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Biodegradation of Sodium Dodecyl Sulphate (SDS) by two Bacteria Isolated from Wastewater Generated by a Detergent-Manufacturing Plant in Nigeria

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Abstract

Sodium Dodecyl Sulphate (SDS) is an anionic surfactant widely used all over the world, and it is an important foaming component of shampoos, toothpaste and detergents. Large quantities of SDS are released to the environment and this can cause problems in sewage treatment facilities due to their foaming capabilities and toxicity. The present study aimed at isolating bacteria capable of utilizing SDS from sediment and wastewater samples of a detergent manufacturing plant and laundry section of a student residential hall. Sediment and wastewater samples were collected and cultured on Phosphate Buffered Medium (PBM) supplemented with SDS (PBM-SDS) as the carbon source. Bacterial identification and growth determination were done using conventional methods and the UV visible light spectrophotometer respectively. The SDS-utilizing bacteria were employed in the degradation of SDS in a batch culture for 10 days on a rotary shaker at 150 rpm. The residual SDS concentration was determined using HPLC. A total of eight bacteria belonging to four genera; *Lysinibacillus*, *Staphylococcus*, *Bacillus* and *Paenibacillus* were obtained. All the bacteria tolerated SDS to a concentration of 1000 mM. Two of the eight SDS-degrading bacteria; *Staphylococcus aureus* WAW1 and *Bacillus cereus* WAW2 were selected for the biodegradation set-up based on their growth consistency. *Staphylococcus aureus* WAW1 was able to degrade 36.8% of SDS at the end of the biodegradation study while *Bacillus cereus* WAW2 was able to degrade 51.4%. The bacteria obtained in this study could prove useful in the bioremediation of wastewater laden with SDS, and cleaning up of surfactant from wastewater generated via laundry activities, detergent-manufacturing and other related activities.

Keywords:Detergent-manufacturing, Sodium dodecyl sulphate, Surfactants, Laundry wastewater

1. Introduction

Sodium Dodecyl Sulfate (SDS) otherwise referred to as Sodium Lauryl Sulphate is the most widely used anionic detergent in household products, such as toothpastes, shampoos, shaving foams, bubble baths, cosmetics and detergents (Dhouib *et al*., 2003). In the industries, however, it is used as leather softening agent, wool cleaning agent, penetrant, flocculating agent, de-inking agent in the paper industry; and it is the major components of fire-fighting devices, engine degreasers, floor cleaners, and car wash soaps.

The occurrence of SDS in the environment stems mainly from its presence in domestic and industrial effluents as well as its release directly from some applications (Fendinger *et al*., 1994). Several authors have reported the toxicity of SDS and its effects on the survival of aquatic animals such as fishes, microbes, like yeasts and bacteria (Singer and Tjeerdema, 1992; Sandbacka *et al*., 2000; Martinez and Munoz, 2007). It has also been reported to be toxic to mammals, like mice and humans though to a lesser extent.

The excessive use of detergents domestically and industrially is becoming a serious problem due to the fact that they have detrimental effects on aquatic organisms via the discharge of surfactant-laden wastewater into water bodies and channels (Chukwu and Odunzeh, 2006; Kumar *et al*., 2007). Liwarska-Bizukojc *et al*. (2005) reported that surfactants are ubiquitous and in many untreated effluents, certain classes of surfactants can be present in sufficient concentrations to constitute toxicity problems to aquatic organisms because most of the massive amounts of surfactants used industrially and domestically end up in wastewater flows. Petterson *et al*. (2000) reported that anionic surfactants have toxic effects on various aquatic organisms even at concentrations as low as 0.0025 mg/L thus necessitating the removal of these compounds before they build up to a considerable high concentration in the environment especially water bodies.

