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Biological Treatment of Hydrocarbon Contaminants: Petroleum Hydrocarbon uptake by *Pseudomonas alkanolytica*

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*Abstract***.** Petroleum-based products are the major sources of energy for industry and daily life. The world production of crude oil is more than three billion tons per year, and about the half of this is transported by sea. Oil spill accidents result in significant contamination of the ocean and shoreline environment. Annually, more than one million tons of petroleum are estimated to end up in the sea.

In this study, a strain *of Pseudomonas alkanolytica* had been examined to degrade oil spill in surface water. Such strain which is isolated from petroleum hydrocarbon contaminated soil has the ability to utilize a variety of hydrocarbons substrates. Experiments were carried out in lab scale and the growth of microorganisms were investigated directly and indirectly. Results showed an extent of biodegradation more than 90% can be achieved within 10 days using this strain**.**

1. Introduction

Petroleum oils can cause environmental pollution during various stages of production, transportation, refining and use. Oil pollution from industrial sources and other activities at the harbor area pose great hazard to terrestrial and marine ecosystems. Petroleum hydrocarbons pollutions, ranging from soil, ground water to marine environment, become an inevitable problem in the modern life.

Although accidental releases account for only a small percentage of the oil released into the marine environment, large oil spills pose a great threat to terrestrial and marine ecosystems and hence, attract much public

attention. The National Research Council in Greece has recently updated its classic "oil in the sea" and now estimates that the total input of petroleum into the sea from all sources is approximately 1.3 Million ton/year out of which almost 50% comes from natural seeps and less than 9% emanates from catastrophic releases. Consumption, principally due to non-tanker operational discharges and urban run-off, is responsible for almost 40% of the input [1].

At present, a number of different technologies exist for oil spill removal, such as chemical precipitation, adsorption, and solvent extraction [2]. However, these methods have several disadvantages, such as incomplete oil removal, expensive equipment and monitoring system requirements, high reagent or energy requirements and generation of toxic sludge or other waste products that require disposal [3].

Although physical collection of the oil with booms, skimmers and adsorbents is generally the first priority of responders it is not very effective after a large spill [1] and current mechanical methods typically recover no more than 10–15% of the oil after a major spill according to the Office of Technology Assessment [3].

The need for alternative and additional responses led to bioremediation that has emerged as one of the most promising secondary treatment options for oil removal since its successful application after the 1989 *Exxon Valdez* oil spill [3]. Bioremediation can offer a less ecologically damaging alternative by taking advantage of the oil degrading microbes [4] and by establishing and maintaining the physical, chemical and biological conditions that favor enhanced oil biodegradation rates in the contaminated environment [3].

The use of bioremediation methods for oil contamination treatment can overcome some of limitations of physical and chemical treatments and provides a means for cost-effective removal. Such method is recently receiving favorable publicity as promising environmentally friendly treatment technologies for the remediation of hydrocarbons [5]. For this reason, bioremediation has been considered a potentially useful tool in the cleaning of oil spill and the treatment of oil residues [6].

Many soil microorganisms transform oil hydrocarbons into non-toxic compounds or mineralize them to inorganic compounds [7]. Hydrocarbons are degraded in soil mainly by bacteria (0.13–50% of the total of heterotrophic soil microorganisms) and fungi (6–82%) [7] and [8].

This natural microbiological activity is applied in bioremediation to reduce the concentration and/or toxicity of various pollutants, including petroleum products [9]. These processes take place in the natural environment, and their end-products are carbon dioxide and water [10].

It has been observed that the saturated components of crude oil (alkanes and cycloalkanes) and particularly the n-alkanes of intermediate lengths (C_{10}-C_{20}) are biodegraded more readily. Alkanes of shorter chain length are more toxic, while those of larger chain length (C_{20}-C_{40}) are hydrophobic solids difficult to degrade due their low bioavailability and aqueous solubility [11]. Polyaromatic compounds especially those of high molecular mass, are degraded slowly and with low efficiency, probably because of their low solubility and the high resonance of their structures [12].

