Abatement of styrene waste gas emission by biofilter and biotrickling filter: Comparison of packing materials and inoculation procedures.

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Abstract

The removal of styrene was studied using 2 biofilters packed with peat and coconut fibre (BF1-P and BF2-C, respectively) and 1 biotrickling filter (BTF) packed with plastic rings. Two inoculation procedures were applied: an enriched culture with strain *Pseudomonas putida* CECT 324 for biofilters and activated sludge from a municipal wastewater treatment plant for the BTF. Inlet loads (ILs) between 10 and 45 g m^{-3} h^{-1} and empty bed residence times (EBRTs) from 30 to 120 s were applied. At inlet concentrations ranging between 200 and 400 mg Nm^{-3}, removal efficiencies between 70 and 95% were obtained in the 3 bioreactors. Maximum elimination capacities (ECs) of 81 and 39 g m^{-3} h^{-1} were obtained for the first quarter of the BF1-P and BF2-C, respectively (IL of 173 g m^{-3} h^{-1} and EBRT of 60 s in BF1-P; IL of 89 g m^{-3} h^{-1} and EBRT of 90 s in BF2-C). A maximum EC of 52 g m^{-3} h^{-1} was obtained for the first third of the BTF (IL of 116 g m^{-3} h^{-1}, EBRT of 45 s). Problems regarding high pressure drop appeared in the peat biofilter, whereas drying episodes occurred in the coconut fibre biofilter. DGGE revealed that the pure culture used for biofilter inoculation was not detected by day 105. Although 2 different inoculation procedures were applied, similar styrene removal at the end of the experiments was observed. The use as inoculum of activated sludge from municipal wastewater treatment plant appears a more feasible option.

Keywords: biofilter, biotrickling filter, inoculation procedures, microflora, *Pseudomonas putida*, styrene

Introduction

Styrene is a volatile organic compound (VOC) classified as a hazardous air pollutant under the Clean Air Act Amendments of 1990 (USEPA 1994). It is predominantly emitted from industries producing polystyrene, styrene copolymers and polyester. The principal uses of styrene include the manufacture of plastics, latex paints and coatings, synthetic rubbers, polyesters and styrene-alkyd coatings. Styrene or its derivative products may be released into the atmosphere during any step in production, storage, transport, or utilisation. Styrene is a pollutant that contributes to tropospheric ozone formation (Derwent et al. 1996) and can affect human health (Kolstad et al. 1995). In fact, styrene has been classified as a potential carcinogen in humans by the International Agency for Research on Cancer (IARC 1987).

Recently, biotechnologies have been developed as alternatives to conventional techniques to treat waste gas with high flow rates and relatively low VOC concentrations. Biotechnologies are cost-effective due to their low operational costs, low energy requirements and an absence of residual products requiring further treatment or disposal (Kennes et al. 2009). Among the available biological gas treatments, biofilters (BFs) and biotrickling filters (BTFs) are of particular interest. These bioreactors work by passing waste air through a bed of packing material where an active biofilm is developed on its surface. The air pollutants, previously transferred from the gas phase, are mainly degraded to H_2O and CO_2 by the microorganisms. In BFs, the reactor is usually filled with organic packing materials. The selection of material is one of the key factors in the successful application of BFs. Therefore, it is essential to choose the material with adequate physical and chemical properties, such as high surface area, long-term physical stability, low pressure drop, low cost, good moisture

retention, pH buffering capacity, nutrients and appropriate adsorbing capacity (Shareefdeen and Singh 2005). In fact, a great variety of packing materials have been tested in BFs, such as peat, compost bark, and wood chips (Ivanpour et al. 2005; Malhautier et al. 2005). BTFs use an inert packing material and a liquid phase that trickles through the bed and provides nutrients to the biofilm. This allows for pH control, yielding a stable operation. The trickling liquid can be fed continuously or discontinuously into the bioreactor (Sempere et al. 2008). Because of their relatively small footprint, BTFs do not need much ground area, but do usually reach high pollutant removals (Kennes and Veiga 2013).

Several studies have proven the successful application of these biotechnologies for the removal of styrene from gas emissions, although the comparison of biofilters and biotrickling filters has not been evaluated. Arnold et al. (1997) investigated styrene removal in a peat biofilter. Inlet concentrations ranging between 50 and 1200 mg m^{-3} were applied and an average elimination capacity (EC) of 12 g m^{-3} h^{-1} with a maximum of 30 g m^{-3} h^{-1} was obtained. Dehghanzadeh et al. (2005) used a biofilter packed with yard waste compost mixed with shredded hard plastic and observed a maximum EC of 45 g m^{-3} h^{-1} at an inlet load (IL) of 60 g m^{-3} h^{-1} and an empty bed residence time (EBRT) of 120 s. Novak et al. (2008) reported a maximum EC of 11.3 g C m^{-3} h^{-1} at an IL of 18.6 g C m^{-3} h^{-1} in a BTF packed with polypropylene Pall rings working with an EBRT of 35s.

Currently, more interest is aimed at opening the black box of biofiltration by unravelling the biodiversity-ecosystem function relationship (Cabrol and Malhautier 2011). In the case of the removal of styrene, the ability of the genus *Pseudomonas* for the degradation of this compound under aerobic conditions has been reported. Okamoto et al. (2003) tested the *Pseudomonas putida* strain ST201 in flask experiments,

achieving complete styrene degradation after 48 h at concentrations up to 600 mg L^{-1}. Jang et al. (2004) used *Pseudomonas* sp. SR-5 as a styrene-degrading bacterium in 2 biofilters packed with peat and a ceramic material, reporting maximum ECs of 236 and 81 g m^{-3} h^{-1} for the peat and ceramic BF, respectively. Paca et al. (2001) observed the predominance of *Pseudomonas aeruginosa* strains working at pH above 5 in four perlite BFs at EBRTs varying from 6.5 s to 26 s and inlet concentrations from 200 to 1000 mg m^{-3}.

Among the inoculation procedures which were applied in the start-up of bioreactors treating styrene emissions, the use of 2 different inoculum sources has been used: enriched cultures with styrene-degrading strains (Okamoto et al. 2003; Jang et al. 2004; Kim et al. 2005) and activated sludge from wastewater treatment plants (Juneson et al. 2001; Sempere et al. 2011). From an industrial point of view, the choice of activated sludge as an inoculum presents advantages due to the ease of implementation and lower operational costs.

The purpose of this work is to investigate the removal of styrene from air emissions by using 2 types of bioreactors and to compare both procedures of inoculation on the basis of their influence on the system performance and the evolution of the microbial community. For this purpose, the following objectives have been taken into consideration: (1) to evaluate the behaviour of one BF packed with peat, another with coconut fibre and one BTF packed with plastic rings. The bioreactors were operated at several EBRTs and at a range of inlet concentrations representative of emissions from industries producing polystyrene; and (2) to analyse the effect on the systems of using 2 sources of inoculum: an enriched culture of the strain *Pseudomonas putida* CECT 324 in the BFs and activated sludge from a municipal wastewater treatment plant (WWTP) in the BTF. To the best of our knowledge, this is the first work which includes a

comparison of the performance and microflora of bioreactors inoculated with an enriched culture or with an activated sludge. The microbial community was studied by fluorescence *in situ* hybridisation (FISH) and denaturing gradient gel electrophoresis analysis (DGGE).

Materials and methods

Inoculation procedures

The inoculation of the BFs was performed with 1 L of enriched culture of the strain *Pseudomonas putida* CECT 324 (named here as inoculum 1). The following protocol was developed for the inoculum preparation: (i) the strain *Pseudomonas putida* CECT 324 was supplied from the Spanish Type Culture Collection (CECT). This species was selected for the inoculation process since previous studies have reported that *Pseudomonas putida* is suitable for degrading styrene (Okamoto et al. 2003). (ii) The strain CECT 324 was grown in 50 mL sterile flasks containing 20 ml of nutrient Broth/Agar II medium (composition: 1 g L^{-1} beef extract, 2 g L^{-1} yeast extract, 5 g L^{-1} peptone, 5 g L^{-1} NaCl, pH: 7.2) at 30ºC in a rotary shaker at 100 rpm (New Brunswick scientific, Edison, USA). (iii) After 1 week, 250 ml of the concentrated pure culture was introduced into one aerated batch reactor and diluted with 750 mL of nutrient Broth/Agar II medium. The batch reactor was aerated using non-sterile air and was continuously fed with styrene at a rate of 0.15 mL h^{-1} for a period of 30 days.

