daily, including the samples for physical-chemical analyses, with the aim to maintain a SRT close to 35-40 days. The same SRT was imposed during the following experimental periods. The excess sludge production was evaluated in terms of observed heterotrophic growth yield (Y_{obs}) and the Y_{obs} obtained in Period 1 was assumed as the reference value to evaluate the sludge minimization efficiency achieved in the following experimental periods. In Period 2 the MBR was operated with AMSR configuration for 49 days with a HRT in the anaerobic reactor of 6 h. When a 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 high SRT value, the achievement of steady state conditions in each periods was evaluated based on the biological performances, kinetic parameters and excess sludge production, instead of considering a duration of three times SRT.

Table 1 summarizes the main operating conditions and the average characteristics of the influent wastewater throughout experiments.

Table 1: 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

2.3 Analytical methods and activated sludge characterization

All the chemical-physical analyses including total and volatile suspended solid (TSS, VSS) concentrations, soluble chemical oxygen demand (SCOD), ammonium nitrogen (NH_4-N), nitrate nitrogen (NO_3-N), nitrite nitrogen (NO_2-N) and orthophosphate (PO_4-P) were performed according to standard methods (APHA, 2005). The chemical oxygen demand (COD), NH_4-N, NO_3-N, NO_2-N and PO_4-P were measured in the influent, in the supernatant of the mixed liquor from each reactor and in the clean-in-place (CIP) tank. Briefly, the aim of CIP tank was to store a portion of permeate for the membrane ordinary backwashing. TSS and VSS were measured in the mixed liquor of all the reactors. Dissolved oxygen (DO) concentration, oxidation-reduction potential (ORP) and pH were measured in all the reactors by means of specific probes (WTW 3310).

The EPS and SMP were extracted from the activated sludge according to the literature (Le-Clech et al., 2006). For each fraction, the polysaccharides and protein concentrations were determined according to the phenol-sulphuric acid method (DuBois et al., 1956) and by the Folin method (Lowry et al., 1951), respectively.

With the aim to give an insight to the membrane fouling mechanisms, specific measurements on the cake layer were performed. In particular, after the cake was manually removed, the amount and composition of EPS was evaluated according to the above reported method, as well as the relative hydrophobicity (Rosenberg, 1984).

2.4 Assessment of biomass growth and heterotrophic kinetics parameters

The Y_{obs} was calculated through a mass balance according to literature (de Oliveira et al., 2018). The evaluation of the heterotrophic kinetic parameters, including the endogenous decay coefficient (b_H), the net growth coefficient (μ_H), the maximum yield coefficient (Y_H) and the active fraction of the ordinary heterotrophic biomass (f_{XH}), was carried out at controlled temperature (20 ± 0.1 °C) according to the literature (Capodici et al., 2016). The calculations of the above mentioned parameters are reported as supplemtary information (SI).

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, if present, 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). Subsequently, samples were taken at regular time intervals (15-20 minutes) and PO_4-P and COD were measured after samples filtration through a 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 external COD in the batch reactor was not completely depleted, whereas the endogenous P-release was measured as the P-release occurred after external COD was completely depleted.

2.5 Membrane fouling analysis

The membrane fouling was investigated by assessing the total resistance to filtration (R_T), the fouling rate (FR) and the specific deposition mechanisms according to a previous study (de Oliveira et al., 2018). The details about the calculation of R_T, FR and RIS model application are reported as SI.

3. Results and Discussion

3.1 Biomass growth and excess sludge production

The trend of TSS concentration as well as the ratio between VSS and TSS throughout experiments are shown in Figure 1.

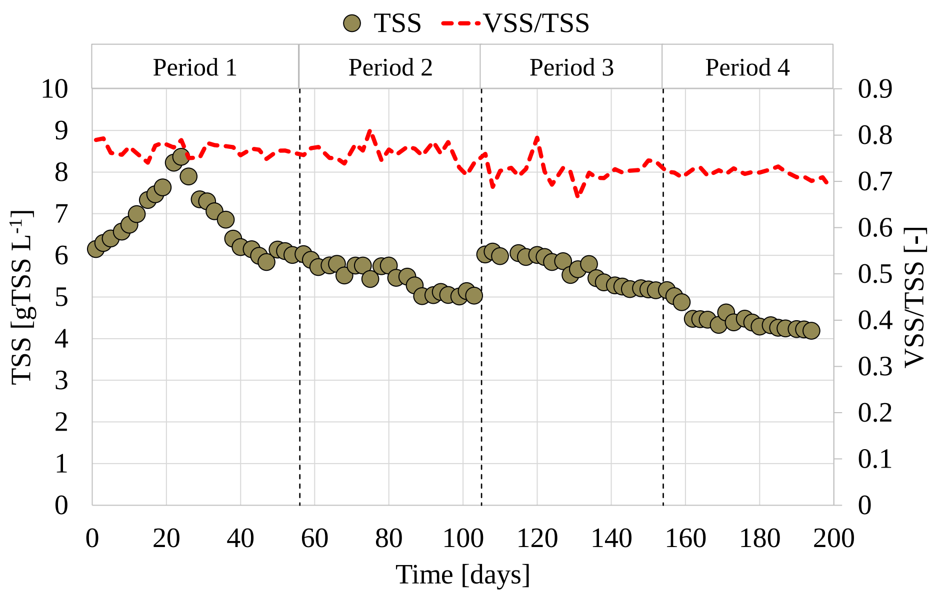


Figure 1: Trends of TSS concentration and VSS/TSS ratio during the experiment

In Period 1, the TSS concentration increased from 6.15 gTSS L^{-1} to a 8.5 gTSS L^{-1}, indicating that the biomass was successfully acclimated to the new operating conditions of the MBR system. When a regular sludge withdrawn was performed, the TSS concentration decreased reaching an almost constant value of 6 gTSS L^{-1}. In Period 2, the TSS concentration showed a slightly decreasing trend, reaching a steady value of 5 gTSS L^{-1} at the end of this period. At the beginning of Period 3, the TSS concentration was increased to 6 gTSS L^{-1} by adding a portion of the sludge wasted in Period 2, in order to achieve similar conditions of Period 1 in terms of TSS and food/microorganisms (F/M) ratio. In Period 3, the TSS concentration decreased according to what observed in Period 2, reaching an almost stable value of 5.20 gTSS L^{-1} at the end of the period. Compared to the previous periods, the decreasing trend observed in 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 result 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 4.22 gTSS L^{-1} at the end of the experiments, showing a similar trend of Period 3.

