International Biodeterioration & Biodegradation 84 (2013) 1–7

Contents lists available at SciVerse ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Hexadecane aqueous emulsion characterization and uptake by an oil-degrading microbial consortium

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ARTICLE INFO

Article history:
Received 27 February 2013
Received in revised form
12 April 2013
Accepted 19 May 2013
Available online

Keywords:
Hexadecane uptake
Oil-degrading consortium
Emulsion

ABSTRACT

In the present work was characterized in abiotic and biotic systems, the droplet size of hexadecane (HXD) in emulsified form. Furthermore it was assessed the uptake of HXD in their both form emulsified (microscopic droplets) and free (macroscopic droplets), using a microbial consortium with the capacity of degrading oil. HXD in emulsified form includes microscopic droplets of 0.1 and 0.5 up to 0.7 μm. In the biotic experiments the kinetic parameters values were determined either by fitting to the Contois model the consumed data of HXD emulsified and by considering also the uptake rate of the free forms of HXD as independent of their own concentration. A comparison of the maximum specific HXD uptake rate (q_{max}) of the oil-degrading consortium when consumes the two forms of HXD, shows to be 53 times greater for the emulsified HXD that for their free form, suggesting that consumption of HXD is realized mostly by the emulsified form. The specific transfer area decreases with the culture time due to that the HXD is emulsified and consumed by the microbial consortium, being the specific transfer area of emulsified forms (microscopic droplets) a parameter that must be considered in the design of biodegradation processes of insoluble organic pollutants.

1. Introduction

The increasing demand of global energy requirements during the last years has resulted in water and soil deterioration by pollution of the oil industry (Bridjanian and Samimi, 2011). The overexploitation of oil wells, associated to uncontrolled spills and improper disposal procedures of hydrocarbons are some common causes of this pollution. Insoluble contaminants in water and soil, particularly oil derivatives, are widely studied due to their constant and persistent negative impact in the environment, as well as the economic importance for the contaminated sites restoration (Saval, 2000). The biotechnological processes are able to remediate contaminated sites and are based on the use of microbial consortia to degrade hydrocarbons (Lizardi-Jiménez et al., 2012).

One of the most important oil related hydrocarbon groups is the alkanes, being the hexadecane (HXD) widely used as a compound model for oil degradation studies because it is easy to monitor and yields high biomass values (Inakollu et al., 2004). The hexadecane biodegradation usually requires the cooperation of several microbial species with different metabolic capabilities, since the use of microbial consortia enhances the consumption rate of this hydrocarbon (Ghazali et al., 2004). In the consortia, some strains possess the genotype to degrade hydrocarbons and others, the ability to produce biosurfactants (Saravanan et al., 2009). In a recent work Tzintzun-Camacho et al. (2012), using HXD as unique source of carbon and energy, cultured a consortium consisting of four microorganisms (*Xanthomonas* sp, *Acinetobacter bouvetii*, *Shewanella* sp and *Defluvibacter lusatiensis*). In this work was emphasized the interactions among the strains, which gave to the consortium a special ability to degrade hexadecane (HXD). For this consortium one of the strains (*Xanthomonas* sp) possesses the gene that coding for the synthesis of alkane mono-oxygenated enzyme (alk-B) and, degrades 46% of the initial HXD. Another strain (*A. bouvetii*) of the same consortium produces a biosurfactant (possibly emulsan) favoring HXD consumption (72%).

The HXD is a substrate of low solubility in water ($\sim$7.10 mg L^{-1}) which in microbial cultures can be found in free forms (macroscopic drops and soluble) and/or emulsified form (microscopic

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http://dx.doi.org/10.1016/j.ibiod.2013.05.018

droplets), (Mehrnia et al., 2005). The HXD can be degraded through the use of emulsified forms (microscopic droplets) or by direct contact with the free HXD (macroscopic droplets) and the soluble HXD. Once bioavailable, the HXD is a source of carbon and energy for many microorganisms in pure cultures (Pepi et al., 2005) and in microbial consortia (Medina-Moreno et al., 2005; Lizardi-Jiménez et al., 2012).

In microbial cultures the apparent concentration of HXD via biosurfactants can be increased to 62 mg L^{-1} (Lizardi-Jiménez et al., 2011), to make the HXD highly bioavailable. Considering that the consumption of HXD could be carried out through emulsification of their free form, three critical stages are distinguished: (i) transporting hydrocarbons from the organic phase to the aqueous phase, (ii) transporting oxygen from the gas phase to the aqueous phase and (iii) the consumption of emulsified hydrocarbons and oxygen by microorganisms, mainly residing in the aqueous phase. Therefore, the rates of mass transfer and consumption are key parameters which determine the successful design and operation of the bioreactor to be used in the process of aerobic biodegradation of hydrocarbons (Quijano et al., 2010). On the other hand, it is considered that the consumption of HXD can also be carried out on it is free form by direct contact with macroscopic droplets and the soluble fraction. Taking in consideration this phenomenon, does not imply a limitation on the mass transfer. The direct contact is not imposing a further limitation which is additional to the emulsification process (where a liquid is dispersed into another form of droplets). For both cases, direct contact and emulsification, is always the area of macroscopic droplets which limit either the HXD consumption or transfer rate. The hexadecane in abiotic systems of aqueous emulsions has been widely characterized; however, there is scarce information available about these systems in presence of microbial consortia. Furthermore for environmental purposes, there is also a lack of information about the uptake of insoluble and emulsified substrates by microbial consortia.

