DOI: 10.24996/ijs.2025.66.8.10

ISSN: 0067-2904

Using *Klebsiella oxytoca* bacteria to treat Sodium Lauryl Ether Sulfate (SLES) Pollution

Hussein Ali Awadh Al-Zamili*, Ithar Kamil Al-Mayaly
Department of Biology, College of Science, Baghdad University, Baghdad, Iraq.

Received: 27/4/2024 Accepted: 24/8/2024 Published: 30/8/2025

Abstract

Sodium lauryl ether sulfate (SLES), a surfactant frequently incorporated into detergent formulations, typically ends up in wastewater treatment facilities after use. The present study aims to investigate the efficacy of bacteria isolated from Iraqi wastewater in removing SLES. Genetic analysis (16S rRNA) revealed that this strain is *Klebsiella oxytoca*. Three temperatures (30, 35 and 40) ºC and pH values (5,7 and 9) were selected for this study, and three concentrations of SLES (25, 50, 100) mg/l were used. The SLES anionic surfactant demonstrated that the best biodegradation by *Klebsiella oxytoca* occurred at 30 ºC and both pH 7 and 9, while the removal percentage for them was 98.32% and 95.4 %, respectively at 25 mg/l of SLES. The outcomes of this study revealed the potential and significance of SLES removal in actual effluents by aerobic biodegradation. The ability of this bacterium to degrade SLES makes it an important tool for bioremediation.

Keywords: *Klebsiella* bacteria, detergents, SLES, biodegradation.

استخدام بكتريا *Klebsiella oxytoca* لمعالجة التلوث بكبريتات لوريل إيثر الصوديوم

حسين علي عوض الزاملي*، إيثار كامل الميالي
قسم علوم الحياة, كلية العلوم, جامعة بغداد, بغداد, العراق

الخلاصة

كبريتات لوريل إيثر الصوديوم (SLES) هو مادة خافضة للتوتر السطحي فعال في التنظيف يتم استخدامه بشكل متكرر في تركيب المنظفات، ينتهي به الأمر عادة في محطات معالجة مياه الصرف الصحي. تهدف الدراسة الحالية إلى التحقيق في فعالية البكتيريا المعزولة من مياه الصرف الصحي العراقية في إزالة كبريتات لوريل إيثر الصوديوم. أظهر التحليل الجيني لـ 16S rRNA أن هذه السلالة هي *Klebsiella oxytoca* . اختيرت ثلاث درجات حرارة 30 و 35 و40 م° وثلاثة قيم من الpH 5 و 7 و 9 لهذا الغرض, واستخدم ثلاثة تراكيز من كبريتات لوريل إيثر الصوديوم (25 ، 50 ، 100) ملغرام / لتر. أظهر الخافض للتوترالسطحي الأنيوني SLES أن أفضل تحلل حيوي بواسطة *Klebsiella oxytoca* حدث عند 30 درجة مئوية ودرجة حموضة 7 و 9، حيث كانت نسبة الإزالة لهما 98.32٪ و 95.4٪ على التوالي عند 25 ملغ / لتر من SLES. كشفت نتائج هذه الدراسة عن إمكانية وأهمية إزالة SLES في النفايات السائلة الفعلية عن طريق التحلل الحيوي الهوائي. إن قدرة هذه البكتيريا على تحلل SLES تجعلها أداة مهمة للمعالجة الحيوية.

*Email: hawad@uowasit.edu.iq

1. Introduction

Anionic surfactants account for 60% of the global surfactant production, with sodium lauryl ether sulfate (SLES) being one of the most commonly used anionic surfactants. SLES is a blend of primary linear alkyl ether sulfates (AES) [1, 2]. The molecular formula for SLES is (CH3[CH2]11[OCH2CH2]n OSO3Na+) which is the most frequently utilized for household and industrial applications. It is used in many products, including detergents, cosmetics, and items for personal care, due to its inexpensive production and strong emulsifying and foaming qualities [3]. Surfactants are widely used, and as a result, they frequently exist in industrial and household wastewater. According to reports, the majority of surfactants are biodegradable to some extent, due to their complex structures and high molecular weights, which frequently involve multiple steps in the biodegradation process [4,5]. The biodegradation of metabolites generated through surfactant biodegradation may be slower than that of the parent substance. In streams and rivers near dams or obstructions, large masses of foam have been developed as a result of incomplete surfactant biodegradation [6,7]. Wastewater treatment involves a diverse range of methods, including chemical, biological, and physical treatment, to remove surfactants. The most effective method for treating wastewater will depend on key factors, including energy consumption, treatment costs, environmental impact, influent and effluent quality, and treatment costs [8]. Physical treatment techniques have the advantages of being robust, chemical-free, and requiring less operational input; however, their main disadvantages are producing secondary waste, having high capital cost (requiring land/space), and having a long retention period [9].