The speedy removal of some *n*-alkanes or branched-alkanes by *Rhodococcus erythropolis* was reported by C.W. Liu *et al.* [13]. This strain was tested under saline conditions, as well as in pure sea water. At an initial NaCl concentration 1.2% and 2.4%, 80 - 90% removal of alkane was achieved within 56 h and 65% removal at 3.6% NaCl concentration within 68 h. Addition of 0.24 g/L nutrient broth (NB) in the medium would further promote the biodegradation rate and cell aggregation phenomenon during the process and more than 80–95% removal of alkane was achieved within 56 - 68 h. In the case of seawater, alkane removal was about 50% after 140 h and 65% with NB addition. The results suggest that strain NTU-1 has considerable ability for the bioremediation processes of *n*-hexadecane in a marine environment.

Pseudomonas species are often isolated from hydrocarbon contaminated sites and hydrocarbon degrading cultures. Members of this genus have broad affinity for hydrocarbon and can degrade selected alkanes, alycyclics, thiophenes and aromatics [14,15].

It was reported that *R. erythropolis* tolerates both water-miscible, such as ethanol and butanol up to 50% v/v, and water-immiscible solvents such as dodecane and toluene (5% v/v) [16]. Tolerance mechanisms to the hydrocarbons involve the adoption of the cell surface to the environment [17] , *e.g.* more mycolic acids or fatty acids in the cell surface making the cells hydrophobic attack oil pollutants and the direct contact between cells and oil pollutants improves the biodegradation rate [18].

In the present study, the main objective is to investigate the extent of biodegradability of petroleum hydrocarbons spilled into natural water.

A strain *Pseudomonas alkanolytica* was used for its ability to utilize a variety of hydrocarbon substrates.

2. Materials and Methods

2.1 *Microorganisms*

The bacterial strain *Pseudomonas alkanolytica* ATCC 21034 was used throughout this study for its ability to adherence to hydrocarbons and to degrade variety of hydrocarbons as it is isolated from soil contaminated with petroleum components in Manali. *Pseudomonas alkanolytica* is a gram-positive bacterium that exhibited the following physiological characteristics: yellow colored colonies on nutrient agar, presence of motility and spore formation and producing coenzyme A utilizing hydrocarbons [19].

2.2 *Hydrocarbon*

Dodecane ($C_{12}H_{26}$) commercialized by Fluka was chosen as the sole carbon source to simplify the study, as the assimilation of a mixture of hydrocarbons is difficult with respect to pure hydrocarbon. Moreover, Dodecane represents 42% of the petroleum pollutants as shown Table 1.

Table 1. Mass percentage of alkanes in some petroleum products.

No. of Carbon atoms	10	11	12	13	14	15	16	17	18	19	20
% n-alkanes in Kerosene	6	39	42	13	trace	-	-	-	-	-	-
% n-alkanes in gasoil	-	trace	trace	4	28	29	22	12	4	1	-
% n-alkanes in paraffins	-	-	1	2	3	5	8	14	21	19	13

2.3 *Culture Medium*

A mineral medium salt proposed by Goswami and Singh was used as the aqueous phase; its composition is shown in Table 2.

Table 2. Composition of the mineral medium salt.

Compound	Quantity
$(NH_4)SO_4$	2 g
Na_2HPO_4	3.61 g
KH_2PO_4	1.75 g
$MgSO_4.7H_2O$	0.2 g
$CaCl_2$	50 mg
$FeSO_4.7H_2O$	1 mg
$CuSO_4.5H_2O$	50 mg
H_3BO_3	10 mg
$MnSO_4.7H_2O$	10 mg
$ZnSO_4.7H_2O$	70 mg
$(NH_4)_6Mo_7O_{24}.4H_2O$	10 mg

Ammonium phosphate commercialized by Fluka was used as the source of nitrogen. pH of such solution was adjusted at 7.4 before sterilization, with a solution of NaOH (1N). The sterilization time at 121^{o}C was 15 minutes for volumes lower than 2 liters and 30 minutes for higher volumes.