The inoculation of the BTF was performed with 1 L of activated sludge (named here as inoculum 2) from a municipal WWTP located in Quart-Benager (Valencia, Spain), which was continuously recirculated over the bed for a period of 24 h.

BFs set-up and operational conditions

Two lab-scale BFs were operated in parallel. One BF (BF1-P) was packed with fibrous peat (ProEco Ambiente, Spain) and another (BF2-C) with coconut fibre (Pure Air, Solutions, The Netherlands). The physical and chemical properties of these materials are shown in Table 1. The peat was acidic, so pH adjustment until neutral pH was achieved by using diluted sodium hydroxide solution prior to the start-up.

The schematic of the experimental set-up of the BFs is shown in Fig. 1a. Each BF was made from methacrylate, with a total length of 97 cm, an internal diameter of 13.6 cm (volume of 14 L) and was equipped with five sampling ports to measure VOC concentrations along the bed of the column, located at 0 (inlet port), 25, 50, 75 and 97 (outlet port) cm of column height. Four additional ports were used to extract packing material samples located at 20, 40, 60 and 80 cm. A 10 cm head space was used for the waste gas inlet and for water/nutrient feed, while a 10 cm bottom space was used for the treated air outlet and leachate. Prior to introduction into the BFs, the compressed, filtered and dry air was passed through the humidifier to assure a relative humidity value of at least 90%. The air stream was contaminated with styrene by using a syringe pump (New Era, infusion/withdraw NE 1000 model, USA) and fed to the BFs through the top of the column flowing downwards into the bed. The gas flow rate was adjusted by a mass flow controller (Bronkhorst Hi-Tec, The Netherlands).

The macro- and micro-nutrients were incorporated by pouring 50 ml per day of a nutrient solution buffered at pH 7 (22.4 g KNO_3 L^{-1}, 2.4 g KH_2PO_4 L^{-1}, 0.4 g K_2HPO_4 L^{-1}, 0.9 g $MgSO_4 \cdot 7H_2O$ L^{-1}, and Ca, Fe, Zn, Co, Mn, Na, Ni, B, I, Se, Cr, Cu and vitamins at trace doses). The moisture content of the packing material was controlled by

adding 50 and 150 ml per day of deionised water on top of the BF with peat and coconut fibre, respectively. This difference in the spraying volume was due to the fact that peat has a higher water-holding capacity than coconut fibre; 88% and 60% respectively.

The 2 BFs were operated in parallel under different operational conditions for more than 5 months under continuous loading. Operational conditions in BFs are shown in Table 2. During the first 3 months, the BFs were operated at EBRTs between 90 and 120 s and ILs between 12 and 24 g m^{-3} h^{-1}. After this period, four different stages (1–4) were performed. EBRTs were set at values of 45, 60 and 90 s, and inlet concentrations ranged between 250 and 1300 mg Nm^{-3} (corresponding to ILs varying from 15 to 45 g m^{-3} h^{-1}, Table 2). *In-situ* emissions from industries producing polystyrene were previously monitored and found to oscillate between 200 and 400 mg Nm^{-3}. Therefore, the inlet concentrations tested on this work covered the typical values of the emissions coming from the industrial manufacturing of polystyrene. Each stage was carried out within a minimum period of 10 days.

BTF set-up and operational conditions

After ending the operation of the BFs, another experiment using a BTF was performed in order to compare the use of different bioreactors and inoculation procedures. The BTF was filled with polypropylene rings (Flexiring, Koch-Glitsch B.V.B.A., Belgium) of 25 mm nominal diameter and a surface area of 207 m^2 m^{-3} whose physical characteristics are shown in Table 1. The schematic of the experimental set-up of the BTF is shown in Fig. 1b. The BTF was built using 3 cylindrical methacrylate modules in series with a total bed length of 123 cm and an internal diameter of 14.4 cm (volume

of 20 L). The BTF was equipped with four sampling ports to measure styrene concentrations along the bed of the column, located at 0 (inlet port), 44, 86, and 123 (outlet port) cm of column height. Three additional ports were used to extract biofilm samples located at 20, 63 and 105 cm. The bioreactor was also provided with 20 cm of top and bottom free spaces. The compressed, filtered and dry air was contaminated with styrene using a syringe pump (New Era, infusion/withdraw NE 1000 model, USA). The polluted air stream was introduced through the bottom of the column and the flow rate was adjusted by a mass flow controller (Bronkhorst Hi-Tec, The Netherlands). A 3 L recirculation tank, partially renewed every week, was used to feed the recirculation solution into the bioreactor in counter-current mode with respect to the air flow using a centrifugal pump at $2.5 - 3$ L minute^{-1} for 15 minutes every 2 h.

A nutrient solution buffered at pH 8 (9.7 g NH_4Cl L^{-1}, 0.9 g $MgSO_4 \cdot 7H_2O$ L^{-1}, 2.2 g $(NH_4)_2HPO_4$ L^{-1}, 0.5 g $NaHCO_3$ L^{-1}, 0.4 g NaOH L^{-1}, 0.6 g KCl L^{-1} and Ca, Fe, Zn, Co, Mn, Na, Ni, B, I, Se, Cr, Cu and vitamins at trace doses) was supplied to the recirculation tank using a peristaltic pump. The nutrient solution flow rate was maintained at a mass ratio of carbon and nitrogen of 25 in order to assure that the nitrogen concentration in the recirculation solution was not limiting the biodegradation process, corresponding to a flow rate of nutrients between 32 and 160 mL d^{-1}.

The BTF was operated for more than 5 months under continuous loading. Operational conditions applied to the BTF are shown in Table 2. In the first 20 days, an EBRT of 60 s and an inlet concentration of 200 mg Nm^{-3} were applied. Afterwards, 6 different stages (1–6) were performed over 5 months. In the first 3 stages, an EBRT of 60 s and inlet concentrations ranging between 300 and 468 mg Nm^{-3} were applied (Table 2). At stages 4 and 5, the EBRT was decreased from 60 to 45 s. The maximum inlet concentration was applied (475 mg Nm^{-3}) in stage 5. At stage 6, the EBRT was

decreased to 30 s in order to evaluate the minimum EBRT that allows high RE and stable performance. Each stage was carried out for a minimum period of 20 days.

Analytical methods

The styrene concentration was measured using a total hydrocarbon analyser (Nira Mercury 901, Spirax Sarco, Spain). The inlet and outlet gas streams were monitored daily while the intermediate ports were monitored at a minimum of 2 times for each stage. The response factor of the total hydrocarbon analyser was determined by gas chromatograph (model 7890, Agilent Technologies, USA) equipped with a 1 mL automated gas valve injection system, a flame ionisation detector and an Rtx®-VMS capillary column (30 m x 0.25 mm x 1.4 µm). The gas carrier was helium at a flow rate of 1.3 mL min^{-1}. The injector, oven and detector temperatures were 250, 100 and 240ºC, respectively. The pressure drop was monitored at a minimum of once a week (KIMO, MP101 model, Spain). The CO_2 concentrations in the inlet and outlet gas streams were measured once a week using a CARBOCAP® carbon dioxide analyser (GM70 model, Vaisala, Finland).

The conductivity and pH of the leachate from BFs were determined daily (pH/Cond 340i, WTW, Germany) and the moisture content of the media was measured at a minimum of 2 times in each experimental stage using the dry weight method at 2 locations: at 20 cm from the inlet (top zone) and at 80 cm from the inlet (bottom zone). The conductivity and pH of the BTF recirculation solution were determined daily (pH/Cond 340i, WTW, Germany). In addition, the total organic carbon and inorganic carbon (TOC-V$_{CHS}$, Shimadzu, Japan), suspended solids (SS), volatile suspended solids

(VSS) and NO_3^-, NH_4^+ and PO_4^{3-} concentrations were measured twice a week (Ionic Chromatograph 883 Basic IC Plus).

