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PRODUCTION AND CHARACTERIZATION OF POLYHYDROXYALKANOATE OBTAINED FROM PINEAPPLE PEEL WASTE

PAWAR P, MODI M AND SHRIVASTAV A*

Assistant Professor, Parul Institute of Applied Sciences, Parul University, Limda, Waghodiya Road, Vadodara-390025, Gujarat

***Corresponding Author: Dr. Anupama Shrivastav: E Mail: anupama.shrivastav82045@paruluniversity.ac.in**

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ABSTRACT

The aim of this study was to produce and characterize the Polyhydroxyalkanoates which includes different pineapple peel waste fermentation conditions. The produced biopolymer was characterized using FTIR.. The use of bioplastics has gained the momentum as it is biodegradable to environment. Use of cost effective and easily available substrates in the production of polyhydroxyalkanoate (PHA) enhances its application and hence PHA can be successfully used as a substitute for plastics. The present work was performed to study the optimum production of polyhydroxyalkanoate (PHA) by bacteria isolated from soil and effluent samples using pineapple peels as carbon source and its efficiency was checked by PHA production at different time intervals. The maximum PHA production was observed with pineapple peel as carbon source. Thus, waste food as peels could be utilized as alternate sources of substrates for PHA production. Further investigations are undertaken.

Keywords and abbreviations: Biopolymers, PHB, Pineapple Fermentation, FTIR

1. INTRODUCTION:

Pineapple agro-industrial waste has been studied regarding glucose syrup production and preparation of high surface area activated carbon with pineapple peel. Regarding food industry applications, determined pineapple peel physicochemical properties, yogurt and vinegar **[1]**. Another alternative to the pineapple peel agro-industrial waste is its use in biopolymer production, such as polyhydroxyalkanoates (PHA), by the fermentation.

PHAs are biopolymers that are synthesized by many gram-positive and gram-negative bacteria that are stored in an intracellular inclusion bodies as an energy reserve, in the response to carbon source excess and under the nutrient-limited conditions **[2-4]**.

Food waste management has now become a great challenge globally. Statistics report that 33-50% of all food produced is not consumed and when food waste is used as landfill, methane is generated, which is more deadly than carbon dioxide **[5]**. Thus, food wastage increases the carbon pollution of the environment.

The aim of this study is to research the polyhydroxyalkanoate production by fermentation using the pineapple agro-industrial waste as substrate, particularly focusing on the fermentation condition effect on biopolymer structural properties.

Hence the food waste management has gained significance and food waste has valuable biomass which could be used as efficient carbon sources to create eco-friendly industrial products. Thus, biotransformation of vegetable and fruit wastes could aid in production of useful industrial products **[6]**. The conversion of food waste to biodegradable eco-friendly plastics could be a good alternative to replace synthetic plastics. Synthetic plastics were favoured for their good mechanical and thermal properties. But its persistence in the environment has raised several problems in the ecosystem. Hence, replacement of non-biodegradable plastics by polyhydroxyalkanoate (PHA), a biodegradable polymer has gained momentum. The increased cost in production of PHA has discouraged the use of the polymer.

However, the use of inexpensive and renewable carbon substrates as an agro wastes and by products can contribute towards the reduction of cost by 50%. Hence the production of vegetable and fruit peels can provide cost effective and environment friendly biodegradable polymer.

There are very few reports on the utilization of starch by bacteria to produce PHA. Hence, the aim of the current study was to explore and evaluate the potential of the isolate to produce PHA from pineapple peels **[9]**.

2. MATERIALS AND METHODS

2.1 Sample Collection

The bacteria used in this study were collected from Soil samples and effluent for screening of best PHA producing bacteria.

2.2 Serial Dilution

Soil and effluent samples were collected in clean bags. One gram of the soil sample is dispensed in 10ml of sterile distilled water. This is then mixed vigorously and 1ml from this is taken out and added to another tube with 9ml sterile distilled water to get a dilution of 10^{-1} **[13]**. This serial dilution is then repeated to get the dilutions of 10-2, 10-3, 10-4, 10-5, 10-6and 10-7. For the isolation of the organisms. 0.1ml of each dilution was plated onto a nutrient rich medium by spread plate method for the propagation of the microbial growth. The plates are incubated at 300 C for 48 hours. Colonies with different characteristic features were maintained as the pure cultures **[14]**.

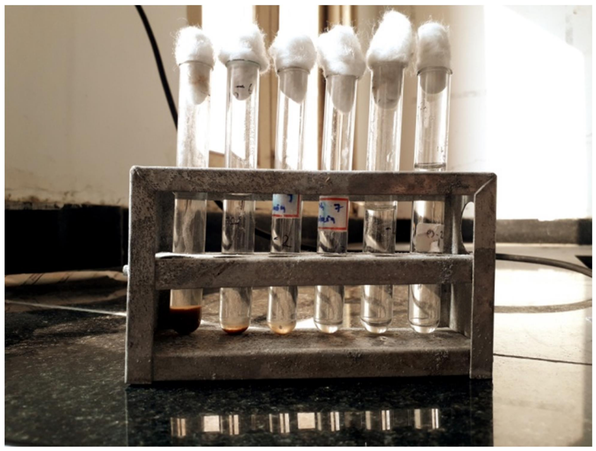

Figure 1: Serial Dilution of Soil Bacterium

2.3 Microscopic Observation-Gram Staining & Motility

The unknown bacteria were subject to Gram staining technique and motility test by hanging drop technique.

