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Cost Effective Production of Biosurfactant Using Banana Peel

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ABSTRACT: The cost of production of biosurfactant is much higher than that of synthetic surfactant due to which synthetic surfactants are in demand. This problem can be solved using agro waste for production of biosurfactants. The present study is carried out to develop cost effective production method using banana peel. Nutritional characteristics of banana peel revealed its potential to be used as raw material for the production of biosurfactant. The potential biosurfactant producing fungi was isolated from oil contaminated soil and was identified as *Aspergillusniger NJP09*. Biosurfactant production was carried out using tap water formulated with insoluble carbon source as used engine oil and banana peel. 3gms of biosurfactant/100g of banana peel was obtained from formulated media. Extracted biosurfactant was found to recover 20% of absorbed used engine oil from sand.

KEYWORDS: Biosurfactant, banana peel, *Aspergillusniger*, Used engine oil.

I. INTRODUCTION

Large consumption of petroleum products results in large amount of hydrocarbon discharge annually in the environment (ArezoDadrasnia *et al.*, 2015). Approximately 8.4 million tons of petroleum products diffuse into the ocean each year making a large impact on aquatic ecosystem and the alkanes present in the petroleum products are proved to be narcotic, irritant, toxic, carcinogenic, teratogenic and mutagenic (Henrique F S *et al.*, 2015; JixiangGuo *et al.*, 2012). Synthetic surfactants are used as main ingredient in lots of commercial products, but the role of synthetic surfactant has been reported as a major pollutant (Chioma Blaise Chikere *et al.*, 2012; Christina Cowan-Ellsberry *et al.*, 2014). Hence, the production of non toxic biological counterpart from raw waste material would be an ideal alternative over chemical surfactant.

II. LITERATURE SURVEY

Biosurfactants are gaining a lot of attention due to their low toxicity, specificity of action and easy production (Ahmed M Elazzazy *et al.*, 2015). Biosurfactants are not commonly used as its production requires costly substrates and provides low yield of product along with undesirable byproducts mixed with biosurfactants (Ahmed M Elazzazy *et al.*, 2015). Production cost of biosurfactant can be reduced by utilizing various renewable materials/wastes along with the use of microbe in utilizing that waste for production of biosurfactant (Randhir S Makkaret *et al.*, 2011). *Yarrowialipolytica*s has reported to grow and produce biosurfactant in media in which traditional carbon sources have been replaced by agricultural waste (Fontes *et al.*, 2012).

Banana peel, pineapple peel and papaya peel are major wastes generated by fruit processing industries contain simple and complex sugars that are metabolizable by microorganisms. Banana is one of the most abundantly available fruit in tropical countries. World production of banana is estimated at 48.9 million tons. The main by-product of the banana processing industry is the peel, which represents approximately 30% of the fruit and contains large quantities of

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nitrogen and phosphorus and its high water content makes it susceptible to modification by microorganisms. The present work is an attempt to develop cost effective production of biosurfactant using banana peel by fungi isolated from oil contaminated soil.

III. MATERIALS AND METHODS

A. Collection and Nutritional characterization of banana peel

Robusta Banana (*Musa acuminata*) was collected from a juice centre located near Ulhasnagar railway station (Thane-District, Maharashtra, India), the peels were immediately transported to laboratory and washed using sterile distilled water to remove undesirable surface materials and studied for its nutrient content.

Carbon and Organic matter content of banana peel were determined by Adams R. C *et al.*, (1951). Banana peel was also studied for its Nitrogen content (Macro Kjeldahl method), Phosphorous content (ANSA method), Carbohydrate content (Phenol sulphuric acid method) by Dubois *et al.*, (1954) and Protein content by Folin-Lowery method (1951).

The micronutrients of banana peel and tap water was determined using Himedia K095 micronutrient testing kit. pH was detected using the liquid universal pH indicator.

B. Isolation and Screening of biosurfactant producing organisms

Soil samples contaminated with petroleum oil were collected from petrol pump. The collected soil was inoculated in MM medium (Minimal mineral medium) supplemented with 10% banana peel (BP) and 1% used engine oil. Biosurfactant activity of the isolates were screened by Drop collapse test and Hemolysis on blood agar as mentioned by Camila Rattes de Almeida Couto *et al.*, (2015) and EI-Shahawy, (2014).

