THE EFFECT OF CULTURAL AND ENVIRONMENTAL CONDITIONS ON BIODEGRADATION AND BIOSURFACTANT PRODUCTION BY *SERRATIA MARCESCENS* UTILIZING WEATHERED DIESEL OIL

Nadem H. Hayder Gumaa, Wameidh M. Potrus and Sahar Imad Mohammed
Biotechnology Department, College of Science, University of Baghdad, Baghdad-Iraq.

Abstract

The capability of a *Serratia marcescens* isolate to biosurfactant production from spilled weathered diesel oil has been studied in batch culture. Several cultural and environmental conditions were analyzed to optimized condition for growth and biosurfactant production. Results showed that the optimized conditions which for growth was, pH 7, incubation period for 72 h, supplementation of the production medium with $(NH_4)_2$ SO_4 0.4 % and weathered diesel oil in a concentration of 5 % which yielded 8.6 g/l biomass. Optimized condition for biosurfactant production was, pH 8, incubation period 96 h, $(NH_4)_2$ SO_4 0.2 % and weathered diesel oil in a concentration of 6 % which yielded 10.5 g/l biosurfactant. Temperature 30 °C was optimum for growth and biosurfactant production. Surface active properties of isolate studied during cultivation with weathered diesel oil at the concentration 6 % (w/v) at different incubation period. The isolate has synthesized extra – cellular compounds which increase the E 24 % emulsion index of culture medium to 58 % and emulsification activity to 0.9. The presence of these substances (crude form) lower the surface tension of the culture until 43 mN/m. Bacterial cell – surface hydrophobicity (BAH) as measured by analyzing cell affinity towards aliphatic, aromatic and mixed hydrocarbons was also determined. The isolate was found to have a surface hydrophobicity (Hydrocarbon affinity) in the following order: aliphatic, mixed and monoaromatic hydrocarbons. The present study concludes possibility of using surface active agents (Biosurfactant) produced by *Serratia marcescens* mainly in: petroleum industry in enhanced oil recovery and in variety of biotechnological applications, including bioremediation of hydrocarbons contaminated sites.

Keywords: Biosurfactant, diesel oil, surface active properties, hydrocarbon affinity.

Introduction

Biosurfactants are surface-active substances derived from living organisms, especially microorganisms. Biosurfactants are amphiphilic compounds, containing hydrophobic and hydrophilic moieties. The hydrophilic moiety can be carbohydrate, amino acid, phosphate group or some other compounds whereas the hydrophobic moiety usually is a long chain fatty acid (1). Biosurfactants are being investigated as replacements for synthetic surfactants because they are biodegradable, less sensitive to extreme environments and can be produced on renewable substrates (2). The potential applications of biosurfactants in industrial include emulsification and foaming for food processing, wetting and phase dispersion for cosmetics and textiles, or solubilization for agrochemicals. In addition, biosurfactants can be use in environmental applications such as bioremediation and dispersion of oil spills (3).

Biosurfactants can be divided into 4 groups based on their overall structures. They are glycolipids, phospholipids, lipoproteins or lipopeptides and polymeric (4).

Commercial viability of biosurfactants is still limited by their high production costs, associated with inefficient recovery methods and with the use of expensive raw materials. These costs can be significantly reduced with the development of cheaper processes and the use of low-cost raw materials, which account for 10-30% of the overall cost (5). Biosurfactants can be commercially produced at levels of up to 100 g/L, as reported for rhamnolipids from *Pseudomonas sp*. This production level, combined with the use of cheap renewable substrates as organic wastes, makes the cost of biosurfactants competitive with the cost of synthetic surfactants (6). Alternative substrates have been suggested for biosurfactant production, especially water-miscible agro-industrial wastes: molasses,

whey, cassava wastewater and distillery wastes. However, there are few examples of the use of hydrophobic wastes as cheap substrates, for instance, waste frying oils, used lubricant oils and oily sludge from petroleum refineries (7).

Diesel oil is an excellent model for studying hydrocarbon biodegradation, since it is constituted of a variety of these molecules, such as paraffin, olefins, and naphtha and aromatic compounds. The molecular weight of the hydrocarbons present in diesel is also variable, with molecules containing from 9 to 20 carbon atoms. There have been several reports on Diesel spills in the environment, besides other pollution problems related to the extensive use of this fuel (8).

