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# Study on Optimization, Degreasing and Destaining Potentials of Glycophospholipid Biosurfactant Produced by *Bacillus Anthracis* S62A

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## Abstract

*Oil-polluted soil is one of the major sources of biosurfactant producing bacteria. This study was to study the optimization, degreasing and de-staining potentials of glycophospholipid biosurfactant produced by B. anthracis strain S62A isolated from spent engine oil-polluted soil. Five (5) bacterial isolates associated with spent engine oil – polluted soil was primarily screened for biosurfactant production using emulsification index test, oil displacement test, and surface tension test. The bacterial isolate with the highest screening test results was selected, characterized and examined for its biosurfactant production optimization, degreasing and de-staining potential on different oil-stained fabric materials. The results showed that the parameters required for the maximum biosurfactant yield were 20 g of slaughter house waste, 10 g of rice bran, 1% of sodium chloride, 9.0 pH, 4 °C temperature, 10% of the test bacterial inoculum and 5 days incubation period. The biochemical profile revealed that the extracted biosurfactant was identified as glycophospholipid. The maximum cleaning and degreasing efficiency of the biosurfactant obtained from B. anthracis strain S62A was observed to possess 78, 72 and 64 % oil removal efficiency from stained polyester, cotton and chiffon fabric materials, respectively at 10 mg/mL critical micelles concentration (CMC). Statistically, significant differences ($P < 0.05$) were detected among the means of all surfactant concentration treatments in comparison to their controls using two-way ANOVA followed by Dunnett comparison test. Therefore, this study suggests that the glycophospholipid biosurfactant from B. anthracis strain S62A could be exploited in laundry industries for the formulation of detergents as well as in textile dye biotreatment.*

**Keywords**: Biotreatment, Glycophospholipid, Biosurfactant, Degreasing, Destaining

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## INTRODUCTION

Modern detergents are complex mixtures containing chemical surfactants, softeners, oxidizing agents, and various enzymes among other ingredients. They are contained in a wide range of industrial cleaning applications, including laundry detergents (Andrade *et al*., 2018).

Surfactants are a versatile group of chemicals with various applications as household detergents, personal care products, pharmaceutical agents, agricultural chemicals, oilfield chemicals, food processing agents, industrial additives, environmental remediation agents and so on (Varjani and Upasani, 2017). They 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). In 2014, the global surfactant market was USD 25.6 billion value, and has been expected to grow at a compound annual growth rate (CAGR) of 4.6% from 2015 to 2020 (Santos *et al*., 2016). The current dominant 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).

Chemically synthesized surfactants are exerting environmental problems as their residues end up in soil, water, or sediment potentially exerting toxic effects on various living organisms (Vijayakumar and Saravanan, 2015). In view of the limitations of the chemical surfactants in the past decades, efforts have been placed in the development of environmentally friendly, renewable and non/less-toxic alternatives such as biosurfactants, which are surfactant molecules produced by microorganisms. Such development efforts have resulted in a thriving global biosurfactant market which was estimated at USD 4.2 billion in 2017 and was projected to reach USD 5.52 billion by 2022, at a CAGR of 5.6 % (Markets and Markets, 2017).

Previous study by Ghribi and Chaabouni (2011), the SPB1 biosurfactant preserves its activity at extreme conditions of pH and temperature which is an extremely interesting feature in view of its potential use in detergent industries. Moreover, Mnif *et al*. (2013) reported that the SPB1 lipopeptide was used to enhance diesel solubility and mobility and to improve textile dye biotreatment. There are limited literatures on the application of biosurfactants on enhanced fabric degreasing and cleaning. The available information in global biosurfactant database especially mostly focuses on the application of biosurfactant to oil and heavy metal recovery and remediation, very less on fabrics degreasing and de-staining; therefore, justifying the need for this study. Thus, the study was carried out to study the optimization, degreasing and de-staining potential of glycophospholipid biosurfactant produced by *Bacillus anthracis* S62A.

## MATERIALS AND METHODS

### Sampling Site

The spent engine oil-polluted soil was sampled from the mechanic workshops located at Uli, Ihiala Local Government Area, Anambra State. The basic anthropological activity in the site was repair and maintenance of damaged vehicles leading to indiscriminate release of waste oils to the ground surface. The site was referenced using Global Positioning System (GPS) App with the coordinates obtained from the sampling points. The coordinates were later used to download the Geoeye Statelite Images from the online archive of digital globe. 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 sampling site as shown in Figure 1:

