Biodegradation of Crude Oil Contaminated Soil in North Oil Refineries/ Kirkuk /Iraq by Using Acinetobacter spp. and Streptomyces spp.

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Abstract

The current work objective was to reveal the Biodegradation of the crude oil by Acinetobacter spp. and Streptomyces spp. oil samples collected from tanks of north oil refineries/in in Kirkuk governorate/Iraq. The current work was determining the crude oil biodegradation under various conditions. This work revealed that led optimum conditions for the biodegrarocesses were pH seven even and temperature 37 0C. Under this optimum condition, the rate of consumption reported, Acinetobacter spp. and Streptomyces spp. The fourth day (pH 7: 28.3 and 28.1 and temperature 30 0C for Acinetobacter sp.: 38.4 and temperature 37 0C for Streptomyces spp. 27.4, respectively). Also, cell numbers reported Acinetobacter spp. and Streptomyces spp. On the fourth day (pH 7: 11.1; 10.8 X log 10 and temperature 30 0C for Acinetobacter spp.: 11.3 and temperature 37 0C for Streptomyces spp. 10.1 respectively). Also, consumption rate reported, Acinetobacter spp. and Streptomyces spp. The Optimum incubation period (third day) is 35.9 and 30.1, respectively, while cell numbers were reported for Acinetobacter spp. and Streptomyces spp. At optimum incubation period (third day) is 11.2 X log 10 and 10.8 X log 10. It was concluded from this study that the optimum conditions of Acinetobacter spp. and Streptomyces spp. to Biodegradation of crude oil were pH 7, temperature 37, and third incubation day.

Key words: *Crude oil; Biodegradation; Acinetobacter spp.; Streptomyces spp*.

INTRODUCTION

The biodegradation process is outlined due to the catalyzed, which causes decreasing in substance quality [Latha and Kalaivani .,2012]. Microorganisms square calculate degrading solely capacity a limited crude variety depends on the appeal of metabolically several microorganism populations [Vinothini et al.,2015]. Bioremediation efficiency of contaminated soil depends on the number of microorganisms that can degrade the hydrocarbon in the soil[Burghal et al.,2015a]. Several types of bacteria are called a good degraders for hydrocarbon these bacteria tolerate a different hydrocarbons concentrations with a high bility for degradation of hydrocarbons like Pseudomonas spp, Mycobacterium, Aeromonas, Bacillus spp, Alcaligenes, Acinetobacter spp, Arthobacter, Rhodococcus and Brevibacterium [ATLAS

R.M. Petroleum microbiology, 1984 ; Płaza et al.,2008].Different Actinomycetes genus can degrade various pollutants such as Mycobacterium spp.,Arthrobacter,Gordonia, Rhodococcus spp.and Streptomyces are called degraders of hydrocarbon [Al-Charrakh etal.,2016 ; Hopwood, 2007]. Streptomyces spp. is a very threadlike fungus: each grows like the branching of hyphae that make a plant structure and unfold via spores which make generative structures called aerial hyphae formed from the surface of the colony into the air [Sette et al.,2004 ; Burghal et al.,2015b]. The Acinetobacter genus utilized in the current study is described as aerobic microorganisms with non-motile and gram-negative bacteria[Doughari.,et al2011; Fatajeva et al.,2014]. So, the aim of current study is determining ability of Acinetobacter spp and Streptomyces spp to Biodegradation of crude oil contaminated soil in north oil refineries/Kirkuk governorate.

MATERIALS & METHODS

SAMPLES COLLECTION

The crude oil samples were collected from tanks of northern oil refineries/ in Kirkuk governorate/Iraq. Dark, bottles were utilized, for collecting of samples.

THE SAMPLE OF SOIL

Soil samples (100mg) utilized in Biodegradation were collected from north oil refineries/Kirkuk governorate.

ISOLATION OF BACTERERIA

100 ml of selective media, MSM was transported to the flask with (1%) crude oil. The flasks after that incubated at 30 0C to 24 hours., 1ml of MSM transporting to dishes that contained Trypticase soya agar (TSA). After that, the dishes were incubated at 30 0C to 24 hr. Bacteria colonies were identified done according to [Padmapriya and Williams, 2012]. The isolate of Acinetobacter spp was maintained on a nutrient agar medium (Difco, India) at 30 C for daily use.

