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# Biosurfactants as Bioremediation Tools Isolated from Bacteria Found in Industrial Wastewaters of the Kakuri Drain Kaduna, Northern Nigeria

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**Abstract**
Biosurfactants are chemical substances produced in living spaces or excreted extracellular hydrophobic and hydrophilic moieties that confer on the organism the ability to accumulate between fluid phases thus reducing surface and interfacial tension. Biosurfactants are produced by several microorganisms which include *Bacillus* sp., *Candida antactica*, *Acinetobacter* and *Psudomonas aeruginosa.* The physiological role of biosurfactant production in microorganisms include among others, the ability to make substrates readily available for uptake by the cells in adverse environmental conditions. Biosurfactant applications in the environmental industries are promising due to their biodegradability, low toxicity, and effectiveness in enhancing biodegradation and solubilization of low solubility compounds. Bacterial strains isolated from the Kakuri drain expressed high Liquid Surface Tension Reducing Activity (LSTRA).Strains were identified as Pseudomonas aeruginosa using the API20E Kit. Strain Ali31 (*P.aeruginosa*) recorded a mean surface tension of 29.033mN/m, while the lowest surface tension recorded amongst the strains was 28.840mN/m by strain Ali13 (*P.aeruginosa*).The model strain recorded a mean of 57.979mN/m. Several factors however influence biosurfactant production, such as nature of carbon source, temperature, pH and aeration.

## INTRODUCTION

Surfactants are compounds that lower the surface tension (or interfacial tension) between two liquids or between a liquid and a solid. Surfactants may act as detergents, wetting agents, emulsifiers, foaming agents and or dispersants. Biosurfactants(BS) are amphilic compounds produced on living surfaces, mostly microbial cell surfaces or excreted extracellularly and contain hydrophobic and hydrophilic moieties that reduce surface tension(ST) and interfacial tensions between individual molecules at the surface and interface respectively. Hence biosurfactants are surfactants that are produced extracellularly or as part of the cell membrane by bacteria, yeast and fungi. Examples include Pseudomonas aeruginosa which produces rhamnolipids, Candida bombicola, one of the few yeast to produce biosurfactants, which produces a lipopeptide called Surfactin.

Biosurfactants have been applied in the environment in the control of oil spills especially, and have been found to have high potentials for degradation of Petroleum hydrocarbons, Pesticides, Heavy metals etc. The effectiveness of a surfactant is determined by its ability to lower the surface tension, which is a measure of the surface free energy per unit area required to bring a molecule from the bulk phase to the surface (Rosen,1978). Due to the presence of a surfactant, less work is required to bring a molecule to the surface and the surface tension is reduced. For example, a good surfactant can lower the surface tension of water from 72 to 35mN/m (Mulligan,2004).

Production of chemically synthesized surfactants, which are non biodegradable and toxic to the environment for commercial purposes, has today exceeded 17million metric tonnes. Surfactants may also bio-accumulate, becoming hazardous to crops and humans. Their toxic effect has led to the switch to surfactants of microbial origin as alternative. Today, several applications of biosurfactants in our environment are noticed and include their applications as , biopesticides, enhancers in the solubility of bio-hazardous chemical compounds, as well as in commercial laundry detergents. They showed good emulsion formation capability with vegetable oils as reported by Das K Mukherjee (2007). In medicine, biosurfactants are used in various roles, for their surfactant nature; as antimicrobial, anticancer, antiviral and anti adhesive agents. They have also found use in the food processing industries, as well as the cosmetic industry.

