PRODUCTION OF POLYHYDROXYBUTYRATE FROM CAFETERIA WASTE : AN ENVIRONMENT FRIENDLY APPROACH

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Abstract

The main objective of the study was the production of polyhydroxybutyrate (PHB) with the help of wastes as carbon sources to reduce the cost of production of PHB. This was accomplished by accessing the potential of PHB production from the waste samples taken from different industries. Four wild type strains named RBS1, RBS2, RBS3 & RBS4 were isolated from leather, textile, chrome, and molasses waste samples which are biochemically tested for PHB production. All four waste samples were tested for BOD measurement, COD measurement and TSS by adopting a novel repeated washing method. The BOD, COD and TSS of all four samples lied between 5 to 1450 mg/l, 23 to 3380 mg/l and 196 to 300 mg/l respectively for the cafeteria waste. The PHB concentration in leather sample was observed to be 3.970g/l. The PHB concentration in chrome sample was observed to be 0.461 g/l but the polymer was degraded with respect to time, hence, it was not appropriate for our objective. The PHB concentration in molasses was observed to be 3.953g/l.The maximum accumulation of PHB was in textile sample. Although accumulation of PHB in molasses was much uniform.

Keywords: PHB, PHA, biodegradable plastics, polymers.

Introduction

Water insolubility and relatively resistant to hydrolytic degradation differentiates PHB from most other currently available plastics, which are either water soluble or moisture sensitive. Polyhydroxyalkanoates (PHAs) are biodegradable polyesters which are synthesized by many bacteria. They are accumulated intracellular as carbon and energy reserves under certain conditions like, in the presence of excess carbon source. The oldest known such polymer is polyhydroxybutyrate (PHB). Biodegradable plastics represent a solution to environmental problems generated by the utilization of plastics from petrochemical sources, which have many undesirable properties such as durability and resistance to biodegradation.

Biodegradable plastics are plastics that will decompose in natural aerobic and anaerobic environments. Biodegradation of plastics can be achieved by enabling micro-organisms in the environment to metabolize the molecular structure of plastic films to produce inert humus like material that is less harmful to the environment. PHB is the most known polyhydroxyalkanoate (PHA). The accumulation of PHA at higher concentration has been observed in a variety of microorganisms such as *Clostridium, Syntrophomonas, Pseudomonas, and Alcaligenes genera.* Some cyanobacteria produce PHB as well but at lower levels [1]. Generally, the nature and the proportion of polymer produced by bacteria are controlled by the carbon source used during culture [2]. Moreover, bioplastic utilization depends on the production costs and on polymer properties. Hence, 40 to 50% of the total production cost is

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related to the raw material [3]. In order to reduce the production cost, the use of cheaper carbon source is needed. In this perspective, many waste materials such as food wastes, potato starch waste water, alpechin and waste water sludge [4, 5] have been used as substrates for bioplastic production. However, the major hindrance in the commercialization of PHA is their high production cost. The cost of 1 kg PHB is about US$ 15-30 as compared to polypropylene which costs about US$ 0.70. Zeneca bio products (Billingham UK) is producing approximately 1000 tones PHA polymer per year and selling under the trade name BIOPOLTM at approximately US$16/kg cost [6]. This study reports that organic pollutants contribute a high pollution process waste e.g. food industry generates 450-2500 m^3/day of waste water. Thus, the improved use of wastes can reduced the pollution load due to the carbon uptake simultaneously in promoting PHB production rate.

Materials And Methods

Isolation of wild strains and growth conditions

The PHB producing microorganisms were isolated from the four samples collected as waste from the Textile industry of Kanpur, Leather industry of Agra, Chrome water from Textile industry of Kanpur and Molasses from Ganga sugar Mills, Muzaffarnagar. The microorganisms were identified as PHB producing by microscopic and biochemical characterization. Four isolated wild strains were named as RBS1, RBS2, RBS3, and RBS4 and are isolated from leather waste, textile waste, chrome waste and molasses respectively are determined as PHB producing microorganisms and were used for further studies for PHB production and as the source for PHB production in an analysis of cafeteria waste.

Identification of samples requirement for cafeteria waste (suited for PHB production)

The waste samples of leather, textile, chrome and molasses were analyzed for BOD, COD, and TSS for suitability of PHB production and then compared with their standard values as shown in table 1.

Parameter	Conc. (mg/l)
BOD	5-1450
COD	26-3380
S	4-185
TSS	196-300
DO	2.2-5.8
Ca^{++}	19-59

Table 1: Standard values of samples for production of PHB.

Now this cafeteria waste is mixed with sewage sludge and tap water in the ratio of 4:1:5. Fed batch cultivation is then done with mixed cultures in feast and famine conditions with 6 hrs duration cycle of anoxic and aerobic period [7].

The wild strains RBS1, RBS2, RBS3, and RBS4 obtained by isolation from leather, textile, chrome and molasses samples were inoculated in YMB (Yeast Mannitol Broth) and the medium was incubated at 30°C rotating at 150 rpm in aerobic mode. The YMB medium was prepared for the growth experiment [8]. The micro-organisms were allowed to grow in the broth medium. Samples were taken at regular interval of one hour during the incubation period and cell growth was determined by estimating the turbidity of the sample spectrophotometrically.

