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EPS and SMP dynamics at different heights of a submerged anaerobic membrane bioreactor (SAMBR)

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ABSTRACT

Membrane bioreactors (MBR) technology for wastewater offers many advantages over conventional technologies such as high effluent quality, less footprint and others. The main disadvantage of membrane bioreactors (MBR) is related to membrane fouling, which is mainly caused by extracellular polymeric substance (EPS) and soluble microbial products (SMP). This research studied EPS and SMP dynamics at different heights of a submerged anaerobic membrane bioreactor (SAMBR). The SAMBR was operated under two organic loading rates (OLR) (0.79 and 1.56 kg/m^3 d) and was fed with synthetic wastewater with glucose as the carbon source. The results showed percentages of chemical oxygen demand (COD) removal above 95% and the highest COD removal rates were observed at the bottom of the reactor (>83%) for both OLR. The EPS showed a stratification with highest quantities in the supernatant. For the SMP the highest concentration was in the bottom of SAMBR where utilization predominated associated products whereas in the SAMBR supernatant predominated biomass associated products. The OLR change led to a significant increase in SMP accumulation but not in EPS. These facts showed that EPS and SMP dynamic in the SAMBR seemed to be mainly influenced by biological activity, total suspended solids concentration and substrate composition.

1. Introduction

The use of membrane bioreactor (MBR) technology for wastewater treatment offers many advantages over conventional alternatives (i.e., activated sludge systems), including the production of a high quality effluent and a reduction in installation costs [1]. Particularly, anaerobic membrane bioreactors (AnMBR) have many advantages over their aerobic counterparts since they do not require aeration, produce less sludge and generate energy in the form of biogas, thereby decreasing operational costs. For these reasons, AnMBR are no longer an emerging technology but have become established as a competitive process in the current market [2], and have been recognized as suitable for the treatment of wastewaters at high [3] and low organic loading rates (OLR) [2].

The main disadvantage of MBRs is related to membrane fouling, which is mainly caused by extracellular polymeric substances (EPS) and soluble microbial substances (SMP) [4]. In submerged anaerobic membrane bioreactors (SAMBR) fouling of the membrane is directly affected by the nature of biomass (suspended solids; particle size distribution; microbial composition), as well as by the amount and characteristics of SMP and EPS that accumulates in the bioreactors [5]. Biomass, SMP and EPS are directly affected by reactor operational parameters, such as sludge retention time (SRT), organic loading rate (OLR), food to microorganism ratio (F/M), temperature, and substrate type and concentration [3].

Concerning the operating parameters of AnMBR, Chu et al. [6] have found that varying the reactor operation temperature between 25 °C and 11 °C led to granule segregation. They also reported that more EPS tended to accumulate on the surface of the membrane than on the granules, and that in both sludge samples carbohydrate EPS were more prevalent than protein EPS. Salazar-Peláez et al. [7] found that EPS and SMP levels grew with an increase in chemical oxygen demand (COD) and a decrease in hydraulic retention time (HRT) and that the percentage of carbohydrates was higher than proteins in the composition of both SMP and EPS. In another study Vyrides and Stuckey [8] reported that EPS concentration in the membrane biofilm was three times higher than in the suspended biomass, and that seemed to adversely affect membrane fluxes.

Besides being affected by EPS and SMP accumulation, membrane fouling is also tightly dependent on biomass aggregation. Gao et al.

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[9] reported that high pH shocks induced biomass flocs dispersion causing an accumulation of biopolymers in the sludge suspension. They also found that the EPS did not have any direct influence on fouling rates, though a decrease in the protein/carbohydrate ratio of the EPS led to low flocculation levels, which could indirectly influence the fouling rate. In another work Gao et al. [10] found that an increase in temperature caused sludge deflocculation and affected the structure of the microbial community and species diversity.

Huang et al. [11] examined the effects of SRT and HRT in an AnMBR and found that a decrease in HRT increased biomass growth and SMP accumulation. At high SRT they found low EPS levels, which reduced flocculation and particle size. Additionally, they reported that increases in OLR led to an increase in the fouling rate. Lin et al. [12] found that high COD in the supernatant influenced cake formation but that the mixed liquor suspended solids did not. Trzcinski and Stuckey. [13] confirmed that the COD in the sludge seems to be the main parameter that governs membrane flux at solids concentrations below 20 g total suspended solids (TSS)/L.

All of these studies show that EPS and SMP are affected by operational conditions and that there are changes in the production of the biopolymeric substances in the sludge cake, supernatant and sludge. These differences were reported to vary in upflow anaerobic sludge blanket (UASB) reactors according to reactor height [14], which could be explained by differences in biomass concentration, the production of intermediary compounds volatile fatty acids (VFA), reactor hydrodynamics, and other aspects that affects the production or consumption of SMP and EPS along the reactor height.