## MATERIALS AND METHODS

In Kaduna, capital of Kaduna state in Northern Nigeria, laying at latitude 10.20N and longitude 7.23E),the huge increase in both human population and human activities (industrial and otherwise), has led to a huge increase in amount of waste discharged into the environment, which results in pollution (Emere, 1996). Most of the industries in the city of Kaduna are located in the southern part in an industrial layout within the Nassarawa-Kakuri area. The Industries which comprises of several textile Industries as well as foam and automobile (PAN) industries and the military factory (DICON), Turner's Asbestos Product (TAP) ,a fertilizer company Federal Superphosphate Fertilizer) CO Ltd, (FSFC) and the Breweries along with others discharge their wastes, treated

or untreated into local streams called drains that eventually empty their contents in to river Kaduna.
The industrial discharge along with domestic waste gets into river Kaduna (FME, 2002) through several drains or tributaries that run across the city,

Wastewater samples from 5 different sampling points along the Kakuri drain in Makera district of Kaduna South, Kaduna, in Northern Nigeria, were analysed and isolated bacteria from the drain were cultured and tested for their biosurfactant-producing ability. The drop collapse technique as explained by Chen,( 2007) was adopted in testing for the biosurfactant production amongst strains. The kakuri drain is an outlet that serves the purpose of conveying waste from the surrounding industries and homes and empties into river Kaduna. Some of the industries include the superphosphate fertilizer company, textile industries, an ammunition and electroplating plant, among others.

Bacterial isolates were characterized and identified by biochemical assays and by the use of the API 20E Kit. Isolates were cultured in 10ml microcosm bottles in a shaking incubator for 72hrs at 37$^{o}$C. 10ul of the culture was then added to fresh 10ml microcosm bottles containing 6ml of KB* culture broth.

**Drop Collapse Assay**

The assay was conducted by placing a drop of the inoculated culture broth (10ul) on a fresh empty petri-dish plate and observed. If the drop from the culture broth collapses and spread on the surface of the petri-dish, then it signifies the presence of biosurfactant. If however the drop retains its spherical shape, as in the uninoculated broth used as control, then it is negative or does not produce biosurfactant.

**Tensiometry**

Isolates that proved positive, for biosurfactant production, were then subjected to the tensiometry. The liquid surface tension reducing activity (LSTRA) of the strains was measured using the Kruss K100, MK2 Tensiometer. Readings included that of the three tested strains, water, uninoculated or sterile KB* culture broth, and water.

**RESULTS**

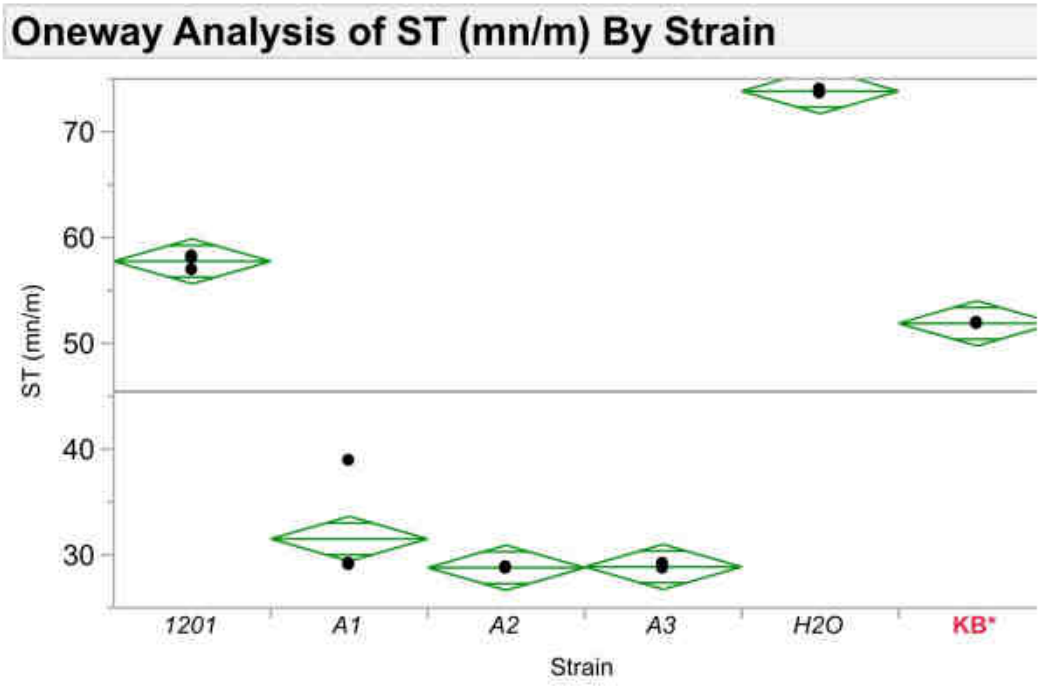
Several bacteria isolates were screened and after the preliminary tests to determine biosurfactant producing ability, the strains were then subjected to the tensiometry .Strains Ali31,Ali13 and Ali28 were subjected to the tensiometry. Strain #1201 was used as a model strain, while the KB* culture broth was the control.

