

Treatment of phenol in an anaerobic fluidized bed reactor (AFBR): continuous and batch regime

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Received: 28 July 2009 / Accepted: 13 January 2010 / Published online: 2 February 2010
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Abstract Results of this study describe the feasibility of anaerobic treatment of highly concentrated phenol synthetic wastewater using an anaerobic fluidized bed reactor (AFBR) in both continuous and batch modes. Wastewater with a maximum load of $2,100 \text{ mg C}\cdot\text{l}^{-1}$ was prepared using phenol (maximum concentration of $1,600 \text{ mg C}\cdot\text{l}^{-1}$) as substrate and a mixture of acetic, propionic and butyric acids ($500 \text{ mg C}\cdot\text{l}^{-1}$) as co-substrate. AFBR reached total organic carbon (TOC) and phenol removal efficiency over 95% treating the highest organic loading rate (OLR) containing phenol studied for this kind of reactor ($5.03 \text{ g C}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$). The phenol loading rate rise caused volumetric biogas rate increase up to $4.4 \text{ l}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$ (average yield of $0.28 \text{ l CH}_4\cdot\text{g}^{-1} \text{ COD}_{\text{removed}}$) as well as variation in the biogas composition; the CO_2 percentage increased while the CH_4 percentage decreased. Morphological examination of the bioparticles at $4.10 \text{ g C}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$, revealed significant differences in the biofilm structure, microbial colonization and bacterial morphological type development. The five batch assays showed that phenol degradation may be favoured by the presence of volatile fatty acids (VFAs) (co-metabolism), whereas VFAs degradation may

be inhibited by phenol. AFBR reached initial phenol degradation velocity of $0.25 \text{ mg C}\cdot\text{l}^{-1}\cdot\text{min}^{-1}$.

Keywords Biodegradation · Anaerobic treatment · Anaerobic fluidized bed reactor · Phenol

Introduction

The growth of industry has brought with it the problem of pollution and hazardous wastewater. The challenges for the industries are to solve these critical problems. Phenolic compounds are hazardous pollutants that enter into the environment through wastewater discharges from a variety of industries such as oil refinery, pharmaceutical, coal conversion, phenol-formaldehyde resin, etc. The concentration of phenols in these effluents ranges from 10 to $17,500 \text{ mg}\cdot\text{l}^{-1}$ (Veeresh et al. 2005). Phenol compounds are toxic, carcinogenic, mutagenic and teratogenic (Autenrieth et al. 1991). For this reason, phenols have been included in the priority pollutant lists of the European Economic Community (EEC) and United States Environmental Protection Agency (USEPA). A phenol concentration of $1 \text{ mg}\cdot\text{l}^{-1}$ or higher affects aquatic life. Therefore, in most cases stringent effluent discharge limit of less than $0.5 \text{ mg}\cdot\text{l}^{-1}$ is imposed (Chang et al. 1995; Tay et al. 2001). For example the maximum permitted concentration level is being $0.5\text{--}1.0 \text{ mg}\cdot\text{l}^{-1}$ for industrial wastewater according to Spanish legislation (González et al. 2001).

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In order to reduce phenol content below permitted level, phenolic wastewater have to be treated before final discharge. For this purpose physic-chemical or conventional biological process are usually used for the treatment effluent with high concentrations of phenol. Nevertheless, biodegradation of phenol in wastewater is generally more cost-effective than the physic-chemical treatment processes (Loh et al. 2000). Conventionally, aerobic biological methods have been used for the treatment of phenolic wastewater (González et al. 2001; Bajaj et al. 2008; Sevillano et al. 2008). However, due to their low-energy requirement and sludge yield, anaerobic biological methods are now being preferred (Veeresh et al. 2005) despite they have been reported since late 80's (Table 1).

Although anaerobic treatment has known advantages, the slow growth of anaerobic microorganisms requires a technology that assures reasonable reactor volume and low hydraulic residence times, accompanied with high active biomass concentration in the reactor. Anaerobic fluidized bed reactors meet these requirements. The application of fluidized bed technology to wastewater has increased due to its advantages over traditional processes. On the one hand, the adhesion of microorganisms over solid carrier with large specific surface areas, leads to high biomass concentration and high reaction rates and because of this, the required reactor volume will be lesser. On the other hand, due to this biomass retention, the growth rate of the microorganisms and the hydraulic retention time are uncoupled. This system permits to work with the highest organic loading rates among all the anaerobic digesters treating wastewater (García-Encina and Hidalgo 2005). Moreover, AFBR applications for the treatment of hazardous wastewater with inhibitory/recalcitrant compounds have widely reported (van Lier et al. 2001; Pérez et al. 2007; Fernández et al. 2008). However, despite these potential advantages, the highest phenol loading rate used in AFBR ($1.37 \text{ g COD} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$) has always been much lower than OLR used in UASB reactors which has attracted the most of attention worldwide (Table 1). Furthermore, most of phenol biodegradation studies have been only carried out in a continuous mode.