2.4 *Growth Conditions*

Microorganisms were cultivated in a mineral medium containing different percentage (v/v) of dodecane as a sole carbon and energy source. To minimize the adoption phase of growth, reactivation of biomass was necessary which was done in two successive steps as follows:

Inoculum of microorganisms from Petri dish was cultivated in 250 ml Erlenmeyer containing 100 ml of the aqueous phase with 2% (v/v) dodecane over a period of 5 days.

One milliliter of such was introduced in another Erlenmeyer containing 100 ml of the aqueous phase with 2% (v/v) dodecane over a period of 2 days.

The cultivation was carried out at 30^{o}C in a rotary shaker incubator with 250 rpm.

2.5 *Experimental Design*

An open cylindrical tank is used in this study, made of Pyrex glass with 22 cm diameter and 10 cm depth, thus a working volume of 3.8 liters was used. The reactor was fed with 3.5 liter of mineral salt [20] with different concentrations of dodecane (2, 4, 6, 10, 15% v/v). Volume of inoculum was determined by calculation in order to have a concentration of biomass in the range of 0.01g/l at the beginning. Growth of microorganisms is realized at 30^{o}C (the optimum temperature for growth of the chosen bacterial strain) for a period of 12 days.

The same procedures were carried out with pre-aerated culture medium. For that purpose, two cylindrical open tanks (Fig. 1) with the same dimensions were used.

The biodegradation process was carried out in the first tank while the other was used for aeration of mineral salt solution which is recycled to the first tank at a rate of 1 ml/s with the aid of peristaltic pump to a hydraulic residence time of 1 hr. The same pump was used to maintain

the effluent flow at the same rate. From the resulting data of dodecane layer thickness measurements, substrate consumption rate could be predicted.

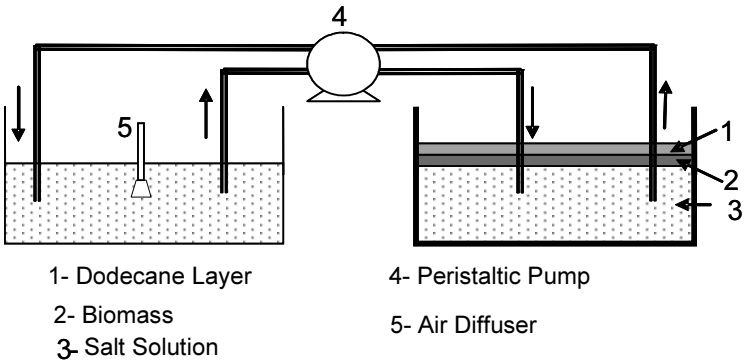


Fig. 1. Schematic diagram of the Apparatus.

3. Analytical Methods

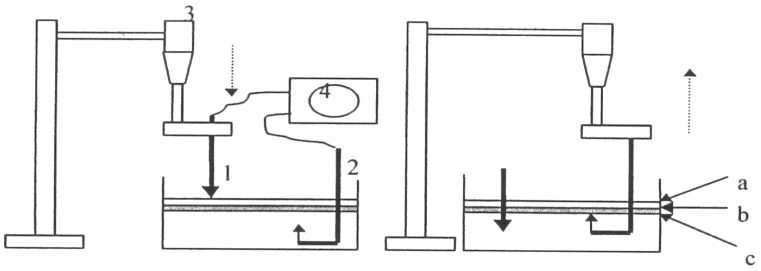
The extent of oil removal was monitored by three different methods:

3.1 *Hydrocarbon Consumption (Direct Method)*

The proposed system used to quantify biofilm thickness is illustrated in Fig. 2. The technique of measurement was carried out in two successive steps:

In the first step, the probe (1) was mounted on a manual micromanipulator, it was introduced from the top of the reactor perpendicular to the biofilm, Location of the probe tip in the layer of hydrocarbon was monitored with the help of binocular microscope. Electrical conductivity was measured through the dodecane layer while the probe (2) is immersed in the medium salt.

A sudden change in the value of electrical conductivity is observed when the probe (1) touches the biofilm [point "b"], then the interface dodecane-biofilm can be identified and the thickness of the hydrocarbon layer can be determined (Step 1).



