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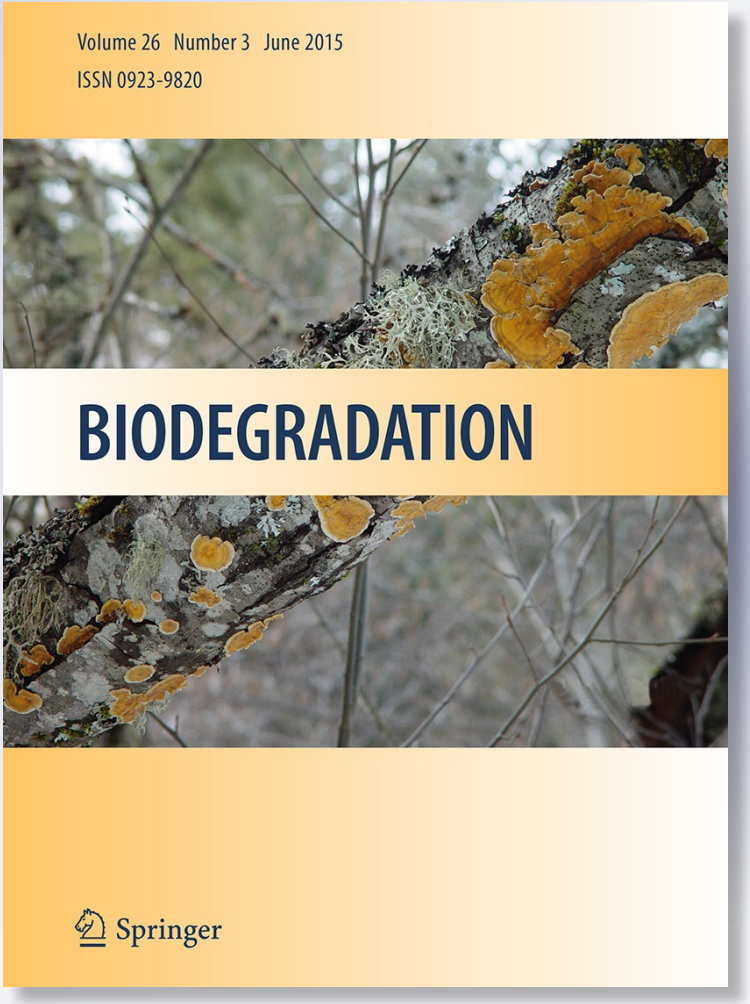

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ORIGINAL PAPER

Simultaneous biodegradation of bisphenol A and a biogenic substrate in semi-continuous activated sludge reactors

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Abstract In this work, the simultaneous degradation of BPA and cheese whey (CW) in semi-continuous activated sludge reactors was studied. The acclimation process and microbial growth on BPA, CW and BPA + CW were analyzed. In addition, the effect of increasing CW concentration on the BPA degradation by acclimated activated sludge was also studied. In order to reduce the factors involved in the analysis of the simultaneous degradation of BPA and CW, the effect of bisphenol A (BPA) on activated sludge not previously exposed to BPA (native activated sludge) was studied. Results demonstrate that BPA concentrations lower than 40 mg l^{-1} had a negligible effect on the growth of native activated sludge. In the semi-continuous reactors, the presence of CW increased the acclimation time to 40 mg l^{-1} of BPA. Once the capability of degrading BPA was acquired, the removal of BPA was not affected by the presence of CW. Increasing the CW concentration did not affect the removal of BPA by the acclimated activated sludge. Additionally, the CW consumption was not modified by the presence of BPA. Kinetic and stoichiometric coefficients reported in the present work can be useful in developing mathematical models to describe the simultaneous aerobic biodegradation of a biogenic substrate, such as CW, and BPA by activated sludge.

Keywords Bisphenol A · Activated sludge · Acclimation · Growth yield

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Introduction

Endocrine disrupting compounds (EDCs) are chemicals that mimic or inhibit the actions of endogenous hormones, and they have the potential to alter the structure and function(s) of the endocrine system (Patisaul and Adewale 2009). One of these EDCs widely found in the environment is bisphenol A (BPA). Animal studies have shown that in utero exposure to BPA produces prenatal and postnatal adverse effects on multiple tissues, including the brain (Richter et al. 2007). Prenatal BPA exposure affects brain development, sexual differentiation, social and anxiety-like behavior, and learning/memory (Kundakovic and Champagne 2011). In humans, emerging

evidence for BPA-associated disruption of neurodevelopment is consistent with the rodent data and has revealed sex-specific effects of gestational BPA levels on emotional regulation and aggression in children (Braun et al. 2009, 2011; Perera et al. 2012).

Despite its potential as an endocrine disruptor, BPA was widely used in the preparation of a wide range of products, including baby bottles, food-storage containers and medical equipment (Benuchour and Aris 2009). Also, BPA is currently used in the production of polycarbonate and epoxy resins in industry and households. As a result, BPA has become one of the most ubiquitous contaminants in the environment (Zhao et al. 2008), and it is very frequently detected in municipal and industrial wastewaters (Stasinakis et al. 2008b).

Although several physicochemical methods to remove BPA from wastewaters have been proposed (Torres et al. 2007; Li et al. 2013; Umar et al. 2013; Yüksel et al. 2013), increasing evidence demonstrates that this xenobiotic can also be effectively removed by biological treatments (Melcer and Klecka 2011; Zhou et al. 2013; Omoike et al. 2013; Yang et al. 2013). One of the main problems regarding the biodegradation of xenobiotics, such as BPA, is that their molecular structure often differs from that of natural microbial substrates, hindering its biodegradation (Chong et al. 2008). Acclimation of a microbial community to a xenobiotic involves the selection of those microorganisms containing existing enzymes and pathways or development of new catabolic pathways for the xenobiotic (Hu et al. 2005). In the case of BPA, the time required to acclimate an activated sludge to this xenobiotic compound could take some days (Ferro Orozco et al. 2013). During this time, in which no BPA consumption occurs, the microbial community is in contact with a xenobiotic concentration that could be toxic, leading to reductions in the performance of the reactor. On the other hand, industrial wastewater treatment plants generally receive effluents comprised of a mixture of xenobiotics and readily biodegradable compounds in different proportions. As a general rule, a given specific competent fraction of the microbial community (e.g., the acclimated microorganisms) is mainly responsible for the removal of an individual xenobiotic. However, this concept does not exclude the possibility that microorganisms acclimated to the xenobiotic could also have the capability to degrade biogenic compounds or primary substrates. In this context, the degradation of BPA could be modified by the presence of readily biodegradable substrates.