DNA isolation, PCR and DGGE

The presence of *Pseudomonas putida* in both BFs was checked by DGGE analysis. DNA was isolated from the pure culture of *Pseudomonas putida* CECT 324, from the enriched culture prior to inoculation (inoculum 1) and from the bed samples of each BF at 105, 142 and 156 days of operation. Bed samples were taken from the bottom port (located at 80 cm from the inlet). The DNA isolation was performed using a FastDNA Spin Kit for soil (MP Biomedicals, Illkirch, France) and was stored at -20ºC until analysis. The extracted DNA was amplified by PCR using 2 universal primers targeting the 16S rRNA gene for eubacteria: primer F357GC containing a CG clamp (5´CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGCCTACGGG AGGCAGCAG3´) and primer R518 (5´ATTACCGCGGCTGCTGG3´). PCRs were performed in a thermal cycler (LongGene Scientific Instruments, Hangzhou) with a 50-µl reaction volume of a mixture containing final concentrations of 1.25 units of Taq DNA polymerase, 0.2 mM dNTPs, 2 mM Mg^{2+} and 0.5 µM of each primer (EuroClone, Italy). PCR conditions (Muyzer and Ramsing, 1993) consisted of 20 cycles of: 94ºC for 1 minute, 65ºC for 1 minute, a touchdown annealing step of 0.5ºC increments from 65ºC to 55ºC for 1 minute, and followed by 72ºC for 3 minutes. For DGGE analysis, 20 µl of PCR product generated from each sample was separated on an 8% acrylamide gel running in a linear denaturant gradient increasing from 35% to 60% using a KuroGel Verti 2020 DGGE System (VWR international Eurolab S.L.). The gel was run at 60ºC for 5 minutes at 50 V, 120 minutes at 150 V and 60 minutes at 200 V.

Fluorescence *in situ* hybridisation (FISH)

The FISH technique was carried out adapting the procedure described by Amann et al. (2001). Bed samples from the bottom port of both BFs at day 105, 142 and 156 were analysed. Biofilm samples taken from the bottom port (located at 20 cm from the inlet) and the top port (located at 105 cm from the inlet) from BTF at day 65 and 165 were analysed. Inoculum samples of the BFs (inoculum 1) and the BTF (inoculum 2) were analysed as well.

The samples (5 mL for the samples of the BFs, 1 mL for the BTF and the inoculum samples) were suspended in 15 ml sterile distilled water and disaggregated with an Ultra-Turrax (IKA$^{®}$ T18 basic, Germany). The samples were fixed using the procedure described by Amann et al. (2001). According to the procedure, Gram-negative cells were fixed with 4% paraformaldehyde and Gram-positive cells with ethanol. Oligonucleotide probes targeting the phylogenetic groups *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria*, *Firmicutes* and *Actinobacteria* were analysed. Furthermore, the evolution of *Pseudomonas* sp. and *Pseudomonas putida* were monitored. An equimolar mixture of EUBI, EUBII, and EUBIII probes were used to detect all eubacteria. Probes sequences and their formamide percentage are summarised in Table 3 (Loy et al. 2007). In the hybridisation, the general EUB$_{mix}$ probe was labelled with Cy5, while taxonomic specific probes were labelled with Cy3-labelled (Thermo Fisher Scientific, Germany). Hybridisation was performed at 46ºC for 2 h. After the hybridisation step, slides were rinsed for 18 minutes at 48ºC, dried, mounted and examined using a confocal laser scanning microscope (FV 1000, Olympus, Japan). Specific probes were quantified as a proportion of EUB$_{mix}$-labelled

bacteria using image analysis based on the methodology developed by Jubany et al. (2009).

Results

BF performance

The performance of the 2 BFs treating styrene emissions, BF1-P packed with peat and BF2-C with coconut fibre is shown in Fig. 2. At day 0, each BF was inoculated with the enriched culture of *Pseudomonas putida* CECT 324 (inoculum 1) whose suspended solid concentration and percentage of volatile suspended solids were 1.7 g L^{-1} and 71%, respectively. Following inoculation, the bioreactors were operated during 84 days at mild conditions, corresponding to EBRTs between 90 and 120 s and inlet concentrations between 300 and 790 mg Nm^{-3}, in order to develop a suitable active biofilm on the surface of the packing material. BF1-P showed a gradual increase in the removal efficiency (RE) until reaching 99% on day 60 (Fig 2a). From this day, a progressive decrease in the removal of styrene occurred until day 64, from which REs fluctuated in 35 ± 8%. Pressure drop measurements in BF1-P showed a sudden increase on day 37 (Fig. 2c). The pressure drop rose from a value of 98 (day 37) to 490 Pa m^{-1} (day 41), and reached a maximum value of 931 Pa m^{-1} on day 52 indicating compaction of the bed (Fig. 2c). BF2-C showed a gradual increase in the RE until reaching 95% on day 52 (Fig. 2b). From days 53 to 84, RE oscillated in 78 ± 13%. No bed compaction was detected in BF2-C, the pressure drop was maintained in stable values as low as 10 Pa m^{-1} from days 1 to 84 (Fig. 2d).

After nearly 3 months of operation, an EBRT of 60 s and an average inlet concentration of 372 ± 14 mg Nm^{-3} were applied in both BFs (stage 1). During this stage, an average outlet concentration of 166 ± 15 mg Nm^{-3} (RE of 55%) was observed in BF1-P from day 84 to day 110 (Fig. 2a). On day 110 the pressure drop retained values as high as 800 Pa m^{-1} (Fig. 2c), so the reactor was opened to decompress the packing material. Upon restoration of operation, the pressure drop decreased to 170 Pa m^{-1} (Fig. 2c) and the RE increased to a value of 95%. From day 110, RE was kept at values higher than 95% (average outlet concentration of 23 ± 9 mg Nm^{-3}) until the end of stage 1 (day 122). In the BF2-C, an outlet concentration of 106 ± 20 mg Nm^{-3} (RE of 72%) was observed for the whole stage 1 (Fig. 2b). The pressure drop in BF2-C was kept below 30 Pa m^{-1} for the entire stage 1 (Fig 2d).

Due to the different performances of both BFs at the end of stage 1 (with a higher RE for BF1-P than BF2-C), different values of EBRTs were chosen for stage 2. It was decided to lower the EBRT in BF1-P and increase it in BF2-C in order to determine the minimum EBRT that allows high efficiency in each system. In stage 2, BF1-P presented REs of ~70% using inlet concentrations of 782 ± 20 mg Nm^{-3} (EBRT of 60 s). BF2-C showed REs >90% (outlet concentrations below 40 mg Nm^{-3}) using lower inlet concentrations (368 ± 15 mg Nm^{-3}) and a higher EBRT (90 s).

In stage 3, the same IL (22 g m^{-3} h^{-1}) was applied in both BFs. The EBRT in BF1-P was decreased to 45 s and the inlet concentrations (282 ± 18 mg Nm^{-3}) were set to apply the same IL value as stage 1, obtaining outlet concentrations of 14 ± 10 mg Nm^{-3} (RE of 95%). The EBRT in BF2-C was maintained for 90 s and the inlet concentrations were increased to 585 ± 18 mg Nm^{-3}, reaching outlet concentrations below 110 mg Nm^{-3} (RE ~80%). At an IL of 22 g m^{-3} h^{-1}, BF1-P working at an EBRT of 45 s achieved higher REs than BF2-C working at twice the EBRT. In stage 4, a

maximum IL of 45 g m^{-3} h^{-1} was applied to both BFs maintaining the same EBRTs as in stage 3. The average inlet concentrations of 580 ± 10 and 1085 ± 67 mg Nm^{-3} were applied in BF1-P and BF2-C, respectively. The RE decreased in both BFs: from 95 to 62% in BF1-P and from 80 to 47% in BF2-C. From day 148, pressure drops fluctuated between 637-784 Pa m^{-1} (Fig. 2c) in BF1-P indicating the presence of compaction problems in the peat bed. Maximum pressure drop in BF2-C was 59 Pa m^{-1} (day 154 , Fig. 2d).

In BF1-P, the moisture content was maintained at ~80% for both sampling ports (top and bottom) when EBRTs greater than 60 s were applied (stages 1–2, average value of 80.6 ± 3.1%, Fig. 2c). On day 162, the moisture content decreased until 63% in the top and 50% in the bottom after 24 days working at 45 s of EBRT (Fig. 2c). In BF2-C, the average moisture content in the start-up period was 51.4 ± 7.4% and from day 84 the average moisture content was 66.6 ± 6.5% and 70.6 ± 11.4% in the top and bottom locations, respectively (Fig. 2d). The average pH of the leachate was 8.4 ± 0.5 and 8.8 ± 0.3 in BF1-P and BF2-C, respectively. The average conductivity of the leachate was 3.8 ± 1.9 and 6.2 ± 1.5 mS cm^{-1} in BF1-P and BF2-C, respectively.

