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Research

Water Treatment: Enhancing Chlorobenzene Biodegradation by Delfia tsuruhatensis Using Water- silicon Biphasic System

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Abstract : *In spite of being read for more than 20 years, little is thought about the components fundamental the treatment of volatile organic compounds (VOCs) from mechanical off-gases in two-liquid phase bioreactors (TLPBs). Late reports have featured a noteworthy confuse between the high abiotic mass exchange limit of TLPBs and the low VOC biodegradation rates now and then recorded, which proposes that a procedure impediment may likewise be found in the microbiology of the procedure. Subsequently, this examination was led to survey the key job of microbial attributes on the execution of VOC biodegradation in a TLPB utilizing three distinctive hexane debasing consortia. At the point when silicone oil 200 cSt (SO200) was added to the frameworks, the relentless state hexane end limits (ECs) expanded by a factor of 8.7 and 16.3 for Consortium A (hydrophilic microorganisms) and B (100% hydrophobic microorganisms), separately. Within the sight of SO200, Consortium C bolstered a first unfaltering state with a 2-overlap increment in ECs pursued by a 16-overlay EC increment after a hydrophobicity move (to 100% hydrophobic microorganisms), contrasted with the framework denied of SO200. This work uncovered that cell hydrophobicity can assume a key job in the effective execution of TLPBs, and as far as we could possibly know, this is the principal report on hydrophobic VOC treatment with selective VOC take-up inside a nonbioavailable non watery stage. At long last, a free arrangement of analyses demonstrated that metabolite collection can likewise seriously hinder TLPB execution regardless of the nearness of SO200.*

Introduction

The natural treatment of unstable natural mixes (VOCs) from off-gases is much of the time tested by the hydrophobic nature of some VOCs (e.g hexane, pentane, and terpenes), which restricts the VOC move from the gas to the watery stage, where biodegradation as a rule takes place.1,2 These frameworks are additionally constrained by lethality issues due to the amassing of dangerous metabolites while treating high VOC stacking rates.3 Two-fluid stage bioreactors (TLPBs) depend on the expansion of a non-watery stage (NAP) with a high proclivity for the objective hydrophobic VOC so as to conquer mass move constraints and microbial inhibition.4 The nearness of a Snooze can give a general higher main impetus for mass move and instigate an expansion in the gas interfacial area,5 which at last upgrades the exchange of hydrophobic VOCs what's more, in this way their biodegradation rates.6,7 Moreover, the NAP can keep up the grouping of poisonous substrates and metabolites delivered underneath subinhibitory levels, therefore improving procedure robustness.8−10 Late examinations on VOC mass exchange and biodegradation in TLPBs demonstrated that increments in the VOC mass exchange due to a NAP expansion under abiotic conditions didn't result in practically identical increments in VOC biodegradation. For example, Hernandez et al.11 saw that the hexane end limits accomplished in a TLPB were fundamentally lower than the hexane move limit of the framework recorded under abiotic conditions. Correspondingly, Rocha-Rios et al.12 detailed no critical upgrades in CH4 biodegradation in a TLPB executed in a transport bioreactor, while the equivalent TLPB under abiotic conditions bolstered a 6-crease increment in the volumetric mass exchange coefficient comparative with a control reactor denied of NAP. Thusly, these ongoing trial discoveries propose that microbial qualities can likewise assume a key job in the VOC biodegradation execution of TLPBs. In such manner, a few creators affirmed that microbial attributes like cell hydrophobicity can emphatically influence the biodegradation execution of hydrophobic contaminations in TLPBs. For example, Ascon-Cabrera and Lebeault13,14 ascribed the higher biodegradation execution of 2,4,6- trichlorophenol in a TLPB (contrasted with a control denied of NAP) to the cell connection to the NAP/watery interface. MacLeod and Daugulis15 announced that Mycobacterium PYR-1 displayed unprecendently high polycyclic fragrant hydrocarbons biodegradation rates likely because of its capacity to develop specially in the NAP, which expanded polycyclic fragrant hydrocarbons bioavailability contrasted with the fluid stage. In spite of the enormous number of studies led to date in the field of TLPBs, the vast majority of them

