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Monitoring the biodegradability of bisphenol A and its metabolic intermediates by manometric respirometry tests

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Abstract As a result of its wide usage in the production of polycarbonate plastics and epoxy resins, bisphenol A (BPA) is commonly detected in wastewaters. Recently, BPA became a major concern because its adverse effects as an endocrine disruptor. In this work, the biodegradation kinetics of BPA and its metabolic intermediates 4-hydroxyacetophenone (4HAP), 4-hydroxybenzaldehyde (4HB) and 4-hydroxybenzoic acid (4HBA) by BPA-acclimated activated sludge was studied using manometric respirometry (BOD) tests. For all the tested compounds, BOD curves exhibited two phases. In the first one, a fast increase of the oxygen consumption (OC) due to the active oxidation of the substrate was obtained. Then, when the substrate was exhausted, the endogenous respiration produced a slower increase of OC. A standard Monod-model with biomass growth was used to represent the OC profiles as a function of time. For all the tested compounds, a good agreement between the proposed model and the experimental data was obtained. According to their biodegradation rates, the tested compounds can be ordered as follows: BPA < 4-HAP ≪ 4HB < 4HBA. Because the oxidation rate of BPA by BPA-acclimated activated sludge limits the rate of the whole biodegradation pathway, the accumulation of metabolic intermediates 4HAP, 4HB, and 4HBA would be negligible. To calculate the dissolved oxygen (DO) concentration (C) during the BOD tests, the oxygen transfer coefficient ($k_L a$) of the BOD bottles was obtained using the sulfite method. Finally, a simple procedure to calculate the minimum DO concentration (C_{min}) based on BOD data was developed. Calculation results demonstrated that under the tested conditions, C values were high enough as not to be the limiting substrate for the microbial growth.

Keywords Bisphenol A · Endocrine disruptor · Activated sludge · Respirometry · Modelling

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Introduction

Bisphenol A (BPA) (2,2-(4,4-dihydroxydiphenyl)propane, BPA) is a widely used monomer for the production of

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polycarbonates plastics and epoxy resins. About 65 % of the produced BPA is used in polycarbonates, and approximately 30 % in epoxy resins (Plastics Europe 2010; Geens et al. 2011). BPA is also used to manufacture a great variety of products, including compact disks, food can linings, thermal paper, safety helmets, plastic windows, car parts, adhesives, protective coatings, powder paints, and the sheathing of electrical and electronic parts (Staples et al. 1998). As a result of its wide usage, BPA is frequently detected in wastewaters (Melcer and Klecka 2011).

In recent years, BPA was identified as an endocrine disruptor and became a major concern (Omoike et al. 2013). Endocrine disrupting compounds are chemicals that mimic or inhibit the actions of endogenous hormones, and have the potential to alter the structure and function of the endocrine system. Several studies demonstrate reproductive, metabolic and neuro-developmental problems in animals exposed to environmentally relevant levels of BPA (Salian et al. 2009; Wei et al. 2011; Wolstenholme et al. 2011). Moreover, increased risk for cardiovascular disease, miscarriages, decreased birth weight at term, breast and prostate cancer, reproductive and sexual dysfunctions, altered immune system activity, metabolic problems and diabetes in adults, and cognitive and behavioral development in young children were associated with the exposure of humans to BPA (Braun et al. 2009, 2011; Lang et al. 2008; Rees-Clayton et al. 2011; Li et al. 2010; Miao et al. 2011; Sugiura-Ogasawara et al. 2005).

Several physicochemical processes for the removal of BPA from wastewaters were reported. Zeng et al. (2006) investigated the adsorption BPA on river sediment. Barbieri (2008) studied photodegradation of BPA. Xiaoying et al. (2009) studied the removal of BPA from water using polyaluminum chloride in coagulation process. The main problems of these processes are related with the use of high cost reactives, the generation of a BPA-containing sludge, and the carefully controlled conditions necessary to achieve an acceptable BPA removal degree. In recent years, several biological systems using pure and mixed cultures have been reported for the detoxification of BPA-containing wastewaters (Keum et al. 2010; Wang et al. 2014; Peng et al. 2015; Ferro Orozco et al. 2013, 2015, 2016). The most frequently detected metabolic products of the aerobic biodegradation pathway of BPA include 4-hydroxyacetophenone (4HAP), 4-hydroxybenzaldehyde (4HB) and the 4-hydroxybenzoic acid (4HBA) (Gao et al. 2010; Spivack et al. 1994; Sasaki et al. 2005; Sakai et al. 2007; Zhang et al. 2007). BPA and 4-hydroxybenzoic acid meet the definition of readily biodegradability (Stasinakis et al. 2008; West et al., 2001; OECD Guidelines for testing of chemicals. Paris 1993; UNEP publications 1999). As a result, these compounds are not expected to be persistent in an activated sludge system. Unfortunately, the information concerning de biodegradation kinetics of 4HAP and 4HB is scarce.

In a previous work, the aerobic biodegradation of BPA using BPA-acclimated activated sludge was studied using an open respirometer (Ferro Orozco et al. 2016). Due to the high biomass concentration employed in those experiments, the initial substrate to biomass ratio (S_0/X_0) ranged from 0.01 to 0.02 $gCOD_S/gCOD_X$. These low S_0/X_0 ratios can be expected in activated sludge reactors, for example. Conversely, in aquatic systems the biomass concentration is frequently several orders of magnitude lower than in activated sludge reactors. For this reason, S_0/X_0 ratios in aquatic systems are much higher than those expected in activated sludge reactors. Because the low initial biomass concentration used in manometric respirometry (BOD) tests, S_0/X_0 ratios in BOD tests are close to those obtained in aquatic systems. Thus, the objectives of this work were: a) to study the biodegradation kinetics of BPA and its metabolic intermediates 4HAP, 4HB and 4HBA by BPA-acclimated activated sludge using a manometric respirometry (BOD) test, and b) to determine if the dissolved oxygen (DO) concentration during the BOD tests is high enough as not to be a limiting substrate for the oxidation rate of the tested compounds.

