International Biodeterioration & Biodegradation 85 (2013) 392–399

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Biodegradation of bisphenol-A (BPA) in activated sludge batch reactors: Analysis of the acclimation process

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ARTICLE INFO

Article history:
Received 29 July 2013
Received in revised form
10 September 2013
Accepted 10 September 2013
Available online

Keywords:
Activated sludge
Bisphenol A
Acclimation
Biodegradation

ABSTRACT

In this work the effect of (a) the sludge age (SA) and the acclimation strategy on the acclimation process of activated sludge (AS) to bisphenol A (BPA), (b) the presence of biogenic substrates on the degradation of BPA, and (c) the acclimation on the degradation of biogenic substrates was studied. AS samples from reactors operated at SA of 30 and 45 d were acclimated to BPA using two strategies: constant or increasing the initial BPA concentration. Although acclimation to BPA was achieved using both strategies, no net biomass growth was detected. Specific BPA degradation rate of acclimated AS ranged between 65 and 90 mgBPA $gTSS^{-1}$ d^{-1}. BPA degradation was not affected by the biogenic substrates cheese whey or acetate; acclimation to BPA did not cause a negative effect on the biodegradation of these substrates. During the degradation of cheese whey, specific mean oxygen uptake rate ($q_{o_2 mean}$) and specific mean substrate consumption rate (q_{Smean}) of non-acclimated AS were similar to those corresponding to acclimated ones. When acetate was tested, $q_{o_2 mean}$ and q_{Smean} values corresponding to acclimated AS were about twofold the values of the non-acclimated ones. This enhancement could occur because acetate is an intermediate of the metabolism of BPA.

1. Introduction

Bisphenol A (BPA) is a widely used industrial organic compound. BPA is used in adhesives, building materials, powder coatings, the inner lining of cans, compact discs, and electronic components (Staples et al., 1998). BPA is an intermediate in the manufacture of polycarbonate plastic, epoxy resin, flame retardants, and other products (Mutou et al., 2006). Due to the wide usage of polycarbonate and epoxy resins in industry and households, BPA has become one of the most ubiquitous contaminants in the environment (Zhao et al., 2008). As a result, this compound is frequently detected in municipal and industrial wastewaters (Stasinakis et al., 2008b).

When a xenobiotic compound, such as BPA, becomes an environmental pollutant, it is often hard-to-treat. The molecular structure of a xenobiotic often differs significantly from that of natural microbial substrates; therefore, its biodegradation is usually more difficult than the corresponding to biogenic substrates (Chong et al., 2008). The microbial population can acquire new metabolic pathways for a xenobiotic degradation through a process called acclimation (Buitron et al., 1998; Chong and Lin, 2007). Acclimation generally includes a period of dormant time (lag phase), followed by a degradation phase of the compound. Lag and degradation phases seem to divide the acclimation process into segments; however, acclimation is a continuous process and it should be analyzed entirely, taking into account both phases (Chong, 2009).

In general, industrial wastewater treatment plants receive effluents comprised by a mixture of xenobiotics and biodegradable substrates in different proportions. In this context, the xenobiotic degradation could be modified by the presence of readily biodegradable substrates. Conversely, the acclimation of the activated sludge system to a xenobiotic, such as BPA, could interfere with the normal degradation of biodegradable substrates. 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. It is important to consider that the acclimation

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http://dx.doi.org/10.1016/j.ibiod.2013.09.005

process depends on several factors, such as, type and concentration range of the xenobiotic compound, type of microorganisms studied in pure or mixed cultures, presence of readily biodegradable compounds, among others (Ye and Shen, 2004; Chong et al., 2008, 2012). For these reasons, the acclimation process must be analyzed on each particular case. Moreover, interactions between the xenobiotic and the biogenic substrates that are present in wastewaters must be considered in order to comply with the effluents requirements.

Although several authors have studied the removal of BPA by pure and mixed cultures, such as activated sludge (Zhang et al., 2007; Zhao et al., 2008; Roh et al., 2009; Fischer et al., 2010; Kabiersch et al., 2011; Li et al., 2012), literature data concerning the acclimation process of activated sludge to BPA are scarce. This is an area that merits much more attention; the information could contribute to achieve a stable BPA removal process from wastewaters. For these reasons, the objectives of the present work were to study: i) the effect of the sludge age and the acclimation strategy on the acclimation process of activated sludge to BPA, ii) the effect of the presence of biogenic substrates on the biodegradation of BPA, and iii) the effect of acclimation of activated sludge to BPA on the degradation of biogenic substrates.

2. Materials and methods

2.1. Biological and chemical materials

Bisphenol A (BPA) was purchased from Sigma–Aldrich (St. Louis, USA). Cheese whey 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 (St. Louis, USA). Activated sludge used in all the experiments were harvested from aerobic laboratory-scale (4.5 l) activated sludge reactors with partial biomass recycle. The hydraulic retention time was 48 h and the sludge age was maintained at 30 d or 45 d by daily wasting of the mixed liquor directly from the reactor; throughout the present work, the activated sludge biomass from those sludge ages are called as X_{30} and X_{45}, respectively. 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. Soluble chemical oxygen demand (CODs) of the synthetic wastewater was 1500 mg l^{-1}. The temperature of the bioreactors 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}, pH was 7.5 ± 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}.

