

# Degradation of surfactant and metal-removal by bacteria from a Nigerian laundry environment

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## ABSTRACT

This study aimed at degrading sodium dodecyl sulphate (SDS), a surfactant in the presence of metals using metal-tolerant bacteria from a laundry site. Metal composition of wastewater and sediments from a laundry environment was determined using atomic absorption spectrometry (AAS). *Paenibacillus amylolyticus* BAL1 (PAB) and *Bacillus lentus* BAL2 (BLB), earlier reported to tolerate 1000 ppm SDS were screened for metal tolerance. The bacteria were employed in the simultaneous degradation of SDS and metal removal in a batch culture set-up containing SDS and metals for 14 days on a rotary shaker at 250 rpm. Residual SDS and metal concentrations were determined using high performance liquid chromatography (HPLC) and AAS. Copper (Cu), zinc (Zn), and cadmium (Cd) were detected in both laundry wastewater and sediment while chromium (Cr) and nickel (Ni) were only detected in the sediments. The MICs of metals on PAB were: Cu and Zn (500 µg/ml), and Cd (100 µg/ml), while for BLB: Cu (500 µg/ml), Zn (400 µg/ml), and Cd (100 µg/ml). PAB degraded 49.90% of SDS and simultaneously removed 8.3% of Cu, 5.1% of Cd, and 6.6% of Zn, while BLB degraded 54.9% of SDS and simultaneously removed 3.1% of Cu, 39% of Cd, and 3.1% of Zn. A combination of the two bacteria led to 44.3% degradation of SDS, and removal of 11% of Cu, 7.7% of Cd, and 9.8% of

Zn. Bacteria from this study possessed both SDS-degradation and metal-removing abilities, and could be useful in the bioremediation of wastewater co-contaminated by surfactants and metals due to their dual tolerance to both compounds.

**Keywords:** Metal-removal; Surfactant degradation; Sodium dodecyl sulphate; Laundry environment; Metal-tolerant bacteria; Dual tolerance; Nigeria.

## 1. INTRODUCTION

Surfactants are chemicals containing polar and non-polar chains and are designed to have solubilization and cleaning properties. They have been used extensively in household detergents, textile industries, mining, pharmaceuticals, personal care products and the pulp and paper industries [1, 2]. The hydrophobic tail of the molecule, which usually consists of a long chain hydrocarbon or fluorocarbon acts by reducing the solubility of the compound in water, while the hydrophilic head confers on the surfactant molecule the opposite effects. This unique property of surfactants gives them numerous applications and versatility in many processes [3].

Depending on the charge on the hydrophilic moiety of the surfactant molecules, they can be classified into: anionic, non-ionic, cationic and amphoteric [4]. In detergent formulations, however,

anionic surfactants are widely used, and they constitute a large percentage of surfactants used worldwide. Examples of the anionic surfactants include; linear alkylbenzene sulfonates and linear alkyl sulfate. Sodium dodecyl sulfate used in this study belongs to the latter [5, 6].

Surfactants constitute a significant portion of wastewater discharge from laundry activities and detergent-manufacturing operations. This could cause severe environmental challenges because the survival of most aquatic organisms is dependent on the surface tension of the water medium. Anionic surfactants for instance could bind to protein molecules such as peptides and enzymes, and to DNA, which may result in the folding of polypeptide chains and change in surface charge eventually leading to alteration in biological functions [2, 7].

In addition to surfactants, detergents and laundry wastewater, have metals in varying concentrations. Household detergents have been implicated as a major contributor of metals such as zinc, cadmium, and chromium, in sewage [8]. Though information on the metallic composition of laundry and detergent-related effluents are relatively limited, the presence of metals in this category of wastewater has been confirmed in few studies. The presence of metals has been reported from the characterization of wastewater from a commercial laundry in Brazil [7]. It should however be stressed that the metals were present in levels that were well below the maximum permissible limit for discharge into the environment, as approved by the National Council for the Environment Ordinance in Brazil. Studies have also shown that metals could also act as inhibitors to pollutant biodegradation via their interaction with the enzymes involved in biodegradation [9]. The ionic form is the one mostly implicated, as it mediates inhibition of pollutant-degrading enzymes in heavy metal contaminated environments. Several metals have been reported to inhibit organic pollutant biodegradation, thus affecting degradation rates, and this may be directly linked to the bioavailability of the metals rather than the total metal concentration [10-12]. There is however a paucity of information in this area of research.

