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Influence of bisphenol A occurrence in wastewaters on biomass characteristics and activated sludge process performance

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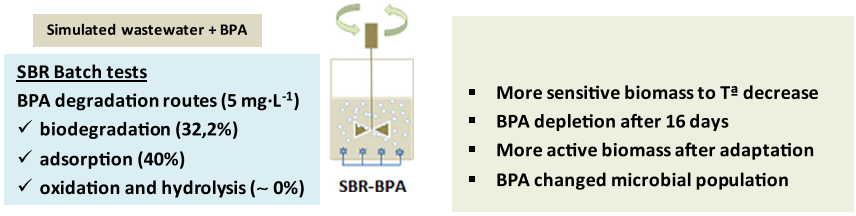
HIGHLIGHTS

- Adsorption is the main BPA mechanism removal for non-adapted activated sludge.
- Once biomass is adapted, a total BPA depletion is achieved.
- Temperature decrease affected at higher extent biomass treating wastewater with BPA.
- Biomass activity is higher in reactor treating wastewater with BPA.

GRAPHICAL ABSTRACT



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ABSTRACT

In this work, the influence of bisphenol A (BPA) on biological wastewater treatment was studied. For it, two sequencing batch reactors (SBRs) were operated for three months. Both SBRs were fed with synthetic wastewater (SW), adding 1 mg·L^{-1} of BPA into the feed of reactor SBR-BPA, while the other one operated without BPA as a control reactor (SBR–B). In addition, batch experiments were performed with adapted and non-adapted activated sludge, simulating the reaction step of SBR-BPA, to determine the pathways for BPA removal. Results of batch experiments showed that adsorption and biodegradation were the only significant BPA removal routes. BPA removal by biodegradation was more efficient when adapted biomass was used in the tests (32.2% and 8.2% with adapted and non-adapted biomass, respectively), while BPA adsorption removal route was similar in both types of activated sludge (around 40%). Regarding the SBRs experiments, after 16 days no BPA concentration was detected in SBR-BPA effluent. In the adaptation process, SBR-BPA biomass was more sensitive to low temperatures resulting in higher effluent turbidity, COD and soluble microbial products concentrations than in SBR–B. However, once temperature increased, adapted biomass from SBR-BPA presented higher activity than SBR-B biomass, showing higher values of sludge production, microbial hydrolytic enzymatic activities and specific dynamic respiration rate. The bacterial community study revealed the increase of abundance of *Proteobacteria* (especially *Thiothrix* species) and *Actinobacteria* (especially *Nocardioides* species) phyla at the expense of *Bacteroidetes* and *Chloroflexi* phyla in SBR-BPA during its operation.

1. Introduction

In the last years, the concern about the increase of the named organic micropollutants compounds has become huge. Plasticizers and plastic additives are included in these groups, being bisphenol A (BPA) one of the most used plastic additives. Regarding this substance, many researchers

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included BPA among the organic micropollutants to be monitored in municipal wastewater treatment plant (WWTP), like Gardner et al. (2013), who reported that BPA was one of the five organic micropollutants with the highest occurrence on wastewaters in United Kingdom (authors studied 16 WWTPs). Once it is clear that BPA is a common organic micropollutant in WWTP, the following question to be answered is about its origin. In this way, Wang et al. (2020) studied the urine contribution as source of BPA in municipal wastewaters. They reported an average intake of 2.5 μg of BPA per person and day, while the nominal exposure levels obtained according to wastewater-based epidemiology was 513.7 μg of BPA per person and day. Thus, BPA concentrations in municipal WWTPs can be higher due to discharges coming from industry and hospitals or especially when landfill leachates are transported to the plants, since BPA in these effluents may reach concentrations higher than 5 $mg \cdot L^{-1}$.

BPA is an intermediate of polycarbonates and epoxy resins productions, and it is present in a great number of plastic goods that are commonly employed in domestic and industrial applications (Margot et al., 2015). Because of wide usage, BPA become one of the most micropollutants detected in the environment (Wang et al., 2019). This compound is an endocrine disrupter with estrogenic activity (Chen et al., 2016; Chouhan et al., 2014), which may cause sperm count reduction, early sexual maturation in females, diabetes and other diseases (Wang et al., 2019). Additionally, it has been proved that BPA affects the gonad morphology of the fish at very low concentrations (1 $\mu g \cdot L^{-1}$) (Kermoysan et al., 2013).

The major source of BPA in surface waters is the effluents of WWTPs. This is due to the fact that this substance is not degraded efficiently through conventional process in the treatment plants (Ratola et al., 2012). In this way, BPA is usually detected in treated wastewater. In addition, operational conditions, influents composition or type of plants drive to different BPA removal efficiencies as reported by several authors. Thus, Lee and Peart (2000) informed that removal efficiencies of BPA in 31 WWTPs from Canada ranged between 37 and 94%, and Margot et al. (2013) reported that some organic micropollutants, including BPA, were degraded by the activated sludge in a percentage less than 30% in absence of nitrification and in more than 60% when nitrification occurred, what had to be related to the sludge retention time. However, Frankowski et al. (2020) described an almost total removal of BPA through activated sludge treatment.

On the other hand, most of works reporting BPA removal efficiencies in WWTP do not specify whether BPA elimination is by degradation or by sorption onto the sludge pathways. In this way, biological treatment with activated sludge can remove the pollution of wastewater through the following strategies (Ferrer-Polonio et al., 2020; Min et al., 2018): biodegradation, adsorption, oxidation, hydrolysis and volatilization . Regarding BPA removal, volatilization pathway can be ignored, due to its low vapour pressure and Henry's constant (5.32×10^{-5} Pa and 10 $\mu Pa \cdot m^3 \cdot mol^{-1}$ at 25 °C) (Groshart et al., 2001; Wang et al., 2019). Considering that a very widespread practice for the sewage sludge disposal is its application to the agriculture soil because it enhances the soil fertility, a previous treatment of sludge is necessary. This could be an important problem since BPA can remain in the sludge. In fact, adsorption could contribute to almost 50% of BPA separation (Huang et al., 2019). This BPA is not completely degraded in the sludge treatment, since sludge stabilization is mainly anaerobic (Limam et al., 2013). Thus, it means that BPA will be present in the final sludge applied to the agriculture (Abril et al., 2020).

