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Optimization of Cultural Conditions to Maximize the Efficiency of Petroleum Crude Oil-Degrading Bacteria

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ABSTRACT

The optimization of cultural conditions to maximize the efficiency of petroleum crude oil bioremediation by three bacterial strains, *Alcaligenes faecalis*, *Microbacterium oxydans* and *Microbacterium Paraoxydans* were isolated from Tebbin, Al Kanater charity and Agiba, respectively. The mineral salt medium was used**,** pH 7.5, temperature 30 ^{o}C and 30 days of incubation showed the highest degradation of crude oil with the isolated three bacterial strains. Ammonium sulphate as a sole N-source and crude oil as a sole C-sources were more suitable for crude oil degradation at the concentration of 0.2 % and 1.0 %, respectively. Inoculum size of 0.8 % was the most suitable inoculum for the degradation. Moreover, the agitation condition was advantages at 100 rpm than the static condition for the crude oil degradation by the three bacterial strains.

INTRODUCTION

Petroleum oil is the major source of energy for various industries and daily life. Releasing petroleum into the environment whether accidentally or due to human activities is the main cause of soil pollution. Soil contaminated with petroleum has a serious hazard to human health and causes environmental problems as well. Petroleum pollutants, mainly hydrocarbon, are classified as priority pollutants (Yuniati, 2018).

Petroleum components have traditionally been divided into four fractions: saturated hydrocarbons, aromatic hydrocarbons, compounds containing nitrogen, Sulphur and oxygen (NSO) and asphaltenes. The relative proportions of these fractions depend on the crude type, and the susceptibility of specific crude to microbial degradation can be predicted from its composition **(**El-Sheshtawy *et al*., 2015**)**.

Releasing petroleum into the environment whether accidentally or due to human activities is the main cause of soil pollution. Many methods for controlling oil contamination have been investigated including physicochemical and biological treatment.

In physicochemical treatment, incineration, thermal desorption, solvent extraction and landfilling are used but they have some disadvantages (Jain *et al*., 2011).

Numerous physicochemical techniques decontamination methods are expensive due to the cost of excavation and transportation of large quantities of contaminated materials for ex-situ treatment. Green technologies for pollutant clean up by biological means are used for bioremediation of petroleum polluted site (s) (Varjani *et al*., 2015).

Bioremediation provides the most cost-effective and eco-friendly measurements for the remediation of petroleum contaminated soil and water to bring back its native environment (Borah *et al*., 2016).

Successful application of bioremediation technology to contaminated systems requires knowledge of the characteristics of the site and the parameters that affect the microbial biodegradation of pollutants (Sabate *et al*., 2004). Understanding the mechanisms and factors which affect biodegradation are of great ecological significance, since the choice of bioremediation strategy depends on it. Microbial degradation processes aid the elimination of spilled oil from the environment. This is possible because microorganisms have enzyme systems to degrade and utilize different hydrocarbons as a source of carbon and energy (Sihag *et al*., 2014).

The present work aims to study the optimization of cultural conditions to maximize the efficiency of petroleum crude oil degrading bacteria, *Alcaligenes faecalis, Microbacterium oxydans* and *Microbacterium Paraoxydans*.

MATERIALS AND METHODS

Microorganisms:

Three bacterial strains *Alcaligenes faecalis, Microbacterium oxydans* and *Microbacterium Paraoxydans* used, were isolated from soil and water contaminated by crude oil from Tebbin, Al Kanater charity and Agiba, respectively.

Medium Used:

The composition of mineral salt medium (MSM) used in this work was (g/L) 2g $(NH_4)_2SO_4$, 4g KH_2PO_4, 6g Na_2HPO_4, 0.2g $MgSO_4.7H_2O$, 0.001g $FeSO_4.7H_2O$, pH 7.5.

Crude Oil:

Crude oil used in this study was obtained from the Egyptian Petroleum Research Institute.

