

**1 Removal of 2-butoxyethanol gaseous emissions by biotrickling filtration packed
2 with polyurethane foam**

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1 **Abstract**

2 The removal of 2-butoxyethanol from gaseous emissions was studied using two
3 biotrickling filters (BTF1 and BTF2) packed with polyurethane foam. Two different
4 inoculum sources were used: a pure culture of *Pseudomonas* sp. BOE200 (BTF1) and
5 activated sludge from a municipal wastewater treatment plant (BTF2). The bioreactors
6 were operated at inlet loads (ILs) of 130 and 195 g m⁻³ h⁻¹ and at an empty bed residence
7 time (EBRT) of 12.5 s. Under an IL of ~130 g m⁻³ h⁻¹, BTF1 presented higher
8 elimination capacities (ECs) than BTF2, with average values of 106 ± 7 and 68 ± 8 g m⁻³
9 h⁻¹, respectively. However, differences in ECs between BTFs were decreased by
10 reducing the irrigation intervals from 1 min every 12 min to 1 min every 2 h in BTF2.
11 Average values of EC were 111 ± 25 and 90 ± 7 g m⁻³ h⁻¹ for BTF1 and BTF2,
12 respectively, when working at an IL of ~195 g m⁻³ h⁻¹. Microbial analysis revealed a
13 significant shift in the microbial community of BTF1 inoculated with *Pseudomonas* sp.
14 BOE200. At the end of the experiment, the species *Microbacterium* sp.,
15 *Chryseobacterium* sp., *Acinetobacter* sp., *Pseudomonas* sp. and *Mycobacterium* sp.
16 were detected. In BTF2 inoculated with activated sludge, the denaturing gradient gel
17 electrophoresis (DGGE) technique showed a diverse microbial community including
18 species that was able to use 2-butoxyethanol as its carbon source, such as *Pseudomonas*
19 *aeruginosa* and *Pseudomonas putida* as representative species. Although BTF1
20 inoculated with *Pseudomonas* sp. BOE200 and higher gas velocity (probably greater
21 gas/liquid mass transfer rate) showed a slight improvement in performance, the use of
22 activated sludge as inoculum seems to be a more feasible option for the industrial
23 application of this technology.

24 **Keywords:** volatile organic compound (VOC), biotrickling filter, inoculation
25 procedures, polyurethane foam, *Pseudomonas* sp.

1 **Introduction**

2

3 2-Butoxyethanol is a volatile organic compound (VOC) of the glycol ether family that is
4 emitted into the atmosphere due to its use as solvent, mainly during surface coating and
5 cleaning activities. This compound is commonly used in industry based on its high
6 water solubility (Dimensionless Henry's constant (H) = $6.5 \cdot 10^{-5}$ at 25 °C) [1], chemical
7 stability and low costs [2]. Aside from its beneficial uses, the exposure to 2-
8 butoxyethanol can cause adverse effects, such as irritation of the nose and eyes,
9 headache, vomiting, dyspnoea, hypotension, declining levels of haemoglobin,
10 haematuria and metabolic acidosis [3]. In addition, VOCs are of significant
11 environmental concern since they are involved in the tropospheric ozone formation.
12 These facts have led to reinforcement of environmental regulations in Europe
13 (2010/75/EU) [4], and thus, treatment technologies are required.

14 The removal of VOCs from waste air emissions through biological processes
15 provides a cost-effective and environmentally friendly alternative to conventional
16 treatment methods [5]. Biological processes utilise microbial metabolic reactions for
17 cleaning of contaminated air, converting the organic pollutants mainly to carbon
18 dioxide, water and biomass. In the case of biotrickling filters (BTFs), which involve a
19 trickling liquid for the nutrient supply and the pH control, the microorganisms are
20 attached on the surface of an inert packing material. Among the synthetic materials that
21 have been tested as packing materials in BTFs, polyurethane foam is one of the
22 purposed materials [6,7] since it offers high mechanical strength, resistant to attack from
23 organic solvents and microbes, easy handling, good regeneration ability, and especially
24 very low cost [8].

1 The effectiveness of the BTF processes in the reduction of VOC emissions has
2 been widely demonstrated from the laboratory up to an industrial scale [9–11].
3 However, the removal of 2-butoxyethanol using biotechnologies has not yet been
4 reported. Several studies have proven the successful application of the biofiltration
5 process for other compounds with high solubility in water and characterised by low
6 Henry's constants ($H < 0.01$). For example in the use of biotrickling filters, Morotti et
7 al. [12] obtained a maximum ethanol elimination capacity (EC) of $46 \text{ g m}^{-3} \text{ h}^{-1}$; San-
8 Valero et al. [13] observed a maximum isopropanol EC of $51 \text{ g C m}^{-3} \text{ h}^{-1}$; and Popov et
9 al. [14] reported average removal efficiency (RE) of 89% treating industrial emissions
10 from a flexographic printing facility.

11 The start-up procedure of bioreactors can be carried out by use of different
12 inoculum sources. From an economic point of view, the use of activated sludge from
13 wastewater treatment plants (WWTP) [15,16] is preferred due to advantages, including
14 ease of implementation and lower operational costs. The use of pure cultures as
15 inoculum sources is also applicable [17,18] due to shorter start-up periods and the
16 prevention of emissions of potentially pathogenic germs. Comparative studies in the
17 performance of bioreactors using different inoculum sources are still scarce [19, 20]. In
18 our previous research, two inoculation procedures were compared regarding the
19 removal of styrene in two types of bioreactors by using an enriched culture of the strain
20 *Pseudomonas putida* CECT 324 and activated sludge [21]. In this study, working at an
21 EBRT of 60 s and an IL of $75 \text{ g m}^{-3} \text{ h}^{-1}$, the bioreactors presented similar EC values of
22 $\sim 40 \text{ g m}^{-3} \text{ h}^{-1}$.

23 Extensive efforts have been made to optimise the BTF process from a design and
24 operational perspective. However, biological information about the structure and
25 dynamics of their microbial communities are still required for a better understanding of

1 the relationship between microbial diversity and the performance of the bioreactor.
2 Biological molecular tools, such as fluorescence in situ hybridisation (FISH) [22,23],
3 polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE)
4 [24,25], cloning and sequencing [26,27] and pyrosequencing [28,29], have been applied
5 in the field of biofiltration. For example, Álvarez-Hornos et al. [30] evaluated the
6 dynamics of the microbial population using the FISH technique in a BTF pilot unit for
7 the treatment of exhaust gases of a plastic coating facility. The pilot unit was inoculated
8 with activated sludge and changes in the bacterial community were observed. The
9 *Betaproteobacteria* group was the most abundant group detected in the inoculum source
10 (relative abundance of 20%). However, after 3 months of operation, the abundance of
11 this group dropped to $8.9 \pm 3.0\%$. Wan et al. [31] analysed the bacterial community by
12 using denaturing gradient gel electrophoresis (DGGE) in a BTF adapted with the
13 commercial available microbial community B350 to remove trimethylamine (TMA)
14 from waste air. Although the bacterial community was clearly sensitive to the TMA,
15 more than 21 initial species were detected in the BTF.

16 The aim of the present study was to investigate the process performance of two
17 BTFs packed with polyurethane foam using different inoculation procedures and
18 microbial community structures in the removal of gaseous emissions of 2-
19 butoxyethanol. For this purpose, the following objectives have been taken into
20 consideration: (1) To evaluate the performance in terms of EC and RE of the two BTFs
21 working both at the same value of EBRT, 12.5 s, and equal ILs: 130 and $195 \text{ g m}^{-3} \text{ h}^{-1}$.
22 The two bioreactors were packed by using the same packing material: polyurethane
23 foam with 10 pores per inch (PPI) and operated in different laboratories. (2) To analyse
24 the influence of two inoculum sources on the performance of the process: a pure culture
25 of the strain *Pseudomonas* sp. BOE200 and an activated sludge from a municipal

1 WWTP were used. The shift in the microbial community was analysed by PCR-DGGE,
2 sequencing of 16S rRNA and by plating methods. To the best of our knowledge, this is
3 the first study regarding 2-butoxyethanol biodegradation from gaseous emissions.

4

5 **Materials and Methods**

6

7 BTF set-ups and operational conditions

8

9 The first experiment, with a bioreactor named BTF1, was carried out in the Department
10 of Biological Waste Air Purification of the University of Stuttgart (Germany) by using a
11 pure culture of the strain *Pseudomonas* sp. BOE200 as the inoculum source. The second
12 experiment, with a bioreactor named BTF2, was carried out in the Department of
13 Chemical Engineering of the University of Valencia (Spain) by using an activated
14 sludge from a municipal WWTP as the inoculum source. Both bioreactors were packed
15 with polyurethane foam with 10 PPI (BTF1: EMW filtertechnik, Germany; BTF2:
16 Modisprem, Spain). The packing material presents a specific surface of $400 \text{ m}^2 \text{ m}^{-3}$, a
17 void fraction of 96% and a bulk density of 23 kg m^{-3} .

