International Journal of Scientific & Engineering Research, Volume 6, Issue 2, February-2015
ISSN 2229-5518

Production of Biosurfactants from Submerged Fermented Fruit Bagasse of Yellow Cashew (*Anacardium occidentale*) Using *Pseudomonas Aeruginosa*

Ogugua Victor N., Okpashi, Victor E.Iroha Okechukwu K., Njoku Obi U.
Department of Biochemistry, University of Nigeria, Nsukka.
Correspondence should be addressed to Ogugua Victor N., oguguavictor@yahoo.com

Abstract: A methodology for producing biosurfactants from Submerged Fermented Fruit Bagasse of Yellow Cashew (*Anacardium occidentale*) Using *Pseudomonas aeruginosa* was determined. This involved controlling agitation, temperature, pH and salt concentration conditions. The emulsification capacity of the biosurfactant was also verified with engine oil, olive oil, kerosene, and petrol. Carbon sources were evaluated with regards to the growth and production. The stability of the rhamnolipid produced by *Pseudomonas sp.* was also evaluated for possible applications in Bioremediation, antimicrobial and emulsions. Its antimicrobial activity was checked against *Staphylococcus aureus, Escherichia coli, Pseudomonas* and *Salmonella typhimurium.* On the basis of high production of biosurfactant, it is suggested that the hydrolysate of yellow cashew bagasse is a good and cheapest source. The study concludes that Fruit Bagasse of Yellow Cashew waste are ideal substrate for biosurfactant production, which may have potential industrial applications.

Key words: Biosurfactant, fermented fruit bagasse, *pseudomonas aeruginosa*, yellow cashew and production

[1]. Introduction

Surfactants are molecules that adsorb at liquid-liquid interfaces to decrease the enthalpy of the overall free energy increase and reduce the tendency of emulsion to destabilize (Okpashi, *et al;* 2014). Surfactants are widely used in various industrial processes such as pharmaceutics, cosmetic, petroleum, and food production. Often, there are synthesized. These chemical compounds are not biodegradable and can be toxic to the environment. Biosurfactants have equivalent emulsification properties compared synthetic surfactant. Biosurfactants are surface active compounds produced by microorganisms in cells using variable substrates, to biodegrade pollutants in aerobic and anaerobic conditions (Van Dyke *et al;* 1993; Kosaric *et al;* 1993; Banat, 1995). The emulsification capacity of these substances and their derivatives are due to the existence of hydrophobic and hydrophilic moiety within the molecule. These allows them to interact between two liquids phases with different physicochemical characteristics. Biosurfactants can be glycolipids, lipopeptides/lipo-proteins, lipopolysaccharides, substituted fatty acids and phospholipids according to their radicals (Duvnjak *et al.,* 1982; Montes and Oca, 1992; Fiechter, 1992; Hommel, 1990). The efficiency and effectiveness of these compounds are determined by ability to reduce the surface tension of aqueous solution and measurement of critical micelle concentration (CMC) (Fiechter, 1992). Rhamnolipids are normally synthesized by microorganisms to facilitate the taking-up of insoluble substrate. For example, when oil spill in a particular areas, the native microorganisms are selected for their assimilation capacity resulting in the availability and incorporation of substrate to the cells for posterior metabolism (biodegradation) (Hommel, 1990; Zhang and Miller, 1994) There are many different microorganisms capable of synthesizing biosurfactants, such as *Pseudomonas fluorescens,* and *P. aeruginosa,* (Kosaric *et al.,* 1993; Hommel, 1990; Fiechter, 1992). Although extensive investigation with promising future of glycolipids have not being commercialized due to increase cost. Rhamnolipids, produced by *Pseudomonas* are glycolipids (Hisatsuka *et al.,* 1971; Hauser and Karnovsky, 1954). Due to their biological origin and functional properties, biosurfactants is used in petroleum industry, including emulsification and de-emulsification, separation, formation of low viscosity, production of emulsions to transport heavy crudes, emulsion washing, formation of slurries, corrosion inhibition, enhance oil recovery and hydrocarbon biodegredation promotion (Kosaric *et al.,* 1983; Díaz, 1991). It is economically competitive, biosurfactants have the potential to replace synthetic surfactants, as they possess similar structural and physical properties, produced by renewable substrates, with the

International Journal of Scientific & Engineering Research, Volume 6, Issue 2, February-2015 ISSN 2229-5518

advantage of being degradable. When analyzing their economic viability is important considering the nutritional requirements of the microorganism. The cost of carbon sources in culture conditions and the design of simple procedure to separate the product is challenging. More so the use of alternative substrates for the production of high value products such as biosurfactants presents an advantage over the traditional processes. The recycling of materials whereupon materials are systematically used and reused to bring about the robust increase in resource productivity, make human activity sustainable is relevant (Clift, 1997; Cammarota and Freire, 2006). Several species of bacteria, yeast and fungi are known to secrete enzymes during growth on hydrophobic low cost substrates, which are important sources of industrial applications. The industrial application of biosurfactants have being useful for a spectrum of transesterification and other reactions (Harwood, 1989; Malcata *et al.,* 1992; Shimizu and Nakano, 2003. The research was aimed at producing biosurfactant from cashew bagasse *anacardium occidentalis* fruits as carbon and energy source by submerged fermentation using *pseudomonas aeruginosa*.