The VSS/TSS ratio showed a slightly decreased trend throughout experiments. 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.

The average Y_{obs} values, the maximum yield coefficient (Y_H), as well as their respective percentage reductions obtained in each experimental period are depicted in Figure 2.

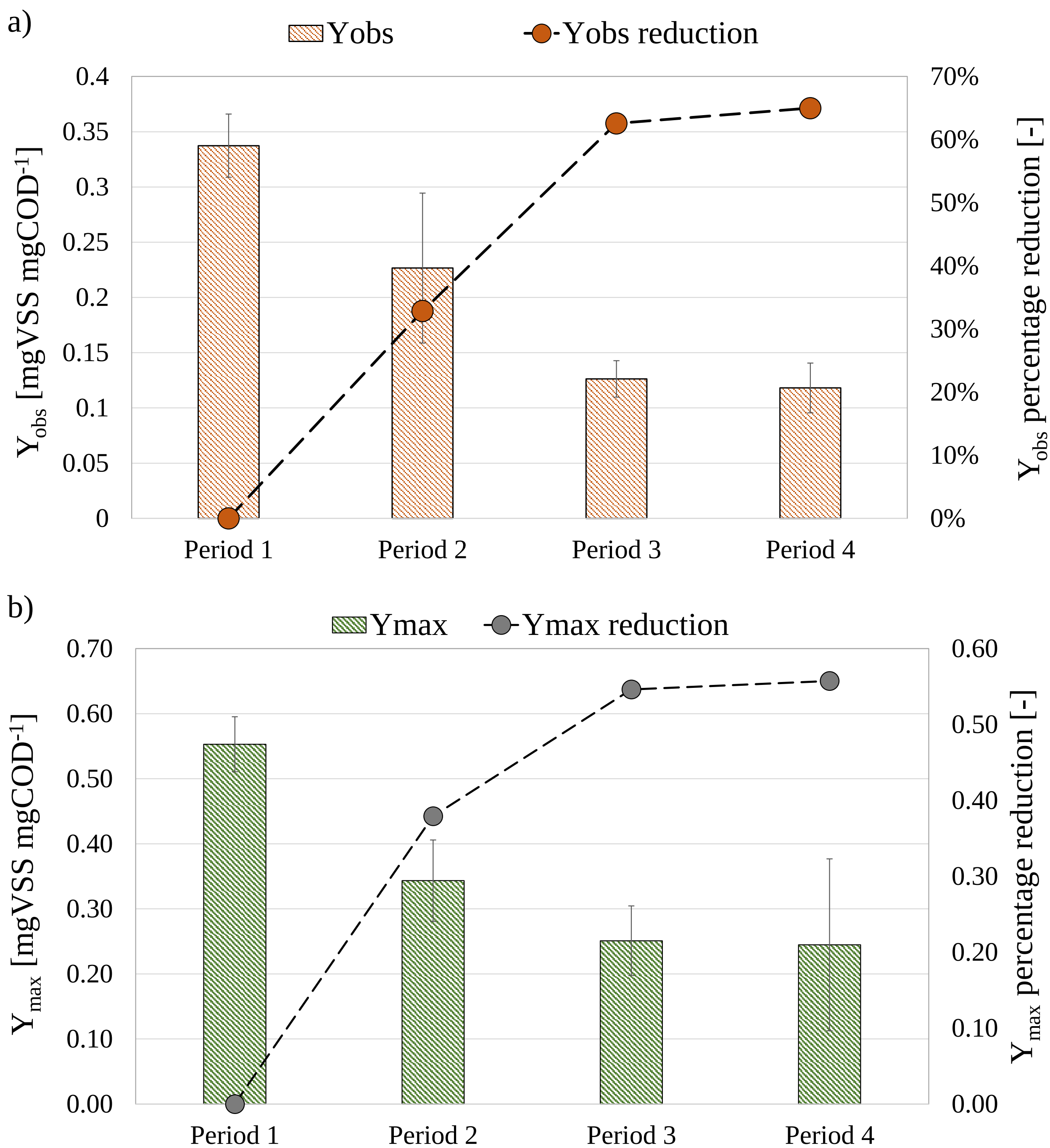


Figure 2: Average values of the observed yield coefficient (Y_{obs}), the maximum yield coefficient (Y_{max}) and their respective percentage reduction obtained in the four experimental periods.

The average value of the Y_{obs} (Fig. 2a) in Period 1 was close to 0.33 kgVSS $kgCOD^{-1}$ that was slightly higher compared with what observed in other MBR systems operated under similar SRT and F/M values (Wang et al., 2013). Nevertheless, the Y_{obs} was very similar to what observed by de Oliveira et al. (2018), who operated with acetate based synthetic wastewater and prolonged SRT. After the plant configuration was changed to AMSR (Period 2), the Y_{obs} decreased to 0.22 kgVSS kgCOD^{-1}, showing a reduction of 33% compared to Period 1. This result was in good agreement with what reported by de Oliveira et al. (2018), thus confirming that it is possible to achieve 30% of Y_{obs} reduction by operating the anaerobic reactor of AMSR configuration at 6 h. 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 the excess sludge production. In Period 4, the increase of the anaerobic reactor HRT did not provide a significant decrease of the sludge minimization. Indeed, the Y_{obs} was approximately 0.11 kgVSS kgCOD^{-1}, which was very close to what achieved in the previous period. This result highlighted that the increase of the anaerobic reactor HRT from 8 h to 10 h did not provide any significant advantage in terms of sludge minimization.

The Y_H 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. 2b). 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 similar trends and values observed for both the Y_{obs} and Y_H indicated that the operating parameters (i.e. SRT) had a negligible role on the overall excess sludge minimization. Therefore, sludge reduction was achieved because of the change in the plant configuration.