concentrated on the choice of the ideal NAP as a key procedure parameter in TLPB execution and overlooked the organic part of this innovation. This examination was along these lines directed to evaluate the key job of microbial qualities on the exhibition of VOC biodegradation in TLPBs. Thus, the exhibition of three diverse hexane corrupting consortia was assessed in a TLPB actualized in a mixed tank. Silicone oil 200 cSt (SO200) and hexane were utilized as model NAP and hydrophobic VOC, individually. What's more, the impact of metabolite collection on hexane biodegradation (because of procedure activity at diverse mineral salt medium trade rates) was further evaluated in an autonomous arrangement of tests.

Materials And Methods

Microorganisms. Three bacterial consortia isolated under different conditions were used in this work: Consortium A was isolated from 2 mL of activated sludge (Valladolid sewage work, Spain) cultured in 1 L-glass bottles containing 100 mL of mineral salt medium and 5 mL of 2,2,4,4,6,8,8-heptamethylnonane. Heptamethylnonane was not directly in contact with the mineral salt medium but placed in a separate compartment in the bottle. This NAP was used for the isolation of Consortium A at low hexane concentrations due to the high affinity of heptamethylnonane for hexane (Gas phase/ heptamethylnonane partition coefficient = 0.0027). The bottles were incubated at 30 °C under magnetic agitation (200 rpm) and supplied with 10 μL of hexane (corresponding to an initial headspace concentration of 2 g m−3) every 3−4 days for 22 days. To furnish fresh inoculum, Consortium A was further enriched as above-described for 20 days prior to experimentation. Consortium B was obtained from a biotrickling filter treating a VOC mixture at trace level concentrations (0.28 ± 0.02 mg m−3 of hexane, 0.22 ± 0.03 mg m−3 of toluene, 0.23 ± 0.03 mg m−3 of α-pinene and 22 ± 2 mg m−3 of methyl mercaptan,) for approximately 5 months. The biotrickling filter was packed with polyurethane foam, inoculated with activated sludge (Valladolid sewage work, Spain) and the operational conditions can be found elsewhere.16 To furnish fresh inoculum, a 2-L glass-bottle containing 1 L of mineral salt medium was inoculated with 2 mL of the biofilm present in the biotrickling filter and continuously supplied with 1 L min−1 of gaseous hexane at 5− 10 mg m−3 . The culture was incubated at room temperature under magnetic agitation (200 rpm) for 40 days.

Chemicals

All chemicals for mineral salt medium preparation were purchased from Panreac (Barcelona, Spain), with a purity of at least 99%. Mineral salt medium was prepared according to Hernandez

et al.11 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 (compatible with biological applications) was purchased from Sigma−Aldrich (Madrid, Spain). Experimental Setup and Operation Mode. A sterile 3-L jacketed glass reactor (Afora S.A., Spain) equipped with two marine impellers was initially filled with 1900 mL of sterile mineral salt medium and 100 mL of the corresponding fresh bacterial inoculum. The system was operated at 300 rpm and 30 °C in the absence of SO200 for 10 days until a steady state was achieved. At day 10, 400 mL of cultivation medium were replaced with 400 mL of sterile SO200 (corresponding to a volume fraction of 20%) and the system was operated under similar conditions for 15 days more. The biomass from the 400 mL of cultivation medium drawn was returned (prior centrifugation under sterile conditions) to the bioreactor in order to avoid a microbial activity limiting scenario. Gaseous hexane at 2.1 ± 0.1 g m−3 was continuously supplied through the aeration (1 L min−1 of air filtered through a sterile 0.2 μm Millex1-FG membrane filter) resulting in a loading rate of 64 ± 1gm−3 h−1 .

Performance of Hexane Biodegradation by Different Microbial Consortia.

