A novel three-stage bioreactor for the effective detoxification of sodium dodecyl sulphate from wastewater

P. S. Ambily, Sharrel Rebello, K. Jayachandran and M. S. Jisha

ABSTRACT

Anionic surfactants like sodium dodecyl sulphate (SDS), due to its extensive disposal to water bodies cause detrimental effects to the ecosystem. Among the various attempts to reduce the after effects of these toxicants, microbial induced bioremediation serves as a promising strategy. The current study aimed to develop a three stage bioreactor to remediate anionic surfactants in wastewater using effective bacterial isolates. Screening of effective SDS biodegraders led to isolation of *Pseudomonas aeruginosa* (MTCC 10311). Treatment of synthetic effluent with an immobilized packed bed reactor at a flow rate of 5 mL h^{-1} resulted in 81 ± 2% SDS eliminations and 70 ± 1% reduction in chemical oxygen demand (COD) in five cycles (6 h per cycle). The hydraulic retention time of the reactor was found to be 6 h. Combinatorial usage of a three stage bioreactor, involving aeration, adsorption with low cost scrap rubber granules and treatment with immobilized *Pseudomonas aeruginosa,* successfully reduced SDS concentrations and COD of wastewater to 99.8 ± 0.1% and 99 ± 1%, respectively, in 18 h by continuous treatment. Half-life of the three stage bioreactor was 72 h. In addition to reducing the surfactant concentrations, this novel bioreactor could resolve the surfactant associated foaming problems in treatment plants, which make it more unique.

Key words | bioremediation, immobilized cells, *Pseudomonas aeruginosa*, scrap granular rubber

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INTRODUCTION

Surfactants occur as silent indispensable partners in people's day to day life (Rebello *et al.* 2013). Among the various groups of surfactants, anionic surfactants such as sodium dodecyl sulphate (SDS) are widely used due to their low cost and excellent foaming properties. Despite its use in cleaning products, it has various applications as additives in food, pharmaceutical preparations, cosmetics, oil industry, agrochemicals petroleum industry and many other industries (Karsa 1987). But this wide array of surfactant applications also results in indiscriminate and raw disposal of surfactants into water bodies. They pose a threat to residing macro and micro populations hampering biological processes such as phosphate solubilization, ammonia reduction, nitrogen fixation, photosynthetic ability (in bacteria, algae and plants); causing cell membrane damage, asphyxiation and death in fishes; disruption of filter feeding ability of bivalves; dermatitis and ocular lens damage in vertebrates, etc. (Rebello *et al.* 2014).

High concentrations of surfactants are commonly encountered in soil washing and other surfactant-based remediation technologies. An average anionic surfactant concentration of 1–10 mg L^{-1} can be found in municipal wastewater treatment dealing with only domestic wastewater (Field *et al.* 1992) but this range is visibly increased when industrial wastes are also treated (Beltran *et al.* 2000). Surfactants can also act on biological wastewater treatment processes and cause problems in sewage aeration and treatment facilities due to their high foaming properties which lower oxygenation potentials leading to final destruction of aquatic flora and fauna (Eichhorn *et al.* 2002). Thus, development of methodologies aiding effective surfactant removal of industrial wastewater gains much relevance.

Bacterial degradation of SDS involves interplay of multiple enzymes viz., alkylsulphatases, dehydrogenases which convert surfactants ultimately to CO_2 and water.

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Degradation of SDS is initiated by alkylsulphatases which hydrolyse SDS to sulphate and 1-dodecanol. The liberated alcohols are subsequently oxidized by alcohol dehydrogenases (Thomas & White 1989). The incorporation of surfactant degrading bacterial cultures in household and industrial sewage could be a cost effective anionic surfactant elimination method reducing the biochemical oxygen demand (BOD), chemical oxygen demand (COD) and methylene blue active levels in the water bodies (Hosseini *et al.* 2007).

The current work describes the prospects and practical use of surfactant degrading bacterial isolates in wastewater treatment plants. Wastewater, however, is a composite mixture containing organic waste, xenobiotics such as surfactants and inherent microflora. Thus, a bioreactor was designed with three stages viz., aeration, adsorption and immobilized bacterial cells. The aeration of the water before treatment promotes the growth of inherent microbial flora by utilizing the organic waste in the water and function as an activated sludge in principle. The second stage of adsorption would aid in the entrapment of such floccules from water and make it ready for final detergent bioremediation. Scrap granular rubber (SGR) used in this study was the waste product of tyres, locally purchased at the cost of Rs. 10 per kg (i.e. equivalent to $0.2 per kg). An enormous amount of waste tyres accumulate in the environment and most of these used tyres are not recycled but simply dumped in open or landfill sites. Therefore, a very low cost scrap rubber in the form of granules was used to remove anionic surfactant from the effluent.

