Biodegradation of 2-fluorobenzoate in upflow fixed bed bioreactors operated with different growth support materials

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Abstract: Three upflow fixed bed bioreactors treating an aqueous stream containing 2-fluorobenzoate were operated for a period of 7 months, during which they were exposed to high organic loading rates and starvation. The reactors contained granular activated carbon (GAC), polyethylene (PE) particles and expanded clay (EC) respectively as growth support for microbial biofilms. The performance of the reactors was compared and the biofilm microbial population was followed by cell counting and denaturing gradient gel electrophoresis (DGGE). The reactor containing GAC always had 100% removal efficiency owing to the adsorption properties of the material combined with biodegradation. The GAC reactor also recovered better after starvation periods in the sense that it showed more stable behaviour than the reactors containing EC and PE. The highest biological elimination capacity was observed for the reactor containing EC, which reached 200 mg day^{-1} L^{-1} during reactor start-up, but during long-term operation the reactor containing GAC showed the highest biological elimination capacity, 140 mg day^{-1} L^{-1}. DGGE analysis indicated that starvation periods seemed to be responsible for shifts in the microbial population.

Keywords: 2-fluorobenzoate; upflow fixed bed bioreactors; granulated activated carbon; expanded clay; starvation periods; DGGE

INTRODUCTION

Halogenated organic compounds are widely used in the chemical and pharmaceutical industries. Biological degradation of these compounds has been reported for many chlorinated compounds;[1] however, fluorinated compounds have received less attention, and less information on their successful biological degradation is available.[2–5] These compounds are special within the group of halogenated compounds because of the unique chemical and physical properties of fluorine, which make them very stable. This stability has resulted in their extensive use in the production of pharmaceuticals, herbicides and pesticides, but their persistence leads to accumulation in the environment.[6] The compound 2-fluorobenzoate is often used in the production of chemical agents and pesticides, since benzoates carrying a halogen in the *ortho* position are known to have herbicidal or fungicidal properties.[7] A few studies have been done on the biodegradation of 2-fluorobenzoate.[8–10]

Biological treatment technologies have been developed and successfully applied in the treatment of waste streams containing xenobiotic compounds. However, a drawback with nearly all biological treatment processes is the sensitivity of the bacteria during changes in substrate load and composition, which can result in periods of bacterial starvation and wash-out, thereby decreasing the effectiveness of the treatment unit. This occurs for example when the production line changes, a common scenario found in batch processing in the chemical industry.[11] However, studies have found that immobilised bacteria remain more active under starvation periods and have shorter lag periods when fed a compound that they can degrade.[12–14] This is thought to be due to the following: (1) biofilm reactors have a high cell density, which is particularly important for slow-growing organisms, which is often the case in the biodegradation of xenobiotic organic compounds;[15] (2) immobilised cells can better withstand changes in environmental conditions such as temperature, pH, substrate concentrations and toxic substances;[16] (3) immobilised bacteria are better retained in the biofilm and will therefore not be washed out during starvation periods.

The aim of this study was to further the understanding of the degradation of fluorinated organic compounds under treatment conditions often found

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at industrial sites. Three upflow fixed bed bioreactors (UFBRs) filled with different growth support materials, namely granular activated carbon (GAC), expanded clay (EC) and polyethylene (PE) Rauschert rings, were used to treat aqueous streams containing 2-fluorobenzoate (2-FB) as a model compound. The reactors were exposed to dynamic treatment conditions, namely shock load and starvation periods. Both the overall treatment performance of the reactors and the development of the microbial community were followed over time.

MATERIALS AND METHODS

Inocula

For inoculation of the UFBRs a 2-FB-degrading culture, enriched from sediments of an industrially contaminated site and named FB2, was used. Pure cultures of FB2, a Gram-negative strain, were grown in sealed flasks containing a minimal salts liquid medium, prepared as previously described,[17] supplied with 100 mg L^{-1} 2-FB.

Reactor design and operation

The reactors consisted of cylindrical stainless steel columns (33 cm height, 3 cm internal diameter) provided with sampling ports at their entrance, exit and middle points. The columns were packed full with three different materials, 30 g of PE Rauschert rings (cut into 3–7 mm pieces), 57 g of EC particles (Filtralite 2/4, 2–4 mm diameter) or 57 g of GAC (0.85–2.4 mm diameter), which led to empty bed volumes of 156, 112 and 120 mL respectively. The specific surface areas of PE and GAC were 5.5 and $8 \times 10^5\ m^2\ kg^{-1}$ respectively, while the EC material had *ca* 75% porosity, according to the technical descriptions of the suppliers (see 'Reagents').

Prior to use the three growth supports were washed several times with deionised water to remove fine residues, dried in an oven at 105 °C and sterilised by autoclaving.

