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Optimization of Immobilization Conditions for Petroleum Oil Biodegradation by *Candida tropicalis* AQ1 using Wood Chips and Wax as Carrier.

Soha Farag[1], Nadia A Soliman[2]*, Yasser R Abdel-Fattah[2]*.

[1]Environmental Biotechnology Department Genetic Engineering and Biotechnology Research Institute, City of Scientific Research and Technological Applications, Alexandria, Egypt
[2]Bioprocess Development Department. Genetic Engineering and Biotechnology Research Institute, City of Scientific Research and Technological Applications, Alexandria, Egypt

ABSTRACT

Crude oil removal by microorganisms is hot choice due to its low cost effective. A locally isolated yeast cell coded as AQ1 showed high potential activity in oil removal and identified as *Candida tropicalis* (ac: KU341838) was used in this study. Screening program for optimization the oil removal was applied including different carries, incubation time and saturation capacity. It reported that, the biodegradation potential increased with increasing the incubation time from 1-5hr. The percentage of crude oil removal reached to 90% by using immobilized AQ1 on wood chips as a carrier (1.75g/58cm^2 surface area of the flask). However, in case of using wax as a carrier 11g/58cm^2, the percentage reached to 70%. A high microbial colonization was observed at 1-3mm thick biofilm of the outer surface of thin wood chip through examination by Scanning Electron Microscopic (SEM). A new semi-continuous fermentation cycle was tested along three days using constant weight of wood chips. The best result of oil removal using oil concentration (1% and /or 3.5%) for each cycle along three days reached to 89.76 and 29.31% respectively. This study explored the role of biocarrier in enhancing biodegradation of hydrophobic contaminants such as crude oil.

Keywords: crude oil; 18Sr RNA; biodegradation; immobilization; wood; wax.

Corresponding author

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INTRODUCTION

In order to manage oil residues and effluents contamination with hydrocarbons various technologies have emerged, recently. Biodegradation is one of the most extensively used because of its high efficiency and low cost [1,2].

Microorganism might be considered brilliant pollutant removal tools in soil, water, and sediments, mostly due to their advantage over other bioremediation procedures [3-6]. Alternatively, cell immobilization techniques have become increasingly important and are being successfully applied in industrial processes such as alcohols, organic acids and enzymes production. Also, it was used in biotransformation of steroids for wastewater treatment, hormone production and food applications (sugars, meat, wine, beer) [7].

In general requisites, freedom in cellular movement can be restricted in two main ways: physical entrapment of cells within the carriers or adsorption of cells (either between the cells themselves or to a solid carrier surface) with physical and chemical bonds [8]. The oxygen transfer in immobilization processes is needed for microorganism and it is considered a limiting factor [9,10].

In any case, it has been widely reported in the literature that immobilization provides bacteria with a special stability against the negative effects of temperature, and pH [11] as well as avoiding the washing-out of the residents of cells upon using high dilution rates in the continuous process. Adsorption to surfaces and encapsulation within gels or porous materials (a particular type of physical entrapment) has been the most widely studied methods for the immobilization of microorganism. The adhesion techniques is related to the ability of microorganisms to secrete a polysaccharide which make it able to stick the surface of the carriers as described by Moonmangmee *et al*., [12].

The adsorption occurs only at the carrier surface when the pore size of the matrix is smaller than the dimensions of the cell, as in the case of diatomaceous earth, clays and other related materials. However, when the carrier has pores that are large relative to the cell dimensions, it is possible to find adhesion within the pores. This situation occurs in materials such as active carbon, polyurethane foam wood chips, wax and sintered glass. The problems associated with oxygen diffusion can be reduced by applying adsorption techniques, which do not suffer from the scale-up drawbacks experienced with encapsulation matrices [13].

Oil adsorbents can adsorb and concentrate floating petroleum and prevent its migration to shorelines and beaches. If oil adsorbents were immobilized with hydrocarbon degraders, bioremediation may occur in situ or ex situ. Of great interest to bioremediation is the potential of immobilizing microorganisms onto polyurethane foams (PUF), alginate and other matrix to degrade hydrocarbons and toxic wastes [14-19].

