Bioremediation of oil-contaminated water by bacterial consortium immobilized on environment-friendly biocarriers

Original Article

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ABSTRACT

Background: Accidental oil spill in the Nile River represents a public health concern in Egypt. Bioremediation of oil-contaminated water is efficiently competitive and economically effective compared with mechanical and chemical methods.

Aim: The current study aimed to; i) assess using environment-friendly materials as carriers for immobilizing oil-degrading bacteria and ii) evaluate applying these biocarriers for removal of oil from water in a batch experiment.

Materials and Methods: Four bacterial strains *Pseudomonas aeruginosa*, *Cronobacter sakazakii* (recently named *Enterobacter sakazakii*), *Klebsiella oxytoca* and *Bordetella bronchispetica* were immobilized on grinded corn qgualh, Egyptian loofah, palm leaf raffia and sponge. Immobilizing bacterial consortium was carried out by incubating bacteria with carriers at 37°C with shaking at 120 rpm for three days. Biofilm formed on biocarriers was checked using scanning electron microscopy. Biocarriers with immobilized bacterial consortium were used for removal of oil. Experiments were carried out in flasks each containing 100 ml of mineral salt broth fortified with 1 g/l light crude oil. Flasks were incubated at 37°C with shaking at 120 rpm for nine weeks. Total viable bacterial count and oil and grease were determined weekly.

Results: Oil and grease measurements showed a significant removal that reached 68.3, 71.5, 77.7 and 81%for grinded corn qgualh, Egyptian loofah, palm leaf raffia and sponge respectively. Flasks inoculated with sponge biocarriers showed rapid oil removal compared with other biocarriers. Gas chromatography-mass spectrometry (GC-MS) showed a percentage removal that reached 87% for compounds C11 to C32 in case of sponge carrier.

Conclusion: Immobilizing bacteria on biocarriers showed a good biofilm formation. Immobilized bacterial consortium showed rapid and efficient oil removal in bioremediation processes. Sponge showed dense bacterial colonization and consequently biofilm formation compared with other biocarriers.

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Key Words: Biodegradation, environment-friendly biocarrier, immobilization, light crude oil.

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INTRODUCTION

Oil contamination has become a global public health problem especially in developing countries. In Egypt, Nile River Transport, Nile Cruise tourism, and oil distribution centers located along River Nile have deteriorated its water quality due to direct or indirect leakage of different effluents into the water body[1,2]. This is due to accidental oil spill which might be derived from failure to control barges, tankers, and boats activities in the Nile River[3,4].

Petroleum hydrocarbons are one of the common toxic organic chemicals being introduced into river streams[5]. Mechanical and chemical methods are densely used for treating petroleum-contaminated water, but these methods are generally expensive and have limited effectiveness[6,7]. Bioremediation of oil-contaminated water is a promising technology due to its economic and environmental effectiveness[4, 8]. Isolation of highly potential bacterial strains from oil-contaminated environment and determining the optimum factors controlling its vitality are factors preceding the biodegradation process[8, 9]. Oil removal in a crude, oil-contaminated water could be enhanced by immobilization of hydrocarbon-degrading bacteria on biocarriers[10]. Biocarriers provide large surface area and strong adsorption capability. In addition, they improve oxygen diffusion and enhance dehydrogenase activity[5]. In Egypt, many agricultural residues can be used as biocarriers[11]. Examples include Egyptian loofah (*Luffa cylindrica*)[6], palm leaf raffia, corn qgualh, and used sponge[5]. These carrier materials are environment-friendly (nontoxic and nonpolluting materials) and economic[12]. Maintaining high biomass of bacterial populations on biocarriers is a key point for bioremediation[10]. Besides,

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immobilization is crucial to improve the survival and retention of bacterial cells[13]. Immobilized cell systems provide high biomass and cell reuse, reducing the costly processes of cell recovery and cell recycle[14,15]. In addition, they have high resistance to toxic chemicals, pH and temperature variation, solvents, and heavy metals and they show a decline in maturation time for some products[5,14].