[2]. Methodology

Yellow cashew (*Anacardium occidentale*) fruits were used as the plant materials for this study.

[2.1] Plant Materials

The cashew apples were collected from Ubogidi cashew plantation in Nsukka Local Government Area, Enugu state, Nigeria.

[3]. Microorganisms

Strains of *Pseudomonas aeruginosa* obtained from the Culture Isolation Unit of the Department of Microbiology, University of Nigeria, Nsukka

[4]. Chemicals

The chemicals used in this study were of analytical grade, products of British drug House BDH England and were acquired from the Department of Biochemistry, University of Nigeria, Nsukka.

[5]. Preparation and Processing of Plant Materials

The yellow cashew fruits were washed with normal saline and manually crushed to remove the juice. The bagasse obtained was air-dried for 4 days and milled into powder. The powdered bagasse was packaged in an air-tight polythene bag and stored at room temperature.

[6]. Proximate Analysis

The proximate composition of the yellow cashew bagasse was determined using the method described by AOAC (1990).

[7]. Moisture Content

A glass Petri dish was placed on a tarred analytical balance and 10.0 g (initial weight) of dried yellow cashew bagasse was placed in it. This was placed in an air-circulating oven set at 105˚C for one hour. At the end of the heating, the container was placed in a desiccator to cool down to room temperature. Ensuring minimum exposure to air, the container and its contents were weighed again (dry weight). The overall procedure was repeated until two consecutive weighing gave a constant value. The moisture content was calculated see equation below:

$$\% \text{ Moisture } = \frac{\text{initial weight} - \text{dry weight}}{\text{initial weight}} \, x \, 100$$

[8]. Fibre Content

A quantity 1.0 g (W_1) of the sample was placed in a beaker. A known volume, (150 ml) of preheated 0.128M H_2SO_4 was added and boiled for 30 minutes and filtered. The residue was washed three times with hot water and placed into the beaker. Preheated at 0.223M KOH with 150 ml, and boiled slowly for 30 munites and filtered. The mixture was washed with hot water and acetone three times. Later, it was dried at 130˚C for one hour and weighed (W_2). The mixture was heated to ash in a furnace at 500˚C for three hours, cooled and weighed (W_3). Crude fibre content was calculated using the equation below.

$$\% \text{ Crude fibre} = \frac{(W2 - W3)}{W1} \times 100$$

[9]. Ash Content

Empty heat resistant crucibles were dried at 500˚C for one hour, and cooled in a dessicator. A quantity 2g of the ground sample was transferred into the dish and the contents ignited, first gently at 500˚C for three hours. The crucible was removed and the lid replaced. It was allowed to cool before reweighing. The weight of the residue was obtained and expressed as percentage of the original sample find the equation below.

Send correspondence to: Oguguavictor @yahoo.com

International Journal of Scientific & Engineering Research, Volume 6, Issue 2, February-2015 ISSN 2229-5518

$$\% \text{ Ash} = \frac{\text{weight of ash}}{\text{original weight}} \times 100$$

[10]. Protein Content

A quantity 0.5 g of oven-dried sample was empty in side 30ml Kjeldahl flask 15.0ml of concentrated H_2SO_4 was added with 1 g of the catalyst mixture. The mixture was heated cautiously in digestion rack under fume cupboard until a greenish solution appeared. After the digest was cleared, it was heated further for 30 minutes and allowed to cool. About 10.0 ml of distilled water was added to avoid caking. The mixture was transferred to the Kjeldahl distillation apparatus. A receiver flask containing 5.0ml of boric acid was placed under the condenser of the distillation apparatus. 10.0 ml of 40% NaOH solution was added to the digested sample. Distillation was stopped when the distillate reached the 35ml in the receiver flask. The distillate was titrated to end point pink colour with 0.1M HCl. The crude protein content was calculated as:

$$\% \text{ Crude protein} = \frac{(\text{titre value} \times 14.01 \times 0.1 \times 100 \times 6.25 \times \text{dilution factor})}{1000 \times \text{sample weight}}$$

[11].
Lipid Content

Soxhlet flask was dried in an oven at 100˚C, allowed to cool and weighed (W_1). 5g (W_2) of the sample was transferred to a thimble and its contents into the Soxhlet extractor. Hexane was used for the extraction. After three hours, the thimble was removed and the solvent distilled. The flask was disconnected and placed in an oven set at 60˚C for two hours, cooled and weighed (W_3). Lipid content was calculated as:

$$\% \text{ Lipid} = \frac{(W3 - W1)}{W2} \times 100$$

[12]. Carbohydrate Content

The carbohydrate content was calculated from the results of the other five parameters since they are all presented in percentages.

[13]. Reactivation of *Pseudomonas aeruginosa* by Subculture

The pure strain of *Pseudomonas aeruginosa* in a Bijou bottle was reactivated by streak-plating on nutrient agar contained in a Petri dish. The serving nutrient agar was prepared by dissolving 2.8 g of powdered nutrient agar in 100 ml of distilled water. The solution was autoclaved at 121˚C for fifteen minutes. Under an aseptic environment, the autoclaved nutrient agar solution was allowed to cool for some time, then poured into a sterile Petri dish and left undisturbed for some minutes to gel. After gelling, the pure strain of the *Pseudomonas aeruginosa* was inoculated by streaking on the gelled nutrient agar in the Petri dish with inoculum from the Bijou bottle containing the stock culture of the organism. The streaking process was achieved by the use of a heated/sterilized wire loop. The inoculated Petri dish was left undisturbed for twenty-four hours at room temperature to enhance the formation of colonies required for the preparation of the inoculum.