The results indicated that the integration of an anaerobic reactor (HRT of 8 h) in the mainstream enabled 62% of the excess sludge reduction. Compared with a previous study, the AMSR configuration enabled a slightly lower excess of sludge minimization compared with the ASSR (62 *vs* 72%) even operating at higher HRT (8 h vs 6 h) (de Oliveira et al., 2018). Nevertheless, it is worth mentioning that in the present study, the SRT was significantly lower (35-40 d *vs* infinite SRT), whereby the contribution of the decay phenomena to the excess sludge minimization was certainly lower. 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 connected 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 with ASSR-MBR systems, the maximum excess sludge reduction (55-58%) was achieved under 10-11 h of HRT in the anaerobic reactor (Saby et al., 2003; Ferrentino et al., 2016). Similarly, Cheng et al. (2017) observed that the sludge yield decreased by 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 to an anaerobic side stream reactor under a HRT of 5.9 h.

The results obtained in the present study suggested that in general the AMSR configuration might enable a higher sludge minimization than the ASSR under similar HRT and SRT.

3.2 Nutrient removal performances

The MBR plant was periodically monitored to evaluate the COD, nitrogen and phosphorous removal performances (Fig. 3).

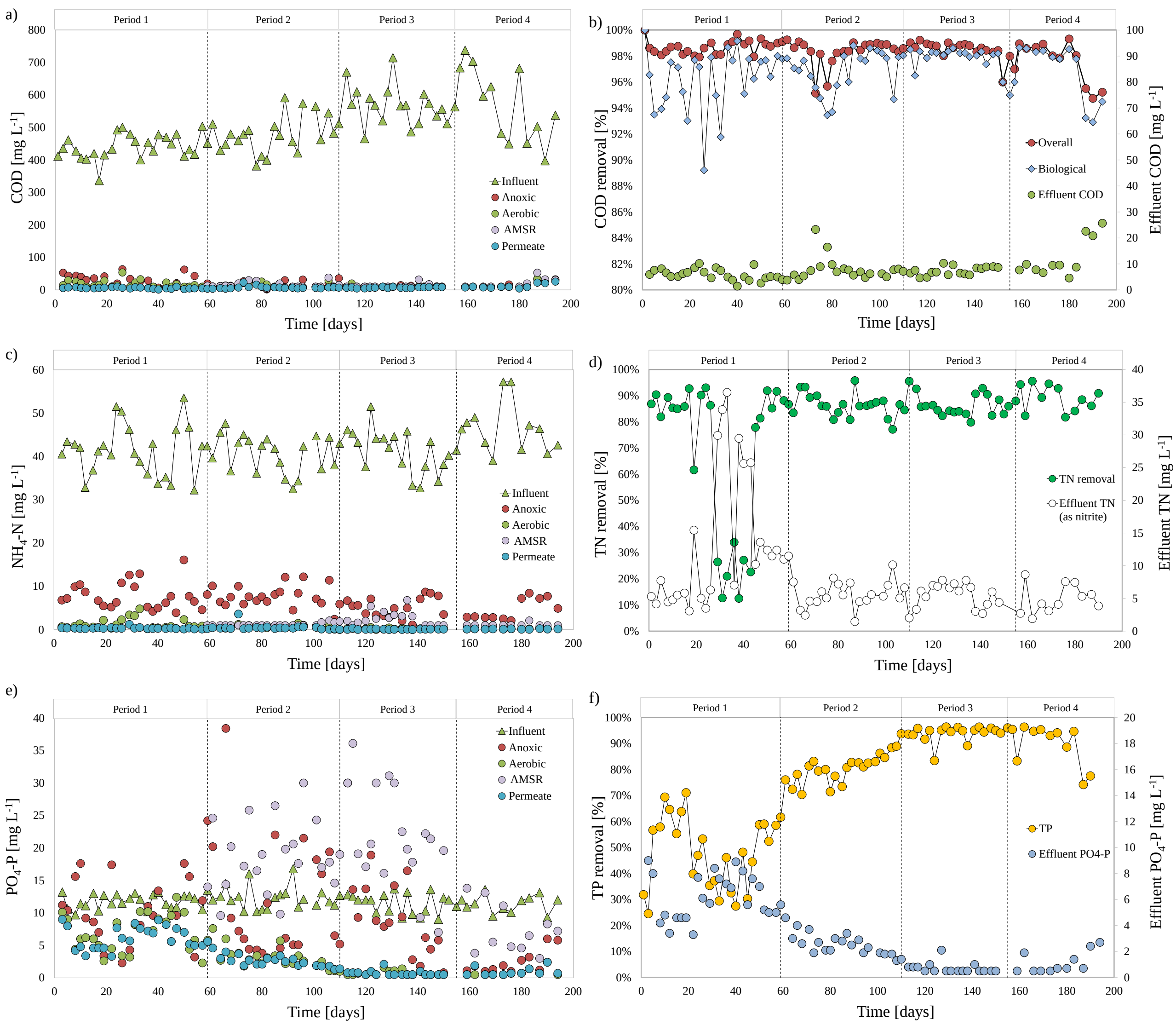


Fig. 3: Trends of the COD concentration in the influent, in the supernatant of the anoxic, aerobic and AMSR and in the permeate (a); biological and overall COD removal efficiency and effluent COD concentration (b); ammonia nitrogen in the influent and in the supernatant of each reactor (c); TN removal 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.

In particular, Fig. 3a shows the COD trend in the influent and in the supernatant of each reactor, whereas Fig. 3b depicts the overall removal of COD including membrane filtration, as well as the COD concentration in the permeate. The influent COD concentration ranged from 80 to 680 mg L^{-1}

in order to maintain a stable F/M ratio according to the TSS variation and the volume increase of the system related to the HRT variation in the anaerobic reactor.

In Period 1, the COD concentration in the supernatant of the anoxic reactor was close to 18 mg L^{-1} (average value) and it slightly decreased after the change of plant configuration, likely due to a more effective use of the organic carbon for denitrification (Capodici et al., 2015). In contrast, the COD concentration remained constant when the HRT was increased in Periods 2-4. The COD concentration in the supernatant of the aerobic reactor decreased during experiments from approximately 17 mg L^{-1} (average value in Period 1) to 10 mg L^{-1} (average value in Period 3), whereas it increased up to 19 mg L^{-1} in Period 4 (average value), 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 suggest the occurrence of bacterial lysis in the anaerobic reactor under prolonged HRT. Nevertheless, it is worth mentioning that the released COD was subsequently degraded in the anaerobic reactor, thus suggesting the biodegradability of COD generated in the anaerobic reactor, causing a negligible impact on the overall COD removal efficiency.

