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Interdependence between the aerobic degradation of BPA and readily biodegradable substrates by activated sludge in semi-continuous reactors

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Abstract The objective of the present work was to analyze the interrelationship between the aerobic degradation of BPA and readily biodegradable substrates by activated sludge (AS) in semi-continuous reactors (SCRs). AS were obtained from three SCRs fed with glucose, acetate or peptone. AS from these reactors were used as inocula for three SCRs that were fed with each biogenic substrate, and for three SCRs that were fed with the biogenic substrate and BPA. In all cases, dissolved organic carbon (DOC), BPA, total suspended solids (TSS) and respirometric measurements were performed. Although BPA could be removed in the presence of all the tested substrates, AS grown on acetate exhibited the longest acclimation to BPA. Reactors fed with peptone attained the lowest TSS concentration; however, these AS had the highest specific BPA degradation rate. Specific DOC removal rates and respirometric measurements demonstrated that the presence of BPA had a negligible effect on the removal of the tested substrates. A mathematical model was developed to represent the evolution of TSS and DOC in the SCRs as a function of the operation cycle. Results suggest that the main effect of BPA on AS was to increase the generation of microbial soluble products. This work helps to understand the relationship between the biodegradation of BPA and readily biodegradable substrates.

Keywords Bisphenol A · Activated sludge · Biogenic substrate · Acclimation · Degradation

Introduction

With an annual production exceeding the 3.8 million tons, bisphenol A (2,2-bis(4-hydroxyphenyl)propane, BPA) is one of the most important anthropogenic contaminant. Mass consumption of BPA-containing products has contributed to the widespread contamination of BPA in the environment (Yang et al. 2014). BPA has been proposed to be an endocrine-disrupting chemical. This xenobiotic has become one of the major environmental concerns because of its estrogenic and genotoxic effects on animals and humans (Oshiman et al. 2007; Zhang et al. 2013). In spite of these potential hazards, BPA is widely used as a

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monomer for the production of polycarbonate plastics, epoxy resins, and flame retardants (Staples et al. 1998; Li et al. 2015).

Biodegradation by bacteria plays a major role in the elimination of BPA from the environment, reducing the risks associated with this compound (Oshiman et al. 2007; Zhang et al. 2013). A variety of BPA-degrading microorganisms have been isolated (Kang and Kondo 2002; Matsumura et al. 2009; Oshiman et al. 2007; Sakai et al. 2007; Kamaraj et al. 2014). Many studies have shown that toxic intermediates accumulate during the biodegradation of BPA, indicating that a single organism may not have the ability to mineralize the xenobiotic (Buitron and Gonzales 1996). For this reason, complex microbial communities, such as activated sludge, are a promising option to remove BPA. Numerous environmental factors such as dissolved oxygen concentration, pH, salinity, temperature, and the presence of co-substrates affect the structure of bacterial communities (Kamaraj et al. 2014). In particular, the wastewater composition is a key factor that determines the population structure of the activated sludge (Grady et al. 1993; Mielczarek et al. 2012, 2013). The type of readily biodegradable substrates determines in a great extent the conformation, in terms of genera of bacteria and metabolic variability, of the microbial community. Microbial communities may respond to a varying supply of substrates either by physiological adaptation or by changes in the community composition (Eiler et al. 2003). Even small additions of organic substrates may trigger a shift in the composition of the microbial community and also an accompanying change in the relative abundance of specific enzymes (Pinhassi et al. 1999; Van Hannen et al. 1999).

When a xenobiotic is in contact with a given microbial community, microorganisms will acclimate to the presence of this new compound, and depending on its metabolic capacities, they will or will not be able to degrade it. Moreover, the time accounted for the acclimation process to the xenobiotic compound is strongly affected by the structure of the microbial population and its metabolic variability potential. Organic compounds that a microbial community has encountered in the past play a crucial role in the response of the community to the presence of new compounds. In this context, microbial communities with different nutritional histories could have different behavior and performance in the degradation of a xenobiotic compound, such as BPA. Thus, the objective of the present work was to analyze the interdependence between the aerobic degradation of BPA and readily biodegradable substrates (glucose, acetate or peptone) by activated sludge in SCRs.

Materials and methods

Biological and chemical materials

Bisphenol A ($\geq$ 99%) was from Sigma-Aldrich. Tested biogenic substrates (individual carbon sources) were glucose, acetate, and peptone. Beef peptone was from Britannia Lab (Argentina). According to the manufacturer specifications, the peptone used in the present work contained 14%(w/w) of total nitrogen. All other reagents used in the present work were commercial products of reagent grade from Sigma-Aldrich.

Activated sludge used in all the experiments were harvested from an aerobic laboratory-scale (4.5 L) activated sludge reactors with partial biomass recycle. The hydraulic retention time was 48 h and the solids retention time was maintained at 33 days by daily wasting of the mixed liquor directly from the reactor. The activated sludge reactors were fed with a synthetic wastewater with the following composition: dehydrated cheese whey 1.5 g, $(NH_4)_2SO_4$ 0.5 g, and $NaHCO_3$ 1.03 g, all dissolved in 1 L of tap water. The soluble chemical oxygen demand (CODs) of the synthetic wastewater was 1500 mg L^{-1}. Aeration was provided by an air pump; air was pumped near the bottom of the reactor. Under steady-state conditions the dissolved oxygen (DO) concentration was above 4 mg L^{-1}, pH was 7.5 $\pm$ 0.4, CODs of the effluent ranged from 30 to 80 mgCOD L^{-1}, and total suspended solid (TSS) concentration ranged from 3700 to 4500 mgTSS L^{-1}.

Operation of semi-continuous reactors

To obtain activated sludge cultures with different nutritional histories, three laboratory-scale (V = 700 mL) semi-continuous reactors (SCRs) were fed with different carbon sources. These SCRs were inoculated with biomass from the activated sludge reactor described in "Biological and chemical materials". All SCRs were filled with the same mineral

basal medium with glucose (Reactor G), acetate (Reactor A), or peptone (Reactor P) as the sole carbon source. The composition of the mineral basal medium was: $(NH_4)_2SO_4$ 0.5 g L^{-1} (nitrogen source), K_2HPO_4 2 g L^{-1}, KH_2PO_4 0.6 g L^{-1}, and 1 mL L^{-1} of micronutrient solutions M1 and M2. The composition of M1 was (g/100 mL): $FeSO_4{\cdot}7H_2O$ 1.5, $ZnSO_4{\cdot}7H_2O$ 0.5, $MnSO_4.H_2O$ 0.3, $CuSO_4{\cdot}5H_2O$ 0.075, $CoCl_2{\cdot}6H_2O$ 0.015, and citric acid 0.6. Solution M2 contained the following (g/100 mL): $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ 0.05, H_3BO_3 0.01, KI 0.01.

During each operation cycle, samples were taken to measure dissolved organic carbon (DOC), total suspended solids (TSS), total ammonia nitrogen (TAN) and pH. The total volume removed for these measurements was V_P = 29.5 mL per cycle. The reactors were aerated continuously and the carbon source was depleted. Then, the next operation cycle started with the addition of a volume V_P of fresh mineral basal medium with an appropriate concentration of the tested substrate (glucose, acetate or peptone) to achieve an initial substrate concentration at the beginning of each cycle S_0 = 500 mgCOD L^{-1}. Because peptone was a source of carbon and nitrogen, in this case, ammonium sulfate was absent in the mineral medium added to the SCR. Once the biomass was acclimated to the tested biogenic substrate, five operation cycles per week could be performed. According to this procedure, the average total time for each operation cycle was t_T = 7/5 days. Considering the initial volume of these reactors (V = 700 mL), and the purged volume per cycle (V_P = 29.5 mL), the solids retention time (SRT) was 33 days.

