Chemical Engineering Journal 143 (2008) 293–298

Contents lists available at ScienceDirect

Chemical Engineering Journal

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

# Novel bioreactor design for decolourisation of azo dye effluents

Gergo Mezohegyi [a], Christophe Bengoa [a], Frank Stuber [a], Josep Font [a], Azael Fabregat [a,*], Agustí Fortuny [b]

[a] Departament d'Enginyeria Química, ETSEQ, Universitat Rovira i Virgili, Av. Països Catalans 26, 43007 Tarragona, Catalunya, Spain
[b] Departament d'Enginyeria Química, EPSEVG, Universitat Politécnica de Catalunya, Av. Víctor Balaguer s/n, 08800 Vilanova i la Geltrú, Catalunya, Spain

ARTICLE INFO

*Article history:*
Received 21 December 2007
Received in revised form 5 May 2008
Accepted 6 May 2008

*Keywords:*
Azo dye
Biological activated carbon
Packed-bed reactor
Redox mediator

ABSTRACT

The anaerobic decolourisation of azo dye Acid Orange 7 (AO7) was studied in a continuous upflow stirred packed-bed reactor (USPBR) filled with biological activated carbon (BAC). Special stirring of BAC and different biodegradation models were investigated. The application of appropriate stirring in the carbon bed resulted in an increase of azo dye bioconversion up to 96% in 0.5 min, compared to unstirred reactor system with ensuring high dye degradation rates at very short space times. In addition, USPBR provided much more reproducible data to make kinetic modeling of AO7 biodegradation. First-order, autocatalytic and Michaelis–Menten models were found to describe the decolourisation process rather well at lower initial dye concentration. AO7 showed significant inhibition effect to biomass beyond inlet dye concentrations of 300 mg $L^{-1}$. Expanding Michaelis–Menten kinetics by a substrate inhibition factor resulted in a model giving good fitting to experimental points, independently on the initial colourant concentration. Processing at very low hydraulic residence time together with higher initial dye concentration resulted in toxicity to bacteria.



## 1. Introduction

Azo colourants make up the largest and most versatile class of dyes with more than 2000 different azo dyes being currently used [1]. A typical drawback of azo dye colouration – mainly occured in textile industry – is that large amounts of the dyestuff are directly spilt to wastewater. These chemicals and their degradation products may cause serious problems of environmental pollution and, in addition, the increased demand for textile products have made textile industry one of the main sources of severe environmental problems worldwide [2]. Relevant factories have deficiencies of treating efficiently these effluents on industrial scale, particularly at higher dye concentrations and at lower energy consumptions.

Up to now, several methods have been found to treat azo dye wastewaters [3–5]. However, among the diverse colour removal techniques, biological methods seem to be the most economic and environmental friendly. Many reviews are available on microbiological decolourisation of dyes and azo dyes [1,6,7–9]. While latter ones can be reduced to the corresponding amines by bacteria under anaerobic conditions, they are difficult to completely breakdown aerobically [10]. On the other hand, the anaerobic breakdown products of azo dyes are more susceptible to biodegradation under aerobic conditions rather than under anaerobic conditions. Complete treatment and efficient biomineralisation process can, thus, be obtained by a sequential anaerobic–aerobic process [8].

These sequential reactor studies have shown that a generally high extent of colour removal can be obtained [11] and several of them furthermore provide evidence for removal of aromatic amines [12,13]. However, anaerobic reduction of many azo dyes can be considered as a relatively slow process [10,12–14] that is, practically the only, but serious disadvantage of biological azo dye decolourisation. To overcome this problem, by using redox mediators during the reduction, anaerobic biodegradation can be enhanced resulting in much higher removal rates. During last years, evidences have been accumulated that quinoid compounds and humic substances can play important roles as redox mediators in anaerobic reduction processes such as biotransformation of azo dyes, polyhalogenated pollutants and nitroaromatics [15]. Among quinones, mostly applied compounds in azo dye degradation as catalytic mediators have been anthraquinone-2,6-disulfonate [16–19] and anthraquinone-2-sulfonate [18,19], both resulting highly efficient azo dye decolourisation. However, homogeneous reaction requires continuous dosing of the redox mediator resulting additional process costs. This problem can be avoided by immobilizing the electron mediator in the bioreactor. Aside from immobilized anthraquinone [20], activated carbon as a possible solid redox mediator containing surface quinonic structures, was reported to be enable to accelerate azo dye reduction [21,22].

* Corresponding author. Tel.: +34 977 559643; fax: +34 977 559667.
*E-mail address:* afabrega@urv.cat (A. Fabregat).

1385-8947/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.cej.2008.05.006

The role of activated carbon as catalyst is diverse in different reactions, related to oxidation, combination and decomposition but not to reduction [23]. Research for dye wastewater treatments by BAC system under anaerobic conditions have not been so widespread either. Upflow anaerobic sludge blanket reactors have been the most commonly used high-rate anaerobic systems that could be used for treatment of dye wastes [24]. To our knowledge, packed-bed-type reactors using biological activated carbon system have never been applied for anaerobic azo dye decolourisation by other authors.

