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ORIGINAL ARTICLE

Biodegradation of Anionic Surfactants by *Klebsiella planticola* isolated from Hospital Wastewater (Case Study: ShahidBeheshti Hospital in Abadan City)

Ghodous Fathi*[1], Reza Jalilzadeh Yengejeh[2], Esmaeil Kohgerdi[3], Farzaneh Janmohammadi[4]
[1] Department of Environmental Engineering, Bushehr Branch, Islamic Azad University, Bushehr, Iran, Email: g_fathi1982@yahoo.com
[2] Department of Environmental Engineering, Khuzestan Science and Research Branch, Islamic Azad University, Ahvaz, Iran
[3] Department of Environmental Engineering, Bushehr Branch, Islamic Azad University, Bushehr, Iran
[4] Department of Environmental Engineering, Khuzestan Science and Research Branch, Islamic Azad University, Ahvaz, Iran

ABSTRACT
Iranian healthcare centers use large volumes of detergents every day. Alkylbenzene sulfonate is a compound widely used in detergents as an ionic surfactant and, if carelessly used, can inflict irreparable damages to the environment and human health if carelessly used. In this, native bacteria in wastewater of Shahid Beheshti Hospital in Abadan were enriched in a mineral culture medium, from which Klebsiella planticola bacteria were isolated and identified. These bacteria were identified using biochemical tests, and their efficiency in removing Alkylbenzene sulfonate was evaluated by employing a model DR 5000 spectrophotometer and using the methylene blue method. Results showed these bacteria could remove 81.5% of alkylbenzene sulfonate within 96 minutes (and 100% of it in 120 minutes) at 40˚C, pH of 8, nitrogen content of 0.5 mg and carbon content of 10 mg.
Keywords: *Ionic surfactant, Alkylbenzene sulfonate, hospital wastewater*

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INTRODUCTION

Hospital wastewater is similar to urban wastewater in quality, but may also contain materials and compounds that can easily reach water resources and may have negative effects on human and environmental health. Therefore, its management is of great importance [1]. Detergents account for a large part of materials used at healthcare centers and surfactants, their main ingredient, are used because of their great wetting and emulsifying power. If hospital wastewater is discharged into the environment, these surfactants will damage aquatic organisms, pollutes water, and endangers human health. Therefore, supervision over, and control of, surfactants in the environment is very important [2]. Among ionic surfactants, Alkylbenzenesulfonate is used more than others due to its greater cleaning power and because of its relatively lower cost. Due to their high foaming capabilities which can cause numerous problems in sewage treatment facilities as well as direct toxic effects on many different organisms in ecosystem; they are generally considered as serious pollutants [3].

Removal of surfactants includes processes such as chemical and bioelectrochemical oxidation, membrane technology, chemical precipitation, photocatalytic degradation, adsorption and biological methods, each of which has its own advantages and disadvantages. The active sludge process can remove 94 to 96% of alkylbenzenesulfonate [4]. The reverse osmosis method, while being very efficient in removing pollutants, is very costly [5]. Chemical methods generally separate pollutants from solutions and transfer them onto adsorbents or into sludge, the consequent disposal of which will be accompanied by environmental

problems. Employment of microorganisms is one of the useful technologies for treatment of wastewater and removal of various pollutants [6], and can be an appropriate and cost effective substitute in removing chemical and microbial pollutants [7]. Extensive research on isolating bacteria and determining their capability has proved their ability in removing pollutant compounds, and has revealed that even dead microbial cells can be useful in biotreatment technologies [8]. Because of their greater resistance to organic compounds, and due to their ability to use these substances as their carbon source, bacteria are well adapted to these compounds and are very suitable for biotreatment [9].

In designing systems for wastewater treatment at hospitals, low cost, high treatment rates, and high efficiency levels must be considered, and these systems should not require chemical materials or specialist operators [10]. Previous research in this area has indicated that treatment with the help of microorganisms meets these requirements well. Rajan et al. noticed *Bacillus subtilis* isolated from soils polluted by petroleum were very efficient in removing hydrocarbon substances [11]. In research conducted by Vilma Cipyinyte et al., Arthrobacter sp. N3 isolated from urban wastewater were able to remove 87.5% of fats and oils within seven days. This research investigated the feasibility of removing surfactants by native bacteria isolated and purified from wastewater at Shahid Beheshti Hospital in Abadan, and various factors were studied at three levels to determine the maximum degree of pollutant decomposition by these bacteria.

MATERIAL AND METHODS

This study aimed at isolating and purifying indigenous bacteria in the wastewater of Shahid Beheshti Hospital treatment plant in Abadan, which are capable of removing anionic surfactants. This research was carried out in seven steps including sampling, enrichment, isolation and purification of bacteria, identification using culturing, reproduction, determination of bacteria efficiency in removing organic materials, and determination of optimal conditions in bacteria growth.