The presence of metals as co-contaminants with SDS in laundry wastewater has raised the need for the employment of metal-resistant bacteria in the

degradation of the latter, as metal stress could be a major hindrance to the degradation of SDS by bacteria. This study therefore aimed to evaluate the simultaneous degradation of SDS and removal of selected metals in a surfactant-metal set-up by metal-resistant bacteria isolated from soil sediments of the laundry section of a student hall of residence within a University community in Ibadan, Nigeria.

## 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. The metal salts and all other reagents used in this study were of the highest available grade at the time of carrying out the study. Nutrient agar was purchased from Oxoid, UK.

### 2.2. Bacteria used for the study

The bacteria used for this study: *Paenibacillus amylolyticus* BAL1 and *Bacillus lentus* BAL2 are SDS-tolerating strains isolated from sediment samples of the laundry section of a student residence hall within a university. They have been screened on SDS-incorporated medium and were able to tolerate 1000 mM of SDS [13]. The bacteria were resuscitated on nutrient agar and further cultured on SDS-incorporated medium for adaptation.

### 2.3. Preparation of metal solutions

The heavy metals used as challenge for the isolates were zinc (Zn), copper (Cu) and cadmium (Cd). Filter sterilized soluble salts of the following metals e.g., CdCl<sub>2</sub>, CuSO<sub>4</sub> and ZnSO<sub>4</sub> were used for the preparation of the metal solutions. Stock solutions of the respective metals (10000 µg/ml) were prepared [14].

### 2.4. Screening of bacteria for heavy metal tolerance

The bacteria were screened on metal-incorporated nutrient medium to check for resistance to increasing concentrations of the three selected heavy metals. Filter sterilized solutions of each

metal were incorporated into sterilized, molten nutrient agar, mixed gently, and poured into sterile plates. An 18-24 hour old culture of the isolates was streaked on the metal-supplemented medium and incubated at  $35\pm 2^{\circ}\text{C}$ . The plates were observed for growth till the 48th hour. The observation of no visible growth on the metal-supplemented medium by the 72nd hour was regarded as 'no growth'. The concentrations of each heavy metal were gradually increased from an initial concentration of  $50\ \mu\text{g/ml}$  with an increment of  $50\ \mu\text{g/ml}$  at a time. The bacteria growing on each concentration was transferred to the next higher concentration until it failed to grow. The lowest metal concentration at which bacteria failed to show any observable growth on the metal-incorporated medium was taken as the Minimum Inhibitory Concentration (MIC) [15, 16].

### 2.5. SDS degradation and metal removal in simulated surfactant-metal set-up

The degradation set-up was carried out in 250 ml conical flasks containing 180 ml simulated wastewater. The inoculum of the two bacteria selected for the set-up was prepared from overnight cultures on nutrient agar plates incubated at  $35\pm 2^{\circ}\text{C}$ . The inoculum was standardized to 0.5 McFarland standard and 10% aliquots were used to inoculate the set-up to a final volume of 200 ml. The simulated set-up contained 10 mM of SDS and  $100\ \mu\text{g/ml}$  each of zinc, cadmium, and copper. The set-up without the bacterial inoculum served as the control. The cultures were maintained at room temperature with shaking at 150 rpm for 14 days [17].

### 2.6. Analysis of the residual SDS concentration

The set-up was centrifuged at 10000 rpm for 10 minutes to remove the bacterial cells, and the residual SDS concentration was determined by high performance liquid chromatography (HPLC) using a Water Alliance 1100 series system fitted with a 1260 Infinite Variable Wavelength detector set at 225 nm and an Agilent ( $3.9\ \text{mm} \times 150\ \text{mm}$ ,  $4\ \mu\text{m}$ ) Waters Novapak C18 column. The isocratic mobile phase gradient of acetonitrile-water (80-20) was conducted at a flow rate  $1.0\ \text{ml/min}$  [18].