Referring to COD removal, in Period 1 the removal efficiency due to the biological process gradually increased suggesting the acclimation of the biomass to the new operational conditions (Fig. 3b). In Period 2, after the startup of the AMSR configuration, the biological contribution to COD removal slightly decreased from 98% to approximately 93%. Nevertheless, it was gradually recovered, reaching a stable value of 98% at the end of Period 2. In Period 3, the biological COD removal stably close to 96%, showing a slight decrease in the last days of operation. From Period 1 to Period 3, the overall COD removal was on average equal to 97%, without any significant variation with the change of plant configuration as well as the HRT increase in the anaerobic reactor. This result confirmed the high MBR robustness, enabling high COD removal even in presence of temporary decreases of biological contribution towards COD removal. In contrast, in Period 4 both the biological and the membrane removal efficiencies decreased in the long-term, suggesting that extended HRT values (higher than 8 h) in the anaerobic reactor could be detrimental in terms of effluent quality.

The above results were in good agreement with previous literature, indicating that the released COD in the anaerobic reactor increased with 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 the present study demonstrated that the COD removal efficiency decreased with HRT higher than 8 h, in good agreement with what reported by Ye et al. (2008). It is possible to speculate that under prolonged anaerobic condition, the decrease of biomass activity was so severe that bacteria resulted unable to cope with the COD release that occurred in the anaerobic reactor.

The trends ammonium concentration in the influent and in the supernatant of each reactor are shown in Fig. 3c. The average ammonium concentration in the anoxic reactor was approximately equal to 10 mg L^{-1} throughout experiments, thus 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 occurred throughout experiments. In the anaerobic reactor a slightly increase in the ammonium concentration occurred only during Period 3, whereas no significant variations were observed in the other periods.

The main nitrogen form in the permeate was represented by nitrates (Fig. 3d), accounting for more than 98% of the total nitrogen in the effluent, whereas nitrites were not detected during the entire experiments duration. In the first two weeks of Period 1, the nitrate concentration in the effluent increased up to a maximum value of 36 mg L^{-1} (38^{th} day), whereas it decreased to 12 mg L^{-1} at the end of Period 1, indicating the achievement of steady state conditions. The TN removal efficiency at steady state was close to 85%. In Period 2, the nitrate concentration in the permeate decreased by 50%, reaching a stable value of 5 mg L^{-1} (average value) until the end of the experimental campaign.

Accordingly, the TN removal efficiency increased from 86% to 91% after the implementation of the anaerobic reactor, but no significant improvements were observed with the HRT increase. The obtained results were in good agreement with previous literature (Semblante et al., 2014; de Oliveira et al., 2018), indicating that the AMSR configuration enabled a significant improvement of nitrogen removal efficiency.

The trend of orthophosphate concentrations in the supernatant of each reactor are depicted in Figure 3e, while Figure 3f shows the removal efficiency. In Period 1, a slight increase of the phosphorous concentration in the supernatant of the anoxic reactor was periodically observed, suggesting that anaerobic condition occasionally occurred likely due to denitrification depletion. Indeed, the P-release in the anoxic reactor occurred when the TN removal efficiency was close to 90% in Period 1. In general, in Period 1 the average TP removal efficiency was almost equal to 50%. In Period 2, a significant release of phosphorous was observed in the anaerobic reactor. Indeed, the average concentration of orthophosphate in the supernatant of the anaerobic reactor was 20 mg L^{-1}, whereas it was significantly lower in the aerobic reactor (3.28 mg L^{-1}) indicating that the integration of the anaerobic reactor in the mainstream enhanced the orthophosphate release and uptake by PAO bacteria. Similarly, in Period 3 the PO_4-P concentration in the anaerobic reactor slightly increased to 25 mg L^{-1}, while in the aerobic reactor it decreased to 0.76 mg L^{-1}, highlighting a further increase of TP removal. As noticeable from Fig. 3f, the average TP removal efficiency in Period 2 was close to 78%, showing an increasing trend, whereas it significantly increased in Period 3 reaching a maximum steady state value of 97%. In Period 4, a significantly lower release of orthophosphates 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 above results demonstrated that the change of plant configuration enabled the achievement of high TP removal performances. In the anaerobic reactor, similar conditions in terms of ORP (< -350 mV) compared to that of EBPR systems occurred (Chudoba et al., 1992). In previous literature, it was observed that the integration of one anaerobic reactor in the plant layout (i.e. OSA process), promoted the selection of PAO (Semblante et al., 2014). However, some contradictory results in terms of phosphorus removal were found 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 a MBR-OSA decreased from 55% to 28% when the ORP in the anaerobic reactor was adjusted to -250 mV. Moreover, Velho et al. (2016) observed that coupling an ASSR in a UCT scheme had negative effects on phosphorous removal. Velho and co-authors emphasized that the main drawback affecting phosphorus removal in ASSR configuration was the huge release of orthophosphate under prolonged anaerobic conditions (ORP < - 250 mV), imposed to maximize the excess sludge minimization.

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

The results obtained in the present study demonstrated that very high TP removal efficiencies 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.

3.3 Behavior of OHO and PAOs kinetics

The main biokinetics parameters of OHO are summarized in Table 2.

Table 2: 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 decreased from 2.51 d^{-1} to 1.43 d^{-1} from Period 1 to Period 2, thereby indicating a decrease of cell synthesis after the startup of the AMSR. When the HRT in the AMSR was increased to 8 h (Period 3), the $\mu_{max,H}$ slightly decreased to 1.19 d^{-1}, whereas it only slightly increased in Period 4 to 1.23 d^{-1}. The decrease of the $\mu_{max,H}$ suggested the occurrence of the uncoupling metabolism. Indeed, it is well known that the sludge cycling between anaerobic and aerobic conditions indices the biomass to use internal energy sources (ATP) for maintenance metabolism. This causes the detachment of catabolism from anabolism cutting off energy for cellular propagation. Consequently, the bacterial growth rate decreased by more than 50% from Period 1 to Period 2. The increase of the anaerobic HRT caused a further decrease (almost 15%) in Period 3, whereas no significant changes were observed in Period 4 characterized by 10 h of HRT in the anaerobic compartment.