GROWTH OF BACTERIA

For the growth of bacteria, MSM media was used. MSM (50ml) transferring to a flask then crude oil (1%) was transported to flasks that contained 1ml of bacterial suspension (6X104). Then, the flasks of suspension bacteria were incubated at 30 0C to report the cell numbers, and the bacteria's capability to biodegrade crude oil [Marins et al.,2002].

BATERIA VIABLE COUNTS

The counts of viable bacteria were reported for 4 days. 1X108 cell/ml for Acinetobacter spp and Streptomyces spp cultured on MSM. Petri dishes were incubated at different pH (5, 6, 7, and 8) and different temperatures (25, 30, 37, and 40). Counts were measured as follows:

Number of cells = colonies number/sample volume X dilution [Teschner and Wehner,1985].

OPTIMUM CONDITIONS

To reactivate the isolates of Acinetobacter spp and Streptomyces spp, broth agar media was utilized at 37 0C for 24 hr.; then, isolates were transported to MSM (100 ml) with 1% crude oil. The shaker incubator was utilized to incubate flasks at 150 rpm at different pH; 5, 6, 7, and 8, and different temperatures 25, 30, 37, and 40 0C. After 4 days, cell numbers of Acinetobacter spp, and Streptomyces spp, were reported [Nnamchi et al., 2006].

RESULTS AND DISCUSSION

Isolation of bacteria

Bacteria were isolated from soils, which contaminated with crude oil. Acinetobacter spp and Streptomyces spp identification are shown in table (1).

Table (1): identification tests

Bacteria isolates / Biochemical tests	*Acinetobacter spp*	Streptomyces *spp*
Gram stain	-	-
Oxidase test	-	+
Catalase test	+	+
Indole test	-	-
MR test	-	-
VP test	-	+
Citrate test	+	+
Urease test	+	+
Nitrate reduction	-	-
Motility test	-	-

As shown in a pouvisly table about the chemical characteristics of Acinetobacter, the results agree with [Al-Dulaimi et al.,,2017]. While the chemical characterized Streptomyces, the results agree with [Al-Shaibani et al., 2016].

OPTIMUM CONDITION

EFFECT OF pH

Isolates of bacteria that cultured on MSM with crude oil (1%) and incubated at 37 0C with 4 degrees of pH (5, 6, 7, and 8). rate of consumption for crude oil by Acinetobacter spp and Streptomyces spp isolates appear in figures. (1-2).

Figure (1): the consumption rate at different pH by Acinetobacter spp

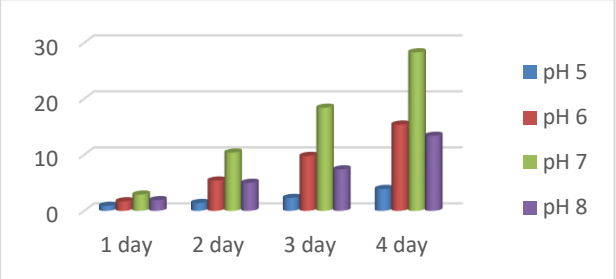
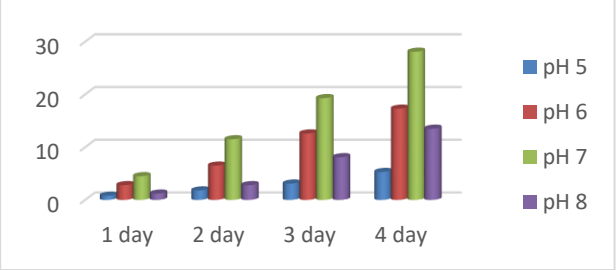


Figure (2): the consumption at differenT pH by Streptomyces spp.



The highest rate of consumption to Acinetobacter spp and Streptomyces spp showed at pH 7 was 28.3 and 28.1 respectively at the fourth day. Numbers of cells demonstrate you elevate at temperature as shown in figures

(3-4). The current finding is agreed with [Yonis 2012], who referred to the Biodegradation and Biosurfactant by Agrobacterium. The results show that Agrobacterium's optimum pH for Biodegradation is 7. Also [Hyder, 2015] referred that the optimum pH to degrade of crude oil by using Acinetobacter spp is 7.