### 2.7. Metal analysis using Atomic Absorption Spectrophotometry (AAS)

The residual metal concentration in the set-up was determined by atomic absorption spectrometry (AAS). Standard concentrations of copper, cadmium, and zinc solutions were prepared from 1000 mg/l stock solutions. The standards solutions were analyzed using the Atomic Absorption Spectrophotometer (UNICAM 929, London Atomic Absorption Spectrophotometer powered by SOLAAR software) for calibration. The metal concentrations in the filtrate were analyzed using the respective cathode lamps [19].

### 2.8. Data analysis

The residual SDS and metal concentrations after the bioremediation study were analyzed using the Analysis of Variance (ANOVA) and separation of was done using the Duncan Multiple Range Test (DMRT) using the Statistical Package for Social Sciences (SPSS) version 22.0.

## 3. RESULTS

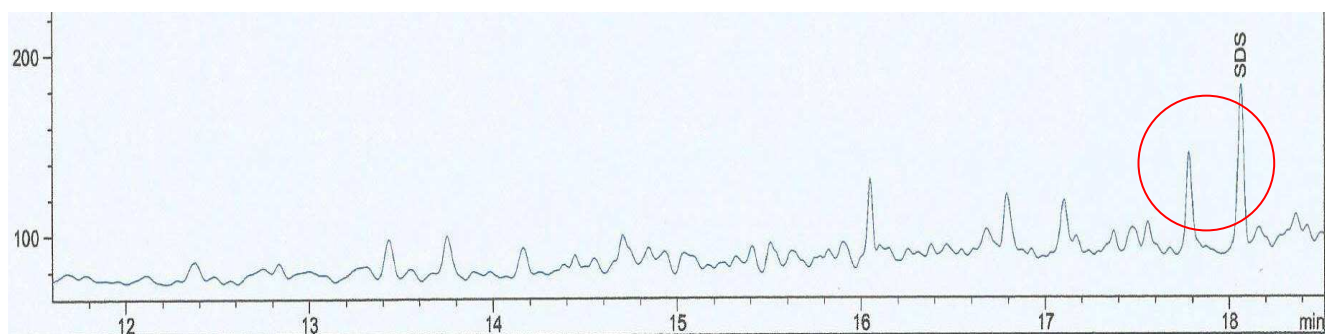
### 3.1. Metal composition of selected laundry wastewater and sediments

Table 1 shows the metal composition of the laundry wastewater and sediment samples used. The concentration of the selected metals in the wastewater was lower than that in sediment samples.

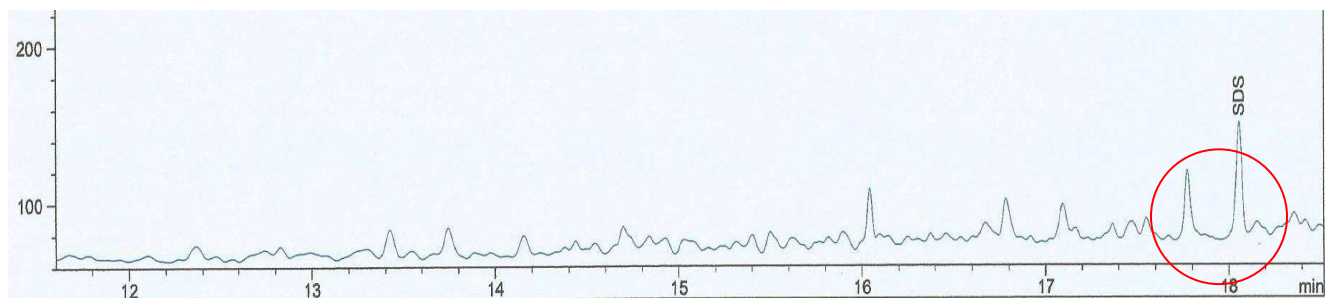
**Table 1.** Metal composition of laundry wastewater and sediments samples.

