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SOLUBLE MICROBIAL PRODUCT (SMP) CHARACTERIZATION IN BENCH-SCALE AEROBIC AND ANAEROBIC CSTRS UNDER DIFFERENT OPERATIONAL CONDITIONS

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Abstract - This work presents results on the production and characterization (by both mass spectrometry and conventional chemical analyses) of Soluble Microbial Products (SMP) that accumulated in aerobic and anaerobic bench scale completely stirred tank reactors (CSTRs) fed with glucose or acetate under different hydraulic retention times (HRT) and temperatures. SMP accumulation varied from 2 to 68% of the influent COD in the aerobic reactor and from 9 to 27% in the anaerobic reactor and increased with the decrease in temperature and with the HRT reduction in the aerobic reactor. On the other hand, in the anaerobic reactor, the organic loading rate and the temperature had little impact on SMP production, implying that the SMP originated from different mechanisms in each system. For both reactors, a higher accumulation of SMPs was observed as the substrate was acetate when compared to glucose, and the chemical analysis showed that the majority of the SMP did not seem to be proteins or carbohydrates. Principal component analysis (PCA) of the mass spectra from positive and negative mode electron-spray ionization (LC-IT-TOF-MS) and results from matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS) confirmed the chemical analyses and showed the absence of proteins in the effluents and the predominance of low molecular weight SMP. The PCA analysis also showed that the majority of the SMP from aerobic and anaerobic reactors did not seem to originate from soluble extracellular polymeric substances (EPS) or cell lysis products.
Keywords: Biological wastewater treatment; Soluble microbial products; Residual COD; Volatile fatty acids; mass spectrometry.

INTRODUCTION

Although there are many wastewater treatment processes, biological systems have already been consolidated in Brazil for sewage and biodegradable industrial wastewater (e.g., food and dairy products, distilleries and beverage industry) treatment. During biological wastewater treatment, either aerobic or anaerobic, microorganisms use the biodegradable organic matter as carbon and energy sources and produce soluble microbial products (SMP) as the result of the interaction of the microorganisms with their environment. As a result, the effluent COD from biological reactors is comprised not only of influent non-degraded compounds, but also of compounds produced and excreted by microorganisms that either play a role in their survival or result from their death (Aquino and Stuckey, 2006).

Many studies on the characterization of biological effluents, conducted by different research groups,

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showed that most soluble organics that accumulate in well-operated (i.e., not stressed) aerobic and anaerobic reactors were not present in the influent, but were produced during treatment instead (Siber and Eckenfelder 1980; Parkin and McCarty 1981; Namkung and Rittmann 1986; Noguera *et al*. 1994; Barker *et al*. 1999; Barker and Stuckey 1999). Such compounds, which come both from endogenous decay (e.g., cell lysis products) and from substrate metabolism (e.g., metallophores, extracellular polymeric substances), make up the majority of soluble organic matter in biological effluents (Jarusutthirak and Amy, 2007) and, for most well-operated systems, determine the limits of biological treatment efficiency (Barker and Stuckey, 2001). In other words, it has been shown that, in systems fed with biodegradable influent in which there is no accumulation of intermediates, such as volatile fatty acids (VFA) produced during anaerobic digestion, the effluent residual COD will be mainly determined by SMP production (Noguera *et al*. 1994; Aquino and Stuckey 2004). As a consequence, some researchers believe that minimizing effluent COD in non-stressed biological systems fed with typically biodegradable sewage is directly connected with minimizing the SMP production (Barker and Stuckey 1999).

Besides the impact on biological effluent quality, SMP production is also an important issue for membrane bioreactors. Several researchers have shown that internal membrane fouling can occur mainly due to these microbial organic compounds (Drews et al., 2007; Fonseca et al., 2007) thereby reducing permeate fluxes and affecting the productivity of the system. Indeed, the importance of SMP is acknowledged in many mathematical models of bioreactors (with or without membrane; aerobic or anaerobic) which have included the concept of production and degradation of such microbial products (Aquino and Stuckey, 2008; Oliveira-Esquerre *et al*., 2006; Rittmann and McCarty, 2001).

There are many factors involved in SMP production; some of them have been observed with pure cultures, whereas others were demonstrated in bench or pilot scale aerobic and anaerobic reactors treating different types of wastewater (Aquino *et al.* 2009; Jarusutthirak and Amy, 2007; Aquino *et al.*, 2006; Barker and Stuckey, 1999; Kuo and Parkin, 1996). Different researches showed that some SMP play a role in the microbial survival strategy, because some of them (e.g., metallophores) have chelating properties that may help in the uptake of metal nutrients and/or in the protection against toxicity (Kuo and Parkin, 1996; Aquino, 2003; Aquino and Stuckey, 2004). SMP might lead to a selection of the microbial community in the system (Chipasa and Medrzycka, 2008; 2004a,b); and might be involved in cell-to-cell communication or quorum sensing (Hastings and Greenberg, 1999; Fuqua e Greenberg, 1998). In addition, SMP might be released by hydrolysis and solubilization of extracellular polymeric substances (EPS) or as a response to a stress condition (E.g., to get rid of electrons not invested in cell growth due to nutrient limitation; release of cell lysis products) as discussed in Aquino and Stuckey (2004).