Therefore, the main objective of this paper was to evaluate the dynamics of EPS and SMP at different heights of a submerged anaerobic membrane bioreactor (SAMBR) operated at two OLR. For this, parameters such as COD, VFA and TSS at different heights and OLR were evaluated, in order to understand the dynamics of EPS and SMP from SAMBR operation. In addition, EPS and SMP were measured as proteins and carbohydrates. Finally unknown fraction of SMP was quantified at different heights of the SAMBR.

2. Materials and methods

2.1. Submerged anaerobic membrane bioreactor (SAMBR)

For this research, an upflow liquid SAMBR was designed (1 m height and 20 cm diameter) to have a working volume of 32 L. Three valves were installed at different heights to allow the collection of distinct samples: P1 (0.145 m), P3 (0.445 m), P4 (0.75 m) and P5 (permeate). Additionally, a recirculation loop was installed at the middle of the reactor to ensure a high degree of mixture. The reactor was equipped with a external heating jacket to maintain the operating temperature at $35 \pm 3\,^{\circ}C$. The system had a level control that regulated the reactor feeding pump, as pictured in Fig. 1. The ultrafiltration membrane module (U-shaped) was submerged in the upper part of the reactor. The membrane was made of hollow fiber polyethersulfone (PES) capillaries and was provided by MEMBRANA® with an effective membrane area of $0.39\,m^2$ and nominal pore size of 0.01 μm.

The reactor was fed daily with synthetic wastewater which contained glucose, peptone and yeast extract in amounts to yield the desired COD concentration. Additionally, the feeding solution contained macro and micronutrients required for the cultivation of microorganisms, according to Zehnder et al. [15]. The reactor was inoculated with sludge from wastewater treatment plant from a fruit juice manufacturing plant. Sludge was acclimated for 4 months with synthetic wastewater using 36 h as TRH. The SAMBR was operated during two different OLR (Table 1), which was changed by increasing the concentration of substrate and keeping a constant flowrate.

Table 1
Operational parameters.

Parameter	OLR 1	OLR 2
Flux (LMH)	3.3 ± 0.13	3.2 ± 0.23
pH	6.94 ± 0.20	6.82 ± 0.24
OLR (kg/m^3 d)	0.79 ± 0.06	1.56 ± 0.35
F/M Ratio (gCOD/gVSS.d)	0.036 ± 0.008	0.095 ± 0.026
Operation (d)	95	66
HRT (h)	24	24

2.2. Analytical methods

Weekly samples were taken from each reactor sampling point and centrifuged at 13,000 rpm for 15 min at 4 °C in order to remove suspended solids. The resulting supernatant was filtered through a Millipore® cellulose ester filter with nominal pore size of 0.22 μm. The resulting filtered samples were then used to measure the soluble COD, VFA and to quantify the protein and carbohydrate contents. SMP levels were estimated according to Eq. (1) [16], which deduces from the SMP pool, the COD due to the main fermentative byproducts (VFA) as well as any remaining substrate (glucose).

$$\mathrm{SMP(COD)} = \mathrm{soluble\,COD} - [\mathrm{glucose\,(COD)} + \mathrm{VFA(COD)}] \quad (1)$$

Eq. (2) was used to convert the concentration of each VFA in terms of COD:

$$\begin{aligned}\mathrm{VFA(COD)} = {} & 1.07 * [\mathrm{acetate}] + 1.51 * [\mathrm{propionate}] \\ & + 1.82 * [\mathrm{butyrate} + \mathrm{isobutyrate}] \\ & + 2.04 * [\mathrm{valerate} + \mathrm{isovalerate}]\end{aligned} \quad (2)$$

The analyses of soluble COD and suspended solids (TSS and VSS) were carried out according to the procedures described in the Standard Methods [17]. EPS extraction was carried out using the steam and formaldehyde methods reported by Zhang et al. [18]. Steam extraction was used to harvest EPS proteins, which were then quantified using the Bradford method [19], whereas formaldehyde extraction was used to obtain carbohydrates which were quantified using the phenol-sulfuric method [20]. VFA were measured by gas chromatography using an HP series 6890 Plus® gas chromatograph equipped with an FID detector, and a 60 m × 0.2 mm INNOWAX column after extracting the target analytes by headspace solid phase micro extraction (HS/SPME) technique.

Statistical tests were carried out for each phase at each sampling point, using the Bio Estat 5.0 software. The Shapiro–Wilk test was employed to evaluate the normality of the data series. Then parametric (ANOVA, Tukey) and non-parametric (ANOVA Kruskal–Wallis, Student Newman) tests were applied, adopting a p-value lower than 0.05 to reject the null hypothesis (Ho).

