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Microbial degradation of sodium lauryl sulfate (SLS) using bacterial consortium isolated from coastline of Alexandria city

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Abstract:
Sodium lauryl sulfate (SLS) is an anionic surfactant that may easily find its way to seawater through domestic and industrial wastewater discharge. The current study was suggested to investigate its fate in relation to the ability of heterotrophic bacteria isolated from samples to degrade SLS surfactant. In addition, the different factors affecting the biodegradation process. Out of nine dominant marine bacteria, only three were able to degrade SLS successfully. They were characterized throughout morphological, biochemical and physiological features as; *Enterobacter gergoviae*, *Enterobacter cloacae* and *Bacillus alvei.* Our data confirmed that the biodegradability of consortium consisting of *E. gergoviae*, *E. cloacae* and *B. alvei* combination was more effective than that of the individuals. The increase in inoculum size, support the increase in the biodegradation. The 30°C as an incubation temperature was the most effective temperature (98% biodegradation). The pH 9 was the optimum for the growth of consortium *(E. gergoviae*, *E. cloacae* and *B. alvei)* and consequently for biodegradation rate (96%). The pH level 7 was more or less near to optimum with biodegradation percentage (89.2%). Glucose as a carbon source and casein as a nitrogen source improved the biodegradation process to 91.9% and 90.3% respectively. The biodegradation percentages showed an inverse relationship between the increase in SLS mass and the extent of its degradation. Under these growth conditions a complete degradation of 1000 ppm SLS biomass was achieved with biodegradation percentage (45.5%). Such findings contribute to a better understanding the fate of the SLS in the aquatic environment.

Keywords: Biodegradation**,** sodium lauryl sulfate (SLS), bacterial consortium

INTRODUCTION

In recent years, surfactant-mediated bioremediation is a research focus.[(1)] Biodegradation of surfactants has been the subject of substantial research works since 1950s, when synthetic detergents came into wide spread use.[(2,3,4)] With the increasing environmental awareness, readily biodegradable surfactants are preferred *in situ* not *in vivo* of remediation applications in terms of environmental biocompatibility[(1,5,6)]. The biodegradation of Alkyl Phenol Ethoxylate (APE) by bacteria in seawater polluted with

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urban sewage is brought about by bacteria of the marine *Pseudomonas* genus. Few other species of Gram-negative bacteria are able to degrade APE with nine to ten ethoxy groups. *Pseudomonas* strains degrade only down to four or five ethoxy groups.[7] It is also likely that surfactant addition will over time alter the microbial population with overall positive or negative consequences on rates and extents of biodegradation.[8]

Biodegradation is most often performed by soil or aquatic microorganisms and leads to generation of water and carbon dioxide gas.[9] Aliphatic alkyl sulfate compounds commonly represented by Sodium Lauryl Sulfate (SLS) are anionic surfactants that may easily find their way to the environment through domestic and industrial wastewater.[10] They are characterized by their linear long chain sulfate ester structure which can be degraded by several bacterial strains through desulfation and further assimilation.[11,12] Some strains of *Acinetobacter* are known to be involved in the biodegradation of different pollutants such as biphenyl or chlorobiphenylaniline, phenol, benzoate, crude oil and acetonitrile.[13] *Acinetobacter calcoaceticus*, a facultative anaerobic strain that was able to degrade more of the alkanes than aromatic fractions of crude oil. It grew well on *n*-alkane up to C_{33} as well as, on the branched chain alkane but failed to grow on cycloalkanes oil. [14] It was investigated that Sodium Lauryl Sulphate (SLS) could be used for stimulation of biodegradation of n-alkanes without residual contamination by the surfactant.[15] The presence of SLS in the culture medium with n-decane as main source of carbon and energy accelerated the growth of *Pseudomonas* C12B. SLS disappeared from the culture medium in early stages of cultivation suggesting preferential degradation by the bacterium, while the consumption of n-decane was accelerated.

Recent studies discussed association of the capacity of SLS to induce decane-mineralization system in *Pseudomonas* C12B or with the ability of SLS to stimulate the

surface attachment of competent bacteria resulting in close proximity of the cells with alkane droplets, and thus, enhanced breakdown of the hydrocarbon pollutant. Two different bacterial consortia were isolated by Khleifat[16] from wastewater treatment plant to be used in the biodegradation of Sodium Lauryl Ether Sulfate (SLES). The two consortia consisted of three members. *Acinetobacter calcoacetiacus* and *Klebsiella oxytoca* in one co-culture (A-K) and *Serratia odorifera* in the second co-culture (S-A), which contains *Acinetobacter calcoacetiacus* as well.

The current study investigated the ability of heterotrophic bacteria isolated from the Alexandria coastline seawaters samples to degrade SLS surfactant. In addition, the different effective factors affecting the biodegradation process were estimated.

2. Materials and Methods:

2.1. Chemicals

Sodium lauryl sulfate surfactant was purchased from Sigma chemicals, USA. The chemicals used for biochemical tests were of pure grade and purchased from Sigma chemicals, USA.

