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Microbial Degradation Of Crude Oila Comparativestudy

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Abstract

Microbial degradation of crude oil is one of the major practices in natural decontamination process.The specific objectives of the present investigation are isolation, screening and selection of microorganisms, batch shake flask studies on the culture growth and biodegradation of crude oil using some reference microbes which are earlier reported to degrade crude oil efficiently.

Soil samples were collected from areas of the Rumelia desert,south of Iraq.Five microbe species were isolated from the soil.The microbe species belongs to the genus,Acinetobacter calcoaceticus (S1), Pseudomonas aeruginosa (S2), Arthrobacter sps(S3), Pseudomonas pictorum(S4) and Pseudomonas resinovorans(S5).Their ability to biodegrade crude oil was tested as single isolates, initial concentration =200ppm,T=30 ^{O}C and pH=7 of incubation in the mineral salts medium, the results showed that the microbe Acinetobacter calcoaceticus (S1) was the best with biodegradation ability reaching 99%,while biodegradation ability for Pseudomonas pictorum(S4) 97%, Arthrobacter sps (S3) 96%, Pseudomonas resinovorans(S5) 94% and Pseudomonas aeruginosa (S2) 92%.Also, the results showed that the time play an important on the biodegradation process, in which the percentage of biodegradation increased with time.

S1> S4>S3>S5>S2

The biokinetic parameters of the microbeswere estimated by fitting the growth data to Monod Kinetic Model. The maximum specific growth (μ_m) and half saturation constant (K_s) has been determined for each initialconcentration of crude oil. It has been observed that with increase in initial concentration of crude oil the (μ_m)decreases owing to toxic nature of crude oil. The data generated from this study were analyzed using Microsoft excel and Chi- square of the Statistical Procedure for Social Science version 22.0 (SPSS, Chicago, IL, USA).

Keywords: *Microorganisms;biodegradation; Rumelia desert;Acinetobacter calcoaceticus;Monod Kinetic Model.*

INTRODUCTION

Crude oil hydrocarbons remain the most important energy and chemical source (Sathish, et al.,2008,Obayori, et al., 2009,Olabemiwo, Adekola, et al.,2014).

The remediation of polluted soils in desert region requires the study of the microorganisms' diversity in the environment and the determination of the ability of different microbes and their consortia to degrade pollutants in the presence of high salt concentration. Biodegradation depends on both physicochemical and biological variables(Olowomofe, et al., 2018).
Microorganisms are able to adapt to the presence of toxic organic compounds by using a whole cascade of adaptive mechanisms. Among the adaptive mechanisms, changesin the fatty acid composition of membrane lipids are the most important reactions of bacteriato membrane-active substances (Atlas,1984).

The wide variety of microorganisms that can aerobically degrade crude oil includepure bacterial cultures such as: AcinebacterSps. (Hank, et al., 2010)and(Janiyani,et al., 1993). Alcaligenes eutrophus (Marquez, et al.(2001), Arthrobacter(Shailubhaiet al., 1985). Bacillus stearothermophilus (Foght,et al., 1989).Pseudomonas aeruginosa(Margesin and Schinner, 1999 and Hommel, 1990). Pseudomonas cepaciaG4 also known as Burkholderiacepacia G4 (Kaczorek, et al., 2005, Bielicka.et al., 2002, Bodour and Maier, 2002), Pseudomonas fluorescens(Vardar, etal., 2000, Christofiand Ivshina, 2002), Pseudomonas pictorum(Banat, 1995) Pseudomonas putida,(DesaiandBanat, 1997), Pseudomonas resinovorans(Mulligan,et al., 2001,Mulligan, 2005).

The specific objectives of the present investigation areisolation, screening and selection of five microbes, Acinetobacter calcoaceticus (S1), Pseudomonas aeruginosa (S2), Arthrobacter.sps(S3), Pseudomonas pictorum (S4), Pseudomonas resinovorans(S5) which efficientlydegrade crude oil.Batch shake flask studies on the culture growth and biodegradation of crude oil using reference microbes which are earlier reported to degrade crude oil efficiently.Laboratory batch studies were used to obtain and provide fundamental biokineticparameters involved in theculture growth and crudeoil biodegradation.