2.2. Acclimation of activated sludge to BPA in consecutive batch assays

The acclimation of activated sludge to BPA was carried out in consecutive batch assays (Ferro Orozco et al., 2010). The inoculum for the first batch was obtained from the aerobic laboratory-scale activated sludge reactors described in Section 2.1; then, the inoculum for the next batch assay was obtained from the previous one. Before starting each batch, the biomass was harvested by sedimentation and washed with phosphate buffer (KH_2PO_4 2 g l^{-1}, K_2HPO_4 0.5 g l^{-1}, pH = 7.0). The washed biomass was re-suspended in fresh culture medium to serve as inoculum for the next batch assay. The composition of the culture medium used to acclimate the biomass to BPA was the following: BPA (40 mg l^{-1} in the case of constant initial BPA concentration, or 40–300 mg l^{-1} when the initial BPA was increased), $(NH_4)_2SO_4$ 220 mgN l^{-1}, KH_2PO_4 2 g l^{-1}, K_2HPO_4 0.5 g l^{-1}, and 1 ml l^{-1} of micronutrient solutions M1 and M2, respectively (Lobo et al., 2013). 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. M2 solution contained the following (g/100 ml): $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ 0.05, H_3BO_3 0.01, KI 0.01. Initial pH of the culture medium was adjusted to 7.0 ± 0.2 using NaOH or H_2SO_4 (1 N) solutions.

Two acclimation procedures were tested. The first acclimation strategy (AST1) was performed maintaining constant the initial BPA concentration at 40 mg l^{-1} in all batch assays. In the second strategy (AST2), the initial BPA concentration of the first batch was 40 mg l^{-1}; then, initial BPA concentration was doubled in each consecutive batch. In both strategies a new batch assay was started when the removal of BPA was higher than 90%; thus, the reaction time was extended the necessary time to achieve that removal efficiency (Moreno and Buitron, 2004). For each assay, samples were taken at different times to determine soluble chemical oxygen demand (CODs), soluble total organic carbon (TOCs), and BPA concentrations. Total suspended solids (TSS) were used as a measure for the biomass concentration (X); duplicate TSS was determined at least at the beginning and at the end of each batch.

The acclimation process was monitored by calculating the specific BPA consumption rate (q_{BPA}, mgBPA gTSS^{-1} d^{-1}) as follows:

$$q_{BPA} = \left(\frac{BPA_o - BPA_f}{X\Delta t}\right) \quad (1)$$

where X is the average biomass concentration on the batch assay, BPA_0 and BPA_f correspond to the BPA concentration at the beginning and at the end of the assay, respectively, and Δt is the time (d) required to achieve removal efficiencies higher than 90%. A *t*-test (Zar, 1999) was performed in order to evaluate the differences between the q_{BPA} values corresponding to X_{30} and X_{45}.

2.3. Acclimation of activated sludge to BPA monitored by a respirometric technique

A respirometric technique was used to monitor the metabolic activity of the activated sludge during acclimation to BPA. In this experiment, a sample of activated sludge (500 ml) was obtained from the aerobic laboratory-scale activated sludge reactor operating at a sludge age of 30 d (X_{30}) (Section 2.1). The biomass was harvested by sedimentation, washed and re-suspended in phosphate buffer (KH_2PO_4 2 g l^{-1}, K_2HPO_4 0.5 g l^{-1}, pH = 7) with the addition of micronutrients solutions M1 and M2. Then, the biomass was placed in the respirometer and 9 consecutive pulses of BPA (40 mg l^{-1}) were added. For each pulse, the oxygen uptake rate of the microbial culture and the BPA consumption were monitored.

An open (flowing gas/static liquid) respirometer was used to monitor the respiratory activity of the activated sludge during acclimation to BPA. The respirometer was a 0.5 l reactor with temperature control (30 ± 0.5 °C) in which the sample of activated sludge was placed. Agitation was provided by a magnetic stir-bar; the respirometer was aerated continuously by an air pump. Air was set to a stable flow rate (0.5 l min^{-1}) using a high precision rotameter (Bruno Schilling model MB 60V, Argentina). The DO concentration (C) as a function of time (*t*) was recorded every 5 s using an optical DO probe (YSI ProODO). Before each pulse of BPA, the oxygen mass transfer coefficient (k_La) was obtained using a non-steady procedure (Ros, 1993). Then, the total oxygen uptake rate (OUR_T, mgO_2 l^{-1} h^{-1}) was calculated from the DO mass balance in the reactor as follows:

$$OUR_T = k_L^a(C_S - C) - dC/dt \quad (2)$$

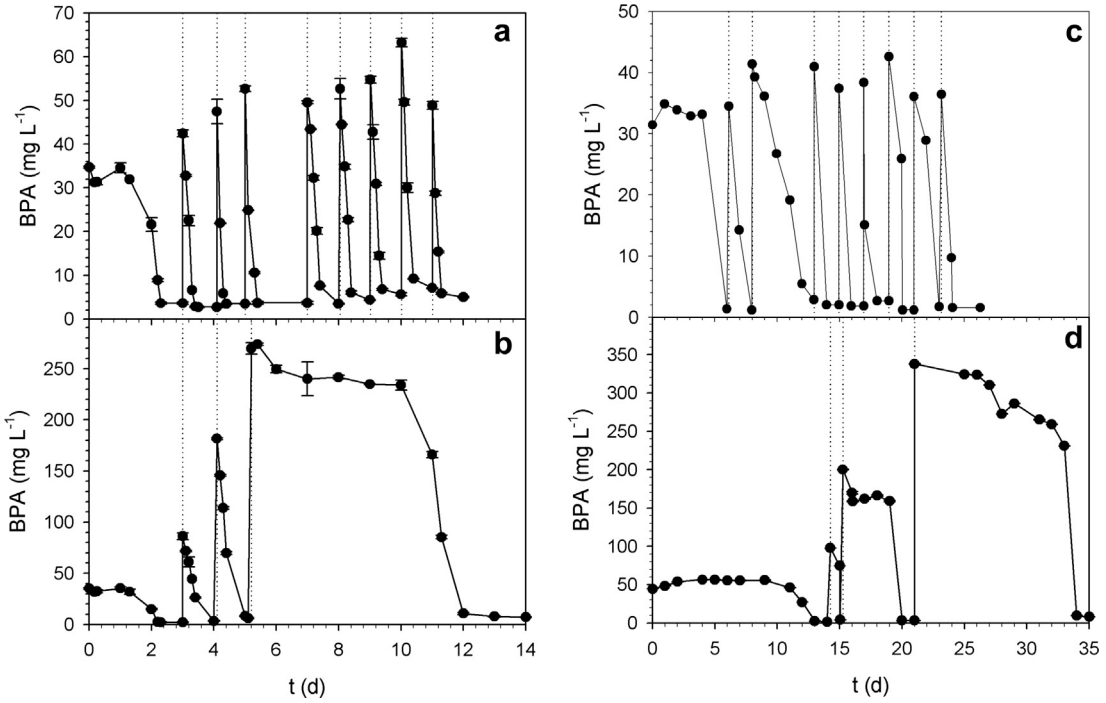

Fig. 1. BPA consumption as a function of time during the consecutive batch assays performed for the acclimation of activated sludge to BPA. (a) AST1 with X_{30}, (b) AST2 with X_{30}, (c) AST1 with X_{45}, (d) AST2 with X_{45}. Dotted lines represent the beginning of each batch assay. Bars indicate one standard deviation.

where C_S corresponds to the DO concentration in the absence of respiration.

2.4. Effect of the presence of biogenic substrates on the biodegradation of BPA

The effect of the biogenic substrates (acetate, cheese whey) on the BPA biodegradation was studied in batch reactors. Activated sludge used in these experiments was harvested from the activated sludge reactor operating at a sludge age of 30 d; this biomass sample was acclimated to BPA using the strategy AST1. Then, batch assays were performed to study the biodegradation of BPA by the acclimated biomass in the presence of biogenic substrates (acetate, or cheese whey). In these experiments, the composition of the culture medium was: 40 mgBPA l^{-1}, 600 mgCOD l^{-1} of the biogenic substrate (acetate, cheese whey), $(NH_4)_2SO_4$ 175 mgN l^{-1}, KH_2PO_4 2 g l^{-1}, K_2HPO_4 0.5 g l^{-1}, and 1 ml l^{-1} of micronutrient solutions M1 and M2, respectively. At different time intervals, samples were taken to measure BPA and CODs concentrations. Additionally, assays in the presence of BPA alone (e.g., without a biogenic substrate) were also performed.

2.5. Effect of the acclimation to BPA on the biodegradation of biogenic substrates

To analyze the effect of the acclimation to BPA on the biodegradation of different readily biodegradable substrates (acetate, cheese whey), a respirometric technique was used. Strategy AST1 was used to acclimate the activated sludge (X_{30}) to BPA. The respiratory activity of acclimated (X_{30a}) or non-acclimated (X_{30na}) activated sludge due to the presence of acetate (A), and cheese whey (CW) was measured using the open respirometer described in Section 2.3. In these experiments, the oxygen uptake rate associated with the oxidation of the exogenous readily biodegradable substrate (OUR_{Ex}) was calculated by assuming that the total oxygen uptake rate (OUR_T) was comprised by two terms. The endogenous oxygen uptake rate (OUR_{EN}) represents the respiration rate in the absence of an oxidizable substrate (S). When a substrate is added, an increase in OUR_T is observed due to the substrate oxidation, in this case $OUR_T = OUR_{EN} + OUR_{Ex}$. Then, when S is depleted, OUR_T returns to a value close to the initial one (OUR_{EN}). The oxygen consumed (OC) due to the oxidation of the exogenous substrate was calculated using the following expression:

$$OC = \int (OUR_T - OUR_{EN})dt = \int OUR_{EX}dt \quad (3)$$

where OUR_{Ex} corresponds to the microbial respiration after the substrate addition (cheese whey or acetate in the present work).