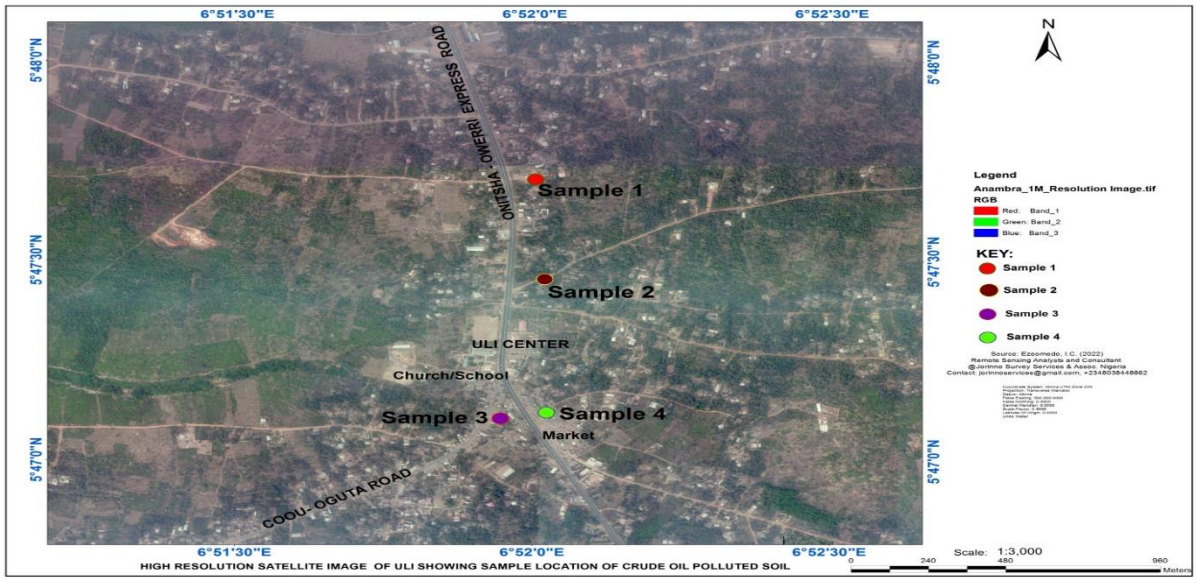


Figure 1: High resolution satellite image showing the location of spent engine oil-polluted soil.
Source: Ezeomedo (2022)

**Collection of Sample**

The spent engine oil-polluted soil samples were collected randomly from four (4) designated shown on Figure 1 at the sampling site in September, 2021. The soil samples were collected using sterile trowel at 5 cm depth into sterile and dry polythene bags. The samples were mixed together to obtain a composite sample and labelled appropriately. They were placed into an ice packed cooler and then transported to Microbiology Postgraduate Laboratory, Chukwuemeka Odumegwu Ojukwu University Uli Campus, Nigeria for subsequent analysis (Jaysree *et al.*, 2011; Uba *et al.*, 2018).

**Isolation of Bacteria Associated with Spent Engine Oil-Polluted Soil**

By adopting the method of Uba *et al.* (2018), modified mineral salt agar (MMSA) was used in isolating bacteria associated with spent engine oil-polluted soil from the composite soil sample collected from the sampling site using spread plate technique after fourteen (14) days of incubation. After incubations, five (5) morphologically distinct colonies that grew on the MMSA plates were selected, purified and subjected to primary biosurfactant screening.

**Screening for the Most Potent Biosurfactant Producing Bacterial Strain**

By adopting the methods of Diab and Shereen (2013) and Sharma *et al.* (2014), five bacterial isolates were inoculated into individual flasks containing 1 mL of spent engine oil and 100 mL MMSM. The flask containing the mixture was subsequently incubated at room temperature for 5 days. After incubation, the media were centrifuged for 20 min at 4,000 rpm. The cell biomasses were discarded while cell supernatants were used for primary biosurfactant screening using oil displacement, emulsification and surface tension tests, respectively (Rabah and Bello, 2015; Sidkey *et al.*, 2014; Uba *et al.*, 2018).

**Identification of the Selected Biosurfactant Producing Bacterial Strain**

The selected bacterial isolate was identified microscopically using Gram staining technique (Cheesebrough, 2006) and molecularly to species level for *16S rRNA* gene identification using Genomic DNA extraction, polymerase chain reaction (PCR), agarose gel electrophoresis and sequencing and blasting techniques (Uba, 2018).

**Optimization of the Best Growth Conditions for the Production of Biosurfactant**

The study on the best growth conditions for biosurfactant production was carried out by adopting the method of Noparat *et al.* (2014). The different cultural conditions are: incubation time (24, 48, 72, 96, 120 and 144 hr); temperature (4, 20, 35 and 45 °C); pH (2, 5, 6, 7, 9, 11 and 13); inoculum volume (1, 2.0, 5.0 and 10% (v/v)); carbon sources: rice bran (5, 10, 15%); cassava peels (5, 10, 15%); and yam peels (5, 10, 15%); nitrogen source: corn steep liquor (5, 10, 15%); plantain peels (5, 10, 15%) and slaughter house liquid waste (5, 10, 15%) and salinity (1, 5, 10, 20, 30%). The effect of these growth conditions described above on the production of biosurfactant was determined using oil displacement method.

**Biosurfactant Production and Extraction**

The MMSM containing 1 mL of spent 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 for 20 min at 4,000 rpm.

**Biochemical Characterization of the Crude Biosurfactant**

In this study, phenol sulphuric, Biuret, phosphate and Cetyl-trimethyl-ammonium-bromide (CTAB) methylene blue tests were adopted in the biochemical characterization of the crude biosurfactant as previously described by Pradhan *et al.* (2014); Jamal *et al.* (2012); Kalyani *et al.* (2014).

**Determination of Critical Micelle Concentration**

**T**he critical micelle concentration (CMC) was determined at different concentrations of the crude biosurfactant (0.05 - 20 mg/mL) using surface tension capillary tube as previously described by Uba *et al.* (2018).