The aim of the current study was to use economical and environment-friendly biocarriers for immobilizing consortium of bacterial isolates to remediate oil-contaminated water in a running batch reactor.

MATERIALS AND METHODS

Crude oil sample

Light crude oil was provided from the Egyptian Institute for Petroleum Research, Ministry of Scientific Research and Technology, Egypt.

Sampling collection sites

Forty water samples were collected from six sites along the Nile River in the Cairo segment (seven samples at El-Hawamdia Sugar factory discharge, seven samples at El-Maasara Bridge, seven samples at El-Hawamdia Bridge, seven samples at Masr El-Qadima bus station, six samples at Cairo University bus station, and six samples at Nadi El-Tagdif). Nine sediment samples were collected from three sites along Ismailia Canal as follows. Mosturod: three samples before discharge of ExxonMobil Company, (Khanka, Qlyubia, Egypt), three samples after discharge of ExxonMobil Company (Khanka, Qlyubia, Egypt) and three samples after discharge of Abouzabal Chemical Fertilizer Factory (Khanka, Qalyubia, Egypt). The samples were collected during the period from October 2014 to January 2015. Chemical screening of aliphatic and aromatic hydrocarbon pollutants and bacteriological examination of the samples were carried out according to APHA[16].

Isolation, screening, and identification of light-crude oil-degrading bacteria

Oil-degrading bacteria were isolated as described by Lin *et al.*[14] It was conducted as follows: 10 g of sediment samples and 1 g of crude oil were coincubated in 100 ml mineral salt broth (7.01 mmol/l K_2HPO_4, 2.94 mmol/l KH_2PO_4, 0.81 mmol/l $MgSO_4$ $7H_2O$, 0.18 mmol/l $CaCl_2$, 1.71 mmol/l NaCl) for 7 days under shaking at 180 rpm at 30ºC. One milliliter of the liquid culture was diluted sequentially to 10^{-7}. One milliliter aliquots of each dilution were spread onto mineral agar which was prepared by adding 1.5% agar into the mineral media. The plates were incubated at 37ºC for 48 h. Bacteria having the ability to use oil as carbon and energy source and consequently showing colony formation were taken as oil-degrading bacteria. They were assessed by viable cell counting. In addition, gravimetric measurement of the residual crude oil remaining in the culture medium was done at the end of incubation time.

Four bacterial strains were screened and purified from total 225 isolates during the first stage of this study[17]. The biochemical identification of bacterial isolates was carried out using Biolog GEN III, (Biolog Int., USA). Each bacterial isolate was inoculated in 94-well Microplates to analyze the phenotypic characters of each microorganism: 71-wells for carbon source utilization assays and 23-wells for chemical sensitivity assays. This test provides a phenotypic fingerprint of the microorganism that can be used to identify it at the species level.

In parallel to biochemical identification, the genetic identification was started. Slant copies were delivered to Macrogen Company (Republic of South Korea) for 16s rRNA sequencing identification. Results were provided on Sequence Scanner Software version 1.0. Sequencing products were resolved on an Applied Biosystems model 3730XL (automated DNA sequencing system Applied Biosystems, USA). The nucleotides BLAST (National Center for Biotechnology Information online program) was used to compare the identity of the strains with databases present on the GenBank.

Preparation of seed and mixed culture

A single colony was transferred from single slant cultures (48 h age) into 50 ml aliquots of trypticase soy broth[16]. Bacterial culture was centrifuged at 2700 rpm and the supernatant was decanted. The sediment was washed using a buffered saline solution containing/100 ml (Na_2HPO_4, 0.81 g, KH_2PO_4, 0.18 g) at pH 7.4 two times. The cell count of each strain was diluted to reach 10^7 colony forming unit per milliliter (CFU/ml). Ten milliliter from each strain suspension was taken to constitute a volume of 40 ml bacterial consortium from the four strains.