2.2. Media

Ingredients of media were all of analytical grade and were obtained from recognized chemical suppliers. The medium for biodegradation activity; a minimal medium (M9).[17] It was used to test the consumption of SLS surfactant as sole carbon source. The composition of this medium involved 3.0 Na_2HPO_4, 1.5 KH_2PO_4, 1.0 NH_4Cl and NaCl that were dissolved in 500 ml of de-ionized water. The medium pH was adjusted to 7.4 before the addition of 0.24 $MgSO_4$, 0.05 $CaCl_2.6H_2O$ and 0.05 $FeCl_3.6H_2O$. After autoclaving, 0.001thiamine-HCl was dissolved in 2 ml- de-ionized water and sterilized by Millipore filter then supplemented to the minimal medium.

2.3. Isolation and purification of bacterial isolates

Seawater samples were collected in 500 ml sterile screw-caped bottles as described by (Austin, 1999).[18] Serial dilutions from 10^{-2}

through 10^{-6} were made using filtered sterilized sea water. A portion (0.1 ml) from each appropriately diluted sample was used to inoculate plates prepared with sea water agar for counting aerobic heterotrophs. Plates were incubated at 30°C for 24 h. Purification of bacterial colonies was carried out by streaking on agar plates of the same medium. The pure colonies obtained were transferred to fresh slants. Sub-cultures were kept under refrigeration for further investigations. Aerobic heterotrophic bacterial isolates were maintained on nutrient agar slants. Colony morphology, cell morphology and Gram staining were performed for the bacterial isolates which exhibited biodegradability towards SLS. The identification of bacteria was achieved according to (Cowan and Steel, and Bergey *et al*.) [19, 20] and API 20E strip system.

2.4. Analysis of Sodium Lauryl Sulfate

Samples of 50 ml were placed in a separating funnel; then, 5 ml of 10^{-3} mol/l methylene blue (MB) solution and 5 ml of chloroform were added, and the mixture was shaken for 1 minute. The separating funnel was allowed to stand one minute and the separated chloroform phase was filtered; the absorbance of the chloroform solution was then measured at 654 nm using UV-Visible double beam Shimadzu spectrophotometer with glass cell length of a 10 mm (1 cm). Each analysis was repeated at least three times.

2.5. Biodegradation process of sodium lauryl sulfate

The culture medium broth was inoculated with single colonies that were previously isolated by an overnight growth of two consecutive shake flask cultures in nutrient broth followed by 3h recovery. Cells were harvested by centrifugation and resuspended in nutrient broth medium (NB) at a final concentration of 0.5 A_{600}. To inoculate culture medium, 1 ml of the harvested cells was suspended in 100 ml of the required medium using 250 ml capacity Erlenmeyer flasks. For preparing mixed culture, an inoculums was made from 50:50 co-cultures of the two

different bacterial isolates [21].

2.6. Optimization of biodegradation process of sodium lauryl sulfate

The optimization experiments were carried out according to (Abbouda *et al.*, 2007) [21] with some modifications appropriating our work. However, seven factors affecting this process were investigated separately as follows.

2.6.1. Effect of biodegradability of the individuals and consortium:

To investigate the effect of the mixed bacterial culture as consortium comparing to the biodegradability of the individuals, the minimal M9 medium with SLS at a concentration of 100 ppm as sole carbon source was utilized. Flasks were inoculated with individual bacterial isolates and also with treatments of; (*E. gergoviae* & *E. cloacae*), (*E. gergoviae* & *B. alvei*), (*E. cloacae* & *B. alvei*) and (*E. gergoviae* & *E. cloacae* & *B. alvei*). All flasks were incubated at 30° C for 15 days with shaking twice daily.

Effect of the inoculum size:

To examine the effect of the inoculum size, the minimal M9 medium with SLS at a concentration of 100 ppm as sole carbon source was utilized. Flasks were inoculated with consortium (*E. gergoviae* & *E. cloacae* & *B. alvei*) at 0.5, 1.0, 1.5 and 2.0 % of 100 ml M9 medium. All were incubated at 30° C.

2.6.3. Effect of the incubation temperature:

To examine the effect of the incubation temperature, the minimal M9 medium with SLS at a concentration of 100 ppm as sole carbon source was utilized. Flasks were inoculated with consortium (*E. gergoviae* & *E. cloacae* & *B. alvei*), then incubated at 30, 35 and 45° C, respectively.

2.6.4. Effect of the pH level:

To test the effect of pH level, the minimal M9 medium with SLS at a concentration of 100 ppm as sole carbon source was utilized. The pH of flasks was adjusted at 5, 7 and 9, respectively, then all treatments were inoculated with consortium (*E. gergoviae* & *E. cloacae* & *B. alvei*)**.**

2.6.5. Effect of different carbon sources:

To investigate the effects of different carbon sources, glucose, sucrose and molass were supplemented individually for each nutrient at final concentration of 0.2% as an additive to the minimal M9 medium. SLS at a concentration of 100 ppm were utilized in this minimal medium. All treatments were inoculated with consortium (*E. gergoviae* & *E. cloacae* & *B. alvei*).