**Fabrics Degreasing and De-staining Performance Analysis**

The modified method of Andrade *et al.* (2018) and Bouassida *et al.* (2018) were employed in determining the oil removal (degreasing) efficiency in fabric materials. Three different materials (chiffon, cotton and polyester) were cut into five (5) pieces each with dimensions 4 x 4 cm using scissors. Thereafter, 1 mL of spent engine oil was impregnated into each piece of materials soaked in a beaker containing different concentrations of crude biosurfactant (½ CMC, 1 x CMC and 2 x CMC), commercial surfactant (Viva detergent) and chemical surfactant (Tween 80) incubated in a rotary shaker at 70 rpm for 12 hr. The percentages of the oil removed were determined using the formula indicated by Grbavcic *et al*. (2015):

$$w = \frac{m_{total} - m_i}{m_{total}} \times 100 \qquad \text{Equation 1}$$

$w$ = Percentage of the oil removed; $m_{total}$ = total mass of impregnated oil, $m_i$ = the mass of residual oil on the materials after treatment.

**Data Analysis**

All the experimental data obtained were expressed in mean ± standard deviation and presented in Tables and Figures. One-way analysis of variance (ANOVA) followed by Dunnett multiple comparison test was used to compare the means of treatments with controls. The $P < 0.05$ was considered statistically significant at 95% confidence intervals using GraphPad Prism version 8.0.2.

## RESULTS AND DISCUSSION

The results of surfactant profile of the biosurfactant producing bacterial strain are presented in Table 1. Out of the five (5) biosurfactant producing 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. Ghayyomi *et al.* (2012) suggested that a single method is not suitable to identify biosurfactant producers, thereby prompting the authors to recommend the combination of methods. In addition, Chandran and Das (2011) used different screening methods, such as emulsification capacity, oil-displacement test, hydrocarbon overlaid agar, and modified drop collapse methods to detect biosurfactant production.

Table 1: Profile of the biosurfactant producing bacterial strains

| Strain code | Emulsification index [$E_{24}$ (%)] | Dispersive index (cm) | Surface tension (mN/m) |
|---|---|---|---|
| S22A | 52.63 ± 1.00 | 9.63 ± 0.00 | 160.80 ± 0.00 |
| S51A | 42.50 ± 0.10 | 0.01 ± 0.00 | 110.20 ± 0.20 |
| S52A | 50.00 ± 1.00 | 3.14 ± 1.00 | 120.90 ± 0.20 |
| S61A | 45.94 ± 0.00 | 0.12 ± 0.02 | 110.20 ± 0.02 |
| S62A | 66.66 ± 0.01 | 23.76 ± 0.10 | 90.10 ± 0.01 |

The results of the molecular features of the most potent biosurfactant producing bacterial strain S62A are presented in Table 2 while the *16S rRNA* gene partial sequence of the most potent biosurfactant producing bacterial strain S62A is shown in Figure 2. The result has shown that the percentage similarities of *16S rRNA* sequence of the closest relative for the selected bacteria strain is 75 % with the *Bacillus anthracis* lar 3. Several researchers and authors have implicated and reported *Bacillus* spp. for their biosurfactant production (Ali *et al*., 2019; Das *et al*., 2019; Hisham *et al*., 2019;).

Table 2: Molecular features of the most potent biosurfactant producing bacterial strain S62A

| Parameter | Observation |
|---|---|
| Closest relative | *Bacillus anthracis* lar 3 |
| Source | Oil-polluted soil |
| Sequence identity | KT 427462.1 |
| Gene | *16S rRNA* |
| Type of genome | Partial sequence |
| Maximum score | 196 |
| Total score | 838 |
| Query coverage | 473 |
| Identity similarity (%) | 75 |

TGTTTATGCGTTAACTTCGGCAGTAACGGCTGGAAAGCCCAACCTCCTAATCAACATCCGTTAACGGGG
GGACTACCCAGGGAATTAAATCCTGTTGCTTCCAACCTTTCGCAGTGAGCGGCCAGCTTTGGACAAGAA
AGCCGCCTTCCCCACTGGAGTCCCTCCAAATTTTTACGATTTTACCCGTTCCCCTGGAAATTCACCTTCCT
TTTTCTGAACTTAAGCCTCCCAGTTTCCATGGACCCTCCACGGTGGACCCGGGGATTTCACCTTCAAATTG
AAAAAACCACCTGCGGGGGTTTAACCCCCAAAAATTCCAGAAAACCTTGGCCCCCTACTATTAACCGGG
GTGGTGGGAACGAATTAACCCGTGCTTTTTTGGCTAGGAACCGCCAAGGAGCCAGTTAATAAACTTAAC
ACTTGCCTTTCCCTAAAAAGAGATTTATACCACCAAAGACTTTTCATCACTCACGGGATGGTTGTCTCAC
GGATTTTCCCCTTGTGCAAAATATCCCCTAGTGGTCTCTCCCATGAGACTGGGGCCCGTGTCTCATCCCCA
GTGGGTGGGAT

Figure 2: *16S rRNA* gene partial sequence of the most potent biosurfactant producing bacterial strain S62A