The endogenous decay coefficient (b_H) increased according to the HRT increase in the AMSR. The endogenous decay coefficient increased by approximately 50% when the AMSR configuration was implemented (Period 2), whereas it almost doubled in Period 3. The maximum value of the b_H was observed in Period 4 (0.291 d^{-1}), although it not significantly increased when the HRT was extended from 8 h to 10 h. The increase of b_H could be related to the intensifying of decay phenomena, thus confirming that under extended substrate-limitation conditions, the biomass decay and cryptic growth mechanisms were favored.

Based on the above results, it can be concluded that the contribution of the uncoupling metabolism to the excess sludge reduction was maximum in Period 2 and it was not significantly affected by the HRT increase in the anaerobic reactor. In contrast, the contribution of decay phenomena and cryptic growth increased with the anaerobic HRT, although showing a not linear relationship at HRT higher than 8 h. The 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 results above discussed, confirming that the maximum efficiency in terms of sludge minimization was observed in Period 3.

According to the above results, 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 HRT increase, reaching a minimum value of 1.14% in Period 4. This result confirmed that the exposure of biomass to stressful conditions caused a significant decrease of the bacterial cells synthesis. Moreover, since the VSS/TSS ratio reamined almost constant thorughout experiments, a significant accumulation of endogenous residue occurred likely due to the membrane retention. This aspect, as better outlined in the following, could have entailed important implications in the phosphorous removal mechanism.

The specific oxygen uptake rate (SOUR) slightly increased from Period 1 (30 mgO_2 $L^{-1}h^{-1}$) to Period 3 (31.7 mgO_2 $L^{-1}h^{-1}$), whereas it significantly arose in Period 4 to approximately 40 mgO_2 $L^{-1}h^{-1}$. The SOUR increase was previously observed in OSA systems (Semblante et al., 2016b). The sharp increase of SOUR was likely related to the alternation of fasting/feasting conditions, which led the starved biomass to use more oxygen when it was returned to the mainstream aerobic reactor where a high substrate availability was ensured by the influent wastewater. However, in the AMSR layout, the biomass flowed from the anaerobic to the aerobic reactor where a poor substrate availability existed. This aspect implied that the alternation of fasting/feasting conditions did not significantly contribute to the excess sludge reduction at least until Period 3. Nevertheless, in Period 4 the SOUR significantly increased (+25%) suggesting that the alternation of fasting/feasting conditions likely occurred in this period. This result was likely due to the increase of the endogenous decay that favored cell lysis phenomena. In this way, the cell lysis in the anaerobic reactor enhanced the availability of soluble and particulate substrates, which were used by other bacteria for cryptic growth in the aerobic reactor, thereby resulting in the increase of the oxygen depletion rate.

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} after 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 8 h to 10 h. These results indicated that the integration of the AMSR was favorable for the growht of bacteria with internal storage ability (i.e., PAO).

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

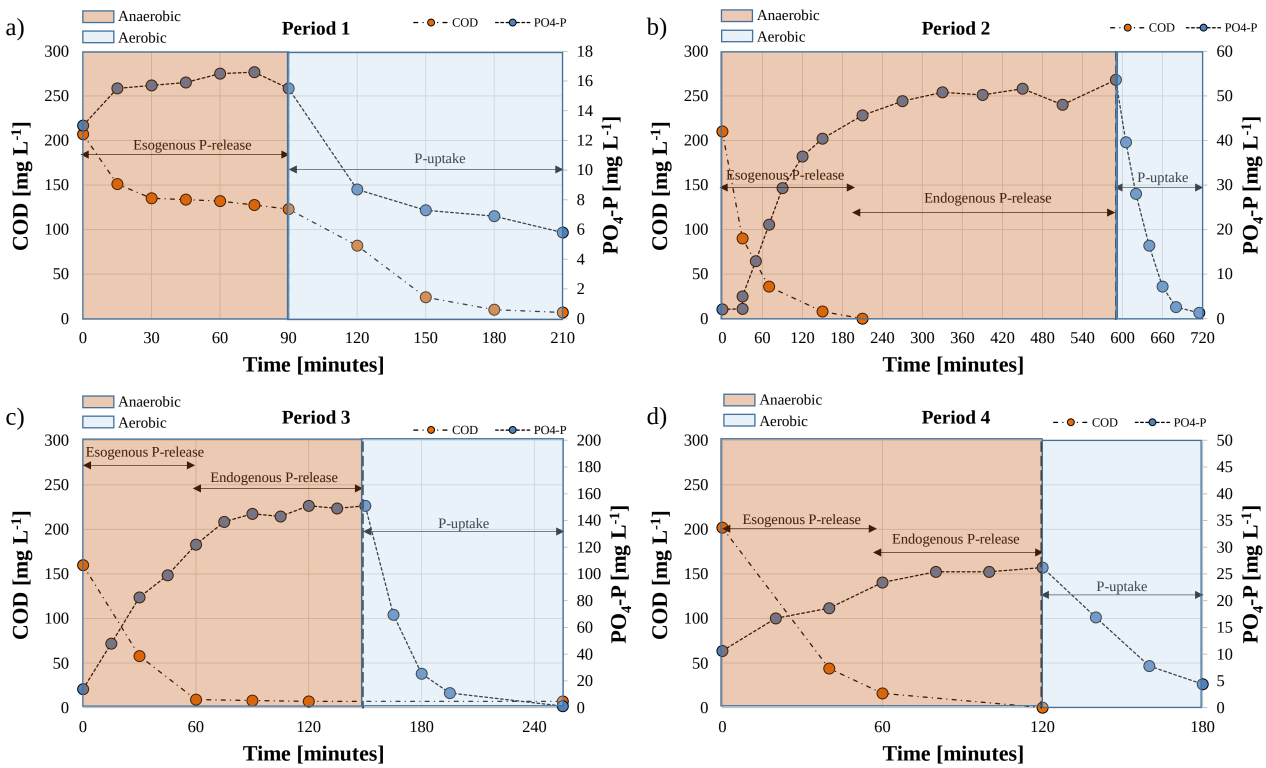


Figure 4: Results of the kinetic tests carried out in Period 1 (a), Period 2 (b), Period 3 (c) and Period (4).