18 The schematic of the set-up for both biotrickling filters is shown in Fig. 1. A
19 summary of their main dimensions and characteristics is detailed in Table 1. BTF1 was
20 built using a cylindrical PVC module with a total bed length of 100 cm and an internal
21 diameter of 15 cm. The bioreactor was equipped with two sampling ports (gas and
22 biomass) located at 0 cm (inlet port) and 100 cm (outlet port). The 2-butoxyethanol was
23 initially dosed and evaporated in an air flow of $0.4 \text{ m}^3 \text{ h}^{-1}$ by a diaphragm metering
24 pump (STEPDOS® 03, KNF, Switzerland) and then remixed with an air flow of a
25 compressor resulting in a total volume flow of $5.0 \text{ m}^3 \text{ h}^{-1}$. A 15-L recirculation tank,
26 partially renewed every week, was used to feed the recirculation solution into the

1 bioreactor in counter-current mode with respect to the air flow using a diaphragm
2 metering pump (Vario HM15-PP, ProMinent GmbH, Germany) at 3.3 L min^{-1} with a
3 frequency of 5 s every 1 min. In the case of the pH adjustment of the recirculation
4 solution, a sodium hydroxide solution (NaOH 1 M) was used. A commercial fertiliser
5 solution (7% N, 3% P_2O_5 , 5% K_2O ; CMI, Germany) was supplied to the recirculation
6 tank (50 mL per week).

7 After ending the operation of BTF1, another experiment operating the other
8 bioreactor (BTF2) was performed using a different inoculum source. As can be
9 observed in Table 1, the design of this reactor was planned to mimic as much as
10 possible the relative dimensions and hydraulic conditions of BTF1, although there is a
11 difference in the gas velocity between both systems (289 m h^{-1} in BTF1 and 208 m h^{-1}
12 in BTF2). BTF2 was built using a cylindrical PVC module with a total bed length of 70
13 cm and an internal diameter of 10.5 cm. The bioreactor was equipped with two gas-
14 sampling ports [0 cm (inlet port) and 70 cm (outlet port)] and two biomass-sampling
15 ports [30 cm (bottom port) and 70 cm (top port)]. The air stream was contaminated
16 using a syringe pump (New Era, infusion/withdraw NE 1000 model, USA) and fed to
17 the bioreactor through the bottom of the column with a gas flow rate of $1.8 \text{ m}^3 \text{ h}^{-1}$. A 5-
18 L recirculation tank, partially renewed every week, was used to feed the recirculation
19 solution through the top of the bioreactor using a diaphragm metering pump (Sigma/2,
20 ProMinent Gugal S.A, Spain) at 1.6 L min^{-1} . Two spraying frequencies were used: a) 1
21 min every 12 min, and b) 1 min every 2 h. For pH adjustment of the recirculation
22 solution, a sodium hydroxide solution (NaOH 0.1 M) was used. A nutrient solution
23 buffered at pH 8 was also supplied to the recirculation tank (20 mL per day) containing
24 (g L^{-1}): NH_4Cl , 9.7; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.9; $(\text{NH}_4)_2\text{HPO}_4$, 2.2; NaHCO_3 , 0.5; NaOH, 0.4 g;

1 KCl, 0.6; yeast extract 0.01; and Ca, Fe, Zn, Co, Mn, Na, Ni, B, I, Se, Cr, Cu and
2 vitamins at trace doses.

3 The operational period of each BTF was 100 days using the same two 2-
4 butoxyethanol loads (130 and 195 g m⁻³ h⁻¹) working at an equal EBRT of 12.5 s.
5 Operational conditions applied to the BTFs are presented in Table 2. Both systems were
6 operated at ambient temperatures ranged from 20 to 24 °C. In the first 41 days (stage 1),
7 an EBRT of 12.5 s and an inlet concentration of 450 mg Nm⁻³ were applied. Afterwards,
8 in stage 2, the inlet concentration was raised until to 680 mg Nm⁻³ at the same EBRT.
9 These inlet concentrations were selected to cover the typical values of emissions coming
10 from industrial sites using 2-butoxyethanol. The weekly purge was set in both systems
11 at 20% of the volume of the recirculation solution. The solvent removal with the purge
12 represented less than 4% of the amount of 2-butoxyethanol that was fed during the
13 whole week. Therefore, the organic carbon removed with the purge was considered
14 negligible for the evaluation of the BTF performance in terms of EC and RE.

15

16 Inoculation source

17

18 The inoculation of BTF1 was performed with 1 L of a pure culture of the strain
19 *Pseudomonas* sp. BOE200, which was formerly isolated by Woiski from an industrial-
20 scale bioscrubber [32]. The bacterial strain was cultivated in 1 L of liquid mineral
21 medium (MM) containing (g L⁻¹): Na₂HPO₄·2H₂O, 3.50; KH₂PO₄, 1.00; (NH₄)₂SO₄,
22 1.00; MgSO₄·7H₂O, 0.20; Ca(NO₃)₂·4H₂O, 0.05g; C₆H₈O₇·FeH₄N, 0.01; trace minerals
23 solution, 1 mL [consisting of (g L⁻¹): H₃BO₃, 0.30; CoCl₂·6H₂O, 0.20;
24 ZnSO₄·7H₂O, 0.10; NaMoO₄·2H₂O, 0.03; MnCl₂·4H₂O, 0.03; NiCl₂·6H₂O, 0.02;
25 CuCl₂·2H₂O, 0.01] and 2-butoxyethanol as the carbon source. The pH of the MM was

1 maintained at 7.1. Incubation took place in 3-L conical flasks placed on a rotatory
2 shaker (150 rpm) at 30 °C and with 7.5 mM 2-butoxyethanol. The culture was fed again
3 at day 5 with ~67% of the initial VOC loading. After 7 days, 1 L of the pure culture (4.3
4 g L⁻¹ of SS and 84% of VSS) was used as the inoculum of BTF1.

5 The inoculation of BTF2 was performed with 1 L of activated sludge (2.8 g L⁻¹
6 of SS and 81% of VSS) from a municipal WWTP located in Paterna (Valencia, Spain),
7 without any previous adaptation of the sludge to the pollutant.

8

9 Analytical methods

10

11 The concentration of 2-butoxyethanol was measured using a flame ionization detector
12 (BTF1: Multi-FID 100, Hartmann & Braun, Germany; BTF2: Nira Mercury 901 total
13 hydrocarbon analyzer Spirax Sarco, Spain). Adequate time intervals of at least 4 h were
14 used for the generation of average values of the outlet concentrations. The pressure
15 losses of both bioreactors (BTF1: AMS 4711-0050 model, AMSYS, Germany; BTF2:
16 MP101 model, KIMO, Spain) and the pH (BTF1: pH Checker, HANNA, Germany;
17 BTF2: pH/Cond 340i, WTW, Germany) of the recirculation solution were monitored at
18 least twice per week. The concentrations of nitrate, ammonium and phosphate were
19 determined weekly either by use of colorimetric kits according to the Standard Methods
20 for Examination of Water and Wastewater [33] in the case of BTF1 or by using an ionic
21 chromatograph (Ionic Chromatograph 883 Basic IC Plus) in the case of BTF2. The
22 suspended solids (SS) and volatile suspended solids (VSS) of the inoculum sources
23 were measured according to the Standard Methods for Examination of Water and
24 Wastewater [33].

25

26 Microbial community analysis in BTF1

1
2 The analysis of the microbial community of BTF1 was carried out by plating methods
3 on day 100, corresponding to the end of the experiment. Sample of biofilm (2 mL)
4 developed on the packing material was taken out of the sampling port at the top of the
5 column, as well as 2 mL liquid sample from the recirculation tank. These samples were
6 scattered either on MM plates with 2-butoxyethanol (each MM plate was composed by
7 liquid mineral medium and 15 μ L of 2-butoxyethanol), in order to evaluate the use of 2-
8 butoxyethanol as carbon source by strains, or on nutrient broth plates (NB) as non-
9 selective media. The composition of NB plates (g L^{-1}) was: beef extract, 3.0; peptone,
10 5.0; agar, 16.0. The pH of the NB was adjusted to 7.0. Incubation took place at 30 °C
11 for 1 week. Single colonies grown under these conditions were transferred to new plates
12 for subsequent DNA isolation and sequencing of 16S rRNA.