The results in Figure 3 reveal that the optimum biosurfactant dispersive activity was observed after 120 hours (5 days) of incubation time. Biosurfactant dispersive activity on 24 and 48 hours had same value. The value obtained for biosurfactant dispersive activity on 120 hours was not similar with those obtained with other incubation time. However, the optimum dispersive activity (23.76 cm) and emulsification activity (66.66%) was observed after 120 hour (5 days) of incubation. This result is similar to research carried out by Patil *et al.* (2014) who reported optimum growth and biosurfactant production after 96 hours of incubation with *Pseudomonas aeruginosa* F23 and Ghayyomi *et al.* (2012) who also reported that optimum production was observed on 120 hours (5 days) after incubation with *Klebsiella pneumoniae*. The results in Figure 4 show that *B. anthracis* strain S62A had the highest biosurfactant dispersive activity (3.14 cm) at 4 $^{o}$C temperature after 120 hours of incubation. At temperatures greater than 4 $^{o}$C, the isolate showed lower biosurfactant-producing ability. Different bacterial species produce biosurfactant at different temperatures ranges. However, most of them produce at the temperature range of 30 – 37 °C (Chander *et al.,* 2012) and any alteration in temperature could affect the chemical and structural constituents of biosurfactants. This result is in contrast with results reported by several authors (Patil *et al*., 2014; Ghayyomi *et al*., 2012). Patil *et al.* (2014) reported maximum biosurfactant production at the temperature of 30 °C for *Pseudomonas aeruginosa* F23 isolated from oil-contaminated soil sample. Ghayyomi *et al*. (2012) also reported similar result at temperature of 30 – 35 $^{o}$C for *K. pneumoniae* isolated from hydrocarbon-polluted soil in Ogoni land.

The results of pH optimization for biosurfactant dispersive activity by *B. anthracis* strain S62A presented in Figure 5 are similar with the results obtained by Hamzah *et al.* (2013). Hamzah *et al.* (2013) reported maximum biosurfactant production by *Pseudomonas aeruginosa* UKMP14T at pH 8 and maximum biomass at pH 9. The results in Figure 5 show that while highest biosurfactant dispersive activity was seen at pH 9, the bacterium also had dispersive activities at slightly acidic and neutral pH. This is in contrast to the findings published by Saharan *et al.* (2011) and Xia *et al.* (2012) who stated that their pH values for optimum production were seen at pH 5 and 6, respectively. Meanwhile, Kannahi and Sherley (2012) reported maximum biosurfactant production at pH below 7. The results in Figure 6 show that the highest dispersive index activity was obtained at 10% inoculum volume while the least dispersive index activity was observed at 1% inoculum volume. These results are similar to the results obtained by Atipan *et al*. (2014) indicating that highest biosurfactant production activity by *Leucobacter komagatae* strain 183 was observed at 10% inoculum while the least was observed at 2% inoculum. The results in Figure 7 reveal that *B. anthracis* strain S62A maximum dispersive activity of 50.27 cm was achieved with rice bran as the carbon source. Although the isolate was able to grow in the presence of other carbon sources (yam peel and cassava peel), rice bran had the highest value of biosurfactant activity. This result is in contrast with the work done by Okuda *et al.* (2014) who studied the use of cassava peel and cashew nut as carbon sources in which cassava peel had the highest dispersive activity.

Nitrogen is also required for microbial growth and production of certain primary and secondary metabolites (Saharan *et al*., 2012). The type of nitrogen existing in the production medium will affect the biosurfactant by microorganisms (Nurfarahin *et al*., 2018). The results in Figure 8 show maximum biosurfactant dispersive activity value of 15.90 cm when grown in a mineral salt medium amended with slaughter house waste. Similar observation was reported by Vishal *et al.* (2017) using *Pseudomonas aeruginosa*. The results in Figure 9 revealed that 1% salinity had optimum dispersive activity of 3.14 cm. These results are in contrast to the study carried out by Hangcheng *et al.* (2015) who reported that 12% NaCl (w/v) supported the maximum growth of *Bacillus subtilis* implying that the organism had good tolerance to the high salinity condition.

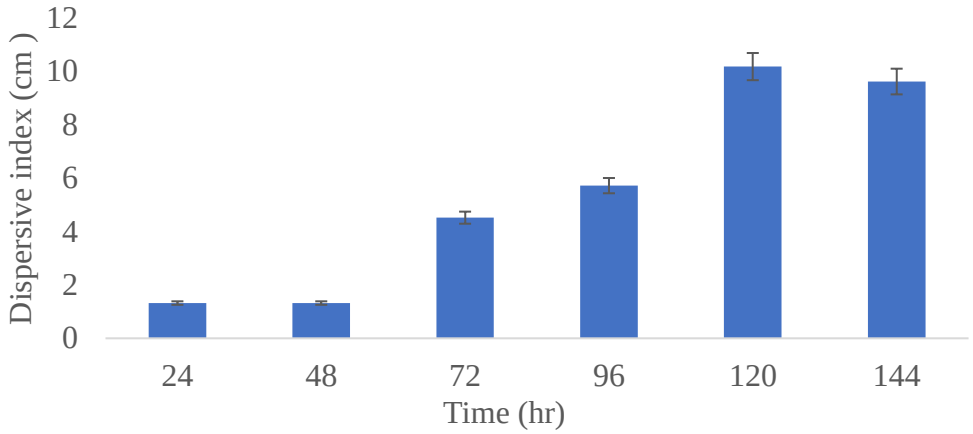


Figure 3: Effect of incubation time on the production of biosurfactant
N.B: Error bar = standard mean deviation, hr = time, cm = centimetre