Based on the respirometric profiles, the total degradation time (t_d, h) can be defined as the time interval between the increase and the decrease of the OUR_T associated with the presence or the depletion of a given tested substrate (Lobo et al., 2013). If the oxygen consumed (OC, mgO_2 l^{-1}) due to the oxidation of the exogenous substrate is known, the specific mean oxygen consumption rate (q_{o_2mean}, mgO_2 $gTSS^{-1}$ h^{-1}) can be estimated as follows:

$$q_{O_2mean} = \frac{OC}{t_d X} \quad (4)$$

where X (gTSS l^{-1}) is the biomass concentration in the respirometer. Similarly, the following expression was used to calculate the specific mean substrate consumption rate (q_{Smean}, mgS $gTSS^{-1}$ h^{-1}) (Lobo et al., 2013):

$$q_{Smean} = \frac{S_0}{t_d X} \quad (5)$$

where S_0 is the initial substrate concentration.

A *t*-test (Zar, 1999) was employed to evaluate the differences between non-acclimated and BPA-acclimated activated sludge with

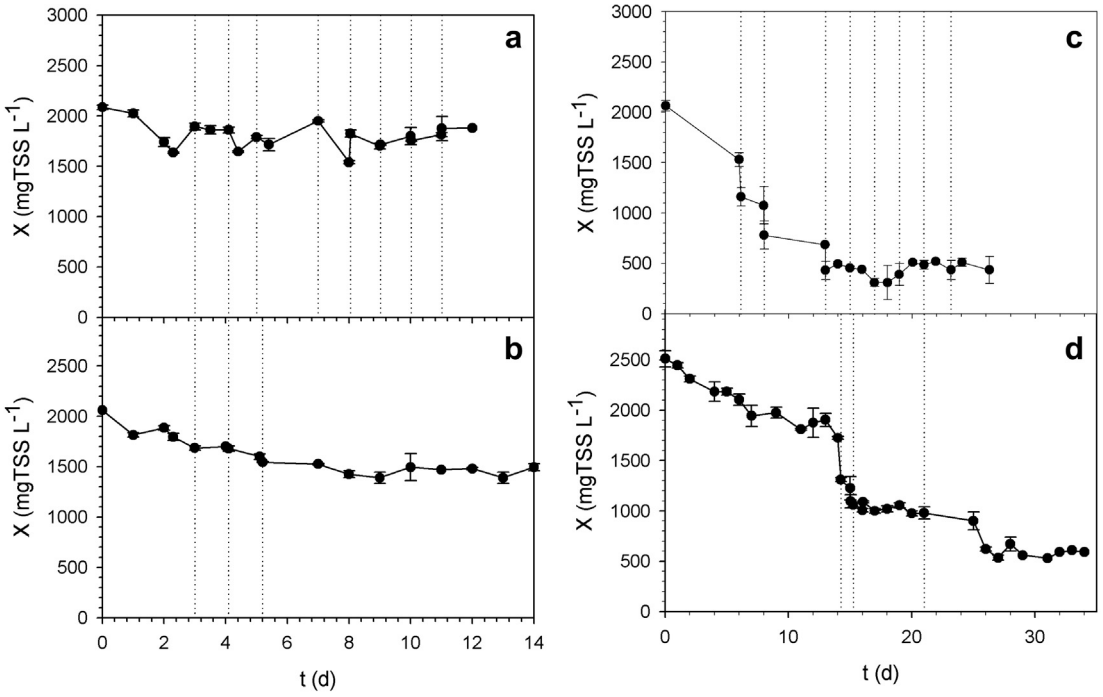

Fig. 2. Changes in the biomass concentration as a function of time during the consecutive batch assays performed for the acclimation of activated sludge to BPA. (a) AST1 with X_{30}, (b) AST2 with X_{30}, (c) AST1 with X_{45}, (d) AST2 with X_{45}. Dotted lines represent the beginning of each batch assay. Bars indicate one standard deviation.

regard to q_{O2mean} and q_{Smean} values during the aerobic degradation of acetate or cheese whey.

2.6. Analytical techniques

Total suspended solids (TSS) were used to measure the biomass concentration in all batch assays (Ferro Orozco et al., 2011). Soluble COD and TOC (CODs, TOCs) concentrations were determined as follows (APHA, 1998): 5 ml of culture samples were centrifuged 5 min at 13000 rpm (Eppendorf 5415C); then, the supernatant was filtered through 0.45 μm cellulosic membranes (Osmonics Inc.). CODs of the filtrate was determined using commercial reagents (Hach Company, Loveland, CO). TOCs concentration was measured using a Shimadzu TOC-VCPN analyzer. BPA concentration was determined by the 4-aminoantipyrine (4-AAP) method (Modaressi et al., 2005). In this method, 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 $NaHCO_3$) are 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. All the experiments presented in this work were performed at least in duplicates.