The immobilized hydrocarbon-degrading microorganisms in inert surfaces encourage crude oil degradation [20,21]. Additionally further protection of the microorganisms from harmful effects of toxic wastes can be achieved and an ability to degrade pollutants at a higher initial concentration for a longer period. Also immobilized cells could be stored for long periods without losing their degrading abilities. The adsorbed oil may even be extracted and the adsorbents reused [18]. Immobilization of oil degraders on oil adsorbents may therefore solve the problem of dilution of microbes and nutrients in open water.

Herein, we described immobilization of yeast isolate by adsorption using two different solid carriers: wood chips and paraffin wax for oil degradation. Three items were considered in carrier selection, the availability, in-situ simple applications and possibility of scaling up for oil removal. As well the immobilization processes were optimized to work properly in situ.

MATERIALS AND METHODS

Sampling site and isolation of yeast.

The yeast isolate used throughout the present work was isolated from a motor oil polluted area of Abou-Qir gulf, Alexandria, Egypt. Samples were collected from sea water and slurry sediments and enriched for three successive times in Nutrient Broth (NB) medium supplemented with 3.4% NaCl, incubated at 30˚C for

three days for each treatment. The enriched cultures were diluted to 10^{-3}, 10^{-4} and 10^{-5} dilutions; different morphotypes were selected and streaked out on the same medium for further purification.

Preliminary test for degradation ability.

Screening of oil degradation ability by selected purified colonies was performed through growing them on (Nutrient Sea Water Agar) NSWA plates' containing petroleum oil (0.5%), incubated at 30˚C for one week. Thereafter, the colonies able to grow and subsequently showed high potency in oil degradation were selected. The best oil degrader isolate named AQ1 was finally selected to continue this study.

DNA isolation from the yeast strain.

An overnight culture of the selected isolate (AQ1) was grown in (Glucose Peptone Yeast) GPY medium containing (w/v %): Glucose 2; Peptone 1 and Yeast Extract 0.5, incubated at 30˚C. The grown cells were spin down and the pellets used for the preparation of genomic DNA. The DNA was isolated using EZ-10 Spin Column Kit (Bio Basic Inc. Canada) according to the manufacturer's instructions.

Identification of the yeast isolate.

The isolate was identified by 18S rRNA gene sequencing using the following universal primers: **18SF 149:** 5'- GGAAGGG(G/A)TGTATTTATTAG -3' and **18SR 1709:** 5'-TCCTCTAAATGACCAAGTTTG-3'**.**

The PCR mixture consisted of 25 pmol of each primer, 10 ng of chromosomal DNA, 200 mM dNTPs and 2.5 U of Taq polymerase in 50µl of polymerase buffer. The PCR was carried out for 30 cycles at 94˚C for 1min, 55˚C for 1min and 72˚C for 2 min. The PCR product was examined on agarose gel electrophoresis [22], and consequently purified using QIAquick PCR purification reagent (Qiagen). The 18S rRNA gene fragment (~1500bp length) was sequenced in both direction and the similarity to other related yeasts from GenBank was carried out by BLAST search (www.ncbi.nlm.nih.gov/blast).

Extraction of residual crude oil.

The culture broth containing residual petroleum oil was extracted three times by 20 ml chloroform and the hydrophobic phase was separated using separating funnel. The chloroform extracts were evaporated and weighed. Negative control of non inoculated medium was extracted using the same extraction procedures and the residual oil was quantified gravimetrically after subtracting the reading from the negative control [23].

Degradation of crude oil by immobilized yeast isolate AQ1 using different carriers.

Physical properties of used carriers

In this experiments two different carrier were used: wood chips (thick and thin) and paraffin wax. The tested carries varied totally in its physical properties. The main characteristics features of used carriers were summarized in **table 1** including: shape, size, thickness, density and melting point.

Preparation of yeast cell for immobilization.