Many studies have reported the concomitant degradation of biogenic and xenobiotic substrates. Biomass growing on mixed substrates may display sequential (diauxic) or parallel (concurrent) substrate utilization (Hu et al. 2005). Besides, biomass growing in a multiple substrate environment may also display xenobiotic removal by fortuitous metabolism or cometabolism. Because beneficial and adverse effects of biogenic substrates on xenobiotic degradation have been reported (Moorman et al. 2001; Lee 2003; Zhao et al. 2008; Chong et al. 2012), the actual behavior depends on the case studied. For this reason, the objective of the present work was to study the simultaneous degradation of BPA and a biogenic substrate CW (cheese whey) in semi-continuous activated sludge reactors. The acclimation process to BPA and the microbial growth on BPA, CW and BPA + CW were analyzed. In this context, it was necessary to employ a higher than ambient BPA concentration, which allowed observing significant bacterial growth and had no toxic effect on the bacterial community. Thus, in order to find the appropriate BPA concentration to be used in semi-continuous reactors, the effect of BPA on activated sludge not previously exposed to BPA (native activated sludge) was studied. In addition, the effect of the CW concentration on the BPA degradation by acclimated activated sludge was also studied.

Materials and methods

Biological and chemical materials

Bisphenol A (≥99 %) was purchased from Sigma-Aldrich. CW was from Food S.A. (Villa Maipú, Argentina). All other reagents used in the present work were commercial products of reagent grade from Sigma-Aldrich. Activated sludge used in all the experiments was harvested from an aerobic laboratory-scale (4.5 l) activated sludge reactor with partial biomass recycling. The sludge age was maintained at 30 days by daily wasting of the mixed liquor directly from the reactor. The hydraulic retention time was 48 h. The reactor was fed with a synthetic wastewater, which contained dehydrated CW as the sole carbon source (Ferro Orozco et al. 2010): CW 1.5 g (1500 mg

COD l^{-1}), $(NH_4)_2SO_4$ 0.5 g and $NaHCO_3$ 1.03 g, all dissolved in 1 l of tap water. Operating temperature of the bioreactor was 20 ± 2 °C. 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}, the pH was 7.5 ± 0.4, the COD of the effluent ranged from 30 to 80 mg COD l^{-1}, and the total suspended solid (TSS) concentration ranged from 3700 to 4500 mgTSS l^{-1}. Throughout the present work, activated sludge that was not exposed to BPA was called native activated sludge (NAS).

Effect of BPA concentration on the native activated sludge growth kinetics

In a first set of experiments, the effect of BPA on activated sludge not previously exposed to BPA (native activated sludge) was studied. The inoculum for these experiments was conditioned as follows. A sample of the mixed liquor from the aerobic laboratory-scale activated sludge reactor described in "Biological and chemical materials" section was placed in an aerated vessel (1 l) that contained the following culture medium: CW 0.5 g l^{-1}, $(NH_4)_2SO_4$ 0.5 g l^{-1}, KH_2PO_4 2 g l^{-1}, K_2HPO_4 0.6 g l^{-1} and 1 ml l^{-1} of micronutrient solutions M1 and M2, respectively. The composition of M1 was (g/100 ml): $FeSO_4{\cdot}7H_2O$ 1.5, $ZnSO_4{\cdot}7H_2O$ 0.5, $MnSO_4{\cdot}H_2O$ 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 and KI 0.01. The vessel was aerated until dissolved organic carbon (DOC) values showed that the biogenic substrate was exhausted. Then, the biomass was harvested by sedimentation and washed with phosphate buffer (KH_2PO_4 2 g l^{-1}, K_2HPO_4 0.6 g l^{-1}, pH = 7.0). The washed biomass was re-suspended in fresh culture medium to serve as the inoculum for the next batch. Four consecutive batch assays were performed using this procedure to ensure that the biomass was growing actively, reducing the lag phase extension to a few hours.

The effect of the initial BPA concentration on the native activated sludge growth kinetics was studied in 500-ml aerated vessels that contained the following culture medium: BPA (0–100 mg l^{-1}), CW (6 g l^{-1}), $(NH_4)_2SO_4$ (1.5 g l^{-1}), KH_2PO_4 (2 g l^{-1}), K_2HPO_4 (0.6 g l^{-1}) and 1 ml l^{-1} of micronutrient solutions M1 and M2, respectively. Then, appropriate volumes of the above-mentioned conditioned inoculum were added to achieve an initial TSS concentration of about 900 mgTSS l^{-1}. At predetermined intervals, samples were taken from the vessels to measure the biomass (as TSS), biogenic substrate (as DOC) and BPA concentrations. Although CW and BPA contributed to the DOC values, under the tested conditions the contribution of BPA was negligible with respect to CW. The carbon contents of CW and BPA are 0.40 and 0.79 gC/g, respectively. Considering that the initial CW concentration was 6 g l^{-1} and the higher tested initial concentration of BPA was 100 mg l^{-1}, contributions of CW and BPA to the initial DOC value were 2400 and 79 mgC l^{-1}, respectively. For this reason, in all cases the DOC values mainly reflected the CW concentration.

Simultaneous biodegradation of BPA and CW in semi-continuous reactors

Three semi-continuous reactors containing the previously described conditioned biomass (see "Effect of BPA concentration on the native activated sludge growth kinetics" section) were filled with the same mineral basal medium but with different carbonaceous substrates. Reactor A contained BPA (40 mg l^{-1}) as the sole carbonaceous substrate; reactor B was fed with CW (500 mg l^{-1}) as the sole carbon source; in reactor C, a mixture of BPA (40 mg l^{-1}) and CW (500 mg l^{-1}) was tested. In all cases, 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 semi-continuous reactors were continuously aerated until the carbonaceous substrates had been depleted. Then, the next operation cycle started with the addition of the specific substrates corresponding to each reactor (BPA, CW and BPA + CW for reactors A, B and C, respectively). Due to the biomass growth, in some cases the total ammonia nitrogen (TAN) concentration decreased below 40 mg N l^{-1}; thus, to avoid the limitation of growth by low nitrogen concentrations, appropriate amounts of solid $(NH_4)_2SO_4$ were added to obtain an initial TAN concentration of about 120 mg N l^{-1}. After 15 days of operation, carbonaceous substrates corresponding to reactors A and C were exchanged. Reactor A (initially fed with BPA) was fed with the mixture BPA + CW; reactor C (initially fed with the

mixture BPA + CW) was fed with BPA. During each cycle, samples were taken at different times to measure BPA, DOC, TAN and TSS concentrations.

Effect of the biogenic substrate concentration on the degradation of BPA by acclimated activated sludge

The effect of the biogenic substrate concentration on the biodegradation of BPA by acclimated activated sludge was studied in 500-ml batch reactors. The composition of the culture media used in the batch assays was as follows: 1, 3 or 6 gCW l^{-1}, 1.5 g$(NH_4)_2SO_4$ l^{-1}, 2 g K_2HPO_4 l^{-1}, 0.6 g KH_2PO_4 l^{-1} and 1 ml l^{-1} of micronutrient solutions M1 and M2, respectively. In all cases the initial BPA concentration was 40 mg l^{-1}. For each tested CW concentration, three batch assays were conducted for comparison purposes: (1) with BPA in the absence of CW, (2) with CW in the absence of BPA and (3) with BPA and CW. The inoculum of these assays was obtained from the semi-continuous reactor C described in the "Simultaneous biodegradation of BPA and CW in semi-continuous reactors" section. At predetermined intervals, samples were taken from the batch reactors to determine the BPA, DOC, TAN and TSS concentrations.