Each parent reactor was operated for about two SRT to ensure the acclimation of the biomass to the tested growth substrate. Then, the biomass from each parent reactor was used as inoculum for two new reactors which were fed with the following carbon sources: glucose (G1), glucose + BPA (G2), acetate (A1), acetate + BPA (A2), peptone (P1) and peptone + BPA (P2). Reactors G1, A1, and P1 were operated in the same manner as the parent reactors. In the case of reactors G2, A2, and P2, the addition of BPA was as follows. For reactors G2 and A2, a stock solution of 280 mg L^{-1} of BPA (220 mgDOC L^{-1}) was prepared in the above mentioned mineral basal medium. At the beginning of each operation cycle, a sample of V_S = 100 mL of the mixed liquor of the tested reactor (G2, A2, or P2) were gently centrifuged and the supernatant was discarded. Then, the biomass was resuspended in 100 mL of the stock solution of BPA and this mixture was returned to the reactor. Finally, a volume V_P = 29.5 mL of the mineral basal medium with appropriate concentrations of the tested biogenic substrate was added to compensate the sampling for measurements. According to this procedure, the resulting initial BPA and biogenic substrate (glucose, acetate, peptone) concentrations were BPA_0 = 40 mgBPA L^{-1} (31.5 mgDOC L^{-1}), and S_0 = 500 mgCOD L^{-1}, respectively. It must be noted that in terms of dissolved organic carbon (DOC), for glucose and acetate this initial COD concentration represented 187 mgDOC L^{-1} while in the case of peptone corresponded to 195 mgDOC L^{-1}.

During each operation cycle, samples were taken to measure pH, dissolved organic carbon (DOC), total ammonia nitrogen (TAN), BPA, and total suspended solids (TSS) concentrations. From the slope of DOC and BPA concentrations as a function of time, the substrate consumption rate (R_S, mgDOC L^{-1} h^{-1}) and BPA consumption rate (R_{BPA}, mgBPA L^{-1} h^{-1}) corresponding to each operation cycle were obtained. For comparative purposes, the specific substrate consumption rate (q_S, mgDOC $gTSS^{-1}$ h^{-1}) and the specific BPA consumption rate (q_{BPA}, mgBPA $gTSS^{-1}$ day^{-1}) were obtained as the ratio between the evaluated rate (R_S or R_{BPA}) and the initial biomass concentration (X_0) of the operation cycle.

Respirometric measurements

A respirometric technique (Lobo et al. 2013, 2014) was used to evaluate the metabolic activity of the activated sludge in all the tested reactors (G1, A1, P1, G2, A2 or P2). Reactors were continuously aerated at a stable flow rate (1.0 L min^{-1}) using a high precision rotameter (Bruno Schilling model MB 60 V, Argentina). Operation temperature was 25 ± 0.5 °C. The DO concentration (C) as a function of time (t) was recorded every 5 s using an optical DO probe (YSI ProODO). Before the addition of the tested substrate (glucose, acetate, peptone), the oxygen mass transfer coefficient of the respirometer (k_La) was obtained using a non-steady state procedure (Lobo et al. 2014). When a stable dissolved oxygen concentration (C) was observed, the reactor was spiked with the corresponding biogenic substrate (glucose, acetate or peptone,

500 mgCOD L^{-1}) and C as a function of time (t) was recorded. The oxygen uptake rate (OUR) associated with the oxidation of the added substrate (OUR_{Ex}) was calculated from the DO mass balance in the respirometer as follows:

$$OUR_{Ex} = k_L a(C_e - C) - \frac{dC}{dt} \quad (1)$$

where C_e is the dissolved oxygen concentration in the absence of an oxidizable substrate, and C is the instantaneous dissolved oxygen concentration. To compare experiments with different biomass concentrations, the specific exogenous respiration rate (q_{O2}, $mgO_2\ gTSS^{-1}\ h^{-1}$) was calculated as the ratio between OUR_{Ex} and the biomass concentration in the respirometer. Based on the obtained OUR_{Ex} profiles, the oxygen consumed (OC) during the substrate oxidation was calculated as follows:

$$OC = \int_0^t OUR_{Ex} dt \quad (2)$$

Then, the oxidation coefficient ($Y_{O/S}$) was calculated as the ratio OC_m/S_0, where OC_m is the maximum amount of oxygen consumed to degrade the added substrate, and S_0 is the initial substrate concentration. For all the reactors, respirometric measurements were performed every two days, at least.

Analytical techniques

Total suspended solids (TSS) were used to monitor the biomass concentration (Contreras et al. 2011). Dissolved organic carbon (DOC) concentration was determined as follows: 5 ml of culture samples were centrifuged for 5 min at 13,000 rpm (Eppendorf 5415C); then, the supernatant was filtered through 0.45 μm cellulosic membranes (Osmonics Inc.). DOC concentration of the filtered samples was measured in a Shimadzu DOC-Vcpn analyzer. BPA concentration was determined using a colorimetric method (Modaressi et al. 2005). This method uses two reagents, 4-aminoantipyrine (4-AAP) (20.8 mM of 4-AAP in 0.25 M $NaHCO_3$), and ferricyanide (83.4 mM of $K_3Fe(CN)_6$ in 0.25 M $NaHCO_3$) as color generating substrates when combined with phenolic compounds. Colored complexes were measured at 510 nm in a Hach DR 2000 spectrophotometer. Calibration curves were performed periodically using BPA as the reference compound. Total ammonia nitrogen (TAN) concentration of the filtrate was measured by the Nessler colorimetric method using commercial reagents (Hach Company, Loveland, CO).

Modeling the semi-continuous reactors (SCRs)

Model development

To represent the evolution of the total suspended solids (TSS) and the dissolved organic carbon (DOC) in the used SCRs, a simplified version of the ASM#1 was employed (Lobo et al. 2016). During a given operation cycle (i) it was assumed that the biomass growth and the substrate consumption can be represented by the following overall reaction:

$$S_s \rightarrow Y_N X + Y_P S_p \quad (3)$$

where S_s represents the limiting substrate for the biomass growth, X is the biomass that consumes S_s, S_p represents a soluble microbial product, Y_N is the net biomass growth yield, and Y_P is the yield of soluble microbial products, both under the tested conditions. A similar approach was used in a previous work by Lobo et al. (2016) for modeling the operation of sequencing batch reactors treating phenol. It must be pointed out that Eq. (3) does not pretend to describe the biodegradation kinetics but only the overall stoichiometry of the growth process.

According to Eq. (3), if the operating conditions were suitable for the complete consumption of S_S within each operation cycle, concentrations of biomass and soluble microbial products at the end of cycle i ($X_{F(i)}$, $S_{pF(i)}$) are a function of their respective initial values ($X_{0(i)}$, $S_{p0(i)}$):

$$X_{F(i)} = X_{0(i)} + Y_N S_{s0} \quad (4)$$

$$S_{pF(i)} = S_{p0(i)} + Y_{Sp} S_{s0} \quad (5)$$

where S_{s0} is the initial substrate concentration of each cycle, which is constant according to the procedure described in "Operation of semi-continuous reactors". At the end of each operation cycle (i), a withdraw of a volume V_P = 29.5 mL of the mixed liquor for measurements and purge was performed. In the case of reactors with BPA, a volume V_S = 100 mL of the mixed liquor was also centrifuged and the supernatant was discarded. Then, the pellet (biomass) was resuspended in 100 mL of the stock solution of BPA

described in "Operation of semi-continuous reactors" and this mixture was returned to the reactor. The next operation cycle (i + 1) started with the addition of a volume V_P of fresh mineral basal medium with an appropriate concentration of the tested substrate (glucose, acetate or peptone) to achieve a constant initial substrate concentration at the beginning of each cycle (S_{s0}). Taking into account this procedure, initial concentrations corresponding to the next operation cycle (i + 1) can be calculated as follows:

$$X_{0(i+1)} = \left(1 - \frac{V_P}{V_T}\right) X_{F(i)} \tag{6}$$

$$S_{p0(i+1)} = \left(1 - \frac{V_P}{V_T} - \frac{V_S}{V_T}\right) S_{pF(i)} \tag{7}$$

where V_T = 700 mL is the total volume, V_P = 29.5 mL is the purged volume per cycle, V_S = 100 mL is the centrifuged volume in the cases of reactors with BPA, and V_S = 0 for reactors without BPA.

