

Comparative Heavy Metal Removal Efficiencies of Biosurfactants Produced by *Odoribacter Splanchnicus* DSM 20712, Bacterium Clone JX981747 and Soil Washing Agents

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Abstract

The application of two biosurfactants in the removal of copper, zinc, and lead from waste metal dumpsite soil with their efficiencies was compared to soil washing agents as KNO_3 , $\text{Ca}(\text{NO}_3)_2$ and NaOH . The test soil samples were also spiked separately with different concentrations (50 mg/L, 250 mg/L, 750 mg/L and 1500 mg/L) of CuSO_4 , ZnSO_4 and $\text{Pb}(\text{NO}_3)_2$. The biosurfactants used were produced by *Odoribacter splanchnicus* DSM 20712 (WBS1) and an unidentified bacterium clone JX981747 (CMS). Five different treatment set up comprising of different ratios (20:1, 15:1, 10:1, 5:1, and 1:1) with the soil solution constant was respectively used. The heavy metal contents were measured using Atomic Absorption Spectrophotometer and the percentage heavy metal removal efficiency was calculated. The highest concentrations of biosurfactant (20:1) at different spiked concentrations of metallic salts recorded the highest values of copper (95.47%, 95.73%, 91.69%, 78.82%); zinc (97.98%, 98.98%, 97.29%, 96.78%) and lead (97.68%, 93.09%, 88.12%, 84.98%) removal. The percentage metal removed in each treatment increased with increasing concentration of the biosurfactants and washing agents (1:1 to 20:1). The chemical structure of the two biosurfactants analyzed using Gas Chromatography Mass Spectroscopy (GC-MS) depict the major component of biosurfactants produced from *Odoribacter splanchnicus* DSM 20712 to be Di-n- amyl phthalate while 9, Octadecanoic acid, methyl ester was from unidentified bacterium clone JX981747. The one dimensional paper chromatography showed presence of galactose/glucose, mannose, ribose, rhamnose in the biosurfactants produced from *Odoribacter splanchnicus* DSM 20712 whereas the unidentified bacterium clone JX981747 produced biosurfactants that contained all sugars except mannose. The test biosurfactants studied showed high levels of copper and lead removal than zinc when compared with the test soil washing agents (KNO_3), $\text{Ca}(\text{NO}_3)_2$ and NaOH used in this study. Biosurfactants have thus shown to have the ability to remove metals hence its use requires scaling up for environmental applications.

Introduction

Soils may become contaminated by the accumulation of heavy metals and metalloids through emissions from the rapidly expanding industrial areas, mine tailings, disposal of high metal wastes, leaded gasoline and paints, land application of fertilizers, animal manures, sewage sludge, pesticides, wastewater irrigation, coal combustion residues, spillage of petrochemicals, hospital effluents, hospital wastes and atmospheric deposition (Roane *et al.*, 1996; Khan *et al.*, 2008; Zhang *et al.*, 2010). Heavy metals constitute an ill-defined group of inorganic chemical hazards, and those most commonly found at contaminated sites are lead (Pb), chromium (Cr), arsenic (As), zinc (Zn), cadmium (Cd), copper (Cu), mercury (Hg), and nickel (Ni) (Ogbulie, 2011). Soils are the major sink for heavy metals released into the environment by aforementioned anthropogenic activities (Ogbulie *et al.*, 2010) and unlike organic contaminants which are oxidized to carbon (IV) oxide by microbial action, most metals do not undergo microbial or chemical degradation (Kirpichtchikova *et al.*, 2006), and their total concentration in soils persists for a long time after their introduction (Adriano *et al.*, 2003). There may be changes in their

chemical forms (speciation) and bioavailability of which soil characterization would provide an insight into these heavy metal speciation and bioavailability (Zhao and Kaluarachchi, 2002).

The effectiveness of washing is closely related to the ability of the extracting solution to dissolve the metal contaminants in soils. However, the strong bonds between the soil and metals make the cleaning process difficult (Gombert, 1994). Owing to the different nature of heavy metals, extracting solutions that can optimally remove them must be carefully sought during soil washing. Several classes of chemicals used for soil washing include surfactants, cosolvents, cyclodextrins, chelating agents, and organic acids (USEPA, 1990; Wood *et al.*, 1990; Chu and Chan, 2003; Gao *et al.*, 2003; Maturi and Reddy, 2008; Zhang *et al.*, 2009). All these soil washing extractants have been developed on a case-by-case basis depending on the contaminant type at a particular site. A few studies have indicated that the solubilization/exchange/extraction of heavy metals by washing solutions differs considerably for different soil types. Surfactants are used on a large scale basis worldwide in everyday household use to industrial cleaning and textile manufacturing. Surfactants are best known for their solubility and cleaning properties which secured them a place among detergents and other cleaning products (Ivanković and Hrenović, 2010). Synthetic detergents used to clean up spillages have often led to more destruction of the environment from an environmental view point (Ogbulie et al., 2008). It is important that the remediation process be as non destructive and environmentally benign as possible if the end product is intended to be a healthy productive ecosystem. Metal-contaminated sites vary according to location, the source of metal contamination, and the history and age of the metal contamination. We had studied the removal of metals from metal dumpsite soil using biosurfactants and other soil washing agents. The metal dumpsite soil is a sink for metals (copper, zinc, lead) and it has a long history of contamination with these metals (Okore, 2017a; b).

