Short Communication

Bioaugmentation of a rotating biological contactor for degradation of 2-fluorophenol

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ABSTRACT

The performance of a laboratory scale rotating biological contactor (RBC) towards shock loadings of 2-fluorophenol (2-FP) was investigated. During a period of ca. 2 months organic shock loadings of 25 mg L^{-1} of 2-FP were applied to the RBC. As no biodegradation of 2-FP was observed, bioaugmentation of the RBC with a 2-FP degrading strain was carried out and, along ca. 6 months, organic shock loadings within a range of 25–200 mg L^{-1} of 2-FP were applied. Complete biodegradation of 50 mg L^{-1} of 2-FP was observed during operation of the reactor. The RBC showed to be robust towards starvation periods, as after ca. 1 month of non-supply of the target compound, the reactor resumed 2-FP degradation. The inoculated strain was retained within the biofilm in the disks, as the 2-FP degrading strain was recovered from the biofilm by the end of the experiment, thus bioaugmentation was successfully achieved.

1. Introduction

The biodegradation of fluorinated compounds, such as 2-fluoro-phenol (2-FP), has been scarcely reported. These compounds are widely used as aerosol propellants, surfactants, agrochemicals, adhesives, refrigerants, fire retardants, pharmaceuticals, among others. These pollutants may occur in wastewaters discontinuously and at low concentrations, leading to the need of developing efficient bioreactors capable to deal with this scenario. Rotating biological contactors (RBCs) are bioreactor systems with high biomass retention that are extremely promising for such situations. These systems constitute a very attractive technology for low cost wastewater treatment due to operational features such as short hydraulic retention times, excellent capacity to deal with shock and toxic loadings, simple design and operation, high treatment efficiency and low energy requirement (Chowdhury et al., 2010; Cortez et al., 2008; Sirianuntapiboon, 2006). RBCs have been successfully used to treat several organopollutants, such as chlorophenols, phenol and trichloroethylene (Alemzadeh et al., 2002; Brar and Gupta, 2000; Majumber and Gupta, 2007; Sahinkaya and Dilek, 2006).

The main aim of this study was to investigate the performance of a laboratory scale RBC towards organic shock loadings with 2-FP before and after bioaugmentation with a specialized strain able to degrade this compound.

2. Methods

2.1. RBC set up and operation

Two 10 L RBC units containing each five stages (5 cm distance; baffles on the bottom) with 10 PVC discs (3 mm thick; diameter 16.5 cm; spaced 1 cm apart; 40% of disc submergence) were operated in series (Fig. 1). The reactor was inoculated with 5 L of activated sludge from the return sludge flow of a conventional wastewater treatment plant and 5 L of synthetic wastewater containing sodium acetate for biofilm formation. During the first 18 days, the RBC was fed every day, in a batch mode, with 200 mg L^{-1} acetate and from day 19 the reactor was operated in a continuous mode (Table 1). The hydraulic loading rate (HLR) applied was 0.25 dm^3 m^{-2} h^{-1} and the rotational speed of the discs was 12 rpm.

2.2. Bioaugmentation with the 2-FP degrading strain

Bioaugmentation of the RBC with a bacterial strain able to degrade 2-FP, named as strain FP1, isolated in our laboratories (unpublished), was performed. FP1 pure cultures were grown in sealed flasks containing a mineral salts liquid medium (Carvalho et al., 2006) and 2-FP at a concentration of 50 mg L^{-1}. The cultures were incubated on an orbital shaker (100 rpm) at 25 °C. The reactor was inoculated with 2 L of an FP1 pure culture with an optical density at 600 nm (OD$_{600}$) of 0.4 and fed with 50 mg L^{-1} of 2-FP, after which it was left for about 2 weeks as a closed system. The feeding regime presented in Table 1 was followed thereafter.