2.6.6. Effect of different nitrogen sources:

To investigate the effects of different nitrogen sources, yeast extracts, casein, ammonium chloride and tryptone were supplemented individually for each nutrient at final concentration of 0.2% as an additive to the minimal M9 medium. SLS at a concentration of 100 ppm were utilized in this minimal medium. All treatments were inoculated with consortium (*E. gergoviae* & *E. cloacae* & *B. alvei*).

2.7. Effect of surfactant concentrations on the biodegradation rate:

To examine the effect of surfactant concentrations on the biodegradation rate, five different concentrations of SLS (200, 400, 600, 800 and 1000 ppm) were applied. The effects of surfactant biomass either on the growth of a mixed consortium in Nutrient Broth medium (NB medium) were determined by absorbance and then dry weight [(22)].

3. Results and discussion:

Several investigations were carried out to determine the degradation of surfactants by bacteria isolated from marine, other aquatic resources, and even wastewater. The biodegradability was observed under aerobic and anaerobic conditions.

3.1. Physico-chemical analysis and SLS concentrations of seawater samples

Physico-chemical analysis and SLS concentrations of the collected seawater samples from different sampling positions along the coastline of Alexandria city during 2009 were estimated (Table 1). Seawater samples were analyzed seasonally for; temperature, pH, dissolved oxygen (DO) and salinity. The data indicated that temperature

readings for seawater samples ranged from 18.5^{o}C in Abu Qir (winter) to 26.7^{o}C in Al Max Bay (summer). The pH values showed in all sites tendency toward alkalinity. Water samples exhibited pH range from 7.8 in Al-Dekhaila (spring) to 8.2 in most station in different season. Salinity value of seawater samples ranged from 37.9% in Eastern Harbour (spring) to 39.8% in Al Dekhaila Harbour (winter). The lowest dissolved oxygen in sea water was recorded in Western Harbour (spring) (6.1 mgO_2/l) followed by that of Abou Qir (spring and winter), Western Harbour (winter) and Al Dekhaila Harbour (spring) (6.2 mgO_2/l). The highest dissolved oxygen was recorded in Al Max Bay (7.6 mgO_2/l).

The observed physical characteristics of seawater in the sites under investigation are similar to those obtained in previous studies carried out in the same areas.[23, 24] It is well known that marine microbial communities are influenced by environmental variables such as temperature, nutrient availability, water salinity and others. [25- 28] So, these factors affect the distribution of marine bacteria. They were detected in our study in the limits allowing the excellent bacterial growth. As well as, the average values of the SLS surfactant concentrations were calculated for all stations, Table (1). The data confirmed that the variations in the SLS concentrations were not highly variable either in the same season or between the different seasons for sampling sites. Moreover, the SLS concentrations ranged from 2.1 to 4.5 ppm for Eastern Harbour in spring and Al Dekhaila Harbour in autumn, respectively. Dentel *et al*. [29]estimated a discharge of over 100 kg/day of anionic surfactants and approximately 300 kg/day of cationic surfactants. In addition, Dentel *et al*. [29] mentioned that although there are wide ranges in some of the values, e.g. values quoted for the load of LAS in treatment plants range from 3 to 21 ppm, it is clear that significant amounts of surfactant are transported into the environment from treatment plants. It

should also be noted that the presence of surfactants in water at concentrations below and above the critical micelle concentration can also lead to the solubilisation of other oil-soluble pollutants such as DDT and trichlorobenzene [30].

Table 1: Physico-Chemical analysis and SLS concentrations of the seawater samples collected from different sampling points along the coastline of Alexandria city during 2009

SLS (ppm)	Salinity (%)	D.O. (ppm)	pH	Temp. (^{O}C)	Season	Stations
3.1	39.2	6.2	8.1	20.1	Sp.	
2.6	39.6	7.3	8.2	26.1	Su.	**Abu Qir**
2.8	39.2	6.8	8.2	22.7	Au.	**Bay**
2.9	39.0	6.2	8.1	18.5	Wn.	
2.1	37.9	7.4	8.2	18.8	Sp.	
2.9	39.2	7.5	8.2	26.4	Su.	**Eastern**
3.0	38.8	6.8	8.2	22.2	Au.	**Harbour**
2.6	38.7	6.5	8.2	19.1	Wn.	
3.2	39.2	6.1	8.1	19.3	Sp.	
3.2	39.4	7.3	8.2	25.7	Su.	**Western**
3.0	39.2	7.0	8.2	22.8	Au.	**Harbour**
2.9	39.0	6.2	8.1	18.7	Wn.	
2.7	38.7	7.1	7.9	20.2	Sp.	
2.7	38.9	7.6	8.0	26.1	Su.	**Al Max**
3.4	38.6	7.1	8.2	22.6	Au.	**Bay**
3.0	38.2	6.4	8.0	18.8	Wn.	
3.1	39.1	6.2	7.8	20.2	Sp.	
3.0	39.4	7.1	8.2	26.1	Su.	**Al Dekhaila**
4.5	38.9	6.8	8.1	22.6	Au.	**Harbour**
2.3	39.8	6.3	8.1	18.8	Wn.	

