BIOLOGICAL TREATMENT OF HYDROCARBON COMPOUNDS IN OIL REFINERY WASTE WATER

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ABSTRCT

Three types of microorganisms are used in this study to show the difference between their ability in consumption of the hydrocarbon residual in the industrial waste water of the Al-Doura refinery. These types are: *Protozoa* (taken from Al-Doura refinery) and two local isolates: *P. aeruginosa* and *P. fluorescens*. These have been already tested boichemically, physiologically and according to API 20E system method. Box-Wilson method is used to find the mathematical relations between the variables (temperature, pH and inoculate amount) with biomass amount during the hydrocarbon residual consumption. The obtained experimental results are fitted to a polynomial function of second order. The experiments are carried out using batch culture for both the three isolates (*Protozoa, P. aeruginosa, P. fluorescens*) and the mixed isolates (*P. aeruginosa*+ *P. fluorescens*). The best incubation period for the three isolates is 72 hours. the optimum operation conditions for mixed isolated are biomass 5.22 g/L, BOD_5 15 mg/L, TSS 95 mg/L, TDS 4500 mg/L and S.T 25 m.N/m. These results have show that mixed isolates are the best for consumption of hydrocarbon residuals.

Key words: Bioreactor, *Pseudomonas*, Waste water, Biotreatment.

المعالجة الحيوية للمركبات الهيدروكربونية في مياه الصرف لمصافي النفط

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الخلاصة

تم في هذه الدراسة إستعمال ثلاث أنواع من الاحياء المجهرية في قدرتها على إستهلاك المخلفات الهيدروكاربونية لمخلفات المياه الصناعية في مصفى الدورة، وهي الـ (*Protozoa*) المأخوذة من مصفى الدورة وعزلتين محليتين تم إجراء الفحوصات الفسلجية والكيموحيوية وإستعمال طريقة API وشخصت على إنها *Pseudomonas aeruginosa* و*Pseudomonas fluorescens*. أستعملت طريقة Box-Wilson لايجاد علاقة رياضية تربط المتغيرات الثلاثة (درجة الحرارة، الدالة الحامضية، كمية اللقاح) مع كمية الكتلة الحيوية Biomass خلال عملية إستهلاك المخلفات الهيدروكاربونية. النتائج العملية التي تم الحصول عليها باستخدام هذه الطريقة تم تمثيلها مع معادلة رياضية من الدرجة الثانية. تم تطبيق نظام الدفعات Batch culture على العزلات المستخدمة في الدراسة وهي الـ *Protozoa* و *P. aeruginosa* و *P. fluorescens* وخليط الاحياء المجهرية (*P.aeruginosa+ P. fluoresens*) كلاً على حدة. وجد أن أفضل مدة حضن للعزلات الثلاث كانت (72) ساعة وان معظم الزيادة في الكتلة الحيوية كانت مصحوبة بخفض قيمة الشد السطحي بعد ثلاثة أيام من الحضن. بالنسبة لخليط الاحياء المجهرية(*P. aeruginosa + P.fluoresens*)). كانت الظروف المثلى التي أعطت أعلى قيم من الفحوصات هي (5.22)g/L للـ Biomass و (15)mg/mL للـ BOD_5 و (95)mg/mL للـ TSS و (4500)mg/mL للـ TDS و (25) m.N/mللـSurface tension. بعد النتائج المتحصلة والتي أثبتت إن خليط الاحياء المجهرية هو أفضل عزلة لاستهلاك المخلفات الهيدروكاربونية من خلال قيم الفحوصات أعلاه.

