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Influence of organic matter type in wastewater on soluble microbial products production and on further ultrafiltration

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Abstract

BACKGROUND: Membrane fouling is the main limiting factor for the application of ultrafiltration (UF) to wastewater treatment as tertiary treatment or in membrane bioreactors. Soluble microbial products (SMP) play the more important role on it. In this work, four sequencing batch reactors were operated in parallel using two different simulated wastewaters under operating conditions that maximizing and minimizing the SMP production. The aim was to study the influence of the wastewater type, which until now is hardly considered, on the SMP production and consequently on the membrane fouling.

RESULTS AND CONCLUSION: Results showed that organic matter type in wastewater greatly influenced on SMP production and composition (Protein/carbohydrate ratio). Food-to-microorganisms (F/M) ratio also influenced significantly on SMP production. The lowest

protein/carbohydrate ratio was achieved for the wastewater containing sodium acetate as organic matter source at a F/M = 0.2. Finally, both mixed liquor and treated effluent were subjected to an UF process and it was checked that the carbohydrate concentration in SMP was the main parameter that influenced on membrane fouling when the reactor effluent was fed to the UF process.

Keywords: proteins, bioreactors, ultrafiltration, membrane, fouling

1. INTRODUCTION

Nowadays, membrane technologies are applied to many industrial processes. In this way, ultrafiltration is used in a wide variety of fields such as water treatment, wastewater reclamation, juice concentration and recovery of nutrients, among others.[1,2] However, the fouling of the membranes during the filtration process still remains a problem limiting the potential of this technique.

An increase in the use of low-pressure membranes in municipal wastewater treatment is foreseen. In addition, there are several aspects like shortage of fresh water or increasingly stringent legislation, which require higher treated water quality. In this way, biological treatment and ultrafiltration (UF) constitute a combination of technologies that obtain disinfected effluents with a high quality.[3,4] Both treatments can be either integrated as secondary treatment (membrane bioreactors, MBR) or consecutively as secondary (conventional activated sludge, CAS) and tertiary treatment (UF). In these processes the main mechanisms of UF membrane fouling are the cake layer formation on the membrane surface and the pore blocking due to colloids and high-molecular-weight solutes.[5] As reported by many researchers, the main foulants of the membranes are the soluble microbial products

(SMP).[6,7] The SMP are the organic compounds released into solution from biomass growth, substrate metabolism and biomass decay, which main components are carbohydrates and proteins.[8]

Feed water characteristics and the operational parameters of the activated sludge process, such as hydraulic retention time (HRT) and food-to-microorganisms ratio (F/M), determine the SMP generation and, consequently, the membrane fouling. In this way, a lot of studies in the bibliography are focused on SMP production under different operational parameters. Huang et al.[9] reported a lineal correlation between the effluent SMP and the influent total organic carbon. In the same way, Xie et al.[10] observed that the SMP production increased when the substrate concentration also increased. On the other hand, longer HRTs increase the endogenous respiration, resulting in a higher biomass decay, which increases the SMP production.[11] However, the wastewater characteristics have been not considered in these studies and, consequently, the comparison among the results of different authors is complicated.

Microbial hydrolytic enzymatic activities offer information about the organic matter hydrolysis in activated sludge systems,[12,13] which may be related to the SMP production. Through biological process only monomers and olygomers can cross the bacterial membrane for intracellular metabolism. Accordingly, the high-molecular-weight compounds must be hydrolyzed by extracellular enzymes to be assimilated. Protease, α-D-glucosidase and lipase activities are very important since proteins, carbohydrates and lipids are around 60-70% of the organic matter fraction in urban wastewater.[14] Additionally, dehydrogenase activity has an important role on oxidative substrate removal and is related with the viable biomass fraction.[15] Thus, all of these enzymatic activities provide valuable information about the biological performance.

In this work, the influence of several operational parameters like F/M ratio and HRT on the SMP production in a biological reactor treating municipal wastewater was studied. The wastewater characteristics (in terms of proteins and carbohydrates concentrations) were also considered. In addition, the relationship of all of these parameters with UF membrane fouling was also studied. For this purpose two different simulated wastewaters (SWW) were treated biologically under operating values that maximized and minimized the SMP productions according to previous results (F/M=0.5 kg COD kg $MLSS^{-1}$ d^{-1} with HRT=24 h and F/M=0.2 kg COD kg $MLSS^{-1}$ d^{-1} with HRT=16 h, respectively). Reactors performance, SMP production and protease, α-D-glucosidase, lipase and dehydrogenase activities were controlled and were related to operational parameters and SWW composition. Additionally, ultrafiltration tests with mixed liquor (ML) and treated effluent from the reactors were carried out in order to evaluate the membrane fouling when membranes work in the secondary treatment (MBR) or as tertiary treatment.

2. MATERIALS AND METHODS

2.1. Biological reactors

The tests were carried out using laboratory sequencing batch reactors (SBRs). Figure 1 shows the main components of each reactor, consisted of a mechanical stirrer, two peristaltic pumps (to feed the SWW and to draw the treated water) and a compressor. The compressor supplied air through two air diffusers located on the reactor bottom.

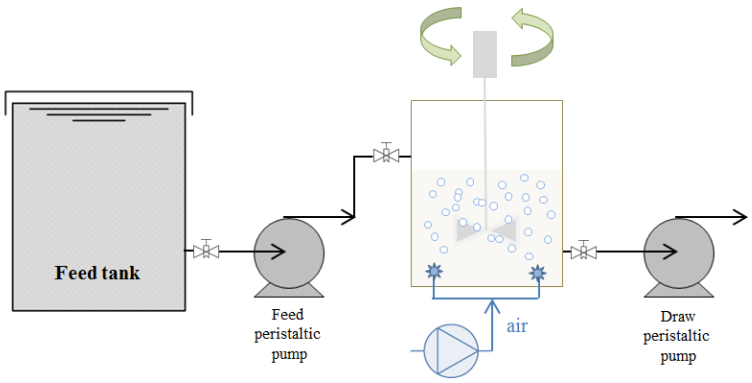


Figure 1. SBR configuration.