Step 1
1, 2 - Electrodes (propes)
3 – Micrometer
4- Conductivity meter

Step 2
[a] Air – Dodecane interface
[b] Dodecane – biofilm interface
[c] Biofilm – salt solution interface

Fig. 2. Proposed technique used for hydrocarbon quantification.

In the second step, the probe (1) is immersed in the salt medium and probe (2) was mounted on the micromanipulator and introduced from the salt medium perpendicular to the bottom of the biofilm. An abrupt decrease in the value of electrical conductivity is observed when the probe (2) reaches the salt medium - biofilm interface [point "c"], which was slowly moved up into the biofilm and then the dodecane layer till the dodecane-air interface which can be identified with the help of a binocular microscope (Step 2). The first step determines thickness of hydrocarbon layer and the second determines thickness of biofilm and hydrocarbon layer, then biofilm thickness can be calculated as the difference between the two values.

3.2 *Dry Mass Technique (Indirect)*

The hydrocarbon consumption by the microorganisms was also followed by determination of biomass dry weight (cell dry weight). However, such technique is considered an indirect method for estimation of the biodegradation of dodecane. For that purpose, a series of experiments were carried out in which biodegradation was allowed to proceed for a certain period (1, 2, 3 ,… and 14 days). Dry weight of biomass was determined according to the following technique: Samples were centrifuged for 10 min at 20,000 rpm at ambient temperature, the resulting supernatant was composed of the aqueous phase and a top viscous layer consisting of dodecane, cells and polymer. The aqueous phase was separated while the pellet and the top layer were treated with 15 ml of a solvent mixture (acetone & petroleum ether; 3: 1 v/v). The

pellet was suspended in osmosed water and filtered through pre-dried cellulose acetate filters (0.2 μm pore size) in a vacuum filtration apparatus. The filters were dried to a constant weight in a vacuum oven at 70 ^{o}C. for 24 h.

3.3 *Nitrogen Consumption (Indirect)*

Determination of the consumption of nitrogen substrate is also a useful method for estimation of the process of biodegradation of dodecane. Since microorganisms consume nitrogen during their growth as it is one of the essential components of their cells (14%) and the only source of nitrogen in the culture media was $(NH_4)_2SO_4$. The performance of the microorganisms can be observed through the determination of the residual content of this substrate. The nitrogen consumption could be estimated by the subtraction of the amount remaining in the aqueous phase of the biphasic media after centrifugation from the initial amount of the ammonium sulfate. One of the most common techniques for determination of mineral nitrogen content is Nessler method in accordance with the standard method [21].

4. Results and Discussion

The present study was initiated to examine the ability of the strain *Pseudomonas alkanolytica* to degrade petroleum hydrocarbons. As bacteria adhere to and grow on interfaces, the growth of biomass in this work was in the form of biofilm, which was observed at hydrocarbon – salt solution interface (Fig. 3).

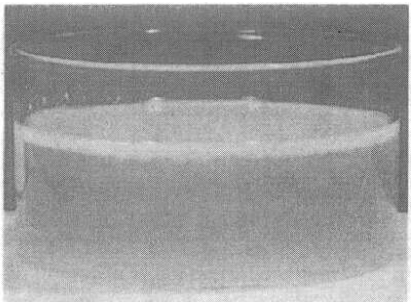


Fig. 3. Formation of bacterial biofilm at hydrocarbon/salt solution interface.

Results of measurements, interpreted and calculated according to the above procedures, are presented in Fig. 4 for both aerated and non aerated medium. Dodecane - biofilm interface is marked as a sudden change in electrical conductivity during the movement of the probe 1 down, while the biofilm – salt medium interface is marked as a sudden decrease in electrical conductivity when the probe 2 reaches the biofilm. Calculated values of hydrocarbon layer thickness, thus its volume during 14 days are illustrated in Fig. 4. These values represent an average of 5 measurements (± 10 μm) at different locations. The value of electrical conductivity through the hydrocarbon layer was the same as that of air, while marked increase in this value was observed when the sensor touches the biofilm and it is still lower than that of the salt medium. This can be explained by the high percentage water content of biofilm (more than 90%).