Effect of Different Concentrations of Carbon Source (crude oil):

In this experiment different concentrations of crude oil 125 µl, 250 µl and 375 µl (represents 0.5, 1.0, 1.5 % v/v which equal 100, 200 and 300 mg) were added to 25 ml of the mineral salt medium, then inoculated with 200 µl of each of bacterial cultures (*Alcaligenes faecalis, Microbacterium oxydans* and *Microbacterium Paraoxydans*) and incubated for 25, 30, 35 days at 30°C in a shaker incubator adjusted to 100 rpm. After incubation periods, the crude oil degradation efficiency was calculated as it will be mentioned later.

Effect of Different Nitrogen Sources:

The most favorable nitrogen source for crude oil degradation by the three strains was determined. The N percent of the mineral salt medium was substituted by the equivalent amount of nitrogen content of different nitrogen sources. The following nitrogen sources were tested: ammonium sulphate, ammonium chloride, sodium nitrate, potassium nitrate, peptone, and urea. Erlenmeyer flasks (100 ml) containing 25 ml of the medium (mineral salt medium) were inoculated with 200 µl of each strain and 1.0 % (v/v) crude oil was added and incubated for 30 days at 30°C in a shaker incubator adjusted to 100 rpm. After the incubation period, the crude oil degradation efficiency was calculated as it will be mentioned later.

Effect of Different Concentrations of the Optimum Nitrogen Source:

Different concentrations of ammonium sulphate (0.15, 0.2, 0.25 and 0.3 g %) were added to the mineral salt medium. The sterilized medium was inoculated with 200 µl of each bacterial culture and 250 µl of

crude oil (1.0 % v/v) was added. The flasks were then incubated for 30 days at 30°C in a shaker incubator adjusted to 100 rpm. After the incubation period, the crude oil degradation efficiency was calculated as it will be mentioned later.

Effect of Incubation Periods:

One hundred ml conical flasks each containing 25 ml of mineral salt medium were used. Two hundred fifty microliter (1.0 % v/v) of crude oil was added to each flask, and then inoculated with 200 µl of each of *Alcaligenes feacalis*, Microbacterium *oxydans* and *Microbacterium paraoxydans*. The flasks were incubated at 30 ^{o}C in a shaker incubator adjusted to 100 rpm. Degradation of crude oil was determined after 20, 30 and 40 days of incubation. After incubation periods, the crude oil degradation efficiency was calculated as it will be mentioned later.

Effect of Inoculum Size:

In this experiment, 100 ml conical flasks each containing 25 ml of mineral salt medium were used. Two hundred fifty microliters of (1.0 % v/v) crude oil were added to each flask, then inoculated with different inoculum sizes (50, 100, 150, 200, 250, 500 and 1000 µl) of each of *Alcaligenes feacalis*, *Microbacterium oxydans* and *Microbacterium paraoxydans*. The flasks were incubated at 30 ^{o}C in a shaker incubator adjusted to 100 rpm for 30 days. After the incubation period, the crude oil degradation efficiency was calculated as it will be mentioned later.

Effect of Initial ph Value:

To determine the most favorable pH values for effective degradation of crude oil, the initial pH value of the mineral salt medium was adjusted to different values ranging from 6.0 to 8.5 before sterilization using 0.1 N NaOH or 0.1 N HCl. Erlenmeyer flasks 100 ml, each containing 25 ml of the medium was adjusted to various pH values and inoculated with 250 µl (1.0 % v/v) of crude oil and 200 µl of each bacterial strains and then incubated for 30 days at 30°C and 100 rpm. After the incubation period, the crude oil degradation efficiency was calculated as it will be mentioned later.

Effect of Different Incubation Temperature:

For this purpose, 100 ml conical flasks, each containing 25 ml of the mineral salt medium at pH 7.5 was used. Two hundred fifty microliter (1.0 % v/v) of crude oil was added to each flask, and then inoculated with 200 µl of each of *Alcaligenes feacalis*, *Microbacterium oxydans* and *Microbacterium paraoxydans.* The flasks were incubated for 30 days in the rotary shaker at 100 rpm and adjusted at different temperatures ranged from 20, 25, 30, 35 and 40°C. After the incubation period, the crude oil degradation efficiency was calculated as it will be mentioned later.