Preparation of biocarrier materials

One gram from each biocarrier material: Egyptian loofah (Luffa cylindrica), palm leaf raffia, and sponge were prepared in symmetric size cubes (nearly one cm dimension). Corn qgualh was ground and 1 g was weighed out. Carrier materials were washed with distilled, sterile water and dried at 37ºC for 4 h before being used for immobilization of bacterial strains (Fig. 1).

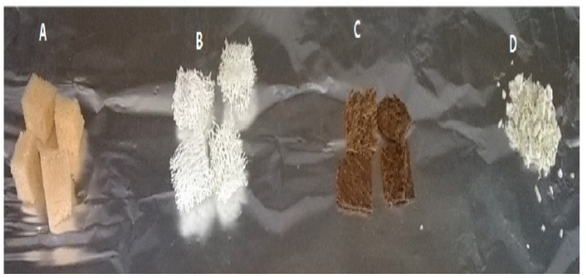

Fig. 1: Carrier materials were prepared in the form of separate pieces, A: sponge was prepared in the form of cubes, with dimensions of nearly 1 cm height × 1 cm width ×1 cm length, B: Egyptian Loofah (*Luffa cylindrica*) was prepared in the form of cubes with dimensions of nearly 1 cm height × 1 cm width ×1 cm length, C: Palm leaf raffia was prepared in dimensions of nearly 1 cm width ×1 cm length, D: Corn qgualh was grinded in variable sizes ≈ 0.1- 0.3 cm dimensions.

Immobilization of bacterial cells

The prepared biocarrier materials were immersed in culture flasks, each containing 150 ml mineral salt broth. Three milliliter (10^7 CFU/ml) of the bacterial consortium was added to each flask. The culture flasks were incubated at 37ºC for 3 days in a shaker at a speed of 120 rpm. At this stage, biocarriers were ready to start the biodegradation experiment after immobilizing the bacterial consortium. Immobilization (either by adhesion and/or entrapment) was checked by scanning electron microscopy (SEM).

Application of immobilized bacteria for biodegradation in a batch reactor

Sterile flasks (250 ml volume) each containing 150 ml of mineral salt broth fortified with 1 g light crude oil were used as bioreactors. One gram from each biocarrier with immobilized bacterial consortium was transferred to each flask. Flasks were incubated at 37ºC under shaking at 120 rpm. The experiment was carried out in two replicates for each carrier. The whole experiment was repeated three times. The maximum incubation time was 9 weeks. Counting bacterial biomass in each flask followed up the biodegradation process. Total viable bacterial count was weekly determined with the pour plate method for 9 weeks (Fig. 2)[16].

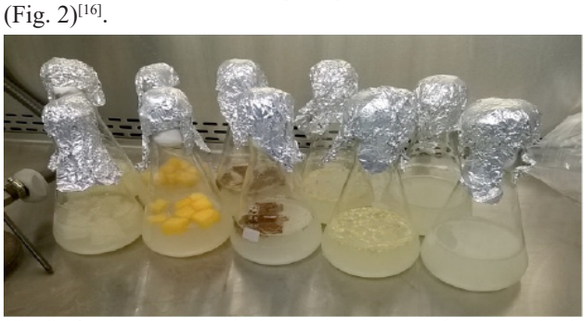

Fig. 2: Batch culture for the biocarriers immersed in mineral salt broth fortified with 1 gm oil/l, incubation at 37° C and shaker 120 rpm

Chemical analysis for oil and grease

Sediment (20 g) and water (30 ml) samples were transferred from the sample container. Sediment samples were weighed on an analytical balance, meshed and ground using a mortar and pestle until the sample was nearly as homogeneous as possible. Oil and grease was measured in water and sediment samples collected from the selected sampling sites according to APHA (2012)[16].

