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THE SELECTION OF BACTERIAL CONSORTIUM FOR THE BIODEGRADATION OF SODIUM DODECYL SULFATE AND LINEAR ALKYIBENZENE SULFONATE.

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ABSTRACT

Sodium Dodecyl Sulfate (SDS) and Linear Alkylbenzene Sulfonate (LAS) are major components of anionic detergent formulations. The main objective of this study was to screen the microorganisms for the biodegradation of SDS and LAS. At first, the screening of microorganism was done from a GenBank database. The microbial enzyme analysis by using bioinformatics tool showed that SDS and LAS could be degraded by *Pseudomonas putida* because the bacterium containing several enzymes, i.e., alkane 1- monooxygenase, alcohol dehydrogenase, aldehyde dehydrogenase, and acyl Co-A synthetase. It was shown that *P. putida* could degrade anionic surfactants resulting in 9.3% degraded SDS and 19% LAS left after two days of the degradation. Once we compared the biodegradation capability of the microorganism recovered from detergent-industrial sludge alone with that of co-culture of the bacterial isolate with *P. putida*, the co-culture of bacterial isolate with *P. putida* yielded better result than that of *P. putida* alone. It was found that the co-culture yielded only 0.03 % of SDS left after two days, whereas 0.39 % of SDS left from the degradation of *P. putida* alone. Similar results were observed when LAS was used as substrate, the co-culture of *P. putida* with the bacterial isolate yielded better result than that of *P. putida* alone, resulting in 4.43 % and 8.33% of left after two days of degradation, respectively.

Keywords: Anionic surfactant, biodegradation, Sodium Dodecyl Sulfate (SDS), Linear Alkylbenzene Sulfonate (LAS), and *Pseudomonas putida*.

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INTRODUCTION

Surfactant or surface active agents are organic compound with at least one lyophilic group and one lyophobic group in the molecule. If the water is used as solvent for the surfactants, the terms "hydrophilic" and "hydrophobic" are used (Farn, 2006). Surfactants are the active ingredients in household detergents, industrial cleaning agents and personal care products. It has been shown that surfactants used in personal care preparations are accounted for an estimated 15–16% of the total surfactant consumption for the time period between 1977 to 1992 (Schelheck et al., 2004). Surfactant consists mainly of three types of ingredients, i.e., anionic, nonionic, and cationic surfactant. Among the different types of surfactants, anionic surfactants are the most widely used as primary surfactants in personal care products. Sodium Dodecyl Sulfate (SDS) and Linear Alkylbenzene Sulphonate (LAS) are anionic surfactants that are widely used as ingredient in several industrial utilities (Karsa, 1987; Swisher, 1987; Trehy et al., 1996). The use of surfactants is gradually increasing. An advanced in sciences and technologies in company with market competition direct to a production of new detergent formulas which have a different extraordinary purpose. In view of environmentalists, laundry and cleaning chemicals, and the environmental risks associated with their manufacture, use and disposal are on focus due to environmentally impacts for many reasons. Detergent products are complex mixtures in which the ingredients tend to biodegrade and undergo partition at different rates. Of detergent products, their ingredients are relatively toxic to aquatic life. They cause the destruction of the function and the structure of the bacterial membranes by increasing their permeability. In the microorganisms, the adsorption of surfactants causes the depolarization of the cellular membranes and decreases consequently the absorption of the nutrients and modifies the release of the substances starting from the cellular metabolism (Ying, 2005).

The aim of this study was to screen microorganisms which had potential to degrade the anionic surfactants.

MATERIALS AND METHODS

Materials

SDS and LAS were kindly offered by Lion Corporation (Thailand) Limited. Most of chemicals were used as best grade and purchased either from Sigma (USA) or Fluka (Switzerland). Other chemicals were of analytical grade and were obtained from commercial suppliers.

Screening of microorganism by using GenBank database (http://www.ncbi.nlm.nih.gov)

In order to screen microorganisms, the biodegradation of the anionic surfactants pathway was studied and the protein sequence alignment of enzymes was conducted. We found that *Pseudomonas putida* has high potential to degrade anionic surfactants. The next step was to investigate the microorganism, which could degrade surfactants by using microorganisms, *P. putida* alone, and in comparison with the mixture of *P. putida* and the microorganism recovered from detergent industrial sludge.

