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Biological nutrient removal in a sequencing batch membrane bioreactor treating municipal wastewater

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ABSTRACT

This study aimed to evaluate the performance of a sequencing batch membrane bioreactor (SBMBR) in wastewater treatment for nutrient removal. The reactor, which was built at a pilot scale with a volume of 15 L, was operated for 240 d and fed with municipal wastewater. The SBMBR was operated under a sequencing batch mode, with a total cycle time of 4 h, including the feeding, anoxic/anaerobic, and aeration/filtration phases. The membrane bioreactor presented high performance on chemical oxygen demand, ammonium, and total nitrogen removal during the whole experimental period, with average removals efficiencies of around 97, 99, and 82%, respectively. Regarding total phosphorus, SBMBR reached the average removal efficiency of 48%. The poor phosphorus removal performance was attributed to the low availability of organic matter during the anoxic/anaerobic phase due to denitrification activity, which limited the P-release process and subsequent P-uptake during aerobic phase. Furthermore, the temperature increase during the summer period further hindered the enhanced biological phosphorus removal process, in which a decrease in the P-release values was observed. Fluorescence *in situ* hybridization analysis revealed a large presence of glycogen-accumulating organisms population on the SBMBR sludge, which contributes to explain the low efficiency obtained in phosphorus removal.

Keywords: Nutrient removal; PAO and GAO; Wastewater treatment; Membrane sequencing batch bioreactor

1. Introduction

The discharge of wastewater without adequate treatment can create several major problems in an aquatic ecosystem, one of which is eutrophication due to excessive nitrogen and phosphorus loading [1]. As a result, more stringent discharge regulations have been observed in recent years that limit the release of total nitrogen (TN) and total phosphorus (TP) to waterways. Brazilian legislation stipulates that ammonia and phosphorus concentrations in a municipal

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wastewater treatment plant's (WWTPs) effluent should be less than 20 and 4 mg L^{-1}, respectively. However, in practice, it is very difficult for WWTPs to attain the rigorous standards for effluent discharge. Consequently, the development of new and reliable technologies for treating municipal wastewater is extremely important to meet effluent discharge standards.

Currently, the sequencing batch reactor (SBR) is perhaps the most promising and viable of the proposed activated sludge modifications made for the removal of organic carbon and nutrients [2]. SBRs are noted for combining the different environmental conditions necessary to remove nitrogen and phosphorus into a single tank, without the need of sludge recirculation.

There has been a significant improvement in the quality of final effluent when SBRs were operated in conjunction with membrane technologies, such as micro or ultrafiltration processes. From this association between membrane technologies and SBRs, the sequencing batch membrane bioreactors have emerged (SBMBR) [3,4].

Nutrients removal in SBMBR is favored due to two aspects: the possibility of anoxic, anaerobic, and aerobic stages in one operating cycle and the high biomass retention by the membrane which enables a complete retention of specific micro-organisms, such as nitrifying, denitrifying, and phosphate-accumulating organisms (PAOs), which are responsible for nitrogen and phosphorus removal [4].

In spite of the advantages reported, only a few studies have previously been published about SBMBR with a focus on simultaneous phosphorus and nitrogen removal [5–7]. Moreover, few studies have evaluated nutrient removal in SBMBR operating with a high solid retention time (SRT). Although the literature suggests that a long SRT can deteriorate the biological phosphorus removal performance, the reactor operation under this condition results in some benefits that should be considered, such as high nitrification rates [8], lower membrane fouling [9], and less sludge production [10]. Thus, the main objective of this study was to evaluate the SBMBR nutrient removal performance operating with a long SRT (80 d).

2. Materials and methods

2.1. Reactor setup and operation

A pilot SBMBR with a 15-L working volume was used in this study (Fig. 1). The SBMBR was operated with two hollow fiber microfiltration membranes (Polymem GF3, with 0.08 μm nominal pore size and total surface filtration area of 0.18 m^2) directly immersed into the MBR tank. Membrane filtration was carried out by a peristaltic pump (Watson Marlow, 505S) under intermittent suction (9 min on and 1 min off). The reading of transmembrane pressure (TMP) was obtained by a digital pressure sensor that was installed at the permeate line.

