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Bacterial proliferation on clay nanotube Pickering emulsions for oil spill bioremediation

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ABSTRACT

Halloysites (tubular aluminosilicate) are introduced as inexpensive natural nanoparticles that form and stabilize oil-water emulsions. Pickering emulsification can proceed with energies low enough to be afforded by ocean turbulence and the stability of droplets extends over more than a week. The oil/water interface is shown to be roughened and bacteria, which are added for oil degradation, are better attached to such oil droplets than to droplets without halloysites. The metabolic activity of *Alcanivorax borkumensis*, alkanotrophic bacteria widely distributed in marine environments, is enhanced by halloysite addition. A halloysite-based dispersant system is therefore environmentally friendly and promising for further optimization. The key elements of the described formulations are natural clay nanotubes, which are abundantly available in thousands of tons, thus making this technology scalable for environmental remediation.

1. Introduction

Pickering emulsions, which are stabilized by solid colloidal particles instead of organic surfactants, are produced by the addition of nano/microparticles positioned on the droplets' interface. These emulsions, named after one of the technique's pioneers S. Pickering [1], may be useful for emulsification of spilled oil [2]. In 2010 an explosion on the Deepwater Horizon (DWH) oil well drilling platform started one of the world's largest marine oil spills, releasing millions of barrels of crude petroleum into the Gulf of Mexico [3]. In an effort to contain the spill, seven million liters of hydrocarbon amphiphilic dispersants were applied at the leak for oil emulsification [4]. The dispersants break the oil plume into minute emulsion droplets and could result in faster bio-remediation of the spill by naturally occurring degrading microorganisms, including *Alcanivorax borkumensis* and *Cycloclasticus pugetti* [5]. These hydrocarbon bio-degraders have been detected in sea waters in close proximity with natural hydrocarbon seepage [5–7]. Most hydrocarbonoclastic organisms in a marine environment are able to produce their own bio surfactants, which also play roles in emulsification of an oil slick. The oil dispersant Corexit EC 9500A, which contains anionic and nonionic surfactants, was deployed in large quantities in response to the DWH spill. This dispersant was found to facilitate formation of "marine oil snow" flakes consisting of bacteria, dispersed oil, and suspended microparticles. The particles of "marine oil snow" thus formed are denser than sea water and precipitate to the bottom [8].

The negative effect of the anionic surfactant sodium dodecyl sulfate and the nonionic surfactant Tween-20, both components, on proliferation of *Alcanivorax borkumensis* was recently shown [9]. Marine bacteria with the ability to degrade components of a dispersant formulation can grow in samples containing up to 100 ppm of dispersants [10]. However, the addition of amphiphilic hydrocarbon dispersant reduces bacterial degradation of crude oil components, as reflected in rates of hydrocarbon oxidation. For example, the degradation rate of hexadecane and naphthalene was 6 and 9 times lower after one week of incubation as compared to the oil sample without dispersants [4]. The major disadvantages of chemical dispersant applications as an oil spill response are the toxicity of the surfactants and harmful solvents such as butoxyethanol present in the formulations [11].

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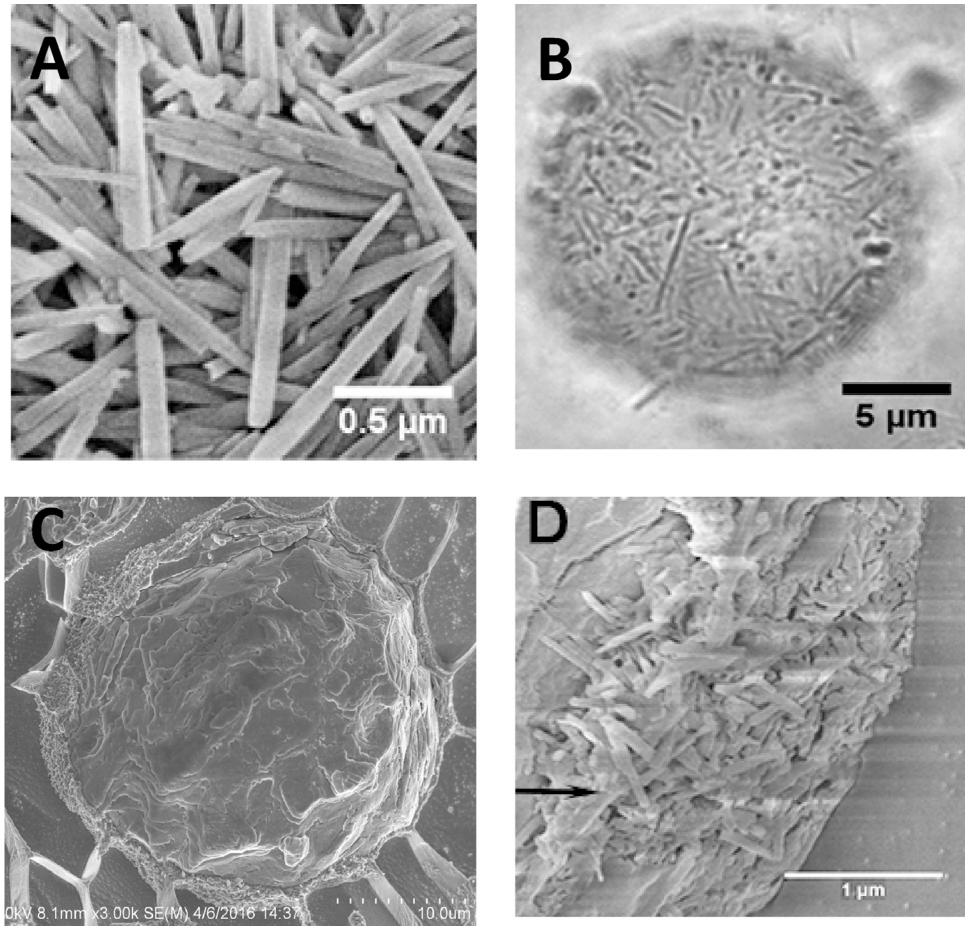