Taking into account these considerations, the aims of this research were to study efficiency and kinetic of phenol degradation in presence of VFAs as co-

substrate using an AFBR at a laboratory scale in both continuous and batch regime. These results can be used to optimize the operation of an industrial scale wastewater treatment plant treating effluents which contain phenol.

Materials and methods

Experimental setup

The experimental setup used in the lab-scale research is shown in Scheme 1. The installation consisted of an AFBR which operated at room temperature ($32 \pm 2^\circ\text{C}$). The reactor is a glass cylinder which had an internal diameter of 74 mm and a total height of 498 mm. It had a conical lower settling zone in order to obtain a homogeneous fluidization. Total working volume in the AFBR was 1.8 l. The bioreactor was filled with solid carrier material Biolite® ($d_p = 250\text{--}315 \text{ }\mu\text{m}$, $\rho_s = 1.25 \text{ kg} \cdot \text{l}^{-1}$, specific surface area $= 0.6 \text{ m}^2 \cdot \text{g}^{-1}$) up to 100 mm of its active volume (0.4 l). The carrier expansion inside the AFBR was maintained around 100%. The high specific area, low density and surface morphology of the Biolite® makes it an excellent solid carrier to be used in biological processes with fluidized beds (Balanguer et al. 1997). This carrier was covered with bacteria which were inoculated from pig slurry (Boltes et al. 2008). The AFBR was in operation for a couple of years using different VFAs mixtures, therefore the experiments were carried out with a mature biofilm.

Wastewater to be treated and recycled was pumped into the bioreactor at the bottom of the AFBR. Gases were collected at the top of the reactor from a gas–liquid–solid separator. Biogas production was measured with a wet gas meter.

The experimental setup had little differences depending on the operating mode. During continuous mode, the wastewater to be treated was pumped by peristaltic pump (101U/R, WatsonMarlow). To ensure stable influent values, e.g. a constant TOC and to prevent contamination by molds, that might produce antimicrobial substances, the feed bottle was refrigerated at 4°C . The effluent was collected from the side opening at the top of the bioreactor. On the other hand, peristaltic pump was only used to load the reactor in batch regime.

Table 1 Studies of anaerobic degradation of pure and mixed phenolic compounds which have been ordered in line with total OLR