Inoculation sludge (MLVSS/MLSS 0.61) was obtained from activated sludge collected from a municipal WWTP. The SBMBR was continuously operated for 240 d and fed with municipal wastewater taken from a sewage collection system using a submerged pump. This pump was installed inside a PVC pipe covered with holes of 0.01 m with the purpose of preventing the entrance of large solid materials into the SBMBR. Table 1 shows the wastewater characteristics.

The MBR pilot was operated under a sequencing batch mode that consisted of the following phases: feeding, anoxic/anaerobic, and aeration/filtration. The total time of each cycle was 4 h, which included 2 min of feeding, 50 min for the anoxic/anaerobic phase, and 188 min for aeration and filtration, simultaneously.

The SRT was maintained at 80 d throughout the experimental period. The temperature in the reactor

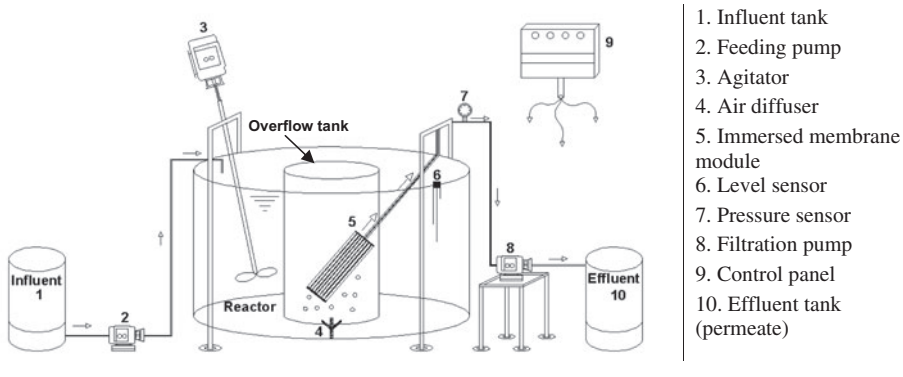

Fig. 1. Schematic representation of the SBMBR pilot plant.

Table 1
Municipal wastewater average characteristics

COD, mg L^{-1}	486 ± 121
OLR, mgCOD L^{-1} d^{-1}	583 ± 145
TN_{in}, mgN L^{-1}	67 ± 10
NH_4^+-N, mg L^{-1}	55 ± 11
TP_{in}, mgP L^{-1}	7.1 ± 1.5
TSS, mg L^{-1}	230 ± 114

Notes: OLR, organic loading rate; TN_{in}, influent TN concentration; TP_{in}, influent total phosphorus concentration; TSS: total solids suspense.

varied according to room temperature, ranging from 19 to 25°C. The volumetric exchange ratio (VER) was held constant at 20%. In Table 2, other operational parameters are presented.

2.2. Analytical procedures

2.2.1. PAO/DPOA tests

In order to investigate the PAOs and denitrifying phosphate-accumulating organisms (DPAOs) activity in more detail, batch experiments were carried out at different times within the experimental run. Sludge samples (2 L), obtained from the SBMBR, were transferred to sealed vessels at the end of the aerobic phase to determine the phosphate uptake and release rates of the PAOs and DPAOs. The sludge was kept in an anaerobic state and incubated in the presence of sodium acetate (0.2 g L^{-1} of AcNa) for 3.5 h to facilitate phosphate release. Subsequently, one of the incubation conditions was exposed to aerobic conditions to measure the aerobic phosphorus uptake rate, and the other incubation condition was exposed to anoxic conditions (20 mg NO_3^--N L^{-1}) to measure the anoxic phosphorus uptake rate. The phosphate uptake rates (PUR) were estimated from the linear regression of phosphate concentrations. The ratio of anoxic PUR to aerobic PUR (anoxic/aerobic PUR ratio) was used to analyze the proportion of DPAOs in PAOs [11,12].

Table 2
Main operating parameters of the SBMBR

Q, L d^{-1}	18
J, L m^2 h^{-1}	6.25
HRT, h	20
SRT, d	80
VER, %	20
AFR, m^3 m^{-2} h^{-1}	5–8

Notes: Q: flow rate, J: filtration flux, HRT: hydraulic retention time, SRT: solids retention time, VER: volumetric exchange ratio, AFR: air flow rate.