The selected yeast isolate AQ1 was grown in GPY medium at 30˚C on a rotary shaker at 150 rpm for 28 hrs. After cultivation the cells were centrifuged at 3000 rpm for 15 min, washed twice with saline solution and re-suspended in the natural sea water for further experiments.

Immobilization using wood chips as carrier.

To immobilize the target isolate (AQ1) on the tested carrier, the overnight cells of AQ1 were incubated with the carrier thin and thick wood chips individually. The cells were allowed to incubate with the carriers for different times (1hr, 2hr, 3hr &5hr) to determine the best time required for cells adsorption (combinations). After cell adsorption under tested conditions, the remainder of GPY medium was removed by decantation. The immobilized cells were incubated under shaking conditions (200rpm, 30˚C) for three days

using natural sea water as a basal medium and 1% crude oil. The efficiency of oil degradation by immobilized yeast cells AQ1 under tested conditions was measured and tabulated.

The effect of using different weights of thin wood chips (0.5, 0.75, 1, 1.25, 1.5, 1.75 g/58cm^2) for cell immobilization at fixed time (3hrs) on oil degradation was investigated. The process was completed by adding of sterilized basal natural sea water as medium then crude oil (1%) to the immobilized cell. This was followed by incubating the tested isolate at 30˚C under shaking for three days. Afterwards, the % of oil consumed was measured gravimetrically.

Microscopic examination of immobilized cells on thin wood chips carrier.

The adsorbed cells AQ1 on thin wood chips and negative control (wood chip with oil) were examined using scanning electron microscope at magnification folds (x500, x1500).

Immobilization using wax as a carrier

This experiment was carried out to investigate the effect of using paraffin wax as cell carrier. The effect of using different weights of wax (5, 7, 9, 11g/58cm^2) for AQ1 cell immobilization at fixed incubation time (3hrs) on oil degradation was tested. The process was completed by adding of sterilized basal natural sea water as medium then crude oil (1%) to the immobilized cell. This was followed by incubating the tested isolate at 30˚C under shaking for three days. Afterwards, the % of oil consumed was measured gravimetrically.

Immobilization process in different panels

At first stage the equivalent carrier weight used to cover the surface area of the flask was used in all experiments in order to obtain comparable results. Initially, the panel I containing 23g of carrier per 402cm^2 panel I surface area was sterilized using wet heat at 120˚C during 20 min in a conventional sterilization system. The use of this method avoids the heating involved in the conventional sterilization process, the effect of which could lead to undesirable effects in the substrate.

In the first stage, 20 ml (1%) oil was inserted into panel I which contains natural sea water as a medium. The immobilization process was developed for successive oil removal by using immobilized AQ1 which incubated 3hrs with the carrier. Initially, the panel I contains 2liter of natural sea water as a medium, 20 ml (1%) crude oil, suitable amount of carrier 23g (according to the surface area of the panel I) and incubated at normal room temperature (25˚C) for constant time one day per run in the bench shaker. The oil removal developed quickly and the medium almost clear, at this point, the first immobilization cycle was taken to be finished.

The second cycle begins with addition of another 20 ml of crude oil to panel I using the same carrier without changing any condition then followed by the third cycle. After that the residual oil in the sea water and the oil stick in the walls of panel I were determined by gravimetrical method.

Other experiment was done by using another panel size in which the carrier weight was 6.5g/ 113 cm^2 of panel II surface area whereas the all conditions were constant.

This process represents the start of a new semi-continuous fermentation cycle in which further immobilization takes place. The whole immobilization process involved successive semi-continuous cycles, with 20 ml (3.5%) crude oil, until the adhered biomass analysis showed that saturation of the carrier had been achieved (stabilization of the number of immobilized cells).

The same procedure was developed in case of wax as a carrier by using weights 20g and 5.5g for 402cm^2 and 113cm^2 of panel I and II surface area respectively.