Chelators are chemical compounds that form complexes with metal ions or other substances and also called chelating agents or sequestering agents. The chelating agent has a ring like center, which forms a complex with the metal ion/substance by two or more bindings and the metal ion is bound and extracted. There are a great number of metal chelators few of these materials are environmentally benign; the acids, alkalis, complexants, other solvents and surfactants examples KNO_3 , $\text{Ca}(\text{NO}_3)_2$ and NaOH (Dermont *et al.*, 2008). The synthetic chemicals such as nitrilotriacetic (NTA), ethylenediamine-tetraacetic acid (EDTA), and diethyl triamine pentaacetic acid (DTPA) are extremely effective at metal complexation. Their xenobiotic nature makes persist in the environment long after they are applied for a remedial measure, pose a threat to the aquatic environment and some are comparatively more toxic to human health. Their use in the field for *in-situ* removal is questionable because of their demonstrated toxicity effects. The strong acids attack and degrade the soil crystalline structure at extended contact times (Dehghan-Noudeh *et al.*, 2005).

The aqueous solutions of biosurfactants can be used to release compounds characterized by low solubility from soil and other media in process called washing. Soil washing performance is highly sensitive to site conditions. The process is most efficient when applied to soils and sediments containing large proportions of sand and gravel and is relatively ineffective when applied to soils having a high silt

and clay content. Further, soils with a relatively high cation exchange capacity (the capacity to exchange cations for those in the polluting substances) tend to bind pollutants more tightly, which can limit the ability of the soil washing process to effectively separate the pollutants from the soil (Oberbremer *et al.*, 1990; Fiebig *et al.*, 1997; Yuste *et al.*, 2000; Pacwa-Plociniczak *et al.*, 2011; Kumari *et al.*, 2012). Therefore, only extractants capable of dissolving large quantities of metals would be suitable for cleaning purposes (Tejowulan and Hendershot, 1998). The identification and quantification of coexisting solid metal species in the soil before and after treatment are essential to design and assess the efficiency of soil-washing technology (Kirpichtchikova *et al.*, 2006).

The usefulness of biosurfactants for bioremediation of heavy metal contaminated soil is mainly based on their ability to form complexes with metals (Ogbulie, 2011). Metal ions are bound to oppositely charged ions or replace the same charged ions (electrostatic interactions or ion exchange) or complex with agents forming chelates on the polar head groups of micelle surface. This makes the metals more soluble in water. Biosurfactants used for soil treatment are also required to have minimal sorptive interactions applied to the soil system; in other words, most of the biosurfactant should remain in the aqueous phase. Biosurfactant sorption in general is likely the reason that high biosurfactant concentrations are required for effective metal removal (Asci *et al.*, 2007; 2008). Logically, the adsorptive behavior of a biosurfactant will depend on their molecular characteristics, such as charge and hydrophobicity, as well as on the soil characteristics. It is important that all substances released into the environment be degradable. Their potential for causing environmental damage should be assessed and the possibility of future harm due to build-up in the environment should be taken into consideration. This study was designed to characterize the biosurfactants produced from *Odoribacter splanchnicus* DSM20712 and unidentified bacterium clone JX981747 with a view to ascertain their metal removal efficiencies and to compare the metal removal efficiencies with some soil washing agents as KNO_3 , $\text{Ca}(\text{NO}_3)_2$ and NaOH .

Methodology

Isolation, screening and identification biosurfactant producing-bacteria

The bacteria species were isolated from waste battery dumpsite soil and cassava mill soil collected at Nekede Imo State, Nigeria. The bacteria species were identified by 16S rRNA gene in Iquaba, Pittsburg, South Africa. Both bacteria were screened for biosurfactant production using emulsification index test, oil displacement test and β hemolysis test using methods described by Okore *et al.*, (2017 a,b,c)

Production and extraction of crude biosurfactant

The colonies of *Odoribacter splanchnicus* DSM 20712 isolated from WBS and an unidentified bacterium clone JX981747 from CMS were inoculated in 500 ml nutrient broth medium in 1000 ml Erlenmeyer flask

and incubated at 30°C for 7 days on a mechanical shaker. After 1 week of incubation, it was filtered by centrifugation for 45 min at 4,000 X g to obtain cell free supernatant and sediments of cell debris. The extraction was performed by acid precipitation with 1 M H₂SO₄ of equal volume to attain a pH of 2.0 (54.3 ml conc. H₂SO₄ poured into 1 L distilled water = 1 M H₂SO₄). This was put in the fridge (4°C) overnight for complete precipitation of the biosurfactant. After 24 h it was centrifuged at 4,000 X g for 45 min to sediment the biosurfactant. The supernatant was filtered off and the sediment stirred continuously for 20 min after which GC-MS chemical analysis and one dimensional paper chromatography was carried out.

Chemical characterization of produced biosurfactant

i. Gas chromatography-Mass Spectrometer

The partially purified biosurfactant obtained as sediment was mixed with chloroform and the chemical component analyzed using Gas chromatography-Mass Spectrometer (GC-MS).

ii. Sugar detection

One dimensional paper chromatography (AOAC, 2005) was employed. The crude biosurfactant was reconstituted in 1 ml of sterile distilled water and hydrolysed by boiling in 5 ml of bench sulphuric acid solution (2 M). The hydrolysate was spotted on Whatman chromatographic paper and ran in two separate solvents Butanol-Acetic acid-Water, 4:1:5 (BAW) and Butanol-Ethanol-Water, 4:1:2.2 (BEW). The separate chromatographs were allowed to run for 18–24 h and the paper was removed and dipped in aniline hydrogen phthalate solution in 1:1 ether: butanol solution. It was then dried for 5 min at 105°C and viewed under UV light and the various spots were marked. The relative fronts (rfs) were calculated and matched against standard relative fronts for sugars (RFS). The matching sugars were deemed detected.

iii. Protein detection

The tests for protein by Ninhydrin and Biuret were based on the standard method of Association of Official Analytical Chemists (AOAC 2005). In Ninhydrin test, 2 ml of sample mixed with 2 ml of Ninhydrin solution was heated, the development of lilac or faint blue colour is positive for presence of protein. In Biuret test, 2 ml of sample was mixed with 2 ml Biuret reagent, the development of purple or violet colour shows presence of protein.

