

Research Article

Biodegradation of sodium lauryl ether sulfate (SLES) contamination by *Pseudomonas aeruginosa* isolates

Hussein Ali Awadh Al-Zamili*, Ithar Kamil Al-Mayaly

Department of Biology, College of Science, Baghdad University, Baghdad, Iraq

*corresponding author: hawad@uowasit.edu.iq

Abstract

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Sodium lauryl ether sulfate (SLES) is a surfactant commonly used in the formulation of detergents, which is typically disposed of in wastewater treatment plants. The current study describes the effectiveness of bacteria isolated from Iraqi wastewater to remove SLES. 16S rRNA genetic analysis revealed that this strain is *Pseudomonas aeruginosa*. Three temperatures (30, 35, and 40°C) and pH values (5, 7, and 9) were chosen for this study, and three concentrations of SLES (25, 50, and 100 mg/L) were used. The SLES anionic surfactant showed that the best biodegradation by *Pseudomonas aeruginosa* was at a temperature of 30°C and both pH 7 and 9, while the removal percentages for them were 98.44% and 96.36%, 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 the bacterium an important tool for bioremediation.

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Introduction

Sixty percent of surfactants produced globally are anionic, and sodium lauryl ether sulfate (SLES) is one of the widely anionic surfactants used, which is a blend of primary linear alkyl ether sulfates (AES) (Holmberg et al., 2002; Al-Ani et al., 2019). The molecular formula for SLES is $(\text{CH}_3[\text{CH}_2]_{11}[\text{OCH}_2\text{CH}_2]_n \text{OSO}_3\text{Na}^+)$, 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 (Caracciolo et al., 2017).

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 because surfactants have complex structures and high molecular weights, and the biodegradation pathways frequently involve multiple steps (Jimenez et al., 1991; van Ginkel, 1996; Al-Shamma and Hassan, 2018).

According to Bennie (1999) and Itrich and Federle (2004), the biodegradation of metabolites generated through surfactant biodegradation could 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 (Jakobi and Lchr, 1987).

Wastewater is treated using a range of methods, including chemical, biological, and physical treatment, to remove surfactants. The best method for treating wastewater will depend on a number of factors, including energy consumption, treatment costs, environmental impact, influent and effluent quality, and treatment costs (Jamaly et al., 2015). Physical treatment techniques have the advantages of being robust, chemical-free, and requiring less operational input; on the other hand, their main disadvantages are producing secondary waste, having a high capital cost (requiring land/space), and having a long retention period (Fakhru'l-Razi et al., 2009).

Chemical methods have advantages, such as reduced sludge generation and high pollutant removal

efficiency, but they also have drawbacks, such as high operational and chemical costs and secondary waste production (Jain et al., 2017). Biological methods have the advantages of low cost and easy application (Shannon et al., 2008). 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 (Qiao et al., 2020).

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* (Khleifat, 2006; Paulo et al., 2017; Najim et al., 2022). According to Budnik et al. (2016), ether cleavage is the primary method of aerobic degradation of SLES. This process produces intermediate compounds that can undergo additional degradation and release sulfate. A further potential mechanism involves the direct cleavage of the AES ester to split the sulfate, which occurs prior to the carbon body's degradation (Hales et al., 1986).

This study aimed to isolate and assess the efficiency of an aerobic bacterial strain that can degrade SLES.

Materials and Methods

Chemicals and media

SLES and other biochemicals were purchased from Sigma (USA), and acetone was used to dissolve SLES and create a stock of 1,000 mg/L. To be used, 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 Singh et al. (2007). The composition of mineral salt medium (MSM) was prepared according to Miller (1972).

Isolation and screening of bacteria that degrade SLES

Samples were taken from three sewage treatment plants that were contaminated with surfactant (in December 2022), which have been given numbers (1, 2, and 3) belonging to Al-Kut City, which is located in the Wasit Governorate/Iraq. For enrichment, sewage samples (10 mL) were added to 250 mL Erlenmeyer

flasks containing 150 mL MSM supplemented with 50 mg/L SLES for enrichment. The flasks were then incubated for five days at 150 rpm and 30°C (Asok and Jisha, 2012). The pour plate method was used for the isolation of bacteria using nutrient agar from the last enrichment culture, according to Andrew et al. (2002).