2.2.2. FISH analysis

Fluorescent in-situ hybridization (FISH) analyses were performed as described by Amann et al. [13]. PAO_{MIX} (comprising equal amounts PAO462, PAO651, and PAO846 probes) and GAO_{MIX} (comprising GAO431 and GAO989 probes) were used to target PAOs and glycogen-accumulating organisms (GAO), respectively. Meanwhile, EUB338 was used to target all eubacteria in the sludge samples. Samples were fixed in a 4% paraformaldehyde-phosphate-buffered saline solution and placed on 0.1% gelatin and 0.01% $KCr(SO_4)_2$ gelatin-coated glass slides. All microbial cells were detected by staining slide samples with 1% 4,6-diamidino-2-phenylindole (DAPI). The slides were examined with an Olympus BX41 microscope. All samples were analyzed against DAPI.

2.2.3. Analytical methods

Chemical oxygen demand (COD), Ammonia nitrogen (NH_4^+-N), TN, and TP were analyzed by spectrophotometry (Hach Lange, DR5000). COD, NH_4^+-N, TN, and TP were measured according to manufacturer instructions using Hach Method 8000, 10031, 10072, and 10127, respectively. Nitrite nitrogen (NO_2^--N), nitrate nitrogen (NO_3^--N), and orthophosphate (PO_4^{3-}-P) were analyzed by chromatography (DIONEX ICS 5000). The diluted sludge volume index (DSVI) was determined by diluting the sludge samples with the SBMBR's permeate, according to standard procedures. Extracellular polymeric substances (EPS) were determined as protein and polysaccharide content by spectrophotometric analysis. Proteins were measured according to the method described by Lowry et al. [14] using bovine serum albumin as a standard. Polysaccharides were measured using the phenol–sulfuric acid method, according to Dubois et al. [15]. Analysis of reactor operating cycle was also performed, which consists of a series of samplings and analyses during the anoxic/anaerobic and aerobic phases to follow the transformations of the nitrogen compounds, phosphate release, and uptake and COD consume along the operational cycle of SBMBR.

3. Results and discussion

3.1. SBMBR performance

3.1.1. Organic matter and nitrogen removal

The SBMBR showed effective COD removal during all operational periods (Fig. 2), with an average COD removal efficiency of 97% and effluent COD concentration of 21.3 ± 5.3 mg L^{-1}.

The organic contents in the supernatant of the feeding, anaerobic, and aerobic phases were 95.6, 55.1,

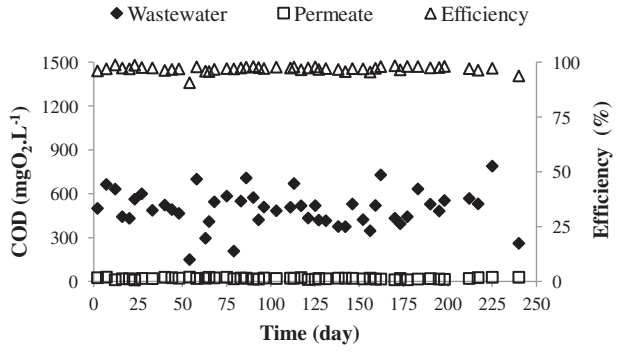

Fig. 2. Wastewater and permeate concentrations and removal efficiency of COD during the operation time.

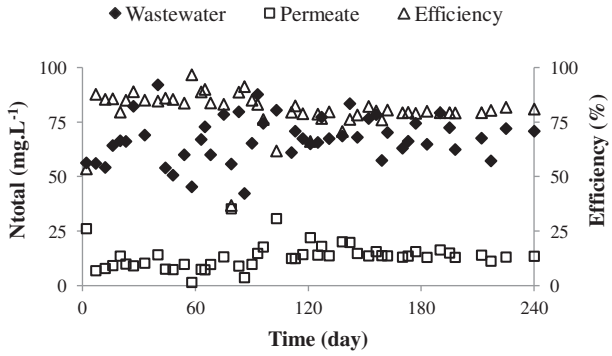

Fig. 3. Wastewater and permeate concentrations of TN and removal efficiency during the operation time.