In Period 1, no significant P-release occurred during the anaerobic phase, suggesting a very poor PAOs activity. In the aerobic phase, a not negligible P-uptake was observed, resulting in a removal of approximately 50%, according to what previously discussed referring to the 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, with 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 one order of magnitude lower compared to the exogenous P-release rate. The P-uptake during the aerobic phase increased up 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}, suggesting the achievement of the maximum activity of PAOs. Even in this case, the P-release under endogenous conditions was observed, with a release rate of 3.08 mgPO_4-P gVSS^{-1} h^{-1}. The P-uptake during the aerobic phase was close to 48.6 mgPO_4-P gVSS^{-1} h^{-1}, significantly higher compared to the previous period. In Period 4, both the exogenous and endogenous P-release rates decreased to 6.10 mgPO_4-P gVSS^{-1} h^{-1} and 1.33 mgPO_4-P gVSS^{-1} h^{-1}, respectively. Also 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. Both the exogenous and the endogenous P-releases and P-uptake increased with the HRT of the AMSR reaching a maximum value at 8 h of HRT.

Comparing the P-release and P-uptake rates obtained in this study with those reported in the literature, it can be stated that the exogenous P-release was 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, (2002). Similarly, the endogenous P-release was 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 could be defined 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 process.

Our findings demonstrated that phosphorous removal was achieved even under endogenous conditions, characterized by 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 experiments because of decay phenomena resulting from AMSR implementation. Nevertheless, the VSS/TSS ratio was almost constant throughout experiments, thereby suggesting that a significant amount of endogenous residues, deriving from bacteria decay, was retained within the system by the membrane. The endogenous residue, 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. Therefore, 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.

The kinetic tests revealed that the HRT of 8 h enabled the highest TP removal efficiency and PAO biokinetics. Based on the above results, the suggestion is that HRT values in the range of 6-8 h are favorable for the selection of slow growing microorganisms (i.e., PAO), whereas prolonged anaerobic conditions in the AMSR might enhance the biomass decay also including PAO bacteria.

3.4 EPS content and composition

Previous studies suggested that a possible mechanism leading to sludge reduction in sludge cycling systems is the destruction of EPS that occurs under anaerobic conditions (Semblante et al., 2014; Wang et al., 2013). The average EPS content during the experimental periods in the mixed liquor of each reactor is shown in Figure 5.

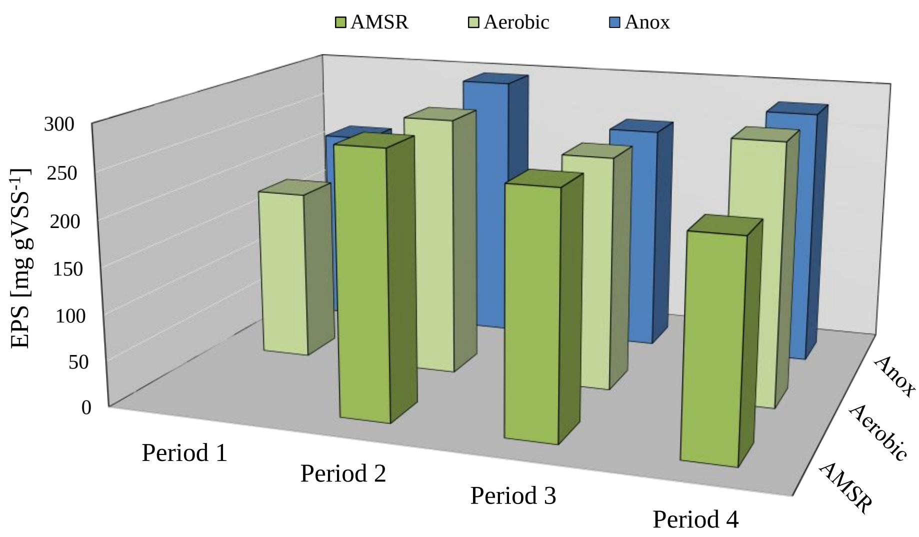


Figure 5: Average EPS content in the anoxic, aerobic and AMSR during the four experimental periods

The average EPSs content in Period 1 was of approximately 200 mgEPS $gVSS^{-1}$ and 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 2 and no significant differences were observed among the anoxic, aerobic and the AMSR reactors. The amount of SMP was negligible (< 2 mgSMP $gVSS^{-}$