Materials and methods

Chemicals

BPA 4-hydroxyacetophenone (4HAP), 4-hydroxybenzaldehyde (4HB) and 4-hydroxybenzoic acid (4HBA) (≥99 %) were from Sigma-Aldrich. Table 1 shows some properties of the tested compounds. Dehydrated Cheese whey was from Food S.A. (Villa Maipú, Argentina). All inorganic salts were commercial products of reagent grade from Anedra (San Fernando, Argentina).

Table 1 Properties of the tested compounds

Compound	CAS	Chemical formula	MW (g/mol)	ThOD (gO_2/mol)	Solubility in water (mg/L)
Bisphenol A (BPA)	80–05–7	$C_{15}H_{16}O_2$	228	576	120
4-hydroxyacetophenone (4HAP)	99–93–4	$C_8H_8O_2$	136	288	10,000
4-hydroxybenzaldehyde (4HB)	123–08–0	$C_7H_6O_2$	122	240	1300
4-hydroxybenzoic acid (4HBA)	99–96–7	$C_7H_6O_3$	138	224	5000

Activated sludge

Activated sludge used in this study was cultured in an aerobic laboratory-scale (4.5 L) activated sludge reactor with partial biomass recycle. The hydraulic retention time was 48 h; the sludge age (SA) was maintained at 30 d by daily wasting of the mixed liquor directly from the reactor. The reactor was fed with the following synthetic wastewater: 1.5 g of dehydrated cheese whey (CW, 1500 mg COD L^{-1}), 0.5 g of $(NH_4)_2SO_4$, and 1.03 g of $NaHCO_3$ dissolved in 1 L of tap water (Ferro Orozco et al. 2010). Operating temperature was 20 ± 2 °C. Aeration was provided by an air pump; air was pumped near the bottom of the reactor and the DO concentration was above 4 mg L^{-1}. Under steady-state conditions pH was 7.5 ± 0.4, COD of the effluent ranged from 30 to 80 mgCOD L^{-1}, and total suspended solids (TSS) concentration ranged from 3700 to 4500 mgTSS L^{-1}.

Acclimation of activated sludge to BPA

Consecutive batch assays were performed to obtain activated sludge acclimated to BPA (Ferro Orozco et al. 2013). The inoculum for the first batch was obtained from the aerobic laboratory-scale activated sludge reactor described in the previous section. Then, the inoculum for the successive batch assays 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 the 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}), $(NH_4)_2SO_4$ 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. The composition of M1 was (in g/100 mL): $FeSO_4.7H_2O$ 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 (in g/100 mL): $(NH_4)_6Mo_7O_{24}.4H_2O$ 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 solutions (1 N). For each batch, samples were taken at different times to determine soluble total organic carbon (TOCs) and BPA concentrations. TSS were used as a measure for the biomass concentration (X); duplicate TSS was determined at the beginning and at the end of each batch. The specific BPA degradation rate (q_{BPA}) was used to evaluate the acclimation degree of the activated sludge. At least three consecutive batch assays were performed to obtain activated sludge acclimated to BPA (see the Supplementary Data, Fig. SD1).

Biodegradation assays

A BOD Sensor System 6 (VELP Scientifica, Italy) was used to study the aerobic biodegradation of BPA 4-hydroxyacetophenone (4HAP), 4-hydroxybenzaldehyde (4HB), and 4-hydroxybenzoic acid (4HBA) by BPA-acclimated activated sludge. Due to the microbial respiration, oxygen is taken from the gas phase of a hermetically sealed bottle. Because the carbon dioxide released from respiration is absorbed by KOH, the reduction in air pressure inside the bottle is proportional to the oxygen consumption (OC). The above mentioned mineral medium with appropriate concentrations of each tested compound were poured into the BOD bottles. Then, 5 mL of BPA-acclimated activated sludge with an appropriate concentration were added to achieve an inoculum concentration of 30 mgTSS/L (40 mgCOD/L). Initial concentrations of the tested compounds were the following: 20 and

40 mg/L for BPA and 20–80 mg/L for 4HAP, 4HB and 4HBA. To prevent nitrification, allylthiourea was added in all flasks at concentration of 10 mg/L (Stasinakis et al. 2008). BOD bottles were incubated at 20 °C in a thermostatic room and OC readings were performed periodically. Experiments without a carbon source were used to assess the OC due to the endogenous respiration. Abiotic experiments were negative for all the tested compounds. In all cases, at least two replicates per experiment were performed.

Analytical techniques

Total suspended solids (TSS) were used to monitor the biomass concentration (Ferro Orozco et al., 2013). Soluble TOCs concentration was determined as follows: 5 mL of culture samples were centrifuged for 5 min at 13000 rpm (Eppendorf 5415C); then, the supernatant was filtered through 0.45 µm cellulosic membranes (Osmonics Inc.). TOCs concentration of the filtered samples was determined 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.

Modeling the oxygen consumption (OC) from the BOD tests

Among the available models to represent the aerobic biodegradation of a given compound, the Monod equation with biomass growth is one of the most used (Simkins and Alexander 1984, 1985). The model used in this work comprises three variables: organic substrate (S, mgCOD L^{-1}), active biomass (X_a, mgCOD L^{-1}) and non-active biomass (X_{na}, mgCOD L^{-1}). While X_a is responsible for the substrate consumption, X_{na} is not able to metabolize S. In the present model it was assumed that S limits the growth rate; thus, all other substrates (such as oxygen, nitrogen or micronutrients, for example) were in excess. To simplify the model, it was assumed that X_a and X_{na} had the same endogenous decay rate constant (b, h^{-1}):

$$\frac{dS}{dt} = -q_{Sm}\left(\frac{S}{K_S + S}\right)X_a \tag{1}$$

$$\frac{dX_a}{dt} = \left[\mu_m\left(\frac{S}{K_S + S}\right) - b\right]X_a \tag{2}$$

$$\frac{dX_{na}}{dt} = -bX_{na} \tag{3}$$

where μ_m (h^{-1}) is the maximum specific growth rate, q_{Sm} (mgCOD $mgCOD^{-1}$ h^{-1}) is the maximum specific substrate consumption rate, and K_S (mgCOD L^{-1}) is the Monod constant.