and 51.5 1 mgCOD L^{-1}, respectively. These results suggest that a major portion of organic influent was degraded in the anoxic/anaerobic phase through denitrification and phosphate release processes, with an average COD removal efficiency of approximately 42%. The remaining COD removal was attributed to the aeration phase (4%) and to the membrane filtration process, which contributes with a COD removal of 32%. The efficiency of soluble COD removal by the membrane is in agreement with that reported by Gander et al. [16], in which the membrane filtration process in MBRs contributes with an additional COD removal rate of approximately 30%.

Due to the high sludge age, the nitrification process was very stable and efficient throughout the entire experimental period [8]. The SBMBR average removal efficiency of NH_4^+-N was 99%, with average effluent NH_4^+-N concentration of 0.4 mg L^{-1}. The fact that ammonia nitrogen was the most prominent compound of TN in the wastewater suggests that the nitrification process was a determining step for efficient TN removal. Moreover, it is important to note that the denitrifying activity reduced the nitrate concentration from approximately 15 mg L^{-1} in the aerobic phase to below 0.3 mg L^{-1} at the end of the anaerobic phase, contributing to the TN removal.

Fig. 3 shows the TN concentrations in both the influent and effluent as well as the removal efficiencies during the operation period. As shown, the SBMBR reached high TN removal, with an average efficiency of 82%, and the TN effluent concentration was always below 15 mg L^{-1}. Ersu [17] observed an average TN removal efficiency of 81% in an MBR with anaerobic and aerobic tanks operating with SRT of 75 d. When the SRT was reduced to 10 d, the TN removal efficiency decreased to 67%. Thus, a long SRT contributes to higher TN removal efficiencies, as seen in the present study in which was used the long SRT of 80 d.

The high TN removal observed in the SBMBR was due to the co-existence of heterotrophic and autotrophic micro-organisms in the bioreactor. Nitrogen removal was carried out initially under aerobic conditions, in which autotrophic nitrobacteria oxidize ammonia to nitrate, and subsequently under anoxic conditions, in which the heterotrophic denitrifying bacteria reduce nitrate to gaseous nitrogen. The SBMBR feeding process during the anoxic/anaerobic phase stimulated the denitrification process, facilitating the nitrate removal and contributing to the high TN removal efficiency.

3.1.2. Phosphorus removal and PAO/DPAO activity

Fig. 4 presents the TP concentration and its respective removal efficiency in the SBMBR during the whole operation period. There is great instability in the phosphorus removal efficiency. The average effluent PO_4^{3-}-P concentration and TP removal efficiency was 4.7 ± 2.8 mg L^{-1} and 48%, respectively.

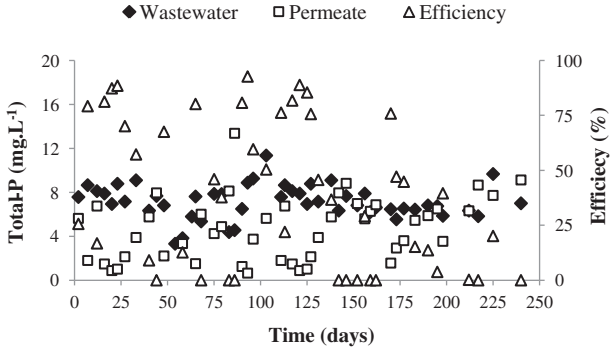

Fig. 4. Wastewater and permeate concentrations of TP and removal efficiencies during the operation time.

Phosphorous can be removed by assimilation for biomass growth and by phosphorus-accumulating organisms (PAOs), through the enhanced biological phosphorus removal (EBPR) process [18]. The low F/M ratio and sludge yield (Y) observed during reactor operation, with average values of 0.08 $gCODgSSV^{-1}$ d^{-1} and 0.16 $gSSVgCOD^{-1}$, respectively, suggest that phosphorus removal for biomass growth was not significant. The TP removal via assimilation was estimated in 3.5 mgP d^{-1}, whilst the removeal by the EBPR process was 31.2 mgP d^{-1}, which indicates a phosphorus removal for biomass growth of approximately 10%.