Previously, biodegradation of SDS in a bioreactor with immobilized cells of *Pseudomonas C12B* on porous glass beads showed 85% efficiency to remove SDS (Jerabkova *et al.* 1999). Different modifications of microbe based synthetic water remediation have developed since then. However, supra-critical micelle concentration (supra-CMC) level of SDS in wastewater cause excessive foaming in reactors, operational difficulties and lead to health hazards in the form of airborne pathogens carried on windblown foams (Zhang *et al.* 1999). The current research also focused on the isolation of bacteria capable of degrading SDS at its supra-CMC level concentrations and to verify the use of them in a reactor for its remediation. Thus, a bioreactor was designed to effectively remediate wastewater with the combinatorial usage of aeration, adsorption and immobilized bacterial cells (active even at supra-CMC levels of detergents).

MATERIALS AND METHODS

Isolation and identification of SDS degrading bacteria

Soil samples were collected from detergent contaminated laundry premises of Meenachil river shore, located in Kottayam, Kerala, India. Isolation of SDS degrading organism was done by successive enrichment of soil bacterial isolates in mineral salt SDS medium (MSSM) to a final SDS concentration of (2.4 g L^{-1}) and incubated at room temperature ($30 \pm 2^{\circ}C$) for 24 h (Ambily & Jisha 2012). Among the nine strains capable of growing in 1.5 g L^{-1} of SDS, the most efficient strain giving maximum SDS removal at minimum time was selected. The SDS degradation potential of the isolates was determined spectrophotometrically by Methylene Blue Active Substance assay using Evolution 201 UV-visible spectrophotometer (Hayashi 1975) and by high-performance liquid chromatography (HPLC) analysis (Farzaneh *et al.* 2010).

The selected isolate was identified based on its morphological, biochemical and cultural characteristics according to Bergey's Manual (Holt *et al.* 1994) and further confirmed by 16S rDNA analysis. Chromosomal DNA was isolated from 5 mL of LB broth culture of isolate incubated at 37°C for 16 h (Pitcher *et al.* 1989). 16S rDNA typing of the isolate was done using universal eubacterial primers 8f–($^{5'}$AGAGTTTGATCCTGGCTCAG$^{3'}$), 1492r–($^{5'}$TACGGATACCTTGTTAGCACTT$^{3'}$) amplifying the 16S rDNA gene in an Eppendorf thermal cycler. Polymerase chain reaction (PCR) product was ligated into Insert-ready PCR-TRAP Cloning vector and transformed into *Escherichia coli* competent cells. The sequence of the insert was determined using the automated DNA sequencing service provided at Institute of Microbial Technology (IMTECH), Chandigarh, India. The isolate was deposited in MTCC with accession number MTCC 10311. The sequences were analyzed using the BLAST (www.ncbi.nlm.nig.gov) search algorithm and aligned to their nearest neighbors. The sequences were deposited in the NCBI gene bank database under accession number HM 214777.

The optimum temperature and pH was 30°C and 7.5, respectively (Ambily & Jisha 2012). This formed the basis of all further experiments described in this paper.

Evaluation on the utility of MTCC 10311 in surfactant removal after prolonged starvation

The starvation experiment was done to evaluate the effectiveness and survivability of the isolate in cases where the

reactor is depleted of nutrients during maintenance or varying concentrations of waste. Starvation experiments were performed in starvation medium with composition (mg L^{-1}) NH_4Cl – 5,400, KH_2PO_4 – 400, $MgSO_4$ – 200, Tris – 6,000 (final pH 7.4) (Roig *et al.* 1998). One percent of cells (1 OD) were inoculated in six sets of 50 mL of starvation medium and incubated at room temperature. SDS (100 mg L^{-1}) was added to the system after different intervals (2 h) of starvation time (0–32 h) and incubated for 48 h.

Optimization of MTCC 10311 mediated surfactant remediation

Prior to the set up of a bioreactor, the effectiveness if the isolate MTCC 10311 at different environmental conditions and different forms were evaluated by treatment of synthetic waste water using free and immobilized cells individual treatments. The use of free and immobilized cell based treatment would help to judge and comparatively evaluate the efficiency of the isolate to work in different forms. The choice of a good adsorbent (activated carbon or SGR) was also evaluated as a preliminary study for further reactor designing. The initial composition of synthetic wastewater (mg L^{-1}) used during the study was SDS – 2,400; $(NH_4)_2SO_4$ – 125, $MgSO_4$. $7H_2O$ – 25, $FeCl_3.6H_2O$ – 0.125, $CaCl_2$ – 1.825, KH_2PO_4 – 131.75, K_2HPO_4 – 267, $MnSO_4{\cdot}H_2O$ – 267, starch -100 (pH- 7.2). The COD and BOD of synthetic wastewater were computed to be 800 mg L^{-1} and BOD-200 mg L^{-1}, respectively, as per standard methods (Clesceri *et al.* 1998). The concentration of SDS was chosen as 2,400 mg/L as we aimed to analyze SDS degradation at supra-CMC level concentrations. As this value is quite above environmentally reported levels, degradation at such high concentrations would imply that lower concentrations could be degraded.