Silicon membranes, permeable to oxygen and pressurised with filtered air, were introduced inside the reactors as a source of oxygen. Each membrane was folded in a helicoidal form around an inert metal support and placed in the centre of the reactor. The total membrane area available for oxygen diffusion was 180 cm^2. The reactors were operated under non-sterile conditions at room temperature (*ca* 20–25 °C) and pH 7. During starvation periods the inlet flow of the reactors was disconnected and the air supply was turned off.

The three UFBRs containing PE, EC or GAC were inoculated with 400 mL of culture with a biomass concentration of 170 mg L^{-1}. The purity of the inoculum was checked by spreading diluted suspensions of the FB2 culture onto nutrient broth (NB) agar plates. The inoculum was recirculated through the packed columns for 8 days at a flow rate of 10 mL h^{-1} to allow colonisation of the support material. The decrease in optical density (OD_{600}) of the recirculating inoculum (>90%) was used as an indication of biomass loading onto the support materials. The three UFBRs were operated under dynamic conditions for almost 7 months (see Table 1); the reactors will be referred to as R-PE, R-EC and R-GAC. Shake flask experiments have shown that growth of FB2 is not nutrient-limited at the concentrations applied to the bioreactor columns. During the time of operation the dissolved oxygen concentration, measured at the outlet of the reactor, was never lower than 4 mg L^{-1} for any of the reactors.

Adsorption studies of 2-FB on PE, EC and GAC

To determine the adsorption capacity of each of the three materials, 0.1 g of GAC or 10 g of PE or EC was added to flasks filled with 50 mL of minimal medium with different concentrations of 2-FB (50–1500 mg L^{-1}). The flasks were sealed and then shaken at 150 rpm in a temperature-controlled (25 °C) shaker for 1 week. Three replicates were carried out for each concentration.

Sampling of support materials

PE, EC and GAC samples (1 g wet weight) were collected from sampling ports of the bioreactors. For extraction of biomass from the carrier the supports were dispersed in 10 mL of NaCl solution (8.5 g l^{-1}) and vortexed for 30 s. The resulting suspensions were used for plate counts and denaturing gradient gel electrophoresis (DGGE) analyses.

Plate counts

Enumeration of bacteria attached to the growth supports was done by plating onto NB agar plates, in duplicate, serial dilutions of samples in NaCl solution (8.5 g l^{-1}). The plates were incubated at 25 °C for 3 days.

DNA extraction

Prior to DNA extraction, *ca* 8.5 mL of the bacterial suspension detached from each support material was centrifuged and washed twice with sterile deionised water. The pellet was kept at −20 °C until DNA was extracted. DNA extraction was performed using the UltraClean Microbial Genomic DNA Isolation Kit (Mo Bio, Carlsbad, CA, USA).

Polymerase chain reaction (PCR) conditions

The primers 341F-GC and 518R, specific for conserved bacterial 16S rDNA, were used for amplification of a fragment corresponding to positions 341–534 of the *Escherichia coli* numbering.[18] Primer 341F-GC, which includes a 39-nucleotide GC-rich sequence, was attached to the 5′ end of the forward primer in order to prevent complete melting of the PCR products during subsequent DGGE analysis. PCR amplification was performed in a total volume of 50 μL; each PCR mixture contained 10 ng of

template DNA, 2.5 mmol L^{-1} $MgCl_2$, 1.5 U of *Taq* DNA polymerase (Bioron, Ludwigshafen, Germany), 250 µmol L^{-1} of each dNTP and 50 µmol L^{-1} of each primer. PCR products were amplified with the following programme: 3 min at 94 °C, followed by 10 cycles of 30 s at 94 °C, 30 s at 65–55 °C (touchdown −1 °C cycle) and 30 s at 72 °C. Twenty additional cycles were performed: 30 s at 94 °C, 30 s at 55 °C and 30 s at 72 °C, followed by a final extension step of 20 min at 72 °C.

DGGE

DGGE was carried out with a DCode™ Universal Mutation Detection System (Bio-Rad Laboratories, Hercules, CA, USA). Samples containing approximately equal amounts (*ca* 300 ng) of the PCR products were loaded onto 8% (w/v) polyacrylamide gels (37.5:1 acrylamide/bisacrylamide) in 0.5× TAE buffer (20 mmol L^{-1} Tris-acetate, pH 7.4, 10 mmol L^{-1} sodium acetate, 0.5 mmol L^{-1} Na_2EDTA) using a denaturing gradient ranging from 35 to 60% (100% denaturant contains 7 mol L^{-1} urea and 40% formamide). Electrophoresis was performed at 60 °C in 1× TAE buffer, initially at 20 V (15 min) and then at 200 V (330 min). The gels were stained with ethidium bromide solution (5 min) and then rinsed in distilled water (20 min). Images were acquired using a Gel Doc 2000 (Bio-Rad Laboratories).

