

Anionic Surfactant Degradation by *Pseudomonas* in Hospital Wastewater. Case Study: Shahid Beheshti Hospital in Abadan City, Iran

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Abstract: The high volume of detergents consumed daily for hospital waste treatment poses a serious threat to both human health and the environment. In this study, attempt made to assess the impacts of impurities by eliminating anionic surfactants through isolating and purifying the indigenous bacteria in the waste produced at Shahid Beheshti Hospital, (Abadan, Iran). Decomposition rate was assessed through the methylene blue method using a Dr5000 spectrophotometer at a wavelength of 650 nm. From the above approaches, *Pseudomonas* species were isolated for incubation period of 144 hours, this bacterial species was able to eliminate 73% of alkylbenzene sulfonate under the following ambient conditions: pH of 7, temperature 30°C, nitrogen and carbon content were 0.25 and 5 mg L⁻¹, respectively. Research conditions in the treatment plant should be maintained for maximum activity of the bacteria. For generalizing the results of the present study to the real world, we also recommend that the elimination efficiency of the studied bacteria be evaluated in an actual treatment plant.

Key words: Anionic surfactant • Hospital wastewater • Alkylbenzene sulfonate • Mineral culture medium

INTRODUCTION

Hospital waste contains large quantities of pathogens and hazardous compounds, which seriously threaten human health as well as the environment [1]. In general, hospital/health centers effluent is, qualitatively, almost similar to that produced by urban sewage; however, they might contain potentially toxic and infectious compounds, which can endanger not only the environment, but also the health of Health Sector staff and the society as a whole [2]. Therefore, it is essential to supervise and manage hospital waste appropriately to prevent environmental pollution [1, 2]. Surfactants are substances with very high solubility, which reduce oxygen transfer through reducing surface tension of water. They are extremely toxic for marine life and cause excessive growth of algae in acceptor water resources (rivers). In addition, detergents disrupt the effective enzymatic activity in respiration of bacteria [3]. Today, detergents are widely used at health centers throughout Iran [4]. Disadvantages of the chemical

methods currently used for eliminating contaminants include increased toxicity for aquatic organisms, increased sludge production, reduced sludge stabilization, increased filamentous growth, decreased sludge dewatering characteristics and increased costs [5]. Using microorganisms is a useful technology for treating sewage of all kind and it can be considered as a useful tool for filtering different types of contaminants [6]. This method can be an appropriate alternative for chemical filtration of pollutants [7]. The process of bioremediation is defined as the highly cost-effective and noninvasive use of microorganisms for detoxification and elimination of contaminants from the environment [8]. Through isolating and purifying the indigenous bacteria which actively decompose alkylbenzene sulfonate in the effluent produced by Shahid Beheshti Hospital in Abadan, we attempted in this study to examine the feasibility of eliminating these compounds by using indigenous bacteria as well as to study different factors at different levels to maximize the associated decomposition rate brought about by these bacteria.

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 in May to September 2015 and 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 for bacteria growth.

MATERIALS AND METHODS

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, 10:00 AM and 13:00 PM; because Abadan is located in a hot and humid region, with 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 Petroleum Engineering College in Abadan city and they were mixed under laboratory conditions. The culture medium used here was a mineral culture medium with K_2HPO_4 , KH_2PO_4 , NaCl, NH_4Cl , Na_2SO_4 , $MgCl_2 \cdot 6H_2O$ compounds were 0.5, 1.5, 0.5, 0.5, 0.14 and 0.15 g L⁻¹ respectively. After preparing the culture medium, its pH was adjusted one molar sodium hydroxide solution and it was sterilized in an autoclave at the temperature of 121°C and pressure of 15 Psi for 15 minutes. Alkylbenzene sulfonate (ABS), which is an anionic surfactant, was used in this culture medium as the only source of carbon [9]. One ppt (Parts-per trillion) 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, 1 mL was taken and added to the new culture medium [10]. 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 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 [11, 12]. The bacteria were reproduced in the mineral 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 Mineral salts medium, transferred to the culture medium and counted using dilution method.

Determination of Bacterial Growth Rate and Analysis of ABS by Bacteria: The methylene blue method, an assay for nonionic surfactants in environmental samples was used [13, 14]. An optical spectrophotometer at a wavelength of 650 nm was used for determining degradation rate of ABS 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 [15]. 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 Mineral salts medium. The optical spectrophotometer was calibrated in an Mineral salts medium free from bacteria.

RESULTS

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 a *Pseudomonas bacterium*.

Pseudomonas bacteria were purified upon enriching the indigenous bacteria isolated from the effluent of Shahid Beheshti hospital. The bacteria removed 73% of the ABS present in the effluent within 144 hours. Studying growth rate of the bacterium in the mineral culture medium containing 10 ppm of ABS sulfonate compound and its modest growth in a culture medium free from ABS as compared with the culture medium containing bacteria indicate that the bacterium used this compound as a carbon source (Figure 2 & 3, respectively).