Because all variables are expressed in oxygen units, the oxygen consumption (OC, mgO_2 L^{-1}) can be obtained from the overall oxygen mass balance:

$$OC = S_0 - S + X_{a0} - X_a + X_{na0} + X_{na} \tag{4}$$

where S_0, X_{a0} and X_{na0} represent the initial concentrations. In the present model two microbial processes are responsible for the OC: the endogenous respiration and the metabolism of the tested compound (exogenous respiration). The total uptake rate (OUR, mgO_2 L^{-1} h^{-1}) due to these processes is

$$OUR = (q_{Sm} - \mu_m)\left(\frac{S}{K_S + S}\right)X_a + b(X_a + X_{na}) \tag{5}$$

Equation (5) can be used to verify if the DO concentration (C) in the BOD bottles is high enough so as not to be the limiting factor in the degradation rate of the tested compound. To calculate C as a function of time, mass balances for the oxygen in liquid and gas phases must be considered:

$$\frac{dC}{dt} = k_L a\left(\frac{P_{O_2}}{H} - C\right) - OUR \tag{6}$$

$$\frac{dP_{O2}}{dt} = -k_L aRT\left(\frac{P_{O_2}}{H} - C\right)\frac{V_L}{(V_T - V_L)}\frac{1}{M_{O2}} \tag{7}$$

where k_La (h^{-1}) is the volumetric mass transfer coefficient for oxygen, P_{O2} (atm) is the partial pressure of oxygen in the gas phase, H = 0.024 L atm mgO_2^{-1} is the Henry's law constant for oxygen in water (Sander, 2015), C is the DO concentration (mgO_2 L^{-1}), OUR (mgO_2 L^{-1} h^{-1}) is the OUR R = 0.082 L atm $°K^{-1}$ mol^1 is the gas constant, T = 293°K is the incubation temperature, V_L (L) is the volume of liquid

in the BOD bottle, $V_T = 0.5$ L is the total volume of the BOD bottle and $M_{O2} = 32000\ mgO_2\ mol^{-1}$ is the molar weight of oxygen. Therefore, if the microbial kinetic parameters and k_La are known, Eqs. (1)–(7) can be used to calculate C and P_{O2} as a function of time.

Assessment of k_La of the BOD bottles

The oxidation of sulfite (0.2 M) by DO in the presence of Cu(II) (1 mM) as catalyst was used to determine k_La of the BOD bottles. Sulfite solutions in the presence of an adequate catalyst, such as copper, are devoid of DO (Contreras, 2008). Because $C \cong 0$, it can be assumed that $\frac{dC}{dt} \cong 0$. Under this condition, the OUR is equal to the oxygen transfer rate (OTR) and Eq. (6) can be rearranged as follows

$$OUR = OTR = k_L a \frac{P_{O_2}}{H}. \tag{8}$$

Besides, under this condition, the integration of Eq. (7) yields the following

$$P_{O_2} = P_{O_2 0} e^{-Zt} \tag{9}$$

where $P_{O20} = 0.21$ atm is the initial partial pressure of oxygen and $Z = \frac{k_L a}{H} \frac{RT}{M_{O_2}} \frac{V_L}{(V_T - V_L)}$ is a constant. Combining Eqs. (8) and (9) and taking into account that, by definition, the oxygen consumed by the reaction with sulfite is the integral of OUR with respect to time

$$\mathrm{OC} = \int_0^t OURdt = OC_\infty \left(1 - e^{-Zt}\right) \tag{10}$$

where $OC_\infty = M_{O2} \frac{P_{O20}}{RT} \frac{(V_T - V_L)}{V_L}$ is the asymptotic OC. According to Eq. (10), for $V_L = 0.25$ L (the used volume in the present work), $OC_\infty = 280\ mgO_2\ L^{-1}$. For short times and low OC values, the value of $Zt < 1$ and $e^{-Zt} \cong 1 - Zt$; using this approximation, Eq. (10) can be rewritten as follows:

$$OC = \frac{P_{O_2 0}}{H} k_L a t. \tag{11}$$

Estimation of the model coefficients and dynamic simulations

The estimation of the coefficients of the mathematical model proposed in this work (Eqs. 1–5) and dynamic simulations were performed using the software package GEPASI 3.30 (Mendes 1993). GEPASI integrates the systems of differential equations with the routine Livermore Solver of Ordinary Differential Equations (LSODA). LSODA algorithm measures the stiffness of the equations and switches the integration method dynamically according to this measure. For non-stiff regions, the Adams integration method with variable step size and variable order up to 12th order is used; for stiff regions the Gear (or BDF) method with variable step size and variable order up to 5th order is used. Among the optimization methods available in GEPASI 3.30, the Multistart Optimization algorithm (with Levenberge-Marquardt local optimization) was selected. Multistart is a hybrid stochastic-deterministic optimization method. Rather than run a single local optimization (e.g. gradient descent methods), Multistart runs several of them, each time starting from a different initial guess. The first start takes for initial guess the parameter values entered by the user. The initial guesses for the subsequent starts are generated randomly within the boundaries for the adjustable parameters. The local optimizer used is the Levenberge-Marquardt method as this method has proved the most efficient gradient optimizer used in GEPASI 3.30. More details can be found in the Supplementary Data, item SD2.

A sensitivity analysis of the proposed model showed that the sensitivity of OC to K_S was much lower than the sensitivity of OC to the coefficients q_{Sm}, μ_m, or b in terms of absolute values. In other words, high variations on the K_S value would have lower effects on the OC profile than low variations on the values of q_{Sm}, μ_m, or b. Hence, q_{Sm}, μ_m, and b values would be always more easily assessed and the relative estimation error of these coefficients is always lower than the relative estimation error of K_S. For this reason, to reduce the fitting errors of coefficients q_{Sm}, μ_m and b, it was assumed that $K_S = 1\ mgCOD\ L^{-1}$ for all the tested compounds. Although the total biomass concentration (X_T) was constant for all the assays, the initial active biomass concentration (X_{a0}) was unknown; for this reason, this value was also fitted. Finally, the initial substrate concentration was adjusted within 2 % of its actual value. This procedure takes into account the degree of uncertainty in the initial conditions due to analytical errors (Mendes and Kell 1998). For more details see Supplementary Data, item SD3.