Analysis of the community fingerprint

Gel images were analysed with Diversity Database Fingerprinting software (Bio-Rad Laboratories). Bands occupying the same position in different lanes of the gels were identified. A binary (1/0) matrix was constructed taking into account the presence or absence of individual bands in each lane. Subsequent analysis was performed using PRIMER v.5 software (PRIMER-E Ltd., Plymouth, UK). The binary matrix was transformed into a similarity matrix using the Bray–Curtis measure. Dendrograms were generated using the group average method.

Analytical methods

The concentration of fluoride ions in the bioreactor minimal medium was measured with a fluoride electrode as described previously.[19] Liquid samples of 2-FB were routinely collected at the inlet and outlet of the UFBRs. 2-FB was analysed by high-performance liquid chromatography (HPLC, Beckman System Gold 126, Fullerton, CA, USA) using the same method as described previously[4] but with a Lichrospher 100 RP18 reverse phase column (250 mm × 4.6 mm, 5 µm particle size; Merck, Darmstadt, Germany). OD_{600} was measured using a spectrophotometer (Heλios Gamma, Unicam Instruments, Cambridge, UK). Dissolved oxygen was monitored periodically at the reactor liquid outlet with an oxygen probe (WTW, Weilheim, Germany).

Reagents

All chemicals used were of analytical grade and were obtained from Sigma-Aldrich Chemie (Steinheim, Germany) or Merck. GAC (8–20 mesh) was obtained from Sigma Chemical Co (St. Louis, MO, USA), the PE Rauschert rings were obtained from Rauschert Verfahrenstechnik GmbH (Steinwiesen, Germany) and EC was obtained from Leca Portugal SA (Avelar, Portugal).

RESULTS

Adsorption studies

The PE and EC growth support materials did not adsorb 2-FB, while GAC was found to adsorb *ca* 180 mg 2-FB g^{-1} GAC.

Colonisation of growth supports

The time required for colonisation was dependent on the support matrix. R-PE and R-GAC had established biofilms after 3 days of inoculum recirculation, while R-EC took 8 days (Fig. 1). For R-EC, OD_{600} in the recirculation flask increased in the beginning, suggesting that the culture only attached to the EC material after the substrate had been consumed.

The reactors started to be operated under continuous mode after bacterial colonisation had occurred in all reactors, i.e. after 8 days (Fig. 1).

Performance of R-PE

Figure 2(a) shows the overall performance of R-PE during the time of operation. During phase I, characterised by a high organic loading rate (OLR), up to *ca* 1141 mg day^{-1} L^{-1} was fed to the bioreactor at a hydraulic retention time (HRT) of 5.7 h (Table 1). High amounts of 2-FB were detected at the outlet (Fig. 2(a)) and the biological removal efficiency was 7%, as indicated by fluoride release (Table 2). During phase II the OLR was reduced to 500 mg $day^{-1}L^{-1}$ by increasing the HRT (Table 1), which induced an increase in biological removal efficiency to 18%. The biological elimination capacity increased slightly from 83 to 87 mg day^{-1} L^{-1} (Table 2), suggesting that the high OLR of 2-FB during phase I may have had an inhibitory effect on the biodegradation process. During phase III the reactor was submitted to a starvation period for 45 days; however, it should be noted that, at the start of phase III, high amounts of

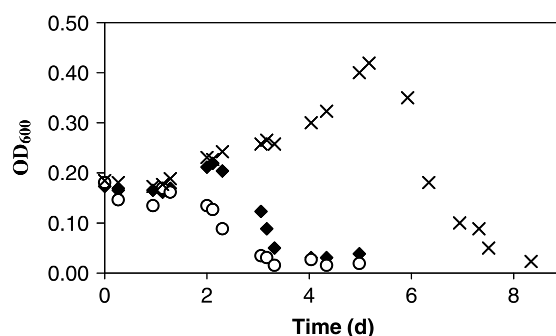

Figure 1. Evolution of OD_{600} in the recirculation flasks during inoculation of UFBRs R-PE (♦), R-EC (×) and R-GAC (○).

