ARTICLE

BIOTECHNOLOGY and BIOENGINEERING

A Comparative Study of Solid and Liquid Non-Aqueous Phases for the Biodegradation of Hexane in Two-Phase Partitioning Bioreactors

María Hernández,[1] Guillermo Quijano,[2] Frédéric Thalasso,[2] Andrew J. Daugulis,[3] Santiago Villaverde,[1] Raúl Muñoz[1]

[1]Department of Chemical Engineering and Environmental Technology, Valladolid University, Paseo del Prado de la Magdalena, s/n, 47011 Valladolid, Spain; telephone: 34-983184934; fax: 34983423013; e-mail: mutora@iq.uva.es

[2]Departamento de Biotecnología y Bioingeniería, Centro de Investigación y de Estudios Avanzados del IPN (Cinvestav), Mexico DF, Mexico

[3]Department of Chemical Engineering, Queen's University, Kingston, Ontario, Canada

Received 25 January 2010; revision received 16 March 2010; accepted 22 March 2010

Published online 8 April 2010 in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/bit.22748

ABSTRACT: A comparative study of the performance of solid and liquid non-aqueous phases (NAPs) to enhance the mass transfer and biodegradation of hexane by *Pseudomonas aeruginosa* in two-phase partitioning bioreactors (TPPBs) was undertaken. A preliminary NAP screening was thus carried out among the most common solid and liquid NAPs used in pollutant biodegradation. The polymer Kraton G1657 (solid) and the liquid silicone oils SO20 and SO200 were selected from this screening based on their biocompatibility, resistance to microbial attack, non-volatility and high affinity for hexane (low partition coefficient: $K = C_g/C_{NAP}$, where C_g and C_{NAP} represent the pollutant concentration in the gas phase and NAP, respectively). Despite the three NAPs exhibited a similar affinity for hexane ($K \approx 0.0058$), SO200 and SO20 showed a superior performance to Kraton G1657 in terms of hexane mass transfer and biodegradation enhancement. The enhanced performance of SO200 and SO20 could be explained by both the low interfacial area of this solid polymer (as a result of the large size of commercial beads) and by the interference of water on hexane transfer (observed in this work). When Kraton G1657 (20%) was tested in a TPPB inoculated with *P. aeruginosa*, steady state elimination capacities (ECs) of $5.6 \pm 0.6\,g\,m^{-3}\,h^{-1}$ were achieved. These values were similar to those obtained in the absence of a NAP but lower compared to the ECs recorded in the presence of 20% of SO200 ($10.6 \pm 0.9\,g\,m^{-3}\,h^{-1}$). Finally, this study showed that the enhancement in the transfer of hexane supported by SO200 was attenuated by limitations in microbial activity, as shown by the fact that the ECs in biotic systems were far lower than the maximum hexane transfer capacity recorded under abiotic conditions.

Correspondence to: R. Muñoz

Contract grant sponsor: Spanish Ministry of Education and Science

Contract grant number: RYC-2007-01667; BES-2007-15840; PPQ2006-08230; CTQ2009-07601; CONSOLIDER-CSD 2007-00055

Biotechnol. Bioeng. 2010;106: 731–740.

KEYWORDS: gas treatment; hexane; liquid solvents; mass transfer limitations; solid polymers; two-phase partitioning bioreactors

Introduction

Hexane is a highly hydrophobic volatile organic compound (VOC) widely used in the food, textile, and pharmaceutical industries (Conkerton et al., 1995; EPA, 1996c; Kim and Yoon, 1990). Hexane contributes significantly to air pollution due to its high toxicity and volatility. In addition, hexane can cause severe human health hazards such us nervous system damage and cancer promotion (Canadian Centre for Occupational Health and Safety, 1985).

Compared to physical/chemical VOCs treatment methods, biological methods often represent the most cost-efficient and environmentally friendly option for the treatment of large flow rates of air contaminated with low VOC concentrations (Van Groenestijn and Hesselink, 1993). Biological gas treatment methods are based on the natural ability of microorganisms to convert the VOCs transferred from the gas phase into carbon dioxide, water, inorganic compounds, and biomass. Of such systems, biofilters, bioscrubbers, and biotrickling filters currently constitute the most commonly used biotechniques (Shareefdeen and Singh, 2005). However, despite their inherent advantages, the performance of biological gas treatment processes is often challenged by the hydrophobicity of some specific VOCs (such as hexane, terpenes), which limits pollutant

transfer from the gaseous phase to the aqueous phase (Devinny et al., 1999; Zhu et al., 2004). Therefore, more research must be devoted to the development of innovative bioreactors capable of supporting a facilitated VOC transfer to the microorganisms.

In this context, the use of two-phase partitioning bioreactors (TPPBs) can enhance the transfer of hydrophobic VOCs (Daugulis, 2001; Daugulis and Boudreau, 2003; Muñoz et al., 2007). This technology is based on the addition to the biological process of a non-aqueous-phase (NAP) with high affinity for the hydrophobic VOCs. A high concentration gradient is thus established between the gas and the NAP, increasing the driving force available for mass transfer, which ultimately improves the transfer of hydrophobic VOCs to the aqueous phase (Muñoz et al., 2007). In addition, the presence of a NAP in the aqueous medium provides an increase in the gaseous interfacial area available for transfer (Quijano et al., 2010a). Thus, the use of TPPBs has resulted in unprecedented biodegradation rates. For instance, Arriaga et al. (2006) recorded hexane biodegradation rates of $160\,g\,m^{-3}_{reactor}\,h^{-1}$ in a fungal biofilter supplied with silicone oil, which were significantly higher than the highest elimination capacities (ECs) reported in classical fungal and bacterial biofilters ($100\,g\,m^{-3}_{reactor}\,h^{-1}$ and $60\,g\,m^{-3}_{reactor}\,h^{-1}$, respectively) (Arriaga and Revah, 2005; Kibazohi et al., 2004). Similarly, maximum hexane removal rates of $135 \pm 17\,g\,m^{-3}_{reactor}\,h^{-1}$ were observed by Muñoz et al. (2006) in a stirred tank TPPB supplied with 10% silicone oil 20 cSt. These rates were approximately five times higher than those recorded in a similar system without an organic phase. Unfortunately, most of the studies carried out in TPPBs for the off-gas treatment of hydrophobic VOCs were based on the use of liquid NAPs, particularly silicone oil. Despite exhibiting high VOCs diffusivities, liquid NAPs present severe operational problems such as foaming, emulsification and a high cost (Quijano et al., 2009). Therefore, a need for cost-effective NAP was identified. Solid NAPs (polymers) could represent an alternative to liquid NAPs based on their low cost (up to 250 times less expensive than liquid organic solvents), nonbiodegradability and potential reuse (Morrish and Daugulis, 2008). Despite the promising results achieved in polymer-based TPPBs with moderately soluble compounds such as BTEX (Boudreau and Daugulis, 2006), there is a lack of comparative studies aiming at evaluate the performance of liquid and solid NAPs during the biodegradation of highly hydrophobic VOCs.