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 as the only source of energy and carbon, as stated by Ghose et al. (2009).
2. Secondary screening was carried out via cultivation on solid (MSM) that were incubated at 30°C for 24 to 96 hours, with SLES serving as the only source of energy and carbon at concentrations between 200 to 2,000 mg/L. The growth of bacterial isolates was based on the bacteria's growth on solid mineral salt media (Singh et al., 2007; AlMamoory and Al-Mayaly, 2017).

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 gram-negative and/or gram-positive bacteria (Harley, 2016). The isolated bacteria were diagnosed by PCR technology, and amplification was done using forward primer 5'-AGA GTT TGA TCM TGG CTC AG - 3' and reverse primer 5'-TAC GGY TAC CTT GTT ACG ACT T -3'. Molecular evolutionary analyses of SLES degrading bacteria based on 16S r-RNA genes were conducted using MEGA version 4 (Tamura et al., 2007).

Laboratory experiment

Sets of 250 mL Erlenmeyer flasks holding 100 mL of the medium were prepared, the pH was adjusted to 7 and autoclaved at 121°C and 1.5 bar for 15 min, then sets of SLES with three concentrations (25, 50, and 100 mg/L) added to flasks, then added inoculum of *Pseudomonas aeruginosa* that was isolated and incubated after being 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 each two days (Abboud et al., 2007). The experiment was repeated for pH 5 and 9 at three different temperature degrees (30, 35, and 40°C) for 15 days, optical density was measured for each one, then 5 mL of the mixture was taken to test the biodegradation efficacy by HPLC analysis.

High-Performance Liquid Chromatography (HPLC)

The concentrations used in the current study were 25, 50, and 100 mg/L, which were measured according to Equation 1.

$$C1 \times V1 = C2 \times C2 \quad (1)$$

HPLC was used to determine the SLES content, and the following equations were used to calculate the percentage of SLES biodegradation (Equations 2 and 3) (Shu and Ding, 2005):

$$\text{Percentage of biodegradation} = \frac{\text{conc. of standard} - \text{conc. of sample}}{\text{conc. of standard}} \times 100 \quad (2)$$

or

$$\text{Percentage of biodegradation} = \frac{\text{beak area of standard} - \text{beak area of sample}}{\text{beak area of standard}} \times 100 \quad (3)$$

Results and discussion

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. *Pseudomonas aeruginosa*, were isolated, purified, and selected based and selected based due to their capability to use SLES as a source

of carbon. The bacterial isolate *P. aeruginosa* was identified based on the nucleotide sequence of the 16S rRNA gene (PCR technology).

Impact of temperature and pH on the growth of *Pseudomonas aeruginosa*

Surfactant biological degradation is typically influenced by a number of factors. It is well known that the rate of degradation is affected by environmental factors such as temperature, pH, shaking, and nutrient availability (Abou-Zeid et al., 2001). The current study examined the effects of temperature and pH on SLES degradation. The results presented in Table 1 demonstrate that notable differences exist in the growth of bacteria at various temperatures and pH levels. The best and most significant means of bacterial growth under 30, 35, and 40°C recorded at pH 7 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°C were 0.360, 0.410, and 0.370 nm, respectively. The mean value of *P. aeruginosa* growth was highest at 30°C and pH 7, which was 0.410 nm, and lowest at 40°C and pH 5, which was 0.081 nm (Figures 1 and 2). Accordingly, the optimal degradation occurs at a temperature of 30°C for isolated bacteria.

Table 1. The mean value of *P. aeruginosa* growth at various temperatures and pH degrees after incubation for 15 days and the LSD value was 25 mg/L of SLES.

Temperatures	pH			LSD value
	pH 5	pH 7	pH 9	
30°C	0.360 ± 0.085	0.410 ± 0.157	0.370 ± 0.165	0.220 NS
35°C	0.205 ± 0.126	0.366 ± 0.121	0.282 ± 0.114	0.122*
40°C	0.081 ± 0.021	0.110 ± 0.024	0.091 ± 0.028	0.053 NS
LSD value	0.086*	0.119 *	0.138 *	---

*(p<0.05), NS: non-significant.