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RESULTS

Isolation and identification of the yeast isolates

In screening program for selection of potent isolate degrading the crude petroleum oil, the isolate AQ1 was selected. Subsequently, the identification of this isolate was performed on molecular bases of sequencing a conserved part of the 18Sr RNA. According to sequence similarities the isolate AQ1 showed a close relation to *Candida tropicalis SZ3* sp. (ac: KT229543) with a 100% identity. The partial sequence of 18Sr RNA of AQ1 isolate has been deposited into GeneBank under accession numbers (ac: KU341838).

Oil degradation by immobilized AQ1 at different incubation times.

Different carries (wood chips & paraffin wax) were used to immobilize the tested strain AQ1, where the physical properties of these carries were summarized in **Table 1.** To determine the best type of wood chips either thin or thick as an immobilized carrier, different incubation times (1hr, 2hr, 3hr& 5hr) with the cells were tested to assign the best combination. After cell adsorption under tested conditions, the remainders were removed by decantation. The immobilized cells were incubated under shaking conditions (200rpm, 30˚C) for three days using natural sea water as basal medium and 1% crude oil. The efficiency of oil degradation by immobilized yeast cells AQ1 under tested conditions was measured and summarized in **Table 2**.

Table 1: The main characteristic of assayed carriers

Properties of used materials	Thick wood chips	Thin wood chips	Paraffin wax
Material shape	Slight curved	Straight	Particles nearly round
Density (g/v)	0.035	0.06	0.025
Material size: Length (cm) Width (cm) Thickness (mm) Diameter (cm)	 2-2.4 1-1.3 0.7 -	 1-1.6 2-3 0.3 -	 - - - 1-1.6
Melting point	ND*	ND*	58-60˚C

* ND: not determined

Table 2: Efficiency of oil degradation by immobilized cell of strain AQ1 using thin and thick wood-chips as carriers at different incubation time for cell adsorption.

Time (h)	Thin wood chip	Thick wood chip
	% of oil consumption by immobilized AQ1	% of oil consumption by immobilized AQ1
1h	60.2	55
2h	65	57
3h	74	64.2
5h	77.5	68

*The used weight of wood chip was 1.25g, which enough to cover the surface area of Erlenmeyer conical flask 250ml (58cm^2).

The results showed that the use of immobilized AQ1 with thin wood chips increased the removal percentage of oil than the thick wood chips. The % removal of crude oil using this carrier increased with increasing the incubation time with yeast cells. After 5hrs incubation the maximal oil removal % was 77.5 for immobilized AQ1. However after 3hrs the oil removal % was 74, subsequently for time consuming 3hrs was selected for the further work.

The used weights of wood chips in this experiment were 1.25 g, this weight enough to cover the surface area of Erlenmeyer conical flask 250ml (58cm^2).

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Degradation of crude oil by immobilized AQ1 at different weights of thin wood chips.

This experiment was carried out to investigate the effect of using different weights of thin wood chips (0.5, 0.75, 1, 1.25, 1.5, 1.75 g/58cm^2) for cell immobilization on oil degradation at fixed incubation time (3hrs). The process was completed by adding of sterilized basal natural sea water as medium then crude oil (1%) to the immobilized cell. This was followed by incubating the tested strains at 30˚C under shaking for three days. Afterwards, the % of oil consumed was measured gravimetrically.

The results **(Fig. 1)** showed that the removal % of oil increased progressively by increasing the weight of wood chips per area and reached to 89% at 1.75g by strain AQ1.

