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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

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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).

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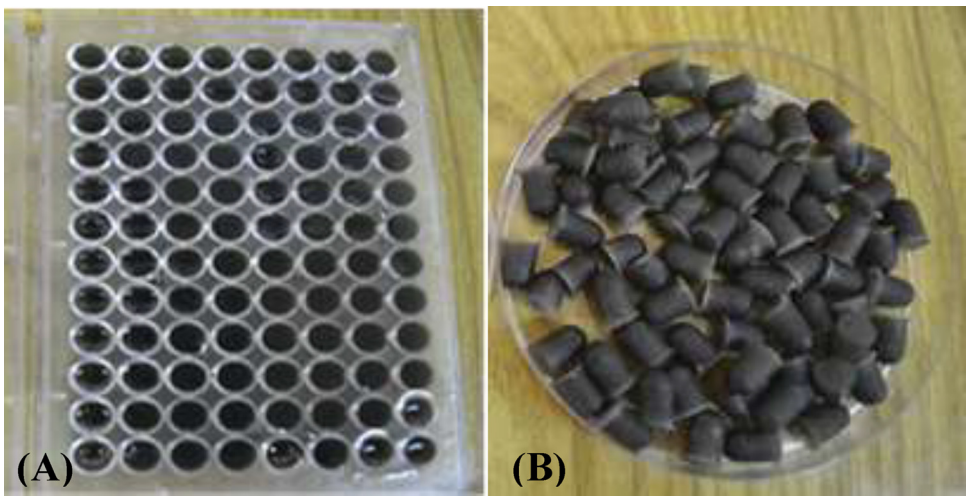

Fig. 1. Immobilized mixed cells in PVA-SA; (A), beads immediately after pouring in micro-plates, (B), beads ready for use.

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.