Surfactant have also been used in cost-effective, contaminant specific treatments to reduce the concentration of individual or mixed environmental pollutants. The ability of a surfactant to enhance the biodegradation of slightly soluble organic compounds depends on the increase in the bio- availability of these organic compounds. The bio-availability could be increased either by the addition of the surfactant during degradation or by applying the biosurfactant producing organisms to the contaminated environment (9).

It is reported in the literature that the genus *Pseudomonas* and *Serratia* are capable of using different substrate, such as hydrocarbons, vegetable oils, olive oils, n-paraffin's to produce biosurfactants (5).

In the present study, we investigate the capability of isolate *Serratia marcescens* to biodegradation and production of biosurfactants from spilled weathered diesel oil under environmental and cultural conditions and study surface active properties of produced biosurfactant.

Material and Methods

Microorganism

Seven isolates of *Serratia marcescens* isolated from soil contaminated with hydrocarbons and its derivatives used for biosurfactant production. The strains obtained from Biology and Biotechnology Department of the Baghdad University.

Media and cultivation conditions

The isolates were grown in nutrient broth for 16–18 h 30 ˚C. This culture was used as a stock culture inoculums at the 1% (v/v) level. For screening of isolates to biosurfactant production, optimum conditions for biosurfactant production and surface active properties of produced biosurfactant, a mineral salt medium with the following composition (g/L) was used (10): K_2HPO_4 (1), KH_2PO_4 (1), Mg SO_4 $7H_2O$ (0.6), Fe SO_4 $7H_2O$ (0.01), NaCl (0.05), $CaCl_2$ (0.02), yeast extract (0.5) and 0.1 mL of trace element solution containing (g/-L): 2.32 g $ZnSO_4$ $7H_2O$, 1.78 g $MnSO_4$ $4H_2O$, 0.56 g H_3BO_3, 1.0 g $CuSO_4$ $5H_2O$, 0.39 g Na_2MoO_4 $2H_2O$, 0.42 g $CoCl_2$ $6H_2O$, 1.0 g EDTA, 0.004 g $NiCl_2$ $6H_2O$ and 0.66 g KI. The pH of the medium was adjusted to 7.0. Carbon and nitrogen sources were added separately. Cultivations were performed in 250 mL flasks containing 50 mL medium at 30 ^oC, and stirred in a rotary shaker incubator (Basal Switzerland) at 150 rpm.

Medium Optimization

The medium optimization was conducted in a series of experiments changing one variable at a time, keeping the other factors fixed at a specific set of conditions. Five factors were chosen aiming to obtain higher productivity of the biosurfactant: pH, temperature, incubation period, nitrogen sources and carbon source. The pH used was (4-9), temperature used were (25-45 ^oC). For incubation period *Serratia marcescens* was growing at different incubation period (1-7) days at pH 7 and 30 ^oC in shaker incubator 150 rpm. To evaluation the most appropriate organic and inorganic nitrogen sources to production of biosurfactant, NH_4Cl, $NaNO_3$, $(NH4)_2SO_4$, urea, yeast extract and peptone were employed at a concentration of 0.2 %. For best concentration of nitrogen source $(NH_4)_2SO_4$ different concentration (0.2, 0.4, 0.6, 0.8 and 1) % was employed. All the experiments above amended with 1 % (w/v) of weathered diesel oil as source of carbon and energy. For appropriate concentration of carbon source for biosurfactant production, different concentration of weathered diesel oil (1, 2, 3, 4, 5, 6, 7) % (w/v) were used, at

optimized condition; pH 8, incubated at 30 ^{o}C, and 150 rpm for 96 h.

Preparation of carbon source

Weathered diesel oil was used as the sole source of carbon and energy. The source of diesel oil is (Al-Dora refinery-Iraq). The refinery produces three type of mineral oil (mineral oil 40, 60, and 150). Mineral oil 60 and 150 are used mainly as diesel oil. The spilled diesel oil (weathered oil), was taken from one of the oil replacement station in Baghdad and used in the present study. Weathered diesel oil used as suspension in the culture medium, and prepared according to the method described by (11), by mixing weathered oil with silica (0.5 mm diameter) until saturation at room temperature. Mixture dried at room temperature to become fine powder and added to the medium before autoclaving. The importance of this procedure is to increase the surface area for microbial attack to the substrate.