INTRODUCTION

The oil industry generates large quantities of oily and viscous residues which are formed during production, transportation and refining. These residues are composed of oil, water, solids and their characteristics are such varied composition that making them of highly recalcitrant and very difficult to reuti1ize. The marked stability of the multiphase system is due to the adsorption of oil into solid particles, producing a highly protective layer (as they tend to settle at the bottom of the tanks), and also to the presence of surface-active compounds, which are responsible for the formation of emulsions (1). In addition to that, the presence of organic polar fractions brings about charge repulsion that impairs the formation of a homogeneous phase. From a chemical point of view, recalcitrance can be ascribed to the presence of aromatic hydrocarbons, polycyclic aromatic hydrocarbons (PAH) and complex compounds with a very high molecular weight, such as asphaltenes (2). Biotechnological processes using specific bacteria to destroy toxic waste offer pollutant over physiological processes. When successfully operated, they are non pollution because such bacteria can completely degrade and oxidize toxic organic compounds to innocuous carbon compounds and are characterized by low costs and offer the possibility of in situ treatments (3). Co-metabolism uses microorganisms growing on one compound to produce an enzyme that chemically transforms another compound on which they cannot grew. Treatability or feasibility is used to determine whether bioremediation would be effective in a given situation. Versatile metabolic activities of microorganisms have played a key role in biodegradation of various toxic organic compounds of aromatic and aliphatic nature, entering natural environment through industrial waste discharges.

The purpose of this work is to study the effect of parameters (temperature, pH and bacteria concentration) on the treatment of hydrocarbons residue and to specify the optimum values of the parameters.

MATERIALS AND METHODS

Experimental Work

The borosilicate glass (beaker 2 L) is filled up with (1L) wastewater and mineral salt medium with 10 ml inoculum are added to the fermenter Figure(1). Non- biodegradable plastic granules is added to make a suspension system in the liquid medium and confirms the bacterial cell. The beaker is used as a batch culture for three days for bacterial isolates growth, then the biomass, BOD_5,TSS,TDS and surface tension are measured (4).The temperature inside the water path is 28C° throughout the batch culture. The oxygen is introduced to the bioreactor and the beaker using air compressor 250 mL/min. The pH is regulated to 7.0 before experiments and measured after that for each experiment(5)(6).

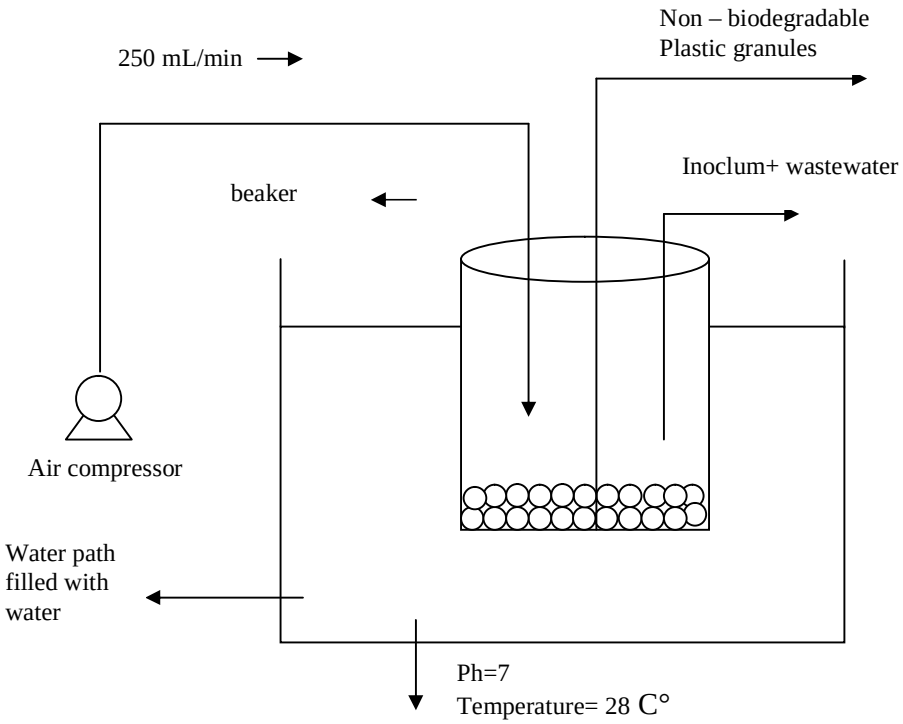


Figure (1): Beaker in batch culture

Experimental Design

Box – Wilson composite rotatable design is a common type of statistical experiments, especially applicable in optimization analysis. In this design, special series of tests are defined. The experimental results of these tests then serve function to represent the relationship between the variable and the response (7) (8) (9). The effect of three variables such as temperature, pH and amount of bacteria with biomass are investigated and analyzed by using the experimental design. Box – Wilson central composite design is used to find a suitable relationship between the three independent variables and the observed response (biomass).