Chemical methods offer advantages such as reduced sludge generation and high pollutant removal efficiency; however, they also present drawbacks, including high operational and chemical costs as well as the production of secondary waste. In contrast, biological methods are characterized by low cost and ease of application [10]. The study of the decomposition of anionic surfactants by microorganisms is considered very important in reducing their environmental impact. Bacterial activity is the main reason responsible for the degradation of surfactants in the ecosystem. In addition to improving the removal of these surfactants from the environment and reducing their impact on ecosystems, biodegradation is an essential process for treating surfactants found in raw sewage in treatment plants. Microbes can co-metabolize surfactants through microbial metabolic reactions or use them as substrates for energy and nutrients during biodegradation [11]. Some recent studies have indicated that SLES can be broken down by the bacterial consortia that were isolated from wastewater and activated sludge. Most of the consortia's identified bacteria were members of the Gammaproteobacteria family, which includes *Aeromonas, Serratia, Pseudomonas, Alcaligenes, Azotobacter, Enterobacter, Klebsiella* and *Acinetobacter* [12,13.14]. Ether cleavage is the primary mechanism of aerobic degradation of SLES. This process yields intermediate compounds that can undergo additional degradation and release sulfate. A further potential pathway involves the direct cleavage of the AES ester to split the sulfate, which occurs before the carbon body's degradation [15]. This study aimed to isolate and assess the efficiency of an aerobic bacterial strain that can degrade SLES.

2. Materials and Methods:

2.1. Chemicals and Media

Sodium lauryl ether sulfate (CAS n. 68585-34-2, 70% purity) and other biochemicals were purchased from Sigma (USA), and acetone was used to dissolve SLES to prepare of the stock of 1000 mg/L. Following, the stock solution was filter-sterilized and stored in the refrigerator. The bacterial strains that degraded SLES were isolated and cultivated using nutrient broth,

nutrient agar and mineral salt media, as stated by [16]. The composition of mineral salt medium (MSM) was prepared according to [17].

2.2. Isolation and screening of bacteria that degrade SLES

Samples were collected from three sewage treatment plants that were contaminated with surfactant (in December 2022), which have been assigned the designations (1, 2, and 3) belonging to sites within Al-Kut City in the Wasit Governorate of Iraq.

For enrichment, sewage samples (10 ml) were added to 250 ml Erlenmeyer flasks containing 150 ml of MSM supplemented with 50 mg/L SLES for enrichment. The flasks were then incubated for five days at 150 rpm and 30 ºC [18]. The pour plate method was employed for the isolation of bacteria using nutrient agar, from the last enrichment culture, according to [19].

Bacterial cultures that SLES degrading were selected by:

1- Primary screening is carried out by cultivating them on nutrient agar plates with 200 mg /L of SLES the sole source of energy and carbon, as described in [20].

2- Secondary screening was conducted through cultivation on solid (MSM) that were incubated at 30 ºC for 24 to 96 hours, with SLES serving as the only source of energy and carbon at concentrations between 200 to 2000 mg/L. The growth of bacterial isolates was based on the bacteria growth on solid mineral salt media [16,21].

2.3. Identification of the bacterial strain that degrades SLES

In addition to studying the shape, color, odor, and margin of the colonies grown on nutrient agar, microscopic analysis of slides stained with gram stain was conducted to distinguish between gram-negative and / from gram-positive bacteria [22]. The isolated bacteria were identified by PCR technology. Total DNA of bacterial isolated was extracted by using the G- spin DNA Extraction Kit (iNtRON biotechnology/Korea, cat.no. 17045). The 16S rRNA gene was amplified by using the universal primer 27F (5'-AGAGTTTGATCCTGGCTCAG- 3') and 1492R (5' GGTTACCTTGTTACGACTT- 3'). The 16SrRNA gene sequence was analyzed and the results were compared with the standard data of similar registered strains in NCBI [23].

2.4. Laboratory experiment

Sets of 250 ml Erlenmeyer flasks holding100 ml of the medium were prepared, the pH was adjusted to 7 and autoclaved at 121ºC and 1.5 bar for 15 min, then sets of SLES with three concentrations 25, 50 and 100 mg/L were added to flasks, then added inoculum of *Klebsiella oxytoca* that isolated and incubated after activated in nutrient broth overnight, the flasks were incubated in a shaker incubator (150 rpm) for 15 days and the growth was measured by recorded values of optical density by spectrophotometer on the wavelength is 600 nanometers every two days [24]. The experiment was conducted at three different temperatures (30, 35, and 40 ℃) and two pH levels (5 and 9) for a duration of 15 days. Optical density measurements were taken for each condition, and 5 ml samples were extracted for HPLC analysis to assess the biodegradation efficacy.

2.5. High Performance Liquid Chromatography (HPLC)

Sodium lauryl ether sulfate concentrations used in the current study were 25, 50, and 100 mg/L .

HPLC was used to determine the SLES content, and the following equations were employed to calculate the percentage of SLES biodegradation (1, 2) [25]:

$$\textbf{Percentage of biodegradation} = \frac{\textbf{conc.of standard} - \textbf{conc.of sample}}{\textbf{conc.of standard}} \textbf{X 100}$$

Or

$$\textbf{Percentage of biodegradation} = \frac{\textbf{beak area of standard} - \textbf{beak area of sample}}{\textbf{beak area of standard}} \textbf{X 100}$$

3. Results and discussion

3.1. Identification of bacteria

In this study, sewage samples from one of the wastewater plants in Wasit Province /Iraq were considered a significant source of locally prevalent bacteria that can mineralize anionic surfactants. The SLES-degrading strain isolated in this study were gram-negative rods. *K. oxytoca*, was isolated, purified, and selected based on their capability to use SLES as a carbon source, The bacterial isolate *K. oxytoca* was identified based on the nucleotide sequence of the 16S rRNA gene (PCR technology).