The effectiveness of free cells *P. aeruginosa* (MTCC 10311) cells in surfactant remediation was evaluated by estimating SDS and COD concentration of the synthetic water incubated with free cells of culture. Briefly, 1% inoculums of 1 OD culture of log phase cells of MTCC 10311 was inoculated in the synthetic water, incubated at $30 \pm 2^{\circ}C$ on a shaker at 150 rpm and aliquoted for analysis at 4 h intervals.

The utility of alginate immobilized *P. aeruginosa* (MTCC 10311) cells in surfactant removal from synthetic water was also evaluated at different flow rates (5 mL h^{-1}, 7 mL h^{-1}, 9 mL h^{-1} and 10 mL h^{-1}). Immobilised alginate beads of culture MTCC 10311 were synthesized by drop wise addition of culture – 4% alginate mixture (1:2) into 0.2 M $CaCl_2$.

Finally, the best adsorbent in surfactant removal was screened with either active carbon (Joseph Leslie dragger, Mumbai, India) or SGR (collected from retreading industry-super tyres, Kottayam, India). The rate of SDS adsorption at different operational conditions of pH, temperature and adsorption material (active carbon and scrap granulated rubber) were evaluated by passing wastewater through the packed bed material in the reactor at different flow rates. The ability of packed bed material in adsorbing SDS and reducing COD were further analyzed at different flow rates (Sarkar *et al.* 2003).

All experiments were conducted in triplicates and values were expressed as mean ± standard deviation throughout the study. Comparison between groups was performed using a student's t-test or a one-way analysis of variance (ANOVA) with post hoc analysis by Tukey's test (Miller & Miller 2000).

Designing and treatment with bioreactor

The three-stage bioreactor (Figure 1) for the removal of SDS was designed in such a way that the first stage consisted of aeration, second stage consisted of adsorption and third stage consisted of treatment with immobilized cells. The three-stage bioreactor was made up of glass and had four subunits. The unit 1 composed of aeration pump, filter disc and bush. The aeration pump was connected to the filter with help of the bushes. Filter discs with a central hole of dia 0.25 cm were meant for upward movement of water. Unit 2 consisted of a hollow covering glass of height 10 cm and 4.1 cm dia and small folding of 0.1 cm for support. Unit 3 was the association of Units 1 and 2. Main reactor system of dia 4.2 cm, height 15.8 cm had the inlet and the outlet of dia 0.2 cm each. Units 2, 3 and 4 were assembled to form the main reactor body to with a lower aeration layer, followed by the adsorbent layer and the top layer of immobilized cells.

The above-mentioned three-stage bioreactor was used for the treatment of synthetic wastewater. The bioreactor was capable of offering treatment in three stages such as aeration, physical adsorption and bacterial treatment. The effluent passed from the bottom of the bioreactor rose through the first reactor (reactor for aeration) and then slowly rose through the SGR in the second stage of the reactor. Finally it went through the immobilized cells in the third stage of the reactor. The bioreactor experiment was conducted at different flow rates of 5 mL h^{-1}, 7 mL h^{-1},

