Archives of Agriculture and Environmental Science 8(2): 116-124 (2023)
https://doi.org/10.26832/24566632.2023.080204

e-ISSN: 2456-6632

This content is available online at AESA

Archives of Agriculture and Environmental Science

Journal homepage: journals.aesacademy.org/index.php/aaes

ORIGINAL RESEARCH ARTICLE

Evaluation of the characterization and heavy metals remediation potential of biosurfactant produced by *Aeromonas hydrophila* S62A

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ARTICLE HISTORY

Received: 28 December 2022
Revised received: 21 February 2023
Accepted: 08 June 2023

Keywords

Aeromonas hydrophilia
Bioremediation
Glycophospholipid biosurfactant
Heavy metals
Imo River

ABSTRACT

The evaluation of the heavy metals remediation potential and characterization of biosurfactant produced by *Aeromonas hydrophilia* strain S62A isolated from water and sediment samples of Imo River, Nigeria was studied. In this study, 12 bacterial isolates were isolated from contaminated water and sediment samples using spread plate technique and primarily screened for biosurfactant production using emulsification index, oil displacement and surface tension tests. Secondary biosurfactant production was carried out in a modified mineral salt medium under optimized conditions for 5 days and the produced biosurfactant was characterized and evaluated for its heavy metals removal efficiencies using standard analytical procedures. The result showed that the bacterial strain identified as *Aeromonas hydrophilia* S62A out of the 12 isolate strains had the highest and lowest values of 66.66 %, 23.76 cm and 90 mN/m for emulsification index, oil displacement and surface tension tests, respectively. The purified biosurfactant was found to be glycophospholipid as confirmed by the gas chromatographic (GC) and Fourier Transformed Infra-Red Spectroscopic (FTIR) profiles with 5 mg/mL critical micelles concentration (CMC). Statistically, significant differences ($P < 0.05$) were detected among the means of all surfactant CMC treatment in comparison to their untreated controls with 2 × CMC lead having the highest (98.92 %) and control (water) having the lowest (2.09 %) heavy metals removal efficiencies. Therefore, the present study has produced glycophospholipid biosurfactant with unique structural and chemical features and composition and could be exploited in environmental remediation of heavy metals contaminated ecosystems.

Citation of this article: Uba, B. O., & Anidu, F. N. (2023). Evaluation of the characterization and heavy metals remediation potential of biosurfactant produced by *Aeromonas hydrophila* S62A. *Archives of Agriculture and Environmental Science*, *8*(2), 116-124, https://dx.doi.org/10.26832/24566632.2023.080204

INTRODUCTION

Globally and especially in Nigeria, the evolution and presence of certain chemical industries causes many problems of heavy metal pollution. Heavy metals are natural metal elements with a density exceeding 5 g/cm^3 (Bouzabata and Djamaa, 2015). The most toxic elements are lead, cadmium, mercury, chromium, nickel, arsenic, zinc, copper, cobalt and manganese. They lead to the toxifying of aquatic and soil environments, plants, animals and humans due to multiple anthropogenic sources. So, urgent need for their elimination has developed (Ines *et al*., 2022). Heavy metals, which are generally more persistent than organic pollutants due to their non-biodegradability and high toxicity even at trace concentrations, are becoming one of the most serious environmental problems today. They may lead to bioaccumulation in living organisms, causing health problems in animals, plants, and human beings if left untreated in the environment (Hisham *et al*., 2019). There are two technologies commonly applied to the treatment of metals contaminated soils. The first consists in immobilizing the heavy metals in a

solid matrix strongly bound to the soil, minimizing the migration. This technology, however, does not consist of a definitive solution to the problem, considering the impossibility of reuse of the soil and the need for long term monitoring. The second technology promotes the mobility of the metal and its migration to the liquid phase by desorption and solubilization (Sarubbo *et al*., 2018).

A potential solution for the remediation of metals contaminated soils is the use of surfactants. Biosurfactants are amphiphilic compounds with both hydrophilic and hydrophobic moieties that align themselves accordingly among diverse interfaces of air, water, oil and solid phases, and affect the properties of these phases (Lamichhane *et al*., 2017). The current dominate players in the market are chemically synthesized surfactants such as Tween 20/80, Triton X-100, and Brij35 (Lamichhane *et al*., 2017; Santos *et al*., 2016). However, such chemically synthesized surfactants generally have concerns of toxicity and low biodegradability (Santos *et al*., 2016). The recent and growing awareness in microbial biosurfactants could be due to the prospect of their production from cheaper wastes and ecofriendly approach. Furthermore, the synthesis of microbial biosurfactants from renewable substrates could help in mitigating the high cost of production, which is attributed to are related to incompetent methods for product recovery and purification (Hisham *et al*., 2019). Nitrogenous and carbon substrates such as slaughter house and rice bran wastes, which are known for their attendant environmental and public health challenges, have been demonstrated to be suitable and cheaper renewable nitrogen and carbon sources for the production of biosurfactants because they contain proper nutritional components which favour microbial growth (Freitas *et al*., 2016; Sarubbo *et al*., 2018). One of the major problems facing environmental and industrial microbiologists is the ability to synthesize biosurfactant with lower cost and higher efficiencies for biotechnological applications.