A total of four SBRs were operated in parallel varying feed composition, F/M ratio and HRT according to the values showed in Table 1. In a previous work (data not shown), it was checked that SMP production decreased in the SBRs with low values of F/M ratio and HRT. Thus, SBR-i and SBR-i* (where "i" is 1 or 2), were operated under conditions that reduced and enhanced the SMP productions, respectively.

Table 1. Operational conditions of biological treatment.

SBR	SWW	F/M (kg COD·kg MLSS^{-1}·d^{-1})	HRT (h)	$V_{feed/draw}$ (L)
SBR-1	SWW1	0.2	16	3
SBR-2	SWW2			
SBR-1*	SWW1*	0.5	24	2
SBR-2*	SWW2*			

All the operated SBRs worked with 3 cycles (8 h) per day. Stirrer and air compressor worked during aerobic reaction phase (6 h), which included the feed time. In the next phase, both systems stopped during 90 min to allow the sludge sedimentation. Finally, the treated effluent was drawn and a new operational cycle started after 10 min of idle phase. The reaction volume of each reactor was 6 L. The mixed liquor suspended solids (MLSS) concentration in the SBRs was maintained around 2500 mg L^{-1}, performing the needed sludge withdrawals for

it. The start-up of the reactors was performed with activate sludge from a municipal wastewater treatment plant located in Valencia (Spain).

2.2. Simulated wastewaters

The compositions of the prepared feeds for the SBRs are presented in Table 2.

Table 2. Synthetic wastewaters characteristics and composition (concentrations of COD, reagents to prepare the SWW, proteins and carbohydrates in mg·L^{-1}).

	SWW1	SWW1*	SWW2	SWW2*
pH	7.4±0.1	7.6±0.2	8.5±0.2	8.7±0.1
Cond (mS·cm^{-1})	1.23±0.08	1.56±0.30	1.72±0.04	2.95±0.13
COD influent	500±32	1250±15	500±12	1250±22
Peptone	225	563	-	-
Meat extract	225	563	-	-
Sodium acetate	-	-	670	1680
Urea	-	-	150	380
K_2HPO_4	28	70	28	70
Proteins	301.1±19.9	657.3±41.0	<3	<3
Carbohydrates	14.9±1.0	32.5±3.5	<3	<3

Simulated wastewaters (SWW) were prepared with peptone and meat extract (SWW1 and SWW1*) and sodium acetate (SWW2 and SWW2*), which provided the biodegradable organic matter. These compounds were selected to have a protein-rich feed (SWW1 and SWW1*) and a feed without proteins and carbohydrates (SWW2 and SWW2*). In terms of organic matter concentration, two levels of F/M ratio were fixed. Thus, SWWi and SWWi* were the simulated wastewaters providing in the SBRs F/M ratios of 0.2 and 0.5 kg COD kg MLSS^{-1} d^{-1}, respectively. The COD of the simulated wastewater to reach these F/M ratios was calculated according to Eq.(1). In this way, 500 and 1250 mg L^{-1} of COD were needed to work with a F/M ratio of 0.2 and 0.5, respectively.

$$F/M = \frac{COD \cdot V_{feed|draw}}{V_R \cdot MLSS} \quad \text{Eq.(1)}$$

where V_R=6 L, MLSS=2500 mg L^{-1} and $V_{feed|draw}$ was calculated according HRT (Table 1)

The relationship between COD:N:P in the SWW was 100:5:1. K_2HPO_4 was added as phosphorus source. Urea was added as nitrogen source to the synthetic wastewaters with a lack of this nutrient (SWW2 and SWW2*). All chemicals were supplied by Panreac and were diluted in tap water in order to have the needed trace elements.

Once the synthetic wastewaters were prepared, proteins and carbohydrates concentrations were measured with the same analytical methods that those carried out for SMP composition (methodology in section 2.4). The measured values are also presented in Table 2 (average and standard deviation of 8 samples prepared during experimental procedure).

2.3. Experimental methodology

2.3.1. SBR performance and SMP production

The following parameters were measured (three times a week): pH, conductivity, turbidity and COD in the SBRs effluent and MLSS and volatile suspended solids (MLVSS) in the mixed liquors. SMPs were characterized (biweekly) through protein and carbohydrates concentrations. Additionally, the sludge production (ΔX) and the sludge retention time (SRT) were calculated using Eq.(2) and Eq.(3).

$$\Delta X = \frac{1}{V_R} \cdot \left(\frac{(MLSS_j - MLSS_i) \cdot V_R}{j - i} + SS_{ef} \cdot Q_{ef} \right) \quad \text{Eq.(2)}$$

where SS_{ef} was the effluent suspended solids, Q_{ef} was the flow rate of effluent (V_{draw}/1 day) and "i" and "j" were two days in which no sludge was withdrawn between.

$$SRT = \frac{MLSS_{average} \cdot V_R}{\Delta X} \quad \text{Eq.(3)}$$

where $MLSS_{average}$ was around 2500 mg L^{-1}.

2.3.2. Membrane fouling

Effluent and mixed liquors of the four SBRs were subjected to UF to compare their behavior from the point of view of the membrane fouling. The commercial UF module Rayflow from Orelis (France) was used for the experiments. Filtrations were performed in cross-flow mode. Flat-sheet polyethersulfone UP150 P membrane from Microdyn Nadir (Germany) with a molecular weight cut-off of 150 kDa was used to carry out the experiments. The effective area was 100 cm^2.