Isolates showing positive activity were further studied for quantitative activity by xylene emulsification assay (Satpute S K *et al.*, 2008).

C. Identification of biosurfactant producing organisms

Selected isolate with potential surfactant activity was identified on the basis of morphological features and PCR (ITS sequencing technique).

D. Cost effective production of Biosurfactant

Cost effective fermentation media was designed using tap water whose mineral content was determined. Mashed banana peel was added to tap water whose pH was adjusted to 6. After autoclaving 3% engine oil was added as insoluble carbon source. Inoculum was prepared by growing isolated and selected biosurfactant producing fungi on Rose Bengal agar medium and spore density was adjusted to 5×10^8 spores/ml by hemocytometer method. 5ml inoculums was added to formulated fermentation medium and incubated at room temperature (27°C) on shaker for 6 days. After every 24 hours of incubation biosurfactant activity was assessed by xylene emulsification assay.

E. Extraction and Application of biosurfactants

The extraction of crude biosurfactant was carried out using chilled acetone following the method described by Pruthi and Cameotra, (1995). Extracted biosurfactant was studied for its carbohydrate content by Phenol sulphuric acid method (Dubois *et al.*, 1954) and protein content by Folin-lowery method. Extracted biosurfactant was used for desorption of used engine oil from sand column: Sand was obtained from seashore, and washed to remove salt and dust. Sand was saturated using known amount of used oil and extracted biosurfactant was used for desorption of adsorbed oil (Eduardo J. Gudina *et al.*, 2015).

IV. RESULTS AND DISCUSSIONS

A. Collection and characterisation of banana peel

Benjamin Anhwange, (2008) reported the presence of 8.50% ash and 91.50% organic matter which included lipids proteins and carbohydrates in banana peel. In the present study, the collected banana peel found to contain 53% % ash,

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62.25% of organic matter while carbon was found to be 36.11%. The adulteration of fruits after harvesting and auto enzyme activity affect the constituents of fruits.

Nitrogen and phosphorous contents of a substrate are important for growth of microorganisms. 0.28% of nitrogen and 2.64% of phosphorous was found to be present in banana peel. HuajunZhenget *al*, (2013) have reported 0.21% nitrogen and less than 2.64% of phosphorous while studying selective adsorption of Au from waste printed circuits using banana peel. High content of phosphorous in media is beneficial for microbes as phosphorous is a constituent of nucleic acid, nucleotides phospholipids and lipopolysaccharides (ShyamalaBellure *et al*, 2011).

ShyamalaBellure *et al*, (2011) reported 0.09-0.419mg carbohydrate/g of banana peel. Benjamin Anhwange *et al*, (2009) reported the presence of 0.9% of protein in banana peel but in the present study banana peel showed 0.37% protein and 0.377mg carbohydrate/g of banana peel. This may be due to improper handling of banana by shop vendor due to which the peels must have been damaged or it can also be due to artificial maturation of banana in the presence of chemicals. Auto degradation and over maturation also effect the protein content of banana peel.

Micronutrients are very important cofactor for various enzymes involved in electron transfer and nitrogen fixation (Trevor *et al*, 1990: Archibald F , 1986), pH of banana peel was found to be 5.5-6.0 and 7.50 that of water. Micronutrient content of banana peel and water is depicted in table 1. Micronutrients like Copper, Molybdenum, Iron, Boron and Zinc have been found to present in banana peel.

Table 1: Micronutrient content and pH value of banana peel and water

Micronutrient content and pH value	Banana peel	Water
Copper	0.0 – 0.20 ppm/5g	0.0 – 0.20 ppm/10ml
Molybdenum	0.0 – 0.05 ppm/5g	0.0 – 0.05 ppm/10ml
Manganese	0.2 – 1.0 ppm/5g	0.2 – 1.0 ppm/10ml
Iron	3.0 – 4.5 ppm/5g	3.0 – 4.5 ppm/10ml
Boron	2ppm/2g	2ppm/10ml
Zinc	2ppm/2g	2ppm/10ml
pH	pH5.5-6.0	pH 7.5

B. Screening and Identification of biosurfactant producing organisms:

Fungal organism isolated from oil contaminated soil was selected for study on the basis of positive drop collapse test, hemolytic activity and higher xylene emulsification property. Similar methods of isolation and screening of the biosurfactant producing organisms have been followed by Adria A B *et al*, (2003): EI-Shahawy , (2014) and Satpute *et al*, (2008).