X_1 = temperature (28 – 50) ^{0}C.

X_2 = pH (2 – 7).

X_3 = amount of Bacteria (10 – 30) mL.

Table (1) shows the coded and real values of the experiments to be conducted according to Box – Wilson method (*P. aeruginosa* + *P. fluorescens*).

RESULTS AND DISCUSSION

The response of experiments conducted according to Box - Wilson method, is represented by biomass (mg/L), and is fitted by a second - order polynomial mathematical model.

Postulating the Mathematical Model

A second polynomial equation is employed in the range of the independent variables. Three variables are used, the general form of second order polynomial equation is:

$$y = B_0 + B_1X_1 + B_2X_2 + B_3X_3 + B_4X_1^2 + B_5X_2^2 + B_6X_3^2 + B_7X_1X_2 + B_8X_1X_3 + B_9X_2X_3 \quad \text{---- (1)}$$

For postulating the best form of the models, the coded data of Table (1) is fitted to equation (1), the regression analysis of central composite design can be applied to the approximating model to obtain the optimum conditions for the process.

B0	Coefficient of Polynomial Equation
B1	
B3	
X1	Coded Variable
X2	
X3	
y	(biomass for *P. aeruginosa*, *P. fluorescens*)

Effect of Different Variables on the Biomass of *P. aeruginosa*, *P. fluorescens*, (*P. aeruginosa*+ *P. fluorescens*) and Optimization of Variables

Effect of Temperature

The biomass increases with the decrease of temperature until it reaches 5.5 g/L at 28 °C and pH of 7, using constant amount of bacteria 10 ml as shown in Figure (2). On the other hand Figure (3) shows that at constant pH equal to 7 the biomass increases with the decrease in temperatures until it reaches 5.5 g/L in 28 °C and amount of bacteria 10 mL. that is due to the effect of temerature on the activity of the microorganisms. It is well known that temperature above 40°C affects the membrane composition of microorganisms, e.g. the phospholipids fatty acid composition changes with temperature and hence affect the enzymetic system of the bacteria. This lead in turn to decrease in biomass with increasing temperature.

Effect of pH

Figures (4),(5) show that biomass increases with increase in pH until it reaches maximum value of 5.5 g/L at pH 7 and (temperature 28 °C, amount of bacteria 10 mL) respectively. This behavior is due to increasing the activity of bacteria as it reaches the value pH to 7. At high value of pH is activates decrease and hence decreasing the production of biomass.

Effect Amount of Bacteria

Figure (6) shows that the biomass increase with the decrease in the amount of bacteria until it reaches 5.5 g/L at temperature 28 °C and pH 7. this behavior is caused by the higher growth rate of microorganisms as the amount of bacteria decreased. The same results are obtained from Figure (7) where the biomass increases with the decreasing amount of bacteria until it reaches 5.5 g/L at temperature 28 °C and pH 7.

From Figures(2,3),(4,5),(6,7) the best conditions to produce maximum biomass of bacteria we can detected, these are temperature 28 C°, pH 7 and amount of bacteria 10 mL.

Optimization of Operating Variables for *Protozoa, P. aeruginosa, P. fluorescens,* (*P. aeruginosa*+ *P. fluorescens*)

The optimum conditions for *Protozoa, P.aeruginosa, P.fluorescens* and (*P.aeruginosa*+ *P.fluorescens*) can be obtained by using a computer program depending on the method of Hook and Jeeves (standard computer program for finding optimum conditions) that lead to obtain the highest possible conversion of the product. The optimum conditions resulting from the application of this method are: for *Protozoa* Temperature = 28 °C, pH = 7, amount of *Protozoa*= 0.25 mg and for *P. aeruginosa, P. fluorescens,* (*P. aeruginosa*+ *P. fluorescens*) are temperature = 28 °C, pH = 7, amount of bacteria= 10 mL.

Experimentally, biomass and other tests are determined under these operating conditions for *Protozoa, P. aeruginosa, P. fluorescens* , (*P. aeruginosa*+ *P. fluorescens*) as shown in table (2),(3),(4),(5) respectively.