3. Results and discussion

3.1. Overall process performance of SAMBR

3.1.1. COD removal

Fig. 2 shows the percentage of COD removal at different reactor heights for both OLR. The percentage of overall COD removal during the process was greater than 95% (P5). These values are consistent with previous research findings in AnMBR [10,21,22]. Fig. 2 also shows the percentage stratification of COD removal. The highest percentage of removal occurred in the bottom of the reactor (P1). Chromatographic analyses did not detect the presence of glucose at any of the points of measurement, so it appears that the glucose was completely consumed in the bottom of the reactor. Significant

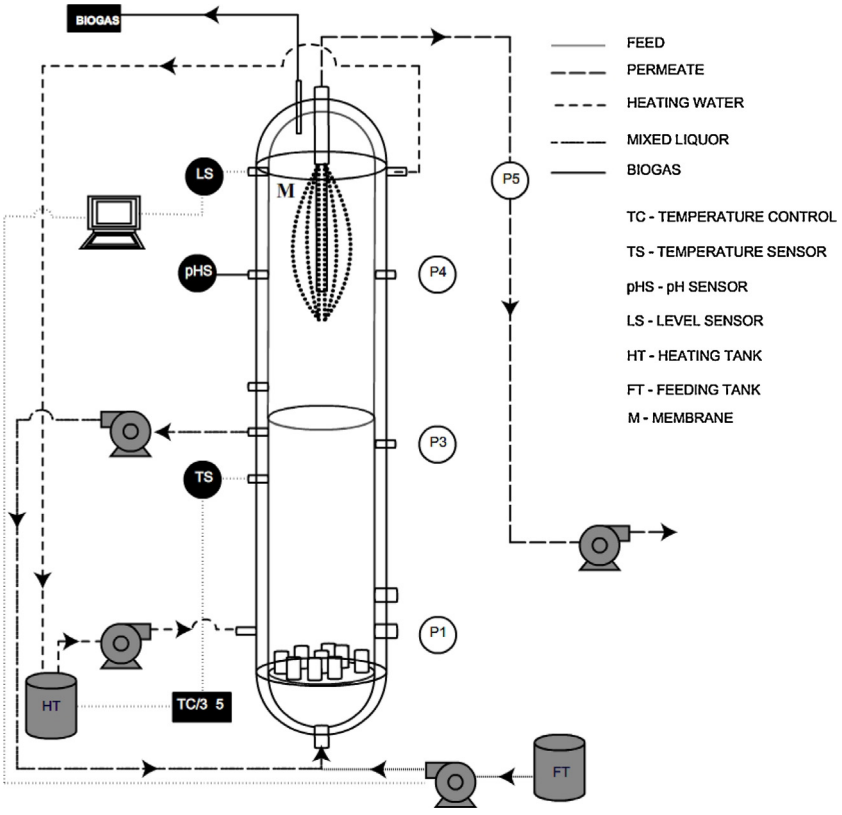

Fig. 1. Experimental set-up.

differences were also found between the values of points P4 and P5 under both test conditions, indicating that the membrane either removed or retained organic material, a phenomenon that has been reported in the literature in MBR [8,23,24]. Increasing the OLR produced higher percentages of COD removal at points P3, P4, and P5, whereas no significant differences occurred in the bottom of the reactor.

3.1.2. Total suspended solids (TSS) and biological activity

The TSS concentration at different reactor heights for both OLR is shown in the top of Fig. 3. The SAMBR presented a stratification of solids regarding height. The highest TSS concentration was obtained at the bottom of the reactor, but was below the detection threshold in the effluent. The augmentation in OLR led to an increase in the TSS concentration in the upper part of the reactor and a decrease in the bottom, attributable to the phenomenon of biomass deflocculation possibly caused by microorganism retention by the membrane, which makes it unnecessary for bacteria to form flocs. This phenomenon was also observed by Liu et al. [25] in AnMBR operated at high F/M ratios. The increase in solids at P4 during OLR 2 may be associated with increased bio-cake on the membrane. Jeison and van Lier [26] found that increased biomass levels in the supernatant contribute to cake formation on the membrane in AnMBR under thermophilic conditions. Gao et al. [27] also reported that increasing the concentration of solids contributes to membrane fouling. Therefore it can be inferred that the increase in