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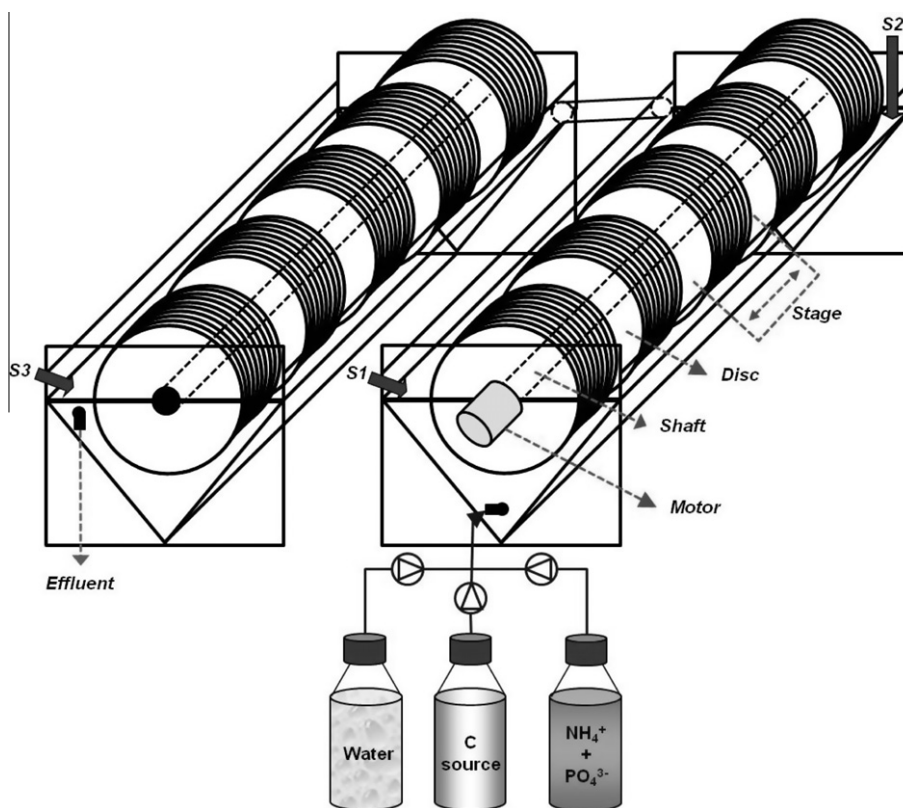
Fig. 1. Schematic representation of the RBC. S1, S2 and S3 are sampling points located in the 1st, 5th and 10th stages, respectively.

2.3. Synthetic wastewater composition

The composition of the influent medium was as described by Beun et al. (2002), but with a sodium acetate inlet concentration of 200 mg L^{-1}. During 2-FP shock loadings, 2-FP was added to the medium in order to reach the range of 2-FP inlet concentrations described in Table 1.

2.4. Analysis

Dissolved oxygen (DO) and pH in the reactor were measured with a WTW 91 handheld multi-parameter instrument 340i. Chemical oxygen demand (COD) was determined according to Standard Method 5220 (APHA, 1998). The fluoride concentration was measured with a fluoride electrode (Carvalho et al., 2006) and 2-FP in filtered samples was analyzed by high performance liquid chromatography (HPLC; Beckman System Gold 126, Fullerton, USA) with a LiChroCART® 25-4 LiChrospher® 100 RP-18 reversed-phase column (5 μm particle size; Merck, Darmstadt, Germany). The mobile phase consisted of 50% (v/v) acetonitrile/water and was used at a flow rate of 0.8 ml min^{-1} (run time – 10 min; injected volume – 20 μl). The compound was detected at 220 nm using a diode array detector.

