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RESEARCH ARTICLE

PRODUCTION OF BIOSURFACTANT BY *PSEUDOMONAS TAENENSIS* USING DIFFERENT SOLID AND LIQUID WASTE AS ENERGY SOURCES

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ABSTRACT

Persistence and recalcitrant of materials in the environment for longer period of time that has surfactant which are main ingredients of some products that are our basic needs e.g. detergents, shampoos, toothpaste, soap e.t.c. are of great concern. These surfactants are mainly of chemical origin which gives the scientists keen interest and reason for replacing chemical surfactant found in the products with biosurfactants. Biosurfactants are surface active compounds that have key roles in various field of applications such as bioremediation, biodegradation, enhanced oil recovery, pharmaceutics, food processing among many others. This study aimed at the production of low cost biosurfactant using different solid and liquid wastes as energy sources. Biosurfactant was produced in the fermentation broth at 24hrs to 168hrs using different solid and liquid wastes and was extracted using solvent extraction method (methanol and chloroform) in the ratio of 2:1. The biosurfactant was purified using open column chromatography and characterized using thin layer chromatography. Result showed that potato peel waste produced the highest biosurfactant yield of 21.0mg/L at 168 hrs while groundnut cake had the lowest biosurfactant yield of 0.09 mg/L at 24 hrs. Eluents of molasses as energy source in the fermentation broth from open column chromatography was the only informative one which showed that the biosurfactant present is a glycolipid and thin layer chromatography showed that the sugars present were: arabinose, glucose and ribose sugar. Result showed the potential of *Pseudomonas taenensis* to successfully producedlow cost biosurfactant that could be used in many industrial sectors.

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INTRODUCTION

Toothpaste, personal hygiene, cosmetic products and other pharmaceutical by-products contain surfactants and emulsifiers that are used in our daily routine basic activities which result to increase and huge in market demand for such products. This products all contain surfactants that are chemically synthesize and general desire to find replacement surfactants to the chemically synthesized compounds with biological products has emerged due to the non-biodegradability, ability to accumulate and toxicity of some of the chemical petroleum based product to the environment (Satpute *et al*., 2010a,b; Marchant and Banat, 2012a,b). Biosurfactants can be said to be biological surface-active agents (surfactants) capable of reducing interfacial tension between liquids, solids and gases, thereby allowing them to mix and disperse readily in water or other liquids. Complex molecules covering a wide range of chemical types including peptides, fatty acids and phospholipids are microbial surfactants (Cooper and Zajic, 1980). Aerobic microorganisms uses carbohydrates, hydrocarbons, animal and vegetable oils or a mixture of them as carbon source to produce microbial surfactants (Bognolo, 1999 and Fiechter, 1992). The main group of biosurfactant-producing microorganisms are bacteria, although they are also produced by some yeast and filamentous fungi (Desai and Banat, 1997). Biosurfactants can be produced intracellularly and/or can be excreted to the culture media. When the biosurfactants are intracellular, their structure includes membrane lipids, and promotes the transport of insoluble substrates through the membrane. But when they are extracellular, the biosurfactantshelp in the substrate solubilization and are usually a complex structure of lipids, proteins and carbohydrates (Adamczak and Bendnarski, 2000; Chayabutra *et al*., 2001). Solubilization beforedegradation or metabolism by microorganisms are required in hydrophobic pollutants present in petroleum hydrocarbons, soil and water environment (Chen *et al.,* 2007). Biological surfactants have many advantages over their chemical similitude's as they are easily degraded by the microorganisms, they have low toxicity, they can be produced from very cheap raw materials, they are

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not easily affected by environmental factors such as temperature, pH, ionic strength and they have the unique property of biocompatibility and digestibility (George and Jayachandran, 2012). Biosurfactant play an important role in various fields like bioremediation, biodegradation, oil recovery, food, pharmaceutics, and many other applications in different industrial sectors (Cameotra and Makkar, 2004; Banat *et al*., 2010; Fracchia *et al*., 2014; Franzetti *et al*., 2014). The structural and functional novelty of such surface active molecules is attracting the attention of many researchers throughout the world. Their synthesis processes take place on water soluble and insoluble substrates (Satpute *et al*., 2010c). Esterases are among enzymes, which are shown to be produced in the culture media when the biosurfactantsproduction is at its peak, thus forming a complex with biosurfactants and this interplay between the two greatly helps in the emulsification of the hydrophobic substrates (Sekhon *et al*., 2011; Tsujita *et al*., 1990). Agro-industrial wastes contain high amounts of carbohydrates and lipids and hence can be used as a rich carbon source for microbial growth (Fiechter, 1992; Lee *et al*., 2008). This study evaluated the production of biosurfactantsusing renewablesolid and liquid waste that are easily available in the environment as sourcesto produce low cost biosurfactant.

MATERIALS AND METHODS

Organism

The organismPsuedomonastaenensis (Gram-negative bacilli) was isolated from soil contaminated with hydrocarbon collected from automobile shops and was chosen as a potential biosurfactant producer after undergoing series of screening test for biosurfactantproduction in theprevious work of Adebajo *et al*., 2016 and was maintained on nutrient agar.