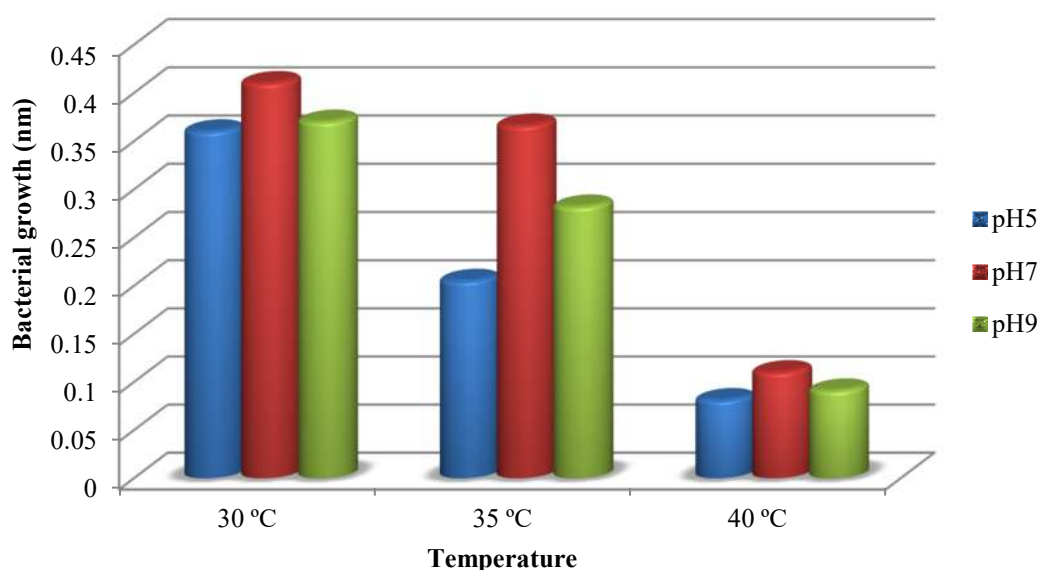


Figure 1. Mean value of *P. aeruginosa* growth at various temperatures and pH values after incubation for 15 days.

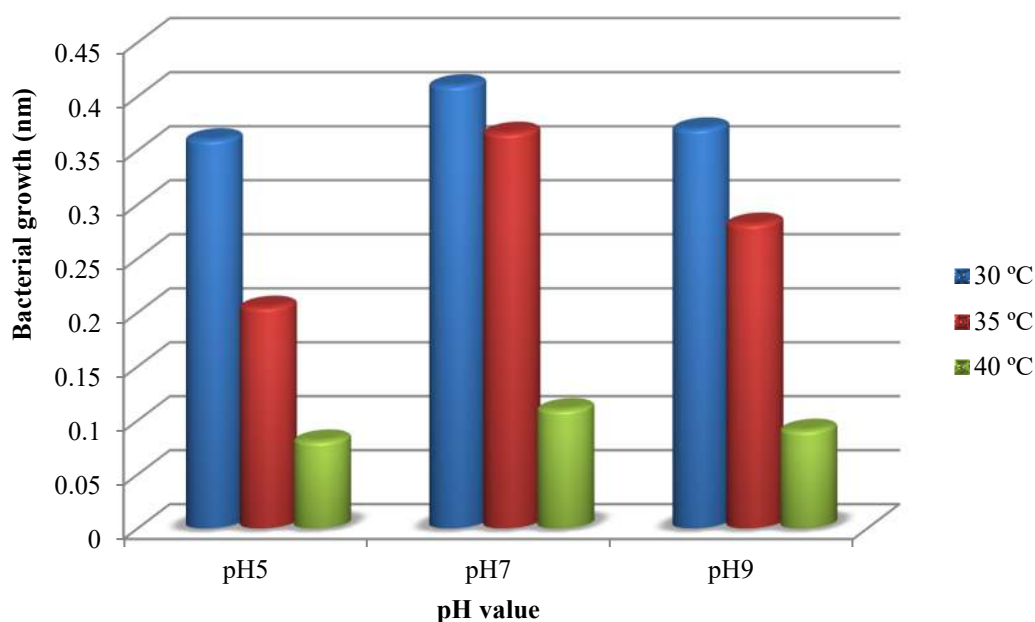


Figure 2. Mean value of *P. aeruginosa* growth at various pH values and temperatures after incubation for 15 days.

