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Biodegradation of anionic surfactants by isolated bacteria from activated sludge

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ABSTRACT: Sodium dodecyl sulphate, (SDS) is an anionic surfactant that widely used all over the world. They will eventually end-up and accumulate in household or industrial sewage. Due to their high foaming capabilities, which can cause numerous problems in sewage treatment facilities as well as direct toxic effects on many different organisms in ecosystem; they are generally considered as serious pollutants. In this survey, two different bacteria were isolated from Tehran municipal activated sludge. Biochemical tests as well as 16S rRNA gene sequencing for identification have been applied. After experiments to optimize the pH and temperature for growth of the two bacterial isolates, the extent of SDS utilization was evaluated by HPLC method. Two bacterial isolates show which ability to rapidly and actively degrade SDS upon using it as their sole source of carbon. The identification tests have indicated the two isolates to be *Acinetobacter johnsoni* and *Pseudomonas beteli*. The *Pseudomonas beteli* and *Acinetobacter johnsoni* isolates were able to degrade 97.2% and 96.4% of the original SDS levels after 10 days of growth; respectively. Mixed culture of the two isolates did not significantly increase SDS utilization, (97.6%). In conclusion, the results of this study suggest that growth of simple bacteria such as Acinetobacter or Pseudomonas in household and industrial sewage can be cost-effective method anionic surfactants elimination.

Key word: *Sodium dodecyl sulphate SDS), biodegradation, activated sludge, anionic surfactant*

INTRODUCTION

Surfactants, due to their favorable physicochemical properties are extensively used in many fields of technology and research, i.e. in pharmacy, in cosmetics, textile industry, agriculture, biotechnology (Sales, *et al*., 1999). After use large quantities of surfactants and their derivatives are released to aquatic and /or terrestrial environment. These compounds can act on biological wastewater treatment processes and cause problems in sewage aeration and treatment facilities due to their high foaming, lower oxygenation potentials and making death of waterborne organisms (Eichhorn, *et al*., 2002). Anionic surfactants such as sodium dodecyl sulphate (SDS) have been use for about 40 years (Lauer, *et al*., 1996). SDS, in particular, is an essential component of shampoos and foaming agent for toothpaste. Principal criterion for the ecological behavior of surfactants is their biodegradability (Cain, *et al*., 1981). Biodegradation is most often performed by soil or aquatic microorganisms and leads to generation of water and carbon dioxide gas (Schleheck, *et al*., 2000). The molecular structure of SDS is composed of three units, (1) A hydrocarbon chain, (C_{11}-C_{14}); (2) A benzene ring attached to the chain; and, (3) A sulphate group attached to the ring (Schleheck, *et al*., 2003). In nature and under standard at very low rate (Juker, *et al*., 1994). In this survey, SDS degrading bacteria were isolated and identified by 16S RNA sequencing from activated sludge of several locations in Tehran. Their single as well as mixed culture surfactant degradation capability in aerobic growth was measured by HPLC method.

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MATERIALS AND METHODS

Bacterial isolation

Activated sludge samples obtained from Gheitarihe sewage treatment company in Tehran was subjected, (5%) to 500 mL basal salt medium, (KH_2PO_4 3.5 g, K_2HPO_4 1.5 g, NH_4Cl 0.5 g, NaCl 0.5 g, Na_2SO_4 0.14 g

, $MgCl_2.6H_2O$ 0.15 g , dissolved in 1 L of distilled water and the final pH adjusted to 7.1) and containing 1.5 mM sodium dodecyl sulphate ($C_{12}H_{25}OSO_3Na$). Its molecular weight is 288g/moL .Critical micellar concentration (cmc) is equal to 2310 mg/L The inoculated media were incubated at room temperature with constant Shaking, (150 rpm). After no foams were visible during growth, (due to surfactants utilization), the liquid culture was transferred to solidified, (1% agar) basal salt medium with 1.5 mM SDS in culture plates. Following three subcultures on the solid media, two different bacterial colonies were isolated and identified. The growth curve of the two bacterial strains, (A and B) in the surfactants containing liquid media as well as pH and growth temperature optima were subsequently determined.

Culture

Each strain was grown, either as single or mixed culture, after an adaptation step in nutrient broth was containing SDS, in basal salt medium, (BSM) containing 1.5 mM SDS as the sole source of carbon. Incubation was performed at optimum pH and temperature with shaking, (150 rpm) for 12 days. Culture samples were collected and analyzed for SDS utilization after 1, 3, 5, 7 and 10 days of growth.