Cell Dry Weight Determination

Ten millitre aliquot from the fermentation broth was centrifuged at 3 000 rpm for 50 min, thereafter the cells were collected, washed with distilled water and then dried to a constant weight at 60^{o}C as described by Aswini *et al.,* 2013

Production of Biosurfactant using Different Waste as Substrate

Potential biosurfactant producer was cultured in 250mL of fermentation medium which contains (g/L): 1.0 K_2HPO_4, 0.2 $MgSO_4$. $7H_2O$, 0.05 $FeSO_47H_2O$, 0.1 $CaCl_2$. $2H_2O$, 0.01 $Na_2MoO_4.2H_2O$, 30 NaCI and 5g each of the solid waste (cassava flour waste, potato waste, peanut cake, molasses and cassava waste peel or 2% liquid waste (cassava waste water, palm oil mill effluent, waste lubricant oil and waste cooking oil) as carbon source were added and autoclaved at 120°C for 15 mins. The pH was maintained at 7. The sterilized medium was inoculated with 5mL of culture broth and the content was mixed properly and incubated at 35°Cin an orbital rotary shaker set at 120rpm min^{-1} for 7 days (Govidammal and Parthasarathi, 2013; Tambekar and Gadakh, 2013).

Extraction of Biosurfactant

Biosurfactant was extracted using solvent extraction method. Cell-free supernatant was obtained by centrifuging the fermentation broth at 13,000 rpm for 30 mins and supernatant was used as crude source. Hydrochloric acid was added to the supernatant in order to precipitate lipid and proteins up to pH 2.0 and kept overnight at 4°C. It was again centrifuged at 10,000 rpm for 20 mins. For further extraction, chloroform and methanol (2:1 v/v) was added to the supernatant in a separating funnel and biosurfactant within the organic layer was collected and evaporated to dryness using rotary evaporator. The remaining residue was dispensed in sodium phosphate buffer (pH 7.0) and stored at 4°C (Suresh *et al*., 2012)

Purification of Biosurfactant

The precipitate or biosurfactant was allowed to run through open column chromatography packed with sephadex LH 20 (Sigma Aldrich) as the stationary phase and methanol as the mobile phase. The velocity of flow was 12 seconds per drop and methanol was the only flow solvent. Eluents from the column was collected in clean glass bottles. At the end of the collection, methanol in the glass bottle was allowed to evaporate leaving the fractionated compound (Qiao and Shao, 2010; Kim *et al.,* 2010).

Characterization of Biosurfactant

Open Column Chromatography

Eluent was assayed for amino acids (Ninhydrin reagent), lipids (Rhodamine B) and carbohydrate (Anthronereagent). Aliquots (0.04ml) of the fractionated biosurfactant was taken with capillary tubes and 0.4 ml of reagents was added, after which they were shaken and observed for colour change. Positive result for amino acids showed purple or pink colour, positive result for carbohydrate showed bluish green colour and positive result for lipids showed purple colour.

Thin Layer Chromatography (TLC)

This was carried out using a modified method of Santhini and Parthasarathi (2014). One gram of sample was weighed and suspended in 10mls of 80% ethanol. The suspension was refluxed for 1 hr and filtered through Whatmann No 1 filter paper. The residue was washed twice with 10 mls of ethanol. The extracts was concentrated to 10mls using a rotary evaporator. Aliquots of 50µL of the extract were spotted on Avicel-crystalline cellulose (Whatman analytical plates) along with 20µL of reference standard mixture. The reference mixture contained sucrose, glucose, lactose, galactose, fructose, maltose, mannitol and raffinose (sigma products) each present at a concentration of 0.1%. One dimensional ascending chromatography was done. The solvent system employed was n-propanol- ethylacetate and water at a ratio of 6: 1: 3vol/vol. After 6 hrs, the oligosaccharides were located by spraying with modified α-naphthol reagent (0.5% α-naphthol in acetone) (Santhini and Parthasarathi, 2014).

RESULTS

Biomass Yield for Biosurfactant Production by *Pseudomonas taenensis*

Cooking oil and waste engine oil at 24 hrs had highest biomass yield and at 48hrs pome produced the highest biomass up to 72 hrs and later reduced at 96 hrs while waste cooking oil had the highest biomass at 96 hrs and 120 hrs. Pome produced the highest biomass at 144 hrs and 168 hrs (Figure 1)