Metal	Wastewater (mg/l)	Sediment (mg/kg)
Copper (Cu)	$0.0114\pm 0.00$	$15.95\pm 0.02$
Zinc (Zn)	$0.0098\pm 0.00$	$83.30\pm 0.07$
Cadmium (Cd)	$0.0094\pm 0.00$	$2.05\pm 0.01$
Chromium (Cr)	ND	$18.30\pm 0.04$
Nickel (Ni)	ND	$6.90\pm 0.02$

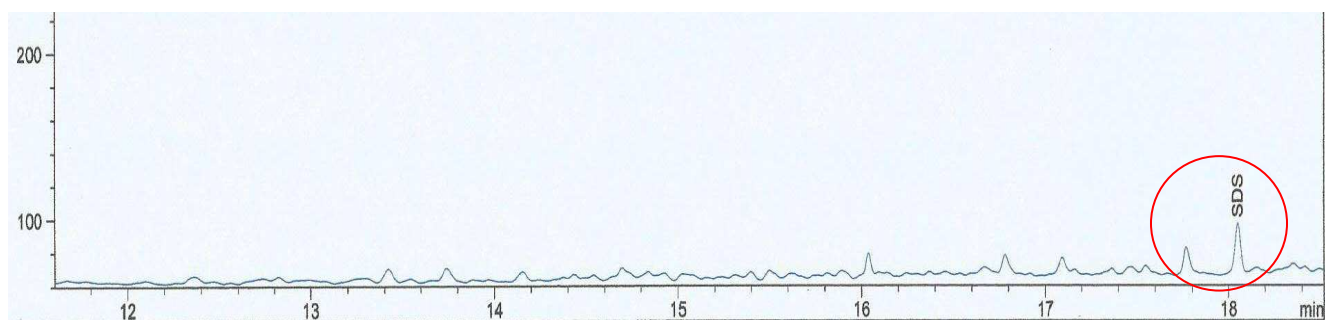
Each value is an average of three samples. ND: Not detected.



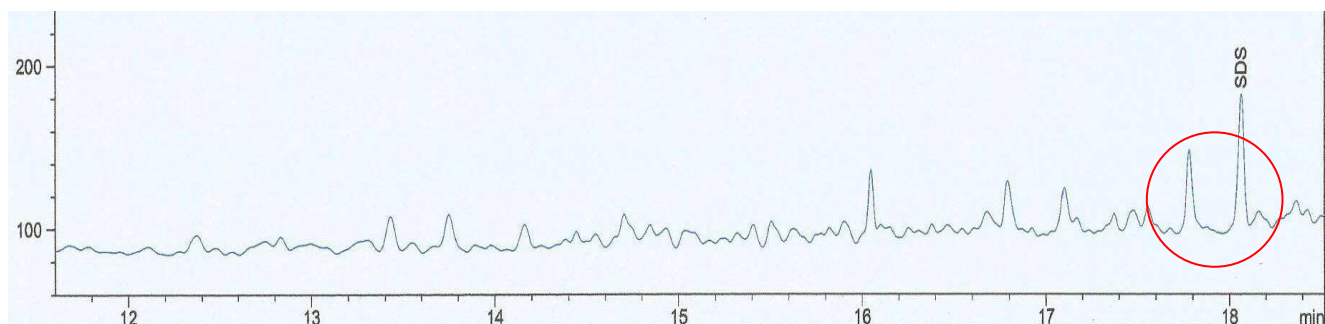
**Figure 1a.** HPLC chromatogram of the surfactant-metal set-up (without bacterial inoculum). The red ring shows the peak corresponding to the SDS concentration in the sample.



**Figure 1b.** HPLC chromatogram of the surfactant-metal set-up treated with *Paenibacillus amylolyticus* BAL1 showing suspected degradation (red ring) of SDS.



**Figure 1c.** HPLC chromatogram of the surfactant-metal set-up treated with *Bacillus lentus* BAL2 showing area of suspected degradation (red ring) of SDS.



**Figure 1d.** HPLC chromatograms of surfactant-metal set-up treated with a combination of *Paenibacillus amylolyticus* BAL1 and *Bacillus lentus* BAL2 showing area of suspected degradation (red ring) of SDS.

However, chromium and nickel were not detected in the wastewater samples. The highest concentration of metal detected in the sediment was zinc (83.30 mg/kg), while the least was cadmium

(2.05 mg/kg). Copper had the highest concentration in the wastewater (0.0114 mg/l) with the lowest being cadmium (0.0094 mg/l).