Determination of consumed hydrocarbons

At the end of the incubation time, the residual hydrocarbons in each culture medium were extracted by using chloroform [three samples: one chloroform (Sigma, Germany)]. Samples were continuously shaken with chloroform in a separating funnel. The contents were allowed to separate, two layers were formed: a watery layer and a chloroform layer containing the residual hydrocarbons, which was left to decant and air dry (ASTM-D-2007). After chloroform evaporation, the residual hydrocarbons were weighted and the consumed hydrocarbons were calculated by subtracting the residual hydrocarbons from the original weight of hydrocarbons (control).

RESULTS

Identification of bacterial strains

A mixed culture of four strains was immobilized using different types of natural environment-friendly materials to be used for biodegradation of oil spills. The isolates were identified using Biolog GEN III and sequencing for the completion of identification[18]. Sequencing analyses for complete genome of bacterial isolates using16S rRNA reported the following bacterial strains with the matched accession numbers: *Pseudomonas aeruginosa* (accession number KF835840.1) with 99% identity, *Cronobacter sakazakii* (recently named *Enterobacter sakazakii*) (accession number NR102490.1) with 96% identity *Klebsiella oxytoca* KCTC 1686 (accession number NC016612.1) with 95% identity, and *Bordetella bronchispetica* 253 (accession number NC019382.1) with 98% identity.

Immobilization of selected strains

Fibrous biocarrier materials of sponge, loofah, palm raffia, and ground corn qgualh were used as biocarriers in this study. The immobilizations of bacterial strains on biocarriers were shown by SEM images. Samples were investigated by SEM after coating with gold (high vacuum mode). Images for the immobilized bacterial consortium are illustrated in Figs 3–6.

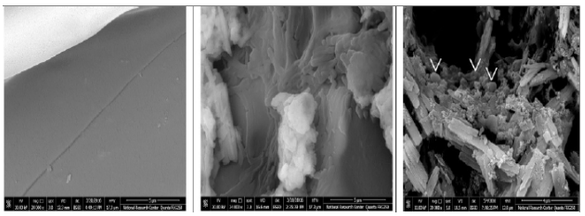

Fig. 3: SEM images for sponge carrier (a, b and c from left to right); a. control without any bacterial attachment (bare 5 µm), b. dense fresh rod-shaped biofilm and white material (exopolysacharides) (bare 5 µm), c. cocci-shaped cells (the arrow) on old sponge carrier material (bare 4 µm)

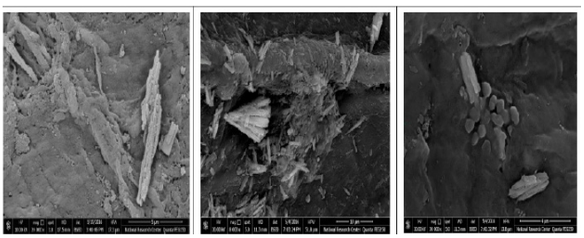

Fig. 4: SEM images of biofilm formed on Loofah biocarrier (a, b and c from left to right); a. control without bacterial attachment (bare 5 µm), b. dense continuous layer of cocci-shaped bacterial cells (bare 10 µm), c. aggregate of cocci-shaped bacterial cells supported on the biocarrier (bare 4 µm)

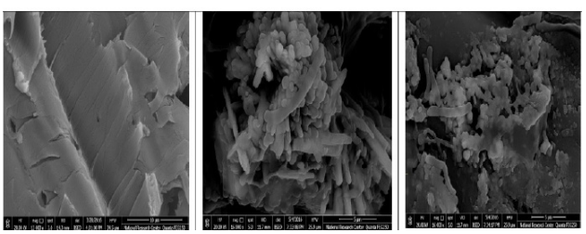

Fig. 5: SEM images of biofilm formed on palm raffia biocarrier (a, b and c from left to right); a. control with media and no biofilm formation (bare 10 µm); b. dense and fresh biofilm of rod-shaped cells (bare 5 µm), c. film of cocci-shaped cells covered with thin layer of exopolysaccharide, and threads of exopolysaccharide to left of the image (bare 5 µm)