In our previous study [22] the results cleared the efficiency of using a solid electron mediator in both continuous upflow packed-bed reactors (UPBR) and discontinuous reactors during anaerobic Acid Orange 7 (AO7) reduction. Moreover, evidences were given that in UPBR with BAC system, the electron conductivity of the active carbon and its specific surface with both functional groups and the carbon's strong adsorption capacity for Acid Orange 7, contribute together to higher azo dye decolourisation rates. Recent study similarly concerns with testing the anaerobic biodegradation of azo dye AO7 in packed-bed reactors containing biological activated carbon. Differences in goals of present study were to develop UPBR reactors (USPBR) to both have more effective treatment and make kinetic modeling possible; to investigate the effect of stirring of BAC; and, to develop a possible model to describe anaerobic azo dye biodegradation in USPBR–BAC system.

## 2. Materials and methods

### 2.1. Chemicals

Azo dye Orange II (C.I. Acid Orange 7) sodium salt (dye content 99%, Sigma, ref. O8126), an acid dye widely used in textile processes, was selected as model azo colourant. Sulfanilic acid, one of the anaerobic degradation products of Acid Orange 7 was supplied by Sigma (min. 99%, ref. S5263). Sodium acetate (99%, Aldrich, ref. 11019-1) was used as co-substrate being both the carbon source for sludge and electron donor for azo reduction. Activated carbon (Merck, granules of 2.5 mm, ref. 1.02518.1000) was used as catalytic support material in upflow stirred packed-bed reactors. Activated carbon was crushed and granules of 25–50 mesh size were separated, washed with distilled water, dried at 104 °C for 15 h and stored under normal conditions. Carborundum granules (Carlo Erba Reagents, ref. 434766) were used as inert diluent for activated carbon. The basal media contained the following compounds (mg $L^{-1}$): $MnSO_4{\cdot}H_2O$ (0.155), $CuSO_4{\cdot}5H_2O$ (0.285), $ZnSO_4{\cdot}7H_2O$ (0.46), $CoCl_2{\cdot}6H_2O$ (0.26), $(NH_4)_6Mo_7O_{24}$ (0.285), $MgSO_4{\cdot}7H_2O$ (15.2), $CaCl_2$ (13.48), $FeCl_3{\cdot}6H_2O$ (29.06), $NH_4Cl$ (190.9), $KH_2PO_4$ (8.5), $Na_2HPO_4{\cdot}2H_2O$ (33.4), $K_2HPO_4$ (21.75).

### 2.2. Upflow stirred packed-bed reactor setup

Fig. 1 shows schematically the continuous and anaerobic experimental system. The upflow stirred packed-bed reactor has a diameter of 15 mm with a volume of 10 mL. It is filled with the mixture of 10 g of carborundum granules as inert and 1 g of activated carbon with size of 25–50 mesh. The reasons of using an inert diluent for activated carbon are that on the one hand, it is required to test unit amount of catalyst, and on the other hand, because of technological reasons, since the stirring system in USPBR requires a minimal bed volume of about 10 mL while 1 g of AC has only about 3 mL of apparent volume. The packed-bed porosity is about 0.3. Two filters were placed into the top and bottom of the reactor to prevent washing out of AC. The temperature was kept constant at 35 °C. The entering feed was 100 mg $L^{-1}$ Acid Orange 7 solution

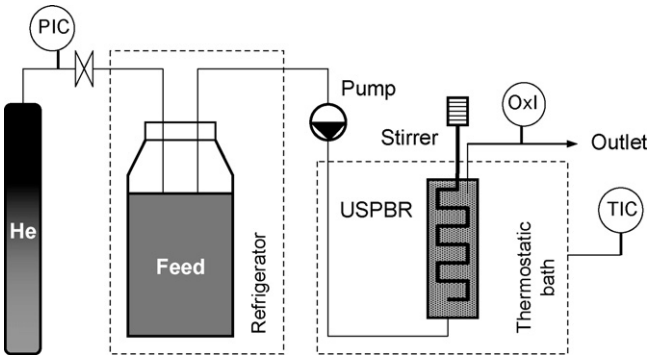


**Fig. 1.** Anaerobic upflow stirred packed-bed reactor setup.

containing 200 mg $L^{-1}$ sodium acetate and the basal media with microelements. The flow rate of the feed was varied between 25 and 350 mL $h^{-1}$ and was ensured by a micro pump (Bio-chem Valve Inc., ref. 120SP2420-4TV). The pH of the outlet solution varied between 6.7 and 7.4 and was measured by a Crison lab pH-meter with a Slimtrode pH electrode (Hamilton, ref. 238150). The anaerobic condition in the feeding bottle (5 L) was maintained by both cooling of the solution (at 5 °C) and bubbling of helium. The establishment of low oxidation–reduction potentials ($\leq -400$ mV) for the system, under anaerobic conditions, is necessary for high colour removal rates [11]. The redox potential was continuously monitored (measured where the outlet immediately left the USPBR) and remained below −500 mV (referred to $Ag^+$/AgCl electrode). The reactor was built together with a stirring system that makes possible to apply a very fine and slow agitation (1 revolution per hour) in the biological activated carbon bed.