Results and discussion

Biodegradation of the tested compounds

Figure 1 shows the OC profiles corresponding to the biodegradation of the tested compounds by BPA-acclimated activated sludge. As a general rule, two phases were clearly noticeable. The first phase comprised a fast increase of the OC value due to the actively degradation of the organic substrate. At a given critical time (t_C) the substrate was depleted. Then, in the second phase the endogenous respiration produced a slower increase of OC. According to the method OECD 301F (OECD, 1993), the amount of oxygen taken up by the microbial population during the biodegradation of the test substance must be corrected for uptake by blank inoculum run in parallel. It must be pointed out that this correction is based on the assumption that the endogenous oxygen uptake rate (OUR_{en}) corresponding to the biodegradation of the tested substance and the blank BOD bottles are constant and similar in both assays. This assumption could be true when the tested substrate cannot be a carbon source for the microbial growth (e.g., when the inoculum is not acclimated to the tested compound). Conversely, in the cases when the increment of the active biomass is noticeable, the initial OUR_{en} value (e.g., the blank test) is lower than the OUR_{en} at the end of the biodegradation of the tested substance.

To verify this assumption, the OUR_{en} after the substrate depletion (OUR_{en}) was obtained from the slope of the line obtained by plotting OC as a function of time during the second phase of the BOD assays. Regardless the substrate, Fig. 2 shows that OUR_{en}

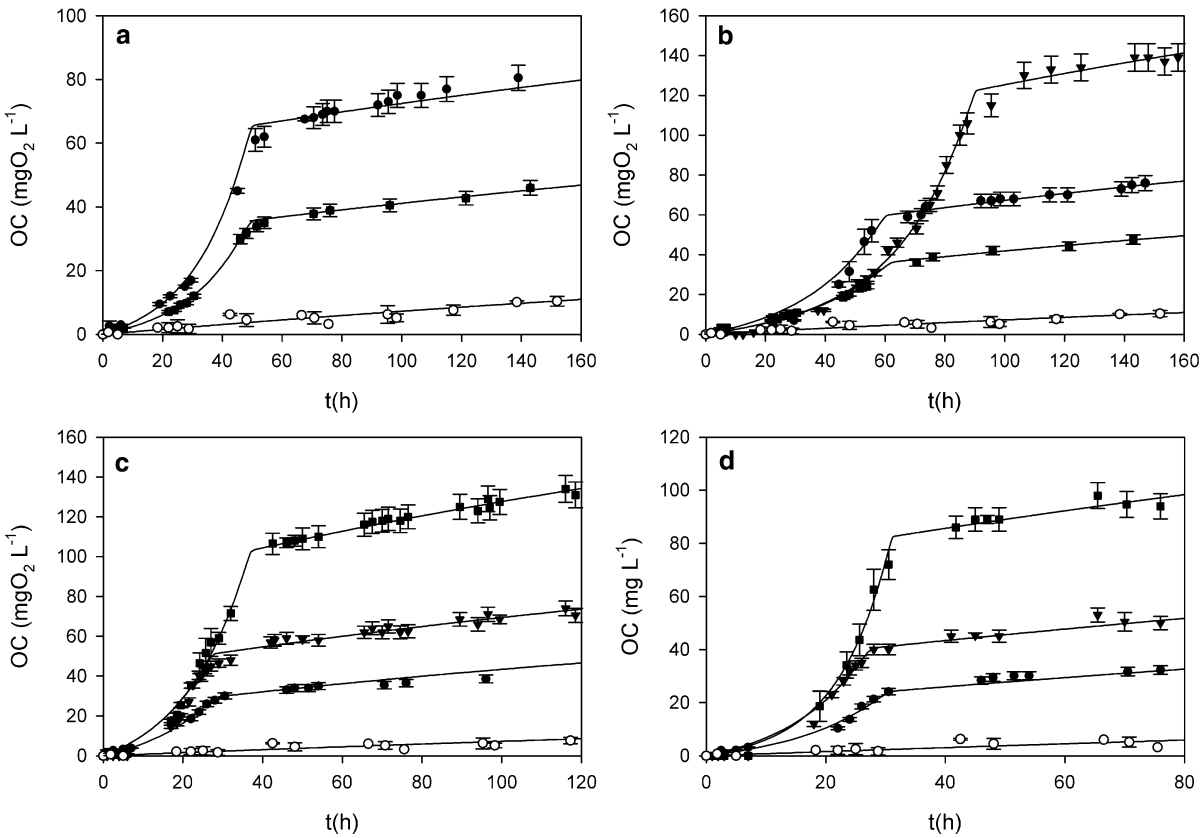

Fig. 1 Oxygen consumed (OC) during the aerobic biodegradation of the following initial concentrations (in mgThOD/L) of the tested compounds: **a** BPA: (*filled circle*) 100, (*filled square*) 50; **b** 4HAP: (*filled inverted triangle*) 170, (*filled circle*) 85, (*filled square*) 43; **c** 4HB: (*filled square*) 157, (*filled inverted triangle*) 78, (*filled circle*) 39; **d** 4HBA: (*filled square*) 130, (*filled inverted triangle*) 65, (*filled circle*) 33. *Open symbols* represent the blank experiments (without a carbon source). *Bars* indicate the standard deviation. In all cases, *continuous lines* represent the proposed model

increased as a function of the initial substrate concentration. Thus, if the OC of the blank test is used to correct the OC obtained from the tested substrate, this procedure may lead to an overestimation of the corrected OC values. Moreover, to evaluate the biodegradation degree of the tested compound, OECD also recommends expressing the results from manometric respirometry tests as a percentage of theoretical oxygen demand (ThOD) or COD of the tested sample (OECD, 1993). However, because the corrected OC values are overestimated, the biodegradation degree of the tested sample is also overestimated. Because the growth of microorganisms, the initial OUR_{en} value is lower than the corresponding to the second phase. Thus, when the OC values of the tested sample are corrected by the OC corresponding to the blank test, the corrected OC values increased with time. When biodegradation percentages are calculated according to the OECD recommendation, results demonstrate that for sufficient long times, erroneous biodegradation percentages higher than 100 % could be achieved (Supplementary Data, Fig. SD4). For these reasons, in this work raw OC values (e.g., not corrected by the OC values of the blank test) were analyzed.