Sampling:

Two 50ml samples were taken from the aerated chamber and settling basin of the active sludge system of ShahidBeheshti Hospital in Abadan. Totally, three samples were taken at 7:30 AM, 10:00 AM, and 13:00 PM; because Abadan is located in a hot and humid region, its temperature fluctuation during the day and the time the research was carried out was between 25°C in the early morning to 50°C in the middle of the day and the activity of different parts of the hospital during a day, which increases or decreases mineral load imposed on the power plant was taken into account.

Bacteria Isolation and Enrichment:

The samples were taken to the laboratory and they were mixed under laboratory conditions. The culture medium used here was a mineral culture medium with 0.5gr K_2HPO_4,1.5gr KH_2PO_4, 0.5gr NaCl, 0.5gr NH_4Cl, 0.14gr Na_2So_4, 0.15gr $MgCl_2.6H_2O$ compounds. After preparing the culture medium, its pH was adjusted to 40 g/l sodium hydroxide (1 M) and it was sterilized in an autoclave at the temperature of 121°C and pressure of 15 Psi for 15 minutes. Alkylbenzenesulfonate, which is an anionic surfactant, was used in this culture medium as the only source of carbon [12]. 1ppt stokes were prepared from the whole compound by solving 10 mg of each compound in 10 ml sterile distilled water [6]. After cooling the culture medium at the room temperature up to 45°C, one ml of the sample was taken and added to it; and then it was kept in a shaking incubator at 30°C and 150 RPM for six days. At the end of this period and observance of the turbidity caused by bacterial growth, one milliliter was taken and added to the new culture medium [13]. These steps were repeated three times. Finally, 0.5 ml of the bacteria-containing culture medium was diluted five times in the final step of enrichment and dipped into a SMS culture medium containing 1% agar to purify the bacteria. It was then put in a normal incubator at 37°C for 72 hours.

Bacteria Identification:

Biochemical tests were used for identifying the bacteria (14). The bacteria were reproduced in the SMS culture medium containing alkylbenzenesulfonate and they were kept in the shaking incubator for six days at 30°C and 150 rpm. To prepare bacterial suspension, the bacteria reproduced in the centrifuge at 3000 RPM for 10 minutes were respectively removed from the culture medium, transferred to the SMS culture medium, and counted using dilution method.

Determination of Bacterial Growth Rate and Analysis of AlkylbenzeneSulfonate by Bacteria:

The methylene blue method described in MBAS, Version 8 [15]. An assay for nonionic surfactants in environmental samples was used. An optical spectrophotometer at a wavelength of 650 nm was used for determining degradation rate of alkylbenzenesulfonate during six days. Five factors were studied at three levels to determine optimal conditions. An optical spectrophotometer at a wavelength of 600 nm was used for determining bacteria growth. The cells used in this stage were made of compressed plastic with

3ml volume. In every measurement, 0.6 ml of the bacterial suspension was diluted by 2.4 ml of the sterile SMS culture medium. The optical spectrophotometer was calibrated in an SMS culture medium free from bacteria.

RESULTS AND DISCUSSION

Alkylbenzenesulfonate degradation and bacterial growth

After taking samples from wastewater at Shahid Beheshti Hospital in Abadan and enriching the bacteria, *Klebsiella planticola* bacteria were purified. Results showed these bacteria could remove 81.5% of alkylbenzenesulfonate in 96 minutes and 100% of it within 120 minutes (Diagram number 2). Similar studies by previous researchers indicated the ability of these bacteria in removing various pollutants. In a study conducted by MilvaPepi, a Klebsiella species isolated from a mixture of olive mill waste was able to remove 40 to 80% of tannic acid within 24 hours [16].

Study of bacterial growth in culture media containing alkylbenzenesulfonate compared to culture media lacking this compound indicated these bacteria used alkylbenzenesulfonate as a carbon source (Diagram number 3).

