

**Biological treatment performance of hypersaline wastewaters with high phenols concentration from table olive packaging industry using sequencing batch reactors**

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**Abstract**

Biological treatment of hypersaline wastewaters such as fermentation brine from table olive processing (FTOP), was carried out using four sequential biological reactors (SBRs). These wastewaters were characterized by conductivities higher than 90 mS·cm<sup>-1</sup> together with COD and total phenols concentration values of more than 15 g·L<sup>-1</sup> and 1000 mg·L<sup>-1</sup>,

respectively. In order to increase the organic removal efficiency and to reduce the hydraulic retention time (HRT), extra nutrients were added and pre-treatment by adsorption was performed. Results showed that the COD/N/P relationship, in the FTOP, of 250/5/1 was appropriate for the biological process reaching COD removal efficiencies of around 80%. The FTOP adsorption pre-treatment with powder activated carbon for the reduction of phenols concentration to 400 mg·L<sup>-1</sup> led to a HRT reduction from 40 to 15 days, maintaining the COD and total phenols removal percentages around 78% and 97%, respectively. On the other hand, *γ-Proteobacteria* was the main bacterial class, representing around 74% of the microbial community in the reactors.

*Keywords:* Hypersaline effluents; Phenols removal; Table olives; SBR.

## **1. INTRODUCTION.**

Industrial activity related to olive processing is concentrated on Mediterranean countries such as Spain, Italy, Greece, Tunisia and Morocco [1]. The average world production of table olives, between 2010 and 2015, was 2,531,600 tonnes [2].

Table olive processing (TOP) is performed in three steps: 1) Debittering; olives are submerged in a NaOH solution (1-2% w/v) during 8-12 hours, and the natural olive bitterness is removed, because oleuropein is hydrolysed. 2) Rinsing; to eliminate the alkali excess. 3) Fermentation; olives are submerged in brine (4–8% w/v) of NaCl for several months. During

this time lactic fermentation is performed and the organoleptic properties of olives are improved [3]. Throughout the TOP, large quantities of wastewater (about 3.9–7.5 m<sup>3</sup> per ton of green olives) are produced [1]. Usually, these effluents are stored in evaporation ponds (risk of surface and groundwater contamination, bad odors...) or even discharged into the sea or rivers. Strict environmental regulations have led to the research on the appropriate treatment of this industrial wastewater.

Wastewater generated in the fermentation brine of table olive processing (FTOP) only represents 20% of the total volume within the global industry. Nevertheless, these effluents contribute to 85% of the global wastewater pollution [1, 4]. Therefore, if FTOPs are treated separately, the overall management of wastewater will be improved.

The FTOP is an acidic effluent (pH around 4) with a very high conductivity (between 70-90 mS·cm<sup>-1</sup>) and high organic matter concentration (between 7 and 20 g·L<sup>-1</sup> in terms of COD), which includes phenolic compounds in concentrations between 700 and 1500 mg·L<sup>-1</sup>. These variations are mainly due to the olive characteristics (variety, maturation degree...) and to the olive processing [5].

Unlike the biological treatment of olive oil mill wastewaters, which have been studied in many investigations [6, 7], there are only a few authors dealing with the FTOP effluents. Some authors have reported results on the treatment of the global wastewater [8], the alkaline wastewaters [9, 10] and the washing water [11]. However, there is a lack of results regarding FTOP biological treatment. There has also been very few works focusing on the removal of the COD and phenolic compounds from other saline wastewaters. Moreover, the studies that have been carried out used simulated wastewater [12, 13]. It is known that the biological treatment of hypersaline wastewater involves great difficulties [14-16], since high salt concentrations can produce plasmolysis and loss of cells activity. In the same way,

hydrophobicity and settlement properties of the activated sludge will decrease. In addition, phenolic compounds can inhibit the biomass because of their bactericidal effect [17, 18]. Thus, the reduction of phenol concentration before the biological treatment could enhance the process performance. For it, adsorption can be tested, since other authors reported successful results of this technique for phenols removal and other organic compounds like dyes from other wastewaters [19-25].

The aim of this work is to perform a direct biological treatment of FTOP wastewater in order to reduce the COD and phenol compounds. The previous biomass acclimation was already reported [26]. In that work COD removal efficiencies were around 88% and phenols were almost completely removed. However, to achieve these results a high hydraulic retention time (HRT = 40 days) was required. In the present work, two strategies were carried out to reduce the HRT to get an economically viable full-scale treatment: extra nutrients were added to the FTOP (nitrogen and phosphorous) and the concentration of COD and phenols in the FTOP were reduced by a previous adsorption with powder activated carbon (PAC).

## **2. MATERIALS AND METHODS.**

### **2.1. Wastewater.**

Fermentation brine was provided by a table olive packaging industry located in Comunidad Valenciana (Spain). Experiments were carried out with four fermentation brine samples, named from FTOP-1 to FTOP-4. The FTOPs were previously filtered in a 60  $\mu\text{m}$  sieve in order to reduce the suspended solids concentration. Additionally, some FTOPs were treated with PAC and these samples were named FTOP-PAC. Before their use, all wastewater samples

were stored at 4°C. Table 1 shows the main characteristics of the different samples of FTOP and FTOP-PAC wastewaters used in the experiments. The parameters were measured in triplicate and the average value has been presented. Filtration was necessary for PAC removal after the adsorption process, which eliminated suspended solids as shown in the table.

**Table 1. FTOPs characteristics.**

<b>Characteristics</b>	<b>FTOP-1</b>	<b>FTOP-2</b>	<b>FTOP-2<sub>PAC</sub></b>	<b>FTOP-3<sub>PAC</sub></b>	<b>FTOP-4<sub>PAC</sub></b>
pH	4.0 ± 0.1	3.7 ± 0.1	3.7 ± 0.1	4.4 ± 0.2	4.5 ± 0.2
Cond (mS·cm <sup>-1</sup> )	94.2 ± 0.2	94.1 ± 0.1	94.1 ± 0.3	78.8 ± 0.3	79.0 ± 0.1
COD (g·L <sup>-1</sup> )	17.70 ± 0.12	21.50 ± 0.08	16.72 ± 0.06	6.71 ± 0.02	5.47 ± 0.03
N <sub>T</sub> (mg·L <sup>-1</sup> )	365 ± 3	352 ± 2	320 ± 8	205 ± 12	247 ± 7
P <sub>T</sub> (mg·L <sup>-1</sup> )	75 ± 1	76 ± 2	65 ± 2	35 ± 5	23 ± 2
Cl <sup>-</sup> (g·L <sup>-1</sup> )	50.01 ± 0.25	44.93 ± 0.32	44.93 ± 0.12	38.48 ± 0.09	40.17 ± 0.11
T.Ph (mg TY·L <sup>-1</sup> )	1109 ± 23	1550 ± 34	400 ± 12	200 ± 13	200 ± 4
SS (mg·L <sup>-1</sup> )	936 ± 15	1237 ± 43	408 ± 21	250 ± 9	407 ± 11
VSS (mg·L <sup>-1</sup> )	466 ± 8	511 ± 22	182 ± 12	117 ± 11	203 ± 9