Effect of Different Agitation Speed:

The effect of agitation speed on degrading the crude oil was carried out in 100ml conical flasks, each containing 25 ml of the mineral salt medium. Two hundred fifty microliter (1.0 % v/v) of crude oil was added to each flask, and then inoculated with 200 µl of each of *Alcaligenes feacalis*, *Microbacterium oxydans* and *Microbacterium paraoxydans.* The flasks were incubated on a rotary shaker adjusted at different speeds 0, 50, 100, 150 and 200 rpm for 30 days. After the incubation period, the crude oil degradation efficiency was calculated as it will be mentioned later.

Determination of Residual Crude Oil Content:

The total crude oil content was analyzed using a standard solvent extraction method (APHA, 1992). Samples (25 ml) of liquid culture were extracted with equal volume of toluene in separating funnel. The clear extracted solution was absorbed at 420 nm spectrophotometer. The total hydrocarbon content concentration was extrapolated with a reference from a standard curve obtained from the graph of produced crude oil at varying concentrations.

Calculation of Degradation Efficiency by the Three Different Bacterial Strains:

At the end of the different previous experiments, the residual crude oil was

determined according to the method described by Sathishkumar *et al*., 2008. The percentage of degradation efficiency of crude oil by any of the studied strains was calculated by subtracting the residual crude oil at the end of the experiment from the initial crude oil content (200 mg) divided by 100.

RESULTS

Effect of Different Concentrations of Crude Oil as The Sole Carbon Source:

This experiment aims at finding the most suitable concentration of crude oil at which its degradation reached its maximum level at three different incubation times (25, 30 and 35 days) in the mineral salt medium for *Alcaligenes faecalis, Microbacterium oxydans* and *Microbacterium Paraoxydans* species. In this study, different crude oil concentrations (0.5, 1.0 and 1.5 %) were added as a sole carbon source to the mineral salt medium at different incubation time intervals.

Results in Table (1) showed that, crude oil concentration in the mineral salt medium had a great effect on its degradation by *Alcaligenes faecalis, Microbacterium oxydans* and *Microbacterium Paraoxydans*. In general, as the crude oil concentration increased, during certain incubation period, the amount of degraded oil increased till it reached the maximum value at 250 µl (1.0 % v/v) crude oil after 30 days of incubation. It was found that a higher increase in the concentration of petroleum oil (1.5 % v/v) or increasing the incubation period (more than 30 days) resulted in a depression effect in the degradation rate. From the above-mentioned data, crude oil at concentration of 250 µl (1.0 % v/v) was the best concentration and it was selected as a sole carbon source, for 30 days of incubation, in the mineral salt medium to get the maximum degradation rate by the three studied bacterial strains and it was used for further experiments.

Table 1: Effect of different crude oil concentrations at different incubation periods on the percentage of crude oil degraded by the three bacterial species.

Incubation period (in days)	Conc. Of initial petroleum oil (% v/v)	Crude oil degradation % (v/v)		
		A. faecalis	*M. oxydans*	*M. paraoxydans*
25	0.5	4	4	4
	1.0	20	42	20
	1.5	2	16	2
30	0.5	10	8	16
	1.0	60	78	50
	1.5	4	18	2
35	0.5	10	8	16
	1.0	60	78	50
	1.5	4	18	2

* Control (medium and crude oil only) recorded zero % of degradation.

Effect of Nitrogen Sources:

This experiment was conducted to study the effect of different nitrogen sources on the degradation of crude oil by *Alcaligenes faecalis, Microbacterium oxydans* and *Microbacterium Paraoxydans*. Nitrogen source of the mineral salt medium was substituted by the equivalent amount of nitrogen content of other nitrogen sources. The following nitrogen sources were tested: ammonium sulphate, ammonium chloride, ammonium nitrate, sodium nitrate, potassium nitrate, peptone and urea.