Production of PHB

The waste samples were supplemented with 0.05%K_2HPO_4, 0.02% $MgSO_4.7H_2O$, 0.01%Nacl, 0.1% Yeast extract for production of PHB [9].The above production medium inoculated with wild strains were incubated for 3 days at 30°C rotating at 150 rpm for oxic and anoxic cycle of 2 hrs and 3 hrs respectively. 0.9%w/v ferric chloride was added in medium to enhance the accumulation of PHB. Samples were taken for PHB determination and estimation at regular intervals of 5 hrs.

Determination of PHB

The samples were centrifuged in polypropylene centrifuge tubes which had been previously washed thoroughly with ethanol and hot chloroform to remove plasticizers. The cell paste was resuspended in a volume of commercial sodium hypochlorite solution equal to the original volume of medium. After 1 hr at 37°C the granules were centrifuged at 10,000rpm for 20 min at 10°C, washed with water, and then washed with acetone and alcohol. Finally, the polymer was dissolved by extraction with three small portions of boiling chloroform, the chloroform solution was filtered, and the filtrate was used for polyhydroxybutyrate assay.

Estimation of PHB

For the spectrophotometric assay of polymer, samples containing polymers in chloroform is transferred to a clean test tube. The chloroform is evaporated and 10 ml of conc.H_2SO_4 is added, tube is capped and heated for 10 min at 100°C in a water bath. The solution is cooled and after thorough mixing, sample is transferred to a cuvette and the absorbance at 235nm is measured against a sulphuric acid blank. PHB curve was plotted and PHB concentration was estimated with respect to standard curve of PHB.

Recovery

10 ml of each sample were taken at regular intervals after anoxic cycle and PHB was determined by John and Ralph method [10]. The brown color was obtained and concentration of PHB was estimated spectrophotometrically at 235 nm. The PHB production process was followed in a sequence of steps. All the samples were mixed with calcium hypochlorite solution and the mixtures were kept for 1 hr at 30°C. Samples were then centrifuged at 10,000 rpm for 20 min at 10°C. The centrifuged pellets were washed with ethanol, acetone and boiling chloroform and then heated with sulphuric acid for 10 min at 100°C in water bath. The brown colored ring appears by treating it with sulphuric acid which confirm presence of PHB as it passes the qualitative test of PHB

Results And Discussion

The main objective of the study was the production of polyhydroxybutyrate (PHB) with the help of wastes as carbon source to reduce the cost of commercial PHB. It was accomplished by accessing the potential of PHB production from the waste samples taken from different industries.

PHB production was carried out in batch fermentation by wild type strains isolated from the samples taken from various industries. Leather, textile, chrome and molasses waste samples were employed to provide the desired concentration of glucose (carbon source) in the fermentation medium. The optimum bacterial growth lead to optimum PHB accumulation is dependent on the fermentation medium. The four samples were subjected to BOD, COD, TSS tests and all lied within the required range for the cafeteria waste. The results are shown in figure 1. All the four isolated wild strain RBS1,

zRBS2, RBS3 & RBS4 are tested for PHB production in YMB medium under aerobic condition, the results are shown in figure 2. RBS 4 gives best result among four and is used for further study of PHB production. Further, leather, textile, chrome and molasses.

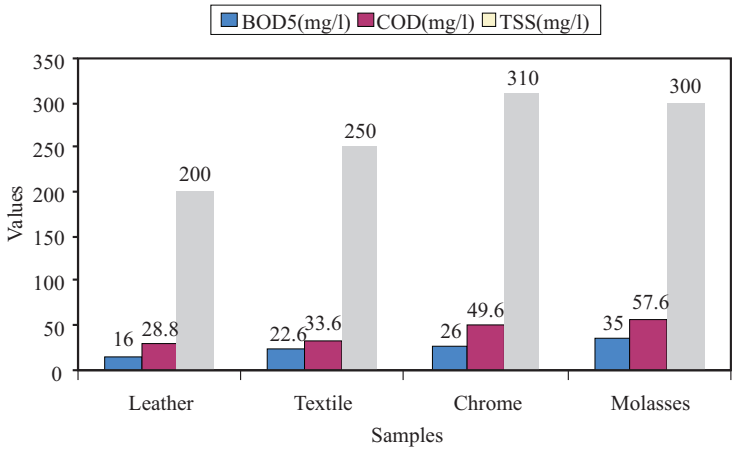

Figure 1: BOD, COD & TSS of various samples

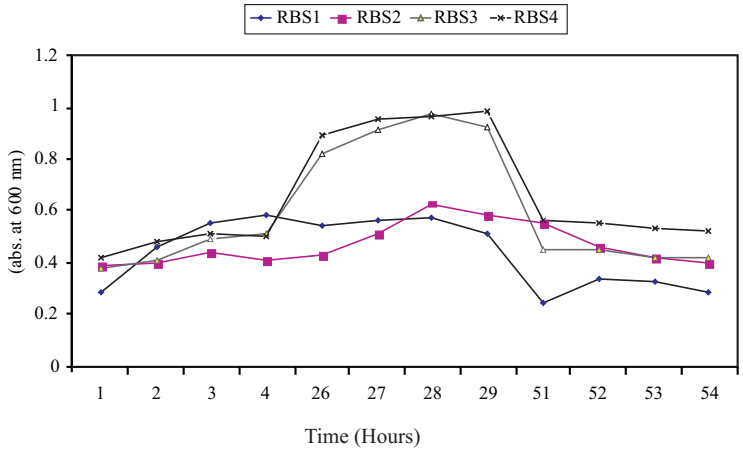

Figure 2: Growth study in aerobic mode of wild strains RBS1, RBS2, RBS3, and RBS4 in YMB medium

