

EFFECT OF pH ON PHA PRODUCTION FROM DIFFERENT STREPTOMYCES SPECIES

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ABSTRACT

The problem of pollution which has been caused by the constant utilization of man-made polymers has drawn substantial attention related to the growth and production of biodegradable polymer. Polyhydroxyalkanoate (PHA) is known to be intracellular addition in some bacteria. PHA has the parallel structural properties of many conventional plastics, but it can be produced from renewable resources which gets biodegraded easily. The present work is an attempt that PHAs can be substitutes to conventional plastics. A research investigation was felt in order to explore the indigenous bacterial culture from PHA production. Four *Streptomyces* species e.g. *Streptomyces diastaticus*, *Streptomyces cyaneus*, *Streptomyces microflavus* and *Streptomyces achromogens* adopted effectively in harsh environment state e.g. pH. The culture medium is used as liquid or gel is designed to support the growth of microorganisms. There are different types of media for growing different types of cells. It was revealed in light of present investigation that there is limitless demarcation for the use of biodegradable polymer which can be allied to various sectors.

KEYWORDS: *Streptomyces*, Harsh Environmental, Rpm, Polyhydroxyalkanoate and Bioplastic

INTRODUCTION

Plastics, they are occupying a unique position in the world of materials as crucial as one of the greatest invention of modern age. They are responsible for molding the modern world as well as for the transformation of life quality through the manufacturing of materials pertaining to clothing, shelter, transportation, communication, entertainment and health care on the whole depend on Plastics. Secondary property of plastic include as they meet a large share of the material needs of man for there possess many attractive properties, such as lightweight, high strength and easy of processing. At present there has been a tremendous growth of development and production of biodegradable polymer.⁵ This in terms will lead to save the progress problem related to green house gases which is a result of the continuous use of man-made polymer.

POLYHYDROXYALKANOATES (PHA)

There has been considerable interest in the development and production of biodegradable plastics knowing to the hazards associated with plastic waste and its environment effects. One significant compound that can be potential replacement to the traditional plastics compounds are polyesters called Polyhydroxyalkanoate (PHAs). Owing to the property of natural biodegradability, PHA are considered as the potential candidates for biodegradable plastics, as they possess material properties resembling to those of synthetic polymers currently in use and are completely biodegradable after disposal. The unique property of PHA has similar to that of polypropylene with unique features, e.g. thermoplastic process ability, 100% resistance to water, and 100% biodegradability stated that PHB is an aliphatic homopolymer which

has melting point of 179°C and is highly crystalline in nature gets (80%)¹. It gets degraded at the temperature above its melting point. The molecular weight of PHA decreases to approximately one half of its original value when it was heated at 190°C for 60 minutes.² PHAs can have wide range of entirely physical properties such as brittle and thermally unstable soft and tough, this depending upon their compositions, i.e., PHV/PHB ratios. The paper is an attempt to indicate that diversity exists among bacteria with regard to synthesis and accumulation of PHA. A research was felt investigation in order to explore indigenous bacterial culture from PHA production. Actinomycetes species adopted successfully in harsh environmental conditions to stress and nutrient imbalance etc. In the natural environment actinobacteria have significant roles in ecosystem sustainability. Hence collection of actinomycetes species and its utilization for PHA synthesis.

OBJECTIVES

Based on the above the main objective of the thesis was:-

- The collection of bacterial culture (Institute of modern biotech, Raipur)
- The preparation of production media (MSM)
- The extraction of PHA from production media (MSM)
- The optimization of PHA production on different parameters pH
- The quantitative estimation of extraction of PHA granules.

MATERIALS AND METHODS

There are four Streptomyces species; bacterial culture was brought from the Institute of Modern Biotech, Raipur in preservative container for prospective of PHA production. These are required to screening of PHA production on different pH.

MEDIA PREPARATION FOR BACTERIAL CULTURE

There is wide range of bacteriological media for the growth for microorganisms. Starch casein agar Medium is a complex medium as it contains ingredients with unknown amounts or types of nutrients such as Soluble starch, casein purified, KNO₃, NaCl, K₂HPO₄.7H₂O, CaCO₃, FeSO₄.7H₂O, agar and distilled water. Starch casein agar consists of nutrients which are necessary for the replication of a large number of microorganisms. Fastidious agar is added in to microbiological media as a solidification agent. Primarily slanted tubes are used for the cultivation of maintenance of pure culture of microorganism. They should be inoculated with the help of an inoculating loop and incubated under the specific state of affairs for the plated medium

MINIMAL SALT MEDIA (MSM) AS A PRODUCTION MEDIA

A growth medium or culture medium is a liquid or gel designed to support the growth of microorganisms or cells. There are different types of media for growing different types of cells. For the production of PHA granules condition apply for pH e.g. 4-8 pH., pH adjusted with the help of added base and salt. Media which has used for the production of PHAs granules is minimal salt media. Those contain the minimum nutrients possible for colony growth, without the presence of amino acids.