Results in Table (2) showed that using ammonium sulphate as nitrogen source for degrading crude oil by *A. faecalis, M. oxydans* and *M. Paraoxydans* strains recorded the highest percentage of crude oil degradation 60, 78 and 50 %, respectively. Using ammonium chloride as nitrogen source by the different strains recorded degradation efficiency ranged between 2 to

26 %. Application of ammonium nitrate as N source in the mineral salt medium led to a degradation efficiency, not more than 22%. In the case of using sodium nitrate as a nitrogen source, the degradation percentage by *A. faecalis* was 2%, while it reached 38 % and 22 % by *M. oxydans,* and *M. paraoxydans.* When potassium nitrate was used, *A. faecalis*, *M. oxydans* and *M. paraoxydans* recorded 8, 36 and 18 % of degradation. The degradation percentage of petroleum oil by *A. faecalis*, *M. oxydans* and *M. paraoxydans* was 22, 44 and 32%, respectively when urea was used as nitrogen source. While for peptone, the degradation percentage by *A. faecalis* was 6 %, but *M. oxydans recorded* 4 % and *M. paraoxydans* showed 8 % of the initial quantity of petroleum oil.

From the above-mentioned data, ammonium sulphate showed to be the best nitrogen source that can be used during the degradation process of petroleum oil present in the contaminated environment. And also it was selected as a sole nitrogen source in the mineral salt medium to get the maximum growth of the three bacterial species in the next experiments.

Table 2: Effect of different nitrogen sources on the percentage of crude oil degraded by the three bacterial species.

Bacterial species	Percentage of degradation at different nitrogen sources (%)						
	Amm. Chloride	Amm. Nitrate	Amm. Sulphate	Sod. Nitrate	Pot. Nitrate	Urea	Peptone
A.faecalis	2	22	60	2	8	22	6
M. oxydans	20	20	78	38	36	44	4
M. paraoxydans	26	22	50	22	18	32	8

Effect of Different Concentrations of Ammonium Sulphate:

The aim of this experiment was to determine the optimum concentration of ammonium sulphate as a sole nitrogen source for crude oil degradation by *Alcaligenes faecalis, Microbacterium oxydans* and *Microbacterium Paraoxydans* strains. Different concentrations of ammonium sulfate 0.15, 0.2, 0.25 and 0.3 % were used.

Results in Table (3) showed that the highest degradation rate by the different bacterial strains was obtained at 0.20 % ammonium sulphate. The percentage of crude oil degraded by *Alcaligenes faecalis, Microbacterium oxydans* and *Microbacterium Paraoxydans* were 60, 78 and 50 %, respectively. At the concentration of 0.15 % the ammonium sulphate, *A. faecalis* degraded only 18 % of the initial petroleum oil, while, both *M. oxydans* and *M. paraoxydans* recorded 20 % of initial crude oil. For 0.25 % concentration of ammonium sulphate, *A. faecalis* recorded 40 % degradation from the initial amount of crude oil.

Table 3: Effect of different concentrations of ammonium sulphate on the percentage of crude oil degraded by the three bacterial species.

Bacterial species	Percentage of degradation at different concentration of ammonium sulphate			
	(0.15%)	(0.2%)	(0.25%)	(0.3%)
A. faecalis	18	60	40	32
M. oxydans	20	78	60	40
M. paraoxydans	20	50	36	34

* Control (medium and crude oil) recorded zero mg of degradation.

The percentage of degradation by *M. oxydans* and *M. paraoxydans* were 60 and 36 % respectively. At concentration of 0.3% ammonium sulphate, *A. faecalis* degraded 32 % of initial crude oil, while, *M. oxydans* and *M. paraoxydans* degraded 40 and 34 % in respective order.

From the above-mentioned data, ammonium sulphate at the concentration of 0.2 % showed the highest effect on the degradation process by the three bacterial strains and thus this concentration was used for the next experiments.