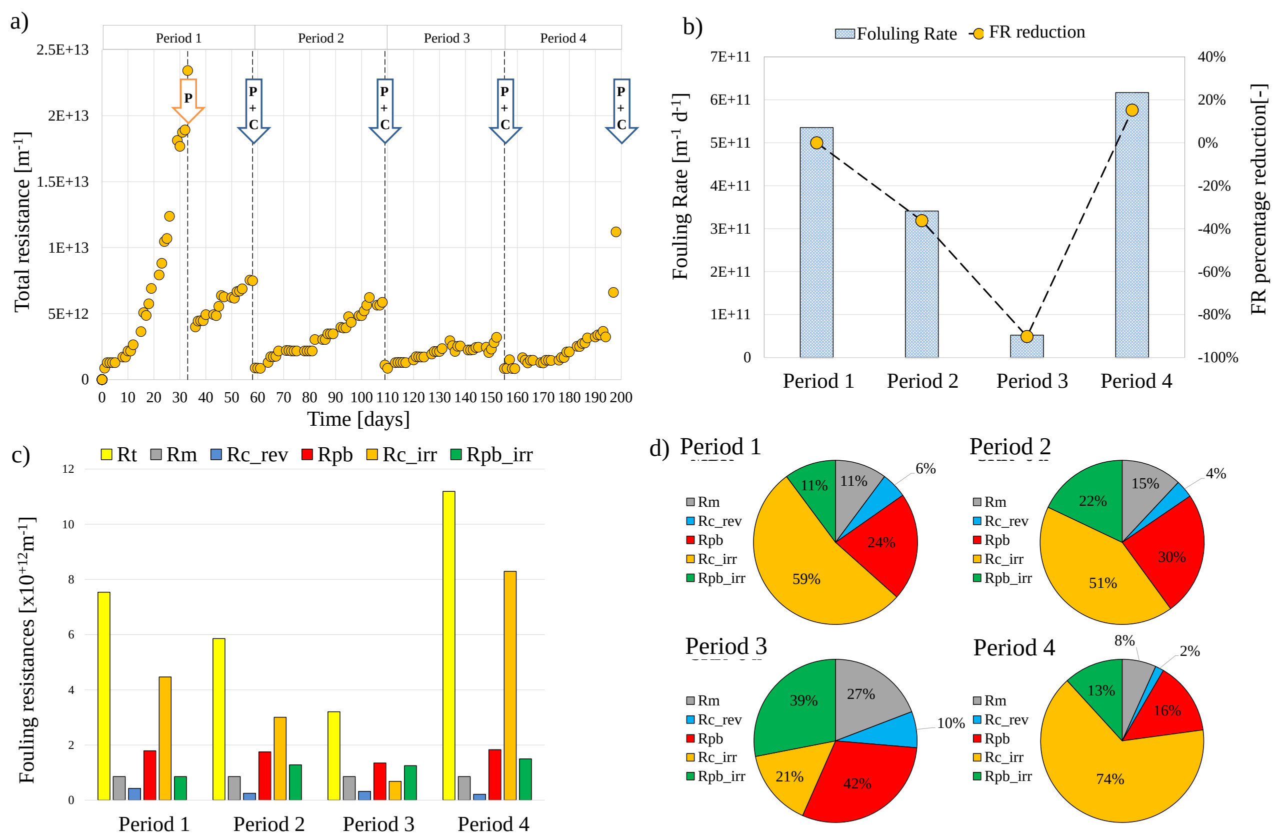
^{1}) indicating that the no destruction of bound EPS occurred after AMSR implementation in the original MBR layout in Period 2. The PN/PS ratio increased to 10.7 because a significant decrease in the carbohydrates (> 40%) amount occurred. In Period 3, the EPS content slightly decreased to approximately 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 showed a 20% decrease in the AMSR, indicating that EPS destruction occurred when the HRT in the anaerobic reactor was higher than 8 h. Nevertheless, no SMP was measured in Period 4 likely due to bacterial consumption. The PN/PS ratio was similar compared to the previous period (5.7), thereby indicating that no significant changes in the composition of EPS matrix occurred.

Based on the obtained results, a more extensively EPS destruction occurred in Period 4, whereas in Period 2 and Period 3 only a partial decrease of specific EPS fractions was observed. More precisely, the amount of carbohydrates slightly decreased under HRT of 6 h, whereas proteins decreased under a HRT of 8 h. These results were in contrast with that previously reported by de Oliveira et al. (2018), who 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 the present study and that of de Oliveira and co-workers was the SRT (35-40 d *vs* infinite SRT), it is possible to speculate that under HRT lower than 8 h, the EPS destruction is mainly driven by prolonged SRT. On the other hand, under lower SRT values the EPS destruction occurred likely due to prolonged HRT in the anaerobic reactor (> 8 h). This finding should be taken into account concerning the effects that EPS destructuration could exert on membrane fouling tendency (Campo et al., 2017).

3.5 Membrane fouling analysis

It was previously emphasized that the sludge minimization must not compromise the effluent quality. Moreover, in a MBR system also the hydraulic performances of the membrane should be taken into account with the aim to optimize the whole process.

Figure 6 depicts the trend of R_T, FR as well as the mechanisms involved in the membrane fouling mechanism.



Legend: P: membrane physical cleaning; P+C: membrane physical+chemical cleaning; Rt: total resistance; Rm: membrane resistance; Rc_rev: reversible cake resistance; Rpb: pore blocking resistance; Rc_irr: irreversible cake resistance; Rpb_irr: irremovable pore blocking resistance

Figure 6: 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 R_T increase was characterized by two different trends (Fig. 6a). Indeed, the R_T rapidly increased when the MBR was operated under a complete sludge retention strategy, whereas it was chacacterized by a slower increase when regular sludge withdrawals were performed. The average FR in this period was $5.3 \cdot 10^{11}$ m^{-1} d^{-1} (Fig. 6b). In Period 2, the R_T increased to $6.3 \cdot 10^{12}$ 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 equal to $3.4 \cdot 10^{11}$ m^{-1} d^{-1} on average. In Period 3, the R_T reached a maximum value 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 compared to what observed in the previous periods. In Period 4, the R_T reached its maximum value of $1.18 \cdot 10^{13}$ m^{-1} after 44 days of operation, thereby showing the highest FR ($6.15 \cdot 10^{11}$ m^{-1} d^{-1}) of the entire experimental campaign. The latter result was in good agreement with the EPS destruction above discussed, indicating that the destructuration of the extracellular polymeric matrix was detrimental towards the membrane fouling tendency.

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

As noticeable from Figure 6c and Figure 6d, 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 24% and 11%. In Period 2, the contribution of the irreversible cake decreased to 51%, whilst those of pore-blocking and reversible cake increased to 30% and 22%, respectively. In Period 3, the irreversible cake contribution significantly decreased (21%), while 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 what observed in Period 1, with the irreversible cake deposition, the pore-blocking and the reversible cake deposition accounting for 74%, 16%, 13% respectively.

Concerning the membrane service-life preservation, it is of paramount importance to minimize the pore-blocking mechanism or, alternatively, maximize the recovery of the membrane permeability with chemical cleaning operations. The highest value of the “irremovable fouling”, defined as the residual portion of fouling after chemical cleanings (Di Bella and Di Trapani, 2019), was observed in Period 4, whereas the lowest in Period 1. This result indicated that the integration of the AMSR might cause a potential decrease in the membrane service-life. Nevertheless, the minimum irreversible pore-blocking resistance was observed in Period 3, suggesting that a HRT of 8 h in the AMSR resulted the most suitable value 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). With 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. S1).