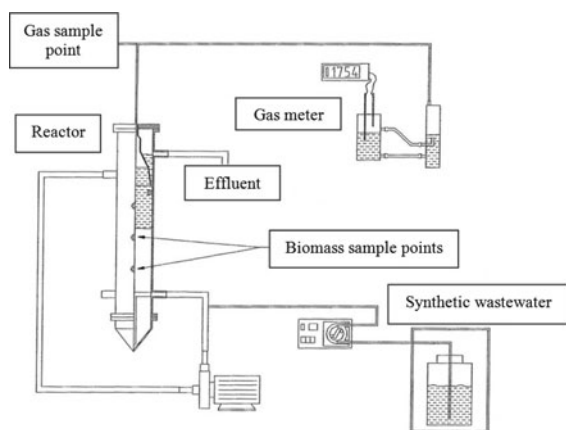
Substrate	Phenol (mg·l ⁻¹)	Phenolic comp. (mg COD·l ⁻¹)	Total OLR (g COD·l ⁻¹ ·d ⁻¹)	Total COD removal (%)	Reactor ^a	Reference
Phenol + <i>m</i> -Cresol	3,000	–	28.5	98 ^b	UASB	Fang and Zhou 1999
Phenol	2,000	4,760	19.04	99	UASB	Lay and Cheng 1998
Phenol + VFAs	2,090	4,976 ^c	15.46	>95 ^d	AFluidizedBR	Present study
Phenol + Sugarcane molasses	1,176	2,800	8	87–99.9	UASB	Gali et al. 2006
Phenol	1,176	4,000	8	81.8	UASB	Mehrotra et al. 2003
Phenol + Glucose	1,260	4,067	8	92 ^b	UASB	Tay et al. 2001
Phenol + <i>p</i> -Cresol	1,200	4,000	7.1	91	UASB	Razo-Flores et al. 2003
Phenol + <i>p</i> -Cresol	1,200	4,000	7	94	UASB	Razo-Flores et al. 2003
Phenol	1,260	3,000	6	98 ^b	UASB	Fang et al. 2004
Phenol	1,260	3,000	6	86	UASB	Tay et al. 2001
Phenol	1,260	3,000	6	86	UASB	Tay et al. 2000
Phenol	1,260	3,000	6	>97 ^b	UASB	Fang et al. 1996
Phenol + Glucose	4,705	11,200	5.7	91–89.5	AFixedBR	Bajaj et al. 2009
Phenol + Glucose	3,764	9,000	5.3	90–93.7	AFixedBR	Bajaj et al. 2009
Phenol	1,200	2,880	5	>80	EGSB-AF	Collins et al. 2005
Phenol + <i>m</i> -Cresol	900	–	4.3	98 ^b	UASB	Zhou and Fang 1997
Phenol + Formaldehyde + Methanol	–	–	3.4	95	Anaerobic AC	Goeddertz et al. (1990)
Phenol	1,200	–	3	99	HAIB	Bolanos et al. 2001
Phenol + <i>o</i> -Cresol + <i>p</i> -Cresol	880	1,980	2.95	81.8	UASB	Razo-Flores et al. 2003
Phenol + <i>m</i> -Cresol + <i>o</i> -Cresol	–	–	2.12	94	Fixed film UAB	Tawfiki et al. 1999
Phenol	1,000	2,379	2	87–98	EGSB	Scully et al. 2006
Phenol	500	1,190	2	>90	UASB	Chang et al. 1995
Phenol	520	–	1.37	97	AFluidizedBR GAC	Claros et al. 1996
Chlorophenol	–	–	1.1	>90	AFluidizedBR	Tseng and Lin 1994
Phenol	1,000	2,512	0.98	>96.4	AFluidizedBR GAC	Lao 2002
Phenol	630	1,500	0.9	>99 ^b	UASB	Fang et al. 2006
Nitrophenol	2,000	–	0.9	>90	AFluidizedBR	Tseng and Lin 1994
Phenol + <i>o</i> -Cresol + <i>p</i> -Cresol + 2,5Dimethylphenol + 3,4Dimethylphenol + 3,5Dimethylphenol	490	–	0.75	86	HUASB	Ramakrishnan and Gupta 2008
Phenol + <i>p</i> -Cresol	–	–	0.66	85	Bioaugmented enriched consortium	Tawfiki et al. 2000
Phenol	2,864	–	0.23	>92	Expanded bed reactor	Wang et al. 1986

^a Anaerobic AC anaerobic activated carbon, A Fixed BR anaerobic fixed bed reactor, A Fluidized BR GAC anaerobic fluidized bed reactor granular activated carbon, DSFF downflow stationary fixed film reactor, EGSB-AF expanded granular sludge bed reactor anaerobic filter, HAIB horizontal flow anaerobic immobilized biomass reactor, HUASB hybri upflow anaerobic sludge, UAB upflow anaerobic reactor, UASB upflow anaerobic sludge blanket

^b Phenol COD

^c 1 mg·l⁻¹ phenol = 2.38 mg COD·l⁻¹ phenol

^d TOC and phenol removal efficiency



Scheme 1 Schematic diagram of experimental setup of the AFBR in continuous operation

Synthetic wastewater

The synthetic wastewater was composed of a mixture of acetic, propionic and butyric acids (2:1:1 TOC proportion) with macro and micro nutrients in line with Evans medium (Evans et al. 1970), maintaining 100:7:1 proportion in C:N:P. The wastewater was buffered with NaHCO_3 up to $\text{pH } 6.0 \pm 0.2$. The AFBR was started (microbial activation stage) with synthetic wastewater prepared only with VFAs as carbon source. These acids had different TOC concentration from 200 to 500 mg l^{-1} . Once reached the value design, the reactor was fed with artificial wastewater prepared using VFAs (500 mg C l^{-1}) and different phenol concentrations which were gradually increased from 10 to $1,600 \text{ mg C l}^{-1}$. The batch assays were carried out using synthetic wastewater with different initial concentrations both VFAs and phenol (Table 2).

Analytical methods

The reactor was monitored for influent flow, pH, temperature, VFAs, phenol and biogas volume and

Table 2 Initial concentration (mg C l^{-1}) of VFAs and phenol in synthetic wastewater used in each batch assay

Assay	VFAs	Phenol
1	450	300
2	300	300
3	150	300
4	600	900
5	300	1,000

composition. Samples in continuous as well as in batch regime were collected and centrifuged (Multi-fuge 3_{L-R}, Heraeus) at 15,000 rpm for 5 min. The supernatant was filtered and used for analysis. Both VFAs ($\text{C}_2\text{--C}_7$) and phenol concentrations were determined by gas chromatography with flame ionization detector (FID), using a Varian 3350 gas chromatograph. A NUKOL fused silica column (30 m, 0.25 mm, 0.25 μm) with the oven temperature rising from 60° to 180°C, an injector and detector temperatures of 200°C and with nitrogen as carrier gas were used.