Effect of Different Incubation Periods:

This experiment was carried out to investigate the effect of different incubation periods on the degradation efficiency of crude oil by three bacterial species, *Alcaligenes faecalis, Microbacterium oxydans* and *Microbacterium Paraoxydans*. The different incubation periods were 20, 30 and 40 days. Results recorded in Table (4) showed that after 20 days of incubation, the percent of crude oil degradation by *A. faecalis, M. oxydans* and *M. Paraoxydans* were 2, 24 and 4 % respectively. After 30 days of incubation, the degradation rate recorded the highest percent of crude oil degradation by all the studied bacterial strains. *A. faecalis, M oxydans* and *M. paraoxydans* recorded the following percentage of crude oil degradation rate 60, 78 and 50 %, respectively. After 40 days of incubation, there was no significant increase in the degradation process by the three bacterial species. From the above-mentioned data, thirty days of incubation showed an optimum growth time for efficient degradation of petroleum oil, and it was used in the next experiments.

Table 4: Effect of incubation period on the percentage of crude oil degraded by the three bacterial species

Bacterial species	Percentage of degradation at different periods of time		
	20 days	30 days	40 days
A. faecalis	2	60	61
M. oxydans	24	78	79
M. paraoxydans	4	50	50

Inoculum Size:

This experiment was carried out to investigate the effect of inoculum size on the degradation of crude oil by three bacterial species. Different inoculum sizes were tested (50, 100, 150, 200, 250, 500 and 1000 µl). Results recorded in Table (5) showed that the least inoculum size used (50 µl) recorded 4, 40 and 2 % of degradation by *A. faecalis*, *M. oxydans* and *M. paraoxydans*, respectively. The degradation rate was increased gradually by the three bacterial species until it reached to 200 µl of inoculum size, where *A. faecalis* recorded 60 % of degradation while *M. oxydans* showed 78 % and *M. paraoxydans* degraded 50 % of total oil present.

Table 5: Effect of inoculum size on the percentage of crude oil degraded by the three bacterial species.

Inoculum size, µl	Percentage of crude oil degraded by each of		
	A. faecalis	*M. oxydans*	*M. paraoxydans*
50	4	40	2
100	22	48	8
150	26	48	12
200	60	78	50
250	56	64	40
500	40	48	26
1000	34	46	24

Further increase in the inoculum size showed decreasing in the degradation process by the three bacterial species. As 0.8 % (200 µl) inoculum size showed the optimum effect on crude oil degradation rate, it was used in the next experiments.

Effect of Initial pH:

The aim of this experiment was to study the influence of the initial pH on the

degradation of crude oil by *Alcaligenes faecalis*, *Microbacterium oxydans* and *Microbacterium Paraoxydans*. The initial pH value of the growth medium adjusted to different values ranging from 6.0 to 8.5.

Results in Table (6) showed that the degradation of crude oil was highly affected by the change of the initial pH. As shown at initial pH 6 the degradation percent did not exceed 2 % by *A. faecalis*, 18 % by *M. oxydans* and 14 % by *M. paraoxydans.* The degradation process began to increase slightly at initial pH 6.5 and 7 until it reached at initial pH 7.5 to 60 % degradation by *A. faecalis*, 78 % by *M. oxydans* and 50 % by *M. paraoxydans.* At higher initial pHs (8 and8.5), the degradation efficiency decreased to lower levels recording percentages ranged from 2 % to 40 % of degradation by the three bacterial species. From the above results, it concluded that, the maximum degradation efficiency of the three bacterial strains was obtained at initial pH 7.5 and therefore, it was used in the next experiments.

Table 6. Effect of initial pH on the percentage of crude oil degraded by the three bacterial species.

Bacterial species	Percentage of crude oil degradation at different pH levels					
	6	6.5	7	7.5	8	8.5
	%	%	%	%	%	%
A. faecalis	2	28	38	60	20	2
M. oxydans	18	28	36	78	30	10
M. paraoxydans	14	36	38	50	40	12

Effect of Incubation Temperature:

This experiment was carried out in order to select the most suitable temperature that supports the highest efficiency of crude oil degradation by three bacterial strains. Temperature ranging from 20^{o}C to 40 ^{o}C was tested for the degradation by the three bacterial strains, *Alcaligenes faecalis*, *Microbacterium oxydans*, and *Microbacterium Paraoxydans.*