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* (Schulz et al., 2000; Dhouib et al., 2003; Khleifat, 2006; Abboud et al., 2007). Temperature and microbial activity were found to be directly correlated during SLES degradation. In addition to potentially affecting growth, a temperature increase above the threshold point of 37°C may also make microbial membranes more toxic. According to the data, 30°C would be the ideal temperature for better SLES degradation, as confirmed by HPLC. In general, temperature influences the SLES compound's chemical and physical characteristics, microbial metabolism, the specific growth average of microorganisms, the rate of the oxidation process's enzymatic activity, and the makeup of the microbial community, all of which have an impact on the SLES biodegradation rate (Abboud et al., 2007).

The degradative activity of bacteria is significantly influenced by pH (Wong et al., 2002). The results indicated that the ideal pH for *P. aeruginosa* growth in MSM containing SLES is 7; this conclusion is corroborated by the findings of Amirmozafari et al. (2007), 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. Dhouib et al. (2003) 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 (Prats et al., 2006).

Impact of incubation time on biodegradation and growth

Table 2 displays the results, which show a significant difference ($p < 0.05$) between the bacterial growth means for various incubation times. After the fifteenth day of incubation, the selected bacteria isolated showed that the highest mean growth was on the ninth day (0.540 nm), and the lowest growth was on the first day (0.230 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 3).

Table 2. Average values \pm standard deviation of bacterial growth at 600 nm at various incubation times and LSD value.

Incubation period Days	Mean \pm SD of bacterial growth <i>P. aerogenosa</i>
First Day	0.230 \pm 0.015
Third Day	0.370 \pm 0.012
Fifth Day	0.420 \pm 0.012
Seventh Day	0.495 \pm 0.010
Ninth Day	0.540 \pm 0.015
Eleventh Day	0.465 \pm 0.010
Thirteenth Day	0.410 \pm 0.021
Fifteenth Day	0.350 \pm 0.031
LSD \leq 0.05	0.029

SLES degradation significantly increased as the incubation times increased. According to Hosseini et al. (2007), after 120 hours, *Pseudomonas beteli* was able to degrade 94% of the SDS levels. It is commonly

known 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 causes a lower media pH and subsequently inhibit bacterial growth (Naveenkumar et al., 2010).