Biogas composition was determined by gas chromatography with thermal conductivity detector (TCD), using a Varian 3350 gas chromatograph. A PORAPAK-N80/100 column (1/8 in. $\times$ 4.5 m) with the oven temperature rising from 40° to 150°C, an injector temperature of 150°C, a detector temperature of 120°C and with helium as carrier gas were used.

SEM was used to study the organization and structure of the anaerobic biofilms in the carrier when OLR was high ($4.10 \text{ g C l}^{-1} \cdot \text{d}^{-1}$). Two samples of Biolite® were collected in the bed: upper and bottom zone. The samples for SEM were fixed with 5% (v/v) glutaraldehyde in 0.2 M cacodylate buffer (pH 7.2) and dehydrated through a graded series of ethanol (25, 50, 70, 90 and 100% ethanol), acetone (100%) and acetone anhydrous solutions. Then, the samples were dried and coated with gold. SEM photographs were made with Zeiss DSM 950 Digital Scanning Microscope (Electronics Microscopy Service at University of Alcalá).

Results and discussion

The experimental performance was carried out in three stages: microbial activation, continuous operation mode and five batch assays. The AFBR operated the two-first stages in a continuous mode with hydraulic retention time (HRT) of 0.43 days, whereas the last one was rated in batch regime.

Phenol concentration reduction during the experimental performance was consequence of its biodegradation due to no significant volatilization (high water solubility and low Henry's constant of phenol), adsorption (low adsorption capacity of Biolite®) or sorption of phenol to the microorganism (low $\log K_{\text{ow}}$ value of phenol).

Microbial activation stage

Owing to phenol is difficult to biodegrade, the AFBR was fed with VFAs as carbon source for activation of the existing microorganisms on surface of the carrier material. The influent used had different OLR from 0.44 to 1.16 $\text{g C}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$, which was increased when the percentage of TOC removal was over 95% and stabilized. The reactor was run in this stage for 32 days. Efficiencies higher than 95% were already obtained at the beginning of the microbial activation stage.

Phenol degradation: continuous operation

During the second stage, the reactor was switched over to phenol containing synthetic wastewater along with VFAs as co-substrate. Thus, the OLR influent was gradually increased from 1.26 to 5.03 $\text{g C}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$ (0.10 to 3.87 $\text{g C}_{\text{phenol}}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$) (Fig. 1). The phenol concentration was increased when the removed percentage was over 95% and stabilized. The AFBR was run in this stage for 212 days.

During the period, the values of phenol removal were tending to percentages close to 100% despite the reactor suffered destabilization episodes at the beginning of the stage (Fig. 1b). At each phenol OLR increment, there was a temporary decrease of removal which improved as soon as the microorganisms were acclimatized to the new OLR. Despite of this, the values of phenol removal are very significant due to the high OLR of a recalcitrant pollutant as phenol, which is inhibitory to the bioactivity of microorganisms (Fang et al. 1996; Veeresh et al. 2005; Bajaj et al. 2008). Others phenol biodegradation studies carried out using AFBR also reached high phenol removal percentages (Tseng and Lin 1994; Lao 2002). However, its phenol loading rate ($<1.5 \text{ g COD}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$) were markedly lower than phenol loading rate of the present study ($15.46 \text{ g COD}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$). Phenol loading rate with the same magnitude order of the present work has been only studied in two cases which were used upflow anaerobic sludge blanket (UASB) reactors (Lay and Cheng 1998; Fang and Zhou 1999) (Table 1).

The values in TOC removal were also closed to 100% (Fig. 1a), nevertheless these experimental data were more robust than those of phenol throughout the period. In spite of these high degradation percentages, a steady decline in TOC removal can be observed

