Enhanced Removal of the Xenobiotic Surfactant Sodium Dodecyl Sulfate from Actual Nondomestic Wastewaters Using Immobilized Mixed Bacterial Cells

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Cell immobilization has been proven to offer noticeable benefits over conventional biological systems using free cells, particularly for recalcitrant compounds. In this study, mixed bacterial cells were alternatively immobilized in sodium alginate (SA) and in sodium alginate-polyvinyl alcohol (SA – PVA) for biodegradation of sodium dodecyl sulfate (SDS). Synthetically prepared SDS-bearing aqueous solution (SWW), as well as actual automobile service station wastewater (AWW) and laundry wastewater (LWW) were used. The results revealed that high removal efficiencies were achieved after 48 h for both types of beads. When SDS concentration in SWW increased from 10 to 1000 mg L–1, SDS degradation using both types of beads were decreased from 99.71 % to 85.12 % using SA beads, and from 99.63 % to 83.29 % using SA-PVA beads. The removal efficiency of SDS in the actual (AWW) were 94.91 % and 93.82 % using SA beads and SA-PVA beads, respectively. While, for SDS-bearing laundry (LWW), the removal efficiencies were 94.39 % and 92.04 % using SA beads and SA-PVA beads, respectively. No decline in the biodegradation capacity of immobilized consortium was noted over its recycling and reuse. Both hydrogel matrices lasted for up to five cycles in the actual wastewaters. These promising results confirmed the validity of using immobilized mixed cells as an efficient and cost-effective approach for SDS biodegradation in real industrial wastewaters.

Keywords:
immobilization, hydrogel matrices, sodium dodecyl sulfate, biodegradation, sodium alginate