Biomass measurement

The dry weight technique was used to quantify microbial growth as bacterial dry weigh. Biomass obtained after centrifugation at (10.000 g, 15 min), then the precipitate cells transferred to weighted container and dried overnight at 105˚C and reweighed.

Recovery of Crude Extract

A crude biosurfactant was extracted from the cell-free culture supernatant of *Serratia marcescens* N3 grown on weathered diesel oil, using the technique described in (5); briefly, at the end of the cultivation in weathered diesel oil, and after the hydrophobic layer located at the surface has been removed, the culture medium was centrifuged at 10. 000 *g* at 4 °C. The supernatant was extracted by means of the organic solvent system chloroform: methanol (2:1, v/v); the extract was concentrated and dried at room temperature and weighted: resulting crude extract.

Surface active properties

Surface tension measurement

The surface tension measurement of cell free supernatant was determined according to the method described by (10) in a K6 tensiometer (Krüss GmbH, Hamburg, Germany), using the du Nouy ring method. The values reported are the mean of two measurements. All measurements were made on cell-free broth obtained by centrifuging the cultures at 10,000 *g* for 15 min.

Emulsification index (E24)

E24 of culture samples was determined by adding 2 mL of a hydrocarbon (kerosene) to the same amount of culture, mixing with a vortex for 2 min, and leaving to stand for 24 h. The E24 index is given as percentage of height of emulsified layer (mm) divided by the height of the hydrocarbon phase (mm) and multiplying to 100 (12).

Emulsification activity

Emulsification activity of the culture sample was determined according to the method described by (13).

Bacterial adhesion to hydrocarbon percent (BATH %)

Bacterial adhesion to hydrocarbon was carried out, using the method followed by (11). Bacteria were harvested from growth cultures by centrifugation (10.00 g, 15 min) at 4 °C. Washed and suspended in 3 ml of sterilized distilled water. Then 0.5 ml of different hydrocarbons were added to cell supernatant, and vortexes in the test tube for 2 min. the phases were allowed to separate during 20 min, and the optical density of the lower aqueous phase was measured at 600 nm. The percentage of cells bound to hydrocarbons (% HYD) was calculated according to the equation: $\% HYD = (1 - A/A_{\circ}) \times 100\ \%$, were $A_{\circ}$ is the absorbance of bacterial suspension without hydrocarbons added and A is the absorbance after mixing with hydrocarbons. All the experiments were done in duplicate.

Results and Discussion

Seven isolates of *Serratia marcescens* obtained from (Department of Biology and Biotechnology / University of Baghdad) were tested for biodegradation and biosurfactant production. The results in Table (1) indicated that the isolate *Serratia marcescens* N3 was the best biosurfactant producing among the other isolates based on the capability of isolates to degrade weathered diesel oil as the sole source of carbon and energy, and biosurfactnt production with emulsification activity.

Table (1)
Screening of Serratia marcescens isolate for biomass and biosurfactant production.

Isolates	Biomass (g/l)	Biosurfactantconcentration (g/l)	Emulsification activity (O.D. at 540 nm)
S. marcescens N1	1.3	2.5	0.32
S. marcescens N2	3	4.5	0.38
S. marcescens N3	3.66	5.5	0.55
S. marcescens N4	2.33	1.6	0.48
S. marcescens N5	1.80	2.5	0.40
S. marcescens N6	1.66	2	0.48
S. marcescens N7	2.7	3	0.36

The isolate *S. marcescens* N3 was selected for the present study according to their higher growth and biosurfactant production.

Effect of cultural and environmental conditions on growth and biosurfactant production:

The effects of pH, temperature, incubation period, weathered diesel oil concentration and nitrogen source on biomass yield and biosurfactant production were investigated. The results in Fig.(1) showed that the maximum biomass yield obtained at pH 7, while biosurfactant production increased with increase in pH. At pH 8 maximum surfactant production was obtained and lowering the surface tension value of the culture to minimum value. Any change in pH to lower or higher level caused drop in the biosurfactant production. At the optimum level of pH 8, the maximum biomass and biosurfactant production obtained with lowering of surface tension of the culture to minimum value at temperature 30 ^{o}C Fig.(2). Any increase of temperature resulted decrease in the growth and biosurfactant production and increase surface tension value. This result indicates that the isolate favored the mesophilic condition.