Analytical techniques

TSSs were used to monitor the biomass concentration (Contreras et al. 2011). The dissolved organic carbon (DOC) concentration was determined as follows: 5 ml of culture samples was centrifuged for 5 min at 13,000 rpm (Eppendorf 5415C); then, the supernatant was filtered through 0.45-μm cellulosic membranes (Osmonics Inc.). The DOC concentration of the filtered samples was determined in a Shimadzu DOC-Vcpn analyzer. The total ammonia nitrogen (TAN) concentration of the filtrate was measured by the Nessler colorimetric method using commercial reagents (Hach Company, Loveland, CO). The 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.

Results and discussion

Effect of BPA on the growth kinetics of native activated sludge

Batch experiments were performed to analyze the effect of the BPA concentration on the growth kinetics of native activated sludge. Figure 1 shows the consumption of the biogenic substrate (CW) and the biomass concentration as a function of time in the presence of different initial BPA concentrations. Regardless of the BPA concentration, no significant lag phase was observed; in all cases, the increase of the biomass concentration and consumption of the biogenic substrate started about 2 h after the addition of the conditioned inoculum. Then, after 35–40 h, the biogenic substrate was depleted and the biomass concentration decreased because of the endogenous decay process; additionally, a slight increase of the DOC values as a function of time due to the release of inert soluble products was observed (Contreras et al. 2011).

For all the tested initial BPA concentrations, the observed specific growth rate (μ) was calculated from the slope of the linear part of the plot of ln (TSS) as a function of time. The biomass yield on CW ($Y_{X/CW}$) was obtained from the slope of TSS as a function of DOC. Then, the specific biogenic substrate consumption rate (q_S) was evaluated as the ratio between μ and $Y_{X/CW}$. Table 1 shows that the increase of the initial BPA concentration caused a slight decrease in μ, $Y_{X/CW}$ and q_S. For example, μ, $Y_{X/CW}$ and q_S values in the presence of the maximum tested BPA concentration were 82, 93 and 88 % of the values obtained in the absence of BPA, respectively. In all cases, the BPA concentration remained almost constant within the first 100 h (Fig. 2), suggesting that the acclimation of the biomass to BPA did not take place during that time.

The effect of BPA on the microbial growth is under discussion. Reported results suggest that the effect of BPA is strongly dependent on several factors, such as the BPA concentration, presence of biogenic substrates and tested species. For example, Omoike et al. (2013) demonstrated that the growth of *Heliscus lugdunensis* (an aquatic fungus) on glucose is not inhibited by the

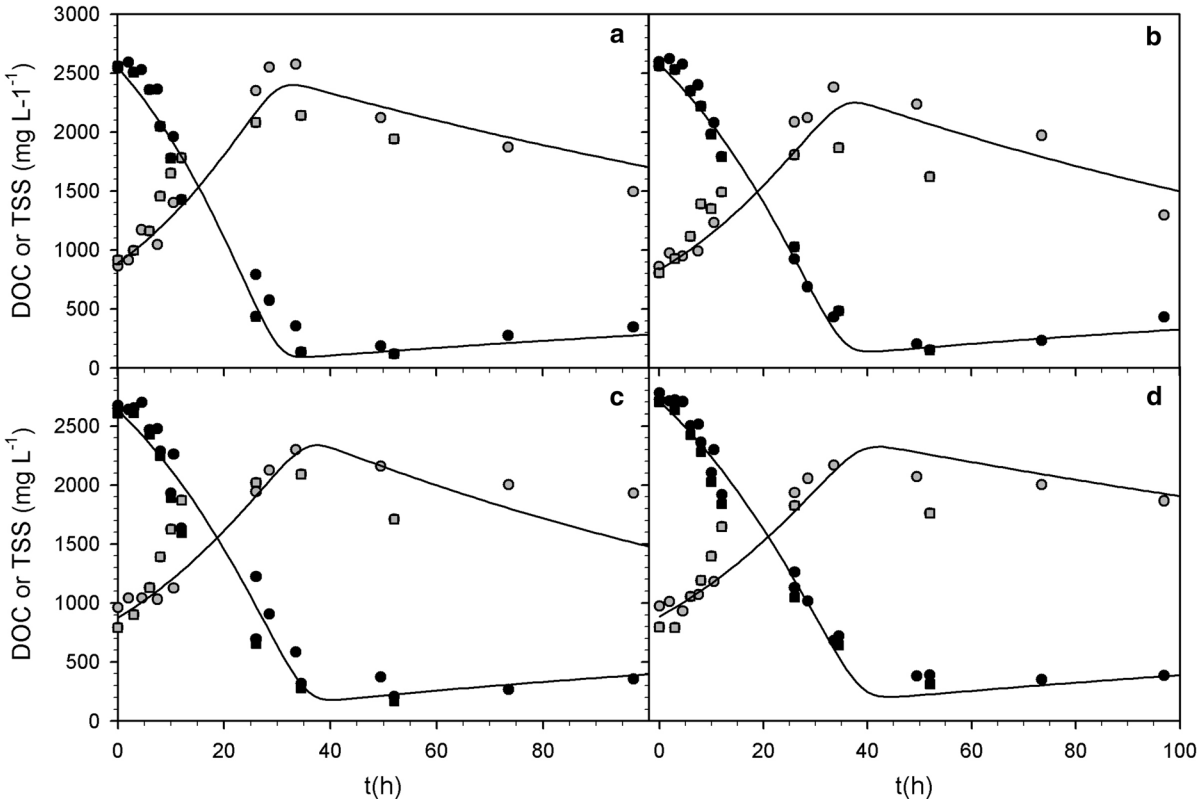

Fig. 1 Consumption of the biogenic substrate (DOC, *black symbols*) and biomass growth (TSS, *gray symbols*) as a function of time under different initial BPA concentrations (in mg/l): **a** 0, **b** 25, **c** 50 and **d** 100. *Squares* and *circles* represent two independent experiments. *Continuous lines* represent trend lines