### 3.2. Minimum inhibitory concentration (MIC) of metals on the two selected bacterial isolates

The metals' MICs for the two bacteria used in this study are shown in Table 2. Both bacteria had the same MIC for Zn (500 µg/ml) and for Cd (100 µg/ml). The MIC for Cu, for *Paenibacillus amylolyticus* BAL1 was 500 µg/ml, and for *Bacillus lentus* BAL2 400 µg/ml.

**Table 2.** MIC of the two selected bacteria employed in this study (µg/ml).

Isolate	Copper	Cadmium	Zinc
<i>Paenibacillus amylolyticus</i> BAL1	500	100	500
<i>Bacillus lentus</i> BAL2	400	100	500

### 3.5. SDS concentration in the control and treatments

The chromatograms of the set-ups treated with either of the two bacteria, both bacteria, and uninoculated (control), are shown in Figure 2. There was an observed reduction of SDS peaks in the bacteria-treated samples compared to the control, suggesting degradation of the compound.

### 3.6. Rate/percentage of SDS degradation by the bacteria

The percentage/rate of degradation of SDS by the two bacteria and their combination is shown in Table 2. The amount of SDS degraded was statistically significant among the three treatments when compared as shown in Table 3. There was

higher degradation of SDS in the set-ups of each of *P. amylolyticus* BAL1 and *B. lentus* BAL2 compared to the combination of the two. From the initial SDS concentration of 6727.67 ppm, *P. amylolyticus* BAL1 degraded 49.9% of SDS; at a rate of 9.98 ppm/h. *B. lentus* BAL2 on the other hand degraded 54.9% of SDS at a rate of 11.00 ppm/h, while the combination of the two bacteria degraded 44.3% of SDS at a rate of 8.86 ppm/h.

### 3.7. Metal removal by the bacteria

The concentration of metals removed by each of the two bacteria and their combination is shown in Table 4. In each case, the concentrations of metal removed were statistically significant for the treatments when compared with the control set-up. The combination of the two bacteria removed the highest amount of zinc and copper (9.71 ppm and 9.29 ppm respectively), while *Bacillus lentus* BAL2 removed the highest amount of cadmium (35.50 ppm).

The percentage metal removal in the after treatment with the two bacteria and their combination is shown in Figure 2. The range of removal for Zn was 3.1-9.8%, with the combination of the two bacteria removing the highest concentration with *B. lentus* BAL2 being the least remover of Zn. There was a 39.96% removal of Cd when the set-up was treated with *B. lentus* BAL2, while the set-up having *Paenibacillus amylolyticus*, able to remove 5.08%. The combination of the two bacteria was able to remove 7.72% of the metal. The combination of the two bacteria removed the highest concentration of Cu (11.01%), with the least being the set-up treated with *Bacillus lentus* BAL2 (3.14%).

**Table 3.** Rate and percentage of degradation of SDS by the two bacteria and their combination.

Isolate	Initial SDS concentration (ppm)	Amount of SDS degraded	Rate of SDS degradation (ppm/h)	Percentage degradation (%)
<i>Paenibacillus amylolyticus</i> BAL1	6727.67	3354.12±0.03 <sup>b</sup>	9.98	49.9%
<i>Bacillus lentus</i> BAL2	6727.67	3696.74±0.01 <sup>a</sup>	11.00	54.9%
Combination of the two bacteria	6727.67	2978.50±0.01 <sup>c</sup>	8.86	44.3%

Note: Values are Means ± Standard deviations of duplicate observations.

Means with same alphabets down each column are not significantly different at  $p \leq 0.05$ .