It can be observed that the FTOPs are acidic effluents. The pH in these five samples was 4.2 ± 0.4. FTOP-1, FTOP-2 and FTOP-2<sub>PAC</sub> had very similar conductivities, around 94 mS·cm<sup>-1</sup>, meanwhile this parameter decreased in FTOP-3<sub>PAC</sub> and FTOP-4<sub>PAC</sub> to 79 mS·cm<sup>-1</sup> (lower concentration of NaCl into the brine preparation). FTOP-2<sub>PAC</sub> was treated with the PAC to obtain a total phenols concentration of 400 mg·L<sup>-1</sup>. Similarly, FTOP-3<sub>PAC</sub> and FTOP-4<sub>PAC</sub> were treated with PAC to reduce the total phenols concentration to 200 mg·L<sup>-1</sup>. The adsorption process also reduced the COD considerably. Thus, COD values of 6710 and 5465 mg·L<sup>-1</sup> were reached. On the other hand, only FTOP-2 required additional nutrients to achieve the relationship COD/N/P of 250/5/1. The mass of urea and KH<sub>2</sub>PO<sub>4</sub> required to meet the stoichiometric ratio of 250/5/1 and 10% in excess of this values was calculated.

The HPLC analysis of the FTOPs showed that the main phenolic compounds in all of the samples were hydroxytyrosol and tyrosol. These results agree with those reported by other authors [27, 28]. Hydroxytyrosol concentration is explained by the acid and enzymatic hydrolysis of oleuropein. Tyrosol may be produced from the hydrolysis of ligstroside. The presence of other phenolic compounds such as caffeic, gallic, p-hydroxyphenylacetic, vanillic..., depend on the cultivar and olive maturation stage [29].

## 2.2. Analysis.

The FTOP samples were characterized by the following analysis: pH, conductivity, soluble COD (filtered to 0.45  $\mu\text{m}$ ), total phenols (T.Ph), phenolic profile (analysis of simple phenolic compounds with HPLC), chloride ( $\text{Cl}^-$ ), total nitrogen ( $\text{N}_\text{T}$ ), total phosphorus ( $\text{P}_\text{T}$ ), turbidity, suspended solids (SS) and volatile suspended solids (VSS).

The parameters measured in the effluents from the SBRs were: pH, conductivity, soluble COD (filtered to 0.45  $\mu\text{m}$ ), turbidity, T.Ph and phenolic profile. In the SBRs mixed liquor suspended solids (MLSS) and volatile suspended solids (MLVSS) were measured and the ratio MLVSS/MLSS was calculated.

The percentages of COD and T.Ph removed during the biologic treatment were calculated from experimental measurements and according to the following equations:

$$\text{COD removal (\%)} = \frac{\text{COD}_0 - \text{COD}_{\text{effluent}}}{\text{COD}_0} \cdot 100 \quad (\text{Eq.1})$$

$$\text{T. Ph removal (\%)} = \frac{\text{T. Ph}_0 - \text{T. Ph}_{\text{effluent}}}{\text{T. Ph}_0} \cdot 100 \quad (\text{Eq.2})$$

where  $COD_0$  and  $T.Ph_0$  were the concentration values ( $mg \cdot L^{-1}$ ) for these parameters in FTOP and  $COD_{effluent}$  and  $T.Ph_{effluent}$  were the concentration ( $mg \cdot L^{-1}$ ) for these parameters in the effluent.

Also, food-to-microorganism ratio (F/M) was calculated as an operating parameter of the SBRs (Eq. 1) [30]:

$$F/M = \frac{COD_0 \cdot Q}{V_R \cdot MLVSS} \quad (Eq.3)$$

where  $Q$  is the daily wastewater volume fed to SBR ( $L \cdot day^{-1}$ ),  $V_R$  was the volume reaction (L) and MLVSS was the mixed liquor volatile suspended solids ( $mg \cdot L^{-1}$ ).

The pH and conductivity measurements were carried out with pH-Meter GLP 21+ and EC-Meter GLP 31+ (CRISON), respectively. Turbidity was determined with a Turbidimeter D-112 from DINKO INSTRUMENTS. The SS and VSS, in the FTOP and mixed liquor, were measured according to APHA, 2005 [31]. Total nitrogen, total phosphorus, chloride and soluble COD were analyzed using kits and a Spectrophotometer DR600 (HACH LANGE) after the appropriate dilutions to avoid interferences.

### ***2.2.1. Phenolic compounds.***

The FTOPs and SBRs effluents were previously treated in order to extract phenols according to El-Abbassi et al. (2011) [32]. The extracts were dried in a rotary evaporator (Rotavapor R-114 from BÜCHI) at 40°C and the residue was dissolved in methanol. T.Ph were measured spectrophotometrically according to the Folin-Ciocalteu method [33]. Sodium carbonate (20% w/v) from PANREAC and Folin & Ciocalteu's reagent from Sigma Aldrich were used for it. The phenolic profile was measured by liquid chromatography. The analysis was carried

out with a Jasco HPLC system equipped with a MD-2018 Photodiode Array detector. The separation was carried out using a Phenomenex Kinetex 5u Biphenyl 100A column (4.6 x 250 mm, 5  $\mu\text{m}$ ). The optimal chromatographic conditions were established: flow rate of 1.5  $\text{mL}\cdot\text{min}^{-1}$ ; injection volume of 10  $\mu\text{L}$ ; solvent system were phase A (1 % acetic acid in water) and phase B (1 % acetic acid in methanol); gradient conditions: the elution started at 5% of B and remained constant for 1 min, it linearly increased up to 80% of B in 25 min and returned to 5% of B in 2 min. The detailed procedure for samples preparations and external calibration by different analytes can be found in a previous work [26].

### ***2.2.2. Fluorescence in situ hybridization (FISH) and microscopic observation to $\gamma$ -Proteobacteria quantification.***

According to the results obtained in the process start-up and to the fact that the predominant class was  $\gamma$ -*Proteobacteria*, it was decided to consider the percentage of this bacterial subclass as biological indicator, in order to relate it with the operational changes, the COD and the phenols removal efficiencies.  $\gamma$ -*Proteobacteria* percentage in the biomass was measured by FISH technique. For it, the oligonucleotide probe GAM42a with the sequence GCCTTCCCACATCGTTT was used. Microscopic observation was carried out using an epifluorescence microscope Olympus BX50, equipped with a CCD camera (Olympus DP12). The detailed procedure can be found in Ferrer-Polonio et al. (2015).