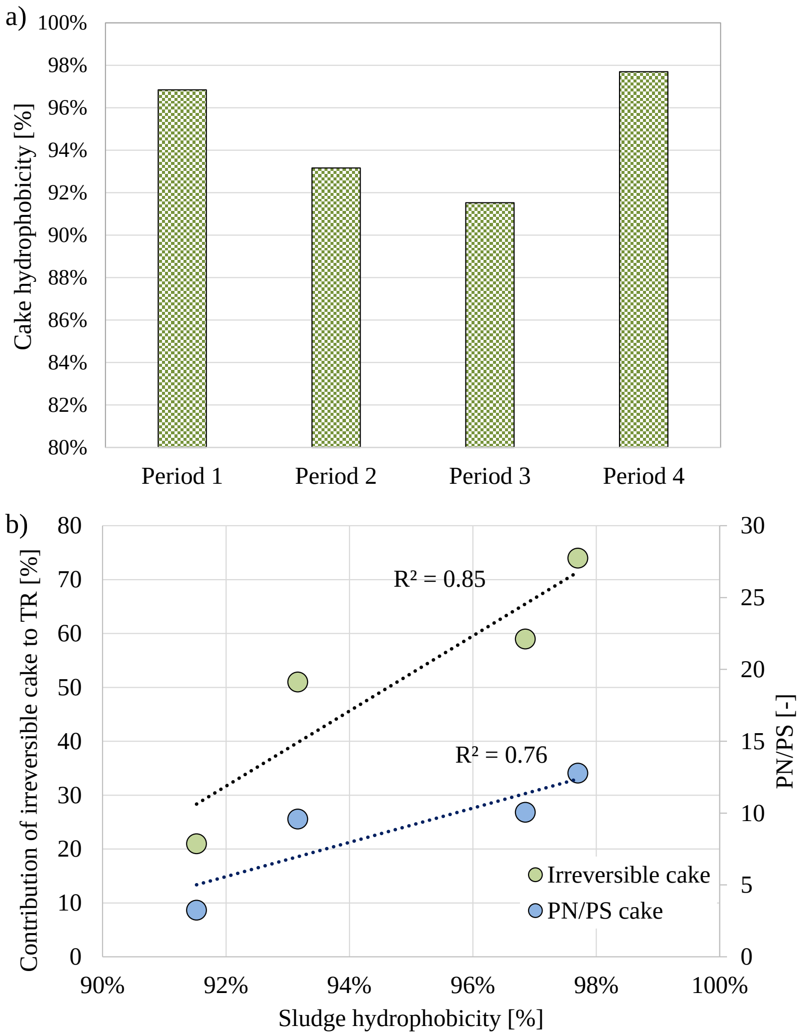


Figure S1: Hydrophobicity of the cake layer during the four experimental periods (a); relationship between the sludge hydrophobicity with the contribution of the irreversible cake to the total resistance and the PN/PS.

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 membrane fouling. In contrast, the maximum cake hydrophobicity was observed in Period 4 (97.7%), when the contribution of the irreversible cake deposition 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 in terms of both bound (3.5) and soluble (4.3) EPS throughout experiments. 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 of the irreversible cake resistance was strictly related to the change of cake composition. The higher was the amount of proteins, the higher resulted the cake hydrophobicity. Indeed, as confirmed by previous studies, proteins are more hydrophobic than carbohydrates; therefore, their predominance resulted in the increase in sludge hydrophobicity (Niu et al., 2016). The high protein concentrations dissolved in the bulk liquid determined a significant presence of proteins on membrane surface, because of the establishment of hydrophobic interactions with the material of the membrane fibers (PDVF).

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

The findings achieved in the present study demonstrated the effectiveness of integrating the AMSR for the simultaneous achievement of sludge minimization and phosphorous removal, enabling higher efficiency compared to ASSR configuration (Kim et al., 2012; Ferrentino et al., 2014; de Oliveira et al., 2018). Based on the above results, the AMSR integration enabled a significant decrease of sludge production with the HRT increase 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 occurring under higher HRT (10 h). The mechanisms involved in sludge minimization were different, which operated simultaneously, 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 the 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. Therefore, the TP removal significantly increased up to 97%, with PO_4-P concentration in the effluent close to 0.5 mg L^{-1}. Besides, kinetic tests highlighted that phosphorous release occurred under endogenous conditions, without an external carbon source. This result was likely due to cell lysis that released biodegradable low molecular weight compounds utilized by bacteria as a secondary substrate, suggesting the existence of the cryptic growth process (Quan et al., 2012). Lastly, it should be taken into account 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 huge increase of proteinaceous EPS and SMP in the membrane cake layer promoted a dramatic increase in the membrane fouling rate and the irreversible cake deposition, thereby limiting the applicable flux.

Our findings 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.

Conclusions

The simultaneous achievement of sludge minimization and phosphorous removal was studied in a novel AMSR-MBR scheme. The AMSR-MBR enabled a sludge reduction of 64% and the highest removal performance of organic carbon (98%), nitrogen (90%) and phosphorous (97%), as well as the lowest membrane fouling tendency ($FR=0.65 \cdot 10^{11}$ m^{-1} d^{-1}). Our findings suggested that the mechanism driving phosphorous removal in the AMSR could involve the cryptic growth process: the use of the biodegradable low molecular weight compounds deriving from the bacterial lysis, as secondary substrate by PAO, favored the simultaneous achievement of phosphorous removal and sludge minimization.

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Figure caption

Figure 1: Trends of TSS concentration and VSS/TSS ratio during the experiment

Figure 2: Average values of the observed yield coefficient (Y_{obs}), the maximum yield coefficient (Y_H) and their respective percentage reduction obtained in the four experimental periods.

Fig. 3: 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.

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

Figure 5: Average EPS content in the anoxic, aerobic and AMSR during the four experimental periods

Figure 6: 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).

Figure S1: 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.

Table caption

Table 1: Summary of the wastewater characteristics and the main operating conditions of the MBR

Table 2: Summary of the main biokinetics parameters of the OHO.