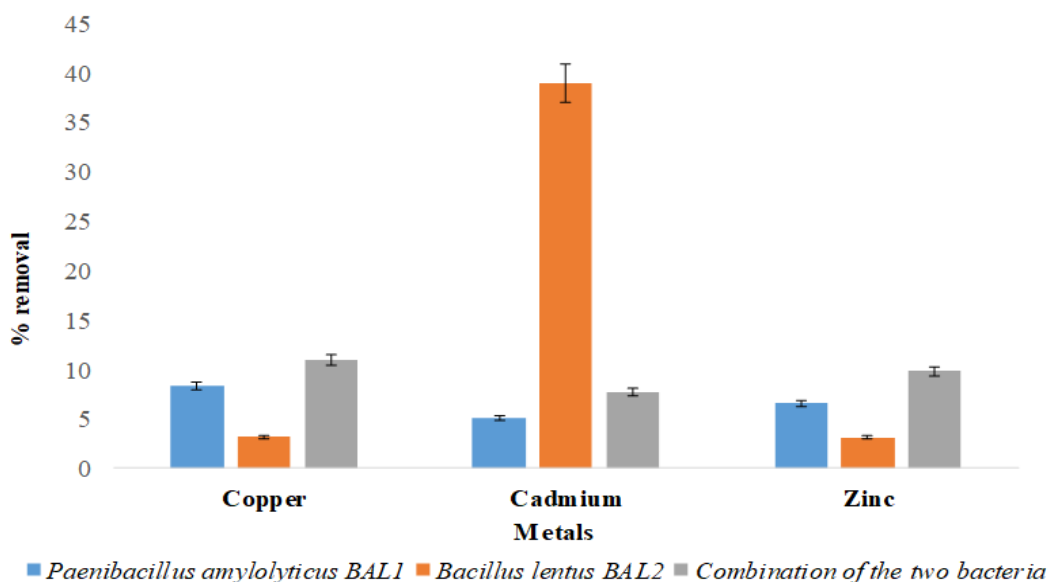


Figure 2. Percentage metal removal by the two bacteria and their combination.

Table 4. Concentration of metal removed by the two bacteria and their combination (ppm).

Metal	<i>Paenibacillus amylolyticus</i> BAL1	<i>Bacillus lentus</i> BAL2	Combination of the two bacteria
Copper	7.30±0.000 <sup>a</sup>	2.77±0.000 <sup>b</sup>	9.71±0.000 <sup>c</sup>
Cadmium	4.63±0.000 <sup>a</sup>	35.50±0.000 <sup>b</sup>	7.04±0.000 <sup>c</sup>
Zinc	6.23±0.003 <sup>a</sup>	2.96±0.000 <sup>b</sup>	9.29±0.001 <sup>c</sup>

Note: Values are Means ± Standard deviations of duplicate observations.

Means with same alphabets across each row are not significantly different at  $p \leq 0.05$ .

#### 4. DISCUSSION

Numerous species of bacteria have been reported to have the ability of degrading surfactants. Bacteria isolated from a detergent-polluted pond have reported to degrade SDS, an important component of detergents [20]. In Nigeria however, two bacteria, isolated from wastewater of a detergent manufacturing plant were able to degrade SDS [13]; while in Malaysia, an SDS-degrading strain of *Klebsiella oxytoca* was isolated from soil and water contaminated by detergents from a car wash facility [21]. Wastewater contaminated with excessive detergents is becoming a serious issue as detergents are known to have adverse effects on aquatic life due to their excessive use and eventual discharge via wastewater into water bodies putting aquatic organisms at risk [22-24].

In this study, two SDS-utilizing bacteria, *Paenibacillus amylolyticus* BAL1 and *Bacillus lentus* BAL2 were employed due to their ability to

tolerate metals. The two bacteria were able to tolerate SDS to a concentration of 1000 mM as reported by Adekanmbi and Usinola [13]. However, the concentration of SDS tolerated by the bacteria in this study (1000 mM) is comparatively lower than the 1500 mM concentration tolerated by four *Pseudomonas* spp. isolated from the wastewater generated by a car wash [25] and *Acinetobacter johnsoni* and *Pseudomonas betelli*, isolated from sewage sludge [26].

Metals are often present in different wastewater at varying concentrations, and these toxic metals cannot be effectively removed by the conventional process of wastewater treatment [27]. Activities involving the use of detergents and other chemicals have all contributed immensely to the release of significant amounts of metals into wastewater. Metal composition analysis of detergent wastewater and sediment in this study revealed the presence of copper, zinc and cadmium. The presence of metals in wastewater of detergent/laundry origin

has been reported especially in Brazil [7]. SDS-utilizing bacteria in this study were able to tolerate copper, zinc and cadmium, which are frequently encountered in both laundry and detergent wastes; and this could be attributed to the ability of several bacterial species to develop resistance to metals present in their immediate environment due to adaptation [28].