In this work, a deep study of BPA removal in laboratory sequencing batch reactor (SBR) was carried out. For it, two SBRs were operated under controlled operating conditions treating synthetic wastewater whit BPA (SBR-BPA) and without it (SBR-B). These experiments allowed studied the influence of BPA presence on the biological wastewater treatment performance, and on the bacterial community of activated sludge. Additionally, batch experiments whit adapted and non-adapted to BPA activated sludge were carried out to have a better understanding of the mechanisms/pathways of BPA removal.

2. Materials and methods

2.1. Biological treatment

A synthetic wastewater (SW) including 1 $mg \cdot L^{-1}$ of BPA was treated by aerobic activated sludge in a sequencing batch reactor (SBR-BPA) for three months. In parallel, another SBR worked under the same operational conditions of SBR-BPA as a control system, treating the same SW without BPA (SBR-B).

Each SBR included the following components: one cylindrical tank (30 × 10 cm; height×diameter), one air compressor with two air diffusers located at the bottom of SBR, one mechanical stirrer with a stirring paddle and two peristaltic pumps. The operational conditions of SBR-B and SBR-BPA can be seen in Table 1. In each SBR cycle, mechanical stirrer and compressor worked during reaction time (6 h) to maintain the homogeneous and aerobic conditions of mixed liquor. After this time, both equipments stopped to allow the sedimentation, draw, and idle steps. Peristaltic pumps worked to fill SW (placed in a tank of 25 L) or to draw treated effluent.

SW composition was formulated to achieve a relationship between COD, nitrogen and phosphorous concentrations of 100:5:1 and the food to microorganisms ratio (F/M) shown in Table 1. For it, peptone (0.23 $g \cdot L^{-1}$), meat extract (0.23 $g \cdot L^{-1}$) and K_2HPO_4 (0.03 $g \cdot L^{-1}$) were mixed with tap water as a COD concentration of 500 $mg \cdot L^{-1}$. In the first 7 days, both reactors were fed with this SW to acclimate the sludge to the new influent. From this day, 1 $mg \cdot L^{-1}$ of BPA was added to the feed of SBR-BPA until the last experimental day.

Both SBRs were inoculated with activated sludge from a municipal WWTP and worked at a room temperature. Sludge withdrawals were performed periodically for the experimental time to maintain the mixed liquor suspended solids (MLSS) concentration in 2.5 $g \cdot L^{-1}$.

2.2. Batch experiments

In this work, batch experiments were performed to study BPA removal mechanisms in the biological treatment process carried out to treat a SW, which contains 5 $mg \cdot L^{-1}$ of BPA (SW + BPA). As commented in introduction section, biodegradation (B), adsorption (Ad), oxidation (O) and hydrolysis (H) were studied. Batch experiments were performed with and without activated sludge, depending on the pathways shown in Table 2. In addition, the tests conducted with sludge were carried out with adapted and non-adapted biomass to BPA presence. The adapted biomass was taken from SBR-BPA in the last

Table 1
Operating parameters in SBR-B and SBR-BPA.

Operating parameters		
Reaction volume	6	L
Hydraulic retention time	24	h
F/M ratio	0.2	kg COD·kg $MLSS^{-1} \cdot d^{-1}$
MLSS	2.5	$g \cdot L^{-1}$
Number of cycles	3	$cycle \cdot d^{-1}$
$Volume_{fed/draw}$ ($V_{feed/draw}$)	2	$L \cdot cycle^{-1}$
Cycle characteristics		
Filling + Aerobic reaction	6	h
Sedimentation	1.5	h
Draw	25	min
Idle	5	min

Feed characteristics

	0–7 day	8–94 day
SBR-B	SW	SW
SBR-BPA		SW + 1 mg BPA·L^{-1}

Table 2
Batch experiments to study the pathways BPA removal.

Activated sludge	Test	Pathways	ML (L)	SW + BPA (L)	Aeration (mg $O_2 \cdot L^{-1}$)	$HgCl_2$ (mg·g ML^{-1})
Adapted	I-A	B + Ad + Ox + H	0.67	0.33	>2	–
	II-A	Ad + Ox + H	0.67	0.33	>2	30
Non-Adapted	I-N	B + Ad + Ox + H	0.67	0.33	>2	–
	II-N	Ad + Ox + H	0.67	0.33	>2	30
Without sludge	III	Ox + H	–	1.00	>2	–
	IV	H	–	1.00	0	–

operational day, and non-adapted biomass was collected from the same municipal wastewater treatment plant from which the initial sludge inoculum was taken. In both cases, the tests with activated sludge (biomass) were performed taken the mixed liquor (ML) of each reactor at the end of the reaction time under endogenous conditions. In this way, it was ensured that there was no biodegradable substrate before the test.

All the batch experiments were performed at 20.5 ± 0.3 °C, for 6 h, using glass beakers and 1 L of sample, which was maintained in continuous stirring. Test I and II simulated the operational conditions of the reaction step in SBR-BPA. Thus, the same volume ratio of mixed liquor and SW + BPA as in this SBR at the initial reaction step was prepared for both tests. To achieve the activated sludge inhibition in test II, to eliminate biodegradation pathway, $HgCl_2$ was added into the sludge (30 mg·g $MLSS^{-1}$) stirring for 2 h as reported by Pierre et al. (2014). After this time, test II was performed. Test III and IV evaluated BPA oxidation removal by dissolved oxygen or by hydrolysis. Thus, only SW + BPA was placed into the beakers. In each test, BPA concentrations were measured every hour during the experimental time.