3.2. Impact of Temperature and pH on growth of Klebsiella oxytoca

Surfactant biological degradation is typically influenced by several factors. It is well established that the degradation rate is influenced by environmental factors such as temperature, pH, shaking, and nutrient availability [26]. The present study investigated the effects of temperature and pH on SLES degradation. The results presented in Table 1 reveal that notable differences exist in the growth of bacteria at various temperatures and pH levels. The most significant bacterial growth was observed at pH 7, with highest values recorded at 30 ºC, 35 ºC, and 40 ºC, which were 0.410, 0.366 and 0.110 nm respectively. Also, the better and more significant means of bacterial growth under pH 5, 7 and 9 measured at 30 ºC were 0.360, 0.410, and 0.370 nm respectively. The mean value of *K. oxytoca* growth was highest at 30 ºC and pH 7, which was 0.410 nm, and lowest at 40 ºC and pH 5 which was 0.081 nm (Figure1).

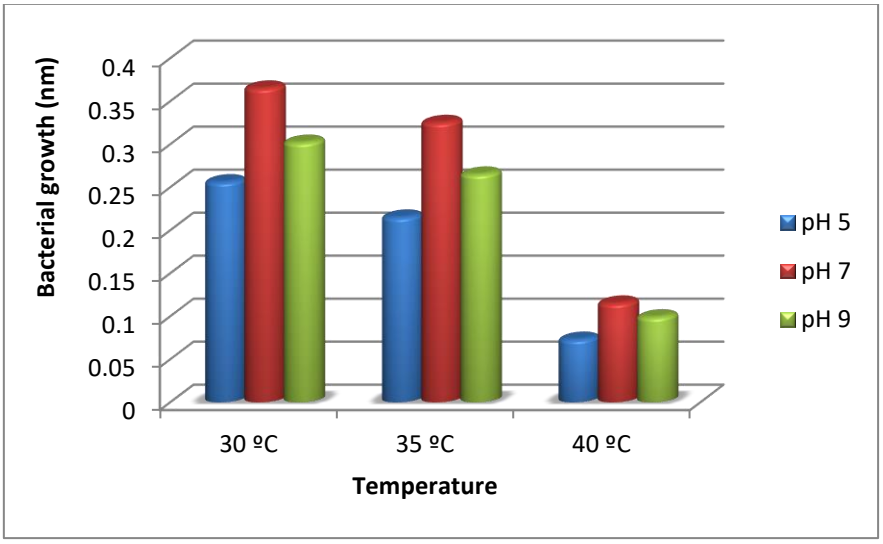


Figure 1 : Mean value of *K. oxytoca* growth of at different temperatures and pH value after 15 days of incubation.

Table 1: Mean value of *K. oxytoca* growth of at different pH and temperatures degrees after 15 days incubation, and LSD value with 25 mg/L of SLES

Temp. \ pH	5	7	9	LSD value
30 ºC	0.253 - 258 0.256 ±0.054 c	0.361 – 0.370 0.365 ±0.097 a	0.298 – 0.308 0.302 ±0.100 b	0.064*
35 ºC	0.211 – 0.218 0.215 ±0.035 c	0.321 – 0.329 0.325 ±0.061 a	0.264 – 0.266 0.265 ±0.064 b	0.126*
40 ºC	0.068 – 0.080 0.073 ±0.019 a	0.113 – 0.117 0.115 ±0.025 a	0.095 – 0.104 0.099 ±0.027 a	0.073 NS
LSD value	0.074*	0.122 *	0.073 *	---

* (P≤0.05), NS: Non-Significant.

Accordingly, the optimal degradation occurs at a temperature 30ºC for isolated bacteria, similar research revealed that 30°C was necessary for the optimal degradation of surfactants by *Citrobacter braakii*, *Pseudomonas*, *Delftia acidovorans*, *Acinetobacter calcoaceticus*, and *Pantoea agglomerans* [12,24.27.28]. Temperature and microbial activity were found to be directly correlated during SLES degradation. A rise in temperature beyond 37°C may not only impact growth but could also increase the toxicity of microbial membranes. According to the data, 30ºC would be the ideal temperature for better SLES degradation, as confirmed by HPLC. Temperature generally affects the SLES compound's physical and chemical properties, microbial metabolism, the rate at which certain microorganisms grow, the rate at which enzymatic activity involved in the oxidation process occurs, and the makeup of the microbial community, all of which affect the rate at which SLES biodegrades [24]. The degradative activity of bacteria is significantly influenced by pH [29]. The results indicated that the ideal pH for *K. oxytoca* growth in MSM containing SLES is 7, this conclusion is corroborated by the findings of [30], who found that isolated strains of Pseudomonas can grow and maintain their capacity for degradation across a broad pH range, with optimal growth occurring at pH roughly. These results in agreement with [27], who reported that many SLES-degrading bacteria, including *Citrobacter braakii*, grow optimally on SLES at a pH of 7. The pH level may have an impact on the enzyme(s) responsible for SLES degradation [31].

Impact of incubation time on biodegradation and growth

Table 2 presents the results, indicating a significant difference ($P < 0.05$) in the mean bacterial growth across various incubation times. After fifteenth day of incubation, the selected bacteria isolated showed that the highest mean growth was on the ninth day (0.490 nm), and the lowest growth was on the one day (0.205 nm). The growth rate of the selected bacteria that were isolated increased gradually from one day to the ninth day, and then started to decrease (Figure 2).