Given the importance of the SMP issue to biological wastewater treatment and the fact that there is limited information on SMP production and particularly on their nature, the main objective of this paper is to present results for the quantitative and qualitative characterization of the SMP that accumulated in anaerobic and aerobic systems operated under different conditions and fed with simple biodegradable substrates.

MATERIAL AND METHODS

Experimental Apparatus and Operational Phases

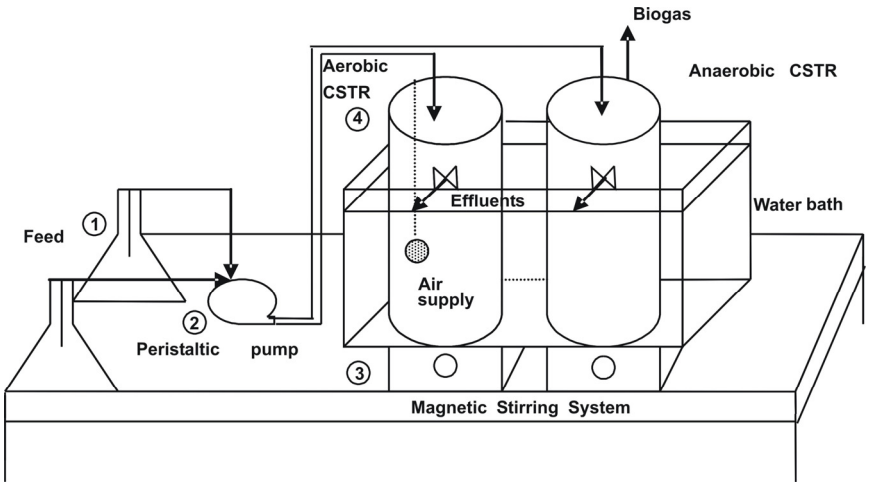
This study employed two completely stirred tank reactors (CSTRs) – with 6 L of working volume – that were operated under aerobic and anaerobic conditions and fed with easily detectable biodegradable substrate (glucose or acetate). The reactors were made of PVC and were continuously fed by means of peristaltic pumps; they were kept under permanent stirring and constant temperature by using, respectively, magnetic stirrer plates and a thermostatic bath. Oxygen was provided in the aerobic reactor by means of aquarium air pumps and porous stones. Figure 1 shows the experimental apparatus and the operation of the aerobic and anaerobic CSTRs was conducted according to the conditions described in Table 1.

The composition of feed biomedia was based on Aquino *et al*. (2007), as shown in Table 2. The feed was always autoclaved and the substrate added, after feed cooling, by sterile injection (0.22 μm disposable filters) to prevent the growth of microorganisms and substrate degradation before reaching the reactor.

The aerobic reactor was seeded with a sample of "returned sludge" from an activated sludge plant (Arrudas sewage treatment plant, Belo Horizonte – MG), whereas for the anaerobic system, the innoculum was obtained from a demonstration scale UASB reactor fed with sewage after preliminary

treatment (Sanitary Training and Research Center, Belo Horizonte – MG). Both reactors were operated continuously for at least three times the hydraulic retention time (HRT) to ensure the steady-state condition (defined by low variation of effluent COD – coefficient of variation < 10% -) had been attained.

During steady-state, effluent samples were collected (daily during a week) for chemical oxygen demand (COD), total and volatile suspended solids (TSS and VSS) and volatile fatty acid (VFA) analyses, so that the SMP amount could be calculated for each operational condition. Temperature, pH and oxygen (for the aerobic CSTR) were also monitored, and all analyses (except VFA) were executed according to the Standard Methods for Examination of Water and Wastewater (APHA, 1998). Volatile Fatty Acids (VFA) and glucose analyses were possible using high performance liquid chromatography (HPLC) on a *Hewlett Packard Series 1050* chromatographic equipment with a *Biorad Aminex HPX-87H* ion exclusion column maintained at 55ºC. For the chromatographic analysis, the injection volume was 40μL and the mobile phase was 0.01 mol/L H_2SO_4 at a constant flow rate of 0.6 mL/min. VFAs were detected with ultraviolet (UV) light at 210 nm, while for glucose the detection was with a refractive index (RI) detector. The VFA methodology was properly validated, achieving the criteria of linearity, precision, selectivity and accuracy within the concentration range of 25 to 1,000mg/L. The limit of detection (LD) was determined as 3 mg/L for all organic acids (C1 to C5) except valeric acid, which showed a higher limit of detection (12.5mg/L).