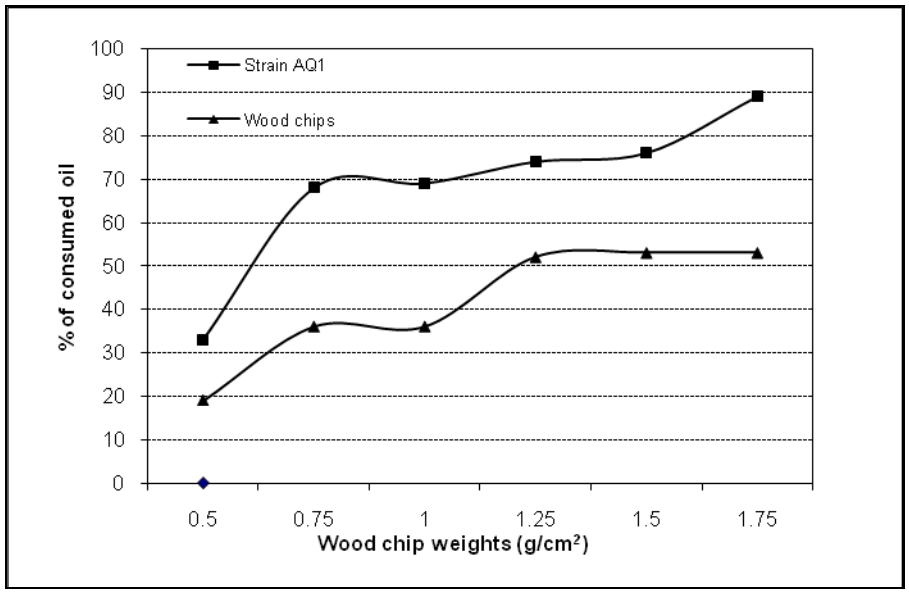

Figure 1: Efficiency of immobilized *Candida tropicalis* AQ1 to remove oil expressed as % using different wood chip weights (thin type) in comparison with a negative control.

It is worth to mention that the wood chips alone able to adsorb oil on its surface as shown in **figure 1**, where the percentage was increased by increasing the weights then steady reached at 1.25g which enough for covering the whole surface area of Erlenmeyer conical flask 250ml equivalent to (58cm^2),where 52% of the oil was removed.

Degradation of crude oil by immobilized AQ1 at different weights of paraffin wax.

This experiment was carried out to investigate the effect of usage different weights of paraffin wax as carrier in immobilization. Different weights of wax 5, 7, 9, 11g/58cm^2 were tested at fixed incubation time between cell and carrier (3hrs) then oil degradation was monitored. The process was completed by adding of sterilized basal natural sea water as medium then crude oil (1%) to the immobilized cell. This was followed by incubating the tested strains at 30˚C under shaking for three days.

The results presented in **figure 2**, showed that by increasing the wax weight per area the removal % of oil by immobilized AQ1 increased gradually by increasing the weight of wax which reach to 63% removal at 9g and 69% at 11g.

It is worth to mention that the wax alone able to adsorb oil on its surface as shown in **figure 2**, where the percentage was increased by increasing the weights and achieved 43% removal at 9g wax alone. We believe that it is due to bonding effect and hydrophobic interaction between oil and wax. It is mentioned that the weights of paraffin wax (7g) is enough for covering the whole surface area (58cm$^{2)}$ for the Erlenmeyer conical flask 250ml.

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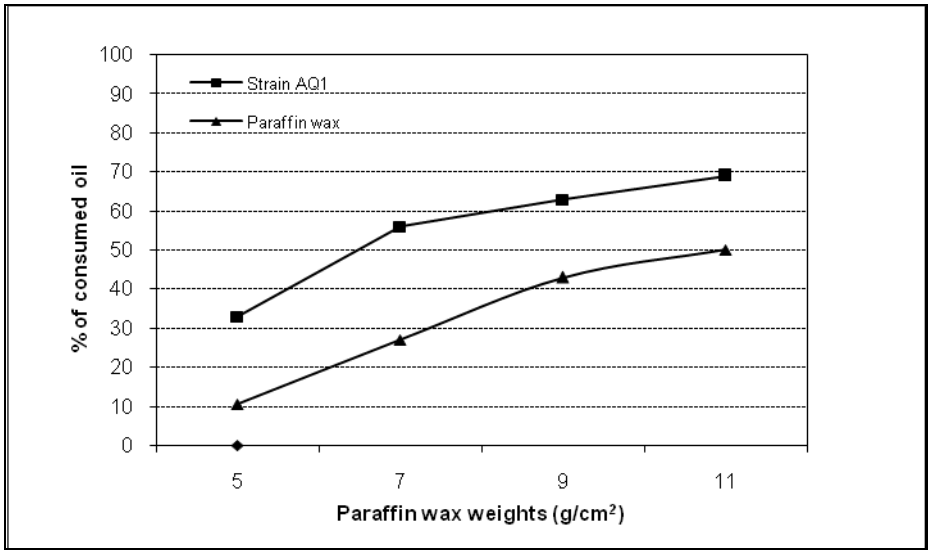

Figure 2: Efficiency of immobilized *Candida tropicalis* AQ1 to remove oil as % using different paraffin wax weights in comparison with a negative control.