3. Results and discussion

3.1. Acclimation of activated sludge to BPA: effect of the sludge age and the acclimation strategy

The acclimation process of activated sludge samples obtained from reactors operated at sludge ages of 30, and 45 d using two acclimation strategies was studied. Acclimation strategy AST1 consisted of consecutive batch assays using a constant initial BPA concentration of 40 mgBPA l^{-1}. Acclimation strategy AST2 involved consecutive batch assays with increasing the initial BPA concentration from 40 to 320 mgBPA l^{-1}. Fig. 1 shows that, regardless the acclimation strategy or the sludge age, the degradation activity began after a lag period. While lag periods corresponding to X_{30} ranged between 1.5 and 2 d, lag periods corresponding to X_{45} ranged from 5 to 10 d. In all cases, after the lag phase, BPA concentration decreased to almost zero. Moreover, the same behavior was observed with regard to TOCs and CODs concentrations (data not shown).

With regard to X_{30}, obtained results using the AST1 (Fig. 1a) showed that, after the lag period of the first batch assay, the activated sludge acquired the capability to degrade BPA. When the AST2 was tested (Fig. 1b), the increase of the initial BPA concentration did not cause observable changes in the BPA degradation up to batch #3. However, during batch #4 a new lag phase of 5 d was observed. This second lag period could be attributed to the toxicity caused by the high tested initial BPA concentration (270 mg l^{-1}). Fig. 1 shows the BPA consumption obtained during AST1 (Fig. 1c), and AST2 (Fig. 1d) when X_{45} was tested. The behavior of X_{45} for both acclimation strategies was similar to the observed for X_{30}; however, when AST2 was carried out, two new lag phases corresponding to batch #3 and #4 were observed. These results suggest that X_{45} is more sensitive to the increase in the BPA concentration than X_{30}.

Toxicity caused by high BPA concentrations was reported by Yamanaka et al. (2007). Those authors reported that BPA in the range of 2–25 mg l^{-1} was effectively degraded by three strains of *Bacillus pumilus*; however, higher concentrations of BPA caused the inhibition of the BPA-degrading activity. Zhang et al. (2007) also reported that the BPA-degrading activity of a culture of *Achromobacter xylosoxidans* was disturbed by increased levels of toxicity at BPA concentrations of 20 and 50 mgBPA l^{-1}. The activated sludge community presents large microbial diversity with a broad physiological capability; for this reason, the bacterial consortium is able to deal with higher BPA concentrations. Therefore, even when new lag phases were observed (batch #4 for X_{30}, and #3 and #4 for X_{45}), the BPA was totally consumed after that period.

Fig. 2 shows the biomass concentration during the acclimation strategies AST1 and AST2 for X_{30} and X_{45}. Considering strategy

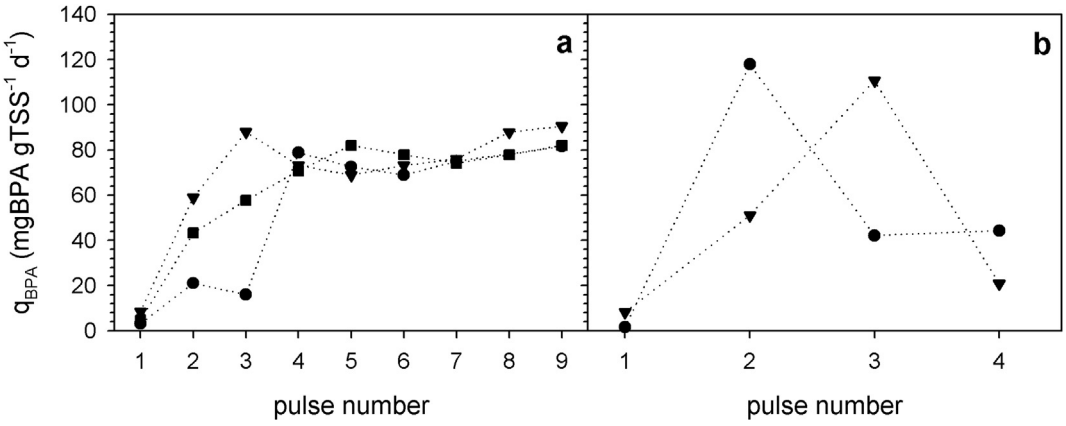

Fig. 3. Specific BPA consumption rate (q_{BPA}) as a function of the pulse number of BPA. (a) strategy AST1: X_{30}, batch assay (▼), X_{45}, batch assay (●), and X_{30}, respirometric assay (■). (b) strategy AST2: X_{30} (▼), and X_{45} (●).

AST1 (Fig. 2a), the biomass concentration corresponding to X_{30} was stable around 1800 mgTSS l^{-1} throughout the 9 consecutive batch assays. When strategy AST2 was carried out (Fig. 2b), a slight decrease in the biomass concentration from 2000 to 1500 mgTSS l^{-1} was observed. These differences in the stability of the biomass concentration could be attributed to the increment in the toxicity of the environment due to the increase in the initial BPA concentrations during the strategy AST2. In contrast to the stability obtained for X_{30}, a marked decrease of the biomass concentration corresponding to X_{45} was observed when both acclimation strategies were employed (Fig. 2c, d). The biomass concentration decreased from 2000 (strategy AST1) or 2500 (strategy AST2) mgTSS l^{-1} to a value around 500 mgTSS l^{-1}; then, X_{45} remained without significant changes until the end of the batch assays. In addition, as it was observed with X_{30}, the decrease of the biomass concentration was higher when the strategy AST2 was carried out.