This study was therefore conducted to systematically compare the ability of the most common liquid NAPs (polydimethylsiloxane 20 and 200 cSt), 2,2,4,4,6,8,8-heptamethylnonane, 1,1,1,3,5,5,5-heptamethyltrisiloxane and the perfluorocarbon FC40™) and solid NAPs (Kraton G1657, G1650, G1642, Desmopan DP9370A and Elvax 360, 880) to enhance hexane biodegradation in a stirred tank TPPB. This systematic evaluation was based on biodegradability, biocompatibility, volatility, partition tests, and hexane mass transfer studies in a stirred tank reactor under abiotic and biotic conditions.

Materials and Methods

Microorganisms and Culture Conditions

Pseudomonas aeruginosa BM-B-450 was kindly supplied by Dr. Marcia Morales (UAM-Cuajimalpa, Mexico). This strain was deposited in the Industrial Culture Collection of the Instituto de Investigaciones Biomedicas (Universidad Nacional Autonomade Mexico) IIBM-UNAM WDCM48 (http://wdcm.nig.ac.jp/CCINFO/CCINFO.xml?48). The culture was maintained at 4°C on agar plates with Mineral Salt Medium (MSM) with hexane as the sole carbon and energy source. The MSM was prepared according to Muñoz et al. (2006). To furnish fresh inoculum, 250-mL E-flasks were supplied with 100 mL of MSM, $1\,g\,L^{-1}$ of glucose, and incubated for 16 h in a thermostated magnetic shaker at 300 rpm and 30°C. A temperature of 30°C was chosen in all experiments herein conducted since the genus *Pseudomonas* exhibits an optimum temperature range of 25–40°C (Madigan et al., 2009). In addition, operation at 30°C would allow the comparison of the results here obtained with previous hexane biodegradation studies carried out at this temperature (Arriaga et al., 2006; Muñoz et al., 2006).

Chemicals

All chemicals were purchased from PANREAC with a purity of at least 99% (Barcelona, Spain). *n*-Hexane PA-ACS grade was obtained from MERCK. The liquid NAPs tested were polydimethylsiloxane (silicone oil) 20 cSt (SO20) and 200 cSt (SO200), 2,2,4,4,6,8,8-heptamethylnonane (HMN), 1,1,1,3,5,5,5-heptamethyltrisiloxane (HMS) and the perfluorocarbon FC40™. The solid vectors tested were Kraton™ G1657, Kraton™ G1650, Kraton™ G1642, (3–4 mm beads of styrene–ethylene/butadiene tribloc copolymer), Desmopan™ DP9370A (3×3 mm cylinders of polyurethane of poly(oxytetramethylene)glycol and methyldiisocyanate), and Elvax™ 360 and Elvax™ 880 (3–4 mm beads of polyethylene-co-vinyl acetate). SO20, SO200, HMN, and HMS were purchased from Sigma–Aldrich (Madrid, Spain). FC40, Kraton, Desmopan, and Elvax were kindly supplied by 3 M, Kraton Polymers, Bayer, and Dupont, respectively. The polymers were used as received, without any washing prior to use.

Experimental

NAPs Selection

The following tests were carried out in order to select the optimal NAP to enhance hexane biodegradation:

Partition tests. Partition tests were conducted in duplicate in 52.5 mL tubes containing 2 mL of the tested NAP and closed with Mininert Teflon valves (VICI Precision Sampling, Inc., Baton Rouge, LA). For each NAP, a set of tubes was supplied with 3, 5, 10, and 15 μL of hexane. The

tubes were maintained under magnetic agitation (500 rpm) in a water bath at 30°C for 24 h in the case of liquid organic phases and 48 h in the case of solid organic phases. The headspace composition of the tubes was monitored by GC-MS following 6 and 24 h in the case of liquid NAP and following 24 and 48 h in the case of solid NAP, in order to ensure that equilibrium was established in all systems. The hexane partition coefficient (K) was defined by the expression:

$$K = \frac{C_g}{C_{NAP}} \quad (1)$$

where C_g and C_{NAP} represent the hexane concentration in the tube headspace and non-aqueous phase, respectively. C_{NAP} was calculated from a mass balance on hexane after the experimental determination of C_g.

NAPs toxicity tests. Glass flasks of 120 mL were supplied with 2 mL of organic NAP, 3 mL of fresh *P. aeruginosa* inoculum and 20 mL of sterile MSM enriched with 1 g L^{-1} of glucose. The flasks were closed with butyl septa, sealed with aluminium caps and incubated under magnetic agitation (500 rpm) in a water bath at 30°C. Tests in the absence of organic phase were also carried to serve as a control. Flask headspace composition (O_2/CO_2) was periodically recorded for 60 h under sterile conditions by GC-TCD. A NAP was considered toxic if CO_2 production in the flasks supplied with the NAP and the enriched MSM was at least five times lower than that in the control systems (Arriaga et al., 2006).

The tests were carried out in duplicate. The same experiment was carried out as above described but adding 10 μL of hexane as carbon source instead of glucose.

NAPs biodegradability tests. Biodegradability tests were conducted in 120 mL glass flasks supplied with 20 mL of sterile MSM, 2 mL of a sterile NAPs and 1 mL of fresh *P. aeruginosa*. The flasks were closed with butyl septa, sealed with aluminium caps and incubated under magnetic agitation (500 rpm) in a water bath at 30°C. Control flasks were prepared and incubated under similar conditions without organic solvent in order to account for bacterial endogenous respiration. The headspace composition (O_2/CO_2) of the flasks was periodically monitored by GC-TCD under sterile conditions for 4 weeks (in order to allow for microbial acclimation; OECD, 1993). A NAP was considered biodegradable if the CO_2 production in the flasks supplied with the NAP was at least five times higher than the CO_2 production in the controls (Arriaga et al., 2006). The tests were carried out in duplicate.

Liquid NAPs volatility tests. Volatility tests for the liquid NAPs (SO20, SO200, HMN, and FC40) were carried out according to Quijano et al. (2010b). Poly(methyl methacrylate) tubes (0.032 m inner diameter, 1 m height) containing 200 mL of liquid NAP were aerated from the bottom using a porous sparger at 3.3×10^{-6} m^3 s^{-1}. The tests were maintained at room temperature for 30 days. Volatilization was estimated by direct measurement of the vector volume decrease. A liquid NAP was considered non-volatile if 99% of the initial amount of solvent was present at the end of the volatility test.

Hexane Mass Transfer Tests Under Abiotic Conditions

Continuous tests in fermentor. A 3-L jacketed stirred glass fermentor (Afora S.A., Barcelona, Spain) operated with a working volume of 2 L and equipped with two marine impellers was used to evaluate hexane mass transfer from the gas phase in a TPPB constructed with MSM and 20% of a NAP (Fig. 1). The hexane transfer enhancing potential of the NAPs selected in the NAPs Selection Section under Experimental Section (SO20, SO200, and Kraton G1657) was independently tested and compared to control tests carried out in the absence of NAP (only MSM). Each series of experiments was performed at 300 rpm and 30°C and supplied with hexane at 2 g m^{-3} and 1 L min^{-1}. Gas samples of 250 μL were periodically taken using Gas-Tight Hamilton syringes from valves A and C (inlet and outlet concentrations, respectively) to monitor hexane absorption from the gas phase during the period needed to reach saturation of the water and NAP-water dispersion.

