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Research Article

PRODUCTION OF BIODEGRADABLE PLASTIC TO COMBAT HAZARDS OF CONVENTIONAL PLASTICS

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ABSTRACT

Plastic is a synthetic biopolymer which possess many attractive properties because of which it has become one of the most prominent products used nowadays. Despite of such undeniable positive effects of plastic on our lives, it still has several disadvantages. In the current project, an effort has been carried out to fight with such flaws by the replacement of such conventional plastics with that of biodegradable plastic. In the current study the potential and easily available bacteria were isolated from different localities and the strains potential for the production of PHB were selected for further studies. Most of the potential isolates were found to be *Bacilli*. The optimum growth and maximum PHB accumulation by isolate MDC happened at 72 h with sugarcane bagasse. Pre-treated sugarcane bagasse was a better carbon source as compared to the general production media. Biopolymer produced was confirmed by FTIR.

INTRODUCTION

During centuries synthetic polymers have been used in a variety of applications in the everyday of human life. They became essential due to its versatility, durability and low cost. However, due to their excessive use, several drawbacks as its resistance to the decomposition, toxicity after incineration and accumulation in the environment induce negative ecological impact in landfills and water contamination. Population growth has led to the accumulation of massive volume of non-degradable waste materials across our planet. The accumulation of plastic waste has become a major concern in terms of the environment[1].Conventional plastics not only take many decades during decomposition, but also produce toxins while degradation.

Simultaneously, the high amount of agro-wastes generated from food industry have been a growing concern, around 1.3 billion tons are leftover, which became an environmental and financial issue. Among these agro-waste stands out, food waste, halum and stems of vegetables/fruits, grains and a seed, from which it is possible to obtain natural polymers.

Bio-plastics are natural biopolymers synthesized and catabolized by various organisms. These get accumulated as storage materials in microbial cells under stress conditions. However, the high production cost and the availability of low-cost petrochemical-derived plastics led to bio-plastics being ignored for a long time. Biopolymer exhibit unique properties and can be produced from plants and crops wastes. Hence, there is need to produce plastics from materials that can be readily eliminated from our biosphere in an "eco-friendly" fashion[2].

These bioplastics can be produced by a variety of natural biopolymers, one of them is Polyhydroxybutyrate.

PHB is a type of polyhydroxyalkanoates, or PHA[3]. PHAs are biodegradable thermoplastics that are synthesized by many different types of bacteria. When bacteria develop in nutrient-deficient environments, bacteria create PHAs as food and energy reserves, which are then stored as insoluble granules in the cytoplasm[3,4].

PHAs are synthesized by many living organisms. The main candidates for the large-scale production of PHAs are plants and bacteria. Plant cells can only cope with low yields [<10% (w/w) of dry weight] of PHA production. High levels [10–40% (w/w) of dry weight] of polymer inside the plant have a negative effect on the growth and development of the plant. At present, this problem has not been overcome[5]. In contrast,

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within bacteria, PHAs are accumulated to levels as high as 90% (w/w) of the dry cell mass[6]. Accumulating PHAs is a natural way for bacteria to store carbon and energy, when nutrient supplies are imbalanced. These polyesters are accumulated when bacterial growth is limited by depletion of nitrogen, phosphorous[7] or oxygen and an excess amount of a carbon source is still present. While the most common limitation is nitrogen, for some bacteria, such as *Azotobacter spp.*, the most effective limitation is oxygen[8].

Thus, the main objective of the study was to isolate some easily available PHB producing living organisms and production of PHB by using low cost agricultural wastes.

MATERIAL AND METHODS

Collection of Sample

Soil samples were aseptically collected from 5 different localities in and around Nagpur city.

Isolation and Identification of Bacillus sp

Bacillus subtilis from soil was isolated by serial dilution method followed by its identification. Purified culture was stored at 4^0C. On the basis of Morphological, Cultural and Biochemical tests *Bacillus sp* was identified.

Screening for PHB Isolates

Two different methods were being used in the current project for the screening of PHB producing bacteria's.