2.5. Recovering of strain FP1 from the RBC

2.5.1. Bacterial isolation and DNA extraction

Biofilm samples from the 1st disc of the 1st stage of the RBC were scrapped with a scalpel. The biofilm was then subjected to sonication for a total time of 1 min (2 cycles of 30 s at 97% of 20 kHz) (SONOPLUS ultrasonic homogenizer HD 2070; BANDELIN electronic, Berlin, Germany). Resulting supernatant was plated as described by Emanuelsson et al. (2008). Bacteria isolation and DNA extraction was performed according to Calheiros et al. (2010).

2.5.2. DNA sequencing analysis

Isolates were identified by 16S rRNA sequencing analysis. Amplification was carried out with the universal primers f27 and r1492 (Lane, 1991), as previously described (Emanuelsson et al., 2008). The amplified fragments were sequenced by Macrogen Inc. (Seoul, Republic of Korea). To determine the phylogenetic affiliation, similarity analyses were performed using the BLAST program (Altschul et al., 1997).

3. Results and discussion

The RBC treatment efficiency was followed through the evaluation of the parameters biological removal efficiency (BRE) and biological elimination capacity (BEC), which were calculated by considering only the fraction of 2-FP biologically degraded.

Throughout reactor operation, DO concentration and pH at the different stages of the RBC were in the range of 3.7–10 mg O$_2$ L^{-1} and 6.6–7.5, respectively. This indicates that none of these parameters were limiting factors for the reactor operation.

3.1. RBC performance – 2-FP degradation by unacclimated sludge

The reactor was operated for 1 year and 5 months with a hydraulic retention time (HRT) of 18.6 h. The overall operational performance of the RBC is shown in Fig. 2. During phase I (16 days), acetate was continuously fed to the reactor with the objective of achieving a stable and thick biofilm. From phase II to phase IX (days 36–126) the reactor was subjected, every 2 weeks, to organic shock loadings of 25 mg L^{-1} 2-FP during 48 h, mimicking scenarios of industrial wastewaters where these micropollutants may occur discontinuously. As it can be observed in Fig. 2, there was no indication of 2-FP biodegradation during the 90 days period of intermittent organic shocks, with no fluoride released. However, 2-FP was not detected in the outlet, indicating that the compound

Table 1
Degradation of 2-FP in the RBC under several operating conditions.

Phase	Length of operation (days)	Days of operation	Inlet carbon sources concentrations (mg L^{-1})		BRE[a,c](%)	BEC[b,c](g_{2-FP} d^{-1} m^{-2})
			NaAc	2-FP		
	0–18	18	200	0	NA	NA
I	19–35	16	200	0	–	–
II	36–38	2	200	25	–	–
III	39–69	30	200	0	–	–
IV	70–72	2	200	25	–	–
V	73–83	10	200	0	–	–
VI	84–86	2	200	25	–	–
VII	87–97	10	200	0	–	–
VIII	98–100	2	200	25	–	–
IX	101–126	25	200	0	–	–
X	127–144	17	Bioaugmentation with strain FP1[d]			
XI	145–152	7	200	0	–	–
XII	153–155	2	200	25	22 ± 7	0.33 ± 0.1
XIII	156–166	10	200	0	–	–
XIV	167–169	2	200	25	20 ± 6	0.30 ± 0.1
XV	169–177	8	200	0	–	–
XVI	178–185	7	200	25	50 ± 6	0.73 ± 0.1
XVII	186–240	54	200	50	81 ± 6	2.4 ± 0.2
XVIII	241–243	2	200	100	82 ± 27	4.8 ± 1.6
XIX	244–254	10	200	50	110 ± 6	3.2 ± 0.2
XX	255–257	2	200	100	64 ± 14	3.8 ± 0.8
XXI	258–261	3	200	50	126 ± 6	3.7 ± 0.3
XXII	262–264	2	200	100	50 ± 1	2.9 ± 0.1
XXIII	265–290	25	200	50	112 ± 8	3.3 ± 0.2
XXIV	291–293	2	200	100	22 ± 2	1.3 ± 0.1
XXV	294–304	10	200	50	69 ± 3	2.0 ± 0.1
XXVI	305–307	2	200	200	16 ± 1	1.9 ± 0.2
XXVII	308–317	9	200	50	86 ± 9	2.6 ± 0.2
XXVIII	318–346	28	Starvation period			
XXIX	347–360	13	200	0	–	–
XXX	361–496	135	200	50	76 ± 5	2.2 ± 0.1