The results in Table (7) showed that crude oil degradation by all studied strains was affected by the variation in incubation temperature. The percent of degradation was increased as the degree of temperature increased up to 30 ^{o}C for 30 days of incubation. At that temperature, the three bacterial species *A. faecalis*, *M. oxydans* and *M. Paraoxydans* recorded 60, 78 and 50 % degradation rate, respectively. The percentage of crude oil degraded by the three bacterial species began to decrease by increasing the incubation temperature above 30^{o}C. In case of using 35 ^{o}C, the degradation process recorded 32 % by *A. faecalis*, 44 % by *M. oxydans* and 26 % by *M. paraoxydans.* Also at 40 ^{o}C, the percent of degradation process ranged from 18 to 22 % only of the total oil present. From the above-mentioned data, the optimum incubation temperature for degrading petroleum oil by the three bacterial species was 30 ^{o} C, thus it was used for the further experiments.

Table 7: Effect of incubation temperature on the percentage of crude oil degraded by the three bacterial strains.

Bacterial species	Percentage of crude oil degradation at different temperature (^{o}C)				
	20 ^{o}C	25 ^{o}C	30 ^{o}C	35 ^{o}C	40 ^{o}C
A. faecalis	2	4	60	32	20
M. oxydans	30	30	78	44	22
M. paraoxydans	2	2	50	26	18

Effect of Agitation:

This experiment was designed to study the effect of different agitation speeds on the degradation of crude oil by *Alcaligenes faecalis*, *Microbacterium oxydans* and *Microbacterium Paraoxydans.* Different agitation speeds (0, 50, 100, 150 and 200 rpm) of rotary incubator shaker were tested.

Result in Table (8) showed that the static state affected negatively the efficiency

of the degradation process; it showed only 8, 4 and 4 % of degradation by *A. faecalis, M. oxydans* and by *M. paraoxydans*, respectively. A slight increase in the degradation process at speed 50 rpm was recorded by the three bacterial species. At 100 rpm, the degradation percent was the highest by the three bacterial strains and recorded 60, 78 and 50 % by *A. faecalis, M. oxydans* and *M. paraoxydans*, respectively. At higher agitation speeds (150 and 200 rpm) the degradation efficiency by the three bacterial species was decreased to low levels of degradation.

From the above-mentioned data, the maximum percentage of crude oil degradation by the three bacterial species was obtained at the agitation speed of 100 rpm, therefore, this speed was selected to get the maximum growth of bacterial strains that could be used in the cleaning up of the polluted environments.

Table 8: Effect of agitation speed on the percentage of crude oil degraded by the three bacterial species.

Bacterial species	Percentage of crude oil degradation at different agitation speed (rpm)				
	Static	50 rpm	100 rpm	150 rpm	200 rpm
A. faecalis	8	10	60	22	20
M. oxydans	4	6	78	42	32
M. paraoxydans	4	16	50	24	16

DISCUSSION

Petroleum as a major source of energy and due to its wide scale production, transport and disposal globally, made it a lead contaminant (Rahman, et. al., 2002). Oil contamination is one of the most dangerous pollution factors known today. It can cause a huge threat to the environment. During accidental spills, action will be taken to remove or remediate the contaminant immediately, whereas in the gasoline and diesel stations the spills due to leakage may be small but continuous and prolonged. Because of its persistence, the chance for groundwater contamination is high.

The maximum biodegradation occurred when conditions are favorable for microorganisms. It is important to know the characteristics of the contaminated site before beginning the treatments. The basic information such as residual oil concentration, population density of hydrocarbon degrading microorganisms and the environmental factors such as pH, temperature are some of the key factors to be considered for bioremediation (Sihag *et al*., 2014).

The present work aims at studying the optimization of cultural conditions in order to maximize the degradation process of petroleum crude oil by bacterial degrading strains such as *Alcaligenes faecalis*, *Microbacterium oxydans* and *Microbacterium Paraoxydans* which were used in this study.