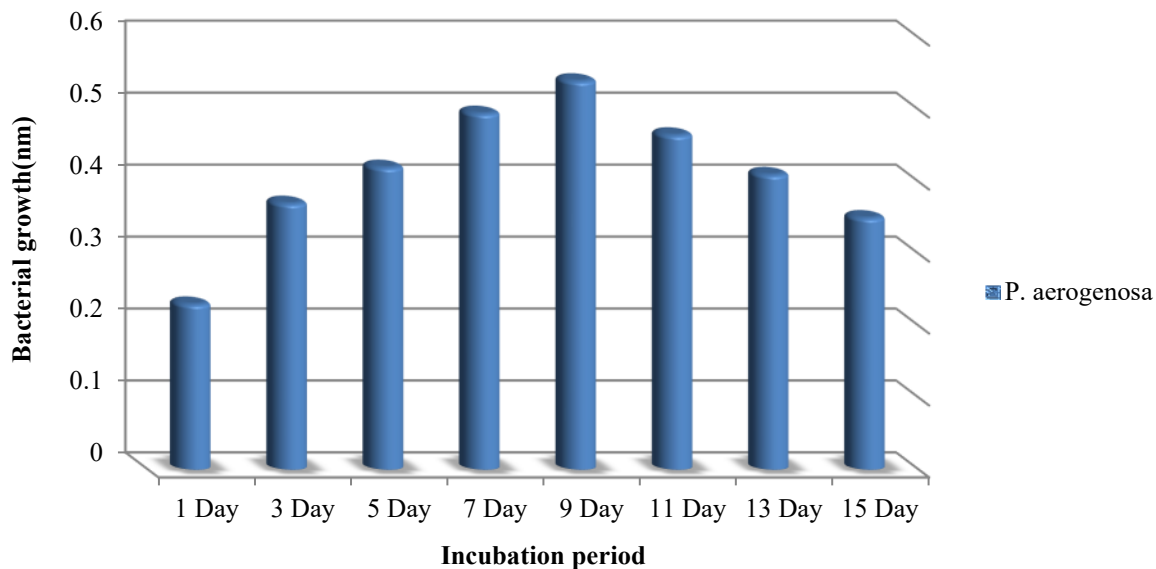


Figure 3. The average value of bacterial growth over various incubation times.

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 salts medium at three different temperature degrees and pH. These samples were analyzed with HPLC system (Fedeila et al., 2018). An analysis of a standard

solution of surfactant was done, where the concentration of the standard solution was 10 mg/L, and the beak area was (1,025.5) per (5.90) minute. The comparing figures of SLES were done according to the concentrations used in the study (Figure 4). The results of the HPLC analysis are mentioned in Table 3, which explains the concentrations of SLES, beak area of samples, percentage of removal, and remaining concentration for each sample.

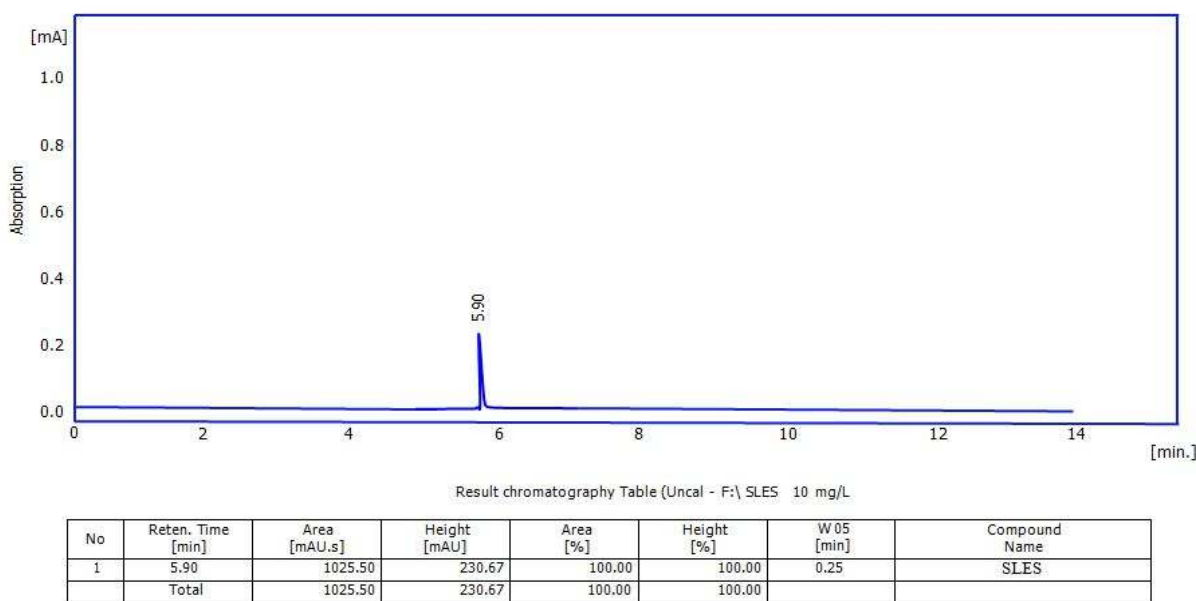


Figure 4. Beak area for 10 mg/L of SLES.

Table 3. Beak area and removal percent of samples.