BTF performance

The performance of the BTF is shown in Fig. 3. At day 0, the BTF was inoculated with the activated sludge (inoculum 2), whose suspended solid concentration and percentage of volatile suspended solids were 7.3 g L^{-1} and 78%, respectively. During the first 20 days, the system was operated at styrene inlet concentrations of 216 ± 14 mg Nm^{-3} and an EBRT of 60 s. After the first week, the BTF was able to reach a RE of 94% (Fig. 3a). In stage 1, the inlet concentration was raised to a value of 290 ± 12 mg Nm^{-3} and the RE

was maintained at ~90%. In stages 2 and 3, average inlet concentrations of 391 ± 13 and 444 ± 39 mg Nm^{-3} were applied, respectively. REs of ~75% were obtained in both stages corresponding to average outlet concentrations of 99 ± 28 and 109 ± 40 mg Nm^{-3}, respectively. It is worth noting that on day 70, the RE dropped to 62% because of the limitation of nutrients, since the phosphate concentration (PO_4^{3-}) in the recirculation tank was <1 mg-P L^{-1} due to a maintenance failure. Afterwards, the phosphate concentration was kept >30 mg-P L^{-1} for the rest of the experiment. The RE was successfully recovered to 77% on day 75 (Fig. 3a). From stage 4, the EBRT was decreased to 45 s and average inlet concentrations of 355 ± 50 mg Nm^{-3} (stage 4) and 512 ± 15 mg Nm^{-3} (stage 5) were applied. Average outlet concentrations of 64 ± 10 mg Nm^{-3} and 115 ± 25 mg Nm^{-3} (REs about 80%) were obtained for stage 4 and 5, respectively. In stage 6, the minimum tested EBRT was applied (30 s). The RE was 80% with an average outlet concentration of 45 ± 11 mg Nm^{-3}. The pressure drop was maintained in low values ranging between 1 and 60 Pa m^{-1} during the whole experimental period (Fig. 3b).

During the operation of the BTF, a liquid volume of 3 L was maintained in the recirculation tank with a weekly purge of 1.5 L. Average values of pH and conductivity were 8.1 ± 0.5 and 7.3 ± 2.4 mS cm^{-1} (Fig. 3b). Average values of ammonium (NH_4^+), nitrate (NO_3^-) and phosphate (PO_4^{3-}) concentrations were 24.6 ± 16.0, 1.7 ± 2.9 and 121.5 ± 80.2 mg L^{-1}, respectively. The average concentration of suspended solids was 480 ± 408 mg L^{-1} with a percentage of volatile suspended solids of 83 ± 8%. The average amount of total organic carbon was 146 ± 59 mg C L^{-1}. In all cases, solvent removal with the purge represented less than 1% of the total amount of added styrene during the week. Thus, the organic carbon in the purge was considered negligible for the evaluation of the BTF performance in terms of EC and RE.

Relationship between EC and IL

The relationships between EC and IL calculated from the inlet to each intermediate sampling port (first quarter, half and three quarter of the BFs and the first third, half and two third of the BTF) and for the whole volume of the bioreactors are plotted in Fig. 4. The 3 bioreactors performed near complete degradation with an IL of 40 g m^{-3} h^{-1} for the whole range of tested EBRT. Maximum ECs of 81 and 39 g m^{-3} h^{-1} were obtained for BF1-P and BF2-C, respectively, with REs ~45% (IL of 173 g m^{-3} h^{-1} at EBRT of 60 s in BF1-P, Fig. 4a; IL of 89 g m^{-3} h^{-1} at EBRT of 90 s in BF2-C, Fig. 4b). A maximum EC of 52 g m^{-3} h^{-1} was obtained for BTF with a RE of 45% (IL of 116 g m^{-3} h^{-1}, EBRT of 45 s, Fig. 4c). The comparison of the behaviour of the 3 reactors when working at an EBRT of 60 s and an IL of ~75 g m^{-3} h^{-1} indicates that BF1-P and BTF presented a similar value of EC ~40 g m^{-3} h^{-1} (Fig. 4a,c) unlike BF2-C, which showed a lower EC of ~30 g m^{-3} h^{-1} (Fig. 4b). By decreasing the EBRT to 45 s, both BF1-P and BTF resulted in similar values with ECs of ~55 g m^{-3} h^{-1} for an IL ~85 g m^{-3} h^{-1} (Fig. 4a,c).

Carbon dioxide production

The evolution of CO_2 production (calculated for the whole bed volume) as a function of the EC is presented in Fig. 5 for the 3 bioreactors. A proportional ratio exists between EC and CO_2 production. Linear regressions for these data resulted in values of 2.57, 2.81 and 2.34 g CO_2/g styrene in BF1-P (Fig. 5a), BF2-C (Fig. 5b) and BTF (Fig. 5c), respectively. These values are comparable to the ratio of 2.78 g CO_2/g styrene reported by Rene et al. (2010) by using a fungal monolith bioreactor.

Assuming a general biomass composition formula of $C_5H_7O_2N$, overall yield coefficients defined as g of dry biomass synthesised per gram of substrate consumed can be determined from the biodegradation reaction balance:

$$aC_xH_yO_z + bO_2 + NH_3 \rightarrow C_5H_7O_2N + cCO_2 + dH_2O$$

resulting in values of 0.42, 0.30 and 0.54 g of dry biomass produced per g of styrene consumed for BF1-P, BF2-C and BTF, respectively. The greater yield coefficient observed in the BTF (0.54 g g^{-1}) indicates that a higher biomass growth occurred. This fact could be associated with the trickling liquid that allows better control of parameters such as pH and better distribution over the bed of nutrients and substrates.

Microbiological studies

Denaturing gradient gel electrophoresis (DGGE)

DGGE was applied in order to check the presence of *Pseudomonas putida* in the BFs. Fig. 6 shows the DGGE banding patterns of samples from: the pure culture (*Pseudomonas putida* CECT 324), the enriched culture of strain CECT 324 prior to inoculation (inoculum 1) and the samples from BFs at days 105, 142 and 156 of operation. DGGE patterns showed 3 dominants bands for the pure culture (bands A, B and C; Fig. 6, lane 1). Similar band intensities of bands A, B and C, corresponding to *Pseudomonas putida* CECT 324, were detected in the enriched culture sample (inoculum 1; Fig. 6, lane 2). In addition, 2 new bands D and E appeared in the inoculum 1 (Fig. 6, lane 2). In contrast bands corresponding to *Pseudomonas putida* CECT 324 did not appear as a dominant species in the BF samples (Fig 6, lanes 3-8). BF samples containing multiple DGGE bands, some of them differed for each sample, although

some common bands were detected. The bands D, G, H and I were observed for all

BF1-P samples over the time period tested, while band J appeared by days 142 and 156

(Fig. 6, lanes 3, 5,7). Bands D, H, I, J, K, L were observed for all BF2-C samples, and

relative intensities of the bands changed between different sampling times (Fig. 6, lanes

4, 6,8). For instance, bands H and I increased at day 142 (Fig. 6, lane 6) and decreased

by day 156 (Fig. 6, lane 8). A new band M appeared by days 142 and 156 (Fig. 6, lanes

6,8) for BF2-C and by day 156 (Fig.6, lane 7) for BF1-P. At the end of the experiment,

both BFs presented 5 common dominant bands (D, H, I, J, M; Fig. 6, lanes 7,8).

Monitoring of the bacterial community by fluorescence *in situ* hybridisation (FISH)

The monitoring of the bacterial community was carried out by FISH for the 2 inoculum

and the 2 BFs: days 105 (stage 1), 142 (stage 3) and 156 (stage 4); and for the BTF:

days 65 (stage 2, top and bottom zones) and 165 (stage 6, top and bottom zones). The

total percentages of all target phyla were 76.5 and 98.4% for inoculum 1 and inoculum

2, respectively (Fig. 7, a,b,c). Total percentages of all target phyla ranged from 92.0 to

96.6%, 55.0 to 64.9%, and 88.8 to 98.8% for BF1-P, BF2-C and BTF, respectively (Fig.