[a] BRE – biological removal efficiency.
[b] BEC – biological elimination capacity.
[c] The values listed are means ± standard error of the mean (SEM).
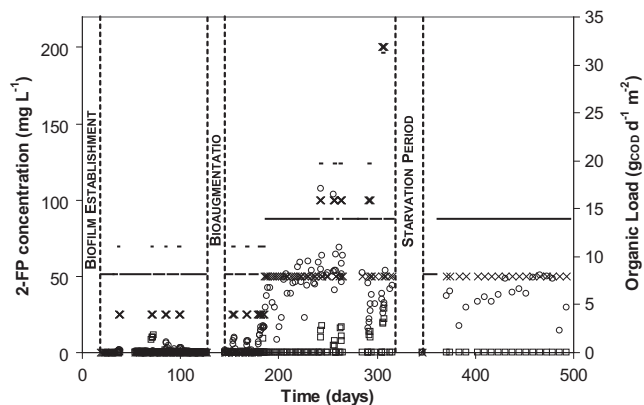[d] Strain FP1 – 2-FP degrading strain; NA – not applicable.

Fig. 2. Biodegradation of 2-FP in the RBC. 2-FP inlet concentration (×), 2-FP outlet concentration (□), 2-FP degraded based on fluoride release (○) and organic load based on acetate and 2-FP COD (-) are indicated.

Fig. 3. Performance of the RBC after bioaugmentation with strain FP1. 2-FP Organic load (×), 2-FP BRE (●) and 2-FP BEC (△) are indicated.

was probably adsorbed to the biofilm matrix. Most of the studies reported in the literature started the acclimatization period with the target pollutant, such as phenol, chlorophenols and trichloroethylene in the feeding and reached complete degradation for the range tested (between 20 and 125 mg L^{-1}) (Brar and Gupta, 2000; Chavan and Mukherji, 2010; Majumber and Gupta, 2007). In this study, the 48 h shocks could have been insufficient for microbial acclimatization, however, even if the time period of the shocks had been longer, acclimatization to 2-FP would have been unlikely to occur due to the highly recalcitrance exhibited by fluorinated
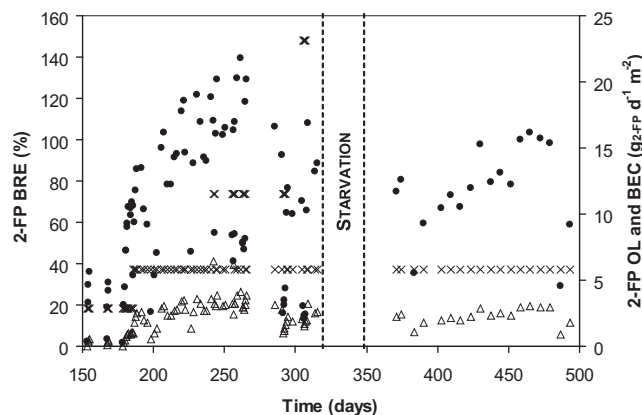
organics. Gentry et al. (2004) reported that bioaugmentation has been shown to improve degradation of various toxic compounds, such as chlorinated compounds and phenols. Therefore, the RBC was, subsequently, bioaugmented with a bacterial strain able to degrade 2-FP, strain FP1.