Introduction

Emerging pollutants such as surfactants may produce adverse impacts on the environment as they exist in the environment through the discharge of untreated or improperly treated wastewater.¹,² In general, there are four types of surfactants: anionic, cationic, non-ionic, and ampholytic surfactants. Anionic surfactants account for about 55 % of worldwide total surfactant production due to the ease and low cost of manufacture.³ Sodium dodecyl sulfate (SDS) is an example of anionic surfactants that is a vital constituent of foaming agent for toothpaste and shampoos.⁴ Sodium lauryl sulfate (SLS) is another known name of SDS, that is a primary alkyl sulfate, which has the chemical formula CH₃(CH₂)₉OSO₃Na.¹ Bioremediation is profitable and favorable technology for the reduction of these pollutants and will result in complete mineralization via microorganisms.⁵ To progress the holding and the survival of the biological mediators in the most polluted places, bacterial cells must be immobilized. It is known that immobilization improves microorganism resistance to unfavorable environmental impacts. Also, they can be recycled, thus decreasing the luxurious techniques of cell recovery and recycle.⁶ Several methods of cell restriction have been used, such as adsorption, encapsulation, entrapment, and covalent binding. Encapsulation is a method in which a combination or one material is entrapped within, or, coated with, a different system or material. The material that is entrapped or coated is known as the core material, actives, internal phase, fill, or payload. On the other hand, the coating material is known as the coating, carrier, wall material, shell, or membrane.⁷ The wall material offers a defending place to the bacterial cells and confirms sustained over a long time of release.⁸ Food grade polymers such as carrageenan, carboxymethyl cellulose, chitosan, alginate, starch, xanthan gum, pectin, and gelatin, are mostly used in diverse micro-encapsulation methods.⁹ Sodium alginate is a natural carrier used most commonly for immobilization by crosslinking agent CaCl₂, owing to the simple gelatinization and high biocompatibility.
Calcium alginate is highly sensitive to the existence of EDTA, lactate, phosphate, citrate, magnesium, potassium, and/or sodium ions because they may contribute in dissolving the gel beads.\textsuperscript{5,9} Natural polymers, such as alginate, possess poor mechanical strength and durability, therefore, they were crosslinked with polyvinyl alcohol (PVA) and boric acid in order to increase the mechanical strength. Bacterial cells exploited from activated sludge were immobilized by the PVA-alginate-borate method. At least 1\% of alginate in the beads were necessary to inhibit bead accumulation. The optimum concentration of PVA used to immobilize sludge was found to be 10–12.5\%.\textsuperscript{10} Degradation of pyrene (PYR) by \textit{Herbaspirillum chlorophenolicum} immobilized in sodium alginate (SA)-diatomite carrier, polyvinyl alcohol (PVA)-diatomite carrier (chemical method), and PVA-diatomite carrier (physical method) was studied. Polyvinyl alcohol (PVA)-diatomite carrier by chemical method proved the most efficient, with a PYR biodegradation of 92.8\% in 10 days.\textsuperscript{11} Photosynthetic hydrogen production from organic wastewaters using immobilized mixed culture with photosynthetic bacteria (PSB) was investigated. A PSB consortium was immobilized by alginate matrix to form granules. The so-yielded granules exhibited minimal diffusional resistances to substrates and to illumination penetration, but still produced more hydrogen from synthetic wastewater than the free cells at identical experimental conditions.\textsuperscript{12} Biodegradation of cationic surfactants, tetradecyl-trimethyl-ammonium bromide (TTAB) and benzalkonium chloride (BAC), was investigated using free and immobilized bacteria \textit{Aeromonas hydrophila} and \textit{Pseudomonas putida} in Ca-alginate.\textsuperscript{13} Biotreatment of real-field petroleum wastewater using mixed microbial cells immobilized in sodium alginate-polyvinyl alcohol in spouted bed bioreactor was studied. The results established that immobilized cells showed a better performance compared to free cells.\textsuperscript{14} Decolorization and biodegradation of reactive blue (RB) in a sequential anaerobic-aerobic processes was studied. Activated sludge was immobilized in alginate-polyvinyl alcohol (PVA). The used beads resulted in 88\%, 87\%, and 87\% maximum COD removals with samples containing RB at initial concentration of 10, 20, and 40 mg L\textsuperscript{-1}, respectively.\textsuperscript{15} Biodegradation of diesel by a mixture of equal proportions of two strains, \textit{Halomonas} and \textit{Aneurinibacillus}, as free and immobilized cells with sodium alginate and straw was explored. The best degradation rate of immobilized cells in straw-alginate beads was 68.68\%.\textsuperscript{16} Biodegradation of three- and four-rings polycyclic aromatic hydrocarbons (PAHs) (phenanthrene [PHE] and fluoranthene [FLU]) was conducted using free and Ca-alginate-immobilized \textit{Sphingomonas pseudosanguinis} strain J1-q (S1) and \textit{Pseudomonas stutzeri} strain (S2) in bench-scale sediment slurry reactors. The effects of sodium alginate (SA) dosage on the characteristics of immobilized bacterial beads were investigated. The results indicated that a 3\% alginate concentration was optimal for immobilizing bacteria for PHE and FLU degradation.\textsuperscript{17} The changes in degradation characteristics and bacterial community structure of immobilized cells in straw-alginate beads in marine environment were investigated. Two diesel-degrading strains were embedded in straw-alginate beads to form immobilized cells. The results revealed that C11–C17 was more degraded by immobilized cells, and straw-alginate beads had appropriate pore structure.\textsuperscript{18}

The biodegradation of SDS by free cells has been extensively investigated, and there are numerous previously published studies in this regard. However, to the authors’ knowledge, very limited, i.e., not more than two studies have dealt with SDS degradation using immobilized mixed cells. The performance of \textit{Pseudomonas} C12B in polyacrylamide gel beads to degrade SDS;\textsuperscript{19} The biodegradation of SDS by \textit{Escherichia coli} in k-Carrageenan matrix.\textsuperscript{1}

This study aimed to investigate the potential of SDS biodegradation in actual SDS-bearing wastewaters by using mixed bacterial cells alternatively immobilized in sodium alginate (SA) and sodium alginate with poly vinyl alcohol (SA-PVA). For comparison purposes with actual wastewaters, SDS biodegradation was investigated in synthetically prepared SDS aqueous solutions. In addition, the experimental investigation was extended to evaluate the recycling of beads for successive cycles.

\textbf{Materials and methods}

\textbf{Inoculum}

Activated sludge obtained from a local wastewater treatment plant was used as the source for the mixed cultures. Analysis of the activated sludge indicated that \textit{Pseudomonas medocina} and \textit{Bacillus} were the initial dominant species in the mixed culture.