MATERIALS AND METHODS

Collection of The Solid Soil Samples

Soil samples were collected from the area Rumelia desert, South of Iraq.

Sampling

A soil profile of 1-10 cm under the surface was sampled using a sterile knife afterremoval of the top layer of the soil up to 1-2 cm. Four subsamples were taken from eachpoint and mixed in a sterile plastic bag. The total mass of the soil sample collected wasaround 250 grams. The soil sample pooled in the container was thoroughly mixed and wasstored in ambient temperature for travelling. Kept at 4ºC prior to experimentation. Standard microbiological procedures were employed in thecollection and handling of the soil samples and analysis was done within 24 hours of collection.

Preparation ofCrude OilStock Solution

The stock solution of crude oil was prepared by adding 10g of crude oil to double distilledautoclaved water and the volume was made up to 1000ml. The final concentration of thestock solution was 1000 ppm (1000 mg/L) and the stock solution was diluted to therequired concentration for its use in the experiments. The stock solution was filter sterilizedby passing it through a 0.25µM syringe filter.

Isolation of Microbes

Isolation of microbes were performed by soil dilution, two grams of dried soil was dissolved in 8 ml ofdistilled water, aqueous dilutions, of the suspension were applied in nutrient agar, the plates wereincubated at 37°C for 24 hours. Different isolates were carried out then selected colonies of microbeswere transferred from mixed culture plates onto respective agar plates and incubated at 37°C for

24 hoursplates containing pure cultures were stored at 4°C until the examination(Wanapaisan, et al. 2018).The bacterium Acinetobacter calcoaceticus (S1), Pseudomonas Aeruginosa (S2) Arthrobacter sps (S3), Pseudomonas pictorium (S4) and Pseudomonas resinovorans (S5) were procured from Basrah Oil Compony Central Laboratory, Pune in lyophilized form.

Growth Medium

Microbes were cultivated in minimal salt medium (MSM). The composition of the MSM is detailed in Table 1. Crude oil (analytical grade) was used as a sole source of carbon and sterilized crude oil solution was added directly to MSM at a concentration of 200 ppm. The media was sterilized by autoclaving and the crude oil was used after filter sterilization (Saravanan, et al. 2008).

Crude Oil Degradation Studies

All biodegradation experiments were performed in 250 ml Erlenmeyer flaskcontaining 100 ml of minimal salt medium (MSM) containing crude oil at concentration ranging from 200 -800 ppm. Upon incubation of the flasks at 30°C and pH=7 under agitation condition(250 rpm), samples were withdrawn at regular time interval, centrifuged (10 ml for 3minutes) and analyzed for residual crude oil concentration. For each concentration triplicateexperiments were performed under the same condition and mean value has been reported. Each experiment was carried out for until the residual concentration of crude oil in flask was found to saturate with time and amount of biomass or it is below the detection limit. The reaction mixture containing all components but devoid of bacterial inoculums were used ascontrol.Crude oil degradation percentage calculate byformula:

$$\boldsymbol{crude\ oil\ degradation}\% = ((\boldsymbol{Initial\ conc.of\ crude\ oil} - \boldsymbol{Final\ conc.of\ crude\ oil})/ \boldsymbol{Initial\ conc.of\ crude}\ oil) \times 100 \quad (1)$$

Screening ofCrude Oil Degradation Strain

All the strains isolated by soil enrichment technique were individually inoculated into 10 ml of the mineral salt crude oil medium with 200 ppm of crude oil. The conical flasks were incubated on orbital shaker incubator at 150 rpm at room at T=30°C and pH=7 for 2880min. The isolates which showed growth in the broth were plated with crude oil containing nutrient agar medium to inoculate individually into mineral salt crude oil medium with 400 ppm of crude oil. The same procedure was repeated with 600 and 800 ppm crude oil containing mineral salt medium. The culture which showed growth in mineral salt crude oil medium with 800 ppm of crude oil was selected as the crude oil degrading strain. The selected culture was purified by repeated streaking and was stored at -20ºC as 30% glycerol stock(Vinithini, et al. 2015).