From Fig. 4, two different phosphorus removal efficiencies profiles can be observed. As can be seen, before the day 125, the reactor showed higher removal efficiencies, with an average of 53%, whilst from day 125 on, this efficiency dropped significantly to the mean value of 19%. Among the factors that deplete the EBPR process, Kuba et al. [19] report the presence of nitrate in the anaerobic phase as one of the most important interferences. The loading nitrate in the anaerobic phase results in consumption of influent organic compounds by denitrifiers, thus decreasing the availability of organic matter for PAO [20]. The reduction in the organic carbon content results in lower anaerobic P-release rates. Looking in more detail in Fig. 3, the TN effluent concentration shows higher values after day 120, indicating a higher amount of nitrate in reactor thereafter. When it is compared to the results shown in Fig. 4, it is evident that the phosphorus removal efficiencies were lower from day 125. The increase in the average nitrate concentration from 9.4 to 14.5 mg L^{-1} in the effluent after day 120 suggests the need of larger amount of organic matter for the denitrifying bacteria. Thus, the higher organic matter consumption by denitrifying bacteria may have limited the carbon substrate availability for PAO, affecting negatively the P-release process, as can be seen in Fig. 5.

It is also important to note that, in addition to higher nitrate concentrations after day 120, it is also the beginning of the summer period, which resulted in the temperature increase in the reactor after day 120. Before day 120, the average bioreactor temperature was 20.4℃, whilst after day 120, the average temperature increased to a mean value of 24.1℃. Several studies have suggested that temperature plays an important role in the biological phosphorus removal process. Whang and Park [21] reported that temperatures above 20℃ facilitate the growth of GAO populations, which have competitive advantages over PAO under such temperature conditions and tend to deteriorate the EBPR. These micro-organisms, similar to PAO, are able to proliferate under alternating

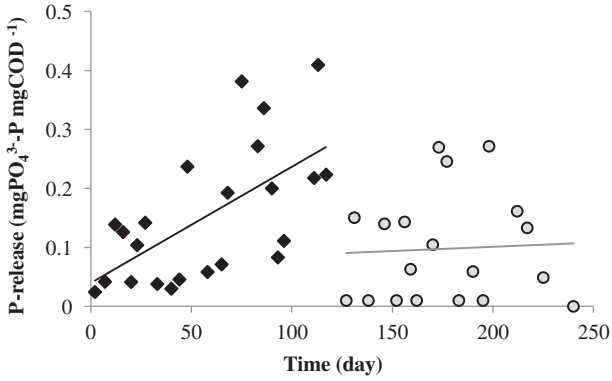

Fig. 5. Phosphate release per COD consumed at anoxic/anaerobic phase during the operational days.

anaerobic and aerobic conditions [22]. However, because they do not store poly-P, intracellular stored glycogen is used as both the energy and carbon source for volatile fatty acids (VFA) uptake, without exhibiting the typical anaerobic P-release and subsequent aerobic P-uptake from PAO [23]. Therefore, the GAO population proliferation negatively affects the biological phosphorus removal process [24].

FISH analyses revealed an increase in the PAO population from day 10 to day 190. However, there is also a significant increase in the GAO population on these same days. Estimations revealed a PAO population of approximately 5 and 15% at days 10 and 190, respectively, while for GAO population, the values were approximately 1 and 30% at the same period. This higher increase in GAO population in SBMBR sludge suggests a possible deterioration in the EBPR mechanism, which can result in low phosphorus removal efficiency [18]. Thus, besides the higher nitrate concentration after day 120, the poor phosphorus removal performance by SBMBR can also be attributed to the large presence of GAO population.