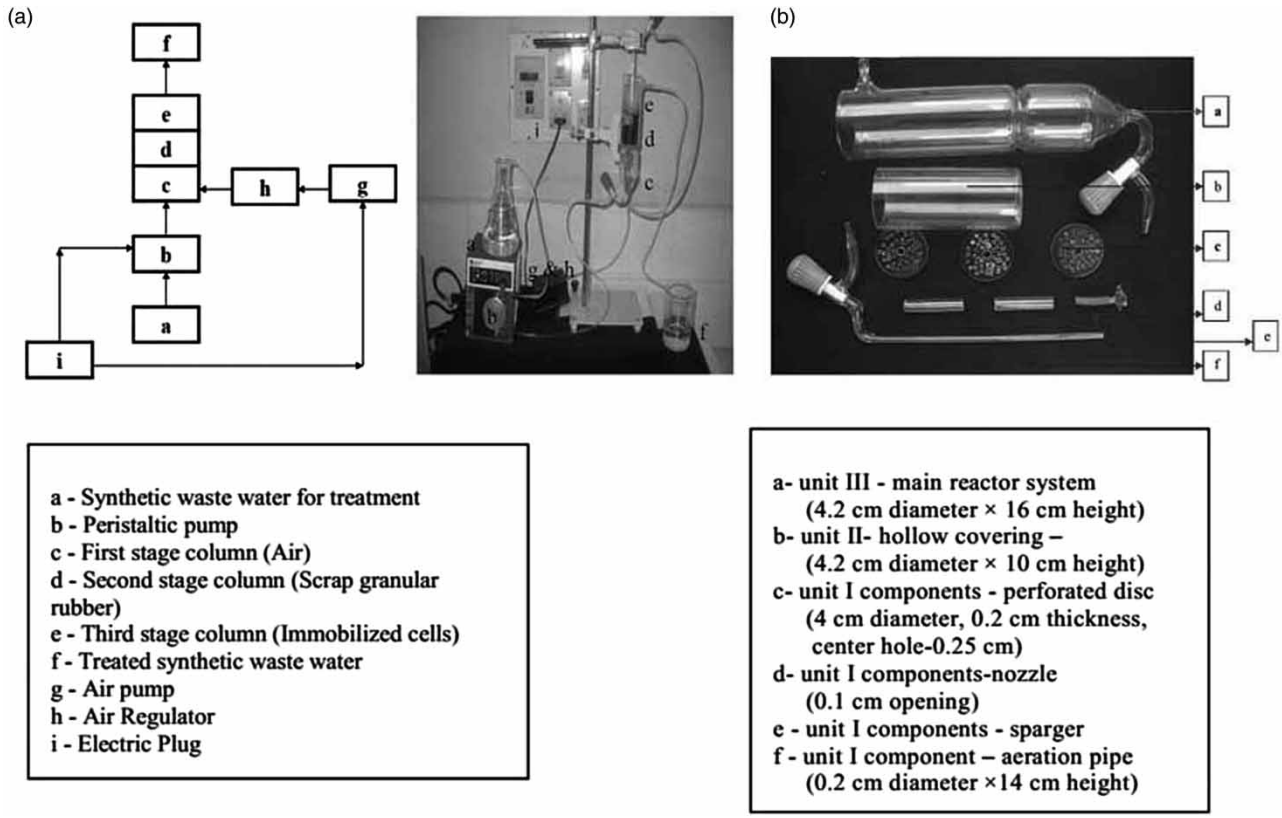

Figure 1 | Treatment of synthetic wastewater in a multistage reactor: (a) schematic diagram with assembled unit and (b) components of multistage reactor.

9 mL h^{-1} and 10 mL $h^{-1}$with a peristaltic pump (Pharmacia). Performance of bioreactor was evaluated at individual stage and combined stage by analyzing the SDS and COD.

Determination of half-life of three-stage reactor

Performance of the packed bed reactor was checked continuously for 108 h and the time required for the performance to become half of the maximum was calculated (Mathew & Jayachandran 2009).

RESULTS AND DISCUSSION

This study succeeded in isolating *P. aeruginosa* (MTCC 10311) capable of degrading 80% of 2.4 g L^{-1} SDS in 24 h and 96% reduction of 1.5 g L^{-1} SDS in 48 h. Figure 2 depicts the HPLC analysis of SDS degradation caused by *P. aeruginosa* MTCC 10311. The area of 0 h biodegradation of SDS in mineral salt medium reduced from 56,156,126 to 432,439 after 48 h of incubation as indicated in the zone marked red in colour. The 16S rDNA sequence of the isolate was generated and deposited in NCBI Gene Bank under accession number HM 214777. The BLAST search of 16S rDNA sequence in the gene bank database confirmed that this organism belonged to *P. aeruginosa*.

Biodegradation of surfactants is most often performed by diverse soil or aquatic microorganisms leading to generation of water and carbon dioxide gas (Schleheck *et al.* 2004). The effective utilization of any biodegradatory process relies on its successful isolation of bioremediatory agents. *Pseudomonas sp.* has been reported many times as a potent SDS biodegrading isolate (Chaturvedi & Kumar 2010; Yeldho *et al.* 2011). Our isolate *P. aeruginosa* (MTCC 10311) strain was capable of degrading comparatively higher SDS concentrations of 2.4 g L^{-1} in a short incubation period while 0.1% to 1% is found to be optimum concentrations of SDS degradation as per published studies (Rebello *et al.* 2013; Rebello *et al.* 2016).

Starvation experiments

Long time starvation (16 h) resulted in more efficient conversion of SDS to degradation products. Highest