13 DNA isolation was performed by mechanical extraction using 0.1 mm zirconia-
14 silica beads for five cycles of 60 s of extraction time in a Bead-Beater (B. Braun
15 Biotech International, Melsungen, Germany) with intercylic cooling of the samples on
16 ice. The supernatant was transferred to a new tube and was stored at -20 °C until
17 analysis. 16S rRNA genes were amplified by PCR using the bacteria-specific primer
18 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and the universal primer 1492R (5'-
19 ACCTTGTTACGACTT-3'). PCRs were performed in a thermal cycler (Bio-Rad
20 Laboratories GmbH, Munich) with a 50- μ L reaction volume containing final
21 concentrations of 1 unit of Taq DNA polymerase, 0.25 mM dNTPs, 2.5 mM Mg^{2+} and 1
22 μ M of each primer (Biomaster GmbH, Windeck, Germany). PCR conditions consisted
23 of 30 cycles of: 94 °C for 0.5 min, 58.5 °C for 1 min, and followed by 72 °C for 1 min.
24 The PCR reactions followed with 10 cycles of: 94 °C for 1 min, 55 °C for 1 min and 72
25 °C for 3 min. A final extension at 72 °C for 8 min was undertaken as the final step. The

1 amplicon was purified using the GenElute™ PCR Clean-Up Kit (Sigma-Aldrich
2 Chemie GmbH, Taufkirchen, Germany) with pure water as solvent, and the products
3 were sequenced by GATC Biotech AG (Köln, Germany). The alignment editing was
4 implemented using MEGA version 6.06 software and the results were compared with
5 those available from the NCBI GenBank database using BLAST software.

6

7 Microbial community analysis in BTF2

8

9 The evolution of the microbial population in BTF2 was analysed by DGGE on days 0
10 (inoculum), 40, 60, 80 and 100. Samples of biofilm (2 mL) developed on the packing
11 material were taken out of the sampling ports at 30 cm (bottom port) and 70 cm (top
12 port) of the column, as well as 2 mL liquid samples from the recirculation tank. In
13 addition, the identification of strains able to use 2-butoxyethanol as carbon source was
14 examined on day 100, corresponding to the end of the experiment, by the combination
15 of plating methods using solid MM, the DGGE analysis of samples from the MM plates
16 and the subsequent sequencing of the predominant DGGE bands.

17 DNA was extracted with the PowerSoil® DNA Isolation Kit (MO BIO
18 Laboratories, USA) using the manufacturer's protocol. The isolated DNA was stored at
19 -20 °C until analysis. 16S rRNA genes were amplified by PCR using the two universal
20 primers F357GC (5'-
21 CGCCCGCCGCGCGCGGGCGGGCGGGGCGGGGGCACGGGGGGCCTACGGGAG
22 GCAGCAG-3') and R518 (5'-ATTACCGCGGCTGCTGG-3'). PCRs were performed
23 in a thermal cycler (LongGene Scientific Instruments, Hangzhou) with a 50-µL reaction
24 volume of a mixture containing final concentrations of 1.25 units of Taq DNA
25 polymerase, 0.2 mM dNTPs, 2 mM Mg²⁺ and 0.5 µM of each primer (EuroClone, Italy).

1 PCR conditions consisted of 20 cycles of: 94 °C for 1 min, 65 °C for 1 min, a
2 touchdown annealing step of 0.5 °C increments from 65 °C to 55 °C for 1 min, followed
3 by 72 °C for 3 min. Subsequent PCR reactions followed with 10 cycles of: 94 °C for 1
4 min, 55 °C for 1 min and 72 °C for 3 min. A final extension at 72 °C for 7 min was
5 undertaken as the final step. For DGGE analysis, 10–20 µL of PCR product generated
6 from each sample were separated on an 8% acrylamide gel running in a linear
7 denaturing gradient (30%–50%) using a KuroGel Verti 2020 DGGE System (VWR
8 International Eurolab S.L., Spain). The gel was run at 60 °C for 5 min at 50 V, 120 min
9 at 150 V and 60 min at 200 V. The DGGE gel was visualised in the MiniBIS Pro system
10 (DNR Bio-Imaging System Ltd., Spain).

11 Predominant DGGE bands were excised in a UV-transilluminator (ECX-20M,
12 Vilber Lourmat, Spain) with a sterile sharp scalpel. Bands were resuspended in
13 microcentrifuge tubes with 30 µL of sterilised Mili-Q water, and stored at 4 °C
14 overnight. After centrifugation, the supernatant was used as the template for PCR
15 amplification of the 16S rRNA genes. PCR was performed using the primers F357GC
16 and R518 in a 50-µL reaction volume containing 1 unit of Taq DNA polymerase, 0.25
17 mM dNTPs, 2.5 mM Mg²⁺ and 0.25 µM of each primer (Integrated DNA Technologies,
18 Spain). The PCR used the same protocol described previously for the DGGE. The PCR
19 product was purified with the High Pure PCR Product Purification Kit (Roche,
20 Barcelona, Spain) and, then, was sequenced by using an automated DNA analyser (3730
21 KL DNA analyzer, Applied Biosystems, Spain). The alignment editing was
22 implemented using MEGA version 6.06 software and the results were compared with
23 those available from the NCBI GenBank database using BLAST software.

24

25 **Results and Discussion**

1

2 BTF performance

3

4 The performance of both BTFs treating 2-butoxyethanol is shown in Fig. 2 from day 20
5 to 100. The inlet and outlet concentrations during the process time, as well as the RE,
6 are presented in Fig. 2a (BTF1) and in Fig. 2b (BTF2). The inoculation of each
7 bioreactor was conducted at day 0 by using 1 L of the inoculum source (pure culture of
8 strain *Pseudomonas* sp. BOE200 in BTF1 and activated sludge in BTF2). From day 0 to
9 41 (stage 1), the bioreactors were operated at a low EBRT of 12.5 s and average inlet
10 concentrations of 450 ± 10 and 474 ± 12 mg Nm⁻³ (IL ~ 130 g m⁻³ h⁻¹) in BTF1 and
11 BTF2, respectively. From day 20 onwards, the RE in BTF1 was kept at values $>79\%$
12 (average outlet concentration of 77 ± 18 mg Nm⁻³, Fig. 2a) whereas the RE in BTF2
13 fluctuated around $54.1 \pm 6.7\%$ (average outlet concentration of 218 ± 35 mg Nm⁻³, Fig.
14 2b).

15 After 41 days of operation the IL was increased to ~ 195 g m⁻³ h⁻¹ at constant
16 EBRT of 12.5 s, corresponding to average inlet concentrations of 699 ± 78 and $742 \pm$
17 12 mg Nm⁻³ in BTF1 and BTF2, respectively. The response of BTF1 to this change was
18 a progressive decrease in the RE, reaching 35% on day 67 and subsequently oscillating
19 around $55.2 \pm 9.3\%$ until the end of the experiment, corresponding with an average
20 outlet concentration of 313 ± 70 mg Nm⁻³. In BTF2, a progressive deterioration of the
21 RE was observed, reaching a value of 21.5% on day 69. At that moment, it was decided
22 to decrease the spraying frequency by a factor of 10 (from 1 min every 12 min to 1 min
23 every 2 h) in order to evaluate the influence of the spraying frequency on the
24 performance of the bioreactor. Working with the new spraying frequency, the RE

1 suddenly increased to 50% on day 71 and was stable at $47.1 \pm 3.9\%$ afterwards until the
2 end of the experiment (average outlet concentration of $393 \pm 32 \text{ mg Nm}^{-3}$).

3 The effect of the spraying frequency on the outlet concentration in BTF2 is
4 presented in Fig. 3 where representative examples of 8-h emission time intervals for
5 both spraying frequency are plotted. Fig. 3a shows an example of outlet emissions on
6 day 53 at high spraying frequency, while Fig. 3b shows emissions on day 80 under low
7 spraying conditions. Both figures directly show the correlation between spraying
8 conditions and high outlet concentrations due to a high loading capacity of 2-
9 butoxyethanol in the aqueous phase and subsequent desorption of the VOC out of the
10 liquid phase. Thus, the decrease in the trickling conditions directly causes a reduction in
11 the number of outlet emission peaks and thus average outlet concentration. As a
12 consequence, 8 h-average values dropped by 35 % from 502 to 325 mg Nm^{-3} . The outlet
13 concentration decreases from the concentration of the peak (550 mg Nm^{-3}) to 250 mg
14 Nm^{-3} during low spraying conditions (Fig. 3b), while the outlet concentration slightly
15 declined to 450 mg Nm^{-3} under high spraying conditions (Fig. 3a). The reduction of
16 average daily emission levels via reduced spraying frequencies was previously reported
17 by San-Valero et al. [13] in a BTF treating isopropanol emissions, a solvent with high
18 solubility in water (Dimensionless $H = 3.3 \cdot 10^{-4}$ at 25 °C) [1]. The authors obtained a
19 decrease in the outlet concentration from 86 to 59 mg C Nm^{-3} when the spraying
20 frequency was changed from 15 min every 1.5 h to 15 min every 3 h. In this study, the
21 decrease in the spraying frequency of BTF2 resulted in 2-butoxyethanol removal that
22 was slightly lower than that obtained in BTF1 ($55.2 \pm 9.3\%$ in BTF1 and $47.1 \pm 3.9\%$
23 in BTF2). Although the spraying frequency was reduced by tenfold, no drying of the
24 packing material was observed. Thus, the results presented here indicate that an

1 optimization of the spraying frequency for the removal of compounds with high water
2 solubility is a key parameter to achieve low emissions.