### 2.3. Biological activated carbon system

To prepare the biological system, anaerobic sludge with mixed culture was filtered by a microfilter with a pore size of 20–25 μm to only have single cells and spores. This filtrate was pumped through the activated carbon for a week. During this period the biofilm was immobilized on AC surface resulting in the so-called biological activated carbon. Then the biofilm was adapted to AO7 by continuous flowing of the dye solution containing both the basal media and carbon source through the reactor. To maintain the same culture of sludge, every new reactor was set by using the outlet of an already operated reactor as the inlet to the new one.

The use of mixed culture instead of a specific strain is reasonable. The large number of azo dyes that can be reduced by many different bacteria indicates that azo dye reduction is a non-specific reaction. So far, no strain has been reported being able to decolourise a wide range of azo dyes. Therefore, the use of specific strains on anaerobic biodegradation does not make much sense in treating textile wastewaters, which are composed of several kinds of dyes.

### 2.4. Analytical methods

Acid Orange 7, sulfanilic acid and acetate were measured by HPLC on a $C_{18}$ Hypersil ODS column in a gradient of methanol-water mobile phase with a flow rate of 1 mL $min^{-1}$. AO7 was determined at 487 nm, sulfanilic acid at 252 nm and acetate at 210 nm. Sulfanilic acid generation is not represented in results, the only reason of measuring that was to check if the AO7 degradation/sulfanilic acid production ratio was appropriate and the colourant and by-products were not used as a carbon source. The other product generated during the anaerobic degradation of AO7,

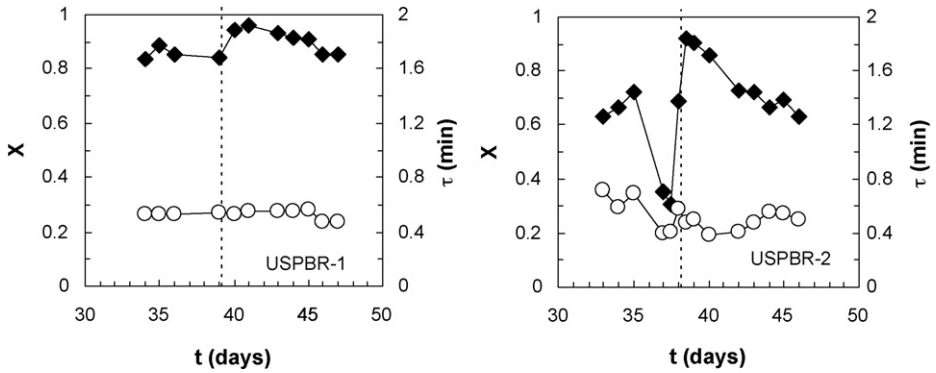


**Fig. 2.** Effect of stirring in BAC in the packed-bed reactors: (♦) Acid Orange 7 conversion ($X$); (○) space time ($\tau$); dotted line represents the start of 24-hour agitation period.

1-amino-2-naphthol (1A2N), was not determined due to its partial precipitation.

## 3. Results and discussion

### 3.1. Stirring of BAC in packed-bed reactor

#### 3.1.1. Agitation effects on biomass

The reason of testing this novel-type reactor is complex. Uncontrolled BAC may lead to an overproduction in biomass that may result head losses because of clogging phenomena and high bacteria levels in the effluent may also be observed [25]. On the other hand, higher density of biomass in the bed pores can inhibit biodegradation near to the activated carbon surface. It was found that after a certain process time, the pressure loss in USPBR was significantly less than in UPBR, meaning that the stirred reactor contained less amount of biomass than the simple packed-bed reactor, supposing no significant activated carbon wash-out. Thus, the stirred packed-bed holds less resistance to the flow and slow agitation of BAC together with continuous flow of dye solution through the bed can help removing the 'superfluous' amount of biomass from the reactor. Moreover, agitation can help keeping a nearly constant amount of biomass in the packed-bed and, also helps eliminating isolated layers of microorganisms, thus, enhancing the performance of the activated carbon.

#### 3.1.2. Periodical stirring

Continuous packed-bed reactors working with catalysts – supposing that the amount of catalyst is rather decisive in the reaction than the reactor volume – can be better characterized by space time than by hydraulic residence time. In upflow packed-bed reactors used in our previous study [22], slow but monotonous decreasing of dye conversion values was observed over the time. This can be explained by the isolation of metabolically active organisms from the activated carbon surface by continuous expansion of biofilm around the catalyst. To avoid this problem, appropriate stirring of BAC was applied in the packed-bed reactor.

