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Predominant mode of diesel uptake: Direct interfacial versus emulsification in multiphase bioreactor

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HIGHLIGHTS

- Strategies focused on mass transfer may not be enough to design multiphase reactors.
- Working without emulsifiers diesel are consumed by direct interfacial contact.
- Direct interfacial, no emulsification, is the predominant mode of diesel uptake.

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ABSTRACT

Hydrocarbon uptake by microorganisms in a multiphase bioreactor can be carried out by two mechanisms: direct interfacial contact of microorganisms with hydrocarbon drops or emulsified microdroplet uptake. Most previous studies have considered uptake mediated by biosurfactants to be the predominant mode of uptake, but scarce experimental information is available about which mechanism actually prevails. The aim of this work was to evaluate the predominant mode of diesel uptake in multiphase bioreactors. In the absence of emulsifiers in bioreactor, day 0 to day 2 of culture time of oil–degrading consortium composed of three bacterial genera: *Pseudomonas*, *Vibrio* and *Diplococcus*, 6870 mg L^{-1} of diesel was consumed, which can only be explained by direct interfacial contact. In the presence of emulsifiers, at a superficial gas velocity (Ug) of 2 cm s^{-1} from day 5 to day 7, 3460 mg L^{-1} of diesel was consumed and the maximum diesel transfer rate (DTR) (16.3 mg L^{-1} h^{-1}) for this Ug could only explain 782.4 mg L^{-1} of diesel uptake. Our study reveals that strategies focused only on mass transfer may not be sufficient to design multiphase bioreactors since direct interfacial, not emulsification, is the predominant mode of diesel uptake.

1. Introduction

Spills of petroleum and petrochemical products, such as diesel, in soil and water are susceptible to microbial degradation processes (Das and Chandran, 2010; Arora and Bae, 2014). Microbial consortia can be cultured in airlift reactors for environmental purposes, such as diesel uptake (Lizardi-Jiménez et al., 2015). Hydrocarbon uptake by a microbial consortium in a bioreactor can be carried out by two mechanisms: uptake by direct interfacial contact of microorganisms with diesel drops (Bouchez-Naïtali et al., 2001; Abbasnezhad et al., 2011) and uptake by emulsified forms of hydrocarbons (Medina-Moreno et al., 2013).

Most studies consider uptake mediated by biosurfactants to be the predominant method carried out by microorganisms (Ron and Rosenberg, 2002; Bento et al., 2005; Cameotra and Makkar, 2010) but because there is scarce information about which of the two uptake mechanisms prevails during the degradation of hydrocarbons (Owsianiak et al., 2009; Bouchez-Naïtali and Vandecasteele, 2008), it is necessary to evaluate both direct interfacial and emulsified uptake, as a criteria for bioreactor operation. Airlift bioreactors produce high rates of hydrocarbon degradation; however, knowledge of the mechanism of uptake is necessary (Lizardi-Jiménez et al., 2012), particularly because the predominant type of uptake could be a key factor for bioreactor design and operation. Understanding the predominant mechanism for the uptake of oil, diesel, and other non-miscible substrates is critical because it is directly related to mass transfer and hydrodynamics in bioreactors, and is therefore an important criteria for their successful operation.

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The aim of this work was to determine the predominant mode of diesel uptake: direct interfacial or emulsification – in an airlift bioreactor.

2. Materials and methods

2.1. Microbial consortium

The indigenous hydrocarbon–degrading bacterial consortium was isolated in a previous work from a hydrocarbon polluted sinkhole in Quintana Roo, south of Mexico, and identified by biochemical tests (Medina-Moreno et al., 2014). The oil–degrading consortium was composed of three bacterial genera: *Pseudomonas*, *Vibrio* and *Diplococcus*. The native consortium was cultured and grown in a sequential batch airlift bioreactor (ALB) with a previously reported mineral medium containing (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%). The pH was adjusted to 6.5 with 1 N HCl (Lizardi-Jiménez et al., 2012).

2.2. Bioreactor set up

A 1.2 L airlift bioreactor (ALB) with cylindrical vessel and draft tube located 1.36 cm above the bottom (stainless steel 1/4 in. internal diameter; 7 orifices, 1.0 mm diameter) was made of glass (Pyrex) (height/diameter = 5; ratio of vessel diameter to draft tube diameter = 0.66). Airflow was supplied through the ALB with an L-shaped air diffuser at 28 °C.

2.3. Physicochemical properties

The surface tension of the cultures was determined with a bubble tensiometer (SensaDyne, USA) at 28 °C with distilled water and ethanol used as reference fluids. Viscosity was determined at 28 °C using a rotary viscometer (Brookfield, USA). Density was calculated using the gravimetric technique based on the weight of 50 mL of the liquid culture.