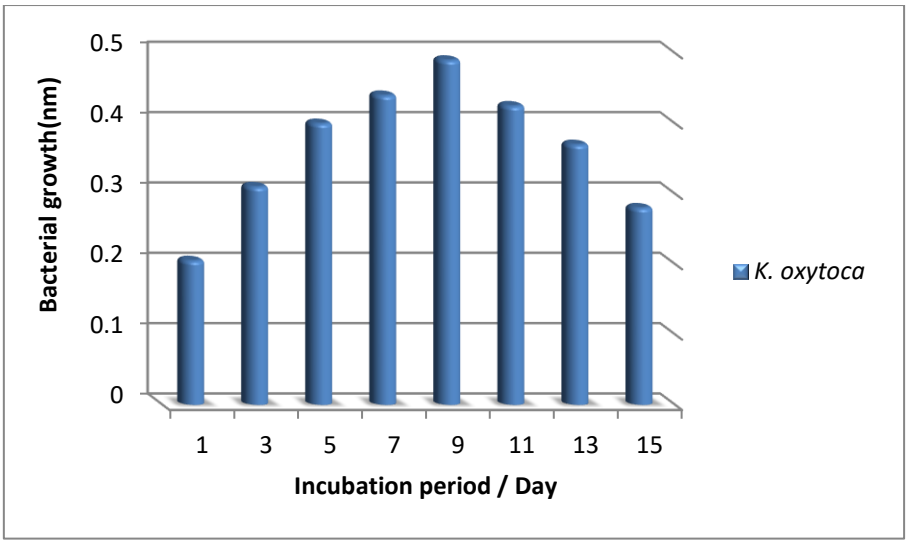


Figure 2: The average value of bacterial growth over various incubation times.

Table 2: Mean values ± SD of Bacterial growth at 600nm at various incubation times, and LSD value.

Incubation period / Days	Mean± SD of bacterial growth *K. oxytoca*
1	0.205 ± 0.012
3	0.310 ± 0.015
5	0.400 ± 0.010
7	0.440 ± 0.012
9	0.490 ± 0.006
11	0.425 ± 0.0105
13	0.370 ± 0.022
15	0.280 ± 0.028
LSD ≤ 0.05	0.022

SLES degradation increased significantly as the incubation time increased. According to [32], after 120 hours, *Pseudomonas beteli* was able to degrade up to 94% of the SDS levels. It is widely recognized that extending the incubation period increases the number of viable organisms, especially on medium with minimal amounts of nutrients, and as a result of contaminant concentration depletion and the production of intermediate substances and metabolic byproducts, which in turn leads to a decrease in media pH and subsequent inhibition of bacterial growth [33].

3.3. HPLC analysis of biodegraded SLES

For the degradation efficacy test, the bacteria were inoculated into a mineral salt medium, with SLES at three concentrations 25, 50 and 100 mg/L; the surfactant was added to 100 mL of mineral salt medium at three different temperature degrees and pH. These samples were analyzed with the HPLC system [34]. An analysis of a standard solution of surfactant was done, where the concentration of the standard solution was 10 mg/L and the peak area was (1025.5) per (5.90) minute. The comparing figures of SLES were done according to the concentrations used in the study (Figure 3).