Analytical Procedures

Measurements of crude oil were done by UV–vis spectrophotometer (Systronics**)** bothin UV and visible range. For measuring biomass, the samples were centrifuged at approximately 6000 rpm for 20 min. The supernatant was used for crude oil determination. The biomass attached to the walls of tubes was resuspended in distilled water and optical density of this suspension was measured against distilled water as reference at 600 nm using UV–vis double beam spectrophotometer. All the transfers were made in UV chamber, and glass wares and medium properly autoclaved. The batch experiments were repeated and the results were found reproducible within acceptable range(Abdulla, et al.,2019).

MICROBIAL GROWTH KINETICS FOR CRUDE OILBIODEGRADATION SYSTEMS

In order to describe the kinetics of crude oil degradation bymicrobes, several kinetic models such as growth-associated models (logarithmic, logisticand Monod with growth), non-growth associated models (zero order, first order and Monod based) and three-half order models have been reported in the literature (Lathaand Kalaivani,2012). To establish the effect of crude oil concentration on growth of microbial culture, specific growth rates of the culture at different crude oil concentrations is calculated as per the following relationship:

$$\mu = \frac{1}{X}\frac{dX}{dt} \qquad (2)$$

Where, μ is the specific growth rate(h^{-1}), X is the biomass concentration (mg/L). Usually, themicrobial growth can be represented by a simple Monod equation:

$$\mu = \frac{\mu_m S}{S + K_S} \qquad (3)$$

Where, S is the limiting crude oil concentration (mg/L), m is the maximum specific growth rate (h^{-1}), K_s is the half saturation constant (mg/L). On rearranging equation, we get,

$$\frac{1}{\mu} = \frac{K_S}{\mu_m}\left(\frac{1}{S}\right) + \frac{1}{\mu_m} \qquad (4)$$

The plots were obtained using Microsoft excel and Chi- square of the Statistical Procedure for Social Science version 22.0 (SPSS, Chicago, IL, USA)
and the kinetic parameters μ_m, K_s wereobtained using the equation (3) & (4). For each microorganism, different μ_m, K_s value is obtained and they were curved along with respective crude oil concentration.

Result & Discussion

Crude Oil Biodegradation byAcinetobacter Calcoaceticus (S1)

The strain Acinetobacter calcoaceticus (S1)was subjected to different initial concentration of crude oil from 200- 800 ppm with mineral salt medium. The crude oil concentration was estimated at regular interval of time. Fig .1 represents the degradation characteristics of the Acinetobacter calcoaceticusat different initial concentration of crude oil. From the figure is observed that the microbe is able to degrade 200 ppm of crude oil within 2880 minutes while it takes 4320minutes for the complete degradation of 600 ppm of initial concentration of crude oil. The microbe has been claimed for its capability to reduce to800 ppm of crude oil below the detection level and the degradation curveis in agreement with the literature(Shahab, et al. ,2017). The microbe is able to degrade 800 ppm of crude oil injust 8640 minutes. Similar results have been reported by**(**Liuet al. 2009) where they have subjectedthe microbe to 800 ppm of crude oil and the microbe was able to degrade it up to 99%.

Crude Oil Biodegradation byPseudomonas Aeruginosa(S2)

The degradation profile of the microbe Pseudomonas aeruginosa(S2)at various concentration of crude used as the sole source of carbon and energy offered in Fig. 2. The microbe is able to degrade up to 600 ppm of crude oil in the media but shows an extended lag phase with increase in the concentration of crude oil. Above 600 ppm of crude oil, the growth of the microbe was inhibited and the microbe was no longer able to degrade crude oil,(Ahmed ,2014).

