**Comparative Biosurfactant Production by *Actinomycetes* Isolated from Hydrocarbon Contaminated Soils, Plastic-Enriched**

**Composting Soil and Ikpoba River Sediments in Benin-City.**

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**Abstract:** This study examined the production of biosurfactants by *Actinomycetes* isolated from hydrocarbon contaminated soils, plastic-enriched composting soil and Ikpoba river sediments. Soil samples were collected from some mechanic workshops in Benin City, from the Edo state waste management dumpsite located at Iyowa in Benin City and from Ikpoba River. Isolation of *Actinomycetes* was done using starch casein agar incorporation with antibiotics incubated for 7 – 10 days at 30oC. Growth on mineral salt medium initiated the production of biosurfactants which was extracted by centrifugation and filtration followed by liquid extraction using chloroform: methanol (2:1v/v). Characterization and stability studies were conducted. From the physico-chemical analyses, the pH of the hydrocarbon contaminated soil was 4.92±0.049; the plastic composted soil had 5.46±0.08 while that of Ikpoba river sediments was 6.62±0.056. The hydrocarbon contaminated soils had the highest concentration of iron (29.97±0.25), the plastic composted soil had the highest value for electricity conductivity (103.88±2.70) while the highest concentration for nitrate was recorded at Ikpoba River (8.34±0.436mg/kg). The isolates were screened for their ability to produce biosurfactant. The biosurfactant produced were screened for its emulsification activity and surface tension reduction ability by subjecting them to varied temperatures, pH and salt concentration. The biosurfactant was found to have a better surface tension of 117.01±0.20 dynes/cm with temperature, 160.04±0.33dynes/cm and 111.00±0.00dynes/cm for the hydrocarbon polluted soil which the highest values. The percentage emulsification activity of the biosurfactant produced from the Ikpoba river sediments recorded the highest value with respect to pH and temperature which were 38.04±0.0 and 32.40±0.00 (%). The hydrocarbon polluted soil recorded the highest percentage emulsification activity (39.01±0.04%) with salt concentration. Aerobic Gram positive rodswithextensive branching were observed confirming growth of *Streptomyces* sp. based on morphological and biochemical test.

**Keywords**: Biosurfactants, Actinomycetes*,* plastics, Contaminated Soils and Ikpoba River.

**1.0 Introduction** Due to the problems associated with plastic waste

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petroleum hydrocarbons is unfortunately a environmental friendly disposal methods (Kumar *et al*., he contamination of soil and groundwater with management, there is an increased interest in more

common phenomenon and has caused serious 2007; Atuanya *et al*., 2012). The degradation of organic environmental problems in Nigeria. The release of these substances is mostly evaluated through chemical and

contaminants to the environment including petroleum and petroleum based/derived products is one of the main causes of global contamination (Rahman *et al*., 2003). It is also a risk for human and animal health, given that many of these contaminants are toxic and carcinogenic (Prabhu *et al.*, 2003). Hydrocarbon molecules that are released into the environment are difficult to remove since they are absorbed to surfaces and are trapped by capillary in the water immiscible phase. Plastics have been preferred to other materials over the years due to its low cost, light weight, high durability, easy to store and transport, comes in an endless variety of textures and shape and can hold almost anything (Karanth *et al*., 1999).

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Copyright © 2017 Nigerian Society for Microbiology physical changes, oxygen consumption or carbon dioxide production (Albertsson and Randy, 1979). Although studies on biodegradation of plastics have shown that more organisms show the potential to degrade these anthropogenic molecules, some xenobiotics have been shown to be unusually recalcitrant (Esteve-Nenez *et al*., 2001).Bioremediation has been considered and proven to be one of the best approaches and a suitable alternative to diminish the effects caused by hydrocarbon and plastic waste contamination of soil and water. This can be accomplished by using the metabolic capacity of microorganisms that can use hydrocarbon as source of carbon and energy, or that can be modified by cometabolism and also for restoration of soil because the technology is cost effective and environmentally safe (Menezes *et al.*, 2005). The efficiency of biodegradation of hydrocarbon and plastic contaminants is directly related to the chemical structure of the compound, its bioavailability (concentration, toxicity, mobility and access) and the physico-chemical conditions present in the environment (Cooper and Gondenenberg, 1987).