The effect of slow agitation was examined in two identical USPBRs. Fig. 2 shows AO7 conversions and referred space times in function of time on stream. During the first 30 days, both reactors were saturated with azo dye to avoid the influence of initial dye adsorption during the initial period of operation. Stirring was first applied on day 39 and 38 in *USPBR-1* and *USPBR-2*, respectively, and was stopped after 1 day of operation. It can be clearly seen that azo dye conversion increased by applying 24-hour long stirring in both reactors. *USPBR-1* worked with space times of 0.47–0.56 min (105–125 mL $h^{-1}$) and *USPBR-2* with space times of 0.39–0.72 min (85–155 mL $h^{-1}$). In case of *USPBR-1*, stirring resulted 10% increase of AO7 conversion at a space time of 0.54 min (110 mL $h^{-1}$) while 55% of increase at space time of 0.40 min (150 mL $h^{-1}$) was observed in *USPBR-2*. When stirring was stopped, conversion started decreasing in both reactors, thus, confirming the positive effect of slow agitation of BAC on decolourisation rates. However, before application of stirring, different conversions of AO7 were found at same space time in the two reactors. This can be explained by having different concentrations of biomass in them. It is very difficult to control biomass growth in the BAC bed. On the other hand, after stirring, similar dye conversions were observed at same space time in both reactors (e.g., 90–95% at a space time of 0.5 min). According to these, an optimal amount of biomass exists that can be mostly ensured by using agitation in the biological activated carbon.

#### 3.1.3. Continuous stirring

Since decolourisation rates slowly decreased by time in UPBRs and, in addition, the microbial concentration may vary depending on both the lifetime of the reactor and the applied flow rate of azo dye solution, it was not possible to examine process kinetics in unstirred reactor accurately. In *USPBR-3* – identical as *USPBR-1* and *USPBR-2* – AO7 decolourisation was tested at a certain space time of 0.5 min (HRT of 1.4 min calculated from the reactor hold-up). Results are shown on Fig. 3. During long time of continuous operation, no significant change in dye conversion was observed. Moreover, nearly the same conversions (90–96%) were achieved than in case of the other two stirred reactors at same space time. These suggest that USPBR gives more representative results for AO7 degradation than UPBR and, in addition, stirred reactor could provide more exact data for kinetic modeling.

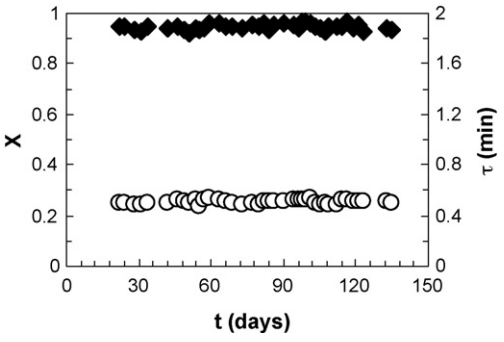


**Fig. 3.** Effect of continuous stirring in BAC in *USPBR-3*: (♦) Acid Orange 7 conversion ($X$); (○) space time ($\tau$).

### 3.2. Modeling AO7 anaerobic biodegradation in USPBR

#### 3.2.1. Determination of reaction rate

The mole balance for the packed-bed reactor is given by (Eq. (1)):

$$\frac{dF_{AO7}}{dm_C} = -r'_{AO7} = \frac{d(c_{AO7} \cdot F_V)}{d(\tau \cdot F_V \cdot \rho)} \quad (1)$$

where $F_{AO7}$ (mmol min$^{-1}$) is the molar flow of azo dye solution, $m_C$ (g) is the mass of catalyst in the reactor, $r'_{AO7}$ (mmol min$^{-1}$ g$^{-1}$) is the rate of the reaction, $c_{AO7}$ (mmol L$^{-1}$) is the dye concentration, $F_V$ (L min$^{-1}$) is the volumetric flow, $\tau$ (min) is the space time and $\rho$ (g L$^{-1}$) is the density of solution. If the flow rate of azo dye solution is kept constant and the density difference between the dye solution and water is neglected, the reaction rate $r_{AO7}$ (mmol min$^{-1}$ L$^{-1}$) will finally be (Eq. (2)):

$$\frac{dc_{AO7}}{d\tau} = -r_{AO7} \quad (2)$$

#### 3.2.2. Kinetic models

To make kinetic modeling possible, a new reactor, *USPBR-4*, was built since in the former ones solely high AO7 conversion values were found even at maximum flow rates of the system (up to 350 mL h$^{-1}$). The reactor *USPBR-4* contained 250 mg of activated carbon. More than one kinetic model was found to describe rather well Acid Orange 7 anaerobic biodegradation in the upflow stirred packed-bed reactor (Fig. 4), namely, first-order model, Michaelis–Menten (MM) model and a second-order autocatalytic model [26]. Table 1 shows the kinetic parameters encountered for these models. The simple first-order model fits well the experimental points (Fig. 4a). According to the standard deviations associated to the model fits (Table 1) although, there are no significant differences among them, the autocatalytic model was found to be the most appropriate to describe AO7 biodegradation (Fig. 4b). This can be explained by the autocatalytic nature of 1-amino-2-naphthol [26,27], being one of the anaerobic degradation products of Acid Orange 7. On the other hand, Michaelis–Menten model is also expected to describe AO7 biodecolourisation since it is a biological process and, also, the amount of consumed acetate by bacteria – providing the electrons to azo reduction – is directly proportional to dye conversion. Indeed, MM kinetics seems to be applicable for modeling Acid Orange 7 degradation in our reactor system (Fig. 4c). According to the very good fitting of all the models, the reaction rate predicted by all then should be similar. This is accomplished when comparing the first-order and autocatalytic model, since the first-order constants are similar and the second part of the autocatalytic model gives relatively small values because of the second-power function of the small dye concentration used. The reaction rates of the first-order and MM model are similar as well, since the first-order constants are similar and the Michaelis-constant is rather big relatively to the outlet dye concentrations, thus MM reduces to first-order model in this case.