Gas-tight tests. Abiotic hexane absorption experiments were also carried out in gas-tight 120 mL serum flasks supplied with 20 mL of MSM +10 mL of NAP (SO200 or Kraton G1657), 20 mL of MSM (control tests) and 10 mL of NAP in order to evaluate the ability of solid and liquid NAPs with similar partitioning coefficients to absorb hexane and to identify any potential interference of the aqueous phase in hexane absorption. The flasks were closed with Teflon-coated butyl septa and supplied with 1.85 mg of hexane in the gaseous state. The time course of hexane headspace concentration was monitored every 6 min by GC-MS for 38 and 83 min in the tests conducted with silicone oil and Kraton G1657, respectively.

NAPs Performance During Hexane Biodegradation in a Mechanically Agitated Bioreactor Operated Under Continuous Mode

The same 3-L jacketed glass fermenter (Afora S.A., Barcelona, Spain) equipped with two marine impellers was used to evaluate the ability of SO200 and Kraton G1657 to enhance hexane biodegradation by *P. aeruginosa* under continuous operation. The reactor was initially filled with 1,880 mL of sterile MSM, and 120 mL of fresh bacterial inoculum and operated at 300 rpm, 30°C and a dilution rate (D) of 0.05 day^{-1} (by daily exchange of 100 mL of culture broth by fresh sterile MSM) unless otherwise specified. Hexane at 2.1 ± 0.1 g m^{-3} was continuously supplied in the gas phase through the aeration (1 L min^{-1} of air filtered through a 0.2 μm Millex®-FG membrane filter) by mixing a hexane-saturated stream with a hexane-free air stream, resulting in a load of 65.6 ± 10.3 g m^{-3} h^{-1}. The system was operated in the absence of a NAP for 12 days until a steady state was reached. Then, 400 mL of cultivation broth were replaced with 400 mL of Kraton G1657 and the reactor was

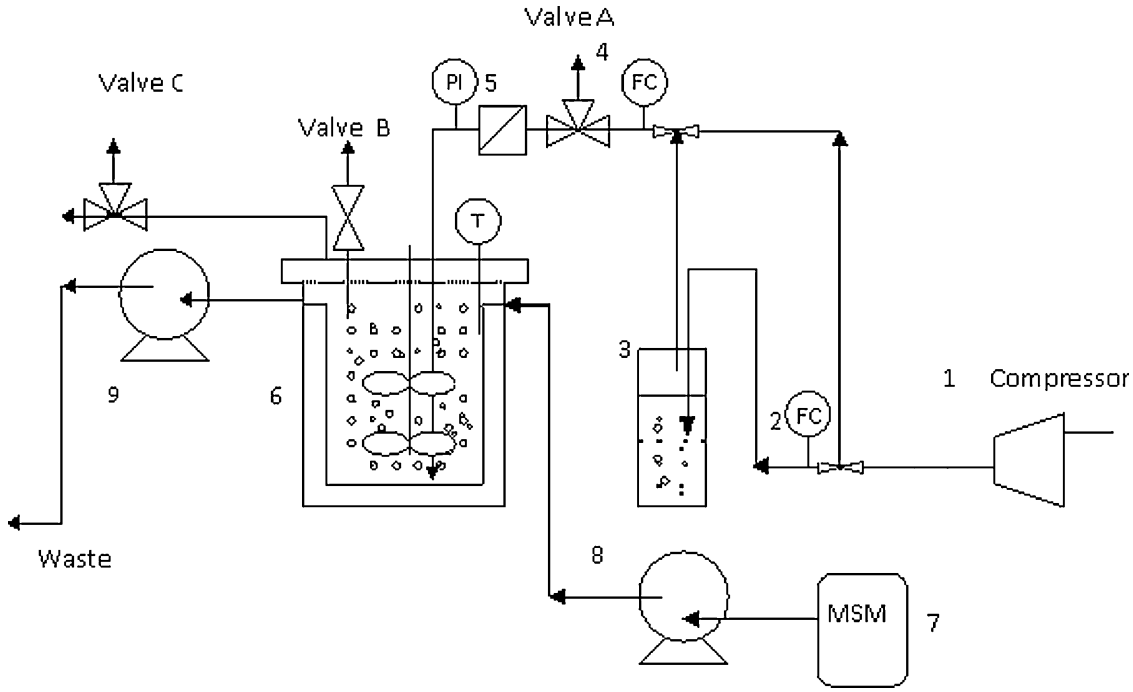

Figure 1. Schematic illustration of the experimental set-up. 1, Compressor; 2, Mass flow controller; 3, hexane evaporator; 4, mass flow controller; 5, air-filter; 6, stirred tank reactor; 7, MSM reservoir; 8, MSM influent pump; 9, waste effluent pump. PI, pressure indicator; *T*, temperature indicator; VALVE A, inlet gas phase sampling; VALVE B, liquid sampling; VALVE C, outlet gas phase sampling.

operated under similar experimental conditions until a new stationary state was reached at day 24. Finally, Kraton G1657 was replaced with 400 mL of SO200 and the process was operated with this NAP until the end of the experimentation. During the last 8 days of experimentation the process was however operated at a D of 0.2 day^{-1} pH was maintained above 5.5 by addition of NaOH 1 M. In addition, sterile distilled water (5 mL day^{-1}) and sterile silicone oil (5 mL day^{-1}) were daily added to minimize water losses by evaporation and silicone oil losses due to medium exchange and sampling, respectively. Silicon oil was autoclaved at 121°C for 25 min with no significant deterioration of its properties (Pol et al., 2006; Quijano et al., 2010b). Gas samples of 250 μL were daily taken to monitor hexane and CO_2/O_2 concentrations. Liquid samples of 15 mL were also drawn to record pH, absorbance, dissolved total organic carbon (TOC) and total nitrogen (TN).

Analytical Procedures

Hexane quantification was performed by gas chromatography (6890, Hewlett-Packard, Palo Alto, CA) coupled with a Mass Spectrometer Detector (5973 MSD, Hewlett-Packard) and a HP-5MS fused silica capillary column (Agilent Technologies, CA). Oven, injector and MS Quadrupole were maintained at 80°C, 250°C, and 150°C, respectively. Helium was used as carrier gas at 1 mL min^{-1}.

CO_2 and O_2 concentrations were determined in a gas chromatograph (CP-3800, Varian, Palo Alto, CA) coupled with a thermal conductivity detector and equipped with a CP-Molsieve 5A (15 m × 0.53 μm × 15 μm) and a CP-Pora BOND Q (25 m × 0.53 μm × 10 μm) columns. Oven, injector and detector temperatures were maintained at 40°C, 150°C, and 175°C, respectively. Helium was used as carrier gas at 13.7 mL min^{-1}.

TOC and TN were measured using a TOC-V_{CSH} analyzer (Shimadzu, Tokyo, Japan) coupled with a TN module based on chemiluminesce detection (TNM-1, Shimadzu). Biomass concentration (mg dry weight L^{-1}) was determined via optical density measurements at 650 nm using a HITACHI U200 UV/visible spectrophotometer (Hitachi, Tokyo, Japan) according to Bordel et al. (2007). When SO200 was present in the cultivation medium, the liquid supernatant was removed after centrifugation for 10 min at 9,200 g and the biomass resuspended in fresh MSM to its original concentration in order to avoid silicone oil interferences in absorbance measurements.