Heavy metal removal from spiked contaminated soil

The experimental set up was done using the soil samples collected from waste metal dumpsite soil spiked with solutions of different concentrations of the metallic salts (Pb(NO₃)₂, Cu(SO₄)₄, and Zn(SO₄)₄ using the modified methods of Mulligan *et al.*, (1999) and Maier *et al.*, (2001). The metallic solutions was prepared by dissolving 50 mg, 250 mg, 750 mg and 1500 mg each of Cu(SO₄)₂, Pb(NO₃)₂ and Zn(SO₄)₂ separately in 1 L distilled water. The Atomic Absorption Spectrophotometer

(AAS) reading of the various metallic concentrations was analyzed. Then 1 g of the soil sample from waste metal dump site was added to equal volumes of each concentrations of the metallic solution (5 ml). Thereafter it was centrifuged and supernatant subjected to AAS for the metal content determination. The results served as the initial metal content without biosurfactant treatment. The biosurfactant producing isolates *Odoribacter splanchnicus* DSM 20712 and the unidentified bacterium clone JX981747 were inoculated into nutrient broth and left for 7 days on the mechanical shaker. Thereafter the broth was centrifuged to separate the bacterial cells from the biosurfactant containing supernatant. The heavy metal removal of the spiked soils with biosurfactant was carried out using soil metallic solution to biosurfactant ratios (1:1, 1:5; 1:10; 1:15 and 1:20). The soil metallic solution with biosurfactant was shaken and left for 3 days. Then the soil was removed by centrifugation (4000 X g, 25 min) and dried. The supernatant was removed and placed into another centrifuge tubes acidified with 5 drops of HNO₃ to precipitate the biosurfactant then refrigerated for 24 h. After refrigeration the precipitated biosurfactant was removed from solution by centrifugation at (4000 X g, 25 min). Then the supernatant analyzed for the metal content using AAS. The amount of metals extracted from the soil by each biosurfactant was then calculated as a percentage of the original metal concentration without biosurfactant using the formula below.

$$\text{Percentage metal removal} = \frac{A - B}{A} \times 100\%$$

Key: A = the amount of metal originally in the soil
B = the amount of metal left in the supernatant

Soil washing of heavy metal contaminated soils (un-spiked)

This was carried out using the method of Mulligan *et al.*, (1999) and Rufino *et al.*, (2011) with modifications. The test soil samples (10 g) dried at room temperature was digested over low flame for 15 min. This was done by initial heating for 3 min with 10 ml of H₂O₂, then another 5 ml of H₂O₂ for another 3 min, followed by 5 ml of H₂O₂ and heating for 3 min. Thereafter 70 ml of concentrated H₂SO₄ was added and heated for 6 min before the sample will be removed from the heat for cooling. Distilled water was added after cooling to make up the volume to 400 ml. The soil sample supernatant was collected after vortexing to attain an equilibrium and centrifuged (5000 X g 10 min). The supernatant was then filtered through a 0.2 µm cellulose acetate filter prior to AAS analysis for metal concentration and this served as the amount of metal originally in the soil sample supernatant. The biosurfactant producing isolate was inoculated into 1000 ml nutrient broth and left for 7 days on mechanical shaker. Thereafter the broth was centrifuged at 4,000 X g 45 min to sediment the bacterial cell and the supernatant used for the soil washings studies. The soil washing with biosurfactant study was carried out using soil sample supernatant to biosurfactant ratios ranging from 1:1 to 20:1 in acid washed plastic centrifuge tubes of 50 ml without varying the pH. The comparison experiment was set up using aqueous solution of similar ionic strength like KNO₃, Ca(NO₃)₂ and NaOH. The experimental set up was left for 1 week before AAS analysis to ascertain the amount of heavy metal left in the supernatant after centrifugation. The percentage of metal removal was calculated using similar equation as above:

$$\text{Percentage metal removal} = \frac{A - B}{A} \times 100\%$$

Key: A = the amount of metal originally in the soil
 B = the amount of metal left in the supernatant

Results

Screening and Identification

The E₂₄ of biosurfactants from *Odoribacter splanchnicus* DSM 20712 recorded is **78.13%** on kerosene while the E₂₄ of the biosurfactants from an unidentified bacterium clone JX981747 is 68.52% on power vegetable oil and 57.41% on crude oil. Both isolates exhibited positive β hemolysis and displaced hydrocarbons used in the oil displacement test (Table 1).

Table 1: Screening test for biosurfactant production

S/N	Isolate	β Hemolysis	Emulsification index (E ₂₄) on Hydrocarbon					Oil displacement test on hydrocarbon (cm)				
			Kerosene	Crude oil	Vegetable oil	Petrol	Diesel	Kerosene	Crude oil	Vegetable oil	Petrol	Diesel
1	WBS1	+ve	78.13	0	0	22.60	0	2.50	3.00	-	2.00	2.00
2	CMS1	+ve	0	57.41	68.52	28.00	0	-	2.00	-	-	-

Key: WBS1 = *Odoribacter splanchnicus* DSM20712 from waste battery dump site
 CMS1 = unidentified bacterium clone JX981747 from cassava mill soil

Production and extraction of crude biosurfactant

The quantity of crude biosurfactant produced from the 500 ml broth culture of biosurfactants from *Odoribacter splanchnicus* DSM 20712 and unidentified bacterium clone JX981747 was respectively 1.53 g and 1.33 g after acid precipitation (Table 2).