It is important to note that in the present work no net biomass growth was observed in the performed consecutive batch assays (Fig. 2), suggesting that BPA cannot be used as carbon source by the tested activated sludge samples. Alternatively, net cellular growth cannot be detected if the decay rate is higher or similar to the growth rate; this case occurs if the specific growth rate is very low. In this sense, Fischer et al. (2010) reported that the specific maximum growth rate of *Cupriavidus basilensis* in a medium containing 34 mgBPA l^{-1} as the sole carbon and energy source was extremely low, about 0.003 d^{-1}. This value for the specific maximum growth rate is lower than the specific decay rate for activated sludge reported by Ferro Orozco et al. (2010), and Contreras et al. (2011).

According to Chong and Lin (2007), and Rezouga et al. (2009), when a mixed culture (such as activated sludge) is in contact with a xenobiotic compound (e.g., BPA), two types of microorganisms, degraders (X_d) and non-degraders (X_{nd}) of the xenobiotic compound, are usually found. Even though degraders initially may be absent, due to the acclimation process a fraction of X_{nd} acquires the capability to degrade the xenobiotic compound turning into X_d; the other fraction of non-degraders disappear from the system due to the endogenous decay process. The force that generates those changes in the X_{nd} population leading to its conversion on X_d is called the adaptive pressure. According to Chong (2009), this pressure is proportional to the product between X_{nd} and the xenobiotic concentration at time *t*. Based on these considerations, in the present work the adaptive pressure in strategy AST2 was bigger than during strategy AST1. On the other hand, the higher the sludge age, the greater the proportion of inert total suspended solids (Orhom and Artan, 1994); thus, X_{45} would contain lower amounts of both X_d and X_{nd}. This characteristic also leads to lower initial degradation rates; as a consequence, contact times with high BPA concentrations would be longer, contributing to enhance the cellular decay process.

The specific BPA degradation rate (q_{BPA}) was used to evaluate the acclimation degree of both activated sludge samples (X_{30}, X_{45}) during the acclimation strategies AST1 and AST2. Fig. 3a shows that for both sludge ages, the acclimation to BPA can be fully achieved between batch #3 and #4 using strategy AST1. For batch #1 to #3, q_{BPA} values for X_{30} were higher than those corresponding to X_{45}. After batch #3, no significant differences ($p < 0.05$) between q_{BPA} values corresponding to X_{30} and X_{45} were found; thus, an overall q_{BPA} value of 77 ± 12 mgBPA gTSS^{-1} d^{-1} was obtained. Although q_{BPA} values corresponding to fully acclimated X_{30}, and X_{45} activated sludge were similar, the total time to complete the batch #3 for X_{30} and X_{45} were 5 and 13 d, respectively (Fig. 1a, c). Thus, the acclimation rate of X_{30} was higher than the acclimation rate corresponding to X_{45}. These results agree with the low concentration of active biomass for high sludge ages (Orhom and Artan, 1994), and also with the decrease of biomass concentration described previously (Fig. 2c).

Fig. 3b shows q_{BPA} values obtained during the acclimation process using strategy AST2. For X_{30}, q_{BPA} values gradually increased with the increment of the initial BPA concentration up to 160 mg l^{-1} (batch #3); then, when 270 mgBPA l^{-1} was added (batch #4), the occurrence of a second lag phase (Fig. 1b) caused a marked decrease of q_{BPA}. Results concerning X_{45} showed a similar behavior; however, the increase of q_{BPA} was observed until an initial BPA concentration of 80 mg l^{-1}. Then, successive increments of the BPA concentration (batch #3 and #4) caused two new lag periods (Fig. 1d), and as a consequence, the decrease of the observed q_{BPA} values.

According to Fig. 3, q_{BPA} values corresponding to fully acclimated activated sludge obtained in the present work, ranged from 65 to 90 mgBPA gTSS^{-1} d^{-1}. These q_{BPA} values are close to those reported by other authors. West et al. (2001) studied the degradation of BPA by activated sludge cultures; those authors reported q_{BPA} values of 16 and 44 mgBPA gTSS^{-1} d^{-1} for initial BPA concentrations of 7 and 25 mgBPA l^{-1}, respectively. Stasinakis et al. (2008a) found a q_{BPA} value of 62 mgBPA gTSS^{-1} d^{-1} for activated sludge samples in the presence of 35 mgBPA l^{-1}. Kim et al. (2007) studied the capability of nitrifying activated sludge to degrade BPA; when ammonium was

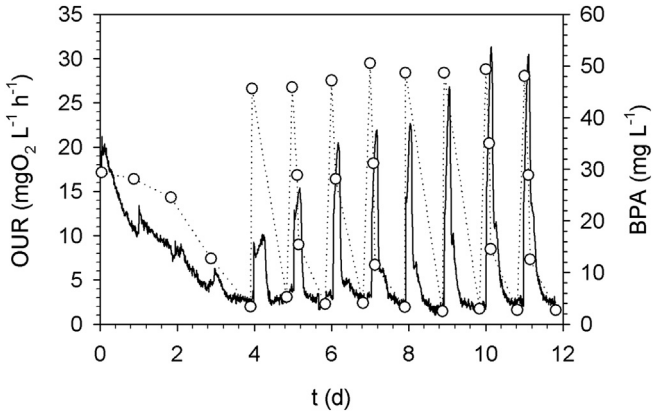

Fig. 4. Oxygen uptake rate (continuous line) and BPA concentration (dotted line, circles) as a function of time during acclimation of activated sludge (X_{30}) to BPA. Each peak corresponds to the addition of 40 mgBPA l^{-1}.