Results

NAPs Selection

In order to select the optimum NAP, a preliminary screening was thus carried out among the most common NAP

Table I. Partition coefficient of NAP tested.

	$K\ (C_g\ C_{NAP}^{-1})$
Liquid NAPs	
FC40	0.0268 ± 0.0021
HMS	0.0029 ± 0.0001
HMN	0.0027 ± 0.0001
SO20	0.0058 ± 0.0001
SO200	0.0058 ± 0.0002
Solid NAPs	
Desmopan DP 9370A	0.0168 ± 0.0005
Kraton G1657	0.0057 ± 0.0003
Kraton G1650	0.0072 ± 0.0007
Kraton G1642	0.0060 ± 0.0004
Elvax 360	0.0135 ± 0.0004
Elvax 880	0.0154 ± 0.0003

reported in literature for VOCs. In this context, partition, toxicity, biodegradability and liquid NAPs volatility tests were carried out. The liquid and solid NAPs tested exhibited a similar range of partition coefficients: in the case of liquid NAPs the experimental hexane partition coefficients ranged from 0.0268 ± 0.0021 for FC40 to 0.0027 ± 0.0001 for HMN, the solid NAPs tested possessed partition coefficients ranging from 0.0168 ± 0.0005 for Desmopan DP9370A to 0.0057 ± 0.0003 for Kraton G1657 (Table I).

None of the NAPs tested was toxic to *P. aeruginosa*, based on the similar final CO_2 concentrations recorded in the flasks supplied with and without NAP (ranging from 87.5 to 101.3 g m^{-3} and from 33.1 to 45.2 g m^{-3} in the tests supplied with glucose and hexane, respectively). Likewise, none of the tested NAPs was biodegradable by *P. aeruginosa*.

The volatilization tests showed that SO20 and SO200 were the only liquid NAPs that were non-volatile.

Hexane Mass Transfer Tests Under Abiotic Conditions

Continuous Tests in Fermentor

Abiotic absorption experiments in a continuous gas phase stirred tank reactor were carried out in order to evaluate the maximum potential for hexane mass transfer of solid (Kraton G1657) and liquid (SO20 and SO200) NAPs (NAPs with similar partitioning coefficients). The hexane transfer performance of SO20, SO200, and Kraton G1657 under continuous aeration was assessed by monitoring the time course of the normalized hexane outlet concentration (C_{outN}), defined as:

$$C_{outN} = \left(\frac{C_{out}}{C_{in}}\right) \quad (2)$$

where C_{out} and C_{in} represent the inlet and outlet hexane gas concentrations in the reactor, respectively.

Thus, C_{outN} values of 0.58 and 0.56 were recorded at minute 1 of operation in tests supplied with and without

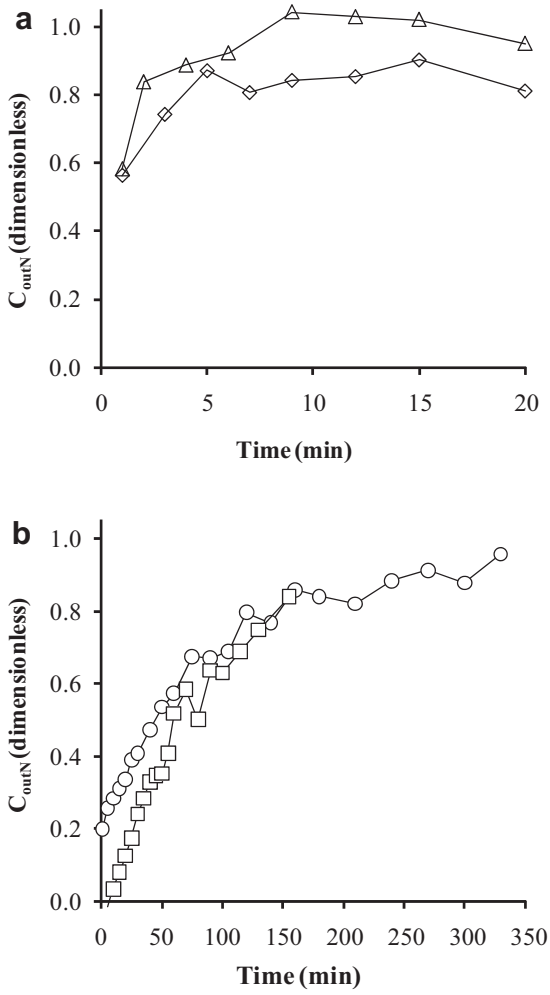

Figure 2. Time course of C_{outN} during the abiotic hexane mass transfer tests conducted in a fermentor without NAP (-Δ-) and with 20% of Kraton G1657 (-◇-) (**a**) and with 20% of SO200 (-□-) and 20% of SO20 (-○-) (**b**).

Kraton G1657 (control test), respectively. Likewise C_{outN} values of 0 and 0.25 were observed at minute 5 for the experiments carried out with SO200 and SO20, respectively (Fig. 2). An extrapolation of C_{outN} values for SO200 and SO20 at minute 1 would result in even lower C_{outN}, which clearly demonstrates that silicon oil (20 and 200 cSt) can support higher maximum hexane transfer rates than Kraton G1657. Regardless of the operational conditions evaluated, C_{outN} gradually increased to finally level off at values close to the saturation of the water and water-NAP dispersion. The elapsed times corresponding to a C_{outN} of 0.85 were 4, 9, 155, and 180 min in the absence of NAP and in the tests supplied with Kraton G1657, SO200, and SO20, respectively (Table II).

Gas-Tight Tests

Abiotic hexane absorption experiments were carried out in enclosed flasks supplied with and without MSM in order to

Table II. Steady state performance[a] during the biodegradation of hexane at 2 g m^{-3} by *P. aeruginosa* under the different experimental conditions evaluated.

	EC (g m^{-3} h^{-1})	CO_2 (g m^{-3} h^{-1})	Biomass (g L^{-1})	pH	TOC (mg L^{-1})	TN (mg L^{-1})	D (day^{-1})
Control	5.9 ± 0.3	4.0 ± 0.3	0.14 ± 0.01	6.8 ± 0.1	28.6 ± 1.2	144.6 ± 17.2	0.05
Kraton G1657	5.6 ± 0.6	5.4 ± 0.5	0.24 ± 0.01	6.5 ± 0.0	40.9 ± 1.4	153.3 ± 0.4	0.05
SO200	8.4 ± 1.2	7.9 ± 0.5	0.61 ± 0.06	5.9 ± 0.1	52.3 ± 10.8	9.0 ± 4.6	0.05
SO200	10.6 ± 0.9	10.9 ± 0.6	0.63 ± 0.06	6.5 ± 0.0	25.2 ± 1.6	55.5 ± 10.7	0.2

[a]Values are given as the average ± standard deviation from the measurements obtained during each steady state.

identify any potential interference of the aqueous phase. When only MSM was present, the hexane gas concentration decreased slightly during the initial stages of the test and remained approximately constant afterwards (91.3 ± 3.2% of the initial value) (Fig. 3). In the experiments carried out with MSM + SO200, the hexane gas concentration sharply decreased to 7.1 ± 0.0% of the initial concentration within the first 6 min and to 6.7 ± 0.0% following 38 min (Fig. 3). Likewise, when MSM + Kraton G1657 were present, the hexane concentration decreased to 52.8 ± 0.7% within the first 6 min and to 19.2 ± 1.5% at minute 83 (Fig. 3). When only SO200 was present in the flasks, the hexane gas concentration rapidly decreased to 6.3 ± 0.1% within the first 6 min and remained constant afterwards. Finally, Kraton G1657 achieved 31.8 ± 1.5% in the first 6 min and 11.7 ± 0.4% after 83 min of experimentation (Fig. 3).