A study carried out by Hisham *et al.* (2019) aimed to produce biosurfactant from renewable feedstock, which is used cooking oil (UCO), by a local isolate, namely *Bacillus* sp. HIP3 for heavy metals removal revealed that the biosurfactant was capable of removing 13.57 %, 12.71 %, 2.91 %, 1.68 %, and 0.70 % of copper, lead, zinc, chromium, and cadmium, respectively, from artificially contaminated water, highlighting its potential for bioremediation. There are limited literatures on the application of microbial biosurfactants for enhanced heavy metal recovery and remediation. To the best of knowledge, the available information especially in Nigeria mostly focuses on the applications of biosurfactants to oil and oil - products recovery and remediation with limited study on heavy metal removal or reduction. The limited studies available examined fewer instead larger number of heavy metals and hence justifies and validates the current study. Thus, considering the severe environmental and public health problems caused by these substrates and heavy metals, this study was undertaken to initially isolate, screen and characterize the biosurfactant producing bacteria with potential of utilizing slaughter house waste and rice bran as source carbon. The study further described the production, stability and structural elucidation of the extracted biosurfactant of *Aeromonas hydrophilia* S62A which contributes to the attempts to minimize the production cost of biosurfactant using cheaper substrates as well as evaluating the efficiency of the extracted biosurfactant of *A. hydrophilia* S62A in the remediation of heavy metal contaminated media.

MATERIALS AND METHODS

Description of the sampling site

The studied area includes Imo River located at Afam in Oyibo Local Government Area Rivers State. There were anthropological activities in this site such as transportation of petroleum products, using speed boats and canoes and repair of damaged vehicles. The site was referenced using Global Positioning System (GPS) App with the coordinates obtained from the sampling points (Uba, 2018). The coordinates were later used to download the Geoeye Statelite Images from the online archive of digital globe (2022). Thereafter, the images were Geo referenced in ILWIS Software version 3.30 and exported to ArcGis 10.20 where the imageries were digitized to produce schematic maps indicating the land cover of different sampling points of the studied area as shown in Figure 1 .

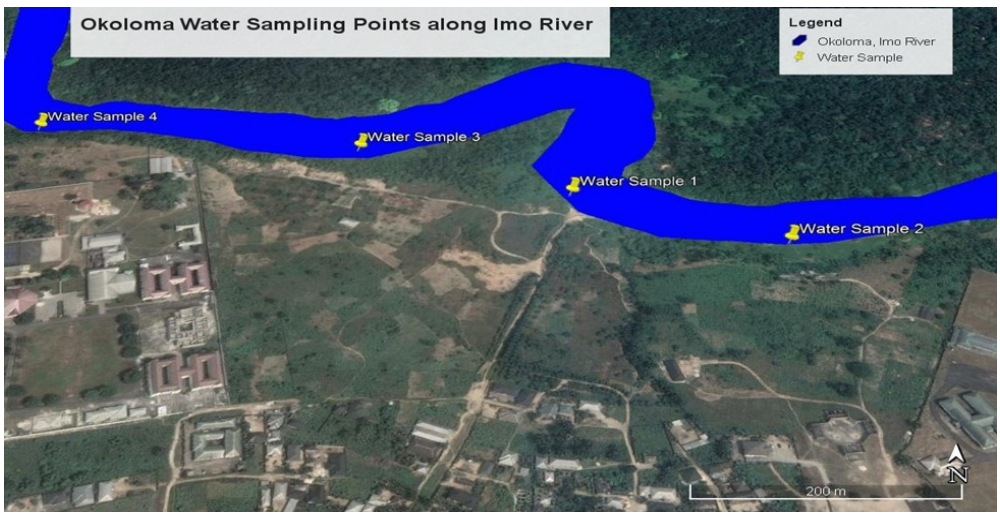

Figure 1. *High resolution satellite image showing water and its sediment sample collection point in Imo River Afam, Oyigbo L.G.A, Rivers State, Nigeria (Source: DigitalGlobe, 2022).*

Sample collection and transportation

Three samples each of the fresh water and its sediments were collected randomly from 4 designated points at the two sampling sites in September, 2021. The sediments samples were collected with a 70 % ethanol- sanitized trower at 5 cm depth into 70 % ethanol-sanitized clean, dry polythene bags. The water samples were collected at the air water interface by hand dipping the 70 % ethanol sanitized clean, dry cylindrical shaped 750 mL plastic container. The containers with lids were slightly opened and rinsed with the samples thrice before aseptically collecting the samples. The samples were mixed together to obtain composite samples of water and sediment. All the composite or representative samples containers were labeled with sample type, date, time and place of collection. They were placed into a sterile polythene bag in ice packed with cooler to keep them under a temperature not more than 4 °C and then transported to Microbiology Postgraduate Laboratory, Chukwuemeka Odumegwu Ojukwu University Uli Campus, Nigeria for further analysis (Uba, 2018).