Each experiment was performed by duplicate. Samples for ultrafiltration were taken after two weeks from the SBRs start-up (between 13rd and 15th day) and at the final part of the experimental period (between 22nd and 24th day). For this purpose 3 L of effluent or ML was taken from the reactors to perform the experimental procedure. ML samples were returned to the SBRs. The experimental procedure carried out was the following: 1) membrane compaction at transmembrane pressure (TMP) of 2 bars during 2 h, 2) initial membrane permeability (with deionised water, at 25ºC and TMP between 1 and 2 bar), 3) membrane fouling with effluent or ML until reaching the stationary permeate flux by the following conditions; TMP=1 bar, feed flow rate=300 L h^{-1} and temperature=25ºC. During this fouling step both retentate and permeate streams were recycled to the feed tank and permeate flux was measured periodically, 4) membrane rinsing (30 min with deionised water without applying TMP at 25ºC), 5) final membrane permeability under the same conditions as step.

Permeate flux (Jp) was determined by measuring the elapsed time to collect a particular permeate volume. To compare the membrane fouling in the experiments, the normalized

permeate flux (Jp/Jp_0) was calculated, where Jp_0 was the initial permeate flux measured in each experiment. In this way, the normalized permeate flux decline varied between 1 and a particular value in all the experiments carried out.

2.4. Analysis

Conductivity and pH were measured with an EC-Meter GLP 31+ and a pH-Meter GLP 21+ both from Crison. To measure COD, N_T and P_T a Spectroquant NOVA 30 and reactive kits, from Merck, were used. MLSS and MLVSS were measured according to APHA, 2005.[16]

Proteins and carbohydrates concentrations were performed by BCA method[17,18] and anthrone method[19], respectively. For this purpose 25 mL of ML were collected from each SBR and were centrifuged at 12000 x g. The clarified liquid was filtered at 0.45 μm to analyse both substances.

Several enzymatic activities were analyzed at the beginning and at the end of the experiment in every SBR. Protease, α-D-glucosidase and dehydrogenase were measured according to Goel et al.[15] using azocasein, 4-Nitrophenyl α-D-glucopyranoside and iodonitrotetrazolium chloride as substrate solution (all from Sigma-Aldrich), respectively. Lipase was determined employing a procedure adapted from Gessesse et al.[20] using 4-Nitrophenyl palmitate from Sigma-Aldrich as substrate solution (incubated at 37 ºC for 30 min). For performed these analysis, samples of mixed liquor were taken and the activities measured were normalized according their MLVSS concentration. P-nitrophenol (pNP) is the reaction product of lipase and α-D-glucosidase activity, which values were measured at 410 nm in Thermo Scientific™ 9423UVG1002E spectrophotometer. In both activities one enzyme unit (EU) is defined as production of 1.0 μmol of pNP in one hour of reaction. For dehydrogenase activity, 1,3,5-Triphenyltetrazolium formazan is the reaction product, which was measured at 490 nm. For this activity the EU is defined as production of 1.0 μmol of formazan in one hour of reaction.

For protease activity the reaction products are unknown and the EU is defined as the absorbance increase (measured at 340 nm) after one hour of reaction.

On the other hand, biological reactor samples were collected weekly and were observed immediately after sampling under a Carl Zeiss phase contrast microscope, Axiostar Plus model (X100). A Nikon D5200 digital camera with special camera adapter T2-T2 DSLR 1.6x was used to take microphotographs of the activated sludge.

2.5. Statistical analysis

An one-way ANOVA analysis (confidence level of 95 %) was carried out with Statgraphics Centurion XVII in order to study the statistical significance of feed composition and operational conditions (F/M ratio and HRT) in the SBR performance and SMP productions. The SBR performance was evaluated through the following parameters: pH, conductivity, turbidity, effluent COD, ΔX and enzymatic activities.

It was studied the effect of feed composition under conditions that minimized (comparing SBRs-i) and that maximized (comparing SBRs-i*) on the SMP productions. Additionally, it was analyzed the statistical significance of operational conditions such F/M ratio and HRT on the SBR performance including enzymatic activities and SMP productions comparing SBR-1/SBR-1* and SBR-2/SBR-2*.

3. RESULTS AND DISCUSSION

3.1. SBR performance and SMP production

3.1.1. Influence of operational parameters and simulated wastewaters on the process performance and enzymatic activities

After 24 days of biological treatment, effluent COD was similar in the SBRs with F/M=0.2 (SBR-i), in which COD average value was 21.4 ± 9.9 mg L^{-1}. Nevertheless, more data dispersion was observed in the SBR-i*, as it can be seen in Figure 2.

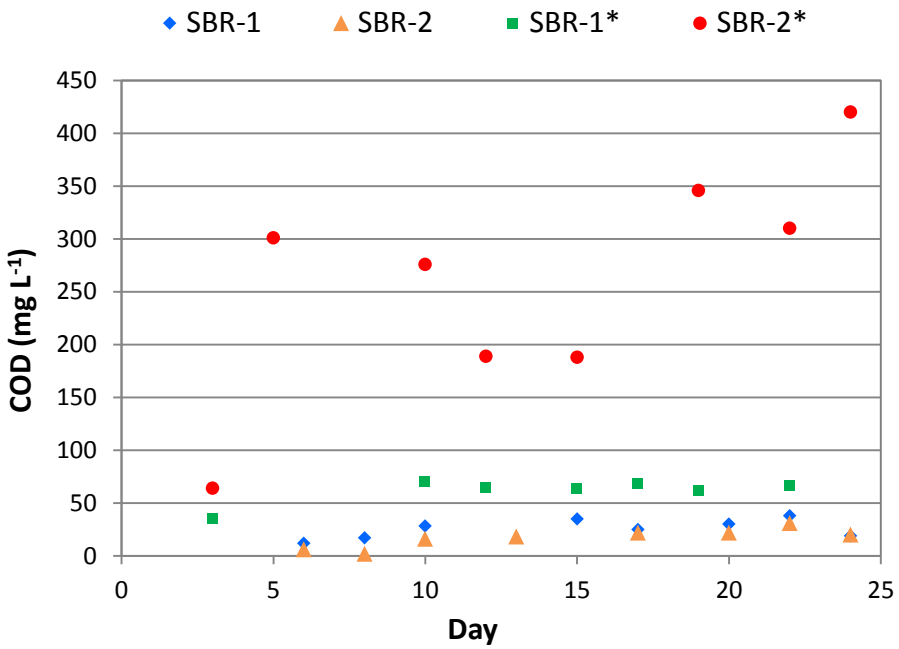


Figure 2. Effluent COD in SBRs with low SMP productions (SBR-1 and SBR-2) and high SMP productions (SBR-1* and SBR-2*).