Table(1): The coded and real values of the experiments to be conducted according to Box – Wilson method (*P. aeruginosa* + *P. fluorescens*).

Exp. No.	Coded variable			Real variable		
	X1	X2	X3	X1	X2	X3
1	1	1	1	45.35	5.94	25.77
2	-1	1	1	32.64	5.94	25.77
3	1	-1	1	45.35	3.05	25.77
4	1	1	-1	45.35	5.94	14.22
5	-1	-1	1	32.64	3.05	25.77
6	-1	1	-1	32.64	5.94	14.22
7	1	-1	-1	45.35	3.05	14.22
8	-1	-1	-1	32.64	3.05	14.22
9	1.732	0	0	50	4.5	20
10	0	1.732	0	39	7	20
11	0	0	1.732	39	4.5	30
12	-1.732	0	0	28	4.5	20
13	0	-1.732	0	39	2	20
14	0	0	-1.732	39	4.5	10
15	0	0	0	39	4.5	20
16	0	0	0	39	4.5	20
17	0	0	0	39	4.5	20
18	0	0	0	39	4.5	20

Table(2):Tests for *Protozoa* under optimum conditions

Test	Value
Biomass	3.55 g/L
BOD_5	33mg/mL
TSS	170 mg/mL
TDS	2800 mg/mL
Surface tension	38 m.N/m
Optical density	0.57

Table(3):Tests for *P. aeruginosa* under optimum conditions

Test	Value
Biomass	4.45 g/L
BOD_5	28 mg/mL
TSS	130 mg/mL
TDS	3000 mg/mL
Surface tension	36 m.N/m
Optical density	0.62

Table(4):Tests for *P. fluorescens* under optimum conditions

Test	Value
Biomass	4.21 g/L
BOD_5	25 mg/mL
TSS	150 mg/mL
TDS	3400 mg/mL
Surface tension	31 m.N/m
Optical density	0.65

Table(5):Tests for (*P. aeruginosa*+ *P. fluorescens*) under optimum conditions

Test	Value
Biomass	5.22 g/L
BOD_5	15 mg/mL
TSS	95 mg/mL
TDS	4500 mg/mL
Surface tension	25 m.N/m
Optical density	0.74

It is concluded from the previous results that mixed culture(*P.aeruginosa*+ *P.fluorescens*) produces maximum amount of biomass =5.22 g/L, $(BOD)_5$=15 mg/mL, TSS=95 mg/mL, TDS=4500 mg/mL and Surface tension=25 m.N/m under optimum conditions table(5) in comparison with *P.aeruginosa* table(3), *P.fluorescens* table(4) and *Protozoa* table(2). So mixed culture (*P.aeruginosa*+ *P.fluorescens*) can be chosen as the best isolate for waste water treatment (10)(11)(12).

Estimation of Coefficients of the Second Order Equations

The coefficients of equation (1) can be determined by using computer software, so equation (1) can be written as follows for *P. aeruginosa, P. fluorescens* and (*P. aeruginosa* + *P. fluorescens*):-

$$y = 6.15756 - 0.154148\ X_1 + 1.091737\ X_2 - 0.119353\ X_3 + 0.002284\ X_1^2 + 0.146811\ X_2^2 + 0.000004\ X_3^2 - 0.013467\ X_1X_2 + 0.000004X_1X_3 - 0.002154\ X_2X_3 \quad \text{---- (2)}$$

Correlation coefficient= 0.982124857
Average absolute error=3.005%

Equation (1) can be written as follows for *Protozoa*:-

$$y = 4.87461 - 0.150138\ X_1 + 1.291737\ X_2 - 0.109313\ X_3 + 0.021064\ X_1^2 + 0.345321\ X_2^2 + 0.000005\ X_3^2 - 0.112366\ X_1X_2 + 0.000005\ X_1X_3 - 0.032892X_2X_3 \quad \text{---- (3)}$$