Morphological features of the isolate were studied by wet mount staining with lactophenol cotton blue. Figure 1 shows mycelia and spore bearing structure of biosurfactant producing fungi. The morphological observation revealed presence of black coloured spores arranged on spherical sporangiospore. The large globose, black conidial heads were found become radiate and split into several loose columns with age.

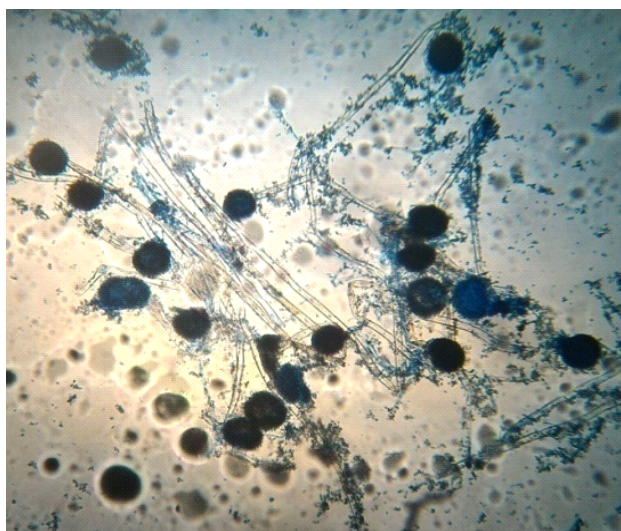
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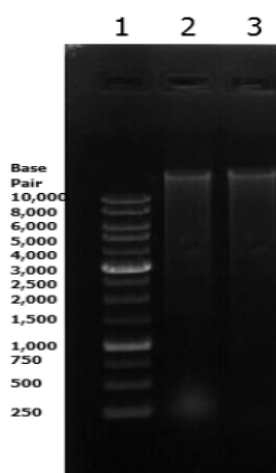
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Figure 1: Morphological structure of biosurfactant producing selected fungi.



PCR identification was done using ITS sequencing technique for which genomic DNA was isolated using CTAB method and purified with lithium chloride treatment. Genomic DNA was loaded on 0.8% agarose gel alongwith ladder. Figure 2 shows the separated bands of the genomic DNA and standard ladder (250-10,000 bp). The separated genomic DNA bands are seen above the 10,000bp band of standard ladder confirming the size of genomic DNA to be greater than 10,000bp.

Figure 2: Genomic DNA isolated by CTAB method and loaded on 0.8% Agarose gel stained with 0.1µg/ml EtBr. Lane 1:ladder, Lane 2: gDNA, Lane 3: gDNA



The total volume of reaction mixture for PCR was 50µl and the reaction was run till 35 cycles. The figure 3 shows the separated bands of the PCR amplified fragment of genomic DNA and standard ladder (400-1000bp). The PCR amplified fragment of genomic DNA corresponds at 700bp band of standard ladder. Gel extraction kit was used for

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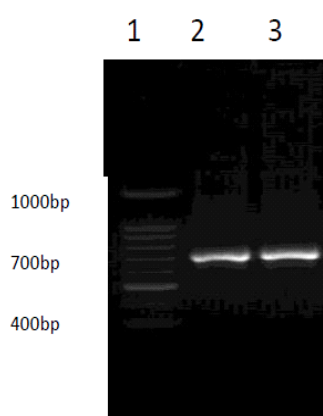
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excision of gel and extraction of PCR product. The gel purified product was then sequenced and the raw sequence was aligned using multalin software and the final sequence was submitted to blast tool of NCBI.

On the basis of morphological features and PCR identification, the selected isolate was identified as *A. niger*NJP09.

