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Hexane biodegradation in two-liquid phase bioreactors: High-performance operation based on the use of hydrophobic biomass

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ABSTRACT

An innovative operation mode in two-liquid phase bioreactors (TLPB) for the treatment of volatile organic compounds (VOC) was investigated. This mode was based on confining the biocatalytic activity exclusively in the non-aqueous phase (NAP) by using hydrophobic microorganisms. The TLPB was implemented in a 2.5 L stirred tank reactor using 10% (v/v) of silicone oil as NAP and hexane as model VOC. A stable elimination capacity (EC) of $21.0 \pm 2.5\ g\ m^{-3}\ h^{-1}$ (corresponding to a removal efficiency of 80%) was recorded for 26 days. The accumulation of inhibitory metabolites resulted in drastic drops in the elimination capacity (EC) and an unstable performance of the system, hexanol being identified as potential inhibitory metabolite. Aqueous culture broth exchange by fresh mineral salt medium at a dilution rate of $0.2\ day^{-1}$ allowed maintaining a high and sustained VOC removal performance. Dissolved oxygen concentration measurements revealed that the oxidative metabolism was strongly stimulated by the aqueous broth exchange. The temporary blockage of the gas/water/NAP transfer pathway for O_2 highlighted the paramount role of this pathway on the performance of the TLPB based on hydrophobic microorganisms.

1. Introduction

Two-liquid phase bioreactors (TLPB) have been used for the biodegradation of volatile organic compounds (VOC) since the early 1990s, with pioneering studies mostly devoted to the treatment of sparingly water-soluble VOC (e.g. benzene, toluene and styrene [1–3], but also few studies early explored the treatment of hydrophobic VOC (e.g. ethene and hexane) [4,5]. However, the treatment of hydrophobic VOC (dimensionless Henry's law constant $H > 1$) in TLPB has gained importance in recent years because conventional biological technologies such as biofilters or biotrickling filters are poorly efficient when treating these VOC [6].

TLPB for gas treatment are based the addition of a liquid non-aqueous phase (NAP) with high affinity for the target VOC in order to improve the biological processes performance by: (i) increasing the VOC mass transfer from the gas phase to the microorganisms, (ii) increasing the O_2 mass transfer, and (iii) buffering drastic changes in the aqueous VOC concentration after sudden load surges [7,8]. Recently, significant advances in the understanding of VOC uptake mechanisms in TLPB have been achieved, cell hydrophobicity being identified as a key parameter affecting the performance of these systems. In this regard, Hernandez et al. [9] found that traditional TLPB operational mode where VOC biodegradation occurs in the aqueous phase can be substantially improved by using microorganisms with high cell hydrophobicity. These findings together with the greater understanding of the mass transfer mechanisms, certainly encourages the application of TLPB at full scale. In the last years, the partial mass transfer quantification (e.g. gas/water and gas/NAP/water), the assessment of the NAP addition effect on the gas/liquid and NAP/aqueous interfacial areas, and the optimization of the NAP percentage in TLPB have increased the understanding of the fundamentals of this technology [10–12]. However, the achievement of stable performance at low power inputs is required prior to the implementation of TLPB at full scale. Unfortunately, there is a lack of systematic studies addressing the stability of TLPB, with the few existing studies reporting a stable hydrophobic VOC biodegradation for only few days [5,6]. As a matter of fact, there are reports where no stable biodegradation performance was achieved during the whole experimental time [13,14].

This work was devised to determine the operational conditions supporting a stable biodegradation performance of hydrophobic VOC in TLPB. Process operation was carried out at low stirring power inputs since the energy required to disperse the NAP is a major limitation for the scale-up of this technology [15]. The performance of the system was controlled by the type of microorganisms (hydrophilic and hydrophobic) and the removal of inhibitory metabolites. In addition, the key role of the gas/water/NAP transfer pathway for O_2 on the performance of the TLPB operated with hydrophobic microorganisms was identified.

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2. Materials and methods

2.1. Chemicals and mineral salt medium

All chemicals for mineral salt medium (MSM) preparation were purchased from PANREAC (Barcelona, Spain) with a purity of at least 99%. n-Hexane (99.0% purity) was obtained from MERCK (Madrid, Spain). Silicone oil 200 cSt (dynamic viscosity = 0.19 kg m^{-1} s^{-1}) and Antifoam 204 (non-silicone polypropylene based polyether) were purchased from Sigma–Aldrich (Madrid, Spain).

The MSM was composed of (g L^{-1}): Na_2HPO_4-$12H_2O$, 6.15; KH_2PO_4, 1.52; $(NH_4)_2SO_4$, 1; $MgSO_4 \cdot 7H_2O$, 0.2; $CaCl_2$, 0.038; and 10 mL L^{-1} of a trace element solution containing (g L^{-1}): EDTA, 0.5; $FeSO_4 \cdot 7H_2O$, 0.2; $ZnSO_4 \cdot 7H_2O$, 0.01; $MnCl_2 \cdot 4H_2O$, 0.003; H_3BO_3, 0.03; $CoCl_2 \cdot 6H_2O$, 0.02; $CuCl_2 \cdot 2H_2O$, 0.001; $NiCl_2 \cdot 6H_2O$, 0.002; $NaMoO_4 \cdot 2H_2O$, 0.003. The final pH of medium was 7.

