



Analysis of biological demulsification process of water-in-oil emulsion by *Alcaligenes* sp. S-XJ-1

Yue Wen, Hang Cheng, Li-Jun Lu, Jia Liu, Yi Feng, Wei Guan, Qi Zhou, Xiang-Feng Huang*

College of Environmental Science and Engineering, State Key Laboratory of Pollution Control and Resource Reuse, Tongji University, Shanghai 20092, China

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ABSTRACT

A demulsifying strain (S-XJ-1) was isolated from petroleum-polluted soil and identified as *Alcaligenes* sp. It showed emulsion breaking ratio of 81.3% for W/O emulsion within 24 h when the cell concentration was 500 mg/L. Evolution of water droplets during the biological demulsification process was investigated using a Turbiscan stability analyzer and microphotography. Further investigation focused on cell surface hydrophobicity and oil–water interfacial properties. The biological demulsification process began with rapid dispersal of the cells into the oil phase and adsorption onto the oil–water interface. This occurred due to high cell surface hydrophobicity and the presence of amphiphilic compounds in the cell walls. The cells had higher interfacial activity than the emulsifier molecules, and they displaced some of the emulsifier molecules, which effectively reduced the interfacial tension gradient. As a result, the interfacial film strength decreased, the water droplets coalesced and eventually phase separation occurred.

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1. Introduction

An emulsion is a dispersion system, in which liquid droplets are dispersed throughout another immiscible liquid. Emulsions are mainly classified by their composition of dispersed and continuous phases as either oil-in-water (O/W) or water-in-oil (W/O). The latter is widely found in paint, pharmaceutical, cosmetic, food and petrochemical industries (Mason et al., 1995). To achieve phase separation in crude oil extraction and petrochemical processing, chemically-derived demulsifiers, which are generally high-molecular-weight polymers, are widely used (Wang et al., 2004). Despite their broad application, chemical demulsifiers have the following disadvantages. Firstly, they are chemically synthesized from crude oil (Mukherjee et al., 2006), and consequently their production costs will fluctuate with the price of international crude oil. It is predicted the cost of chemical demulsifiers will increase as crude oil stocks are consumed and the cost of crude oil rises. Secondly, water-soluble chemical demulsifiers, which preferentially partition to the water phase, will be removed with separated water and inevitably cause environmental contamination when discharged to the environment. Demulsifying bacteria are an innovative solution to this problem and have attracted increasing attention due to their low toxicity, biodegradability and high efficiency in extreme conditions (Das, 2001). Demulsifying bacteria can demulsify the emulsion with their cells or extracellular compounds. To date, the

following demulsifying bacteria have been reported: *Nocardia amarae*, *Corynebacterium petrophilum*, *Rhodococcus aurantiacus*, *Bacillus subtilis*, *Micrococcus* sp., *Torulopsis bombicola*, *Acinetobacter calcoaceticus*, *Alteromonas*, *Rhodococcus*, and *Aeromonas* (Das, 2001; Duvnjak and Kosaric, 1987; Janiyani et al., 1994; Singh et al., 2007). Moreover, some biologically-produced agents such as acetoin, polysaccharide, glycolipid, glycoproteins, phospholipid and rhamnolipid exhibit demulsifying properties (Singh et al., 2007).

A thorough understanding of the demulsification process will facilitate the application of demulsifiers. To date, most demulsification theories are based on studies of the chemical demulsification process, including changes in dispersed droplets, the relationship between interfacial properties and demulsification effectiveness, and model establishment. With the aid of microphotography, it was found that the sizes of water droplets increased while total number of droplets decreased as demulsification progressed (Al-Sabagh et al., 2008; Zhang et al., 2004). Therefore, it was proposed that the demulsification of crude oil emulsion took place in three steps: the replacement of asphaltene in the oil–water interface, flocculation, and coalescence of water droplets. Interfacial properties have an important influence in demulsification, and have been intensively studied. For instance, in the comparison of demulsification performance of different chemical demulsifiers, interfacial elasticity was found to profoundly affect the strength, lifetime and thickness of the interface (Kang et al., 2006). Moreover, a decreased degree of interfacial elasticity was positively correlated with demulsification performance (Kang et al., 2006). It was assumed that the partial replacement of

* Corresponding author. Tel./fax: +86 21 65982592.

E-mail addresses: hxf@tongji.edu.cn, ly008150@online.sh.cn (X.-F. Huang).

emulsifier molecules by the demulsifier resulted in the decrease of interfacial elasticity and eventually destabilization on the emulsion. After studying the effect of various demulsifiers on the elasticity of crude oil and water interfaces, *Ese et al. (1999)* also found that a decrease of interfacial tension increased demulsification efficiency. An efficient demulsifier is generally able to decrease the interfacial tension gradient and interfacial viscosity, which helps increase the film thinning rate and shorten the time taken to reach critical film thickness. *Krawczyk et al. (1991)* found that interfacial activity, adsorption kinetics and oil–water partitioning were three key factors governing demulsification. Among these factors, interfacial activity was correlated with demulsification performance but interfacial shear viscosity was not. *Kim and Wasan (1996)* found that demulsifiers with good oil–water partitioning properties would efficiently reduce the interfacial tension gradient and accelerate film drainage. In addition, these emulsifiers also exhibited high static and dynamic interfacial activity, low interfacial shear viscosity, and low film dilatational modulus, with both a high adsorption rate and excellent demulsifying capability. Some interfacial properties were incorporated into liquid-to-liquid coalescence modeling, which was verified by *Krawczyk et al. (1991)* and *Kim and Wasan (1996)*. The model indicated that the interfacial activity of demulsifiers should be high enough to suppress the interfacial tension gradient and the Marangoni–Gibbs stabilizing effect in order to accelerate the film drainage rate and achieve demulsification.