Table 2: Yield of biosurfactant produced by WBS1 and CMS1

Isolate	Volume of broth culture (ml)	Weight of biosurfactant (g)
WBS1	500	1.53
CMS1	500	1.33

Key: WBS1 = *Odoribacter splanchnicus* DSM 20712 from waste battery dump site soil
 CMS1 = unidentified bacterium clone JX981747 from cassava mill soil

Chemical characterization of produced biosurfactants

1) Gas chromatography analysis

A chromatogram depicting the real time of peaks generated as the separate components pass through the detector was recorded. The GC-MS fatty acids analysis of *Odoribacter splanchnicus* DSM 20712

showed peaks each corresponding to long chain poly aliphatic and unsaturated compounds consistent with fatty acid methyl esters linked with benzene ring (Table 3). On the other hand, the GC-MS fatty acids analysis of the unidentified bacterium clone JX981747 showed peaks corresponding to long chain poly aliphatic and unsaturated compounds consistent with fatty acid methyl esters linked mainly with Decanoic acid (Table 4).

The chromatograms of the biosurfactants produced by both test isolates are as shown in Figs. 1 and 2 respectively depicting a major peak of the likely compound at varying retention times (*Odoribacter splanchnicus* DSM 20712: Retention time of 12.470; an unidentified bacterium clone JX981747: Retention time of 13.705) whereas the other low peaks are the contaminants.

The Gas Chromatography library search result of biosurfactants produced by the test isolates showed the retention times of compounds detected, the components that exhibited peaks, their individual mass spectrum and chemical structures as shown on Tables 3, and Table 4 for each of the respective biosurfactants.

2. Sugar test

The presence of these sugars (Tables 5 and 6) Glucose/Galactose, Mannose, Ribose, Rhamnose, were found in biosurfactant from *Odoribacter splanchnicus* DSM 20712, Glucose/Galactose, Ribose and Rhamnose found in biosurfactant from the unidentified bacterium clone JX981747. This result was confirmed from the two mobile solvents used in the one dimensional paper chromatography method.

3. Protein test

Tests for protein by Ninhydrin and Biuret both show faint colours hence indicating traces of protein. The same result was obtained for both samples.

Metal analysis result on spiked soil

The percentage metal removed increased with higher concentrations of the biosurfactants used. Copper recovery rates were as high as 95.47%, 95.73%, 91.69% and 78.82% in solutions respectively spiked with 50 mg/L, 250 mg/L, 750 mg/L and 1500 mg/L of copper and treated with 20:1 concentration of biosurfactant produced by *Odoribacter splanchnicus* DSM 20712 (Fig. 3a). Figure 4b also showed that 97.98%, 98.98%, 97.29% and 96.78% of Zinc was recovered in solutions spiked with 50 mg/L, 250 mg/L, 750 mg/L and 1500 mg/L of zinc respectively and treated with 20:1 concentration of biosurfactant from an unidentified bacterium clone JX981747. Lead on the other hand equally recorded high recovery rates of 97.68%, 93.09%, 88.12% and 84.98% in solutions spiked with 50 mg/L, 250 mg/L, 750 mg/L and 1500 mg/L of lead respectively and treated with 20:1 concentration of biosurfactant an unidentified bacterium clone JX981747 (Fig. 4c). Comparatively, there was obvious steady increase in copper removal rate in all the concentration ratios in relation to using biosurfactants from *O. splanchnicus* DSM

20712 than with BC JX981747 with the peak recorded in the highest treatment ratio (20:1) whereas Zinc and Lead removal was high in the later [Figures 3 (a-c) and 4(a-c)].

Table 3: The components of biosurfactant from *Odoribacter splanchnicus* DSM 20712 depicting peaks by GC-MS analysis

S/N	Retention Time	Name of compound/ Library /ID	Molecular formular	Molecular weight	Quality %
1	5.494	Iso propyl Benzenamine or N, N-diethylaniline	C ₁₀ H ₁₅ N	149.120449	93
2	7.583	4 -Bromo-3-chloroaniline 2,5-Dimethoxy-4-ethylamphetamine	C ₁₃ H ₂₁ NO ₂	223.157228	38
3	7.978	Phenol, 2,4-bis- (1,1-dimethylethyl)	C ₁₄ H ₂₂ O	206.167066	93
4	10.804	Octadecane	C ₁₈ H ₃₈	254.297351	96
5	12.355	n-Hexadecanoic acid	C ₁₇ H ₃₄ O ₂	270.25558	86
6	12.470	Di-n- amyl phthalate	C ₁₈ H ₂₆ O ₄	306.18311	92
		1,2-Benzenedicarboxlic acid butyl 2-methyl propyl ester OR 1,2-Benzenedicarboxlic acid butyl cyclohexyl ester	C ₁₆ H ₂₄ O ₄	278.15181	90
7	12.738	Octadecane, 3-ethyl-5-(2-ethylbutyl) Heptacosane	C ₂₆ H ₅₄ C ₂₇ H ₅₆	366.707 380.48202	83 81

Table 4: The components of biosurfactant from an unidentified bacterium clone JX981747 depicting high peaks by GC-MS analysis