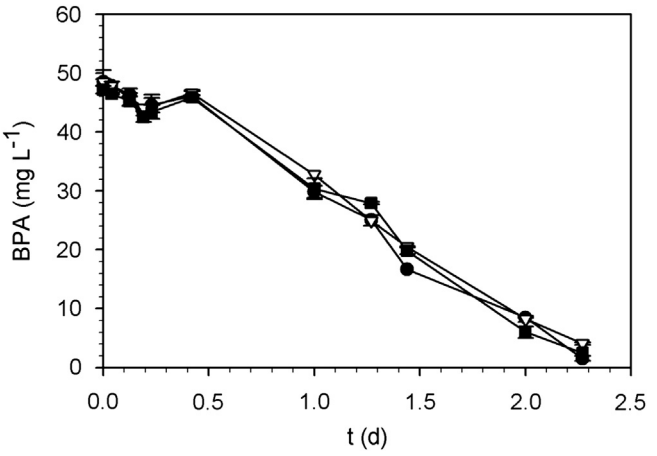

Fig. 5. BPA removal in the absence and presence of biogenic substrates by activated sludge (X_{30}) acclimated to BPA. Symbols correspond to batch assays with the addition of: BPA (●), BPA + acetate (∇), and BPA + cheese whey (■). Initial BPA and biogenic substrate concentrations were 40 mg l^{-1} and 600 mgCOD l^{-1}, respectively. Bars indicate one standard deviation.

used as the energy source, those authors reported q_{BPA} values of 8 and 30 mgBPA $gTSS^{-1}$ d^{-1}, corresponding to initial BPA concentrations of 10 and 100 mg l^{-1}, respectively.

Results obtained in the present work demonstrate that, although both activated sludge samples (X_{30}, X_{45}) had the capability to acclimate and degrade BPA, the biomass obtained from the activated sludge reactor operating with a sludge age of 30 d had a higher stability to BPA and that its acclimation rate was also higher. For these reasons, all the further experiments were performed using activated sludge samples from the reactor operated with a sludge age of 30 d.

3.2. Monitoring the acclimation of activated sludge to BPA by respirometry

A respirometric technique was employed to monitor the acclimation process of the biomass obtained from the activated sludge reactor operated with a sludge age of 30 d (X_{30}) to BPA. This technique allows determining the oxygen uptake rate (OUR) on a continuous base throughout the acclimation process. Nine consecutive additions of 40 mgBPA l^{-1} to the respirometer were performed. Fig. 4 shows that the first pulse of BPA caused a gradual decrease of OUR; additionally, the BPA concentration was almost constant for the first 2 d; then, BPA decreased to almost depletion on day 4. After the first BPA pulse, in the subsequent additions of BPA an increase of the OUR was observed. Then, oxygen uptake rate markedly decreased to reach the endogenous level due to the BPA depletion. The total degradation time (t_d, h) was calculated as the time interval between the increase and the decrease of the OUR. Fig. 3 shows that $q_{BPAmean}$ values (Eq. (3)) based on respirometric data (Fig. 4) were similar to q_{BPA} values (Eq. (1)) calculated from the consecutive batch assays (Fig. 1a). A *t*-test demonstrate no significant differences ($p < 0.05$) between $q_{BPAmean}$ values based on respirometric measurements and those from consecutive batch assays, confirming that using strategy AST1 for the acclimation of activated sludge to BPA can be fully achieved after batch #4.

3.3. Effect of the presence of biogenic substrates on the biodegradation of BPA

The biodegradation of BPA alone or in the presence of acetate, or cheese whey was studied. These experiments were carried out in batch reactors using a sample of activated sludge (X_{30}) that was previously acclimated to BPA using the AST1. Fig. 5 shows that the consumption of BPA in the absence of biogenic substrates was similar to the BPA removal in the presence of cheese whey or acetate. Several authors reported that the presence of a readily biodegradable substrate, such as glucose, acetate, peptone and others, can delay the biodegradation of BPA (Sasaki et al., 2005; Urase and Kikuta, 2005; Zhao et al., 2008; Stasinakis et al., 2008a). Thus, the degradation of the xenobiotic may be hampered by the diauxic growth condition, in which the microbial supplies for growth and energy can be obtained from the readily degradable substrate (Chong and Chen, 2007). On the other hand, there are some study cases describing the beneficial effect of the biogenic substrates on a xenobiotic degradation (Moorman et al., 2001; Lee, 2003; Chong et al., 2012). In the present work, the BPA-degradation activity was not modified due to the addition of 600 mgCODs l^{-1} as acetate or cheese whey (Fig. 5). These results suggest that it is possible to use activated sludge to remove BPA from wastewaters in the presence of readily biodegradable substrates.