Crude Oil Biodegradation byArthrobacter sps (S3)

The crude oil biodegradation behavior of the Arthrobacter sps(S3) at different initial concentration of crude oil was depicted in Fig. 3. The microbe is able to degrade up to 96 % and 52% of the initial crude

oil when the initial concentration of the crude oil in the media is 200ppm and 800 ppm respectively. Even if the microbe is not able to utilize the crude oil completely, it is able to tolerate such high concentration of crude oil which makes it a promising candidate in the field of crude oil biodegradation(Ahmed, 2014).

Crude Oil Biodegradation byPseudomonas Pictorum(S4)

The degradation pattern of the microbe Pseudomonas pictorum(S4) is shown in Fig. 4. Itis notable to see that the microbe is also not able to degrade the crude oil completely but hasthe potential to withstand such high concentration of crude oil as 600 ppm. The microbedegrades only 85 % of 600 ppm of crude oil and on increasing the concentration of the crude oil its capacity to degrade the crude oil decreases but the microbe is able to grow even in theenvironment. From Fig. 4itcan be seen that the microbe exhibits a lag phase athigher concentration of crude oil thus implicating that the crude oil at higher concentration islethal to the microbe(Li, et al., 2016).

Crude Oil Biodegradation byPseudomonas Resinovorans (S5)

The degradation profile of the microbe Pseudomonas resinovorans (S5) was represents in Fig. 5. Contradicting the earlier theory available on its biodegradation potential the microbe is able to tolerate up to 600 ppm of crude oil with an extended lag phase but a linear degradation curve. The microbe shows an efficient degradation potential up to 400 ppm of crude oil where it completely degrades the crude oil in 5760 minutes(Li, et al.,2016and Kumar, el al., 2014).

Degrading Ability of Soil Microbes Isolate

The five selected isolated found to have crude oilFig. 6 shows their ability to biodegrade crude oil was tested as single isolates, initial concentration =200ppm,T=30 ^{O}C and pH=7 of incubation in the mineral salts medium, the results showed that the microbe Acinetobacter calcoaceticus (S1) was the best with biodegradation ability reaching 99%,while biodegradation ability for Pseudomonas pictorum(S4) 97%, Arthrobacter sps (S3) 96%, Pseudomonas resinovorans(S5) 94% and Pseudomonas aeruginosa (S2) 92%.

S1> S4>S3>S5>S2

Determination of The Biokinetic Parameters

The maximum specific growth (μ_m) and half saturation constant (K_s) has been determined for each initial concentration of crude oil. It has been observed that with increase in initial concentration of crude oil the $\mu_m$decreases owing to toxic nature of the crude oil. The plots were obtained using Microsoft excel and Chi- square of the Statistical Procedure for Social Science version 22.0 (SPSS, Chicago, IL, USA) and the kinetic parameters μ_m, $K_s$were obtained using theequations (3&4). For each microorganism, different μ_m,$K_s$value is obtained and were listed in the Table 2. In this study crude oil is used as the growth limiting factor since it is the sole source of carbon and energy. But even if it is a sole source for the microbe, its increasing concentration is toxic for the microorganism. Hence instead of increasing, the specific growth rate of the microbe decreases with each unit of increase in concentration. From the Table.2 it can be noted that with increasing concentration of the crude oil the maximum specific growth ratedecreases and the half saturation constant increases. After a certain point the half saturation constant for the microorganism increases drastically.

CONCLUSION

The results of this study appeared that the five selected isolated showed a good biodegradation efficiency. The microbe Acinetobacter calcoaceticus (S1) was the best with biodegradation ability reaching 99%, while biodegradation ability for Pseudomonaspictorum(S4) 97%, Arthrobacter sps (S3) 96%, Pseudomonas resinovorans(S5) 94% and Pseudomonas aeruginosa (S2) 92%. Also, the results showed

that the time play an important on the biodegradation process, in which the percentage of biodegradation increased with time.