Surfactant degradation

HPLC with a C-18 column, (18 cm length and 4 mm width) using an isocratic mobile phase gradient of acetonitrile-water, (80-20) was conducted at a flow rate 1ml/min. Eluent absorption was detected with a UV spectrophotometer at 220 nm.

Bacterial identification

Initial identification schemes were performed with biochemical tests as suggested by the Bergeys Manual of Systematic Bacteriology. For final and specific identification, 16S rRNA sequencing was performed after PCR with specific primers.

RESULTS

The two bacteria, (A and B) isolated from activated sludge grow well in BSM media with SDS as their sole carbon source. The optimum pH values for the growth of A and B strains in the basic medium at 30ÚC were 7.4 and 8.0, respectively. Based on morphologic and biochemical characteristics, (Fig.1 and Table 1), as well as 16S rRNA gene sequencing, with the nearest phylogenic relatedness (99% homology), the A and B strains are members of *Pseudomonas betelli* and *Acinetobacter johnsoni* strains, respectively. Fig. 1 shows electron micrographs of the two bacteria in activated growth. HPLC analysis indicated that A strain had the highest surfactant degrading potential, (Table 2). The B strain was able to decrease SDS level in the growth media from an original of 522 mg/L to the extent of 93.6% within 5 days; whereas, the other strain did so to the extent of 84.6%, (Fig. 2 and Table 2). However, following 10 days of incubation, A strain showed greater degradation, (97.2%) potential relative to the B strain, (96.4%). The highest peak of SDS degradation occurred during the logarithmic phase of bacterial growth (Figs. 3 and 4). Co-culture of the two strain did not significally increase the degradation potential, (97.6%) SDS degradation after 10 days of growth) relative to single bacterial growth, (Table2).

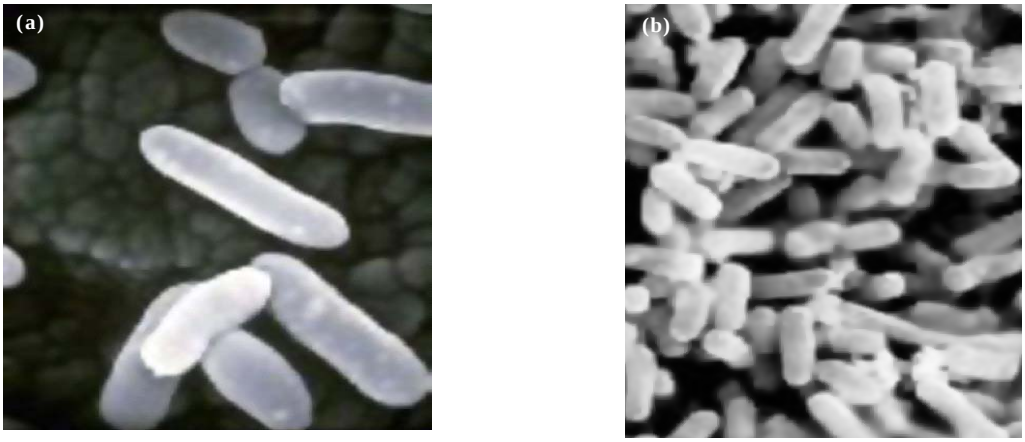

Fig . 1: Electron micrographs of A strain (a), and B strain (b)

