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Bioremediation of anionic surfactants in hospital wastewater. Case study: Shahid Beheshti Hospital in Abadan City, Iran

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Abstract. Hospital wastewater contains many chemicals, which attenuate its microbial load and have negative effects on biological processes. Among them, anionic surfactants are highly regarded; because a large volume of them is consumed. In this study, the feasibility of removal of alkylbenzene sulfonate by these bacteria was studied using indigenous bacteria isolation in hospital wastewater in a mineral culture medium and their purification. Alkylbenzene sulfonate disintegration rate was determined by the extracted bacteria using a spectrophotometer through the methylene blue method at a wavelength of 650 nm. Research results led to purification of four bacterial species. Among them, *Enterobacter aerogenes* type II had maximum capability (95.7%) for removing this compound at 30°C, with a pH of 7, nitrogen concentration of 0.5 mg L^{-1}, carbon content of 10 mg L^{-1}, and retention time of 72 hours.
Key Words: anionic surfactant, hospital wastewater, alkylbenzene sulfonate, mineral culture medium.

Introduction. Hospitals and health centers in Iran consume a high volume of detergents every day, which flow into wastewater easily. They attenuate wastewater microbial load and have negative impacts on biological processes in treatment plants, which may threat environment and human health severely (Majlesi Nasr & Yazdanbakhsh 2008). These compounds reduce suspended solids removal in water, absorb pathogenic microbes, and cause difficulty in coagulation, settlement and filtration, eutrophication of rivers, and undesirable taste and odor of water (Ivanković & Hrenović 2010). They also reduce absorption of foodstuff in intestines, skin restorability, eczema skin, local erythema, skin allergy and denatured proteins of body skin (Abd El-Gawad 2014). Anionic surfactants – similar to alkylbenzene sulfonate - are extensively used today due to their low cost and high efficiency (Asok & Jasha 2012). Environmental impacts of these materials were detected in the USA in the early 1960 (Eniola 2012). Such compounds cause some problems in wastewater aeration and its treatment stages and reduce oxygenation potential by creating foam (Louvado et al 2010). At present, the method of activated sludge with extended aeration is employed for treating hospital wastewater in Iran (Majlesi Nasr & Yazdanbakhsh 2008). However, there are some doubts about the performance of these systems because of the type of the load imposed on treatment plants, which attenuate microbial load due to bactericidal properties.

Sequencing batch reactors are capable of removing 94% of detergents (Jokerst et al 2012), but these systems are not utilized much in treatment centers due to frequent need to continuous supervision and controls, maintenance, and wear of the parts. Literature showed that Fenton chemistry was able to remove 9.58% of anionic surfactants within 80 minutes (Rouhullah et al 2013). Yet, chemical methods generally isolate pollution from liquid; enter them into an absorbent or sludge, the disposal of which will subsequently cause many environmental problems. The use of microorganisms is one of the appropriate technologies for purifying different forms of wastewater and it

can be a suitable tool for treating types of pollutants (Moghbeli et al 2011; Louvado et al 2010). Earlier studies in this field proved that bacteria and even their dead microbial cells might be highly suitable in biodegradation technology (Syed Mohd et al 2010). Due to their resistance to mineral compounds and their use as a source of carbon, bacteria cope with these compounds well and they are very suitable for biodegradation (Louvado et al 2010). Low cost, high speed of treatment, high efficiency of treatment, and no need for chemicals and skilled labors should be considered in designing systems for hospital wastewater treatment (Jafrudeen & Ahsan 2012). Literature showed that purification using microorganisms satisfies these conditions well. *Bacillus subtilis* bacteria isolated from oil-contaminated soil showed a considerable ability in removing hydrocarbon compounds (Rajan 2010). *Arthrobacter* sp. N3 bacterium isolated from municipal wastewater was capable of removing 5.87% of oil and fat within seven days (Čipinytė et al 2009). In this study the indigenous bacteria in wastewater of Shahid Beheshti Hospital in Abadan, which were resistant to the toxicity of alkylbenzene sulfonate compound, were enriched; and their ability to remove this compound and different factors effective in their optimal growth were assessed.

