# Extraction of Polyhydroxyalkanoate from Waste Water

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*Abstract-* **In wastewaters which are generated in different operations in a chemical process plant, huge amount of organic compounds are present. These compounds generate COD level of wastewater from thousands to lakhs. The degradation of these compounds is done in a conventional process in secondary treatment. After treatment dead microbes coming in waste streams can be utilized to produce (PHA) polyhydroxyalkanoates. In the thesis extraction of PHA with solvents will be tried and optimum conditions will be find. Characterization of the PHA will be done.**

*Index Terms-* **Polyhydroxyalkanoates, extraction, Production cost, separation, solvents**

## I. INTRODUCTION

### 1.1 General Aspects of PHAs

Various types of Biopolymers are under development like Polylactides, Polyglycolic acids, Polyhydroxyakanoates (PHAs), etc. Natural renewable polymers include porous sponges (from cellulose wood fibres), fibres (made from natural fibres), hydrogels, starch, cellulose, chitin, lignin and proteins. Here, PHA is being considered as the most potential renewable material to petrochemical plastics because of its resemblance to commercially available plastic in context to physical and chemical properties. PHAs are microbiologically produced polyesters that combine high functionality with low environmental impact (biodegradability), making them promising candidates for sustainable polymer production[5]. Their properties range from brittle thermoplastics to elastomers and can be controlled by the choice of substrate, bacteria and fermentation conditions. PHAs can potentially substitute polypropylene, polyethylene and polystyrene, which are the three main polymers of the global polymer market in recent.

In contrast, PHAs are both compostable and biodegradable in marine environment. This is an important difference to other bio-based polymers such as polylactic acid (PLA), which is compostable, but may remain in marine environments for up to thousand years. Naturally occurring prokaryotes such as bacteria (e.g. Cupriavidus necator, Alcaligenes latus, Aeromonas hydrophila, Pseudomonas oleovorans[7]) decompose PHAs into carbon dioxide and water, which are consumed during plant growth. In addition, PHAs naturally occur in human blood and tissues and are non-toxic. This biocompatibility enables new applications to be developed from PHAs to for the medical field.

PHAs were discovered at the beginning of the 20th century by Lemoigne (1926) when observing poly(3-hydroxybutyrate) (PHB) granules inside the Gram-positive bacterium Bacillus megaterium. PHAs are composed of hydroxy fatty acids and represent a complex class of intracellular storage polymers synthesized by various bacteria and archaea. PHAs are produced in the presence of excess carbon source while growth is inhibited due to limited nutrient availability. PHAs are deposited as water-insoluble granules inside the cells.However, under carbon starvation conditions granule-associated PHA depolymerizing enzymes degrade the PHA to provide carbon and energy. More than 150 different monomers can be combined within this family to give materials with extremely different properties. These plastics are biodegradable and are used in the production of bioplastics. Most microorganisms produce PHB which is composed of (R)-3-hydroxybutyrate with a molecular weight ranging from about 500000 to several millions. Purified PHB is a crystalline, rather brittle thermoplastic material which has been considered for bulk application to replace commodity oil-based products. The second major class of naturally produced PHAs is composed of medium-chain length (MCL) (R)-3-hydroxyfatty acids (6-14 carbon atoms) with molecular weights ranging from about 100000 – 500000. Short chain length or SCL-PHA that they have 3–5 monomers are weak and solid, medium chain length or MCL-PHA that they have 6–14 carbon atoms are more noteworthy in having flexibility and are more biocompatible.