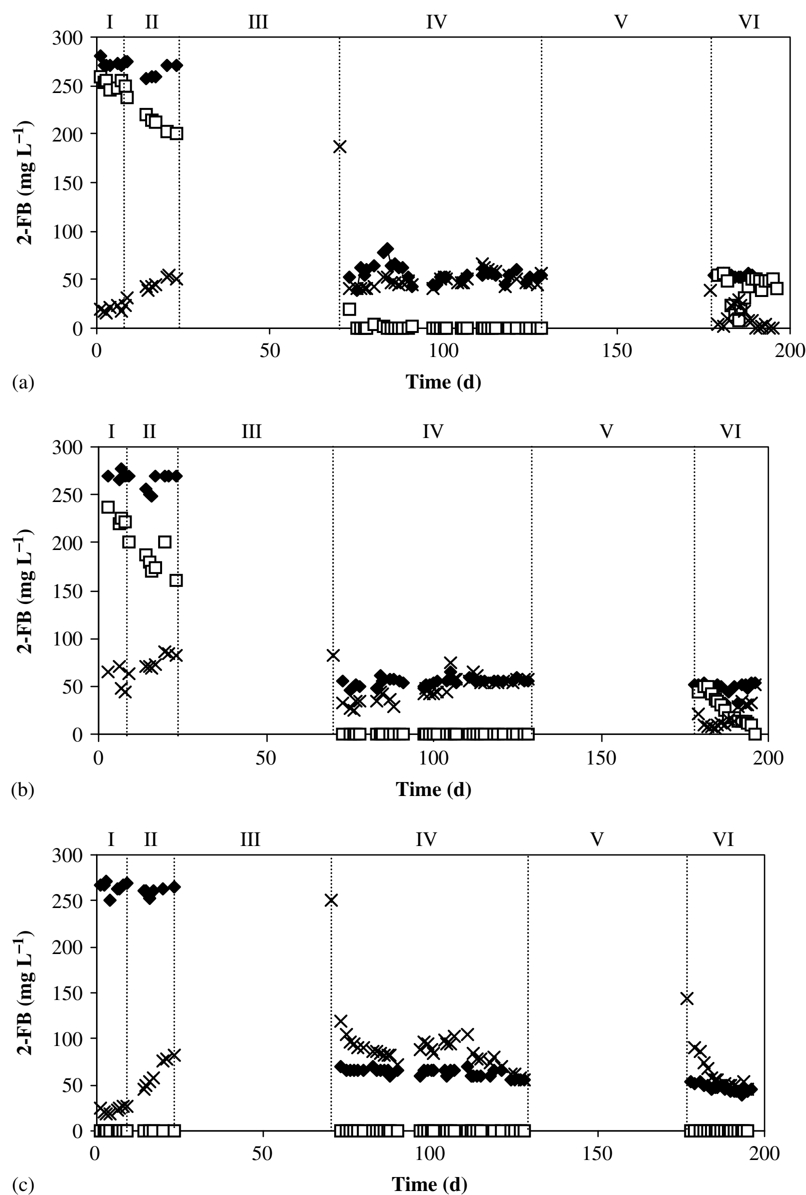

Figure 2. Degradation of 2-FB in UFBRs (a) R-PE, (b) R-EC and (c) R-GAC operated in continuous mode under a dynamic treatment scenario: 2-FB inlet concentration (⧫), 2-FB outlet concentration (□) and 2-FB degraded based on fluoride release (×). The different phases of operation (I–VI) are indicated.

Table 1. Summary of operating parameters used in continuous UFBRs

Phase	Days	Number of days	Organic loading rate (mg day^{-1} L^{-1})			Hydraulic retention time (h)		
			R-PE	R-EC	R-GAC	R-PE	R-EC	R-GAC
I	1–8	8	1141 ± 30	980 ± 45	900 ± 25	5.7 ± 0.2	6.6 ± 0.4	7.1 ± 0.1
II	9–24	16	500 ± 6	480 ± 18	414 ± 27	12.6 ± 0.3	13.0 ± 0.4	15.1 ± 0.8
III	25–72	48	0	0	0	0	0	0
IV	73–128	56	108 ± 9	92 ± 10	107 ± 11	12.4 ± 0.8	14.5 ± 1.9	13.8 ± 0.6
V	129–177	49	0	0	0	0	0	0
VI	178–196	19	90 ± 14	82 ± 18	91 ± 12	13.8 ± 1.2	14.5 ± 2.1	12.5 ± 1.0

2-FB were still detected at the outlet (Fig. 2(a)). At the end of the starvation phase (day 72) a higher fluoride concentration was observed than at the start of the phase (Fig. 2(a)), suggesting that biological activity was maintained during this phase. When the feed was restarted on day 73 (phase IV, Table 1) with a lower OLR (108 mg day^{-1} L^{-1}; Table 2), no 2-FB was detected at the outlet of R-PE, indicating biological degradation of 2-FB. On day 77, 100% removal efficiency was observed for R-PE (Table 2). Between days 73 and 93, although no 2-FB was detected at the outlet, not all the 2-FB fed to the reactor was

Table 2. Performance evaluation of continuous UFBRs under varying operating conditions. The presented values are averages for each operational phase