2.3. Effluents physicochemical analysis

In both SBRs the following effluent parameters and measurement frequencies were considered:

- Three times a week; pH (GLP 21+ from Crison), conductivity (GLP 31+ from Crison), turbidity (D-122 from Dinko), dissolved COD (reagent kits and Spectroquant NOVA 30 from Merck).
- Once a week; total nitrogen (N_T) and the other nitrogen forms (NO_3-N; NO_2-N; NH_4-N), total phosphorous (P_T) and phosphate (PO_4-P) (reagent kits and Spectroquant NOVA 30 from Merck).

Additionally, soluble microbial products (SMP) and BPA concentrations were analyzed also once a week. SMP were evaluated through proteins and carbohydrates concentrations since these substances are the main compounds generated during biomass growth and decay (Namkung and Rittmann, 1986). Carbohydrates were measured according to anthrone method (Frølund et al., 1996) and proteins by BCA method (Zuriaga-Agustí et al., 2013). High performance liquid chromatography (HPLC) was performed to quantify BPA on effluent and influent of SBR-BPA and samples of batch experiments. For it, HPLC from Japan Spectroscopy Corporation (Jasco) and Kinetex C18 (1.7 μm; 50 mm × 2.1 mm) column from Phenomenex were used. The mobile phase was acetonitrile and Milipore-water (50:50, v/v) at a flow rate of 1 mL·min^{-1} with 20 μL of filtered sample (0.22 μm pore size syringe filter from Labbox). BPA detection was achieved at wavelength of 214 nm and a retention time of 2.3 min.BPA limit detection was 0.01 mg·L^{-1}.

2.4. Biomass analysis

2.4.1. Mixed liquor

Biomass concentration of both reactors was controlled three times a week measuring suspended solids and volatile suspended solids of mixed liquor (MLSS and MLVSS). This information allowed calculating the sludge production (ΔX; Eq.(1)) in the time periods in which no withdrawal sludge was performed:

$$\Delta X_{i-j} = \frac{(MLSS_j - MLSS_i) \cdot V_R}{j - i} \quad (1)$$

where i and j were two consecutive days without withdrawal sludge and V_R was the SBR reaction volume.

Respirometric techniques provide valuable information of biomass activity (Bina et al., 2018; Di Trapani et al., 2011). The rate at which microorganisms consume oxygen in the substrate biodegradation process is related to biomass activity. In this way, the specific dynamic respiration rate (Rsp; $mgO_2 \cdot gVSS^{-1}.h^{-1}$) was measured in initial, middle, and final experimental time using BM-Advance equipment from Surcis under the following operational conditions: 20.0 ± 0.1 °C, air flow rate of 0.90 ± 0.01 L·min^{-1} and 2000 rpm of stirring rate. Endogenous conditions of activated sludge were achieved before test. In addition, autotrophic biomass was inhibited adding Allyl-Tiourea (3 mg ATU·g $MLVSS^{-1}$). Sodium acetate (25 mL; COD = 300 mg·L^{-1}) was added to 1 L of ML, finishing the respirometric tests once substrate had been consumed.

On the other hand, four microbial hydrolytic enzymatic activities (MHEA) were measured. Lipase, protease, α-glucosidase and dehydrogenase were quantified spectrophotometrically every two weeks following using 4-Nitrophenyl palmitate, Azocasein, 4-Nitrophenyl-α-D-glucopyranoside and 2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2H-tetrazolium-chloride (Sigma-Aldrich). Lipase was measured following Gessesse et al. (2003), and the other activities were quantified by Goel et al. (1998) method.

2.4.2. Microbial community

To have a holistic knowledge of bacterial community and its changes throughout the experimental procedure, MiSeq sequencings of 16S rRNA genes according to methodology reported by Ferrer-Polonio et al. (2019), were performed in both reactors in the days 24, 53, 66 and 94. Briefly, total genomic DNA was isolated from each SBR sample using FastDNA® SPIN kit for soil (MP Biomedicals, USA), according to protocol supplied by the manufacturer. DNA samples were sent to FISABIO sequencing service (Valencia, Spain) for Illumina MiSeq sequencing. Amplicon libraries were created using PRO341F and PRO805R 16S V3-V4 primers. Raw data were analyzed using QIIME™ 1.9.1 (Caporaso et al., 2010), applying additional scripts available in MicrobiomeHelper virtual box v0.4 (Comeau et al., 2017). The most abundant operational taxonomic unit (OTU) was elected as its representative and it was assigned against MiDAS v3.6 (Nierychlo et al., 2020a) at 97% identity. Alpha diversity indices were calculated to assess microbial diversity in SBR-B and SBR-BPA.

2.5. Statistical analysis

To evaluate BPA influence on the biological treatment, statistical analysis of several parameters measured was carried out with one-way ANOVA (Statgraphics Centurion XVII). In this analysis F-ratio and *p*-value were achieved with a confidence level of 95%.

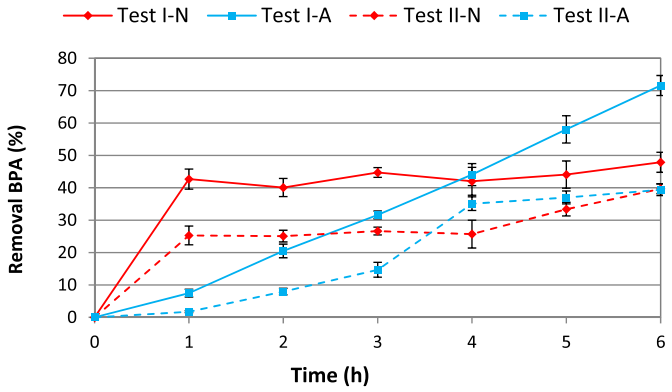

Fig. 1. BPA removal percentages in batch experiments performed with adapted (A) and non-adapted (N) sludge.

