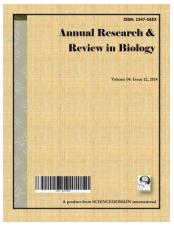

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Enhancement the Biodegradation of Sodium Dodecyl Sulfate by *Pseudomonas aeruginosa* and *Pseudomonas otitidis* Isolated from Waste Water in Saudi Arabia

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Authors' contributions

This work was carried out in collaboration between both authors. Author AGI designed the study, managed the literature searches, carried out practical experiments, wrote the protocol and wrote the first draft of the manuscript. Author HEAE performed the statistical analysis and some practical experiments. Both authors read and approved the final manuscript.

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ABSTRACT

Sodium dodecyl sulfate, (SDS) is an anionic surfactant that widely utilized in industry and households. Which represent toxic effects to the health and aquatic organisms. The bacterial strains *Pseudomonas aeruginosa* and *Pseudomonas otitidis* were isolated from the water samples from waste disposal sites (Taif Governate, Kingdom of Saudi Arabia). So, in the present study, we have made an attempt to improve the biodegradation of SDS by *Pseudomonas aeruginosa* and

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Pseudomonas otitidis by different methods such as mutation (Physically and chemically), physically by exposure of bacterial strains to ultraviolet radiation (UV) and chemically by using chemicals such as ethidium bromide (EtBr), also biodegradation rate of SDS can be increased by immobilization technique. The bacterial strains were immobilized in alginate beads, and its SDS degradation efficiency was observed to increase many fold than free strains.

Keywords: Sodium dodecyl sulphate (SDS); biodegradation; Pseudomonas sp.; mutation; ethidium bromide; UV; immobilization; calcium alginate.

1. INTRODUCTION

Mainly because of their widespread use and high utilization, surfactants and their products have been distinguished at various concentrations in surface waters, sediments and sludge-amended soils [1,2].

Contaminants of groundwater by surfactants is caused generally by leaching from industrial and municipal sewage systems but can be introduced to the environment by domestic and professional effluents from dumped cleaning products [3,4].

Among the essential anionic surfactants (by generation volume) are the sodium dodecyl sulphate (SDS), linear alkylbenzene sulfonates (LAS), alcohol ethoxy sulfates (AES), and alkyl sulfates (AS) [5].

Sodium dodecyl sulfate, is widely used anionic detergent, and it is the most widely used in several products such as, shampoos, cosmetics, cleaning agents, herbicides, and utilized in oil-spill cleanups, in paper industry as penetrant, flocculating agent, de-inking agent, in building construction as additive of concrete, firefighting devices, engine degreasers [6,7], it is also now widespread in biochemical research involving electrophoresis [8].

It is reported that SDS is toxic and influences endurance of aquatic animals such as fishes. All detergents destroy the external mucus layers that protect the fish from microorganisms and parasites; plus they can cause extreme harm to the gills. Most fish will die when detergent concentrations approach 15 parts every million. Additionally, it is toxic to mammals like mice and humans, which consume drinking water contaminated with it [1,3].

In recent years, the use of bioremediation for the elimination of anionic detergents has been a successful elective to other distinctive strategies, due to its ease and low cost, and the absence of damage to the environment and human. Now, there is a trend to use thermophilic bacteria besides to mesophilic bacteria to degrade organic pollutants with a higher degradation rate [9].

Immobilization of microbial cells is now gaining more importance than the immobilized enzymes since it decreases the need for release of intracellular enzymes and subsequent filtration [10] steps. Moreover, reuse or continuous use of biocatalysts is made possible by the immobilization procedure [11,12]. Immobilization technique could increase the efficiency of cell viability and/ or growth [13]. To improve the efficiency of biological treatment of SDS, the present study focused on the optimization of bioremediation process using Ca-alginate immobilized cell.

Bioremediation of anionic detergent (SDS) is necessary due to its ease and low cost, and the absence of damage to the environment and human [14]. So, Isolation, screening and identification of the most efficient bacteria (*Pseudomonas aeruginosa* and *Pseudomonas otitidis*) degrading SDS were obtained in our previous study [15]. Members of the family P*seudomonas* were found capable of degrading SDS and utilizing it as a carbon source [16]. In the present study, an attempt was made to biodegradation of SDS by *Pseudomonas aeruginosa* and *Pseudomonas otitidis* by different methods such as mutation and immobilization technique.