Phase	Inlet conc. ($mg L^{-1}$)	Biological elimination capacity ($mg day^{-1} L^{-1}$)			Biological removal efficiency (%)			Total removal efficiency (%)		
		R-PE	R-EC	R-GAC	R-PE	R-EC	R-GAC	R-PE	R-EC	R-GAC
I	269 ± 12	83 ± 10	209 ± 59	76 ± 10	7 ± 1	21 ± 5	8 ± 1	7 ± 2	16 ± 3	100 ± 0
II	261±10	87 ± 9	141 ± 11	98 ± 28	18 ± 1	29 ± 2	23 ± 5	20 ± 5	31 ± 6	100 ± 0
III	0	0.52	0.20	0.63	–	–	–	–	–	–
IV	57±6	95 ± 15	78 ± 17	141 ± 25	88 ± 14	86 ± 18	127 ± 16	99 ± 6	100 ± 0	100 ± 0
V	0	0.12	0.120	0.350	–	–	–	–	–	–
VI	49±4	16 ± 19	32 ± 18	111 ± 35	16 ± 19	42 ± 27	121 ± 21	22 ± 29	46 ± 31	100 ± 0

being degraded (Fig. 2(a)) and the biological removal efficiency was 88%. After day 93 the biological removal efficiency was 100% and remained so throughout phase IV. The reactor was then exposed to another starvation period (phase V, Table 1), this time with no 2-FB being detected at the reactor outlet at the start of the starvation period (Fig. 2(a)). After the second starvation period, no significant increase in fluoride ion concentration was observed (Fig. 2(a)). When the 2-FB feed was reintroduced (phase VI) at an OLR similar to that of phase IV (Table 1), high levels of 2-FB were detected at the outlet and the maximum biological removal efficiency was 50%, but that declined rapidly thereafter and complete inactivation was observed after day 190.

Performance of R-EC

The overall performance of R-EC is shown in Fig. 2(b). When a high OLR was fed to the reactor during phase I (Table 1), R-EC showed the highest biological removal efficiency and biological elimination capacity during both phases I and II (Table 2). At the start of the starvation period (day 25, phase II, Table 1), 2-FB was detected at the outlet of the reactor. After the starvation period, no 2-FB was detected at the outlet, although the fluoride level was no different from that observed at the start of this phase. As in the case of R-PE, not all 2-FB being fed to the reactor was degraded during the first 15 days of operation after the first starvation period (Fig. 2(b)). After day 88 and during the rest of phase IV the biological removal efficiency was maintained at 100%. At the start of the second starvation period (phase V, Table 1), no 2-FB was detected at the outlet of the reactor (Fig. 2(b)). When the 2-FB feed was reintroduced (phase V), high amounts of 2-FB were detected at the outlet. The outlet concentration decreased with time and, on day 194, no 2-FB was detected (Fig. 2(b)) and 100% biological removal efficiency was maintained thereafter.

Performance of R-GAC

Figure 2(c) shows the overall performance of R-GAC during its operation. 2-FB was not detected at the outlet of R-GAC at any time during operation of the reactor owing to the adsorption properties of GAC combined with biodegradation. At high OLR during phase I (Table 1) the biological removal efficiency was 8% and, when the HRT was changed from 7.1 to 15.1 h during phase II (Table 1), the biological removal efficiency increased to 23% (Table 2). Also, the biological elimination capacity increased at lower OLR. After the first starvation period (phase III, Table 1) the fluoride concentration observed in the reactor was higher than that observed at the start of this period (Fig. 2(c)). During phase IV a lower OLR was fed to the reactor (107 $mg day^{-1} L^{-1}$; Table 2). A mass balance of the reactor performance showed that, in addition to the 2-FB fed to the reactor, 2-FB adsorbed on the GAC was also degraded, hence a biological removal efficiency above 100% was measured (Table 2). After the second starvation period (phase V) an increase in fluoride concentration was observed (Fig. 2(c)) similar to that observed in phase III. In phase VI an OLR similar to that in phase IV was fed to the reactor and a high concentration of fluoride was detected at the reactor outlet, which stabilised after approximately 10 days and could be a wash-out of fluoride ions accumulated within the GAC during the starvation period. The biological removal efficiency was maintained at 100% until the end of phase VI.

Microbial community in biofilm reactors

Microbial community analyses were performed throughout the operation of the UFBRs. Bioreactor populations were quantified by counting the viable bacteria extracted from the support materials. The biomass attached to the support materials did not suffer significant variations over time in any of the reactors. A slight decrease can be noted during and immediately after the starvation periods for all three reactors (Fig. 3).

Bacterial suspensions extracted from the support materials were also analysed by DGGE. The inoculated strain FB2 was present in R-PE and R-EC throughout the operation of the reactors, although the intensity of the band varied over time. The total numbers of band positions detected in the gels for R-PE, R-EE and R-GAC were 19, 21 and 17 respectively. The number of bands in each lane varied in the range 4–13 for R-PE (Fig. 4(a)), 4–11 for R-EC (Fig. 4(b))

and 6–12 for R-GAC (Fig. 4(c)). Cluster analysis was performed aimed at characterising the similarity between the DGGE profiles. Two clusters of samples could be identified in all gels. In gels R-PE and R-GAC, one of the clusters included samples collected at time points T1–T4 and was clearly separated from the cluster that included samples collected at T5–T8. In contrast, in gel R-EC the sample collected at T5 clustered with samples collected at T1–T4. Thus a major shift in bacterial assemblage was identified between samples collected at T4 and T5 (Fig. 5(a) and (c)) or between samples collected at T5 and T6 (Fig. 5(b)).