\textbf{Mineral salt medium}

The mineral salts medium (MSM) used for pre-cultivation of the mixed cells consisted of (in g L\textsuperscript{-1}): \textit{KH}_2\textit{PO}_4 (1.36), KNO\textsubscript{3} (0.5), (NH\textsubscript{4})_2\textit{SO}_4 (7.7), Na\textsubscript{2}HPO\textsubscript{4} (1.39), CaCl\textsubscript{2} (0.01), and MgSO\textsubscript{4} (0.01). The MSM also contained trace elements (0.01 g) of: MnCl\textsubscript{2} 4H\textsubscript{2}O, ZnSO\textsubscript{4} 7H\textsubscript{2}O, FeSO\textsubscript{4} 2H\textsubscript{2}O, COCl\textsubscript{2} 6H\textsubscript{2}O, NaMoO\textsubscript{4} 2H\textsubscript{2}O, CuCl\textsubscript{2} 2H\textsubscript{2}O, and H\textsubscript{3}BO\textsubscript{4}.\textsuperscript{20}
Substrates

Two types of substrates were individually used in this study; (1) aqueous solutions of sodium dodecyl sulfate (SDS) prepared by dissolving different concentrations of SDS including 10, 20, 50, 100, 300, 500, and 1000 mg L\(^{-1}\) in MSM, (2) actual freshly collected wastewaters, i.e., laundry wastewater (LWW) and automobile service station wastewater (AWW) having SDS concentration of 121.5 ± 20 mg L\(^{-1}\) and 25.5 ± 5 mg L\(^{-1}\), respectively.

Immobilization procedure

Two forms of hydrogel beads were individually prepared, sodium alginate (SA) and sodium alginate-poly vinyl alcohol (SA–PVA). The SA beads were prepared by dissolving 2 g of SA in 100 mL sterilized distilled water. Seven mL of bacterial cells was added to the SA solution and stirred for 10 min, taking into account no bubbles were trapped inside to produce a smooth surface. The prepared solution was added to 4 % (w/v) CaCl\(_2\) solution, and kept to harden at room temperature for 30 min.

One gram of SA with 10 g PVA were dissolved in certain volume of distilled water, and then the mixture of SA-PVA was cooled down to 40 °C. Seven mL of the mixed cell was added to the mixture of SA-PVA. The resulting solution was dropped into CaCl\(_2\) (1 % w/v) and saturated boric acid solution to form beads. The beads were moderately stirred in this solution for 24 h to complete solidification, and then washed with distilled water to remove any trace boric acid left on the beads. Sodium chloride of 0.9 % concentration was used to preserve the beads in the refrigerator at 4 °C. Fig. 1 illustrates both types of beads which were prepared with SA and SA-PVA.

To examine the dominant mechanism rather than biodegradation that may govern the SDS removal and disappearance from the aqueous solutions, a group of beads were prepared free of bacterial cells. A set of experiments was carried out using those free cells–beads with synthetically prepared SDS-loaded solution, and with the actual wastewaters.

Experimental procedure

Three sets of experimental assays were carried out in this study. The first set was performed using the synthetically prepared SDS-loaded aqueous solution at different SDS concentrations. This set was carried out alternatively with free cell, SA-beads, and SA-PVA beads. The second set was conducted with actual laundry wastewater (LWW) using free cell, SA-beads, and SA-PVA beads, and the third set was carried out using actual automobile service station wastewater (AWW) using free cell, SA-beads, and SA-PVA beads. The experimental procedure of the aerobic biotreatment assays involved setting up 100 mL-Erlenmeyer flasks as bench scale-bioreactors. The experiments were alternatively conducted with free and immobilized cells. The flasks were aerated for 48 h using an air pump to provide the required O\(_2\) for aerobic biotreatment besides adequate mixing. At specific time intervals, 10 mL were taken from each flask, centrifuged at 10000 rpm for 10 min, filtered through 0.22 micron, and then analyzed by a reliable and quick solvent extraction spectrophotometric method.
Fig. 2 – Biodegradation of different concentrations of SDS in synthetically prepared solution by free and immobilized cells.
Recycling of beads

To determine the long-life action and the feasibility of the prepared beads, recycling of the used beads was carried out in this study for successive cycles. After each cycle of cultivation, the reused hydrogel beads were washed many times with distilled water to remove any residuals and then transferred into fresh SDS-contained solutions.

Methods of analysis

The remaining concentrations of SDS were measured by UV/VIS spectrophotometer (Model: T80+) at \( \lambda_{\text{max}} = 499 \text{ nm} \). The concentration of SDS was determined using equation (1). Each experiment was achieved in duplicate with lower than 3 % standard deviation. The results represent the mean values. The removal efficiency of SDS was calculated according to the following formula:

\[
\text{% Removal efficiency} = \frac{\gamma_i - \gamma_f}{\gamma_i} \cdot 100 \quad (1)
\]

where: \( \gamma_i \) and \( \gamma_f \) (mg L\(^{-1}\)) are the initial and remaining concentration of SDS, respectively. Constituents including COD, TSS, Cl\(^-\), SO\(_4\)\(^{2-}\), NO\(_3\)\(^-\), PO\(_4\)\(^{3-}\) were measured according to the procedures outlined in the Standard Methods.\(^{26}\) Chemical oxygen demand (COD) was measured using COD analyzer type Lovibond COD/RD/125.