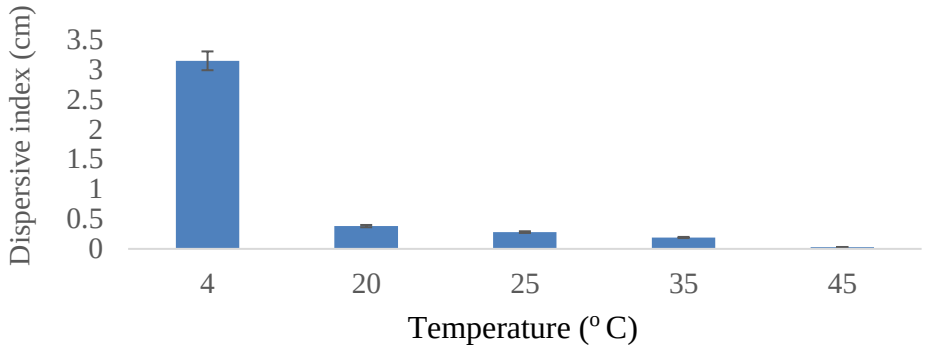


Figure 4: Effect of incubation temperature on the production of biosurfactant
N.B: Error bar = Mean standard deviation, °C = Degree centigrade, cm = Centimetre

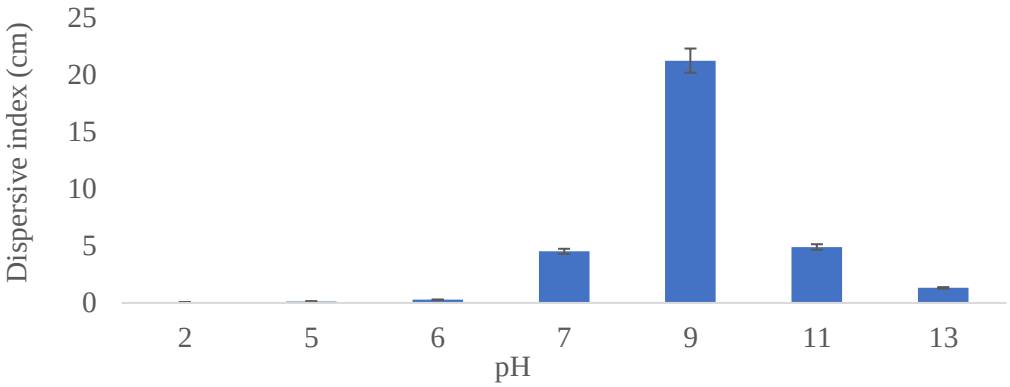


Figure 5: Effect of incubation pH on the production of biosurfactant
N.B: Error bar = Mean standard deviation, cm = Centimetre

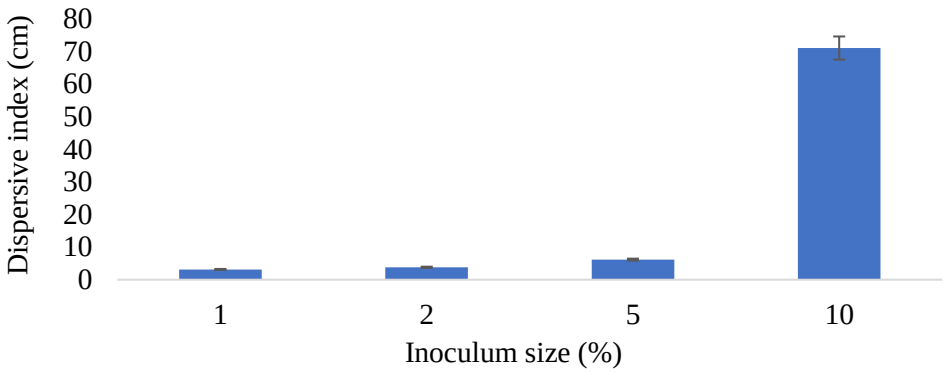


Figure 6: Effect of inoculum size on the production of biosurfactant

N.B: Error bar = Mean standard deviation, % = Percentage, cm = Centimetre

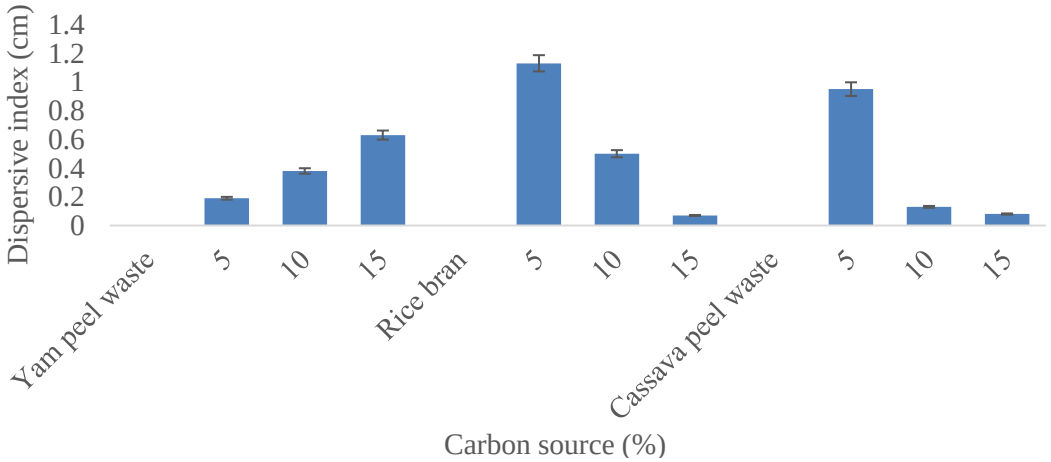


Figure 7: Effect of carbon source on the production of biosurfactant
N.B: Error bar = Mean standard deviation; % = Percentage; cm = centimetre