The aim of this work was to characterize in abiotic and biotic systems, the droplet size of HXD in emulsified form via the Tween 20 surfactant, as well as the assess uptake of HXD in their both emulsified and free form (macroscopic droplets), using a microbial consortium with the capacity of degrading oil. The HXD dispersion in emulsions was characterized by measuring the diameter of microscopic droplets to determine the specific transfer area of HXD (a_{HXD}, defined as the ratio of the sum of the area of all HXD droplets, between the volumes of aqueous system, Torres-Martínez et al., 2009) to different saturation concentrations of emulsified hexadecane. Moreover, the consumption of hexadecane emulsified was assessed through to compare the kinetic parameter values obtained by the experimental data of the Contois model, with kinetic parameter values determined from experimental data of the consumed free form of HXD.

2. Materials and methods

2.1. Microbial consortium

A rhizospheric bacterial oil-degrading consortium (*Xanthomonas* sp, *A. bouvetii*, *Shewanella* sp and *D. lusatiensis)* was cultivated in a previously reported mineral medium (Lizardi-Jiménez et al., 2012) added with 13 g L^{-1} of HXD (Sigma–Aldrich, 99.7%). A 10-Liters airlift bioreactor (ALB, Fig. 1) was used in this work to cultivate and maintain the rhizospheric bacterial oil-degrading consortium. An ALB cylindrical vessel was made of Pyrex glass (0.14 m diameter; 1.0 m height) provided with a draft tube (0.09 m diameter; 0.54 m height) located 0.035 m above the bottom. Air was sparsed through the draft tube with an L-shaped perforated (7 orifices; 1.0 mm diameter) stainless steel 1/4 inch internal diameter.

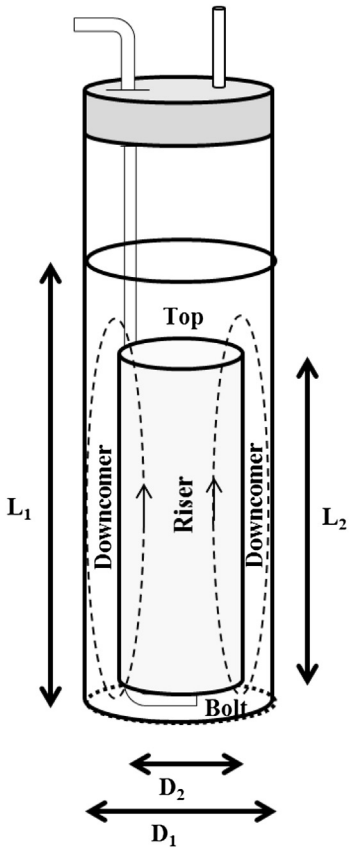

Fig. 1. Airlift Bioreactor (ALB) used to cultivate and maintain the rhizospheric bacterial oil-degrading consortium.

Geometrical relations and the flow pattern are shown in Fig. 1, in brief: D_1 and D_2 are reactor (0.14 m) and draft tube (0.09 m) diameters respectively; L_1 and L_2 represent reactor (0.70 m) and draft tube (0.54 m) heights; riser, top clearance, down comer and bottom clearance are identified. Geometrical relations: $D_2/D_1 = 0.65$, $L_2/L_1 = 0.77$ and $L_1/D_1 = 5$ were used (Lizardi-Jiménez et al., 2012).

2.2. Abiotic medium

In order to adjust the bulk HXD concentration under saturation ($\boldsymbol{C}^*$HXD) up to 62 mg HXD L^{-1}, as have been reported (Bai et al., 1997; Lizardi-Jiménez et al., 2011), an abiotic medium was designed by adding different Tween 20 (0–0.15 mL L^{-1}) concentrations, HXD (13 g L^{-1}) and a mineral medium (g L^{-1}): 6.75, $NaNO_3$ (J. T. Baker, 99. 9%); 2.15, K_2HPO_4 (J. T. Baker, 99.3%); 1.13, KCl (J. T. Baker, 99.9%) and 0.54, $MgSO_4 \cdot 5\ H_2O$ (J. T. Baker, 100.1%). pH was adjusted to 6.5 with 1 N HCl. Surface tension was measured in order to compare the chemical surfactant (Tween 20) to the biosurfactant emulsification ability.

2.3. Emulsion characterization and HXD uptake assessment

With the aim of characterizing under abiotic conditions the emulsified hexadecane, the next experiments were carried out. 125 baffled flasks were filled with 20 mL of the abiotic medium at minor or equal HXD saturation concentration ($\boldsymbol{C}^*$HXD) of 62, 45, 30, 15 y 5 mg HXD L^{-1} using Tween 20 as surfactant and maintaining the flasks to 150 rpm and 28 °C. 2 mL of each flask were taken and analyzed with a particle size meter (Zetasizer, Nano-ZS, Malvern, R.U.). The particle size meter determines the size by first measuring