Table 1 Effect of BPA on the growth kinetics of activated sludge on cheese whey

BPA (mg l^{-1})	μ (h^{-1})	$Y_{X/CW}$ (mgTSS $mgDOC^{-1}$)	q_S (mgDOC $mgTSS^{-1}$ h^{-1})
0	0.040 ± 0.002	0.630 ± 0.040	0.063 ± 0.003
25	0.035 ± 0.002	0.600 ± 0.040	0.058 ± 0.002
50	0.034 ± 0.002	0.614 ± 0.050	0.056 ± 0.003
100	0.030 ± 0.001	0.576 ± 0.040	0.052 ± 0.002

presence of 10 mgBPA l^{-1}. However, toxicity caused by high BPA concentrations was reported, even for microbial cultures that were previously acclimated to this xenobiotic compound. Yamanaka et al. (2007) reported that several strains of *Bacillus pumilus* showed neither degradation activity nor cell growth in the presence of 100 mgBPA l^{-1}. Zhang et al. (2007) also reported that the BPA degrading activity and cell growth of *Achromobacter xylosoxidans* were inhibited at high BPA concentrations (50 mg l^{-1}). In the present work, even the presence of 100 mgBPA l^{-1} during 100 h caused only a slight inhibition of the growth of activated sludge. These results could be explained considering that a bacterial consortium, such as the tested activated sludge, presents a large microbial diversity with broad physiological capabilities in comparison with a pure culture. For these reasons, activated sludge was more tolerant to higher BPA concentrations than pure cultures.

Simultaneous biodegradation of BPA and CW in semi-continuous reactors

According to the results reported in "Effect of BPA on the growth kinetics of native activated sludge"

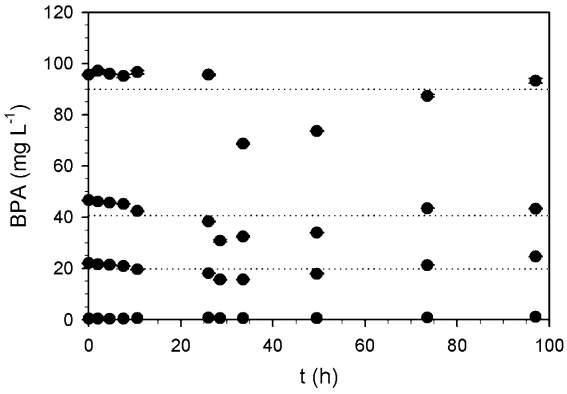

Fig. 2 Concentration of BPA as a function of time corresponding to the different tested initial BPA concentrations. In each case, *dotted lines* indicate the average value

section, a BPA concentration of 40 mg l^{-1} had a negligible effect on the growth of native activated sludge. For this reason, this BPA concentration was selected for the study of the simultaneous biodegradation of BPA and CW in semi-continuous reactors. Three semi-continuous reactors (A–C) were used to study the simultaneous degradation of BPA and a biogenic substrate (CW) by native activated sludge. These reactors were inoculated with the conditioned activated sludge described in "Effect of BPA concentration on the native activated sludge growth kinetics" section. The experiment consisted of two phases. During the first 15 days (phase I), reactor A was fed with a mineral basal medium with BPA (40 mg l^{-1}) as the sole carbonaceous substrate; reactor C was fed with a mixture of BPA (40 mg l^{-1}) and CW (500 mg l^{-1}). After 15 days, the feeding corresponding to reactors A and C were exchanged; thus, in phase II reactor A received the mixture BPA + CW, while reactor C was fed with BPA only. These conditions were maintained for 10 days. Reactor B was fed with CW (500 mg l^{-1}) as the sole carbon source for 25 days; this reactor was considered the control experiment.

Figure 3a shows that during phase I, regardless of the presence of CW, after about 3–4 days of operation, the activated sludge acquired the capability to degrade BPA. In the absence of CW, BPA was exhausted in 5 days (reactor A). Conversely, under the presence of CW, about 8 days were necessary to achieve a BPA consumption of about 95 % (reactor C). Then, subsequent additions of BPA to reactors A and C were depleted within 24 h. Figure 3b–d shows that regardless of the presence of BPA, DOC consumption, biomass production and TAN uptake corresponding to reactors B and C were similar. These results suggested that the presence of CW did not affect the removal of BPA.

Although BPA was actively consumed in reactor A (Fig. 3a), Fig. 3c, d shows that within the first 2 days of phase I, the biomass concentration decreased and TAN increased, indicating that the decay of non-acclimated microorganisms to BPA prevailed over the growth of acclimated ones. The lowest value corresponding to biomass concentration was 460 mgTSS l^{-1} at day 3; then, the TSS slowly increased up to 995 mg l^{-1} at day 15. Moreover, from day 3 to 15, a gradual decrease of TAN values was observed. These results suggested that once the acclimation was achieved, activated sludge could use BPA as the sole carbon and energy source.

Phase II comprised the last 10 days of the operation (Fig. 3). During this phase, the feed of reactor A was changed from a culture medium with BPA as the sole carbonaceous substrate to a mixture of BPA and CW. The addition of CW to reactor A did not affect the removal of BPA (Fig. 3a). Although during phase I reactor A was initially fed with a culture medium devoid of CW, the consumption of CW (Fig. 3b) started almost immediately after the addition of the mixture BPA + CW (phase II). Then, subsequent additions of the mixture BPA + CW determined the increase of the biomass concentration (Fig. 3c) and the corresponding assimilation of the nitrogen source (Fig. 3d). Note that the obtained results regarding CW and TAN consumptions and biomass production in reactor A during phase II were close to those corresponding to reactor B and to reactor C in phase I, confirming that the presence of BPA has a negligible effect on the growth of activated sludge on CW.

With regard to reactor C, during phase II the feed was changed from a medium with BPA + CW to BPA alone. Figure 3a shows that the removal of BPA was not affected by the absence of CW. However, a slight decrease of the biomass concentration (Fig. 3c) and a release of nitrogen (Fig. 3d) due to the endogenous decay process during phase II in reactor C were observed. Although these results seem to contradict those obtained from reactor A in phase I (e.g., a slight increase of biomass concentration and TAN consumption), it must be considered that during phase I, the biomass concentration in reactor C increased because