S/N	Retention Time	Name of compound/ Library /ID	Molecular formular	Molecular weight	Quality %
1.	12.046	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270.25558	99
2.	12.469	Di-n- amyl phthalate OR 1,2-Benzenedicarboxylic acid, butyl 1cyclohexyl ester OR 1,2-Benzenedicarboxylic acid, butyl 1,2-ethylhexyl ester	C ₁₈ H ₂₆ O ₄ C ₁₆ H ₂₄ O ₄	306.18311 278.15181	92 90
3.	13.654	9, 12-Octadecadienoic acid (z-z) -, methyl ester 10, 13-Octadecadienoic acid , methyl ester	C ₁₉ H ₃₄ O ₂	294.479	99 99
4.	13.705	9-Octadecenoic acid, methyl ester (E)	C ₁₉ H ₃₆ O ₂	296.271	99
5.	13.751	11-Octadecenoic acid , methyl ester/Methyl stearate OR 9-Octadecenoic acid, methyl ester (E) OR Cis-13- Octadecenoic acid, methyl ester	C ₁₉ H ₃₆ O ₂ C ₁₉ H ₃₆ O ₂	296.271 296.487	99 99
6.	13.917	Octadecanoic acid , methyl stearate	C ₁₉ H ₃₈ O ₂	298.287	99

Table 5: Sugar detection by RFS using BAW

	S/N	WBS1	CMS1	Detected sugar
i		12.0	12.02	Glucose/Galactose
ii		16.96	-	Mannose
iii		26.94	26.98	Ribose
iv		32.01	32.00	Rhamnose

Legend:

Sample WBS1 = biosurfactant from *Odoribacter splanchnicus* DSM 20712

Sample CMS1 = biosurfactant from an unidentified bacterium clone JX981747,

BAW = Butanol-Acetic acid-water, 4:1:5,

RFS = standard relative fronts for sugars

Table 6: Sugar detection by RFS using BEW

	S/N	WBS1	CMS1	Detected sugar
i		16.03	16.00	Glucose/Galactose
ii		23.00	-	Mannose
iii		36.03	35.97	Ribose
iv		37.00	37.03	Rhamnose

Legend:

Sample WBS1 = biosurfactant from *Odoribacter splanchnicus* DSM 20712;

Sample CMS1 = biosurfactant from an unidentified bacterium clone JX981747,

BEW = Butanol-Ethanol-water, 4:1:2.2,

RFS = standard relative fronts for sugars

Comparative Heavy metal removal efficiencies of biosurfactant WBS1- from *Odoribacter splanchnicus* DSM 20712, CMS1 - from bacterium clone JX981747 and soil washing agents

The percentage metal removal efficiency of the biosurfactants and other soil washing agents used are shown on Figs. 5a-c. The trend observed showed that the percentage metal removal efficiency recorded increased with higher concentrations of the biosurfactants and the test soil washing agents used.

In Fig. 5a, the highest removal percentage observed in 20:1 concentration for copper was 97.49% (WBS1) followed by 97.38% (CMS1); of the 15:1 concentration is 96.78% ($\text{Ca}(\text{NO}_3)_2$) followed by 95.95% (WBS1); of 10:1 concentration is 95.95% ($\text{Ca}(\text{NO}_3)_2$ and NaOH) followed by 95.35% (CMS1) in that order. The varying range of copper removal at 5:1 concentration is 92.49%, 91.42%, 91.06% and 90.58% for $\text{Ca}(\text{NO}_3)_2$, KNO_3 , WBS, CMS1 and NaOH respectively. However, WBS1 removed more copper (91.06%) for this concentration than CMS1 (90.58%). In addition, at the lowest concentration of 1:1, the soil washing agents removed higher concentration of copper than the biosurfactants. Generally, there is negligible significance difference in copper removal by all the biosurfactants and soil washing agents.

The percentage lead removal is as shown in Fig. 5b. WBS1 removed the highest concentration of lead (90.22%, 87.43%, 84.83%, 79.24% and 57.09%) in all the treatment ratio followed by CMS1 (90.02%,

87.03%, 84.23%, 78.24% and 56.89%) for 20:1, 15:1, 10:1, 5:1 and 1:1 ratios respectively. However, of the soil washing agents used in lead removal, NaOH had the highest values of lead removal at higher treatment concentrations (Fig. 5b).

The zinc removal percentage observed in this study is as shown in figure 5c. Comparatively, of the 20:1, 15:1, 10:1 and 5:1 concentrations NaOH removed highest concentration of zinc followed by KNO_3 , $\text{Ca}(\text{NO}_3)_2$, CMS1 and WBS1 in that order except at 1:1 ratio where $\text{Ca}(\text{NO}_3)_2$ had the highest value.

Comparatively, the test biosurfactants used in this study removed copper and lead more than the soil washing agents whereas the later removed zinc more than the former.

Discussions

Crude Biosurfactant production and characteristics

Biosurfactants are indeed good bio-products with great diversity and broad spectrum of functions and environmental applications. Their various sources could be from contaminated water, soil and most importantly from microorganisms (Reis et al., 2013). In this study, crude biosurfactants were obtained from two major isolates amongst others namely *Odoribacter splanchnicus* DSM 20712 and an unidentified bacterium clone JX981747. The possible production and recovery of crude biological surfactants from microorganisms as observed in this study corroborates with the findings of Anandaraj and Thivakaran, (2010) who obtained 0.122 g of biosurfactant from *Pseudomonas* sp. grown in 50 ml of R2B broth with 1 ml of petrol as carbon source. It also supports the findings of Maneerat et al., (2006) who also proved the possibility of extracting crude biosurfactant from isolates during his experiment with marine bacterium *Myroides* sp and extracted 2.64 g of a crude oil emulsifier from 10 L of cultured strain SMI. This current study is in agreement that different yields of crude biosurfactant can be precipitated and extracted from the culture broth.