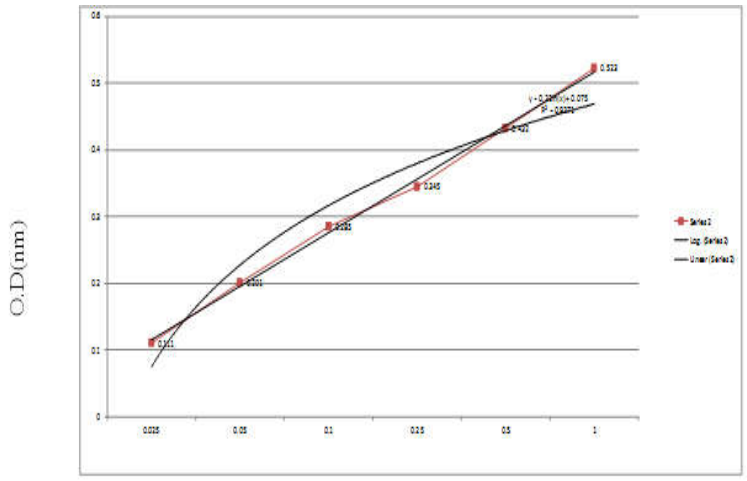
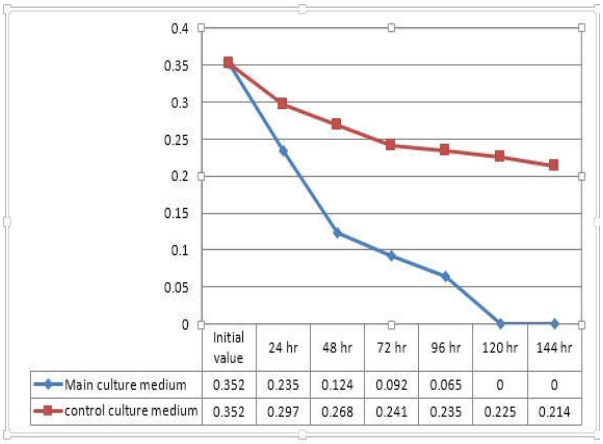


Diagram 1. Drawing of a calibration curve to restore alkylbenzenesulfonate concentrations



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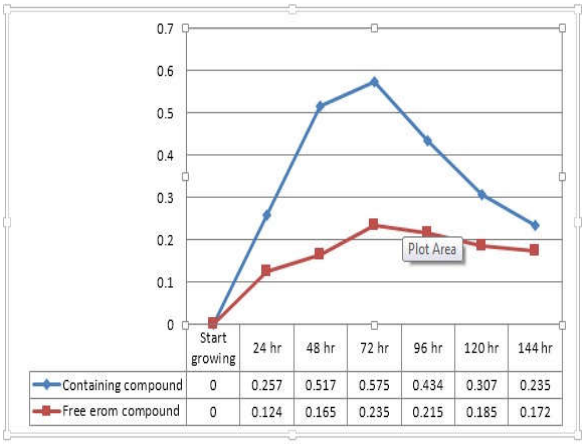


Dig 2

Diagram 2. Changes of alkylbenzenesulfonate concentrations by *Klebsiellaplanticola*

Diagram 3. The growth curve of *Klebsiellaplanticola*at PPM10 concentration of alkylbenzenesulfonate compound

Results related to optimal conditions for the bacteria

Table 1 shows results of bacterial identification by biochemical tests. As shown in the table, these Gram-negative, rod-shaped, and facultative aerobic bacteria were *Klebsiella planticola*.

Table (1) The results of identification of bacteria using biochemical tests

Lysine	Lactose	Oxidase	Indole	SIM	TSI	S.C	MR/VP	U	Bacteria name	The bacteria tested	Row
+	+	-	-	$S^{-}I^{-}M^{-}$	A/A+Gas	-	- +	-	*Klebsiellaplanticola*	E_3	2

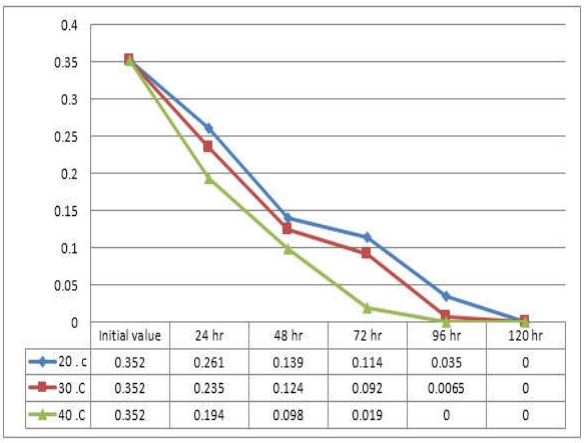
Effects of temperature and pH

These bacteria were most active at 40˚C, and their activity considerably declined at lower temperatures and reached its minimum at 20˚C. The type of weather conditions in Abadan and the adaptation of the bacteria to these weather conditions were probably the reasons for the obtained results (Diagram number 4). *Klebsiellaplanticola* bacteria removed maximum alkylbenzenesulfonate at pH = 8, and the degree of removal decreased at lower pH values and reached its minimum at pH=5 (Diagram number 5). K Sivashanmugam et al. isolated *Klebsiella pneumoniae* from tannery effluent of leather industries, and studied their activity in tannin removal in the pH and temperature ranges of 4-8 and 30-60˚C, respectively. Results indicated these bacteria exhibited their highest efficiency at pH = 7 and temperature of 37˚C (17).Research by Jadhav, U. et al. yielded similar results (18). The *Klebsiella* bacteria they isolated from soil samples were able to remove 98% of tannic acid within 40 hours at pH=7 and temperature of 35˚C.