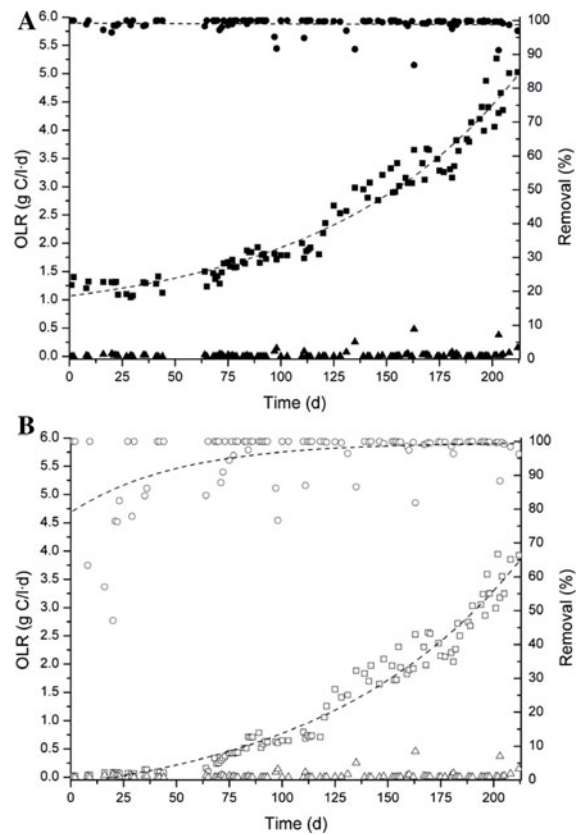


Fig. 1 Variation in TOC (closed circle) and phenol TOC (opened circle) removal efficiency with the OLR (closed square influent, closed triangle effluent, opened square phenol influent, opened triangle phenol effluent) throughout continuous operation time in the AFBR

when OLR was higher than $4.50 \text{ g C}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$ ($3.34 \text{ g C}_{\text{phenol}}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$), as a result of which there was a decline in phenol removal during the last days of the stage.

The phenol OLR increment caused both a variation in the biogas composition and a volumetric biogas rate increase as can be seen in the Fig. 2. The CH_4 percentages decreased (90–75%) despite the methane production rate increase. However, this CH_4 production increase was lower than volumetric biogas rate rise. This fact has been shown by others (García-Calderón et al. 1998; Pérez et al. 1999). The CO_2 percentages increased (10–25%) due to the overloading phenomenon. In this situation, the concentration of acids such as valeric or isobutyric suffered an increment in the reactor (data not shown) which could be due to cellular lysis and proteins hydrolysis (Bajaj et al. 2009; Feng et al. 2009). Moreover, phenol in

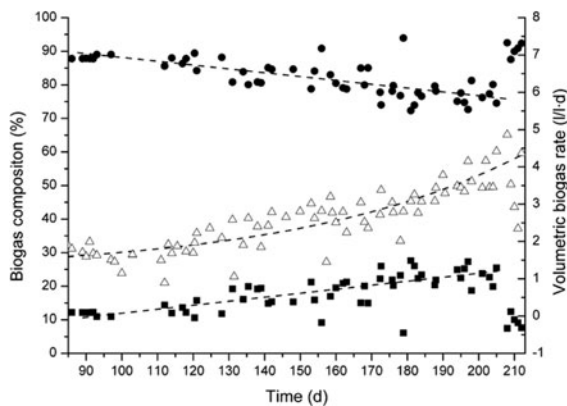


Fig. 2 Variation in biogas composition (closed circle CH_4 , closed square CO_2) and volumetric biogas rate (opened triangle) throughout continuous operation time (86–205 days) in the AFBR

this reactor might be degraded in line with the anaerobic pathway suggested by Kobayashi et al. (1989) which is formed VFAs as valeric. The acids formed did not decline the pH (6.6–7.5) of the AFBR as consequence of buffered synthetic wastewater. The acids reacted with the bicarbonate that was present in the media and the result was the generation of new CO_2 which passed mainly to the biogas (García-Encina and Hidalgo 2005). On the other hand, volumetric biogas rate increased from 1.4 to $4.4 \text{ l} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$ as consequence of biodegradation of increasing OLR (1.68 to $5.03 \text{ g C} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$) in CH_4 and CO_2 .

The AFBR had a CH_4 yield of $0.28 \text{ l CH}_4 \cdot \text{g}^{-1} \text{ COD}_{\text{removed}}$ which was determined from $\text{OLR}_{\text{removed}}$ calculated and express as COD as well as generated CH_4 volume (Fig. 3). The data scattered is due to the estimation was carried out throughout the overloading period which had high variability in generated CH_4 volume. Owing to theoretical value in steady-state conditions is $0.35 \text{ l CH}_4 \cdot \text{g}^{-1} \text{ COD}_{\text{removed}}$, the reactor CH_4 yield may be reflected that anabolism pathway (biological synthesis) was significantly more important than catabolic pathway (methane productions) (Michaud et al. 2005). In addition the friction and abrasion forces, which appear inside the reactor when it is working with high expansion as this study (100%), controlled biomass accumulation (Hidalgo and García-Encina 2002).