Figure (3): the cell number at different pH by Acinetobacter spp

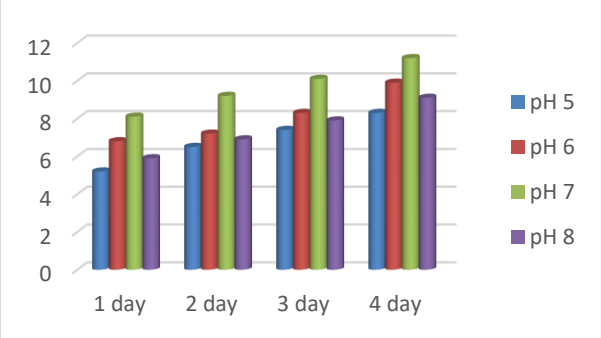
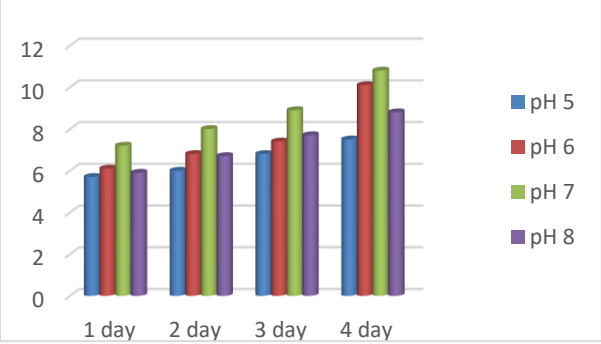


Figure (4): the cell number at different pH by Streptomyces spp



EFFECT OF TEMPRETURE

Isolates of bacteria cultured on MSM, containing, of crude oil, (1%) and then incubated at seven pH with temperatures (20, 30, 37, and 40 0C). Crude oil consumption rate by bacteria was shown in figures (5-6).

Figure (5): the consumption rate at different different temperature by Acinetobacter spp.

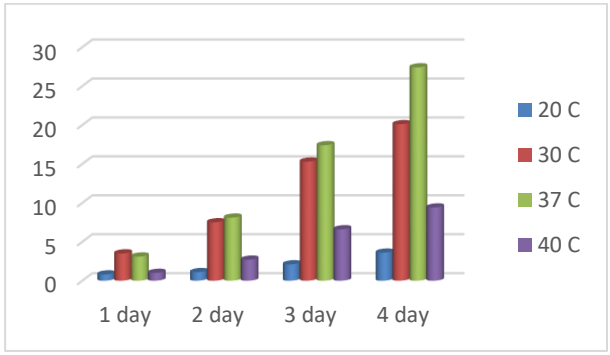
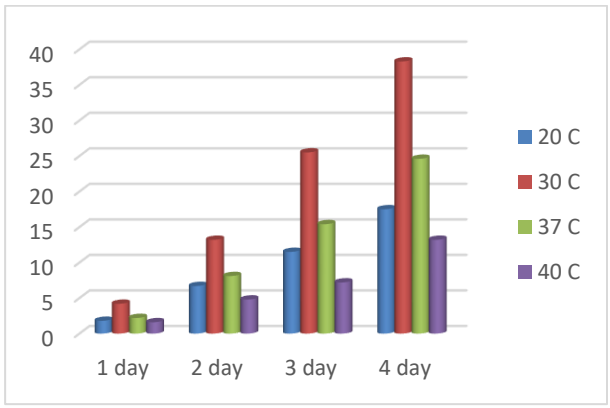


Figure (6): the consumption rate at Temperature Streptomyces spp.



The highest consumption rate reported for Acinetobacter spp at a temperature of 30 0C was 38.3, and Streptomyces spp at 37 0C was 27.4 on the fourth day. The number of cells shown increased at different temperatures, as shown in figures(7-8).

Figure (7): the cell number at different temperature Acinetobacter spp.