[14]. Preparation of Basal Mineral Medium

The basal mineral medium (BMM) was prepared as described by Atlas *et al.,* (2010). The trace element solution was prepared first by adding components (0.232 g H_3BO_3, 0.174 g $ZnSO_4.7H_2O$, 0.116 g $FeSO_4$ $(NH_4)_2SO_4.6H_2O$, 0.096 g $CoSO_4.7H_2O$, 0.022 g $(NH_4)_6Mo_7O_{24}.4H_2O$, 8.0 mg $CuSO_4.5H_2O$, 8.0 mg $MnSO_4.4H_2O$) to distilled water and bringing its volume to 1.0 L. Finally, the basal mineral medium was prepared by adding components (12.5 g K_2HPO_4, 3.8 g KH_2PO_4, 1.0 g $(NH_4)_2SO_4$, 0.1 g $MgSO_4.7H_2O$ plus 5.0 ml of the trace elements solution) to distilled water and bringing the volume to the 900.0 ml mark. The solution was mixed thoroughly, gently heated and brought to boiling. It was then autoclaved for fifteen minutes at 15 psi pressure-121˚C and cooled to 45-50˚C.

[15]. Preparation of Media Composed of Basal Mineral Medium and Yellow Cashew Bagasse

A quantity, 14 g of the yellow cashew bagasse was added to 100 ml of distilled water in a conical flask. The flask and its contents were sterilized by autoclaving for fifteen minutes at 15 psi pressure-121˚C. This sterile carbon source was added to a

IJSER

International Journal of Scientific & Engineering Research, Volume 6, Issue 2, February-2015 ISSN 2229-5518

freshly prepared basal mineral medium, and then poured into 250 ml flasks in triplicate and labeled (BMM/YC 1, BMM/YC 2 and BMM/YC 3).

[16]. Preparation of Media Composed of Basal Mineral Medium, Yellow Cashew Bagasse and Glucose

A quantity, 14 g of yellow cashew bagasse and 14 g of anhydrous glucose was added to 100 ml of distilled water in a conical flask. The flask and its contents were sterilized by autoclaving for fifteen minutes at 15 psi pressure-121˚C. This sterile carbon source was added to a freshly prepared basal mineral medium and then poured into 250 ml flasks in triplicate and labeled (BMM/YC/Glu 1, BMM/YC/Glu 2 and BMM/YC/Glu 3).

[17]. Preparation of Media Composed of Basal Mineral Medium and Glucose (BMM/Glu)

A quantity, 14 g of anhydrous glucose was added to 100 ml of distilled water in a conical flask. The flask and its contents were sterilized by autoclaving for fifteen minutes at 15 psi pressure-121˚C. This sterile carbon source was added to a freshly prepared basal mineral medium and then poured into 250 ml flasks in triplicate and labeled (BMM/Glu 1, BMM/Glu 2 and BMM/Glu 3).

[18]. Preparation of *Pseudomonas aeruginosa* Culture

This process was achieved by adopting the method described by Atlas *et al.,* (2010). Under a sterile working environment provided by a disinfected work bench and Bunsen flame, a loopful of *Pseudomonas aeruginosa* colony from the culture medium in the Petri dish was inoculated into a 100.0 ml nutrient broth contained in a 250 ml conical flask. The inoculum was shaken and left undisturbed for four hours after which 1 ml of the inoculum was collected in a flamed environment and the absorbance was read at 600 nm wavelength using a spectrophotometer blanked to 0.00 reading with distilled water. The readings were taken three consecutive times at two hours interval. Growth was indicated by the increase observed in absorbance. This primary inoculum was grown until the optical density reached to 1.459 and was then used to inoculate the various fermentation media at 2% (v/v); that is 2ml of inoculum for every 100ml of media.

[19]. Inoculation of *Pseudomonas aeruginosa*into fermentation media

Each of the previously prepared medium was inoculated with inoculums from the seed culture as described by Atlas *et al.,* (2010). In an aseptic working environment provided by disinfection of the bench tops and flaming of the air around the work space, 2 ml of inoculum was inoculated into each of the twelve 250ml conical flasks containing the different media prepared. The culture broths (media plus inoculum) were left for fifteen days at room temperature with continuous shaking.

[20]. Screening the Culture Broth for Growth of *Pseudomonas aeruginosa*

Growth of the organism was monitored by taking plate counts on nutrient agar. The plates were inoculated with 0.1ml of serial dilutions using the pour plate method described by Willey *et al*., (2013). The plates were incubated for twenty-four hours before the colonies were counted.

[21]. Screening of the Supernatant for Biosurfactant Activity

The various culture supernatants were screened for biosurfactant activity using the following tests.

[22]. Oil Spreading Test

The oil displacement test is a method used to measure the diameter of the clear zone, which occurs after dropping a surfactant-containing solution on an oil-water interphase. The binomial diameter allows an evaluation of the surface tension reduction efficiency of a given biosurfactant. The oil displacement test was done by adding 50 ml of distilled water to a Petri dish with a diameter of 15 cm. After that, 20 μl of oil was dropped onto the surface of the water, followed by the addition of 10 μl of cell culture supernatant. The diameter and the clear halo visualized under visible light were measured after thirty seconds (Rodrigues *et al*., 2006).