Compared with chemical demulsification, the research on biological demulsification lags far behind. Most current studies only focus on the relationship between bacterial properties and demulsifying effectiveness. For example, *Gray et al. (1984)* found that cells with low hydrophobicity broke W/O emulsions efficiently, while cells with high hydrophobicity destabilized O/W emulsions efficiently. In another study the demulsification capability of a demulsifying strain, PR-1, was also found to be associated with its hydrophobic cell surface (*Ma et al., 2006*). *Kosaric et al. (1987)* found that the entire demulsification process was dependent on the hydrophobic–hydrophilic balance of the cell wall, which arises from species-specific genetic potential. *Krawczyk et al. (1991)* found that demulsifiers with partition coefficients approaching unity exhibited superior demulsification performance. However, the biological demulsification process was rarely studied.

In this study, an efficient demulsifying strain (S-XJ-1), which was isolated from petroleum-polluted soil in our previous study (*Huang et al., 2009*), was used to study the biological demulsification process. The strain S-XJ-1 was identified as *Alcaligenes*

2. Methods

2.1. Test strain and culture medium

The strain S-XJ-1 was kept at $-4\text{ }^{\circ}\text{C}$ on agar slant plate medium containing 5.0 g/L beef extract, 10.0 g/L peptone, 5.0 g/L NaCl, and 15.0 g/L agar powder (pH 7.0). It was revived and inoculated in 100 mL of broth culture for 72 h in a rotary shaker at 130 rpm and $35\text{ }^{\circ}\text{C}$. The broth culture contained (L^{-1}): beef extract 3.0 g, peptone 10.0 g, and NaCl 5.0 g at pH 7.0–7.2. After enrichment, a 10 mL aliquot was transferred into 100 mL of modified mineral salts medium (MMSM) with liquid paraffin (4%, v/v) as the carbon source for an 8-day cultivation. The composition of MMSM was (L^{-1}): NH_4NO_3 4.0 g, K_2HPO_4 4.0 g, KH_2PO_4 6.0 g, $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$ 0.2 g, and trace mineral solution 1 mL at pH 9.0–9.2. The trace mineral solution contained (L^{-1}): $\text{CaCl}_2\cdot 2\text{H}_2\text{O}$ 1.0 g, $\text{FeSO}_4\cdot 7\text{H}_2\text{O}$ 1.0 g, and EDTA 1.4 g. After the cultivation, the whole culture was centrifuged at 12,000 rpm for 15 min. The wet cells obtained were suspended in a measured volume of sterilized distilled water to make cell suspensions of various concentrations. Each cell suspension was then dosed in the demulsification test and the dry cell weight was measured after lyophilization at $-50\text{ }^{\circ}\text{C}$ for 24 h.

2.2. Demulsification test

W/O model emulsion preparation mainly followed the previously published protocol (*Liu et al., 2009*). Distilled water (containing 1.9% w/v Span 80) and aviation kerosene (containing 0.1% w/v Tween 80) were mixed at a volume ratio of 3:2 at 10,000 rpm for 3.5 min with the aid of high speed emulsifying machine (WL-500CY, Shanghai Wei Yu Mechanical and Electrical Manufacture Ltd. Co., China). Span 80 and Tween 80 were purchased from Shanghai Shenyu Pharmaceutical and Chemical Ltd. Co. (China). The emulsion type was identified by Oil Red O test as described elsewhere (*Lee and Lee, 2000*). The fresh emulsion had an emulsion breaking ratio of <10% at $35\text{ }^{\circ}\text{C}$ within 24 h.

In the demulsification test, 2 mL of cell suspension (25–5000 mg/L) was added to a 20 mL graduated test tube containing 18 mL of the model emulsion (*Akit et al., 1981*). The test tubes were vigorously inverted 120 times to achieve complete mixing and then left undisturbed in water baths at $35\text{ }^{\circ}\text{C}$. The volume of separated oil (top layer), separated water (bottom layer) and residual emulsion (middle layer) were recorded at set time intervals. Demulsification performance was evaluated by the oil separation ratio, water separation ratio and emulsion breaking ratio according to the following equations:

$$\text{Oil separation ratio} = \left(\frac{\text{oil volume (on the top)}}{\text{oil volume in original emulsion}} \right) \times 100\% \quad (1)$$

$$\text{Water separation ratio} = \left(\frac{\text{water volume (on the bottom)}}{\text{water volume in original emulsion} + \text{added cell suspension volume}} \right) \times 100\% \quad (2)$$

$$\text{Emulsion breaking ratio} = \left(1 - \frac{\text{remaining emulsion volume}}{\text{original emulsion volume} + \text{added cell suspension volume}} \right) \times 100\% \quad (3)$$

sp. with the aid of physiochemical tests and 16S rDNA analysis. The information was uploaded to GenBank as entry No. EF117974 (*Huang et al., 2010*). Emulsion stability analyzer Turbiscan Lab[®] expert was used to visualize demulsification process. The phenomena observed were further explained by cell surface hydrophobicity and the changes in interfacial properties.

The blank test was conducted with 2 mL of distilled water, and it had an emulsion breaking ratio <10% within 24 h.

2.3. Emulsion destabilization analysis with Turbiscan Lab[®] expert

After adding 2 mL cell suspension (5000 mg/L) into 18 mL of model emulsion, the sample was vigorously inverted 120 times

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