Evaluation of a New Millifluidic Device for the Consistent Determination of Oil Droplet Biodegradation Kinetics

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Abstract
Natural seeps and accidental releases of crude oil in the sea result in swarms of droplets that are carried away by underwater sea currents. The droplets may be created either at the sea surface during the breakup of an oil slick by sea waves, or at the seafloor during the extrusion of crude oil from natural cracks or broken wellheads. A high concentration of oil droplets in seawater disturbs the established ecosystem dynamics and poses a significant risk of toxic effects to fish and other marine animals. The fate of underwater droplet swarms is determined by natural attenuation processes, mainly dissolution into the seawater and biodegradation by oil-eating microbes. Using microfabrication techniques (photolithography and 3D printing), we have developed a new millifluidic device that enables the generation of oil droplet populations with desired size and, subsequently, the entrapment, long-term incubation and microscopic imaging of the droplets while they undergo microbial degradation. Here, we will present experimental results on the biodegradation of hexadecane droplets by Marinobacter sp. microbes in synthetic saltwater.
Keywords: hexadecane, biodegradation, droplet microfluidics, emulsification

1. Introduction

After a natural or accidental release of crude oil in the sea, part of the oil ends up in the form of droplets moving through the seawater column. The droplets may be formed either at the sea surface through the action of sea waves that breakup floating oil layers (Li et al., 2017), or at the seafloor during the vigorous extrusion of crude oil from a natural crack or a broken wellhead (Zhao et al., 2017). The latter case occurred, for instance, after the blowout of the Deepwater Horizon rig in the Gulf of Mexico where the addition of the chemical dispersant Corexit in the leaking crude oil resulted in swarms of droplets travelling underwater along with sea currents (Camilli et al., 2010). Large amounts of oil droplets in the seawater column disturb the established ecosystem dynamics and pose an imminent risk of toxic effects from various crude oil components to many marine species (invertebrates, fishes, mammals, etc.) (Almeda et al., 2014). At present, there are no practical means for the collection or in situ treatment of dispersed oil droplets in vast bodies of marine waters and, thus, their removal relies mainly on dissolution and biodegradation. With regard to the biodegradation of oily substrates by microbial species, three major strategies have been identified: interfacial degradation by oleophilic microbes, degradation in the bulk aqueous phase by suspended chemotactic microbes, and degradation in a microbial biofilm formed around the oil droplet (Kapellos, 2017; Kapellos et al., 2018). Mechanistic understanding and accurate quantification of the biodegradation of oil droplets in marine waters will be a major enabler towards developing efficient technologies and mitigating the pertinent adverse effects.

2. Objective & Preliminary Results

In this paper, a new approach is presented for the consistent determination of droplet biodegradation kinetics. The approach is based on a combination of micro/milli fluidic devices and particle size analysis methods (microscopic image analysis and dynamic light scattering). With a toolbox of various microfabrication methods (lithography, xurography and 3D printing), we have developed a fluidic devices that enable the generation of a droplet population with desired size distribution, the long-term incubation with specific microbial species and the monitoring of the droplet size distribution. A primitive version of such a device is presented in Figure 1 and consists of two fluidic elements: a droplet generator and a droplet incubator. Preliminary experiments have been carried out with a model oil-microbe-water system consisting of hexadecane (biodegradable aliphatic hydrocarbon) as the model oily phase, a pure culture of *Marinobacter* ATCC 49840 as the model marine microbial species, and a synthetic seawater (Gauthier et al., 1992) as the model aqueous phase. It has been observed that these microbes are able to form biofilms on the surface of hexadecane and produce excessive amounts of biosurfactants that break down the oil into a highly polydisperse emulsion (Figure 2). It is thus expected that these microbes uptake emulsified sub-micron droplets, rather than uptaking hydrocarbon molecules directly from the oily surface.