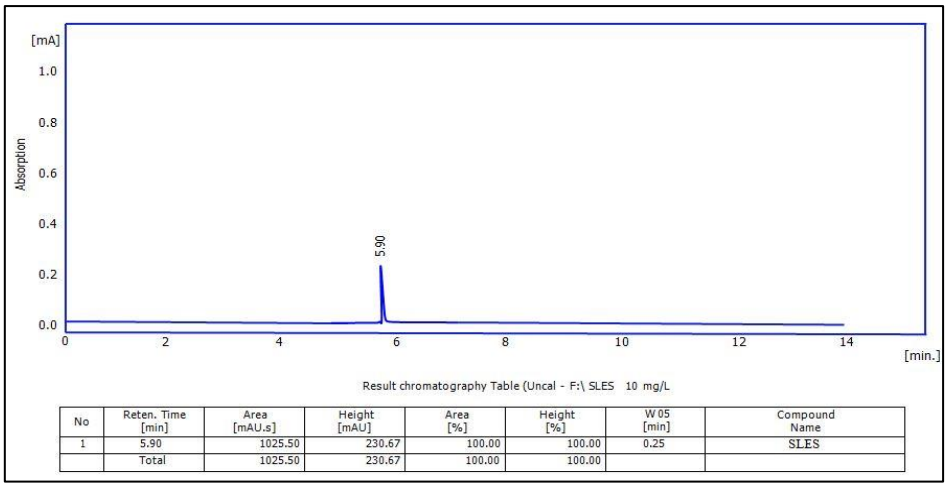


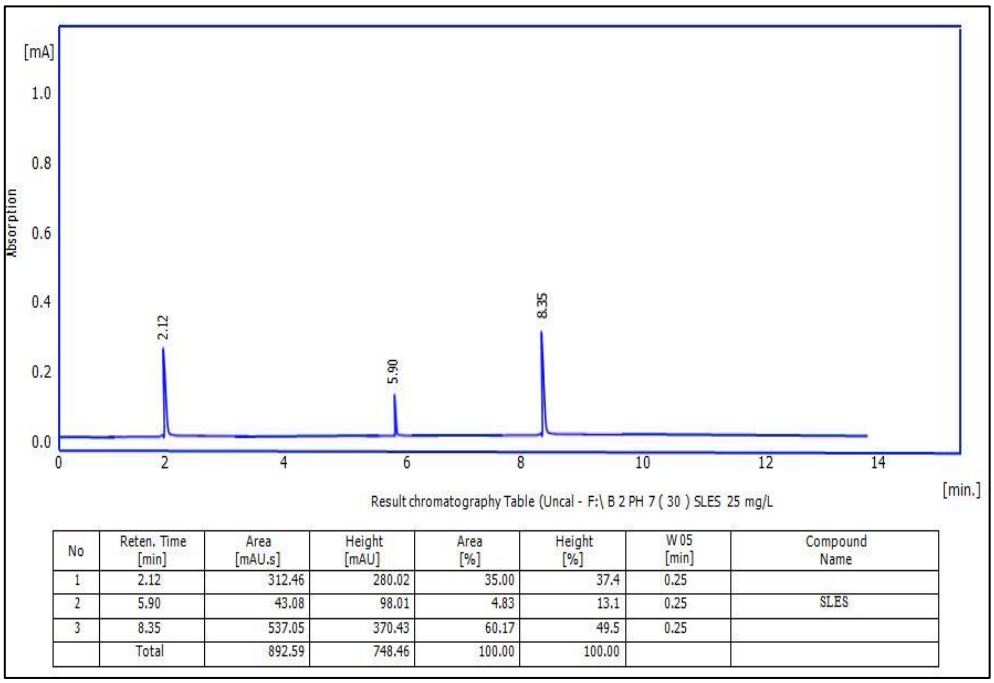
Figure 3: Peak area for 10 mg/L of SLES

The results of the HPLC analysis are mentioned in Table 3, which explains the concentrations of SLES, peak area of samples, percentage of removal, and remaining concentration for each sample.

Table 3: Removal percentage of SLES samples by different temperature, pH and concentration

Temperature ◦C	Concentration of SLES (mg/L)	pH	Peak area (mAU.s)	% removal	Remain conc. (mg/L)
30	25	9	117.75	95.4	1.15
		7	43.08	98.32	0.42
		5	204.27	92	1.5
	50	9	966.24	81.16	9.42
		7	612.19	88.06	5.97
		5	1281.87	75	12.5
	100	9	3058.02	70.18	29.82
		7	2358.15	77	23
		5	3988.48	61.1	38.9
35	25	9	382.22	85.08	3.73
		7	153.06	94	1.5
		5	560.21	78.12	5.47
	50	9	1536.03	70.04	14.98
		7	915.16	82.16	8.92
		5	1845.55	64	18
	100	9	3589.25	65	35
		7	2650.55	74.2	25.8
		5	5126.75	50	50
40	25	9	2051.00	20	20
		7	1987.17	22.48	19.38
		5	2127.75	17	20.75
	50	9	4299.35	16.16	41.92
		7	4204.15	18	41
		5	4459.48	13.02	43.49
	100	9	8706.35	15.1	84.9
		7	8500.88	17.1	82.9
		5	9126.54	11	89

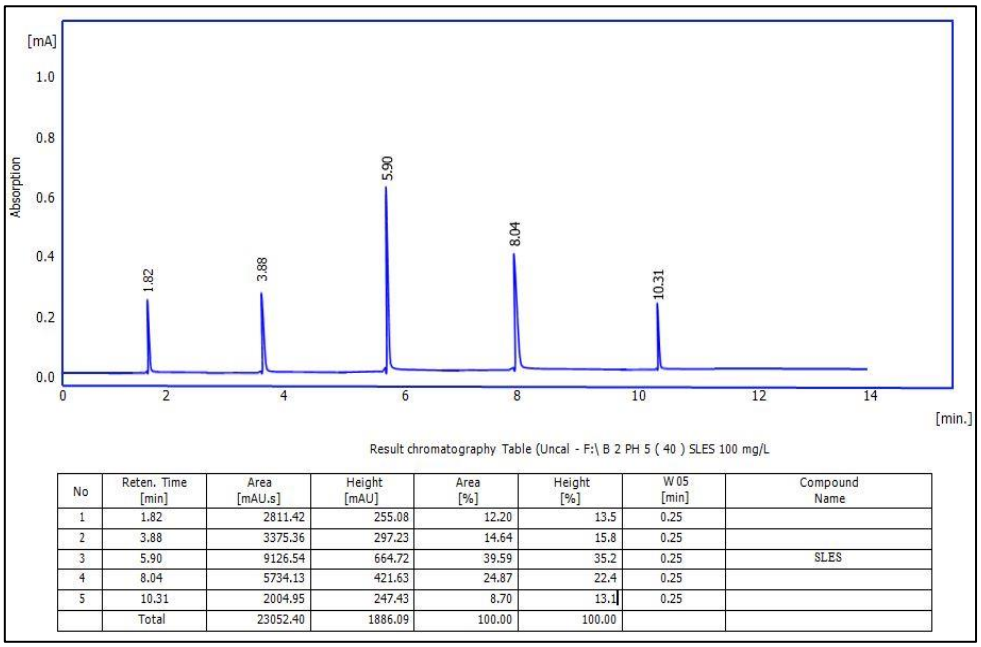
In this table, the dissociation of SLES surfactant in different concentrations with different temperatures and pH, where the best result was the dissolution of the SLES into its secondary components at a temperature of 30 ° C and pH 7 (Figure 4) with the concentration used 25 mg/L, and the lowest results were at temperature 40 ° C at pH 5 with the concentration used 100 mg/L as in (Figure 5). This mean that the higher the temperature and concentration of SLES and the lower the pH value, the less efficient this bacteria is in degrading the surfactant.