1. feeding erlenmeyers;
2. pumping;
3. temperature control;
4. aerobic and anaerobic CSTRs.

Figure 1: Schematic of the experimental setup used.

Table 1: Operational phases of the bench scale CSTRs.

Phase	T (ºC)	HRT (d)	Substrate type	Aerobic			Anaerobic		
				S_0 [1]	OLR [2]	BL [3]	S_0 [1]	OLR [2]	BL [3]
I (63 days)	25	4	Glucose	4,700 n = 36	1.17	0.65	4,700	1.17	0.14
II (59 days)	25	10	Glucose	5,033 n = 36	0.50	0.39	4,900	0.49	0.06
III (63 days)	25	16	Glucose	5,390 n = 36	0.34	0.12	5,290	0.33	0.04
IV (43 days)	15	10	Glucose	5,890 n = 33	0.59	0.11	6,090	0.60	0.15
V (42 days)	15	10	Acetate	5,047 n = 30	0.50	0.12	5,080	0.50	0.37
VI (33 days)	25	10	Acetate	5,113 n = 33	0.51	0.15	4,947	0.49	0.16

[1]median of experimental values (mgCOD/L);
[2]organic loading rate (kgCOD/m^3.d);
[3]biological load (kgCOD/kgVSS.d)

Table 2: Feed composition for influent COD of 5,000mg/L

MACRONUTRIENTS (mg/L)	
NH_4Cl	1,112
$(NH_4)H_2PO_4$	153.2
$(NH_4)_2HPO_4$	44.5
$MgCl_2$	250.0
$CaCl_2$	189.0
$NaHCO_3$	2,500
MICRONUTRIENTS (mg/L)	
Yeast extract	125.0
$FeCl_3.6H_2O$	5.0
$ZnCl_2$	0.13
$MnCl_2.4H_2O$	1.25
$(NH_4)_6Mo_7O_{24}.4H_2O$	1.60
$AlCl_3.6H_2O$	0.13
$CoCl_2.6H_2O$	5.0
$NiCl_2.6H_2O$	13.0
H_3BO_3	3.0
$CuCl_2.2H_2O$	8.0
HCl	1.0 mL/L

Estimation of SM Accumulation

VFA were analyzed to allow SMP calculation, according to equations 1 and 2 (Aquino and Stuckey, 2003).

$$COD_{VFA} = 0.35\times[\text{formiate}] + 1.07\times[\text{acetate}] + 1.51\times[\text{propionate}] + 1.82\times[\text{butyrate} + \text{isobutyrate}] + 2.04\times[\text{valerate} + \text{isovalerate}] \quad (1)$$

$$COD_{SMP} = [COD_{Sup}] - [COD_{VFA} + COD_{ResSub}] \quad (2)$$

where, COD_{VFA} = COD due to the volatile fatty acids; COD_{SMP} = COD due to soluble microbial products; COD_{ResSub} = residual substrate (glucose or acetate) COD; and COD_{sup} = COD of the supernatant free from suspended solids (after centrifuging at 5,000 rpm for 15 minutes).

SMP Chemical Characterization

The protein amount was assessed using Lowry's modified method (Lowry *et al*., 1951) and carbohydrates were determined by the phenol-sulfuric acid method, based on Dubois *et al*. (1956). Extracellular polymeric substances (EPS) extraction procedures were performed with an ion-exchange resin, according to Frolund *et al*. (1996), and cell lysis was carried out through sonication, according to Guerlava *et al*. (1998).

Lyophilized (Liotop equipment, São Carlos, SP, Brazil) effluent samples from operational phases I and II were sent to the Multiuser Proteomic Laboratory at Unifesp (São Paulo, Brazil) for matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) analysis in an attempt to identify the high molecular weight SMP present in the biological effluents. The analyses were carried out on an Axima CFRplus Shimadzu equipment using two matrices (α-cyano-4-hydroxycinnamic acid (CHCA) and sinapinic acid) both dissolved in acetonitrile (50%) and trifluoracetic acid (0.05%). The samples (0.5 μL) were deposited directly on the MALDI metal plate onto which were applied 0.5 μL of the matrix solution and then allowed to dry. The equipment was calibrated using peptides with known MW which varied from 757.40 Da for bradicinine to 39,212.28 Da for aldolase. The equipment was set with an ion gate of 700 Da; the shot energy varied from 110 to 180 and the results were analyzed using the software LaunchPad – Shimadzu Biotech MALDI-MS (version 2.8).