[23]. Emulsification Test

The emulsification test was carried out as described by (Balogun and Fagade; 2010). Sterile biosurfactant solution (1.0 ml) was added into each test-tube (in a set of three) containing 2.0 ml of the substrates (crude oil, olive oil and kerosene). The content of the tubes were vigorously shaken for uniformity for two minutes and left undisturbed for twenty-four hours. The volume of oil that separated after twenty-four

IJSER

International Journal of Scientific & Engineering Research, Volume 6, Issue 2, February-2015 ISSN 2229-5518

hours was measured, that is, the ability of a molecule to form a stable emulsion. Their emulsification index after twenty-four hours (E_{24}) was determined and expressed as percentage of height of emulsified layer in centimeter divided by total height of the liquid column in centimeters. Emulsification index of value greater than 50% was indicative of a positive result.

$$E24 = \frac{\text{Height of emulsified layer}}{\text{Total height}} \times 100\%$$

[24]. Statistical Analysis

All investigations were carried out in triplicate and data obtained were presented as mean ± standard deviation using descriptive statistics. Analysis was conducted using SSPSS version v16, for the determination of mean values.

[25]. Results and Discussions

The biosurfactant producing capacity of *Pseudomonas aeruginosa* using yellow cashew (*Anacardium occidentale*) fruit bagasse as carbon source was investigated in this study. An attempt to reducing the cost of biosurfactants production as alternatives to the synthetic surfactants. The approach utilized carbon source which is a byproduct of cashew fruits, thus reducing the volume of wastage and finding useful applications for a material usually and always considered as waste. Proximate analysis of *A. occidentale* (yellow cashew) bagasse shows that the under-utilized by-product, contains appreciable quantities of carbohydrates 49.37 ± 0.60%, lipids 26.67 ± 0.66%, protein 11.34 ± 0.16%, ash 2.70 ± 0.04, fibre 2.86 ± 0.09 and moisture content 5.78 ± 0.17% respectively as shown table 1.

Table 1. Production of Biosurfactants and proximate analysis

Parameter	Proximate composition (%)
Moisture	**5.78 ± 0.17**
Fibre	**2.86 ± 0.09**
Ash	**2.70 ± 0.04**
Protein	**11.34 ± 0.16**
Lipid	**26.67 ± 0.66**
Carbohydrates	**49.37 ± 0.60**

IJSER

Culture	Carbohydrate(mg/L)	Lipid(mg/L)	Protein(mg/L)
Yellow cashew	5.23	0.15	Not detected
Yellow cashew + glucose	5.05	0.21	Not detected
Glucose	4.77	0.33	Not detected
Nutrient broth	4.23	0.29	Not detected

Table 2. Production of Biosurfactants and Biochemical Composition of from the Various Broth Supernatants

This is in agreement with the results obtained by Sivagurunathan *et al*., 2010; Adebowale *et al*., 2011; Gordon *et al*., 2012). As the carbohydrate dominate in all the culture media with 5.23mg/L, 5.05mg/L, 4.77mg/L and 4.23mgL for yellow cashew, yellowcashew + glucose, glucose and nutrient broth respectively as shown in table 2. These nutrients make good carbon source that support growth of *P. aeruginosa* in a fermentation media as shown in figure 1. The highest growth pattern was observed day 3.7 at about 90 hours with cell count 80cfu/ml in glucose media. But in the yellow cashew media, the growth pattern showed 69cfu/ml in 76 hours in day 3.

International Journal of Scientific & Engineering Research, Volume 6, Issue 2, February-2015 ISSN 2229-5518

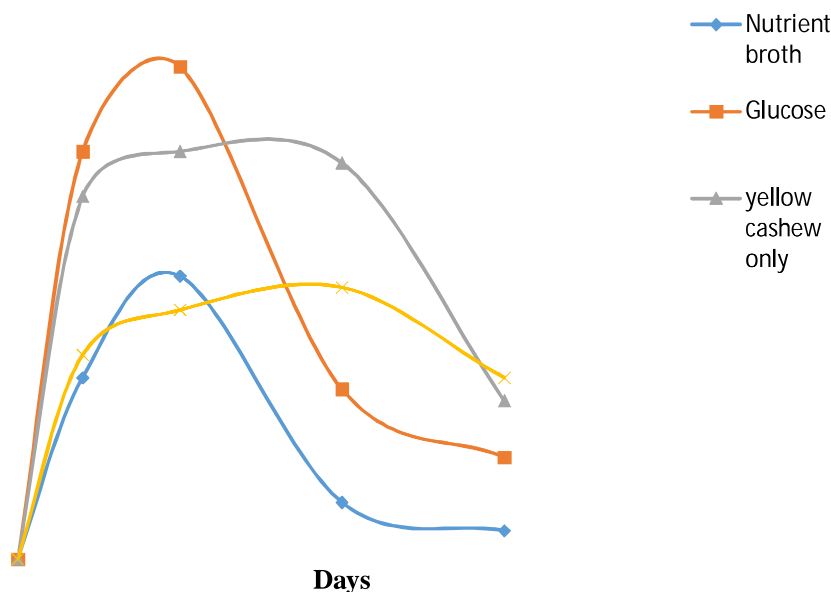

Figure 1. Production of biosurfactant and Growth pattern of *Pseudomonas aeruginosa* in the various fermentation media