Screening

Individual isolates were streaked on nutrient agar plates. Plates were incubated for 24 hrs at 37° C. Ethanolic solution of 0.3% (w/v in 70% ethanol) Sudan Black B was spread over the colonies. Plates were kept undisturbed for 30 min and were de-stained by washing with 96% ethanol. The colonies which retained their black color after de-staining were attributed as PHB producing strains

Screening under microscope

Heat fixed smear was Stained with 5% Sudan Black for 5 minutes. The stain was washed off with tap water. The counter stain saffranine (5%) was then applied and kept for 5 minutes. Counter stain was washed off with tap water. Slide was observed under light microscope. Cells appearing black were considered as PHB positive strains.

Preparation of Production media

General Production Media

Mineral salts medium (MSM) [composition (g/L): Urea (1.0), Yeast extract (0.16), KH_2PO_4 (1.52), Na_2HPO_4 (4.0), $MgSO_4{\cdot}7H_2O$ (0.52), $CaCl_2$ (0.02), Glucose (40), and trace element solution 0.1 ml] was used for the production of PHA by the selected isolates. The trace element solution contained (g/L): $ZnSO_4{\cdot}7H_2O$ (0.13), $FeSO_4{\cdot}7H_2O$ (0.02), $(NH_4)_6Mo_7O_{24}.4H_2O$ (0.06) and H_3BO_3 (0.06). Both glucose and trace element solution were autoclaved separately, and reconstituted prior to inoculation. The culture was prepared by sub culturing the isolates twice in nutrient broth. Then one ml of a 24 h old culture was inoculated into 100 ml production medium and incubated at 37 °C and 150 rpm for 48 h.

Production Media with Added Agro-Waste

Locally collected sugarcane bagasse was shredded into pieces and was hydrolyzed using distilled water at 60-65^{o}C.The reduced sugar contents were then extracted from the hydrolysate and 10 ml of hydrolysate was added to 100 ml of general production media. Then one ml of a 24 h old isolated culture was inoculated into 100 mL production medium and incubated at 37 °C and 150 rpm for 72 h.
(In the current project the hydrolysates of agro-waste, sugarcane bagasse were used as an excess carbon source.)

Extraction and Quantification of PHB

Ten mL of culture was centrifuged at 10,000 rpm for 15 min. The supernatant was discarded and the pellet was treated with 10 mL sodium hypochlorite and the mixture was incubated at 30 °C for 30 min. The mixture was centrifuged at 5000 rpm for 15 min and then washed with distilled water and acetone respectively. The pellet was dissolved in 5 mL boiling chloroform and evaporated by pouring the solution on sterile glass tray kept at 4 °C and weighed. The relative PHB accumulation by the different isolates was compared to help in identification of the best producer.

Measurement of Dry Biomass

For dry biomass measurement the culture was centrifuged at 10,000 rpm for 15 min, and the pellet was dried in an oven at 55 °C to constant weight.

FTIR spectrophotometer analysis of PHB

About 1 mg extracted sample of PHB was dissolved in 5 ml chloroform. After pellet was formed by adding KBr, spectra were recorded at 4000–400 cm−1 range by Spectrum 65 FT-IR[9].

Preparation of Biopolymer/ Stretch of Bioplastic

A total of 45 mg of each of the produced PHB was dissolved in 5 mL of hot chloroform and was evenly distributed in petridishes. The dishes were then maintained at 30^{o}C for complete evaporation of solvent i.e. chloroform. The evaporation of chloroform resulted in the production of PHB particles in the petridishes.

RESULTS AND DISCUSSION

The amount of plastic waste increases every year and the exact time needed for its biodegradation is unknown. Ecological awareness has impelled the development of new biodegradable materials. Bio-plastics can be defined as plastics made of biomass such as corn and sugarcane. These substances have been increasingly highlighted as means for saving fossil fuels, reducing CO_2 emission and plastic wastes. Biodegradability of bio plastics has been widely publicized in society and the demand for packaging is rapidly increasing among retailers and the food industry at large scale.