The biodegradation pathway and enzymes which were used through the destruction of chemical of anionic surfactants by metabolic activity of microorganisms were studied. The protein sequence alignment of the microorganisms were obtained from the GenBank database and subsequently used to compare with the protein sequence alignment of the isolates.

Determination of the critical micelle concentration and standard curve

One tenth g of SDS was dissolved in LB medium to make a final volume of 100 ml (concentration =1 mg/ ml). SDS solution was subsequently diluted in the LB medium to make the final concentrations of 0.2, 0.4, 0.6, 0.8, and 1.0 mg/ml, respectively. LAS in the amount of 0.238 g was dissolved in LB medium to make the final volume of 100 ml (concentration= 1 mg/ml), and serial dilutions were made in LB medium to make the final concentrations of 0.2, 0.4, 0.6, 0.8, and 1.0 mg/ml, respectively. The temperature of diluted solutions was adjusted to 37 °C. The surface tension was determined using a tensiometer, with the unit of the surface tension was expressed as mN/m. Results of the value of surface tension were plotted against the concentration of the solution.

Biodegradation of SDS and LAS by *P. putida*

P. putida TISTR 1522 was grown in 3 ml of LB medium, and subsequently immobilized on the filter paper with the pore size of 0.45 micron. The immobilized microorganism was transferred into LB medium containing the surfactants solution at the concentration equal to critical micelle concentration (CMC). The solution was shaken continuously under 37 °C conditions. The culture was collected and filtered through the 0.45 micron filter paper. The surface tension of the filtrate was determined by tensiometer everyday for seven days.

Selection of the microorganism recovered from the detergent industrial sludge

A sample of the detergent industrial sludge was serially diluted, spread on LB agar containing 10 mg/ml surfactants, and incubated for 18 h, at 37 °C. The colonies of bacteria that grew on the surface of the selected media were selected and subcultured for the future use.

Biodegradation of SDS and LAS

A mixed culture of *P. putida* and the bacterial isolate was obtained by using a ratio of 50:50 between the culture of *P. putida* and of the bacterial isolate recovered from the sludge. The mixture was co-immobilized onto the 0.45 micron filter paper. Then, the immobilized culture was transferred into LB medium containing the surfactants, with the concentration equal to CMC. The solution was shaken continuously under 37 °C conditions. A sample of solution was randomly collected and passed through the 0.45 micron filter paper. The surface tension of the filtrate was determined by tensiometer in the following day.

RESULTS

The experiment was carried out in five steps, i.e., the determination of the critical micelle concentration of SDS and LAS, the determination of standard curve of each surfactant, testing the degradation of surfactants using *P. putida* alone, selecting microorganisms recovered from wastewater treatment ponds, and screening for the ability to degrade the surfactant in comparison with that of *P. putida*. Those isolates that had the ability to degrade surfactants faster were selected.

The CMC of SDS was found at 0.1 mg/ml because the value of surface tension of SDS was the same from the concentration of 0.1 to 2.5 mg/ml (data not shown). It was found that the CMC of LAS was 0.05 mg/ml since the surface tension was the same from concentration of 0.05 to 2.5 mg/ml (data not shown). The concentrations of SDS and LAS, which were used for the study of degradation by bacteria, were equal to CMC.

Biodegradation of SDS and LAS by *P. putida*

The primary biodegradation of LAS and SDS by the individual culture of *P. putida* was established by measuring the disappearance of LAS and SDS in the cultures everyday for nine days. The results of SDS biodegradation are summarized in Figure 1. It showed the percentage of SDS left in the broth medium over time by using LB broth. The percentage of the degradation of SDS ranged between 30 % and 8 % in 1 and 2 days, respectively. The results of LAS biodegradation are summarized in Figure 2. It showed the percentage of LAS left in the broth medium over time by using LB broth. The percentage of the degradation of LAS ranged between 43 %, 19 %, and 8 % at 1, 2, and 3 days, respectively.