As can be seen figure 1, the log phase of growth was observed in the first three days. In B.M.M + Yellow cashew and B.M.M + Yellow cashew + Glucose media, stationary growth phase was noticed from the fourth day till the tenth day after which decline in growth started. The decline in growth rate started from the fourth day for the B.M.M + Glucose and Nutrient broth media.The phenolic components could interfere with the fermentation process by imparting cloudiness and pH variations. It considerably reduced the processing technique of pressing out the fruit juice. Despite this step in the fruit processing, the resulting by-product still contained nutrients that is explore in fermentation media. Its utilization in fermentation is a contribution to the search for cheap substrates and starting materials in biosurfactant production at the industrial and commercial scale.

Culture	Weight of biosurfactant (g/L)
Yellow cashew	0.71
Yellow cashew + glucose	0.93
Glucose	0.63
Nutrient broth	0.12

Table 3. Production of Biosurfactant Yield from the Various Broth Supernatants

The yield of biosurfactants in different culture media was determine results indicate that a consortium of yellow cashew + glucose was 0.93g/L and lowest in nutrient broth 0.12g/L, while yellow cashew only o.71g/L and glucose 0.63g/L as seen table 3 above.

The *P. aeruginosa* used for this study was first maintained in nutrient broth culture at 37ºC after sub-culturing. Growth determination of the microbial culture by measurement of absorbance at 600 nm showed gradual increase with time. The increase in absorbance with time indicates growth of *P. aeruginosa* and its viability for subsequent inoculation into the various fermentation media. For successful fermentation, the microorganism of choice, which is an important factor to be considered. The process of sub-culturing the microorganism serves to revive it from dormancy as new microorganisms are formed from cell divisions. The result is a generation of primed organisms ready for use in fermentation (Volkering *et al;* 1995).

Demain (1998) points out that secondary metabolism in microorganisms is brought by exhaustion of nutrient, biosynthesis or addition of

International Journal of Scientific & Engineering Research, Volume 6, Issue 2, February-2015 ISSN 2229-5518

an inducer, and/or by a growth rate decrease. These events generate signals which effect a cascade of regulatory events resulting in chemical differentiation and morphological differentiation. The signal is often a low molecular weight inducer which acts by negative control, i.e. by binding and inactivating a regulatory protein (repressor protein/receptor protein) which prevents secondary metabolism and morphogenesis during rapid growth and nutrient sufficiency (Ducret *et al.*, 1995).

Biosurfactant activity was tested in the various fermentation cultures using emulsification index, oil spreading and haemolysis tests. The emulsification ability was analyzed on some vegetable and hydrocarbon oils. From the emulsification index test, appreciable biosurfactant activity was observed as the various fermentation culture supernatants were able to form stable emulsions with kerosene, olive oil and engine oil. Stable emulsion formation with petrol was poor; less than 40% as seen in table 4.

Culture Supernatant	E24 with Kerosene	E24 with Olive oil	E24 with Petrol	E24 with Engine oil
Medium I (Yellow cashew)	53.50±1.19	61.20±1.37	33.40±0.45	52.10±0.86
Medium II (Yellow Cashew and Glucose)	61.10±1.10	59.3±0.93	44.30±0.99	44.30±0.41
Medium III (Glucose)	60.40±0.91	58.50±1.09	40.30±1.32	47.70±0.52
Medium IV (Nutrient broth)	48.70±1.23	40.6±1.12	49.40±0.91	30.97±1.17
SDS	60.3±0.57	30.6±1.15	36.3±1.47	59.0±1.20

Values are Mean ± SD, where n=3.

Table 4. Production of Biosurfactants and Emulsification indices (%) of culture broth supernatant and SDS.

Emulsification index was calculated as height of emulsified layer (cm)/total liquid column (cm) × 100%.This may be caused by the inability of the biosurfactants to stabilize the microscopic droplets of the vegetable and hydrocarbon oils. The emulsification indexes were generally lower than the values in the various studies carried out on biosurfactant production. Emulsification indices obtained by Adebusoye *et al*., 2008; Chandran and Das, 2010; and Anyanwu *et al*, 2011 were high (well above 50%) though the variations in carbon source, duration of incubation period and the nature of vegetable or hydrocarbon oils could be the contributing factors. The use of culture supernatants in place of pure biosurfactant solution in the emulsification index analysis could also have negative effects on the results due to the presence of some interfering substances in solution. These substances could be cellular components released during mechanical forces employed for cell disruption during centrifugation. Table 3. Shows the antibacterial activity of biosurfactants from the B.M.M + Yellow cashew broth supernatant against

International Journal of Scientific & Engineering Research, Volume 6, Issue 2, February-2015 ISSN 2229-5518

some Gram negative and Gram positive bacteria indicated by the diameter of zone of growth inhibition. The biosurfactant inhibited the growth of four bacterial species namely *Bacillus cereus, Klebsiella*, *Staphylococcus aureus* and *Escherichia coli*. The antibacterial activity screening was negative for *Pseudomonas aeruginosa* and *Salmonella as in table* 5.