Many azo dyes may have strong adsorption affinity to activated carbons depending on the surface chemistry of the carbon [28]. It can be interesting to mention that the so-called Langmuir–Hinshelwood equation – describing the rate law for surface catalysed reactions where the overall reaction rate is proportional to the surface coverage of the substrate over the catalyst – is analogous with the MM model and differences only are between the kinetic constants. Hereby, the former equation may also be used to describe our system suggesting that strong adsorption capacity of the carbon for AO7 can play an important role during this complex biological decolourisation process.

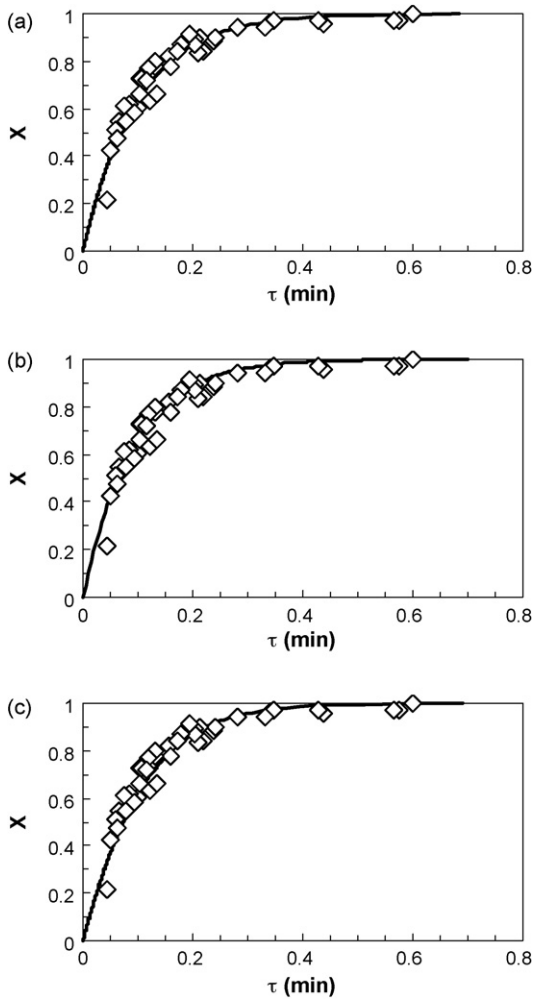


**Fig. 4.** Kinetic modeling of Acid Orange 7 anaerobic biodegradation in *USPBR-4*: line shows the fitting to (a) first-order kinetic model, (b) autocatalytic model and (c) Michaelis–Menten model.

### 3.3. Substrate inhibition and toxicity effects

50 days after measuring experimental points in *USPBR-4*, the reproducibility of the reactor system was checked by measuring AO7 conversions again, at certain space times. The previously determined Michaelis–Menten model fitted still well the newly measured points. After that, the inlet dye concentration was increased from 100 mg L$^{-1}$ to 300 mg L$^{-1}$ to check if higher AO7 concentrations may have inhibition or toxicity effects to the biomass. Fig. 5 shows that the MM model set before shows significant deviation from experimental points at initial concentration of 300 mg L$^{-1}$. This suggests that AO7 possesses concentration-dependent inhibition effects for bacteria in the reactor. For this, MM model was expanded by an inhibition factor and this model with 3 kinetic constants describes well the degradation process, independently on the initial dye concentrations (Fig. 5). The substrate inhibition was found to be significant since the value of the constant ratio $k_i/k_2$ is less than 10. Table 1 also shows the standard deviation value associated to experimental points involving both initial dye concentrations of 100 and 300 mg L$^{-1}$. However, the recalculated kinetic constants – including both inlet concentrations – differ from the former ones. This can be explained by having not only inhibition but also toxicity effects to the biomass at higher inlet dye concentrations. Indeed, using very high flow rate