According to the proposed model, initial total suspended solids ($TSS_{0(i)}$), and initial and final dissolved organic carbon corresponding to the cycle i ($DOC_{0(i)}$, $DOC_{F(i)}$) are given by:

$$TSS_{0(i)} = X_{0(i)} \tag{8}$$

$$DOC_{0(i)} = S_{s0}(1 - f_{ads}) + S_{p0(i)} + BPA_0 \tag{9}$$

$$DOC_{F(i)} = S_{pF(i)} \tag{10}$$

The term f_{ads} takes into account that for low initial substrate to biomass ratios (S_{s0}/X_0) (such as the employed in the present work) a significant fraction of the added substrate is rapidly adsorbed onto the biomass during its degradation (Rensink and Donker 1991; Novak et al. 1995; Majone et al. 1999).

Evaluation of model coefficients

The proposed model was fitted to the available data ($TSS_{0(i)}$, $DOC_{0(i)}$, $DOC_{F(i)}$) as follows. Combining Eqs. (4), (6), and (8) the following expression was obtained:

$$TSS_{0(i+1)} = \left(1 - \frac{V_P}{V_T}\right)\left(TSS_{0(i)} + Y_N S_{s0}\right) \tag{11}$$

Because the initial biomass concentration corresponding to the first operation cycle is known, Eq. (11) was solved for different values of Y_N. Then, the Y_N value that minimizes the root mean squared error (RMSE) between experimental and calculated TSS was obtained.

A similar procedure was used to obtain the coefficients f_{ads} and Y_{Sp}. In this case, from the combination of Eqs. (5), (7), (9), and (10) the following expressions were obtained:

$$DOC_{0(i+1)} = S_{s0}(1 - f_{ads}) + \left(1 - \frac{V_P}{V_T} - \frac{V_S}{V_T}\right) DOC_{F(i)} + BPA_0 \tag{12}$$

$$DOC_{F(i+1)} = \left(1 - \frac{V_P}{V_T} - \frac{V_S}{V_T}\right) DOC_{F(i)} + Y_{Sp} S_{s0} \tag{13}$$

where for reactors without BPA, V_S = 0, and BPA_0 = 0. Conversely, for reactors G2, A2, and P2, V_S = 100 mL, and BPA_0 = 31.5 mgC L^{-1}.

Knowing the DOC concentration at the end of the first cycle, Eqs. (12) and (13) were solved for different values of f_{ads} and Y_{Sp}. Then, from the comparison between experimental and calculated $DOC_{0(i)}$ and $DOC_{F(i)}$, the pair of values f_{ads} and Y_{Sp} that minimizes the root mean squared error (RMSE) was obtained. The fitting procedure was implemented in the software Sigma Plot Version 10.0.

Results and discussion

Performance of the SCRs in terms of dissolved organic carbon (DOC) and total suspended solids (TSS)

Figure 1 shows the evolution of $DOC_{0(i)}$, $DOC_{F(i)}$ and $TSS_{0(i)}$ corresponding to the tested SCRs under the absence (G1, A1, P1) and the presence (G2, A2, P2) of BPA. The whole data corresponding to the evolution of DOC as a function of time for all the tested reactors can be found Fig. 6 in the Appendix. Because the inoculum of these reactors came from a parent reactor that was fed with the same substrates (glucose, acetate or peptone) as the sole carbon source during two SRT (60 days), the biomass was fully acclimated to these

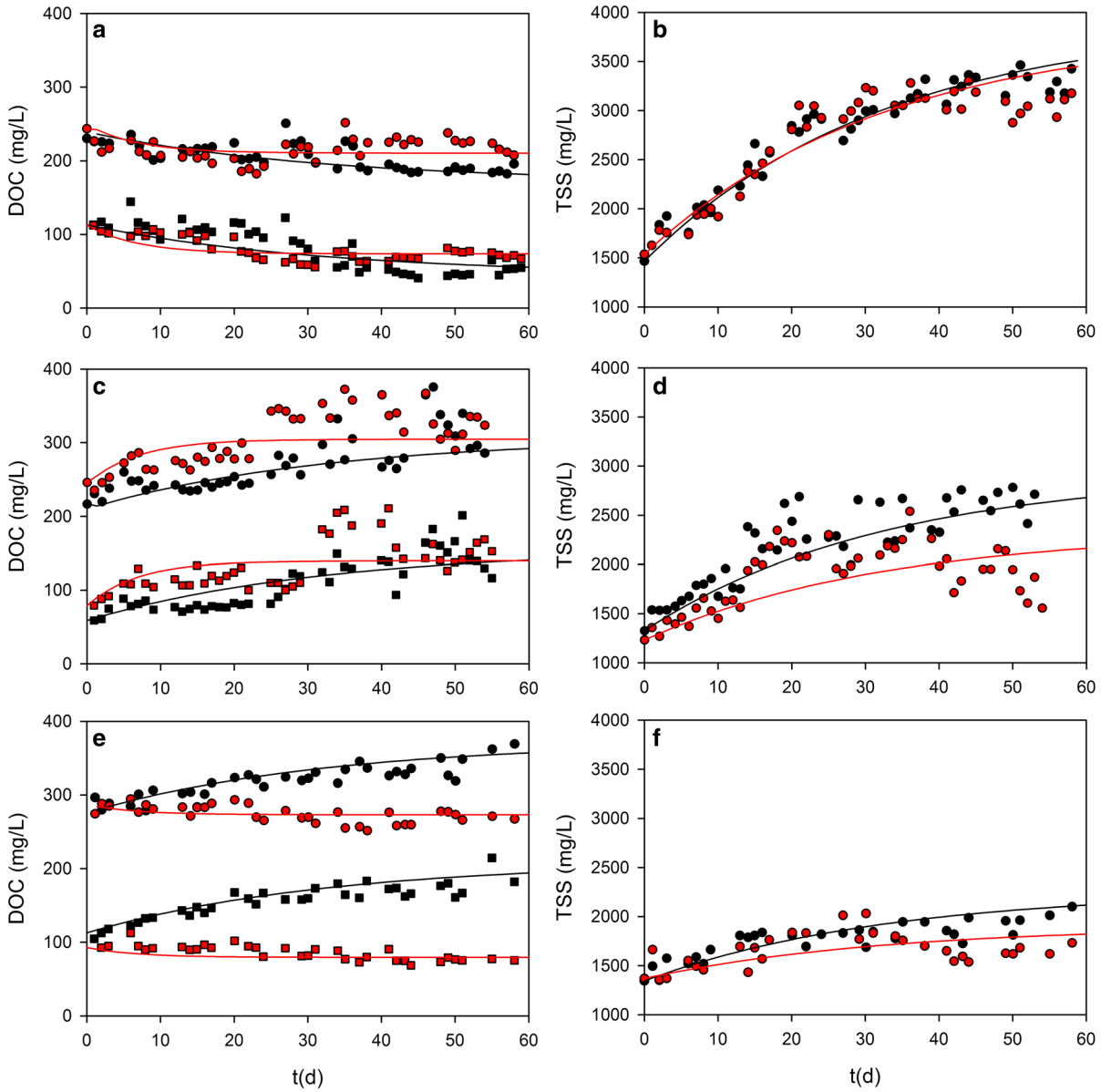

Fig. 1 Initial DOC (circles) and final DOC (squares) (**a**, **c**, **e**) and initial TSS (**b**, **d**, **f**) for each operation cycle as a function of time corresponding to the reactors fed with glucose (**a**, **b**), acetate (**c**, **d**) and peptone (**e**, **f**). Black symbols represent reactors without BPA (G1, A1, P1), red symbols correspond to reactors with BPA (G2, A2, P2). In all cases, lines represent the proposed model (Eqs. 11–13) using the coefficients shown in Table 1

compounds. For this reason, biomass growth and DOC consumption in all reactors started from the first operation cycle.