Isolation of hydrocarbon degrading bacteria

The hydrocarbon degrading bacteria were isolated from sediments and water samples of the two sampling sites using spread plate technique on modified mineral salt agar (MMSA). After series of sample dilution and inoculation on sterile solidified MMSA plates, the colonies that grew on MMSA plates were sub-cultured into new MSM plates and incubated for another 14 days. After incubations, 12 morphological distinct colonies that grew on these plates were selected and subjected for biosurfactant screening (Uba, 2018).

Screening of biosurfactant producing strain

A loopful of each of the 12 bacterial isolates was inoculated into each flask containing 100 mL of the MMSM and 1 mL of waste engine oil. The broths were then incubated at laboratory temperature for 4 days. At the end of the incubation period, the media were centrifuged at 4,000 rpm for 20 min for the removal of cells. The cell free broths (supernatants) were later tested for the production and activity of biosurfactant using screening methods (Uba *et al*., 2018; Ali *et al*., 2019).

Screening for selection of most potent biosurfactant producing bacterial strain oil displacement test

In this test, 30 mL of distilled water was added to the Petri plate followed by addition of 2 mL of crude oil to the surface of water. Then 0.5 mL of cell free culture broth was dropped on the crude oil surface (Ali *et al*., 2019).

Emulsification test

In this test, the E_{24} of culture samples were determined by adding 4 mL of waste engine oil and 4 mL of the cell-free broth in test tubes. The mixtures were vigorously shaken for 5 min and centrifuged at 4,000 rpm for 10 min. The percentage of emulsification index was calculated using the following equation (Ali *et al*., 2019):

$$E_{24} = \frac{\text{Height of emulsion formed}}{\text{Total height of the solution}} \text{ X } \frac{100}{1}$$

Surface tension measurement

The surface tension of the supernatant and the solution of extracted biosurfactant was determined using a capillary tube method at room temperature as described by Uba *et al*. (2018). After 5 days of incubation, the cell free culture broth was obtained using centrifugation technique. The supernatants were transferred to a glass tube and a capillary tube was dipped in this liquid. This procedure was done at 28 ± 2 °C. The height reached by the liquid through the capillary tube was measured in triplicates and surface tension calculated according to the following formula:

$$\gamma = ½ rh\delta g$$

Where: γ = Surface tension (mN /m); δ = Density (0.99 g /mL); g = Gravity (980 cm /s^2); r = Capillary radius (0.09 cm); h = Height of the liquid column (cm).

Identification of the selected biosurfactant producing bacterial strain

The isolated colony was identified on the basis of morphology by colonial characteristics by inoculating bacteria on Nutrient Agar to note the shape, elevation, margin, optic, surface, colour and size (Pakpour and Horgan, 2023). Gram staining involves staining the bacterial cell to indicate if the bacteria is Gram positive or negative. Also, biochemical by testing catalase, indole, methyl red, Voges Proskauer (MR-VP), starch hydrolysis, citrate, oxidase, sugar fermentation, urease, nitrate reduction, motility and hydrogen sulphide production test (Pakpour and Horgan, 2023). The isolate was identified using key of identification as contained in Bergey's Manual of Systematic of Archaea and Bacteria by Whitman (2015).

Biosurfactant production and extraction

The (MMSM) containing 1 mL of waste engine oil, and supplemented with optimized parameters of 20 g of slaughter house waste, 10 g of rice bran, 1 % of sodium chloride with pH of 9.0 was sterilized at 121 ° C and 15 psi for 15 min. The sterilized medium was then inoculated with 10 % of the test bacterial culture and incubated at 4 ° C for 5 days. After incubation, the fermentation broth sample was centrifuged at 4,000 rpm for 20 min.

Biosurfactant stability determination

The effect of pH, temperature and salinity on biosurfactant stability was studied to determine the stability of biosurfactant with changing conditions. The stability studies were carried out using the crude surfactant (cell-free broth). The pH of cell-free broths was adjusted to 3, 5, 7, 9, and 11 pH ranges using 5 M HCl or 5 M NaOH solutions and then incubated at 4°C for 5 days. The salt concentration of the cell free broth was adjusted to 2, 5, 8, 11, and 14 g salinity ranges using NaCl. The effects of

different temperature ranges (4, 28, 45, and 75 °C) on stability of the biosurfactant was also determined. After incubation, Oil displacement, emulsification indices of these cell-free broths were determined (Silva *et al*., 2018).

Critical Micelle Concentration (CMC) Determination

The CMC was determined using different concentrations of the crude biosurfactant (0.05, 0.1, 0.5, 1.0, 1.5, 2.0, 2.5, 5, 7, 10, 15 and 20 mg/ mL) and the surface tension of each concentration was determined using a glass capillary technique as previously described at room temperature after which the CMC of the biosurfactant was then determined (Uba *et al*., 2018).

Recovery technique of biosurfactant

The extraction techniques involve the use of acid precipitation and solvent extraction methods. The crude biosurfactant was treated by acidification to pH 2.0 using concentrated HCl and the acidified supernatant was left for 24 hr. at 4 °C (Odalys *et al*., 2017). After 24 hr, the acidified supernatant was centrifuged for 20 min and grey white precipitate was collected. Chloroform and methanol in the ratio (2:1 v/v) were added to precipitate the pellet. The precipitated biosurfactant was air - dried and the mass noted (Odalys *et al*., 2017).