2. MATERIALS AND METHODS

2.1 Screening of the *Pseudomonas aeruginosa* and *Pseudomonas otitidis* for SDS Degradation in Liquid Culture

Biodegradation was achieved by methylene blue active substance (MBAS) [17,18]. Autoclaved mineral media (50 ml), was taken in sterile conical flasks. 2% detergent was added along with a loop full of inoculum and incubated in a

rotary shaker at 30°C for 150 rpm. At the end of 3 days, 4ml of this sample, 4 ml of chloroform and 4 ml methylene blue was mixed well and allowed to settle. The absorbance was measured at 651 nm. Absorbance obtained is a direct indication of the amount of residual surfactant present in the solution.

2.2 Effect of Mutation on the Biodegradation of SDS by *Pseudomonas aeruginosa* and *Pseudomonas otitidis*

A). Physical mutation by ultraviolet (UV)

Bacteria were grown in Lauria Britani (LB) medium and incubated overnight at 30±2°C. The culture were centrifuged at 5000 rpm per 10.0 min., and the obtained pellets were resuspended in an equal volume of mineral salt medium (MSM). Five ml aliquots from the suspension were then transferred into a polyethene dish and subjected to UV at 312 nm on dual-intensity transilluminator for different intervals (5, 10, 15, 30 and 60 min.). Irradiated bacteria were spread on MSM agar containing SDS and incubated overnight at 30±2°C. The survival cells were then selected and cultivated on LB broth medium [19]. The activity of isolates for degradation was monitored by the methylene blue active substance (MBAS).

B). Chemical mutation by ethidium bromide (EtBr)

The bacteria were grown as previously described and pelleted by centrifugation at 5000 rpm for 10 min., the resulted pellets were resuspended in solution of 3% NaCl (w/v).

Subsequently, the various EtBr concentration at 50, 100, 150 and 200 µg/ml was added to the bacterial cells. After the examined exposure period (24 hours), cells were pelleted again, washed by phosphate buffer saline at pH 6.8, and inoculated into mineral salt agar containing SDS, then incubated overnight at 30±2°C. The survived cells were then selected and cultivated on LB broth [20,21]. The activity of isolates for degradation was monitored by the methylene blue active substance (MBAS).

2.3 Immobilization of the *Pseudomonas aeruginosa* and *Pseudomonas otitidis*

Encapsulation of *Pseudomonas aeruginosa* and *Pseudomonas otitidis* into Ca-alginate beads was performed. Liquid cultures were centrifuged at 2,500 g for 10 minutes in a 50-ml plastic centrifuge tube at room temperature for 10 min, and the supernatant was discarded. The pellets were resuspended with a previously autoclaved sodium alginate solution at a final concentration 4% (w/v) and 10% (v/v) bacterial biomass. Sodium alginate-bacterial mixture was added dropwise with a sterile syringe (20 ml) fitted with a wide bore needle (1 mm diameter) into an autoclaved solution of 3% (w/v) calcium chloride, adjusted to pH 7.0, where beads (calcium alginate included the bacteria) was formed immediately. The beads were left in this strengthening solution overnight at 4°C before being collected by filtration [12].

Degradation experiments were performed under shaking in flasks containing SDS and immobilized bacterial cells as well as free cells. Control (Ca-alginate beads without bacteria in MSM medium containing SDS) was also performed.

3. RESULTS

3.1 Degradation Rate of SDS by *Pseudomonas aeruginosa* and *Pseudomonas otitidis* without any Improvment

Pseudomonas aeruginosa and *Pseudomonas otitidis* were isolated from waste disposal sites (Taif Governate) in our previous study. Mineral salt medium (MSM) containing SDS as the sole carbon source was used, and the degradation rate of these strains were appeared in Fig. 1.

3.2 Acceleration the Biodegradation rate of SDS

There were many attempts to accelerate the biodegradation process by mutation and immobilization technique.

3.2.1 Effect of mutation on the biodegradation of SDS by *Pseudomonas aeruginosa* and *Pseudomonas otitidis*

A. Physical mutation by UV

This experiment demonstrated that the degradation rate of SDS was increased with exposure to UV as shown in Fig. 2.