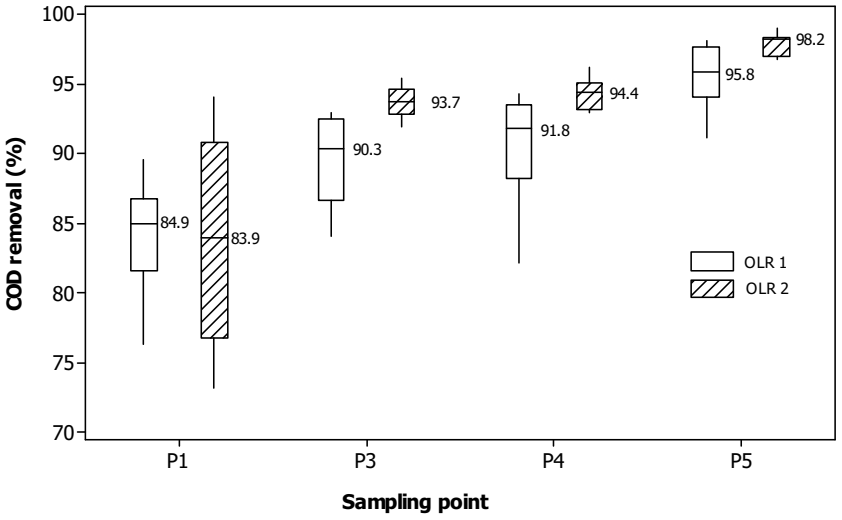

Fig. 2. COD removal at different heights.

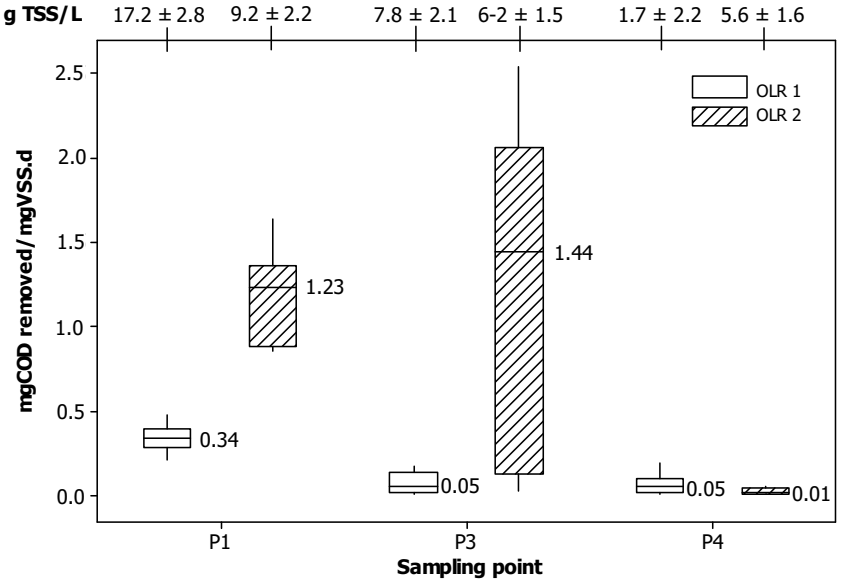

Fig. 3. Total suspended solids and biological activity at different heights.

OLR led to sludge deflocculation, which contributed to cake formation on the membrane (fouling).

Fig. 3 also shows the biological activity in terms of organic matter consumption in SAMBR, expressed as mg COD removed/mg VSS.d at different SAMBR heights for both OLR. The biological activity also presented stratification. Most biological activity occurs in the bottom of the reactor. Increasing the OLR led to a significant increase in biological activity at point P1, which explains the absence of significant differences in COD removal at point P1 between OLR 1 and OLR 2 despite the decrease in solids during OLR 2. Moreover, the low concentration of solids during OLR 2 reduces apparent viscosity in this part of the reactor. As a result, the mixing conditions and contact between biomass and substrate are improved, leading to increased biological activity.

3.1.3. *Volatile fatty acids (VFA)*

Fig. 4 shows the VFA concentration at the different reactor heights for both OLR. As occurred with the above-mentioned parameters, VFA concentration presented stratification at the different heights. The highest VFA accumulation occurred in the bottom of the reactor (P1), which is consistent with COD removal because all the glucose in the bottom was converted to VFA. However, Fig. 4 also shows percentage of VFA as total COD. These percentages were low (maximum value of 30.4% at P1 for OLR 2), indicating that VFA accounted for only a part of the total COD at the different heights.

Increasing the OLR led to an increase of VFA at all points of measurement, which can be attributed to the increase in F/M ratio for OLR 2 (P1: 1.49 mg COD/mg VSS.d) as compared with OLR 1 (P1: 0.39 mg COD/mg VSS.d). Another aspect that should be highlighted is the VFA accumulation between points P4 and P5. No significant differences were observed for the low OLR, allowing VFA to pass through the membrane. In the case of the high OLR, VFA were degraded or adsorbed by the cake formed on the membrane, increasing effluent quality. This effect of the membrane-bound biofilm is consistent with that reported by Martinez-Sosa et al. [23].

Fig. 4 also shows the composition of VFA. Acetic and propionic acids were the main VFA accumulated in the SAMBR. Consistent with these data and taking into account the absence of glucose, it can be inferred that acidogenic bacteria producing acetic and propionic acids predominated in the bottom of the reactor. The population of acetogenic and methanogenic organisms was higher at points P3 and P4.