3. Results and discussion

3.1. Batch experiments: removal pathways of BPA

Batch experiments of test III and IV showed that oxidation and hydrolysis did not contribute to BPA removal in the biological treatment, since BPA concentrations remained constant for the 6 h of the experiments (data not shown).

Since Ox and H pathways were negligible, test II evaluated only Ad pathway, while test I evaluated B + Ad. As it can be observed in Fig. 1, both non-adapted and adapted biomass reached similar BPA removal percentages (39.7% and 39.4%, respectively) at the end of the test II. This value agrees with other ones reported by other researchers (Stasinakis et al., 2010; Zhao et al., 2008). It can be commented that BPA had lower sorption comparing it with other endocrine disruptors like triclosan and 4-n-nonylphenol, which showed removals of around 80% due to the sorption route (Stasinakis et al., 2010). This fact is due to higher hydrophilicity of this compound, which results in a low octanol–water partition coefficient (log K_{ow} = 3.32) (Groshart et al., 2001). Regarding test I results, it can be seen in Fig. 1 that BPA removal depended on the biomass adaptation. After 6 h of test I, 47.9% and 71.6% of BPA were removed with non-adapted and adapted biomass, respectively. Taken into account the BPA removal due to Ad route, only 8.2% was associated to biodegradation pathway in non-adapted biomass, while 32.2% of BPA was eliminated by biodegradation route when the process was performed with adapted sludge. This fact suggests that changes on microbial community have been occurred due to BPA presence.

3.2. Biological treatment of wastewaters with BPA

For three months several physicochemical parameters of both reactors (SBR-B and SBR-BPA) were monitored to evaluate the influence of BPA occurrence in wastewaters at a concentration of 1 mg·L^{-1} on the biological process. The average values of these parameters in the SBR effluents in this period are presented in Table 3.

Table 3
Effluents parameters average of SBR-B and SBR-BPA for 94 experimental days.

Effluent parameter	SBR-B	SBR-BPA
pH	7.4 ± 0.3	7.4 ± 0.2
Conductivity (mS/cm)	1.17 ± 0.12	1.07 ± 0.05
Turbidity (NTU)	0.82 ± 0.96	1.48 ± 1.76
COD (mg/L)	13.4 ± 4.1	23.7 ± 8.9
N_T (mg/L)	38.6 ± 8.9	43.4 ± 5.0
N-NH_4^+ (mg/L)	≈0	≈0
N-NO_3^- (mg/L)	31.5 ± 15.1	36.3 ± 4.8
P_T (mg/L)	5.7 ± 1.4	5.6 ± 2.2
P-PO_4^{3-} (mg/L)	5.5 ± 1.2	5.4 ± 2.0
Mixed liquor parameters	SBR-B	SBR-BPA
MLSS (g·L^{-1})	2.40 ± 1.75	2.59 ± 1.17
MLVSS/MLSS	0.87 ± 0.03	0.90 ± 0.02

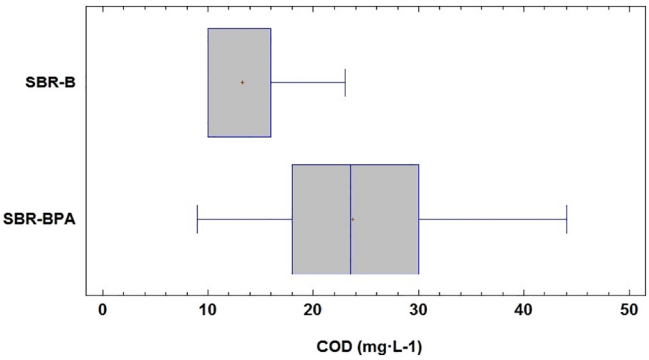

Fig. 2. Tukey diagram for effluent COD of SBR-B and SBR-BPA.

After one-way ANOVA analysis of all values measured throughout the 94 days according to Section 2.3 (taken the reactor as factor), only soluble COD presents a statistical significance (F = 34.3; *p*-value<0.0001). Fig. 2 shows that this parameter presents higher values in SBR-BPA than in SBR–B. Thus, it can be concluded that BPA addition led to an increase of effluent COD. In this way Stasinakis et al. (2008) reported the same effect of BPA on substrates removal. In an activated sludge system, BPA presence increased the biodegradation time to remove a readily biodegradable compound like sodium acetate trihydrate, which reduces its removal efficiency. Leyva-Díaz et al. (2017) also reported the same effect of BPA on sodium acetate biodegradation under certain operating conditions.

Regarding the sludge production, it can be seen in Fig. 3 that SBR-B remained stable in the first 38 days (0.58 ± 0.16 g SS·d^{-1}), while ΔX decreased in the other reactor after BPA addition. The same behavior was reported by Ferro Orozco et al. (2013) during acclimation of activated sludge to BPA, under two acclimation strategies (constant and increasing concentration of BPA addition). Independently of strategy followed, these authors reported a significant initial decrease of sludge production.

On the other hand, BPA was found in the effluent of SBR-BPA in the initial period, as shown in Fig. 4, ranging from 0.22 mg·L^{-1} to negligible values (below detection limit) after 16 days of BPA addition. This period was similar to the ones reported by other authors like Press-Kristensen et al. (2008), who reported periods between 10 and 40 days (depending on temperature, hydraulic retention time, and pre-exposure of the biomass) to remove 1 mg·L^{-1} of BPA. Other authors reported periods around 10 days (Zhao et al., 2014) and 15 days (Ferro Orozco et al., 2013) to achieve BPA concentrations below the limit of detection of

