Polyhydroxyalkanoates preparation by Fusarium moniliforme using sugarcane as substrate

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Abstract

Continuous usage of conventional plastic cause waste accumulation and green house gases emission.Due to this reason development of biogreen materisl has been enhanced as they exert negligible side effects on environment. Biogreen materials include polylactic acid (PLA), naturally occurring zein and polyhydroxyalkanoates. Now a days, polyhydroxyalkanoates has been attracting major interest due to its compatibility with synthetic plastic. PHA is synthesized by a number of microorganisms as intracellular inclusion. Fusarium moniliforme is one of the microorganism that accumulate PHA using sugarcane juice as carbon substrate and ammonium ferrous sulphate as nitrogen substrate. Its production cost is comparatively less as compared to that in which glucose is used as substrate. But the overall production cost is high which is the major drawback of this method of PHA preparation. That's why efforts are going on in developing some mutant strain or using some inexpensive substrate in order to reduce the production cost.

Introduction

Synthetic plastic has replaced glass wood and other constructional materials and even metals in much industrial, domestic and environmental application not only due to their mechanical and thermal properties but also due to its stability and durability. But their persistence in environment causes high cost of solid waste disposal as well as the potential hazards from waste incineration such as dioxin emission from PVC resulted waste management problem. That'why recent technologies are directed towards the development of biodegradable plastic. PHAs have been attained major interest due to its similar material properties with conventional plastic and its biodegradability.

General structure of PHA:

[-O-CH(R)-(CH2)n-CO-]100-30000

n=1 R = hydrogen poly-3-hydroxypropionate

methyl poly-3hydroxybutyrate

ethyl poly-3-hydroxyvalerate

propyl poly-3hydroxyhexanoate

pentyl poly-3-hydroxyoctanoate

nonyl poly-3-hydroxydodecanoate

n=2 R = hydrogen poly-4-hydroxybutyrate

n=3 R = hydrogen poly-5-hydroxyvalerate

It is known to accumulate by a number of microorganisms. Fusarium moniliforme a fungus is also known to accumulate PHA. It causes bakanae and foot rot disease in rice plant i.e. isolated from the infected roots of rice plant.

Material and method:

Extraction and culture of Fusarium moniliforme

F.moniliforme a fungus is found in the infected roots of rice plant. It causes bakanae and foot rot disease in rice plant resulting in enhanced growth and decreased yield.

For the culture of F.moniliforme infected portion of the root is cut and inoculate in petridish having potato sugarcane agar media. Placed this petri dish in BOD at temperature 250^0C for 8 days. After 8days colonies came.

Preparation of media

For media boiled 300g potato 1L distilled water for the extraction of starch and add 2ml sugarcane juice as C-source instead of glucose and 0.2g agar agar for solidification of media.

PHA production in Fusiform moniliforme

PHA production was carried out in 500ml Erlenmeyer flask having 100ml liquid media contained different C:N ratios such as $(NH_4)_2SO_4FeSO_4$ 1g/l with different amount of sugarcane juice like 10ml, 20ml, 30ml or 20ml/l sugarcane juice with 2g, 4g, & 6g of $(NH_4)_2SO_4FeSO_4$. Incubations were carried out for 72 hours at 250rpm and 30^0C temperature.

Biomass & Polysaccharides estimation

After centrifugation of culture media cell biomass was collected and dried in airflow drier at temperature 70^0C for biomass estimation. For polysaccharides estimation isopropanol was used. Polysaccharides were dried at 70^0C upto a constant weight.

PHA estimation:

For the extraction of PHA from biomass solvent extraction was used. The solvent employed for this was chloroform. As this method of extraction is very simple and effective to separate PHA granules from biomass. PHA obtained by this method was highly purified and without any degradation of PHA molecule.

PHA production with varying concentration of carbon and nitrogen:

PHAs are mainly synthesized in the presence of excess of carbon with limiting amount of other nutrients like N, O, S, P etc. Production of PHA increases with increasing concentration of substrate that provides carbon and decreases with increasing concentration of substrate that provides nitrogen. Results are shown in the following table:

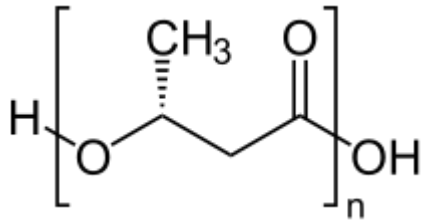
Table1- Production of PHA with varying concentration of carbon and nitrogen

Sugarcane juice (Carbon source) (ml/l)	Ammonium ferrous sulphate (Nitrogen source)(g/l)	Biomass (g dry wt/l)	PHA (g/l)
10	1	3.5	1
20	1	4	1.3
30	1	5	1.5
20	2	4	1
20	4	2.0	0.8
20	6	1	0.5

Result and discussion

PHA synthesized by this method is mainly polyhydroxybutyrate(PHB).

Structure of PHB



And its IR spectra and NMR spectra gives the following data:

IR spectra : 1098 (C-O), 1670 (C=O aliphatic), 2650 (CH2), 2927 (C-H streching)

NMR : 1.45 (3H,d,-CH3), 1.874 (2H,d,-CH2-), 6.49(1H,m,-CH-)

The 1H NMR were recorded in the indicated solvent on a Varian 500 MHz and 200 MHz spectrometer with TMS as internal standard. All chemical shifts (δ) were reported in ppm from internal TMS. Infrared spectra were recorded in KBr medium. The infrared spectra and proton NMR data clearly suggest the molecule obtained is Polyhydroxybutyrate (PHB).

Use of sugarcane juice instead of sucrose/ glucose as carbon substrate is relatively cheap.

Properties of PHA

- Water insoluble and relatively resistant to hydrolytic degradation. This differentiates PHB from most other currently available biodegradable, which are either water soluble or moisture sensitive.
- Good oxygen permeability.
- Good ultra-violet resistance but poor resistance to acids and bases.
- Soluble in chloroform and other chlorinated hydrocarbons.[8]
- Biocompatible and hence is suitable for medical applications.
- Melting point 175°C., and glass transition temperature 2°C.
- Tensile strength 40 MPa, close to that of polypropylene.
- Sinks in water (while polypropylene floats), facilitating its anaerobic biodegradation in sediments.
- Nontoxic.
- Less 'sticky' when melted, making it a potentially good material for clothing in the future.

Biodegradability of PHA synthesized

PHB synthesized degrades in microbially active environment. Degradation occurs most rapidly in anaerobic sewage and slowest in seawater. Microorganisms colonize on the polymer surface and secrete enzyme that convert PHB into hydroxybutyrate (HB). These monomer units are then utilized by cell as carbon source.

Degradability rate depends on various factors like surface area, pH, temperature, pressure of other nutrients and moisture etc. In aerobic environment carbon dioxide and water are the end products of PHB degradation.

Applications of PHB

The perspective area of PHB application is development of implanted medical devices for dental, craniomaxillofacial, orthopaedic, hernioplastic and skin surgery.

A number of potential medical devices on the base of PHB: (A) bioresorbable surgical suture; (B) biodegradable screws and plate for cartilage and bone fixation; (C) biodegradable membranes for periodontal treatment; (D) surgical meshes with PHB coating for hernioplastic surgery , wound coverings have been developed.

Future outlook

PHAs production has drawn major attention due to its biocompatibility and biodegradability with conventional plastic. The production cost of PHAs through this method is less as compared to method in which glucose/sucrose used as carbon substrate.To prodyce it commercially researches are continuously going on by developing some mutant strains and using some alternative carbon sources.

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