DISCUSSION

Biofilm colonisation

R-GAC and R-PE led to the formation of biofilms faster than R-EC. This may be explained by the fact that EC holds a negative charge.[20] The duration of adherence of bacterial cells on materials is dependent on several factors, such as the nature of the support, the type and concentration of the feed and the material surface charge, as adherence occurs immediately on positively charged surfaces but can be delayed on negatively charged surfaces.[21] It was also observed that, owing to the EC material, the pH in the recirculation flask for R-EC increased during the recirculation period from 7 to over 8 (data not shown); similar behaviour has been seen for other

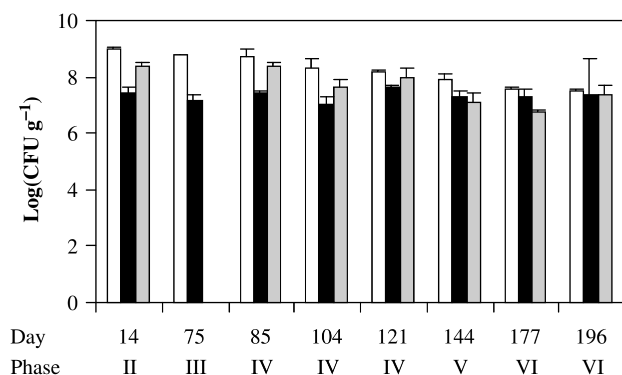

Figure 3. Biofilm composition of UFBRs R-PE (□), R-EC (▧) and R-GAC (■) analysed by plate counts and expressed in colony-forming units (CFU). Support matrix samples were analysed during the 197 days of UFBR operation.

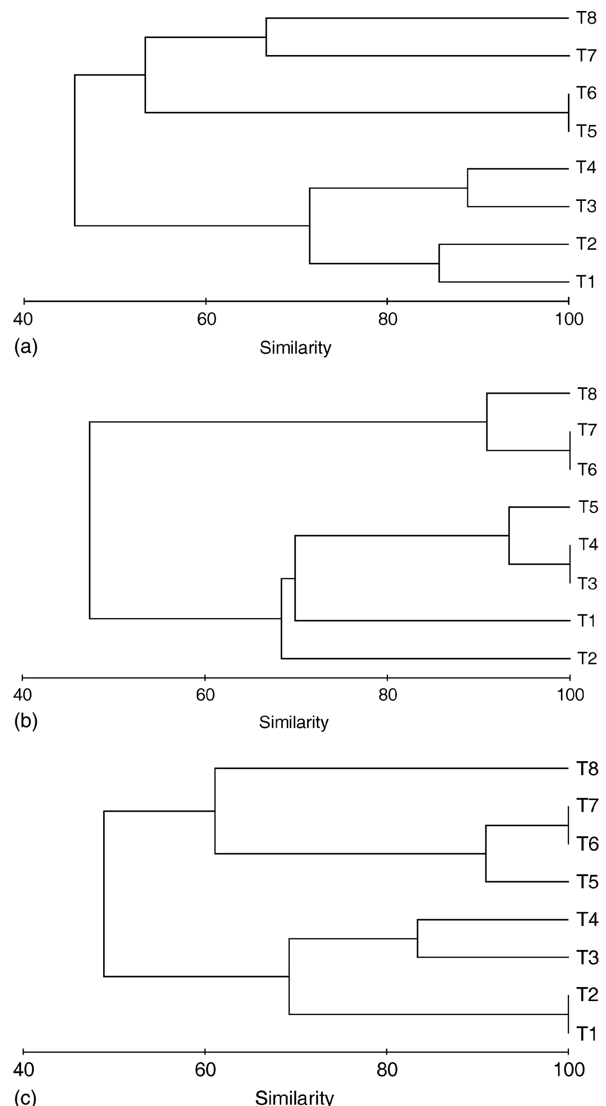

Figure 5. Cluster analysis of bacterial communities of UFBRs (a) R-PE, (b) R-EC and (c) R-GAC based upon DGGE profiles. Similarities were calculated using the Bray–Curtis measure.