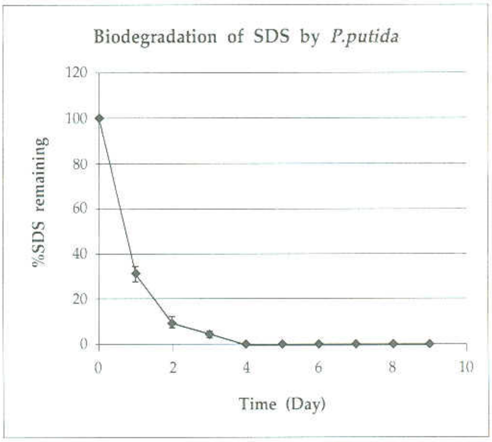

Figure 1. Results of biodegradation of SDS by *P. putida* alone.

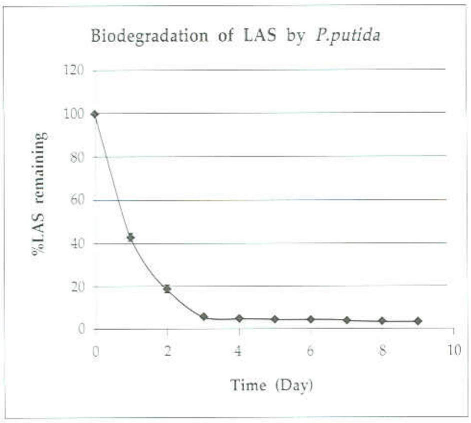

Figure 2. Results of biodegradation of LAS by *P. putida* alone.

Screening of the microorganisms recovered from detergent industrial sludge

The characteristics of microorganisms recovered from detergent industrial sludge that selected from the selected medium containing SDS and LAS are summarized in Tables 1 and 2, respectively.

Table 1. The characteristics of microorganisms that selected from SDS selected medium.

Name.	Surface colonies plate culture				Streaking morphology	Optical character	Color
	Form	Elevation	Surface	Consistency			
SDS-01	Circular	Raised	Smooth	Butyrous	Filiform	Opaque	White opaque
SDS-02	Circular	Flat	Smooth	Butyrous	Filiform	Translucent	White opaque
SDS-03	Circular	Raised	Smooth	Butyrous	Filiform	Opaque	White opaque
SDS-04	Circular	Raised	Smooth	Butyrous	Filiform	Opaque	White opaque
SDS-05	Circular	Raised	Smooth	Butyrous	Filiform	Opaque	White opaque
SDS-06	Circular	Flat	Smooth	Butyrous	Filiform	Opaque	White opaque
SDS-07	Circular	Flat	Smooth	Butyrous	Filiform	Opaque	White opaque
SDS-08	Circular	Effuse	Smooth	Membranous	Filiform	Translucent	Yellow crystal

Table 2. The characteristics of microorganisms that selected from LAS selected medium.

Name.	Surface colonies plate culture				Streaking morphology	Optical character	Color
	Form	Elevation	Surface	Consistency			
LAS-01	Circular	Flat	Smooth	Butyrous	Filiform	Opaque	Orange mud
LAS-02	Circular	Flat	Smooth	Butyrous	Filiform	Opaque	White opaque
LAS-03	Filamentous	Raised	Rugose	Butyrous	Filiform	Opaque	White opaque
LAS-04	Circular	Raised	Smooth	Butyrous	Filiform	Opaque	White opaque
LAS-05	Circular	Flat	Smooth	Butyrous	Filiform	Opaque	White opaque
LAS-06	Filamentous	Flat	Rugose	Viscid	Spreading	Opaque	Translucent white
LAS-07	Circular	Flat	Smooth	Butyrous	Filiform	Opaque	White opaque
LAS-08	Circular	Flat	Smooth	Butyrous	Filiform	Opaque	White opaque

Biodegradation of SDS and LAS by *P. putida* and microorganism from sludge

The capability of biodegradations of SDS and LAS by the individual culture and bacterial consortium were established by measuring the disappearance of SDS and LAS in the cultures. The capability of degradation of SDS from all microorganisms recovered from sludge, especially the isolate SDS-05, had higher percentage of degradation than *P. putida*, as shown in Figure 3. The co-cultures that had higher percentage of degradation were the co-culture of *P. putida* and the isolate SDS-05, the co-culture of *P. putida* and the isolate SDS-08, with the remaining value of surface tension of SDS as 0.39% and 0.49%, respectively, as shown in Figure 4.