Cserhati *et al*. (2002) reported that SDS and other surfactants are considered to be biodegradable by aerobic

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processes; however, the mass loadings of these compounds into water bodies suggest that, even at these natural removal rates, appreciable amounts of surfactants are released into receiving waters to the extent that a variety of surfactants has been identified in both surface and drinking water (Isobe *et al*., 2004). This has necessitated the need for a system capable of degrading surfactants discharged into water system as a means of augmenting the natural biodegradation of these compounds.

Numerous studies have been carried out in the Nigerian environment on bacteria isolated from wastewater generated by laundry and detergent-manufacturing, and their ability to grow on/degrade different washing detergents; however , none has focused on the ability of bacteria isolated from the same source to subsist on SDS, a surfactant present in detergents. This present study, therefore, investigated the ability of bacteria isolated from laundry and detergent-manufacturing sediments and wastewater to utilize SDS as a sole source of carbon and degrade SDS in a batch culture system spiked with SDS as the carbon source.

2. Materials and Methods

2.1. Chemicals, Culture Media and Reagents

Sodium Dodecyl Sulphate (SDS) was purchased from Merck (Pty) Ltd, Gauteng, 1645, South Africa. Nutrient agar was purchased from Oxoid, UK. Other chemicals, salts and reagents used were of the highest grade available at the time of carrying out the present study. The composition of the Phosphate Buffered Medium (PBM) was (g/L): K_2HPO_4 1.0, KH_2PO_4 1.0, NH_4Cl 1.0, $MgSO_4.7H_2O$ 0.20, NaCl 0.5 and $CaCl_2$ 0.02, (pH 7.5). The medium also contained trace elements (1 mL of stock) having (g/L): $FeCl_3 \cdot 6H_2O$ 0.24, $CoCl_2 \cdot 6H_2O$ 0.04, $CuSO_4 \cdot 5H_2O$ 0.06, $MnCl_2 \cdot 4H_2O$ 0.03, $ZnSO_4 \cdot 7H_2O$ 0.31, $Na_2MoO_4 \cdot 2H_2O$ 0.03. After sterilization, SDS was added to the medium as the sole source of carbon.

2.2. Description of the Study Site

The present study was carried out in Ibadan. Ibadan is located in South-western part of Nigeria. The sampling sites were the wastewater disposal channel of a detergent manufacturing plant located in an industrial estate; and the laundry section of a hall of residence located within the University of Ibadan, Nigeria. The description of the sampling points is shown in Table 1.

Table 1. Description of the Sampling points and GPS readings

Description of sampling point	Source	Type of samples collected	Latitude	Longitude
Point 1	Effluent disposal channel of a detergent manufacturing plant	Soil sediment and wastewater	7.2119N	3.511E
Point 2	Laundry section of a hall of residence	Soil sediment and wastewater	7.2659N	3.5333E

2.3. Sample Collection

Soil sediments were collected in aluminum foil, while wastewater samples were collected in pre-sterilized sample bottles. The samples were transported to the Environmental Microbiology and Biotechnology Laboratory, Department of Microbiology, University of Ibadan, and analyzed within one hour of collection.

2.4. Enrichment and Isolation of SDS-Degrading Bacteria

The SDS-degrading bacteria were isolated from the sediment and wastewater samples using PBM as the enrichment medium. Aliquot (10 mL) of the wastewater sample and ten gram of soil sediment were added separately to 100 mL sterilized PBM supplemented with SDS (100 ppm) in different culture flasks. The set-up was incubated at room temperature for 96 h on a rotary shaker at a speed of 150 rpm. After the incubation period, morphologically distinct colonies of bacteria were picked and repeatedly subcultured on PBM supplemented with SDS (100 ppm) to obtain pure culture (Chaturvedi and Kumar, 2010). The purified cultures of the bacteria were stored in glycerol broth at -80°C. The identity of the SDS-degrading bacteria was determined using conventional morphological and biochemical tests according to Sneath (1996).