Using the standard rod method at 20$^{o}$C (Fechner 2011), the liquid surface tension,*Y (Lambda)* of deionised water was estimated to be 73.2mNm$^{-1}$, while that of sterile KB* was 54.4mNm$^{-1}$. The *Y* of the model strain, #1201, was estimated to be 57.75mNm$^{-1.}$

The table below shows values *Y* , for the strains , model, control and water.

Table 1 –Tensiometry Results -

| Strain | Replicate | Surface tension (mN/m) |
|---|---|---|
| A1 | 1 | 29.171 |
| | 2 | 29.033 |
| | 3 | 28.983 |
| | 4 | 38.884 |
| A2 | 1 | 28.815 |
| | 2 | 28.84 |
| | 3 | 28.681 |
| | 4 | 28.837 |
| A3 | 1 | 29.161 |
| | 2 | 28.962 |
| | 3 | 28.69 |
| | 4 | 28.674 |
| 1201 | 1 | 58.204 |
| | 2 | 57.979 |
| | 3 | 57.905 |
| | 4 | 56.906 |
| H2O | 1 | 73.984 |
| | 2 | 73.824 |
| | 3 | 73.59 |
| | 4 | 73.906 |
| KB* | 1 | 51.877 |
| | 2 | 51.808 |
| | 3 | 51.927 |
| | 4 | 51.946 |




## Oneway Anova

### Summary of Fit

| | |
|---|---|
| Rsquare | 0.989556 |
| Adj Rsquare | 0.986654 |
| Root Mean Square Error | 2.022681 |
| Mean of Response | 45.44113 |
| Observations (or Sum Wgts) | 24 |

### Analysis of Variance

| Source | DF | Sum of Squares | Mean Square | F Ratio | Prob > F |
|---|---|---|---|---|---|
| Strain | 5 | 6977.2418 | 1395.45 | 341.0821 | <.0001* |
| Error | 18 | 73.6423 | 4.09 | | |
| C. Total | 23 | 7050.8841 | | | |

## Oneway Analysis of ST (mn/m) By Strain

### Oneway Anova

#### Means for Oneway Anova

| Level | Number | Mean | Std Error | Lower 95% | Upper 95% |
|---|---|---|---|---|---|
| 1201 | 4 | 57.7485 | 1.0113 | 55.624 | 59.873 |
| A1 | 4 | 31.5178 | 1.0113 | 29.393 | 33.642 |
| A2 | 4 | 28.7933 | 1.0113 | 26.669 | 30.918 |
| A3 | 4 | 28.8718 | 1.0113 | 26.747 | 30.996 |
| H2O | 4 | 73.8260 | 1.0113 | 71.701 | 75.951 |
| KB* | 4 | 51.8895 | 1.0113 | 49.765 | 54.014 |

Std Error uses a pooled estimate of error variance

### Means Comparisons

#### Comparisons with a control using Dunnett's Method

Control Group = KB*

#### Confidence Quantil

| \|d\| | Alpha |
|---|---|
| 2.76150 | 0.05 |

#### LSD Threshold Matrix

| Level | Abs(Dif)-LSD | p-Value |
|---|---|---|
| H2O | 17.99 | <.0001* |
| 1201 | 1.909 | 0.0029* |
| KB* | -3.95 | 1.0000 |
| A1 | 16.42 | <.0001* |
| A3 | 19.07 | <.0001* |
| A2 | 19.15 | <.0001* |

Positive values show pairs of means that are significantly different.