3.2. Counting the bacteria capable of degrading SLS

The data shown in Table 2 reveal that the count of aerobic heterotrophs recorded on seawater agar varied from a highest count (16 x 10^4 $\pm$ 0.005 CFU/ml) in Abu Qir Bay to a lowest count (2.5x $10^4\pm$ 0.005 CFU/ml) in Western Harbour. The physico-chemical parameters, previously detected, had no considerable effect on the total counts of marine bacteria. The counts obtained in this study for aerobic heterotrophs are similar to

those obtained by Cavello *et al.* [31] in Lonian Sea in Italy. The variation of count observed in different sites represents the responses of heterotrophic bacteria to environmental changes. Bacterial abundance is now known to vary at the millimeter scale. [32] In addition, the bacterial community structure can change dramatically within weeks and may form blooms of bacteria.[33] The factors controlling the distribution of bacteria in marine environments are complex and vastly different from those experienced by terrestrial bacteria.[18] Several studies have concluded that, the marine microbial communities are influenced by environmental variables such as temperature, nutrient availability, salinity, length of day, algal bloom and hydrostatic pressure.[25-28] In order to obtain the highest recovery of aerobic heterotrophic marine bacteria, sea water nutrient agar was selected. Various microbiological media were used to evaluate the microbial quality of seafood and strongly suggested the use of marine agar. However, the highest count observed along coastline of Alexandria city appears to be a result of combination of continuous effluent input and hydrographic dynamics which affect *in situ* microbial community. These sites experience varieties of human interference (domestic/industrial/ fishing). Dispersion and dilution of industrial and/or domestic wastes create a favorable situation for bacteria and other microbial heterotrophs.[34]

Table 2: Average values of bacterial count (CFU/ml) and SLS biodegradation percentage in seawater samples containing heterotrophic bacteria from different sampling points

Biodegradation %[a]	Bacterial count (CFU/ml x 10^4)	Stations
58	16 ± 0.005	Abu Qir Bay
78	4.5 ± 0.003	Eastern Harbour
64	2.5 ± 0.005	Western Harbour
68	4.8 ± 0.002	Al Max Bay
67	8.5 ± 0.001	Al Dekhaila Harbour

a= Initial SLS surfactant concentration was 100 ppm

3.3. Isolation of dominant bacteria capable of degrading SLS:

Only six dominant bacterial isolates growing on SLS as a sole carbon source were obtained from seawater samples (Table 3). Three of these isolates (MH1, MH2 and MH3) were isolated from the Eastern Harbour station. From Abu Qir Bay station; MH4 and MH5 were isolated. The bacterial isolate MH6 was of Western Harbour. Al Dekhaila Harbour had two isolates coded; MH8 and MH9. Al Max Bay had the isolate MH7 as dominant one. Bacteria capable of degrading SLS were examined to degrade SLS individually. The biodegradation percentage showed that three isolates coded as; MH3, MH5 and MH6 had the ability for SLS biodegradation as individuals with percentages of 46, 27.3 and 26.7%, respectively (Figure 1). These bacterial isolates were selected for furthermore biodegradation in the optimization step. Microorganisms have evolved different natural methods of uptake of hydrophobic compounds, in general reflected by their capacity to generate hydrophobic cell surfaces or to secrete bio-surfactants into the surrounding medium [(8)]. In a study of linear alkylbenzene sulfonated (LAS) biodegradation by bacterial cultures originating from an estuarine site (Krka river estuary) it was found that the rate of biodegradation depended on the origin of the culture, temperature and the structure of the alkylbenzene group.[(35)]

Table 3: Dominant bacteria isolated from different samples grown on SLS surfactant (100 ppm) as a sole carbon source

Dominant bacterial isolate	Stations
MH4, MH5	Abou Qir Bay
MH1, MH2, MH3	Eastern Harbour
MH6	Western Harbour
MH7	Al Max Bay
MH8, MH9	Al Dekhaila Harbour

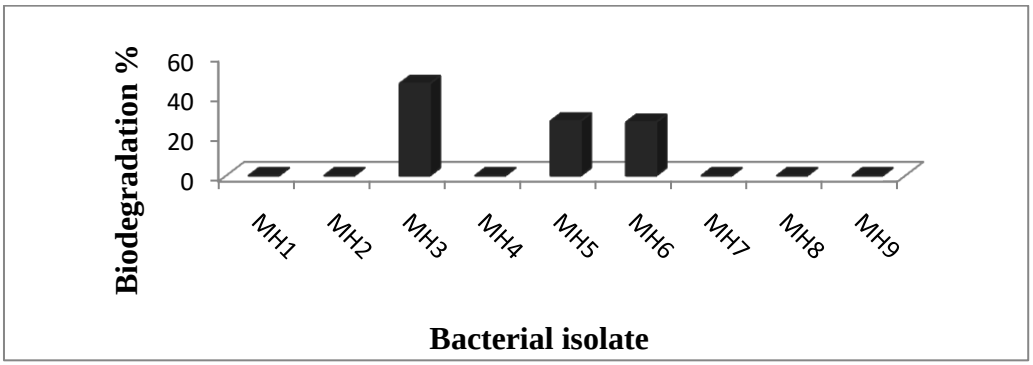

Figure 1: Biodegradation % of SLS surfactant by dominant bacterial isolates as individuals