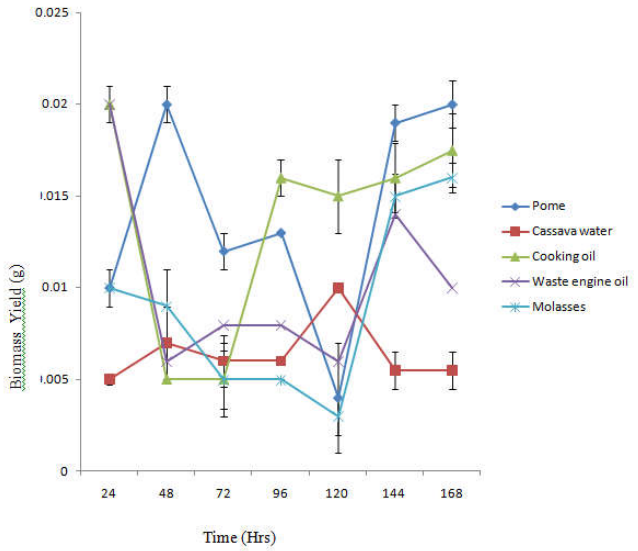

Figure 1. Biomass Yield for Biosurfactant Production by *Pseudomonas taenensis*

Production of Biosurfactant by *Pseudomonas taenensis*

Ability of *Pseudomonas taenensis*to utilize different solid waste: groundnut cake, cassava flour waste, cassava peel, potato peel and liquid waste: palm oil mill effluent, molasses, cassava water, cooking oil and waste engine oil as energy sources varies to different degree. Biosurfactant production yield of *Pseudomonas taeanensis* (1.92×10^4 CFU/mL) in the fermentation broth (Figure 2) was observed to be highest at 168 hrs of 21.0 mg/L when potato waste was used as substrate and lowest in groundnut cake of 0.09 mg/L at 24 hrs.

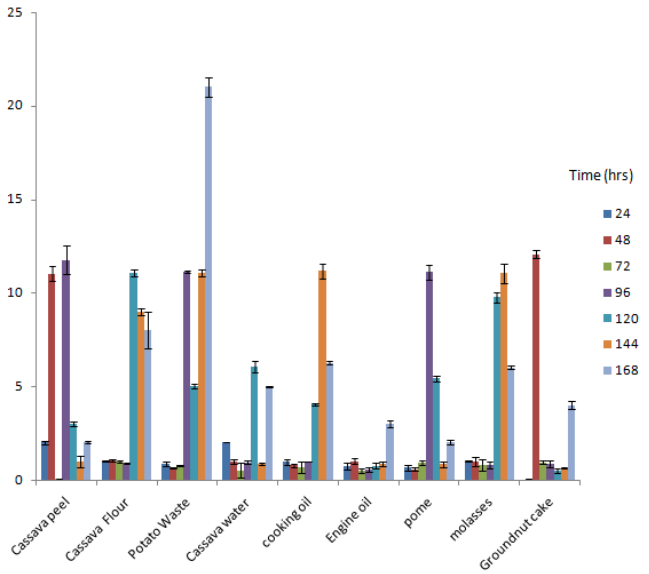

Figure 1.Biosurfactant produced by *Pseudomonas taenensis* at different time interval

Characterisation of Biosurfactant using open column and thin layer chromatography

Eluents were collected when biosurfactant produced by the bacterial isolate was passed through open column chromatography using sephadex LH 20 as the stationary phase and methanol as the mobile phase. A total of 22 eluents were collected when waste engine oil and waste cooking oil were characterized, 40 eluents were collected when waste cassava flour waste, waste cassava peel and waste potato peel and 54 eluents were collected when waste cassava water, palm oil mill effluent, groundnut cake and molasses were characterized. Nihydrin reagent did not react to any of the eluents. Of all the eluents collected, eluent 7 to 35 collected from the biosurfactant produced by P*seudomonas taenensis* were the most informative one when molasses was used as substrate (Table 1).

Table 1. Characterisation of Biosurfactant using Open Column Chromatography Packed with Sephadex with Molasses as Substrate

Isolate	Anthrone	Nihydrin	Rhodamine B	Name of biosurfactant
Pseudomonas taenensis	+	-	+	Glycolipid

Key: - Negative, + Positive

Morealso, TLC result analysis of biosurfactant produced by P*seudomonas taenensis*when molasses was used as substrate (A) showed that the sugars present were: glucose, arabinose and ribose sugar (Plate 1).

Plate 1. Characterisation of Biosurfactant using Thin layer Chromatography for Sugar Analysis

DISCUSSION

The capabilities of the organisms to increase biomass yield using different waste as substrate may be an indication of the different microbial activities and the nutritional constituents of the substate. Agro-industrial waste contains high amount of carbohydrates, lipids and hence, can be used as a rich carbon source for microbial growth. Achieving cost effective biosurfactantproduction depends on the development of

cheaper processes and the provision of low cost substrate raw material.Different quantity of biosurfactant was produced at different time interval. The quantity of biosurfactant production by *Pseudomonas* sp, however disagrees with the work of Varadharajan and Subramaniyan, 2011 which couldbe because of the percentage of carbon source that was used (1% carbon source was used for their biosurfactant production). *Pseudomonastaenensis* had its maximiumbiosurfactant production at 168hrs which also was in accordance with the work of Sriparna *et al.* (2011). Biosurfactantproduced by waste engine oil was the least among all the wastes used and this may be improved when all the parameters of production were optimized. The biosurfactant produced was of carbohydrate and lipid based and was a glycolipid which is similar to the work of Bhavani and Hemashenpagam (2013).

Conclusion and Recommendations

In this study, the potential of *Pseudomonas taenensis* for biosurfactant production had been investigated, the present study thereby recommend the use of *Pseudomonas taenensis* MS-3 for producing biosurfactant that further could be used for many industrial purposes.

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