In addition, lyophilized samples of influent and effluent from all operational phases were redissolved in a methanol solution (30% v/v aqueous solution) and then analyzed in positive and negative modes (molecular weight range from 100 to 4,000 Da) in a Shimadzu high resolution liquid chromatography ion trap time-of-flight mass spectrometry equipment (LC-IT-TOF-MS), installed in the Chemistry Department of the Federal University of Ouro Preto (UFOP). The injection volume was 5μl at a flow rate of 0.2mL/min (isocratic mode for 5 minutes with

70:30 v/v of water/methanol as mobile phase), using argon as collision gas in a full scan mode, according to other detailed equipment configurations presented elsewhere (Leite *et al*., 2009).

RESULTS AND DISCUSSION

Influence of Operational Conditions on SMP Accumulation

Figure 2 shows that the median influent COD were in the same range from both reactors (from 4,700 to 5,890 mg/L in the aerobic reactor and from 4,700 to 6,090 mg/L in the anaerobic reactor) and that the residual COD from aerobic reactor was always lower when compared (phase by phase) to the anaerobic one. These results were expected given the metabolic differences that favor the aerobic microrganisms, which have higher substrate utilization and microbial growth rates when compared to the anaerobic ones.

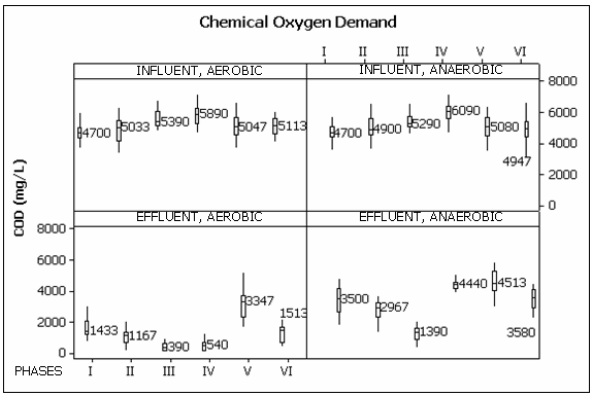


Figure 2: Influent and effluent COD in aerobic and anaerobic effluents for each operational phase (median values shown).

The analysis of glucose during the phases in which it was used as substrate (I to IV) showed that it was not detected in the effluents, implying its complete removal in both reactors under the different HRT and temperatures employed. Therefore, the effluent soluble COD under such conditions was comprised of intermediate VFAs (mainly in the anaerobic reactor) and SMPs. During phases V and VI, when acetate was used as substrate, a high accumulation of acetic acid was observed, which represented from 98 to 99% of the total measured VFAs in the anaerobic reactor and nearly 88% of the VFAs measured in the aerobic effluent during phase V (Figure 3). Such results might be due to the indirect production of acetate in both reactors from SMPs released into the media following the change of substrate (from glucose to acetate), as was observed mainly in the aerobic reactor (Figure 4). The reason for the increase in SMP production in the aerobic reactor in phases IV and V (from SMP/S_o = 0.085 in phase IV to SMP/S_o = 0.68 in phase V) is not clear but might be related to greater cell lysis resulting from the decrease in biomass concentration (as VSS) that was observed in these phases (from 5,290 mg/L in phase IV to 4,115 mg/L in phase V and 3,390 mg/L in phase VI). A reduction of biomass concentration might have happened due to the change from a more energetic (glucose) to a lower energetic (acetate) substrate which might also have led to some cell starvation and death.

Figure 3 shows that, in the anaerobic reactor, a large part of the effluent COD was comprised of VFA, especially in phases I, IV and V, where stressful conditions (low HRT and/or low temperature) were applied. The accumulation of VFA might have occurred due to both kinetic and thermodynamic constraints (Aquino and Chernicharo, 2005) since, at a lower HRT, a greater washout of slow-growing microorganisms (e.g., methanogenic and acetogenic) should occur; at lower temperatures, such microorganisms cannot accompany the pace of the fast-growing acidogenic fermentative bacteria.

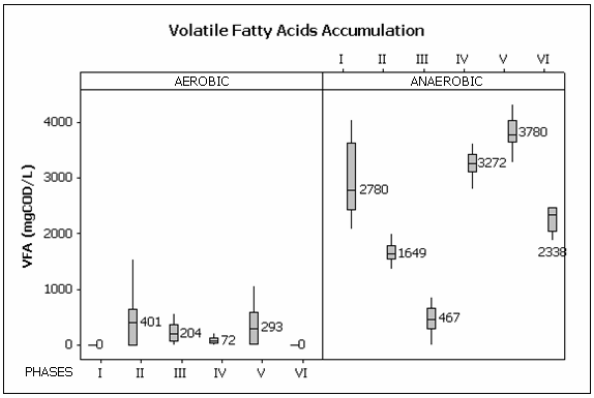


Figure 3: Volatile fatty acids (VFA) accumulation in the aerobic and anaerobic effluents for each operational phase (median values shown).