7a,b,c). In all samples, the phylum *Proteobacteria* (*Alphaproteobacteria*,

Betaproteobacteria, *Gammaproteobacteria* and *Deltaproteobacteria*) was more

abundant than the phyla *Firmicutes* and *Actinobacteria*. Among bacterial groups from

both inocula, members of the *Gammaproteobacteria* (inoculum 1: 30.6 % and inoculum

2: 37.1%; Fig. 7a,c) followed by *Alphaproteobacteria* (inoculum 1: 25.8 % and

inoculum 2: 20.5 %; Fig. 7a,c) were the most abundant groups. Inoculum 2 showed a

higher proportion of *Betaproteobacteria* (14.4%), *Firmicutes* (10.5%) and

Actinobacteria (10.6%) (Fig. 7c). Regarding the evolution of the bacterial groups, their

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composition changed over time. For BF1-P, *Alphaproteobacteria* (32.5%), and *Gammaproteobacteria* (22.4%) were the most abundant groups on day 105, but they declined on day 142 in which more abundance of *Actinobacteria* (26.5%) *and Deltaproteobacteria* (23.2%) was observed (Fig. 7a). On day 156, *Gammaproteobacteria* (37.0%) predominated among other phylogenetic groups. For BF2-C, *Alphaproteobacteria* (16.3%) and *Gammaproteobacteria* (16.2%) were the most abundant groups by day 105 (Fig. 7b). However, the abundance of *Alphaproteobacteria* (7.8%) declined on day 142, and *Gammaproteobacteria* (17.5%) and *Betaproteobacteria,* (13.1%) were the most abundant groups. At the end of the experiment (day 156), there was a predominance of *Gammaproteobacteria* (20.4%). For the BTF, *Gammaproteobacteria* (bottom: 41.7%; top: 43.7%) was the most abundant group on day 65, with slightly higher values than in the inoculum 2 (Fig. 7c). As shown in Fig. 7c, a predominance of *Gammaproteobacteria* was maintained (bottom: 24.8%; top: 30.6%) till the end of the experiment (day 165), but with a lower relative abundance. Similar results in relative abundances of phylogenetic groups from the bottom and top of the BTF for each sampling day showed that the bacterial community was quite homogeneous along the BTF.

Regarding the relative abundance of *Pseudomonas putida,* the enriched culture (inoculum 1) showed an abundance of 25.1% (Fig. 7d), while the activated sludge from a municipal WWTP (inoculum 2) showed a percentage of 1.0 % (Fig. 7f). A different evolution of percentages of *Pseudomonas putida* over time was observed for both inoculation procedures. In the BFs, the relative abundance of this species was lower than in the inoculum. For BF1-P the relative abundance was 14.7%, 8.1% and 24.8% on days 105 (stage 1), 142 (stage 3) and 156 (stage 4), respectively (Fig. 7d). For BF2, a gradual decrease from a value of 25.1% in the inoculum 1 to 9.2% in stage 4 for BF2-C

was observed (Fig. 7e). In BTF, the relative abundance of *Pseudomonas putida* increased from a value of 1.0% in the inoculum 2 to 14.1% and 7.7% in stage 6 in the bottom and top, respectively (Fig. 7f).

Discussion

Two inoculation procedures were compared on the basis of their influence on the performance and the microflora of two types of bioreactors (biofilter and biotrickling filter) in the removal of styrene from air emissions. A slow start-up was observed for the 2 biofilters packed with peat and coconut fibre and inoculated with an enriched culture of *Pseudomonas putida* CECT 324 (inoculum 1). The adaptation period lasted for 60 days (BF1-P) and 52 days (BF2-C) during which the styrene RE gradually increased from nearly zero removal up to 99% for BF1-P and 95% for BF2-C, respectively (Fig. 2a,b). Other authors have studied styrene removal by using biofilters inoculated with activated sludge and reported shorter adaptation periods. Arnold et al. (1997) only needed 12 days to achieve 80% RE with inlet concentrations ranging from 50 to 115 mg m^{-3} in a peat BF inoculated with enriched bacteria obtained from activated sludge of a petrochemical plant. Bina et al. (2004) observed that during a start-up period with inlet concentrations of 315 ± 51 mg Nm^{-3} and EBRT of 360 s, the RE attained 80% at day 6 in a yard waste compost BF inoculated with activated sludge from municipal WWTP. The slower adaptation period in our work cannot be related to the different inlet concentration, both authors obtained adaptation periods shorter than 2 weeks by using lower or similar inlet concentrations than those used in the present work (540 mg Nm^{-3} for days 1 to 20 and 350 mg Nm^{-3} for days 21 to 60; Fig. 2a,b). It can be inferred that in spite of using a strain of *Pseudomonas putida* cultivated during 30 days

with styrene as a sole carbon source, this inoculation strategy resulted in longer adaptation periods in comparison with using inocula from activated sludge coming from petrochemical or from municipal WWTP. This fact was corroborated by comparing results of BFs and the BTF. In the BTF, a start-up period as short as 7 days was observed by using activated sludge from municipal WWTP as inoculum. The shortness of the adaptation period in comparison with that obtained in the BFs could be associated to the high bacterial diversity of the activated sludge used as inoculum in BTF.

The results obtained in our study demonstrate that these 2 types of bioreactors have a good capacity to remove styrene gas emissions at the typical inlet concentrations of emissions from industries producing polystyrene. At inlet concentrations varying from 200 to 400 mg Nm^{-3}, REs between 70 and 95% were obtained in the 3 bioreactors (outlet emissions values ranged between 15 and 125 mg Nm^{-3}), demonstrating its suitability as an end-of-pipe control technique.

The comparison of the performance of the 2 biofilters indicates that BF1-P showed higher REs than those observed in BF2-C, even when the peat biofilter operated at half the value of EBRT than coconut biofilter. For example, at stage 3 for an IL of 22 g m^{-3} h^{-1}, BF1-P presented a RE of 95% with an EBRT of 45 s and BF2-C showed a RE of 80% with EBRT of 90 s. A maximum EC of 81 g m^{-3} h^{-1} (RE of 45%, Fig. 4a) was obtained in the peat BF at EBRTs of 60 s while a maximum EC of 39 g m^{-3} h^{-1} (RE of 45%, Fig. 4b) was achieved in the coconut fibre BF, but at a higher EBRT of 90 s. Juneson et al. (2001) reported ECs between 69 and 118 g m^{-3} h^{-1} (REs ranged from 65 to 75%) by using a BF packed with composted wood bark and yard waste working at EBRTs ranged between 30 and 60 s. Dehghanzadeh et al. (2005) observed maximum ECs of 39 and 27 g m^{-3} h^{-1} (REs of 63% and 43%, respectively) for EBRTs of 60 and 30 s, in a BF packed with yard waste compost mixed with shredded hard plastics. Dion St-

Pierre et al. (2009) obtained a maximum EC of 105 g m^{-3} h^{-1} (RE of 80%) at an EBRT of 55 s in a BF filled with inorganic media. The maximum EC obtained in the peat biofilter is included in the range reported by Juneson et al. (2001) and is slightly lower than the maximum EC observed by Dion St-Pierre et al. (2009). The coconut fibre biofilter, which had a lower maximum EC than the obtained in the peat one, presented a maximum EC similar as those reported by Dehghanzadeh et al. (2005), although in our study the biofilter with coconut media operated at higher EBRTs.

Elimination capacity of styrene differed for the 2 packing materials tested (peat and coconut fibre) due to their differences in chemical and physical properties. Peat has a higher specific surface area, 13.4 m^2 g^{-1}, and water holding capacity, 88%, than coconut fibre, 0.9 m^2 g^{-1} and 60%, respectively. Higher specific surface area is favourable for biofilm growth, allowing higher elimination capacities to be achieved. Although the peat biofilter achieved higher removal capacities than the coconut fibre biofilter, one of the main operational costs in a full scale bioreactor is the energy consumption in the blower related to the pressure drop in the bed. The main drawback of using peat media is the fast bed compaction problems which tended to lead to high pressure drops. In this study, compaction problems were detected during day 37, with an increase of 5-fold pressure drop by day 41 (from 98 to 490 Pa m^{-1}; Fig. 2c). Bed compaction in a laboratory-scale biofilter can be easily solved by opening the biofilter to decompress the bed (pressure drops decreased from 800 to 170 Pa m^{-1} on day 110), but this maintenance strategy is difficult to implement on industrial full scale biofilters. The industrial use of peat media would demand a careful control of water spraying and/or periodic turns to keep pressure drop controlled at low values. The coconut fibre did not show this compaction problem, pressure drop slightly increased with time, but values were kept <59 Pa m^{-1} for the whole experimental period. From the point of view

of energy consumption, coconut fibre showed lower consumption and a more reliable performance, making it more suitable for industrial application. However, due to the low water holding capacity of the coconut fibre, BF2-C demanded a high water addition (150 mL/day) to avoid excessive drying.