Furthermore, an increase in the bed viscosity and adhesion on the reactor walls from ORL of $4.10 \text{ g C} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$ ($3.00 \text{ g C}_{\text{phenol}} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$) can be observed. This was accompanied by stratification of

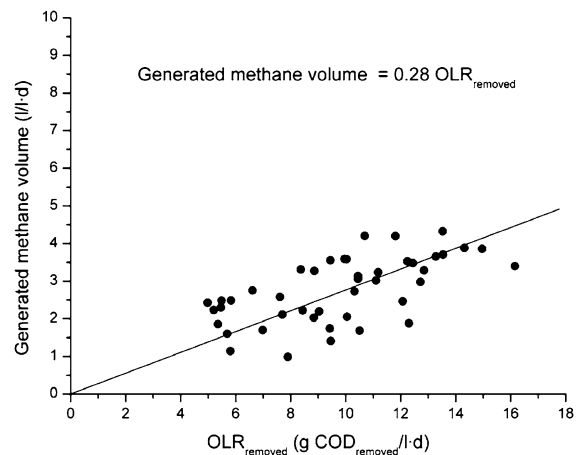


Fig. 3 Variation in generated methane volume with $\text{OLR}_{\text{removed}}$ throughout continuous operation time (86–205 days) in the AFBR

the bed into two zones. The SEM revealed important differences in the biofilm structure, microbial colonization and bacterial morphological type development, besides the great superficial porosity of Biolite® which confers high specific surface (Fig. 4). SEM photographs showed that there was an accumulation of microorganisms inside the cavities and the other regions of the carrier surface sheltered from hydraulic shear forces in the bottom stratum. These forces caused biomass losses, avoiding the development of a wide biofilm around carrier (Díez et al. 1999). The photographs A and B showed that microorganisms colonized Biolite® in this stratum predominate in filamentous forms. On the contrary, the carrier material surface was fully covered in the upper stratum as can be seen in photographs C and D. The bacterium communities had different morphological types (coccos, coccobacillus, streptobacillus and filamentous). SEM photographs suggest that a significant syntrophic association was established inside the thicker biofilm which permit faster phenol degradation according to Zache et al. (1989). Moreover, the syntrophic microcolonies were together with the extracellular material which is synthesised by the bacteria. This extracellular can form a protective shield for cells against the adverse influence of the external environment and act as a diffusion barrier to delay or prevent toxicants from reaching the microorganisms (Wingender et al. 1999). The biofilm growth modified the density of the upper stratum bioparticles allowing it to float over the

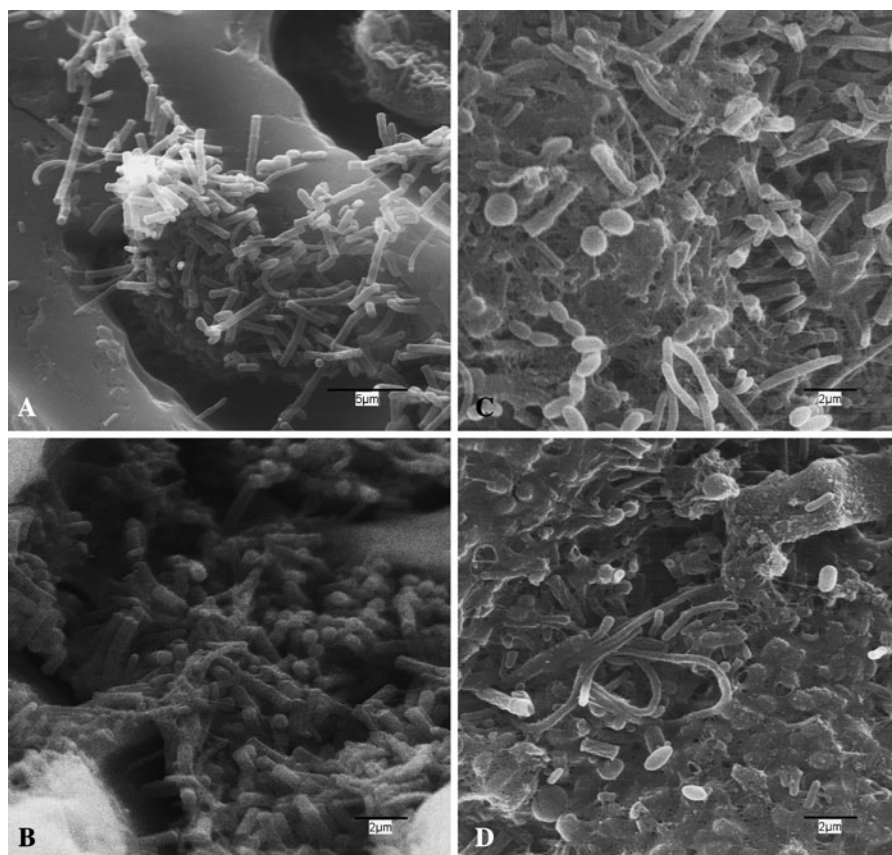


Fig. 4 SEM photographs of the upper stratum (**a** and **b**) and the bottom stratum (**c** and **d**) in continuous operation time with phenol ORL of $4.10 \text{ g C} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$ in the AFBR

bottom stratum bioparticles (Díez et al. 1995). This fact contributed to bed segregation which biomass concentration is higher upwards as consequences of the lower importance of shear stress and attrition (Díez et al. 1999; Seok and Komisar 2002). In addition the physical properties of the extracellular material also caused increase in the bed viscosity and adhesion to the reactor walls. These facts impeded thereby the correct fluidization and under these circumstances mass transfer resistance was increased. These fluidization problems caused decrease in the removed percentages of phenol in the last days of the stage (Fig. 1b) and therefore, they disable to increase the OLR above $5.03 \text{ g C} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$ ($3.87 \text{ g C}_{\text{phenol}} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$).