Table 1: The results of identification of bacteria using biochemical tests

Simmons Citrate	Methyl red/Voges-Proskauer	Oxidase	Indole	Urea	Bacteria name	The bacteria tested	Row
-	-	-	-	-	<i>Pseudomonas</i>	E ₁	1
Lysine	Gelatin	Lactose	SIM	Triple Sugar Iron	-	-	-
-	-	-	-	ALK/ALK	“	“	“

Results of ABS disintegration rate and bacterial growth.

Calibration curve to restore ABS concentrations is shown in Figure 1.

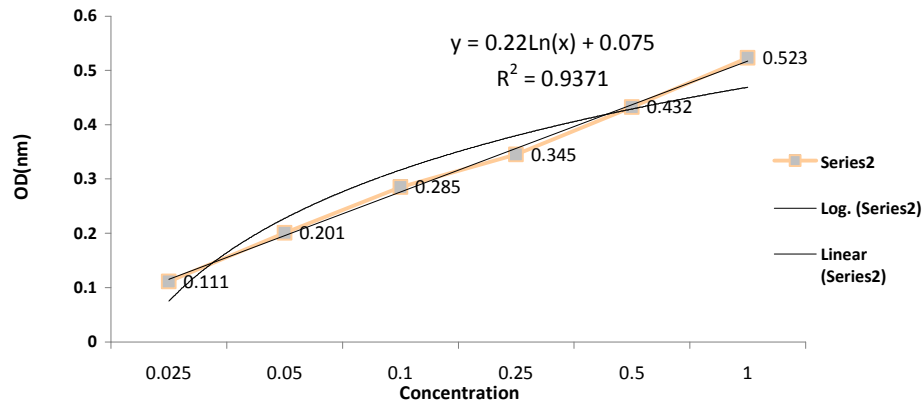


Fig. 1: Calibration curve to restore ABS concentrations is shown in.

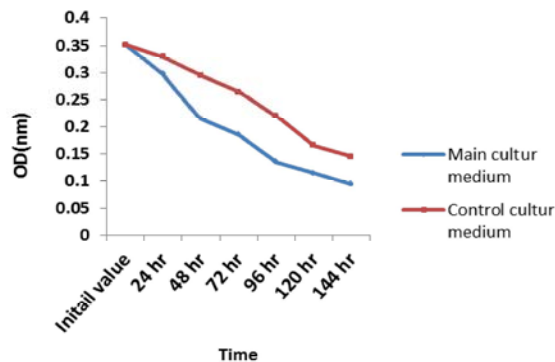


Fig. 2: Changes of ABS concentrations by *Pseudomonas* bacteria.

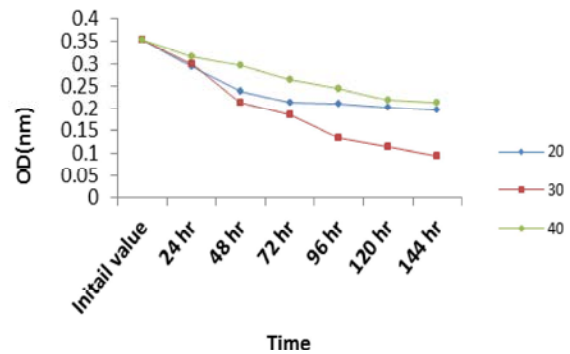


Fig. 4: Comparison of ABS removal rates at different temperatures using *Pseudomonas* bacteria.

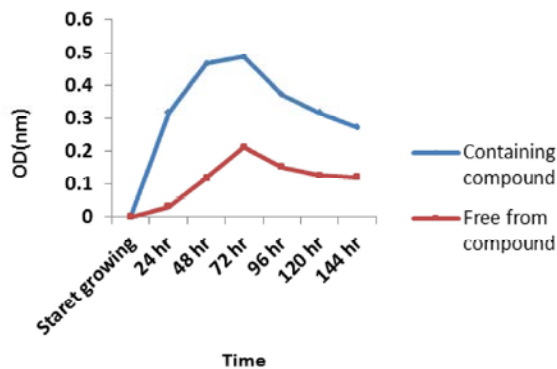


Fig. 3: The growth curve of *Pseudomonas* bacteria. at ppm 10 concentration of ABS compound.

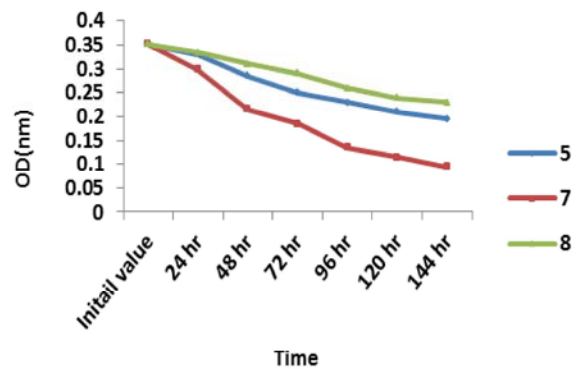


Fig. 5: Comparison of ABS removal rates at different pHs using *Pseudomonas* bacteria.