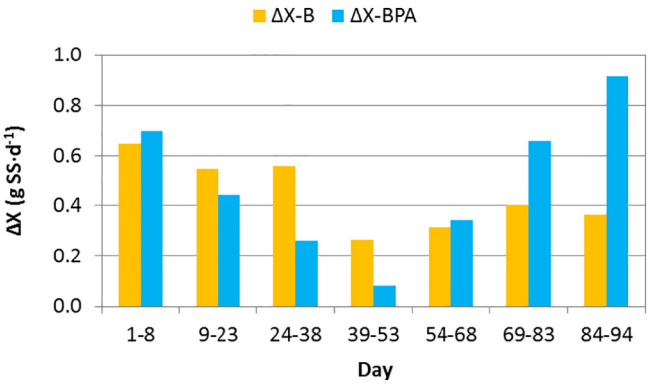

Fig. 3. Sludge production (ΔX) of SBR-B and SBR-BPA.

their analysis. Thus, this fact also confirmed that biomass adaptation occurred in SBR-BPA, although ΔX continued to decline until day 53. In addition, SBR-B also showed a ΔX decrease from 39 day, as it can be seen in Fig. 3. This fact could be due to decrease of room temperatures due to the season. The maximal average temperature in 94 experimental days was 18.2 ± 3.5 °C. However, greater variations in minimum temperatures were registered in the following periods: from day 1 to 38 (period A) the average temperature was 10.9 ± 1.5 °C; from day 39 to 66 (period B) it was 5.5 ± 1.7 °C; from day 67 to 94 (period C) it was 10.3 ± 0.7 °C. It is well-known that activated sludge characteristics in wastewater treatment plants are affected by seasonal temperatures variations (Jones and Schuler, 2010; Krzeminski et al., 2012). In this way, low temperatures in period B could result in lower biomass growth, worse settleability and higher polysaccharide and protein concentrations in the supernatants of both reactors (Wang et al., 2010). Fig. 5 shows the SMP substances (as sum of proteins and carbohydrates), in SBR-B and SBR-BPA. Regarding these results, statistically significant differences of SMPs were observed between both reactors in period B ($F = 9.48$; p-value $= 0.0370$), unlike the rest of the experiment (period A and C) where significance was not detected. Thus, SBR-BPA seems to be more affected to low temperatures. The main causes of SMP increase can be associated to substrate metabolism, biomass growth (UAP; utilization-associated products) and to biomass decay (BAP; biomass-associated products) (Barker and Stuckey, 1999; Namkung and Rittmann, 1986). As no changes in substrate and no biomass growth occurred, it could be concluded that SMP increase was due to biomass decay.

Additionally, in the same period (B) it was observed an increase of both COD and turbidity measured in SBR-BPA effluent, meanwhile this low temperature period had little impact on SBR–B, as can be verified from Table 4 data. Thus, it can be concluded that BPA presence is associated to a more sensitive to low temperatures activated sludge.

In period C, when temperatures increased to values like period A, ΔX of SBR-B remained stable in 0.38 ± 0.05 g SS·d^{-1}. Conversely, SBR-BPA experienced a progressive increase of the sludge production together with an improvement of effluent quality, since a decrease of SMP, COD and turbidity values in the effluent were observed. As commented previously, an increase of the sludge production should be correlated with an increase of SMP associated to UAP substances, which was the contrary to what was observed. This behavior can be explained by the fact that biomass became more active, as MHEA analysis corroborated. It is known that dehydrogenase activity is related to microbial respiration and it could also report information about the biodegradation of organic compounds in biological processes (Bian et al., 2019; Hu et al., 2014). In this way, Fig. 6 illustrates the decrease of dehydrogenase activity in SBR-BPA in periods A and B due to both acclimation process and low room temperatures. After these periods, dehydrogenase rapidly increased until reaching values very similar to those measured in SBR-B.

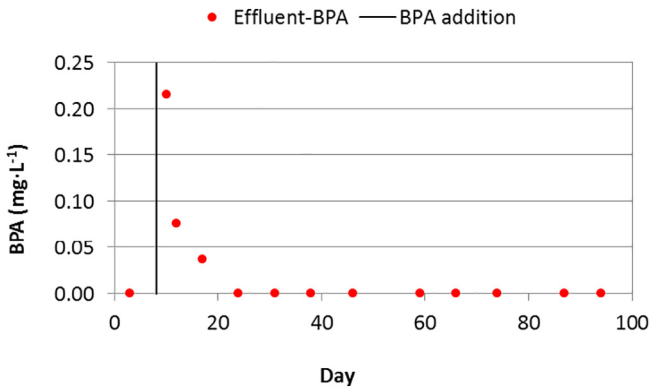

Fig. 4. Effluent BPA concentration of SBR-BPA.

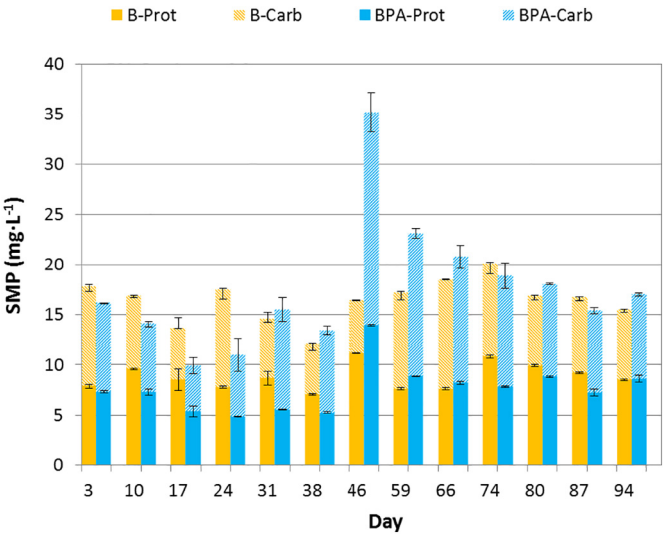

Fig. 5. SMP (sum of proteins and carbohydrates) in SBR-B and SBR-BPA.