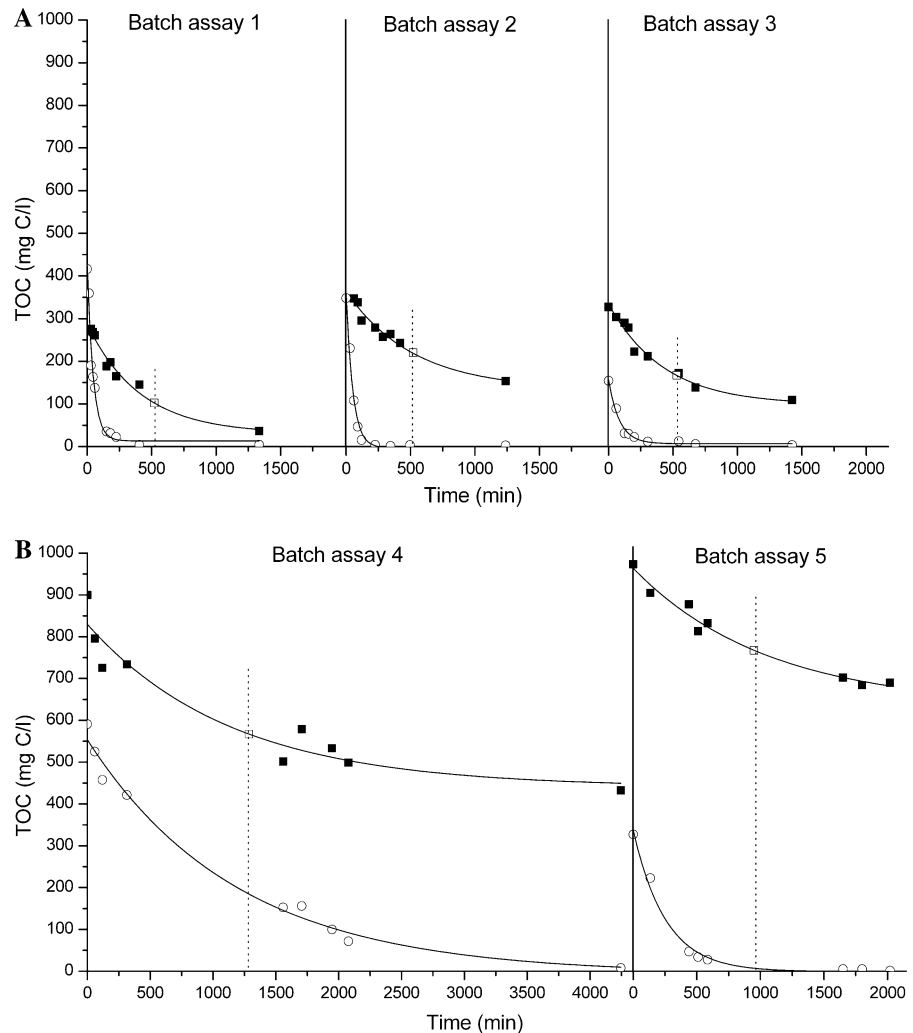
Phenol degradation: batch operation

Five assays were carried out in order to study kinetic degradation of phenol and VFAs. For each one, the reactor was loaded with synthetic wastewater with

different initial concentrations of phenol and VFAs (Table 2). Phenol and VFAs degradation profiles were determined for each assay (Fig. 5). As can be observed, the complete removal of VFAs occurred before maximum sharp curve of the phenol degradation profile (bisector), except to the assay 4. From the VFAs removal complete, the profile slope (phenol concentration per minute) decreased. This fact was caused by co-metabolism which allow that a substrate difficult to degrade such as phenol may be effectively degrade in the presence of other easily removal compounds (co-substrate) (Veeresh et al. 2005).