SMP monitoring showed that their accumulation (as SMP/S_o) in the aerobic reactor was very dependent on the HRT and type of substrate used, whereas in the anaerobic reactor the SMP

accumulation was more uniform throughout all six operational phases (Figure 4). Figure 4 also shows the accumulation of SMP normalized in relation to the total amount of biomass present in the reactor (SMP.Q/VSS.V), where an increase of SMP accumulation can be seen in both systems following the change from glucose to acetate. As discussed before, it is possible that such SMPs are biomass associated products (BAPs) released into the medium as a result of increased starvation and cell death.

Figure 4 shows that SMP/S_o ranged from 2 to 68% in the aerobic reactor, decreasing with the increase of HRT; for the anaerobic reactor, the SMP/S_o varied from 9 to 27% and was only partially affected by the HRT change. Indeed, low HRT values impose a higher organic load (in this case ~1.25 gCOD/L.d), which could derive from an excessive metabolic activity and excretion of SMP related to substrate utilization. Hence, the SMPs accumulated in the aerobic reactor are possibly utilization associated products (UAPs). For the anaerobic reactor, the same trend was observed, except for the shortest HRT (4 days), which could be explained by the high VFA production resulting from the stressful condition, which was confirmed by the low VSS accumulation and tendency to decrease of the pH (Figure 5). Such results might indicate that the SMP produced in the anaerobic reactor during phases I, II and III are more likely to be cell lysis products (BAPs), as discussed by Aquino and Stuckey (2004).

Overall, the results confirm that, in anaerobic reactors under stress, the importance of SMP is minimized due to the increased accumulation of intermediate VFA, which are not included in SMP pool. On the other hand, for the aerobic reactor, adverse conditions can contribute to SMP accumulation, since the microbial population could respond to the adverse conditions by altering their metabolism and excreting organic compounds into the medium, as well as releasing cell lysis products.

As far as the influence of operational temperature is concerned, Figure 6 shows that temperature decrease resulted in higher SMP accumulation, especially in the aerobic system. In the aerobic reactor, about 33.8% of the influent COD were converted into SMP at 15°C, while at 25°C this figure was 18.7%; for the anaerobic reactor, this trend was not verified since SMP/S_o was essentially the same at both temperatures (18.1% at 15°C and 18.3% at 25°C). The higher accumulation of SMP in the aerobic reactor at 15°C might be a combination of a reduction in the SMP degradation rates and the increase in the excretion of metallophores to scavenge metal nutrients whose solubility was reduced by the temperature decrease. One hypothesis as to why this did not occur in the anaerobic reactor is that the excretion of metallophores is already higher in anaerobic systems, no matter the temperature, since the metal nutrients are normally associated with poorly-soluble sulphide salts (e.g., FeS, NiS).

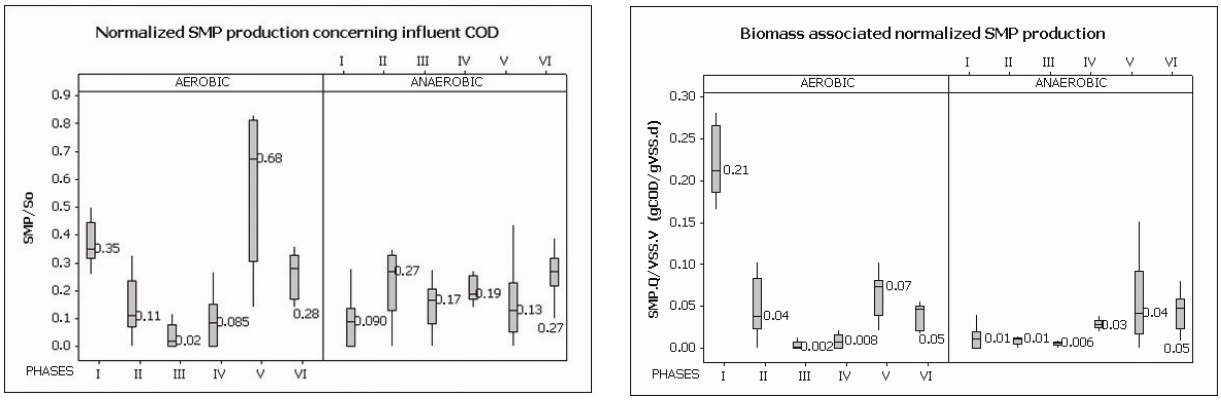


Figure 4: Normalized SMP accumulation (as COD) in relation to substrate (SMP/So) and biomass (SMP.Q/VSS.V) in the aerobic and anaerobic CSTRs for each operational phase (median values shown).