*Actinomycetes* are Gram positive bacteria with a distinctive feature of possessing filamentous hyphae that do not normally undergo fragmentation and produce asexual spore. They degrade enormous number and variety of organic compounds and are extremely important in the mineralization of organic matter. The success of bioremediation technology is also dependent upon a microbial ability to access the complex hydrocarbon mixture (Margesin and Schinner, 2001) which are compounds with low water solubility and thus not readily available to microorganisms. Because of this, bacteria consortia display a wide array of metabolic mechanisms for coping with the breakdown of oil components including the production of surface active agents and emulsifiers (Willumsen and Karlson, 1965). These agents are small surfactant molecules with a hydrophilic portion and a hydrophobic portion and these complex structures provide them with amphipathic properties enhancing the bacteria growth and bioremediation rate.

Biosurfactants are structural diverse group of surface active molecules synthesized by microorganisms. Virtually all surfactants are chemically synthesized (Banat, 1995). In recent years, much attention has been directed towards biosurfactant owing to their advantages such as low toxicity, high biodegradability, better environmental compatibility, high foaming capacity/capability, higher selectivity, specific activity at extreme temperature, pH, salinity and ability to synthesize them from removable food stocks (Cameotra and Makkar, 1998). Surfactants are amphiphilic agents which by accumulating at interphase between immiscible phases and can reduce surface and interfacial tensions. The significant reduction of interfacial tension caused by biosurfactant increases the solubility and emulsification of the immiscible phases and bioavailability of the insoluble substrate for the microorganisms (Banat *et al.*, 2010). Biosurfactant with such surface properties make good candidates for enhanced oil recovery (EOR). The most effective biosurfactant reduce surface tension (si) of water from 72dyness/cm to values in the range of 25-30 dyness/cm (Ron and Rosenberg, 2001). They are also known to have therapeutic application (Rodrigues *et al.,* 2006). Recently, biosurfactant have been widely used in environmental protection including EOR, oil spill control, bio-degradation and detoxification of oil contaminated industrial effluents and soil (Cameotra and Prothi, 2003). Another important application of biosurfactants is the stimulation of oil production in marginal wells that have approached the economic limitation of operation (Banat *et al.*, 2010). Among the many classes of biosurfactants, lipopeptides represent a class of microbial surfactants with remarkable surface properties and biological activities such as surplus crude oil recovery. Therefore, this research was designed to study and compare the production of biosurfactants by *Actinomycetes* isolated from hydrocarbon contaminated soils, plastic-enriched composting soil and Ikpoba river sediments.

# 2.0 Materials and Methods Sample collection

Soil samples were collected from hydrocarbon contaminated soils in mechanic workshops located in various parts of Benin City and river sediments were collected from Ikpoba River in Edo state. Plastic enriched composting soil samples were collected from Edo state waste management dumpsite located at Iyowa community Benin City. Soil samples were collected at different locations at a depth of 0-15cm with a standard soil-auger in sterile plastic bags and tagged before transported to the laboratory. In the laboratory, soil samples were spread out on plastic trays and kept on laboratory bench to air-dry, served and stored in accordance with (Karthik *et al.*, 2010). The soil samples were used for actinomycetes isolation according to methods of Desai and Banat (1997).