Growth and biosurfactant production increased with incubation period and reached maximum at 72 h, for biomass production, while maximum biosurfactant production obtained at 96 h of incubation which causes to lowering surface tension of the culture to minimum value, beyond which biomass and biosurfactant production were decreased and surface tension value were increased Fig.(3).

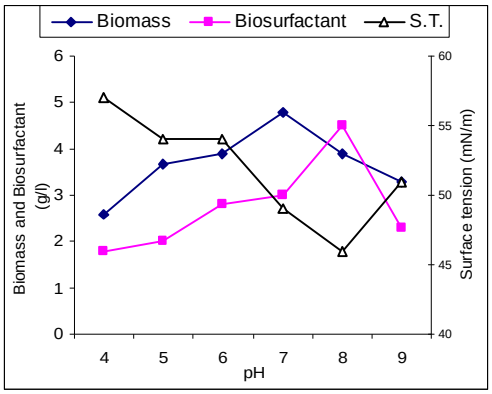

Fig. (1): Effect of pH on growth rate and biosurfactant production of Serratia marcescens N3 at 30 °C in shaker incubator at 150 rpm for 72 h.

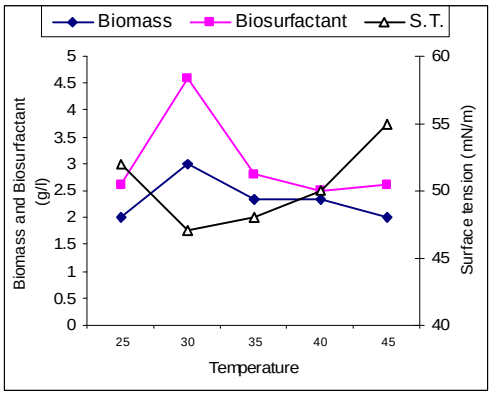

Fig.(2): Effect of temperature on growth rate and biosurfactant production of Serratia marcescens N3 at 30 °C, pH 8 in shaker incubator at 150 rpm for 72 h.

Nitrogen limitation has been shown to play an important role in surfactant production. The nature of nitrogen source also affects biosurfactant production (9). In present study among different organic and inorganic nitrogen sources tested, the microorganisms were found to prefer ammonium sulfate $(NH_4)_2$ SO_4 as source of nitrogen, resulted higher growth rate and biosurfactant production Fig.(4). For the best concentration of nitrogen source, the results in Fig.(5) cleared that maximum growth rate achieved using $(NH_4)_2SO_4$ at concentration of 0.4%, while maximum biosurfactant production obtained at concentration 0.2% of $(NH_4)_2SO_4$.

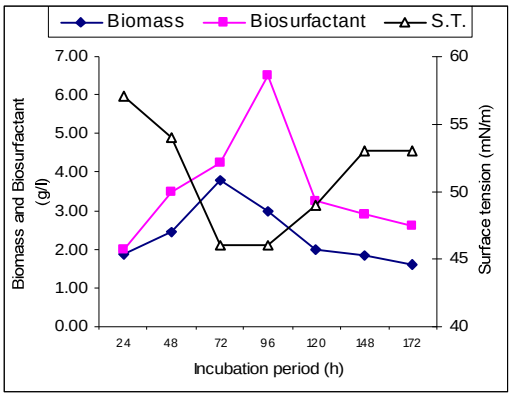

Fig. (3): Effect of incubation period on growth rate and biosurfactant production of Serratia marcescens N3 at 30 ℃, pH 8 in shaker incubator at 150 rpm for 7 days.

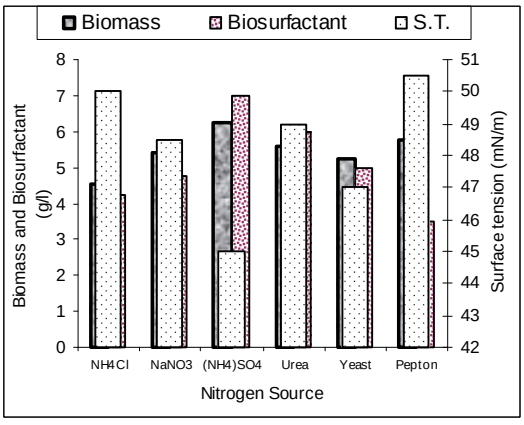

Fig.(4): Effect of nitrogen source on growth rate and biosurfactant production of Serratia marcescens N3 at 30 ℃, pH 8 in shaker incubator at 150 rpm for 4 days.