### **2.3. Biological reactors.**

The experiments were carried out in four sequencing batch reactors: SBR-1, SBR-2, SBR-3 and SBR-4. SBRs were operated in 24 hours cycles. Table 2 summarizes the HRT,  $V_R$  and

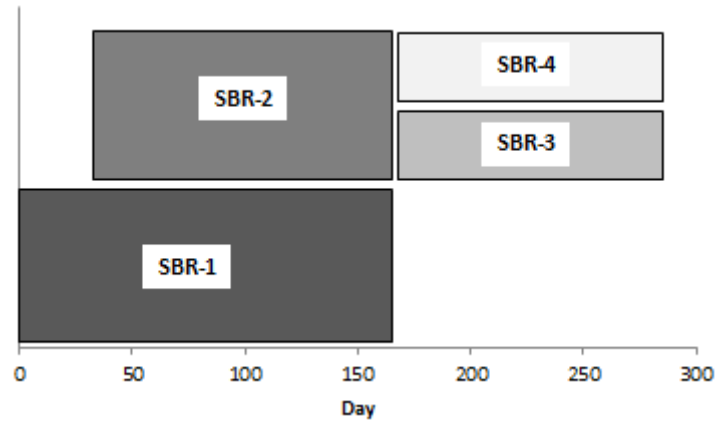


daily volume fed to the four reactors. In this table, the duration of each phase of the cycle is also included. In the oxic reaction phase, the oxygen concentration ranged between 1.5 and 2.5 mg·L<sup>-1</sup>.

**Table 2. SBRs operation characteristics.**

<b>Operation characteristics</b>	<b>SBR-1</b>	<b>SBR-2</b>	<b>SBR-3</b>	<b>SBR-4</b>
Reaction volume (L)	6	6	3	3
Feed volume (mL·L <sup>-1</sup> ·d <sup>-1</sup> )	25	33	33	67
HRT (days)	40	30	30	15
<b>Cycle characteristics</b>				
<b>Phase</b>	<b>Time</b>			
Filling	2 min			
Reaction (anaerobic/aerobic)	1 h / 21 h			
Sedimentation	1.5 h			
Draw	2 min			
Idle	26 min			

SBR-1 start-up was carried out in a previous work, where the mechanical components and scheme of the SBR plant (identical for all SBRs) can be found [26]. This reactor was operated for 166 days. The start-up strategy for SBR-2 consisted of filling it gradually with withdrawn sludge from SBR-1 and FTOP until achieving 6 L of  $V_R$ . The volume of the added FTOP was calculated daily for maintaining the same organic load ratio as in SBR-1 until  $V_R$  was 6 L. At the start-up, neither sludge nor treated water was withdrawn from the SBR. After 33 days, SBR-2 reached the targeted volume. SBR-2 was operated during 133 days. After that, SBR-2 was split into two new reactors, SBR-3 and SBR-4 (116 operating days), to achieve more quickly two reactors with mixed liquors of identical characteristics. The reaction volume in both reactors was 3L, adapting the feed/draw volumes to the new  $V_R$ . Figure 1 illustrates the time distribution of the SBRs operation in the experiments.



**Figure 1. SBRs experimental time**

The sludge retention time (SRT) values for the four SBRs were different due to the variation of the F/M ratio and HRT parameters. Thus, the SRT was around 90 days for SBR-1, 75 days for SBR-2 and SBR-3 and 43 for SBR-4. The high values for the SRT are caused by the low sludge production. This can be explained by the high salinity in mixed liquor that leads to high energy consumption for cell maintenance instead of cell growing.

## **2.4. Hydraulic retention time reduction**

### ***2.4.1. First strategy: The effect of nutrients addition in FTOP on process efficiency.***

The requirement of nutrients (nitrogen and phosphorous) in wastewater was evaluated comparing with the relationship: COD/N/P of 250/5/1 [34]. According to COD,  $N_T$  and  $P_T$  calculated in the FTOPs and FTOP-<sub>PAC</sub>, the external nutrients to be added were calculated, in order to obtain the required amounts. The chemicals used to provide nitrogen and phosphorus

were  $\text{CH}_4\text{N}_2\text{O}$  (urea) and  $\text{KH}_2\text{PO}_4$ , respectively. Besides, 10% of nutrients excess were added in SBR-1, during the last 48 days, in order to compare the COD and total phenols removal efficiencies under stoichiometric conditions (250/5/1) and under excess of N and P.

**2.4.2. Second strategy: The effect of adsorption treatment with powder activated carbon (PAC) to reduce COD and phenols concentrations in FTOP.**

Adsorption is an appropriate process to remove the COD and phenolic compounds from wastewaters [35]. Another advantage of PAC pre-treatment is that it achieves a more stable composition of FTOP, since the concentration of COD and phenolic compounds are variable. It depends on several factors such as cultivar [5, 36] and maturation degree [37]. Also, these parameters are closely linked to olive processing, mainly to the debittering and fermentation processes [36, 38].

In a previous work, the COD and phenols adsorption in the FTOPs with four different PACs were reported [39]. Table 3 shows the main characteristics of these carbons and the T.Ph removal percentages achieved after 4 hours of adsorption time with  $4 \text{ g}\cdot\text{L}^{-1}$  of each PAC.

**Table 3. Total phenols removal efficiencies in the adsorption test with  $4 \text{ g}\cdot\text{L}^{-1}$**

<b>Activate carbon</b>	<b>Characteristics</b>		<b>T.Ph removal</b>
<b>Clarimex 061 CAE</b>	Pinewood activated with phosphoric acid	macroporous	49.8 %
<b>Clarimex 061 GAE</b>	Modified from CAE (pH 4.5-6.5)		33.8 %
<b>BM8</b>	Bituminous activated with water vapour	micro-	57.5 %
<b>CCP80</b>	Coconut shell activated with water vapour	mesoporous	66.9 %

The FTOP used in these tests had a T.Ph. concentration of  $1391 \text{ mg}\cdot\text{L}^{-1}$ . The selected PAC (BM8 from PUROLITE) has been used for this work. Pre-treatment was performed in a jar-test equipment. PAC was mixed with 0.5 L of FTOP under the following operational conditions; stirring rate of 150 rpm during 60 minutes at room temperature. After the adsorption process, PAC was separated from the FTOP by filtration (cellulose filter of  $40 \mu\text{m}$  sieve).