Antibacterial Activity of Biosurfactants from the B.M.M + Yellow Cashew Broth Supernatant

Microorganisms	Zone of inhibition diameter (mm)
Bacillus cereus	28.70 ± 1.70
Escherichia coli	38.70 ± 1.30
Staphylococcus aureus	38.00 ± 2.94
Klesbsiella	31.00 ± 2.20
Pseudomonas aeruginosa	Not detected
Salmonella	Not detected

Values are mean ± SD, where n = 3.

Table 5. Production of Biosurfactants and Antibacterial Activity Test

IJSER

The emulsification indices of the various culture supernatants were compared to sodium dodecyl sulphate (SDS), a synthetic surfactant, it was observed that the emulsification index for SDS were higher than the emulsification index of the culture supernatants. This is as a result of the purity of the synthetic surfactant and condition associated. The ability of biosurfactants to emulsify fats, oil and hydrocarbon-water mixtures has been demonstrated to increase hydrocarbon degradation significantly and is potentially useful in oil spill, enhanced oil recovery and potential application as cleaning and emulsifying agent in the food industry (Anyanwu *et al*., 2011; Kapadia and Yagnik, 2013). The ability of the isolated biosurfactant to form stable emulsions with different hydrocarbon and vegetable oils suggests its potential applications in the petroleum, food and pharmaceutical industries (Dandik *et al*., 1993; Jaeger *et al*., 1997; Jaeger and Reetz, 1998)
Oil spreading or displacement ability was present in all the culture supernatants as a sign of biosurfactant activity. The oil displacement test is indicative of the surface and wetting activities of a surfactant, thus a wide diameter represents high surface activity (Chandran and Das, 2010). Supernatants from the culture containing basal mineral medium and yellow cashew had the widest diameter of oil displacement while supernatants from the culture containing nutrient broth showed the thiner diameter of oil displacement. These values show high oil spreading activity compared values recorded by (Umeji *et al*; 2010) which had 1.2cm as the widest diameter of the spread oil. This ability to disperse oil is due to the tension-active properties of the biosurfactant molecules in the various culture supernatants (Saharan *et al*., 2011). This property thus gives reason for the application of biosurfactants in pollution control especially in oil spillages. As would be expected, the diameter of oil spreading with sodium dodecyl sulphate (SDS), an artificial surfactant, was (8.27±0.21cm) compared to the values obtained for the microbial surfactants. This result corroborated the high values for oil displacement obtained for SDS and Tween 80 by (Chandran and Das 2010). This explained purity of the synthetic surfactant.
All the culture broth supernatants were tested for haemolytic activity. This is regarded by some authors as indicative of biosurfactant production and activity (Umeji *et al*., 2010). The supernatants were positive to the haemolysis test and gave clear zones on the solid blood agar medium. This implies that all the culture media produced biosurfactants.The emulsification index of

International Journal of Scientific & Engineering Research, Volume 6, Issue 2, February-2015 ISSN 2229-5518

biosurfactants were also measured at different temperatures, pH and salt (NaCl) concentrations. When these culture supernatants were subjected to various temperatures (30ºC to 110ºC) for temperature stability test, the emulsifying ability of B.M.M + yellow cashew was considerably stable as the emulsification index did dropped below 50% showing that at increase temperature, the biosurfactants activity is not inhibited, suggesting that the biosurfactants might be useful in stress environments such as temperate marine compartments and industrial systems where variable temperature degrees are factors find table 6 below.

Temperature (°C)	Biosurfactant	Sodium dodecyl sulfate (SDS)
30	50.00 ±0.57	36.60 ±0.76
50	55.00 ±1.15	40.00 ± 0.87
70	55.00 ±0.50	40.00±0.28
90	50.00 ±0.29	35.00 ±1.04
110	60.00 ±0.76	36.00±0.77

Values are Mean ± SD, where n=3.

Table 6. Production of Biosurfactants and Temperature stability test

There was a decrease in the emulsification index of SDS as the temperature increase implying that high temperatures affect the activity of the synthetic surfactant. Thus suggesting the preference of biosurfactants over the synthetic ones. In the pH stability test, the emulsifying ability of the B.M.M + yellow cashew bagasse supernatant were not affected at pH 6. The results showed that emulsification index is favoured by near neutral pH and that the biosurfactants might also be useful in acidic and alkaline environment conditions since it will retain its activity over a pH ranges from 2 to 10 as in table 7.

pH	Biosurfactant	Sodium dodecyl sulfate (SDS)
2	40.00 ±0.67	37.00 ±0.86
4	45.00 ±1.05	38.00 ± 0.87
6	48.00 ±0.52	50.00±0.29
8	46.00 ±0.79	40.00 ±1.84
10	42.00 ±0.74	35.00 ±0.78

Values are Mean ± SD, where n=3.

Table 7. Production of Biosurfactants and pH Stability Test

When the effect of salt concentration on the emulsifying capacity of the culture supernatants from the B.M.M + Yellow cashew medium was analyzed, it was observed that there was decrease in emulsification index as the concentration of salt increased. This suggests that the biosurfactant might not be useful in marine environments and other systems where salt concentration is above physiological level though there are reports on some biosurfactants produced by bacteria showing stability in the presence of high salt concentration (Samadhan, *et al*; 2014) shown in table 8.