A critical value of substrate consumption was observed in the two cases in a period of 10 days, it can be explained by the decrease in substrate transfer rate through the biofilm which leads to suppress cell growth and hence formation of extracellular polymer. Such critical value was higher in case of pre-aerated medium than in the other case. Hence, it can be concluded that aerated medium results in increasing of substrate consumption. It can be explained by the fact that, limitation of oxygen in non-aerated system leads to a decrease in the biological reaction rate.

As can be seen from Fig. 4, percentage hydrocarbon removal of about 90 and 68% of the initial amount of hydrocarbon for pre-aerated and non-aerated medium respectively were achieved. These percentages correspond to an average value of consumed dodecane which was 0.168 ml/cm^2 of the oil spill surface area in 240 h with a rate of 6.8×10^{-4} $ml/cm^2.h$ for pre-aerated medium and 0.12 ml/cm^2 in 240 h with a rate of $5x10^{-4}$ $mg/cm^2.h$ for non-aerated medium.

After 240 h the rate of hydrocarbon consumption was almost zero, at the same time an excess of dodecane was measured, this ensure that dodecane is not limiting factor as it was not completely consumed, it gives an impression about the limitation phenomena. The most probably explanation of these phenomena is the difficulty of biomass to get its substrate requirements from two different phases as the thickness of biofilm increases.

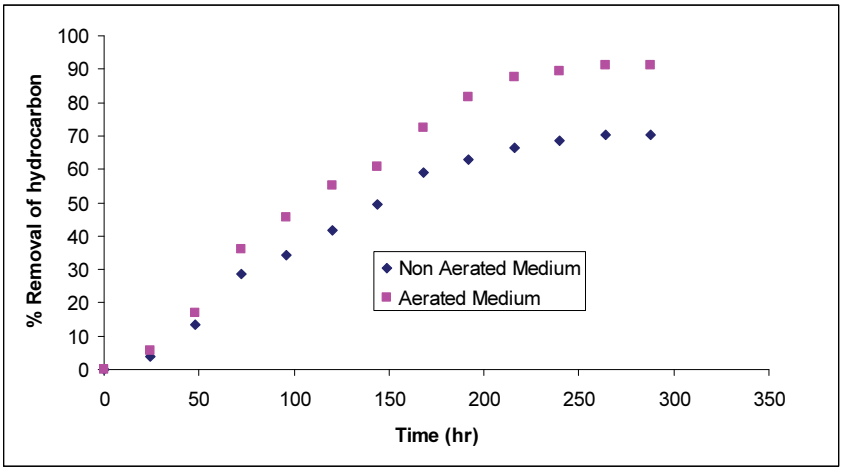


Fig. 4. Effect of aeration on the substrate uptake (dodecane concentration of 2%).

To ensure the accuracy of dry weight method in determination of substrate consumption, three samples were withdrawn and tested in parallel to each other. Figure 5 shows the typical growth of biomass. A lag phase of 24 h, where no increase in biomass was observed. The peak value of dry weight was 140 mg/cm^2 for aerated medium and 80 mg/cm^2 for non-aerated medium. After 240 h, a stationary phase is achieved, thus no growth occurs.

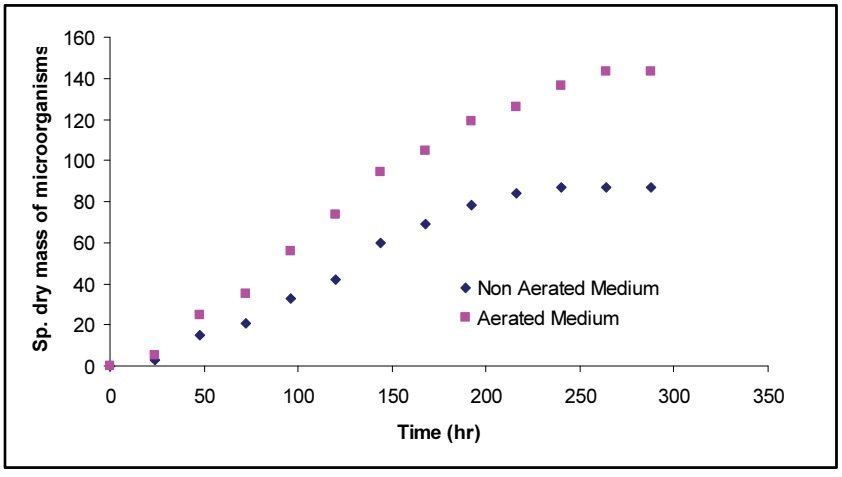


Fig. 5. Effect of aeration on the growth of microorganisms (dodecane concentration of 2%).