3.4. Characterizations of dominant bacteria capable of degrading SLS

Out of nine dominant marine bacteria, only three were able to degrade SLS successfully. They were characterized throughout morphological, biochemical and physiological features for Gram +ve bacteria and by using API-20E test for Gram –ve bacteria with negative oxidase test. However, they were tentatively identified as; *Enterobacter gergoviae*, *Enterobacter cloacae* and *Bacillus alvei* (Table 4).

The biodegradability was observed under aerobic and anaerobic conditions. Some researches suggested that Secondary Alkane Sulphonates (SAS) are readily ultimately degradable under aerobic conditions[36,37]. Others recorded that Alcohol Ether Sulphates (AES) degraded well under aerobic conditions with comparable primary and ultimate degradation rates to AS (rates of 96% in 30 days in a closed bottle test)[38]. Marcomini *et al.* [39] reported the biodegradation of Alkyl Phenol Ethoxylate (APE) anaerobically by digested sludge (rate of 900-1100 mg/kg). While Torslov *et al*. [40] screened the biodegradation of APE aerobically by digested sludge (0.3 mg/kg). Nguyen and Sigoillot[7] concluded that the biodegradation of APE by bacteria in seawater

polluted with urban sewage is brought about by bacteria of the Genus marine *Pseudomonas*. Jones and Westmoorland [41] observed a 98% Nonyl Phenol Ethoxylate (NPE) degradation in composted sludge collected from wool scouring in 100 days. Bautista *et al*.[42] used *Enterobacter sp.* in the biodegradation of Polycyclic Aromatic Hydrocarbons (PAHs) with Tween-80 surfactant as a carbon source. Abbouda *et al*.[21] isolated several bacterial combinations from wastewater. They obtained that a mixed culture of the facultative anaerobic bacteria *Pantoea agglomerans* and *Acinetobacter calcoaceticus* grown on NB was found most effective in breaking down the surfactants Linear Alkylbenzene Sulfonates (LAS) and Sodium Dodecyl Sulphate(SDS). Hosseini *et al*. [43] isolated two different bacteria from Tehran municipal activated sludge. They were identified as; *Acinetobacter johnsoni* and *Pseudomonas beteli*. They showed the ability to rapidly and actively degrade SDS as their sole source of carbon, with rate of 97.2% and 96.4% of the original SDS levels after 10 days of growth, respectively.

3.5. Optimization of different variables affecting the biodegradation process of SLS:

In order to improve the efficiency of biodegradation for SLS surfactants, the effects of different conditions on this biodegradation process were further investigated. The isolates *Enterobacter cloacae*, *Enterobacter gergoviae* and *Bacillus alvei* were proved to be the most potent in the biodegradation of SLS surfactant. Therefore, they were selected for further study to maximize and evaluate the efficiency of the biodegradation process. Biodegradation process is influenced by several factors such as medium composition and medium optimization may result in a significant increase in the productivity.[41] Optimization of physicochemical conditions is inevitable in any bioprocess development and usually performed by varying the levels of one independent variable while, fixing other variables at a certain level.

Table 4: Morphological and biochemical characters of the dominant isolated marine bacteria

MH6	MH5	MH3	Character
+ve	-ve	-ve	Gram stain
rods	rods	Rods	Shape
-	+	+	Catalase
+	-	-	Oxidase
+	-	-	Endosopores
+	+	+	O/F
-	-	+	Motility
+	-	+	Indole
+	+	+	V.P.
+	-	-	M.R.
-	-	-	H_2S
+	-	-	Gelatin
+	-	+	Starch
+	-	-	Casein
-	-	-	Nitrate
-	+	-	Arginine
-	+	+	Ornithine
-	-	-	Lysine
-	-	+	Urease
-	+	+	Citrate utilization
			Production of acid from:
+	+	+	Arabinose
+	+	+	Cellobiose
+	+	+	Glucose
+	-	+	Glycerol
+	-	-	Lactose
+	+	+	Sucrose
+	+	-	Sorbitole
+	-	+	Xylose
+	+	+	Maltose
+	+	–	Mannitol
			Growth on:
+	+	+	0% NaCl
+	+	+	3% NaCl
+	+	+	5% NaCl
+	+	+	6.5% NaCl
+	+	+	7% NaCl
			Growth at:
+	+	+	35 ºC
+	+	+	45 ºC
Bacillus alvei	***Enterobacter cloacae***	***Enterobacter gergoviae***	**Probable species**