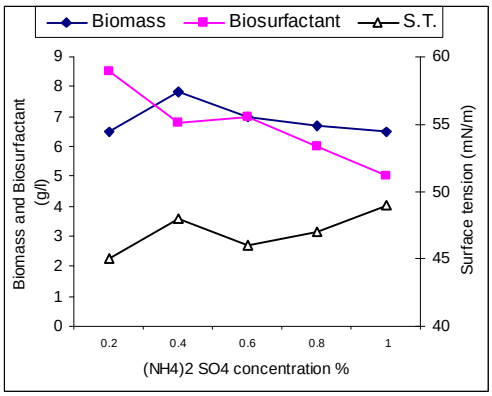

Fig.(5): Effect of nitrogen source concentration on growth rate and biosurfactant production of Serratia marcescens N3 at 30 ℃, pH 8 in shaker incubator at 150 rpm for 4 days.

The results observed in the present study agreement with that obtained by (9), where nitrogen source $(NH_4)_2SO_4$ at concentration of 0.5g/l produced higher glycolipid biosurfactant production by isolate *Serratia marcescens* among different nitrogen sources used. They also found that at higher $(NH_4)_2SO_4$ level, the growth increased while surfactant production did not show significant changes. Also (14) mentioned that higher growth and biosurfactant production in continuous culture by *Pseudomonas aeruginosa* achieved using $(NH_4)_2SO_4$ as nitrogen source. They also mentioned that the addition of $(NH_4)_2SO_4$ accelerated the biodegradation rate of petroleum hydrocarbons.

When applying the optimum level of pH, temperature, incubation period (96h) and nitrogen source $(NH_4)_2SO_4$ (0.2 %) maximum yield of biomass (8.6 g/l) was obtained at a substrate (weathered diesel oil) concentration of 5% (w/v), while substrate at 6% (w/v) yielded maximum surfactant production 10.5 g/l Fig.(6). The surfactant production increased with increase in substrate concentration. The results obtained indicated that the surfactant production is a linear function of growth and substrate concentration.

Types of carbon sources play a major role in biosurfactant production. Different carbon sources such as molasses, corn steep liquor, way wastes, n-alkanes. Agricultural and food

industry wastes rice, starch waste liquors, whey, and domestic waste (9 and 10).

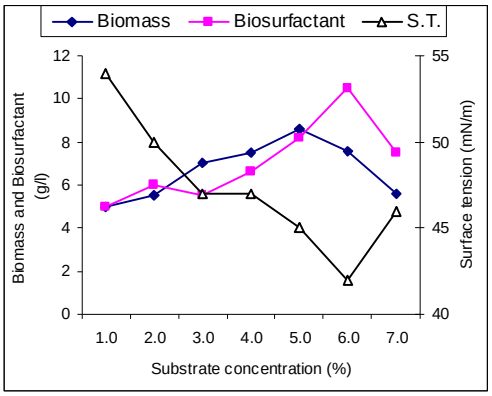

Fig.(6): Effect of Weathered oil concentration on growth rate and biosurfactant production of Serratia marcescens N3 at 30 ºC, pH 8 in shaker incubator at 150 rpm for 4 days.

Municipal sludge waste has also been proposed to be utilized for biosurfactant production. The type of carbon source used has been shown to play a key role in the type of biosurfactant produced. When n- alkane was used as a carbon source, a raminolipid was produced by *Pseudomonas sp.* (Kosaric, 1984). In a similar investigation to the results observed in the present study the strain *Pseudomonas aeruginosa* LBI was capable to producing the raminolipid biosurfactant using diesel oil, crude oil, oily sludge, kerosene and glycerol (7). Also in the study (9) they mentioned the capability of the isolate *Serratia marcescens* DT to produce glycolipid biosurfactant containing arabinose sugar, in which maximum biosurfactant (20 g/l) produced at the optimum condition (pH 8, temperature 30 ^{o}C, incubation period 8 days and glycerol concentration 5% v/v).

The optimum conditions, which were obtained for biomass production (biodegradation rate) was, pH 7, temperature 30 $^{\circ}$C, incubation period 72 h, nitrogen source $(NH_4)_2SO_4$ (0.4%) and weathered diesel oil concentration (5% w/v). While pH 8, temperature 30 ^{o}C, incubation period 96 h, $(NH_4)_2SO_4$ (0.2%) and weathered diesel oil concentration (6% w/v) for biosurfactant production.