The concentrations of microbial cells were estimated as the volatile suspended solids (VSS) concentration based on the procedure described in the Standard Methods.\(^{26}\) Field emission scanning electron microscope was used to provide detailed images of the surfaces of cells and developed microorganisms inside the beads.

Results and discussion

SDS removal in synthetically prepared solution

Results of SDS biodegradation of SDS in the synthetically prepared aqueous solutions used free cell, SA-beads, and SA-PVA beads are given in Fig. 2. The results demonstrated that after 48 h, the removal efficiency of SDS at 10 mg L\(^{-1}\) initial concentration were 99.93 %, 99.71, and 99.63 % using free cells, SA beads, and SA-PVA beads, respectively, indicating that no tangible difference in the SDS removal efficiency was observed. By increasing the SDS concentration from 10 to 1000 mg L\(^{-1}\), SDS removal efficiencies after 48 h were 94.89 %, 85.12 %, and 83.29 %, using free cells, SA beads, and SA-PVA beads, respectively. This reduction in the SDS removal efficiencies could be attributed to the fact that longer time may be required for complete degradation of SDS by immobilized cells due to restriction of mass transfer, since the bacterial cells were trapped in a casing that must be pierced by the substrate. However, after 82 h, the SDS removal efficiencies using SA beads and SA-PVA beads increased to 99.12 % and 99.32 %, respectively, whereas, when using free cells, it remained constant at 94.89 % even by increasing the time to 82 h. Both types of beads exhibited high removal efficiency of SDS at all concentration levels.

On the other hand, Pseudomonas and Bacillus, which were the dominant bacterial cells in the used mixed biomass, have been reported in many studies as a potential species for degrading SDS.\(^{27,29}\) Ajithkumar et al., suggested a degradation of 0.5 %. Sixty percent of the dissolved SDS within 5 days at 0.20 % initial concentration using Bacillus strain isolated from activated sludge for the degradation of SDS.\(^{30}\) Hosseini et al. reported SDS removal efficiency of 96.4 % and 97.2 % after 10 days growth of two bacterial strains, Pseudomonas beteli and Acinetobacter johnsonii, respectively, isolated from activated sludge.\(^{31}\)

SDS removal in actual wastewaters

As mentioned in Section Substrates, SDS biodegradation in automobile service station wastewater (AWW) and laundry wastewater (LWW) samples was examined alternatively. The results demonstrated that the SDS removal efficiencies in the AWW after 48 h were 98.78 %, 94.91 %, and 93.82 % using free cells, SA beads, and SA-PVA beads, respectively (Fig. 3). However, after 60 h, the SDS removal efficiencies using immobilized cells increased to 99.12 % and 99.45 % for SA beads and SA-PVA beads, respectively. Again, the observed delay for complete removal of SDS using immobilized cells could be due to mass transfer restriction, since the bacterial cells were trapped in a casing that had to be pierced by the substrate to be in direct contact with the cells and substrate. In addition, actual wastewaters typically comprise more than one species; thus, knowing how these systems exhibit in a multisubstrate environment is of considerable importance. As total organic content increased, the dynamics of each substrate consumption was expected to be different compared to a single type of substrate.\(^{32}\) SDS removal efficiencies in the actual LWW after 48 h were 92.31 %, 94.39 %, and 92.04 % using free cells, SA beads, and SA-PVA beads, respectively (Fig. 3). Ambily and Jisha reported 96 % removal efficiency of SDS detergent contaminated wastewater by Pseudomonas aeruginosa MTCC 10311.\(^{32}\) Nair and Swarnalatha investigated the efficiency of Bacillus cereus sp. for biodegradation of surfactants in synthetic laundry wastewater sample. The maximum reported removal efficiency of surfactants was 95 % under optimum conditions.\(^{33}\)
Fig. 3 – Profiles of SDS removal efficiency in actual wastewaters by free and immobilized cells.