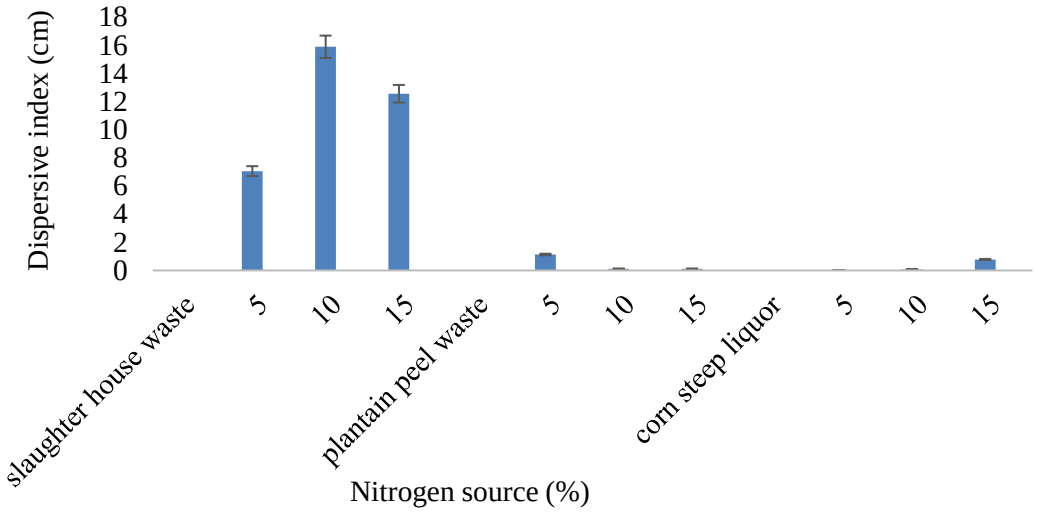


Figure 8: Effect of nitrogen source on the production of biosurfactant
N.B: Error bar = Mean standard deviation; % = Percentage; cm = Centimetre

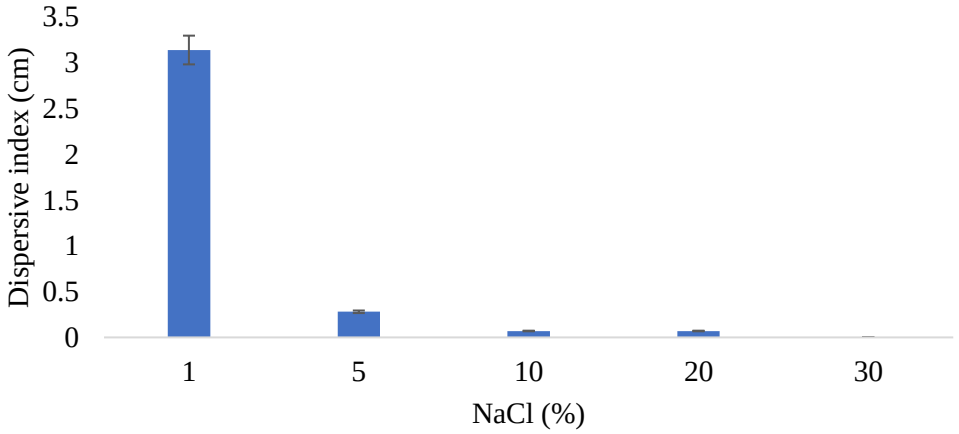


Figure 9: Effect of salinity on the production of biosurfactant
N.B: Error bar =Mean standard deviation; % = Percentage; cm = Centimetre

Preliminary performance of the biosurfactant carried out, excluded the presence of rhamnolipids and lipopeptide, with a positive result for glycophospholipid using phenol and phosphate tests. Phosphate test has been applied by Kalyani *et al.* (2014) in determining the presence of phospholipid biosurfactants. They reported that the formation of yellow colour, which may be followed by slow formation of a fine yellow precipitate indicated the presence of phospholipid biosurfactant. The results of the biochemical profile of the produced biosurfactant are presented in Table 3. It can be observed from the results that the produced biosurfactant was positive to phenol and phosphate tests but negative to Biuret and CTAB tests, respectively. The results in Table 3 further confirm that *B. anthracis* strain S62A is a producer of glycolipid and phospholipid. This study is similar to the study conducted by Nwaguma *et al.* (2016) using *Klebsiella pneumoniae* strain 1VN51 in which the produced biosurfactant was negative to Biuret and methylene blue agar test but positive to phosphate test. Their study confirmed the phospholipid nature of the biosurfactant but is in contrast to the study by Kalyani *et al.* (2014) on actinomycetes (PLS-1 and NDYS-4). The isolates were positive to phenol acid test and CTAB test but negative to phosphate and Biuret tests.