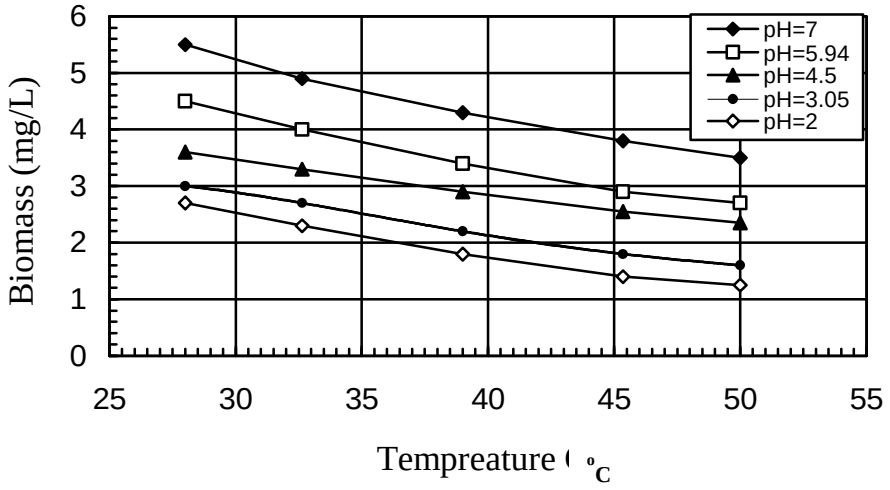
Correlation coefficient= 0.972104985
Average absolute error=3.514%

CONCLUSIONS

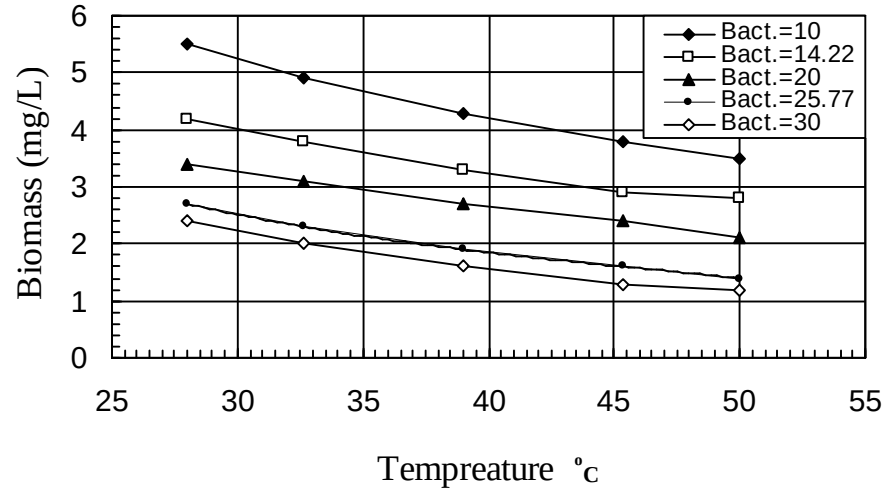
1- Microorganisms *Pseudomonas* (which is Gram-negative) is efficient in consumption of hydrocarbon wastes and spread into the contaminated soils and water.
2- Local isolates of microorganisms are more effective than imported in the waste consumption.
3- Mixed isolates is more efficient than one type isolate in hydrocarbons waste consumption.
4- *P. aeruginosa* seems to be more efficient than *P. fluorescens.*
5- Mixed isolates are more efficient in view of rate of growth and biomass increase as well as decreasing the surface tension and the values of BOD_5, TSS, TDS when the process is carried out in batch or continuous operation.
6- The optimum conditions for growth of local mixed isolates are: amount of bacteria = 10 mL, pH=7 and temperature = 28ºC.
7- At optimum operating conditions for the growth of local isolates and the value of tests are: Biomass = 5.22 g/L, BOD_5 = 15 mg/mL, TSS=95 mg/mL, TDS = 4500 mg/mL, S.T=25 m.N/m.

8- The optimum conditions for growth of *P. fluorescens, P. aeruginosa* and *Protozoa* and the value of tests are less than that for local mixed isolates.