The results showed that the optimum condition for biomass production did not yield highest biosurfactant production. Similarly, optimize conditions required for higher biosurfactant production did not yield highest biomass. The results in present study agree with that found by (9), which mentioned that the optimized condition for biodegradation of glycerol by *Serratia marcescenes* differ from optimized condition for glycolipid production.

Surface active properties of produced bioemusifier:

In this part of the work, we describe the use of weathered diesel oil by *Serratia marcescens* as sole carbon and energy source for producing surface active agents. This production was detected by: emulsion index (E24%), emulsification activity, surface tension lowering and cell hydrophobicity percent when the microorganisms was cultivated in mineral salt medium amended with weathered diesel oil at concentration of 6% (w/v).

Fig.(7) shows the emulsion index and emulsification activity of the culture medium compared to the bacterial growth measured through dry weight of the culture medium. The E24% increase from 8% after 3 days of incubation to 46% at the end of the active growth phase and emulsification activity from 0.2 to 0.6. It means that the bacterial exponential growth phase is related by the production of an emulsifying substance, which indicates that the microorganisms assimilated the weathered diesel oil. The process can have acted according to two ways (5): either making cellular surface more hydrophobic or the biosurfactant enhanced the aqueous solubilization and dispersion of the weathered diesel oil. During the stationary phase, the E24% increases from 46% to 58% and emulsification activity from 0.6 to 0.9. The results obtained in present study was in agreement with that found by (5), in which E24% increases from 4% after 3 day to 54% and reached 63% at the stationary phase, when they studied the capability of *Rhodococcus* strain to utilize sun flower frying oil at the concentration of 3% (v/v) to produce bioemulsifier.

The results in the Fig.(7) also cleared the surface tension of the bacterial culture compared with the dry weight of the bacterial growth. The surface tension of the culture medium decreases from 60 to 48 mN/m during the exponential growth, and reaches the value 43 mN/m at the stationary phase, this indicated to the presence of a surface active substances in the culture medium. From the results mentioned we can conclude that the increase of E24% and lowering the surface tension of the culture medium during exponential and stationary phase indicated that the produced biosurfactant is of extra-cellular nature (15).

More water – soluble hydrocarbons, (2) direct contact of cells with large hydrocarbon drops – in this mechanisms microbial cells attach to the surface of hydrocarbon drops that are much larger than cells; the availability of substrate surface area for cell attachment is a limiting factor.

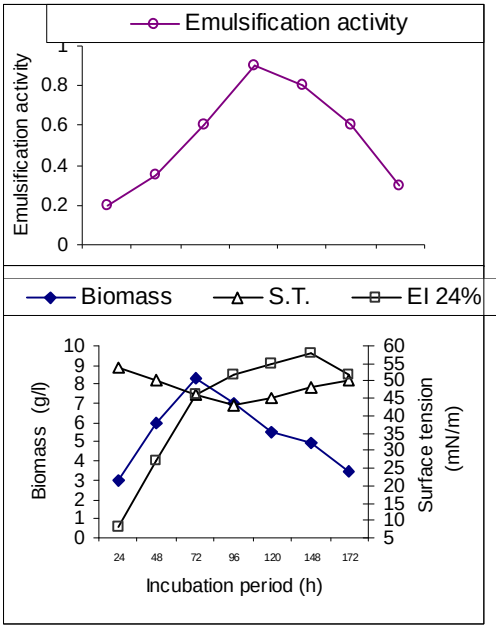

Fig.(7): The effect of surface active agents on growth rate of Serratia marcescens in weathered diesel oil at the concentration 6% at 30 ℃, pH 8 in shaker incubator at 150 rpm for 7 days.