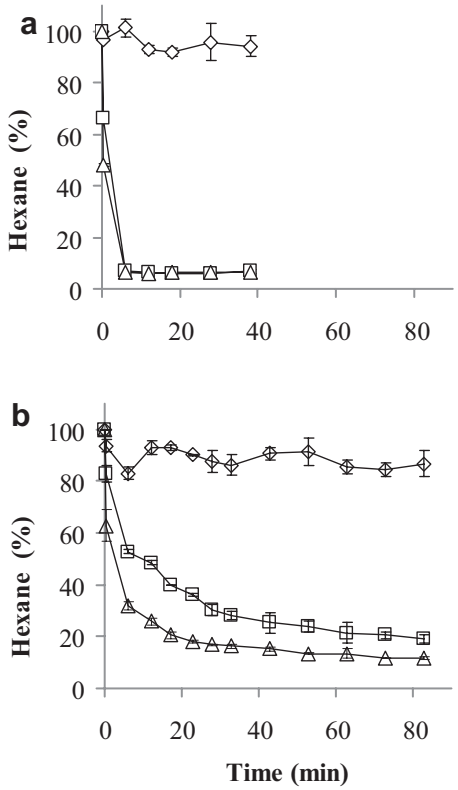

Figure 3. Time course of the hexane headspace concentration in the gas-tight tests carried out with SO200 (**a**) and KratonG1657 (**b**) as NAP. Diamonds, squares, and triangles represent systems supplied with MSN, MSN + NAP, and NAP, respectively. Vertical bars represent standard deviations.

NAPs Performance During Hexane Biodegradation in a Mechanically Agitated Bioreactor Operated Under Continuous Mode

In order to compare the ability of Kraton G1657 and SO200 to enhance hexane biodegradation, biodegradation tests with *P. aeruginosa* were carried out in a continuous mechanically agitated bioreactor. When the system was operated without a NAP, the process was initially characterized by an increase in hexane EC and CO_2 production concomitant with a decrease in cultivation pH (Fig. 4a and b). Steady state ECs of 5.9 ± 0.3 g m^{-3} h^{-1}, CO_2 productions of 4.0 ± 0.3 g m^{-3} h^{-1} and biomass concentrations of 0.14 ± 0.01 g DW L^{-1} were recorded in the absence of NAP. Likewise, the steady state values for pH, TOC, and TN were 6.8 ± 0.1, 28.6 ± 1.2 mg L^{-1}, and 144.6 ± 17.2 mg L^{-1}, respectively.

When Kraton G1657 was present at 20%, process performance remained similar to the single phase case. Thus, steady state EC of 5.6 ± 0.6 g m^{-3} h^{-1}, CO_2 of 5.4 ± 0.5 g m^{-3} h^{-1}, biomass concentration of 0.24 ± 0.01 g DW L^{-1} and pH of 6.5 ± 0.0 were achieved from days 12 to 24. The values of TOC and TN were 40.9 ± 1.4 mg L^{-1} and 153.3 ± 0.4 mg L^{-1}, respectively.

Finally, the addition of SO200 prompted a rapid increase in hexane EC, biomass concentration and CO_2 production, which achieved maximum values of 19.4 g m^{-3} h^{-1}, 0.66 g DW L^{-1} and 20.9 g m^{-3} h^{-1}, respectively, at day 32. This increase was also concomitant with a rapid decrease in the cultivation pH to 5.5 and a gradual decrease in TN concentration. However, from this moment on, process performance declined significantly to finally reach a pseudo-steady state at day 36 with EC of 8.4 ± 1.2 g m^{-3} h^{-1}, CO_2 production rates of 7.9 ± 0.5 g m^{-3} h^{-1}, biomass concentration of 0.61 ± 0.07 g DW L^{-1} and pH of 5.9 ± 0.1. The TN concentration also stabilized at 8.7 ± 4.6 mg L^{-1}.

An increase in D at day 40 resulted in a gradual recovery of the biodegradation performance. A new steady state was achieved at day 45 with values of EC, CO_2 production and biomass concentration of 10.6 ± 0.9 g m^{-3} h^{-1}, 10.9 ± 0.6 g m^{-3} h^{-1} and 0.63 ± 0.06 g DW L^{-1}, respectively.

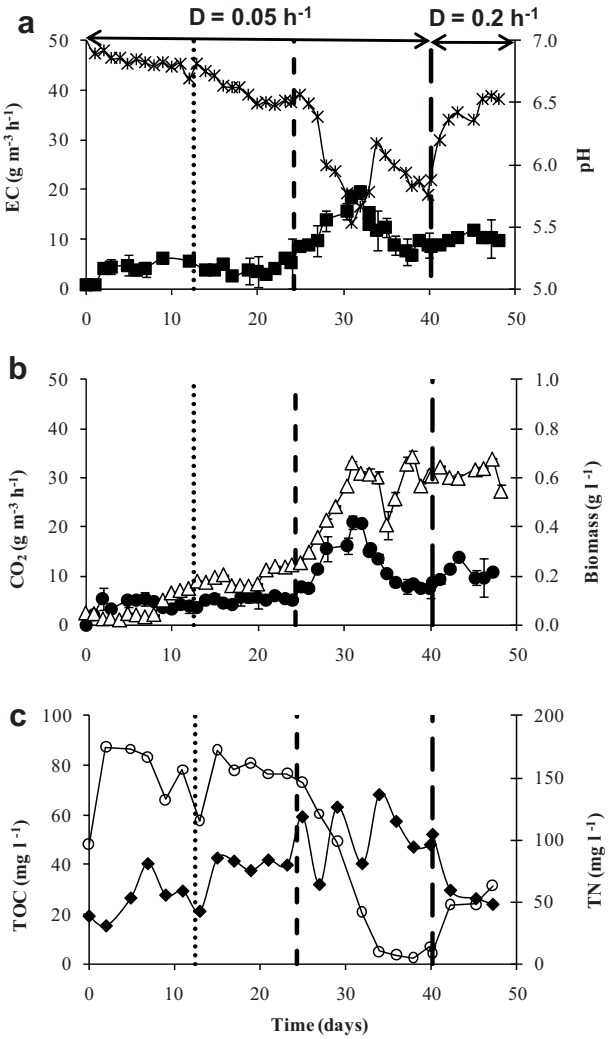

Figure 4. Time course of EC (-■-), pH (-*-), CO_2 production rates (-●-), biomass concentration (-Δ-), TOC (-◆-), and TN (-○-) during hexane biodegradation by *P. aeruginosa* without a NAP (days 0–12), with Kraton G1657 (days 12–24) and with SO200 (day 24 and onwards). Vertical bars represent standard deviation.