Figure 1a, c, e shows that the evolution of the DOC concentration at the end of each operation cycle ($DOC_{F(i)}$) strongly depended on the tested substrate. In the case of reactors G1 and G2, $DOC_{F(i)}$ decreased from 110 to about 60 mgDOC L^{-1} at 60 days (Fig. 1a). Conversely, $DOC_{F(i)}$ increased from 70 to 170 mgDOC L^{-1} in reactors A1 and A2 at the end of the experiment (Fig. 1c). Finally, while $DOC_{F(i)}$ in reactor P1 increased up to about 180 mgDOC L^{-1}, in reactor P2 almost constant $DOC_{F(i)}$ values were obtained (Fig. 1e).

In all cases, $TSS_{0(i)}$ increased up to a stationary value which depended on the type of carbon source

Table 1 Average oxygen consumed during the aerobic biodegradation of the tested substrates under the absence (OC_1) or the presence (OC_2) of BPA

	Substrate		
	Glucose	Acetate	Peptone
OC_1 ($mgO_2\ L^{-1}$)	176 ± 18	226 ± 20	257 ± 27
OC_2 ($mgO_2\ L^{-1}$)	239 ± 26	267 ± 17	344 ± 28
OC_2–OC_{BPA} ($mgO_2\ L^{-1}$)	179 ± 32	207 ± 26	284 ± 39
$Y_{O/S}$ ($gO_2\ gCOD^{-1}$)	0.36 ± 0.07	0.43 ± 0.07	0.54 ± 0.09

In all cases, the concentration of the biogenic substrate was 500 mgCOD L^{-1}. For calculation purposes, it was assumed that the consumption of oxygen for to the degradation of 40 mgBPA L^{-1} (OC_{BPA}) is 60 ± 12 $mgO_2\ L^{-1}$ (Ferro Orozco et al. 2016a, b, c)

and on the presence of BPA (Fig. 1b, d, f). Moreover, the time at which this stationary value was achieved depended on the difference between TSS at the beginning of the experiment and its corresponding stationary value.

For example, Fig. 1b shows that during the first 40 days the biomass in reactors G1 and G2 increased from 1500 to 3100 mgTSS L^{-1}. Then, from day 40 to 60 the biomass concentration remained approximately constant. Conversely, in the case of reactors fed with peptone (Fig. 1f), the biomass increased from 1300 to 1800 mgTSS L^{-1} within the first 15 days. Figure 1b, d, f also shows that as a general rule, TSS in reactors with BPA (G2, A2, P2) were a lower than TSS values measured in the reactors under the absence of BPA (G1, A1, P1). In this sense, Ferro Orozco et al. (2013) reported that during the acclimation of activated sludge to BPA in the absence of another carbon source, an enhancement of the biomass decay rate was observed.

With regard to reactors with the presence of BPA, Fig. 2a shows that reactor G2 did not show a lag phase in terms of BPA consumption. Although the biomass of reactor G2 could degrade BPA from the first addition, the time required to achieve a consumption of BPA of about 90% was 6 days. Then, in the second operation cycle BPA was exhausted in 2.2 days, and in the following cycles, the xenobiotic was depleted within 24 h. In a previous work, Ferro Orozco et al. (2015) studied the removal of BPA using activated sludge in the presence of cheese whey as the biogenic substrate. Those authors informed that about 8 days were necessary to achieve a depletion of 95% of the initial BPA concentration by activated sludge not acclimated to this xenobiotic.

Figure 2b shows that the activated sludge from a reactor that was fed with acetate as the sole carbon source exhibited a quite long acclimation period to BPA. The acclimation process of the biomass to BPA in reactor A2 lasted 34 days. Each addition of BPA was depleted in about 10 days. For this reason, within this period only three additions of BPA could be performed. However, after the third addition the activated sludge was acclimated to BPA and from the fourth addition of BPA to the end of the experiment, each addition of BPA was depleted within 24 h.

Among the tested reactors, the best performance concerning the removal of BPA corresponded to reactor P2 (Fig. 2c). As in the case of reactor G2, the removal of BPA in reactor P2 started from the first addition of the xenobiotic. However, from the first addition of BPA to the end of the experiment, the time required to achieve a consumption of BPA of 95% of each addition was less than 24 h. Moreover, while in reactors G2 and A2 the minimum achieved BPA concentration was about 7 and 4 mg L^{-1}, respectively, only 2 mg L^{-1} of BPA was measured at the end of each operation cycle in reactor P2.

Figure 3a shows that, as a general rule, specific substrate consumption rates (q_S) measured in reactor G2 (with BPA) were lower than those corresponding to reactor G1 (without the xenobiotic). However, regardless the experimental variations, the ratio between $q_{S\text{-}G2}$ and $q_{S\text{-}G1}$ increased from about 0.5 (at the beginning of the experiment) to approximately 1.0 (Fig. 4), demonstrating the acclimation of the biomass of reactor G2 to the presence of BPA. In the case of reactors fed with acetate (A1, A2) or peptone (P1, P2), q_S values measured in reactors with BPA were close to

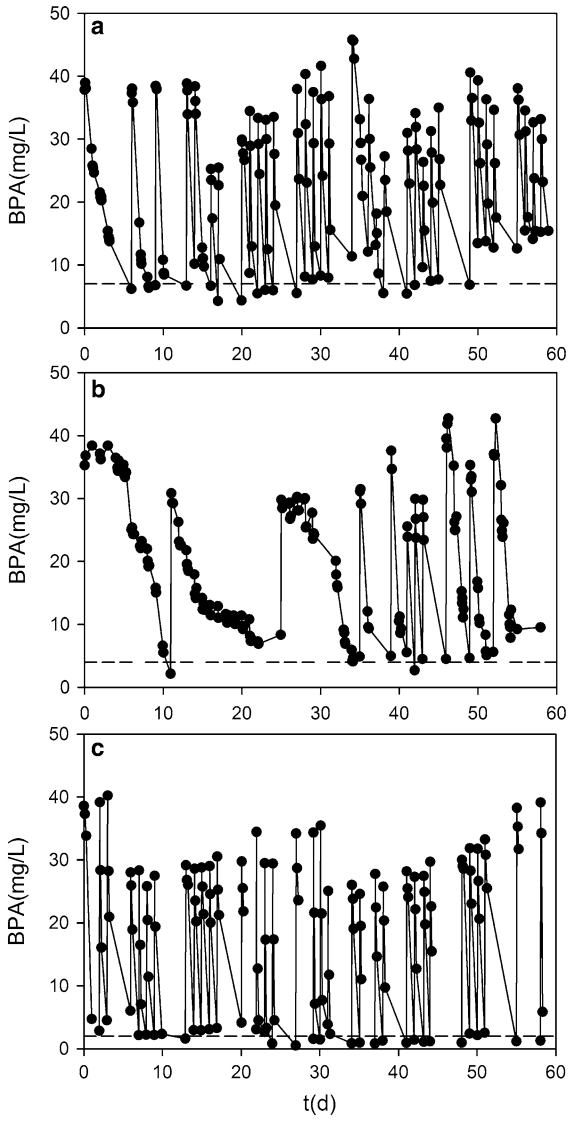

Fig. 2 Concentration of BPA as a function of time corresponding to reactors **a** G2, **b** A2, and **c** P2. Dashed lines represent the average of minimum BPA concentrations achieved during each operation cycle

those obtained in reactors without the xenobiotic (Fig. 3b, c). Although the ratio q_{S2}/q_{S1} ranged from 0.5 to 1.2 for acetate, and 0.6 to 1.0 for peptone (Fig. 4), observed changes were attributed to a random variation in the measured values of q_S instead of a real trend. These results suggest that the presence of BPA had a negligible effect on the metabolization rate of these biogenic substrates. Similar results were reported in a previous work (Ferro Orozco et al. 2015) when acclimation and degradation of BPA in semi-continuous activated sludge reactors was studied.