Temperature (°C)	Concentration of SLES (mg/L)	pH	Beak area (mAU.s)	% removal	Remaining concentration (mg/L)
30	25	9	93.27	96.36	0.91
		7	40.05	98.44	0.39
		5	205.61	91.98	2.005
	50	9	763.48	85.1	7.45
		7	460.95	91	4.5
		5	1,384.01	73	13.5
	100	9	2,947.06	71.26	28.74
		7	2,152.33	79	21
		5	3,691.22	64	36
35	25	9	332.25	87.04	3.24
		7	230.52	91	2.25
		5	509.85	80	5
	50	9	1,281.45	75	12.5
		7	871.57	83	8.5
		5	1,583.06	69.12	15.44
	100	9	3,794.05	63	37
		7	2,661.99	74.04	25.96
		5	5,112.05	50.15	49.85
40	25	9	2,075.12	19.08	20.23
		7	2,047.18	20.15	19.96
		5	2,127.58	17	20.75
	50	9	4,296.81	16.2	41.9
		7	4,186.05	18.36	40.82
		5	4,409.55	14	43
	100	9	8,818.75	14	86
		7	8,603.46	16.1	83.9
		5	9,023.46	12	88

In Table 3, 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 5) 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 6).

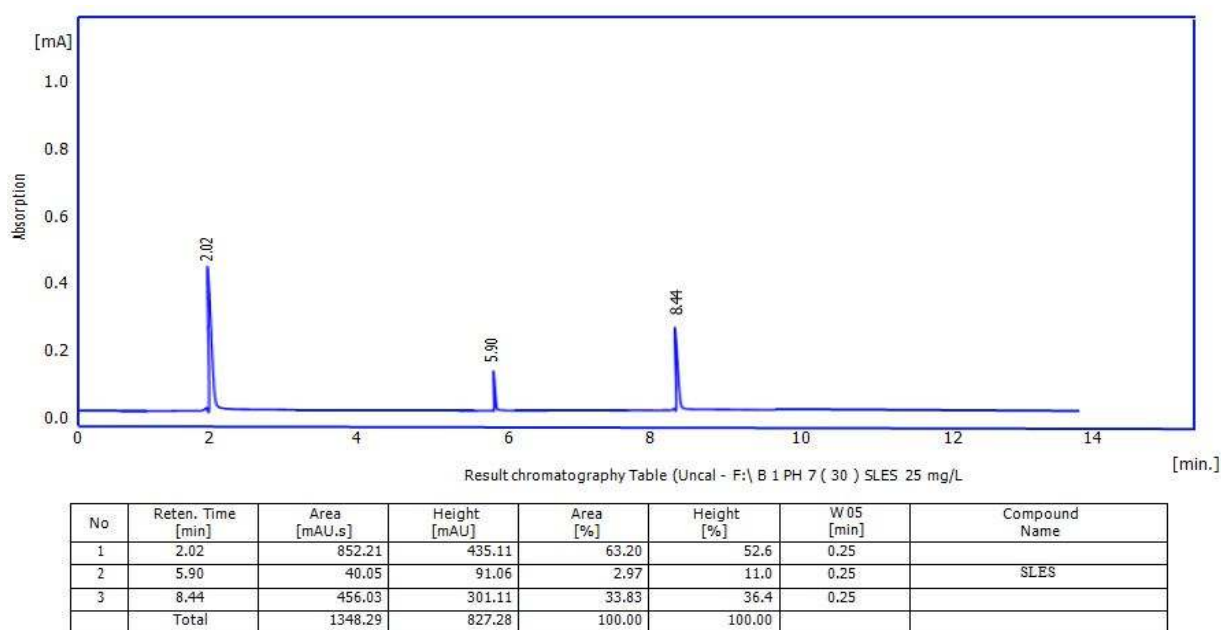


Figure 5. The highest removal of SLES.