3 The monitoring of additional parameters of both BTFs is summarised in Table 3.
4 The pH value was kept at normal values (between 7 and 8). Nitrate, ammonium and
5 phosphate in the recirculation tank were kept at appropriate concentrations to make sure
6 that nutrients were not limiting the bioprocesses. The pressure drop was maintained at
7 low values ($<5 \text{ Pa m}^{-1}$) in both reactors during the 100 days of operation. In fact,
8 biomass clogging problems were not observed over this operational period.

9

10 Relationship between EC and IL

11

12 The relationship between IL and EC, calculated for the whole volume of each
13 bioreactor, is presented in Fig. 4 based on the experimental data of the last three
14 operational weeks. In the case of BTF1, an average EC of $106 \pm 7 \text{ g m}^{-3} \text{ h}^{-1}$ (RE of 82.6
15 $\pm 4.5\%$) was obtained for the lower IL $\sim 130 \text{ g m}^{-3} \text{ h}^{-1}$ (stage 1). When the IL was
16 increased to $\sim 195 \text{ g m}^{-3} \text{ h}^{-1}$ (stage 2), the EC level was practically maintained without
17 differences with an average value of $111 \pm 25 \text{ g m}^{-3} \text{ h}^{-1}$ (RE of $55.4 \pm 10.1 \%$), although
18 a maximum EC value of $154 \text{ g m}^{-3} \text{ h}^{-1}$ (RE of 65.0%) for an IL of $237 \text{ g m}^{-3} \text{ h}^{-1}$ was
19 observed. In the case of BTF2, due to the high influence of the spraying frequency on
20 the performance, the EC was increased from $68 \pm 8 \text{ g m}^{-3} \text{ h}^{-1}$ (RE of $54.1 \pm 6.0\%$) for
21 the IL of $\sim 130 \text{ g m}^{-3} \text{ h}^{-1}$ to $90 \pm 7 \text{ g m}^{-3} \text{ h}^{-1}$ (RE of $45.4 \pm 3.6\%$) for the IL of $\sim 195 \text{ g m}^{-3}$
22 h^{-1} . The comparison of the performance of both bioreactors, pointed out that the BTF1
23 showed slightly better removal efficiencies than the BTF2. This fact could be associated
24 mainly to two factors: (i) the use of a greater gas velocity (289 m h^{-1} in BTF1 and 208 m
25 h^{-1} in BTF2) that can enhance the gas/liquid mass transfer and (ii) the use of a pure

1 culture of a 2-butoxyethanol degrader instead of activated sludge without previous
2 adaptation. Therefore, the system that presented better characteristics in terms of mass
3 transfer and biological degradation showed relatively small improvement in
4 performance, so that, activated sludge is preferable due to its ease of implementation
5 and the reduction of operational costs in future industrial applications.

6 Woiski [32] identified 2-butoxyacetic acid, n-butanol and butanoic acid as
7 central intermediates of the biodegradation of 2-butoxyethanol by *Pseudomonas* sp.
8 BOE200. However, no data related to the biodegradation of 2-butoxyethanol in a
9 bioreactor for treatment of waste air have been previously published in the literature. An
10 attempt to compare the data of this work with those previously reported for other
11 compounds with high water solubility has been carried out. For example, Pielech-
12 Przybylska et al. [17] investigated the biodegradation of acetone in a trickle-bed biofilter
13 inoculated with two strains: *Pseudomonas cepacia* and *Acinetobacter baumannii*. A
14 maximum EC of $96 \text{ g m}^{-3} \text{ h}^{-1}$ (RE of 99%) was achieved at an IL of $97 \text{ g m}^{-3} \text{ h}^{-1}$ and an
15 EBRT of 75 s. Chang and Lu [34] investigated a biotrickling filter for the removal of
16 isopropanol and acetone mixtures inoculated with activated sludge. Working at an IL of
17 $80 \text{ g m}^{-3} \text{ h}^{-1}$ and an EBRT of 20 s, ECs between 60 and 80 (RE > 75%) were achieved.
18 San-Valero et al. [13] studied a BTF with activated sludge as inoculum for the removal
19 of isopropanol, and achieved an EC $\sim 40 \text{ g C m}^{-3} \text{ h}^{-1}$ (RE $\sim 60\%$) under an IL of 65 g C
20 $\text{m}^{-3} \text{ h}^{-1}$ and an EBRT of 14 s.

21

22 Isolation and identification of the strains in BTF1

23

24 Plating methods were applied in BTF1 in order to identify strains present in the
25 bioreactor at the end of the experiment (day 100). The MM was used as selective media

1 in order to isolate strains able to use 2-butoxyethanol as carbon source, and NB was
2 used as non-selective media to identify further strains. From the MM, a unique strain
3 with 100% sequence similarity to *Pseudomonas* sp. B1_64 was isolated. This result
4 shows the presence of species belongs to *Pseudomonas* sp. genus after more than 3
5 months of operation. Previous studies have pointed out that *Pseudomonas* is one of the
6 typical bacterial species found in biofilms from bioreactors treating VOC emissions
7 [35,36].

8 Six different strains were isolated in NB medium and their nucleotide sequences
9 were determined. The analysis indicated that these strains have 99–100% sequence
10 similarity with *Microbacterium* sp. 111H3b, *Chryseobacterium* sp. CHKOV-5M,
11 *Acinetobacter* sp. Ooi24, *Pseudomonas* sp. B1_64, *Sphingobacterium* sp. P031 and
12 *Mycobacterium* sp. SWH-M4. Table 4 shows the accession numbers, similarities to
13 related GenBank sequences and the phylum and class for each strain. The presence of at
14 least six species after 100 days of operation indicates the evolution of a complex
15 microbial community consisting of primary degraders of 2-butoxyethanol, such as
16 *Pseudomonas* sp., and other non-biodegrading bacteria in the biofilm during VOC
17 degradation. The presence of these other species can be explained by the non-sterile
18 operational conditions in the bioreactor. In addition, there are microorganisms
19 observable in nature that cannot be cultured using conventional techniques [37];
20 therefore, the plating method could underestimate the diversity of the microbial
21 community since other species can be present in the biofilm making more complex the
22 population.

23 In fact, microbial communities in technical ecosystems can adapt and change
24 their composition in accordance with variable physicochemical operational conditions
25 [28]. The bacterial diversity observed in the biofiltration studies may be explained by

1 the resource availability and by the large numbers of saprophytic microorganisms
2 dominating the bioreactor and consuming cellular products or extrapolymeric
3 substances [38].

4

5 DGGE profile, sequencing and analysis of 16S ribosomal DNA in BTF2

6

7 DGGE was applied in BTF2 in order to monitor microbial population evolution. Fig. 5a
8 shows the DGGE banding patterns of samples from days 0 (inoculum), 40, 60 and 80.

9 The spatial distribution of the bacterial community along the reactor was studied by
10 including biofilm samples from the bottom port (30 cm) and the top port (70 cm).

11 Additionally, samples from the recirculation tank were also analysed in parallel. Fig. 5b

12 shows the DGGE profile for the samples taken at the end of the experiment (day 100)

13 from: the top port of the bioreactor, a MM plate cultivated with a sample from the
14 recirculation solution and a MM plate cultivated with a sample from the top port. The

15 samples from the MM plates allowed the identification of bands that matched with

16 species able to use 2-butoxyethanol. In addition, in Fig. 5 the Shannon diversity indices

17 for samples of inoculum, biofilm and recirculation tank have been also indicated. This

18 diversity index was calculated taking into account both the number of DGGE bands and

19 their relative intensity [39].

20 The predominant bands of the native samples (named with numbers in Fig. 5a)

21 of the DGGE profile from day 40 to 80 were excised and sequenced. DGGE band

22 designation, accession numbers, similarities to related GenBank sequences and the

23 phylum and class of each strain are summarised in Table 5. The corresponding

24 sequences exhibited a similarity of >96% with: *Pseudomonas aeruginosa* (band 1),

25 *Pseudomonas putida* (band 2), *Sphingobacterium mizutaii* (band 3), *Lactobacillus*

1 *brantae* (band 4), *Lactobacillus curvatus* (band 5), *Pedobacter koreensis* (band 6),
2 *Marivirga tractuosa* (band 7), *Rubrobacter naiadicus* (band 8) and *Acidovorax avenae*
3 (band 9).