Material and Method. This study aimed at isolating and purifying indigenous bacteria in the wastewater of Shahid Beheshti Hospital treatment plant in Abadan, Iran which are capable of removing anionic surfactants. This research was carried out between May and September 2014 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 50 mL samples were taken from the aerated chamber and settling basin of the active sludge system of Shahid Beheshti 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 of oil college in Abadan city and they were mixed under laboratory conditions. The culture medium used here was a mineral culture medium with 0.5 g K_2HPO_4, 1.5 g KH_2PO_4, 0.5 g NaCl, 0.5 g NH_4Cl, 0.14 g Na_2SO_4, 0.15 g $MgCl_2.6H_2O$ compounds. After preparing the culture medium, its pH was adjusted to 40 g L^{-1} 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. Alkylbenzene sulfonate, which is an anionic surfactant, was used in this culture medium as the only source of carbon (Amirmozafari et al 2007). One ppt stokes were prepared from the whole compound by solving 10 mg of each compound in 10 mL sterile distilled water (Moghbeli et al 2011). 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, 1 mL was taken and added to the new culture medium (Chaturvedi & Kumar 2010). 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 salt mineral (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 (Patrao et al 2012). The bacteria were reproduced in the SMS culture medium containing alkylbenzene sulfonate 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 alkylbenzene sulfonate by bacteria. The methylene blue method described in MBAS, Version 8, i.e. an assay for nonionic surfactants in environmental samples, was used (Shahbazi et al 21013). An optical spectrophotometer at a wavelength of 650 nm was used for determining degradation rate of alkylbenzene sulfonate 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 3 mL 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. Table 1 shows the results of identifying bacteria through biochemical tests. The results showed that this is a gram-negative, rod-shaped, facultative aerobic bacterium. It was an Enterobacter aerogenes bacterium type II.

Table 1

The results of identification of bacteria using biochemical tests

Lysine	*Lactose*	*Oxidase*	*Indole*	*SIM*	*Triple Sugar Iron*
-	-	-	-	-	ALK/ALK
+	+	-	-	$S^-I^-M^-$	A/A+Gas
	-	+	-	$S^-I^-M^-$	A/A

Simmons Citrate	*Methyl Voges-/ red Proskauer*	*Urea*	*Bacteria name*	*The bacteria tested*	*Row*
-	-	-	95% *Pseudomonas*	E_1	1
-	- +	-	*Klebsiella planticola*	E_3	2
+	+ -	-	*E. aerogenes* bacterium type II	E_4	3

Results of alkylbenzene sulfonate disintegration rate and bacterial growth. Calibration curve to restore alkylbenzene sulfonate concentrations is shown in Figure 1. Totally, three bacterial colonies were isolated and purified in the SMS culture medium after sampling and enriching wastewater of Shahid Beheshti Hospital in Abadan. Among them, strain E_2 showed considerable removal efficacy of alkylbenzene sulfonate compound, as it diminished its amount to about 95.7% during 72 hours and brought its content to zero within 96 hours (Figure 2). Comparing biological removal of alkylbenzene sulfonate compound by the three bacteria is shown in Figure 3.

Hosseini et al (2007) isolated *Acinetobacter johnsonii* bacterium. They showed the removal efficacy of anionic surfactants as much as 6.093%. Chaturvedi & Kumar (2010) isolated *Pseudomonas alcaligenes and P. mendocina* bacteria from municipal wastewater, which were able to remove respectively 99% and 98% of anionic surfactants. *Klebsiella* sp. was capable of disintegrating 80% of anionic surfactants within four days. It could remove 100% of the compound within ten days (Patrao et al 2012).

Studying growth rate of the bacterium in a mineral culture medium containing 10 PPM of alkylbenzene sulfonate compound and its modest growth in a culture medium free from alkylbenzene sulfonate as compared with the culture medium containing bacteria indicate that the bacterium uses this compound as a carbon source (Figure 4).

