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Production Of PHB From Cupriavidus Necator Using Calophyllum inophyllum seed oil as Carbon Source

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***Abstract: Calophyllum inophyllum* is a large evergreen plant, low-branching and slow-growing tree with a broad and irregular crown and it is native to Africa, southern coastal India. It is best known as an ornamental plant, trees often grow in coastal regions, as well as nearby lowland forests. The oil was extracted from dried seeds of *Calophyllum inophyllum* using Soxhlet extraction column and used as carbon source for the growth of *Cupriavidus necator*. This organism was fed in batch mode and the culture was utilized for the production of Polyhydroxybutarate (PHB). The microorganism was procured from MTCC Chandigarh [MTCC No-1472]. The fermentation time for the production of PHB was observed to be 72hr. The intracellular PHB from the cells was extracted using various organic solvents and analyzed using Fourier Transform Infra-red spectroscopy [FTIR] and the peaks are compared with the standard peaks.**

I. INTRODUCTION

The increasing awareness of environmental hazards arising from plastic wastes, especially those originating from non-renewable natural resources, presents new opportunities for the development of bio-based and biodegradable polymer materials[1].The utilization of agro-industrial wastes allowing the production of biopolymers with adequate properties from low-cost, renewable resources[2].Poly(3-hydroxybutyrate) (PHB) is the best characterized PHA.Gavin says “polyhydroxybutyrate is the stuff of the future: renewable materials that just break down to water and carbon dioxide when degraded by microorganisms found in the soil.”PHB, however, is both bio-derived and biodegradable. A bio-derived substance is created entirely from raw materials found in nature.

Polyhydroxyalkanoates (PHAs) are natural, renewable and biocompatible biopolymers, produced intracellular in bacteria. They can be made into plastic materials with properties that are similar to petrochemical plastics and can replace these materials in many applications[3]. However, the high production cost of biopolymers and the availability of low-cost petrochemical equivalents make polyhydroxyalkanoates economically unattractive. Concern over plastic waste and increasing environmental awareness has put bioplastics into the attention of research and industry. In order to make the production of PHAs economically more attractive, the use of inexpensive substrates has been investigated thoroughly[4].PHA are polyesters of various hydroxyalkanoates that are synthesized by many gram-negative and gram positive bacteria ,bacteria that accumulate them in the cytoplasm.[5]. These polymers are accumulated intracellularly to levels as high as 90% of the cell dry weight under conditions of nutrient stress and act as a carbon and energy reserve[6].Polyhydroxyalkanotes (PHAs), the eco-friendly biopolymers produced by many bacteria, are gaining importance in curtailing the environmental pollution by replacing the non-biodegradable plastics derived from petroleum[7,8,9] .

The present investigation is production of PHB from Cupriavidus necator using *Calophyllum inophyllum* seed oil as carbon source.Cupriavidus necator (formerly known as Ralstonia eutropha) is a versatile organism for the production of PHAs[10].Out of the many different bacterial cultures that can produce PHAs[11] *Cupriavidus necator* has been most extensively studied[12]. At present, bacterial fermentation of *Cupriavidus necator* is used widely in industrial processes towards PHAs[13].

The oil extracted from Calophyllum inophyllum is called as Tamanu oil[14]. The nuts yield 70–75% of the greenish-yellow inedible oil. C. inophyllum yields fatty acid methyl ester, the oil which is extracted from the seeds is used as major biodiesel requirements in the United States, and European Union (EN 14214). The average oil yield is 11.7 kg-oil/tree or 4680 kg-oil/hectare. In the northwest coastal areas of Luzon island in Philippines, the oil was used for night lamps. This widespread use started to decline when kerosene became available, and later on electricity. It was also used as fuel to generate electricity to provide power for radios during World War II.Now, it is widely cultivated in all tropical regions of the world.Because of its decorative leaves, fragrant flowers, and spreading crown, it is best known as an ornamental plant[15].

II. METHODS AND METHODOLOGY

- ***Extraction of Oil:***

Calophyllum.inophyllum seeds were collected from Visakhapatnam city, near Andhra University and sun dried for 1 week. Powder was obtained from the dried seeds by using blender and oil was extracted from this powder using Soxhlet extractor. The extracted oil was used as a carbon source.

- ***Preparation of cultures and Media:***

Cupravidus necator was used as microorganism for the production of PHB, which was procured from MTCC Chandigarh. The lyophilized culture is revived and was cultured in nutrient media. From the revived culture, stock culture was prepared and from the stock culture, starter culture was prepared. This starter culture was used for the production of PHB. For preparation of Media, extracted oil from seeds is used as a carbon source and other sources are also added to this media 5 % of starter culture is inoculated and incubated for 72 hours at 30 ^{0} C and p ^{H} 6 with continuous shaking at 120rpm. After 72hrs the PHB accumulated inside the cells was extracted from the biomass.