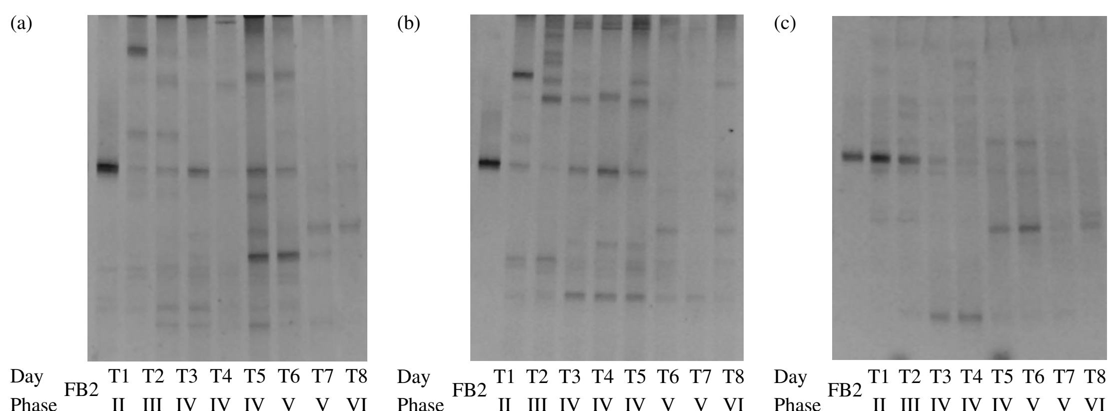

Figure 4. DGGE community fingerprints of UFBR populations. Gel lanes contain PCR-amplified bacterial 16S rDNA fragments. (a) R-PE, (b) R-EC and (c) R-GAC sampled at different time points, namely T1 = day 14, T2 = day 75, T3 = day 85, T4 = day 104, T5 = day 121, T6 = day 144, T7 = day 177 and T8 = day 196.

types of clay materials.[22] During continuous operation of the reactors the pH was always stable at 7.2. A study done to test the initial adherence of cells in rotating biological reactors containing different materials showed that GAC worked better as a surface for biofilm formation, reaching 10 mg dry matter (DM) cm^{-2} after 4 days, compared with sand, which had 9 mg DM cm^{-2}, and diatomite earth, which reached 7 mg DM cm^{-2}. Even lower biofilm growth was seen for PVC and stainless steel, reaching only 2 mg dry weight cm^{-2}.[23]

Reactor performance

A total balance on 2-FB shows that from the 3890, 2530 and 2600 mg fed to R-PE, R-EC and R-GAC respectively, 1280 mg (33%), 1075 mg (42%) and 1517 mg (58%) were degraded (Table 3). During the time of operation, R-GAC showed on average the highest biological elimination capacity; during phase IV, when all three reactors showed stable performances, R-GAC had an average biological elimination capacity of 141 mg day^{-1} L^{-1}, while values of 95 and 78 mg day^{-1} L^{-1} were determined for R-PE and E-EC respectively (Table 2). A similar mean value of biological elimination capacity (122 mg day^{-1} L^{-1}) was reported during treatment of an aqueous stream containing fluorobenzene,[24] but low biological elimination capacities were found under high loads of fluorobenzene. Similar behaviour was also observed in the present study, namely during phase I, when the organic load applied to the reactors was high and the biological removal efficiency was low for all three reactors. During phase II, when the OLR decreased, the biological elimination capacity increased in all three reactors, suggesting that the high OLR had an inhibitory effect on biodegradation activity. R-EC showed the highest biological removal efficiency during phases I and II, particularly during phase I, when the biological removal efficiency of R-EC was twice that of R-PE and R-GAC. The fact that biofilm colonisation took longer in R-EC may have contributed to the formation of a relatively more active biofilm in comparison with the biofilm already established for a few days in the other two reactors. After the first starvation period, higher amounts of fluoride were detected in R-GAC than in R-PE and R-EC, suggesting that 2-FB adsorbed on the GAC was utilised during the starvation period. When the feed was reconnected, degradation of 2-FB occurred immediately, since no 2-FB was observed at the outlet of the reactors, and biological removal efficiencies higher than 100% were quantified for R-GAC, while lower biological removal efficiencies of 50–60% were observed in R-PE and R-EC. During this phase, R-GAC showed significantly higher biological elimination capacity (Table 2). In both R-PE and R-EC, even though no 2-FB was detected at the outlet, not all 2-FB fed to the reactors was utilised by the bacteria (Table 3). Since adsorption experiments showed that PE and EC did not adsorb 2-FB, there was either adsorption of 2-FB to the biofilm, so-called biosorption, or incomplete mineralisation of 2-FB generating fluorinated intermediate compounds. Upon reaching a stable performance in phase IV for 20 days with an OLR of 100 mg day^{-1} L^{-1}, with 100% biological removal efficiency, another starvation period was introduced.