Equations 1–5 were fitted to the obtained OC profiles as a function of time corresponding to the tested compounds; fitting results are shown in Table 2. Details concerning fitting results can be found in the Supplementary Data, items SD4 and SD5. Table 2 shows that the more oxidized is the tested compound, the faster its biodegradation. Although the lowest μ_m and q_{Sm} values were obtained when 4HAP was tested, it must be considered that the inoculum used in the BOD tests was BPA-acclimated activated sludge. For this reason, μ_m on BPA was higher than the one obtained with 4HAP. From μ_m and q_{Sm} values depicted in Table 2, the oxidation coefficient of each tested compound ($Y_{O/S}$, $gO_2/gThOD$) can be calculated as follows:

$$Y_{O/S} = \frac{q_{Sm} - \mu_m}{q_{Sm}}. \quad (12)$$

Regardless the substrate, $Y_{O/S}$ values obtained in the present work (Table 3) demonstrate that about 60 % of the available electrons of the tested compounds were transferred to molecular oxygen. Table 3 also shows that the obtained $Y_{O/S}$ values expressed in mol units are in accordance with those obtained from the aerobic biodegradation pathway of BPA that it was proposed in a previous work (Ferro Orozco et al. 2016).

For comparison purposes, the specific mean substrate consumption rate (q_{Smean}) was calculated as the ratio between the initial substrate concentration and the total degradation time (t_C) normalized by the initial biomass concentration (Ferro Orozco et al., 2016; Lobo et al. 2013). Expressing q_{Smean} values in mol units, Table 3 shows that the tested compounds can be ordered as follows: BPA < 4HAP ≪ 4HB < 4HBA. Therefore, for a given biomass concentration, the oxidation rate of BPA limits the rate of the whole biodegradation pathway and the accumulation of the metabolic intermediates 4HAP, 4HB, and 4HBA would be negligible. It must be pointed out that in a previous work (Ferro Orozco et al. 2016), the aerobic degradation of BPA and its metabolic intermediates was studied in an open respirometer where the initial substrate to biomass ratio (S_0/X_0) ranged from 0.01 to 0.02 $gCOD_S/gCOD_X$. Conversely, in the present work the ratio S_0/X_0 ranged between 1 and 6 $gCOD_S/gCOD_X$, about two orders of magnitude higher than the one used in the previous work. The agreement between both studies indicates that the relative biodegradation rates and the stoichiometry of the oxidation of the tested compounds are intrinsic features of BPA-acclimated activated sludge.

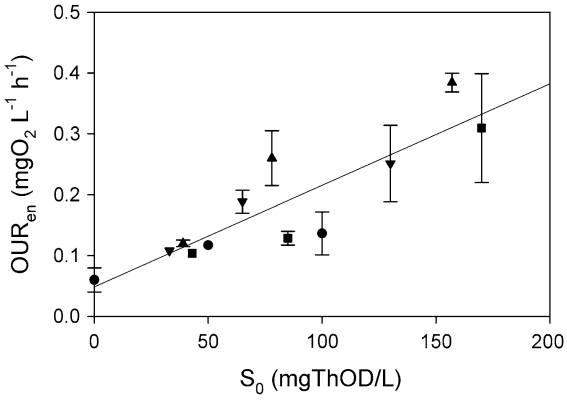

Fig. 2 Endogenous oxygen uptake rate after the substrate depletion (OUR_{en}) as a function of the initial substrate concentration: (*filled circle*) BPA; (*filled square*) 4HPA; (*filled triangle*) 4HB; (*filled inverted triangle*) 4HBA. *Bars* indicate the standard deviation

Assessment of the DO concentration (C) during the BOD assays

In general, activated sludge not acclimated to the tested compound is usually used as inoculum for the

Table 2 Kinetic coefficients corresponding to the aerobic biodegradation of the tested compounds

Compound	μ_m (h^{-1})	q_{Sm} ($gCOD\ gCOD^{-1}\ h^{-1}$)	$b \times 10^3$ (h^{-1})	r^2	m[a]	n[b]	RMSE[c] ($mgO_2\ L^{-1}$)
BPA	0.061 ± 0.004	0.142 ± 0.033	2.6 ± 0.6	0.9970	7	106	1.3827
4HAP	0.044 ± 0.004	0.118 ± 0.044	3.2 ± 1.1	0.9958	8	123	2.5904
4HB	0.089 ± 0.004	0.216 ± 0.034	4.4 ± 0.8	0.9969	10	202	2.2242
4HBA	0.120 ± 0.006	0.289 ± 0.085	3.7 ± 1.0	0.9963	9	133	1.8513

[a] Number of independent experiments

[b] Total number of data

[c] Root mean square error

Table 3 Comparison of kinetic (q_{Sm}) and stoichiometric ($Y_{O/S}$) coefficients obtained from manometric respirometry (this work) and open respirometry (Ferro Orozco et al. 2016)

Compound	q^a_{Smean} ($\mu mol\ gCOD^{-1}\ h^{-1}$)	q^b_{Smean} ($\mu mol\ gCOD^{-1}\ h^{-1}$)	$Y^a_{O/S}$ ($gO_2/gThOD$)	$Y^a_{O/S}$ ($molO_2/mol$)	$Y^b_{O/S}$ ($molO_2/mol$)
BPA	6.9 ± 2.0	8–11	0.57 ± 0.13	10.3 ± 2.3	10.0 ± 0.5
4HAP	12.8 ± 3.3	7–108	0.63 ± 0.23	5.6 ± 2.1	5.2 ± 0.2
4HB	34.8 ± 9.7	15–350	0.59 ± 0.09	4.4 ± 0.7	3.7 ± 0.2
4HBA	31.7 ± 8.9	15–380	0.58 ± 0.17	4.1 ± 1.2	3.0 ± 0.1

[a] This work

[b] Open Respirometry experiments (Ferro Orozco et al. 2016)

BOD test (OECD, 1993). As a result, lag phases of up to 15 days and slow degradation rates are frequently observed (Reuschenbach et al. 2003; Stasinakis et al. 2008). It must be noted that kinetic parameters obtained from these experiments mainly reflect the adaptation process of the used microbial consortium to the tested compound. Conversely, in this work BPA-acclimated activated sludge were used as inoculum for the BOD tests, leading to shorter lag phases and higher biodegradation rates. As in all aerobic process, the DO concentration (C) may limit the growth rate of the microorganisms in the BOD test. To avoid this problem, the volumetric mass transfer coefficient for oxygen (k_La) must be high enough as to satisfy the total OC rate (OUR) of the microorganisms throughout the assay. For this reason, it is crucial to know the value of k_La of the BOD bottles.