Fig. 6 shows the results of an operating cycle of the SBMBR, in which the P-release and P-uptake process during the anox/anaerobic and aerobic phases, respectively, can be observed. As can be seen, the P-release process stops at 20 min of anoxic/anaerobic phase, when the soluble COD reached its minimum value of 65 mg L^{-1}. Thus, the low organic matter content available during the anoxic/anaerobic phase limited the phosphate release process by PAO. The low nitrate concentration in the beginning of the anoxic/anaerobic phase indicates that the denitrification process occurred predominantly during the reactor feeding, consuming part of the substrate that could be used by PAO during the anaerobic phase. Under anaerobic conditions, the VFA are rapidly metabolized and stored by PAOs as poly-b-hydroxyalkanoates

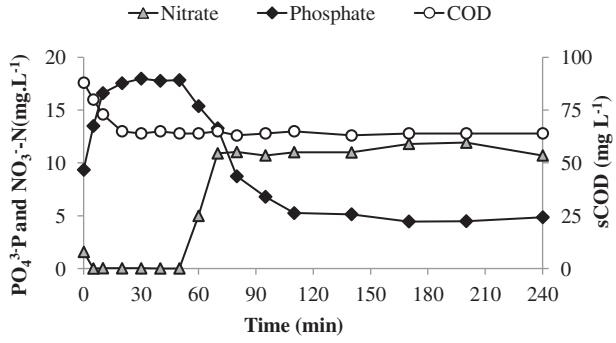

Fig. 6. NO_3^--N, PO_4^{3-}-P and COD profiles during the operational cycle of SBMBR on day 220.

(PHA) using stored poly-P as an energy source [20]. Subsequently, during the aerobic phase with a rather low organic concentration, PAOs use the PHA as carbon and energy sources to grow and to assimilate P, restoring poly-P chain [25]. This way, if insufficient organic matter is available, the PHA storage by PAOs will not occur properly, and then the P-uptake process will deteriorate [26]. According with P-PO_4^{3-} values observed during the aerobic phase (Fig. 6), the P-uptake process does not occur satisfactorily. There is a significant velocity reduction in the P-uptake process after 110 min, which results in an incomplete P-uptake, with PO_4^{3-}-P concentration in the mixed liquor of about 5 mg L^{-1} at the end of the cycle. This incomplete P-uptake process deteriorated the reactor phosphorus removal efficiency.

The average volumetric organic loading rate (VOLR) of 586 mgCOD L^{-1} d^{-1} applied to SBMBR during the experimental period did not seems to be sufficient for denitrification and EBPR process simultaneously. Xu et al. [27] evaluated the use of 400, 800 and 1,600 mgCOD L^{-1} d^{-1} in a MBR operated in sequencing batch and obtained the P-release values of 3.7, 6.4, and 8.7 mgP h^{-1}, respectively. Furthermore, the reactor phosphorus removal efficiency increased to 85% when the VOLR of 1,600 mgCOD L^{-1} d^{-1} was applied. These results show that, for a good EBPR process, performance is essential the use of suitable organic loading rate.

To better understand the EBPR process in the SBMBR, batch tests were carried out with the sludge mixed liquor to measure the specific PAOs and DPAOs activity at different operating days. The results are shown in Table 3.

PAOs that utilize oxygen as the electron acceptor showed higher P-uptake activity, as has been observed in other studies [12,28]. As can be seen, there is a progressive increase in the P-uptake and P-release and until day 120, both for PAO and DPAO, and subsequently, a significant decay in these parameters at day 240. At day 120, the P-uptake reached 8.38 mgP g^{-1} SSV h^{-1} for PAOs and 5.09 mgP g^{-1} SSV h^{-1} for DPAOs, whereas at day 240, these values decreased to 3.62 and 0.89 mgP g^{-1} SSV h^{-1}, respectively. The lower P-uptake observed in these batch tests at day 240 confirm the lowest SBMBR performance in the phosphorus removal verified from day 120.

It is also important to note that the temperature increase resulted in a decrease in DPAO/PAO ratio from 61% (at day 120) to 24% (at day 240). These results suggest that the growth of DPAOs at temperatures above 20°C is even more limited than that of the PAOs, and therefore its contribution to phosphorus removal in a reactor exposed to high temperatures is less expressive.

3.2. Membrane fouling

Fig. 7 shows the TMP values during the experimental period. Two profiles can be identified from the TMP behavior.

Note that, between days 50 and 150, the TMP increased slowly with fouling rate of 0.34 mbar d^{-1}. On the other hand, from days 150 to 240, the TMP increased more intensely, presenting fouling rate of 1.2 mbar d^{-1}, four times greater than that observed during the previous period.