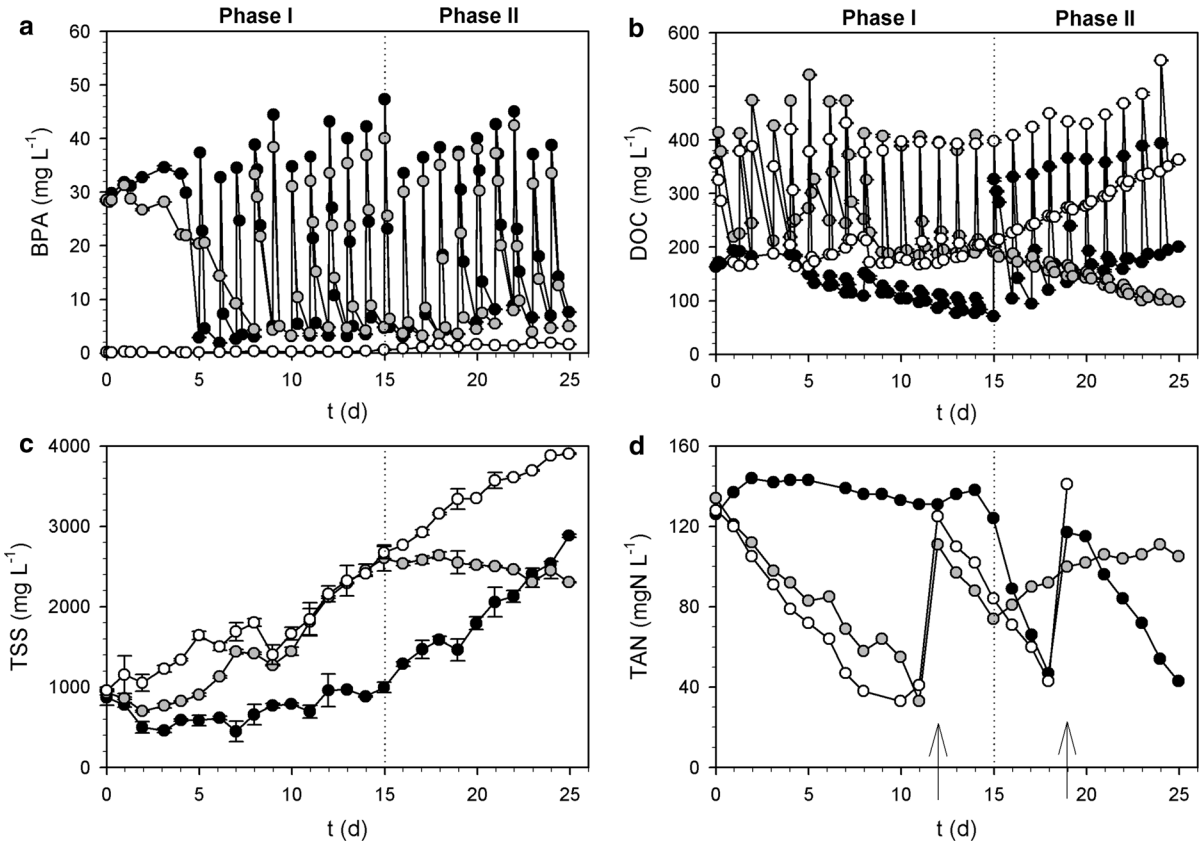

Fig. 3 Evolution of **a** BPA, **b** DOC, **c** TSS and **d** TAN as a function of time corresponding to reactors A (*black*), B (*white*) and C (*gray*). *Dotted lines* indicate the time when feeds corresponding to rectors A and C were exchanged. *Arrows* in **d** indicate new additions of the nitrogen source

of the repeated additions of CW (500 mg l^{-1}) and BPA (40 mg l^{-1}). Taking into account the carbon content of CW (0.40 gC/gCW) and BPA (0.79 gC/gBPA), CW represented 87 % of the total carbon of the feed. According to several authors, the wastewater composition is a key factor that determines the population structure of mixed cultures, such as activated sludge (Grady et al. 1993; Mielczarek et al. 2012, 2013). In this sense, the biomass in reactor C at the end of phase I could be composed mainly of microorganisms that use CW as the carbon source. When reactor C was fed with BPA alone (phase II), the decrease of the biomass concentration and release of nitrogen (Fig. 3c, d) suggest that BPA alone could not sustain the growth of the fraction of activated sludge that utilized CW as the carbon and energy source.

The biomass yield on CW ($Y_{X/CW}$) was calculated using the results obtained from the semi-continuous reactor B. Because this reactor was fed with repeated additions of 500 mg l^{-1} of CW, the low initial CW concentration also produced a low increase of the biomass concentration in comparison with the biomass concentration at the beginning of a given batch. Taking into account the experimental errors corresponding to the measurements of biomass and DOC concentrations, $Y_{X/CW}$ values calculated as the ratio between biomass production and DOC consumption during a single batch were quite variable. Instead, a more robust method developed by Lobo et al. (2013) was used; according to these authors, $Y_{X/CW}$ can be obtained from the plot of the TSS concentration as a function of the cumulative DOC consumption (ΔDOC) (Fig. 4a). Using this procedure, the obtained $Y_{X/CW}$ value was 0.66 $\pm$ 0.03 mgTSS $gDOC^{-1}$, which was close to that obtained from the growth of non-acclimated activated sludge in the absence of BPA (Fig. 1a; Table 1). Figure 3d shows that due to the biomass growth in reactor B, the nitrogen source was

actively consumed. From the plot of the TAN concentration as a function of TSS (Fig. 5), the nitrogen content of the suspended solids ($i_{N,TSS}$) in reactor B was $i_{N,TSS} = 89 \pm 7$ mg N $gTSS^{-1}$. According to Ramdani et al. (2012), when an activated sludge is fed with a biodegradable substrate as the sole carbon source, a typical value for the nitrogen content of the biomass is 86 mgN $gTSS^{-1}$. Thus, the agreement between the values obtained in the present work with those reported by Ramdani et al. (2012) confirms that CW was used as the carbon source for the growth of activated sludge.

Using the data obtained from the semi-continuous reactors that were fed with BPA alone (e.g., reactor A, phase I; reactor C, phase II), the biomass yield on BPA ($Y_{X/BPA}$) can be evaluated. However, because BPA could not sustain the growth of microorganisms that use CW as the carbon source, the decay of these microorganisms in the absence of CW in reactor C during phase II masked the growth of BPA-acclimated

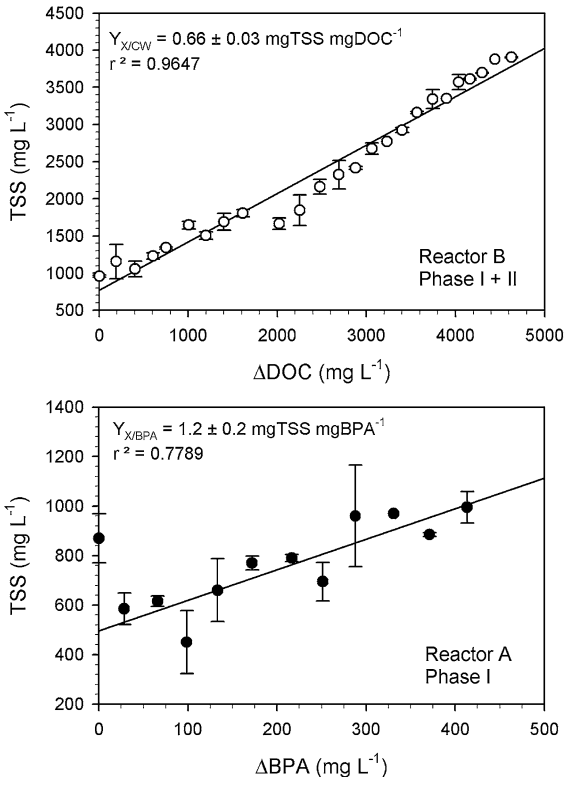

Fig. 4 Calculation of the growth yield on CW ($Y_{X/CW}$) and on BPA ($Y_{X/BPA}$). *Bars* indicate the standard deviation of TSS values. Yields were obtained from the slope of the *straight lines*