In SBR-1* effluent COD was maintained around 66 mg L^{-1}, while in SBR-2* stationary conditions were not reached and this parameter increased up to 420 mg L^{-1}. Table 3 shows the average values with their standard deviations of some parameters measured in the effluents.

Table 3. Effluent characterisation (average value and standard deviation of 24 operational days).

	SBR-1	SBR-2	SBR-1*	SBR-2*
pH	7.7±0.1	8.3±0.2	7.9±0.2	8.7±0.1
Cond ($mS \cdot cm^{-1}$)	1.27±0.08	1.74±0.04	1.70±0.30	3.15±0.13
Turb (NTU)	0.03±0.01	0.03±0.01	0.19±0.25	45.83±17.48
COD ($mg \cdot L^{-1}$)	25.6±9.0	15.9±9.2	65.7±2.9	261.7±110.9

According to these results, it can be concluded that the different feed compositions only had influence on the SBR performance under high F/M conditions. In this way, a statistical significant difference was observed in the effluent COD of the SBR-i* ($F=9.41$; p-value=0.0154), while it was not found in the SBR-i.

On the other hand, it can be observed that the effluent COD values were the highest in the reactors with F/M=0.5, as expected, since the COD removal efficiency decreases with the increase of the organic matter load. The lower performance achieved in SBR-2* could be influenced by the higher conductivity in the mixed liquor of this reactor (more than 3 mS cm^{-1}), which was related to the feed characteristics (Table 2). This fact affected both the physical and biochemical properties of the activated sludge, driving to worse sludge sedimentation and bioflocculation[21], contributing to higher turbidity values in the effluent. Additionally, it can be commented that sodium acetate is a very easily biodegradable compound, resulting in the appearance of free-swimming bacteria, which was enhanced by high F/M ratio conditions[22,23]. In Figure 3A and Figure 3B it can be seen the free-dispersed bacteria in the ML of SBR-1* and SBR-2* (F/M = 0.5 and SWW1 and SWW2), respectively. It can be observed that free-swimming bacteria are almost negligible when peptone and meat extract mixture was used as organic matter source. Both high conductivity and high free-dispersed bacteria, contributed to the increase of the turbidity values in SBR-2*, which is in concordance with the high COD measured in the effluents of this reactor.

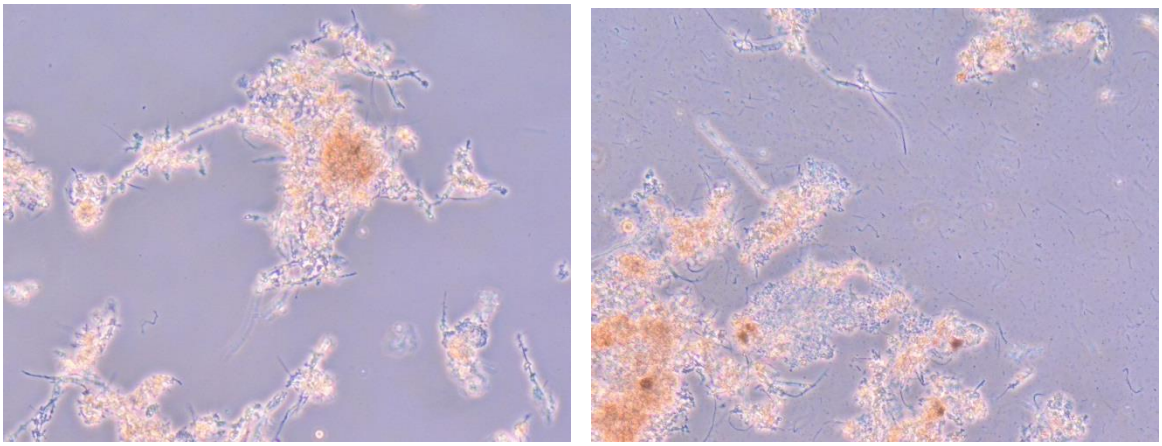


Figure 3. Microphotographs of activated sludge of SBR-1* (A) and SBR-2* (B) with microscope Axiostar Plus model (X100).

Additionally, it was checked that the biomass growth was enhanced under high F/M conditions, as expected.[24] A statistical significance between F/M ratio and biomass growth was observed when comparing SBR-1/SBR-1* ($F=18.71$; p-value=0.0050) and SBR-2/SBR-2* ($F=11.60$; p-value=0.0144). Thus, the average ΔX was higher in SBR-i* (1.54 ± 0.12 g MLSS d^{-1}) than in the SBR-i (0.91 ± 0.08 g MLSS d^{-1}). This fact implied more frequent sludge withdrawals in SBR-i* to maintain the MLSS around 2500 mg L^{-1}, driving to a lower sludge retention time (SRT). In this way, the average SRT values along 24 operational days were 10.0 ± 0.0 and 27.5 ± 6.4 days in SBR-i* and SBR-i, respectively. No relationship was observed between feed source and ΔX (comparing SBR-1/SBR-2 and SBR-1*/SBR-2*).