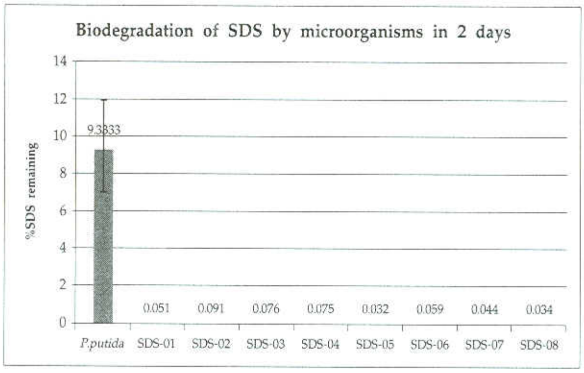

Figure 3. Results of biodegradation of SDS by microorganisms that recovered from detergent industrial sludge.

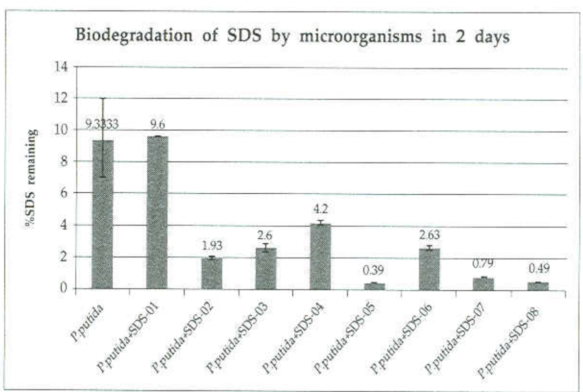

Figure 4. Results of biodegradation of SDS by microorganisms that recovered from detergent industrial sludge co-cultured with *P. putida*.

The capabilities of all microorganisms that recovered from sludge and *P. putida* to degrade LAS were also studied, and the results are summarized in Figures 5 and 6. All individual cultures had higher percentage of degradation than those of the co-culture. The isolate LAS06 had higher percentage of degradation than the co-culture of *P. putida* and the isolate LAS06, with the remaining value of surface tension of SDS as 4.434% and 8.33%, respectively.

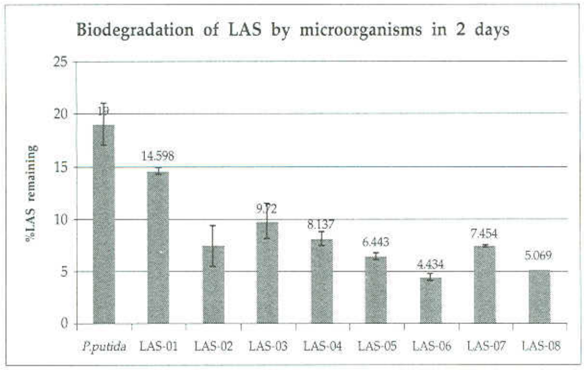

Figure 5. Results of biodegradation of LAS by microorganisms that recovered from detergent industrial sludge alone.

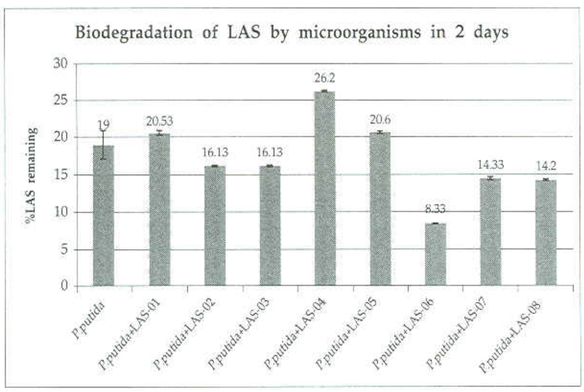

Figure 6. Results of biodegradation of LAS by microorganisms that recovered from detergent industrial sludge co-cultured with *P. putida*.