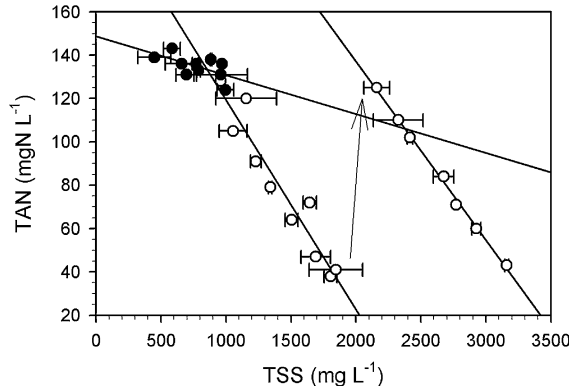

Fig. 5 Total ammonia nitrogen (TAN) consumption as a function of the total suspended solid (TSS) concentration in reactor A during phase I (*black symbols*) and reactor B (*open symbols*). *Bars* represent the standard deviation. The *arrow* indicates a new addition of the nitrogen source to reactor B

microorganisms; thus, the net effect was a decrease of the TSS concentration in this reactor (Fig. 3c). For this reason, only data obtained from reactor A during phase I was considered in the calculus of $Y_{X/BPA}$. Figure 4b shows that the TSS value after the first addition of BPA in reactor A was lower than the initial TSS one. Due to the acclimation process of the biomass to BPA, the first batch lasted about 5 days; thus, the initial decrease of TSS could be attributed to the decay of microorganisms not acclimated to BPA. However, once the activated sludge acquired the capability to degrade BPA, subsequent additions of BPA produced an increase of the TSS values (Fig. 3c, 4b). From the slope of the straight line obtained by plotting TSS as a function of the cumulative BPA consumption (ΔBPA), the biomass yield on BPA ($Y_{X/BPA}$) was 1.2 ± 0.2 mgTSS $mgBPA^{-1}$. Figure 5 shows that when BPA was the sole carbon source, the nitrogen content of the suspended solids was $i_{N,TSS} = 18 \pm 8$ - mgN $gTSS^{-1}$. This value was about five times lower than the value reported by Ramdani et al. (2012) or that obtained in the present work when CW was the carbon source ($i_{N,TSS} = 89 \pm 7$ mgN $gTSS^{-1}$). The low nitrogen consumption obtained when BPA was the sole carbon source (Fig. 3d) suggests that the observed increase of TSS values could be attributed to the synthesis of some kind of internal storage product along with the growth of actual microorganisms. Spivack et al. (1994) reported that BPA can be metabolized to procedure 4-hydroxybenzoic acid and 4-hydroxyacetophenone. While 4-hydroxybenzoic

acid is further oxidized and finally enters the citrate cycle, 4-hydroxyacetophenone is oxidized by a monooxygenase to yield 4-hydroxyphenyl acetate. Then, this compound is hydrolyzed to hydroquinone and acetate (Moonen et al. 2008; Rehdorf et al. 2009). Taking into account that the role of acetate on the production of polyhydroxybutyrate (PHB) by activated sludge is well recognized (Johnson et al. 2010; Cavaillé et al. 2013; Wang et al. 2013), the presence of acetate as an intermediate during the metabolism of BPA could favor the synthesis of internal storage products, such as PHB, for example. Because these storage products are devoid of nitrogen, the accumulation of such products determines an increase of the TSS but not the consumption of the nitrogen source. Thus, the accumulation of storage products could be the reason for the low nitrogen content of the suspended solids when BPA was the sole carbon source.

Several authors reported the growth of microorganisms on BPA. However, in most of those studies growth was monitored by measuring the optical density (Sasaki et al. 2005; Sakai et al. 2007; Yamanaka et al. 2007; Zhang et al. 2007; Fischer et al. 2010; Zhou et al. 2013). Although some valuable information can be extracted from those works, none of them report the actual biomass yields on BPA or the nitrogen consumption associated with the degradation of BPA.

Biomass yields on CW ($Y_{X/CW}$) and on BPA ($Y_{X/BPA}$) obtained in the present work can be useful for predicting the TSS concentration in reactors A and C as a function of the cycle number (n) (Fig. 6). Phase I in reactor A consisted of 11 cycles (n_{API}) with an initial BPA concentration (BPA_0) of 40 mgBPA l^{-1}. In phase II, reactor A was fed with a mixture of 40 mgBPA l^{-1} along with 500 mgCW l^{-1} (CW_0 = 200 mgC l^{-1}). Considering both phases, the TSS concentration in reactor A (TSS_A) as a function of the cycle number (n) can be calculated as follows:

$$TSS_A = \begin{cases} TSS_{A0} + Y_{X/BPA}\, n\, BPA_0 & n \le n_{API} \\ TSS_{A0} + Y_{X/BPA}\, n\, BPA_0 + Y_{X/CW}\,(n - n_{API})\, CW_0 & n > n_{API} \end{cases}, \quad (1)$$

where TSS_{A0} is the initial TSS concentration in reactor A and $n_{API} = 11$ is the number of cycles performed during phase I.

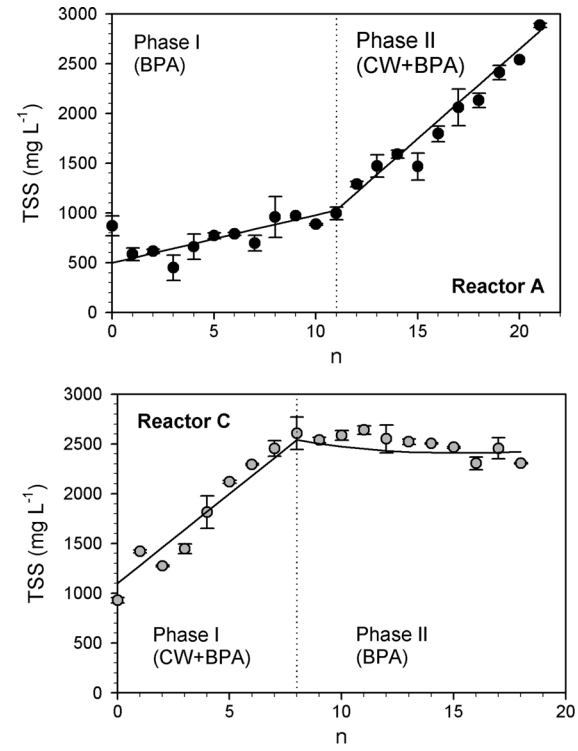

Fig. 6 Total suspended solid (TSS) concentration as a function of the batch number (n) corresponding to reactors A and C. *Bars* represent the standard deviation. *Continuous lines* represent Eqs. (1) (reactor A) and (6) (reactor C)