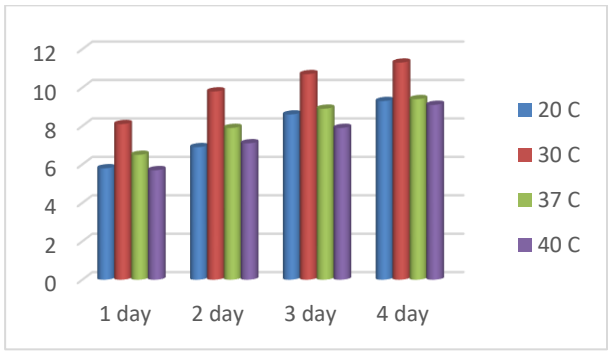
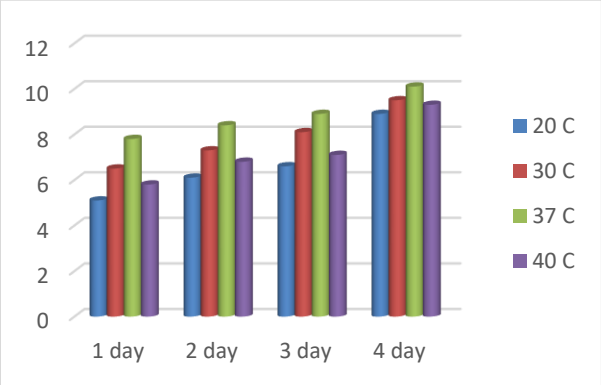


Figure (8): the cell number at different temperature Streptomyces spp



The highest cell numbers reported to Acinetobacter spp at a temperature of 30 0C was 11.3, and Streptomyces spp at 37 0C 10.1 on the fourth day. The present outcome was similar to[Al-Janabi, 2009], who found that the optimum temperature to degrade crude oil is 30 0C. [Farid, 2012] referred that that the optimum temperature to degrade of crude oil by using Streptomyces spp is 37 0C. Also, [Hyder, 2015] referred that the optimum temperature to degraded of crude oil by using Acinetobacter spp is 30 0C

INCUBATION PERIOD

Isolates of bacteria that were cultured, on, MSM, which contains of crude oil (1%), and then incubated at 7 pH with temperatures 30 and 370C for Acinetobacter spp and Streptomyces spp. respectively) at different incubation periods (1-4 days). Consumption rate by both types of bacteria was demonstrated in figures (9-10).

Figure (9): the rate of consumption at incubation period by Acinetobacter spp.

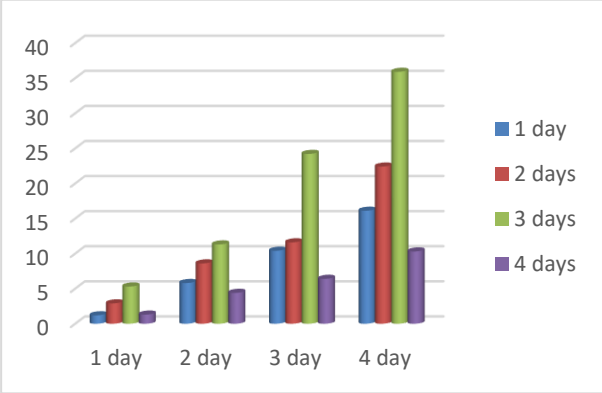
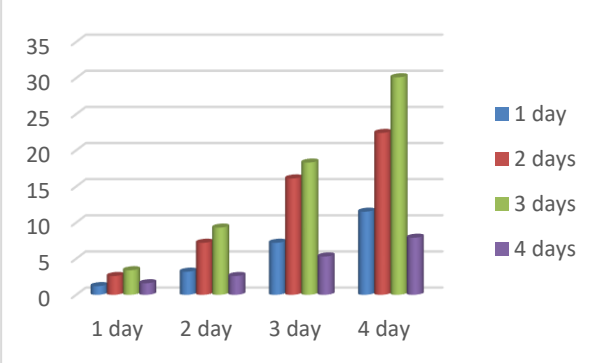


Figure (10): the rate of consumption at incubation period Streptomyces spp.



Highest rate of consumption was reported for Acinetobacter spp. and Streptomyces spp. On the third incubation day. The number of cell show was increased at different temperatures, as shown in figures (11-12).

Figure (11): the cell number at incubation period Acinetobacter spp.