Table 3: Biochemical profile of the produced crude biosurfactant

| Test | Reaction |
|---|---|
| Phenol | + |
| Biuret | - |
| Phosphate | + |
| CTAB | - |

N.B: + = Positive reaction, - = Negative reaction, CTAB = N-Cetyl-N, N, N – trimethyl ammonium bromide

The CMC is an important physiochemical parameter used to evaluate biosurfactant activity, which indicates the minimum concentration of biosurfactant necessary to achieve the lowest stable surface tension. Efficient surfactants have low CMC values, i.e., less surfactant is required to decrease surface tension (Araujo *et al*., 2019). The results of the critical micelles concentration (CMC) of the produced biosurfactant are shown in Figure 10. It can be seen that 5 mg/mL concentration is the CMC of the produced biosurfactant which decreased surface tension from 72 to 20 mN/m. These results corroborate with the published work of Ravindran *et al.* (2020) but much lower than Ali *et al*. (2019) and relatively lower than some other biosurfactants produced by some other *Bacillus* species.

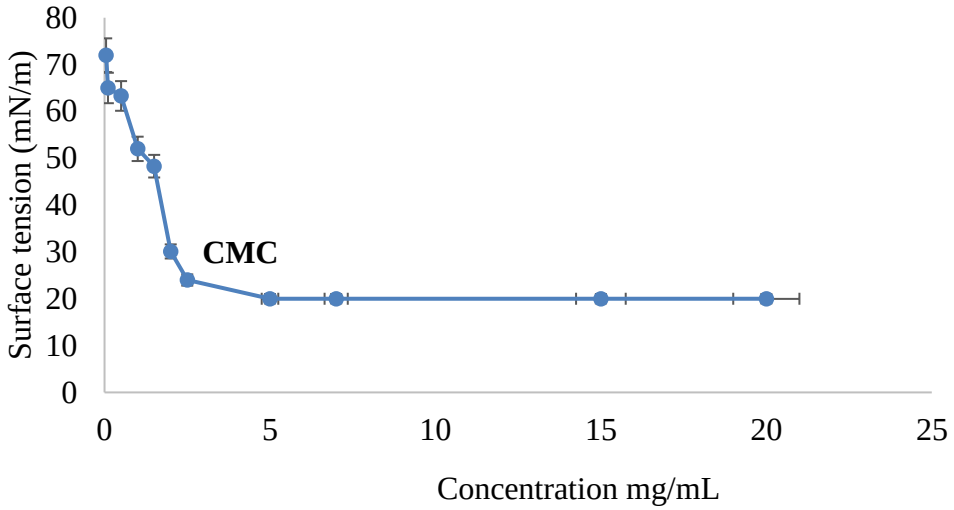


Figure 10: Critical micelle concentration of the produced biosurfactant

The industrial biotechnological application of the biosurfactant produced by *B. anthracis* strain S62A was explored in the degreasing and washing of different fabrics materials. The results of the oil washing performance of the biosurfactants produced by *B. anthracis* strain S62A, chemical surfactant (Tween 80) and commercial detergent (Viva detergent) on different fabric material are shown in Figure 11. The results show that the different materials had significant physical washing performance by different concentrations of the biosurfactant and was found to be comparable to the chemical surfactant (Tween 80) and commercial surfactant (Viva detergent). The control treatment (water) had significant of spent engine oil stain on different fabric materials (polyester cotton and chiffon) respectively after treatment. Also, the results of the oil cleaning efficiency of the glycophospholipid biosurfactant produced by *Aeromonas hydrophilia* strain S62A, chemical surfactant (Tween 80) and commercial detergent (Viva

detergent) on different fabric materials are shown in Figure 12. From the results, ½ CMC was able to remove 53, 70 and 45% of oil from polyester, cotton and chiffon, respectively; 1x CMC removed 60, 70, and 58% of oil from polyester, cotton and chiffon, respectively while 2 x CMC removed 78, 72 and 64% of oil from polyester, cotton and chiffon, respectively. The chemical detergent (Tween 80) removed 75, 88 and 67% of oil from polyester, cotton and chiffon. The commercial detergent (Viva detergent) removed 60, 68 and 66% of oil from polyester, cotton and chiffon, respectively. Water which was used as a negative control removed 11, 20, and 17% oil from the materials as previously mentioned. The results further reveal that both chemical (Tween 80), biological (glycophospholipid) and commercial surfactant (Viva detergent) were found to be efficient in degreasing and washing of different fabric materials, in terms of oil removal. However, in various points the oil stains were still there. On the contrary, after the use of the biosurfactant of *B. anthracis* strain S62A in washing of the dirty polyester, cotton and chiffon fabrics with spent engine oil, the fabrics showed characteristics similar to the clean cotton fabrics because the stain was totally removed. According to the percentage of oil removed from the materials, the results indicate that the Viva detergent and the biosurfactant of *B. anthracis* strain S62A were similar in their degreasing and washing potentials. Statistically, significant differences ($P < 0.05$) were detected among the means of all surfactant concentration treatment in comparison to their controls using two-way ANOVA followed by Dunnett comparison test. The resultant observation was almost similar to the study carried out by Bouassida *et al.* (2017) who reported that the biosurfactant removed 81% of spent engine oil while the commercial detergent removed 34%, respectively. Bouassida *et al*. (2018) reported that their published results demonstrated that the biosurfactant acted additively with a commercial detergent and enhanced their performances from 33 to 45% and 57 to 64% in removal oil and tea stains, respectively and contradicts the observations made in this study.