3.5.1. Effect of biodegradability of the individuals and consortium:

Data in Table 5 confirmed that the biodegradability of consortium consisting of *E. gergoviae*, *E. cloacae* and *B. alvei*, combinations were more effective than that of the individuals. The biodegradation percentage of SLS by this consortium was 95.1%, followed by that of *E. gergoviae* and *B. alvei* combination (88%). The lowest biodegradation % was of individual *B. alvei* (18%). The complete biodegradation of surfactants requires a consortium of bacteria due to the limited metabolic capacities of individual microorganisms [(45)]. The opportunity for commensalism (benefit to one microorganism) and synergism to develop exists in a consortium. Such interactive effects lead to more effective biodegradation than is possible by any individual microorganism. The biodegradation of LAS requires a four member consortium, three members of which oxidize the alkyl chain but synergism amongst the four members was essential for mineralization of the aromatic ring.[(46)]

In other studies, bacterial consortium consisted of two members, *Pantoea agglomerans* and *Serratia* odorifera 2*,* cells were grown evenly together in a minimal medium (M9) and nutrient broth (NB). The bacterial consortium was able to grow in the minimal medium containing LAS as the only carbon source. The percentage degradation of 200 ppm LAS by this bacterial consortium was better when cells were grown in NB (~70%) than in the M9 medium (36%). Also, the degradation ability by the bacterial consortium was very much higher than by its individual cells. An incubation temperature of 32^{O}C, an agitation rate of 250 rev min^{-1}, and the addition of different carbon and nitrogen sources all independently caused complete mineralization of 200 mg/L LAS within 48–72 h [(16)]. Swindoll and Aelion [(47)] also found that several types of organisms may be required to degrade some xenobiotic compounds sequentially. As each species may have its own particular nutrient requirements, a

number of nutrients may be influencing metabolism by a heterogeneous population at any given time. Therefore, the concept of a single limiting nutrient may not be applicable to heterogeneous microbial populations.

Table 5: SLS biodegradation % for consortium isolates

Biodegradation %	Bacterial isolate
85.2	*E. gergoviae* & *E. cloacae*
95.0	*E. gergoviae* & *B. alvei*
89.9	*E. cloacae* & *B. alvei*
95.1	*E. gergoviae* & *E. cloacae* & *B. alvei*

a= Initial SLS surfactant concentration was 100 ppm

3.5.2. Effect of the inoculum size on SLS biodegradation:

To detect the best inoculum size of the consortium of *E. gergoviae*, *E. cloacae* and *B. alvei*, for SLS biodegradation, four ratios were examined. The results shown in Figure 2 reveal that the increase in inoculum size is accompanied with the increase in biodegradation. The ratio of 2.0 % was the most effective inoculum size in the biodegradation (95.9 %), while the lowest effective inoculum size towards biodegradation was the 0.5 % (81.2 %).

3.5.3. Effect of the incubation temperature on SLS biodegradation:

The consortium of *E. gergoviae*, *E. cloacae* and *B. alsvei* completed the next experiments in optimization. Five temperature degrees were examined to detect the best one for SLS biodegradation. The results in Figure 2 showed that the 30°C was the most effective temperature (97.2% biodegradation), followed by the 35°C (76.5% biodegradation). The lowest effective temperature towards biodegradation was the 45°C (53.7% biodegradation). Our results were in agreement with the work of Abbouda *et al*. [21] who found the combined bacterial culture to grow better at 30 or 37°C for LAS biodegradation. Similarly they obtained that the incubation temperatures 30 and 37°C provided the best biodegradation extent of

SDS. In addition, the data of Khleifat [16] showed that a temperature of 32°C would be optimum for complete degradation of LAS. Bautista *et al.* [42] observed that the different bacterial cultures were performed better at 25°C in evaluation of the effect of several non-ionic surfactants (Tween-80, Triton X-100 and Tergitol NP-10) on the ability of bacteria (*Enterobacter sp., Pseudomonas sp. and Stenotrophomonas sp.*) to degrade polycyclic aromatic hydrocarbons (PAHs).

On the contrary, Hosseini *et al*. [43] found that mixed culture of the two isolates; *Acinetobacter johnsoni* and *Pseudomonas beteli* did not significantly increase SDS utilization, (97.6% biodegradation).

3.5.4. Effect of the pH level on SLS biodegradation:

A range of pH buffers 5–9 was used to examine the effect of mixed culture pH on the biodegradation process. From data shown in Figure 2, the pH 9 was the optimum for the growth of consortium *(E. gergoviae*, *E. cloacae* and *B. alvei)* and consequently for biodegradation rate (96%). The pH level 7 was more or less near to optimum with biodegradation percentage (89.2%). On the contrary, pH 5 showed sharp decrease in the biodegradation percentage (19.4%). Abbouda *et al*. [21] observed that maximum degradation of both LAS and SDS was at high pH (8.5). Other pH level like 7.5 and 6.5 gave intermediate effect on the degradation ability of both surfactants while pH 5.5 produced the lowest extent of degradation. Similarly, Khleifa [21] stated that a pH of 8.5 would be the optimum level for maximum LAS degradation by the combined bacteria.