For estimation of the biomass activity, consumption of mineral nitrogen was also studied. Since microorganisms consume nitrogen during their growth as it is one of the essential components (14%) of their cells and the only source of nitrogen in the culture media was $(NH_4)_2SO_4$.

As can be seen from Fig. 6, the average value of consumed nitrogen was 400 mg/l in 240 h with a rate of 1.67 mg/l.h for pre-aerated medium and 340 mg/l in 240 h with a rate of 1.4 mg/l.h for non-aerated medium. These values represent 95 and 81% of the initial amount of nitrogen in the biphasic for pre-aerated and non-aerated medium respectively. After 240 h the rate of nitrogen consumption was almost zero, at the same time an excess of nitrogen was measured, this ensure that nitrogen is not a limiting factor. Since the nitrogen substrate was not completely consumed, it gives an impression about the limitation phenomena. The most probable explanation of these phenomena is the difficulty of mass transfer through the biofilm.

The extent of dodecane degradation and the time required for degradation varied as a function of the initial dodecane concentration in the pre-aerated medium as shown in Fig. 7.

An increase in biodegradation time was observed as the initial concentration was increased. For higher concentrations, a little reduction in dodecane degradation was observed. This behavior is probably due to the complexity of the biofilm mechanism formed at the hydrocarbon-saline solution which inhibits microbial growth.

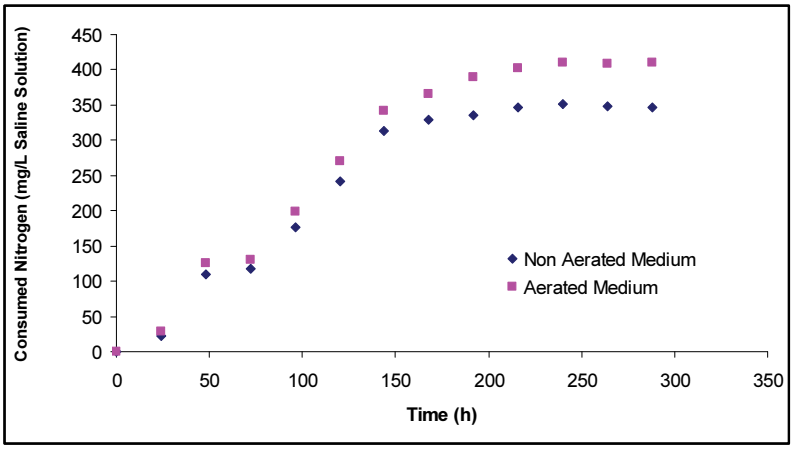


Fig. 6. Consumption of nitrogen as a function of time (dodecane concentration of 2%).

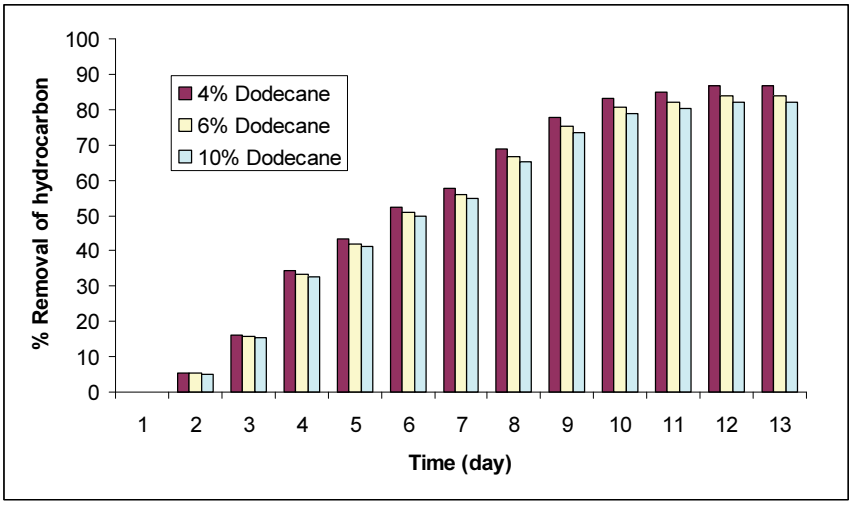


Fig. 7. Effect of hydrocarbon initial concentration on the percentage removal.