With regard to enzymatic activities (EA), it was observed a relationship between these parameters and F/M ratio. When comparing the initial and final activities, it can be concluded that EA increased at a higher rate in the reactors with higher F/M ratio. In this way, it can be seen in Figure 4 that all the EA increased in SBR-i* (except for α-D-Glucosidase in SBR-1*, which was maintained almost constant). In contrast to it, in SBRs under the lowest F/M values, only protease in SBR-1 and protease and lipase in SBR-2 increased during the SBRs

operation. In this way, EA were directly related to the F/M ratio and, consequently, to the SMP production.

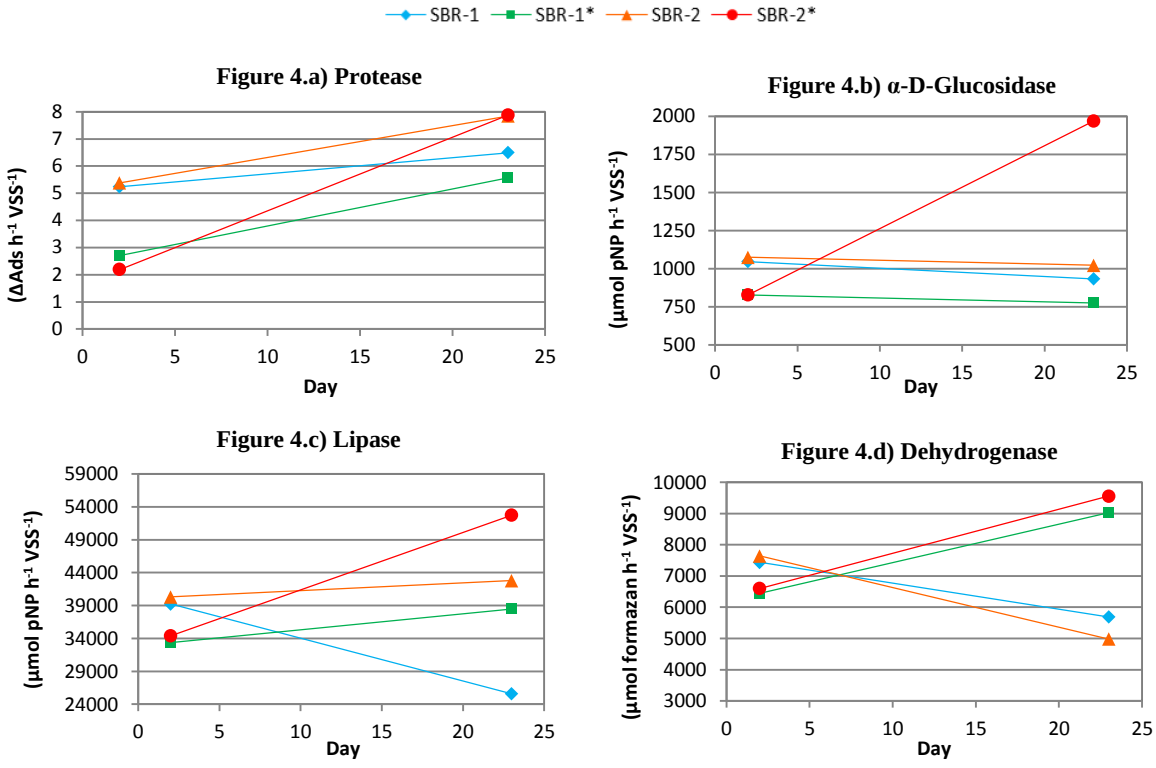


Figure 4. Enzymatic activities.

On the other hand, no influence of the wastewater type was observed on the EA except for protease activity. The final protease activity of SBR-2 and SBR-2* was higher than that measured in the other SBRs. This was due to the fact that sodium acetate is a more rapidly biodegradable organic matter than the mixture peptone-meat extract. It implied that endogenous respiration occurred earlier in these reactors. This fact resulted in the appearance of more cellular debris, which composition is characterized by around 50% of proteins[25], increasing the protease activity.In addition, as explained above, the free-dispersed bacteria grew more in SBR-2* than in SBR-2, due to high F/M ratio conditions. This is the reason why α-D-Glucosidase and lipase could increase in a high rate in SBR-2*.

3.1.2. Influence of operational parameters and simulated wastewater on the SMP production

Figure 5 show the SMP productions during the experimental time in SBR-i and SBR-i*. Data of protein and carbohydrate concentrations can be observed, representing the sum of them the total height of the bars. The average values of these SMP concentrations for each reactor are presented in Table 4.

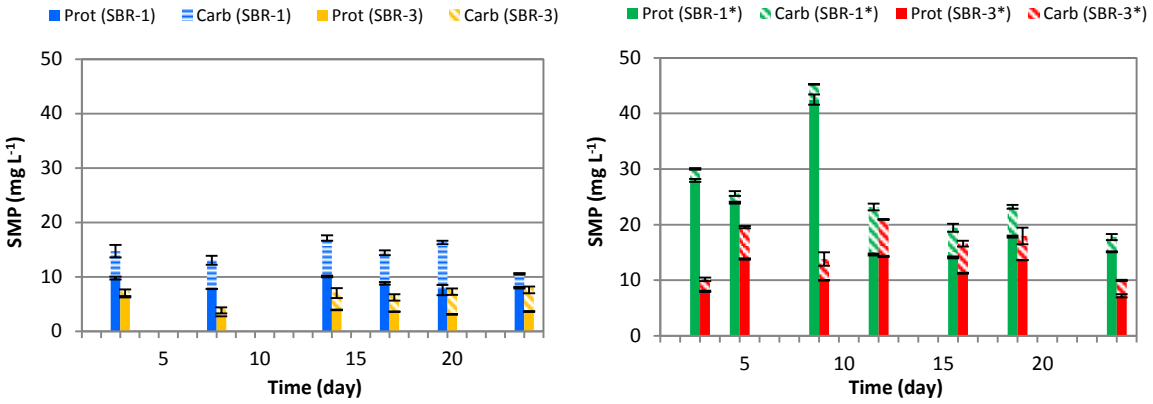


Figure 5. SMP productions of SBR-1 and SBR-2 (left panel) and SBR-1* and SBR-2* (right panel).