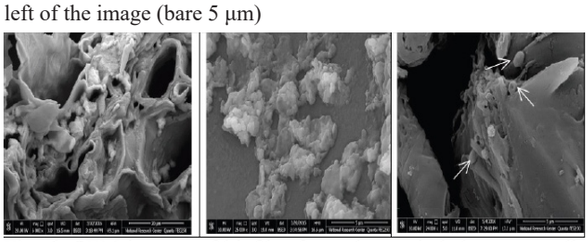

Fig. 6: SEM images of biofilm formed on grinded corn qgualh biocarrier (a, b and c from left to right); a. control with media and no biofilm (bare 20 µm), b. bacterial cocci-shaped cells on surface of qgualh and exopolysaccharides (bare 5 µm), c. cocci-shaped cells attached to qgualh surface (bare 5 µm)

Controls of different carriers are illustrated in Figs 3a, 4a, 5a and 6a where SEM showed no biofilm on sponge, loofah, palm raffia, and qgualh, respectively. Figure 3b shows the biofilm of bacillary cells partially covered with exopolysaccharides. Figure 3c shows coccoid cells on sponge tissue. Figure 4b shows dense coccoid cells of bacterial biofilm formed and attached on loofah biocarriers. Fig. 4c shows aggregate of cuboidal cells. Figure 5b and c show sufficiently strong interaction between bacterial cells coherent on the biocarrier surfaces, and the interconnection between individual bacterial cells via extracellular polysaccharides, suggesting that the bacterial cultures were tending to form thick biofilms. Figure 6b and c show coccoid cells forming biofilm attached on the ground corn qgualh surfaces. Bacterial biofilm synthesized exopolysaccharide support attachment to the biocarriers. The changes of bacterial numbers and activity in water are shown in Figs 3–7.

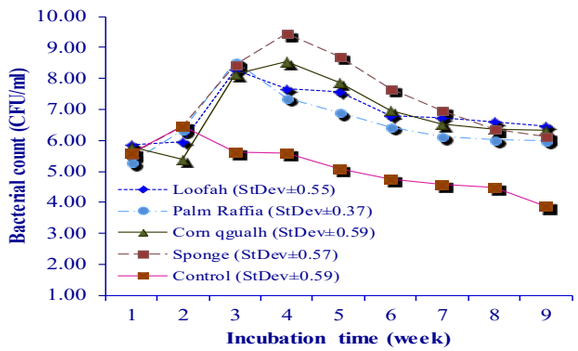

Fig. 7: Bacterial consortium count (cfu/ml) in batch flasks containing bio-carriers incubated at 37° C and shaken at 120 rpm, bars on each curve show the standard deviation

The number of bacteria entrapped was critical to the biodegradation effectiveness of the immobilized cells. The four different carriers were loaded with a bacterial consortium of up to 1.8 x 10^6 CFU/ml; sponge was more effective compared with other carriers. Bacterial count increased by 2–3 logs (1.24 x 10^8 - 1.6 x 10^9 CFU/ml).

Comparison of growth curves of bacterial consortium with and without carrier material.

In the absence of crude oil as the sole carbon source, the growth curve of bacterial count consortium decreased from 6.0 x 10^6 to 8.0 x 10^2 CFU/ml within 9 weeks in the batch reactor. When comparing the control with the other carrier growth curves, results showed no growth decline for all carriers. When using sponge carrier, bacterial consortium count reached the maximum after 3 weeks. The bacterial biomass produced in the case of sponge was higher (8 x 10^9CFU/ml) in comparison with other biocarriers. Palm leaf raffia, Egyptian loofah, and ground corn qgualh showed a bacterial count increase from 1.2 x 10^8 to 6.8 x 10^8 CFU/ml in a way similar to the sponge carrier (Fig. 7).