Likewise, pH, TOC and TN were 6.5 ± 0.0, 25.1 ± 1.6, and $55.5 \pm 10.7\ mg\ L^{-1}$, respectively.

An analysis of the average carbon flow within the reactor revealed that $88 \pm 3\%$ of the carbon left the system as non-degraded hexane, $0.2 \pm 0.1\%$ was transformed into TOC, $3 \pm 1\%$ was converted to CO_2 and $1 \pm 0.5\%$ was transformed into biomass. Regarding TN concentration, when the system was operated without a NAP and with Kraton G1657, TN concentration ranged from 96.3 to $174.7\ mg\ L^{-1}$. The addition of SO200 prompted a rapid decrease of TN concentration (concomitant with the rapid increase of hexane EC), which achieved average values of $8.7 \pm 4.6\ mg\ L^{-1}$ from days 34 to 40. An increase in D at day 40 resulted in a gradual rise of TN concentrations up to average concentrations of $55.5 \pm 10.7\ mg\ L^{-1}$.

Discussion

A high affinity of the NAP for hexane is crucial to support a high concentration gradient and therefore high VOC transfer rates. The K recorded for the tested NAPs (ranging from 0.0268 to 0.0027) were substantially lower (up to five orders of magnitude) than the partition coefficients of hexane in water ($K = 74$, Sander, 1999), which highlights the high affinity of the tested NAPs for the hexane. The results herein obtained are comparable to the K values reported by Arriaga et al. (2006) in diethyl sebacate and tetradecane (0.0115 and 0.0026, respectively) and by Abraham and Acree (2004) in undecane and dodecane (0.0013 and 0.0019, respectively).

Toxicity and biodegradability constitute process-specific NAP selection criteria that must be evaluated for each particular VOCs-degrading microbial consortium. In this study, none of the tested NAPs was toxic nor biodegradable by *P. aeruginosa*. This can be attributed to the low bioavailability as a result of the large molecular size, poor solubility and resistance to hydrolysis of these materials.

Thus, based on their high partition coefficient, biocompatibility, resistance to microbial attack and non-volatility, the NAPs SO200, SO20 and Kraton G1657 were selected for further evaluation.

Traditionally, the partition coefficient has been considered as the main NAP selection criterion; however, recent studies have shown that other properties of the NAP could be even more critical in the enhancement of gaseous compounds mass transfer. Quijano et al. (2010b) showed that the hydrodynamic behavior of the NAPs in the bioreactor is a key selection parameter, which must be evaluated for each particular bioreactor configuration. In this sense, the ability of the three NAPs selected (exhibiting similar K) was firstly assessed in a stirred tank reactor supplied with a continuous hexane-laden air stream. The values of C_{outN} recorded at the beginning of the absorption process corresponded to the maximum hexane transfer rates of the system (maximum driving force at the point of minimum liquid hexane concentration: water or NAP-water dispersion). The high initial values of C_{outN} recorded in the control test and in the presence of Kraton G1657 clearly indicated that the presence of Kraton did not increase hexane mass transfer compared to the tests carried out in the absence of NAP. Maximum hexane transfer rates of 26 and $28\ g\ m^{-3}\ h^{-1}$ were recorded for MSM and Kraton, respectively. This poor hexane mass transfer in Kraton-supplemented TPPBs was also confirmed by the low elapsed time to achieve a C_{outN} of 0.85. Hence, while in 9 min the total amount of hexane supplied to the system accounted for ~17 mg, the amount of hexane accumulated in a system in equilibrium at C_{outN} of 0.85 would be approximately 112 mg. The low initial C_{outN} values obtained using SO200 and SO20 confirmed the potential of liquid NAPs to enhance the transfer of hydrophobic VOCs and highlight the fact that NAP with comparable K can exhibit a dramatically different performance. In this context, the lower initial C_{outN}

values and elapsed times to reach a C_{outN} of 0.85 recorded with SO200 suggest a higher hexane transfer rate than in the case of SO20, despite its higher viscosity (lower diffusivity of hexane and therefore lower mass transfer coefficient). Maximum hexane transfer rates of 52 and 61 g m^{-3} h^{-1} were recorded for SO20 and SO200, respectively. Similar results were reported by Littlejohns and Daugulis (2007) who observed three times higher volumetric mass transfer coefficients (*kla*) for oxygen when using Nylon 6.6 (O_2 diffusivity = 1.6×10^{-9}, bead diameter = 2.6 mm) instead of silicone rubber (O_2 diffusivity = 3.4×10^{-5}, bead diameter = 2.5 mm) as NAP in a stirred tank reactor. Higher viscosities entail to lower diffusivities and therefore lower mass transfer coefficients (*kl*). However, there are other NAPs properties (e.g., those determining NAP dispersion in the bioreactor) that could increase the value of the interfacial area (*a*) and therefore increase the value of the *kla* and the mass transfer rate (Quijano et al., 2010a).

Hexane absorption in gas-tight flasks confirmed the lower performance of Kraton G1657 compared to SO200 (Fig. 3). Thus, hexane depletion is the headspace was almost complete within the first 6 min of experimentation with SO200 (regardless of the presence of water), while 83 min were necessary to reduce hexane concentration to 12% of the initial value in the systems supplied with Kraton. It is also clear from Figure 3b that the presence of water in the tests supplied with Kraton severely hindered hexane absorption.

Several of the mechanisms proposed in literature to describe VOCs transfer in TPPBs can explain the results obtained in the present study. Firstly, the size of the NAP particles or droplets is known to influence the mechanisms involved in VOCs transfer. Thus, the smaller the size of the NAP droplet or particle, a larger surface area is available for VOCs transfer. According to Cesário et al. (1997a), the interfacial area NAP/water (a_{NAP}) in the case of spherical particles or droplets is:

$$a_{NAP} = \frac{6 f_{NAP}}{d_s} \tag{3}$$

where f_{NAP} and d_s represent the NAP fraction and the Sauter diameter, respectively.

On the other hand, the droplet diameter for liquid NAPs can be estimated as:

$$d_s = 0.047 \left(\frac{\sigma_{vw}^{0.6}}{\rho_w^{0.6} N^{1.2} D^{0.8}} \right) (1 + 2.5 f_{NAP}) \tag{4}$$

where σ_{vw}, ρ_w, N, and D are the liquid NAP/water interfacial tension (kg s^{-2}), the density of water (kg m^{-3}), the stirring speed (s^{-1}) and the impeller diameter (m^{-1}).

According to Equation (4), under the particular conditions of this study, oil droplets present a d_s of ~0.17 mm. However, Kraton particles present d_s of ~3–4 mm, which results in approximately a_{NAP} 23.5 times lower in the case of Kraton for the same f_{NAP}. This difference might explain part of lower hexane transfer rates recorded. In addition, if the NAP droplets are sufficiently small, VOCs grazing at the liquid side of liquid–gas interface could take place: microscopic NAP droplets penetrate within the liquid film at the gas–liquid interface (where VOC absorption is higher due to the higher VOC concentrations), pick the VOCs up and return to the bulk liquid where VOC desorption occurs. Grazing has been shown to be one of the mechanisms involved in the enhancement of gaseous substrates transfer in activated carbon slurry bioreactors and TPPBs (Alper et al., 1980; Cents et al., 2001; Zhang et al., 2006).