3.5.5. Effect of different carbon sources on SLS biodegradation:

Various carbon sources including glucose, sucrose, and maltose were added separately at a fixed concentration of 0.2% to broth medium for growing consortium (*E. gergoviae*, *E. cloacae* and *B. alvei*). The biodegradation was clearly observed in presence of glucose and molase as carbon sources with biodegradation percentage 94.9 and 87.2,

respectively (Figure 2). On the other side, the supplementation of sucrose did not improve the biodegradability of microbial SLS. Moreover, a positive control of mixed culture growing under similar conditions but lacking additional supplementation of carbon sources has failed to produce similar degree of biodegradation. Our results were parallel to that of Abbouda *et al*. [21] who found that the supplementation of either surfactant as sole carbon source to the mixed culture growing in minimal medium produced 10% degradation of SDS and 60% degradation of LAS, respectively. These data are discussed in terms of differences between the enzymatic induction of LAS and SDS bacterial biodegradation. In addition, Khleifat [16] observed that all of the carbon sources being tested caused an increase in the LAS biodegradation rate. Sucrose, maltose and glucose were better carbon sources, although all carbon sources being tested produced better and more complete degradation activity than the positive control (without C-sources). This complete degradation was achieved within 72 h.

3.5.6. Effect of different nitrogen sources on SLS biodegradation:

In SLS biodegradation, such enhancement by nitrogen sources (yeast extract, tryptone, ammonium chloride and casein) was more lagging using selected consortium (*E. cloacae*, *E. gergoviae* and *B. alvei*). All nitrogen sources were highly effective in the SLS biodegradation as shown in Figure 2. The yeast extract showed a considerable enhancing effect on SLS degradation (96.6% biodegradation), followed by tryptone, ammonium chloride and casein with biodegradation percentages of (92.6, 90.6 and 90.3, respectively). Our data were in agreement with that of Abd-Allah and Srorr[48] who observed that surfactant (XP-100) biodegradability was faster with yeast extract than without, and Abbouda *et al*. [21] who obtained that all nitrogen sources produce improvements in LAS biodegradation compared with a positive control of mixed

culture grown in NB medium but was deprived of additional nitrogen source supplementations. The supplementation with nitrogen nutrients has increased the biodegradation extent of LAS from 60% to 90%. Additionally, Khleifat[16] found that all nitrogen sources being tested produced the greatest rate of LAS degradation, which was completed within 48 h. Khleifat[16] discussed that the nature of the carbon and nitrogen sources may affect both growth biomass and enzymes involved in LAS degradation. Based on our data, we may share him the same conclusion. In contrast, such supplementation of the mixed culture with carbon and nitrogen nutrients had adverse effects on the induction of SDS degradation [21].

3.6. Effect of different concentrations of SLS on the biodegradation rate:

Such effect was studied using several amounts of SLS surfactant ranging from 200 to 1000 ppm to be degraded by selected consortium (*E. cloacae*, *E. gergoviae* and *B. alvei*), incubated for 14 days at 30ºC. The biodegradation percentages showed, as in Figure 3, an inverse relationship between the increase in SLS mass and the extent of its degradation. Under these growth conditions a complete degradation of 1000 ppm SLS biomass was achieved with biodegradation percentage (45.5%). Our results were matched with that of Abbouda *et al*.[21] who observed an inverse relationship between the increase in LAS and SDS mass and the extent of its degradation. Moreover, they obtained that the mixed culture produced about 60% of LAS degradation when this surfactant was added at 300 ppm level compared with 10% degradation at 700 ppm surfactant supplementation. In contrast, higher ranges of SDS concentration (3000–8000 ppm) were degraded by the same mixed culture but still an inverse relationship existed between the increase in mass and the extent of degradation. On the contrary, Abd-Allah and Srorr[48] observed that increased surfactant (XP-100) concentration did not inhibit its biodegradation over the period studied