Figure 3a shows the OC as a function of time due to the oxidation of sulfite corresponding to different volumes of liquid (V_L) in the BOD bottles. In accordance with Eq. (11), a linear increase of OC as a function time was observed. From the slope of the regression line obtained by plotting OC as a function of time (Eq. 11), k_La values corresponding to the different tested V_L were obtained. Results show that k_La decreased from 7.90 ± 0.07 to 2.00 ± 0.05 h^{-1} as V_L increased from 100 to 400 mL; for V_L = 250 mL (the used volume of liquid in all the biodegradation tests of the present work), k_La was 3.68 ± 0.04 h^{-1}. Figure 3a also shows that the achieved maximum OC was about 120 $mgO_2\ L^{-1}$; considering the stoichiometry for the oxidation of sulfite to sulfate by oxygen, this OC value represents a consumption of 7.5 mM of sulfite, which is less than 4 % of the initial sulfite concentration (200 mM). Finally, Fig. 3b shows that within the obtained range of OC values, the coefficient of variation of OC (CV_{OC}) was always less than 9 % of the measured value; the overall average CV_{OC} value was 5 %.

The slope of the lines depicted in Fig. 3a corresponded to the initial oxygen transfer rate per unit volume of liquid (OTR_0) that can be delivered from the gas to the liquid phase. For example, when

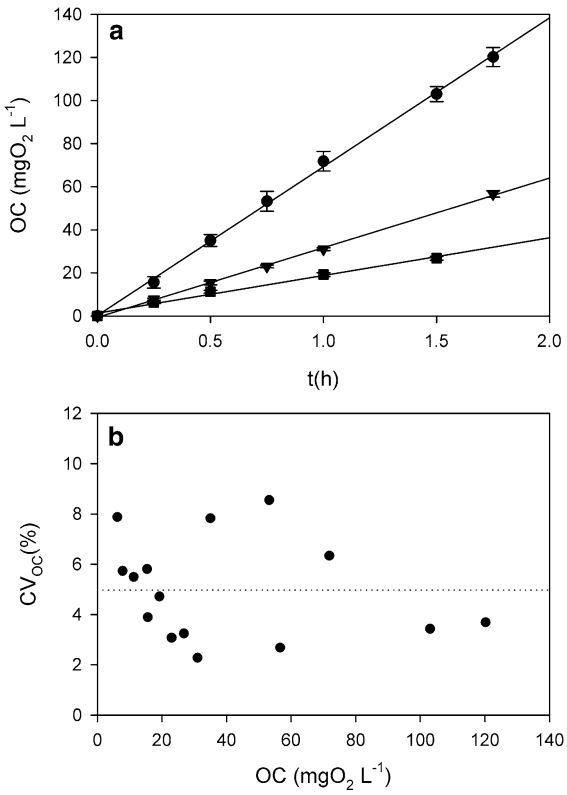

Fig. 3 **a** Oxygen consumed (OC) during the oxidation of sulfite (0.2 M). *Bars* indicate the standard deviation of OC (σ_{OC}) corresponding to five replicates. *Lines* represent the regression line. Different symbols correspond to the following V_L in the BOD bottles (V_L): (*filled circle*) 100 mL, (*filled inverted triangle*) 250 mL, (*filled square*) 400 mL. In all cases, the total volume (V_T) was 500 mL, T = 20 °C, initial sulfite concentration = 0.2 M, Cu(II) concentration = 1 mM. **b** Coefficient of variation of OC (CV_{OC}) as a function of OC values. *Dotted line* indicates the overall average CV_{OC} value

$V_L = 250$ mL, OTR_0 was 32 $mgO_2\ L^{-1}\ h^{-1}$. Thus, if during a given BOD assay the slope of OC as a function of time (e.g., the OUR) is close to OTR_0, this fact is an indication that degradation kinetics could be limited by oxygen depletion. Moreover, according to Eq. (8), OTR is proportional to P_{O2}. Due to the microbial respiration (Eq. 5) always P_{O2} decreases as a function of time, and therefore, OTR also. Moreover, if the tested compound can serve as a carbon and energy source for the microbial growth, OUR will increase as a function of time. As a result, rate limiting DO conditions could also be attained during the BOD test.

Once the value of k_La for the BOD bottles was obtained, Eqs. (1)–(7) along with the coefficients depicted in Table 1 were used to simulate the DO concentration (C) as a function of time. Figure 4a shows typical OC profiles as a function of time corresponding to the tested compounds. In all cases, OC was lower than 140 $mgO_2\ L^{-1}$. Although OC values were always less than 50 % of the maximum value (e.g., $OC_\infty = 280\ mgO_2\ L^{-1}$, Eq. 10), these OC caused a significant decrease on P_{O2} (Fig. 4b). Moreover, a sharp decrease on C associated with a change of the OUR was obtained. However, within the tested time interval, in all cases C was always higher than 4 $mgO_2\ L^{-1}$ (Fig. 4c). As a general rule, this value is considered high enough as to avoid DO limiting conditions (Orhon and Artan 1994; Henze et al., 2002). This result demonstrates that kinetic coefficients determined from the BOD assays performed in this work (Tables 2 and 3) were obtained under conditions of an excess of DO.