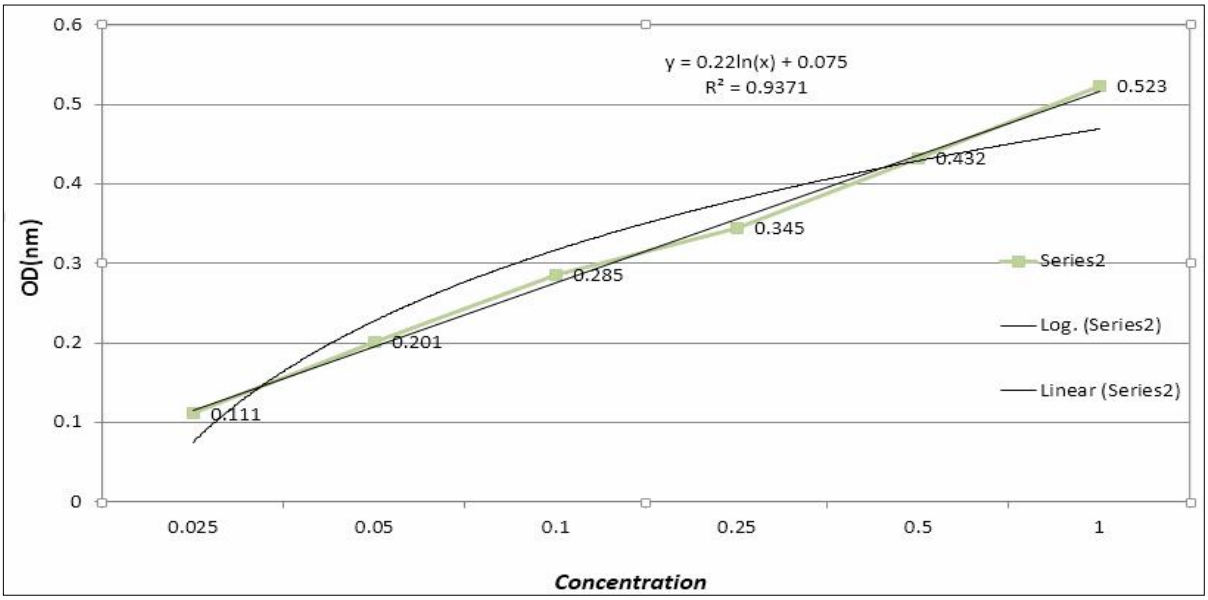

Figure 1. Drawing of a calibration curve to restore alkylbenzene sulfonate concentrations.

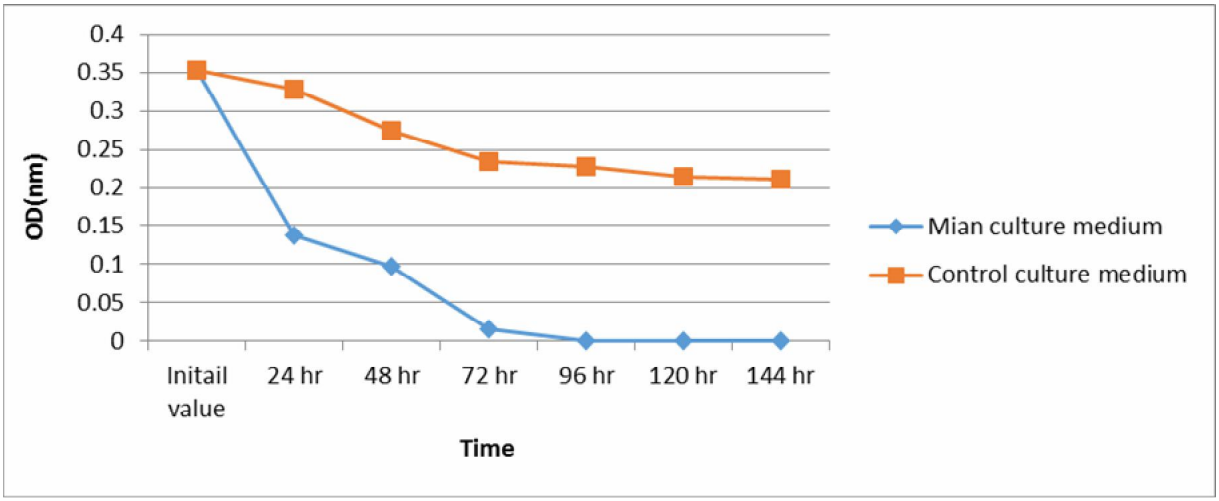

Figure 2. Changes of alkylbenzene sulfonate concentrations by *E. aerogenes* bacterium type II.