In the case of reactor C, phase I consisted of eight cycles ($n_{CPI} = 8$) in which the mixture of BPA and CW was added. Then, from cycle 9 to 18 (phase II) only BPA was fed to reactor C. Because BPA alone could not sustain the growth of activated sludge that utilized CW as the carbon source, a decay term of these microorganisms was included as follows. In reactor C, at the end of phase I, the concentration of microorganisms that used CW as the carbon source (X_{CWmax}) can be calculated as follows:

$$X_{CWmax} = Y_{X/CW}\, n_{CPI}\, CW_0 \quad (2)$$

During phase II, this biomass decays because of the absence of CW in reactor C. In general, the endogenous decay process is usually modeled as a first order reaction with respect to the biomass concentration (Contreras et al. 2011). Thus, under the absence of CW, X_{CW} at the end of the first cycle of phase II was:

$$X_{CW(n_{CPI}+1)} = X_{CWmax} \exp(-b t_B) = X_{CWmax} f_D \quad (3)$$

where b is the endogenous decay coefficient, t_B is the total time of the cycle, and f_D is the fraction of biomass that decayed during t_B. In a similar manner, at the end of the second cycle of phase II, X_{CW} can be calculated as follows:

$$X_{CW(n_{CPI}+2)} = X_{CW(n_{CPI}+1)} \exp(-bt_B) = X_{CWmax} f_D^2 \quad (4)$$

In general, expressing Eq. (4) in terms of the total number of cycles ($n > n_{CPI}$) and combining with Eq. (2), X_{CW} at the end of a given cycle of phase II is:

$$X_{CW(n)} = Y_{X/CW}\, n_{CPI}\, CW_0\, f_D^{(n-n_{CPI})} \quad (5)$$

Thus, the TSS concentration in reactor C (TSS_C) as a function of the cycle number (n) can be calculated as follows:

$$TSS_C = \begin{cases} TSS_{C0} + Y_{X/BPA}\, n\, BPA_0 \\ \quad + Y_{X/CW}\, n\, CW_0 & n \le n_{CPI} \\ TSS_{C0} + Y_{X/BPA}\, n\, BPA_0 \\ \quad + Y_{X/CW}\, n_{CPI}\, CW_0\, f_D^{(n-n_{CPI})} & n > n_{CPI} \end{cases}, \quad (6)$$

where TSS_{C0} is the initial TSS concentration in reactor C and $n_{CPI} = 8$ is the number of cycles performed during phase I. Because cycles were performed on a daily basis, it can be assumed that the average t_B value was 24 h. Besides, considering that the endogenous decay coefficient for microorganisms that were grown on CW is $b = 0.0033\ h^{-1}$ (Contreras et al. 2011), the value corresponding to f_D is 0.92. Figure 6 shows that Eqs. (1) and (6) adequately represent the evolution of TSS in reactors A and C over the whole assay. The accordance between predicted and experimental TSS values confirms that BPA cannot sustain the growth of microorganisms that use CW as the carbon source. These results demonstrate that the presence of BPA during or after the acclimation process has a negligible effect on $Y_{X/CW}$. The growth of microorganisms that use BPA as the carbon source was also not affected by the utilized CW concentration.

Figure 7 shows the BPA degradation rate (R_{BPA}) and specific BPA degradation rate (q_{BPA}) of reactors A and C as a function of time. While in reactor A the capability of BPA degradation appeared within the first 5 days, 7 days were necessary in reactor C (Fig. 7a). Then, in both cases a similar increment of R_{BPA} values as a function of time was observed.

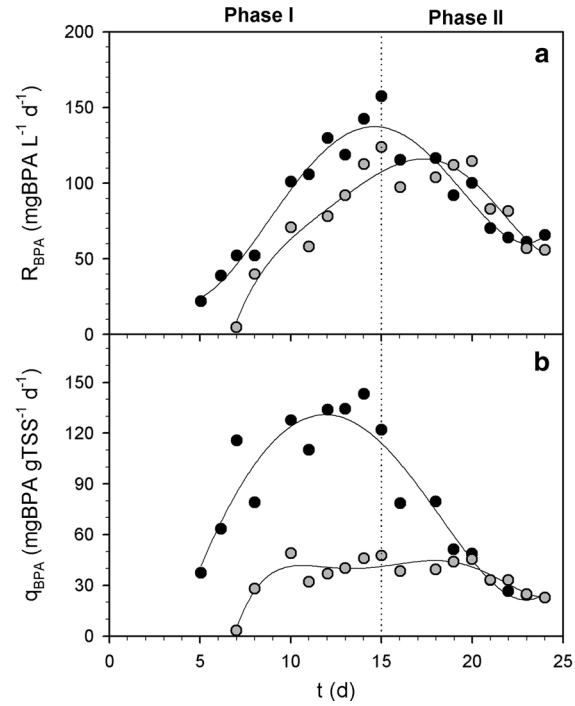

Fig. 7 **a** BPA consumption rate (R_{BPA}) and **b** specific BPA consumption rate (q_{BPA}) as a function of time in reactors A (*black symbols*) and C (*gray symbols*). *Continuous lines* represent trend lines

Assuming that R_{BPA} values were proportional to the amount of microorganisms that use BPA as the carbon source, it can be concluded that the presence of CW did not affect the growth of these microorganisms. Thus, the effect of CW in reactor C was only to extend the beginning of the acclimation process to BPA.

At $t = 15$ days (phase II) feeds of reactors A and C were exchanged. Figure 7a shows a decrease of R_{BPA} values as a function of time in both reactors. The decrease of R_{BPA} in reactor A indicates that in the presence of BPA and CW, some microorganisms preferred a readily biodegradable substrate, such as CW, instead of a xenobiotic compound (BPA). Besides, the presence of CW in reactor A determined a loss of the capability to degrade BPA to some extent. With regard to reactor C, the transition from phase I to phase II involved the change from a complex culture medium (BPA + CW) to a mineral medium with BPA as the sole carbon source, producing a loss of the BPA degrading activity.

The specific BPA degradation rate (q_{BPA}) was used to evaluate the acclimation degree of the biomass in reactors A and C during the first 15 days of operation

(phase I). Figure 7b shows that the acclimation to BPA was fully achieved on both reactors after the fourth batch. From day 10 to 15, q_{BPA} values in both reactors were approximately constant; the average q_{BPA} values corresponding to reactors A and C were 128 ± 12 and 42 ± 7 mgBPA $gTSS^{-1}$ day^{-1}, respectively. The average q_{BPA} value in reactor A was close to that obtained in a previous work (Ferro Orozco et al. 2013). During phase II, the presence of CW in reactor A favored the growth of microorganisms (Fig. 3c) and the loss of BPA degrading activity (Fig. 7a); these two factors contributed to the abrupt decrease of q_{BPA} values in reactor A (Fig. 7b). Conversely, during phase II q_{BPA} values in reactor C were about constant. The absence of CW in reactor C during phase II caused a decrease of the biomass concentration (Fig. 3c) and a loss of the BPA degrading activity (Fig. 7a) approximately to the same extent. For this reason, the average q_{BPA} value in reactor C during phase II was 35 ± 7 mgBPA $gTSS^{-1}$ day^{-1}, which was similar to the value obtained in phase I (42 ± 7 mgBPA $gTSS^{-1}$ day^{-1}).