The performance of Consortium A, B, and C was evaluated independently using the experimental setup and operational protocols described above. In order to elucidate whether hexane transfer from the gas to the liquid Environmental Science & Technology Article 4060 dx.doi.org/10.1021/es204144c | Environ. Sci. Technol. 2012, 46, 4059−4066 phase was limiting the process performance, the bioreactor was operated at a higher stirring rate (500 rpm) from days 22 to 25 and subjected to a 3 h step increase in hexane loading rate (by doubling the hexane inlet concentration and maintaining the air flow constant at 1 L min−1) at the end of each experiment. During the 3 h step increase, gas samples were taken every 1.5 h to monitor the inlet and outlet hexane and CO2 concentrations. Hexane biodegradation performance was evaluated in terms of elimination capacity (EC, g m−3 h−1) and removal efficiency (RE, %). The main operational conditions of this section are summarized in Table 1.

Table 1. Operational Conditions for Consortia A, B, and C

stage	period (days)	D (day^{-1})	agitation rate (rpm)	hexane concentration ($g\ m^{-3}$)
control	0–10	0.025	300	2
20% SO200	10–11	0.025	300	2
	11–15	0.35	300	2
	15–17	0.70	300	2
	17–22	1.0	300	2
	22–25	1.0	500	2
	the last 3 h of the experiment	1.0	500	4

Results And Discussion

The isolation strategy was mainly based on the exposure of the microorganisms to different gaseous hexane concentrations, which resulted in consortia with different macroscopic characteristics (e.g., different hydrophobicities). Other studies have shown that the operation mode can influence the cell hydrophobicity.14 Unfortunately, in this particular study the authors could not, a priori, establish a clear relationship between the isolation conditions and cell hydrophobicity. The hydrophobicity definition was based on the behavior of the cultures in the presence of the NAP in the bioreactor, since it was not possible to measure directly the cell hydrophobicity because the biomass was totally adhered to the NAP (even at a centrifugal force of 45 000 × g the biomass could not be separated from the silicone oil). However, culture absorbance measurements in the bioreactor follow the same methodology used in the BATH method, which is a well-known technique to quantify the cell hydrophobicity.18 In addition, despite no molecular-biology based characterization of the microbial consortia was performed, optical microscopic observations confirmed the bacterial nature of all consortia here tested.

Performance of Hexane Biodegradation by Consortium

A. Steady state ECs of 3.4 ± 0.5 g m−3 h−1 (corresponding to REs of 5.7 ± 0.6%), CO2 production rates of 4.3 ± 0.7 g m−3 h−1 and culture absorbance values of 0.27 ± 0.01 were recorded for Consortium A in the absence of SO200 (days 1−10) (Figure 1). During this stage, the pH values decreased from 7.0 to 6.2, total nitrogen decreased from 180 to 173 mg L−1 while TOC ranged from 12 to 78 mg L−1 . The TOC values increased by days 9−10 likely due to the accumulation

of hexane biodegradation metabolites as a result of the low dilution rate used in this stage (0.025 day−1).