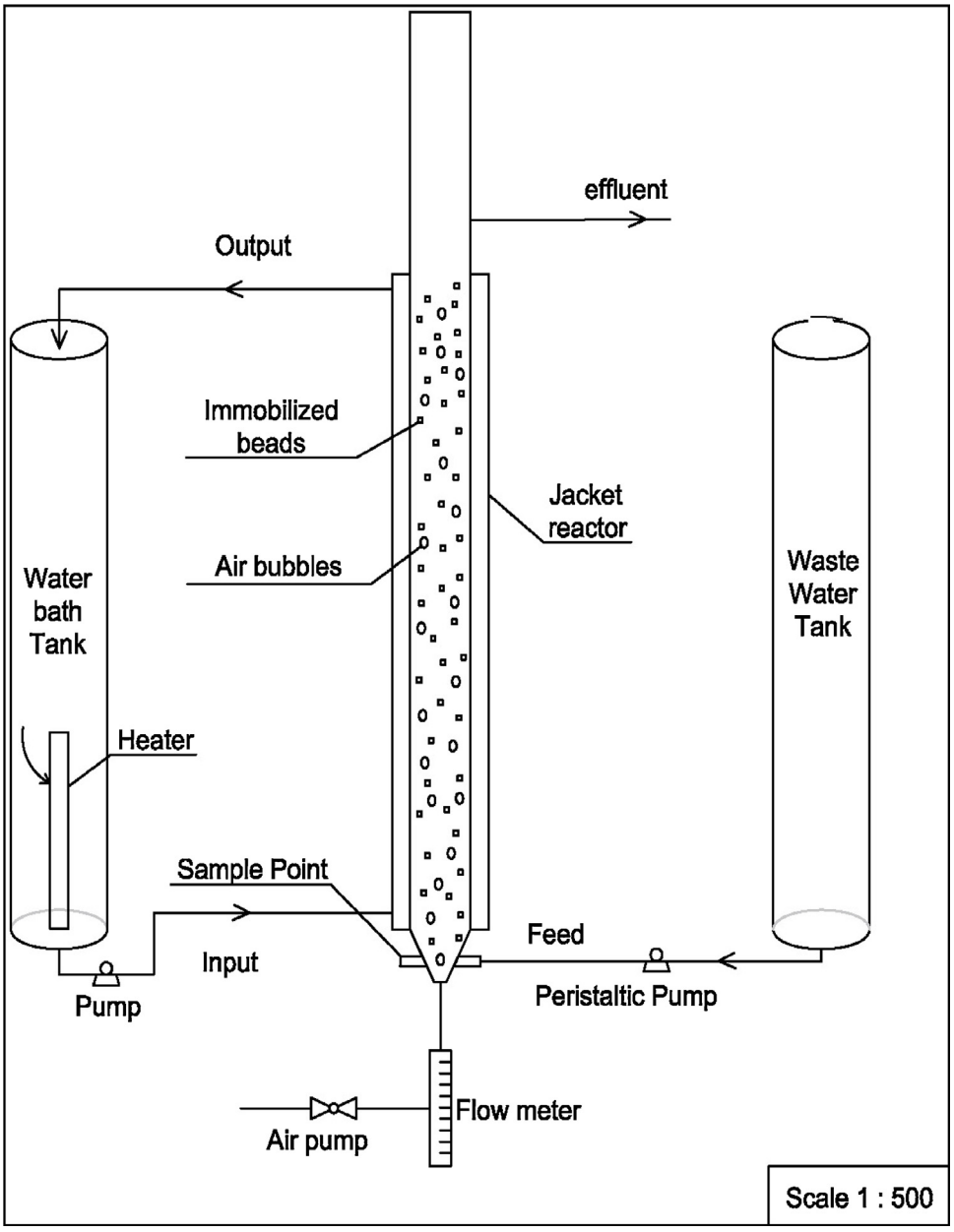

Fig. 2. Schematic diagram of the lab-scale pilot plant.

2.2. Bio-carrier materials and immobilization protocol

Sodium alginate, an algal polysaccharides derivative was used as the bio-carrier material. It was prepared by the reinforcement and cross-linking of sodium alginate with polyvinyl alcohol (PVA) to improve and increase its mechanical stability of beads. Na-alginate (SA) solution was prepared in sterilized distilled water and combined with PVA. Then according to Bai et al. [23], biomass inoculum (5% v/v) was added to the PVA-SA slurry, and stirred for 10 min to get a uniform mixture taking into consideration no bubbles were entrapped inside. The slurry was poured in sterile hypodermic syringe. The alginate solution was dropped into ice cold mixture of calcium chloride (4%) and boric acid (6%). Beads were formed in $CaCl_2$ solution that was incubated overnight for curing. The cured beads were washed with sterilized distilled water 3–4 times. According to Kumar et al. [17] when the beads were not being used, they were preserved in 0.9% sodium chloride in the refrigerator. Samples of the prepared immobilized cells in PVA-SA are given in Fig. 1.

2.3. Analytical techniques

The performance of the suggested process was analyzed by measuring the various parameters. Concentrations of COD were measured by using the COD analyzer (Model: Lovibond, RD 125). However, measured values of COD could be less than the total COD. This observation probably occurred due to the high concentration of oil in the samples and inability to obtain representative dilutions of samples due to its immiscibility with water. Similar observation was reported by Chavan & Mukherji, [18] for the treatment of hydrocarbon-rich industrial wastewater in bioreactors.

Total petroleum hydrocarbons (TPH) concentrations in aqueous samples were measured by Gas Chromatography-Mass (GC/MS) (Model: PACKARD 438A). Phenol and furfural concentrations in