The two selected organisms, *Paenibacillus amylolyticus* BAL1 and *Bacillus lentus* BAL2 in addition to their combination were able to degrade SDS at different rates. The degradation was evident in the reduction of the SDS peak in comparison with the control (un-inoculated) set-up. The residual concentration of SDS in the set-up showed a reduction in the SDS concentration after the 14-day degradation period. *Paenibacillus amylolyticus* BAL1 degraded 3354.12 ppm of the initial SDS at the rate of 9.98 ppm/h and eventually degrading 49.90% of the initial SDS concentration, while *Bacillus lentus* BAL2 degraded 3375.55 ppm of SDS at a rate of 11.00 ppm/h thereby degrading a total of 54.9, while the combination of the two bacteria in the set-up degraded 3749.17 ppm of the initial SDS at the rate of 8.86 ppm/h leading to a 44.3% reduction in the concentration of SDS. The percentage degradation of SDS in a 14-day period by the two bacteria in this study, singly and in combination, was higher than that reported by Adekanmbi and Usinola [13]. The higher degradation rate in the present study might be as a result of the increased incubation period rather than the presence of metals in the set-up.

Prior to the work of Adekanmbi and Usinola [13], Hosseini et al. [26] had isolated two bacteria, *Pseudomonas betelli* and *Acinetobacter johnsoni*, from a detergent polluted pond, demonstrating high SDS degradation potential. *Acinetobacter johnsoni* degraded 93.6% of 522 mg/l of SDS within 5 days, while *Pseudomonas betelli* degraded 84.6% at the same conditions. However, following 10-day incubation, *Pseudomonas betelli* showed greater degradation (97.2%) potential relative to *Acinetobacter johnsoni* (96.4%). In addition, a *Klebsiella oxytoca* strain was isolated from SDS-polluted water samples from Malaysia was able to degrade approximately 80% of 0.2% SDS after 4 days of incubation and 100% in 10 days of incubation [21]. Hence, these species were more efficient at SDS

degradation than the isolates used in the present study, although the presence of metals could be a factor in the reduced degradation observed in this study.

According to Sigoillot and Nguyen [29], mixed cultures of different bacteria could improve biodegradation potential significantly. Contrary to this however, the combination of the two bacteria in the present study, did not cause any significant increase in SDS degradation when compared to the degradation rates of the single isolates. This is in accordance with the findings of Hosseini et al. [26], who reported that a mixed culture of two bacterial isolates (*Pseudomonas betelli* and *Acinetobacter johnsoni*) in their study did not significantly increase SDS degradation.

Many studies have reported the microbial degradation of SDS, as a safe and effective means of SDS remediation. However, there is a dearth of information on the biodegradation of SDS along with simultaneous removal of heavy metals. This is necessary because many polluted sites contained not only organic pollutants but also inorganic pollutants in the form of metals and other compounds. Rusnam and Gusmanizar [30] reported the inhibition of SDS degradation by metals such as mercury, silver, and copper, with additional information that the bacterium from their study, *Enterobacter* sp. strain Neni-13 could degrade SDS in the presence of molybdenum. The bacteria from the present study have shown the ability to cope with the stress posed by three metals, while degrading SDS at the same time. Based on the literature at the time of this study, this is the first report on the simultaneous degradation of SDS and removal of metals by metal-adapted bacteria isolated from a laundry environment in Nigerian.

## 5. CONCLUSION

The bacteria from this study possessed both SDS-degradation and metal-removing abilities and could be useful in the bioremediation of wastewater co-contaminated by surfactants and metals. Further studies should be geared towards the degradation of surfactants and metal-removal on a larger scale using immobilized cells in controlled ponds.

## AUTHORS' CONTRIBUTIONS

This work was carried as collaboration among the authors. AOA designed and supervised the study. WOO and AVO managed the laboratory analysis. AOA managed the data analysis and literature searches, and prepared the first draft of the manuscript. AOA revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

## CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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