4 The dominant bands of the samples from the MM plates on day 100 were also
5 excised and sequenced (named with numbers in Fig. 5b). Table 6 summarises the
6 DGGE band designation, accession numbers and the phylum and class of each strain.
7 These bands belong to species that present the ability to use 2-butoxyethanol as carbon
8 source. The corresponding sequences of bands exhibited a high level of similarity
9 (100%) with: *Pseudomonas aeruginosa* strain SNP0614 (band 10), *Alcaligenes faecalis*
10 strain NBRC 13111(band 11), *Pseudomonas putida* F1 strain F1 (band 12), *Alcaligenes*
11 *aquatilis* strain LMG 22996 (band 13), *Bacillus flexus* strain NBRC 15715 (band 14)
12 and *Fictibacillus phosphorivorans* strain Ca7 (band 15).

13 The DGGE profile of BTF2 revealed patterns in the composition of the bacterial
14 community. Several bands that appear in the inoculum (activated sludge) disappeared
15 on day 40, while other bands, such as band 6, were conserved during the whole
16 experiment. This change in the microbial community is corroborated by the decrease in
17 the Shannon index from 2.31 (inoculum, day 0) to 2.21 (biofilm, day 40), indicating a
18 slight deterioration of the microbial diversity. In contrast, the majority of bands
19 appearing from days 40 to 100, such as bands 1, 2, 4, 5 and 8, were not found in the
20 original inoculum. As a consequence, the bacterial community from days 40 to 100 was
21 composed of species that were either dominant in the original inoculum or of low
22 relevance. The appearance of new bands caused an increase in the microbial diversity in
23 the bioreactor with Shannon index values, from day 60 onwards, ranging from 2.4 to
24 2.5. Several authors have previously described the divergence between the original
25 inoculum and the microbial community developed in a technical system after an

1 acclimatisation period [40,41] and the deterioration of the microbial diversity during
2 this period [42]. The bacterial classes, including the species involved in the removal of
3 2-butoxyethanol from day 40, such as *Gammaproteobacteria*, *Actinobacteria* or
4 *Sphingobacteria*, have been found in bacterial communities previously analysed for the
5 treatment of VOCs emissions [23,43–45].

6 As can be seen in Fig. 5a, two bands (band 1 and 2) detected in BTF2 match
7 species able to use 2-butoxyethanol as carbon source (band 10 and 12). Bands 1 and 10
8 belong to the species *Pseudomonas aeruginosa* and bands 2 and 12 to the species
9 *Pseudomonas putida*. The bands 1 and 2, associated with the *Pseudomonas* species,
10 appeared in the BTF on day 40 despite not being detected in the inoculum source. The
11 capability of *Pseudomonas* species to degrade VOCs and to emerge in bioreactors has
12 been demonstrated previously, even though *Pseudomonas* sp. was not used as an
13 inoculum of the bioreactors [21,46,47]. The results suggest that *Pseudomonas* species
14 can easily proliferate in a 2-butoxyethanol-degrading environment, independently of
15 whether they were found in the inoculum or not.

16 Regarding the operational changes carried out in the bioreactor, the increase in
17 the IL on day 42 (from 130 to 195 g m⁻³ h⁻¹) caused slight changes in the DGGE profile
18 between days 40 and 60, within this period the Shannon varied from a value around 2.2
19 to ~2.4, indicating the increase on the microbial diversity. As can be seen in Fig. 5a
20 bands 4 and 5 tended to disappear after day 40, but new bands (bands 3 and 7) appeared
21 on day 60. Bands 1 and 2 corresponding to *Pseudomonas* species, presented the highest
22 intensity at day 60. Although the bacterial community showed smooth shifts during the
23 days 40 to 60, with Shannon indices maintaining practically in values around 2.4, no
24 significant changes in the performance of the bioreactor were observed. Interestingly,
25 the change in the spraying frequency on day 69 had no effect on the DGGE profiles

1 between days 60 and 80. Thus, the improvement in the performance of the bioreactor
2 from day 69 onwards was related to the physical phenomena involved in the decrease of
3 the spraying frequency in the case of VOCs with high water solubility, such as 2-
4 butoxyethanol.

5 The analysis of the DGGE profile shows a similar banding pattern along the
6 filter bed and the recirculation tank, thus indicating a homogeneous bacterial
7 composition caused by the recirculation solution. This fact has previously been
8 observed [48]. In contrast, conventional biofilters usually present a stratification of
9 bacterial communities along the filter height [37,38] due to the use of a low flow rate of
10 nutrient solution, which is usually sprayed infrequently (e.g. once per day), among other
11 factors. Regarding Shannon diversity indices in the BTF samples (Fig. 5), the lower
12 values calculated for the recirculation tank in comparison with those obtained in the
13 biofilm during the whole experimental period indicates lower microbial diversity. This
14 different diversity could be explained by the fact that inside the reactor is produced a
15 better transfer of oxygen and/or substrate to the biofilm, so that, more quantity of
16 substrate and oxygen may be accessible causing a greater growth of different
17 microorganisms.

18 This study shows that two different microbial communities developed from two
19 different inoculum sources were able to remove similar quantities of 2-butoxyethanol,
20 even with a system with slightly better gas/liquid mass transfer rate, demonstrating the
21 irrelevance of the origin of the inoculum. Therefore, the use of activated sludge without
22 prior acclimation (the most simple inoculation procedure) is an advantageous strategy
23 for industrial applications.

24

25 **Conclusions**

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With the exception of Woiski [32], the aerobic biodegradation of 2-butoxyethanol by biotrickling filtration has not been reported previously. Two different BTFs worked at an equal EBRT of 12.5 s, and same inlet loads (130 and 195 g m⁻³ h⁻¹). The systems were started with different inoculum sources, a pure culture of *Pseudomonas* sp. BOE200 (BTF1) as a 2-butoxyethanol-degrading strain and activated sludge (BTF2), and operated for 100 days. Despite using different inoculum sources and BTF1 presented a greater gas velocity (probably resulting in a better gas/liquid mass transfer rate), similar average EC values were achieved with values of 111 and 90 g m⁻³ h⁻¹ for BTF1 and BTF2, respectively, at an IL of 195 g m⁻³ h⁻¹.

The outcomes of the microbial analysis in each bioreactor indicated that a complex bacterial community was developed throughout the experimental time and it was different from that observed in the inoculum sources. In the case of BTF1, different strains were identified after 100 days with *Pseudomonas* sp. as the only 2-butoxyethanol degrading-strain. In the case of BTF2, 2-butoxyethanol-degrading strains, such as *Pseudomonas putida* and *Pseudomonas aeruginosa*, were observed from day 40 onwards, despite the fact that these species were not identified as predominant in the inoculum source, confirming the large degradation potential of *Pseudomonas* species.

Acknowledgements

Authors gratefully acknowledge the financial support by Ministerio de Economía y Competitividad (Project CTM2014-54517-R) and Generalitat Valenciana (PROMETEO/2013/053), Spain. M.C. Pérez acknowledges the Ministerio de

1 Educación, Cultura y Deporte, Spain for her FPU contract (AP2009-2645). We would
2 like to thank Dr. Kevin Portune for his assistance in the microbiological tests at the
3 University of Valencia. Finally, we would also like to thank the staff of the Department
4 of Biological Waste Air Purification of the University of Stuttgart, especially Christine
5 Woiski for pre-isolation of the strain *Pseudomonas* sp. BOE200 and for allowing us to
6 use this strain.

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1 **Table 1** Main dimensions and characteristics of the two BTFs.

	BTF1	BTF2
Bed length, cm	100	70
Diameter, cm	15	10.5
Ratio length to diameter	6.7	6.7
Recirculation tank volume, L	15	5
Ratio tank volume to reactor volume	0.8	0.8
Air flow rate, m ³ h ⁻¹	5	1.8
Recirculation flow rate, L min ⁻¹	3.3	1.6
Spraying frequency	5 s every 1 min	1 min every 12 min 1 min every 2 h
Gas velocity, m h ⁻¹	289	208
Liquid velocity, m h ⁻¹	11	11
EBRT, s	12.5	12.5

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1 **Table 2** Operational conditions of BTFs.