# Soil analysis

The physico-chemical parameters of the soil samples were analyzed which include: pH, temperature, total organic carbon, silt and sand composition, nitrate, phosphorus, calcium, magnesium, sulphate, potassium, vanadium and moisture content of the soil samples. These parameters were analyzed using standard procedures (APHA, 1998; Kalra and Maynard, 1991; Adelekan and Abegunde, 2011; Maniyar *et al*., 2011). The pH reading was obtained with the aid of a Hanner microprocessor pH meter which was earlier standardized with buffer 4.0, 7.0 and 9.0. Soil moisture content was calculated and recorded as percentage weight of respective soil samples. Particle-size distribution was obtained using hydrometer method (Onyeonwu, 2000). Heavy metals aspirated on Atomic absorption spectrometer (AAS) PG550 model. Nitrate, sulphate and aluminum, nitrogen from soil was obtained colorimetrically after optical density (OD) of the samples and standards were taken at specific wavelengths. Total organic carbon (TOC) was obtained using dichromate with titration method (Onyeonwu, 2000). Total nitrogen was obtained using micro kjeldahl digestion and colorimetric method. Available phosphorus content was obtained colorimetrically after the absorbance of the solution was read at 660nm. Total hydrocarbon content (THC) was obtained after nhexane extraction and absorbance read colorimetrically and THC calculated according to Akpoveta *et al*.

(2011).

# Isolation and Enumeration of *Actinomycetes*

For each collected hydrocarbon polluted soil, plastic enriched composting soil and the Ikpoba river sediment, 1g of soil was suspended in 100ml of physiological water (NaCl), and then incubated in an orbital shaker incubator at 28oC with shaking at 200rpm for 30mins. Mixtures were then allowed to settle and serial dilutions up to 106 were prepared. An aliquot of 0.1ml of each dilution was taken and spread evenly over the surface of the starch casein agar (Narendra *et al*., 2010). The combination of 5.0 μg/ml of griscofulvin and 1.0ug/ml of sodium penicillin was observed to inhibit the growth of microbial contaminants (bacteria and fungi) as actinomycetes are slow growers. The plates were incubated for 7 – 10 days at 28±02oC (Zaki *et al.,* 2013; Lakshmipathy *et al.*, 2010). Plates showing morphological differences were selected, purified by pour plate technique and maintained for further studies (Narendra et al., 2010). The ability of biosurfactant producing *Actinomycetes* to utilize hydrocarbon was determined by measuring the turbidity of the *Actinomycetes* inoculation into the Bushnell Hass broth containing crude oil as carbon source of mineral salt medium (Lakshmipathy *et al.*, 2010).

**2.1 Production and Extraction of Biosurfactants** The samples selected were inoculated in a 250ml Erlenmyer flask containing 250ml sterile mineral salts medium consisting of (Na2HPO4 6g, KH2PO4 3g, NH4CL 1g, MgSO4. 7H2O 0.24g and CaCL2 0.01g,

Daeto-agar 15g, distilled water (l) with 20g glucose pH 7 soybean oil 0.19g as carbon source, 5.0g glutamic acid. Trace elements solution containing (g/l): ZnSO4. 7H2O 2.32; MnSO4. 4H2O 1.78; H3BO3 0.56; CySO4. 5H2O 1.0; Na2MO4. 2H2O 0.39; CaCL2. 6H2O 0.42; EDTA 1.0; NiCL2. 6H2O 0.004 and KI 0.66; and Bushnell Hass medium with 3% forcados blend crude oil as carbon source. The broth culture was incubated on a reciprocal shaker at 120rpm for 5-7 days at 30oC (Sabina et al., 2010; Moliterni *et al.*, 2012). The biosurfactant was extracted from the culture by centrifugation and filtrations. The cell free broth was extracted by liquid – liquid extraction from the cell–free supernatant using mixture of chloroform: methanol (2:1v/v). The extracts was divided in rotating evaporator, weighed and quantified (Jain *et al.*, 1991).