DISCUSSION

Bacteria are the primary agents that have capabilities to degrade surfactants. A bacterium or a consortium of bacteria that can be used for the treatment of wastewater loaded with surfactants must be able to destroy the surfactants properties of the molecule by their enzymes, and also be able to use the breakdown products as a source of carbon and energy (Ying, 2005). Scott and Jones reviewed the literature concerning the degradation of surfactants and described the biodegradation pathway as primary and/or ultimate pathway (Scott and Jones, 2000). Primary degradation can be defined as to have occurred when the structure has changed sufficiently for a molecule to lose its surfactant properties due to the degradation of the parent substance and consequential loss of surface-active property. Ultimate degradation means the level of biodegradation achieved when the surfactant is totally used by microorganisms resulting in its breakdown to inorganic end-products, such as CO_2, water and mineral salts, or any other elements present (mineralization), and new microbial cellular constiturent (biomass) (Swisher, 1987; Kuhnt, 1993; Trehy et al.,1996). The mechanism of breakdown of the anionic surfactants is initiated by the breakdown of alkyl chain (ω-oxidation) and followed by successive β-oxidation (Scott and Jones, 2000).

LAS biodegradation intermediates are mono- and dicarboxylic sulfophenyl acids (SPC) that are formed by ω-oxidation; the alkyl chain starts with the oxidation of the terminal methyl group to the alcohol by enzyme alkane monoxygenase, alcohol to the aldehyde by enzyme alcohol dehydrogenase, and aldehyde to the carboxylic acid by enzyme aldehyde dehydrogenase. The carboxylic acid is further degraded via β-oxidation and the two carbons fragment enters the tricarboxylic acid cycle as acetylCo-A. The protein sequence alignment of enzymes was conducted by searching from a GenBank database, and we found that *Pseudomonas putida* had high potential to degrade anionic surfactants because it had enzyme alkane 1- monooxygenase, alcohol dehydrogenase, aldehyde dehydrogenase, and acyl Co-A synthetase (data not shown).

The primary biodegradation of SDS and LAS by the individual culture *P. putida* was established by measuring the disappearance of SDS and LAS in the cultures everyday for nine days. The concentrations of SDS and LAS used were equal to CMC. The results in Figures 1 and 2 show the percentage of SDS left in the broth medium over time by using LB broth.

The percentage of the degradation of SDS (Figure 1) ranged between 30% and 8 % at 1 and 2 days, respectively. These data indicated that the single bacterial culture contributed enzymatic activity for cleavage SDS which agreed with the previous result. It showed that P. putida had enzymes, alkane 1-monooxygenase, Alcohol Dehydrogenase, Aldehyde Dehydrogenase, and Acyl-CoA Syntertase that could degrade SDS. The results in Figure 2 show the percentage of LAS left in the broth medium over time by using LB broth. The percentage of the degradation of LAS ranged between 43, 19, and 8 % at 1, 2, and 3 days, respectively. These data indicated that the *P. putida* contributed enzymatic activity for cleavage LAS which agreed with the previous result. It was shown that *P. putida* had alkane 1-monooxygenase enzyme which could degrade LAS.

Figures 3 and 4 show the percentage of SDS left after two days when LB medium was used. All individual cultures had higher percentage of degradation than that of the co-culture, especially the isolate SDS05, which had higher percentage of degradation than that of *P. putida* co-culture with the isolate SDS05. The value of surface tension of SDS was 0.032% and 0.39%, respectively. The result also showed that the isolate SDS-05 alone was the most degradability than that of the co-culture. These data indicated that SDS-05 could contribute to the enzymatic activity for cleavage SDS and yielded better result than that of *P. putida* or had some SDS degrading enzyme, whereas *P. putida* did not have.

The results in Figures 5 and 6 show the percentage of LAS left after two days by using LB medium. The co-culture which rendered higher percentage of degradation was *P. putida* co-cultured with the isolate LAS-06, where the value of surface tension of the remaining LAS was 8.33% (Figure 6). These data indicated that the two bacterial components of the mixture had the ability to degrade anionic surfactants better than that of *P.. putida* alone because the co-culture contributed to the enzymatic activity for cleavage of the benzene ring (Swisher, 1987; Sigoillot and Nguyen, 1990; Jimenez et al., 1991). It was shown that the isolate LAS-06 alone had better activity than that of the co-culture of *P. putida* with LAS-06, which was 4.434% of LAS remaining (Figure 5). The present data suggest that both bacterial strains of the consortium (*P. putida* and the isolates recovered from sludge) complement each other in their biodegradation action on SDS and LAS. It was observed that *P. putida* alone had failed to produce the same degree of surfactants degradation obtained by the mixed consortium.

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