**DISCUSSION**

Biosurfactant-producing bacteria have been surveyed in the past, but only recently was an attempt made to predict the minimum liquid surface tension produced by these bacteria. The tensiometry test allows for prediction of Bacterial Liquid Surface Tension Reducing Activity (LSTRA). In Biosurfactant-producing Bacteria as established by Fechtner, *et. al.* (2012).All the strains tested for biosurfactant producing ability using the drop collapse method, proved positive. Hence, all strains tested were capable of producing biosurfactants. The tensiometry test further proved how much quantity of the biosurfactant the strains could produce and to what extent they could lower the surface tension of the media in which they thrive. The tensiometry readings obtained from the strains shows the degree to which the strains greatly lower the surface tension of the media and therefore act as biosurfactants with great commercial value. As surface active chemicals expressed by a range of organisms that reduce liquid surface tension (*y*) of aqueous and aqueous-hydrocarbon (oil) mixtures. Biosurfactants are widely used in biotechnology including agriculture, cosmetics, food, pharmacology, bioremediation and oil recovery (Mulligan, 2005;Merchant *et al* 2012;Lawniczak *et al* 2013;Sachdev and Cameotra, 2013) .Fakruddin (2012) defined Microbial surfactants ( Biosurfactants ) as amphiphillic compounds produced in living spaces or excreted extracellular hydrophilic moieties that confer on the organism the ability to accumulate between fluid phases thus reducing surface and interfacial tension The biological roles for bacterial biosurfactants include motility and virulence, the inhibition of nematode, and protist predation, lysis of fungi and oomycetes and the induction of systemic resistance in plants (Raaijmakers *et al*, 2010) as well as modifying

water distribution in soil pore networks (Spiers, *et. al*. 2011). The physiological role of biosurfactant production in microorganisms includes antimicrobial activity and the ability to make substrates readily available for uptake by the cells in adverse environmental conditions. The main physiological role of biosurfactants is to permit microorganisms to grow on water-immiscible substrates by reducing the surface tension at the phase boundary thus making the substrate more readily available for uptake and metabolism (Desai *et al*, 1997). Another physiological role of biosurfactants is their antimicrobial activities towards various micro-organisms. As a rule different surfactant inhibits different taxonomy. In addition, biosurfactants have been shown to be involved in cell adherence which imparts greatest stability under hostile environmental conditions and virulent and in cell desorption when organisms need to find new habitats for survival (Desai *et al* ,1997).

Rahman *et al*, (2002) and Magdalene, (2011) showed that addition of rhamnolipid produced by Pseudomonas sp DS10-129 along with poultry litter and coir pith enhanced ex situ bioremediation of a gasoline-contaminated soil while Southam *et al*,(2001) studied the effect of biosurfactants on waste hydrocarbons. To degrade hydrocarbons bacteria must adsorb onto the surfactant-oil interface which is 25-50 nm in thickness. Due to the anionic nature of rhamnolipids they are able to remove metals from soil and ions such as cadmium, copper, lanthanum, lead and zinc due to their complexation ability.

## CONCLUSION

Microorganisms isolated from the Kakuri drain which carries industrial wastes from the various companies and industries within the Makera-Kakuri industrial layout were found to show great ability to lower surface tension. Hence they hold great promise if and when considered for large scale applications in order to solve industrial pollution or oil spill problems. Compared to strains isolated from non-industrial sites, the strains obviously have been playing a great part in naturally degrading the waste, discharged into the environment by the industries.

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