2.5. Screening of Bacteria on Increasing Concentration of SDS

The obtained bacteria were screened on PBM containing an increasing concentration of SDS with agar as the solidifying agent. The culture growing on the previous concentration is transferred to the next higher concentration until the final screening concentration of 1000 ppm of SDS. Apart from growth on solid medium, the growth of the SDS-utilizing bacteria was also monitored using a UV-Visible light spectrophotometer, and two bacteria showing consistent increase in OD_{540} within 120 h were selected for the degradation study.

2.6. SDS Degradation Set-up

The degradation study was carried out in 150 mL conical flasks containing 99 mL of PBM supplemented with 10 mM SDS. The inoculum was prepared from overnight cultures of the selected bacterial isolates on nutrient agar plates incubated at 35±2°C. Two-three (2-3) identical colonies of each bacterium were then selected and suspended in saline. The saline suspension was standardized (0.5 McFarland Standard) and 1 mL was used to inoculate PBM-SDS medium to a final volume of 100 mL. The PBM-SDS medium without the bacteria served as the control. The cultures were incubated at room temperature with shaking at 150 rpm for 10 days according to the methods of Rusconi *et al*. (2001) with slight modifications. The rate of degradation was calculated using the formula below:

$$(A-B) \div C$$

where: A= Initial SDS concentration (ppm)

B= Final SDS concentration (ppm)

C= Experimental duration (hour)

2.7. Analysis of the Residual SDS Concentration

The cultures were centrifuged at 10000 rpm for 5 min to remove the bacterial cells and the residual SDS concentration in the growth medium was determined by HPLC using a Water Alliance 1100 series system fitted with a 1260 Infinite Variable Wavelength detector set at 225 nm and an Agilent (3.9 mm × 150 mm, 4 µm) Waters

Novapak C18 column. The isocratic mobile phase gradient of acetonitrile-water, (80-20) was conducted at a flow rate 1.0 mL/min.

3. Results

3.1. Bacterial Isolation, Screening and Characterization

Preliminary screening on PBM-SDS medium gave a total of eight bacteria capable of utilizing SDS as carbon source. All the eight isolates obtained showed consistent and visible growth on PBM-SDS solid medium to a concentration of 1000 mM SDS. The eight bacterial isolates belonged to four genera, which were: *Lysinibacillus* (1), *Staphylococcus* (2), *Paenibacillus* (1) and *Bacillus* (4). The identity of the bacteria and the source of isolation are highlighted in Table 2.

Table 2. Identity of the SDS-utilizing bacteria and their source of isolation

Isolate and code	Sample	Source of isolation
Staphylococcus aureus WAW1	Wastewater	Detergent-manufacturing plant
Bacillus cereus WAW2	Wastewater	Detergent-manufacturing plant
Bacillus firmus WAW3	Soil sediment	Detergent-manufacturing plant
Bacillus siamensis WAW4	Soil sediment	Detergent-manufacturing plant
Paenibacillus amylolyticus BAL1	Soil sediment	Laundry section of a hall of residence
Bacillus lentus BAL2	Soil sediment	Laundry section of a hall of residence
Lysinibacillus sphaericus BAL3	Soil sediment	Laundry section of a hall of residence
Staphylococcus sciuri BAL4	Soil sediment	Laundry section of a hall of residence

3.2. Selection of Bacteria for the Biodegradation Study

Two bacteria, namely *Staphylococcus aureus* WAW1 and *Bacillus cereus* WAW2, were selected for the biodegradation study, based on their consistent increase on PBM-SDS monitored at an optical density of 540 nm. Both bacteria showed no appreciable growth within the first 24 hours of growth. However, there was a noticeable increase in the absorbance from the 48th to the 96th h, before growth started declining again till the end of the experimental duration (120th h) as shown in Figure 1.

