Isolation of Bacterial Isolates From Municipal Wastewater For Bioremediation of Anionic Surfactants

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Abstract - An anionic surfactant that widely used all over the world is known as Sodium dodecyl sulphate, (SDS). They are generally considered as serious pollutants 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. In this study, three different bacteria were isolated from municipal wastewater. Biochemical tests have been applied for identification of unknown bacterial isolates. After experiments to optimize the pH and temperature for growth of the three bacterial isolates, the extent of SDS utilization was evaluated by Optical Density (220nm) and CFU of bacterial strains. Three bacterial isolates showed which ability to rapidly and actively degrade SDS upon using it as their sole carbon source. The identification tests have indicated the three isolates to be Serratia sp., Acinetobacter johnsoni and Pseudomonas beteli. The Serratia sp., Pseudomonas beteli and Acinetobacter johnsoni isolates were able to degrade 91.3%, 94.2% and 92.4% of the original SDS levels after 10 days of growth; respectively. Mixed culture of the three isolates increased significantly SDS utilization, (97.6%). In conclusion, the results of this study reveal that growth of simple bacteria such as Serratia, Acinetobacter and Pseudomonas in household and industrial sewage can be cost-effective for anionic surfactants elimination.

Key word: Anionic surfactant, Sodium dodecyl sulphate SDS), Biodegradation, Municipal wastewater.

I. INTRODUCTION

Surfactants are extensively used in many fields of technology and research, i.e. in pharmacy, in cosmetics, textile industry, agriculture, biotechnology (Sales, *etal*., 1999) due to their favorable physicochemical properties. After use large quantities of surfactants andtheir 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 sodiumdodecyl 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 iscomposed 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, *etal*., 2003). In nature and under standard at very lowrate (Juker, *et al*., 1994). In this study, SDS degrading bacteria were isolated and identified by biochemical testsfrom municipal wastewater of several locations in Allahabad. Their single as well as mixed culture surfactant degradation capability in aerobic growth was measured by Optical Density (220nm) and CFU of bacterial strains.

II. MATERIALS AND METHODS

Bacterial isolation and identification

Municipal wastewater collected from railway pulliya MNNIT Campus in Allahabad 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, three different bacterial colonies were isolated and identified. The growth curve of the three bacterial strains, (A, B and C) in the surfactants containing liquid media as well as pH and growth temperature optima were subsequently determined. Initial identification schemes were performed with biochemical tests as suggested by the Bergeys Manual of Systematic Bacteriology.

Culture and Surfactant degradation

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.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.

III. RESULTS

The three bacteria, (A, B and C) isolated from municipal wastewater grow well in BSM media with SDS as their sole carbon source. The optimum pH values for the growth of A, B and C strains in the basic medium at 30ÚC were 6.8, 7.4 and 8.0, respectively. Based on morphologic and biochemical characteristics, (Fig.1 and Table 1), the A and B strains are members of *Serratia sp.,Pseudomonas betelli* and *Acinetobacter johnsoni* strains, respectively. Fig. 1shows electron micrographs of the three bacteria in activated growth. HPLC analysis indicated that B strain had the highest surfactant degrading potential, (Table 2). However, following 10 days of incubation, B strain showed greater degradation, (94.2%) potential relative to the A strain, (91.3%) and C strain, (92.4%) respectively. The highest peak of SDS degradation occurred during the logarithmic phase of bacterial growth (Table 3, 4 and 5). Co-culture of the three strains increased significally the degradation potential, (97.6%) after 10 days of growth, (Table 6).