3.2. *EPS dynamic*

The stratification of the previously described parameters directly influences biomass properties [3]. As a result, EPS dynamics also presented stratification at the different reactor heights. Fig. 5 shows that the highest EPS production occurred at point P4.

The EPS stratification is related to TSS stratification. According to Lin et al. [21], small flocs excrete a greater amount of EPS than agglomerated flocs. It is also possible to extract a greater amount of EPS from smaller flocs. The EPS extracted in this study were cell-bound EPS. According to Wingender et al. [28], cell-bound EPS can be classified as either tightly bound (TB-EPS) or loosely bound (LB-EPS). The TB-EPS is typically found in the inner layer of the cell surface and LB-EPS, in the outer layer. According to Zhan and Fang [29], most of the EPS found in anaerobic sludge are distributed in the outer layer. In agreement with this, most of the EPS extracted from the bottom of the reactor were type LB-EPS. It is also easier to extract TB-EPS from smaller flocs, but small flocs also have greater surface area, which increases the amount of EPS type LB-EPS and contributes to a higher EPS concentration in the top of the reactor.

EPS stratification was also related to COD stratification at the different reactor heights, because microorganisms may excrete more EPS under adverse conditions [30]. Conditions at the top of the reactor were considered the most adverse, with available COD being much lower than at the bottom of the reactor, which led to stress conditions and increased EPS production.

The EPS stratification can also be attributed to SAMBR functioning. The low EPS concentration in the bottom of the reactor promotes mass transfer between substrate and biological agglomerates [30] because high EPS levels reduce floc permeability [31]. This also explains why the highest biological activity and the highest levels of COD removal were reported in the bottom of the reactor.

No significant differences in EPS production occurred with increasing OLR (Fig. 5), but increasing OLR did play an important role when associated with SAMBR operational parameters. Cell adhesion is one of the main functions of the EPS, creating the matrix for granule formation and biofilms [32]. The EPS adherence as flocs

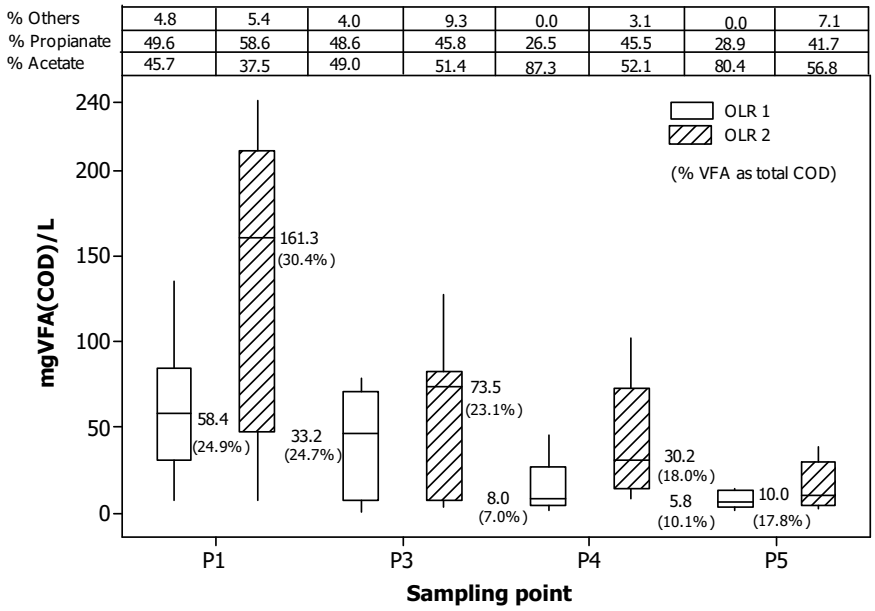

Fig. 4. Volatile fatty acids and composition at different heights.

in the bottom of the reactor may explain the increase in solids at point P4 for OLR 2 as a low EPS content (mainly LB-EPS), weakens floc structure, increases porosity, and decreases sedimentability [5], which in turn favors deflocculation [11]. The adhesion function of biofilm EPS played an important role at point P4 where the lowest concentration of solids occurred (Fig. 3) and the highest caking on the membrane in OLR 2 was reported. Several studies [6,8,12,33] have found that the EPS tend to adhere more to membrane surfaces than to microbial flocs. Therefore, a high EPS concentration at P4 served to induce caking on the membrane [4,33].

Fig. 5 also shows the composition of EPS as proteins and carbohydrates. The bulk composition of these in SAMBR was EPS as carbohydrate at all points of measurement. The literature shows differences in EPS composition. Some authors reported proteins to be the main component of EPS [5,32] and others, carbohydrates [6,7]. This difference has been attributed to extraction method, biomass type, and substrate characteristics. EPS composition also determines the hydrophobic or hydrophilic characteristics of the EPS. According to Jorand et al. [34], protein content mainly determines hydrophobic characteristics and carbohydrate content, hydrophilic. Therefore, in this study, the EPS had hydrophilic properties.