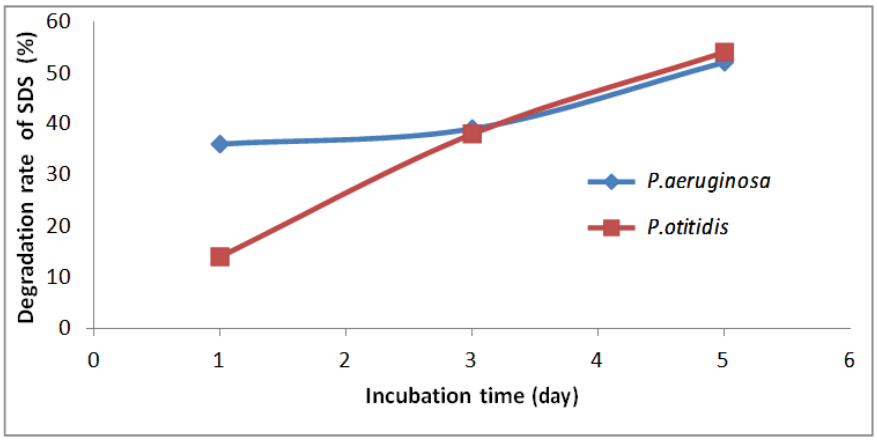

Fig. 1. Degradation rate of SDS (%) by *Pseudomonas aeruginosa* and *Pseudomonas otitidis*

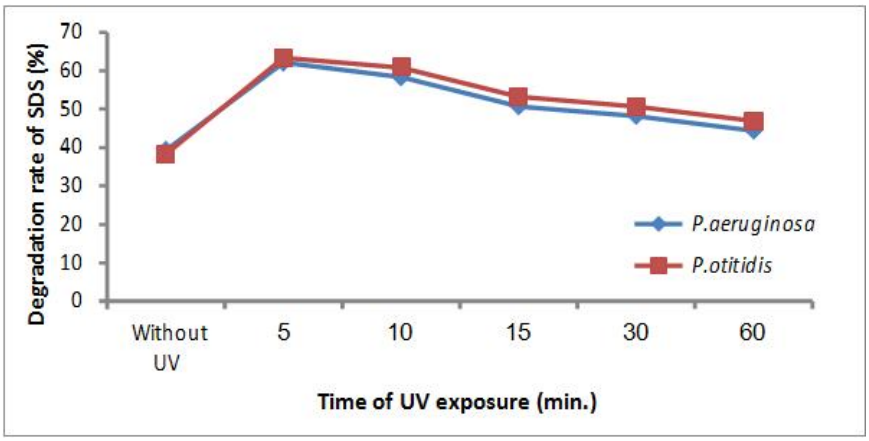

Fig. 2. Effect of exposure to UV (different time) on SDS degradation rate by *Pseudomonas aeruginosa* and *Pseudomonas otitidis*

B. Chemical mutation

Data presented in Fig. 3 showed that the degradation rate of SDS increased by increasing concentration of ethidium bromide (EtBr) with *Pseudomonas otitidis* and *Pseudomonas aeruginosa*

3.3 Immobilization of the *Pseudomonas aeruginosa* and *Pseudomonas otitidis*

Encapsulation of the *Pseudomonas aeruginosa* and *Pseudomonas otitidis* into Ca- alginate beads was performed and the results shown in Fig. 4, revealed that the degradation rate of SDS was increased by immobilized cells more than the free cells. Controls (Ca-alginate beads without bacterial cells in MSM medium containing SDS) demonstrating that SDS cannot adsorb by these beads and therefore no degradation occurred.

4. DISCUSSION

For obtaining a high rate of degradation of the SDS, the wild-type strains (*Pseudomonas aeruginosa and Pseudomonas otitidis)* were subjected to physical [18], and chemical [20,21] mutations. There are many causes of mutation some of which can lead to a complex and unusual type of mutations [22]. Bacterial mutations can also occur naturally for adaptation to new environmental factors (direct mutation). Also, external factors radiation have potentially resulted in mutations [21]. The most common mutation caused by antibiotics and resulting new types of bacteria have more resistance to the antibiotic. The degradation rate was increased with the selected strains as well as chemically

mutant obtained from *Stenophormanus* sp EF105546, also gave high degradation rate more than the wild type.

Reiser and Somerville, Kim and Sundin [23,24], reported that the production of poly hydroxyl butyrate (PHB) by the mutant strains was exceeded more than 50% than the wild-type. Moreover, [20,25], concluded that physical mutation is considered one of the more effective molecular tools due to its low costs less and easily performance as compared to the construction of gene libraries.

Many papers reported that UV mutagenesis has a positive effect on increasing the biodegradation ability of strains [26,27]. Yet, another study showed that UV mutagenesis had no effect on degradation efficiency [28]. Similar results were obtained by Zhen et al. [29], who indicated that the mutant strain *Pseudochrobactrum* sp. XF1 has more tolerant over a wide range of pH values than the wild strain.