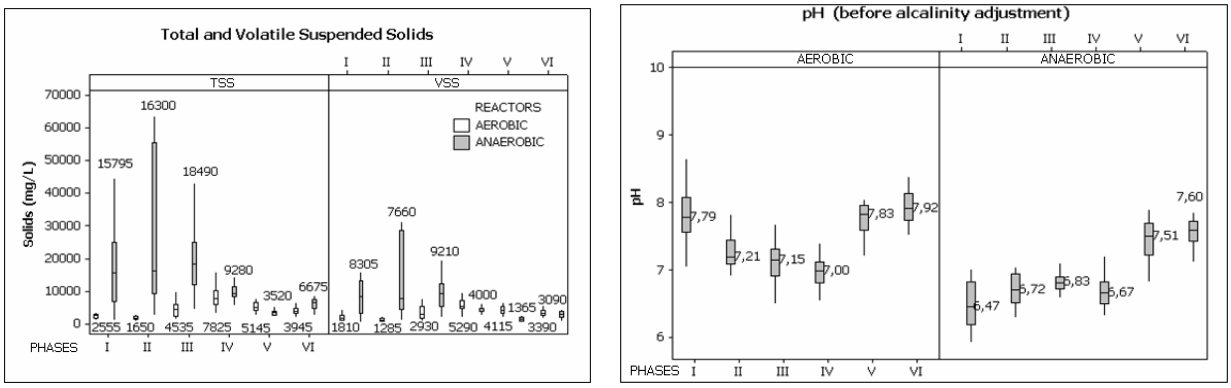


Figure 5: Variation of pH and volatile suspended solids (VSS) in the aerobic and anaerobic CSTRs.

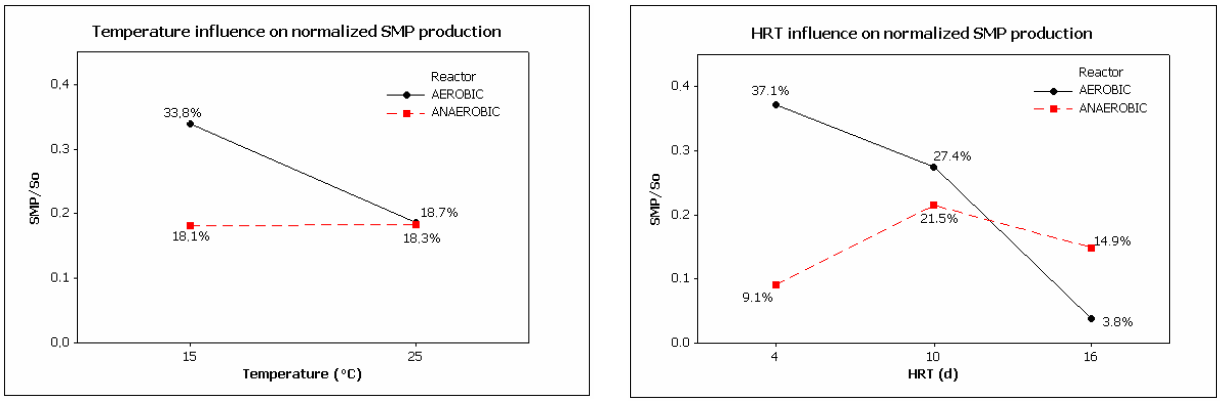


Figure 6: Temperature, hydraulic retention time (HRT) and substrate type influence on the normalized SMP production (SMP/So) in the aerobic and anaerobic CSTRs.

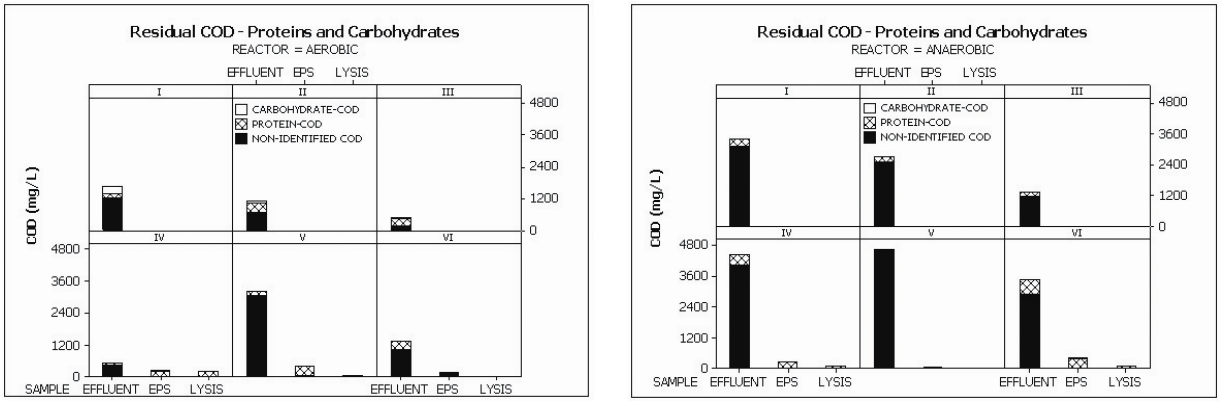


Figure 7: Chemical characterization in terms of protein and carbohydrate in samples of effluent, EPS and cell lyses products for the aerobic and anaerobic CSTRs.