The EPS composition can also determine EPS charge, which is generally negative [35]. A positive charge is attributed to protein content because of the amino groups [30]. Fig. 5 suggests that the negative EPS charge can be attributed to the high carbohydrate content found and this could also explain the higher caking during OLR 2. Because biofilm EPS are distributed in layers throughout the entire biofilm [36], the dominant negative charge in the SAMBR led to an increase in the repulsive forces between cells, decreasing granule sedimentation. Low EPS levels in the form of protein on the biological agglomerate surface not only make it permeable to water, but also weaken it, generating a high amount of LB-EPS [37]. This also explains the deflocculation process that occurs in the bottom of the reactor. The EPS composition also shows that LB-EPS and TB-EPS have a carbohydrate nature. As discussed, the highest amount of EPS was found at point P4 during OLR 2, mainly in the form of LB-EPS as a result of deflocculation. Because of their carbohydrate nature and because they are easier to release, LB-EPS may be the main cause of caking in OLR 2.

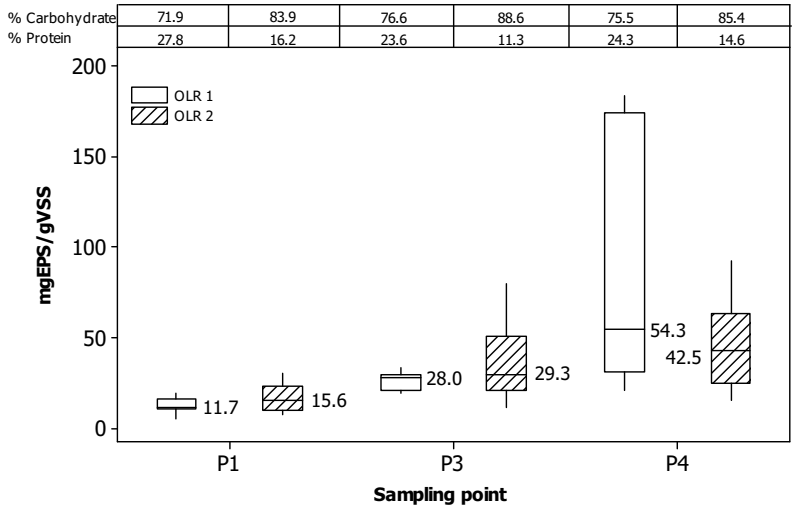

Fig. 5. Extracellular polymeric substances and composition at different heights.

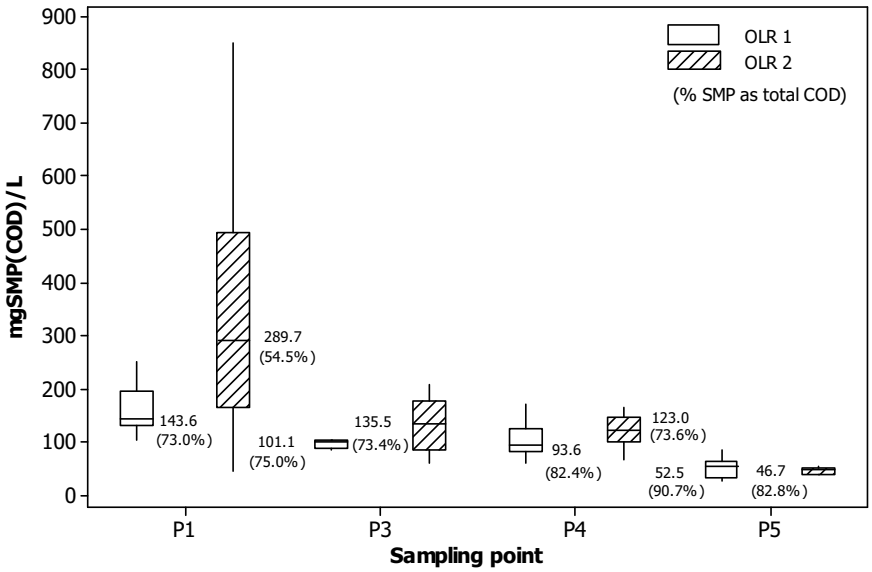

Fig. 6. Soluble microbial products at different heights.