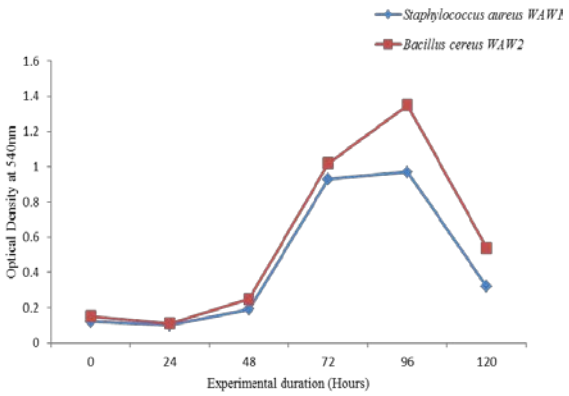

Figure 1. Growth of the two selected SDS-degrading bacteria in PBM-SDS medium

3.3. Residual SDS Concentration

HPLC analysis of the residual concentration of SDS in the samples showed that there was a reduction in the SDS concentration after the 10-day degradation set-up. *Bacillus cereus* WAW2 degraded 5055.70 ppm of the initial SDS in the setup at a degradation rate of 21.07 ppm/h, and eventually degrading 51.4% of the initial SDS concentration, while *Staphylococcus aureus* WAW1 degraded 3615.37 ppm of SDS at a rate of 15.06 ppm/h, leading to a 36.8% reduction in the initial concentration of SDS in the set-up (Table 3).

Figure 2 shows the chromatogram of the control (uninoculated sample) and the samples treated with the two bacteria. The reduction in the height of the peaks in the treated samples in comparison to the uninoculated control gave evidence to the degradation of the surfactant by the two bacterial isolates.

Table 3. Rate of degradation of SDS by the SDS-utilizing bacteria

Bacterial isolate	Amount of SDS degraded (ppm)	Rate of SDS degradation (ppm/h)	Percentage degradation (%)
Staphylococcus aureus WAW1	3615.37	15.06	36.8
Bacillus cereus WAW2	5055.70	21.07	51.4

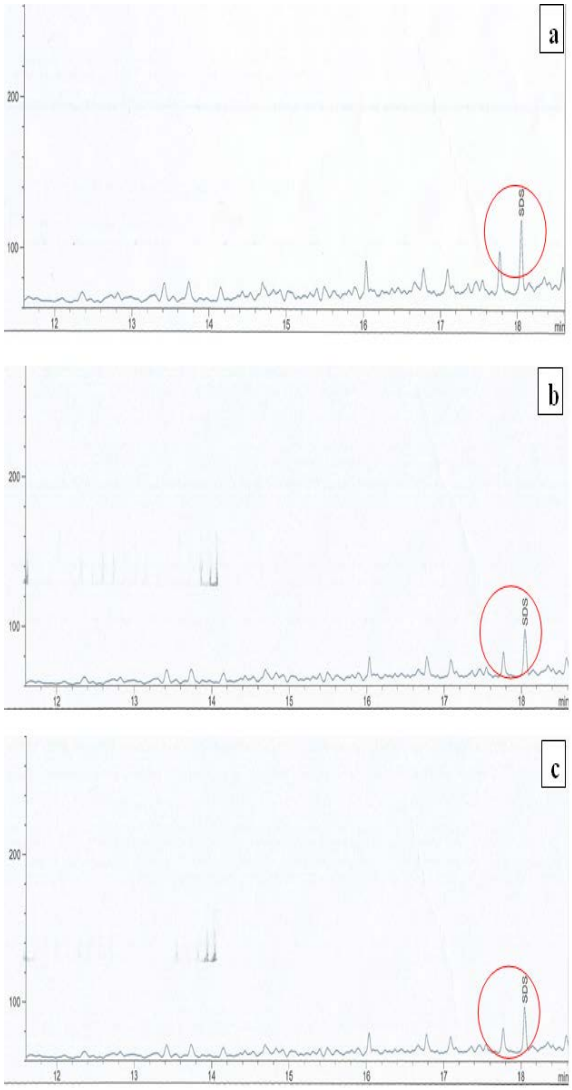

Figure 2. HPLC chromatograms of the (a) uninoculated control (b) *Staphylococcus aureus* WAW1 (c) *Bacillus cereus* WAW2 showing suspected area of degradation (red rings)