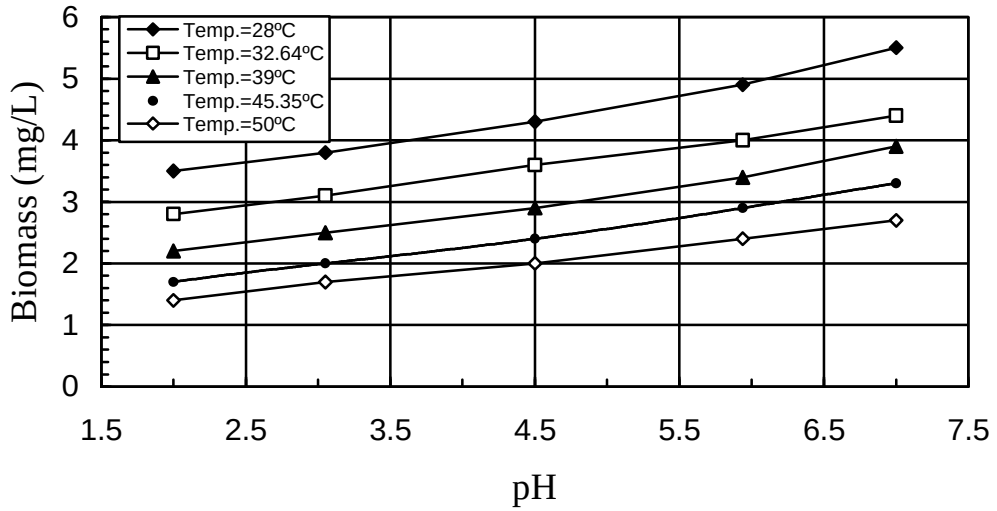
Figure(2):Variable pH at constant amount of bacteria 10 mL



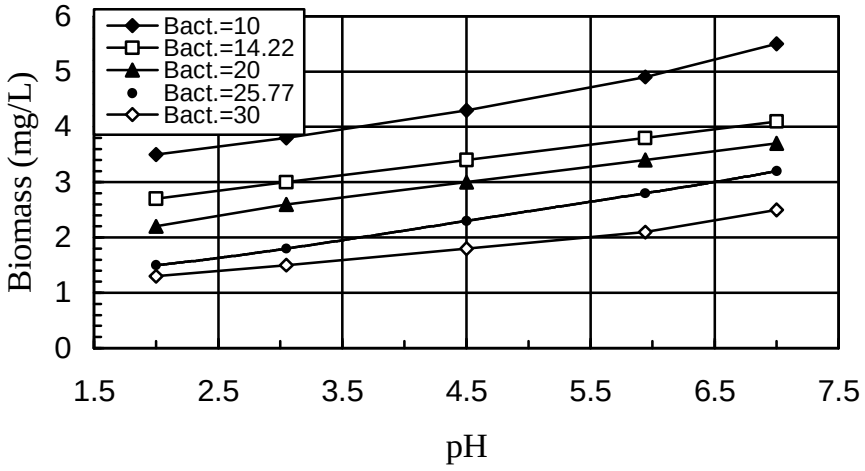
Figure(3):Variable amount of bacteria at constant pH 7



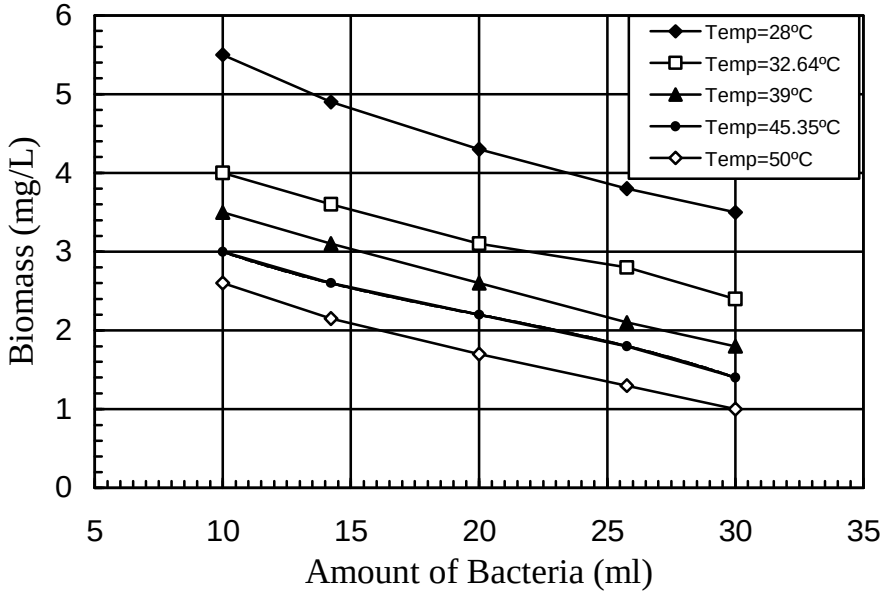
Figure(4):Variable temperature at constant amount of bacteria 10 mL