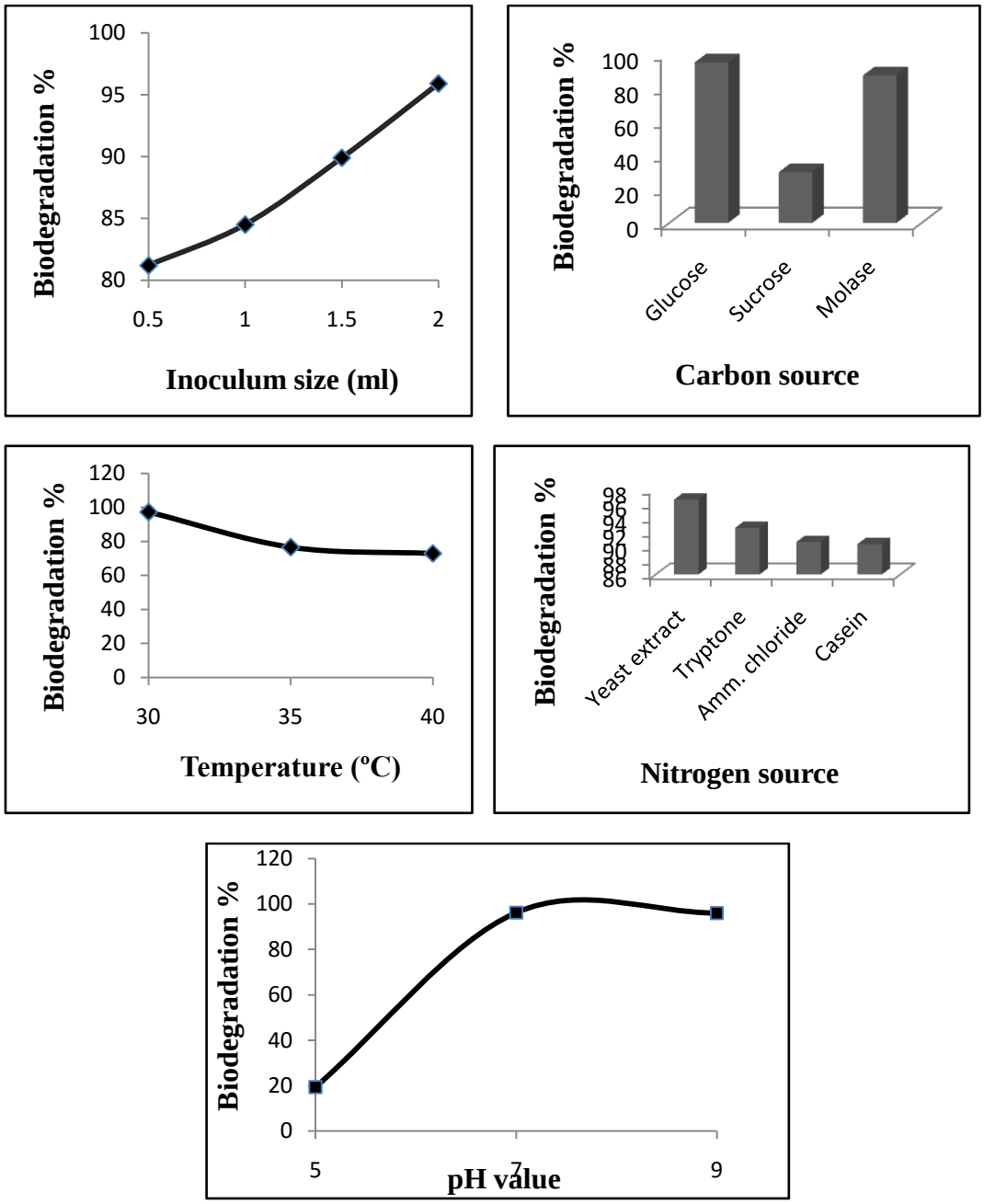

Figure 2: Optimization of different variables affecting the biodegradation process of SLS

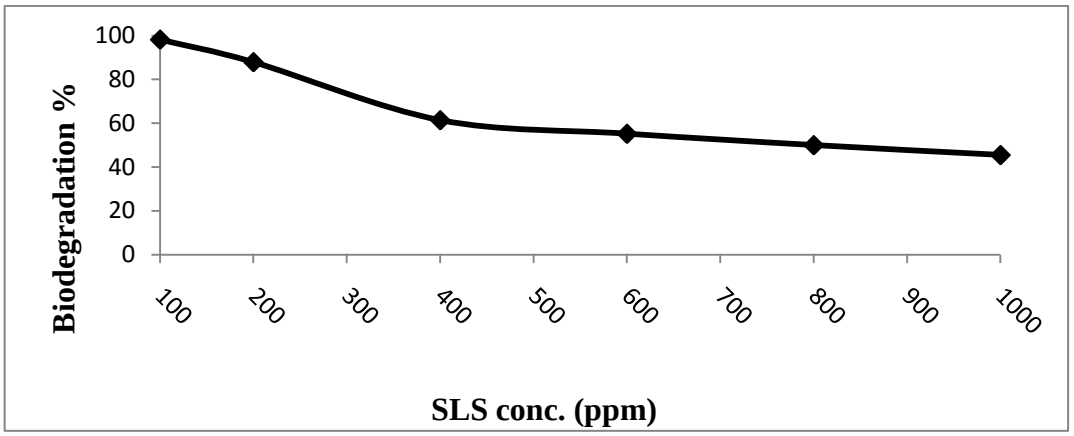

Figure 3: Biodegradation % in different concentrations of SLS by bacterial consortium

CONCLUSIONS

The current study concluded the following points:

1. Cooperation between several bacterial species is needed in order to accomplish complete biodegradation of SLS surfactants.
2. The selection of optimum incubation conditions like temperature, pH, carbon sources and nitrogen sources managed to improve the degradation efficiency of SLS and the process was completely achieved when the mixed consortium was supplemented.
3. Experimental results indicated faster surfactant biodegradation with yeast extract than without.

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