The biokinetic parameters of the microbes were estimated by fitting the growth data to Monod Kinetic Model. The maximum specific growth μ_m and half saturation constant (K_s) has been determined for each initial concentration of crude oil. It has been observed that with increase in initial concentration of crude oil the $\mu_m$decreases owing to toxic nature of the crude oil.

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NOMENCLATURE

Symbol	**Description**	**Units**
K_s	Half Saturation Coefficient	mg L^{-1} h^{-1}
mg/L	Milligram Per Liter	
ppm	Parts Per Million	
μ_{max}	Maximum Specific Growth	h^{-1}
RPM	Rotation Per Minute	
S	The limiting substrate concentration	mg/L
X	The biomass concentration	mg/L

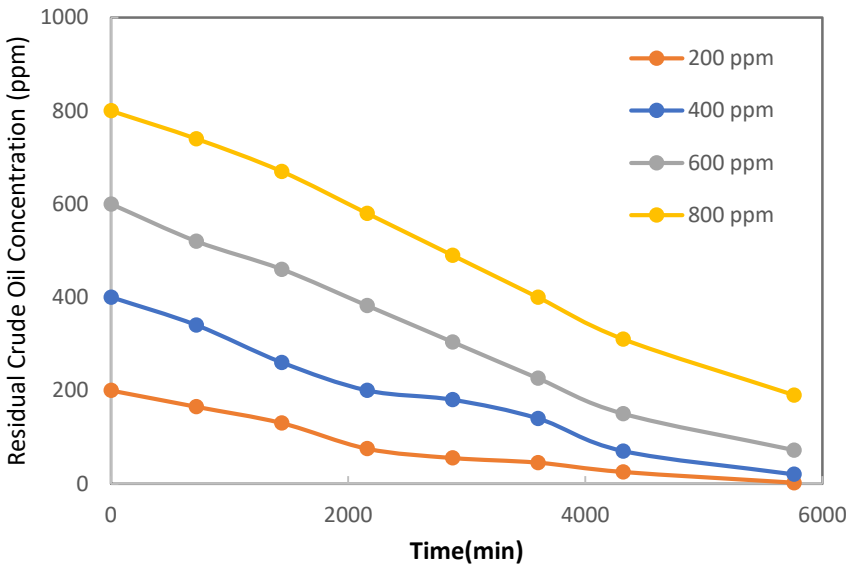

Figure 1. Degradation of S1at different initial concentration of crude oil (T=30 ºC, pH =7)

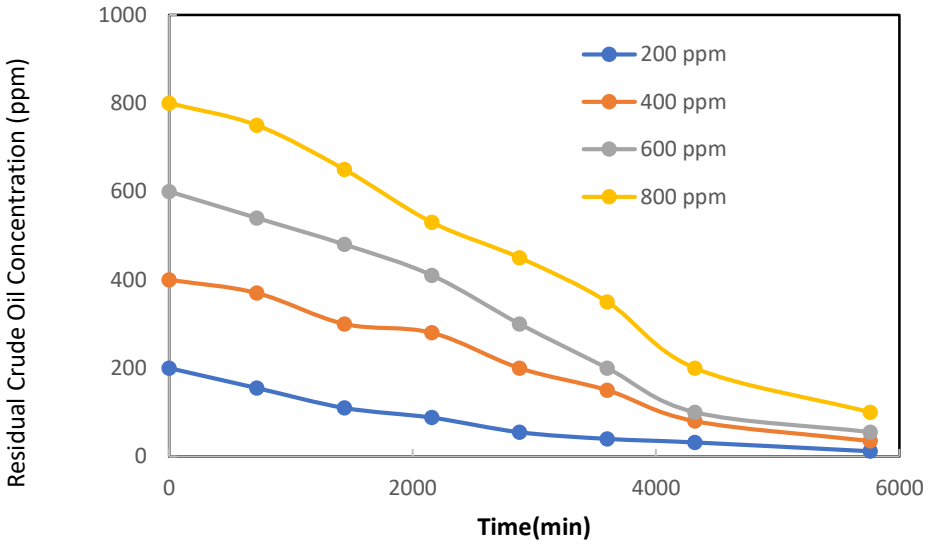

Figure 2. Degradation of S2at different initial concentration of crude oil (T=30 ºC, pH =7)