Calculating the minimum DO concentration (C_{min}) based on BOD data

In most cases, the biodegradation kinetics of a given compound is not known. However, the DO concentration in equilibrium with P_{O2} (C_{eq}) can be calculated from the OC data as a function of time obtained during a typical BOD test as follows. The total amount of oxygen consumed by the microorganisms is the difference between the initial amount of oxygen within the bottle and the amount at a given time t:

$$OCV_L = M_{O_2}[(n_{G0} + n_{L0}) - (n_G + n_L)] \tag{13}$$

where n_G and n_L represents the amount (mol) of oxygen in the gas and liquid phase, respectively, and the sub index 0 represents the initial values. n_L can be easily obtained from the product between the volume of liquid (V_L) and the DO concentration (C). Although C depends on the balance between oxygen transfer and respiration rates, according to the Henry's law, its maximum value (C_{eq}) is determined by P_{O2}:

$$C_{eq} = \frac{P_{O_2}}{H} \geq C \tag{14}$$

Thus, the maximum amount of oxygen in the liquid phase (n_{Lm}) is:

$$n_{Lm} = V_L \frac{P_{O_2}}{HM_{O2}} \geq n_L \tag{15}$$

Assuming ideal gas for oxygen in the gas phase and combining (13) to (15), the following can be obtained:

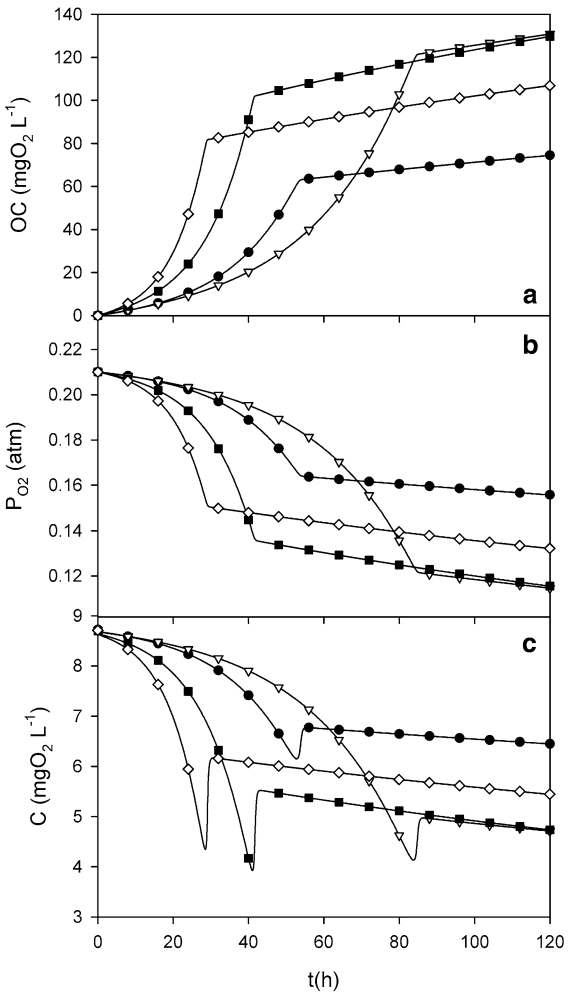

Fig. 4 **a** Oxygen consumption (OC), **b** partial pressure of oxygen (P_{O2}), and **c** DO concentration (C) as a function of time calculated by Eqs. 1–7 using the coefficients depicted in Table 2. Initial concentrations (mgThOD/L): (*filled circle*) BPA = 100, (*open inverted triangle*) 4HAP = 170, (*filled square*) 4HB = 157, (*open diamond*) 4HBA = 130. In all cases: $X_{v0} = 2$, $X_{nv0} = 38$, $k_La = 3.68\ h^{-1}$, $P_{O20} = 0.21$ atm

$$OCV_L = M_{O_2}(n_{G0} - n_G)\left(1 + \frac{V_L}{(V_T - V_L)}\frac{RT}{HM_{O_2}}\right) \cong M_{O_2}(n_{G0} - n_G). \tag{16}$$

The term $\frac{V_L}{(V_T - V_L)}\frac{RT}{HM_{O_2}}$ represents the ratio between n_{Lm} and n_G. In the present work $V_L = ½\ V_T$ and T = 293 °K; using these values, the ratio n_{Lm}/n_G is 0.03. Because $n_L \leq n_{Lm} \ll n_G$, it can be concluded that under the conditions employed in the present work, the contribution of n_L on the value of OC (Eq. 13) is negligible.

The change of P_{O2} can be related with the change of n_G as follows

$$(P_{O_20} - P_{O_2})(V_T - V_L) = (n_{G0} - n_G)RT \tag{17}$$

Combining Eqs. (16), (17) and solving for P_{O2}, the following can be obtained

$$P_{O_2} = P_{O_{20}} - \frac{V_L}{(V_T - V_L)}\frac{RT}{M_{O_2}}OC \tag{18}$$

Finally, according to the Henry's law (Eq. 14), the DO concentration in equilibrium with the oxygen in the gas phase (C_{eq}) is

$$C_{eq} = \frac{1}{H}\left(P_{O20} - \frac{V_L}{(V_T - V_L)}\frac{RT}{M_{O2}}OC\right) \geq C. \tag{19}$$

It must be noted that C_{eq} represents the upper limit of C. However, to obtain the minimum value of C during the biodegradation of the tested compound (C_{min}), the maximum respiration rate (OUR_{max}) must be considered. By definition (Eq. 10), OUR is the derivative of OC with respect to time. Thus, based on the profile of OC as a function of time, OUR_{max} can be easily obtained by several numerical techniques, such as a moving regression window, for example. During the BOD test, at a certain t_C OUR reaches its maximum value (OUR_{max}) and the DO concentration attains its minimum level ($C = C_{min}$, $\frac{dC}{dt} = 0$). Under these conditions and combining Eqs. (6)–(19), the following is obtained

$$C_{min} = \frac{1}{H}\left(P_{O_{20}} - \frac{V_L}{(V_T - V_L)}\frac{RT}{M_{O2}}OC_c\right) - \frac{OUR_{max}}{k_La} \tag{20}$$

where OC_C is the value of OC at $t = t_C$.