With regard to the specific BPA consumption rate (q_{BPA}), Fig. 3d shows that during the first addition of BPA to reactor G2, q_{BPA} increased from 4 at the beginning of the experiment to 30 mgBPA $gTSS^{-1}$ - $days^{-1}$ after 12 days. Then, q_{BPA} slowly decreased and stabilized around 15–20 mgBPA $gTSS^{-1}$ $days^{-1}$ until the end of the experiment. Conversely, reactor A2 exhibited quite low q_{BPA} values during the first 30 days. However, when a full acclimation of the activated sludge to BPA was achieved, the average value of q_{BPA} increased to about 10 mgBPA $gTSS^{-1}$ - day^{-1} (Fig. 3e). Finally, Fig. 3f shows that activated sludge from a reactor fed with peptone had the capability of degrading BPA from the beginning of the experiment. However, high variations in q_{BPA} were measured in reactor P2. After reaching a maximum value close to 60 mgBPA $gTSS^{-1}$ day^{-1} at 30 days of operation, q_{BPA} decreased gradually for the next 30 days until reaching 15 mgBPA $gTSS^{-1}$ day^{-1}.

Ferro Orozco et al. (2013, 2015) reported that in the absence of a biogenic substrate q_{BPA} values corresponding to fully BPA-acclimated activated sludge ranged from 65 to 90 mgBPA $gTSS^{-1}$ day^{-1}. Thus, although activated sludge that grew on glucose, acetate or peptone had the capability of degrading BPA, in all cases q_{BPA} values were lower than those corresponding to activated sludge obtained with BPA as the sole carbon source. Urase and Kikuta (2005) reported that a lower initial readily biodegradable substrate content resulted in a higher BPA degradation rate. Moreover, the decrease of the BPA degradation rate in the presence of biogenic substrates has been reported by other authors (Lobos et al. 1992; Hu et al. 2005; Zhao et al. 2008; Kamaraj et al. 2014).

In a previous work (Ferro Orozco et al. 2015), a SCR was used to acclimate activated sludge to BPA in the presence of cheese whey. Those authors reported an average q_{BPA} value of 42 ± 7 mgBPA $gTSS^{-1}$ - day^{-1} corresponding to fully BPA-acclimated activated sludge. It must be noted that in this previous work the used cheese whey contained 40% of lactose (a disaccharide composed by glucose and galactose) and 12% of proteins. For this reason, q_{BPA} values obtained in the present work corresponding to reactors G2 and P2 were close to q_{BPA} measured in SCRs containing cheese whey. The concomitant presence of both biogenic substrates (proteins and glucose) when

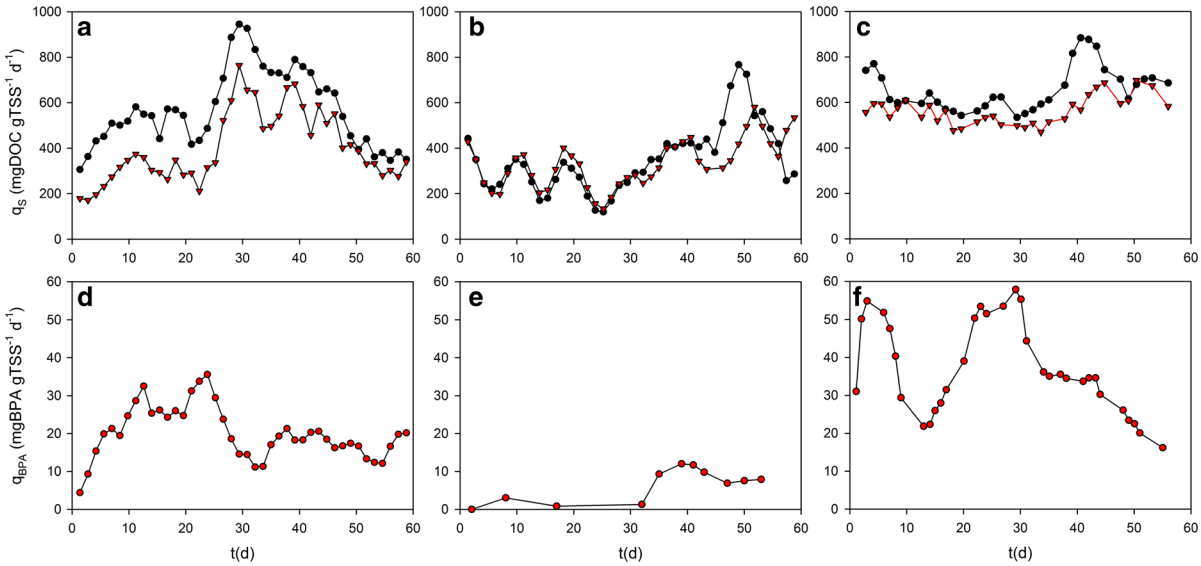

Fig. 3 **a**, **b**, **c** Specific substrate (q_S) and **d**, **e**, **f** Specific BPA (q_{BPA}) consumption rates as a function of time corresponding to the reactors fed with glucose (**a**, **d**), acetate (**b**, **e**) and peptone (**c**, **f**). Black symbols represent reactors without BPA (G1, A1, P1) while red symbols correspond to reactors with BPA (G2, A2, P2)

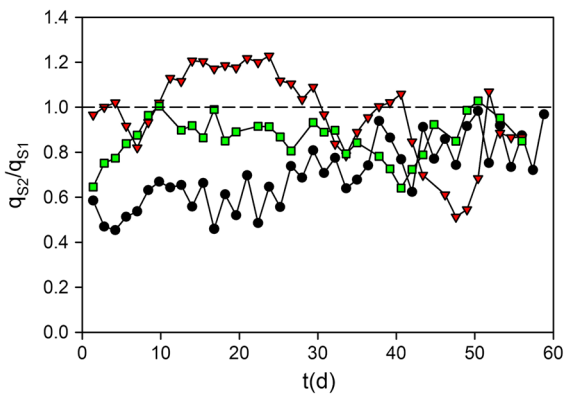

Fig. 4 Ratio between the specific substrate consumption rate in the presence (q_{S2}) and the absence (q_{S1}) of BPA as a function of time. Tested substrates (S) were: glucose (black circles), acetate (red triangles), and peptone (green squares)

SCR was fed with cheese whey leads to the development of a bacterial community that shares some characteristics of those observed in reactors G2 and P2 of the present work.

Respirometric measurements

Figure 5 shows typical examples of the obtained respirometric profiles corresponding to the tested reactors; in all cases, respirometric profiles showed two phases. During the first one, OUR_{Ex} values increase very fast to a maximum and then, respiration rate slowly decreased. Insets in Fig. 5 shows that the length of the first phase was coincident with the time at which DOC values remained constant, indicating the depletion of the readily biodegradable DOC.

According to several authors, when the readily biodegradable substrates are sugars or acetate, these respirometric profiles represent the accumulation (phase I) and utilization (phase II) of storage compounds (Fig. 5a–d). When the microorganisms are subjected to a feast and famine regime, storage of biopolymers is frequently detected in SCRs (Insel et al. 2007; Hernández et al. 2008). Respirometric profiles corresponding to reactors fed with peptone (P1, P2) showed an initial OUR peak with sequential drops afterward, giving an indication of peptone fractions with different biodegradation rates (Fig. 5e, f). Although DOC attains low values within the first 4–5 h (insets in Fig. 5e, f), OUR values reached the endogenous level in the following 15 h, suggesting the oxidation of an endogenous carbon source during phase II. Obtained OUR profiles are typical for peptone biodegradation, and they were in agreement with those reported by other authors (Orhon et al. 2009, 2010; Cokgor et al. 2011).