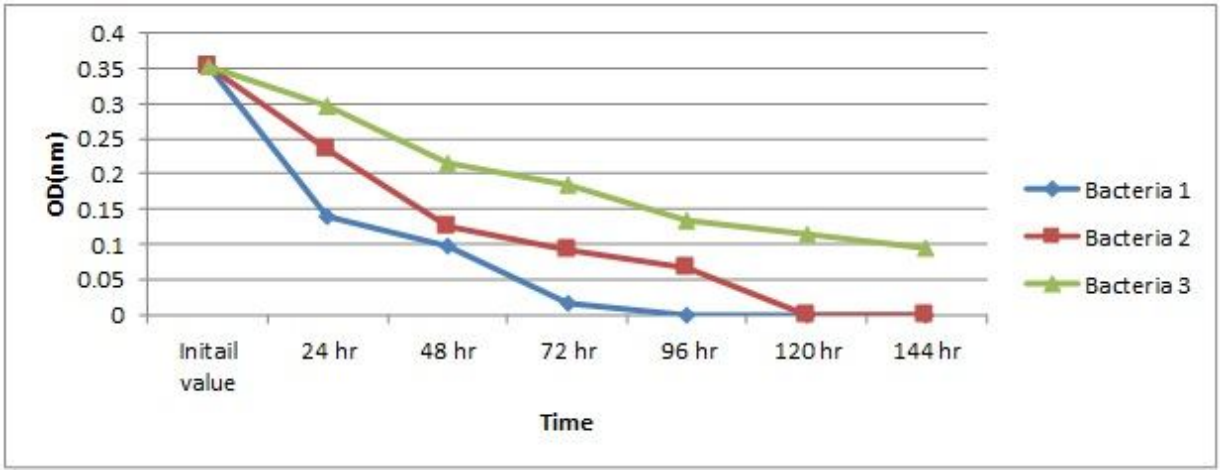

Figure 3. Comparing biological removal of alkylbenzene sulfonate compound by the three bacteria.

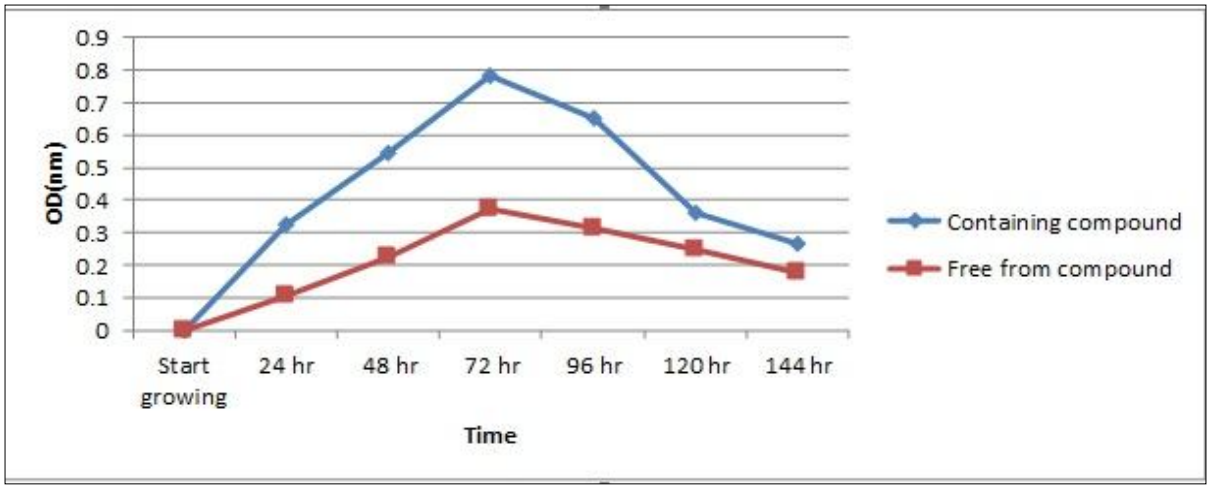

Figure 4. The growth curve of *E. aerogenes* bacterium, type II at Ppm10 concentration of alkylbenzene sulfonate compound.