The biosurfactants produced from *Odoribacter splanchnicus* DSM 20712 and the unidentified bacterium clone JX981747 in this study showed the presence of rhamnose sugar, and fatty acid component. The major component of *O. splanchnicus*, di-n- amyl phthalate as regards its structure will remove more metals by chelating and less of hydrocarbon because of the short aliphatic chain whereas the major component of bacterium clone JX981747, which is 9-Octadecenoic acid- a methyl ester, will remove less metals and function better as hydrocarbon removal because of the chain length.

Heavy metal removal from spiked soil using crude biosurfactant

Heavy metal removal from terrestrial environment is one of the key areas in environmental management and clean up operations where the significance of biosurfactants has not been explored in depth (Luna et al., 2016). The result of this study evidently depict that the test heavy metals (copper, lead and zinc)

removal rate was effective and high with increase in concentration of the biological surfactants tested. This corroborates with the report made by Maier *et al.*, (2001) who studied biosurfactant removal of metals from spiked sewage sludge which was anaerobically digested. They equally had high rate of copper recovery in solution spiked with 2000 mg/kg treated with 50 mM rhamnolipid. The findings of this study equally established this fact, and support the findings made by Mulligan *et al.*, (2001; 2004). In their study, they also observed high copper removal (70%) proving the feasibility metal removal with amnionic biosurfactants in batch washing experiments.

Furthermore, the high rate of metal removal as observed in this study have shown that surfactants could remove metals by ion exchange precipitation, dissolution and counterion association with the crude sugar surfactant, depicting superior performance similar to previous reports (Mulligan *et al.*, 1999, 2001, 2004 and Qi *et al.*, 2018). Generally, the concentration of biosurfactant have been observed to be a critical factor influencing heavy metal removal efficiency in this study and elsewhere (Mulligan *et al.*, 2001 and Qi *et al.*, 2018).

Efficiencies of biosurfactant and soil washing agents in heavy metal removal

Mulligan *et al.*, (1999) showed the feasibility of removing cadmium, lead, and zinc with 2% anionic biosurfactants surfactin from soil that is low in exchangeable metal fractions. The use of NaOH alone removed 20% copper, 10% zinc while surfactin biosurfactant removed 70% copper and 25% zinc. Chakrabarti, (2015) noted lead removal using biosurfactant produced by a *Bacillus* sp. SJ301 which removed nearly 2–3% lead from a highly toxic 100 ppm lead solution. This current study equally established the feasibility of Cu, Pb, Zn removal with biosurfactant depicting increase in the concentration of biosurfactant and its additional effect on the percentage metal removal efficiency. This also in agreement with the findings of Hong *et al.*, (2002) and Mulligan *et al.*, (2007) who observed that the removal percentage of Cu, Zn and Ni in the soil increased linearly with increasing concentration of rhamnolipid used. The recorded values are indications that biosurfactant can be used to remove metals from contaminated samples. Maier *et al.*, (2001) in their study also observed the effects of biosurfactants rhamnolipid and surfactin in metal removal in a historically contaminated soil by 10 mM purified rhamnolipid adjusted to pH 7.1 compared with other soil washing agents, KNO₃ solution adjusted to the same ionic strength as the rhamnolipid solution and 50 mM Ca(NO₃)₂. The rhamnolipid removed a total of 14 to 15% of the lead from each historically contaminated soil in 10 extractions. Their study in consonance with the findings of this study showed that rhamnolipid containing biological surfactant greatly enhanced metal removal than with chelators (KNO₃ or Ca(NO₃)₂). This study, irrespective of the source and history of contamination of soil established the feasibility of biosurfactant removal of copper, zinc and lead contaminants.

Conclusion

The two biosurfactants produced from *Odoribacter splanchnicus* DSM 20712 and unidentified bacterium clone JX981747 recorded the highest emulsification index and equally showed higher levels of percentage metal removal. Biosurfactant from *O. splanchnicus* efficiently removed copper more than that from BC JX981747 whereas that from unidentified bacterium clone JX981747 removed both zinc and lead efficiently more than does the former in the spiked soil. Nevertheless, both significantly removed the test metals with negligible difference achieved possibly by forming complex at the polar end of the micelles with the metals and as a result these metals are desorbed out of the contaminating matrix. Furthermore, the test biosurfactants studied showed high levels of copper and lead removal than zinc when compared with the test soil washing agents (KNO_3), $\text{Ca}(\text{NO}_3)_2$ and NaOH used in this study. Biosurfactants have therefore proved to be efficient in metal removal and since they are biodegradable, the culture medium for the optimum production of these biosurfactants should be formulated to enhance high production yield. In addition, its exploitation should be encouraged for large scale industrial and environmental applications.

Abbreviations

WBS1- biosurfactant from *Odoribacter splanchnicus* DSM 20712 isolated from waste battery dump soil

CMS1 - biosurfactant from bacterium clone JX981747 isolated from cassava mill soil

GC-MS - Gas Chromatography Mass Spectroscopy

E_{24} - Emulsification index

Declarations

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Authors' contributions

The corresponding author (TEO) conceptualize the idea, designed the work, conducted analysis with her team at different stages, interrelated data and results for this work, and wrote this paper. TEO and the other authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work hence the essence of a team comprising a microbial biotechnologist and chemist. The authors read through and I approved the final manuscript. The authors are prepared to take public responsibility for the work.

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Availability of data and materials

The data that support the findings of this study are available from the corresponding author (TEO), upon reasonable request.

Ethics approval and consent to participate

This research does not contain any studies on human participants or animals performed by any of the authors.

Consent for publication

I, Toochukwu Ekwutosi OGBULIE (TEO), the corresponding author declare that it is my study and I developed the manuscript titled 'Comparative heavy metal removal efficiencies of biosurfactants produced by *Odoribacter splanchnicus* DSM 20712, bacterium clone JX981747 and soil washing agents". The authors hereby grant Journal of Bioresources and Bioprocessing full right to the manuscript to publish, revise, reproduce and distribute. The authors are agreeing that the submitted article retains the property/ copyright of Bioresources and Bioprocessing Journal.