The BTF presented a maximum EC of 52 g m^{-3} h^{-1} (RE of 45%) working at an EBRT of 45 s (Fig. 4c). Novak et al. (2008) reported a maximum EC of 11.3 g C m^{-3} h^{-1} (RE of 61%) in a BTF packed with polypropylene Pall rings working with an EBRT of 35s. Sempere et al. (2011) obtained a maximum EC of 57.6 g m^{-3} h^{-1} (RE of 88.3%) at an EBRT of 60 s in a BTF packed with Flexiring. The maximum EC obtained for the BTF in this study is a 10% lower than the maximum value observed by Sempere et al. (2011), but the EBRT in this study is lower. In terms of styrene removal, the BTF of this study showed a similar performance compared to the peat biofilter and a better performance than coconut fibre biofilter. Regarding pressure drop, values in the BTF were always kept below 59 Pa m^{-1} (Fig 3b). In terms of energy consumption, the BTF presented similar values of pressure drop as the coconut fibre BF and substantially lower than the peat BF. Moreover, BTF units can be cost-effective built in 8-10 m heights due to the low density and structural strength of the plastic packing materials while the usual bed height in BF units ranges from 1 to 2 m. This enables the construction of BTF units with smaller footprints than BF units. In conclusion, BTFs appear to be the optimal option, combining factors of styrene removal capacity, energy consumption and requirements of footprint.

From the results regarding styrene removal, similar styrene removals were obtained by using both an enriched culture of the strain *Pseudomonas putida* CECT324 in a BF1-P and activated sludge from a WWTP in a BTF. Therefore, no advantage in using pure cultures for inoculation of bioreactors would be obtained. In addition, application of

activated sludge for start-up of bioreactors in full scale would be practical and economical instead of using pure or enriched consortium (Bina et al. 2004).

The influence on the microflora of the 2 inoculation procedures was analysed by DGGE and FISH. Although 3 bands were observed for the pure culture *Pseudomonas Putida* CECT324 in the DGGE gel, these multiple bands can be attributed to different alleles within the same strain. For example, the sequenced genome of the strain *Pseudomonas putida* KT2440 revealed two alleles differing by only one nucleotide in the region amplified by DGGE which produced two bands (Bodilis et al. 2012). These authors pointed out that many *Pseudomonas* strains had at least two different 16S rRNA alleles. Further, heteroduplexes have been observed in DGGE gels as potential PCR artifacts, thereby producing false positive bands for pure bacterial isolates. The banding patterns of DGGE for the enriched culture used for start-up of the BFs (inoculum 1) revealed that *Pseudomonas Putida* CECT324 was preserved after 30 days of cultivation under non-sterile aerated conditions, but other species appeared to have contaminated the culture (2 new dominant bands). This demonstrates that these other species could survive and grow in an environment with styrene as the sole carbon substrate. Although nutrient broth was used as the initial media for cultivation, only styrene was used as the carbon source during 30 days of cultivation. Among BFs samples, only one species from the inoculum survived in all samples, but the strain *Pseudomonas Putida* CECT324 was not detected in further sampling time points. DGGE fingerprints for BFs indicate that they were colonized by complex communities that changed over time. As Cabrol et al. (2011) points out, in the case of organic packing material (already colonized by endogenous microflora), both the inoculation and the operating conditions are sufficient to induce substantial modifications of the endogenous community structure. These authors indicated that emergence of species which were not detected in

the inoculum proceeded from (a) the inoculum wherein their abundance was below detection limit, or (b) the endogenous reservoir of the organic packing material, and/or (c) the polluted gaseous effluent. The bands appearing in only one of the BFs (F, G for BF1-P, and K, L for BF2-C, Fig. 6) could indicate origin from the packing material. The largest number of common bacterial populations in both BFs (bands D, H, I, J, M, Fig. 6), which occurred at the end of the experiment, could suggest that the origin of these species were in the inoculum or in the polluted gaseous effluent. The polluted gaseous effluent constituted itself a cross-inoculation mechanism between both BFs.

FISH results showed that the phylum *Proteobacteria* represented more than half of the total fluorescence obtained by the general eubacterial probes in every sample. It is worth noting that bacteria from the phylum *Proteobacteria* have previously been identified as the dominant phyla in BTFs degrading mixtures of VOCs including styrene (Lebrero et al. 2012; Li et al. 2012). *Gammaproteobacteria* was detected as a large fraction in all samples. In BFs, members of the other *Proteobacterial* classes (*Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria* and *Deltaproteobacteria*), *Firmicutes* and *Actinobacteria* showed variations of dominant phylogenetic groups over time between both BFs. This can be explained on the basis of the dynamic changes of the community structure related to the different packing material used in BF1-P and BF2-C and the variations in the operational conditions. Although FISH data showed a large percentage of *Pseudomonas putida* in both BFs (ranging between 8.1 to 24.8%), DGGE revealed that *Pseudomonas putida* CECT324 was not a dominant strian in the BFs since day 105 (after the acclimatisation period) till the end of the experiment. This suggests that other strains of *Pseudomonas putida* proliferated in the BFs instead of the pure culture strain chosen for the start-up. Surprinsingly by day 65, the BTF samples showed little differences in the relative

predominance of the *Proteobacterial* classes (*Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria* and *Deltaproteobacteria)*, *Firmicutes* and *Actinobacteria* with the activated sludge used as inoculum. The similarity in relative abundances of detected phylogenetic groups from the inoculum and samples from day 65 can be explained by the fact that the BTF was filled with plastic rings, which can be considered as a negligible source of endogenous microflora. This is a surprising result; large variations in the relative abundance of phylogenetic groups will be expected after 65 days of exposure to styrene. Although the activated sludge used as an inoculum of the BTF contains only 1% of *Pseudomonas putida* as detected by FISH, the percentage of this species increased over time, reaching values of 14.1% (bottom) and 9.8% (top). These values are within the range of the abundance of *Pseudomonas putida* found in BFs. This demonstrates that *Pseudomonas putida* can grow to substantial abundances in a styrene degrading environment, independently of the inoculum source and the packing material (organics or plastic).

Acknowledgements

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Table 1 Physical and chemical properties of packing materials.

	Peat	Coconut fibre
pH	4.8	6.8
Water-holding capacity, %	88.0	60.0
Bulk density, kg m^{-3}	133.0	60.0
Organic matter content, %	95.0	93.8
Specific surface area BET, m^2 g^{-1}	13.4	0.9
Chemical analysis:		
Carbon,%	48.5	47.8
Hydrogen, %	5.8	5.8
Oxygen, %	40.0	42.8
Nitrogen, %	0.6	0.1
Sulphur, %	0.1	0.0
	Flexiring rings	
Specific surface area, m^2 m^{-3}	207.0	
Void fraction, %	92.0	
Bulk density, Kg m^{-3}	71.0	
Ring diameter, mm	25.0	

Table 2 Operational conditions in both BFs and in BTF for the removal of styrene.

	Stages	**Start-up**	**1**	**2**	**3**	**4**
BF1 Peat	day	0-84	84-122	122-138	138-152	152-162
	Inlet conc., mg Nm^{-3}	300-790	335	750	275	560
	EBRT, s	90-120	60	60	45	45
	IL, g $m^{-3}h^{-1}$	12-24	22	45	22	45
BF2 Coconut fibre	day	0-84	84-114	114-129	129-142	142-162
	Inlet conc., mg Nm^{-3}	300-790	335	375	550	1125
	EBRT, s	90-120	60	90	90	90
	IL, g $m^{-3}h^{-1}$	12-24	22	15	22	45

	Stages	**Start-up**	**1**	**2**	**3**	**4**	**5**	**6**
BTF Flexiring rings	day	0-20	20-45	45-67	67-87	87-109	109-133	133-155
	Inlet conc., mg Nm^{-3}	200	300	375	468	350	475	180
	EBRT, s	60	60	60	60	45	45	30
	IL, g $m^{-3}h^{-1}$	12	18	22	28	28	38	22

Table 3 List of oligonucleotide probes used in this study

Probe	Specificity	Sequence (5'–3')	Formamide (%)
EUB338[a]	*Most Bacteria*	GCTGCCTCCCGTAGGAGT	0 – 50
EUB338-II[a]	*Other Bacteria not detected by EUB338*	GCAGCCACCCGTAGGTGT	0 – 50
EUB338-III[a]	*Other Bacteria not detected by EUB338*	GCTGCCACCCGTAGGTGT	0 – 50
ALF968	*α–Proteobacteria*	GGTAAGGTTCTGCGCGTT	20
BET42A	*β–Proteobacteria*	GCCTTCCCACTTCGTTT	35
-	*Competitor for BET42A*	GCCTTCCCACATCGTTT	35
GAM42A	*γ –Proteobacteria*	GCCTTCCCACATCGTTT	20
-	*Competitor for GAM42A*	GCCTTCCCACTTCGTTT	20
DELTA495A[b]	*δ–Proteobacteria*	AGTTAGCCGGTGCTTCCT	35
DELTA495B[b]	*δ–Proteobacteria*	AGTTAGCCGGCGCTTCCT	35
DELTA495C[b]	*δ–Proteobacteria*	AATTAGCCGGTGCTTCCT	35
-	*Competitor for DELTA495A*	AGTTAGCCGGTGCTTCTT	35
-	*Competitor for DELTA495B*	AGTTAGCCGGCGCTTCKT	35
-	*Competitor for DELTA495C*	AATTAGCCGGTGCTTCTT	35
HGC69A	*Actinobacteria (high G+C gram positive bacteria)*	TATAGTTACCACCGCCGT	25
-	*Competitor for HGC69A*	TATAGTTACGGCCGCCGT	25
LGC354A[c]	*Firmicutes (low G+C gram positive bacteria)*	TGGAAGATTCCCTACTGC	35
LGC354B[c]	*Firmicutes (low G+C gram positive bacteria)*	CGGAAGATTCCCTACTGC	35
LGC354C[c]	*Firmicutes (low G+C gram positive bacteria)*	CCGAAGATTCCCTACTGC	35
PS56a	*Pseudomonas sp.*	GCTGGCCTAGCCTTC	20
PPu56a	*Pseudomonas putida*	GCTGGCCTAACCTTC	20