Impacts of temperature and pH. The bacterium exhibited further efficacy at 30°C. Activity of the bacterium increased considerably with decrease in the temperature, as it reached the minimum amount at 20°C. Weather conditions of Abadan city and adaptability of the bacterium to such weather conditions might be a reason for the results (Figure 5). Maximum and minimum rates of alkylbenzene sulfonate removal by the bacteria occurred at pH = 7 and pH = 5, respectively (Figure 6). Similar studies on the bacterium in removing different pollutants showed varied results of the optimal values of temperature and pH. Buranasilp & Charoenpanich (2011) showed a considerable reduction in removing acrylamide by *E. aerogenes* bacterium at a temperature less than 25°C. The highest rate of removal in this study occurred at pH between 6 and 9. However, pH = 7 and temperature = 25-30^{o}C was the best condition for removing pyrethroids pesticide by the bacterium in the study of Liao et al (2009). *Pseudomonas mendocina* bacterium in the study of Peressutti et al (2008) and Klebsiella oxytoca bacterium in the study of Shukor et al (2009) exhibited the highest removal efficacy of anionic surfactants at 37°C. Amirmozafari et al (2007) showed the highest efficiency of *Pseudomonas aeruginosa* bacterium in removing anionic surfactants at pH = 7.4.

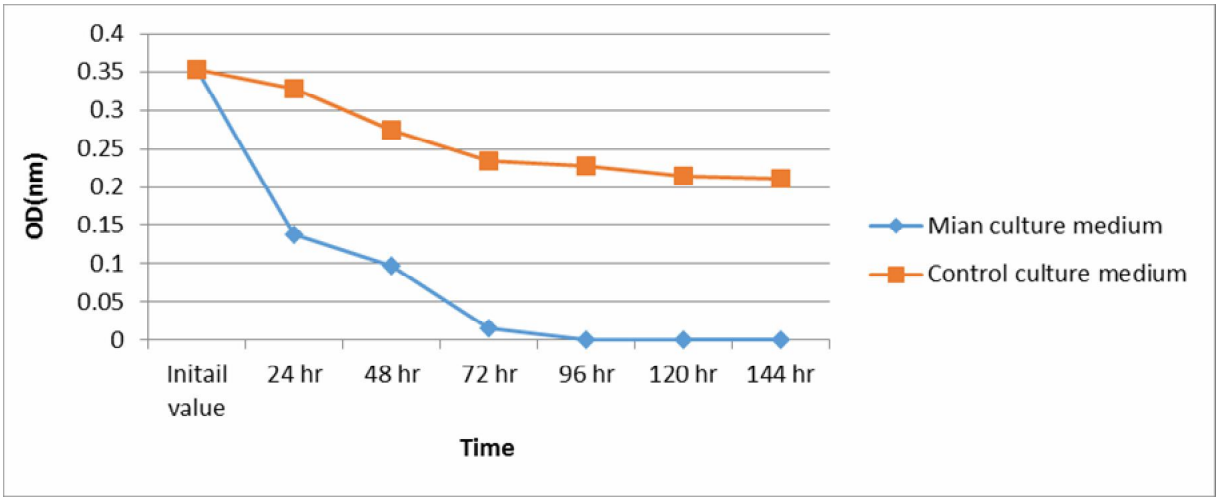

Figure 5. Comparison of alkylbenzene sulfonate removal rates at different temperatures using *E. aerogenes* bacterium, Type II.