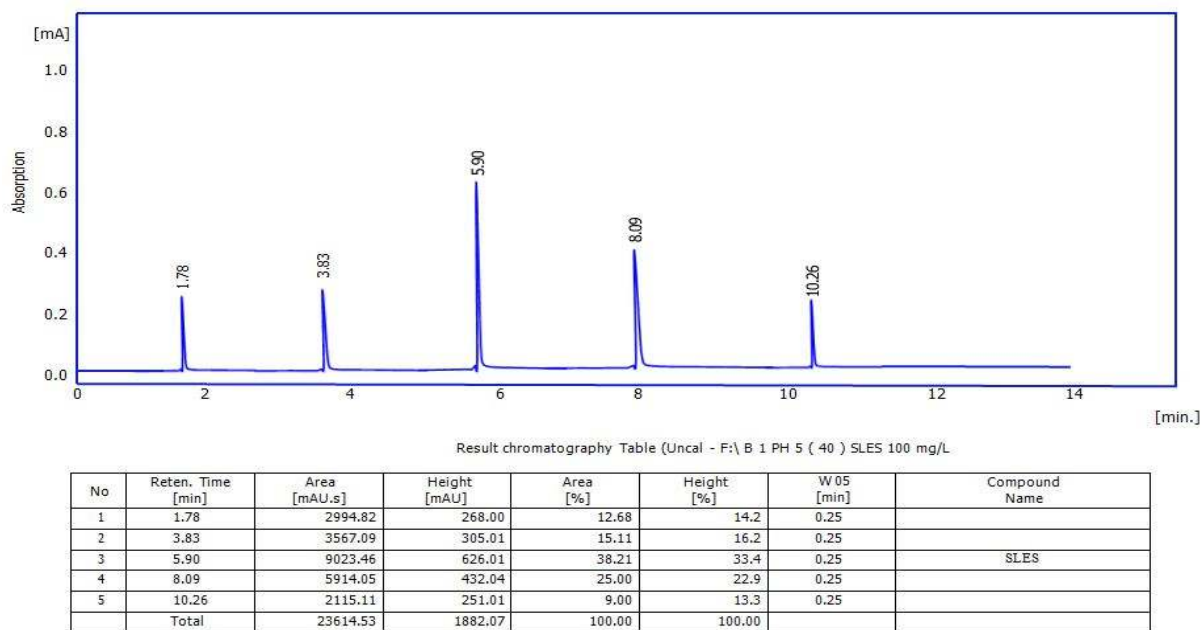


Figure 6. The lowest removal of SLES.

This means that the higher the concentration of SLES and the lower the pH value, the less efficient this bacterium is in degrading the surfactant. The degradation percentage increased with 25 mg/L of SLES and reduced at 100 mg/L, Fedeila et al. (2018), which shows more efficiency degrading at a neutral degree of pH; however, at acidic pH, with nearly 50% degrading rate, while at alkaline pH gave nearly 80% degrading rate at different concentrations and amounts of surfactant. Also, Asok and Jisha (2012) showed that neutral environments cause more degradation than alkaline and acidic environments because in acidic environments, the stability of bonds increases. Numerous bacterial strains that are able to break down comparable substances have been isolated from environments contaminated with surfactants (Peressutti et al., 2008).

In this study, high levels of degradation were attained with low concentrations of SLES. The percentage of SLES degradation of *P. aeruginosa* at a concentration of 25 mg/l was 98.44%, while it was 91% and 79 % for concentrations of 50 and 100 mg/L, respectively. 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 (Yadav et al., 2001).

SLES 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 microorganisms' viability will eventually decline

(Odahara, 2004; Abboud et al., 2007). The *P. aeruginosa* bacteria were able to degrade SLES with good efficiency, indicating that this bacterium presents a good option for SLES disposal from these contaminated environments.

The results agreed with Dhouib et al. (2003), Khleifat (2006), Chaturvedi and Kumar (2010), and Anaukwu et al. (2016), which used bacteria to degrade SLES and showed 75-100% percentage of degradation to SLES explained by HPLC analysis. The slight difference in the percentage of biodegradation is due to the efficiency of the type of bacteria, the isolated strain type, and its adaptation to the environment (Ran et al., 2016).

Conclusion

In this study, SLES biodegradation at aerobic conditions using isolated microbial cells was investigated from wastewater plants. A SLES-degrading bacterium was isolated from an Iraqi water sample that was contaminated with SLES. In order to promote a higher degradation rate and enable the application of the optimized parameters in the field, growth optimization studies involving the isolate were conducted on a variety of physicochemical parameters. *Pseudomonas aeruginosa*, which has a strong SLES degradation ability, was isolated, purified, and selected based on its capability to use SLES as a source of carbon and enrich it from sewage. The bacterial isolate was identified based on the nucleotide sequence of the 16S rRNA gene (PCR technology). The strain's growth characteristics demonstrated that it could degrade more than 80% of SLES at concentrations less than 100 mg/L. The optimal values of temperature and pH for biodegradation were 30°C and pH 7.

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