Elucidation and structural characterization of the biosurfactant

Gas chromatographic (GC) analysis of fatty acid profile: In this analysis, 1 mL of crude biosurfactant was dissolved in 50 mL of chloroform to dryness after which 20 % volume benzene and 55 % volume methanol were added, sealed and heated at 40 °C in a water bath for 30 min. The organic sample was extracted with hexane and water, The mixture was vigorously shaken manually for 2 min and later centrifuged to break stable emulsion. The top hexane phase was transferred to a test tube for injection into GC column: SP™-2560, 100 m × 0.25 mm I.D., 0.20 µm; oven: 60 °C (1 min), 15 °C/min to 165 °C (1 min), 2°C. After analysis of the sample, the trans fatty acids were identified (AOAC, 1990).

Fourier transform, infrared spectroscopic analysis: In this study, an approximately 1.0 g of sample and 0.5 mL of Nujol were mixed properly and placed on a salt pellet. The FTIR spectra was obtained at frequency regions of 4,000 – 600 cm^{-1} and co-added at 32 scans and 4 cm^{-1} resolution during measurement. The FTIR spectra were displayed as transmitter values (AOAC, 1990).

Applicability of the produced biosurfactant

Biosurfactant in heavy metal remediation: Biosurfactant at different concentrations (1/2 × CMC, 1 × CMC, 2 × CMC) were used to remediate 100 ppm of six heavy metals in their salt form (mercury, copper, zinc, lead, cadmium and cobalt) as well as chemical surfactant (Tween 80 (10 %)). The medium without biosurfactant and organisms served as control. They were properly mixed, incubated at 28 °C for 120 hr after which the solutions were drawn and centrifuged at 4,000 rpm for 20 min in order to separate the metal biosurfactant complex precipitate formed and the supernatant containing unbound metal ions. The centration of the unbounded metal ions was then analyzed using atomic absorption spectrophotometer. The metal removal efficiency was therefore calculated as (Hisham *et al*., 2019):

$$\text{Metal removal (\%)} = \frac{\text{Concentration of heavy metals in the supernatant}}{\text{Total concentration of heavy metals}} \text{ X } 100\ \%$$

Data management

All the experimental data obtained were expressed in mean ± standard deviation and presented in Tables and Figures. GraphPad Prism version 8.0.2 was employed in the one factor analysis of variance (ANOVA) followed by Dunnett multiple comparison test was used to compare the means of treatment with respect to their controls. The level of probability less than ($P < 0.05$) were considered statistically significant at 95 % confidence intervals.

RESULTS AND DISCUSSION

The result of the distribution of the hydrocarbon degrading bacteria in the Imo River sampling site is presented in Table 1. From the result obtained, the highest total bacterial count was 2.49 x 10^6 ± 0.12 CFU/mL obtained from Imo River water sample while the lowest bacteria count was 1.45 x 10^6 ± 0.01 CFU/g obtained from the Imo River sediment sample. The reason for this could be due to the high nutrient concentration on the surface of the river than the sediment which the favoured the growth of these microbes. The result is similar to the finding of Uba (2018) who reported that Nembe water had the highest significant (P =.05) mean TNHUB count of log 18.95 ± 0.04 CFU /mL with highest percentage of occurrence (32.40 ± 0.16 %).

The result of the emulsification, displacement and surface tension profile of the biosurfactant producing bacterial strain is presented in Table 2. From the result, out of the 12 biosurfactant bacterial strains, bacterial strain S62A demonstrated the highest ability of oil displacement activity at 23.76 ± 0.10 cm, emulsification activity at 66.66 ± 0.10 % and lowest surface tension activity at 90.10 ± 0.01 mN/m. A higher dispersive, emulsification and lower surface tension properties observed with spent engine oil in this study, suggest the possible application of this biosurfactant in enhanced oil recovery and environmental biotechnology. Previous studies by Ali *et al*. (2019) reported that the biosurfactant produced from *Bacillus* species had an emulsification index for hydrocarbons such as kerosene, hexadecane, tridecane, tetradecane, diesel, crude oil, pristane and heptane in the range of 50 % – 64 %. The observation was almost similar to the results obtained in this study.

Table 1. Distribution of hydrocarbon degrading bacteria in the Imo River sampling site.

Sample code	CFU/mL or CFU/g (10^6)	Log CFU/mL or CFU/g
CPW	2.29 ± 0.12	6.35 ± 0.00
CPSE	1.45 ± 0.01	6.16 ± 0.20

N.B: CPW = Crude oil polluted water, CPSE = Crude oil polluted sediments, CFU = Colony forming unit, Log = Logarithm, mL milliliter, g = Gram

Table 2. Emulsification, displacement and surface tension profile of the biosurfactant producing bacterial strain.