In the degradation pathway suggested by Kobayashi et al. (1989), a co-substrate such as VFAs keep methanogens in active phase and the acclimatized biomass bring about the hydrogenation, fission and fragmentation of the phenolic ring (Veeresh et al. 2005; Subramanyam and Mishra 2007). However, co-metabolism was not observed in the assay 4. This fact could be produced by the excess availability of easily

Fig. 5 Variation of phenol (closed square) and VFAs TOC (opened circle) in each assay throughout batch operation in the AFBR. Bisector of phenol degradation profiles (opened square)



assimilable food (VFAs) whose concentration in this assay was the highest carried out in the experimental performance ($600 \text{ mg C} \cdot \text{l}^{-1}$) (Subramanyam and Mishra 2007).

In the same way, the initial degradation velocity of acetic, butyric and propionic acids and phenol were determined for each assay. The initial degradation velocity of phenol had constant values around $0.25 \text{ mg C} \cdot \text{l}^{-1} \cdot \text{min}^{-1}$ which were independent of initial concentration of phenol (Fig. 6). The degradation velocity of phenol in batch regime was lower than those in a continuous mode. This fact may be due to phenol shock loading because the concentration of phenol in batch assays was markedly higher than continuous mode where the concentration is the same than in the effluent. Acute decrease in phenol

removal as consequence of shock loading has been reported in other studies (Bajaj et al. 2009).

On the other hand, the degradation velocity of VFAs had values which decreased according to an exponential profile when initial concentration of phenol was increased. This fact may be consequence of VFAs degradation was inhibited by phenol. Phenol is growth inhibitory to bacteria at concentrations above $0.11 \text{ g} \cdot \text{l}^{-1}$ and bactericidal at concentrations of about $0.45 \text{ g} \cdot \text{l}^{-1}$, if not adapted to phenol (Bajaj et al. 2008). This inhibition did not observed at continuous mode due to the phenol OLR was gradually increased which permitted microorganisms acclimatization to phenol. Furthermore, the phenol concentration inside the reactor in continuous mode was close to zero (effluent experimental data).

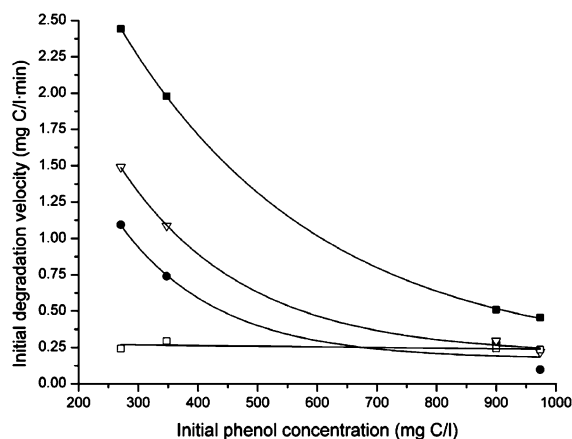


Fig. 6 Variation of initial degradation velocity of acetic (closed square), propionic (closed circle) and butyric (inverted opened triangle) acids and phenol (opened square) throughout the initial concentration of phenol in batch operation in the AFBR

Conclusion

The results allow us to conclude that phenol containing wastewater was effectively degraded in an anaerobic fluidized bed reactor (AFBR) at loading rate up to $5.03 \text{ g C}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$ ($3.87 \text{ g C}_{\text{phenol}}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$). Over 95% of phenol was removed at 32°C , pH 6.6–7.5 and a HRT of 0.43 days for phenol concentrations up to $1,600 \text{ mg C}\cdot\text{l}^{-1}$. However, there were indicator facts during the continuous operation such as variation in the biogas composition (the CO_2 percentages increased) or decline in phenol removal percentages with OLR higher than $4.50 \text{ g C}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$ which may indicate that the highest OLR added is close to the maximum treatment capacity of the system. Moreover, it can be observed that there was an increase in the bed viscosity and adhesion to the reactor walls from ORL of $4.10 \text{ g C}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$. These effects in the bed were accompanied by stratification into two zones. SEM revealed important differences in the biofilm structure, microbial colonization and bacterial morphological type development. SEM photographs showed that in the bottom stratum, the microorganisms (predominant presence filamentous forms) colonized in the cavities of the Biolite® surface, whereas in the upper stratum, bacterium communities (with higher variability of bacterium morphological types) together with the extracellular material were widely located around the carrier.

On the other hand, it could be determined with the batch assays that phenol was degraded with initial degradation velocity around $0.25 \text{ mg C}\cdot\text{l}^{-1}\cdot\text{min}^{-1}$. Phenol degradation may be favoured by the presence of VFAs (co-metabolism). However, VFAs degradation might be inhibited by phenol. Further research must be carried out in order to determine in this kind of reactor (i) if the threshold factor is the incorrect fluidization or the insufficient degradation rate of the microorganisms (ii) the optimum concentration of VFAs in order to optimize the phenol degradation.

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