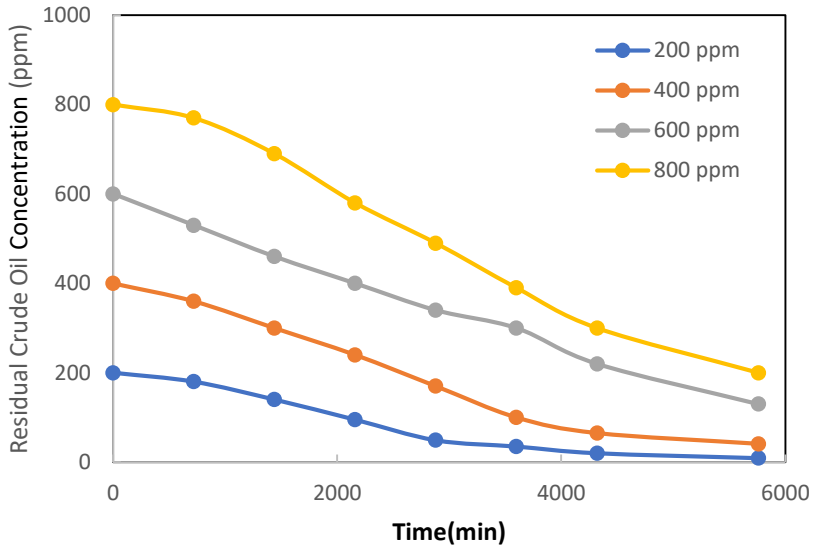

Figure 3. Degradation of S3at different initial concentration of crude oil (T=30 ºC, pH =7)

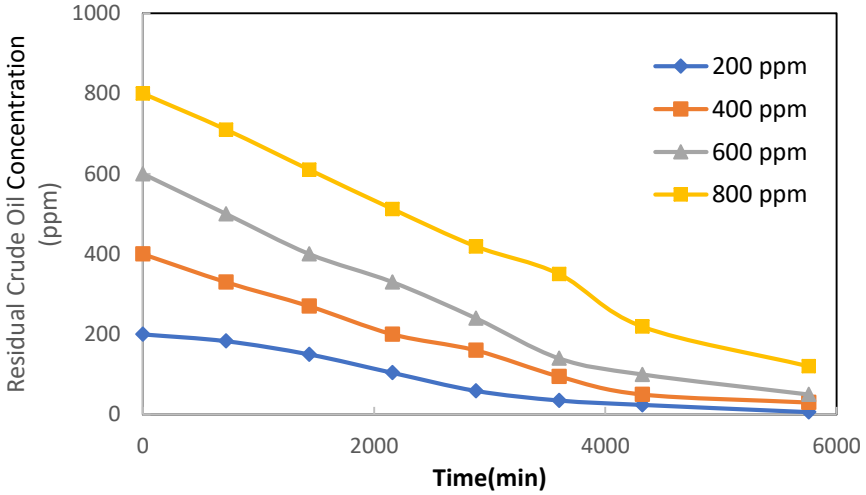

Figure 4. Degradation of S4 at different initial concentration of crude oil (T=30 ºC, pH =7)