In our particular case, grazing could occur in SO dispersions at high stirring rates, where microdroplets of few microns are likely to be present, but not in Kraton dispersions due to the large size of the beads. Secondly, other physical properties of the NAP such as the lyophobicity (wettability) can significantly influence the VOCs transfer mechanism. Thus, wettable particles tend to repel the gas interface, which hinders direct hexane transfer from the gas phase to the NAP (Rehmann, 2007; Ruthiya et al., 2003). In our particular case, Kraton particles might present this type of behavior, which is supported by lower hexane transfer rates in the gas-tight tests carried out with Kraton G1657 in the presence of water compared to tests containing Kraton exclusively. Therefore, a transport in series (gas → water → NAP) model seems to describe hexane mass transport in systems containing Kraton G1657 (beads larger than the liquid film thickness at the gas–liquid interface). No significant enhancement of the overall volumetric mass transfer in the presence of a NAP would be expected in this particular scenario (Cesário et al., 1997b; Littlejohns and Daugulis, 2007). In this context, future research must be devoted to assess the influence of polymer wettability on VOCs biodegradation performance. Finally, it must be stressed that absorption in the NAPs rather than adsorption take place during VOCs removal in TPPBs. In this context, Prpich and Daugulis (2005) observed different specific phenol uptakes with EVA polymers of the same surface area with different polymer composition (different vinyl acetate percentages), which showed that chemical affinity rather than surface affinity was involved in VOC removal in TPPBs.

The performance of Kraton G1657 and SO200 was finally assessed during the continuous biodegradation of hexane by a *P. aeruginosa* strain in a stirred tank reactor. When the system was operated in the absence of a NAP, the ECs recorded were comparable to those achieved in the presence of Kraton G1657 at 20% (v/v) (≈5.8 g m^{-3} h^{-1} corresponding to removal efficiency (RE) of 9%). These results were not surprising based on the high hydrophobicity of hexane and the low enhancement potential of Kraton shown during the abiotic hexane mass transfer experiments. Similar results were obtained by Littlejohns and Daugulis (2009), who reached BTEX removal rates at steady state of 18.3 ± 4.3 and 18.5 ± 4.9 g m^{-3} h^{-1} in a two-phase partitioning airlift bioreactor supplied, respectively, without and with 10% of silicone rubber beads. Despite the poor performance of the solid NAPs during steady operation, these polymers have

shown an excellent performance to handle sudden fluctuations in VOCs loadings. Daugulis and Boudreau (2008) observed that a TPPB supplied with 16% (v/v) styrene butadiene (SB) polymer exhibited superior performance over the course of a 20-fold increase in toluene loading rates (RE of 83% with a solid NAP vs. 59% in the absence of a NAP). This result was explained by the ability of the NAPs to provide a buffering capacity against high toluene transient loadings by the moderately high solubility of toluene. However, when SO200 was added to the microbial culture, both the hexane EC and CO_2 production rapidly increased by a factor of 4 confirming the higher enhancement potential of well-dispersed liquid NAPs. However, hexane biodegradation (and consequently biomass and CO_2 production) gradually declined to steady state ECs of $8.4 \pm 1.2\,g\,m^{-3}\,h^{-1}$, which suggests that microbial activity, rather than hexane mass transfer, could limit hexane biodegradation. The presence of a toxic metabolite excreted as a result of the increased availability of hexane mediated by the addition of SO200 might have inhibited microbial activity and therefore hexane biodegradation. This hypothesis is supported by the increase in EC when increasing process *D*, which probably promoted the wash-out of any accumulated metabolite(s). In addition, the increase in D could speed up the biodegradation kinetics as a result of the higher growth rates of the bacteria. However, a limitation mediated by the absence of an essential elements (others than nitrogen and phosphorous) cannot be ruled out. In addition, the hexane RE achieved in the continuous biodegradation study in the stirred tank (16%) was significantly lower than the maximum hexane mass transport ability recorded in the abiotic experiment carried out under comparable conditions (100% within the first 5 min of experimentation), which confirms that microbial activity rather than mass transfer governed process performance. These results are in agreement with those reported by Muñoz et al. (2006) who observed an increase in the hexane EC compared to the control (up to 5 times higher) followed by a gradual decrease to finally reach EC approximately three times higher than in the absence of the NAP under comparable conditions (stirred tank reactor, SO200, and *P. aeruginosa*).

The lower ECs herein recorded compared to other studies (i.e., Muñoz et al., 2006) were due to the lower bioreactor height, the absence of baffles and the lower stirring rates used, which allowed to record any potential increase in EC due to the addition of a NAP. In addition, it must be stressed that the main purpose of this study was the comparison between solid and liquid NAPs during hexane biodegradation and not the achievement of unprecedented hexane removal rates.

Unfortunately, the volumetric power input was not measured in this study, which would provide a clear picture of the performance of TPPBs when scales-up. It can be however anticipated that the mixing scenario would be slightly different in full-scale applications, where both technical and economical limitations will decrease the performance of this promising technology. For instance, mixing at 600–800 rpm, commonly found in TPPB publications in 1–5 L reactors, cannot be feasible for scale-up due to the high energy requirements involved. For this reason, this study was conducted at maximum stirring rates of 300 rpm and in the absence of baffles in order to mimic a more realistic fluid dynamic pattern in full-scale systems. Finally, it must be noticed that significant differences in the performance of solid and liquid NAPs would be expected under operation at lab and full scales. Thus, the lower density of kraton ($0.9\,kg\,L^{-1}$) would certainly hinder the full dispersion of this NAP in water under the conditions of limited mixing found in large scale applications. In addition, the phenomenon of grazing, that occurs in the presence of liquid NAPs at high stirring rates in lab scale systems, would probably occur in a lower extent at full-scale, which would decrease hexane mass transfer to the aqueous phase.

Conclusion

This study confirmed the need to review the traditional criteria used for the selection of the optimal NAP in TPPBs. Despite exhibiting a similar affinity for hexane, SO200 and SO20 showed a superior performance than Kraton G1657 to support both hexane mass transfer and biodegradation enhancement. The low interfacial area of this solid polymer (as a result of the large size of commercial beads), together with the interference of water on hexane transfer observed in this work, might explain the poor performance of Kraton. Similarly, liquid solvents of a similar nature and very similar properties (i.e., SO200 and SO20) also exhibited significant differences in regards to their ability to enhance hexane mass transfer, with SO200 supporting the highest enhancement despite its higher viscosity. Finally, this study showed that the enhancement in the transfer of hexane supported by SO200 was attenuated by limitations in microbial activity, as shown by the fact that the ECs in biotic systems were far lower than the maximum hexane transfer capacity recorded under abiotic conditions.

This research was supported by the Spanish Ministry of Education and Science (RYC-2007-01667 and BES-2007-15840 contracts, and projects PPQ2006-, CTQ2009-07601 and CONSOLIDER-CSD 2007-00055). Dr. Marcia Morales is also gratefully acknowledged for kindly supplying the bacterial strain used in this investigation.