Stages	1	2
Day	0–41	42–100
Inlet concentration, mg Nm ⁻³	450	680
EBRT, s	12.5	12.5
IL, g m ⁻³ h ⁻¹	130	195

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1 **Table 3** Parameters of trickling water in BTFs

	BTF1				BTF2			
	Stage 1		Stage 2		Stage 1		Stage 2	
	Aver.	SD	Aver.	SD	Aver.	SD	Aver.	SD
pH	7.9	0.3	7.6	0.3	7.1	0.3	7.2	0.4
N-NO ₃ , mg L ⁻¹	0.5	0.2	7.3	4.7	16.7	5.5	10.3	7.9
N-NH ₄ , mg L ⁻¹	26.7	11.3	45.9	27.6	60.2	20.1	99.5	23.1
P-PO ₄ , mg L ⁻¹	30.3	12.2	51.7	30.0	66.7	48.3	43.9	15.1
Pressure drop, Pa m ⁻¹	4.1	3.6	4.8	2.2	4.5	7.1	4.7	8.2

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1 **Table 4** Accession numbers in GenBank, levels of similarity and corresponding
 2 phylogenetic groups of the strains identified and isolated by plating methods in BTF1.

Closest organism in GenBank (accession No.)	Similarity (%)^a	Phylogenetic group^b
<i>Microbacterium</i> sp. 111H3b (KJ744028)	99	Actinobacteria/ Actinobacteridae
<i>Chryseobacterium</i> sp. CHKOV-5M (KF499317)	100	Bacteroidetes/ Flavobacteria
<i>Acinetobacter</i> sp. Ooi24 (AB933637)	100	Proteobacteria/ Gammaproteobacteria
<i>Pseudomonas</i> sp. B1_64 (KC306412)	100	Proteobacteria/ Gammaproteobacteria
<i>Sphingobacterium</i> sp. P031 (KC252768)	99	Bacteroidetes/ Sphingobacteria
<i>Mycobacterium</i> sp. SWH-M4 (KJ729254)	100	Actinobacteria/ Actinobacteridae

3 ^aSequences were matched with the closest relative from the GenBank database.

4 ^bPhylum/class.

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1 **Table 5** DGGE band designation, accession numbers in GenBank and levels of
 2 similarity to related organisms according to Fig. 5a.

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DGGE band	Closest organism in GenBank (accession No.)	Similarity (%) ^a	Phylogenetic group ^b
1	<i>Pseudomonas aeruginosa</i> strain SNP0614 (NR_118644.1)	100	Proteobacteria/ Gammaproteobacteria
2	<i>Pseudomonas putida</i> F1 strain F1 (NR_074739.1)	100	Proteobacteria/ Gammaproteobacteria
3	<i>Sphingobacterium mizutaii</i> strain NBRC 14946 (NR_113705.1)	99	Bacteroidetes/ Sphingobacteria
4	<i>Lactobacillus brantae</i> strain SL1108 (NR_125575.1)	100	Firmicutes/ Bacilli
5	<i>Lactobacillus curvatus</i> (DQ336384.1)	100	Firmicutes/ Bacilli
6	<i>Pedobacter koreensis</i> strain NBRC 101153 (NR_113980.1)	100	Bacteroidetes/ Sphingobacteria
7	<i>Marivirga tractuosa</i> strain DSM 4126 (NR_074493.1)	95	Bacteroidetes/ Cytophagia
8	<i>Rubrobacter naiadicus</i> strain RG-3 (NR_125704.1)	96	Actinobacteria/ Rubrobacteridae
9	<i>Acidovorax avenae</i> strain ATCC 19860 (NR_102856.1)	100	Proteobacteria; Betaproteobacteria

4 ^aSequences were matched with the closest relative from the GenBank database.

5 ^bPhylum/class.

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1 **Table 6** DGGE band designation, accession numbers in GenBank and levels of
 2 similarity to related organisms according to Fig. 5b.

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DGGE band	Closest organism in GenBank (accession No.)	Similarity (%) ^a	Phylogenetic group ^b
10	<i>Pseudomonas aeruginosa</i> strain SNP0614 (NR_118644.1)	100	Proteobacteria/ Gammaproteobacteria
11	<i>Alcaligenes faecalis</i> strain NBRC 13111 (NR_113606.1)	100	Proteobacteria/ Betaproteobacteria
12	<i>Pseudomonas putida</i> F1 strain F1 (NR_074739.1)	100	Proteobacteria/ Gammaproteobacteria
13	<i>Alcaligenes aquatilis</i> strain LMG 22996 (NR_104977.1)	100	Proteobacteria/ Betaproteobacteria
14	<i>Bacillus flexus</i> strain NBRC 15715 (NR_113800.1)	100	Firmicutes/ Bacilli
15	<i>Fictibacillus phosphorivorans</i> strain Ca7 (NR_118455.1)	100	Firmicutes/ Bacilli

4 ^aSequences were matched with the closest relative from the GenBank database.

5 ^bPhylum/class.

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1 **Figure captions**

2 **Fig. 1** Schematic BTFs set-up.

3 **Fig.2** Evolution of the RE (■), inlet concentration (○) and outlet concentration (●) in
4 the BTFs: **a)** BTF1 and **b)** BTF2.

5 **Fig. 3** Influence of the spraying frequency on the outlet pattern emission: **a)** spraying
6 for 1 min every 12 min (day 53); **b)** spraying for 1 min every 2 h (day 80).

7 **Fig. 4** EC versus IL in BTF1 and BTF2. Stage 1 BTF1 (○); stage 2 BTF1 (□); stage 1
8 BTF2 (●); stage 2 BTF2 (■).

9 **Fig. 5** DGGE profiles from samples of the BTF2 including their Shannon diversity
10 indices. **a)** Samples at days 40, 60 and 80 from the recirculation solution, bottom and
11 top port, **b)** Samples at day 100 from the top port, a MM plate cultivated with a sample
12 from the recirculation solution and a MM plate cultivated with a sample from the top
13 port.