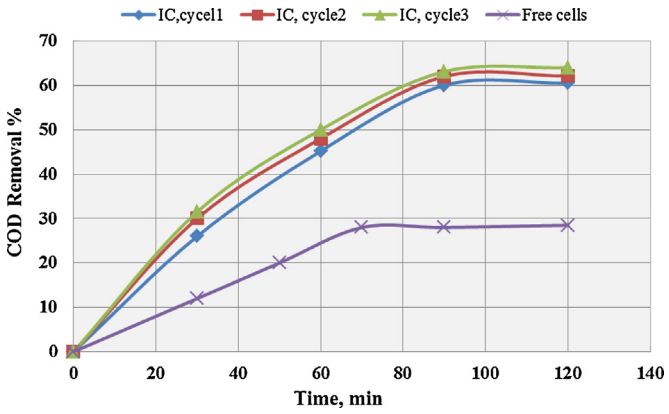

Fig. 3. Efficiency of COD removal from real-field petroleum wastewater in SBBR using non-adopted immobilized mixed cells and free cells.

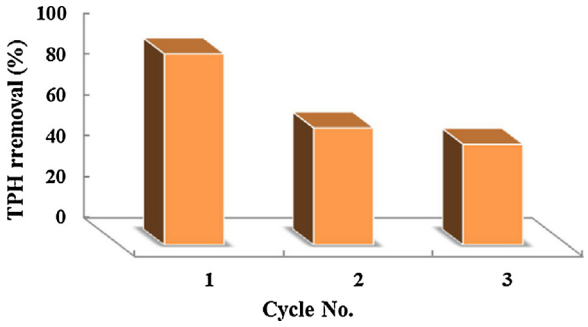

Fig. 4. TPH reduction (%) as a function of cycle of immobilized cells of activated sludge in PVA-GA beads reuse.

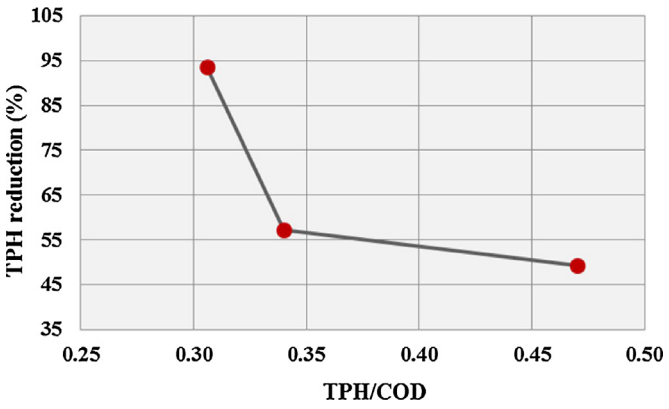

Fig. 5. TPH reduction (%) as a function of (TPH/COD) ratio.

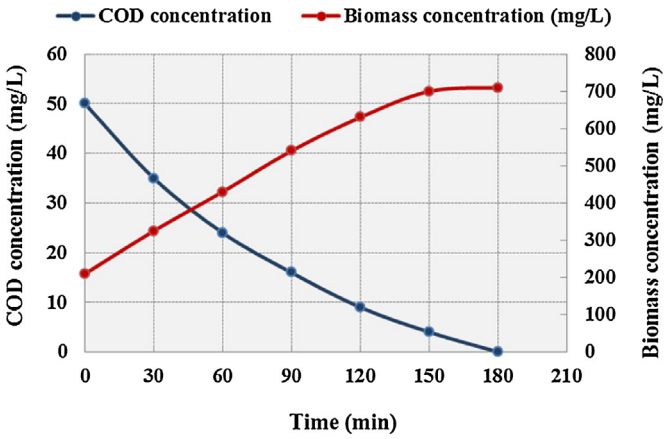

Fig. 6. Biomass growth versus COD degradation in PVA-GA beads.

aqueous samples were determined by T80 UV–vis Spectrophotometer (Model: T80 UV/VIS Spectrophotometer PG Instrument Ltd, UK) at 270 and 278 nm, respectively. Total suspended solids were measured according to the procedure outlined in the *Standard Methods* [19].