Respirometric results confirmed this behavior. Rs, which has a positive correlation with the biomass activity (Andreottola et al., 2002; Di Trapani et al., 2011), reached the following values on days 1, 50 and 94: SBR-BPA 9.26, 7.82 and 14.42 (mg $O_2 \cdot g^{-1} \cdot h^{-1}$); SBR-B 9.26, 8.24 and 8.72 (mg $O_2 \cdot g^{-1} \cdot h^{-1}$). It should be highlighted that in the final period biomass of SBR-BPA was even more active than the biomass of SBR-B, which confirms the stimulation effect of BPA after its acclimation. Regarding α-glucosidase, protease, and lipase activities (Fig. 6), stable values were measured in the entire experimental period in SBR-B. On the contrary, similar variations were observed in the three MHEA in SBR-BPA. In period B, when cellular lysis occurred due to worse environmental conditions, α-glucosidase, protease, and lipase concentrations increased (sample of day 52) to degrade this cellular material (SMP associated to BAP). However, as it is known, BAP substances are less biodegradable than UAP (Maqbool et al., 2017; Ni et al., 2010; Wei et al., 2019) Thus, both proteins and carbohydrates increased in SBR-BPA in this period (shown Fig. 5) despite increasing the three MHEA. Finally, when ΔX increased in period C, SMP associated with UAP had to increase, and an increase in SMP of SBR-BPA should have been noted. However, these substances remained stable in this period, as can be seen in Fig. 5, while α-glucosidase, protease, and lipase activity decreased achieving stable values. This behavior can be explained because soluble substances from debris associated with UAP are more biodegradable than BAP.

The fact that biomass becomes more active due to BPA has been also reported by other authors. Ferro Orozco et al. (2013) showed higher acetate biodegradation removal capabilities by BPA acclimated activated sludge, due to metabolic pathway of BPA, which is metabolized in 4-hydroxyacetophenone which finally results in hydroquinone and acetate. The acetate, as intermediate substance, could be the responsible for the observed enhancement of the metabolic activities of acclimated sludge. Zhao et al. (2014) also detected, once the initial toxic shock to the sludge from the BPA diminished, an improvement of the organic substances removal (from synthetic wastewater with peptone, $NaHCO_3$, urea and other trace substances). According to these authors, this improvement was due to BPA estrogen effect. In our work, the results of the study of MHEA are provided as a novelty to confirm this BPA effect on the biomass.

3.3. Bacterial community

Eight Illumina libraries (shown in Table 5) of bacterial 16S rRNA gene yielded 705,048 reads after quality filtering and removal of

Table 4
Average values of SMP, COD and turbidity of SBR-B and SBR-BPA in three periods of experimental time.

Time period	SMP ($mg \cdot L^{-1}$)		COD ($mg \cdot L^{-1}$)		Turbidity (NTU)	
	SBR-B	SBR-BPA	SBR-B	SBR-BPA	SBR-B	SBR-BPA
A (01–38)	15.4 ± 2.3	13.4 ± 2.5	12.3 ± 2.8	17.7 ± 3.2	0.56 ± 0.03	0.63 ± 0.13
B (39–67)	17.4 ± 1.0	26.4 ± 4.1	12.3 ± 3.0	32.9 ± 7.2	0.66 ± 0.12	3.69 ± 1.20
C (68–94)	17.3 ± 1.9	17.3 ± 1.5	15.3 ± 2.1	23.9 ± 3.1	1.25 ± 0.08	0.95 ± 0.25

chimeric sequences. Results of these measurements indicated similar species richness (ACE, Chao1 and Jackniffe estimators) and species diversity (Shannon index) in both reactors at day 24. However, lower species richness and diversity was observed in SBR-BPA comparing with SBR-B from the sample corresponding to day 53.

On the other hand, Fig. 7 shows the bacterial phyla identified in both reactors. In this figure, it can be seen the phyla representing around 90% of bacterial community. The rest of the bacterial community phyla can be consulted in Supplementary Material S1 (data from SBR-B) and Supplementary Material S2 (data from SBR-BPA). The greatest number of OTU belonged to *Proteobacteria*, *Bacteroidetes*, *Actinobacteria* and *Chloroflexi* phyla, as can be seen in Fig. 7. *Proteobacteria* remained quite stable in the 94 experimental days of SBR-B (25.1 ± 2.0%), while BPA addition improved abundance of this phylum increasing from 26.9% on day 24 to 41.3% on day 94. As it is known, *Proteobacteria* is able to utilize a wide range of aromatic compounds as substrates (Joshi et al., 2016). Additionally, this phylum was detected as the most abundant group of microbial community by other authors (Gómez-Acata et al., 2017). *Actinobacteria* phylum also increased in SBR-BPA. Its abundance ranged from 9.6% to 18.1%, while decreased in SBR-B from 14.9% to 10.3%. Zaborowska et al. (2020) observed the same behavior for bisphenol F (BPF) and bisphenol S (BPS), whose abundance increased by 8.9% and 15.2% compared with the control sample, respectively. On the contrary, both *Bacteroidetes* and *Chloroflexi* decreased in SBR-BPA during experimental time (from 27.4% to 16.2%, and from 9.2% to 1.2%, respectively), while remained constant in SBR-B (22.6 ± 1.9% and 11.7 ± 2.1%, respectively). Thus, according to the results of this work, BPA addition improved abundance of *Proteobacteria* and *Actinobacteria*, while *Bacteroidetes* and *Chloroflexi* reduced their abundance.