Declaration of conflict of interest

The authors declare that they have no competing interests.

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Figures

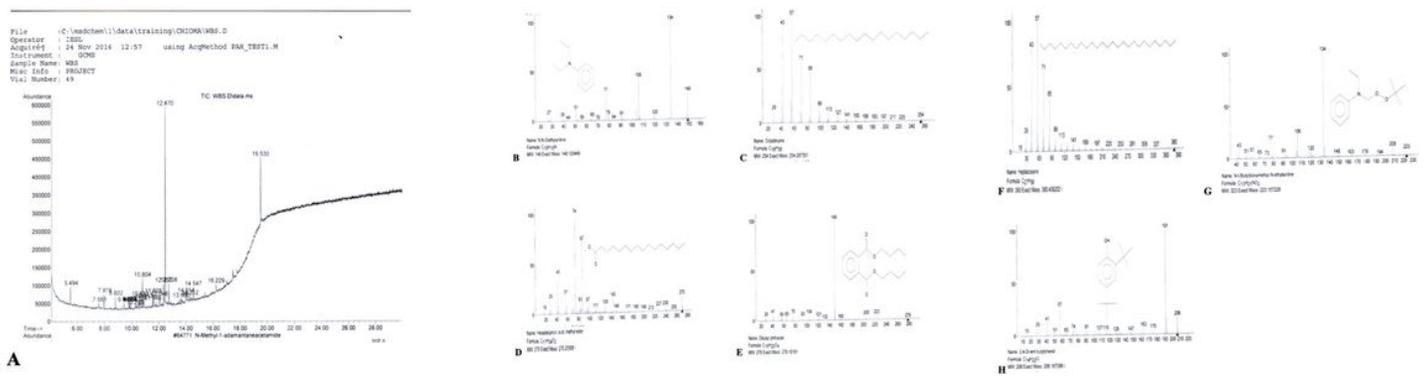


Figure 1

A: Chromatogram of biosurfactant from *Odoribacter splanchnicus* DSM 20712. (B-H): Mass spectral of the components of biosurfactant from *Odoribacter splanchnicus* DSM 20712

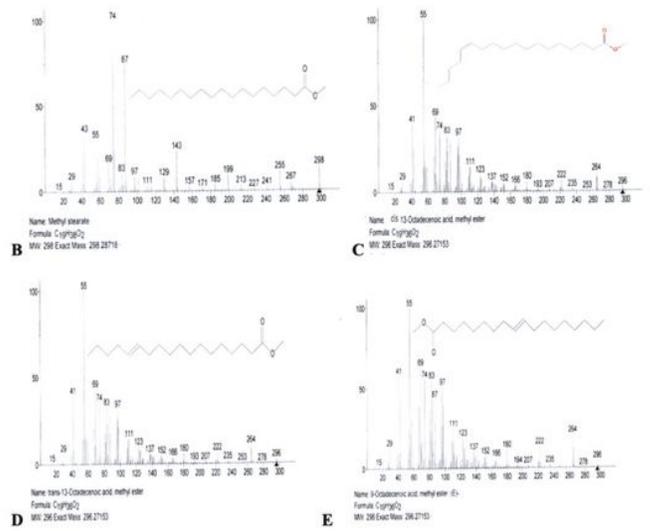
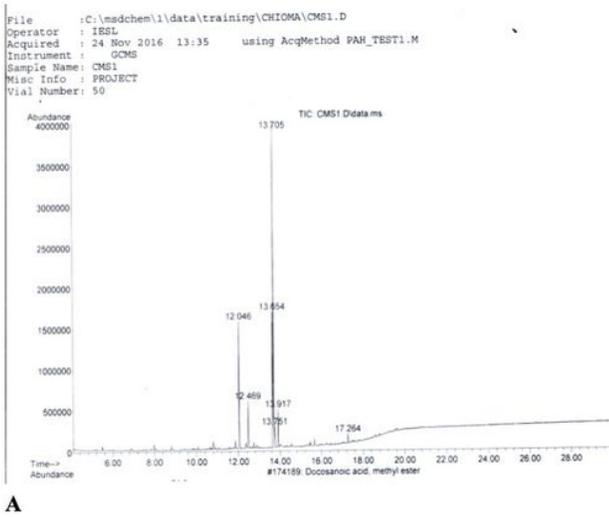


Figure 2

A: Chromatogram of biosurfactant from the unidentified bacterium clone JX981747. (B-E): Mass spectral of the components of biosurfactant from the unidentified bacterium clone JX981747

%age copper removal



Fig 3a. Biosurfactant from *O. splanchnicus* facilitated removal of...

conc 1:1 conc 5:1
conc 10:1 conc 15:1

%age Zinc removal

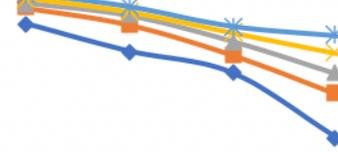


Fig 3b. Biosurfactant from *O. splanchnicus* facilitated removal of zinc from waste...

conc 1:1 conc 5:1 conc 10:1

%age Lead removal



Fig 3c. Biosurfactant from *O. splanchnicus* facilitated removal of lead from waste...

conc 1:1 conc 5:1

Figure 3

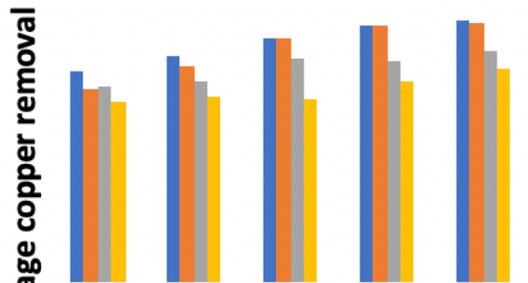


Fig 4a. Biosurfactant from BC JX981747 facilitated removal of copper from waste...

