Biochemical Engineering Journal 100 (2015) 76–82

Contents lists available at ScienceDirect

Biochemical Engineering Journal

journal homepage: www.elsevier.com/locate/bej

Biodegradation of tetradecane using *Acinetobacter venetianus* immobilized on bagasse

Jiajiang Lin [a], Li Gan [a], Zuliang Chen [a,b,*], Ravendra Naidu [b]

[a] *Fujian Key Laboratory of Pollution Control and Resource Reuse, School of Environmental Science and Engineering, Fujian Normal University, Fuzhou 350007, China*

[b] *Cooperative Research Centre for Contamination Assessment and Remediation of Environments, University of South Australia, Mawson Lakes, SA 5095, Australia*

ARTICLE INFO

Article history:
Received 30 December 2014
Received in revised form 2 April 2015
Accepted 20 April 2015
Available online 21 April 2015

Keywords:
Biodegradation
Bioremediation
Immobilized cells
Waste-water treatment
A. venetianus
n-Alkane

ABSTRACT

Immobilized cells present advantages for degrading organic pollutants compared to free suspended cells. However, the immobilization of cells with reference to agricultural waste is still limited. This study investigated using bagasse as a carrier to immobilize *Acinetobacter venetianus* (*A. venetianus*) for the degradation of *n*-alkane. The degradation of alkane based on both adsorption and biodegradation by immobilized *A. venetianus* was observed, where 93.3%, 77.7% and 24.0% of tetradecane (400 mg L^{-1}) were removed by the immobilized cells, free cells and bagasse after 36 h incubation, respectively. Additionally, alkane (C_{10}–C_{25}) in diesel was effectively degraded by the immobilized cells, of which 800 mg L^{-1} of alkane was completely degraded after 60 h. Furthermore, results showed: (1) tetradecane served as the optimal carbon source at the concentration of 100 mg L^{-1}; and (2) cells immobilization reached its peak efficiency with the cells-to-bagasse ratio of 0.5 mg_{cell} $g^{-1}{}_{bagasse}$. Cells strongly immobilized on bagasse retained a high degradation rate of 77.6% after shaking at 1500 rpm. Bagasse protected the cells from pollution stress including cadmium (Cd(II)) and phenanthrene. After 8 reuse cycles, immobilized cells became more stable and efficient. Bagasse-*A. venetianus* has potential for future application in remediating diesel contaminants.

1. Introduction

Persistent crude oil exploration in China's Yellow River Delta poses a serious threat to the environment, ecosystems, and people's health due to increased soil contamination [1]. The effective removal of oil spills is a problem of great global importance and in the interests of society [2]. Aerobic alkane degradation is a widespread phenomenon in nature, and several microbial species/strains and enzymes involved in alkane degradation have been identified, isolated and studied in detail [3]. Alkane hydroxylases play an important role in the microbial degradation of alkane under aerobic conditions [4].

Freely suspended cells used in biodegradation have limited biodegradation ability [5]. A promising technique that has gained increasing attention recently is cell immobilization [6]. Many immobilized microorganisms have been successfully used for bioremediation of crude oil [7]. Immobilized cells present specific advantages over free cells, such as longer cell retention times, partial cell protection against environmental conditions such as pH and toxic compounds [1,8,9]. Immobilization prevents bacteria from being washed away [10]. The correct selection of an immobilization carrier is essential for designing an effective system that suits each particular purpose.

Natural polymeric matrices such as agar, alginate, carrageenan, chitosan as well as synthetic matrices such as polyvinyl alcohol (PVA) and polyurethane, have been utilized in the bioremediation of petroleum compounds [9]. In recent years, increasing attention has been paid to the use of renewable resources particularly of plant origin to assuage ecological concerns [11]. Reusing and recycling these residues can minimize the environmental problems associated with their build-up and reduce the use of expensive starting materials [12]. Many studies have been published on using natural materials to remove oil [2]. The most common natural oil sorbents are kapok fiber, sugarcane bagasse, rice husk, barley straw, cotton, wool, wood residues, and various plant and animal materials [13].

Bagasse is a kind of agricultural waste from sugar production [11]. It contains about 50% cellulose, 25% hemicellulose and 25%

* Corresponding author at: Cooperative Research Centre for Contamination Assessment and Remediation of Environments, Mawson Lakes, SA 5095, Australia. Tel.: +86 591 83465689; fax: +86 591 83465689.
E-mail address: zlchen@fjnu.edu.cn (Z. Chen).

http://dx.doi.org/10.1016/j.bej.2015.04.014
1369-703X/

lignin [14]. Bagasse from sugar production is twenty-eight percent of the biomass. Consequently, huge amounts of bagasse are and will continue to be generated and the utilization of this material is of growing importance [15]. The study of bagasse as an absorbent of environmental pollutants has also received attention recently [13,15,16]. Raw bagasse has excellent sorption capacity on both hydrophilic and hydrophobic compounds [15]. A number of studies have examined using bagasse to remove heavy metal, dyes and oil from wastewater [13,16,17]. However, the contaminant adsorbed onto the bagasse requires further processing which unfortunately increases operational costs. Hence, we assume the intergradations of adsorption of oil from wastewater onto the bagasse and following biodegradation of oil on the bagasse by the immobilized strain. Furthermore, bagasse contains many carbohydrates that can serve as the carbon source in biodegradation [18].

To date, not much study has been done on combining bagasse and microorganisms to remediate oil contamination. For the reason, in this study, untreated sugarcane bagasse was selected as a carrier for cells immobilization. A highly efficient alkane-degrading bacterial strain was immobilized on the bagasse to biodegrade tetradecane. This study investigated the following: (1) the preparation of *A. venetianus* immobilized on bagasse; (2) the conditions affecting the immobilization; and (3) the degradation capability of immobilized *A. venetianus*.