### 1.2 Material Properties

PHA polymers are thermoplastic and can be processed on conventional processing equipment and depending on their composition, ductile and more or less elastic. They differ in their properties according to their chemical composition. They are UV stable, in contrast to other bioplastics from polymers such as polylactic acid, temperatures up to 180 °C, and show a low permeation of water. The crystallinity can lie in the range of a few to 70%. Process ability, impact strength and flexibility improves with a higher percentage of valerate in the material. PHAs are soluble in halogenated solvents such chloroform, dichloromethane (MDC). PHB is similar in its material properties to polypropylene (PP), has a good resistance to moisture and aroma barrier properties. Polyhydroxybutyric acid synthesized from pure PHB is relatively brittle and stiff. PHB copolymers, which may include other fatty acids such as beta-hydroxyvaleriate acid, may be elastic. PHAs can consist of short-chain length (scl, 3–5 carbon atoms) and medium-chain-length (mcl, 6–14 carbon atoms) hydroxyalkanoic acid monomers, depending on strain, carbon substrate, and culture conditions provided[9,10].

### 1.3 Application of PHA

- Packaging films, bags, containers, paper coatings
- Disposable items such as razors, utensils, cosmetics containers, shampoo bottles, cups etc
- Textile materials such as fibers
- Medical applications – Surgical pins, staples, wound dressings, bone replacements & plates and blood vessel replacements, heart valves, cardiovascular applications, matrices for skin regeneration,etc
- According to their excellent properties of biocompatibility and degradability, PHAs are widely exploited in various areas including bioplastics, tissue engineering and drug delivery systems[1]

## 2. PRODUCTION COST

Initially, the price for commercial PHAs was 15-18 times higher than the major petroleum-based polymers[12]. Metabolic engineering improved fermentation conditions, and higher production capacities were able to reduce the cost to around Rs. 322 per kg in which was still three times higher than the price for polypropylene[6]. PHAs therefore still have a limited market, despite their potential to substitute 33% of commercial polymers. Overall, the most important factors contributing to the final PHA price are productivity (g per L per h), yield per substrate, cost of raw materials and the recovery method[11]. Furthermore, total manufacturing costs decrease with process scale-up, which also increases the fraction of raw material costs, dominated by the carbon source, on the final PHA price. However, low substrate costs were only effective at similar process efficiencies. Consequently, reducing PHA production costs at a large scale depends on overall process optimization rather than improvement of individual indicators. It was frequently noticed that the application of inexpensive substrates delivers lower PHA productivity. Low concentration of carbon source in the feed stream renders the PHA production process less effective especially during fed-batch cultivations.[2]

## 3. METHODS

### 3.1 PHA production methods

The biosynthesis of PHA is usually caused by certain deficiency conditions (lack of phosphorus, nitrogen and oxygen) and the excess supply of carbon sources[8]. Once the population has reached a substantial level, the nutrient composition is changed to force the micro-organism to synthesize PHA. Polyesters are deposited in the form of granules in cells. PHA granules are then recovered by disrupting the cells, which is known as lysis process. Various processes like chemical, mechanical are available to extract PHA. One of the extraction process can be applied to extract the PHA like Dispersion method, Acetone Extraction, etc.[4]

### 3.2 Biomass Formation

Sugar Factory waste water is used for experimental propose with bacterial culture of Pseudomonas. Three days aging time of waste water and pseudomonas mixture gave Biomass at bottom. These biomass which is mainly Pseudomonas bacteria, is further used to extract PHA using extraction process.

### 3.3 Extraction of PHA from Biomass

In this modified method of extraction, the biomass was mixed with NaOCl. PHA-containing biomass can be disintegrated using sodium hypochlorite at high pH-value. Most cell components are oxidized by hypochlorite and become water soluble. Cell debris was separated from PHA containing Sodium Hypochlorite using filter paper. PHA containing Sodium Hypochlorite was heated using Oven at 60 °C. Remaining powder was washed using water. Final product will further dried using oven.