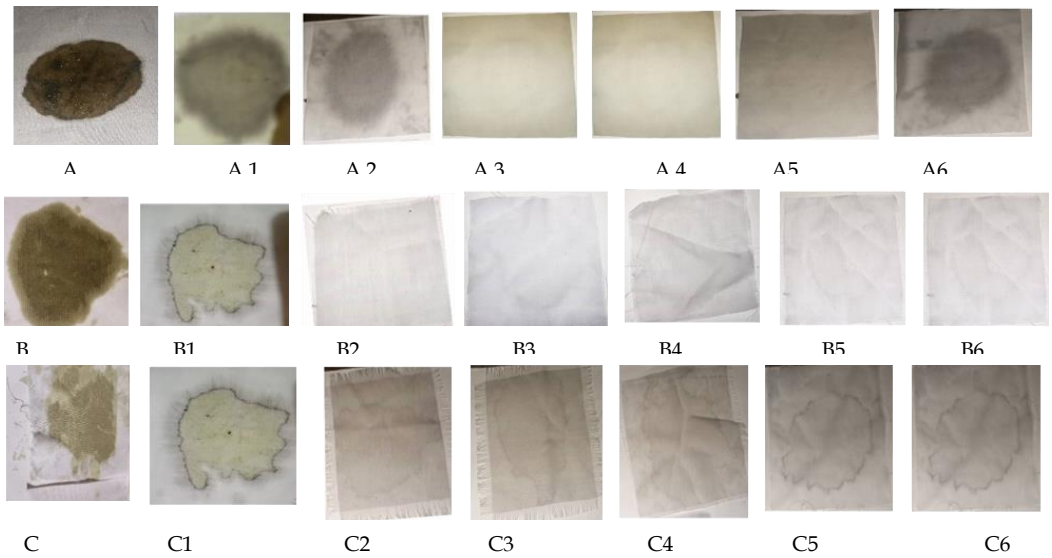


Figure 11: Oil washing performance of the biosurfactant produced by *Bacillus anthracis* S62A, chemical surfactant (Tween 80) and commercial detergent (Viva detergent) on different fabric material
N.B: A = Polyester stained with oil, B= Cotton stained with oil, C= Chiffon stained with oil; A2 – A6 are polyester material washed with ½ x CMC, 1 x CMC, 2 x CMC, Tween 80 and Viva detergent, respectively; B2 – B6 are cotton materials washed with ½ x CMC, 1 x CMC, 2 x CMC, Tween 80 and Viva detergent; C2 – C6 are chiffon materials washed with ½ x CMC, 1 x CMC, 2 x CMC, Tween 80 and Viva detergent; A1, B1, C1 are polyester, cotton and chiffon washed with water respectively (Control)

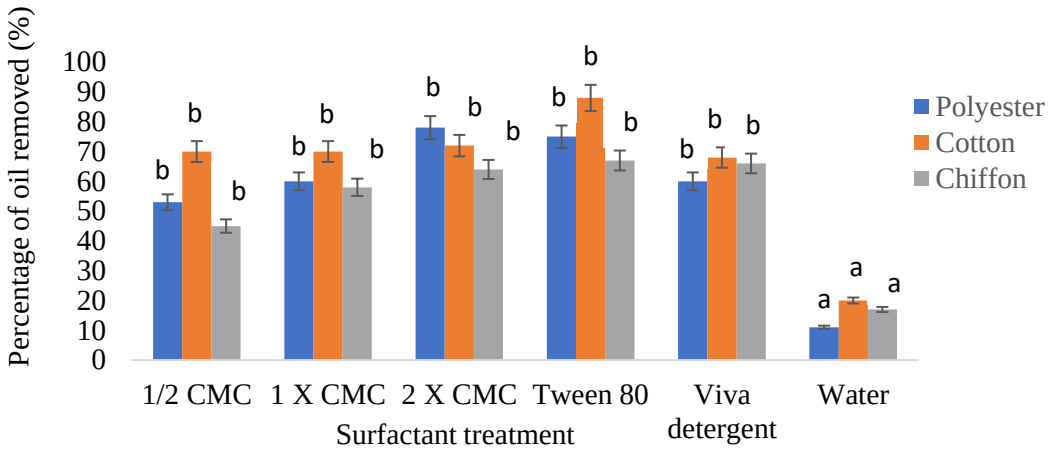


Figure 12: Oil cleaning efficiency of the glycophospholipid biosurfactant produced by *A. hydrophilia* S62A, chemical surfactant (Tween 80) and commercial detergent (Viva detergent) on different fabric material

N.B: CMC = Critical micelles concentration; % = Percentage; Error bar = Standard deviation; column with similar letters is non - statistically significant; columns with different letters are statistically significant at $P < 0.05$.

## CONCLUSION

This study has shown that the bacterium *B. anthracis* strain S62A isolated from spent engine oil-polluted soil was capable of producing biosurfactant and the biosurfactant it produced was a glycophospholipid based on the results obtained from biochemical test profile. In addition, it was observed that temperature, pH, incubation time, inoculum size carbon sources, nitrogen sources and salinity all impacted on the ability of the isolate to produce biosurfactant and its stability. The biosurfactant produced was also found to demonstrate efficient emulsification, degreasing and washing against spent engine oil - stained fabric materials which is attractive for application in the biotechnological industries. The results of the optimization process can be useful in enhancing the production of surface-active agents, making them attractive options for application at industrial levels.

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