Optimization of PHA Production on Different pH

- pH :- optimization the pH 4-8 in media for the production of PHA.
- Extraction of PHA From Bacterial Culture

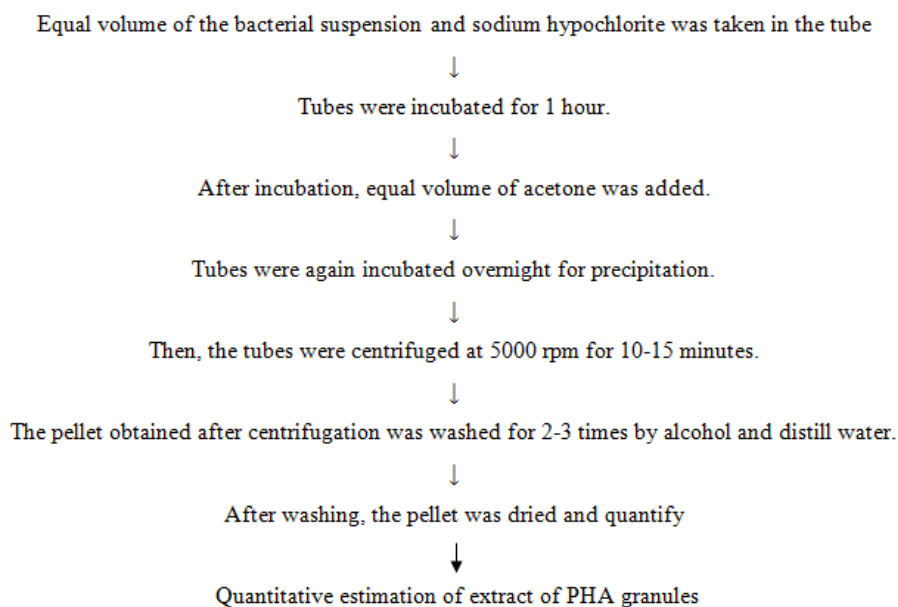


Figure 1: Systematic Representation of Production of PHA from Bacterial Culture

OBSERVATION TABLE

Table 1: Effect of pH on the Production of PHA from the Different Streptomyces species

Total Amount of PHA Gm/L in Different pH					
Streptomyces Species	pH 4	pH 5	pH 6	pH 7	pH 8
Streptomyces Diastaticus	0.067g	0.31g	0.27g	0.068g	0.32g
Streptomyces Cyaneus	0.13g	0.12g	0.28g	0.057g	0.024g
Streptomyces Microflavus	0.063g	1.33g	0.28g	0.07g	0.014g
Streptomyces Achromogens	0.073g	0.2g	0.24g	0.076g	0.039g

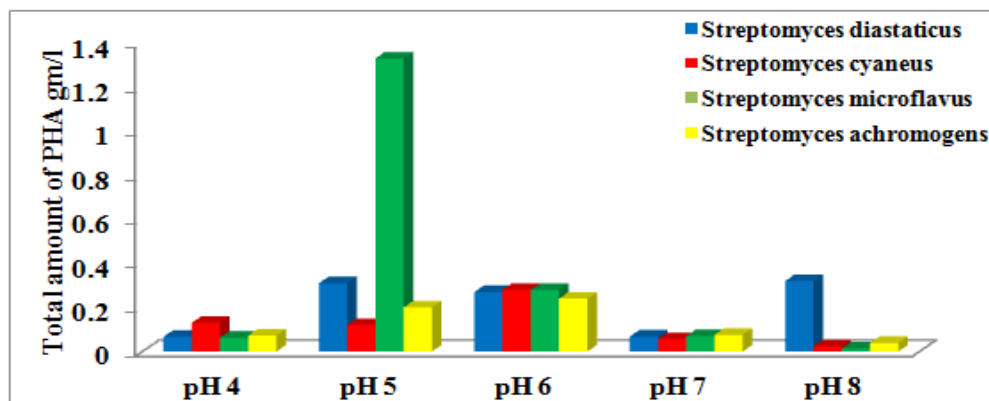


Figure 2: Total Amount of PHA Production from Different Streptomyces Species

RESULTS AND DISCUSSIONS

There are two important biopolymers Polyhydroxyalkanoates (PHA) and exo polysacchrids (EPS) mostly hope for future. Under the unbalanced growth condition bacteria produce Polyhydroxyalkanoate by fermenting sugar and lipids as a mechanism of store carbon and energy. PHA is biodegradable and biocompatible green thermoplastic, synthesized by wide variety of bacteria as an intracellular carbon and energy storage in the middle. The current interest in these biopolyesters is stimulated by the search for cost-effective capitalized production they are used as alternative petroleum derived plastics. This research attempts to reach maximized production rate of substrate. Molasses from agro-industrial waste was employed for the production of PHA from recombinant *E.coli* in batch culture. The yield PHA in molasses ($3.06\text{g/L} \pm 0.05$ -75.5%) was higher as compared to that of sucrose ($2.5\text{g/L} \pm 0.05$ - 65.1%). The study revealed enhanced PHA production (molasses) in recombinant *E.coli*.³ Being biodegradable and biocompatible green thermoplastics, PHA are synthesized by wide variety of bacteria as an intracellular carbon and energy storage intermediate. The current interest in these biopolyesters is stimulated by the search for cost-effective capitalized production. Thus this paper is an attempt to achieve maximum rate of production from recombinant system using cheap substrate. Being an agro-industrial waste, Molasses was used to produce PHA from recombinant *E.coli* in batch culture. The research work revealed that molasses enhanced PHA production in recombinant *E.coli*.⁴

CONCLUSIONS

There seems to be limitless number of the uses of biodegradable polymer materials.⁵ Various sectors such as agriculture, automotives, medicine, and packaging all require environmentally friendly polymers. Every industry can create its own idea material on the basis of biodegrading level. Disposal methods of materials can be tailored as per the specific needs. This can prove key note to industrial specification. Environmental responsibility is constantly increasing in importance to both consumers and industry. For those who produce biodegradable plastic materials, this is a key advantage. Biopolymers limit carbon dioxide emissions during creation, and degrade to organic matter after disposal. Although synthetic plastics are a more economically feasible choice than biodegradable ones, an increased availability of biodegradable plastics will allow many consumers to choose them on the basis of their environmentally responsible disposal.

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