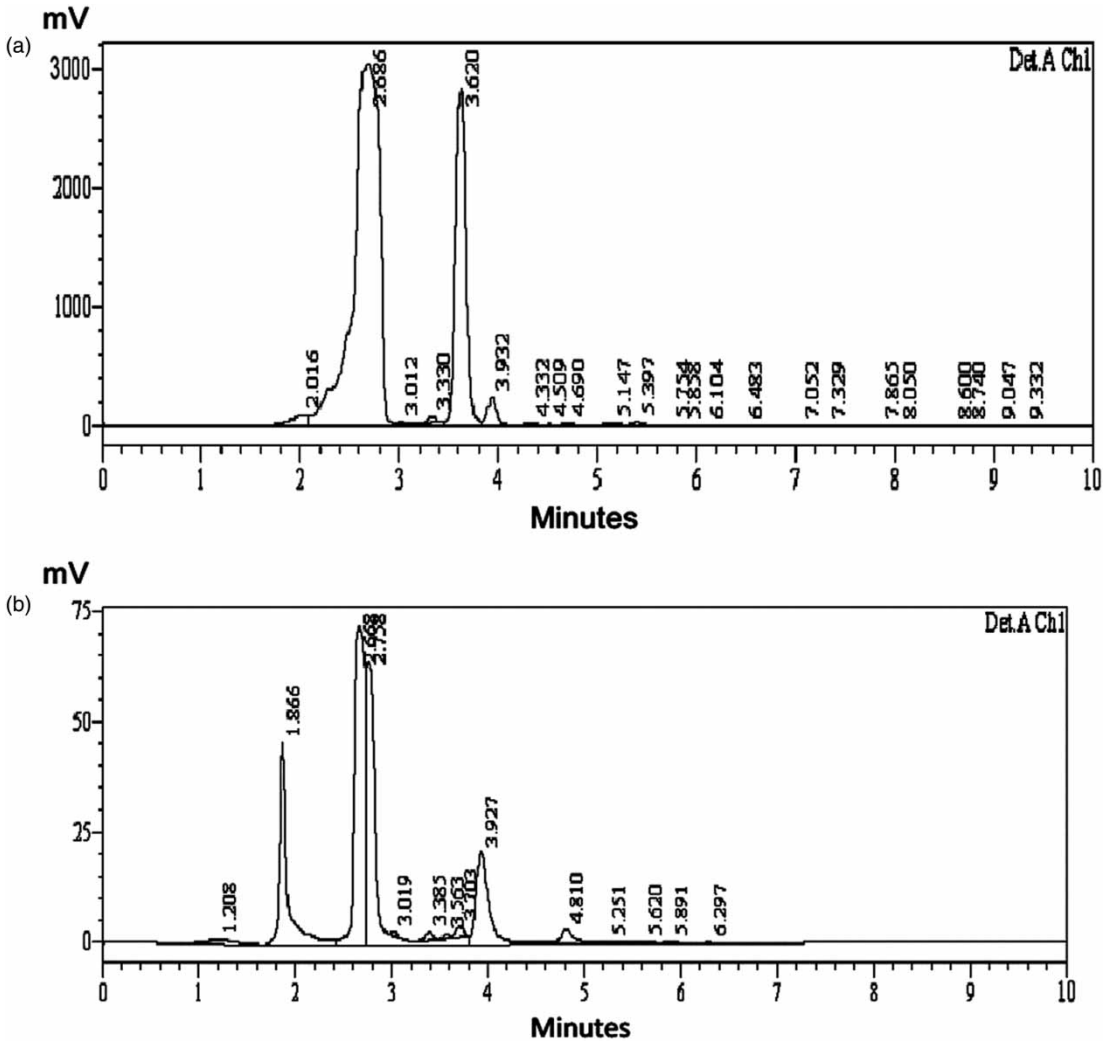

Figure 2 | HPLC analysis of SDS degradation with retention time of 2.686 min. (a) Control, (b) after 48 h of incubation.

Table 1 | Effect of starvation on the biodegradation of SDS by *Pseudomonas aeruginosa* MTCC 10311 on starvation medium containing SDS

	Incubation period (h)			
Starvation time (h)	**18**	**24**	**30**	**36**
Percentage reduction in SDS				
0	74 ± 2	80 ± 2	79 ± 3	81 ± 3
2	74 ± 2	81 ± 2	79 ± 2	80 ± 2
4	69 ± 3	82 ± 2	83 ± 2	82 ± 3
8	68 ± 3	79 ± 2	84 ± 2	83 ± 1
16	65 ± 1	78 ± 2	86 ± 1	85 ± 2
32	34 ± 2	41 ± 1	45 ± 1	44 ± 1

Values are means ± SE of at least three replicates.
ANOVA analysis indicated significance of results.

biodegradation rate of SDS (87 ± 1%) was observed in the case of 16 h of starvation but starvation beyond that lead to gradual decrease of biodegradation (Table 1). However, the longtime starved cells required longer time incubation for SDS degradation (30 h). The starvation conditions would probably prepare the cells to utilize the xenobiotics at a higher rate in the absence of any other carbon source. Similar starvation induced increase in xenobiotic degradation rates was also observed in case of dihexyl Sulphosuccinate (DHSS) degradation by *Comamonas terrigena* (Roig *et al.* 1998).

