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Research article

Immobilization of biofilms of *Pseudomonas aeruginosa* NY3 and their application in the removal of hydrocarbons from highly concentrated oil-containing wastewater on the laboratory scale

Maiqian Nie*, Hongyun Nie, Meili He, Yingying Lin, Lei Wang, Pengkang Jin, SenYuan Zhang

School of Environmental and Municipal Engineering, Xi'an University of Architecture and Technology, No. 13 Yanta Road, Xi'an 710055, Shaanxi Province, PR China

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ABSTRACT

To explore the potential of *Pseudomonas aeruginosa* NY3 for the treatment of highly concentrated crude oil-contaminated water, the immobilization of strain NY3 on the surface of polyurethane foam (PUF), the conditions for using these biofilms and the possibility of recovering the used biofilms were studied. The results demonstrated that the biofilm formation process for strain NY3 was quick and easy. Under optimum conditions, the biomass immobilized on the PUF surface could reach 488.32 mg dry cell/g dry PUF. The results demonstrated that when the degradation time was 12 h, the average oil removal rate in 2 g crude oil/L contaminated water was approximately 90% for 40d. Meanwhile, the biofilms could be recovered for reuse. The recovery ability and the high and steady oil removal rate facilitated the application of the biofilms for the removal of concentrated oil from wastewater.

1. Introduction

Oil-contaminated water or produced water may originate from a variety of sources, such as oil and gas production, oil refining, petroleum utilization, car washing, and accidental oil spillage and leakage (Wang and Fingas, 1995). The primary contaminants may include almost all types of hydrocarbons (from C1 to C40 straight chain, C6 to C8 branched-chain, cyclohexanes, aromatics) and other organic pollutants such as sulfur-, nitrogen-, and oxygen-containing organic compounds, radioactive substances, humus and surfactants (Wang and Fingas, 1995). These contaminants may be a serious threat to halobios and may destroy the ecological balance, which may take years or even decades to recover. Therefore, the treatment of such contaminated water has always been a significant issue for the environment.

Methods for treating water contaminated with concentrated oil and greasy materials require pretreatment processes, including gas flotation processes, coagulation flotation, gravity separation, and/or filtration (Danie et al., 2005), that often have to be applied first to reduce the amount of oil in the water samples to an acceptable level (often lower than 100 mg/L) for the subsequent biotreatment units (Zhang et al., 2011; Zhao et al., 2006). These pretreatments not only rendered the collected oil difficult to recycle when that oil was blended with flocculants and emulsifying agents but also frequently caused problems such as difficulty disposing of the oily sludge, difficulty recycling the oily adsorption materials and filter fouling. In attempts to solve these problems, researchers (Zhao et al., 2006) introduced several commercial inoculants that were developed exclusively for oil degradation in biotreatment units to enhance the oil removal rates (Richard and Vogel, 1999; Dfaz et al., 2000; Li et al., 2005). Using these special microbes in their free or immobilized forms, a high concentration (up to 10 g/L) of crude oil in the biotreatment unit influent could be allowed. The treatment process often took 5 to 6d or longer to reduce the concentration of the oil pollutants to an acceptable level for remediation of the body of water. However, in traditional wastewater treatment processes, the allowable hydraulic retention time (HRT) is often 24 h or less. Therefore, an acceptable degradation time is the most difficult problem in the application of the commercial inoculants to the traditional wastewater treatment processes.

The oil concentrations in wastewaters from different effluents

* Corresponding author.
E-mail address: niemaiqian@xauat.edu.cn (M. Nie).

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usually range from 20 to 3000 mg/L, which is often difficult to collect for oil recycling (Freire et al., 2001; Qi et al., 2013). To substitute for the traditional pretreatment units, biotreatment units should be able to remove 20–3000 mg/L of oil contaminants in less than 24 h (Beun et al., 1999). Previous studies showed that free NY3 cells could grow rapidly on hydrocarbons at high concentrations (Nie et al., 2010; Chang et al., 2013). To benefit from the oil-degrading ability of strain NY3 and to optimize the process for practical wastewater treatment, this paper developed a method for the application of strain NY3 in its immobilized form to treat concentrated oil in wastewater. The supporting materials and the cell immobilization conditions, the water treatment conditions (especially the degradation time), and the possibility of recycling the immobilized NY3 cell strain were studied on the laboratory scale when the initial concentration of crude oil in the influent was 2 g/L, which was equivalent to the quality of most of the inflow water in pretreatment units.

2. Materials and methods

2.1. Microorganisms and chemicals

Pseudomonas aeruginosa NY3 (NCBI GenBank accession number GU377209) was isolated in our laboratory from petroleum-contaminated soil samples collected in Shaanxi, China.

Crude oil was generously supplied by the PetroChina Changqing Oilfield Company (Shaanxi, China). Unless otherwise stated, the organic solvents, media, medium ingredients, salts and acids were purchased from various suppliers (Sigma–Aldrich. VWR and Fisher, USA) and used as received.