Salt concentration (%w/v)	Biosurfactant	Sodium dodecyl sulfate (SDS)
0	48.00 ±0.97	50.60 ±0.78
5	48.00 ±1.15	52.00 ± 0.80
10	46.00 ±0.50	50.00±0.26
15	45.00 ±0.29	48.00 ±1.54
20	45.00 ±0.76	48.00 ±0.77

Values are Mean ± SD, where n=3.

Table 8. Production of Biosurfactant and Salt concentration stability test

These characteristics confirmed the interest in microbial-derived surfactants over the synthetic and correspond with results obtained by (Chandran and Das, 2010; Anyanwu *et al.*, 2011). The four different fermentation media gave varying yields of biosurfactants upon evaporating supernatants to dryness. The yield was highest in B.M.M + Yellow cashew + glucose and lowest in nutrient broth. The high yield observed in the B.M.M +Yellow cashew + glucose could be attributed to high substrate availability in the medium. It is also possible that the depletion of substrates in the other fermentation media led to low yields. The partial solubility of the cashew

International Journal of Scientific & Engineering Research, Volume 6, Issue 2, February-2015 ISSN 2229-5518
bagasse affected its absorption and assimilation. It is inferred that optimization of the B.M.M + Yellow cashew medium by supplementing with glucose enhance the product yield though this may not be a cost-effective.

After serial dilutions to the sixth power, 0.1ml of microbial suspension was taken and spread on the surface of freshly prepared nutrient agar plates and incubated for colony formation.

Number of days	Culture	Number of colony forming units (cfu)
2	Nutrient broth	32
	B.M.M + Glucose	72
	B.M.M + Yellow cashew	64
	B.M.M + Yellow cashew + Glucose	36
5	Nutrient broth	50
	B.M.M + Glucose	87
	B.M.M + Yellow cashew	72
	B.M.M + Yellow cashew + Glucose	44
10	Nutrient broth	10
	B.M.M + Glucose	30
	B.M.M + Yellow cashew	70
	B.M.M + Yellow cashew + Glucose	48
15	Nutrient broth	5
	B.M.M + Glucose	18
	B.M.M + Yellow cashew	28
	B.M.M + Yellow cashew + Glucose	32

Table 9. Production of Biosurfactants and colony formation

IJSER

Nutrient broth is a media for temporal storage and transporting microorganisms. The result thus shows that yellow cashew bagasse could serve as cheap carbon source for biosurfactant production, though the net product yield was low as only about 5% of the starting material was converted to products.

[26]. Conclusion

This study attempt to find the applicability of economically cheaper carbon sources for the production of biosurfactant using *pseudomonas aeruginosa*. Results obtained from biosurfactant production with agriculture waste showed the possibility and reliability for the industrial production of biosurfactant using yellow cashew bagasse. It's cheaper, available economical and abundant carbon sources. Satisfactory emulsification capacity of the biosurfactant against different oils indicated its diverse applicability. Finally, purification, structural characterization of biosurfactant and genetic modulation of biosurfactant production are in perspective.

References

Adebowale, B. A., Olubamiwa, O., and Ogunjobi, M. A. K. (2011). Substitution value of sundried cashew apple bagasse in the diets of *Clarias gariepinus*. *International Research Journal of Agricultural Science and Soil Science,* **1**(7):268-272.

Adebusoye, S. A., Amund, O. O., Ilori, M. O., Domeih, D. O. and Okpuzor, J. (2008). Growth and biosurfactant synthesis by Nigerian hydrocarbon-degrading estuarine bacteria. *International Journal of Tropical Biology,* **56**(4):1603-1611.

Anyanwu, C. U., Obi, S. K. C., and Okolo, B. N. (2011). Lipopeptide biosurfactant production by *Serratia marcescens* NSK-1 strain isolated from petroleum-contaminated soil. *Journal of Applied Sciences Research*, **7**(1):79-87.

Balogun, S.A. and Fagade, O.E. (2010). Emulsifying bacteria in produce water from Niger Delta, Nigeria. *African Journal of Microbiology Research*, **l4** (9): 730-734.

International Journal of Scientific & Engineering Research, Volume 6, Issue 2, February-2015 ISSN 2229-5518

Banat, I. M., (1995). "Biosurfacts production and possible uses in microbial enhanced oil recovery and oil pollution remediation: A review", Bioresource Technology, 51: 1-12.

Cammarota, M.C. and D.M.G. Freire. (2006). A review on hydrolytic enzymes in the treatment of wastewater with high oil and grease content. *Bioresour. Technol*., 97: 2195-2210.

Chandran, P. and Das, N. (2010). Biosurfactant production and diesel oil degradation by yeast species *Trichosporon asahii* isolated from petroleum hydrocarbon contaminated soil. *International Journal of Engineering Science and Technology*, **2**(12):6942-6953.

Clift, R. (1997). Clean technology: the idea and the practice. *J.Chem. Biotechnol*., 68: 347-350.

Dandik, L., G. Arioglu and H.A. Aksoy. (1993). the enzymatic hydrolysis of used frying oil by native lipase. Appl. *Biochem. And Biotechnol*, 42: 119-126.

Demain, A. L. (1998). Induction of microbial secondary metabolism. *International Microbiology*, **1**:259-264.

Díaz, M. P., (1991). "Estudio preliminar de la aplicación de biosurfactantes en el manejo de crudos pesados". Tesis de Maestría, UIS.