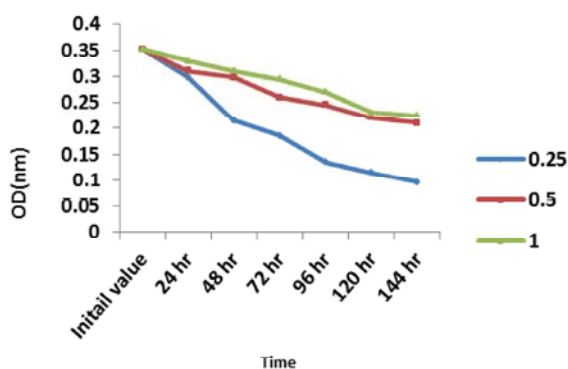


Fig. 6: Comparison of ABS removal in different values of nitrogen by *Pseudomonas bacteria*.

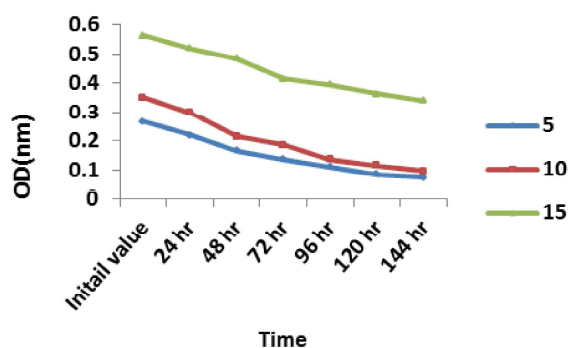


Fig. 7: Comparison of ABS removal in different values of alkylbenzene sulfonate by *Pseudomonas bacteria*

Impacts of Temperature and pH: The studied bacteria showed maximum efficacy in eliminating alkylbenzene sulfonate at 30°C and a pH of seven (Figure 4 & 5, respectively). The bacterial activity was reduced at decreased temperatures, reaching a minimum at 20°C. This might be due to the climatic conditions in Abadan and the consequent adaptation of the bacteria to such weather conditions.

Nitrogen and Carbon Source: Bacterial behavior was assessed for six days in a culture environment containing NH₄Cl as a source of nitrogen at three levels (0.25, 0.5 and 1 mg L⁻¹) as well as ABS as a source of carbon also at three levels (5, 10 and 15 mg L⁻¹). The obtained results showed that the studied bacteria exhibited their maximum efficiency in eliminating ABS composition at the nitrogen and carbon contents of 0.25 mg L⁻¹ and 5 mg L⁻¹ respectively. Bacterial activity decreased considerably upon decreasing the nitrogen and carbon contents. (Figure 6 & 7, respectively).

DISCUSSION

In this study, *Pseudomonas bacteria* was isolated and purified for the purpose of isolating and identifying indigenous bacteria in the effluent produced by Shahid Beheshti Hospital, Abadan and examining the potential biological decomposition of the organic matter ABS in the presence of this bacterial species. The bacteria were identified using biochemical culture method. The results showed that they could effectively eliminate 73% of the ABS composition within 144 hours. Chaturvedi V *et al* [10] isolated *Pseudomonas alcaligenes* and *P. mendocina* bacteria from municipal wastewater, which were able to remove respectively 99 and 98% of anionic surfactants. Amirmozafari *et al* [9] showed that *Pseudomonas beteli* was capable of eliminating 96.4% of ABS within ten days.

According to the obtained results, the optimal conditions for *Pseudomonas bacteria* to eliminate 73% of the ABS composition with a probability of 95% were: nitrogen content = 0.25 mg L⁻¹, pH = 7, temperature = 30 °C and carbon content = 5 mg L⁻¹. Ambily *et al.* [16] showed that maximum efficiency of *Pseudomonas bacteria* in eliminating anionic surfactants occurred at 37°C. Shahbazi [17] proved that *Pseudomonas bacteria* had the maximum impact on eliminating phenol and acid benzoic acid; however, according to the study by Karimi *et al.* [18] the maximum efficiency in eliminating naphthalene occurred at a pH of 8. Syed M *et al.* [19] 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 Hamzah *et al.* [20]. It is recommended that the research conditions in the treatment plant be maintained for maximum activity of the bacteria. For generalizing the results of the present study to the real world, we also recommend that the elimination efficiency of the studied bacteria be evaluated in an actual treatment plant.

CONCLUSIONS

In this study, *Pseudomonas bacteria* from Shahid Beheshti Hospital (Abadan, Iran) wastewater species were isolated. The bacteria were identified by biochemical tests and its ability to remove the ABS composition at DO 650 nm by a spectrophotometer Dr 5000 assessments.

Results revealed for incubation period of 144 hours, this bacterial species was able to eliminate 73% of ABS under the following ambient conditions: pH of 7, temperature 30°C, nitrogen and carbon content were 0.25 and 5 mg L⁻¹, respectively.

The results showed that *Pseudomonas* bacteria have got high ability to remove the ABS compound so with proper management of hospital wastewater treatment and maintain the results of research in order to maximize the growth of bacteria studied, Can minimize contamination from the anionic surfactants.

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