Application of immobilized cells for biodegradation of hydrocarbons

Average counts of bacterial consortia (10^7–10^8 CFU/ml) were immobilized on carriers. The trial was repeated three times and represented as log values in

Fig. 7. Results showed that the free indigenous bacterial cultures were quickly adapted to the environment and showed rapid multiplication and increase of total microbial activity within 2–3 weeks. In the immobilized systems, bacterial isolates in the batch reactor showed similar growth kinetics of degrading microorganisms and total microbial activity.

Average start counts of the three trials were 5.7 x 10^5 - 1.41 x 10^6 CFU/ml. After 2–3 weeks, the consortium count has shown an increase to 7.7 x 10^8 CFU/ml, while the control flask showed a count of 7.0 x 10^5 by the end of the third week. Counts increased gradually after the second week and reached the maximum in week 3. This count continued for further 5 weeks, then started to decrease by one log. Results in Fig. 7 show that the highest bacterial biomass was generated in the sponge carrier followed by palm leaf raffia, loofah, and then ground corn qgualh.

The percentage degradation of the crude oil was calculated by comparison of the gas chromatography-mass spectrometry (GC-MS) analysis of the nondegraded oil (control) and the sample after incubation with bacterial consortium, carrier, and bacterial consortium immobilized on the sponge carrier (Fig. 8) for each experiment.

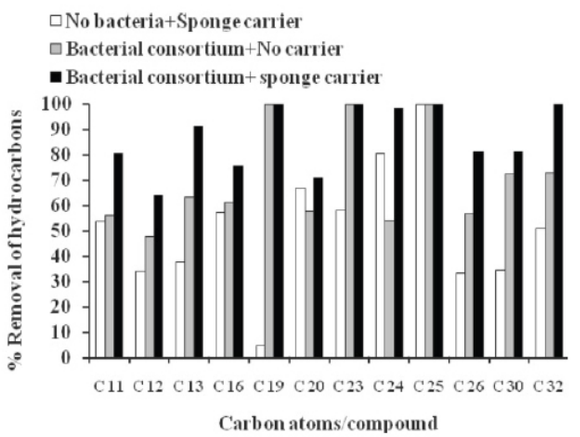

Fig. 8: Hydrocarbons removal (C11 - C32) by bacterial consortium immobilized on sponge carrier incubated at 37° C and shaken

Percentage removal of hydrocarbons was the maximum after treating the sample with the bacterial consortium immobilized on the sponge carrier. The results indicate a significant decrease of hydrocarbons C32, C25, C23, and C19 during the biodegradation process. After 9 weeks, the GC-MS results showed that immobilized bacteria were capable of removing more hydrocarbons than the suspended bacteria. Results have shown that average removal of the suspended bacteria consortium reached 70%. Immobilizing bacterial consortium on sponge has increased the hydrocarbon removal (C11–C32) to 87%.

In parallel to GC-MS analysis, the crude oil was measured as oil and grease concentration along the incubation time. The initial concentration in all flask reactors was adjusted to 1 g/l. The concentration of residual oil and grease was monitored biweekly for all carriers and is illustrated in Table 1. The highest removal rate was seen in the ninth week for sponge and palm leaf raffia with a removal percentage of 81 and 77.7%, respectively. The rate of oil removal was detected via an increase of microbial consortium activity, time, and carrier types. The removal rate of crude oil was calculated after comparing the oil and grease concentrations in the control group and in other groups using different carriers.

Table 1: Percentage removal of oil and grease using immobilized bacterial consortium on different biocarriers

Carrier	Initial oil conc. (g/l)	Residual oil (g/l)	Removed oil (g/l)	% of removal
Control (no carrier)	1	0.99	0.01	1*
Loofah	1	0.285	0.715	71.5
Palm leaf raffia	1	0.223	0.777	77.7
Grinded corn qgualh	1	0.317	0.683	68.3
Sponge	1	0.19	0.810	81

*Non-actual removal percentage, it shows trace of oil adhered on the inner walls of the flasks