2.4. Immobilized cell concentration measurements

The growth estimation of immobilized cells involves the removal of cells from the immobilization matrix. In this study, according to the procedure reported by Cruz et al. [20], the beads were dissolved by immersing them in 4% $NaHCO_3$ solution for 30 min. Samples of microorganisms residing in the wastewater were used without additional treatment. Routine estimation within the beads was carried out by the traditional approach of volatile suspended solids (VSS, g/L) outlined in the *Standard Methods* [19].

2.5. System configuration and set up

The experimental system consisted of a specially designed spouted bed bioreactor (SBBR) made of Perspex column of 50 mm inner diameter and 70 mm height with 45° conical base. The SBBR was outfitted with a Perspex jacket of 80 mm inner diameter for temperature control. A water bath was provided to continuously circulate the water at a desired temperature of 30 °C. The water bath consisted of 6 L-cylindrical Perspex tank, occupied with heater and water pump to circulate the water into the reactor jacket. Based on the results of a preliminary set of experiments to investigate the optimum wastewater air flowrates into the SBBR (data not shown), the wastewater was fed to the SBBR at a rate of 20 mL/min using a peristaltic pump. The air was injected at a rate of 4 L/min from the bottom of the reactor by an air pump through a 6 mm-orifice in order to provide an intense mixing and maintain aerobic environment into the SBBR. A flow meter and controller were provided to control the air flow into the system. The experimental setup is given in Fig. 2. The experimental work was accomplished in a continuous mode. At a regular time interval, samples were withdrawn from the sampling point located at the bottom of the SBBR and centrifuged at 6000 rpm for 10 min, and then the supernatant was filtered using filter paper (type: Whatman, 0.22 μm), and the filtrate was analyzed for the residual concentration of pollutants. One of the major advantages of using immobilized cells is their ability to be recycled for further biodegradation of the considered contaminants. Accordingly, the viability of immobilized cells was examined by recycling the beads for excessive biodegradation experiments. For the recycling assays, after each experiment the beads were collected and washed with distilled water to remove free cells sloughed from the beads. For comparison purposes between the efficiency of immobilized and free cells, the experimental system was individually operated with free mixed cells at similar operating conditions.

In order to confirm the inverse relationship between the growth of immobilized cells and the organics removal, the experimental work was extended and an assay was conducted using PVA-SA immobilized mixed cells for biotreatment of an aqueous solution having an initial COD concentration of 50 mg/L. The aqueous solution was prepared with a mixture of phenol and furfural as models of TPH which are commonly found in petroleum refinery wastewater.

2.6. Storage stability of immobilized cells (IC)

As reported by Wu et al. [21], storage stability is an essential factor for practical application of immobilized cells system. To estimate the immobilized cells stability for biodegradation activity, both free and immobilized cells were stored in 100 mL physiological saline (0.85% NaCl, w/v) at 4 °C for 10, 20, 30 and 40 days.

2.7. Beads morphology

The photomicrographs of immobilized cells in gel beads were obtained using a scanning electron microscope (Model: Inspect S50 SEM) to determine the surface morphology of immobilized cells before and after biodegradation. According to the procedure outlined by Wu et al. [21], the gel beads treated with glutaraldehyde (2.5%) for 3 h followed by dehydration in acetone (50–100) % for 30 min. Then, the pretreated gel beads were coated with gold by sputtering to improve the image quality.

3. Results and discussion

3.1. Removal of COD

COD is the most widely used parameter to estimate the level of contamination in organic-loaded wastewater. The results of a preliminary set of experiments (data not shown) clearly revealed that the reduction rate of COD concentration (or the biodegradation rate) depends on the air flowrate. In this study, this dependency seems to diminish for flow rates higher than 4 L/min. By increasing the air flowrate, the mixing rate will increase, and then the relative velocity of beads will increase. Thus, the thickness of the stagnant layer decreases and so will the external mass transfer resistance. However, at air flowrates higher than 4 L/min, the biodegradation rate almost becomes constant. This can be attributed to the negative effect of air bubbles coalescence that takes place at high air flowrates. The results revealed that 62% and 28% of COD concentration was removed from the real-field petroleum wastewater in the SBBR using non-adopted immobilized mixed cells and free cells as given in Fig. 3. It is well observed that the efficiency of COD removal in real-field petroleum refinery wastewater using immobilized cells was significantly higher when using free cells which were unrecyclable. Immobilized cells were used up to 3 cycles without losing their efficiency.