Optical microscopy images revealed a significant increase in the amount of filamentous bacteria in the SBMBR sludge between days 150 and 240. Likewise, during this period, poor settling properties were observed with an increase in the DSVI mean value to

Table 3
$P_{release}$ and P_{uptake} rates at days 10, 65, 120, and 240 for PAOs and DPAOs

	Parameter	Units	Day 10	Day 65	Day 120	Day 240
PAO test	$P_{release}$	mgP g^{-1} SSV h^{-1}	0.42	1.93	3.24	1.61
	P_{uptake}	mgP g^{-1} SSV h^{-1}	0.99	3.76	8.28	3.62
DPAO test	$P_{release}$	mgP g^{-1} SSV h^{-1}	0.40	1.84	4.12	1.51
	P_{uptake}	mgP g^{-1} SSV h^{-1}	0.20	0.76	5.09	0.89
% DPAO		–	20	20	61	24

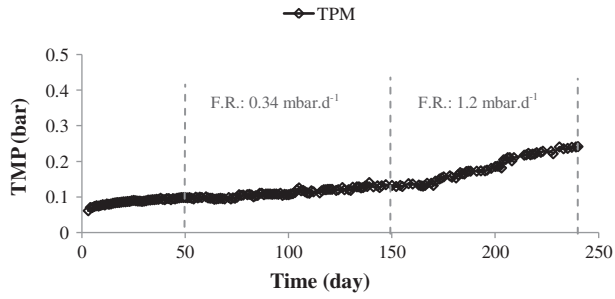

Fig. 7. TMP evolution. F.R.: Fouling rate.

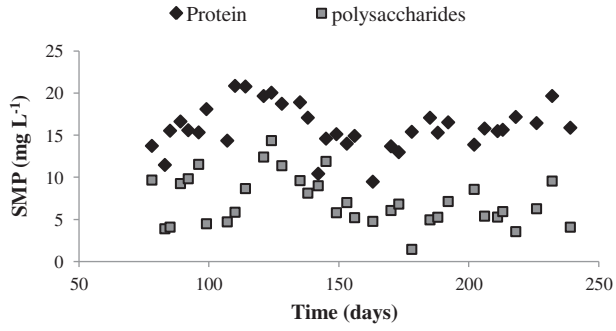

Fig. 8. Protein and carbohydrate concentrations in the mixed liquor of SBMBR during the operation time.

170 mL g^{-1}, whereas between days 50 and 150, the DSVI values remained below 130 mL g^{-1}. The results suggest a deterioration of SBMBR sludge settleability, which also reflected negatively on its filterability.

Several studies have identified EPS as the major cause of membrane fouling in MBRs [29,30]. The membrane fouling potential increased with the increase in the concentration of EPSs, such as protein and carbohydrates [31]. As shown in Fig. 8, the EPS concentrations in the SBMBR were relatively stable, with no tendency to increase over time. This behavior suggests that the concentration of EPSs is not related to the higher fouling rate observed between days 150 and 240, which supports the hypothesis that the increase in fouling rate was due to the growth of filamentous bacteria. Meng et al. [32] reported that excessive growth of filamentous bacteria, also known as "bulking" filamentous, affects membrane performance in MBRs, leading to the formation of a dense cake on the membrane surface due to the attachment of these microorganisms.

4. Conclusions

The SBMBR showed good performance on organic matter and TN removal during the whole experimental period, reaching COD and TN average removal efficiencies of 97% and 82%, respectively. The phosphorus removal performance was negatively affected by low organic matter content during the anoxic/anaerobic phase, resulting in an average removal efficiency of 48%. Furthermore, the higher nitrate concentration in the reactor and the temperature increase during the summer period further hindered the EBPR process after day 120, in which lower phosphorus removal efficiencies and decrease in the P-release values were observed. Similar results were obtained from the PAO/DPAO batch tests. FISH analysis revealed a significant increase in the GAO population from day 10 to day 190 on SBMBR sludge, which contributes to explain the poor EBPR process performance.

Acknowledgments

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