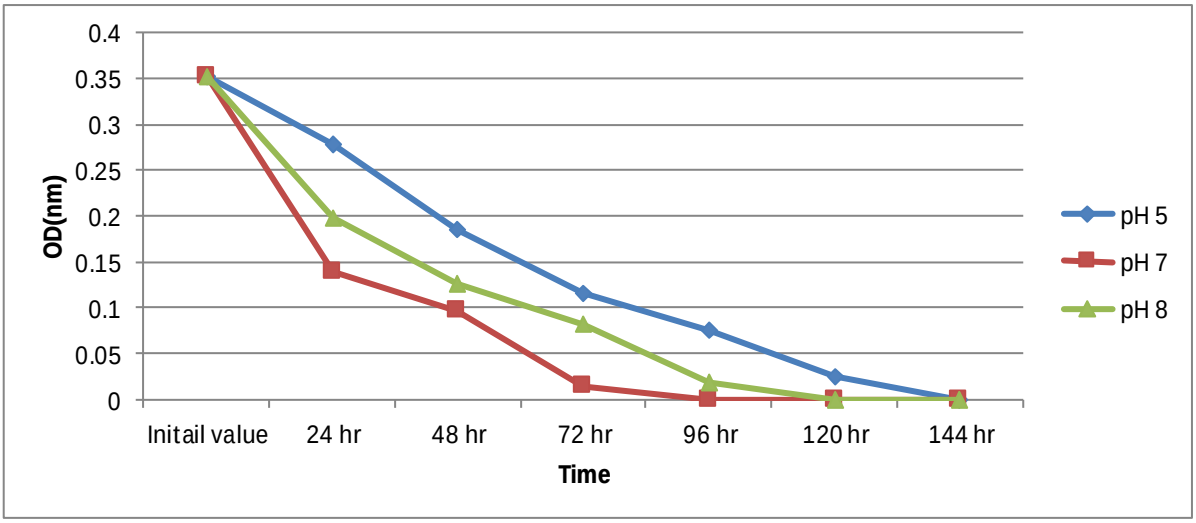

Figure 6. Comparison of alkylbenzene sulfonate removal rates at different pHs using *E. aerogenes* bacterium, Type II.

Nitrogen and carbon source. Bacterium behavior in NH_4Cl-containing culture media, as a nitrogen source at three levels of 0.25, 0.5, and 1 g L^{-1}, showed that *E. aerogenes* bacterium in nitrogen content of 0.5 mg L^{-1} has the highest efficacy in the removal of alkylbenzene sulfonate compound (Figures 7 and 8). Ability of *E. aerogenes* bacterium in different contents of alkylbenzene sulfonate compound, as the only source of carbon for disintegrating this compound, was tested and showed that the bacterium in the primary content of alkylbenzene sulfonate - as 10 mg L^{-1} – has the best efficacy in removing this compound. Activity of these bacteria reduced considerably with the nitrogen and carbon source increasing, which might be due to the increased toxicity of these compounds for the bacterium. Syed Mohd et al (2010) studied anionic surfactants removal by *P. aeruginosa* bacterium and proved that the highest removal occurs in C/N = 1.8 ratio. However, this bacterium showed its highest efficacy in C/N = 1.14 ratio in the study of Ainon et al (2013).