Strain code	Emulsification index (E_{24} (%)	Dispersive index (cm)	Surface tension (mN/m)
S21A	55.55 ± 1.00	13.85 ± 1.00	170.00 ± 0.05
S21B	51.40 ± 1.52	5.73 ± 0.07	150.30 ± 1.20
S22A	52.63 ± 1.00	9.63 ± 0.00	160.80 ± 0.00
S22B	47.36 ± 0.10	0.38 ± 0.10	110.20 ± 0.10
S51A	42.50 ± 0.10	0.01 ± 0.00	110.20 ± 0.20
S51B	45.94 ± 0.00	0.12 ± 0.01	110.00 ± 0.00
S52A	50.00 ± 1.00	3.14 ± 1.00	120.90 ± 0.20
S52B	48.64 ± 1.10	0.78 ± 1.35	240.70 ± 1.50
S61A	45.94 ± 0.00	0.12 ± 0.02	110.20 ± 0.02
S61B	42.50 ± 1.10	0.78 ± 0.10	210.00 ± 1.50
S62A	66.66 ± 0.01	23.76 ± 0.10	90.10 ± 0.01
S62B	45.58 ± 0.21	0.12 ± 0.01	110.00 ± 0.05

Table 3. Colonial, microscopic and biochemical features of the most potent biosurfactant bacterial strain S62A.

Parameter	Observation
Shape	Circular
Margin	Entire
Elevation	Flat
Optic	Translucent
Surface	Smooth
Colour	Creamy
Gram stain	-
Cell shape	Rod shaped
Cell arrangement	Single and in pair
Catalase	+
Indole	+
Methyl red	+
Voges Proskauer	+
Starch hydrolysis	+
Citrate	+
Oxidase	+
Arabinose	+
Fructose	+
Xylose	+
Mannitol	-
Galactose	+
Maltose	+
Lactose	+
Sucrose	+
Glucose	+
Nitrate reduction	+
Motility	+
Hydrogen sulphide production	+
Tentative identity	*Aeromonas hydrophilia*

N.B: + = Positive reaction, - = Negative reaction

The results of the colonial, microscopic and biochemical features of the most potent biosurfactant producing bacterial strain S62A are presented in Table 3. The result revealed that bacterial strain S62A formed round in shape, entire in margin, flat in elevation, translucent in optics, moist and smooth surface texture and creamy in colour on the screening plates. Also, bacterial strain S62A is a Gram-positive rod-shaped bacterium capable of utilizing glucose, lactose, fructose, arabinose, xylose, galactose and maltose. It was positive for oxidase, nitrate, indole, methyl red, Voges Proskauer, motility, starch hydrolysis, catalase, citrate activity but negative for mannitol and urease tests. Bergey's Manual of Determinative Bacteriology was used for the identification of the isolated bacterial strain S62A and on the basis of this manual, the strain S62A was tentatively identified as a member of genus *Aeromonas hydrophilia.* Several Gram-negative rod -shaped bacterial genera have been implicated, identified and documented as excellent biosurfactant producers (Nalini, and Parthasarathi, 2018; Uba *et al*., 2018; Araújo *et al*., 2019; Gomaa and El – Meihy, 2019; Ntshingila *et al*., 2022) and similar observation was made in this study.

The application and suitability of biosurfactants in various industrial areas are depended on their stability against variable or extreme environmental settings of temperature, pH and salinity (Araújo *et al*., 2019). In this study, the result of the stability profile of the produced biosurfactant under pH, salinity and temperature conditions is presented in Table 4. From the results, pH 9, 2 % salinity and 4 °C temperature conditions had the highest dispersive index values of 33.19, 3.14 and 12.57 cm and highest emulsifying indices values of 74.82, 51.20 and 70 % while pH 3, 14 % salinity and 75 °C temperature conditions had the lowest dispersive indices values of 0.19, 0.19 and 1.77 cm as well as lowest emulsifying indices values of 33.46, 36.67 and 41.49 %, respectively. These findings suggest the usefulness of this glycophospholipid biosurfactant for environmental or industrial purposes in extreme conditions.

Table 4. Stability profile of the produced biosurfactant under pH, salinity and temperature.

Condition	Dispersive index (cm)	Emulsification index (E_{24})
pH		
3	0.19	38.46
5	7.07	53.38
7	15.90	61.11
9	33.19	74.82
11	19.63	68.42
Salinity (w/v)		
2	3.142	51.20
5	1.77	48.10
8	0.64	47.70
11	0.38	40.44
14	0.19	36.67
Temperature (°C)		
4	12.57	70.00
28	4.91	54.34
45	3.14	44.44
75	1.77	41.49

Table 5. Functional components of biosurfactant by gas chromatographic spectrum.