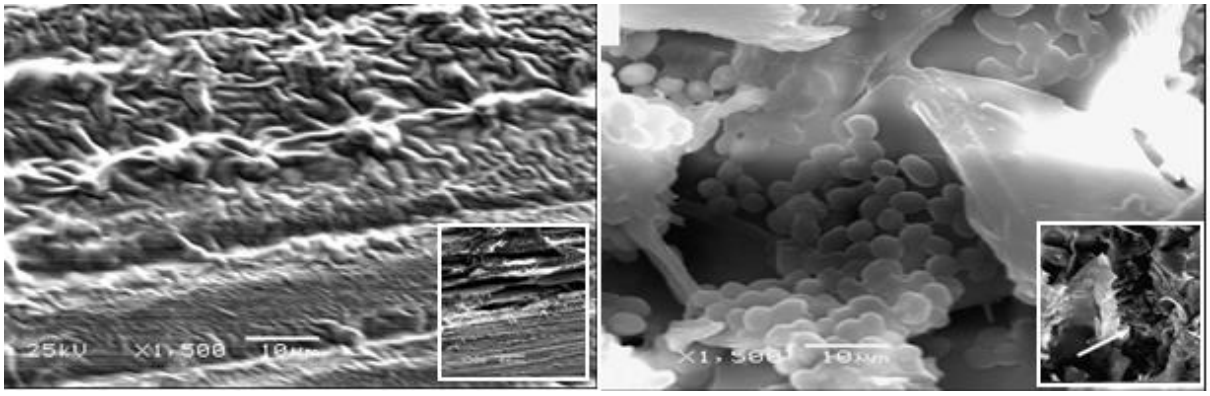

Figure 3: Electron micrographs of the wood chip outer surface at (500x: small box) and (1500x: large box) amplification power.
Left side: negative control (wood chip mixed with oil): Right side: immobilized AQ1 on wood chip.

Microscopic examination of immobilized cells on thin wood chips carrier

The adsorbed cells (AQ1) on thin wood chips were examined microscopically using SEM at different magnification folds (x500 and x1500), where the cell agglutination was easily recognized on the solid surface of the wood chips for tested strain AQ1 **(Fig. 3)**. Macroscopic examination for negative control at different magnification forces showed oil droplets attached with the solid surface of the assayed wood carrier **(Fig. 3)**.

Immobilization process using panels

Table 3, shows the data for the immobilization cycles for each of the two panels which have two different surface areas (402 cm^2& 113 cm^2), at identical conditions for the two carriers: Wood chips and wax. The percentages of crude oil removal were measured during the semi-continuous cycles for every panel. Twenty ml (1%) of the oil was added at each cycle in the panel I without considering the surface area of the reactor but the carrier weights were added in relation to the surface area of the used panels.

Table 3: Percentage of oil removal using wood chip in panel I after one and three runs.

Wood chip panel I, day/run	Residual oil (gm)	Blank oil (gm)	Oil removal % by immobilized cells
First run	0.218	12.2	98.213
After third run	3.749	36.6	89.76

* 20ml (1%) oil was added each run equivalent to 12.2 g
* Weight /surface area equals 23g/402cm^2

Table 4: Percentage of oil removal using paraffin wax in panel I after one and three runs

Wax panel I, day/run	Residual oil(gm)	Blank Oil (gm)	Oil removal % by immobilized cells
First run	0.373	12.2	96.94
After third run	6.854	36.6	81.27

* 20ml (1%) oil was added each run equivalent to 12.2 g
* Weight /surface area equals 20g/402cm^2

Table 5: Percentage of oil removal using wood chip in panel II after one and three runs.