Table 4. SMP characterisation (average value and standard deviation of 24 operational days).

	SBR-1	SBR-2	SBR-1*[a)]	SBR-2*
SMP (mg·L^{-1})	14.1±2.2	6.7±1.5	20.9±2.7	15.6±4.5
Protein (%)	64.2	52.4	73.8	71.8
P/C ratio	1.7/1	1/1	2.8/1	2.5/1

a) Average values between 10 and 24 day (without instable period).

As it can be observed, the SMP production was higher in SBR-i* than in SBR-i. This difference was statistically significant between SBR-1 and SBR-1* ($F=13.47$; p-value=0.0080) and between SBR-2 and SBR-2* ($F=19.31$; p-value=0.0023).

The SMP production is proportional to the biomass concentration (due to biomass decay and cell lysis during endogenous decay).[26] As MLSS remained constant in all the SBRs (around

293 2500 mg L^{-1}), differences in the SMP production were related to F/M ratio and SRT. The
294 higher F/M ratio improved the metabolic activity (as also checked in the above commented
295 EA analysis) and microbial growth, which increased the SMP production.[27] However, the
296 SMP increase was not due to carbohydrate concentrations since its concentration was
297 maintained in 4.3 ± 0.8 mg L^{-1} in the four reactors. In other words, proteins were accumulated
298 in the SBRs due to the fact that lower SRT implied a worsening of the hydrolysis of
299 macromolecules and, consequently, of the organic matter degradation.[28,29]

300 The low value of SMP measured in SBR-2* in the last analysis (23rd day) was related to the
301 decrease of the process performance of this SBR. In fact, although the MLSS concentration
302 was maintained at 2500 mg L^{-1}, the percentage of volatile solids in the mixed liquor
303 decreased from 85% to 60%, which implied biological process deterioration. This explained
304 that no stationary conditions were reached in this reactor and that the effluent COD increased
305 progressively (Figure 2).

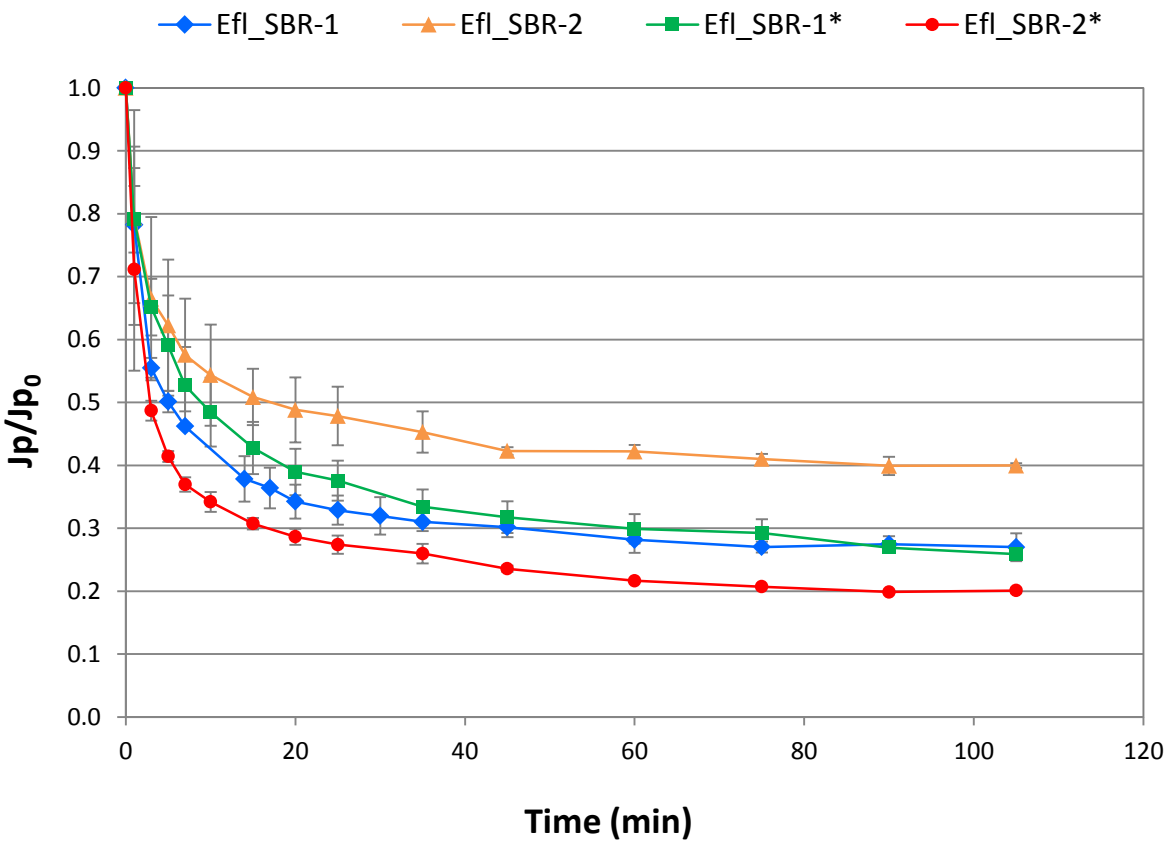
306 Independently of the F/M ratio, the average SMP production was the highest in the SBRs fed
307 by the SWW containing peptone and meat extract as it can be seen in Table 4. Nevertheless,
308 although it was statistically significant comparing SBR-1/SBR-2 ($F=31.11$; p-value=0.0008),
309 this was not achieved in SBR-1*/SBR-2* ($F=4.68$; p-value=0.0739), which was probably due
310 to the operations problems at the end of the test in SBR-2* (caused by the combination of the
311 highest F/M rate with the most rapidly biodegradable substrate).