It must be pointed out that the acquisition of the capability to degrade BPA does not exclude the ability to degrade the biogenic substrate. For this reason, the increase in the TSS concentration in the cases when BPA and CW were present showed that q_{BPA} values under this condition were lower than those obtained when BPA was the sole carbon source. However, the BPA consumption rate (R_{BPA}) was approximately the same in both cases, indicating that the presence of CW neither hampers nor enhances the degradation rate of BPA.

Effect of the biogenic substrate concentration (cheese whey) on the degradation of BPA by acclimated activated sludge

Results obtained from the semi-continuous reactors ("Simultaneous biodegradation of BPA and CW in semi-continuous reactors" section) demonstrated that when the initial CW concentration was 500 mg l^{-1}, the growth of microorganisms that use BPA as the carbon source was not affected by the presence of CW. However, considering that in actual wastewater the concentration of biogenic substrates is highly variable, this conclusion may not be valid for higher CW concentrations. For this reason, the effect of the initial CW concentration on the BPA biodegradation by acclimated activated sludge was studied. Figure 8 shows that within the tested CW concentrations, the removal of BPA was not affected by the presence of CW. Moreover, insets in Fig. 8 demonstrate that the presence of BPA did not modify the CW consumption by acclimated activated sludge. It must be pointed out that if CW and BPA exerted a competitive effect with

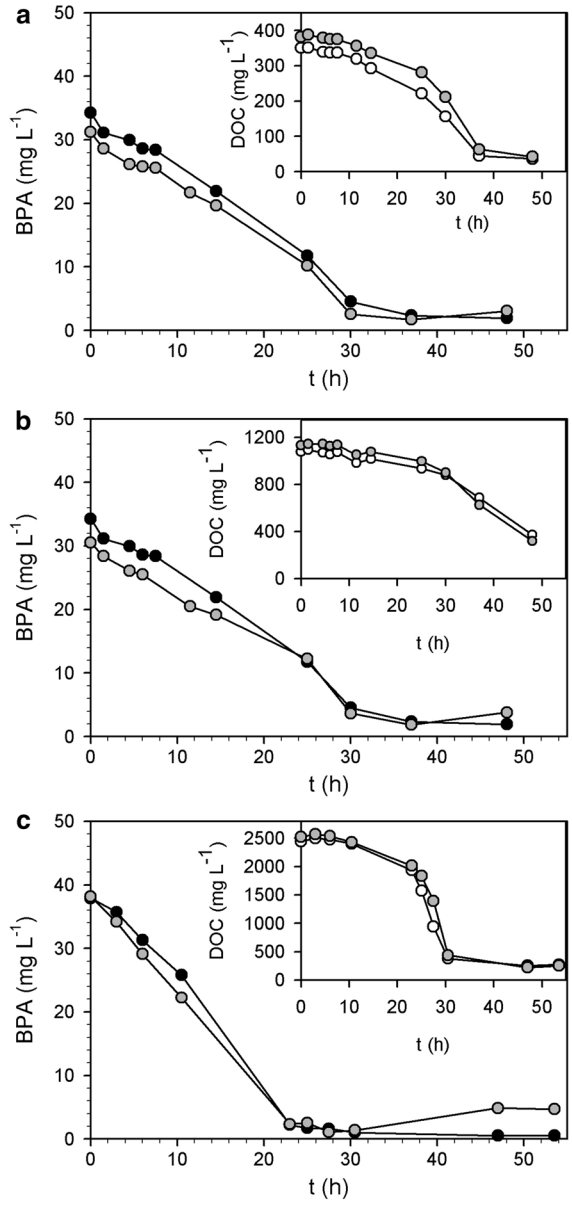

Fig. 8 Removal of BPA and cheese whey (CW) (*insets*) by acclimated activated sludge corresponding to the following systems: BPA alone (*black symbols*); CW alone (*white symbols*); BPA + CW (*gray symbols*). Initial cheese whey (CW) concentrations were: **a** 1000; **b** 3000; **c** 6000 mgCW l^{-1}

regard to the removal rate of each other, this effect was not noticeable under the tested concentrations.

Results obtained in the present work demonstrate that the effect of CW was related more to the acclimation process of the biomass to BPA ("Simultaneous biodegradation of BPA and CW in semi-continuous reactors" section) than to a competitive effect of CW and BPA ("Effect of the biogenic substrate concentration (cheese whey) on the degradation of BPA by acclimated activated sludge" section). This conclusion has a great impact on the development of mathematical models to represent the degradation of BPA in the presence of a biogenic substrate, such as CW. Such models should put the emphasis on several issues concerning the acclimation process and the relative amount of biomass capable of degrading BPA and CW, for example. In this sense, the q_{BPA} and yield values reported in the present work are crucial parameters for such models.

Conclusions

From the obtained results the following conclusions were drawn:

- The acclimation time of activated sludge to BPA depended on the presence of CW. In the absence of CW, BPA was exhausted in 5 days. Conversely, 8 days were necessary to achieve a BPA consumption of 95 % under the presence of CW. However, once the capability of degrading BPA was acquired, the removal of BPA was not affected by the presence of CW.
- The activated sludge growth yield on BPA ($Y_{X/BPA}$) as the sole carbon source was 1.2 ± 0.2 gTSS $gBPA^{-1}$. The low value of the nitrogen content of the biomass obtained when BPA was the sole carbon source ($i_{N,TSS} = 18 \pm 8$ mgN $gTSS^{-1}$) suggested that the increase of TSS was due to the synthesis of some internal storage product along with the growth of actual microorganisms.
- While the BPA degradation rate (R_{BPA}) was not affected by the presence of CW, the specific BPA degradation rate (q_{BPA}) values in the absence of CW ($q_{BPA} = 128 \pm 12$ mgBPA $gTSS^{-1}$ day^{-1}) were higher than those obtained in the presence of CW ($q_{BPA} = 42 \pm 7$ mgBPA $gTSS^{-1}$ day^{-1}).
- Increasing the CW concentration did not affect the removal of BPA by the acclimated activated sludge. Additionally, the CW consumption was not modified by the presence of BPA.
- The kinetic and stoichiometric coefficients reported in the present work can be useful in developing mathematical models to describe the aerobic biodegradation of CW and BPA by activated sludge.

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