Fig. 1. A) SEM images of halloysite nanotubes. Optical (B) and cryo-SEM (C & D) images of HNT emulsions with oil (hexadecane) showing halloysites on oil-water interface.

Formation of oil in water Pickering emulsions stabilized by nano or micro particles can be an alternate approach to increasing the surface area of spilled oil by breaking it into very small droplets. The choice of particles that can assemble at the oil-water interface and lower the surface energy ranges from silica, latex, clay to even bacterial cells [12–14]. Halloysite clay nanotubes (HNT) are an interesting candidate for such Pickering emulsions as they have been shown to work with crude oil [15–17]. Halloysite is a natural tubular aluminosilicate clay mineral that can be mined from deposits in thousands of tons at purity of 95–98% [18]. The nanotubes vary in length between 400 and 1500 nm, with a diameter of 40–60 nm and inner lumen of 15–20 nm (Fig. 1A). In pristine form halloysite has electric zeta-potential of about −30 mV and water contact angle of $13 \pm 2^\circ$; after silanization with aliphatic chain endings it becomes more hydrophobic with increasing contact angle to $99 \pm 3^\circ$ [17]. The unique properties of halloysite including low cost, environmental friendliness, biocompatibility and hollow structure has made it possible to use these nanotubes as a cargo for loading and slow release of active molecules such as drugs, anticorrosion agents and surfactant [18–21]. By loading the lumen with surfactants like DOSS (dioctyl sodium sulfosuccinate salt), Span 80 and lecithin, Pickering emulsions were combined with traditional amphiphile emulsions [11]. The synergy between the two methods of forming emulsions improved the dispersibility of crude oil [11].

Using high aspect ratio (length to diameter) clay nanotubes for oil emulsification has an advantage as compared with the spherical nanoparticles of same size and chemistry (e.g., silica) [16]. Hydrophobization of the halloysite surface by silane coupling increases the resulting emulsion stability [17,22]. The arrangement of the nanotubes parallel to the interface allows for larger contact area and the mechanical stiffening may supress fluctuations that otherwise might facilitate droplet coalescence.

Alkanes constitute the largest portion of crude oil by mass; hence, the biodegradation of this fraction is quantitatively an important process in the removal of crude oil from the environment. In this study, we first describe the application of pristine and hydrophobized halloysite nanotubes for *n*-hexadecane (model alkane) emulsification, and subsequently demonstrate that the approach works with Macondo crude oil as well. Moreover, we demonstrate that halloysite nanotubes attract the alkane-degrading bacteria *A. borkumensis* to emulsions formed with hexadecane as well as with crude oil, and demonstrate that halloysites stimulate the viability of *A. borkumensis* cultured with hexadecane or crude oil as the sole carbon source.

2. Materials and methods

2.1. Materials

Halloysite clay of 95–98% purity (hereafter referred to as halloysite or HNT) was obtained from Applied Minerals Inc., NY. Hexamethyldisilazane (HMDS), octadecyltrimethylsilazane (ODTMS), decane, dodecane, *n*-hexadecane, naphthalene, hexane, fluorescein diacetate, resazurin sodium salt and acridine orange were purchased from Sigma-Aldrich. *Alcanivorax borkumensis* (*A. borkumensis*) bacteria was purchased from American Type Culture Collection (ATCC® 700651™). The crude oil tested was collected from the Macondo basin post at the Deepwater Horizon oil spill.