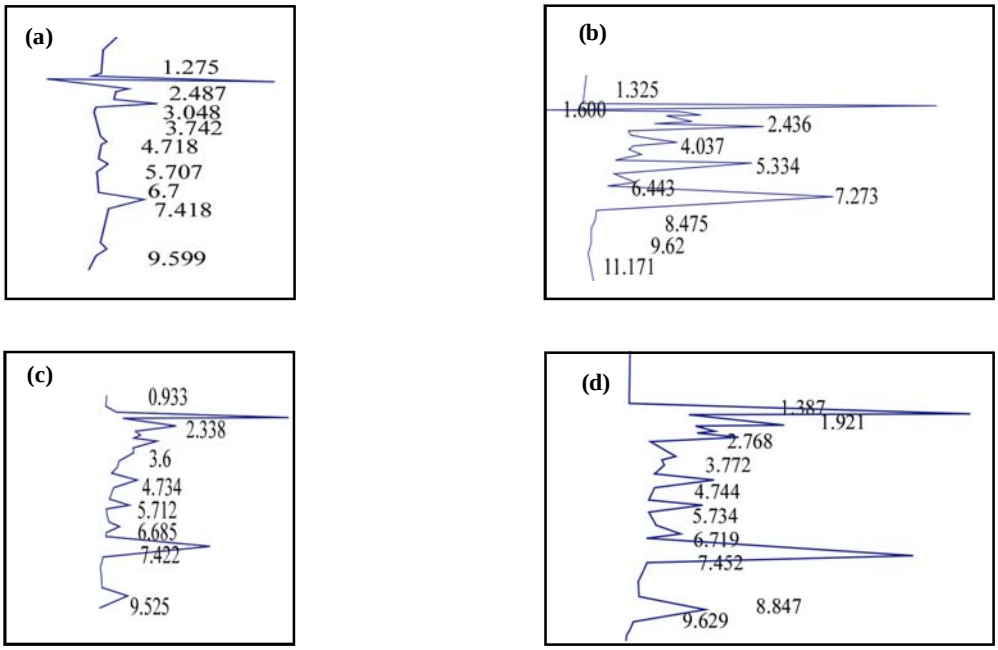

Fig. 2: HPLC analysis of SDS degradation in, (a) A strain at 1 day, (b) A strain at 10 days, (c) B strain at 1 day, (d) B strain at 10 days of incubation

Table 1: Morphologic and biochemical characteristics of isolated strains

Tests	A	B
Gram staining	Slightly curved rods 0.5-1.0 μm in diameter, 1.5-5.0 μm in length, Gram-negative	Straight rods 0.9-1.6 μm in diameter, 1.5-2.5 μm in length, Gram-negative
Motility	+	+
Capsule	+	+
Oxidase	+	-
Catalase	+	+
Growth in:		
4° C	+	-
42° C	+	-
Haemolysis	+	-
Citrate	+	+
Acid from glucose	-	-
Nitrate reduction	+	+
Tryptophanase	-	-
Arginin dihydrolase	+	+
Gelatinase	+	-
Lysine hydrogenase	-	-
Pigment production	-	-
Utilization of:		
D-Lactose	-	+
Glutamate	+	-
Malonate	+	-
L_Ornithine	+	-
L-Leucine	-	-

Table 2: Results of HPLC analysis depends on concentration and removal percentage of SDS from culture

	Co- culture		A isolate		B isolate	
Incubation period (day)	LABS level (g/L)	Utilization LABS (%)	LABS level (g/l)	Utilization LABS (%)	LABS level (g/L)	Utilization LABS (%)
0	0.500	00.0	0.500	00.0	0.500	00.0
1	0.432	13.6	0.436	12.8	0.429	14.2
3	0.236	52.8	0.341	31.8	0.350	30.0
5	0.035	93.1	0.077	84.6	0.032	93.6
7	0.016	96.8	0.018	96.4	0.025	95.0
10	0.012	97.6	0.014	97.2	0.018	96.4