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Figure1

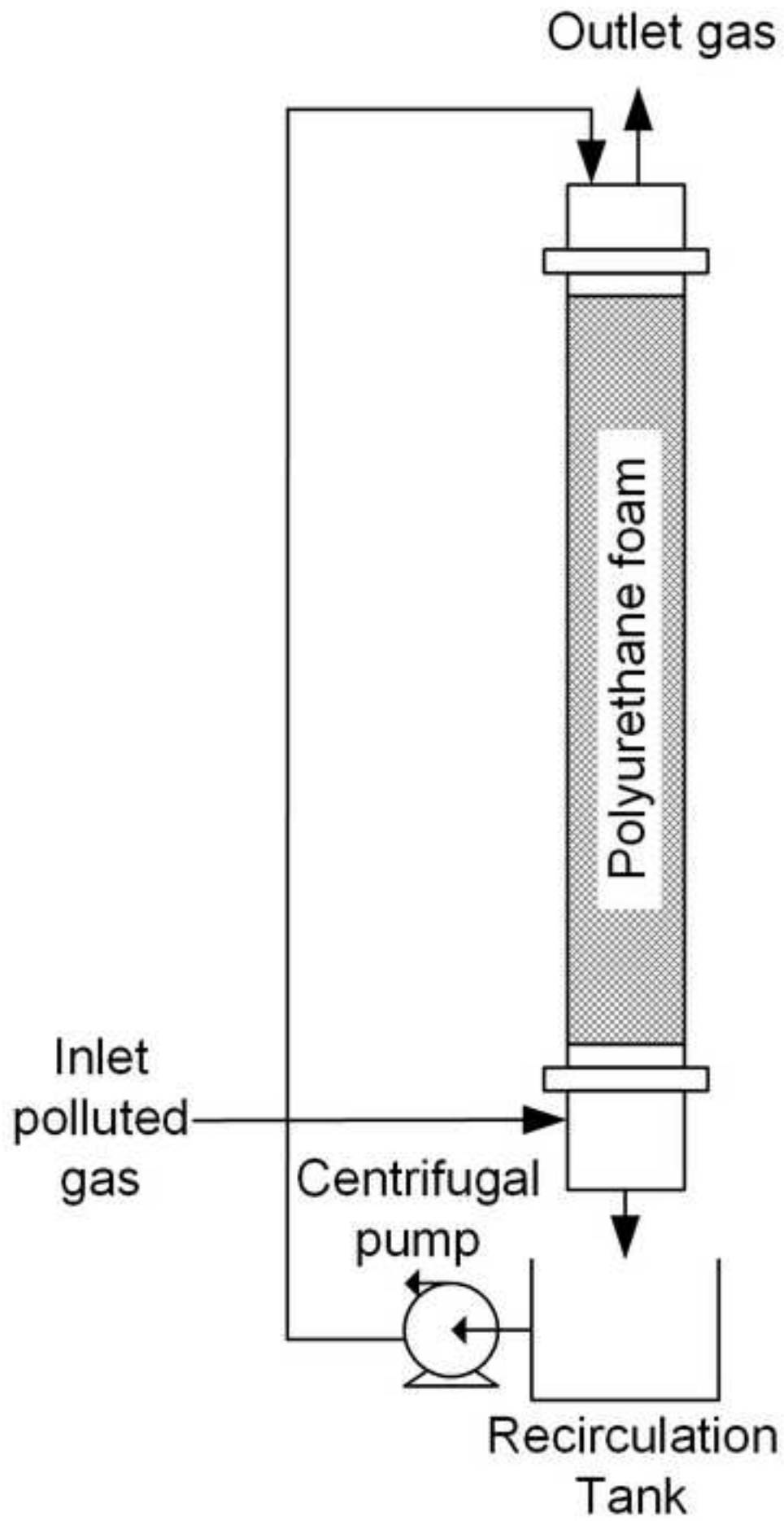
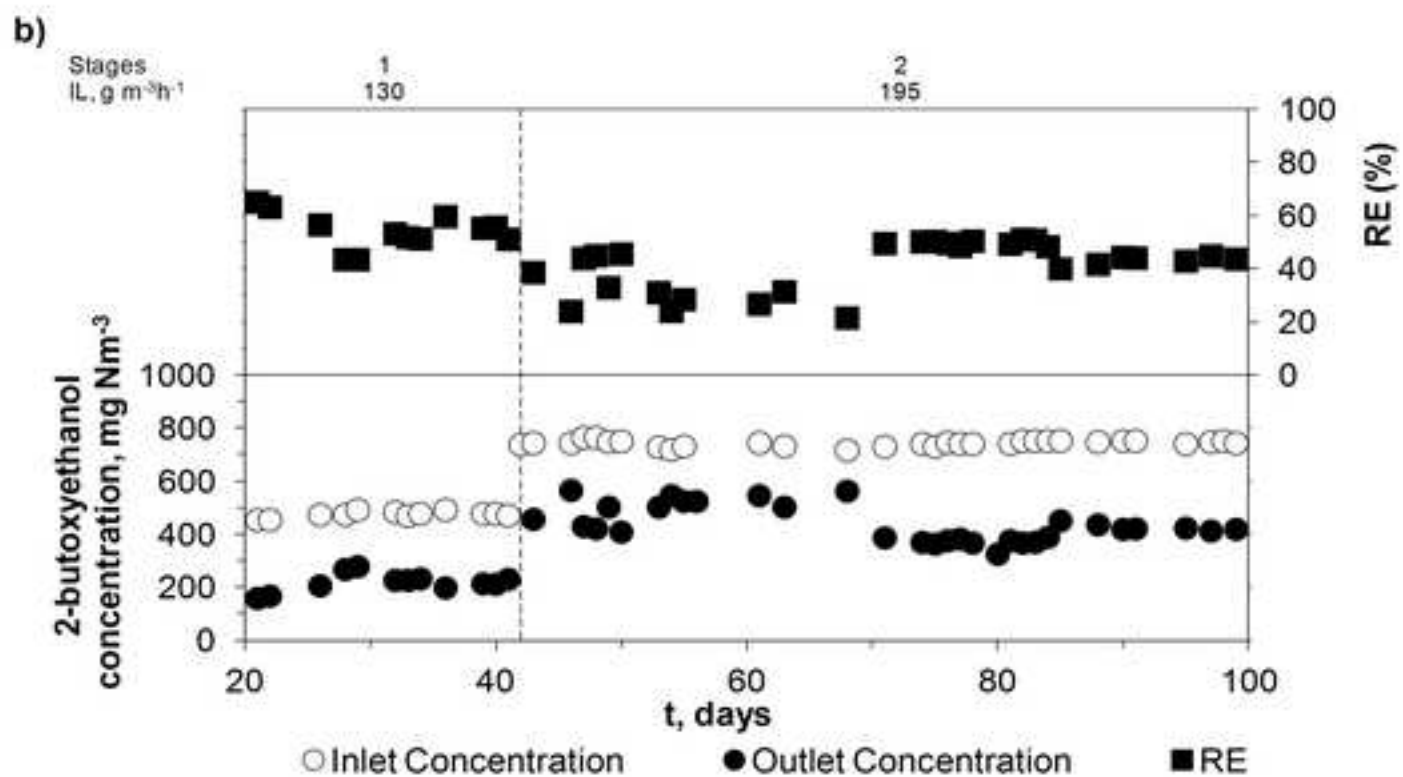
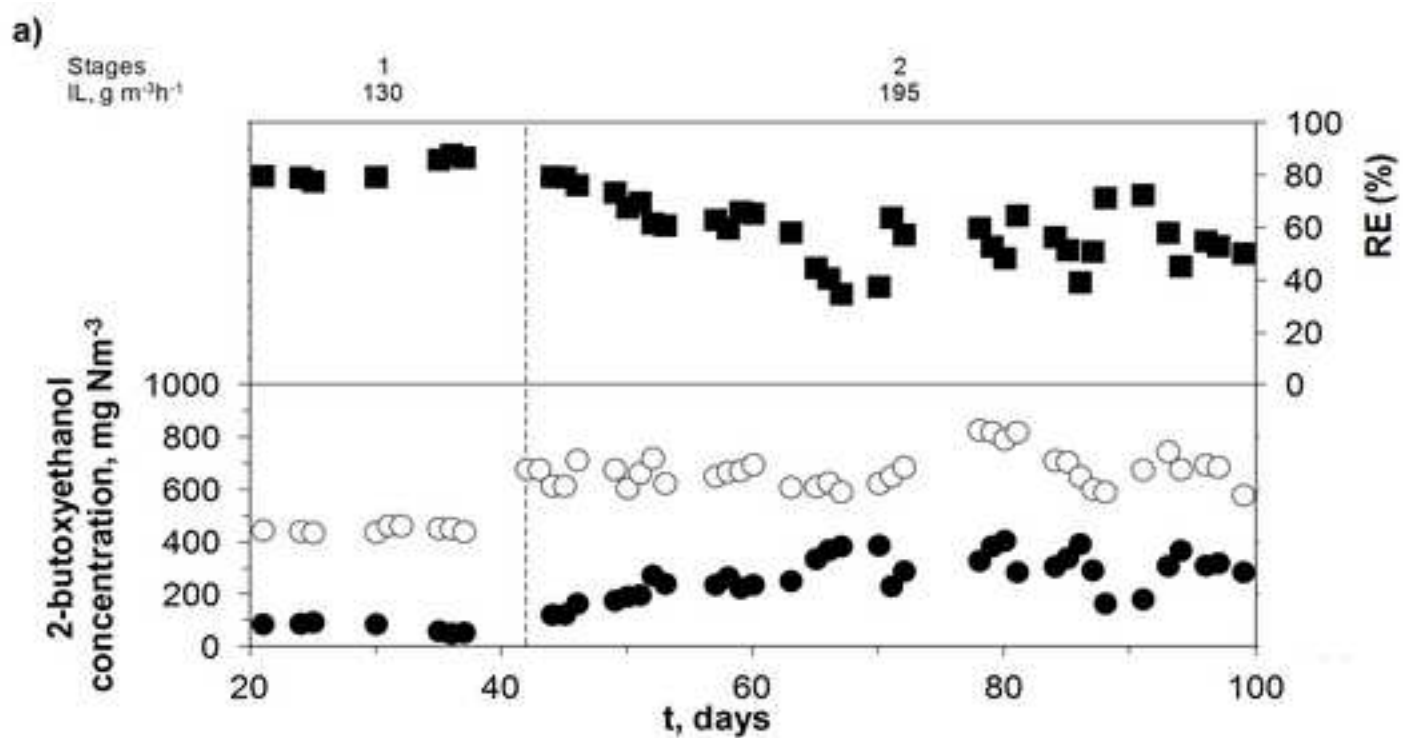
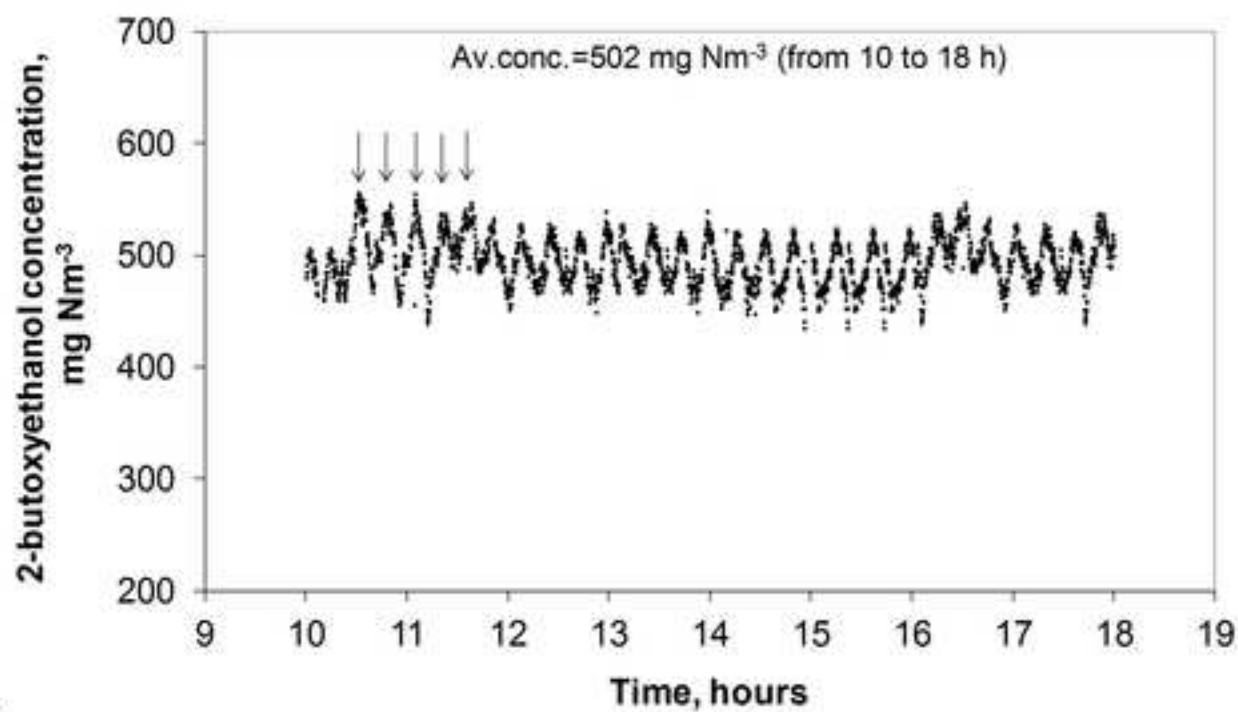