3.4. Effect of the acclimation of the activated sludge to BPA on the biodegradation of biogenic substrates

Respirometric assays were also performed to analyze the degradation of two biogenic substrates (acetate, cheese whey) by X_{30} activated sludge samples before and after acclimation to BPA. In all cases, the specific mean oxygen uptake rate (q_{o_2mean}) and the specific mean substrate consumption rate (q_{Smean}) were calculated. Considering the degradation of cheese whey, no significant differences ($p < 0.05$) between q_{o_2mean} or q_{Smean} values corresponding to non-acclimated and BPA-acclimated activated sludge were obtained (Fig. 6). Thus, the acclimation to BPA did not cause a negative effect on the degradation of a readily biodegradable substrate, such as cheese whey, by activated sludge. With regard to the degradation of acetate, both metabolic activities (q_{o_2mean}, q_{Smean}) of BPA-acclimated activated sludge were about twofold the values corresponding to non-acclimated ones; in this case, a *t*-test showed that those differences were significant ($p < 0.05$). These differences could be attributed to the metabolic pathway of BPA. Spivack et al. (1994) reported that BPA can be metabolized to form 4-hydroxybenzoic acid and 4-hydroxyacetophenone. While 4-hydroxybenzoic acid is further oxidized and finally enters to the citrate cycle, 4-hydroxyacetophenone is oxidized by a monooxygenase to yield 4-hydroxyphenyl acetate, which is hydrolyzed

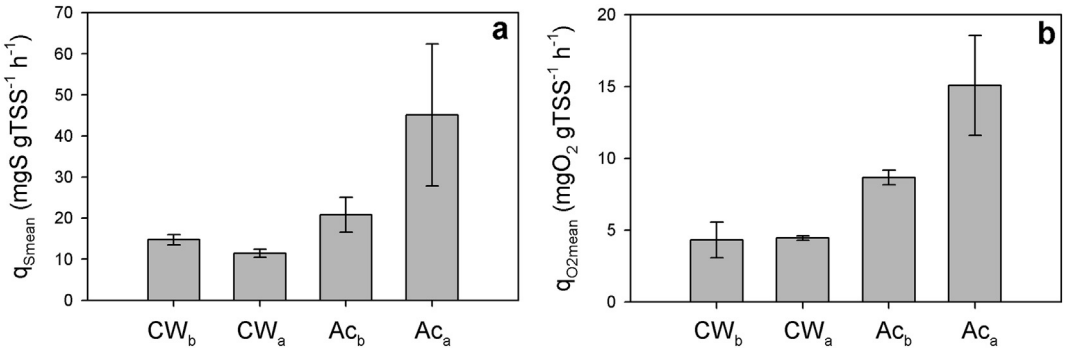

Fig. 6. Degradation activity of cheese whey (CW) and acetate (Ac) by activated sludge (X_{30}) before (CW_b, Ac_b) and after (CW_a, Ac_a) acclimation to BPA. (a) Specific mean substrate consumption rate (q_{Smean}), (b) specific mean oxygen uptake rate (q_{o_2mean}). Bars indicate one standard deviation.

to hydroquinone and acetate (Moonen et al., 2008; Rehdorf et al., 2009). The presence of acetate as an intermediate during the metabolism of BPA could cause an acclimation of the biomass to this substrate. Thus, this indirect acclimation to acetate could be the responsible for the observed enhancement on the metabolic activities (q_{o_2mean}, q_{Smean}) of the degradation of acetate by BPA-acclimated activated sludge.

4. Conclusion

In this work, information concerning the acclimation process of activated sludge to bisphenol A (BPA) was presented. The importance of the sludge age and the acclimation strategy on the degradation of BPA by activated sludge was established. Although biomass samples obtained from activated sludge reactors operated at sludge ages of 30 and 45 d (X_{30}, X_{45}) had the capability to acclimate to BPA, X_{30} had a higher stability, and higher acclimation rate to BPA than X_{45}. The specific BPA degradation rate corresponding to fully acclimated activated sludge ranged from 65 to 90 mgBPA gTSS^{-1} d^{-1}. Acclimation strategy using a constant initial BPA concentration (AST1) was more effective than increasing concentrations (AST2); in the latter, lower specific BPA consumption rates were obtained due to the toxic effect of the high initial BPA concentrations employed. Consumption of BPA by acclimated activated sludge was not affected by the presence of cheese whey or acetate; additionally, acclimation of activated sludge to BPA did not cause a negative effect on the biodegradation of these substrates. During the degradation of cheese whey, no differences between the specific mean oxygen uptake rate (q_{o_2mean}) and the specific mean substrate consumption rate (q_{Smean}) corresponding to non-acclimated and acclimated activated sludge were observed. With regard to the degradation of acetate, both metabolic activities (q_{o_2mean}, q_{Smean}) corresponding to acclimated activated sludge were about twofold the values of non-acclimated ones. This enhancement in q_{o_2mean} and q_{Smean} values could occur because the acetate is an intermediate during the metabolism of BPA.

Acknowledgements

Authors gratefully acknowledge the financial support given by Universidad Nacional de La Plata, Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET) and Agencia Nacional de Promoción Científica y Tecnológica (ANPCYT).

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