DISCUSSION

In this study, the four identified isolates [*P. aeruginosa* (accession number KF835840.1)], *C. sakazakii* (recently named *E. sakazakii*) (accession number NR102490.1), *K. oxytoca* KCTC 1686 and *B. bronchispetica* 253 using 16s rRNA were multiplied and immobilized on environment-friendly carriers for remediation of oil-contaminated water. Several studies found that *P. aeruginosa* had superiority for crude oil biodegradation[15,17,19]. This might be due to their metabolic diversity, abundance in microbial communities, and their resistance to chemical remediation agents[17,19]. Many studies have mentioned that *E. sakazakii* and *K. oxytoca* have the ability to degrade the crude oil and can be used for bioremediation purposes[3,15]. In the present study, we isolated *B. bronchispetica* from Nile water with the ability to degrade crude oil. One disadvantage of using *B. bronchiseptica* for biodegrading oil as it was encountered both as a commensal and a cause of respiratory tract disease in many wild and domestic animals[20].

The selection of a carrier is important for using in immobilization. An ideal carrier should be nontoxic, nonpolluting, nonbiodegradable, having high cell mass loading capacity, biologically and chemically stable, having a long shelf life, low cost, diffusible by bacteria, easy to handle from media, and easy to regenerate[5,15]. In this study, loofah, palm leaf raffia, and corn-ground qgualh were selected as carriers from agricultural residues, in parallel with the sponge material. The suitability of carriers for immobilization of the bacterial consortium was investigated on weekly intervals. After 3 weeks of incubation, the immobilization of the bacterial consortium on carriers was shown using a SEM. SEM showed different shapes of the biofilm formed and attached on the surface of carriers, in addition to the production of exopolysaccharides[21,22].

The sponge, loofah, palm leaf raffia, and corn-ground qgualh carriers can serve as a suitable material for immobilizing bacteria, because they have better chemical and thermal stability[23]. For immobilization of bacteria, the sponge carrier supports the higher biomass density and microbial activity yield than the other three carriers. The maximum growth count of mixed culture strains immobilized on sponge averaged as 8 x 10^9 CFU/ml. This might be attributed to the greater mass transfer of the substrate (and oxygen) between the bulk solution and the bacteria immobilized on carrier[19,24,25].

Degrading bacterial consortium population and activity dropped and reached equilibrium in all treatments by the fourth and the fifth week. This was possibly due to the fact that the bulk of labile hydrocarbons and other nutrients had been consumed before 4 weeks[9,26]. In addition, the productions of toxic intermediates were likely to have an inhibitory influence upon bacterial consortium by the end of the third week[9,27].

Biofilms are superficial growths constituting of aerobic and anaerobic bacteria arranged in multiple layers embedded in hydrated matrices[28]. Bacterial immobilization could occur via adsorption, covalent binding, entrapment, and encapsulation[5,22]. Excellent degradation is enhanced by high immobilization efficiency of bacterial cells onto the carrier material and the high affinity between the hydrophobic immobilization material and the substrates[5, 22]. The availability of the substrates for bacterial cells and cohesive interaction between the substrates and the immobilized cells could accelerate the degradation rate[29]. Hsu *et al.*[21] stated that immobilization by adsorption is mild, quick, simple, and economically advantageous, since there is no need for chemical additives, in addition to easy performance of the process with possible reuse of the carrier for other treatments. It has the advantage of enzyme leak from the biofilm layer adsorbed onto the carrier to the suspending oil[21]. Moreover, cell immobilization by adsorption provides a direct contact between nutrients and immobilized cells. Absorptivity and stability of biocarriers are two major criteria for the selection of a suitable supporting material[21]. Finally, the major advantages of an adsorption system for water pollution control are less investment in terms of initial cost and ease of operation[30]. High-rate bacterial growth in carriers especially in sponge could be due to the sponge material which accelerates the oxygen transfer and provides protection and a favorable niche for bacterial consortium to utilize the hydrocarbons[31]. Also, the growth of the consortium bacterial strains was highest with the sponge carrier, showing its optimistic supporting properties[23]. Some compounds in petroleum can be toxic above a certain concentration[4], inhibiting the metabolism of the microorganism; this explains the results we obtained for control flasks.