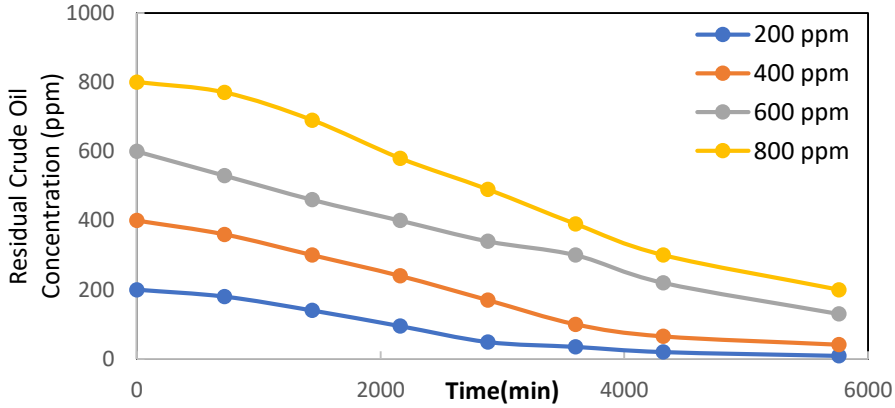

Figure 5. Degradation of S5 at different initial concentration of crude oil

(T=30 ºC, pH =7)

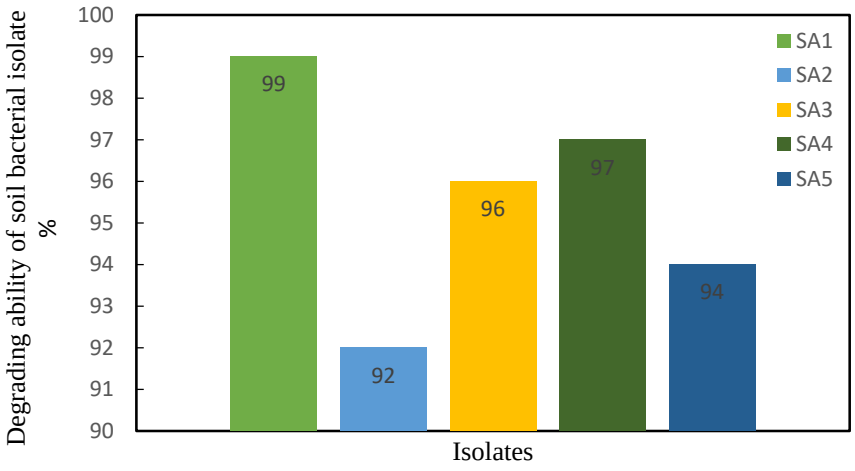

Figure 6. Degrading ability of soil bacterial isolate (Initial concentration =200 ppm, T=30 ^{O}C and pH=7).

Table 1. Mineral Salt Medium

Composition	**Quantity**
K_2HPO_4	500 mg
KH_2PO_4	250 mg
NaCl	0.5g
NH_4SO_4	230 mg
$CaCl_2.2H_2O$	7.5 mg
$MgSO_4.7H_2O$	100 mg
$MnSO_4.7H_2O$	100 mg
$FeCl_3$	1 mg
Double Distilled Water	1000 ml

Table 2. Biokinetics parameters of the different microorganism at various initial concentration of crude oil.

Microorganism	**Concentration (ppm)**	**Maximum Specific Growth(μ_m) (h^{-1})**	**Half Saturation Constant (K_s) (mg $L^{-1}h^{-1}$)**
Acinetobacter calcoaceticus	200	0.053	3.753
	400	0.152	43.659
	600	0.005	60. 091

	800	0.004	68.958
Pseudomonas aeruginosa	200	0.059	60.340
	400	0.051	348.672
	600	0.011	417.456
	800	----	---
Arthrobacter sps	200	1.050	30.492
	400	0.713	169.796
	600	0.419	231.929
	800	0.0426	395.761
Pseudomonas pictorum	200	0. 278	70.062
	400	0.112	108.628
	600	0.019	748.982
	800	0.014	698.73
Pseudomonas resinovorans	200	0.059	12.853
	400	0.011	13.910
	600	0.004	91.038
	800	---	---