Several investigations have been conducted to explore the appropriate concentration of crude oil as a sole carbon source for the optimal degradation of crude oil. In the present study, 250 µl (1.0 % v/v) of crude oil after 30 days of incubation was the best concentration of C-source for crude oil degradation by the three bacterial strains *Alcaligenes faecalis*, *Microbacterium oxydans* and *Microbacterium Paraoxydans*. On the other hand, high concentrations of highly soluble or volatile organic compounds may be detrimental to microbial forms due to their toxicity. Dibble and Bartha (1979) found that biodegradation activities in oil sludge occurred between oil concentrations of 1.25 and 5.0 % and were best at 5.0%. Oil loadings greater than 5.0 % lead to a decline in microbial numbers due to increase in toxicity. In addition to the toxicity, high concentration also inhibits the microbial growth by upsetting the C: N: P ratios. Sihag *et al*., 2014 revealed that oxygen is one of the basic requirements for the biodegradation and its concentration will depend upon the choice of the microorganism used.

The effect of nitrogen sources on crude oil degradation was also examined; the present result showed that ammonium

sulphate was found to be the best nitrogen source for crude oil degradation by the three bacterial strains *Alcaligenes faecalis*, *Microbacterium oxydans* and *Microbacterium Paraoxydans.*

The nitrogen level in the culture medium is critical for the degradation. In the present study, the maximum degradation level was obtained at 0.20 % ammonium sulfate for the three bacterial strains. Further increase in ammonium sulfate concentrations resulted in a stable rate for the crude oil degradation by the three bacterial species.

Different incubation periods were studied for the degradation of crude oil by *Alcaligenes faecalis*, *Microbacterium oxydans* and *Microbacterium Paraoxydans*. The highest yield of crude oil degradation produced by the three species was pronounced after 30 days of incubation period.

The degradation of crude oil was found to be influenced by the initial pH of the medium. The optimum initial pH of the mineral salt medium for crude oil degradation by the three bacterial strains was at pH 7.5. The percentage of crude oil degraded by the strains *Alcaligenes faecalis*, *Microbacterium oxydans* and *Microbacterium paraoxydans* were 60, 78 and 50 % of initial amounts, respectively. However, the lowest amount of degradation was obtained in medium with pH levels other than 7.5. Yuniati (2018) reported that extreme pH is expected to have a negative influence on the ability of microbial populations to degrade the hydrocarbons.

Crude oil degradation was affected by incubation temperature. In the present study, the maximum degradation was obtained at 30 ^{o}C after 30 days of the incubation period for the three species *Alcaligenes faecalis*, *Microbacterium oxydans* and *Microbacterium Paraoxydans*. Further increase or decrease in the temperature degree resulted in an obvious reduction in the percentage of degradation by the three bacterial strains. Rahman *et al*., (2002) reported that extreme temperature conditions affect greatly the microbial ability to degrade crude oil. At low temperature, the viscosity of the oil increases, the volatilization is reduced and their water solubility is decreased, delaying the onset of biodegradation. In addition, microbial growth rates are a function of temperature, and rates of degradation decrease with decreasing temperature. Higher temperature increases the hydrocarbon metabolism to a maximum, typically in the range of 30 to 40 ^{o}C (Leahy and Colwell, 1990).

The performance of the microbial crude oil degradation can be strongly influenced by the choice of inoculum size. Our results showed that the highest degree of crude oil degradation was obtained when the inoculum size used was 200 µl per 25 ml medium (0.8 %). The agitation condition of the system is also considered as a vital step for the degradation process. In the present study, the optimum agitation speed for the crude oil degradation by the three species *Alcaligenes faecalis*, *Microbacterium oxydans* and *Microbacterium paraoxydans* was 100 rpm.

CONCLUSION

The optimization of cultural conditions to maximize petroleum crude oil bioremediation by *Alcaligenes faecalis, Microbacterium oxydans* and *Microbacterium Paraoxydans* were studied. The medium used was mineral salt medium with pH 7.5, temperature 30 ^{o} C, agitation speed at 100 rpm, 0.8 % inoculum size and 30 days of incubation showed the highest degradation of crude oil by the three bacterial strains. Ammonium sulphate as a sole N-source and crude oil as a sole C-sources were more suitable for crude oil degradation at the concentration of 0.2 % and 1.0 %, respectively.

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