Ducret, A., Giroux, A., Trani, M. and Lortie, R., (1995). "Enzymatic preparation of biosurfactants from sugars or sugar alcohols and fatty acids in organic media under reduced pressure", Biotechnology and Bioengineering. **48** (3): 214 - 221.

Duvnjak, Z., Cooper, D. G. and Kosaric, N. (1982). "Production of surfactant by Arthrobacter paraffineus ATCC 19558" Biotechnology and Bio-energetic., 24: 165 - 175.

Fiechter, A., (1992). "A review. Biosurfactants: moving towards industrial application", Tibtech, 10: 208 - 217.

Gordon, A., Friedrich, M., Matta, V. M., Moura, C., F. and Marx, F. (2012). Changes in phenolic composition, ascorbic acid and antioxidant capacity in cashew apple (*Anacardium occidentale* L.) during ripening. *Fruits*, **67**:267-276.

Harwood, J. 1989. The versatility of lipases from industrial uses. *Trends in Biochemical Sciences*, 14: 125-126.

Hauser, G. and Karnovsky, M., (1954). "Studies on the production of glycolipids by Pseudomonas aeruginosa", Biotech, 68: 645 - 654.

Hisatsuka, K.; Nakahara, T.; Sano, N. and Yamada, K., (1971). "Formation of rhamnolipid by *Pseudomonas aeruginosa* and its function in hydrocarbon fermentation", Agric.Biol. Chem., 35 (5): 686 - 692.

Hommel, R., 1990. "Formation and physiological role of biosurfactants produced by hydrocarbon utilizing microorganisms", Biodegradation, 1: 107-119.

Jaeger, K.E. and M.T. Reetz. (1998). Microbial lipases from versatile tools for biotechnology. *Reviews Trends*

Jaeger, K.E., B. Schneidinger, F. Rosenau, M. Wemer, D. Lang, B.W. Dijkstra, K. Schimossek, A. Zonta and M. Reetz. (1997). Bacterial lipases for biotechnological applications. *Journal of Mol. Catalysis B: Enzymatic*, 3: 3-12.

Kosaric, N., Cairns, W. L. and Gray, N.C.C. (1993)."Surfactant science series", Biosurfactants and biotechnology, Marcel Dekker, Mew York Basel, 25.

Malcata, F.X., H.R. Reyes, H.S. Garcia and C.G. Hill. (1992). Kinetics and mechanisms of reactions catalyzed by immobilized lipases. *Enzyme Microb. Technol*., 14: 426-445.

Montes de Oca G, M. A., (1992). "Producción de biosurfactantes microbianos", Revista del Instituto Mexicano del Petróleo, **24** (4): 68 - 78.

Okpashi, Victor. Eshu, Ogana, Joy; Bayim, Peter-Robins Bayim and Obi-Abang M, (2014). Emulsion produced from fluted pumpkin (telfiaria occidentalis hook f) seeds oil and physico-chemical properties. International Journal of Scientific and Engineering Research, **5**(10), pp: 1234-1293 ISSN 2229-5518.

Rodrigues, L.R., Teixeira, J.A., Van der Mei, H.C. and Oliveira, R. (2006). Physiochemical and functional characterization of a biosurfactant produced by *Lactococcus lactis* 53. *Colloidal Surface. B*., **49**(1):79-86.

International Journal of Scientific & Engineering Research, Volume 6, Issue 2, February-2015 ISSN 2229-5518

Saharan, B. S., Sahu, R. K. and Sharma, D. (2011). A review on biosurfactants: fermentation, current developments and perspectives. *Genetic Engineering and Biotechnology Journal*, **29**:1-14.

Samadhan Waghmode, Chandrashekhar Kulkarni, Sneha Shukla, Priyanka Sursawant, Chaitanya Velhal, (2014). Low Cost Production of Biosurfactant from different Substrates and their Comparative Study

Sivagurunathan, P., Sivasankari, S. and Muthukkaruppan, S. M. (2010). Characterization of cashew apple (*Anacardium occidentale* L.) fruits collected from Ariyalur District. *Journal of Biosciences Research,* **1**(2):101-107.

Umeji, A. A., Onwurah, I. N. E. and Anyanwu, C. U. (2010). Isolation and characterization of biosurfactant produced by a diculture of octadecane". Applied and Environmental Microbiology, **60** (6)pp: 2101-2106.

Pseudomonas spp and *Azotobacter vinelandii. Nigerian Journal of Biochemistry and Molecular Biology,* **25**(2):78-85.

Van Dyke, M. I., Couture, P., Brauer, M., Lee, H. and Trevors, J.T., (1993). "*Pseudomonas aeruginosa* UG2 rhamnolipid biosurfactants: structural caracterization and their use in removing hydrophobic compounds from soil". Journal of Microbiology, 39: 1.071 - 1.078.

Volkering, F., Breure, A. M., Van Andel, J. G. and Rulkens, W. H., (1995). "Influence of nonionic surfactants on bioavailability and biodegradation of polycyclic aromatichydrocarbons", Applied and Environmental Microbiology, **61**(5), pp: 1699 - 1705.

Zhang, Y. and Miller, R. M., (1994). "Effect of a *Pseudomonas* rhamnolipid biosurfactant on cell hydrophobicityand biodegradation of

IJSER