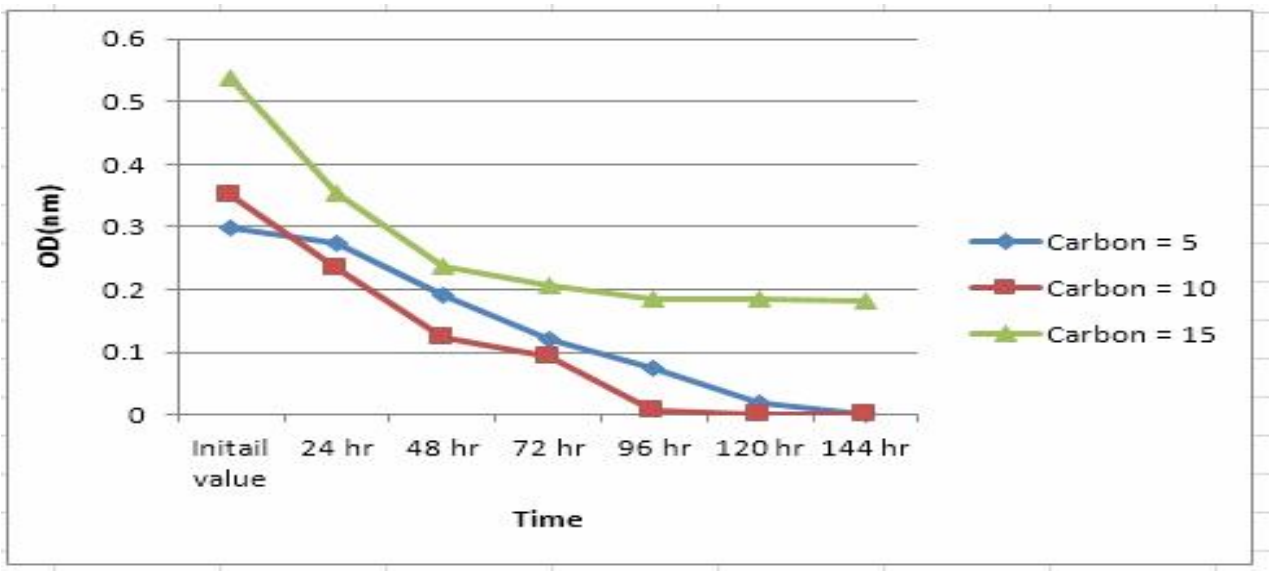

Figure 7. Comparison of alkylbenzene sulfonate removal in different values of nitrogen by *E. aerogenes*.

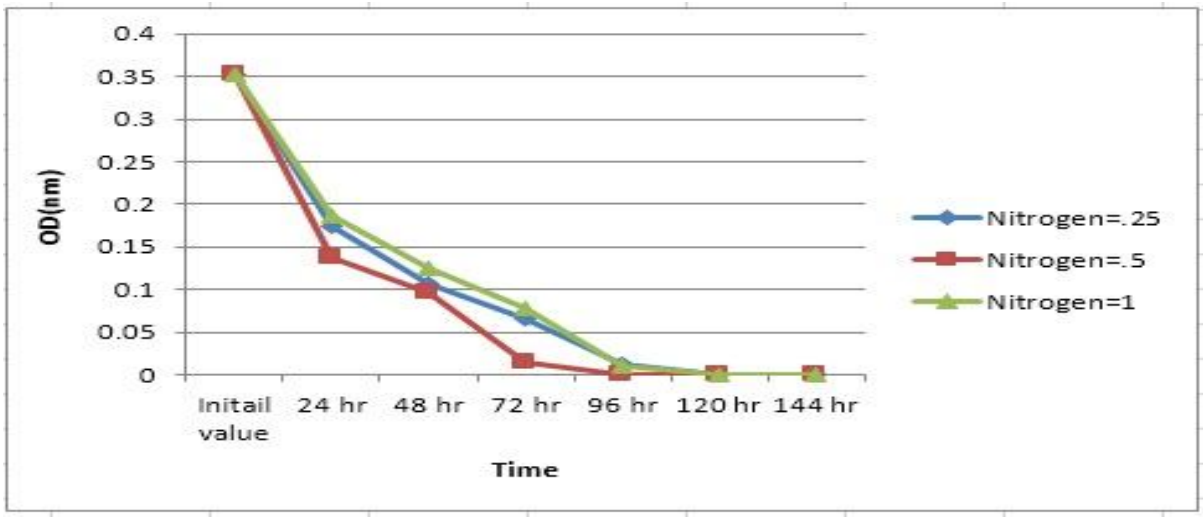

Figure 8. Comparison of alkylbenzene sulfonate removal in different values of alkylbenzene sulfonate by *E. aerogenes*, type II.

Conclusions. This research aimed at isolating and identifying indigenous bacteria of wastewater of Shahid Beheshti Hospital in Abadan and studying their biological degradation potential in biodegrading alkylbenzene sulfonate. The bacteria isolated from the wastewater were identified using biochemical culture method. Among the three strains, *E. aerogenes*, type II had a considerable capability for removing the organic matter, i.e. 81.6% within four days.

The results obtained from determining optimal conditions of the bacterium showed that *E. aerogenes* type II at pH =7 and 30°C, nitrogen content of 0.5 mg L^{-1}, carbon content of 10 mg L^{-1} could remove 95.7% of alkylbenzene sulfonate compound within 72 hours and brought its content to zero within 96 hours.

With respect to the studies, it is proposed to study on the maximum growth of the bacterium in order to maintain C/N ratio and temperature and pH values obtained in the research. This way, attempts should be made to study the function of isolated bacteria consortium and their function under normal conditions of a treatment plant in the future research and to compare the results with the experimental data.

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