Biosurfactant source	Chemical formula	Functional component	Relative abundance %	Formula
Aeromonas hydrophilia	C20:2	Eicosadienoic	2.69	$C_{20}H_{36}O_2$
	C22:6	Docosahexaecnoic or DHA	4.76	$C_{22}H_{32}O_2$
	C16	Palmitic acid	21.87	$C_{16}H_{32}O_2$
	C20:3	Dihomo-γ–linolenic acid	4.54	$C_{20}H_{34}O_2$
	C14	Myristic acid	4.82	$C_{14}H_{28}O_2$
	C18:2	Linolenic acid	8.19	$C_{18}H_{32}O_2$
	C18:3	3- α linolenic acid	25.77	$C_{18}H_{30}O_2$
	C18:1	Oleic acid	6.91	$C_{18}H_3O_2$
	C20:4	Arachidonic acid	9.6	$C_{20}H_{32}O_2$
	C18	Stearic acid	10.85	$C_{18}H_{36}O_2$

N.B: % = Percentage

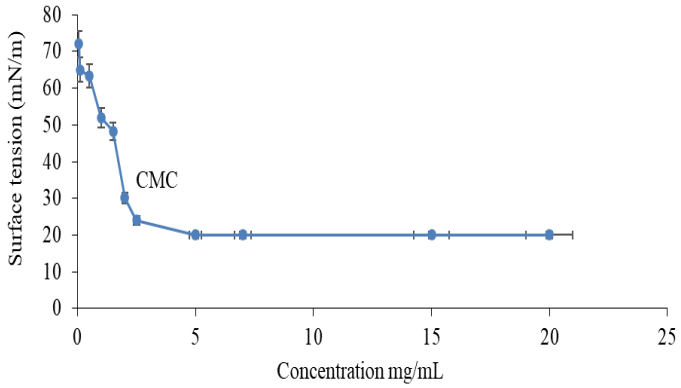

Figure 2. *Critical micelle concentration of the produced biosurfactant.*

This result is in contradiction with the study carried out by Kiran *et al.* (2017) and Ravindran *et al.* (2020) who stated that MSI 54 biosurfactant and *Bacillus subtilis* biosurfactant, respectively were not affected at higher temperature, pH and salinity suggesting its applicability in the environment such as heavy metal remediation and recovery from the complex using ultrafiltration. Araújo *et al.* (2019) reported that the biosurfactant produced from *S. marcescens* strains have demonstrated stability in a wide range of pH, temperature and salinity.

The ability to reduce surface tension depend on the specific concentration of surface-active compound, i.e., the CMC which is defined as the minimum concentration of biosurfactant required to give maximum surface tension reduction of water and initiate micelle formation. The result of the critical micelles concentration (CMC) of the produced biosurfactant is shown in Figure 2. From the figure, the 5 mg/mL concentration was found to be the CMC of the produced biosurfactant which decreases surface tension from 72 to 20 mN/m. The low CMC value of the biosurfactant observed in this study could be due to its excellent formation and aggregation ability (Guo *et al.*, 2022). This result is lower than the published report of Uba *et al.* (2018) which stated that *Bacillus cereus* PYR9 had the highest reduction of 22.10 ± 8.9 mN /m in surface tension followed by *Serratia marcescens* XYL7 with 23.00 ± 8.50 mN /m and *Alcaligenes faecalis* PYR5 had the least reduction of 26.60 ± 8.90 mN /m. In another study by Ravindran *et al.* (2020), they reported that the CMC from *Bacillus subtilis* was found to be 10 mg/mL which reduced the surface tension from 69.0 to 30 mN/m and that the decrease in surface tension could be as a result of sensitize biosurfactant from the optimized parameters.

The results of the gas chromatogram of fatty acid profile of the purified biosurfactant by *A. hydrophilia* strain S62A as well as the functional components of biosurfactant by gas chromatographic (GC) spectrum are shown in Figure 3 and Table 5.

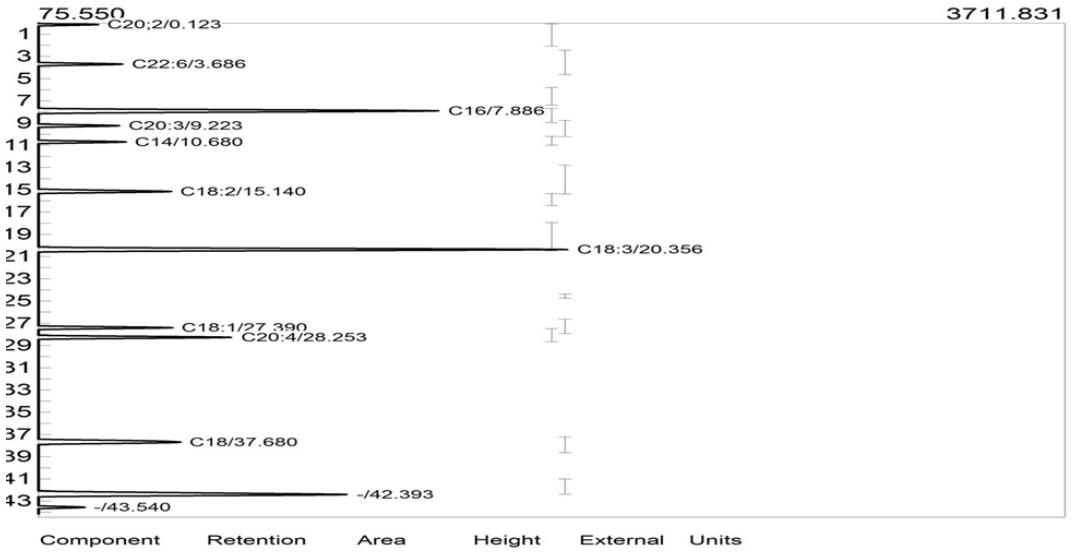

Figure 3. *Gas chromatography of the fatty acid profile of the produced biosurfactant by Aeromonas hydrophilia strain S62A.*