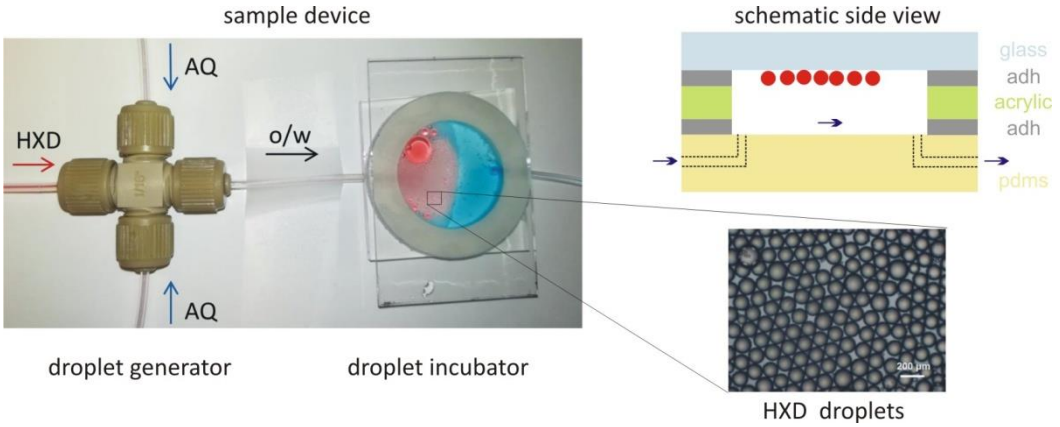

Figure 1. Two-part fluidic device for the study of the biodegradation of oil droplets. The first part (cross) is the droplet generator, and the second part (circular well) is the droplet incubator. The schematic shows the key concept: light oil droplets rise to the upper part of the well, while water flows underneath them. The inset shows hexadecane droplets (~100 μm) generated with this setup.

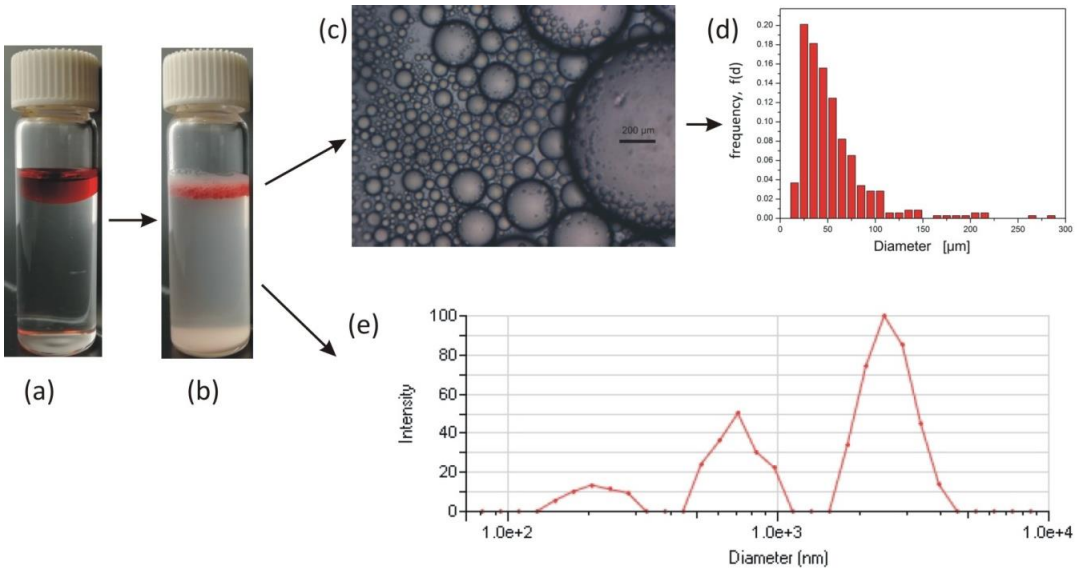

Figure 2. Biodegradation and emulsification of hexadecane by *Marinobacter* microbes. (a) well separated hexadecane (stained red for contrast) and synthetic seawater; (b) oil-in-water emulsion after 6 wks; (c) polydisperse droplets floating on top of the aqueous phase; (d) size distribution of the floating droplets (measured by image analysis); (e) size distribution of suspended particles (measured by dynamic light scattering).

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