5. Conclusions and Recommendations

From the results of the present laboratory scale investigations, the following conclusions can be drawn:

- Direct quantification of dodecane degradation shows that, the extent of removal up to 91% can be achieved after 240 h at a rate of 6.8×10^{-4} ml/cm^2.h for pre-aerated medium. This rate has been declined to 5×10^{-4} mg/cm^2.h for non-aerated medium.

- In dry weight method, a growth of biomass with a rate of 0.6 mg/cm^2.h for pre-aerated medium and 0.36 mg/cm^2.h for non-aerated medium can be achieved after 240 h.

- The average value of consumed nitrogen was 400 mg/l in 240 h for pre-aerated medium and 340 mg/l in 240 h for non-aerated medium. These values represent 95 and 81% of the initial amount of nitrogen in the biphasic for pre-aerated and non-aerated medium respectively.

- The results indicate the high ability of the strain Pseudomonas alkanolytica to degrade hydrocarbon, hence, it can be very useful for environmental protection from oil spill risks.

- More investigations are required about the biofilm formed at the hydrocarbon – saline solution interface in order to achieve 100% hydrocarbon biodegradation.

Acknowledgement

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References

[1] **Prince, R. C., Lessard, R. R.** and **Clark, J. R**., Bioremediation of marine oil spills stimulated biodegradation of oil slicks using oleophilic fertilizers, *Oil and Gas Science and Technology,* **58**:463–468(2003).

[2] **Tjessen, K., Pedersen, D.** and **Aaberg, A.,** On the environmental fate of a dispersed Ekofisk crude oil in sea-immersed plastic columns, *Water Res*., **9**: 1129-1136(1984).

[3] **Zhu, X., Venosa, A. D., Suidan, M. T.** and **Lee, K.**, *Guidelines for the Bioremediation of Marine Shorelines and Freshwater Wetlands,* US Environmental Protection Agency (2001).

[4] **Xia, W. X., Li, J. C., Zheng, X. L, Bi, X. J.** and **Shao, J.L.,** Enhanced biodegradation of diesel oil in seawater supplemented with nutrients, *Engineering in Life Sciences,* **6**: 80–85 (2006).

[5] **Desai, J. D.** and **Banat, I. M.,** Microbial production of surfactants and their commercial potential, *Microbiol Mol Rev.,* **61**:47-64(1997).

[6] **Balba, M. T., Al-wadhi, N.** and **Al-Daher, R.,** Bioremediation of oil contaminated soil: microbiological methods for feasibility assessment and field evaluation, *J. Microbiol. Methods*, **32**:155-164(1998).

[7] **Leahy, J. G.** and **Colwell, R. R.,** Microbial degradation of hydrocarbons in the environment, *Microbiol. Rev.,* **54**: 305–315(1990).

[8] **Wolicka, D.** and **Suszek, A.,** Bioremediation of contaminated area by monocyclic hydrocarbons, *Mineral Econ. Res.,* **24**: 59–66(2008).

[9] **Dua, M., Singh, A., Sethunathan, N.** and **Johri, A. K.,** Biotechnology and bioremediation: successes and limitatio,ns, *Appl. Microbiol. Biotechnol.,* **59**: 143–152(2002).

[10] **Olliver, B.** and **Magot, M.,** *Petroleum Microbiology*, ASM Press, USA (2005).

[11] **Singer, M. E.** and **Finnerty, W. R.,** Microbial metabolism of straight chain and branched alkanes, *Petroleum Microbiology* (Atlas R.M., Ed) pp: 1-59, Macmillan, New York, 1984.