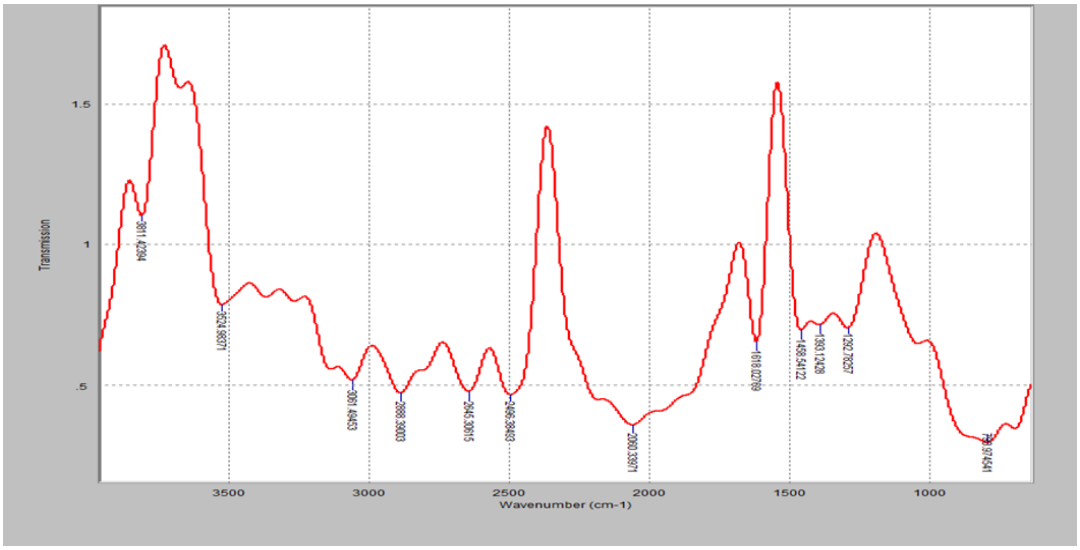

Figure 4. *FTIR spectral profile of the purified biosurfactant produced by Aeromonas hydrophilia strain S62A.*

From the results, C18:3 (Alpha linolenic acid) with retention time of 20.356 s had the highest fatty acid content of 25.78 % while C20:2 (eicosadienioc acid) with retention time of 27.390 s had lowest fatty acid content of 0.27 %, respectively. The results revealed that *A. hydrophilia* strain S62A biosurfactant had ten fatty acidscomponents and out of the components, C18:3 (3- α linolenic acid) had the highest composition of 25.77 % while C20: 2 (eicosadienoic acid) had the lowest composition of 2.69 %. There were more stearic and octadecanoic acids in the GC profile of the biosurfactant than other fatty acids components thereby confirming the assertion by Andrade *et al.* (2018) that stearic acid or octadecanoic acid is the main fatty acid chain found in various glycophospholipid biosurfactants. Similar observation also made for this biosurfactant by Regina *et al.* (2020).

Fourier transform infrared (FT-IR) is a powerful tool that has been widely used to study the diverse forms of biomolecules especially biosurfactants (Araújo *et al.*, 2019). Fourier transform –infrared spectroscopy (FT-IR) was used to analyze the functional groups and composition of the purified biosurfactant. The result of the FTIR spectral profile of the purified biosurfactant produced by *A. hydrophilia* S62A is shown in Figure 4. From the result, the spectrum showed a broad absorbance peak centered around 2060 cm^{-1}, which is a typical feature of the stretching mode, indicating alkyne (C≡C) bonds. The absorption peaks around 2888 and 1458 – 1292 cm^{-1} correspond to the presence of bonds occurring in aromatic chains with asymmetric vibrations of amide bond II. Peaks at 1618 cm^{-1} are expected to feature of the stretching of CO-N, N–H, and N=O bonds are assigned to peptide. The absorption peak at 3524 corresponds to OH stretching present in alcohol and phenol. The peak at 794 indicates an amide group of C-H stretching. Based on the data of the spectrum obtained, the bonds confirm the cyclic glycophospholipid nature of the biosurfactant as phosphatidylethanolamine. This study is in contrast to the result obtained by Hangcheng *et al.* (2015) who studied on *Bacillus* sp. ZG0427 and classified its biosurfactant as a lipopeptide. Guo *et al.* (2022) reported a glycolipid type biosurfactant structure produced by marine bacteria *Planococcus* sp. XW -1 isolated from the yellow sea of China.