Wood chip panel II, day/run	Residual oil (gm)	Blank oil (gm)	Oil removal % by immobilized cells
First run	4.72	12.2	61.31
After third run	25.872	36.6	29.31

* 20ml (3.5%) oil was added each run equivalent to 12.2 g.
* Weight /surface area equals 6.5g/113cm^2

Table 6: Percentage of oil removal using paraffin wax in panel II after one and three runs

Wax panel II, day/run	Residual Oil (gm)	Blank Oil (gm)	Oil removal % by immobilized cells
First run	6.217	12.2	49.04
After third run	30.986	36.6	15.34

* 20ml (3.5%) oil was added each run equivalent to 12.2 g.
* Weight /surface area equals 5.5g/113cm^2

From the data given in **Table 3& 5** it can be seen that since the process already persisted during three days, saturation of the carriers was reached under the used conditions (stirring, aeration, carrier quantity, etc.). The percentage of oil degradation using wood chips as carrier reached to 89.76% and 29.31% in the panel I and II surface area 402 cm^2 and 113 cm^2, respectively after three cycles. The percentage of oil degradation by using wax as a carrier reached to 81.27% and 15.34% in the panel I and II surface, respectively after three cycles as shown in **Table 4& 6**.

DISCUSSION

A marine yeast AQ1 which isolated from Abou-Qir gulf of Alexandria, Egypt was selected due to its pronounced crude petroleum oil biodegradation ability. The isolate AQ1 was identified as *Candida tropicalis* (ac: KU341838).

The progress of immobilized biomass on thin wood chips vs. time is perceptibly different from that registered for thick wood chips. The number of cells in this carrier increases progressively with time, fitting a logarithmic trend in accordance with typical adsorption profiles. This situation is characteristic of regular surfaces in which the pore structures do not vary significantly in size, but all the pores in the carrier have a similar accessibility. In this case, carrier saturation is the key factor and points to the asymptotic limit of maximum immobilization capacity.

Under the experimental conditions used in this study, the maximum quantity of biomass that wood chips can immobilize is recorded after 5hrs of operation. Table (2) represent that the % of oil consumption using thin wood chips reached to 74%, 77.5% after 3hrs, 5hrs incubation time respectively by using strain AQ1. While the oil consumption decreases when thick wood chips were used and reached to 64.2%, 68% after 3hrs, 5hrs incubation time respectively by tested isolate (AQ1). Fig. 3 shows a SEM of one of the units10-30 µm thick biofilm on the outer surface of biocarrier (wood chips) at different magnification folds, where it was easily recognized cell agglutination on solid surface of one of the units, which illustrate the compact yeast agglutination on solid surface aggregates on the wood chips.

Levison *et al*. [24] reported that only biofilms within 10–20 µm thickness could ensure easy diffusion of substrates and oxygen for metabolic activities. As such, the bacteria attached on the carriers examined in his study are expected to be able to sufficiently utilize the hydrocarbon substrates in the soil.

Ideal porous carriers for immobilization of bacteria should have a large specific surface area for the adherence and growth of bacteria, not toxic and cost-effective [25,26]. Other results of Liang *et al*., [27] showed that activated carbon is a better carrier than zeolite for the enrichment of bacteria. This may be due to higher adsorption capacity of activated carbon and the larger specific surface area, which may endorse the microbial aggregation by: (i) adsorbing of sufficient phosphorus and nitrogen for microbial metabolism, (ii) supplying an available surface area to support bacterial growth, and (iii) accelerating the diffusion of gases to increase oxygen as an electron acceptor.

Four criteria are the most relevant to establish the appropriate comparisons between the different carriers assayed: immobilization capacity, time for immobilization process, degradation rate with immobilized biomass and, finally, mechanical stability of the carrier. Moreover, a fifth important characteristic will be considered: the economic cost of each carrier. Based on these criteria, we optimized the oil degradation using different weights at optimum incubation time 3hrs (according to the former result) for the immobilization of AQ1.