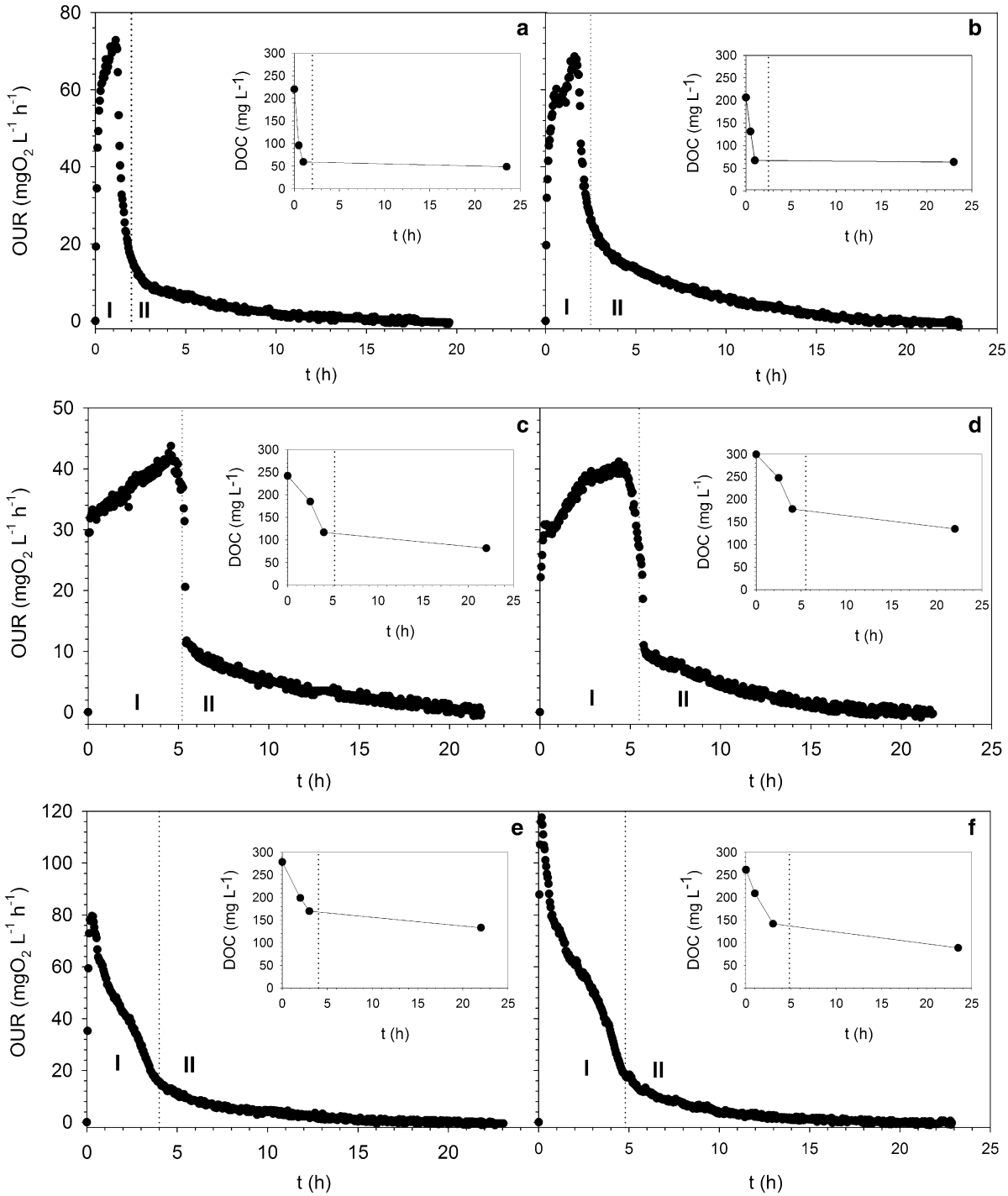

Fig. 5 Typical examples of respirometric profiles corresponding to reactors G1 (**a**), G2 (**b**), A1 (**c**), A2 (**d**), P1 (**e**), and P2 (**f**). Insets represent the change of DOC values as a function of time corresponding to each tested reactor

For each respirometric profile, the total oxygen consumption (OC) corresponding to each tested reactor was obtained according to Eq. (2). Table 1 shows that OC measured in reactors with BPA (G2, A2, P2) were higher than those corresponding to reactors without the xenobiotic (G1, A1, P1). These differences can be attributed to the oxygen consumed during the oxidation of BPA.

The aerobic biodegradation of BPA and of its metabolic intermediates by BPA-acclimated activated sludge was studied by Ferro Orozco et al. (2013, 2015, 2016a, b, c) using different techniques. According to the biodegradation pathway of BPA proposed by Ferro Orozco et al. (2016c), respirometric profiles mainly reflects the oxidation of BPA by mono and dioxygenases which are widely expressed in BPA-acclimated activated sludge. Moreover, even when the biodegradation kinetics of BPA is strongly affected by many factors (e.g., sludge age, acclimation degree, initial concentration), the oxygen consumed per unit of degraded BPA remains quite constant, regardless the experimental conditions. According to the above mentioned works, the consumption of oxygen during the metabolism of BPA by BPA-acclimated activated sludge is 1.5 ± 0.3 gO_2 $gBPA^{-1}$ (Ferro Orozco et al. 2016a, b, c). Considering that in the case of reactors with BPA (G2, A2, P2) the feed contained 40 mgBPA L^{-1}, the consumption of oxygen due to the utilization of BPA (OC_{BPA}) in these reactors was 60 ± 12 mgO_2 L^{-1}. Table 1 shows that the difference between measured OC values in reactors with BPA (OC_2) and OC_{BPA} were close to that obtained in reactors without the presence of the xenobiotic (OC_1). For this reason, it can be concluded that under the tested conditions, the presence of BPA did not affect the metabolism of the tested substrates. It is important to note that this conclusion is in accordance with the negligible effect of the presence of BPA on the metabolization rate of the tested biogenic substrates (Fig. 3).

Fitting the proposed model

For all the tested reactors, the model proposed in the present work (Eqs. 11–13) was fitted to the data depicted in Fig. 1. Fitting results are shown in Table 2. Figure 1 shows that the proposed model along with the coefficients depicted in Table 2 represents reasonably well the evolution of $TSS_{0(i)}$, $DOC_{0(i)}$, and $DOC_{F(i)}$ as a function of time for all the tested reactors.

Tables 1 and 2 show that as a general rule the higher $Y_{O/S}$ the lower Y_N. This trend is caused by the higher fraction of the substrate carbon that is oxidized to obtain energy. If more carbon is oxidized for energy then less is available for biomass growth. According to Ramdani et al. (2012), the carbon content of a typical activated sludge is 0.48 gC $gTSS^{-1}$. Combining this value with Y_N values depicted in Table 2, growth yields in terms of the fraction of the substrate carbon that is incorporated to the biomass carbon can be calculated. According to these calculations, about 44% of the glucose carbon was incorporated to the biomass while for peptone this fraction was only 22%.

The main effect of the presence of BPA was on the coefficient Y_{Sp}. The higher accumulation of products in reactors with BPA could be attributed to the higher decay rate of the biomass, to the formation of metabolic products associated with the metabolization of BPA, or both. In this sense, Table 2 shows that growth yield in reactors G1 and G2 were similar while in the case of reactors fed with acetate or peptone, the presence of BPA caused reductions of 20% and 16% in Y_N with respect to the growth yields obtained in reactors without the xenobiotic.