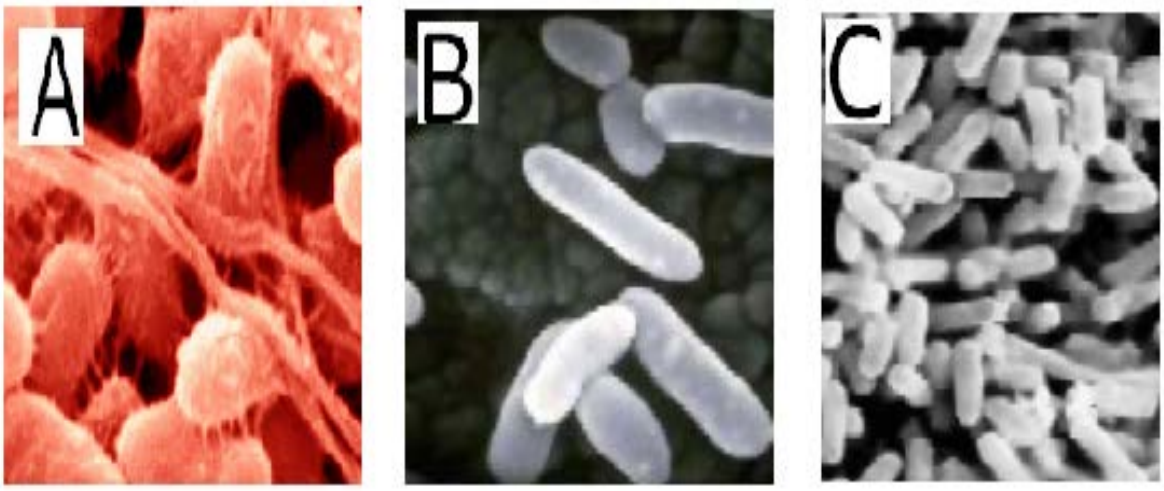

Fig . 1: Electron micrographs of A strain, B strain and C strain

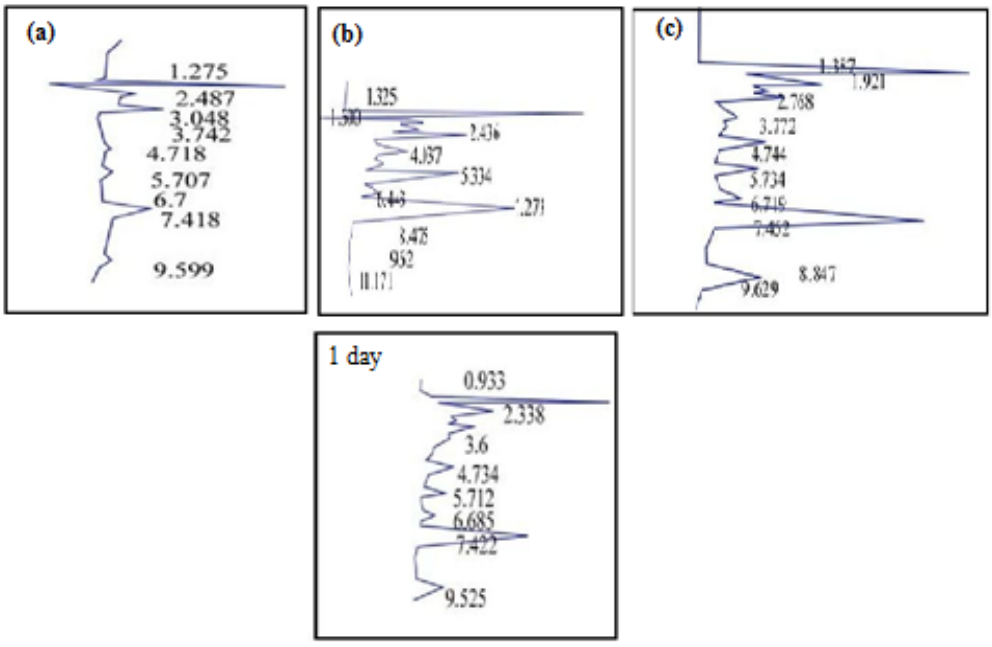

Fig. 2: HPLC analysis of SDS degradation in, (a) A strain at 10 days, (b)B strain at 10 days, (c) C strain at 10 days B strain, (d) strain at 1 day of incubation

Table 1: Morphologic and biochemical characteristics of isolated strains

Tests	A	B	C
Gram staining	Gram-negative	Gram-negative	Gram-negative
Motility	+	+	+
Capsule	+	+	+
Oxidase	-	+	-
Catalase	+	+	+
Growth in:			
4^o C	+	+	-
42^o 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	C isolate
Incubation period (day)	LABS level (g/L)	Utilization LABS (%)	Utilization LABS (%)	Utilization LABS (%)	Utilization LABS (%)
0	0.500	00.0	00.0	00.0	00.0
1	0.432	13.6	12.8	14.2	12.3
3	0.236	52.8	31.8	30.0	33.2
5	0.035	93.1	67.6	80.6	53.2
7	0.016	96.8	84.4	91.1	62.6
10	0.012	97.6	91.3	94.2	92.4

Table 3: Growth of A strain in relation to SDS degradation

Incubation Period(day)	1	2	3	4	5	6	7	8	9	10
OD(220nm)	0.18	0.21	0.26	0.31	0.38	0.41	0.45	0.59	0.67	0.82
SDS(g/l)	0.2	0.16	0.11	0.09	0.06	0.06	0.04	0.03	0.02	0.02

Table 4: Growth of B strain in relation to SDS degradation

Incubation Period(day)	1	2	3	4	5	6	7	8	9	10
OD(220nm)	0.14	0.19	0.32	0.42	0.53	0.67	0.79	0.88	0.91	0.92
SDS(g/l)	0.2	0.19	0.18	0.09	0.05	0.05	0.02	0.02	0.01	0.01

Table 5: Growth of C strain in relation to SDS degradation

Incubation Period(day)	1	2	3	4	5	6	7	8	9	10
OD(220nm)	0.21	0.26	0.37	0.41	0.55	0.59	0.66	0.71	0.89	0.93
SDS(g/l)	0.2	0.2	0.12	0.08	0.07	0.05	0.03	0.01	0.01	0.01

Table 6: Growth of Co-culture of the three strains in relation to SDS degradation

Incubation Period(day)	1	2	3	4	5	6	7	8	9	10
OD(220nm)	0.22	0.29	0.39	0.48	0.55	0.60	0.66	0.79	0.91	0.99
SDS(g/l)	0.2	0.11	0.06	0.04	0.02	0.01	0.01	0.00	0.00	0.00

IV. 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 activatedsludge cultures in order to isolate heterotrophic anionic surfactant degrading bacteria. In this study, aerobic cultures of municipal wastewater were performed in order to isolate anionic surfactant degrading bacteria. Three different bact

eria 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 forsurfactant degrading bacteria identification. Whereas, Dhuib, *et al., 2003*, have relied solely on biochemical tests in order to identify their isolated bacteria. In this study, we have used biochemical tests to identify the three 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 activatedsubstances (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 A, B and C 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 study, 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, found same that co-culture of the three isolated strains caused a dramatic rise in surfactant utilization.The obtained results are shown that anionic surfactants significantly biodegraded by bacteria. The results of this study suggest that growth of simple bacteria such as *Serratia, Acinetobacter and Pseudomonas* in household and industrial sewage can be cost-effective for anionic surfactants elimination.

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