Result chromatography Table (Uncal - F:\ B 2 PH 7 (30) SLES 25 mg/L

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W 05 [min]	Compound Name
1	2.12	312.46	280.02	35.00	37.4	0.25	
2	5.90	43.08	98.01	4.83	13.1	0.25	SLES
3	8.35	537.05	370.43	60.17	49.5	0.25	
	Total	892.59	748.46	100.00	100.00		

Figure 4: The highest removal of SLES



Result chromatography Table (Uncal - F:\ B 2 PH 5 (40) SLES 100 mg/L

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W 05 [min]	Compound Name
1	1.82	2811.42	255.08	12.20	13.5	0.25	
2	3.88	3375.36	297.23	14.64	15.8	0.25	
3	5.90	9126.54	664.72	39.59	35.2	0.25	SLES
4	8.04	5734.13	421.63	24.87	22.4	0.25	
5	10.31	2004.95	247.43	8.70	13.1	0.25	
	Total	23052.40	1886.09	100.00	100.00		

Figure 5: The lowest removal of SLES

The degradation percentage increased significantly at 25 mg/L of SLES, but, decreased at 100 mg/L [34], which indicates a higher efficiency of degradation at a neutral pH. However, acidic pH with nearly 50% degrading rate, while alkaline pH gave nearly 80% degrading rate at different concentrations and amounts of surfactant. Additionally, previous studies [18], have shown that the neutral environments promote more degradation than alkaline and acidic because in acidic environments the stability of bonds increased. Several bacterial strains that can break down comparable substances have been isolated from environments contaminated with surfactants [35]. In this study, high levels of degradation were attained with low concentrations of SLES. The high percentage of SLES degradation of *K. oxytoca* at a concentration of 25 mg/l in the medium was 98.32%, while it was 88.06% and 77 % for concentrations of 50 and 100 mg/L respectively at pH 7, Temperature 30. Higher concentrations resulted in a noticeable slowdown in the rate of degradation, which, appears to be related to the surfactant's harmful effect on bacterial growth [36]. Sodium lauryl ether sulfate can be poisonous and harmful to microorganisms at certain concentrations. This is due to the SLES adsorption causing the cell membrane of microorganisms to depolarize, which reduces the absorption of supplements and alters the release of material from cell metabolism. When the concentration of SLES is high, it is deadly to the bacteria because it removes the lipopolysaccharide outer layer of gram-negative bacteria. The viability of microorganisms will eventually decline [24,37]. *K. oxytoca* bacteria demonstrated effective degradation of SLES, suggesting that this bacterium is a viable option for the disposal of SLES in contaminated environments. The results agreed with [12,26, 38], which use bacteria to degrade SLES and show 75 – 100 % percentage of degradation to SLES explained by HPLC analysis. The slight difference in the percentage of digestion is due to the efficiency of the type of bacteria, the isolated strain type, and its adaptation to the environment [39].

Conclusion

The discharge of synthetic surfactants into the water bodies hinders aeration due to their high foaming and low oxygenation capacity. Microbes are shown to be an efficient degrader of anionic surfactants. The bacteria obtained in the present study are capable of utilizing SLES as their carbon source and this makes the bacterium an important tool in the bioremediation of wastewater contaminated with surfactants. *Klebsiella oxytoca* showed a high degradation rate of SLES. The optimum temperature for *Klebsiella oxytoca* was found to be 30°C and pH was found to be 7 but the degradation rate decreases with increase SLES concentration.

Acknowledgements

The authors thank the Central Laboratory in Department of Biology/ College of Science / University of Baghdad, and the Water Analysis Laboratory of the Ministry of Science and Technology for their help in this research.

Conflict of interest: The authors declare that they have no conflicts of interest whatsoever.

References

[1] K. Holmberg, B. Jonsson, B. Kronberg, and B. Lindman, "Surfactants and polymers in aqueous solution," 2nd edn. Wiley, Chichester, West Sussex, 2002.

[2] R.R. Al-Ani, F.M. Hassan, and A.M.J. Al-Obaidy, "Quantity and quality of surfactants in sediment of Tigris River, Baghdad, Iraq," *Desalination and Water Treatment*, vol. 170, pp. 168-175, 2019, doi: 10.5004/dwt.2019.24679.

[3] A. Barra Caracciolo, M. Cardoni, T. Pescatore and L. Patrolecco, " Characteristics and environmental fate of the anionic surfactant sodium lauryl ether sulphate (SLES) used as the main component in foaming agents for mechanized tunnelling," *Environmental pollution*, vol. 226, pp. 168-175, 2017.

[4] C. G. van Ginkel, " Complete degradation of xenobiotic surfactants by consortia of aerobic microorganisms," *Biodegradation*, vol. 7, pp. 151-164, 1996.

[5] A. Al-Shamma, and R. A Hassan, " Pollution Assessment of Surface and Drainage Water by Heavy Elements in Al Ahrar District, Wasit Governorate, Iraq," *Iraqi Journal of Sciences*, vol. 59, no. 2B, pp. 886-891, 2018, doi:10.24996/ijs.2018.59.2B.9.

[6] D. T. Bennie, " Review of the environmental occurrence of alkylphenols and alkylphenol ethoxylates," *Water Quality Research Journal of Canada*, vol. 34, no. 1, pp. 79-122, 1999.