Regarding filamentous bacteria, a heat map (Fig. 8) was constructed from the genera representing the phylotypes known to contain species with filamentous morphology according to Nierychlo et al. (2020b). As can be seen in this heat map, *Thiothrix* (from 0.0% to 17.6%) and *Nocardioides* (from 0.1% to 8.9) abundance increased in SBR-BPA throughout the experiment, while in SBR-B these abundances remained at values of 0.0% and 1.0% on day 94, respectively. This behavior is in agreement with that reported by other authors. Chen et al. (2017)

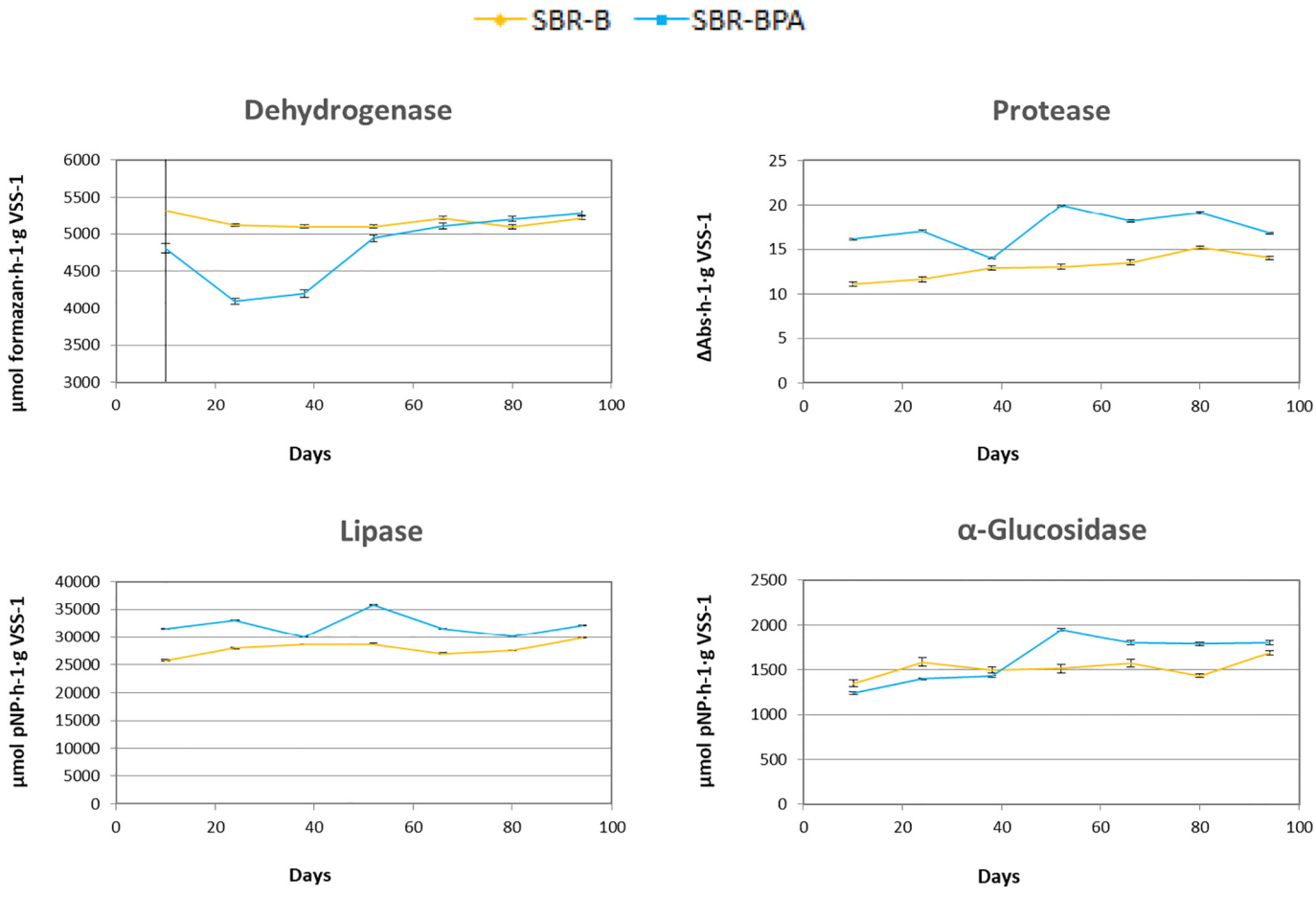

Fig. 6. Microbial hydrolytic enzymatic activities of SBR-B and SBR-fBPA.

Table 5
Microbial community diversity indices of 16S rRNA gene amplicon analysis for SBR-B and SBR-BPA in four sampling days.

Reactor	Day	Clean reads	OTU	ACE	Chao1	Jackniffe	Shannon	Phylogenetic diversity	Coverage
SBR-B	24	92,980	1958	2139	2090	2281	5.466	2755	0.930
	53	93,049	1415	2221	2166	2355	5.790	2782	0.930
	66	63,348	1425	1838	1791	1962	5.604	2425	0.921
	94	93,196	2423	1999	1945	2128	5.562	2629	0.932
SBR-BPA	24	88,860	1903	2024	1965	2118	5.438	2625	0.998
	53	81,504	1354	1517	1446	1584	4.626	2056	0.997
	66	93,247	1316	1463	1403	1534	4.668	1943	0.998
	94	91,284	1342	1479	1428	1554	4.720	1910	0.998

found that *Thiothrix* was largely produced in activated sludge treating phenol wastewater. Zaborowska et al. (2020) reported that *Nocardioides*, *Agromyces*, *Spinghomonas* and *Devosia* were identified in soil exposed to BPA, BPF and BPS, and Futamata et al. (2004) reported that *Actinobacteria* class, mainly *Nocardioides* and *Rhodococcus* genera, degraded aromatic compounds in polluted soils.

Microbial analysis at species level provided a deeper understanding of BPA effects addition on the community structure. In Table S1 and S2 it can be seen that eight *Nocardioides* species have been identified, being s__midas_s_1344 the species that achieved the high increase (from 0.03% on day 24 to 8.6% on day 94) due to presence of BPA. With regards *Thiothrix*, s__midas_s_6986 was the specie which presented higher abundance increase in SBR-BPA (from 0.0% on day 24 to 17.6% on day 94) of the four identified.