[a] EUB338, EUB338-II, EUB338-III were used in the mixture called EUB$_{mix}$
[b] DELTA495A, DELTA495B, DELTA495C were used in the mixture called LGC$_{mix}$
[c] LGC354A, LGC354B, LGC354C were used in the mixture called LGC$_{mix}$

Fig.1 Schematic of experimental setup: **a)** BFs system **b)** BTF system
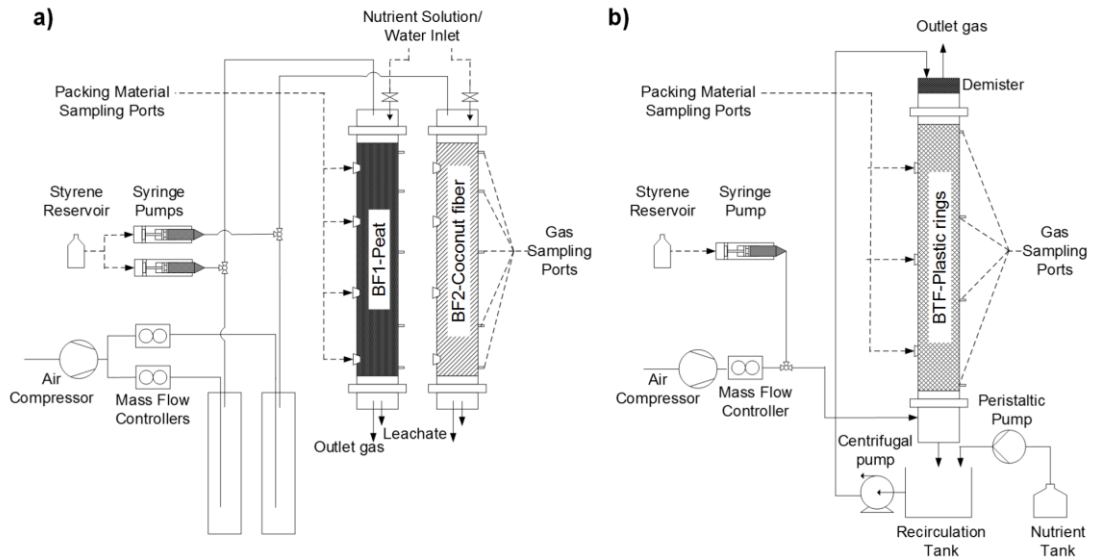
Fig.2 Evolution of the RE (◆), inlet concentration (◇) and outlet concentration (◆) in the BFs: **a)** peat BF (BF1-P) and **b)** coconut fibre BF (BF2-C). Evolution of pressure drop (◆) and moisture content in the top (◇) and in the bottom (◆) for the BFs: **c)** peat BF (BF1-P) and **d)** coconut fibre BF (BF2-C)
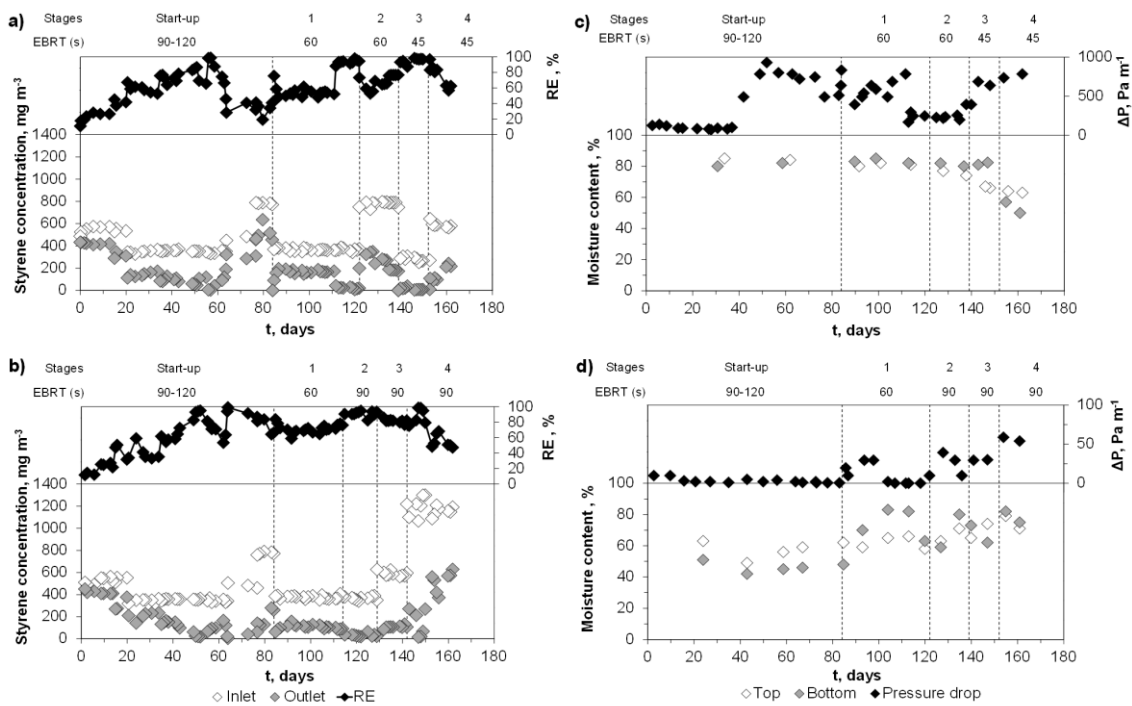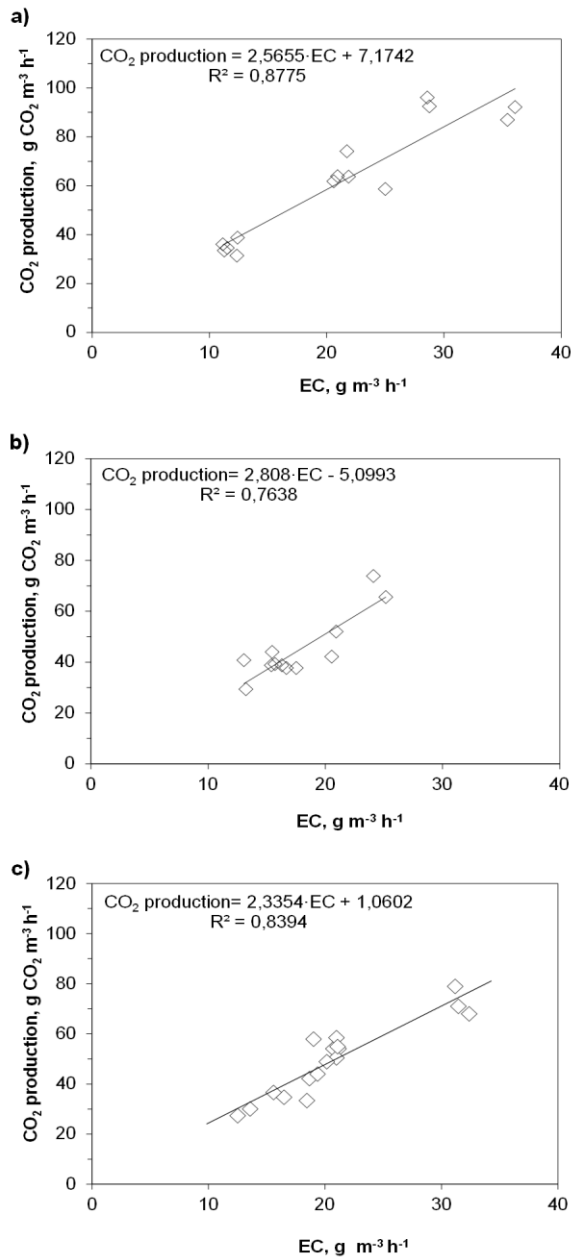
Fig.3 a) Evolution of the RE (◆), inlet concentration (◇) and outlet concentration (◆)