3.2. TPH performance removal

The experimental results indicated that the concentrations of TPH gradually reduced throughout the subsequent cycles. The removal of TPH was 93.46, 57.24 and 49.24% for 1st, 2nd and 3rd cycles, respectively as shown in Fig. 4. However, the overall reduction in TPH concentration was 66.6%. Table 2 presents the initial and final concentrations of the total petroleum hydrocarbons in the real-field petroleum refinery wastewater treated in the SBBR using non-adopted immobilized mixed cells. As reported by Darsa et al. [3] some of simple petroleum hydrocarbons can be degraded by various kinds of bacteria but the ability to degrade more complicated hydrocarbons is found in fewer species. No single bacterium can make all the various enzymes. Bacterial cells have evolved catalytic enzymes which are specific for certain and particular biodegradation reactions.

3.3. The effect of TPH/COD ratio on the removal of TPH in SBBR

During the recycling of immobilized cells in the subsequent cycles, certain enzymes in the bacterial biomass are induced so that they are available for taking part in the metabolism reactions.

Table 2
Remaining concentrations of Total Petroleum Hydrocarbons (TPH) in real-field petroleum wastewater using mixed cultures for biodegradation in SBBR.

Retention time (min)	Hydrocarbon conc. (mg/L)			
	Influent conc. (mg/L)	Effluent conc. (mg/L)		
		1st cycle	2nd cycle	3rd cycle
0.287	0.08	–	–	–
0.358	0.02	–	–	–
0.392	0.02	–	–	–
1.582	18.5	–	–	–
1.850	43.79	–	–	–
2.067	48.43	–	–	–
2.287	71.13	–	–	–
2.587	101.13	–	–	–
3.011	126.07	0.87	–	–
3.358	148.15	30.7	4.44	–
3.841	215.72	0.65	–	–
4.213	112.3	2.11	0.84	–
4.462	92.0	9.75	3.368	
4.870	206.77	14.5	2.78	1.45
5.544	290.78	13.33	6.314	4.002
5.900	176.89	16.05	3.11	1.35
6.733	213.14	26.84	24.6	12.34
7.320[a]	3.5	0.6	–	–
7.862	82.61	17.3	15.04	12.66
8.78	90.68	13.07	3.428	1.02
9.72	211.4	5.52	1.06	0.419
10.85[b]	4.6	0.8	0.504	–
11.45	38.46	1.05	–	–
13.312	41.89	–	–	–
14.25	4.94	–	–	–
Total	2343	153.14	65.484	33.241

-Not detected.
[a] The peak at the retention time (7.32 min) represents furfural.
[b] The peak at the retention time (10.85 min) represents phenol.

This is much more important when dealing with toxic compounds such as phenol and other refractory organic compounds found in PRW at high concentrations. As depicted in Fig. 5, increasing the TPH/COD ratio decreases the percentage of TPH removal. These results were in a good agreement with the findings reported by Safa et al. [22].This could be attributed to the inhibition of bacterial cells caused by the petroleum hydrocarbons which deform the cellular metabolism of microorganisms and prevent them from using carbon for their metabolism and reproduction.

3.4. Immobilized cells growth versus COD removal

Fig. 6 illustrates the relation between the biomass growth and COD removal in the SBBR using non-adopted immobilized mixed cells. A significant rate of COD removal by immobilized mixed cells was clearly observed. This could be due to the existence of different types of bacterial strains in the mixed cultures which promotes the degradation performance. The obtained results confirmed the normal inverse relation between the biomass growth and substrate removal.

