Biochemical Engineering Journal 131 (2018) 17–23

Contents lists available at ScienceDirect

Biochemical Engineering Journal

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

Regular article

Biotreatment of real petroleum wastewater using non-acclimated immobilized mixed cells in spouted bed bioreactor

Zainab Z. Ismail[a,*], Haneen A. Khudhair[b]

[a] Department of Environmental Engineering, University of Baghdad, Baghdad, Iraq
[b] Department of Environmental Engineering, University of Tikrit, Salahaldeen, Iraq

ARTICLE INFO

Article history:
Received 30 August 2017
Received in revised form 5 November 2017
Accepted 5 December 2017
Available online 14 December 2017

Keywords:
Petroleum wastewater
Activated sludge
Immobilized cells
Bio-carrier
Spouted bed bioreactor
Hydrocarbons

ABSTRACT

This study investigates the biotreatment of real-field petroleum wastewater by using non-acclimated immobilized mixed cells in spouted bed bioreactor. Activated sludge was used as the biocatalyst immobilized in bio-carrier matrices prepared by the reinforcement of a natural polysaccharide sodium alginate with polyvinyl alcohol. For comparison purposes, mixed free cells were also tested. The results demonstrated that the percentage removal of COD and total petroleum hydrocarbons in the real-field petroleum wastewater were 61.7% and 66.6%, respectively. The immobilized cells were used up to 3 cycles without losing their efficiency for COD removal. On the other hand, only 28% removal of COD was observed by using non-recyclable mixed free cells. Also, the results proved that the storage stability of immobilized cells maintained at 90% after being stored for 35 days at 4 °C, whereby, the free cells became inactive after 28 days. The effectiveness factor μ was found to be 0.991.

1. Introduction

Oil and its derivatives from oil manufacturing plants including petroleum wastewater have received attention because of their widespread use. Petroleum refinery effluents present very specific problems in terms of detoxification and suitable treatment which would render them acceptable for discharge into the receiving stream due to the dissolved organic materials causing high COD, almost of toxic nature including hydrocarbons, phenol, and oils [1]. The efforts towards effective handling and treatment of petroleum effluents have been intense not only because of their extremely diverse, toxic, and inhibitory effects, but also due to of their large volume, as the oil-refining and processing industry represents one of the largest and most expanding areas of industrial enterprise. The biotechnology methods are known to be very effective in dealing with significant environmental problems associated with realizing organic-loading wastewater including petroleum wastewater due to the ability of bacteria to degrade organic compounds in wastewater [2,3]. A more recent technique in biodegradation is cells immobilization which has the potential to degrade toxic refractory compounds faster than conventional treatment systems since high densities of specialized microorganisms are used in immobilized cells systems. This technique facilitates separation and recovery of microbial cells as well as making the application reusable which reduces the overall cost [4]. Compared to free suspended cells, immobilized cells possess beneficial properties which are; resistance to negative environmental factors high viability, simple reuse of the biomass, increased catalytic activity, easier liquid–solid separation, the solid residence time (SRT) could be increased with minimal clogging in continuous-flow systems. Also, they provides increased protection from the concentration of recalcitrant organics that are toxic to free cells and prevents the active cells from entering the mobile phase which caused washout of the free cells. This technology was considered as an effective innovative approach in considering the environmental challenges associated with conventional bioremediation of petroleum-refinery wastewater [5–7]. Many studies have been reported on the utilization of immobilized cells for biodegradation of aromatic compounds and hydrocarbons products. Wilson & Bradely [8] used free and immobilized cells of *Pseudomonas* sp. for biodegradation of petrol in aqueous system. The results proved that immobilized cells resulted in a combination of increased contact between cell and hydrocarbon droplets and enhanced the biodegradation of hydrocarbons. Gonzalez et al. [9] studied the biodegradation of high phenolic concentration up to 1000 mg/L from industrial wastewater by *Pseudomonas Putida* previously adapted to the toxic chemical and immobilized in calcium-alginate gel beads. The results showed phenol degradation efficiency higher than 90%. Moslemy et al. [10] suggested that immobilization of activated sludge in gellan gum microbeads enhanced the biological activity of microbial cells for the

* Corresponding author.
E-mail addresses: zismail9@gmail.com, zismail3@gatech.edu (Z.Z. Ismail).

https://doi.org/10.1016/j.bej.2017.12.005
1369-703X/

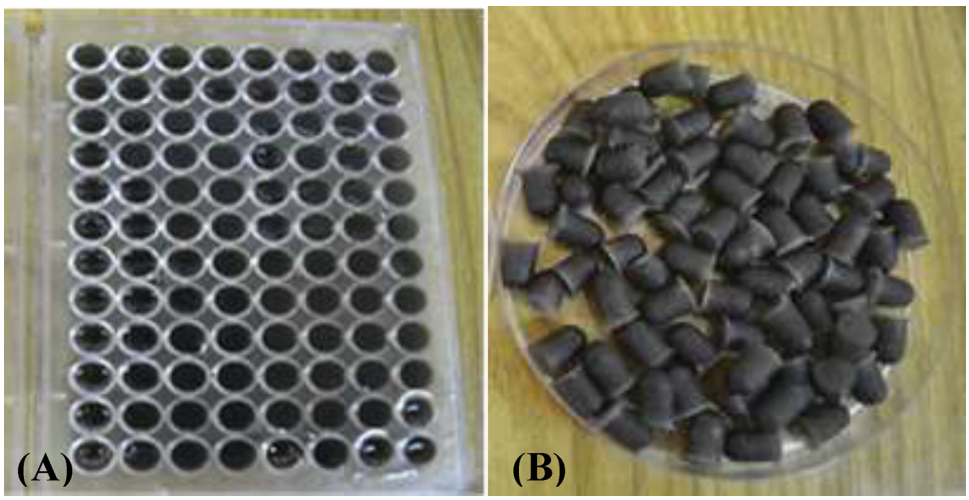

Fig. 1. Immobilized mixed cells in PVA-SA; (A), beads immediately after pouring in micro-plates, (B), beads ready for use.