The experiment with FTOP-PAC was carried out in SBR-2, SBR-3 and SBR-4. For SBR-2 the PAC concentration was calculated to achieve  $400 \text{ mg}\cdot\text{L}^{-1}$  of total phenols concentration in FTOP2-PAC. To keep a similar F/M ratio in SBR-1 and SBR-2, the HRT in SBR-2 was decreased to 30 days. SBR-1 and SBR-2 performances were compared during a 132 days period. SBR-3 and SBR-4 were fed with FTOP-3<sub>PAC</sub> and FTOP-4<sub>PAC</sub>, which were treated with higher PAC amounts to achieve  $200 \text{ mg}\cdot\text{L}^{-1}$  of total phenols concentration. In these reactors different HRT and organic load values were set to evaluate the effect of these parameters on the reactors performance. Thus, the HRT was 30 days in the SBR-3 and 15 days in the SBR-4. Therefore, these SBRs were operated with different F/M ratio.

## **2.5. Statistical analysis.**

According to the experimental procedure described, some parameters the reactors performance have been studied. These parameters were the relationship COD/N/P, the HRT and the F/M ratio. To study the statistical significance of these factors for the COD removal yield, an ANOVA simple analysis (confidence level of 95 %) was carried out with STATGRAPHICS Centurion XVI.

The variance analysis of the HRT and F/M ratio were carried out with 74 experimental values registered during the experimental time in the four reactors. Nevertheless, only experimental values of SBR-1 (22 data) were evaluated to achieve the variance analysis for the relationship COD/N/P in the FTOPs, because in the other reactors the value of this parameter was always the stoichiometric one.

As stated above, the dependent variable was the removal percentage of the effluent COD. Three levels were evaluated for the HRT: 40, 30 and 15 days. Due to the F/M ratio data scattering, this parameter was split into three ranges of values: Range A) F/M ratio > 0.185, Range B)  $0.184 > \text{F/M ratio} > 0.089$  and Range C) F/M ratio < 0.088. The relationship COD/N/P was classified into three categories: “low”, “stoichiometric” or “excess” according to nitrogen and phosphorus concentration and COD in the FTOP.

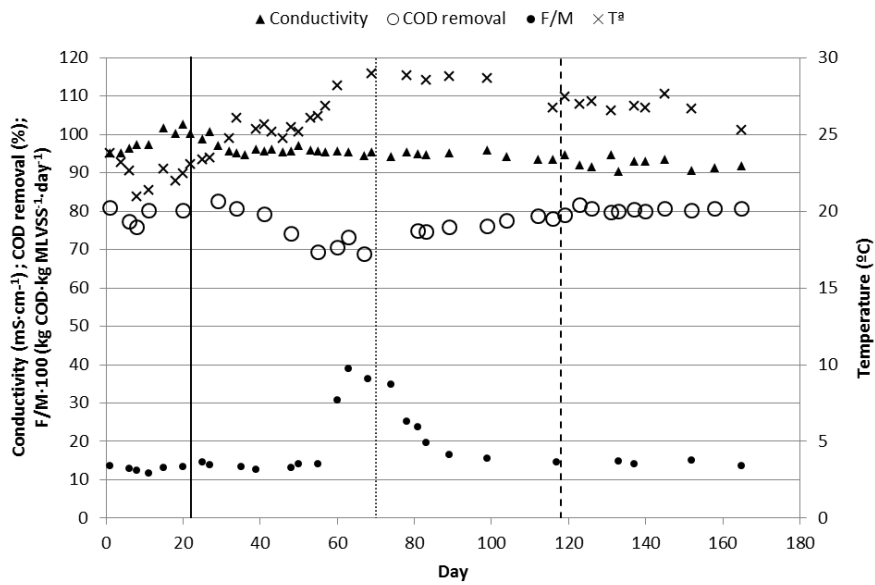
### **3. RESULTS AND DISCUSSION.**

#### **3.1. The effect of nutrients addition in FTOP on the process efficiency.**

The effect of adding nutrients on the FTOP process efficiency was evaluated in SBR-1. The experimental period was 166 days and during this time the HRT was 40 days. This reactor was fed with FTOP-1 and FTOP-2. SBR-1 was operated at room temperature and effluent pH remained at values of  $8.2 \pm 0.1$ . This pH could seem very high if it is considered that the pH of the FTOPs was around 4. This phenomenon can be explained as the FTOPs contain organic acids (mainly lactic, malic, formic and acetic acid) [22], which throughout the biological treatment are oxidized to CO<sub>2</sub> and H<sub>2</sub>O. Thus, the pH values of the ML and the

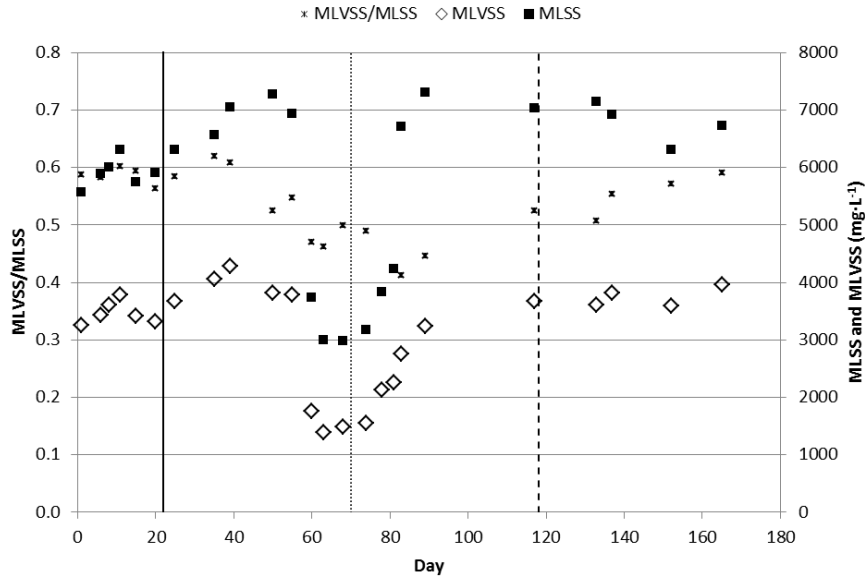
effluent increase. The pH in ML also remained constant while feeding due to the small FTOP volume added into reactor (low volume exchange ratio).

Figure 2 represents the conductivity, temperature, COD removal efficiency and the F/M ratio in SBR-1. In figure 3 the evolution of the MLSS, MLVSS and the ratio MLVSS/MLSS can be seen. The vertical lines in both figures indicates the FTOP change: FTOP-1 was fed during the first 22 days, FTOP-2 without nutrient addition from the 23<sup>rd</sup> to 69<sup>th</sup>, FTOP-2 with nutrient addition until reaching the ratio COD/N/P of 250/5/1, from the 70<sup>th</sup> to 117<sup>th</sup> and the last 48 days FTOP-2 after adding 10% on nutrients excess was fed.