3.5. Scanning Electron Microscopy

Scanning Electron Microscopy (SEM) was carried out to investigate the morphological and topological changes of the immobilized mixed cells before and after the biodegradation process. The SEM images shown in Fig. 7 present (a) the blank PVA-GA beads, (b) immobilized cells before biodegradation, and (c) immobilized cells after biodegradation. The beads with immobilized cells become tight and rough after biodegradation process when the bacterial cells penetrated the pores (Fig. 8c). Bai et al. [23] reported that

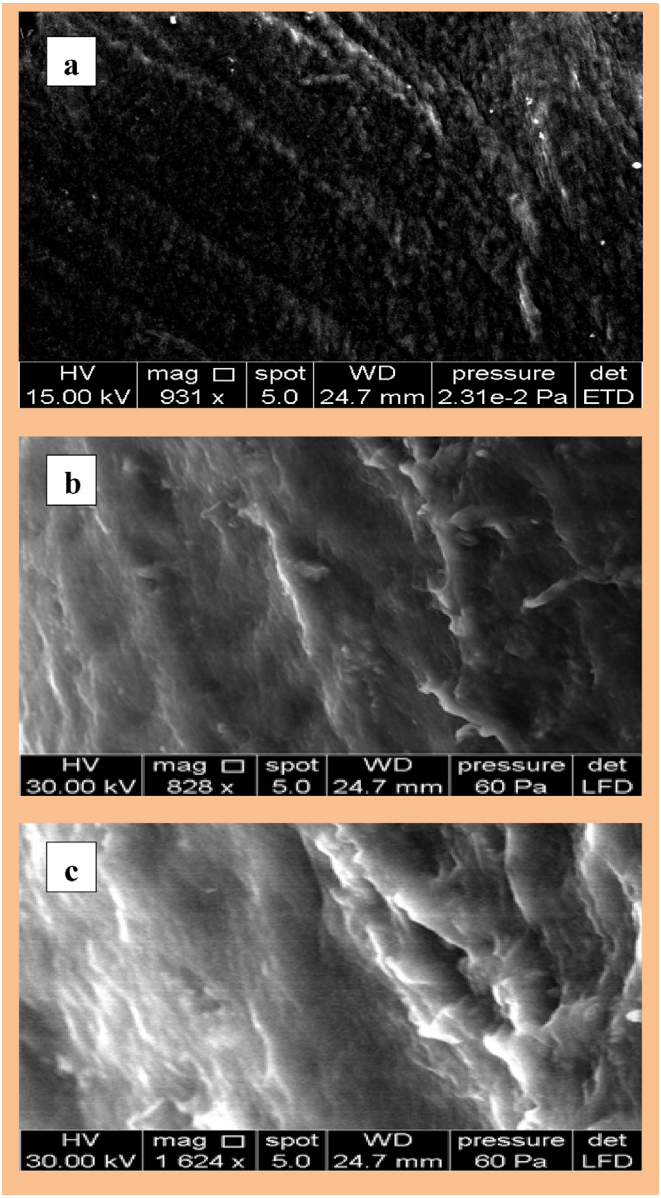

Fig. 7. SEM for the beads; (a) blank beads, (b) beads with cells before biodegradation, and (c) beads with cells after biodegradation.

the difference in microscopic structure of the PVA-SA beads could be attributed to the different cross-linking degrees formed in the bead before and after biodegradation process. The outside of the carriers can sufficiently contact with boric acid and form compact structure, which could not be destroyed by the CO_2 bubble in pore-forming process. Conversely, the inside of the carrier was easily destroyed by the pore-forming bubble and formed larger pore by sequent reuse cycles. The pore size inside the carrier typifies the macro-porous structure.