removal of gasoline hydrocarbons. Immobilized sludge enhanced the biodegradation activity compared with free cells even at 4.4 times lower level of overall biomass loading. Zhiguo et al. [6] isolated a bacterial strain *Pseudomonas* sp. that is capable of degrading nitrobenzene, phenol, aniline, and other aromatics. Strain *Pseudomonas* sp. was immobilized in the mixed carrier of polyvinyl alcohol and sodium alginate. The immobilized cells had stable degradation activity and good mechanical properties in the recycling tests. They could completely degrade 300 mg/L nitrobenzene within 10 h with 150 mg/L aniline and 150 mg/L phenol. Partovinia & Naeimpoor [11] exploited activated sludge from an oil refinery as the microbial consortium for biodegradation of phenanthrene by immobilized cells in aqueous phase entrapped in polyvinyl alcohol-cryogel prepared by freeze-thaw method. Complete removal of 100 and 250 ppm of initial concentration of phenanthrene were observed. Bao et al. [12] explored free and immobilized two bacterial strains, identified as *Rhosococcus* sp. and *Bacillus cereus* sp. to degrade heavy oil. The oil degradation rates were about 34.6% and 45.3% for free cells of *Rhosococcus* sp. and *Bacillus cereus* sp., respectively after 5 days. The best biodegradation rate of immobilized cells reached above 78%, which is 33% higher than of free *Bacillus cereus* sp. Surkatti & El-Naas [13] investigated the biodegradation of simulated wastewater containing *p*-cresol using *Pseudomonas putida* immobilized in PVA gel. Continuous biodegradation results indicated that *P. putida* had high potential for the biodegradation of *p*-cresol up to 200 mg/L, with a removal efficiency of more than 85%. Wang et al. [14] studied the degradation of carbazole, by immobilized *Sphingomonas* sp. strain. Four types of polymers; agar, alginate, k-carrageenan, and gellan gum were evaluated. The immobilized cells were reused for eight cycles. The results showed that the immobilized cells can degrade carbazole at concentration 250 mg/L in 36 h. El-Gendy & Nassar [15] isolated the marine diesel-oil degrading bacterium, *Pseudomonas aeruginosa NH1* and examined its ability to degrade diesel oil-contaminated seawater as immobilized cells by entrapment in Ca-alginate. The biodegradation rate of different components of diesel oil was enhanced by immobilization indicating the improved tolerance of the immobilized cells towards different toxic components of diesel oil and environmental conditions. The reusability tests revealed that the immobilized cells can be effectively reused for two batches of 56 days. Banerjee & Ghoshal [16] studied the microbial degradation of actual petroleum wastewater collected from oil refinery in India. The biodegradation was carried out in a packed bed reactor by *Bacillus cereus* immobilized in Ca-alginate. The performance of the system was evaluated in terms of COD, TOC, phenol, PO_4^{-3}, and NH_4^+ removal. The results demonstrated successful implication of the immobilized species. It may be important to mention here that using real-field wastewater as a substrate source offers a promising option for efficient scale up of the suggested treatment system. Also, using non-acclimated immobilized cells for biodegradation of refractory organics and hydrocarbons in the real-field petroleum wastewater represents a big challenge worth to be investigated.

This study aimed to investigate and evaluate the biotreatment of real-field petroleum wastewater using non-acclimated immobilized mixed cells in spouted bed bioreactor. The storage stability and effectiveness factor of the immobilized mixed cells were also estimated.

Table 1
Characteristics of real-field petroleum refinery wastewater.

Constituents	Average value	Unit
COD	1250 ± 30	mg/L
TPH	2300 ± 50	mg/L
TSS	175 ± 10	mg/L
Phenol	10 ± 0.5	mg/L
Furfural	12 ± 1	mg/L
pH	7.2–7.5	–
Cl^-	68 ± 2	mg/L
PO_4^{-3}	7.5 ± 0.2	mg/L
SO_4^{-2}	55 ± 5	mg/L
NO_3^-	30 ± 2	mg/L

2. Materials and methods

2.1. Wastewater and biocatalyst

In this study, real-field petroleum wastewater (PRW) was freshly collected from the drainage outlet of AL-Dora refinery, located in Baghdad, Iraq. The average measured concentrations of contaminants in real-field PRW samples are given in Table 1. Freshly collected activated sludge obtained from a local municipal wastewater treatment plant was used as the mixed cells biocatalyst. This sludge was characterized in terms of the major content of microorganisms. The dominant types of cultures in this activated sludge were found to be *Pseudomonas, Bacillus*, and *E.coli* at concentrations of 3×10^9, 7×10^9, and 8×10^9 Cell/ml, respectively.