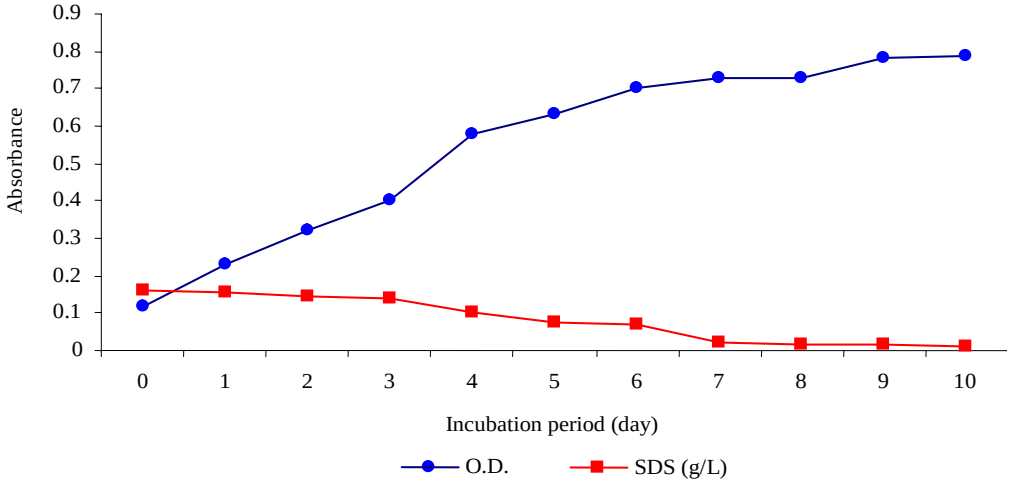

Fig. 3: Growth of A strain in relation to SDS degradation

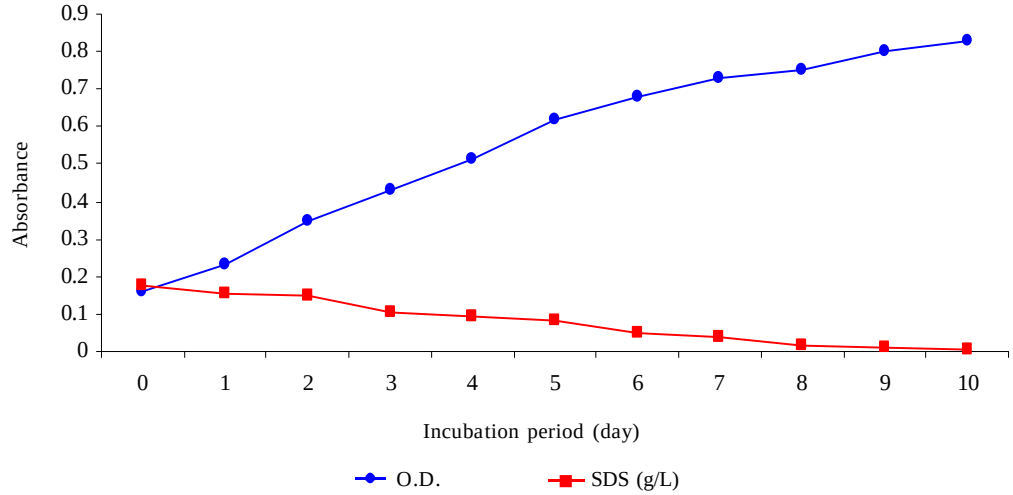

Fig. 4: Growth of B strain in relation to SDS degradationz

DISCUSSION AND CONCLUSION

Past experiences have demonstrated those anionic surfactants biodegradations are exclusively conducted by bacteria (Cain, *et al*., 1981 and Juker, *et al*., 1994). Investigators such as Schleheck, *et al*., 2000, Jimenez, *et al*., 1991 and Dhuiib, *et al*., 2003, have used activated sludge cultures in order to isolate heterotrophic anionic surfactant degrading bacteria. In this survey, aerobic cultures of activated sludge from Tehran municipality were performed in order to isolate anionic surfactant degrading bacteria. Two different bacteria were isolated after subsequent growth in basal salt media containing SDS as the sole carbon and energy source. Schleheck, *et al, 2003, have* used 16S rRNA gene sequencing for surfactant degrading bacteria identification. Whereas, Dhuib, *et al., 2003,* have relied solely on biochemical tests in order to identify their isolated bacteria. In this survey, we have used both biochemical as well as molecular methods in order to identify the two strains. The maximum is in agreement with the observation of Dhuib, *et al, 2003,* and Schleheck, *et al.*, 2000. Hyashi, *et al*., 1975, have used methylene-blue activated substances (MBAS) method for determination of anionic surfactant biodegradation in aquatic environments. This chromatographic method was originally proposed in 1976 and was subsequently used by many other investigators (Kertesz, *et al*., 1994, Jerabkova, *et al*., 1999, and Dhuib, *et al, 2003*). Jerebkova, *et al*., 1999, have used this technique to valuate anionic surfactant elimination by Pseudomonas biofilms. In later years, Schleheck, *et al, 2000,* and Schulz *et al*., 2000, have suggested that the presence of contaminating ions and intermediate compounds can inhibit precise detection of SDS levels by the methylene- blue assay. They suggested that HPLC is a superior technique for SDS identification. Continued growth and biomass accumulation of the bacteria were coincidental in the via. This indicates that the bacteria are actually utilizing SDS as their sole carbon source. This is in agreement with the results of other investigators (Di Cocia *et al*., 1994, Jimenez *et al*., 1991, and Sigoillot *et al*., 1992). During stationary phase, (7^{th} till 10^{th} days of growth), no significant decrease in SDS levels was detected, indicating that bacterial growth had begun to level off. This was true for both A and B strains. Jerebkova *et al*., 1999, have noted that Pseudomonas cultures in continuous bioreactors have contributed to a 70% decrease in surfactant levels after 20 days. Other studies have noted different levels of surfactant utilization in closed cultures. For instance, over 90% of surfactant usage was noted by locally isolated Citrobacter spp. after 35 hours of growth (Schleheck *et al*., 2003). In this survey, the B strain was able to utilize 94% of the original SDS levels after 120 hours. The biodegradation rate was the highest between 3 and 5 days. Sigoillot *et al*., 1992, have reported that mixed cultures of different bacteria can dramatically improve the biodegradation potential. In this study, co-culture of the two isolated strains did not cause a dramatic rise in surfactant utilization.

The obtained results shown that anionic surfactants significantly biodegraded by bacteria. The results of this study suggest that growth of simple bacteria such as Acinetobacter or Pseudomonas in household and industrial sewage can be a cost-effective method of anionic surfactant elimination.

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