■ 50 mg/kg ■ 250 mg/kg

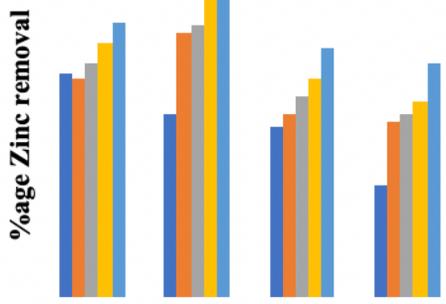


Fig 4b. Biosurfactant from BC JX981747 facilitated removal of Zinc from waste metal dumpsite soil spiked with...

■ 1:1 conc ■ 5:1 conc ■ 10:1 conc

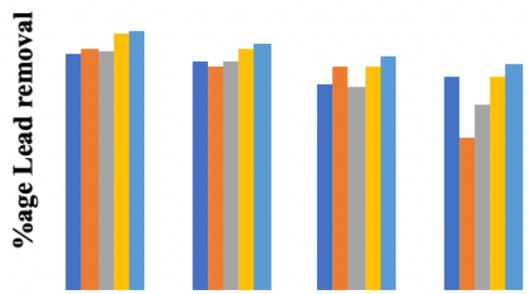


Fig 4c. Biosurfactant from BC JX981747 facilitated removal of Lead from waste metal dumpsite soil spiked with different...

■ 1:1 conc ■ 5:1 conc ■ 10:1 conc

Figure 4

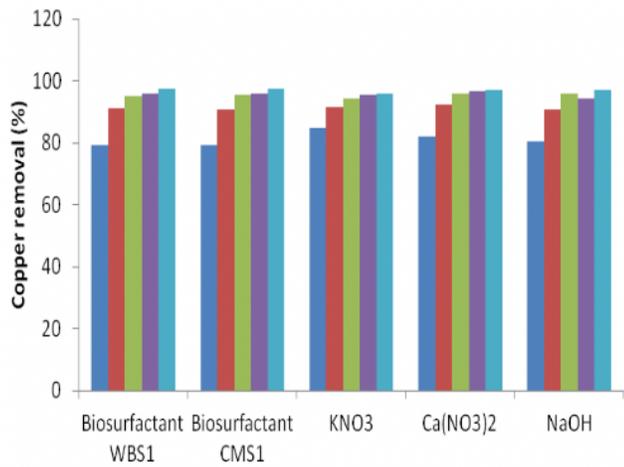


Fig. 5a: Efficiencies of Biosurfactants and soil washing agents in removal of copper from test soil

■ conc 1:1 ■ conc 5:1 ■ conc 10:1 ■ conc 15:1 ■ conc 20:1

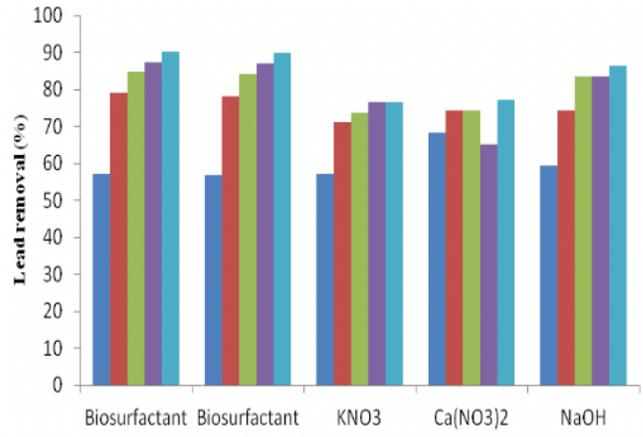


Fig. 5b: Efficiencies of Biosurfactants and soil washing agents in removal of lead from test soil

■ conc 1:1 ■ conc 5:1 ■ conc 10:1 ■ conc 15:1 ■ conc 20:1

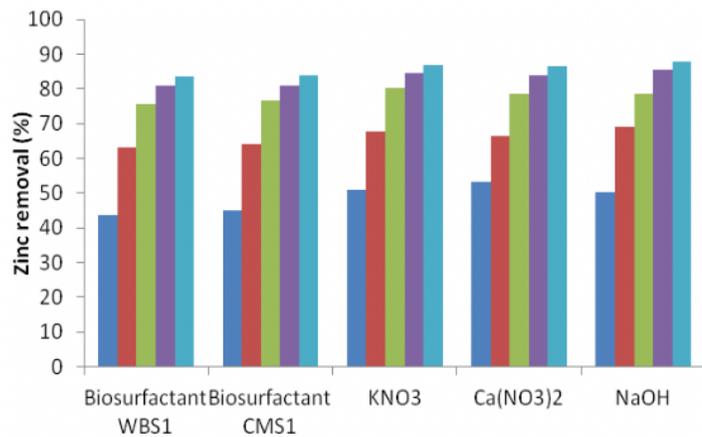


Fig. 5c: Efficiencies of biosurfactants and soil washing agents in removal of zinc from test soil

■ conc 1:1 ■ conc 5:1 ■ conc 10:1 ■ conc 15:1 ■ conc 20:1

Figure 5

WBS1- biosurfactant from *Odoribacter splanchnicus* DSM 20712. CMS1 - biosurfactant from bacterium clone JX981747.

Supplementary Files

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