The changes in bacterial cell surface hydrophobicity (BAH) as measured by analyzing cell affinity towards aliphatic, aromatic and mixed hydrocarbons are showed in Table (2). The differences in the affinities of *Serratia marcescens* for hydrocarbons were demonstrated. The strain showed higher affinity towards aliphatic and alicyclic hydrocarbons (hexane, heptane, dodecane and cyclohexane) ranged from 81% to 68%. Then the values of cell affinity decreased slightly with mixed hydrocarbons (kerosene and diesel oil-40) ranged from 65% to 75%. Lower affinity absorbed towards aromatic hydrocarbons (benzene, toluene and xylene) and ranged from 63% to 50%. The increase of cell hydrophobicity contributes to the increase of the cell interaction with hydrocarbons, and ultimately increases of biodegradation rate. Biosurfactant modulate the hydrophobicity of the cell surface, which appears to be an important factor for cell adhesion (16). In similar study found by (12), they mentioned three models of hydrocarbon transport to microbial cells and are generally: (1) interaction of cells with for microbial growth; biosurfactant /bioemulsifier produced by hydrocarbon utilizing bacteria cause the dispersion of hydrocarbon droplets in the aqueous medium and thereby increase the surface area; addition of biosurfactant to the hydrocarbon medium stimulate growth of microorganisms, and (3) microbial cells interact with particles of solubilized, micro-emulsified hydrocarbons.

Our absorbed results in the present study was agreement with the results obtained in the study of (12), which mentioned that the strain *Alcaligenes picchaudii* have a surface hydrophobicity in the following order: higher affinity towards aliphatic hydrocarbons ranged from 87% to 67%, mono aromatic and poly aromatic hydrocarbons ranged (68 to 54%) and (46 to 15%) respectively.

We can conclude from the results obtained in the present study that the isolate *Serratia marcescens* N3 is able to grow in the presence of diesel oil and can be used to reduce wastes generated by using diesel oil in different applications. Also to convert weathered oil (a cheap renewable material) in to higher value products biosurfactant. So that crude preparation of produced biosurfactant could be applied for: enhanced oil recovery, cleaning oil storage tanks, in a variety of biotechnological applications including bioremediation of hydrocarbons contaminated sites.

Table (2)
Hydrophobicity (%) of Serratia marcescens N3 towards different hydrocarbons.

Hydrocarbons	% cell transferred to hydrocarbon phase (mean value)
Hexane	81
Heptane	82
Dodecane	68
Cyclohexane	77
Benzene	63
Toluene	58
Xylene	50
Kerosene	65
Diesel oil-40	75

References

[1] Suwansuko,P.; Rukachisirrkul, V.; Kawai, F. and H-Kittikum, A. 2007. Production and application of biosurfactant from *Bacillus subtilis* MUV4. J. sci. technol. 30(1): 87-93.

[2] Gautam, K.K. and Tyagi, V.K. 2006. Microbial surfactants: A review. J. oleo. Sci. 55(4): 155-166.

[3] Calvo,C. ; Toledo, F. L. ; Pozo, C. ; Martinez - Toledo, M. V, and Gonzalez - Lopoz, J. 2004. Biotechnology of bioemulsifiers produced by microorganisms. J of food, Agriculture and Environ. 2 (3 & 4): 238 – 243.

[4] Hearly, M. G., Devine, C. M. and Murphy, R. 1996. Microbial production of biosurfactant. Resource Conserve. Recycling. 18: 41 – 57.

[5] Sadouk, Z.; Hacene, H. and Tazerouti, A. 2008. Biosurfactant production from low cost substrate and degradation of diesel oil by a *Rhodococcus* strain. Oil and Gas science and Technology. Institute Frances petrol: 1-7.

[6] Makkar R. S.; Cameotra, S. S. 2002An update on the use of unconventional substrates for biosurfactant production and their new application. Appl. Microbiol. Biot. 58:428 – 434.

[7] Mariano, A.P.; Binotto, D. M.; Angelis, D. F.; Pirollo, M.P. and Contiero, J. 2008. Use of weathered diesel oil as a low – cost raw material for biosurfactant production. Brazilian J. of Chemo. Engin. 25 (2): 269 – 274.

[8] Bicca, F.C.; Fleck, L. C. and Ayub, M. A. 1999. Production of biosurfactant by hydrocarbon degrading *Rhodococcus rubber* and *Phodococcus erythropolis*. Revista de Microbiology. 30: 231 – 236.

[9] Bidlan, R.; Deepthi, N.; Rastogi, N.K. and Manonmani, H.K. 2007. Optimized production of biosurfactant by *Serratia marcescens* DT – IP. Res. J. of Microbiol. 2(10): 705 – 716.

[10] Abouseoud , M; Maachi, R,; Amrane, A. ; Boudergua, S. and Nabi, A. 2008. Evaluation of different carbon and nitrogen sources in production of biosurfactant by *Pseudomonase flourescens*. Desalination 223: 143 – 151.