**Figure 2. SBR-1: Conductivity, temperature, COD removal efficiency and F/M ratio.**

**Vertical lines: [—] FTOP-2, [.....] FTOP-2+nutrients ratio 250/5/1, [- - -] FTOP-2+10% excess nutrients**



**Figure 3. SBR-1: MLSS, MLVSS and MLVSS/MLSS ratio.**

**Vertical lines: [—] FTOP-2, [.....] FTOP-2+nutrients ratio 250/5/1, [- - -] FTOP-2+10% excess nutrients**

Temperature was varying slightly throughout the experimental time as the experiment was carried out at room temperature. Regarding effluent conductivity, it can be observed that this parameter remained around  $95 \text{ mS}\cdot\text{cm}^{-1}$  from FTOP-2 feeding.

While FTOP-1 was fed to SBR-1, COD removal efficiency remained around 80%. The sudden increase in the feed COD, when FTOP-2 was fed, implied a fast increase of the MLSS above  $4000 \text{ mg}\cdot\text{L}^{-1}$  in the first days. In spite of this, COD removal efficiency went on to decrease to a value of 68.8% (between 41<sup>st</sup> to 69<sup>th</sup> days). This fast decline in SBR-1 performance was mainly due to the fact that nitrogen and phosphorous concentrations in FTOP-2 were lower than what is required for the stoichiometric nutrient ratio and thus the microbial activity was limited [34]. The result was that the MLSS and MLVSS concentrations decreased markedly from the 41<sup>st</sup> day until nutrients were added. Through this period the MLSS decreased from  $7050 \text{ mg}\cdot\text{L}^{-1}$  and the MLVSS from  $4280 \text{ mg}\cdot\text{L}^{-1}$ . It was due to the cell death caused by the lack of nutrients. As expected, the F/M

ratio was increased from an average value of  $0.133 \pm 0.008$  to  $0.362 \text{ kg COD} \cdot \text{kg MLVSS}^{-1} \cdot \text{d}^{-1}$  in the 68<sup>th</sup> day, because of MLVSS decrease.

From 70<sup>th</sup> day, nutrients were added in FTOP-2 in a ratio of 250/5/1 (COD/N/P). It can be observed in figure 2, that from that day on, the COD removal efficiency, MLSS and MLVSS increased to reach values of 80%,  $7028 \text{ mg} \cdot \text{L}^{-1}$  and  $3670 \text{ mg} \cdot \text{L}^{-1}$  (117<sup>th</sup> day), respectively.

From the 118<sup>th</sup> day a 10% nutrients excess was added in FTOP-2. During 48 days COD removal was maintained at  $80.3 \pm 0.7\%$ , the F/M ratio was  $0.144 \pm 0.007 \text{ kg COD} \cdot \text{kg MLVSS}^{-1} \cdot \text{d}^{-1}$ , MLSS ranged between 6310 and 7140  $\text{mg} \cdot \text{L}^{-1}$ , and MLVSS ranged between 3600 and 2960  $\text{mg} \cdot \text{L}^{-1}$ . These values show that the excess of nutrients did not improve the SBR-1 performance. In contrast, other authors reported that for the biological treatment of hypersaline wastewater more nitrogen and phosphorous should be added. Moussavi et al. [1] recommended a ratio of 150/5/1 to treat synthetic saline wastewater containing phenol using an aerobic granular SBR. Dinçer and Kargı [40] suggested 100/10/1 for synthetic saline wastewater in a rotating biological disc system. Nevertheless, for FTOPs the additional expense in nutrients would not be justified.

The relationship MLVSS/MLSS in SBR-1 in the first 41 days was  $0.59 \pm 0.02$ . From the 42<sup>nd</sup> to 83<sup>rd</sup> day the ratio of MLVSS/MLSS decreased to 0.41, because the low nutrients concentration in FTOP-2 increased the flocs mineralization. After 47 days of adding nutrients, the ratio of MLVSS/MLSS increased to 0.52 (117<sup>th</sup> day), which was maintained in  $0.51 \pm 0.01$ . This low MLVSS/MLSS ratio is explained by the high amount of MLSS, between 6500 and 7500  $\text{mg} \cdot \text{L}^{-1}$ , which enhances endogenous respiration. As expected, it can be observed that the sludge settleability was maintained during the experimental period, despite the high MLSS, due to the formation of aerobic granules. Although no data on the size of the granules was reported, their formation was evident during the reactors start-up by



direct observation of the sedimentation phase of the SBR cycles. Aerobic granulation in SBRs has been already reported by many researchers [41], including saline wastewater [42, 43]. These microbial structures are denser and consequently settle faster. However, their formation mechanism is a complex topic and no relationship has been found between granule growth and bacterial species, morphology and adaptation mechanism.

On the other hand, the total phenols removal was also affected by nutrients concentration. From FTOP-2 addition without nutrients enrichment, the percentage of total removed phenols decreased from 97.7% to 84.9% in the 53<sup>th</sup> day. Once nitrogen and phosphorous were added, phenols performance increased to 98.5% on the 147<sup>th</sup> day, which was maintained until the end of the experiment. These results agree with those reported [40]. In that work, authors increased the phenols removal performance from 60% to 78%, after nutrients addition ( $\text{KH}_2\text{PO}_4$  and  $\text{NH}_4\text{NO}_3$ ) in olive mill wastewater, which was also treated in a SBR.

As a conclusion, it can be stated that the limiting factor for increasing the COD and total phenols removal efficiency is not the nutrient addition, once stoichiometric dosing is performed. For this reason, a pretreatment with activated carbon was proposed in order to reduce the non-biodegradable COD. The results are described in the following section.

### **3.2. The effect of adsorption treatment with powder activated carbon (PAC) to reduce the COD and phenols in the FTOP. Influence of the F/M ratio on reactor performance.**

#### ***3.2.1. Reactors performance with and without PAC pre-treatment.***

The experimental period of operation of SBR-2 was 132 days. This reactor was fed with FTOP-2 in the first 45<sup>th</sup> days (HTR = 40 days). From that day on, it was fed with FTOP-2<sub>PAC</sub>.

To keep the daily COD load, the HRT was reduced to 30 days. After the adsorption pre-treatment the ratio COD/N/P was found to be the stoichiometric one. The SBR-2 was also operated at room temperature and as in SBR-1, pH effluent remained at  $8.6 \pm 0.3$ .