in the BTF. **b)** Evolution of pressure drop (◆) and pH (●)
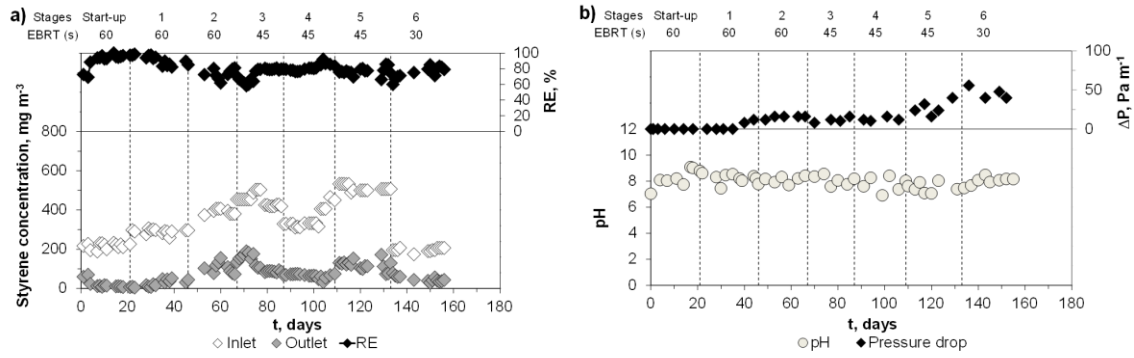
Fig.4 EC versus IL for different EBRT 30 s (✕), 45 s (▲), 60 s (◇) and 90 s (●): **a)** peat BF (BF1-P), **b)** coconut fibre BF (BF2-C) and **c)** BTF
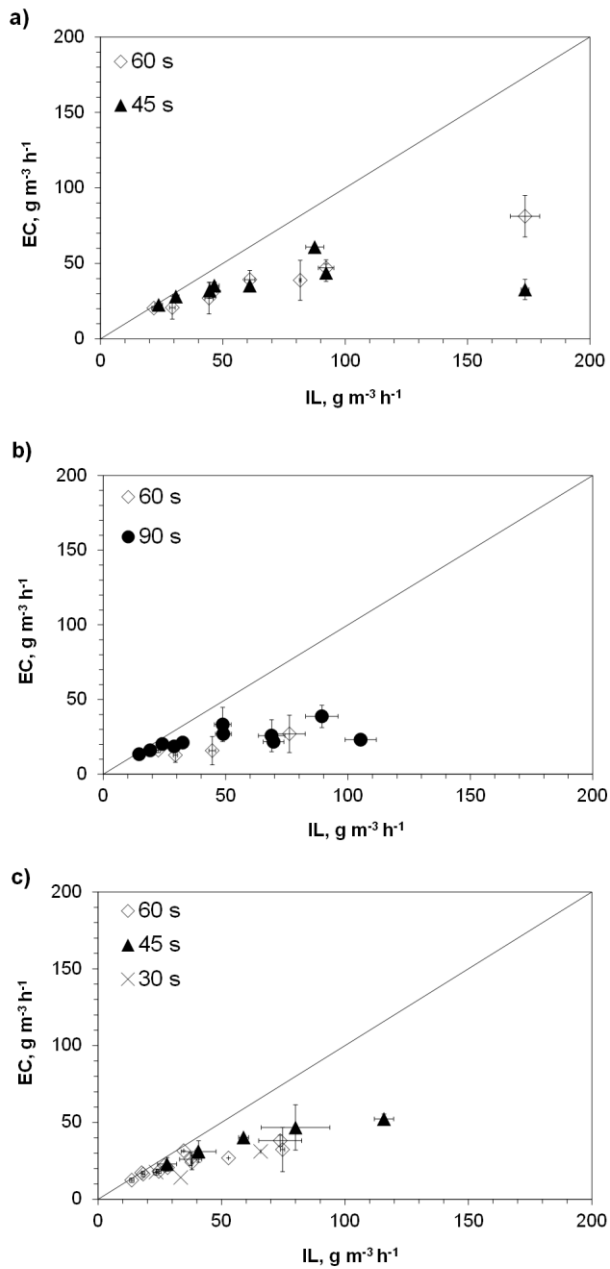
Fig.5 Production of carbon dioxide vs. EC obtained during experimental period: **a)** peat BF (BF1-P), **b)** coconut fibre BF (BF2-C) and **c)** BTF

a)

CO_2 production = $2,5655 \cdot EC + 7,1742$
$R^2 = 0,8775$

x-axis: EC, g m^{-3} h^{-1}
y-axis: CO_2 production, g CO_2 m^{-3} h^{-1}

b)

CO_2 production = $2,808 \cdot EC - 5,0993$
$R^2 = 0,7638$

x-axis: EC, g m^{-3} h^{-1}
y-axis: CO_2 production, g CO_2 m^{-3} h^{-1}

c)

CO_2 production = $2,3354 \cdot EC + 1,0602$
$R^2 = 0,8394$

x-axis: EC, g m^{-3} h^{-1}
y-axis: CO_2 production, g CO_2 m^{-3} h^{-1}

Fig.6 DGGE image of the *Pseudomonas putida* strain CECT 324, the enriched culture with *Pseudomonas putida* strain CECT 324 at day 0 (inoculum 1) and BFs samples on days 105, 142 and 156 of experimental period
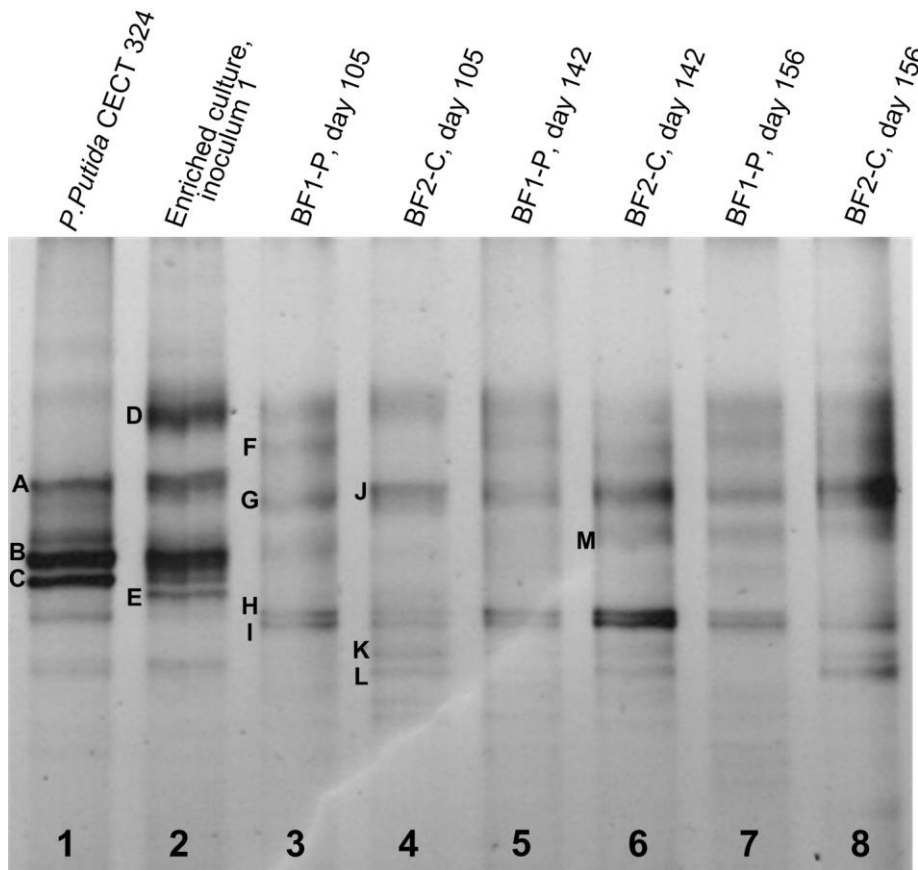
Fig.7 Time-dependent changes in bacterial community. Results corresponding to general FISH probes in: **a)** peat BF (BF1-P), **b)** coconut fibre biofilter (BF2-C) and **c)** BTF; and specific FISH probes in **d)** peat BF (BF1-P), **e)** coconut fibre biofilter (BF2-C) and **f)** BTF. Bars represent the upper and lower bounds of the error of the technique.