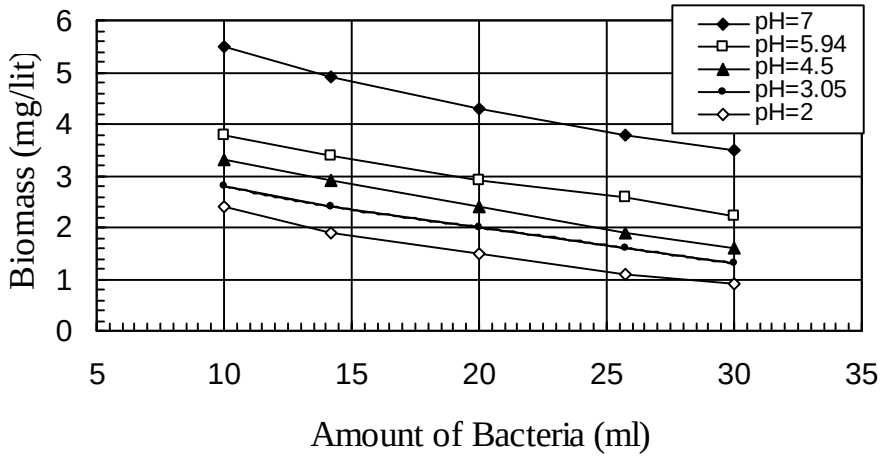
Figure(5):Variable amount of Bacteria at constant pH 7



Figure(6):Variable temperatures at constant pH 7



Figure(7):Variable pH at constant temperature 28 °C



REFERENCES

1- Soriano, A. U. (2007). Oily Sludge Biotreatment. Peters Research Center (CENPES), Rude Janeiro, Brazil.
2- Pandya, M.T. (2006). Biotechnology Applications in the Treatment of Industrial Waste Water. *Water and Water Asia*, June, 2006.
3- Aiba, S.; Humphrey, A. E. and Millis, N. F.; (2000). Instrumentation For Environmental Control Biochemical Engineering, 2nd ed., New York and London, Academic Press Inc.
4- Maefacld, J. F. (2000). Biochemical Test for Identification of Medical Bacteria. 3rd ed. M .G. Lawrence (ed.), lippin cott of Williams, New York.
5- Altas, R-M.; Parks, C. L. and Brown, A. E. (1995). Laboratory Manual of Experimental Microbiology. Department of biology, University of Louisihe.
6- Manresa, M. A; Bastida, J. and Guinea, J. (1991). Kinetic Study on Surfactant Production by *Pseudomonas aeruginosa*. 44 Tl. *J. Ind. Microbiology*, 8: 133-136.
7- Box, G. E, and Hunter, J .C. (1998) .Ann. Math .3 P: 195-210.
8- Montgomery, D, G (1999). Design and Analysis of Industrial Experiments. John Wiley and sons, New York.
9- Peter, M. S and Timmerhouse, R. (2001). Plant Design and Economics for Chemical Engineers. 2nd ed. McGraw –Hill Book CO. Petroleum Oily Sludge".
10- Ray, G. (1994). Bioremediation End its Application to Exxon Valdes Oil Spill in Alaska. Biotechnology –Biological Fundaments, 33:65-70.
11- Zhang, T. and Miller, R. M. (2006). Effect of *Pseudomonas* Rhamnolipid Biosurfactant on Cell Hydrophobicity and Biodegradation Of Octadecane. *Environ. Microbiol*., 60: 2101-2106.
12- Al – Khazaly, E. H. (2000). A study on *Pseudomonas aeruginosa* Capability to Degrade Hydrocarbons and Productions of Bioemulsifiers. MSc. thesis, University of Baghdad.