Figure 4 shows the conductivity, temperature, COD removal efficiency and the F/M ratio in SBR-2. In figure 5 the evolution of MLSS, MLVSS and the ratio of MLVSS/MLSS in SBR-2 can be observed. The vertical lines show in both figures the change of FTOP-2 (without PAC pre-treatment) and FTOP-2<sub>PAC</sub>.

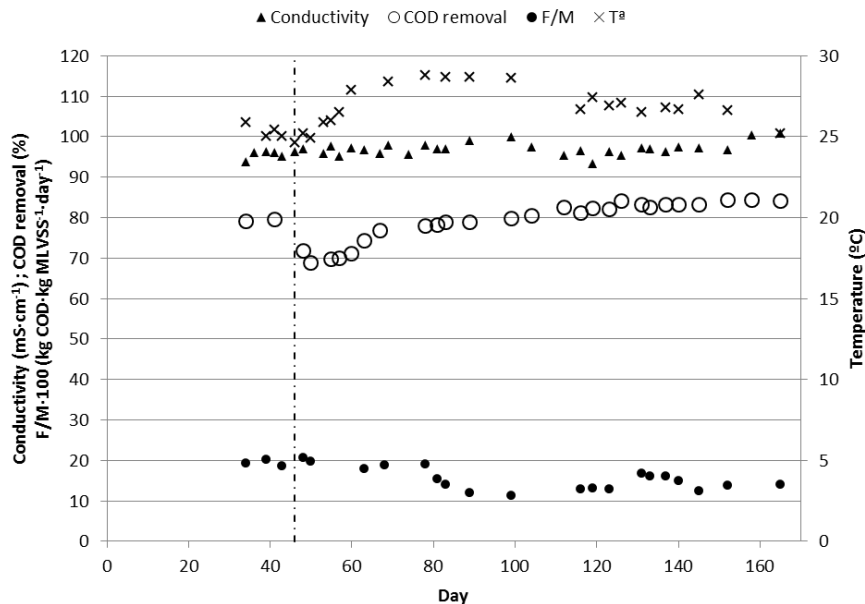
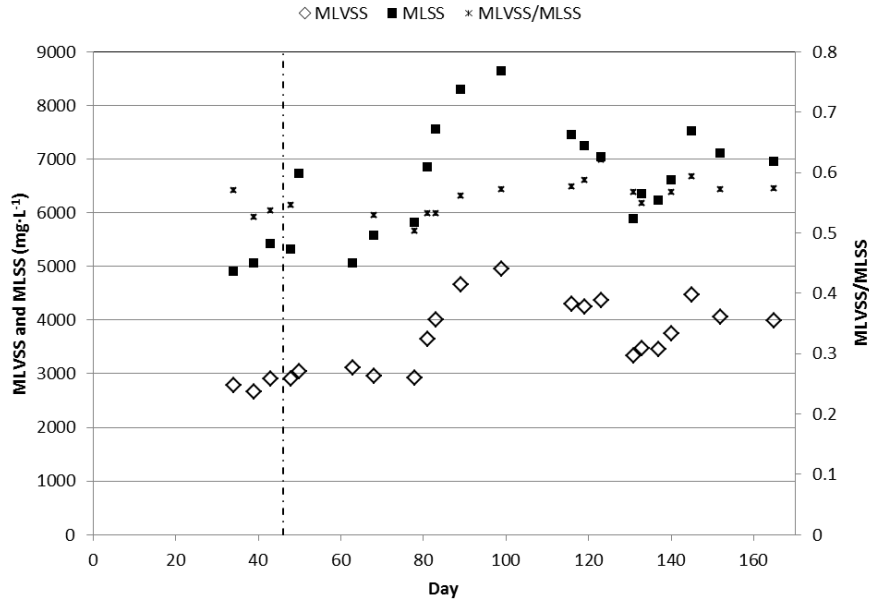


Figure 4. SBR-2: Conductivity, temperature, COD removal efficiency and F/M ratio.

Vertical lines: [- · -] FTOP-2<sub>PAC</sub>.



**Figure 5. SBR-2: MLSS, MLVSS and F/M ratio.**

**Vertical lines: [- · -] FTOP-2<sub>PAC</sub>.**

As both SBR-2 and SBR-1 were operated at a room temperature, the temperature profile in SBR-2 was identical to SBR-1. The effluent conductivity, which remained at  $96.7 \pm 1.6$   $\text{mS}\cdot\text{cm}^{-1}$ , was similar to that obtained in SBR-1, since the FTOP feeding both reactors was the same.

While FTOP-2 was fed in the first days, the COD removal in SBR-2 was similar to that achieved in SBR-1 (around 80%). Nevertheless, when FTOP-2<sub>PAC</sub> was added, it can be observed that the COD removal decreased. This phenomenon was not due to a decrease in the reactor performance, but to the decrease of the influent COD. If Eq.1 is applied, the COD in FTOP-2<sub>PAC</sub> was lower than FTOP-2, but the effluent COD in the first days was still very similar to that achieved when FTOP-2 had been fed. Therefore, it was observed that the COD removal efficiency went down to 68.8%. Afterwards, the COD removal yield gradually increased reaching values around 80% after a few days.

In the first 78 days, the F/M ratio in SBR-2 was  $0.193 \pm 0.009 \text{ kg COD} \cdot \text{kg MLVSS}^{-1} \cdot \text{d}^{-1}$ , which was higher than in SBR-1 (around 0.133 in the first 55 days). This was due to the fact that the MLSS and MLVSS concentrations in SBR-2 were smaller than those of SBR-1. To increase them up to similar values to those registered in SBR-1, no sludge was withdrawn until the 99<sup>th</sup> day. From the 89<sup>th</sup> to 166<sup>th</sup> day practically equal F/M ratios were maintained in both reactors. If the performances in both reactors from the 89<sup>th</sup> day (when operational parameters became similar) are compared, it can be observed that the COD removal efficiency in SBR-2 was higher than in SBR-1;  $83.5 \pm 0.6\%$  in SBR-2 and  $79.3 \pm 1.7\%$  in SBR-1, despite the HRT was reduced 10 days. This can be explained as the ratio of MLVSS/MLSS in SBR-2 ( $0.56 \pm 0.03$ ) was higher than in the SBR-1 ( $0.51 \pm 0.02$ ) and this means that more bacterial cells could contribute to the organic matter removal.

Total phenols removal in SBR-2 was above 97% during all the experimental period; thereby no effect of the adsorption pre-treatment and diminution of the HRT in SBR on phenols removal efficiency were observed.

### ***3.2.2. The effect of HRT reduction, and influence of the F/M ratio on the process performance.***

The experimental period with SBR-3 and SBR-4 was 116 days, during which FTOP-3<sub>PAC</sub> and FTOP-4<sub>PAC</sub> were fed (no nutrients were added). The HRT in SBR-3 was maintained in 30 days and it was reduced to 15 days in SBR-4.