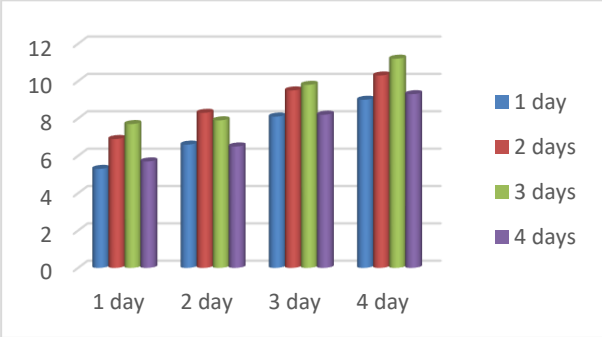
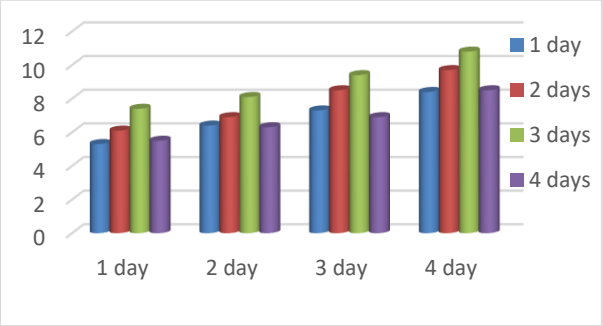


Figure (12): the cell number at incubation period Streptomyces spp.



Highest number of a cell for Acinetobacter spp. and Streptomyces spp. were reported on the third incubation day. The present finding agrees with the study of [Yonis,2012] who referred to the Biodegradation and Biosurfactant by Agrobacterium. Also[Hyder 2015] mentioned that the optimum incubation period for degraded crude oil using Acinetobacter spp is 72.

BACTERIA ABILITY TO DEGRADATION

Viable count of measurement bacteria cultured on MSM media estimated the ability of both types to degrade crude oil. spp. and Streptomyces spp. Growth was continuous throughout the experiment time. The number of all both types that are used is 1X108 cells/ ml—viable count of Acinetobacter spp. and Streptomyces spp—increased on the first day. After that, the cell number decreased until the end figures (13-14).

Figure (13): Acinetobacter spp viable count

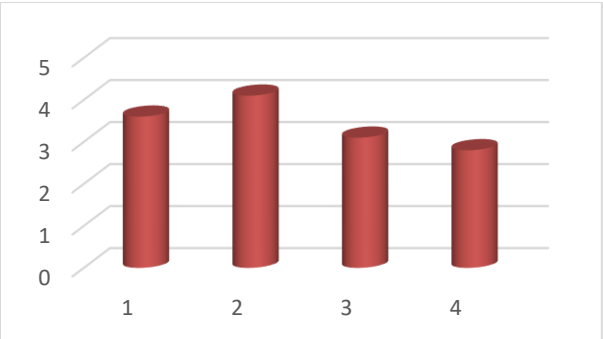
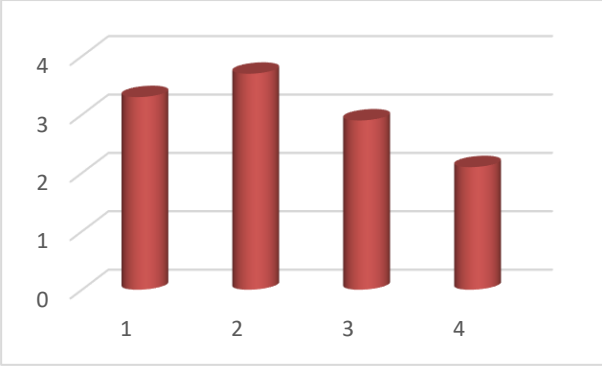


Figure (14): Streptomyces spp viable count



CONCLUSIONS

The highest rate of consumption to Acinetobacter spp and Streptomyces spp showed at pH 7 was at fourth day. the optimum temperature to degrade of crude oil by usingAcinetobacter spp was at temperature 30 0C, while the optimum temperature to degrade of crude oil by using Streptomyces spp is 37 0C. The highest rate of consumption reported to Acinetobacter spp. and Streptomyces spp. at third incubation day while the highest number of cell for Acinetobacter spp. and Streptomyces spp. were reported at third incubation day.The number of all both types that used is 1X108 cell/ ml.Viable count of Acinetobacter spp. and Streptomyces spp. increased at first day after that the number of cell were began to reduce until the end.The optimum conditions of Acinetobacter spp. and Streptomyces spp. to biodegradation of crude oil were pH 7, temperature 37 and third incubation day.

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