**Table 1**
Kinetic data of models used for anaerobic Acid Orange 7 degradation in *USPBR-4*

| Model type | Model equation | Kinetic constants | S.D.[a] |
|---|---|---|---|
| First-order | $r_{AO7} = -k\, c_{AO7}$ | $k = 10.1\ min^{-1}$ | 0.048 |
| Autocatalytic | $r_{AO7} = -k_1\, c_{AO7} - k_2\, c_{AO7}\,(c_0 - c_{AO7})$ | $k_1 = 10.8\ min^{-1}$<br>$k_2 = 1.05\ L\,mmol^{-1}\,min^{-1}$ | 0.047 |
| Michaelis–Menten | $r_{AO7} = -\dfrac{k_1 \cdot c_{AO7}}{k_2 + c_{AO7}}$ | $k_1 = 10.8\ mmol\,L^{-1}\,min^{-1}$<br>$k_2 = 0.94\ mmol\,L^{-1}$ | 0.054 |
| Michaelis–Menten with substrate inhibition | $r_{AO7} = -\dfrac{k_1 \cdot c_{AO7}}{k_2 + c_{AO7} + (c_{AO7}^2/k_i)}$ | $k_1 = 11.7\ mmol\,L^{-1}\,min^{-1}$<br>$k_2 = 1.15\ mmol\,L^{-1}$<br>$k_i = 4.38\ mmol\,L^{-1}$ | 0.048 |
| | | $k'_1 = 6.18\ mmol\,L^{-1}\,min^{-1}$<br>$k'_2 = 0.55\ mmol\,L^{-1}$<br>$k'_i = 0.09\ mmol\,L^{-1}$ | 0.056[b] |

[a] Standard deviation associated to the model fitting: S.D. $= \sqrt{\Sigma(X - X^{MOD})^2/(n-1)}$ where n is the number of experimental points.

[b] Standard deviation associated to $k'$ values calculated from experimental points involving both initial dye concentrations of 0.286 and 0.857 mmol $L^{-1}$ (100 and 300 mg $L^{-1}$, respectively).

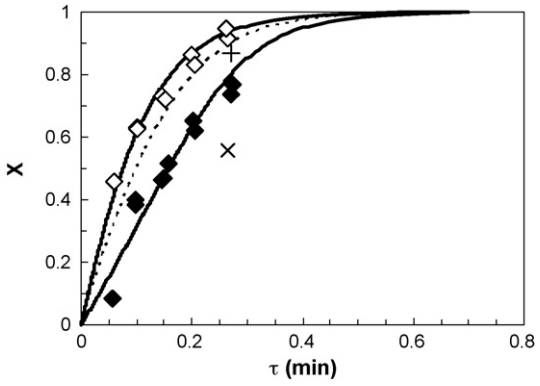


**Fig. 5.** Substrate inhibition and toxicity effects during Acid Orange 7 decolourisation in *USPBR-4*: (◊) shows repeated experimental points with initial Acid Orange 7 concentration of 100 mg $L^{-1}$; (♦) shows experimental points with initial AO7 concentration of 300 mg $L^{-1}$; (*X*) shows AO7 conversion, 1 day after biomass toxicity; (+) shows AO7 conversion, 6 days after biomass toxicity; dotted line represents the Michaelis–Menten model supposing no substrate inhibition at initial AO7 concentration of 300 mg $L^{-1}$; continuous lines show the fitting to expanded Michaelis–Menten model with inhibition factor at initial AO7 concentrations of 100 and 300 mg $L^{-1}$.

in *USPBR-4* at 300 mg $L^{-1}$ of initial dye concentration resulted toxicity, i.e., the redox potential was increased from −485 mV up to −180 mV in 3 h after changing the flow of dye solution from 150 to 260 mL $h^{-1}$. Then, to avoid the irreversible deactivation of microbes, the flow was set back to 55 mL $h^{-1}$ and, in addition, after 2 days the initial AO7 concentration was changed back to 100 mg $L^{-1}$. After 5 more days, the redox potential decreased back to −486 mV and AO7 conversion nearly returned to the value as it was before the toxicity to biomass (Fig. 5).

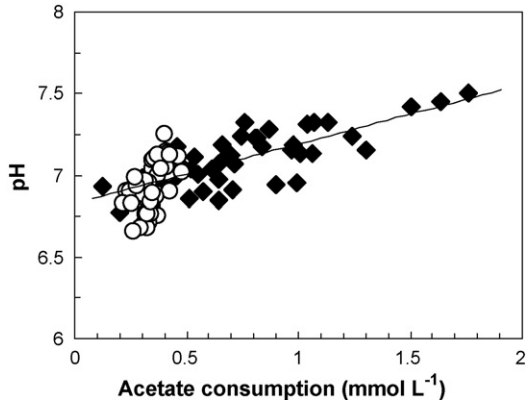


**Fig. 7.** The change of pH of the outlet solution at different acetate consumptions in *USPBR-3* (○) and *USPBR-4* (♦).

It is worth to mention that sulfanilic acid is a toxic product of anaerobic reduction of AO7 – even more toxic than the initial azo dye itself – such as many aromatic amines, originating from the anaerobic degradation of several azo dyes. Recent study only focuses on the reduction of an azo dye as being the first step of a sequential process. The following step of the complete treatment is to remove the (often) toxic anaerobic degradation products that can be done either by aerobic biodegradation or chemical/physical oxidation processes.