The second starvation period seemed to affect R-PE and R-EC in a more severe way. At the time the first starvation period was started (phase III), high concentrations of 2-FB were measured inside R-PE and R-EC (Fig. 2(a) and (b)), so these reactors may have not experienced complete starvation. High concentrations of fluoride ions were detected in the reactors by the end of the starvation period, indicating that the bacterial community biodegraded the 2-FB present in the reactors. However, at the time the second starvation period was imposed (phase V), no 2-FB was detected at the outlet of the reactors, indicating that the reactors may have been exposed to harsher conditions. In fact, the fluoride concentrations measured at the end of this starvation period were lower than those observed after the first starvation period, which may help to explain why R-PE did not survive the second starvation period. R-GAC showed stable behaviour during both starvation periods, maintaining in general the highest biodegradation activities. GAC functions as an accumulator of pollutant and, during starvation periods, this helps in keeping the biomass active.[25] Another study has shown that a biofilm reactor containing GAC and treating an effluent containing 4-chlorophenol recovered fully within 1 day after a 5 month starvation period and showed stable performance thereafter.[26]

Table 3. Degradation of 2-FB in R-PE, R-EC and R-GAC during continuous operation

	2-FB in influent (mg)			2-FB in effluent (mg)			2-FB degraded (mg)		
Phase	R-PE	R-EC	R-GAC	R-PE	R-EC	R-GAC	R-PE	R-EC	R-GAC
I	1408	880	864	1272	736	0	112	185	78
II	1264	864	800	992	592	0	256	250	192
III	0	0	0	0	0	0	25	9	30
IV	952	616	728	8	0	0	840	530	952
V	0	0	0	0	0	0	6	6	18
VI	266	171	209	209	114	0	46	74	247

Owing to its adsorption capacity, GAC has been used as a buffer of variable load regimes, particularly by adsorbing high concentrations of influent pollutant,[27,28] thus preventing the accumulation of high pollutant concentrations and the potential inhibition or even inactivation of the microbial consortia, which could result in reactor downtime or failure.[24] The use of GAC was also investigated under low OLR or even starvation periods. During these periods the adsorbed pollutant is released and then biodegraded, thus allowing for the maintenance of the biological activity in the reactors. The performances of R-PE and R-EC were fairly similar throughout phases I–V of the experiment; however, after the second starvation period, R-PE did not recover. Although EC does not adsorb 2-FB, the bacterial community was able to withstand the starvation period and recovered completely by the end of phase IV. Both EC and GAC have a higher density than PE particles and can be packed in a more compact way, allowing less empty bed volume and a larger surface area for bacterial attachment, which can play a role in the performance of bioreactors under dynamic conditions. When comparing different materials, including plastic and clay, in batch reactors treating wastewater from the manufacture of cellulose pulp, with a chemical oxygen demand (COD) of $40\,g\,l^{-1}$, no great differences were seen between different materials, but a complementary process would be required for full purification.[29] Clay has been shown to work well as a growth support in an anaerobic fluidised bed reactor, and high concentrations of phospholipids were thought to improve the concentration of biofilm onto the material and therefore increase the activity of the biofilm.[30]

Microbial community

Reactor performance and community structure are two important components in a biodegradation process. In this study the composition of the bacterial communities was analysed by both plate counts and DGGE. No significant differences in bacterial numbers during bioreactor operation were found (Fig. 3). DGGE analysis provided data on the microbial diversity within the UFBRs (Fig. 4). The major shift in the microbial community structure observed in R-PE was between time points T4 and T5 (phase IV), when the reactor was fed during continuous operation (Fig. 5). A less significant shift was also observed during the second part of the second starvation period, after which no major changes were observed until the end. Strain FB2 was present during the entire operation of the reactor. For R-EC the microbial community was stable during continuous operation and the major shift was observed during the second starvation period between T5 and T6 (phase V), where less than 45% similarity between the communities was observed (Fig. 5). Strain FB2 was present in the reactor throughout the experiment. For R-GAC the most significant microbial shift occurred in phase IV during continuous feeding (between T4 and T5; Fig. 5), so this cannot be related to any operational change. From the DGGE gels it was not possible to infer whether FB2 was present in the reactor until the end. The microbial dynamics observed, with the appearance of bacteria other than the original inoculum in the UFBRs, suggests that these bacteria were able to tolerate the presence of 2-FB, growing on 2-FB or, most probably, at the expense of 2-FB intermediates. The total number of bands was similar in all reactors, and all three reactors showed a fairly stable community, with major shifts observed at specific time points. Stable microbial communities during long-term operation have been observed in bioreactor communities when subject to operational perturbations such as changes in loading rates and composition of effluent.[31] However, large variations in microbial populations have also been noted in bioreactor communities when subject to perturbations such as changes in loading rates and composition of effluent.[32]

Biofilm reactors have been proven to be very effective when treating wastes with periods of starvation[33,34] and effluents with very low hydraulic retention time.[15] This study confirmed the effectiveness of biofilm reactors but also highlighted the importance of the type of material used. The presence of GAC in the bioreactor improved the long-term performance and robustness of the reactor owing to the adsorption properties and large surface area of GAC. The DGGE results showed that the bacterial communities remained fairly stable during the operation of the reactors, although starvation periods induced shifts in the microbial population.

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