[7] N. R. Itrich, and T. W. Federle, " Effect of ethoxylate number and alkyl chain length on the pathway and kinetics of linear alcohol ethoxylate biodegradation in activated sludge," *Environmental Toxicology and Chemistry*, vol. 23, no. 12, pp. 2790-2798, 2004.

[8] S. Jamaly, A. Giwa, and S.W. Hasan, "Recent improvements in oily wastewater treatment: progress, challenges, and future opportunities," *Journal of Environmental Sciences*, vol. 37, pp. 15–30, 2015, doi: 10.1016/j.jes.2015.04.011.

[9] A. Fakhru'l-Razi, A. Pendashteh, L.C. Abdullah, D.R.A. Biak, S.S. Madaeni, and Z. Z. Abidin, "Review of technologies for oil and gas produced water treatment," *Journal of Hazardous Materials*, vol. 170, pp. 530–551, 2009, doi: 10.1016/j.jhazmat.2009.05.044.

[10] P. Jain, M. Sharma, P. Dureja, P.M. Sarma, and B. Lal, "Bioelectrochemical approaches for removal of sulfate, hydrocarbon and salinity from produced water," *Chemosphere*, vol. 166, pp. 96–108, 2017, doi: 10.1016/j.chemosphere.2016.09.081.

[11] K. Qiao, W. Tian, J. Bai, J. Zhao, Z. Du, T. Song, M. Chu, L. Wang, and W. Xie, "Removal of high-molecular-weight polycyclic aromatic hydrocarbons by a microbial consortium immobilized in magnetic floating biochar gel beads," *Marine Pollution Bulletin*, vol. 159, 111489, 2020, doi: 10.1016/j.marpolbul.2020.111489.

[12] K.M. Khleifat, " Biodegradation of sodium lauryl ether sulfate (SLES) by two different bacterial consortia," *Current Microbiology*, vol. 53, no. 3, pp. 444-448, 2006, doi: 10.1007/s00284-006-0266-4.

[13] A. M. S Paulo, R. Aydin, M. R. Dimitrov, H. Vreeling, A. J. Cavaleiro, P. A. García- Encina, A. J. M. Stams, and C. M. Plugge, "Sodium lauryl ether sulfate (SLES) degradation by nitrate-reducing bacteria," *Applied microbiology and biotechnology*, vol. 101, no. 12, pp. 5163–5173, 2017, doi: 10.1007/s00253-017-8212-x.

[14] A.A. Najim, Z.Z. Ismail, and K.K. Hummadi, "Biodegradation potential of sodium dodecyl sulphate (SDS) by mixed cells in domestic and non-domestic actual wastewater:Experimental and kinetic studies," *Biochemical Engineering Journal*, vol. 180, 108374, 2022, doi: 10.1016/j.bej.2022.108374.

[15] I. Budnik, J. Zembrzuska, and Z. Lukaszewski, "). Bacterial strains isolated from river water having the ability to split alcohol ethoxylates by central fission," *Environmental science and pollution research international*, vol. 23, no. 14, pp. 14231-14239, 2004, doi: 10.1007/s11356-016-6566-8.

[16] A. Singh, J. D. Van Hamme, and O. P. Ward, "Surfactants in microbiology and biotechnology," *Biotechnology Advances*, vol. 25 (1), pp. 99–122, 2007, doi: 10.1016/j.biotechadv.2006.10.004.

[17] J.H. Miller, "Experiment in molecular genetics," Cold Spring Harbor laboratory Press, New York,1972.

[18] A.K. Asok, and M. S. Jisha, "Biodegradation of the Anionic Surfactant Linear Alkylbenzene Sulfonate (LAS) by Autochthonous Pseudomonas sp.," *Water, Air and Soil Pollution*, vol. 223, no. (8), pp. 5039–5048, 2012, doi:10.1007/s11270-012-1256-8.

[19] E. J. Andrew, S. G. Hales, N. G. A. Ur-Rehman, and G. F. White, "Novel alkylsulphatases required for the biodegradation of the branched primary alkyl sulphate surfactant 2-butyloctyl sulphate," *Applied and Environmental Microbiology*, vol. 68, no. 1, pp. 31–36, 2002, doi: 10.1128/AEM.68.1.31-36.2002.

[20] N. C. Ghose, D. Saha, and A. Gupta, "Synthetic detergents (surfactants) and organochlorine pesticide signatures in surface water and groundwater of Greater Kolkata, India*," Journal of Water Resource and Protection*, vol. 4, pp. 290–298, 2009, doi: 10.4236/jwarp.2009.14036.

[21] M. H. AlMamoory, and I. K. Al-Mayaly, "Biodegradation of Cypermethrin by Two Isolates of Pseudomonas aeruginosa," *Iraqi Journal of Science*, vol. 58, no. 4C, pp. 2309-2321, 2017, doi: 10.24996/ ijs.2017.58.4C.6.

[22] J. P. Harley, "Laboratory Exercises in Microbiology," 10th ed. McGraw Hill Higher Education. New York, 2016.

[23] C. S. Miller, K. M. Handley, K. C. Wrighton, K. R. Frischkorn, B. C. Thomas, and J. F. Banfield, "Short-Read Assembly of Full-Length 16S Amplicons Reveals Bacterial Diversity in Subsurface Sediments," *Public Library of Science One*, vol. 8, no. 2, e56018, 2013, doi:10.1371/journal.pone.0056018.