3.3. SMP dynamics

Fig. 6 presents the results of SMP dynamics at different reactor heights. SMP also presented stratification. The highest SMP concentration, expressed as COD/L, occurred at point P1. SMP stratification can be explained based on SAMBR functioning. The high biological activity in the bottom of the reactor completely consumed the glucose. As a result, the substrate available at points 3 and 4 was composed of VFA, mainly acetic and propionic acids (Fig. 4), indicating that the substrate in the bottom of the reactor was more complex (glucose) than elsewhere in the reactor. SMP stratification was accordingly influenced by substrate complexity. Complex substrates also produce more SMP because of their higher amount of Gibbs free energy [38]. Complex substrates also produce more biomass, leading to increased SMP production because of the increase in microbial activity [39]. Furthermore, high microbial activity (Fig. 3) may cause endogenous decay and cell lysis, which trigger the release of significant amounts of SMP. These phenomena occurred in the bottom of the reactor, generating the highest SMP concentration. The levels of SMP were lowest at point P4 because of the predominance of VFA (simpler substrate) and the lower biomass concentration.

Another possible explanation for the higher SMP concentration at point P1 is that EPS were converted into SMP via hydrolysis [40]. EPS monitoring has shown that the concentration of bound EPS, especially LB-EPS, was low in the bottom of the reactor (P1). LB-EPS is the most predominant form of EPS on the biomass surface [37,41], indicating that these were hydrolyzed to form SMP.

Fig. 6 also shows percentage SMP as total COD at different reactor heights. The highest percentage of SMP as total COD was found in the effluent (P5), which could indicate a recalcitrant fraction of COD produced by the anaerobic treatment. The comparison of the percentages of SMP as total COD with the percentages of VFA as total COD (Fig. 4) indicated that SMP are the main organic compounds in both reactor and effluent [24,39,42]. This highlights the importance of SMP during the biological process because these were the major components of soluble COD at all points of measurement in the SAMBR. Fig. 6 shows a decrease in SMP concentrations in function of height, suggesting that microorganism consumption of SMP is greater than microorganism production of SMP. As a result, microorganisms at points P3 and P4, where the substrate was limited, used the SMP as source of carbon.

According to Barker and Stuckey [42], the SMP can be either substrate utilization-associated products (UAP), which are generated during the metabolic decay of the substrate, or biomass-associated products (BAP), which are formed as byproducts of self-oxidation or cell lysis. The UAP are more biodegradable than the BAP [4], explaining the high percentage of SMP produced as UAP at point P1, which agrees with substrate availability and complexity (glucose) and high biological activity found at this point of measurement of the reactor (Fig. 3). The decrease in SMP at points P3, P4, and P5 of the reactor largely corresponds to the SMP consumption as UAP. Therefore, total COD content at points P4 and P5 corresponded mainly to the most recalcitrant SMP (SMP as BAP).

In Fig. 6, points P4 and P5 show significant differences for both OLR. The decrease in SMP in the upper part of the SAMBR highlights the important role played by cake formation on the membrane. The fouled membrane helped degrade or retain part of the SMP depending on cake thickness and membrane pore size. These values confirm the predominance of BAP in this part of the reactor. These SMP are compounds presenting high molecular weight (>10 kDa) and hydrophilic properties [43] and can therefore be retained by the membrane [4]. Only a small fraction of the UAP was degraded by the active part of the cake [24]. Significant differences in VFA were observed between points P4 and P5, but only in the case of OLR 2 (Fig. 4). Consequently, the membrane was observed to remove COD as SMP for OLR 1 and as both SMP and VFA for OLR 2. This highlights the importance of SMP in the form of BAP as the main compounds that lead to membrane fouling [4].

The increase in OLR only had a significant effect on SMP in the bottom part of the reactor. The increase in SMP concentration at point P1 can be attributed to the significant increase in biological activity at higher OLR, which in turn led to increased UAP production. Barker and Stuckey [42] associated this to excess energy.

Fig. 7 shows the composition of SMP (proteins, carbohydrates, unknown COD) at different reactor heights. Most SMP components were unknown. Proteins and carbohydrates presented low significance at all points of measurement. Protein-based SMP were not detected at point P5 for OLR 1 or at any point for OLR 2. Unknown SMP maintained SMP dynamics at the different reactor heights, suggesting that most UAP in the bottom part of the reactor were unknown. Therefore, the UAP consumed at points P3 and P4 were unknown SMP. The significant differences observed between points P4 and P5 for both OLR indicate that the membrane retains or