During experimental time, the pH remained around  $8.7 \pm 0.1$  and  $8.6 \pm 0.1$  in SBR-3 and SBR-4, respectively. Conductivity was around  $88 \text{ mS} \cdot \text{cm}^{-1}$  in both reactors and the temperature ranged between 18 and 24°C.

In figure 6 and figure 7, the evolution of COD removal, F/M, MLSS and MLVSS of SBR-3 and SBR-4, respectively, can be seen. The vertical line shows the change from FTOP-3<sub>PAC</sub> to FTOP-4<sub>PAC</sub>.

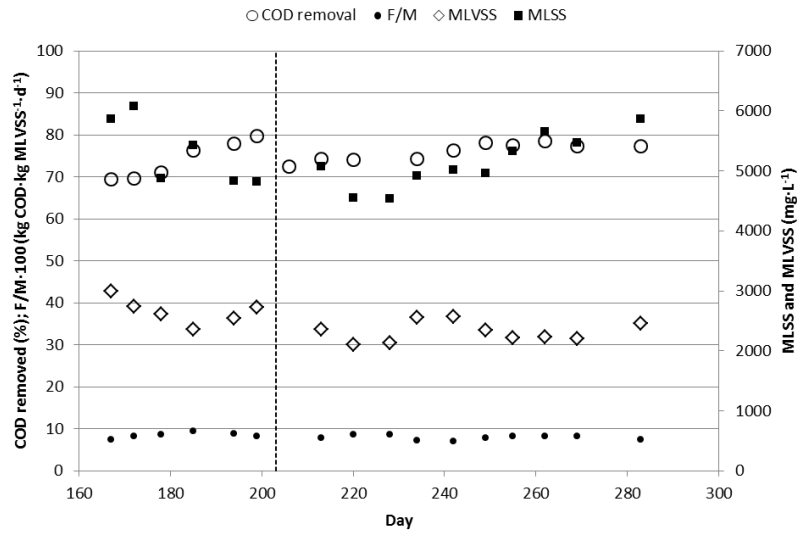


Figure 6. COD removal, F/M ratio, MLSS and MLVSS in SBR-3.

Vertical lines: [- - -] FTOP-4<sub>PAC</sub>.

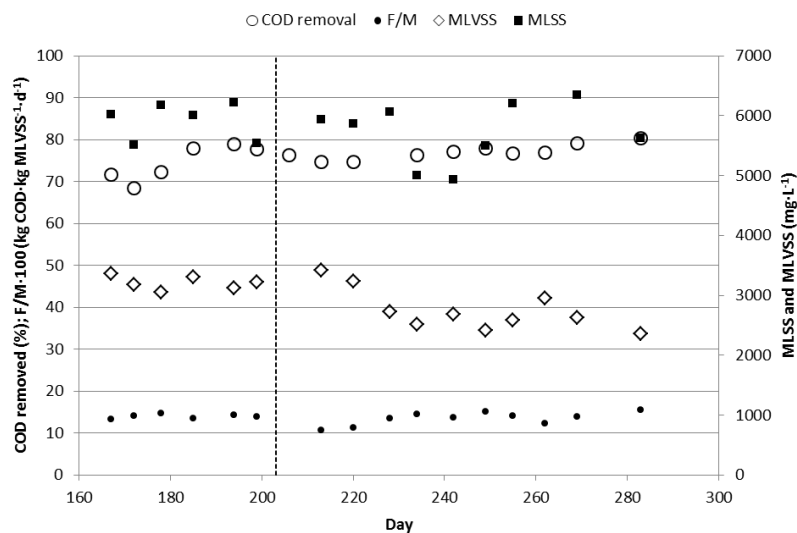


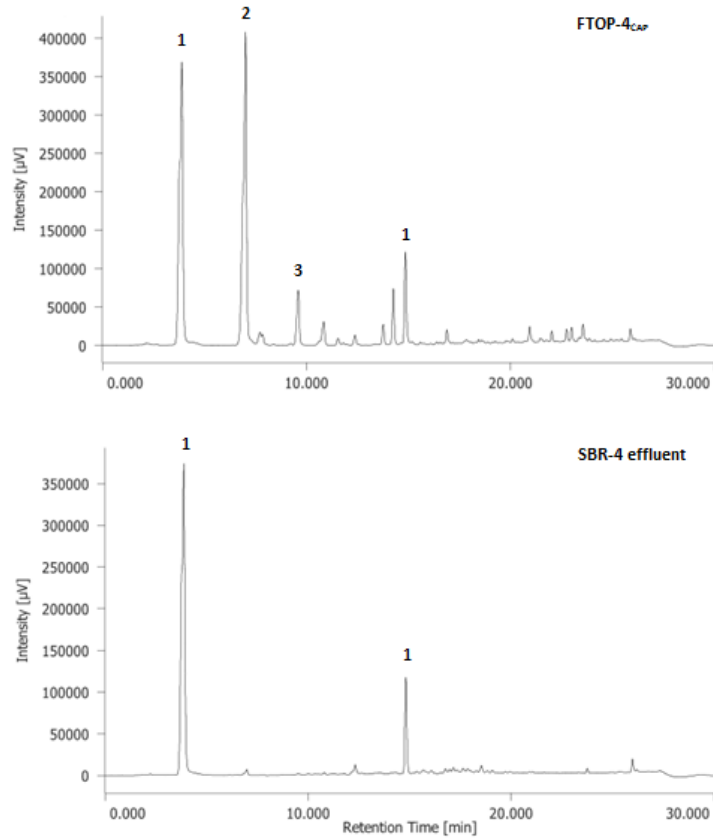
Figure 7. COD removal, F/M ratio, MLSS and MLVSS in SBR-4.

Vertical lines: [- - -] FTOP-4<sub>PAC</sub>.

In these figures, it can be seen the initial decrease in the COD removal efficiency, because the effluent COD was not yet adapted to the new influent COD. For the remaining experimental time, the COD removal yield was similar in both reactors despite the HRT was the half in SBR-4. In the last 50 days, the COD removal percentage remained in  $77.1 \pm 1.4\%$  and  $77.8 \pm 1.5\%$  in SBR-3 and SBR-4, respectively. The MLSS and MLVSS were higher for SBR-4 than SBR-3 during the 116 experimental days, since at the same time sludge withdrawal was carried out in both reactors. This was due to the differences between the F/M ratios.