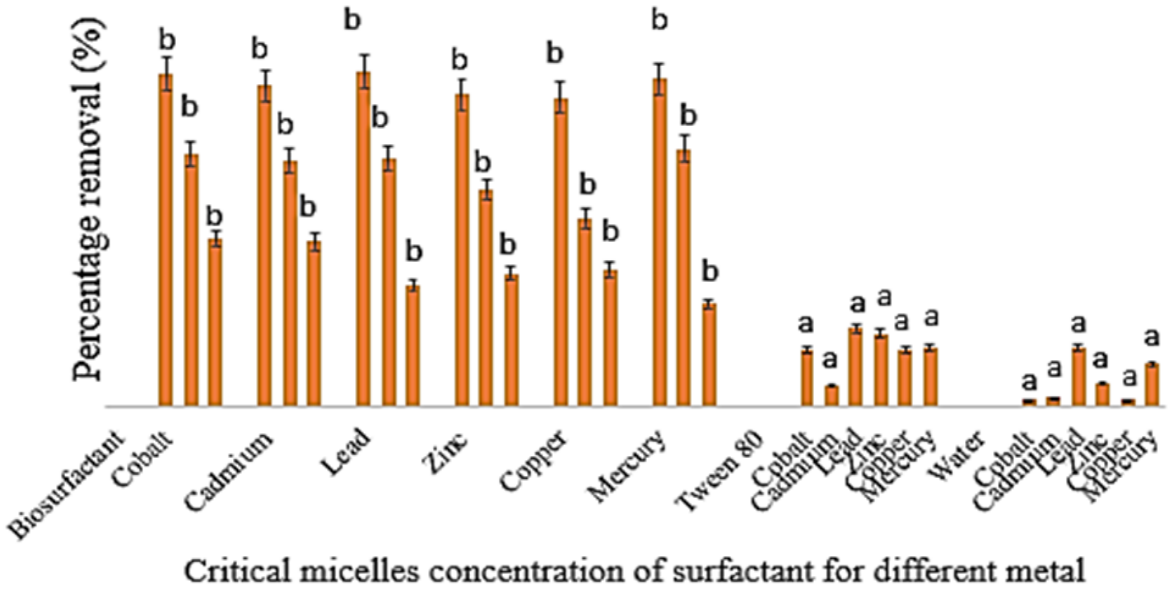

Figure 5. *Heavy metal removal efficiency at different critical micelles concentration of the glycophospholipid surfactant produced by A. hydrophilia strain S62A, Tween 80 and Control (water); N.B: CMC = Critical micelles concentration of the biosurfactant (2×CMC, 1×CMC, ½×CMC; % = Percentage; Error bar = Standard deviation; column with similar letters is non - statistically significant; columns with different letters are statistically significant at P < 0.05.*

The application of the biosurfactant produced by *A. hydrophilia* strain S62A in this study was also explored in the bioremediation of heavy metal solutions. The result of the heavy metal removal efficiency of the glycophospholipid produced by *A. hydrophilia* strain S62A on different metal salt solution is shown in Figure 5. From the result, it was revealed that 2 x CMC of lead had the highest metal removal efficiency of 98.92 % while the control (water) with cobalt had the lowest metal removal efficiency of 2.09 %, respectively. The result in Figure 5 also revealed that the removal efficiencies achieved was almost similar for the six metals solutions (mercury, copper, zinc, lead, cadmium and cobalt), indicating similar affinities to form complexes with the produced glycophospholilpid biosurfactant although lead had the highest removal efficiency of 98.92 % by 2 x CMC biosurfactant. The other possible reasons for metal removal efficiency could be due to solubilization – dissolution and ion exchange mechanisms (Ines *et al*., 2022). Statistically, there were significant differences ($P < 0.05$) detected among the means of all surfactant concentration treatment in comparison to their controls using two-way ANOVA followed by Dunnett comparison test. Gomaa and El - Meihy (2019) reported that biosurfactant produced by *Citrobacter freundii* MG812314.1 was found to be effective in heavy metal removal from wastewater viz 80, 67, 66, 55, 45, 44 and 41 % for aluminum, lead, zinc, cadmium, iron, copper and manganese respectively under two inoculum potentials and two contact times. Ravindran *et al.* (2020) who reported the removal efficiencies of 75.50, 97.73, 89.50 and 99.93 % for mercury, lead, manganese and cadmium, respectively at 2 x CMC (10 mg/L ppm) of lipopeptide biosurfactant. The above studies are similar to the results obtained in this study in which ½ CMC, 1 x CMC and 2 x CMC were used to remediate six metal and the biosurfactant produced by *Aeromonas hydrophilia* strain S62A remediated 30 - 40 %, 60 - 80 % and 90 – 99 % of the metals, respectively.

Conclusion

This study has shown that the oil contaminated water and sediment samples collected from Imo River Afam, Oyibo LGA, Rivers State is a reservoir of excellent biosurfactant producers with *A. hydrophilia* strain S62A having the highest dispersion activity at 23.76 ± 0.10 cm, emulsification activity at 66.66 ± 0.10 % and lowest surface tension activity at 90.10 ± 0.01 mN/m. The biosurfactant produced by *A. hydrophilia* strain S62A was found to be glycophospholipid based on the confirmation from GC and FTIR profiles. The biomolecule had demonstrated stability in a wide range of pH, temperature and salinity with 5 mg/mL CMC. The biosurfactant produced was also found to demonstrate efficient removal capacity (75.50, 97.73, 89.50 and 99.93 %) at 2 X CMC against each heavy metal contaminated water solution (mercury, lead, manganese and cadmium) which suggest its potential in the remediation of heavy metal polluted media especially under extreme environmental conditions.

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