**2.2 Biosurfactant Characterization Surface Tension Measurement.**

The surface tension of the biosurfactant was measured by the ring method using the Du–Nony tension meter (Kruss type 8451). A measurement of surface tension from distilled water was used as negative control. The concentration at which micelles began to form was represented as the CMC. At the CMC, a sudden change in surface tension was observed. The CMC was determined by plotting the surface tension as a function of biosurfactant concentration and surface tension at this point was designated as 7cmc (Khopade *et al.,* 2012).

**Emulsification Activity and Foaming Properties.**

The foaming properties were evaluated by monitoring the stability of the foam formed by hand shaking of the crude biosurfactant for 2 hours. Emulsification index of culture sample was determined by adding 2ml of a hydrocarbon to the same amount of culture/same volume of biosurfactant in a graduated screw cap test tube and then vortexed for 2min and left 24hours. The E24 index was given as a percentage of height of emulsified layer (mm) divided by the height of the liquid column (mm) (Khopade *et al.,* 2012).

E24 (%) = Total height of the emulsified layer x 100% Height of total evolution

# 2.3 Stability Characterization

To determine the thermal stability of the biosurfactant, about 4ml of the culture supernatant were stored at 4 and 25oC heated at 70oC, 100oC and 21oC for 15mins and then cooled to room temperature. The surface and E24 value of each treatment was performed as described (Techaoei *et al.,* 2007). The pH change effect was determined by adjusting the culture supernatural with acid (in HCL) or (in NaOH) to pH values ranging from 1-10 prior to filter sterilization to monitor the surface tension and E24 are measured and determined (Techaoei *et al.,* 2007). The effect of salinity was measured by adding 5-20% NaCl then the sample was subjected to surface tension test measured at E24.

# 2.4 Biochemical and Antimicrobial Activity

The protein content of surfactant was estimated using burette and ninhydrin method and lipid content estimate by isolated and purification method, total carbohydrates were calculated using molish test. The crude biosurfactant was tested for antimicrobial activity using diffusion method and area of zone was calculated. Active compounds were tested against *Escherichia coli*, *Bacillus stubtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albican.*

# 3.0 Results

From the physico-chemical analyses, the pH of the hydrocarbon contaminated soil was 4.92±0.049; the plastic composted soil had 5.46±0.08 while that of Ikpoba river sediments was 6.62±0.056. The hydrocarbon contaminated soils had the highest concentration of iron (29.97±0.25), the plastic composted soil had the highest value for electricity conductivity (103.88±2.70) while the highest concentration for nitrate was recorded at Ikpoba River (8.34±0.436mg/kg) (Table 4). The biosurfactant was found to have a better surface tension of 117.01±0.20 dynes/cm with temperature, 160.04±0.33dynes/cm and 111.00±0.00dynes/cm for the hydrocarbon polluted soil which the highest values (Table 5-7). The percentage

emulsification activity of the biosurfactant produced from the Ikpoba river sediments recorded the highest value with respect to pH and temperature which were 38.04±0.0 and 32.40±0.00 (%) (Table 1 and 2). The hydrocarbon polluted soil recorded the highest percentage emulsification activity (39.01±0.04%) with salt concentration (Table 3). Aerobic Gram positive rodswithextensive branching were observed confirming growth of *Streptomyces* sp. based on morphological and biochemical test (Table 8).

**Table 1:** Effects of pH on the emulsification Activity of biosurfactants produced by actinomycetes isolated from the test samples

 Hydrocarbon

pH contaminated soil Ikpoba River Sediment Plastic composted soil

2 23±0.19 30±0.04 22.00±0.01

4 28±0.03 34±0.09 28.00±0.07

1. 34±0.49 36±0.01 37.67±0.01
2. 36±0.05 46±0.00 34.33±0.02

11 32±0.04 38±0.03 30.44±0.00

**Table 2:** Effects of temperature on the emulsification Activity of biosurfactants produced by actinomycetes isolated from the test samples

|  |  |  |  |
| --- | --- | --- | --- |
|   Temperature (OC)  |  Hydrocarbon contaminated soil  | Ikpoba River Sediment  |  Plastic composted soil  |
|  70  |  117.01±0.20  | 81.00±0.12  | 101.40±0.19  |
|  100  |  105.00±0.04  | 70.20±0.09  | 68.33±0.14  |
|  121  |  99.20±0.32  | 65.40±0.00  | 53.00±0.49  |