### 3.4. Acetate consumption

Azo dye decolourisation should linearly increase with the consumption of acetate by bacteria. Theoretically, 0.5 mol of acetate is

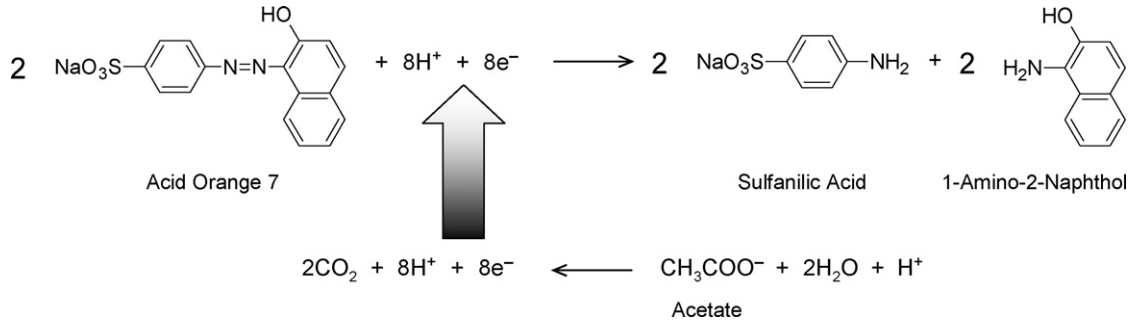


**Fig. 6.** Theoretical consumption of acetate for Acid Orange 7 reduction.

needed for 1 mol AO7 to decolourise (Fig. 6) that means an acetate consumption: AO7 reduction molar ratio of 0.5. In *USPBR-3*, working with dye solution flow rate of about 120 mL $h^{-1}$ (space time of 0.5 min), this ratio was found to be higher than the expected one. Probably, acetate consumption was overestimated since 200 mg $L^{-1}$ of sodium-acetate concentration was supposed to be in the feeding bottle. However, this concentration could be lower by time since anaerobes could consume acetate. Some analysis of the feed solution supported this fact and acetate was found to be totally consumed in 5–6 days in the bottle. To confirm the proposed electron transfer (Fig. 6) both in *USPBR-3* and *USPBR-4*, the pH of the outlets were measured. Fig. 7 clearly shows that higher acetate consumptions resulted higher pH values, thus, suggesting the consumption of $H^+$ during the oxidation of the electron donor.

## 4. Conclusions

To the best of our knowledge, a continuous upflow stirred packed-bed reactor with biological activated carbon was applied for the first time for anaerobic azo dye decolourisation. The application of special stirring in the carbon bed resulted in an increase of Acid Orange 7 bioconversion compared to unstirred reactor system with ensuring high dye degradation rates at very short space times/hydraulic residence times. Moreover, USPBR provided much more reproducible data to make kinetic modeling of AO7 biodegradation possible. First-order, autocatalytic and Michaelis–Menten models were all found to give good fittings to experimental points of dye conversion at lower inlet dye concentration. On the other hand, AO7 showed significant inhibition effects to the biomass at higher initial concentration and, also, processing at very low hydraulic residence times together with high initial dye concentration resulted in toxicity to bacteria. It can be assumed that a general model, describing the anaerobic biodegradation of diverse azo dyes in USPBR–BAC system, will be made up of the combination of dye inhibition and possible autocatalytic effects together with Michaelis–Menten kinetics.

## Acknowledgements

The authors gratefully acknowledge the fellowship from Universitat Rovira i Virgili, the financial support provided by the Spanish Ministry of Science and Education (CTM2005-01873) and by the Catalan government (2007ITT-00008).