Nitrogen and Carbon sources

Bacterial behavior during a period of six days in culture media containing NH4Cl at 0.25, 0.5, and 1 mg/l as the nitrogen source showed that *Klebsiellaplanticola* bacteria were most active in removing alkylbenzenesulfonate at NH4Cl concentration of 0.5 mg/l (Diagrams 6 and 7). Furthermore, these bacteria were most efficient at removing alkylbenzenesulfonate (as the source of their carbon) when its concentration was 10 ppm, and their activity significantly declined when its concentration was raised to 15 ppm. Reduced bacterial activity with increases in the concentrations of carbon and nitrogen sources was probably due to the toxicity of these sources for the bacteria.

In another research, a *Klebsiella* species (*K. oxytoca*) that was isolated from soil samples and from a carwash facility wastewater was able to remove 90% of the ionic surfactant within three days. These bacteria were most efficient at removing this compound at pH=7.2, temperature of 37˚C, and nitrogen at 0.2g/l and carbon at 10 g/l (19).



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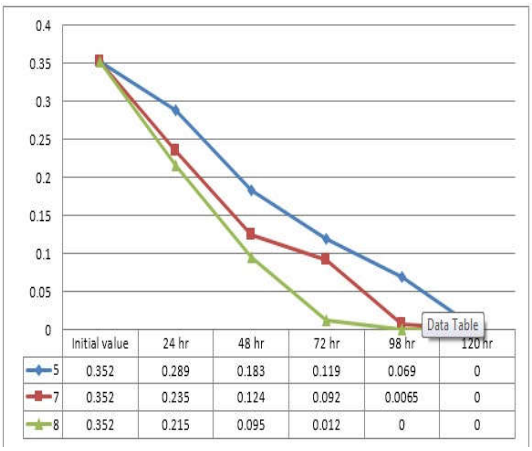
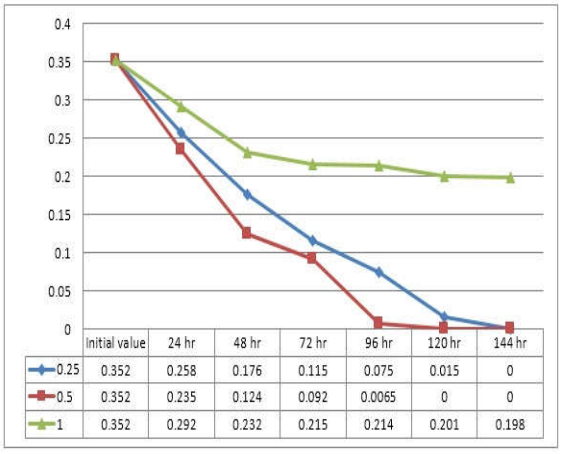


Dig5

Diagram 4. Comparison of alkylbenzenesulfonate removal rates at different temperatures using *Klebsiella planticola*

Diagram 5. Comparison of alkylbenzenesulfonate removal rates at different pHs using *Klebsiella planticola*



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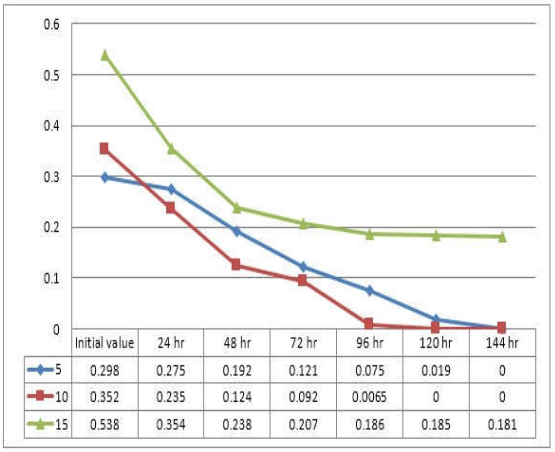

Dig7

Diagram 6. Comparison of alkylbenzenesulfonate removal in different values of nitrogen by *Klebsiella planticola*

Diagram 7. Comparison of alkylbenzenesulfonate removal in different values of alkylbenzenesulfonate by *Klebsiella planticola*

CONCLUSIONS

This research isolated and identified bacteria in wastewater of ShahidBeheshti Hospital in Abadan and studied their potential in biodegradation of alkylbenzenesulfonate. Biochemical tests identified the bacteria as *Klebsiella planticola*. These bacteria removed 81.5% of alkylbenzenesulfonate within 96 hours and 100% of it in 120 hours. The optimal conditions for *Klebsiellaplanticola* in removing alkylbenzenesulfonate were pH value of 8, temperature of 40˚C, and nitrogen at 0.5mg/l and carbon at 10 mg/l. Considering the results of the research, we suggest that in future research the same carbon to nitrogen ratio, temperature, and pH be used to obtain maximum bacterial growth.

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