Fig. 4 – Recycling of beads for SDS biodegradation in synthetically prepared solution; (a) SA beads (b) SA-PVA beads.
Recycling of beads

One of the most important benefits of immobilized cells is their capability to be reused for successive cycles unlike free cells. The recycling of immobilized cells also increases the biodegradation process efficiency, and decreases its operating costs by making the bacterial cells active and stable for a longer time. In this study, results of recycling the SA beads and SA-PVA beads for SDS biodegradation are shown in Figs. 4 and 5. The SA beads were recycled three times in the synthetically prepared solution (SDS-loaded MSM) without losing their form; but in the fourth cycle the SA beads had dissolved. This observation could be related to the fact that the MSM consists of several salts, which may play a role in dissolving the SA beads. The swelling and subsequent dissolving of the beads in MSM solution could be due to the ion-exchange process between sodium, potassium, and magnesium ions in solution and calcium ions on the beads surface.

As given in Fig. 4-Panel A, the removal efficiency of SDS in the third cycle using SA beads, decreased from 100 % to 95.64 % as the initial SDS concentration increased from 10 to 1000 mg L⁻¹. On the other hand, the SA-PVA beads lasted up to five cycles in the MSM-bearing SDS (Fig. 4-Panel B) without losing their form. It is worth mentioning that the existence of PVA in the texture of the SA-PVA beads strengthened the beads and protected them from swelling and subsequent dissolution. However, in the fifth cycle, the removal efficiency SDS decreased from 100 % to 92.38 % as the initial SDS concentration increased from 10 to 1000 mg L⁻¹.

For the actual wastewaters, the SA and SA-PVA beads showed no decline in their shape and performance during the five cycles examined in this study. The removal efficiencies of SDS in LWW were 96.52 % and 96.21 % for SA and SA-PVA, respectively, during the fifth cycle. Whereas, for AWW, the removal efficiencies of SDS were 99.65 % and 99.13 % for SA and SA-PVA, respectively, during the fifth cycle, as shown in Fig. 4.

Mechanism of SDS removal

Unlike the high removal efficiency of SDS obtained when using immobilized cells, results of the experiments using the blank beads (free of bacterial cells) revealed very limited removal of SDS, less than 3 % of its initial concentration (data not shown). This very low removal efficiency may be attributed to the limited adsorption of SDS on the beads surface. This observation indicated that the dominant or even the sole mechanism for SDS removal was biodegradation, since the contribution of
the adsorption process could be considered negligible. This suggestion was supported by the qualitative analysis of the biomass. For SA and SA-PVA beads, the initial biomass concentrations were 8820 mg L\(^{-1}\) and 8840 mg L\(^{-1}\), respectively, whereas the biomass concentrations after use were 12710 mg L\(^{-1}\) and 10320 mg L\(^{-1}\), respectively. These results revealed the growth of bacterial cells inside the beads, confirming that SDS was a favorable substrate for the bacterial cells during the biodegradation process. Fig. 6 illustrates the SEM images for the SA and SA-PVA beads, which clearly show the heavy growth of biomass after treatment. On the other hand, as mentioned in Section *Inoculum*, the qualitative analysis demonstrated that the initial dominant bacterial species were *Pseudomonas fluorescens*, *Bacillus*, while the analysis of bacterial cells in the used beads revealed the presence of a new additional strain, *Klebsiella oxytoca* confirming the occurrence of SDS biodegradation.\(^{37}\)

**Conclusion**

The biodegradation and removal of SDS in aqueous solutions was carried out using mixed culture immobilized alternatively by SA and SA-PVA. Three types of aqueous solutions were examined:
synthetically prepared SDS-loaded solution (SWW), actual automobile service station wastewater (AWW), and laundry wastewater (LWW). Results demonstrated that, by increasing the SDS concentration from 10 to 1000 mg L⁻¹ in SWW, SDS degradation decreased from 99.71 % to 85.12 % using SA beads, and from 99.63 % to 83.29 % using SA-PVA beads. The removal efficiencies of SDS in AWW and LWW were 94.91 % and 94.39 % using SA beads, 93.82 % and 92.04 % using SA-PVA beads. Recycling of the prepared beads for successive cycles was studied to examine the reusability and performance of the beads, which makes it a superior technique compared to free cells. The SA beads lasted for 3 cycles before being dissolved in the SWW, whereas, SA-PVA beads lasted for 5 cycles. On the other hand, both SA and SA-PVA beads lasted for 5 cycles in both actual wastewaters, LWW and AWW.

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