P. aeruginosa (MTCC 10311) also retained the biodegradation ability after depletion of surfactants for 16 days. The microbes used in wastewater treatment plants happen to encounter varied xenobiotic concentrations especially

during weekends when the wastewater treatment is not done, thus exposing them to a period of xenobiotic or nutrient starvation. For this reason, it was necessary to apply such microorganisms which are able to overcome this period of time. The bacterial isolate of this study, proved to be ideal candidates for SDS degradation as it degraded high levels of xenobiotics and this trait was stable, being expressed even after intervals of xenobiotic starvation. In general, microorganisms do not respond to depletion of nutrients by simply arresting all metabolic activity and stopping the growth. Instead, they carry out starvation-induced programs that allow them to exit from the cell cycle, maintain viability during starvation and resume growth when nutrients become available (Siegele & Kolter 1992).

Treatment of synthetic wastewater with free and immobilized cells

A comparison of the degradation of the synthetic wastewater revealed that $80 \pm 2\%$ SDS removals was achieved by 18 h of treatment with immobilized cells where the same performance was achieved by 24 h of treatment in the case of free cells (Figure 3). This implied that immobilization of *P. aeruginosa* enabled it to remediate SDS at a greater level than its free state. The immobilization on adsorbents indirectly promotes the formation of biofilms in the bacteria and cell aggregation helps bacteria to overcome the stress caused by SDS loaded environment (Klebensberger *et al.* 2007). Moreover, washout biomass is a limiting factor to detergent biodegradation in industrial wastewater treatment due to foaming and surfactant biocide effect, can be overcome by selecting suitable immobilization media for supporting microbial attachment and growth (Mutiara & Suhardi 2011).

Immobilized cells brought about a COD reduction of $54 \pm 3\%$ in 18 h (maximum $70 \pm 1\%$ in 30 h) where the free cells gave in $52 \pm 4\%$ COD reduction in 24 h of treatment (maximum $70 \pm 0.8\%$ in 36 h). Reduced incubation time for the reduction of surfactant and COD was a possible indication of a better activity of the immobilized cells (Figure 3). In the batch/recirculation treatment of the effluent at different flow rates using packed reactor with immobilized *Pseudomonas aeruginosa*, $80 \pm 2\%$ removal of SDS and $70 \pm 1\%$ COD reduction were observed at 5 mL h^{-1} flow rate in five cycles. The hydraulic retention time of the reactor in each cycle was computed to be 6 h. Wastewater with lower COD indicates that a substantial part of organic matter is easily degraded biologically.

Treatment of synthetic wastewater with adsorbent

SGR and active carbon were studied for evaluating their adsorption capacity of SDS in the synthetic wastewater (Table 2). The maximum adsorption capacity of SGR was evaluated for 70 h. The capacity for SGR for the reduction of SDS and COD were 30% and 10%, respectively, at a flow rate of 5 mL h^{-1}. It was observed that 30% adsorption of SDS occurred within 5 h of wastewater treatment. The significant challenge in the bacterial treatment of detergent containing wastewater is high concentration of SDS (2,400 mg/L). The use of adsorbent helped to reduce the high concentration of SDS, enabling the bacterial system in the third stage reactor to the complete removal of SDS at a faster pace with 30% reduction caused by adsorbent alone. Although the free and immobilized cells are capable

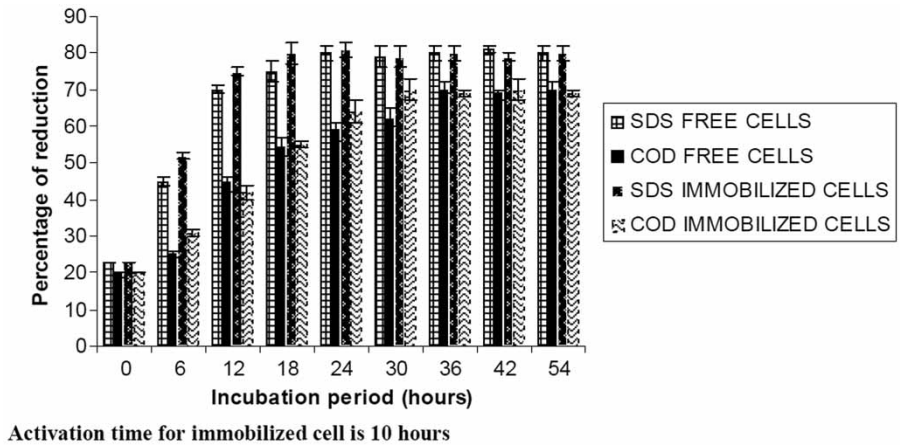

Figure 3 | Treatment of synthetic waste water with free cells and immobilized cells of *Pseudomonas aeruginosa*.