Chemical Characterization of SMP

Chemical characterization results (Figure 7) indicated that there was a low protein and carbohydrate content in the effluent samples from both reactors; as a result most of the effluent soluble COD from all operational phases remained unidentified. An exception is phase II in the aerobic CSTR, in which protein represented 63% of effluent COD. Overall, in the aerobic reactor, the protein content (as COD) represented from 5% (phase V) to 63% (phase III) of effluent COD and carbohydrate (as COD) was always below 17%. For the anaerobic reactor, the protein (as COD) reached its maximum

concentration in phase VI, when the reactor was fed with acetate at 25°C, representing 16% of effluent COD. In all anaerobic samples analyzed the carbohydrate content was always below the limit of detection of the method, estimated in 5 mg/L.

The low protein and carbohydrate content in the effluent samples is in agreement with the MALDI-TOF-MS results obtained with samples from phases I and II. The mass spectrometric results (not shown) indicated the absence of macromolecules in the molecular weight range of 20,000 to 80,000 Da. This result suggests that the protein-like material detected by the Lowry method in the aerobic and anaerobic effluents from phases I and II were either of higher MW (> 80 kDa) or fragments of proteins (small peptides) lower than 20 kDa that would fall outside the working range of the MALDI-TOF-MS equipment.

The predominance of low molecular weight compounds in the SMP pool is not surprising. Aquino *et al*. (2009), studying the SMP production in an UASB reactor, observed that, despite the fact that the molecular weight distribution of the dissolved compounds in the UASB effluent was dependent on the operational conditions, the majority of the organics had molecular weights lower than 1,000 Da. According to Jarusutthirak and Amy (2007), utilization of substrate associated products (UAP) is expected in abundant substrate conditions, whereas biomass associated products (BAP) would prevail under substrate deficiency. In addition, Shin and Kang (2003) suggested that the degradation of high molecular weight SMP in ceramic membrane bioreactors would produce compounds of molecular weight lower than 10,000Da as long as the biomass is acclimated (i.e., during longer sludge retention times). In the present study, all samples were obtained after biomass acclimatization. Hence, the predominance of low molecular weight compounds in the effluents could be explained by the degradation and/or hydrolysis of high molecular weight BAP, except perhaps in phase I, when the UAP production was likely to be high due to the shortest HRT (highest organic load) and high observed substrate utilization rate (there was no glucose accumulation).

For lysis and EPS samples, basically all their content could be characterized as protein, whereas the carbohydrate content was nearly zero in almost every sample. The carbohydrate was present only in EPS coming from aerobic sludge in phase IV and from anaerobic sludge in phase VI, at the concentrations of 18 and 29mgCOD/L, respectively. The low carbohydrate values observed in EPS samples are in accord with specialized literature. Her *et al.* (2003), using an excitation-emission matrix fluorescence technique (EEM), stated that, compared to proteins and humic substances, carbohydrates could be neglected. In addition, Sheng and Yu (2006) confirm that the main fluorescence signals for EPS are those corresponding to proteins and humic substances.

Figure 5 also shows that the carbohydrate content in the effluent samples (lower than 17% of aerobic effluent COD; and not detected in the anaerobic effluent) was higher than in EPS samples (mostly not detected for both aerobic and anaerobic biomass). This suggests that the carbohydrates that accumulates in the effluent do not come from extracellular polymers. Since no carbohydrate was detected in the cell lysis samples, part of the effluent carbohydrate might be utilization associated products (UAP), a type of SMP release proportional to the rate of substrate utilization. This agrees with the literature, which reports that BAPs would be hard to degrade and would have higher molecular weights, while UAPs are easier to degrade and have low molecular weights (Barker and Stuckey, 2001).

Overall, samples from the anaerobic reactor showed a greater protein content when compared to its aerobic counterpart. In addition, the results indicate that EPS samples had more protein content when compared to cell lysis, although the amount of protein in the cell lysis increased greatly in phases V and VI, when acetate was used as substrate at 25°C, especially for the aerobic reactor. These results suggest the hypothesis that, during phases V and VI, cell lysis products are released into the medium reflecting some degree of starvation (due to the change of substrate from glucose to acetate) and contributing considerably to the increased SMP accumulation in the aerobic reactor.