4. Conclusions

Results showed that BPA elimination through oxidation, hydrolysis and volatilization routes in a biological treatment was negligible. Regarding the adsorption route, BPA removal in batch experiments was the same working with adapted and non-adapted activated sludge. However, biodegradation route was highly influenced by the effect of adaptation to BPA. In this way, adapted sludge was able to remove 32.2% of BPA, while non-adapted activated sludge could only reduce BPA concentrations by 8.2%.

From the SBRs experiments, it can be concluded that biomass adapted to BPA under the rested operational conditions. From 16th day on, no BPA was detected in effluent reactor. However, biomass of SBR-BPA was more sensitive to temperature decrease, resulting in higher effluent turbidity and higher COD and SMP concentrations than those measured in SBR-B in the same period. In the last month of the experimental period, BPA presence resulted in an activation of adapted biomass of SBR-BPA. In this way, values of MHEA, sludge production and Rsp were higher in this reactor than in SBR-B. Regarding the bacterial community, BPA presence enhanced *Proteobacteria* and *Actinobacteria* phyla abundance, while *Bacteriodetes* and *Chloroflexi* phyla decreased.

As a general conclusion, it can be stated that BPA favors the biomass activity after an adaptation period without jeopardizing the biological process. This means that BPA does not have a negative effect on reactor performance after adaption of biomass. However, future works should focus on the adsorbed but not degraded BPA by the biomass, since it has been proven that a significant concentration of BPA is adsorbed on the activated sludge. It means that BPA can be transferred to the soil when sludge is used for agricultural purposes.

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CRediT authorship contribution statement

E. Ferrer-Polonio: Conceptualization, Investigation, formal analysis, writing, **C. Bretas Alvim:** Investigation, **J. Fernández-Navarro**: Investigation biological part, **R. Mompó-Curell**: Investigation, **J.A. Mendoza-Roca**: Funding acquisition, Supervision, Conceptualization **A. Bes-Piá**: Funding acquisition, Supervision, Conceptualization, **J.L. Alonso-Molina**: Investigation biological part, writing, **I. Amorós-Muñoz:** Investigation biological part.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

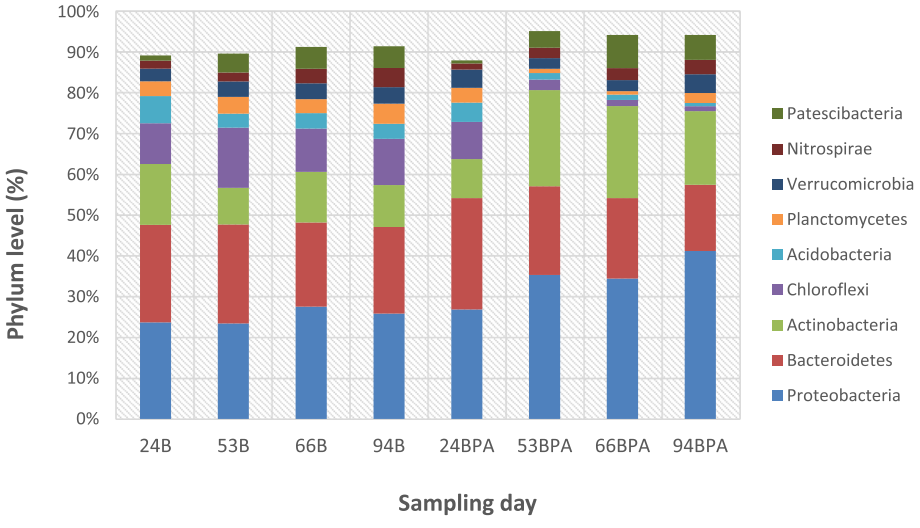

Fig. 7. Percentage of bacterial community at phylum level by MiSeq sequencing in SBR-B (B) and SBR-BPA (BPA). The number before B or BPA indicates the sampling day.

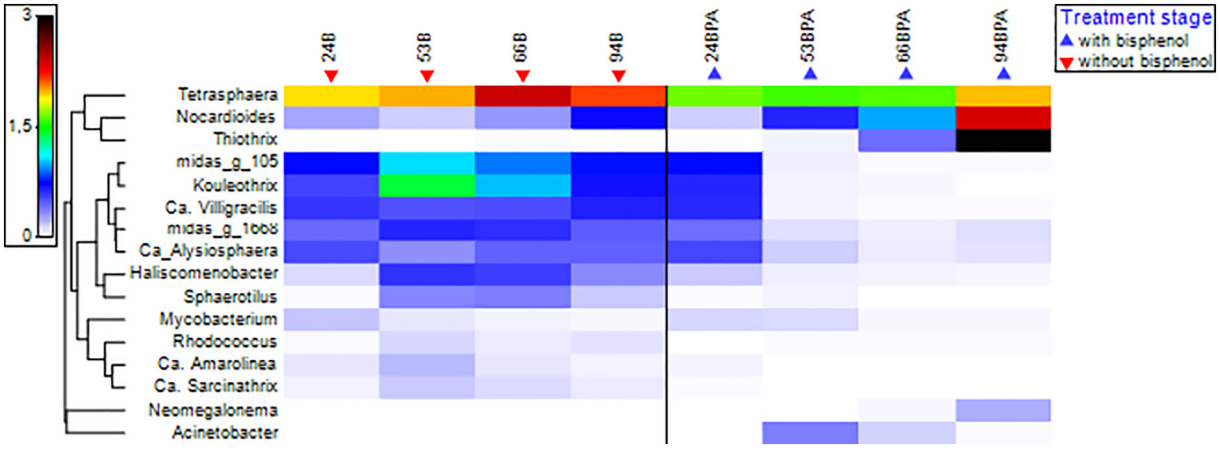

Fig. 8. Heatmap representing the percentage read abundance of the 16 most abundant filamentous bacteria genera in SBR-B and SBR-BPA samples over a 3-month period. The number before B or BPA indicates the sampling day. If no genus name could be assigned MIDAS classification is provided. The colour intensity in each panel shows the percentage of a genus in a sample, referring to colour key at the left side.

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