2.2. Microorganisms

Acclimated hydrophobic and hydrophilic microbial consortia were used. The hydrophobic consortium was obtained from a biotrickling filter treating a VOC mixture at trace level concentrations (0.28 ± 0.02 mg hexane m^{-3}, 0.22 ± 0.03 mg toluene m^{-3}, 0.23 ± 0.03 mg α-pinene m^{-3} and 22 ± 2 mg methyl mercaptan m^{-3}) for 5 months. The hydrophobic nature of the microbial consortium was early reported by Hernandez et al. [9]. The hydrophilic consortium was isolated from an activated sludge (Valladolid sewage work, Spain) [9]. Each consortium was cultured in gas-tight 1.2-L glass bottles at 25 °C under magnetic agitation (300 rpm) for 14 days prior to inoculation. The bottles, containing 100 mL of MSM, were supplied with 5 μL of n-hexane as the sole carbon and energy source each 2 days.

2.3. Experimental set-up and operation mode

A 3-L jacketed glass reactor (Afora S.A., Spain) equipped with two marine impellers (0.05 m diameter) was initially filled with 2.25 L of MSM and 0.25 L of hydrophobic consortium inoculum. Gaseous hexane at 0.50 ± 0.04 g m^{-3} was continuously supplied through the aeration (2 L min^{-1}) resulting in a loading rate (LR) of 24 ± 2 g m^{-3} h^{-1}. The system was operated at 300 rpm and 25 °C. On-line measurements of the dissolved oxygen concentration (DO) and pH were acquired each 4 h with calibrated probes connected to a multiparametric analyser C-3020 (Consort, Belgium). The pH value was maintained at 7.0 ± 0.2 by automatic addition of NaOH 1 M (homemade PID controller).

The reactor was initially operated in the absence of silicone oil until steady hexane biodegradation was reached (10 days). At day 11, 250 mL of cultivation medium were replaced with 250 mL of silicone oil (corresponding to a volume fraction of 10%). The aqueous broth from the bioreactor was periodically replaced by fresh MSM as described below to avoid nutrients limitations and to evaluate the effect of removing potential inhibitory metabolites on the hexane removal performance. From days 1 to 20, no MSM renewal was performed, while from days 21 to 27 a MSM dilution rate (D) of 0.2 day^{-1} was established. At day 28, 250 mL of aqueous culture broth were drawn from the reactor and replaced with 250 mL of hydrophilic consortium inoculum. Additionally, the aqueous medium renewal was stopped from days 28 to 42. Finally, a D of 0.2 day^{-1} was established again from day 43 until the end of the experiment (day 80). Stable performance in the present study was considered as a period with EC/RE data without variations higher than 20% relative to the mean.

Gaseous samples for hexane and CO_2 determination (250 μL and 100 μL, respectively) were periodically taken from sampling ports located at the inlet and outlet of the bioreactor using gas-tight syringes (HAMILTON, USA). Aqueous samples of 50 mL were also periodically drawn to record culture absorbance at 650 nm (OD_{650}) and filtered (0.2 μm filters, Whatman, USA) to measure dissolved total organic carbon (TOC) and total nitrogen (TN). TOC measurements were used to estimate the accumulation of metabolites in the aqueous phase. Moreover, the concentration of potential metabolites accumulated in the NAP was not measured due to the high affinity of silicone oil for organic compounds, which makes metabolite extraction extremely difficult for any solvent. Distilled water (50 mL) was added every 2 days to compensate volume losses by sampling and evaporation.

2.4. Biodegradation performance

Hexane biodegradation performance was evaluated in terms of elimination capacity (EC, g m^{-3} h^{-1}) and removal efficiency (RE, %) as defined by Arriaga et al. [6].

2.5. Determination of the NAP/water partition coefficient for 2-hexanol

The partition coefficient (K) of 2-hexanol between silicone oil and MSM was determined in 120 mL-glass bottles completely filled with 2 mL of silicone oil and 118 mL of MSM initially containing 2-hexanol at 75, 140 and 430 mg L^{-1} (corresponding to TOC values of 50, 100 and 300 mg L^{-1}). The bottles were closed with butyl septa, sealed with aluminum caps and allowed to equilibrate under magnetic agitation (300 rpm) in a water bath at 25 °C. After 24 h, TOC concentrations of the aqueous phase (C_W) were measured and the corresponding concentration in the silicone oil (C_{NAP}) was obtained from the following basic mass balance:

$$C_{Tot} = \frac{V_W}{V_{Tot}} C_W + \frac{V_{NAP}}{V_{Tot}} C_{NAP} \quad (1)$$

where C_{Tot} is the total TOC concentration in the bottle volume (V_{Tot}), while V_w, and V_{NAP} are the volumes of MSM and silicone oil, respectively. Finally, K was determined as follows:

$$K = \frac{C_{NAP}}{C_W} \quad (2)$$

2.6. Oxygen gas/water/NAP transfer pathway

The role of the gas/water/NAP transfer pathway for O_2 on the hexane biodegradation performance of the TLPB was assessed by temporarily blocking this pathway. First, the non-toxic concentrations of sodium sulfite and cobalt sulfate were determined for the hydrophilic and hydrophobic consortia according to Quijano et al. [16]. Then, at day 74 (steady state conditions) Na_2SO_3 and $CoSO_4$ were supplied from stock solutions to the reactor reaching SO_3^{2-} and Co^{2+} concentrations of 4 g L^{-1} and 1 mg L^{-1}, respectively. The DO was recorded each 30 s and samples of gaseous hexane were periodically taken.

2.7. Analytical methods

The hexane gas concentration was determined using an Agilent 6890 gas chromatograph (Palo Alto, USA) equipped with a flame ionization detector and a HP-5MS fused silica capillary column (30 m × 0.250 mm × 0.25 μm) (J & W Scientific, USA). The injector, detector and oven temperatures were set at 200 °C, 200 °C and 140 °C, respectively. Helium was used as the carrier gas at 2 mL min^{-1}. CO_2 concentration was determined in a Varian CP-3800 gas chromatograph (Palo Alto, USA) coupled