samples were subjected to fermentation for PHB accumulation with RBS4 and the concentration of PHB was estimated to be 3.944g/l,3.970g/l, 0.461g/l and 3.953g/l respectively. The graphs were plotted correspondingly and the nature of the graphs was tending to linear.

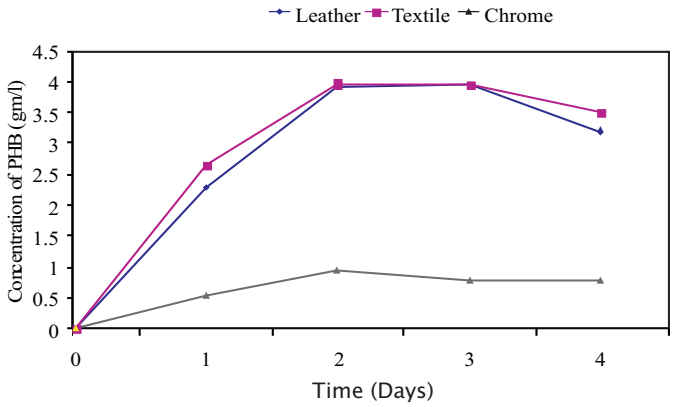

Figure 3: Concentration of PHB accumulated with respect to time in the samples taken

The accumulation of PHB was maximum in textile waste and minimum in leather waste. The chrome water sample was observed to be degraded with respect to time. The course of PHB concentration over time and the specific PHB formation rate are shown in graphs. During the comparative study of four samples the more uniform production was in the case of molasses. Higher specific PHB formation rate was observed at the initial fermentation period but PHB concentration was lower at late exponential phase. Therefore, industrial effluents can be used as an approach to substantially reduce the pollution load and simultaneously produce value-added product such as bio-fertilizer and PHB. Therefore, cost of PHB production can be substantially reduced by the use of these low priced samples used in the present study.

References

1. Fiechter, A. Plastics from bacteria and for bacteria: Poly (beta-hydroxyalkanoates) as natural, Biocompatible and Biodegradable Polyesters. Springer-Verlag, New York, 1990: pp. 77-93.
2. Steinbuchel A., Haywood G.W., Anderson A.J. ,Williams D.R., Dawes E.A. and Ewing D. F. Accumulation of a poly (hydroxyalkanoate) copolymer containing primarily 3-hydroxyvalerate from simple carbohydrate substrates by Rhodococcus sp.NCIMB40126. *International journal of biological macromolecules.* 13(2); 1991: 83-88.
3. Choi J., & Lee S.Y. Process analysis and economic evaluation for poly (3-hydroxybutyrate) production by fermentation. *Bioprocess Engineering.* 17 (6); 1997: 335-342.
4. Yu P.H.F., Chua H., Huang A. L., Lo W.H. and Ho K. P. Transformation of industrial food waste into polyhydroxyalkanoates. *Water science and Technology.* 40(1); 1999: 365-370.
5. Dave H., Ramakrishna C., and Desai J. D. Production of polyhydroxybutyrate by petrochemical activated sludge and Bacillus sp. IPCB-403. *Indian Journal of Experimental Biology.* 34(3); 1996: 216-219.
6. Khanna S. and Srivastava A.K. Polyhydroxybutyrate –an alternative to plastics. *Biobeam*; 2007: 24-26.
7. Chiang S.H. Polyhydroxybutyrate (PHB) production from cafeteria wastes under anoxic and aerobic conditions in sequencing batch reactor (Thesis) Universiti Teknology Malayasia 2006: 4-5.
8. Arun A., Murrugappan R.M., David Ravindran A. D., Veeramanikandan V. Utilization of Various industrial wastes for the production of poly-β-hydroxybutyrate (PHB) by Alcaligenes eutrophus, *African journal of Biotechnology.* 5 (17); 2006: 1524-1527.
9. Chaijamrus S. and Udpay N. Production and characterization of Polyhydroxybutrate from Molasses and corn Steep Liquor produced by Bacillus megaterium ATCC6748. *CIGR e-journal.* Vol. X; 2008: 4-5.
10. Law H. and Slepecky R.A. Assay of poly-1, 3-hydroxicbutyric acid. Department of Chemistry, Harvard University, Cambridge, Massachusetts, and Department of Biological Sciences, Northwestern University, Evanston, Illinois,, 1960; 2-4.