Table 2 | Performance of activated charcoal and SGR in the reduction of COD and SDS for the adsorption of synthetic wastewater

Adsorbents	% SDS* removal (mg/l)	% COD (mg/l)*
Activated charcoal	9 ± 0.2	13 ± 0.2
Sieved SGR particle size (cm)		
1	30 ± 2	38 ± 1
2	23 ± 2	28 ± 2
3	18 ± 1	23 ± 2

*Values expressed as mean ± standard deviation.

of degrading such high SDS concentrations, an inclusion of an adsorption based remediation step in the reactor would increase the pace of degradation by contributing to surfactant reduction by adsorption, as well as removal of floccules in the wastewater generated in preliminary stages of processing.

The sizes of the sieved adsorbed granular rubber used were 1 cm, 2 cm and 3 cm. It was observed that within 1 h, the adsorption of SDS was the same for all the three sizes (20%). But after 5 h the removal was found to be more in the case of smaller one (30%), while it was only about 23% and 18% for the other pieces. This may be due to the increase in the surface area contributed by particles of less size. The optimal adsorption conditions are 30°C temperature, pH 7.5 and SGR particle size is 1 cm. Previous studies with rubber granules packed in a column indicated that rubber could serve as a good adsorbent for anionic surfactants (Purakayastha *et al.* 2003). The use of adsorbents in the treatment of wastewater has been widely accepted and practiced as reviewed by Bhatnagar & Minocha (2006).

Treatment of wastewater with bioreactor

In the three-stage reactor, the effluent was treated with air at first, then with adsorbent packed reactor, and finally with an immobilized cell packed column. Performance of each stage was evaluated individually at different flow rates, of which 5 mL h^{-1} showed a better performance (Figure 4). However, on increasing the flow rate from 5 mL h^{-1}–10 mL h^{-1} both the SDS and COD reduction rate progressively got reduced. The increased flow rate resulted in reduced residence time and hence showed poor performance in both removal of SDS and reduction in COD. The three stages of the reactor individually contributed to the reduction of SDS concentration following different principles viz., aeration (by breaking the bubbles generated from bottom of reactor), adsorption treatment (causing physical removal of SDS) and finally by immobilized cells (accounting for biological SDS removal).

The first aeration unit of the reactor enhanced the biodegradation of SDS and COD (8% and 7% reduction respectively) at a flow rate of 5 mL h^{-1}. The aerators mainly account to provide oxygen to the water to reduce the chemical oxygen demand as well aid in the breaking of the released bubbles from the bottom of a reactor aiding better mass transfer (Rosso & Stenstrom 2006). The second reactor involved treatment of wastewater with SGR. The immobilized cells were packed inside the third reactor and the efficiency of this system to reduce the SDS of the effluent was evaluated with aeration and SGR packed reactor in series. When connected in series with the SGR packed aerated reactors, the immobilized cells in

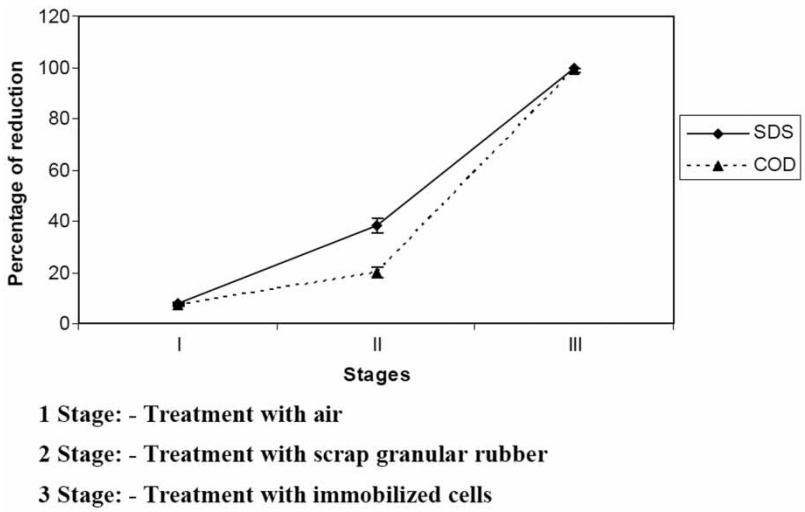

Figure 4 | Evaluation on the effectiveness of continuous treatment of synthetic wastewater with three-stage packed bed reactor involving aeration, SGR adsorbent and immobilized cells.

the third stage were receiving wastewater, which had already been exposed to physical adsorption and aeration.