To improve the efficiency of biological treatment of SDS, the present study focused on the optimisation of bioremediation process using Ca-alginate immobilized cells [12].

Entrapment of bacterial cells using insoluble calcium alginate is a rapid method, non-toxic, inexpensive and versatile. More than 80% of cell immobilization process is still carried out using alginate [30].

Immobilization of microbial cells is now gaining more importance than the immobilized enzymes since it eliminates the need for the release of intracellular enzymes and subsequent purification steps [10].

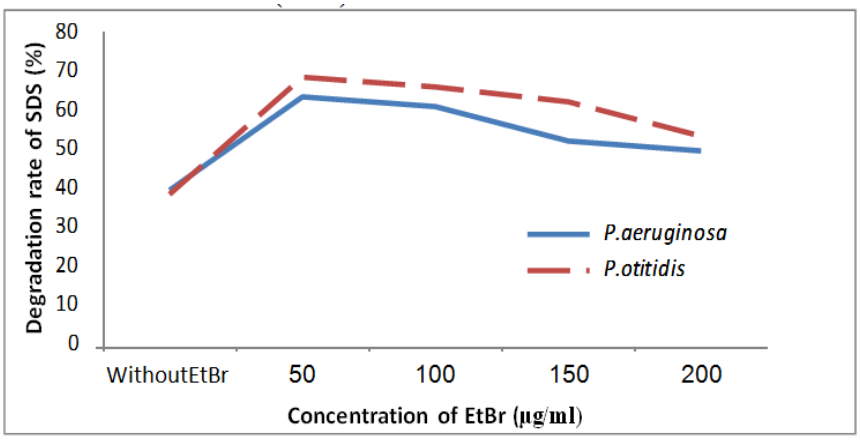

Fig. 3. Effect of different concentration of EtBr on SDS degradation rate *Pseudomonas otitidis* and *Pseudomonas aeruginosa*

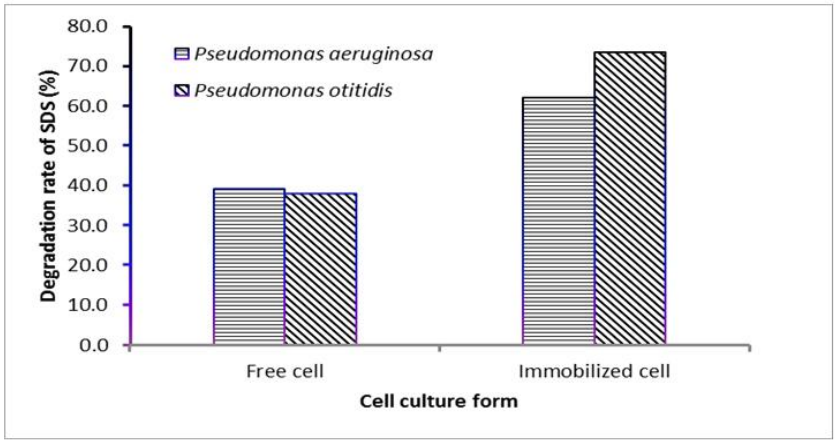

Fig. 4. Degradation rate of SDS by free and immobilized *Pseudomonas aeruginosa* and *Pseudomonas otitidis*

Immobilization could increase the efficiency of the microbial cell viability, growth and some properties [13]. Also Wiesell et al. [31] reported that immobilization is considered to promote better survival and activity of some microorganisms. These observations confirmed our results on SDS degradation performed in shake flasks with immobilized and free bacterial cells, as well as control (Ca-alginate beads without bacteria). SDS cannot adsorbd control (beads without bacteria), while the immobilized cells showed higher degradation rate as compared with free cells [12].

Moreover, immobilisation of the degrading bacteria enhances the SDS degradation rate. Immobilization of the microbes in a solid matrix is an advantage over the freely suspended ones since they can repeatedly be used, giving the treatment process an enhanced cost effective rate of degradation and tolerance to the toxic substrate [32].

5. CONCLUSION

Synthetic surfactants discharged into the water and prevented aeration due to its high foaming and low oxygenation capacity.

Microbes are shown to be an efficient degrader of anionic surfactants.

Pseudomonas otitidis showed a high degradation rate of SDS.

The degradation rate was increased with mutation and immobilization methods.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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