3.6. Effectiveness factor μ

The effectiveness factor (η) is defined as the ratio of the observable reaction rate of the immobilized cell system over the reaction rate of the system under negligible mass transfer resistance. It was assumed that the intrinsic characteristics of the cells remained unchanged after immobilization, so the reaction rate under negligible mass transfer conditions was equal to the reaction rate of the free cells. When the observable reaction rate of the immobilized

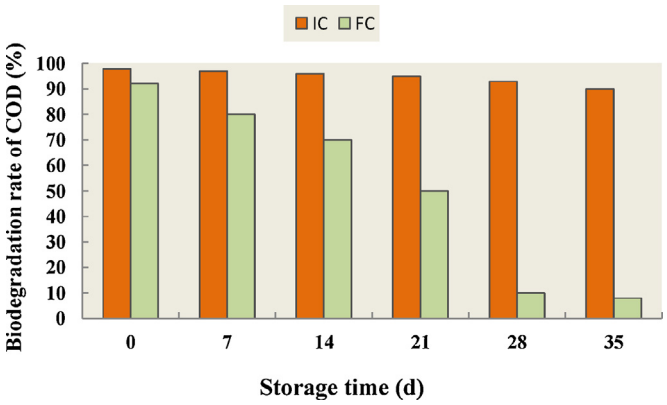

Fig. 8. Storage stability of COD biodegradation activity by immobilized cells of activated sludge in PVA-GA beads.

cells approaches the rate of reaction of free cells, then the effectiveness factor approaches 1. This indicates a negligible intraparticle diffusion resistance, and consequently the rate of the process is determined by the biochemical reaction rate. On the other hand, if the effectiveness factor approaches 0, the rate of substrate diffusion into the particle becomes determining. The effectiveness factor (η) can be estimated as given by the following equation [24]:

$$\eta = R_{actual}/R_{withoutmasstransfer}$$

In this study, η value was estimated to be 0.991. Since η value approached 1, this indicated that the internal mass transfer had less effect on the biodegradation rate and the biodegradation reaction step controlled the overall rate. These conditions indicated that the biodegradation rate at the center and the surface of the bead was the same, and thus all of the volume of bead is fully effective.

3.7. Effects of storage stability

As mentioned earlier in Section 2.6, the experimental sets were conducted using free and immobilized cells to investigate the effect of storage on the mechanical strength and biodegradation activity of these cells. The results suggested that the storage stability of immobilized cells was better than that for the free cells and the immobilization technique is a potential approach for actual applications of bio-treatment, in particular when accidental spills occur. As shown in Fig. 8, in case of immobilized cells (IC), the removal rates of organics represented by COD slightly decreased with the extension of storage time and maintained at 90% after being stored for 35 days at 4 °C. In addition, the beads maintained their physiological stability and had high mechanical strength. In contrast, in case of free cells (FC), the removal rate of COD decreased sharply when they had been stored at 4 °C for more than 20 days, and became almost inactive after 28 days which was 10%. These results are in a good agreement with those outlined by Liu et al. [25] who reported that *Bacillus myciodes* immobilized in the PVA-SA-kaolin beads maintained its biodegradation activity of TNT (Trinitrotoluene) at 91.3% after being stored for 42 days at 4 °C, while the degradation activity of free cells became inactive after 35 days.

4. Conclusion

This study evaluated the performance of non-acclimated immobilized mixed cells for biotreatment of real-field petroleum wastewater in spouted bed bioreactor. The results demonstrated that immobilized cells exhibited better performance compared to free cells in regard with the removal rate of COD, duration of

biodegradation process, as well as the ability for recycling. The results indicated that by using immobilized mixed cells in SBBR, the overall percentage removals of COD in real-field petroleum wastewater was 61.7%, whereby only 28% removal of COD was observed by using non-recyclable free cells. The TPH concentration in real-field petroleum wastewater was reduced by 66.6% using immobilized cells in SBBR. The immobilized cells were used up to 3 cycles without losing their efficiency for COD. 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. Based on the effectiveness factor (η) value which was estimated to be 0.991 for the mixed cells, the biodegradation rate at the center of bead is the same at the surface, and thus all of the volume of bead is fully effective. Future work will be extended to develop a mathematical model including the influence of the fluid-dynamic and mass transfer of the process.

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