2.2. Culture medium

The autoclaved medium for cell immobilization consisted of (per liter): 3 g beef extract, 10 g peptone and 5 g NaCl. The initial pH was adjusted to 7.3–7.5. The mineral salt medium (MSM) for oil degradation consisted of (per liter): 1.3 g NH_4NO_3, 5.0 ml 1 M $MgSO_4 \cdot 7H_2O$ solution, 0.1 ml 1 M $CaCl_2 \cdot 2H_2O$ solution, and 4.6 ml phosphate buffer solution (PBS). The composition of the PBS was (g/L): 81.22 g $K_2HPO_4 \cdot 3H_2O$, 42.9 g $NaH_2PO_4 \cdot 2H_2O$, and 1.0 ml of a trace element solution. The trace element solution contained (per liter) 4.5 g $FeSO_4 \cdot 7H_2O$, 0.148 g $ZnSO4 \cdot 7H_2O$, 0.3 g $MnCl_2 \cdot 4H_2O$, 0.024 g $CoCl_2 \cdot 6H_2O$, 0.024 g $NiCl_2 \cdot 6H_2O$, 0.017 g $CuCl_2 \cdot 2H_2O$, 0.109 g $Na_2MoO_4 \cdot 2H_2O$ and 0.062 g H_3BO_3. The pH of the medium was adjusted to 7.0–7.5 using 1 mol L^{-1} NaOH and autoclaved at 121 °C for 30 min.

2.3. Supporting materials

Polyurethane foam (PUF) cut into cubes (side length of approximately 5.0 mm), crushed corncobs with irregular sizes (approximately 0.2–0.8 cm in diameter) and thin wood chips (approximately 0.2–0.6 cm in length) were pretreated by the method described in previous reports (Pensri and Alissara, 2009; Manohar et al., 2001). The PUF pieces were successively eluted with 5% NaOH solution and 5% HCl to remove the small soluble molecules, washed with distilled water to pH 7.0 and finally dried at 60 °C under vacuum before use. Crushed corncobs or wood chips (300 g) were boiled in 3000 ml of 1% NaOH for 3 h, then washed three times with 3000 ml of distilled water and soaked in distilled water overnight. The pretreated supporting materials were autoclaved prior to use.

2.4. Immobilization of the strain NY3 cells

Immobilization of the NY3 cells was performed as the cells grew on the surfaces of the pretreated supporting materials (PUF, wood chips and corncobs) using the method described by Pensri and Alissara (2009). Cell immobilization was carried out in a 1000 ml Erlenmeyer flask that contained 4.0 g of sterilized supporting material, 400 ml of autoclaved immobilization medium, and 4 ml (OD_{600nm} 2.0) of strain NY3 cell suspension. Cultures were incubated on a rotary shaker at 140 rpm (or other rpm, as indicated in Fig. 2e) for a certain time. Next, the NY3 cells immobilized on the surfaces of the supporting materials were collected and washed three times with 0.85% NaCl. The dry weight of the biomass of the immobilized cells was measured by comparing the dry weights of the supporting materials before and after immobilization. The free cells suspended in the incubation medium during immobilization were also measured using a spectrophotometer (DR5000) and expressed as OD_{600nm}. All processes were performed under sterile conditions at ambient air temperature (28–35 °C) (Iqbal and Saeed, 2007).

2.5. Repeated batch operations for oil degradation

The ability of the immobilized cells to perform oil degradation was evaluated by repeated batch operations under aerobic conditions (Eduardo and Jorge, 2012). Batch operations were performed in 250 ml Erlenmeyer flasks, each containing 100 ml of man-made water samples (100 ml MSM supplemented with 0.2 g of crude oil as the sole carbon and energy source) and 1.40 g of immobilized NY3 cells (with PUF as the supporting material). The flasks were incubated on a rotary shaker under the same conditions (30 °C and 151 rpm) for certain degradation times. Then, the immobilized cells in the parallel flasks were taken out and put into new flasks that contained fresh man-made water samples, as above. After the first five periods, three of the parallel flasks were used for the first sampling, and the cultures in the other parallel flasks continued to repeat the cycles. After the second five periods, another three replicates were selected for sampling as above, until the last three cultures were sampled. For each degradation time testing sequence, the immobilized cells were freshly prepared.

All of the residual oil in each sampling flask, including the oil contained in the culture and adsorbed on the surfaces of the immobilized cells and the supporting materials, was extracted

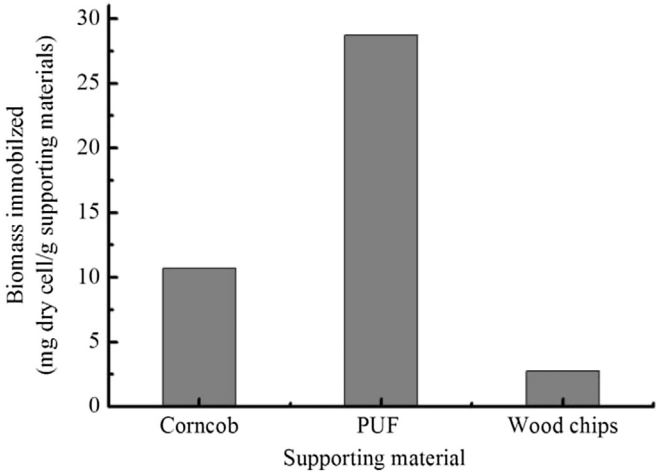

Fig. 1. Immobilization of strain NY3 on different supporting materials. (1.5-column fitting image).