- ***Extraction of Poly Hydroxy Butyrate (PHB):***

The bacterial cultures were harvested by centrifugation at 5000 rpm for 10 min. The cell pellet was suspended in sodium hypochlorite solution and incubated at 37°C for 2 hr for complete digestion of cell components except PHB, where lipids and proteins were degraded. The mixture was centrifuged to collect PHB granules and the supernatant was discarded. The sediment was washed twice with 10 ml of distilled water and centrifuged. The PHB granules in the sediment were washed twice with acetone, methanol and diethyl ether (1:1:1) respectively. The polymer granule was dissolved by boiling in chloroform and was evaporated by air drying, to yield dry powder of PHB.

The extracted PHB samples were evaluated for identification of their functional groups through FTIR analysis (Fig. 31). The functional groups were identified as –OH, $–CH_2$, C=O ester, C=O amide protein, CH_3, –C–O– and alkyl halides

III. FTIR ANALYSIS RESULTS

The peak at 3483 cm^{-1} indicated stretching strong H bond created by the terminal OH groups Similar results have been reported in other works [20, 21]. The peak 2927 cm^{-1} is assigned to C–H stretching methyl groups . These are comparable with results obtained by Kumalaningsih et al. [22] (2925.81 cm^{-1}) and Anish et al. [23] (2932 cm^{-1}).

According to Randriamahefa et al. [24] the absorption band of 1740cm^{-1} (observed in Fig. 1) is PHA marker band allocated to carbonyl C=O stretches of the ester groups located in the chain of exceedingly ordered crystalline structures. The peak at 1632 cm^{-1} indicate a weak C=O bond extended for conjugated carbonyl or amide group. This was similar with peaks obtained by Kumalaningsih et al. [22].

The peak at 1468 cm^{-1} accounts for $–CH_2$. The peak 1165 cm^{-1} represents –C–O– polymeric group .. Stretching other peaks (723, 565 cm^{-1}) correspond to the presence of Alkyl halides [25]. These all prominent absorption bands confirm that the polymer extracted from the samples was poly-β-hydroxybutyrate.

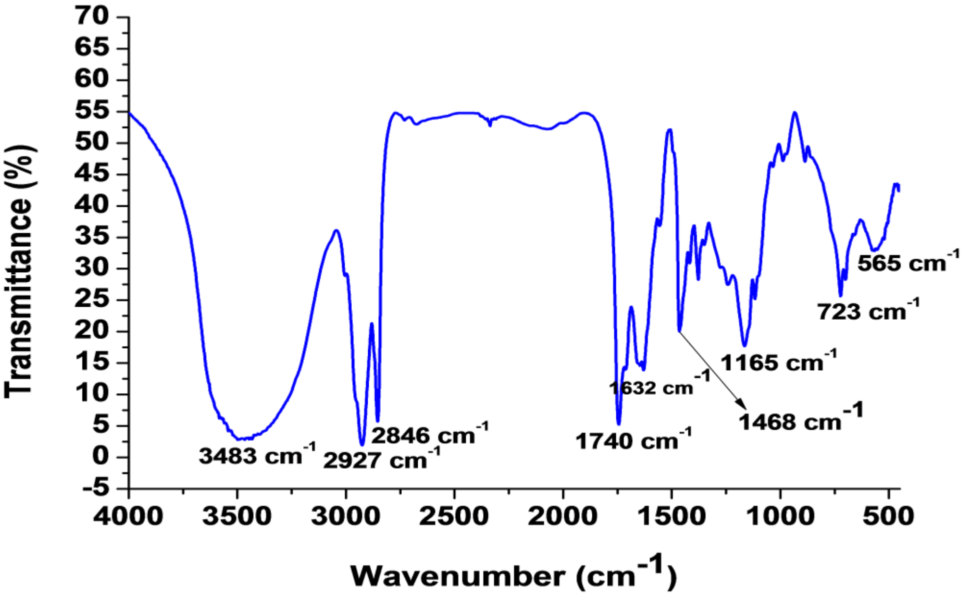

Figure: 1

IV. CONCLUSION

Cupriavidus necator is the efficient culture which can consume plant oil and efficiently produce PHB. The plant oil which is extracted from *Calophyllum.inophyllum seeds* is widely, abundantly and cheaply available carbon source which is effectively used by *Cupravidus necator* for the efficient production of PHB.. The production is maximum at optimum process conditions of 72 hours of production time, at 30 ^{0}C and pH 6. The FTIR analysis confirms the PHB production.

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