312 On the other hand, the feed type was related to SMP composition in the reactors working
313 with F/M=0.2, achieving higher protein/carbohydrate ratio (P/C ratio) in the SMP of SBR-1
314 than in SBR-2. In fact, a statistically significance between SBR-i and P/C ratio of SMP was
315 found ($F=214.52$; p-value < 0.0001). This behavior was also observed by Arabi and Nakhla[30],
316 who reported that high feed P/C ratio resulted in an increase in SMP productions due to the

317 increase of protein concentration (carbohydrate concentrations in SMP remained constant).
318 In the SBR-i* no significant difference was observed between the reactors in terms of SMP
319 composition. The high biomass growth rate in these reactors also implied the accumulation of
320 cellular debris in the mixed liquor, whose composition determined the P/C ratio on the SMP
321 for both SBR-i*.

322 **3.2. Membrane fouling**

323 The results of the UF experiments performed with the SBR effluents and MLs to assess the
324 membrane fouling are presented in Figure 6 and Figure 7, respectively. In order to quantify
325 the membranes fouling, Jp/Jp_0 decline throughout the experiments has been plotted.



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327 **Figure 6. Membrane fouling experiment (TMP=100 kPa; feed flow rate=300 L·h^{-1}; 25ºC) with effluent of**
328 **SBR with F/M=0.2 (SBR-1 and SBR-2) and F/M=0.5 (SBR-1* and SBR-2*).**

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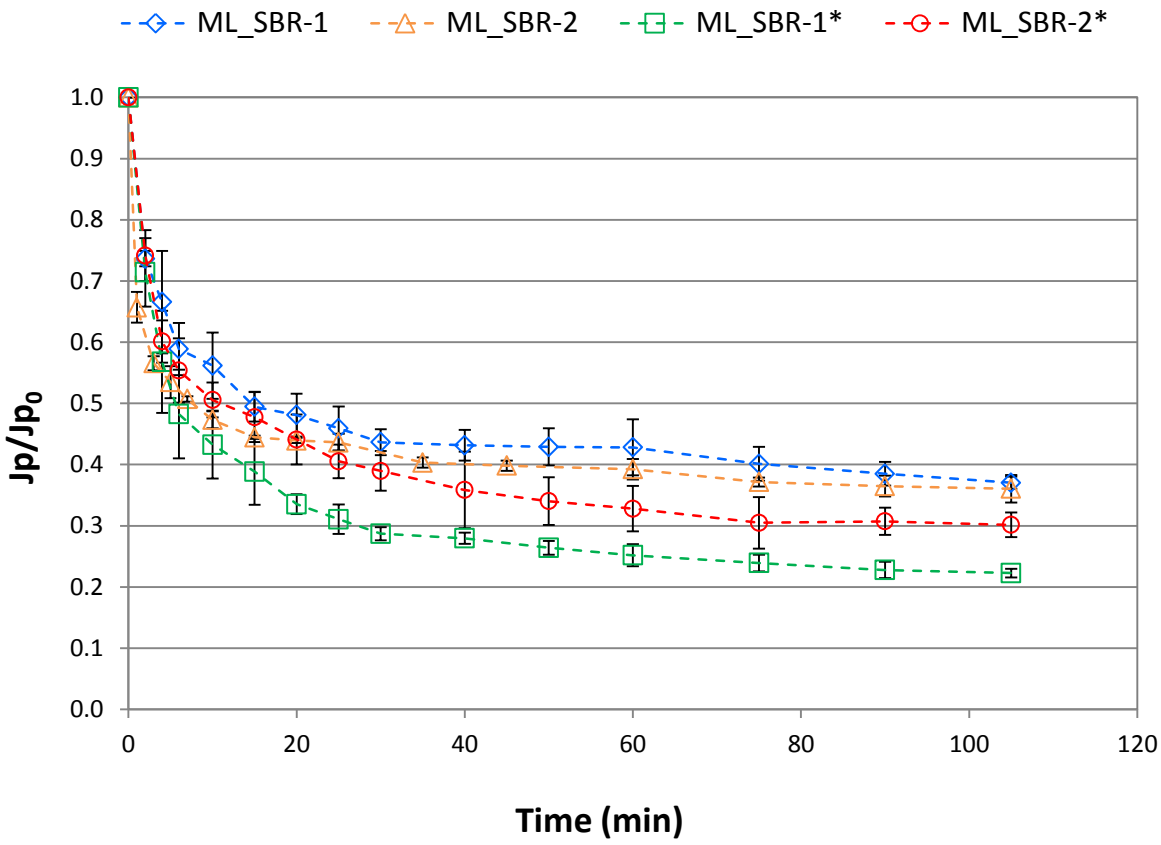

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331 **Figure 7. Membrane fouling experiment (TMP=100 kPa; feed flow rate=300 L·h^{-1}; 25ºC) with ML of SBR**
332 **with F/M=0.2 (SBR-1 and SBR-2) and F/M=0.5 (SBR-1* and SBR-2*).**

333

334 In Figure 6 it can be observed that for F/M=0.2 the membrane fouling was higher for SBR-1
335 effluent than for SBR-2 effluent. In this way, it was confirmed a positive correlation between
336 SMP concentrations and membrane fouling, considering these substances as the major
337 foulants, as other authors had already reported.[31,32] Nevertheless, this behaviour was not
338 observed in the reactors with F/M=0.5 since ultrafiltration was affected by other parameters
339 like turbidity due to the high amount of free-dispersed bacteria (section 3.1.1). On the other
340 hand, it can be commented that stationary Jp/Jp_0 was similar in SBR-1 and SBR-1* although
341 SMP concentration was higher in SBR-1*. This fact was related to SMP composition,
342 specifically on carbohydrates concentration. In this way, Yigit et al.[33] studied the membrane
343 fouling in a MBR under different operational conditions and reported that carbohydrate
344 fraction of SMP contributed to fouling more than protein. Fan et al.[34] also reported the same

345 behaviour. This fact can explain that the effluent of SBR-1 (SMP_{carb}=5.3 ± 1.7 mg L^{-1})
346 resulted in a similar fouling than SBR-1* (SMP_{carb}=4.0 ± 2.5 mg L^{-1}), despite of the low
347 global SMP concentration.