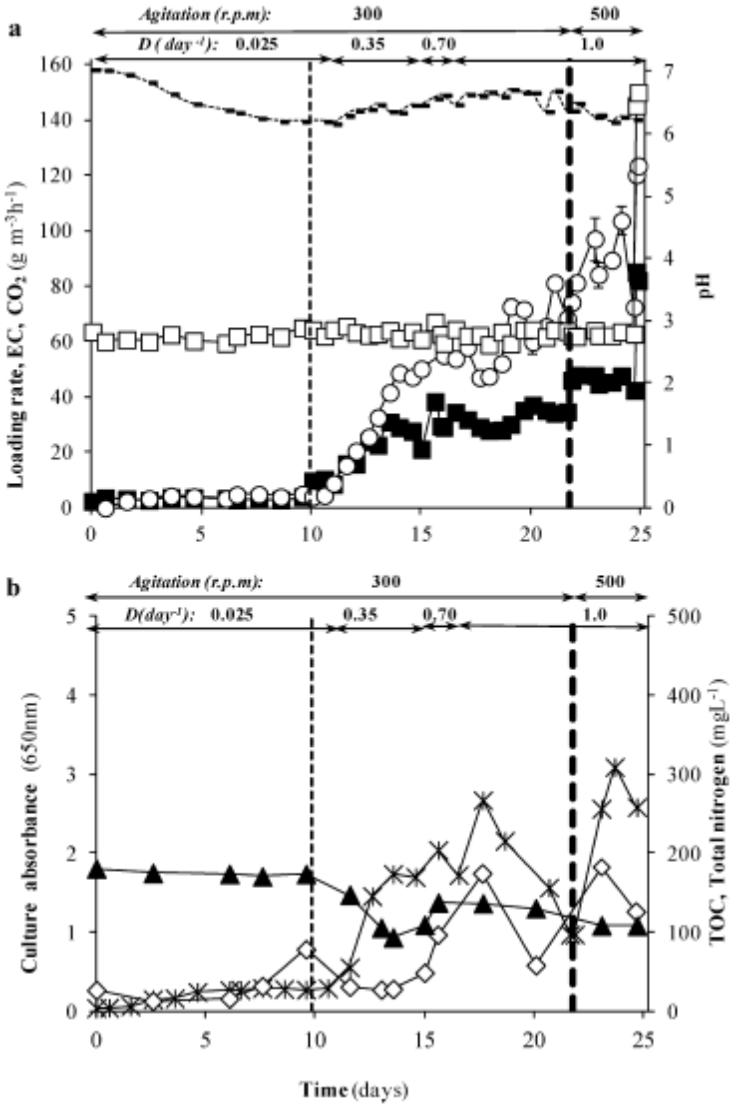
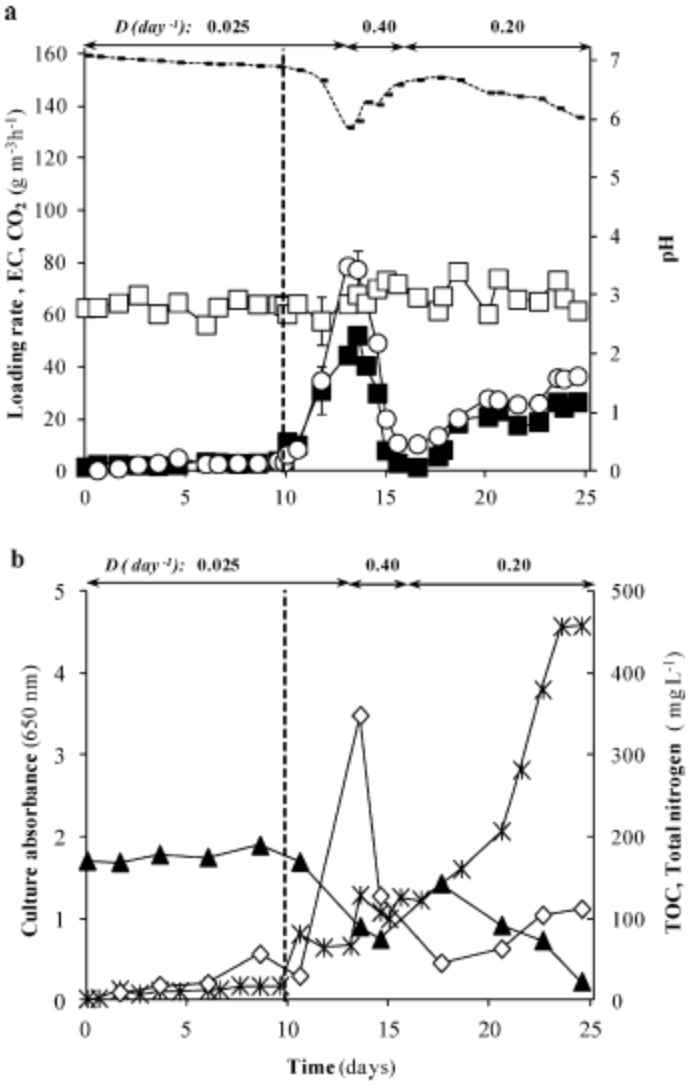