4. Discussion

In the present study, a total of eight bacteria capable of utilizing/degrading Sodium Dodecyl Sulphate (SDS) as the sole carbon source were isolated. All the bacteria obtained in the present study were gram-positive organisms; and this is not in agreement with the work of Ojo and Oso (2008) who reported the isolation of a larger percentage of gram-negative organisms capable of growing on detergents. They were of the view that the gram-positive strains showed more tolerance than the gram-negative. This is, however, different from the report of Higgins and Burns (1975) who asserted that gram positive bacteria are noticeably affected by surfactant concentration of 10-20 ppm while gram negative organisms can tolerate several thousand ppm concentration of surfactants without any adverse effect. This is also corroborated by Chaturvedi and Kumar (2010) who reported the isolation of two *Pseudomonas* strains (gram negative bacteria) from a detergent-polluted pond in Varanasi city, India.

Gram positive bacteria similar to the ones obtained in this present study have been reported to utilize surfactants. Anaukwu *et al*. (2016) reported the isolation of *Staphylococcus scuiri* and *Bacillus cereus* capable of degrading or utilizing surfactants as their carbon source. This is not however in accordance with the report of Schleheck *et al*. (2013) who reported the isolation of *Citrobacter* sp., a gram negative bacterium capable of utilizing over 90% surfactant in 35 h of growth in a closed culture. The isolation of gram positive organisms in the present study is not in agreement with the work of Jerabkova *et al*. (1999), who reported the isolation of *Pseudomonas* strains capable of decreasing surfactants concentration by 70% in 20 days; nor is it in agreement with the work of Shukor *et al*. (2009) who reported the isolation and characterization of an SDS-degrading *Klebsiella oxytoca*, also a gram negative bacterium.

The two bacteria, i.e., *Staphylococcus aureus* WAW1 and *Bacillus cereus* WAW2 selected for the biodegradation experiment in the present study have been reported in previous studies. Notable among them was Singh *et al*. (1998) who reported the degradation of SDS by *Bacillus cereus*, which was the first report of biodegradation of SDS by any gram-positive bacterium. Ojo and Oso (2008) also reported the isolation of *Staphylococcus* strain capable of degrading detergent in their study on the isolation and characterization of synthetic degraders from wastewater.

Staphylococcus aureus WAW1 in the present study degraded 36.8% of SDS from an initial concentration of 10 mM in 10 days, and this is lower than the degradation of SDS by *Pseudomonas betelli* (97%) in 10 days as reported by Hosseini *et al*. (2007). In addition, *Bacillus cereus* WAW2 obtained in the present study degraded 51.4% of SDS within the same number of days, which is not in concordance with 96.4% degradation of SDS by *Acinetobacter johnsonii* in 10 days as reported by Hosseini *et al*. (2007). The difference in the degradation could be attributed to several factors including; disparity in the type of bacteria used, concentration of SDS used in the degradation set-up, source of isolation, geographical location and several other environmental factors. Shukor *et al*. (2009) reported that *Klebsiella oxytoca*, a gram negative bacterium isolated from soils and water contaminated with detergent from a car wash outlet in Malaysia was able to degrade 80% of SDS from an initial concentration of 2 g/L within 4 days. This level of degradation is still higher than what was obtained in this present study (36.8% and 51.4%) despite the degradation set-up in the present study being run for longer period (10 days).

In conclusion, the bacteria obtained in the present study are capable of utilizing SDS as their carbon source and could be helpful in the remediation of wastewater contaminated with surfactants. However, more studies need to be carried out on the optimization of culture conditions for the degradation of SDS, degradation of surfactant simultaneously with the removal of other toxicants and the molecular genetics of the SDS-degrading bacteria.

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