In this study, when applying both free and immobilized cells, GC-MS showed that the removal of crude oil from contaminated water increased by the application of hydrocarbon-degrading bacteria immobilized on the carrier[9]. This was proved via the difference in count found between control flasks and other flasks[28]. Also, at the early stage, bacterial growth and replication within the matrix may release some bacteria to the surrounding environment[28], resulting in low degrading bacterial count and activity in the reactor. After 2–3 weeks both bacterial count and activity increased[32] resulting from both massive cell releases associating with oil degradation and their subsequent growth in the batch reactor[6]. In this context, the biocarrier like sponge, loofah[23] provides a large surface area and strong adsorption capability. Tissue structure of palm raffia and ground qgualh provides an internal area to entrap the bacterial cells. In addition, it improves oxygen diffusion and enhances dehydrogenase production, thus reducing the toxic effect of petroleum compounds. Wiesel *et al.*[29] reported that a mixed immobilized bacterial culture exhibited good growth, and demonstrated equivalent degradation potential of polycyclic aromatic hydrocarbons compared with freely suspended cells[29].

The hydrophilic properties of sponge fibers and oleophilic characters allowed the contact between the crude oil particles with the immobilized bacterial cells[5,33]. This combination facilitates more exposure of the absorbed oil to the immobilized bacterial consortium. The adherence of oil to the bacterial consortium coated biocarriers is a simple way to boost the substrate uptake speed[15]. The high rate growth of bacterial consortium in carriers especially sponge could be due to the acceleration of oxygen transfer, providing protection and a favorable niche for bacterial consortium to utilize hydrocarbons[27].

In this study, after applying the immobilized consortium for biodegrading light crude oil, the mixed consortium of bacterial strains degraded 81% of oil using the sponge carrier, followed by 77% in case of palm leaf raffia carrier. Loofah and ground corn qgualh showed 71.5 and 68.3% degradation, respectively. This comes in parallel with Wang *et al.*[32] who found that the consortium bacteria degraded a maximum of 77% crude oil followed

by 69% by *Pseudomonas spp.* BPS1-8, 64% by *Bacillus spp.* ISS1-7, 45% by *Pseudomonas spp*. HPS2-5, and 41% by *Gorynebacterium spp.* BPS2-6 at 1% crude oil concentration[27].

In our study, we used a mixed culture of four selected strains for the biodegradation of light crude oil for the synergistic mechanism resulting from interaction of consortium bacteria with their diverse enzymatic activities under suitable conditions[34]. It is possible that each species of the consortium was able to remove the toxic metabolites that may hinder the activity of the other strains; consequently they assemble an integrative simple process to remove oil[35]. Loofah is a good biocarrier for bacterial strains and effective natural biosorbent for oil spills[3,36]. However, in a trial to apply fed-batch cultures, a bioreactor was used to immobilize 75.67 and 73.83% of aliphatic and aromatic hydrocarbons, respectively; cells on loofah were repeatedly used to degrade diesel oil[23]. A decrease in degradation capacity was reported and could be the result of intercellular oxygen and nutrient competition commonly associated with high cell concentrations.

CONCLUSION

Palm leaf raffia, sponge, loofah and ground corn qgualh can serve as suitable materials for immobilizing bacteria. For this purpose, sponge carrier supports a higher biomass density and microbial activity yield than palm leaf raffia, loofah, and ground corn qgualh carriers.

P. aeruginosa, *C. sakazakii* (recently named *E. sakazakii*), *K. oxytoca*, and *B. bronchispetica* have shown a high ability for utilizing oil as carbon source, but *B. bronchiseptica* is not recommended for biodegrading oil because it is encountered as a cause of respiratory tract disease in many wild and domestic animals.

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CONFLICT OF INTEREST

There are no conflicts of interest.

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