2. Material and methods

2.1. Chemical and solution

Tetradecane (>98% purity), naphthalene (>98% purity), and phenanthrene (>98% purity) were obtained from Aladdin Industrial Inc. The other analytical grade chemicals were obtained from Sinopharm Chemical Reagent Co., Ltd., Shanghai, China. In the current study, the sugarcane bagasse fiber (hereafter referred to as bagasse) was obtained as sugar production waste from Fuzhou, Fujian, China. The bagasse had been air dried, ground and sieved to a size ranging between 0.6 and 0.85 mm before use.

2.2. Microorganismal strains and immobilization of cells

The relevant microorganism strain was obtained from a return sludge of a refinery wastewater treatment plant using tetradecane as substrate. The sequences of the strain's 16S rRNA gene were determined and compared to the NBCI database for the closest matches using BLAST which identified the most likely species as *Acinetobacter venetianus* with 99% similarity. Details of the method of isolation and identification were described in our previous report [19]. The microorganism strains were stored on Luria–Bertani (LB) plates at 4 °C. The LB medium used to grow microorganisms contained (g L^{-1}): peptone, 10; yeast extract, 5 and NaCl, 10; pH 7.0. The mineral medium was prepared as follows (g L^{-1}): K_2HPO_4, 1.0; KH_2PO_4, 1.0; NH_4NO_3, 1.0; $MgSO_4$, 0.3; $CaCl_2$, 0.03 and 1% micronutrient solution (g L^{-1}): $FeSO_4$, 0.5; $ZnSO_4$, 0.2; $MnSO_4$, 0.02. The pH was adjusted to 7.0.

One liter volume culture of the strain was created using the LB medium in a flat-bottom flask under forced aeration for 1 day. Cultivation was carried out at 25 °C. Following cultivation, the microorganism cells in the supernatant were harvested by centrifugation at 6000 rpm for 10 min. Cell pellets were washed three times with sterilized mineral medium and finally resuspended in a small volume of sterilized mineral medium.

The immobilization was carried out by mixing microorganism cells with bagasse (0.01 mg g^{-1}) in a mineral medium, and followed by incubation in a gyratory incubator at 150 rpm in the dark. After 24 h, the immobilized cells were collected and washed three times with sterilized mineral medium and finally stored at 4 °C for further use.

2.3. Batch experiments

The degradation capability of immobilized cells was conducted as follows: 0.2 g (dry weight) immobilized cells were added into a 50 mL ground conical flask containing 20 mL mineral medium with two tetradecane concentrations: (a) 50 mg L^{-1}, (b) 400 mg L^{-1}, then sealed the flask with a ground glass stopper. Control set with tetradecane alone, free cells (0.0002 g, dry weight) and bagasse (0.2 g, dry weight) were also inoculated in order to compare them to the immobilized cells.

Additionally, the degradation capability of immobilized cells on other alkanes was investigated using supplied diesel at concentrations of 100, 400, and 800 mg L^{-1}. Three Treatments (I–III) were performed in the batch experiments as shown in Table 1. The removal routes of alkanes in mineral medium by immobilized cells are considered to be biodegradation (B), adsorption (A), and volatilization (V) [20]. In Treatment I, all three removal routes occurred (Table 1). In Treatment II, biodegradation was excluded because the activity of cells was inhibited by NaN_3. For Treatment III, only volatilization accounted for the removal of alkanes.

To enhance immobilization efficiency, the factors affecting the immobilization of cells were examined. Batch experiments were conducted using the following various conditions: firstly, the influence of carbon source was investigated at the concentration of 5 g L^{-1} by adding cane sugar, white sugar, yeast extract, glucose, molasses, tetradecane, and diesel; secondly, using tetradecane as the only carbon source for immobilization, the influence of tetradecane concentrations was studied, these being 10, 50, 100, 200, 500 mg L^{-1}; and thirdly, the influence of inoculums (the ratio of cells to bagasse) was analyzed at the values of 0.01, 0.1, 0.5, 2.0, and 10.0 (dry weight, mg g^{-1}). The immobilization method was the same as that described in Section 2.2.

At the end of immobilization, the immobilized cells were collected and transferred into a 50 mL flask containing 20 mL sterilized MM medium with initial tetradecane concentrations of 50 mg L^{-1}. Then, the cells were incubated in a gyratory incubator at 150 rpm in the dark. The concentrations of tetradecane were monitored after 8 h.

Various factors were examined to investigate the degradation capability of immobilized cells. These included the strength of immobilized cells which performed by controlling the agitator's shaking speed at 0, 300, 600, 900, 1200, and1500 rpm; pollution stress resistance by adding Pb (50 mg L^{-1}), Cd (50 mg L^{-1}), naphthalene (200 mg L^{-1}), and phenanthrene (200 mg L^{-1}). The repeated degradation of tetradecane degradation by reusing immobilized cells was also assessed. The immobilized cells were put into a 20 mL MM medium containing 50 mg L^{-1} tetradecane. After 8 h biodegradation, the previous medium was decanted and immobilized cells were washed with sterile water before being transferred into a 20 mL fresh medium.

Table 1
Batch experimental of alkanes removal in diesel.

Treatment	Immobilized cells	Diesel	0.1%[c] NaN_3	Removal routes
I	+[a]	+	–	B + A + V[d]
II	+	+	+	A + V
III	–[b]	+	+	V

[a] "+" indicated "with" or "presence".
[b] "–" indicated "without" or "absence".
[c] NaN_3 was used to inhibit the activity of cells.
[d] B-biodegradation; A-adsorption; V-volatilization.