Table 2 Coefficients of the proposed model (Eqs. 11–13) corresponding to the tested reactors

Reactor	Y_N (gTSS gC^{-1})	f_{ads}	Y_{Sp} (gC gC^{-1})	RMSE[a] (mg L^{-1})		
				TSS_0	DOC_0	DOC_F
G1	0.92 ± 0.02	0.32 ± 0.01	0.010 ± 0.003	102	8.7	15.4
G2	0.90 ± 0.03	0.36 ± 0.06	0.073 ± 0.006	161	13.1	8.4
A1	0.69 ± 0.03	0.16 ± 0.02	0.035 ± 0.003	154	16.5	15.8
A2	0.55 ± 0.03	0.15 ± 0.10	0.138 ± 0.015	185	24.7	20.3
P1	0.51 ± 0.01	0.13 ± 0.01	0.045 ± 0.002	80	7.8	6.8
P2	0.43 ± 0.02	0.09 ± 0.03	0.075 ± 0.005	135	8.2	7.4

[a]Root mean square error

Comparison between microbial communities with different nutritional histories with regard to the acclimation and degradation of BPA

Results obtained in this work demonstrate that the compounds that a given microbial community has encountered in the past can play a crucial role in the response of the community to the presence of new substrates. Aromatic compounds comprise a wide variety of low-molecular-mass natural molecules, including several amino acids (e.g., phenylalanine, tryptophan, tyrosine) that are present in peptone.

Many authors reported that the main aerobic pathway for the metabolism of BPA comprises the sequential action of oxygenases to form 4-hydroxybenzaldehyde and 4-hydroxyacetophenone (Lobos et al. 1992; Spivack et al. 1994; Sakai et al. 2007; Fischer et al. 2010). Besides, a microbial community that grows in the presence of natural aromatic compounds (e.g. amino-acids from peptone) already has the oxygenases to initiate the degradation of other aromatic compounds (Hu et al. 2005). For this reason, phenol has been widely used as co-substrate to enhance the degradation of different phenolic compounds (Aktas 2012; Tobajas et al. 2012). In this sense, the presence of aromatic intermediates as a result of the aromatic amino acids degradation that were present in peptone could promote a faster acclimation process and degradation of BPA.

The presence of BPA as the sole carbon source implies the acclimation of the entire microbial community to the xenobiotic. After acclimation, the microbial population will be composed by those bacteria which possessed a very specific degradation capability. When biogenic substrates (such as glucose, acetate or peptone) and xenobiotics are both present, biogenic compounds are preferred as the carbon and energy source for growth. For this reason, a substantial fraction of the biomass is adapted to use readily biodegradable substrates and only a small fraction is responsible for the removal of the xenobiotic compound. Besides, microorganisms acclimated to the xenobiotic could also have the capability to degrade the biogenic substrate and vice versa. In this context, the degradation of BPA could be modified and reduced, when compared with results obtained in the absence of a biogenic substrate. Because industrial wastewaters are usually comprised of mixtures of xenobiotic compounds and readily biodegradable substrates, the study of the concomitant degradation of these types of compounds is a more realistic approach in order to apply the obtained results to actual wastewater treatment systems.

Conclusions

In the present work, the acclimation and concurrent biodegradation of BPA and different biogenic substrates (glucose, acetate or peptone) by activated sludge was studied using SCRs. Besides, a mathematical model to represent the evolution of $TSS_{0(i)}$, $DOC_{0(i)}$, and $DOC_{F(i)}$ as a function of the operation cycle was proposed.

All the SCRs fed with the tested substrates could remove BPA. However, the activated sludge from a reactor that was fed with acetate exhibited a quite long acclimation period to BPA in comparison with SCRs fed with glucose or peptone. Although the reactor fed with peptone attained the lower biomass concentration, this biomass had the higher specific BPA degradation activity in comparison to the reactors fed with glucose or acetate. Besides, according to the measured specific removal biogenic substrate rates and to respirometric measurements it can be concluded that the presence of BPA had a negligible effect on the metabolism of all the tested biogenic substrates.

The model proposed in the present work adequately represented the evolution of $TSS_{0(i)}$, $DOC_{0(i)}$, and $DOC_{F(i)}$ as a function of time for all the tested reactors. According to the proposed model, the main effect of the presence of BPA was on the generation of microbial soluble products that increased the DOC concentration at the end of each operation cycle. This work helps to understand the interrelationship between the biodegradation of BPA and readily biodegradable substrates such as glucose, acetate or peptone.

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Appendix

See Fig. 6.

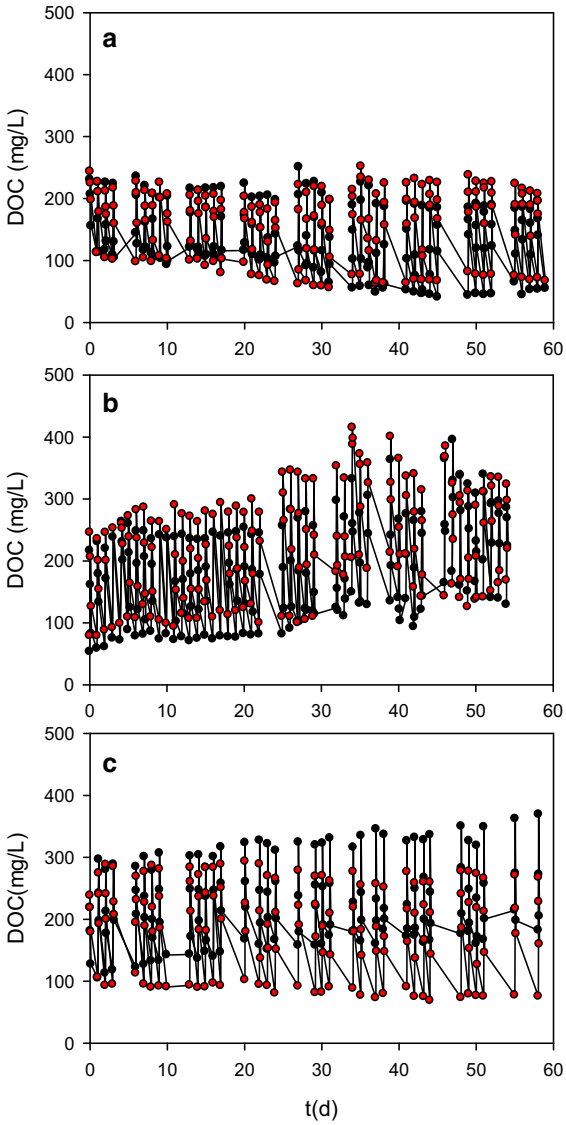

Fig. 6 Evolution of DOC concentration as a function of time corresponding to the reactors fed with glucose (**a**), acetate (**b**) and peptone (**c**). Black symbols represent reactors without BPA (G1, A1, P1), red symbols correspond to reactors with BPA (G2, A2, P2)

References

Buitron G, Gonzales A (1996) Characterization of the microorganisms from an acclimated activated sludge degrading phenolic compounds. Water Sci Technol 34:289–294

Cokgor EU, Insel G, Katipoglu T, Orhon D (2011) Biodegradation kinetics of peptone and 2,6-dihydroxy benzoic acid by acclimated dual microbial culture. Biores Technol 102:567–575

Contreras EM, Ferro Orozco AM, Zaritzky NE (2011) Biological Cr(VI) removal coupled with biomass growth, biomass decay, and multiple substrate limitation. Water Res 45:3034–3046

Eiler A, Langenheder S, Bertilsson S, Tranvik LJ (2003) Heterotrophic bacterial growth efficiency and community structure at different natural organic carbon concentrations. Appl Environ Microbiol 69:3701–3709

Ferro Orozco AM, Lobo CC, Contreras EM, Zaritzky NE (2013) Biodegradation of bisphenol-A (BPA) in activated sludge batch reactors: analysis of the acclimation process. Int Biodeterior Biodegrad 85:392–399