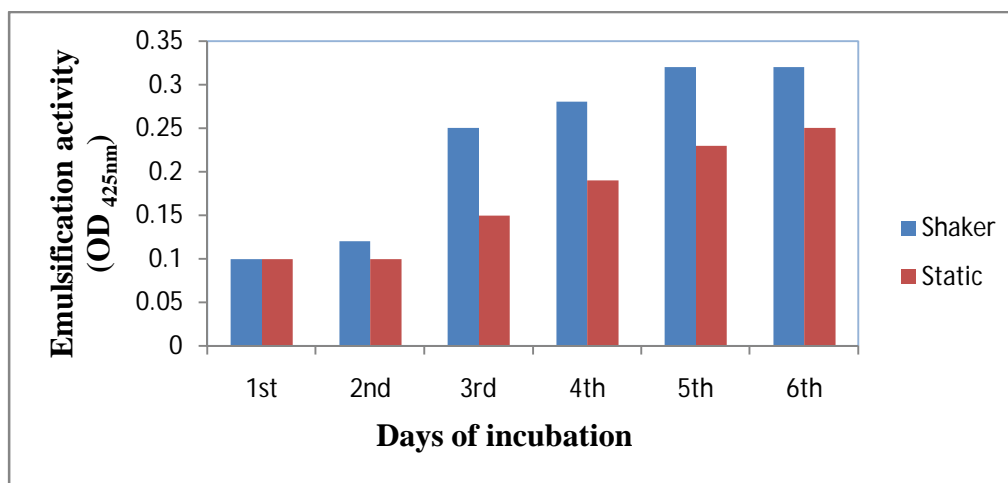
Figure 3: PCR products loaded on 1.5% Agarose Gel stained with 0.1µg/ml EtBr. Lane 1: Ladder 100bp, Lane 2: PCR product, Lane 3: PCR product.



C. Production of biosurfactant using banana peel

Insoluble hydrocarbons are reported to induce biosurfactant production, thus in order to make the biosurfactant production cost effective instead of mineral medium base tap water was used, the mineral content of tap water is shown in (Table 1). Akintunde T *et al.*, (2015) had reported 0.28gm of biosurfactant from 50ml mineral medium supplemented with crude oil while Vilasis G M *et al.*, (2009) had reported biosurfactant production using 1% diesel oil by *A. fumigates*. *Rhodococcus wratislaviensis* has reported to utilize n-hexadecane as a carbon source for trehaloselopid production when incubated at 28°C after 7 days on shaker condition (B. Tuleva *et al.*, 2008).). In the present study, biosurfactant activity was found to increase with incubation period upto 5 days, but on 6th day the emulsification was almost same as that on 5th day (Figure 4). A slight increase in biosurfactant activity was observed after 4th day of incubation. Incubation on shaker showed higher biosurfactant production compared to static condition. Highest growth and biosurfactant production have been reported at a temperature range between 24 – 37°C (Passamani F R *et al.*, 2014).

Figure 4: Biosurfactant production using banana peel by isolated *Aspergillus niger* NJP09



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D. Extraction and characterization of biosurfactant

Extracellular biosurfactant produced by *Aspergillusniger* was extracted using chilled acetone and 3gm of crude biosurfactant was obtained from 100gm of banana peel. Production of biosurfactants using pure carbon sources like hydrocarbons and sugars is very expensive. In the present study, though the amount of biosurfactants produced was less compared to the reported value by Pruthi V, S SCameotra, (1995), but the process is cost effective. Extracted biosurfactant was found to contain 53.27mcg of carbohydrate and 71.33mcg of protein per gram of biosurfactant.

E. Application of extracted biosurfactant in oil recovery

Desorption of insoluble oil (used engine oil) was studied according to the protocol mentioned by Eduardo J. Gudina *et al.*, (2015). 0.1mg/ml concentration of biosurfactant was able to recover $9\% \pm 0.05\%$ of oil whereas 1mg/ml concentration was able to recover $20\% \pm 0.05\%$ of oil. Addition of biosurfactants can be expected to enhance hydrocarbon biodegradation by mobilization, solubilization or emulsification (Nguyen T Tet *et al.*, 2008; Deziel E *et al.*, 1996; Bai G Y *et al.*, 1997; Rahman K S M *et al.*, 2003; Urum K *et al.*, 2004; Nieves M L *et al.*, 2008).

V. CONCLUSION

The cost of production of biosurfactant is much high than that of synthetic surfactants due to which synthetic surfactants are in demand. This problem can be solved by using agro waste for the production of biosurfactant, which will help to reduce the cost and also to utilize the agro waste which is left in the nature which can cause pollution problems. The use of cheap raw materials and wastes contribute immensely towards the reduction of production cost. Consequently, the potential applications of biosurfactants on a commercial scale should be encouraged. In addition, the selected *Aspergillusniger* in present study reflects its potential towards not only in utilization of waste from agro industry but can also be exploited for recovery of oil and remediation of oil contaminated site.

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