[12] **Sugiura, K., Ishihara, M., Shimauchi, T.** and **Harayamas, S.,** Physicochemical properties and biodegradability of crude oil, *Environ Sci Technol*, **3**:351-368(1996).

[13] **Liu, C. W., Chang, W. N.** and **Liu, H. S.,** Bioremediation of *n*-alkanes and the formation of biofloccules by *Rhodococcus erythropolis* NTU-1 under various saline conditions and sea water, *Biochemical Engineering Journal*, **45**: 69–75(2009).

[14] **Vankateswaran, K., Hoaki, T., Kato, M.** and **Murayama, T.,** Microbial degradation of resins fractionated for Arabian light crude oil, *Canadian Journal Microbiology*, **41**: 418–424(1995).

[15] **Allen, C. R., Boyd, D. R., Larkin, M.J., Reid, K. A., Sharma, N. D.** and **Wilson, K.,** Metabolism of naphthalene, I-naphthol, idene and indole by *Rhodococcus* strain NCIMB 123038, *Applied and Environmental Microbiology*, **63**: 151–155(1997).

[16] **De Cavalho, C.C., Da Cruz, A. Pons, M. N., Pinheiro, H., Cabral, J. M. S., Da Fonesca, M. M. R., Ferreira, B. S.** and **Fernandes P.,** *Mycobacterium* sp., *Rhodococcus erthyroplis*, and *Pseudomonas putida* behavior in the presence of organic solvents, *Microsc. Res. Tech*. **64**:215-222(2004).

[17] **Pieper, D.H.** and **Reineke, W.,** Engineering bacteria for bioremediation, Curr, *Opin Biotechnol.,* **11**:262-270(2000).

[18] **Bouchez, M., Rakatozafy, H., Marchal, R., Leveau, J. Y.** and **Vandescasteele, J. P.** Diversity of bacterial strains degrading hexadecane in relation to the mode of substrate uptake, *J. Appl. Microbiology*, **86**:421-428(1999).

[19] **Nakao, Y.** and **Kuno, M.,** *Method for the Production of Coenzyme A*, US Patent 3,669,836 dated Jun 13 (1972).

[20] **Goswami, P.** and **Singh, H. D.,** Different modes of hydrocarbons uptake by two Pseudomonas species, *Biotechnology and Bioengineering*, **37**: 1-11(1991).

[21] **American Public Health Association**, *issue* (1995).

المعالجة البيولوجية للملوثات الهيدركربونية: استهلاك المركبات البترولية بواسطة فصيلة *بيسيدومونا الكانوليتيكا* البكترية

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المستخلص. يعتبر البترول من أهم مصادر الطاقة للصناعة أو للحياة اليومية ولذا فإن عملية نقل البترول الخام من مناطق استخراجه إلي معامل التكرير أو أماكن استخدامه في تزايد مستمر، ومن المشاكل التي تنتج عن ذلك حدوث التسرب البترولي وما ينتج عنه من تلوث للمسطحات المائية أو للتربة.

والغرض من هذا البحث هو دراسة تطبيق الطرق الحيوية لمعالجة البقع الزيتية الناجمة عن التسرب البترولي، وتحديدا باستخدام فصيلة بيسيدومونا الكانوليتيكا، والتي يتم عزلها من التربة في مناطق ملوثة بالمركبات البترولية، وقد أثبتت الأبحاث السابقة أن تلك الفصيلة يمكنها الاعتماد علي عديد من المركبات الهيدروكربونية كمصدر وحيد للكربون اللازم لحصولها علي الطاقة.

وتم إجراء التجارب علي نطاق معملي، وتمت دراسة نمو البكتريا علي الوسط الهيدركربوني، وذلك بالطرق المباشرة وغير المباشرة. وقد أثبتت نتائج هذه الدراسة إمكانية استخدام هذه الفصيلة للتخلص من الملوثات الهيدركربونية في الأوساط المائية، حيث وصلت كفاءة إزالة المركب الهيدركربوني إلي أكثر من ٩٠٪ في غضون ١٠ أيام.