Figure2



a)



b)

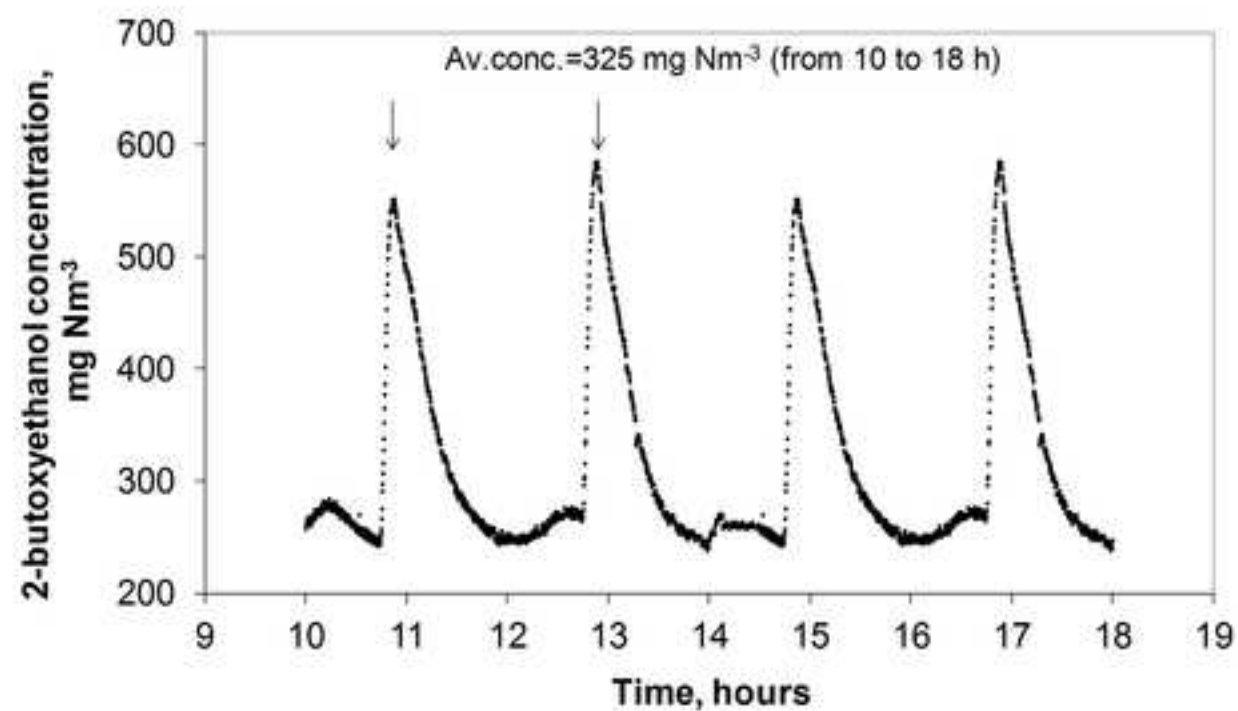


Figure4

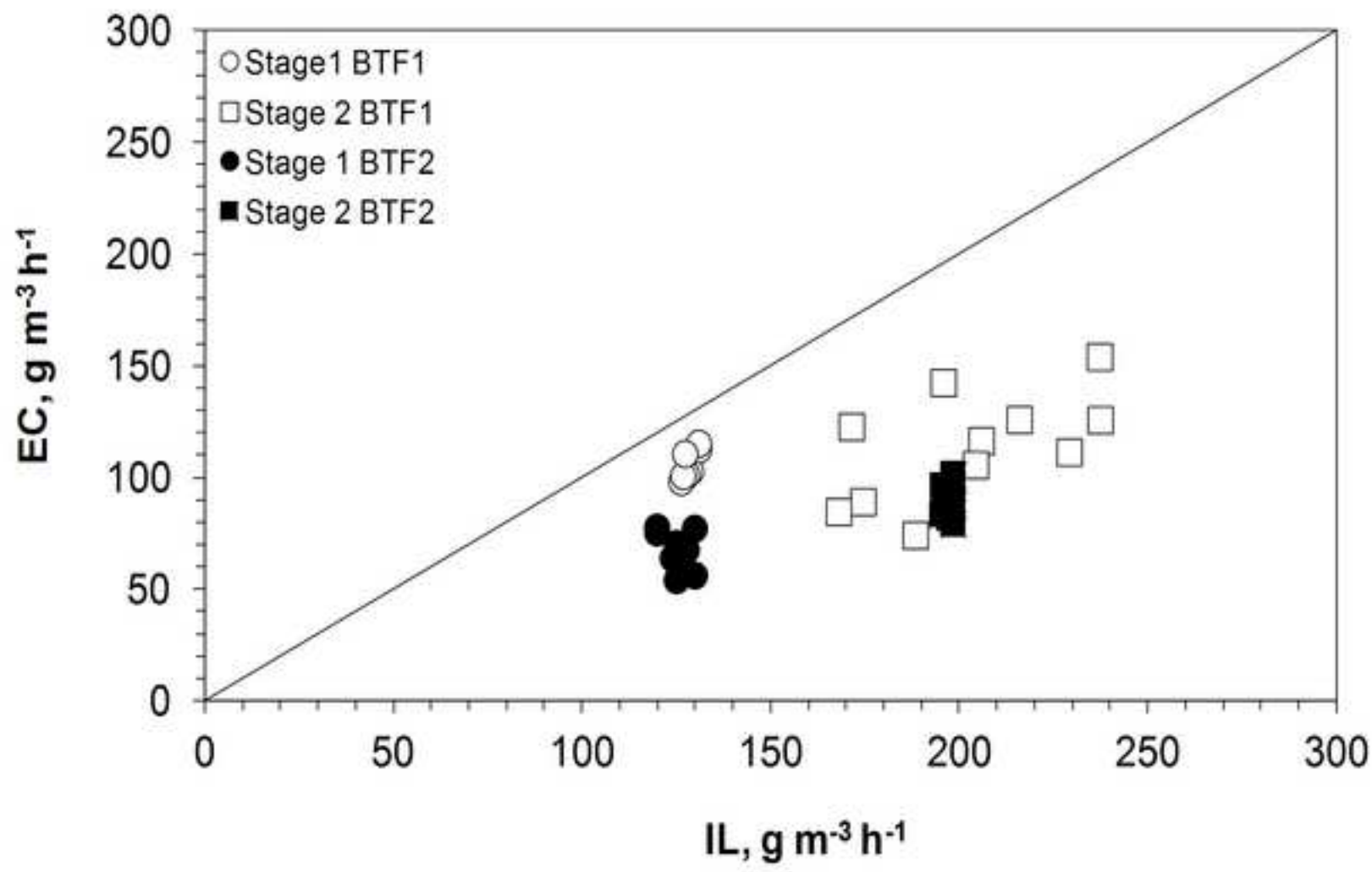


Figure5