The treatment of effluent in the first and second reactor reduced the SDS and COD levels to 38 ± 2% and 35 ± 2% respectively. Therefore, the third stage (immobilized cells packed) reactor received the synthetic wastewater with the concentration of 1.488 g L^{-1} (5.15 mM), surfactants become more bioavailable in this phase than supra-CMC level. The increasing surfactant concentration from sub- to supra-CMCs in wastewater significantly decreases primary biodegradation and foam degradation, which could be due to the limited bioavailability of surfactants in the micelle phase as compared to the monomeric surfactants (Zhang *et al.* 1999). Hence, the immobilized system could exhibit a better efficiency than when it was used as a single system for the treatment of the wastewater. Three-stage reactor could bring a 99.8 ± 0.1% at 99 ± 0.9% SDS removal and COD, respectively. Performance of the three-stage packed bed reactor was challenged continuously with fresh synthetic wastewater for 108 h. The half-life period of the reactor was observed as 72 h as depicted in Table 3. The variation in pH during the process was also analyzed. It was found oxidation reaction caused the pH to decrease and after 72 h pH become 6.5. But our selected strain was found capable of degrading SDS in the pH 6.5.

The present study strongly suggested that immobilized *P. aeruginosa* MTCC 10311 could be effectively used for the safe deposal of the wastewater discharge once appropriate technology was developed for large scale treatment. Many wastewater treatment plants are designated as immobilized cell bioreactors. Immobilization for microbial cultures has proven to be advantageous in municipal and industrial sewage treatment because of high degradation efficiency and good operational stability. The surfactant degrading bacterium *Pseudomonas C12B* in immobilized state has been used in several investigations for removing anionic surfactants (Thomas & White 1991). The flexibility of reactor design and the improved thermal and operational stability are further advantages of using immobilized cells.

Table 3 | Performance and water treatment efficiency of the three-stage packed bed reactor for 108 h at a flow rate of 5 mL/h

Treatment time point in h	Percentage of SDS remaining	Percentage of COD
18	100 ± 0.01	100 ± 0.03
36	99 ± 0.5	98 ± 0.25
54	86 ± 0.2	82 ± 0.41
72	48 ± 0.4	40 ± 1
90	38 ± 1.2	28 ± 0.9
108	27 ± 1.8	22 ± 1.2

The performance of the designed bioreactor and the system was evaluated based on its half-life period. It represents the period during which the performance of the reactor is brought to half. When the bioreactor system was put into operation in continuous mode treatment the reactor could bring 99.8 ± 0.1% SDS removal in 18 h. The performance continued up to 36 h and the hydraulic retention time was found to be 6 h. However, the efficiency progressively decreased after 36 h and was brought to half (48 ± 0.4% of SDS reduction) in 72 h. The time of 18 h was considered as the breakpoint of the reactor. This reduction in efficiency may be due to the diffusion limitation induced by the contaminants present in the wastewater. On reaching half-life, the reactor system is to be repacked and revived to eliminate channeling and diffusion limitation.

The concentration of surfactants employed in this bioremediation study was typically much higher than its CMCs, at a supra-CMC level of SDS is 8.311 mM (2.40 g L^{-1}), while its CMC concentration is 1 mM (Yu *et al.* 2007). Previous studies report that supra-CMC level of SDS is 1–8 mM (2.31 g L^{-1}) (Zhang *et al.* 1999). The characteristics of wastewater released from industry vary with the type of cosmetic treatment plant, sampling time and process condition. Hence, the present study was carried out with synthetic detergent wastewater having SDS concentration 2.4 g L^{-1}, 800 mg L^{-1} COD and 200 mg L^{-1} BOD. With the initial stages of aeration and adsorption on SGR, the SDS concentrations exposed to immobilized cells are lowered to values much lower than their supra-CMC levels and, thus, they become more bioavailable to immobilized cells for bioremediation. The greater bioavailability is witnessed by the greater degradation rates.

The major constraint in using biological processes such as activated sludge for surfactant removal is due to the low kinetics of degradation and excessive foam production (Dhouib *et al.* 2003). Many researchers used one stage bioreactor for the removal of surfactant from the wastewater (Mortazavi *et al.* 2008; Farzaneh *et al.* 2010), and succeeded to remove about 99.8% levels of 40–200 mg/L SDS. However, the use of the three-stage bioreactor finds an efficient solution for surfactant removal up to 99.8% levels even at concentrations of 2,400 mg/L, which makes it more advantageous. To the best of our knowledge, the possibility of using a three stage bioreactor for the complete removal of anionic surfactant is not studied so far.

CONCLUSIONS

In this study, *P. aeruginosa* MTCC 10311 with an efficient SDS degradation spectrum was isolated. It showed better degradation kinetics in the three-stage bioreactor than with the conventional biological systems. The integration of the treatment of immobilized cells with adsorption and aeration permitted the enhancement of SDS degradation rate from 81 ± 2% to 99.8 ± 0.1%. A very low-cost scrap rubber in the form of granules had been selected to adsorb SDS because of its better adsorption efficiency (30 ± 2%). The simplicity of the technique makes the process quite acceptable and the results of this study suggest that the usage of three-stage bioreactor in industrial sewage can be a cost effective method of complete SDS elimination.

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