3.2. RBC performance after augmentation with the 2-FP degrading strain

The bioaugmentation of the RBC, phase X (days 127–144), was performed in batch mode, in order to avoid loss of strain FP1 and

to promote the attachment of the bacterial strain to the biofilm. During phases XI–XVI, the operational strategy used in the RBC was similar to that used before bioaugmentation and from phase XVII, the RBC was continuously fed with 2-FP (25–$200 \, \text{mg L}^{-1}$) (Table 1). The performance obtained in each phase throughout the operation of the RBC after bioaugmentation is summarized in Table 1. A step increase in fluoride release was observed and 2-FP complete biodegradation was reached by day 206 (phase XVII), indicating that bioaugmentation was successfully achieved (Fig. 2). Accordingly, a step increase in the BRE and BEC was also observed, reaching in phase XVII a value of ca. 81% and ca. $2.4 \, \text{g d}^{-1} \, \text{m}^{-2}$, respectively (Table 1 and Fig. 3). With the exception of phase XVIII, where the RBC showed the highest BEC value (ca. $4.8 \, \text{g d}^{-1} \, \text{m}^{-2}$), a decrease in the BRE and BEC was observed in phases XX, XXII and XXIV, indicating that the RBC was not able to deal with the applied concentrations, probably due to an acute toxic effect of 2-FP at higher concentrations. Operational conditions, like HRT and HLR, could be optimized in order to achieve higher degradation rates when treating higher 2-FP concentrations ($>50 \, \text{mg L}^{-1}$); it is possible that a more gradual increase of the concentration of 2-FP could have led to the acclimatization of the biomass to higher concentrations, however in the present work the main objective was to investigate the behavior of the RBC towards 2-FP shock loadings, mimicking an effluent where this kind of micropollutants may occur discontinuously. BRE values higher than 100% were obtained in phases XIX, XXI and XXIII, indicating that the biofilm was highly active, biodegrading both the 2-FP fed in the influent and the 2-FP previously adsorbed onto the biofilm matrix.

Periods of substrate absence can affect biofilm survival and, mainly, maintenance of microbial activity. In phase XXVIII, a starvation period of 28 d was applied to the RBC, in order to mimic a period of plant shutdown. The feed was then re-established at day 347 (phase XXIX) with acetate as sole carbon and energy sources and from phase XXX, the RBC was fed again with $50 \, \text{mg L}^{-1}$ of 2-FP. During phase XXX, the obtained BRE and BEC values were similar to those achieved in phase XXVII, before the starvation period, indicating that the degrading biofilm remained active after the period of substrate absence (Fig. 3). Immobilized reactors have demonstrated capacity to preserve biofilm survival and activity after starvation periods (Carvalho et al., 2006; Emanuelsson et al., 2008; Sahinkaya and Dilek, 2006).

The 2-FP degradation occurring at the different stages of the RBC was investigated (data not shown). It was observed that the 1st stage of the RBC had the higher contribution to the removal of 2-FP, suggesting that strain FP1 was mainly present in the 1st stage. Therefore, only the area of the 1st stage was taken into account for the BEC calculation.

3.3. Recovering of strain FP1 from the RBC

In order to assess the success of the bioaugmentation biofilm samples were collected and plated in NA and recovered isolates were characterized through 16S rRNA gene analysis. According to Blast results strain FP1 was recovered from the reactor, which strongly indicates the success of the bioaugmentation. Successfully incorporation of bioaugmented strains within granular activated carbon biofilm reactors has been reported (Carvalho et al., 2006; Emanuelsson et al., 2008).

4. Conclusions

This study demonstrated the applicability of RBCs in the biological treatment of wastewaters containing toxic compounds, like 2-FP.

- After bioaugmentation with strain FP1, the RBC showed good removal performances for 2-FP concentrations up to $50 \, \text{mg L}^{-1}$, reinforcing that bioaugmentation is often necessary when biodegradation of highly recalcitrant compounds is targeted;
- The RBC was able to deal with a substrate absence period, mimicking a plant shutdown season, being capable to recover its performance;
- After ca. 350 days of operation, strain FP1 was successfully recovered from the RBC biofilm.

Acknowledgements

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