In the present study the efficiency for oil removal by immobilized AQ1 on wood chips reached to 89%, that is better than using wood chips alone (53%) due to the effect of microbial biodegradation added to the physical addition of the oil to the surface area of the wood chips. The % of removal in case of wood chips as a carrier is steady from weight 1.25g to 1.75g in all cases therefore we used the least amount of weight (1.25g) which cover the surface area of Erlenmeyer flask 250 ml ($58cm^2$).

In case of using wax as an immobilized agent to AQ1, the degradation of oil at different weights showed steady state in all cases from 9g to 11g, the maximum percentage of oil removal was 69 and 50% for immobilized AQ1 and wax carrier as a blank respectively.

On the other hand, several studies reporting that the free cells showed lower degradation rate than immobilized cells [17,19]. The better and faster degradation rate was due to the high immobilization efficiency of the cells onto the immobilization material and the high affinity between the hydrophobic immobilization material and the substrates. Enhancing the degradation rate might be related to the strong synergistic effects between the substrates and the immobilized cells [28,29].

Based on the abovementioned criteria applied to the assayed solid supports results and the immobilization capacity it is clear that wood chips would be the most suitable carrier because it allows the immobilization of higher number of cells per milligram of solid. In addition to the values discussed above it is very important to take into account the time required to reach the maximum immobilization capacity. In this sense, the two carriers (wood chips and wax) have the ability to reach an acceptable quantity of adhered biomass in a substantially lower time (3hrs). The porous structure is highly uniform in the wood chips, a fact

that facilitates rapid cellular adhesion. Thus, making the operation more profitable as mentioned also by [13]. Finally, other economic aspects must also be considered. Wood chips are, without a doubt, the cheapest material and it is also readily and widely available. Wood chips can be obtained either from old ageing casks or by direct acquisition [13].

So the formation of semi-continuous cycles by different surface area size panels` was important as an application study. Thus from achieved result by using of immobilized AQ1 on wood chips or wax the percentage of oil removal give best result in large surface area panel I (402cm^2) than the small surface area panel II (113cm^2) after 3cycles due to the using of higher oil concentration in small panel II (3.5%) compared to 1% in the large panel I, the same result found in case of using the wax as a carrier. The % of oil removal in case of wood chips is better than the wax due to the differ in physical properties of them as a carrier.

Khondee *et al*., [30] reported that in a semi-continuous batch experiment, the immobilized bacteria *Sphingobium* sp. P2 were able to remove 80–90% of the 200 mg L^{-1} total petroleum hydrocarbons (TPH) from both synthetic and carwash wastewater. Also Liu and Liu, [31] described that in batch cultures with 10,000 ppmv diesel or crude oil reached approximately 90% oil removal within 4 days (about 30% of biodegradation and 60% of biosorption) by using *Rhodococcus erythropolis* strain NTU-1. In bioreactors with an intermittent feed of 35,000 ppmv diesel or crude oil resulted in more than 90% removal within 2 weeks (about 20% of biodegradation and 74% of biosorption).

Nevertheless, it is important to note the overall decrease in the fermentation yield as these cycles progress, a decrease that is due to saturation of carriers.

With the oil-adsorbing capability of wood chips, wood chips immobilized cells can be used to prevent migration of floating petroleum products from an oil spill to beaches and shorelines. The adsorbed petroleum products can then be left to be biodegraded in situ or ex situ. The technique described in this study could therefore complement existing methods of mitigating oil spills in an open marine environment.

CONCLUSION

This work will eventually help in bioremediation studies to clean up marine oil spills in an affected environment by the use of endogenous marine yeast strain with desirable degradable activities. We believe that bioremediation in the form of adding microorganisms to affected areas will play an important role in the future as environmentally safe and cost-effective response to marine oil spills. Out of the selected two carriers, the best for immobilization of oil degrading yeast strain and open processes is wood chips. It is an inert material and is very cheap, thus making the process potentially suitable for oil removal in- situ.

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