In the current project the study was carried out for the selection of different easily available species of bacteria which can process the production of PHB and the production was optimized with specific agro-waste (used as a major substrate) for maximum PHB production. For the current study different species of bacteria were isolated from different localities of

Nagpur and the cultures of these organisms were maintained at lab scale on nutrient agar slants.

After isolation and screening, blue black colored colonies were observed which showed the presence of PHB granules produced by the bacterial species.

Among different strains of bacteria isolated from five different localities only four isolates were found to be PHB producers with different relative PHB accumulation. Most of the producers identified belonged to the genus *Bacillus* and only one of the potential genus was found to be *Pseudomonas.*

Isolate MDC was found to produce maximum amount of PHB by measuring the dry biomass of accumulated PHB contents. Dry biomass measurement also revealed that the addition of excess carbon source or the sugarcane hydrolysates resulted in higher production of PHB as compared to the amount of PHB produced in general production media (MSM). Formation of a biopolymer was seen as shown in fig 1

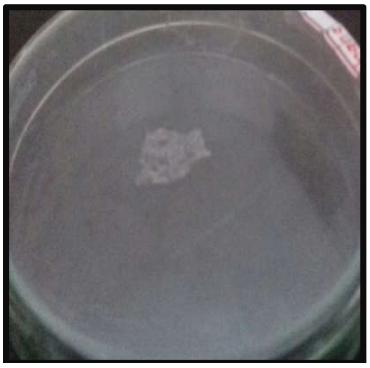

Fig 1 Sample stretch of a bioplastic film

The extracted PHB samples were then evaluated for identification of their functional groups through FTIR analysis.

In the FTIR analysis results, the peaks at 3435.22, 3473.80 indicated the stretching strong H-bond created by terminal -OH group found in sugarcane bagasse produced PHB sample as shown in fig 2.

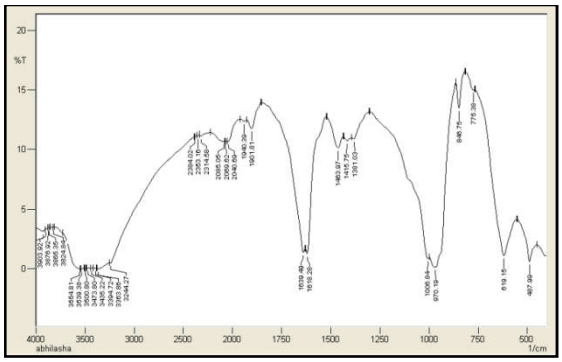

Fig 2 Peaks obtained from FTIR

The peak at 1415.75 is assigned to C-H stretching in methyl groups. The peaks at 1618, 1639.49 indicated weak C=O bond extended for carbonyl or amide group for the sample sugarcane bagasse. The peak at 1381.03 accounts for -CH2. The peak in between 1250-1300 cm-1 represents -C-O polymeric group in the sample sugarcane bagasse. Stretching other peaks 487.99, 619.15, 775.38, 848.75, 970.19, 1006.84 correspond to the presence of alkyl halides.

These all prominent absorption bands confirm that the polymer extracted from the samples were Poly beta hydroxybutyrate.

Comparable results were reported by Adwitiya *et al*[10] from *R. sphberoides* N20glucose as carbon source. Glucose is an easily assimilable carbon source that encourages bacteria to produce more PHB[11,12].

In a study revealed by Anteneh Getachew and Fantahun Woldesenbet[13] potential PHB accumulating bacteria showed the optimum growth and the maximum PHB accumulation by isolate AWW happened at 48 h. This shows biomass and PHB production were concomitant with growth conditions and PHB production of a particular strain is related to its biomass. As biomass increases the bacteria starts accumulating PHB to the maximum level and the accumulated PHB decreases after the peak biomass production. This might be due to nutrient depletion, which forces the bacteria to use the accumulated PHB as energy source.

Hence, it can be concluded that the production bio-plastic though still a lengthy and expensive procedure can be transformed into a low cost one by using easily available microbial cultures and low cost agricultural by-products which can be further degraded into the environment again by the microorganisms and will somewhere contribute in reducing the environmental pollution caused due to conventional plastic.

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