[24] M.M. Abboud, K.M. Khleifat, M. Batarseh, K.A. Tarawneh, A. Al-Mustafa, and M. Al-Madadhah, "Different optimization conditions required for enhancing the biodegradation of linear alkylbenzosulfonate and sodium dodecyl sulfate surfactants by novel consortium of Acinetobacter calcoaceticus and Pantoea agglomerans," *Enzyme and Microbial Technology*, vol. 41, no. 4, pp. 432–439, 2007, doi: 10.1016/j.enzmictec.2007.03.011.

[25] W.C. Shu, and W.H. Ding, "Determination of fluorescent whitening agents in laundry detergents and surface waters by solid-phase extraction and ion-pair high performance liquid chromatography," *Journal of Chromatography A*, vol. 1088(1-2), pp. 218–223, 2005, doi: 10.1016/j.chroma.2005.01.032.

[26] D. Abou-Zeid, R. Muller, and W. Deckwer, "Degradation of natural and synthetic polyesters under anaerobic conditions," *Journal of biotechnology*, vol. 86, no. 2, pp. 113–126, Mar. 2001, doi: 10.1016/s0168-1656(00)00406-5.

[27] A. Dhouib, N. Hamad, I. Hassari, and S. Sayadi, "Degradation of anionic surfactants by Citrobacter braakii," *Process Biochemistry*, vol. 38, no. 8, pp. 1245–1250, 2003.

[28] S. Schulz, W. Dong, U. Groth, and A.M. Cook, "Enantiomeric Degradation of 2-(4-Sulfophenyl) Butyrate via 4-Sulfotechol in Delftia acidovorans SPB1," *Applied and Environmental Microbiology*, vol. 66, no. 5, pp. 1905–1910, 2000, doi: 10.1128/AEM.66.5.1905-1910.2000.

[29] J. W. C. Wong, K. M. Lai, C. K. Wan, K. K. Ma, and M. Fang, "Isolation and optimization of PAHS degradative bacteria from contaminated soil for PAHs bioremediation," *Water Air and Soil Pollution*, vol. 139, pp. 1–13, 2002, doi: 10.1023/A:1015883924901.

[30] N. Amirmozafari, F. Malekzadeh, F. Hosseini, and N. Ghaemi, "Isolation and identification of anionic surfactant degrading bacteria from activated sludge," *Iranian Biomedical Journal*, vol. 11, no. 2, pp. 81-86, 2007.

[31] D. Prats, C. Lopez, V. Diana, V. Pedro, and V. M. Leon, "Effect of temperature on biodegradation of linear alkylbenzene sulfonate and alcohol ethoxylate," *Journal of Surfactants and Detergaens*, vol. 9, no. 1, pp. 69–75, 2006, doi: 10.1007/s11743-006-0377-8.

[32] F. Hosseini, F. Malekzadeh, N. Amirmozafari, and N. Ghaemi, "Biodegradation of anionic surfactants by isolated bacteria from activated sludge," *International Journal of Environmental Science & Technology*, vol. 4, no. 1, pp. 127-132, 2007, doi: 10.1007/BF03325970.

[33] S. Naveenkumar, N. Manoharan, S. Ganesan, S. Manivannan, and G. Velsamy, "Isolation, screening in vitri mutational assessment of indigenous soil bacteria for enhanced capability in petroleum degradation," International journal of environmental sciences, vol. 1, pp. 498–513, 2010.

[34] M. Fedeila, Z. Hachaïchi-Sadouk, L. F. Bautista, R. Simarro, and F. Nateche, "Biodegradation of anionic surfactants by Alcaligenes faecalis, Enterobacter cloacae and Serratia marcescens strains isolated from industrial wastewater," Ecotoxicology and Environmental Safety, vol. 163, pp. 629–635, 2018, doi: 10.1016/j.ecoenv.2018.07.123.

[35] S.R. Peressutti, N. L. Olivera, P. A. Babay, M. Costagliola, and H. M. Alvarez, "Degradation of linear alkylbenzene sulfonate by a bacterial consortium isolated from the aquatic environment of Argentina," *Journal of Applied Microbiology*, vol. 105, pp. 476-484, 2008, doi: 10.1111/j.1365-2672.2008.03771.x.

[36] J.S. Yadav, D.L. Lawrence, B.A. Nuck, T.W. Federle, and R.C. Adinarayana,"Biotransformation of linear alkylbenzene sulfonate (LAS) by Phanerochaete chrysosporium: oxidation of alkyl side-chain," *Biodegradation*, vol. 12, no. 6, pp. 443–453, 2001, doi: 10.1023/A:1015056530264.

[37] T. Odahara, "Stability and solubility of integral membrane proteins from photosynthetic bacteria solubilized in different, detergents," *Biochimica et biophysica acta*, vol. 28, pp. 80–92, 2004.

[38] C.G. Anaukwu, A.I. Ekwealor, C.C. Ezemba, V. N. Anakwenze, K. C. Agu, A. S. Nwankwegu, B. C. Okeke, and B. C. Awah, "Influence of anionic, cationic and non-ionic surfactants on growth of hydrocarbon utilizing bacteria," *American Journal of Current Microbiology*, vol. 4, pp. 10–16, 2016.

[39] Z. Ran, J. Zhu, L. Zhou, and R. Ji, "Study on degradation kinetics of co-metabolic biodegradation of linear alkylbenzene sulfonate strains," *International Forum on Energy, Environment and Sustainable Development*, vol. 75, pp. 1071-1074, 2016, doi: 10.2991/ifeesd-16.2016.192.