2.4. Emulsifying activity

The emulsifying activity was determined from samples (5 mL) taken from the reactor at different times. Samples were centrifuged at 4000*g* at 4 °C for 25 min, then 100 μL of supernatant (free cells), 2.6 mL of buffer TRIS-HCl (20 mM, pH 7) and $MgSO_4$ (10 mM) were added. 100 μL of a mixture containing 2-phenyl-naftaleno: diesel 1:1 (v/v) was then added immediately. Finally, the samples were sonicated for 5 min in order to form an emulsion. The samples were allowed to stand for 24 h after which the optical density was measured at 600 nm. One Emulsifier Unit (EU mL^{-1}) was defined as a 0.1 change in absorbance units at 600 nm under assay conditions using standard as a reference.

2.5. Mass transfer parameters

2.5.1. Oxygen transfer parameters

The dynamic numerical method was used to determine oxygen transfer volumetric coefficient (k_La_{o2}). The concentration of dissolved oxygen (DO) in the bulk of bioreactor was measured with a polarographic oxygen sensor (ADI dO2, Applisens, The Netherlands) and a DO meter (model DO-40, New Brunswick Scientific, USA). Maximum oxygen transfer rate (OTR) was calculated as the product of k_La_{O2} and the oxygen saturation concentration.

2.5.2. Diesel transfer parameters

Gas chromatography was used to evaluate the transferred diesel and consequently the diesel transfer coefficient ($k_{Ldiesel}$). Diesel transfer rate (DTR) was computed by using the balance $DTR = k_La_{diesel}\cdot(C^*_{diesel} - C_{diesel})$ where C^*_{diesel} and C_{diesel} are the saturation diesel-aqueous interphase and diesel aqueous phase concentration, correspondingly and k_La_{diesel} is the diesel transfer volumetric coefficient. The image analyses method allowed determining the resulting specific mass transfer area (a_{diesel}).In brief: k_La_{diesel} and DTR were evaluated using a recently reported novel technique (Lizardi-Jiménez et al., 2011) as follows: an abiotic, transparent, liquid-phase was designed considering biotic values for surface tension (68.9 dynes cm^{-1}) and viscosity (1.15 cP) by adding Tween 20 to a final concentration of 0.081 mg L^{-1}. The abiotic medium was added to the ALB, adding diesel at 13 g L^{-1} in the case of experiments to determine transfer area. A stainless steel cylinder (2.8 mL) was filled with diesel and introduced into the downcomer with the open side downward. Once aeration in ALB reached steady state, 5 mL of abiotic medium were withdrawn every 60 min. The transferred diesel to the aqueous phase was recovered by successive extractions with a sample to solvent ratio of 1:5 (v/v) using a diesel/isotonic solution (1:3, v/v) as solvent. The diesel transfer coefficient ($k_{Ldiesel}$) was obtained from the stainless steel cylinder mass transfer area. k_La_{diesel} was obtained as the product of $k_{Ldiesel}$ and specific mass transfer area of the diesel droplets (a_{diesel}). a_{diesel} was calculated by Eq. (1)

$$a_{diesel} = \frac{6}{d_{32diesel}}\varnothing \tag{1}$$

where $d_{32diesel}$ is the Sauter mean diameter of diesel droplets and Ø is the diesel dispersed phase fraction (dimensionless). $d_{32diesel}$ was measured with a digital camera and image analysis software (Image Pro Plus 4.1., Media Cybernetics, USA). The diesel transfer rate (DTR) was calculated as the product of the volumetric mass transfer coefficient (k_La diesel) and the diesel gradient concentration measured in the aqueous phase. According to previous work (Lizardi-Jiménez et al., 2012) the resulting hydrocarbon-specific area measured in the downcomer can be recognized as the mass transfer-specific area (a_{HXD}) in the whole bulk, riser included. Our work assumes that the concentration measured in the sample is representative of an average concentration in the ALB and the global spatially average diesel volume fraction should change softly.

2.6. Suspended solids

The concentration of suspended solids was determined using the gravimetric method; 5 mL containing free cells from supernatant samples described in Section 2.4 were homogenized and placed in porcelain capsules previously set to a constant weight. The samples were placed in an oven at 100–105 °C for 1 h. The samples were calcined in a muffle at 500 °C for 20 min. The total solid content was calculated based on the weight difference between the samples before and after calcination. The results are reported in g L^{-1}.

2.7. Residual diesel

Gas chromatography (Thermo Scientific model 1310, USA) with a flame ionization at 300 °C, a TR-5 column (30 × 0.00025 m; J&W Scientific), and helium as the carrier gas detector was used to detect and quantify residual diesel in samples taken from the airlift bioreactor. Cromatographic method was: 120 °C for 1 min; rise by 10 °C min^{-1} until 150 °C (3 min); then by 15 °C min^{-1} until 170 °C (1.5 min). The detection limit was 0.003 g L^{-1}. Evaporation of diesel was quantified. Commercial diesel (Pemex) was used as a standard reference.