**Table 3:** Effects of salt concentration on the emulsification Activity of biosurfactants produced by actinomycetes isolated from the test samples

 Hydrocarbon Ikpoba River Plastic

Salt content (%) contaminated soil Sediment composted soil

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| 2 8 15  | 25.04±0.00 27.00±0.01 30.20±0.32  |     | 27.00±0.00 30.03±0.19 32.40±0.00  |   26.00±0.00  29.67±0.33  31.67±0.33  |

**Table 4:** Physico-chemical qualities of hydrocarbon contaminated soil, Ikpoba River Sediments and Plastic Composted Soil

 Hydrocarbon Ikpoba River Sediment Plastic Composted

 Parameters contaminated soil Soil

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| pH CL-, mg/kg SO42-, mg/kg NO3-, mg/kg PO43-, mg/kg Na+, mg/kg K+, mg/kg Ca2+, mg/kg Mg2+, mg/kg Fe3+, mg/kg Zn2+, mg/kg Mn2+, mg/kg Cu2+, mg/kg Ni2+, mg/kg Cd2+, mg/kg V2+, mg/kg Cr6+, mg/kg Pb2+, mg/kg Hg+, mg/kg As , mg/kg EC, us/cm TDS, ppm T-Carbon, % T-Nitrogen, %  |  6.62±0.05  5.89±0.13 3.50±0.01 8.03±0.02  1.13±0.00 4.58±0.01  8.97±0.02 9.95±0.03  3.02±0.01  29.97±0.05  10.97±0.04  4.22±0.01 5.56±0.06  5.35±0.01 4.1±0.02  3.13±0.23 1.26±0.11  8.23±0.02 <0.001  1.11±0.025 66.91±0.41  33.95±0.73  5.80±0.58  2.06±0.01  |                         |   | 4.92±0.09 16.98±0.08 1.717±0.02 2.34±0.46 0.36±0.01 1.35±0.02 2.16±0.04 2.97±0.05 0.81±0.01 6.38±0.42 3.16±0.01 0.35±0.02 1.54±0.06 2.72±0.02 0.72±0.06 0.47±0.00 0.73±0.23 3.89±0.09 <0.001 0.31±0.025 40.85±0.26 10.92±0.16 0.33±0.38 0.74±0.56  |  5.46±0.14  8.10±0.33 4.837±0.05 4.09±0.11  1.18±0.03 2.13±0.06 4.00±0.10  7.12±0.19 2.38±0.34 15.39±0.14  8.50±0.22  3.65±0.04 7.76±0.02 4.72±0.02  2.23±0.01 1.36±0.05 1.73±0.04  5.36±0.12  <0.001 2.11±0.25 53.88±2.27 20.92±0.16 2.09±0.05 2.21±0.02  |

**Table 5:** Effects of temperatureon the Surface Tension reduction ability of biosurfactants

 Hydrocarbon Plastic

Temperature (oC) contaminated soil Ikpoba River Sediment composted soil

 70 111.00±0.09 83.00±0.01 64.33±0.33

**Table 6:**

Effects of pH on the

Surface Tension reduction ability of biosurfactants



100

104.00±0.04

76.03±0.02

61.67±0.33

121

100.00±0.03

71.12±0.04

53.67±0.88

**Table 7:** Effects of salt concentrationon the Surface reduction ability of biosurfactants

Salt content Hydrocarbon Ikpoba River Plastic

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| 2 8 15  |  39.01±0.04  35.03±0.09  34.11±0.07  |     |  35.00±0.12  38.01±0.01  36.02±0.04  |  23.67±0.33  25.67±0.33   29.00±0.04  |