References

Abraham MH, Acree WE. 2004. Correlation and prediction of partition coefficients between the gas phase and water, and the solvents dodecane and undecane. New J Chem 28:1538–1543.

Alper E, Wichtendahl B, Deckwer W-D. 1980. Gas absorption mechanism in catalytic slurry reactors. Chem Eng Sci 35:217–222.

Arriaga S, Revah S. 2005. Improving hexane removal by enhancing fungal development in a microbial consortium biofilter. Biotechnol Bioeng 90:107–115.

Arriaga S, Muñoz R, Hernandez S, Guieysse B, Revah S. 2006. Gaseous hexane biodegradation by fusarium solani in two liquid phase packed-bed and stirred-tank bioreactors. Environ Sci Technol 40:2390–2395.

Bordel S, Muñoz R, Díaz LF, Villaverde S. 2007. New insights on toluene biodegradation by *Pseudomonas putida* F1: Influence of pollutant concentration and excreted metabolites. Appl Microbiol Biotechnol 74:857–866.

Boudreau NG, Daugulis AJ. 2006. Transient performance of two-phase partitioning bioreactors treating a toluene contaminated gas stream. Biotechnol Bioeng 94:448–457.

Canadian Centre for Occupational Health and Safety. 1985. n-hexane. In: Chemical hazard summary, Vol. 11. Hamilton, Ontario: The Centre.

Cents AHG, Brilman DWF, Versteeg GF. 2001. Gas absorption in an agitated gas–liquid–liquid system. Chem Eng Sci 56:1075–1083.

Cesário MT, Beverloo WA, Tramper J, Befftink HH. 1997a. Enhancement of gas–liquid mass transfer rate of apolar pollutants in the biological waste gas treatment by a dispersed organic solvent. Enzyme Microb Tech 21:578–588.

Cesário MT, de Wit HL, Tramper J, Befftink HH. 1997b. Dispersed organic solvent to enhance the overall gas/water mass transfer coefficient of apolar compounds in the biological waste-gas treatment. Modeling and evaluation. Biotechnol Progr 13:399–407.

Conkerton EJ, Wan PJ, Richard OA. 1995. Hexane and heptane as extraction solvents for cottonseed: A laboratory-scale study. J Am Oil Chem Soc 72:963–965.

Daugulis AJ. 2001. Two-phase partitioning bioreactors: A new technology platform for destroying xenobiotics. Trends Biotechnol 19:459–464.

Daugulis AJ, Boudreau NG. 2003. Removal and destruction of high concentrations of gaseous toluene in a two-phase partitioning bioreactor by *Alcaligenes xylosoxidans*. Biotechnol Lett 25:1441–1444.

Daugulis AJ, Boudreau NG. 2008. Solid–liquid two phase partitioning bioreactors for the treatment of gas-phase volatile organic carbons (VOCs) by a microbial consortium. Biotechnol Lett 30:1483–1587.

Devinny JS, Deshusses MA, Webster TS. 1999. Biofiltration for air pollution control. Boca Raton: Lewis Publishers. ISBN: 1-56670-289-5.

EPA. 1996c. 1994. Toxics Release Inventory: Public Data Release. Office of Pollution Prevention and Toxics. June.

Kibazohi O, Yun S-I, Anderson WA. 2004. Removal of hexane in biofilters packed with perlite and a peat-perlite mixture. World J Microb Biotechnol 20:337–343.

Kim IH, Yoon SK. 1990. Effect of extraction solvents on oxidative stability of crude soybean oil. J Am Oil Chem Soc 67:165–167.

Littlejohns JV, Daugulis AJ. 2007. Oxygen transfer in a gas-liquid system containing solids of varying oxygen affinity. Chem Eng J 129:67–74.

Littlejohns JV, Daugulis AJ. 2009. A two phase partitioning airlift bioreactor for the treatment of BTEX contaminated gases. Biotechnol Bioeng 103:1077–1086.

Madigan MT, Martinko JM, Dunlap PV, Clark DP. 2009. Brock biology of microorganisms. Upper Saddle River, NJ: Prentice Hall. p 413.

Morrish JLE, Daugulis AJ. 2008. Inhibitory effects of substrate and product on the carvone biotransformation activity of *Rhodococcus erythropolis*. Biotechnol Lett 30:1245–1250.

Muñoz R, Arriaga S, Hernandez S, Guieysse B, Revah S. 2006. Enhanced hexane biodegradation in a two phase partitioning bioreactor: Overcoming pollutant transport limitations. Process Biochem 41:1614–1619.

Muñoz R, Villaverde S, Guieyesse B, Revah S. 2007. Two phase partitioning bioreactors for treatment of volatile organic compounds. Biotechnol Adv 25:410–422.

OECD Organization for Cooperation and Development. 1993. Guidelines for Testing of Chemicals. Test 302B: Zahn-Wellens/EMPA: Test 302 C: Modified MITI Test (II).

Pol VG, Pol SV, Gedanken A, Lim SH, Zhong Z, Lin J. 2006. Thermal decomposition of commercial silicone oil to produce high yield high surface area SiC nanorods. J Phys Chem B 110:11237–11240.

Prpich GP, Daugulis AJ. 2005. Enhanced biodegradation of phenol by a microbial consortium in a solid-liquid two phase partitioning bioreactor. Biodegradation 16:329–339.

Quijano G, Hernandez M, Thalasso F, Muñoz R, Villaverde S. 2009. Two-phase partitioning bioreactor in environmental biotechnology. Appl Microbiol Biotechnol 84:829–846.

Quijano G, Rocha-Rios J, Hernandez M, Villaverde S, Revah S, Muñoz R, Thalasso F. 2010a. Determining the effect of solid and liquid vectors on the gaseous interfacial area in two-phase partitioning bioreactors. J Hazar Mater 175:1085–1089.

Quijano G, Hernandez M, Villaverde S, Thalasso F, Muñoz R. 2010b. A step-forward in the characterization and potential applications of solid and liquid oxygen transfer vectors. Appl Microbiol Biot 85:543–551.

Rehmann L. 2007. Delivery of hydrophobic substrates to degrading organisms in two phase partitioning bioreactors. PhD Thesis. Queen's University.

Ruthiya KC, Van der Schaaf J, Kuster BFM, Schouten JC. 2003. Mechanisms of physical and reaction enhancement of mass transfer in a gas inducing stirred slurry reactor. Chem Eng J 96:55–69.

Sander R. 1999. Compilation of Henry's Law Constants for Inorganic and Organic Species of Potential Importance in Environmental Chemistry (http://www.mpch-mainz.mpg.de/∼sander/res/871henry.html).

Shareefdeen Z, Singh A. 2005. Biotechnology for odour and air pollution. Heidelberg, Germany: Springer Verlag.

Van Groenestijn JW, Hesselink PGM. 1993. Biotechniques for air pollution control. Biodegradation 4:283–301.

Zhang GD, Cai WF, Xu CJ, Zhou M. 2006. A general enhancement factor model of the physical absorption of gases in multiphase systems. Chem Eng Sci 61:558–568.

Zhu X, Suidan MT, Pruden A, Yang C, Alonso C, Kim BJ, Kim BR. 2004. Effect of substrate Henry's constant on biofilters performance. J Air Waste Manage Assoc 54:409–418.