The F/M was maintained in  $0.081 \pm 0.007 \text{ kg COD}\cdot\text{kg MLVSS}^{-1}\cdot\text{d}^{-1}$  in SBR-3 and  $0.135 \pm 0.012 \text{ kg COD}\cdot\text{kg MLVSS}^{-1}\cdot\text{d}^{-1}$  in SBR-4. The organic removal efficiency, sludge properties and microbial composition are influenced by the F/M ratio. Higher F/M values imply that the metabolic activity and microbial growth are improved [44]. Conversely, if F/M ratio is too low the cell growth is limited [45]. Specialized literature reports values between 0.05 and  $0.30 \text{ kg COD}\cdot\text{kg MLVSS}^{-1}\cdot\text{d}^{-1}$  for sequential reactors [45] and around  $0.07\text{-}0.08 \text{ kg COD}\cdot\text{kg MLVSS}^{-1}\cdot\text{d}^{-1}$  to treat wastewater containing  $400 \text{ mg}\cdot\text{L}^{-1}$  of total phenols,  $1400 \text{ mg}\cdot\text{L}^{-1}$  of COD and  $20 \text{ g}\cdot\text{L}^{-1}$  of chlorides [46].

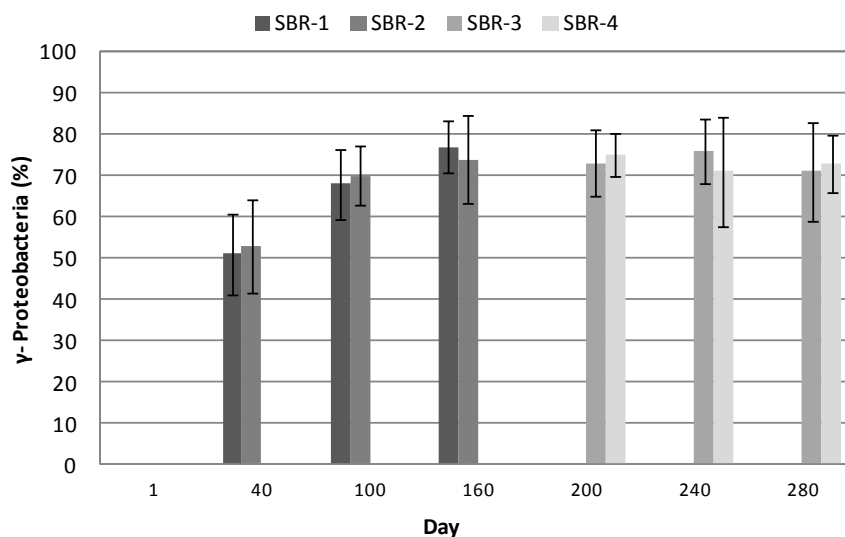
Regarding total phenols removal, the concentration in the effluent was below  $6 \text{ mg TY}\cdot\text{L}^{-1}$  in both reactors, which implies around 97% of removal efficiencies. The phenolic profiles for FTOP-4<sub>PAC</sub> and SBR-4 effluent, at the end of experimental days, are shown in figure 8. In this figure it can be observed that hydroxytyrosol and tyrosol were the main phenolic compounds in FTOP-4<sub>PAC</sub> (as in the others FTOPs). Hidroquinone was used as an internal standard. The phenolic compounds were degraded through the biological treatment as observed in the HPLC analysis of the effluent from SBR-4, which confirms the results of total phenols measurement analysed by Folin-Ciocalteu method.



**Figure 8. Phenolic profile to FTOP-4<sub>PAC</sub> and SBR-4 effluent. Peak identification:**  
**(1) Hidroquinone (internal standard); (2) Hydroxytyrosol; (3) Tyrosol.**

### 3.3. Microbial community analysis.

As commented in methodology section, *γ-Proteobacteria* was quantified in the SBRs. In figure 9, the percentage and uncertainty of this bacterial class, isolated from the total population, is represented. It can be seen that *γ-Proteobacteria* was the predominant class in the microbial community of the four SBRs during all the experiments.



**Figure 9. Quantification of  $\gamma$ -Proteobacteria in the SBRs.**

In the period from 40<sup>th</sup> to 160<sup>th</sup> day,  $\gamma$ -Proteobacteria percentage increased in SBR-1 and SBR-2 from 51% and 53% to 77% and 74%, respectively. In SBR-3 and SBR-4 the percentages remained in  $73.3 \pm 2.5\%$  and  $73.0 \pm 2.0\%$ . Thus, it can be concluded that independently of the nutrients added and on the tested HRT, the percentage of  $\gamma$ -Proteobacteria in the microbial community remained unaltered. The main role of  $\gamma$ -Proteobacteria in the hypersaline environments has also been reported in other research works [47, 48]. Dosta et al. [49] treated hypersaline wastewaters with phenolic content by MBR system, also reporting that  $\gamma$ -Proteobacteria was the predominant bacterial class in the microbial community. Other authors as Wang et al. [12] studied the SBR performance in the removal of phenol under conditions of high salinity ( $80 \text{ g}\cdot\text{L}^{-1}$ ). These researchers also concluded that *Proteobacteria* strains were able to tolerate high concentration of phenolic compounds ( $1200 \text{ mg}\cdot\text{L}^{-1}$ ).

### 3.4. Statistical analysis.



The p-values obtained for the variances analysis of HRT, F/M ratio and relationship COD/N/P, when COD removal percentage was the dependent parameter, were 0.3681, 0.0004 and 0.0678, respectively. These results showed that the F/M ratio was the only statistically significant factor since the p-value was lower than 0.05. It is important to note that the relationship COD/N/P in the FTOP took a p-value near to this set limit.

#### **4. CONCLUSIONS.**

In this work, it can be concluded that the biological treatment of FTOP is technically feasible since the SBRs have treated the FTOP during 10 months successfully.

The main conclusions are the following:

- The appropriate relationship COD/N/P in the FTOP is 250/5/1. An excess of organic matter in relation to nutrients, implied a COD removal efficiency diminution. An excess of nutrients over 250/5/1 ratio did not improve the COD and total phenols removal efficiency.
- The PAC adsorption was an effective pre-treatment to reduce the COD and phenolic compounds in the FTOPs, which can lead to reduce the HRT of the reactors. Another advantage of PAC treatment is the achievement of more homogeneous SBR influents in terms of composition. The PAC concentration can be varied according to the initial COD and phenols values and the required final concentrations.
- The experiments proved that the F/M ratio have to be around  $0.14 \text{ kg COD} \cdot \text{kg MLVSS}^{-1} \cdot \text{d}^{-1}$  for the FTOPs treatment.

- The adsorption pre-treatment let the HRT to reduce from 40 to 15 days maintaining the biological treatment performance. Thus, the COD and total phenols removal percentages were around 78% and 97%, respectively.
- *γ-Proteobacteria* was the main bacterial class, which represented around 74% of the microbial community, in the four SBR studied.

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