Ferro Orozco AM, Contreras EM, Zaritzky NE (2015) Simultaneous biodegradation of bisphenol A and biogenic substrate in semi-continuous activated sludge reactors. Biodegradation 26:183–195

Ferro Orozco AM, Contreras EM, Zaritzky NE (2016a) Biodegradation of Bisphenol A and its metabolic intermediates by activated sludge: stoichiometry and kinetics analysis. Int Biodeterior Biodegrad 106:1–9

Ferro Orozco AM, Contreras EM, Zaritzky NE (2016b) Monitoring the biodegradability of bisphenol A and its metabolic intermediates by manometric respirometry tests. Biodegradation 27:209–221

Ferro Orozco AM, Contreras EM, Zaritzky NE (2016c) Factors affecting the degradation of Bisphenol A by activated sludge. A.M. Lambert Academic Publishing. OmniScriptum GmbH & Co. KG. Germany, p 73. ISBN: 978-3-659-86581-7

Fischer J, Kappelmeyer U, Kastner M, Schauer F, Heipieper HJ (2010) The degradation of Bisphenol A by the newly isolated bacterium *Cupriavidus basilensis* JF1 can be enhanced by biostimulation with phenol. Int Biodeterior Biodegrad 64:324–330

Grady CPL Jr, Cordone L, Cusack L (1993) Effects of media composition on substrate removal by pure and mixed bacterial cultures. Biodegradation 4:23–38

Hernández MA, Mohn WW, Martínez E, Rost E, Alvarez AF, Alvarez HM (2008) Biosynthesis of storage compounds by *Rhodococcus jostii* RHA1 and global identification of genes involved in their metabolism. BMC Genom 9:1–13

Hu Z, Ferraina RA, Ericson JF, Smets BF (2005) Effect of long-term exposure, biogenic substrate presence, and electron acceptor conditions on the biodegradation of multiple substituted benzoates and phenolates. Water Res 39:3501–3510

Insel G, Celikyilmaz G, Ucisik-Akkaya E, Yesiladali K, Cakar ZP, Tamerler C, Orhon D (2007) Respirometric evaluation and modeling of glucose utilization by *Escherichia coli*

under aerobic and mesophilic cultivation conditions. Biotechnol Bioeng 96:94–105

Kamaraj M, Sivaraj R, Venckatesh R (2014) Biodegradation of bisphenol A by the tolerant bacterial species isolated from coastal regions of Chennai, Tamil Nadu, India. Int Biodeterior Biodegrad 93:216–222

Kang JH, Kondo F (2002) Bisphenol A degradation by bacteria isolated from river water. Arch Environ Contam Toxicol 43:265–269

Li K, Wei D, Zhang G, Shi L, Wang Y, Wang B, Wang X, Du B, Wei Q (2015) Toxicity of bisphenol A to aerobic sludge in sequencing batch reactors. J Mol Liq 209:284–288

Lobo CC, Bertola NC, Contreras EM (2013) Stoichiometry and kinetic of the aerobic oxidation of phenolic compounds by activated sludge. Biores Technol 136:58–65

Lobo CC, Bertola NC, Contreras EM (2014) Error propagation in open respirometric assays. Braz J Chem Eng 31:303–312

Lobo CC, Bertola NC, Contreras EM (2016) Approximate expressions of a SBR for wastewater treatment: comparison with numeric solutions and application to predict the biomass concentration in real cases. Process Saf Environ Prot 100:65–73

Lobos HJ, Leib TK, Su T-M (1992) Biodegradation of Bisphenol A and other bisphenols by a Gram-negative aerobic bacterium. Appl Environ Microbiol 58:1823–1831

Majone M, Dircks K, Beun JJ (1999) Aerobic storage under dynamic conditions in activated sludge processes. The state of the art. Water Sci Technol 39:61–73

Matsumura Y, Hosokawa C, Sasaki-Mori M, Akahira A, Fukunaga K, Ikeuchi T (2009) Isolation and characterization of novel bisphenol A degrading bacteria from soils. Biocontrol Sci 14:161–169

Mielczarek AT, Kragelund C, Eriksen PS, Nielsen PH (2012) Population dynamics of filamentous bacteria in Danish wastewater treatment plants with nutrient removal. Water Res 46:3781–3795

Mielczarek AT, Nguyen HTT, Nielsen JL, Nielsen PH (2013) Population dynamics of bacteria involved in enhanced biological phosphorus removal in Danish wastewater treatment plants. Water Res 47:1529–1544

Modaressi K, Taylor KE, Bewtra JK, Biswas N (2005) Laccase-catalyzed removal of bisphenol-A from water: protective effect of PEG on enzyme activity. Water Res 39:4309–4316

Novak L, Larrea L, Wanner J (1995) Mathematical model for soluble carbonaceous substrate biosorption. Water Sci Technol 31:67–77

Orhon D, Cokgor EU, Insel G, Karahan O, Katipoglu T (2009) Validity of Monod kinetics at different sludge ages—Peptone biodegradation under aerobic conditions. Biores Technol 100:5678–5686

Orhon D, Cokgor EU, Katipoglu T, Insel G, Karahan O (2010) Fate of 2,6-dihydroxybenzoic acid and its inhibitory impact on the biodegradation of peptone under aerobic conditions. Biores Technol 101:2665–2671

Oshiman K, Tsutsumi Y, Nishida T, Matsumura Y (2007) Isolation and characterization of a novel bacterium, *Sphingomonas bisphenolicum* strain AO1, that degrades bisphenol A. Biodegradation 18:247–255

Pinhassi J, Azam F, Hemphala J, Long RA, Martinez J, Zweifel UL, Hagstrom A (1999) Coupling between bacterioplankton species composition, population dynamics, and organic matter degradation. Aquat Microb Ecol 17:13–26

Rensink JH, Donker HJGW (1991) The effect of contact tank operation on bulking sludge and biosorption processes. Water Sci Technol 23:857–866

Sakai K, Yamanaka H, Moriyoshi K, Ohmoto T, Ohe T (2007) Biodegradation of bisphenol A and related compounds by *Sphingomonas* sp. Strain BP-7 isolated fron seawater. Biosci Biotechnol Biochem 71:51–57

Spivack J, Leib TK, Lobos JH (1994) Novel pathway for bacterial metabolism of bisphenol A. Rearrangements and stilbene cleavage in bisphenol A metabolism. J Biol Chem 269:7323–7329

Staples CA, Dorn PB, Klecka GM, O'Block ST, Harris LR (1998) A review of the environmental fate, effects, and exposures of bisphenol A. Chemosphere 36:2149–2173

Tobajas M, Monsalvo VM, Mohedano AF, Rodriguez JJ (2012) Enhancement of cometabolic biodegradation of 4-chlorophenol induced with phenol and glucose as carbon sorces by *Comamonas testosteroni*. J Environ Man 95:116–121

Urase T, Kikuta T (2005) Separate estimation of adsorption and degradation of pharmaceutical substances and estrogens in the activated sludge process. Water Res 39:1289–1300

Van Hannen EJ, Mooij W, Van Agterveld MP, GonsHJ Laanbroek HJ (1999) Detritus-dependent development of microbial community in an experimental system: qualitative analysis by denaturing gradient gel electrophoresis. Appl Environ Microbiol 65:2478–2484

Yang Y, Wang Z, Xie S (2014) Aerobic biodegradation of bisphenol A in river sediment and associated bacterial community change. Sci Total Environ 471:1184–1188

Zhang WW, Yin K, Chen LX (2013) Bacteria-mediated bisphenol A degradation. Appl Microbiol Biotechnol 97:5681–5689

Zhao J, Li Y, Zhang C, Zeng Q, Zhou Q (2008) Sorption and degradation of bisphenol A by aerobic activated sludge. J Hazard Mater 155:305–311