## References

[1] A. Stolz, Basic and applied aspects in the microbial degradation of azo dyes, Appl. Microbiol. Biotechnol. 56 (2001) 69–80.
[2] P.C. Vandevivere, R. Bianchi, W. Verstraete, Treatment and reuse of wastewater from the textile wet-processing industry: review of emerging technologies, J. Chem. Technol. Biotechnol. 72 (1998) 289–302.
[3] Y.M. Slokar, A.M.L. Marechal, Methods of decoloration of textile wastewaters, Dyes Pigments 37 (1998) 335–356.
[4] T. Robinson, G. McMullan, R. Marchant, P. Nigam, Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative, Bioresour. Technol. 77 (2001) 247–255.
[5] E. Forgacs, T. Cserháti, Gy. Oros, Removal of synthetic dyes from wastewaters: a review, Environ. Int. 30 (2004) 953–971.
[6] C.I. Pearce, J.R. Lloyd, J.T. Guthrie, The removal of colour from textile wastewater using whole bacterial cells: a review, Dyes Pigments 58 (2003) 179–196.
[7] I.M. Banat, P. Nigam, D. Singh, R. Marchant, Microbial decolorization of textile-dye-containing effluents: a review, Bioresour. Technol. 58 (1996) 217–227.
[8] W. Delée, C. O'Neill, F.R. Hawkes, H.M. Pinheiro, Anaerobic treatment of textile effluents: a review, J. Chem. Technol. Biotechnol. 73 (1998) 323–335.
[9] F.P. van der Zee, S. Villaverde, Combined anaerobic-aerobic treatment of azo dyes—a short review of bioreactor studies, Water Res. 39 (2005) 1425–1440.
[10] P. Nigam, I.M. Banat, D. Singh, R. Marchant, Microbial process for the decolorization of textile effluent containing azo, diazo and reactive dyes, Process Biochem. 31 (1996) 435–442.
[11] N.D. Lourenço, J.M. Novais, H.M. Pinheiro, Effect of some operational parameters on textile dye biodegradation in a sequential batch reactor, J. Biotechnol. 89 (2001) 163–174.
[12] C. O'Neill, A. Lopez, S. Esteves, F.R. Hawkes, D.L. Hawkes, S. Wilcox, Azo-dye degradation in an anaerobic-aerobic treatment system operating on simulated textile effluent, Appl. Microbiol. Biotechnol. 53 (2000) 249–254.
[13] P. Rajaguru, K. Kalaiselvi, M. Palanivel, V. Subburam, Biodegradation of azo dyes in a sequential anaerobic–aerobic system, Appl. Microbiol. Biotechnol. 54 (2000) 268–273.
[14] N. Supaka, K. Juntongjin, S. Damronglerd, M.-L. Delia, P. Strehaiano, Microbial decolorization of reactive azo dyes in a sequential anaerobic–aerobic system, Chem. Eng. J. 99 (2004) 169–176.
[15] J.A. Field, F.J. Cervantes, F.P. van der Zee, G. Lettinga, Role of quinones in the biodegradation of priority pollutants: a review, Water Sci. Technol. 42 (2000) 215–222.
[16] F.J. Cervantes, F.P. van der Zee, G. Lettinga, J.A. Field, Enhanced decolourisation of acid orange 7 in a continuous UASB reactor with quinones as redox mediators, Water Sci. Technol. 44 (2001) 123–128.
[17] F.P. van der Zee, R.H.M. Bouwman, D.P.B.T.B. Strik, G. Lettinga, J.A. Field, Application of redox mediators to accelerate the transformation of reactive azo dyes in anaerobic bioreactors, Biotechnol. Bioeng. 75 (2001) 691–701.
[18] J. Rau, H.-J. Knackmuss, A. Stolz, Effects of different quinoid redox mediators on the anaerobic reduction of azo dyes by bacteria, Environ. Sci. Technol. 36 (2002) 1497–1504.
[19] A.B. dos Santos, I.A.E. Bisschops, F.J. Cervantes, J.B. van Lier, Effect of different redox mediators during thermophilic azo dye reduction by anaerobic granular sludge and comparative study between mesophilic (30 °C) and thermophilic (55 °C) treatments for decolourisation of textile wastewaters, Chemosphere 55 (2004) 1149–1157.
[20] J. Guo, J. Zhou, D. Wang, C. Tian, P. Wang, M.S. Uddin, H. Yu, Biocatalyst effects of immobilized anthraquinone on the anaerobic reduction of azo dyes by the salt-tolerant bacteria, Water Res. 41 (2007) 426–432.
[21] F.P. van der Zee, I.A.E. Bisschops, G. Lettinga, J.A. Field, Activated carbon as an electron acceptor and redox mediator during the anaerobic biotransformation of azo dyes, Environ. Sci. Technol. 37 (2003) 402–408.
[22] G. Mezohegyi, A. Kolodkin, U.I. Castro, C. Bengoa, F. Stuber, J. Font, A. Fabregat, A. Fortuny, Effective anaerobic decolorization of azo dye Acid Orange 7 in continuous upflow packed-bed reactor using biological activated carbon system, Ind. Eng. Chem. Res. 46 (2007) 6788–6792.
[23] F. Stüber, J. Font, A. Fortuny, C. Bengoa, A. Eftaxias, A. Fabregat, Carbon materials and catalytic wet air oxidation of organic pollutants in wastewater, Top. Catal. 33 (2005) 3–50.
[24] C. O'Neill, F.R. Hawkes, D.L. Hawkes, S. Esteves, S.J. Wilcox, Anaerobic–aerobic biotreatment of simulated textile effluent containing varied ratios of starch and azo dye, Water Res. 34 (2000) 2355–2361.
[25] M. Scholz, R.J. Martin, Ecological equilibrium on biological activated carbon, Water Res. 31 (1997) 2959–2968.
[26] F.P. van der Zee, G. Lettinga, J.A. Field, The role of (auto)catalysis in the mechanism of an anaerobic azo reduction, Water Sci. Technol. 42 (2000) 301–308.
[27] D. Méndez-Paz, F. Omil, J.M. Lema, Modeling of the Acid Orange 7 anaerobic biodegradation, Water Sci. Technol. 48 (2003) 133–139.
[28] M.F.R. Pereira, S.F. Soares, J.J.M. Órfão, J.L. Figueiredo, Adsorption of dyes on activated carbons: influence of surface chemical groups, Carbon 41 (2003) 811–821.