Figure 1. Time course of (a) the hexane loading rates (□), ECs (■), CO2 production rates (○), pH (−) and (b) culture absorbance at 650 nm (*), TOC (◊), and total nitrogen (▲) during hexane biodegradation by Consortium A without SO200 (days 1−10) and with SO200 (days 10−25). Vertical bars represent the standard deviation of duplicate measurements and horizontal arrows the medium exchange rates and the stirring rates.

Assessing the Effect of Metabolite Accumulation on Hexane Biodegradation. An additional hexane biodegradation experiment using Consortium A as model microbial community was conducted in order to assess the potential inhibitory effect of metabolite accumulation on hexane

biodegradation. Consortium A was selected since it was exposed to a known metabolite concentration and because it grew preferentially in the aqueous phase. When the system was operated without SO200 (days 1−10), process performance remained similar to the previous experiments conducted without an NAP (steady state ECs and CO2 production rates of 3.5 ± 0.4 g−3 h−1 and 3.2.0 ± 0.4 g−3 h−1 , respectively)



(Figure 4). However, when SO200 was added, the EC and CO2 production rate rapidly increased up to a maximum value of 52.1 g m−3 h−1 (RE of 76.6%) and 78.6 g m−3 h−1 , respectively (day 13). This intense hexane biodegradation (1.7% higher than the steady state ECs in the previous experiment conducted with Consortium A at higher Ds) was concomitant with a rapid increase in the TOC values, which suggested an important accumulation of metabolites (maximum value of 347 mg L−1). Thereafter, the EC decreased to values even lower than those observed in the

absence of SO200 (1.6 g m−3 h−1). From day 18 onward, a gradual recovery in the EC was observed, achieving a final steady state at 22.6 ± 3.6 g−3 h−1 (RE of 34.1 ± 6.2%) by day 20. In this context, microbial inhibition due to pH or nitrogen limitation must be ruled out since pH and total nitrogen values remained above 6 and 23 mg L−1 , respectively, during the whole experiment. However, the steady state ECs obtained in this experiment were 1.4 lower than those obtained in the first experiment conducted with Consortium A at higher D values (1400−2000 mL day−1) under comparable experimental conditions (Figure 1a). This suggests that hexane biodegradation could have been inhibited partially due to the presence of metabolites excreted as a result of the increased availability of hexane mediated by the addition of SO200. At this point, it is Figure 4. Time course of (a) the hexane loading rates (□), ECs (■), CO2 production rates (○), pH (−) and (b) culture absorbance at 650 nm (*), TOC (◊) and total nitrogen (▲) during hexane biodegradation by Consortium A without SO200 (days 0−10) and with SO200 (days 10−25) in a system operated at a lower D in order to assess the effect of metabolite accumulation. Vertical bars represent the standard deviation of duplicate measurements and horizontal arrows the medium exchange rates. Environmental Science & Technology Article 4064 dx.doi.org/10.1021/es204144c | Environ. Sci. Technol. 2012, 46, 4059−4066 important to highlight that despite hexane biodegradation deteriorated, microbial growth at the expenses of previously excreted metabolites and also due to a significant hexane removal is still possible and might explain the final increase in turbidity (Figure 4). In addition, the final ECs obtained in this experiment (Figure 4) were 2 times lower than those obtained in the experiment conducted with Consortium B (Figure 1) with similar TOC concentrations at the end (around 100 mg L−1), which ruled out the hypothesis of inhibition in the experiment carried out with consortium B and confirmed the key role of the cell hydrophobicity in this study. As a result of the large D required in these systems to avoid metabolite accumulation and therefore a metabolite-mediated inhibition, an alternative operation mode including a settler in series with the bioreactor might be used. The settler implementation would allow for the phase separation (biomass containing NAP from the aqueous broth) and consequently a continuous exchange of mineral salt medium could be performed. This integrated process has recently been proposed by Darraq et al.25 Further experiments in gastight serum bottles also supported the hypothesis of a deteriorated hexane biodegradation performance due to metabolite accumulation. The hexane biodegradation rate at a ratio 30:0 (broth from bioreactor: fresh medium) was similar to that at ratio of 15:15, while hexane degradation in fresh mineral medium (ratio 0:30) was