Due to the fact that, in the aerobic (36% to 95%) and anaerobic (84% to 92%) effluents, there is an important fraction of COD that does not seem to be protein- or carbohydrate-like material, mass spectrometry techniques were used in an attempt to characterize such unidentified compounds, as well as to shed some light on their origin. Figure 8 shows the principal components analysis (PCA) profile obtained from the results of mass spectrometry in the positive and negative mode. Three major groups or clusters can be identified (cell lysis, EPS and aerobic effluent), and it is clear that the cell-lysis and the EPS cluster differ from the effluents and that the spectrometric data of the anaerobic effluent samples are more scattered when compared to the aerobic ones.

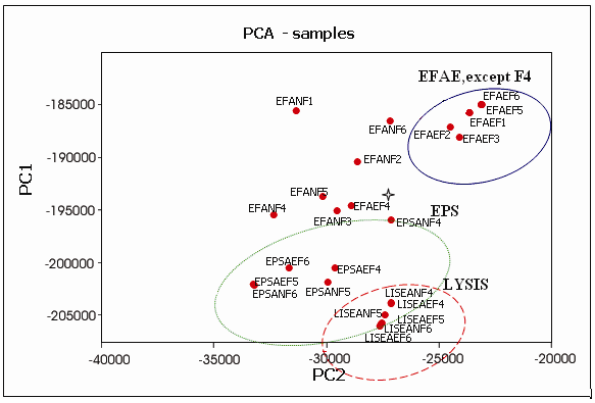


Figure 8: Principal components analysis (PCA) of mass spectrometry results for aerobic and anaerobic samples (AF – influent; EF – effluent; AE – aerobic; AN – anaerobic; EPS – extracellular polymeric substances; LISE – cell lysis products; F – operational phase.

These results indicate that the chemical profile (in the m/z range of 100 to 4,000 Da, which is the working range of the LC-MS/MS used) of the anaerobic samples differ, as expected, from the aerobic ones, as well as from the EPS and cell lysis products. Because the chemical profile of cell lysis products differs a lot from the effluents, it implies that, in the operational phases where cell lysis might have occurred (e.g., phase VI, as discussed above), the BAP-SMP released into the media were chemically changed (hydrolyzed or degraded) into lower MW compounds, as suggested by Shin and Kang (2003).

In phase IV, when the temperature was reduced from 25°C to 15°C, the extracted EPS seemed to be chemically different from the EPS extracted in other phases, especially for the anaerobic biomass. In addition, the aerobic effluent from phase IV differs considerably from the other aerobic effluent samples, being chemically closer to the EPS extract. These results may suggest that the adverse conditions (lower temperature; input of a lower energy substrate such as acetate) in the aerobic reactor contributed to SMP accumulation derived from the release of EPS as a response to the imposed stress.

For the anaerobic effluents no particular trend was noticed apart from the fact that the SMP produced were chemically different from lysis and EPS samples, as well as different from each other for each operational phase. This result suggests that, due to the complexity of anaerobic biochemistry, each operational phase will determine different chemical characteristics of the SMP, which do not seem to be cell lysis products or soluble EPS.

CONCLUSIONS

The results presented in this paper showed that, in the aerobic effluent, nearly all the residual COD during all operational phases was due to SMP, whereas in the anaerobic effluent the accumulation of VFAs reduced the SMP importance as a result of the stress conditions imposed (low temperature or low HRT). In the anaerobic reactor, the influent organic load did not interfere in the accumulation of SMP (as SMP/So), probably due to the higher biomass concentration in the reactor, suggesting that biomass associated products (BAP) prevail under anaerobic conditions, during all phases. On the other hand, in the aerobic reactor, SMP accumulation was directly proportional to the influent organic load, indicating the predominance of utilization associated products (UAPs). SMP/So in the aerobic reactor varied from 0.02 to 0.68 reaching its maximum when the glucose was replaced by acetate as the sole substrate. On the other hand, in the anaerobic reactor, the SMP/So was less affected by the operational phases, varying from 0.09 to 0.27. The SMP accumulation increased from 18.7% to 33.8% in the aerobic reactor when the temperature was reduced from 25°C to 15°C, while in the anaerobic reactor the temperature change did not affect the influent COD conversion into SMP (SMP/So equal to 18.1% at 15°C and 18.3% at 25°C). The use of acetate as substrate resulted in a higher SMP accumulation in both reactors, when compared to the use of glucose. Protein and carbohydrate amounts in the effluent samples were low and most of the effluent COD in all phases could not be related to these compounds; in contrast, for the ECP and cell lyses products, protein was an important component. The mass spectrometry results (MALDI-TOF-MS and LC-MS/MS) indicated the predominance of low molecular SMP, and the PCA analysis of the data showed that the SMP produced in each operational phase did not seem to be either cell lysis products or soluble EPS.

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