To evaluate the actual maximum biodegradation rate of a given compound, experimental conditions must be selected to maintain the DO above a certain critical concentration (C_C). In this sense, Eq. (20) can be used to assess if C_{min} was higher than C_C throughout the BOD test. According to Eq. (20), not only high OUR values but also high OC may lead to low DO concentrations. Figure 5 shows examples of these two conditions. During the biodegradation of 4HBA (Fig. 5a–c), an OUR_{max} value of about 7 $mgO_2\ L^{-1}\ h^{-1}$ (Fig. 5b) was achieved at $t_c = 30$ hs; at this time, the critical OC value was $OC_C = 80\ mgO_2/L$ (Fig. 5a). According to the kinetic coefficients shown in Table 1, the biodegradation of 4HAP must be

slower than the corresponding to 4HBA. For this reason, when 4HAP was tested, the experimental OUR_{max} value was 3.5 $mgO_2\ L^{-1}\ h^{-1}$ (Fig. 5e) and the critical time ($t_C = 90$ hs) was longer than the obtained from the experiments with 4HBA. Because the initial concentration of 4HAP (170 mgThOD/L) was higher than 4HBA (130 mgThOD/L), OC_C for 4HAP was 120 mgO_2/L (Fig. 5d). Using these values in Eq. (20), the calculated minimum DO concentration for 4HBA and 4HAP were 4.3 and 4.0 mgO_2/L, respectively. Figure 5c,f show that in both cases these values were close to the minimum C value calculated using the full model (Eqs. 1–7). This agreement demonstrates that Eq. (20) could be a valuable tool to assess C_{min} during a BOD test.

Conclusions

In this work, the biodegradation kinetics of BPA 4-hydroxyacetophenone (4HAP), 4-hydroxybenzaldehyde (4HB) and 4-hydroxybenzoic acid (4HBA) by BPA-acclimated activated sludge was studied using manometric respirometry (BOD) tests. From the obtained results, the following conclusions were withdrawn:

- For all the tested compounds, BOD curves exhibited two phases. In the first one, a fast increase of the OC due to the active oxidation of the substrate was obtained. Then, when the substrate was exhausted, the endogenous respiration produced a slower increase of OC.

- A standard Monod-model with biomass growth was used to represent the OC profiles as a function of time. For all the tested compounds, a good agreement between the proposed model and the experimental data was obtained. From the fitting of the model to the experimental data, the following coefficients were obtained: maximum specific growth rate (μ_m), maximum specific substrate consumption rate (q_{Sm}) and endogenous decay coefficient (b).

– According to their biodegradation rates, the tested compounds can be ordered as follows:

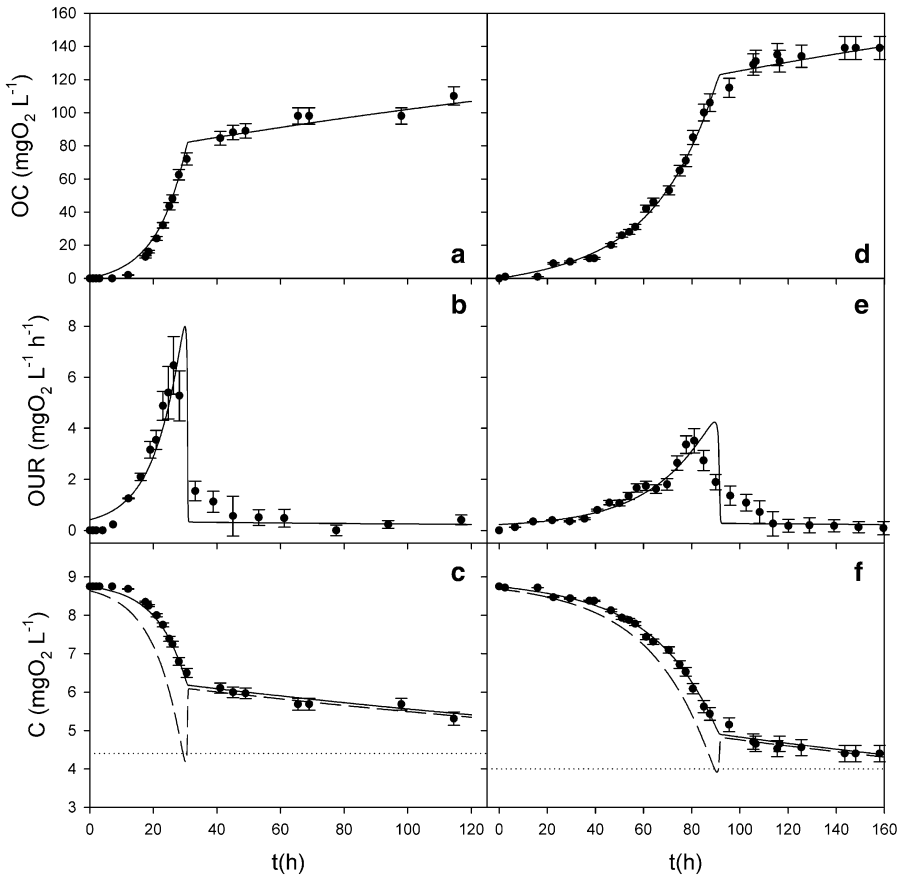

Fig. 5 Examples of calculation of the DO concentration in equilibrium with the oxygen in the gas phase (C_{eq}) and the minimum DO concentration (C_{min}) during the biodegradation of 4HBA (**a**–**c**) and 4HAP (**d**–**f**). Experimental OUR values **b**, **e** were obtained from OC data **a**, **d** using a moving regression window. C_{eq} as a function of time **c**, **f** was calculated using Eq. (19). *Continuous lines* represent the proposed model (Eqs. 1–7); *dashed lines* in **c**, **f** symbolize the simulated DO concentration. *Dotted lines* represent the C_{min} value calculated using Eq. (20). In all cases, *bars* represent the standard deviation

BPA < 4HAP ≪ 4HB < 4HBA. Because the oxidation rate of BPA by BPA -acclimated activated sludge limits the rate of the whole biodegradation pathway, the accumulation of metabolic intermediates 4HAP, 4HB, and 4HBA would be negligible.

– To calculate the DO concentration (C) during the BOD tests, the oxygen transfer coefficient (k_La) of the BOD bottles was obtained using the sulfite method. Calculations show that in all cases C values were high enough as not to be a limiting substrate for the microbial growth.
– A simple procedure (Eq. 20) to calculate the minimum DO concentration (C_{min}) based on BOD data was developed. A good agreement between C_{min} values calculated by the developed procedure and the values from simulation results were obtained.

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