 (%) contaminated soil Sediment composted soil

**Table 8:** Biochemical analysis of the Biosurfactant

Carbohydrate abscent

Protein present

Fats and oils present

# 4.0 Discussion

The results from this study showed a wide range of difference in the physico-chemical parameters of the hydrocarbon contaminated soil, plastic-enriched composted soil and Ikpoba River sediment which influenced the production of biosurfactant by *Actinomycetes*. It was observed that the pH of Ikpoba River sediment recorded the highest value which is 6.62± 0.056 . The electrical conductivity of the hydrocarbon contaminated soil recorded the highest value. The values for sulphate, nitrate, phosphate, sodium, potassium, magnesium, zinc, iron, total nitrogen and total carbon content were higher in the hydrocarbon contaminated soil and the plastic enriched composting soil than that of Ikpoba River sediments, but the chlorine value for Ikpoba River was higher than that of the hydrocarbon contaminated soil (Table 2). There was extensive branching of the mycelium observed in the Petri dish which was observed to be Gram positive rod, non-motile, oxidase negative and catalase positive. The organism grew on mineral salt medium incorporated with crude oil. The organism was identified as *Streptomyces* sp. (Karthik *et al.*, 2010). Biosurfactant production was observed by the presence of foam while on the rotator shaker (Moussa *et al.,* 2006; Khopade *et al.,* 2012). It was also observed that biosurfactant production was independent on growth phase (Chakraborty *et al.,* 2009).

There was no significant difference in the mean emulsification activity of the biosurfactants produced from the three sites across different pH (p>0.05), but with increase in pH between 2 and 8, there was significant difference in the emulsification activity from the extracted biosurfactants from the three sites (Table 1). A significant difference was observed in the mean pH surface tension of the biosurfactants produced from the three sites with the hydrocarbon contaminated soil from the mechanic work shop having a higher surface tension (Table 5). Temperature majorly affects the biosurfactant surface tension. This thermal studies has revealed that the biosurfactant is thermal stable across the temperature range studied (Zaki *et al.*, 2013). (Khopade *et al.,* 2012), reported that the applicability of surfactants in several fields depends on its stability at different temperature and pH value. Heating of the biosurfactant to 100oC caused no significant effect on the biosurfactant performance. This activity indicates its possible usefulness in food industries and pharmaceuticals where heating to stability is of paramount importance (Abouseoud *et al.*, 2008; Mulligan and Gibbs, 1989). Ikpoba River had a slightly higher temperature compared to the other sites, there was no significant difference in emulsification activity of the biosurfactant, although there was an increase from 70oC to 100oC and slightly decreased when the temperature reach 121oC (Table 4). The mean surface tension of the biosurfactant produced from the three sites was not was significant (p<0.05) as there was a gradually decreased in the surface tension. It was observed from the salinity test that the tree sites had similar emulsification values, also there was significant difference in the surface tension activity of the biosurfactants across the three sites with difference salt concentrations (Tables 3 and 5). Boisurfactants are generally stable across salt concentration range of 220% and that salt concentration did not affect biosurfactant emulsification (Khopade *et al.,* 2012; Sarubbo *et al*., 2007). The biosurfactant has stability at an alkaline pH and slightly higher makes it a useful tool in the bioremediation of marine crude oil spill (Prieto *et al*., 2008). The boisurfactant produced in this research possessed foaming ability, amino acids, peptides, fats and oil without carbohydrate.

# 5.0 Conclusion

Results from this research have shown that *actinomycetes* have the potentials of producing a wide range of beneficial compounds. *Actinomycetes* have complex enzymatic mechanism that aids hydrocarbon mineralization and thus increases the potential for biosurfactant production. Biosurfactants are stable across temperature ranges and are not majorly affected by salt concentration; this property aids its potential usage in decontamination of oil contaminated areas in the Niger Delta region of Nigeria.

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