significantly ($p > 0.05$) higher than at the two former ratios (Figure S6, Supporting Information). A preliminary characterization of the aqueous phase according to the extraction protocol given by Bordel et al.,26 allowed the identification of hexanol and acetic acid as intermediates of the hexane biodegradation. Hexanol is a common metabolite of the aerobic hexane biodegradation pathway,27 whose partitioning coefficient NAP-water is ~8, which indicates that hexanol was preferably dissolved in the NAP. However, the presence of acetic acid in the aqueous phase could be the responsible of the pH drop. It is important to stress that a potential inhibition due to hexane accumulation was ruled out for all consortia since the TOC values in the aqueous phase were much higher than the maximum aqueous hexane concentration (0.03 mg L^{-1}) at an inlet gaseous hexane concentration of 2 g m^{-3} . However, the increased loading experiments showed that the systems were limited by mass transfer in the presence of silicone oil, which means that hexane concentration was 0 in the aqueous phase for consortium A and 0 in the NAP for consortia B and C. Likewise, a potential inhibition by low pH values was ruled out since the initial pH of the three test media ranged from 6.5 to 7 and the experiments only lasted for 4 h. Foaming is one of the most important operational problems reported in TLPBs constructed with liquid NAPs such as 2-undecanone, dodecane, hexadecane, and silicone oil.28−30 In our particular study, while hexane biodegradation by Consortium A was characterized by an intense foaming at high EC values, which was partially controlled by antifoam addition (maximum concentration of 250 μL/Lreactor). Consortium B supported a foam-free degradation with the subsequent benefit on process operation, which suggests that biosurfactants/ bioemulsifier production was not significant in this particular experiment. However, Consortium C was characterized by an intense foaming following SO200 addition, which disappeared right after the hydrophobicity shift. This rapid dissapearance of foaming suggests that biosurfactants/bioemulsifier production was limited when microorganisms were confined in the NAP. Therefore, this study suggested that foaming also depends on the specific interactions between the microbial community supporting VOC biodegradation and the NAP. The mechanisms controlling the diffusion of nutrients, water (and therefore pH), and metabolites between the aqueous phase and the NAP were not assessed in this work. However, these issues deserve further research. In summary, the performance of the three microbial consortia evaluated in the absence of SO200 was similar and in accordance with the low solubility of hexane in water (low hexane transfer rates). When SO200 was added to the systems, the steady state hexane ECs increased by a factor of 8.7 and 16.3 in the case of Consortia A and B, respectively.

However, Consortium C supported a first steady state with a 2-fold increase in ECs followed by a 16-fold EC increase after the hydrophobicity shift. Our results suggest that the ability of Consortia B and C to grow immersed in SO200 (where the hexane solubility is ∼13 000 times higher than in the aqueous phase), as a result of their hydrophobic nature, supported an enhanced substrate bioavailability (by using the entire potential of the gas-NAP transfer pathway). This study revealed that cell hydrophobicity can play a key role in the successful performance of TLPBs, and to the best of our knowledge, this is the first report on hydrophobic VOC treatment with exclusive VOC uptake within a nonbioavailable NAP. Finally, it was shown that metabolite accumulation might inhibit TLPB performance despite the presence of SO200.

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Conflicts of Interest

There are no conflicts to declare.