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RESULTS AND DISCUSSION:

Extraction of PHA from Bacterial cells

The PHA produced by the bacterial cells were extracted by mass culturing. Fresh culture of the selected bacterial strain (16 h) of the selected potential bacterial isolate was inoculated (3-5% inoculum) in different media, such as 1 litre of nutrient broth with pineapple peel extract, water with pineapple peel extract and incubated at 370C for 96 h in a rotary shaker. Pineapple peels were used in the study. After growth, the biomass obtained in the culture flask was subjected to PHA extraction using boiling chloroform as solvent since it is considered to be efficient for extracting polymer **[11, 12] (Figure 2)**.

Disruption of cells by Chemical Methods and PHA Estimation

Nutrient broth was prepared in test tubes and inoculated with cultures. The medium was incubated at room temperature for 24-96 hours.PHA was estimated at every 24 hours interval **[7] (Figure 3)**.

About 5 ml of culture was taken and centrifuged at 10,000rpm for 10 minutes.

The Supernatant was discarded and the pellet was suspended in 2.5 ml of sodium hypochlorite and 2.5 ml of chloroform and it was incubated at 30ºC for 1 hour. The above content was centrifuged at 1500 rpm for 10 minutes at the room temperature **(Figure 4)**.

The upper hypochlorite phase, the middle chloroform containing undisturbed cells and the bottom chloroform phase with PHA were obtained. The upper and middle phases were separated the contents were again centrifuged at 1500 rpm for 10 minutes at room temperature and the phase other than chloroform with PHA was removed carefully **[15]**.

Concentrated sulphuric acid was added to the chloroform phase containing PHA **(Figure 5)**.

Biopolymer extraction

The supernatant was removed and the decanted fraction was freeze-dried. The biopolymer was extracted from the dried biomass using chloroform in a ¼ biomass/chloroform ratio, under vigorous agi-tation, for 12 h at 30◦C. Subsequently, dispersion was filtered on no. 1 Whatman paper. After filtration, the material was added to a Chloroform/Ethanol (1/4) solution and maintained under stirring for 5 min. Finally, the dispersion was filtered under vacuum using a 25m paper, while the solid material, constituted by the biopolymer, was dried overnight . Once the polymer was dry, this was stored in glass contain-ers at room temperature in a dark place, until further use **[7] (Figure 6)**.

Biopolymer characterizations

Fourier transform infrared (FTIR)

FTIR spectra were obtained through a Perkin Elmer equipment, Spectrum One model, using a DTGS detector with 16 scans, resolution of 4 cm−1 and wave range of () 3500–500 cm−1 at 22 ◦C. 3320.2 confirmed the presence of unbounded hydroxyl group C=c.1645 ,c-o-c. 1078.33.-C-H.880.56.c=N **(Figure 7)**.

Figure 2: Pineapple Peel

Figure 3: Nutrient Broth Culture after Feeding Bacteria

Figure 4: separation of supernatant

Figure 5: pellet formation and Pellet after centrifugation

Figure 6: Sample Film of Bioplastic

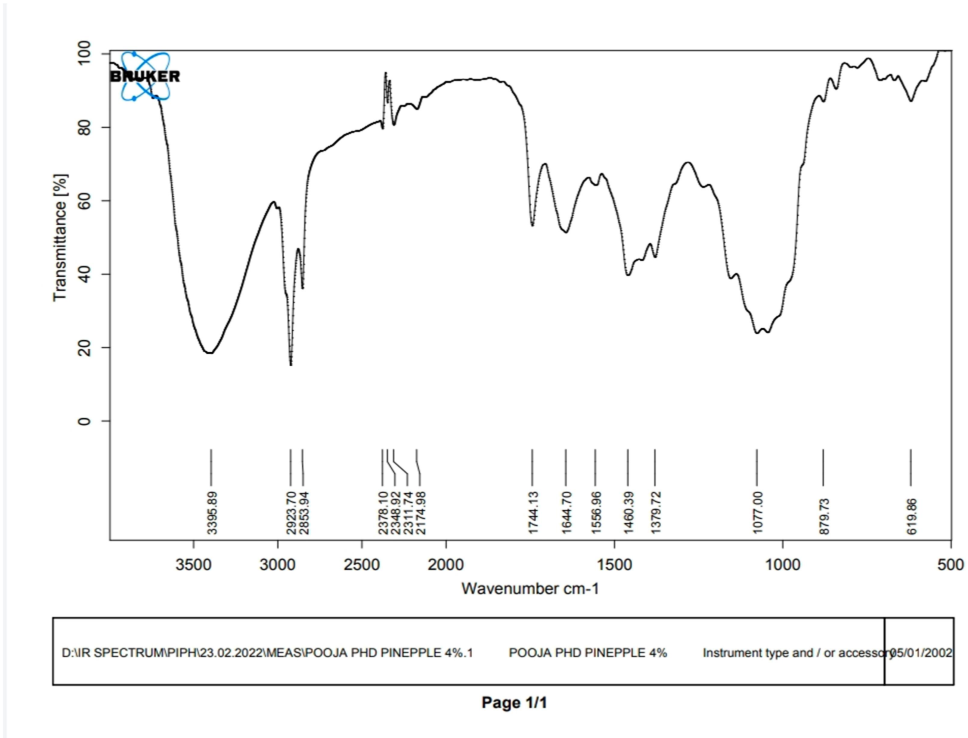

Figure 7

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