[11] Resenberg, E. and Gutnick, D. L.1981. The hydrocarbon – oxidaizing bacteria. In: the prokaryotes, V (1): 903 – 912. starr, M. P. stolp, H., Truper,H.G. Balosus , A. and Schlegel, H. G.(eds).Springer – velage. Co., Berlin, Heidelberg – New York.

[12] Plaza, G. A.; Ulfig, K. and Brigmon, R.L. 2005. Surface active properties of bacterial strains isolated from petroleum hydrocarbon bioremediated soil. Polish, J. of Microbiol. 54(2): 161 – 167.

[13] Patel, R.N.; Desai, A.N. and Desai, A. J. 1997. Surface active properties of reminolipid from *Pseudomonase aeruginosa* G S3. J Basic Microbial. 32:518 – 520.

[14] Bauer. E. Pennersofer, C. ; Renner, S.; Slavica, B. and Braun, R. 1994. Factors influencing biological Soil decontamination. Proceeding of the European Congress on Biotechnology. 1223 – 1226.

[15] Banat, I. M.; Makkar, R. S. and Cameotra, S. S. 2000. Potential commercial applications of microbial surfactants. Appl. Microbiol. Biot. 53 : 495 – 508.

[16] Prabhu, Y. and phale, P.S.2003 Biodegradation of phenanthrene by *Pseudomonas sp*. Strains PP zinovel metabolic pathway role of biosurfactant and cell surface hydrophobicity in hydrocarbon assimilation. Appl. Microbiol. Biotechnol. 61:343 – 351.

التقنيات الحيوية المختلفة والتي تتضمن المعالجة الحيوية للهيدروكاربونات في موقع التلوث.

الخلاصة

تمت في هذه الدراسة اختبار قدرة بكتريا *Serratia marcescens* في انتاج المستحلبات الحياتية من مخلفات زيت المحركات المحروق في مزرعة الدفعة الواحدة. تم تحليل الظروف البيئية والزرعية المختلفة للوصول الى الظروف المثلى لمعدل النمو وانتاج المستحلب الحياتي. بينت النتائج بان الظروف المثلى للنمو كانت عند الرقم الهيدروجيني مساو الى 7، فترة الحضانة 72 ساعة، تركيز المصدر النيتروجيني $(NH_4)_2SO_4$ % 0.4 وتركيز الزيت المحروق % 5 (وزن/حجم)، حيث تم الحصول على كتلة حيوية بلغت 8.6 (غم/لتر). اما الظروف المثلى لانتاج المستحلب الحياتي فكانت عند الرقم الهيدروجيني 8، فترة الحضانة 96 ساعة، وتركيز المصدر النيتروجيني $(NH_4)_2SO_4$ % 0.2 وتركيز الزيت المحروق % 6 (وزن/حجم)، اذ تم الحصول على المستحلب الحياتي بتركيز 10.5 (غم/لتر). درجة الحرارة °C 30 كانت هي المثلى للنمو وانتاج المستحلب الحياتي. كما درست خصائص السطوح الفعالة للعزلة وذلك بحضنها مع زيت المحركات المحروق وبالتركيز الامثل % 6 (وزن/حجم) ولفترة 172 ساعة، وجدت قدرة العزلة على تصنيع مركبات ذات الافراز خارج الخلوي (Extra-cellular compounds)، مما ادت الى زيادة محتوى الاستحلاب (Emulsion index) للوسط الزرعي الى % 58 وفعالية الاستحلاب الى 0.9. كما ان وجود هذه المواد في الوسط الزرعي ادت الى خفض قيمة الشد السطحي للوسط الى 43 ملي نيوتن/ متر. تم تحديد كراهية سطوح الخلايا المايكروبية للماء (Hydrophobicity)، والتي قيست من خلال الفة الخلايا تجاه الهيدروكاربونات المختلفة. وجدت بات العزلة لها سطوح كارهة للماء والفة للهيدروكاربونات ووفق الترتيب الاتي: الهيدروكاربونات الاليفاتية، الخليطة ثم الهيدروكاربونات الاروماتية احادية الحلقة. من خلال النتاج تم الاستنتاج حول امكانية استخدام المركبات الفعالة سطحيا (المستحلبات الحياتية) المنتجة من قبل بكتري *Serratia marcescens* بصورة عامة، في شركات النفط في زيادة استخراج النفط، استخدامها في