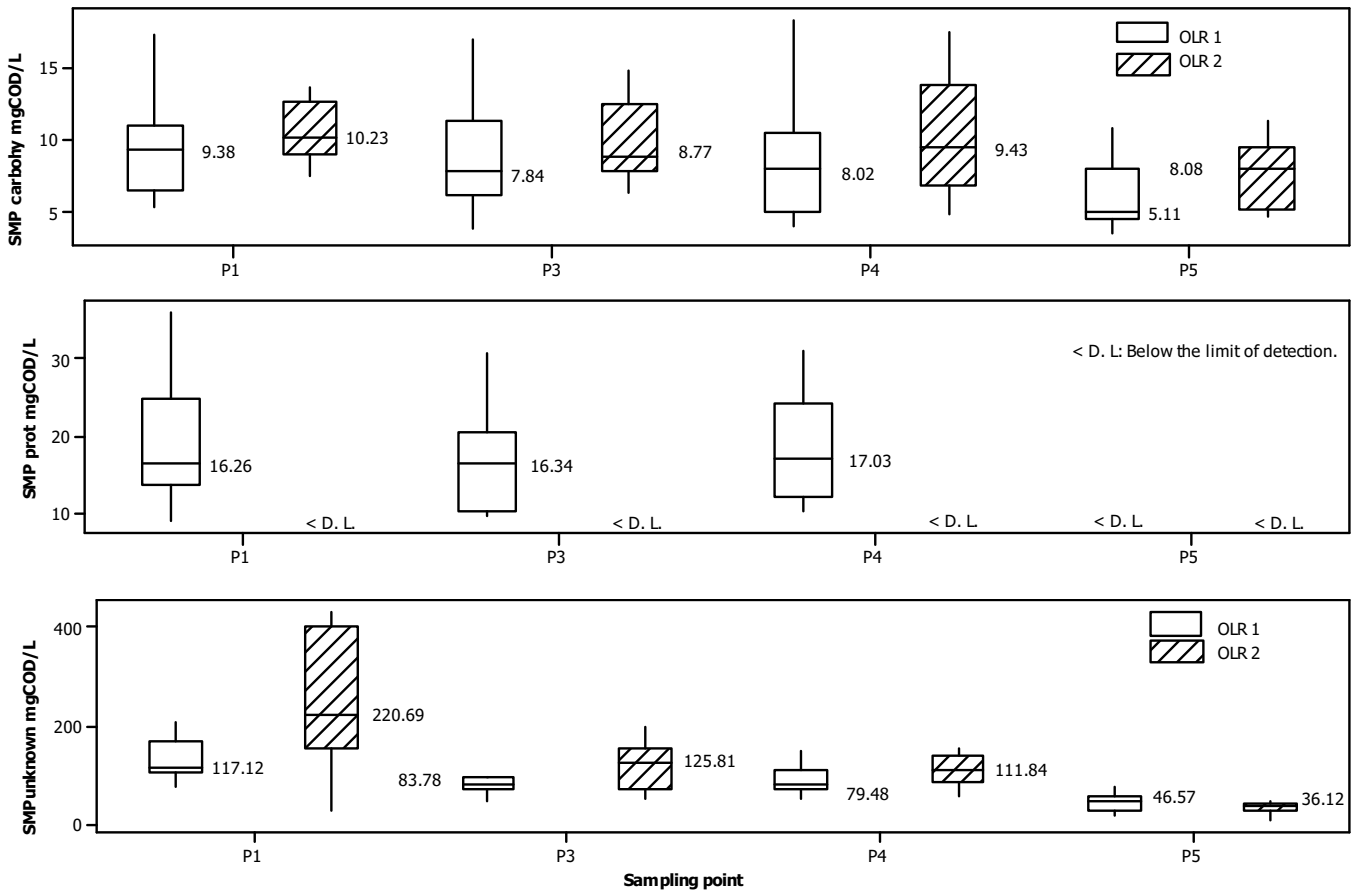

Fig. 7. Soluble microbial products composition at different heights.

degrades BAP, which in a large percentage correspond to unknown SMP. The effluent also presented a high percentage of unknown BAP, indicating that the recalcitrant material in the effluent is unknown. Based on these results, further research is necessary to identify unknown SMP, evaluate the role played by the membrane in SMP removal or retention, and assess the recalcitrant potential of the effluent.

4. Conclusions

This paper presents new results in terms of EPS and SMP stratification deeply discriminated in an anaerobic reactor with immersed membrane. In this study, an EPS dynamics was established shown at different reactor heights. This stratification depended on TSS concentration, particle size, and COD concentration. A low level of EPS, mainly LB-EPS, was found in the bottom of the reactor, where this lower EPS level favored mass transfer, which led to greater COD removal and high biological activity. The increase in OLR had no effect on EPS concentration, but their low level in the bottom of the reactor led to biomass deflocculation. High EPS and TSS levels in the top of the reactor led to increased caking on the membrane due to EPS's adhesion function in biofilms. Because these EPS are mainly carbohydrate-based, it can be inferred that they have hydrophilic characteristics and a negative charge, and that carbohydrate-based LB-EPS contribute to caking. EPS dynamics also showed stratification, which was associated with substrate complexity and biological activity. The SMP were the main COD components both inside the reactor and in the effluent. The SMP were used as source of carbon under limiting substrate conditions in the top of the reactor. The UAP predominate in the bottom of the rector, whereas BAP predominate in the top of the reactor and in the effluent. Increasing the OLR increased SMP concentration in the bottom of the reactor, and the membrane played an important role in retaining BAP and in degrading UAP.

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