348 Analysing the results obtained for the mixed liquor, it can be concluded that in general terms
349 SBR-i ML resulted less foulant than SBR-i* ML. The average Jp/Jp_0 values in the stationary
350 conditions for the UF of SBR-1 and SBR-2 ML were 15.4% and 16.4% higher than those
351 achieved for SBR-1* and SBR-2* ML, respectively. This fact was due to the higher SMP
352 concentrations in the SBR with the highest F/M ratios, which increased the membrane
353 fouling.[27,35] However, other parameters should be taken into account. In this way, it can be
354 seen that SBR-1 and SBR-2 ML had similar stationary Jp/Jp_0 although the SMP
355 concentration was higher in SBR-1. This behaviour can be explained considering that in the
356 reactors with lower SMP concentrations (below 15 mg L^{-1}; SBR-1, SBR-2 and SBR-2*) the
357 cake layer formed by the sludge flocs is the main mechanism of fouling of the membranes as
358 reported by other authors.[35,36]

359 Finally, it has to be taken into account that MLSS concentration was around 2500 mg L^{-1},
360 which is lower than MLSS concentrations from which sludge rheological properties could
361 reduce the flux dramatically.[37] Then, the low MLSS concentration, together with the fact that
362 mixed liquor might hinder the convective transport of the SMP to the pores minimizing the
363 internal pore blocking. This fact may explain that flux decline when mixed liquor and SBR
364 effluents reached a similar order of magnitude.

365

366 **4. CONCLUSIONS**

In this work, the SMP production and composition in terms of concentration and P/C ratio in SBRs fed by two different simulated wastewater and operated under two F/M ratios have been assessed. In addition, the fouling produced by the UF of both treated effluents and mixed liquors has been studied.

The first conclusion is that higher F/M ratios resulted in higher SMP concentrations and higher microbial hydrolytic enzymatic activities. On the other hand, it was observed a relationship between the SMP productions and reactors performance with the feed type. In the reactors with low F/M ratio peptone-meat extract increased the SMP concentrations. In the reactors with high F/M ratio an increase in free-dispersed bacteria was observed in the reactor fed with sodium acetate resulting in operational problems (high COD and turbidity in the effluent). Thus, it can be concluded that wastewater composition affects both SMP generation and performance system. This fact could explain contradictory data found in the bibliography reporting relationships between SMP and operating conditions. As an example, according to our results, the SMP productions are quite similar for SWW1 operated at F/M=0.2 and for SWW2 operated at F/M=0.5.

In the UF experiments a direct relation between the increases of SMP concentration and the membrane fouling was observed when effluent was filtrated, playing carbohydrates concentration the main role. On the contrary, no relation between SMP and membrane fouling was found when mixed liquor was filtrated.

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Tables

Table 1. Operational conditions of biological treatment.

SBR	SWW	F/M (kg COD·kg MLSS^{-1}·d^{-1})	HRT (h)	$V_{feed/draw}$ (L)
SBR-1	SWW1	0.2	16	3
SBR-2	SWW2			
SBR-1*	SWW1*	0.5	24	2
SBR-2*	SWW2*			

520 **Table 2. Synthetic wastewaters characteristics and composition (concentrations of COD, reagents to**
521 **prepare the SWW, proteins and carbohydrates in mg·L^{-1}).**

	SWW1	SWW1*	SWW2	SWW2*
pH	7.4±0.1	7.6±0.2	8.5±0.2	8.7±0.1
Cond (mS·cm^{-1})	1.23±0.08	1.56±0.30	1.72±0.04	2.95±0.13
COD influent	500±32	1250±15	500±12	1250±22
Peptone	225	563	-	-
Meat extract	225	563	-	-
Sodium acetate	-	-	670	1680
Urea	-	-	150	380
K_2HPO_4	28	70	28	70
Proteins	301.1±19.9	657.3±41.0	<3	<3
Carbohydrates	14.9±1.0	32.5±3.5	<3	<3

522

523

524

525 **Table 3. Effluent characterisation (average value and standard deviation of 24 operational days).**

	SBR-1	SBR-2	SBR-1*	SBR-2*
pH	7.7±0.1	8.3±0.2	7.9±0.2	8.7±0.1
Cond (mS·cm^{-1})	1.27±0.08	1.74±0.04	1.70±0.30	3.15±0.13
Turb (NTU)	0.03±0.01	0.03±0.01	0.19±0.25	45.83±17.48
COD (mg·L^{-1})	25.6±9.0	15.9±9.2	65.7±2.9	261.7±110.9

526

527

528 **Table 4. SMP characterisation (average value and standard deviation of 24 operational days).**

	SBR-1	SBR-2	SBR-1*[a)]	SBR-2*
SMP (mg·L^{-1})	14.1±2.2	6.7±1.5	20.9±2.7	15.6±4.5
Protein (%)	64.2	52.4	73.8	71.8
P/C ratio	1.7/1	1/1	2.8/1	2.5/1

529 **a)** Average values between 10 and 24 day (without instable period).

530