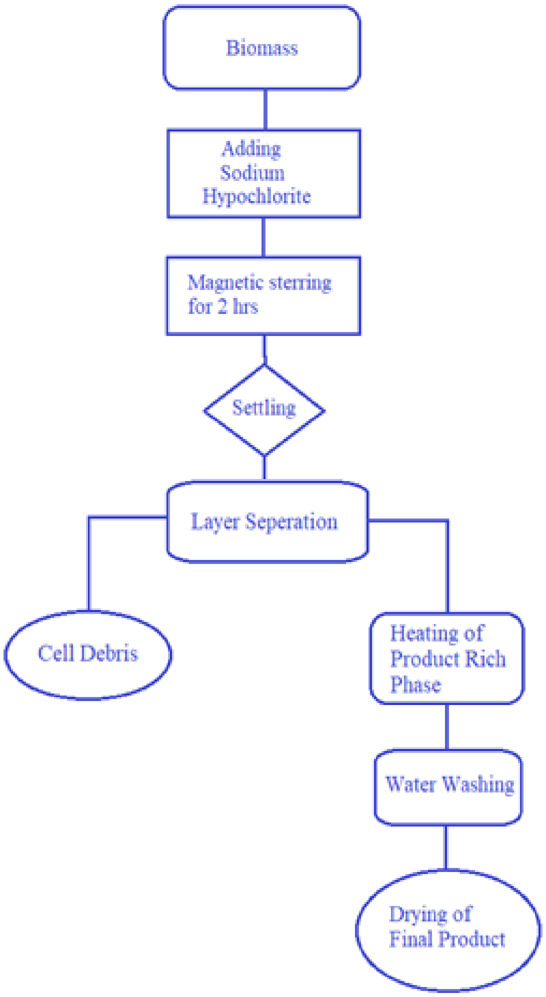


Fig 1. Flowdiagram of Process

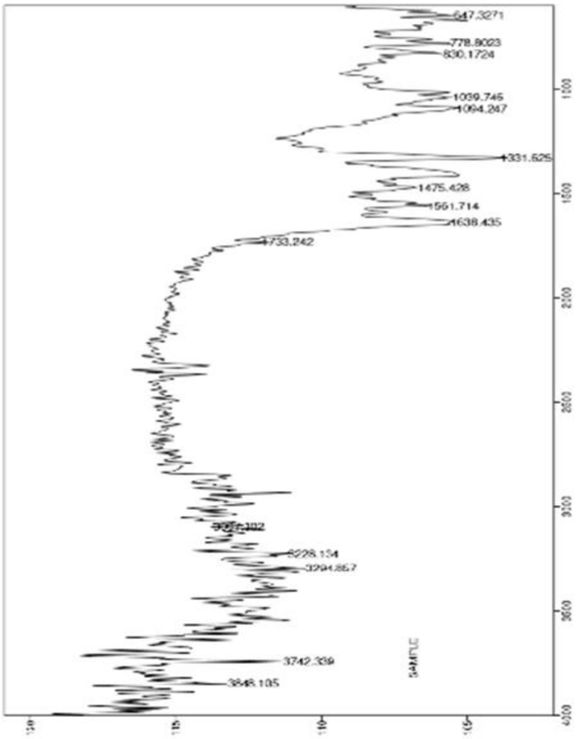
Using various quantity of Sodium Hypochlorite, practicals are done. Practical data is shown on table 1. Here, based on data experiment number 1 shows higher yield.

| No. Exp. | Qty of Wet Biomass (ml) | Qty of NaOCl (ml) | Qty of Water (ml) | Dry Weight (gm) | Output (gm) | Yield (%) |
|---|---|---|---|---|---|---|
| 1 | 40 | 90 | 10 | 4.2 | 3 | 71.4 |
| 2 | 40 | 70 | 30 | 4.2 | 2.85 | 67.9 |
| 3 | 50 | 45 | 5 | 2.9 | 1.8 | 62.1 |
| 4 | 50 | 40 | 10 | 2.6 | 1 | 38.5 |

1. Experimental Data

## 4. RESULT AND DISCUSSION

Pseudomonas bacteria was successfully grown in Sugar factory waste water without adding any external carbon source. Biomass quantity was sufficient for PHA extraction using specific quantity of waste water and aging time was three days. In this experient we have used sodium hypochlorite and water solution without using chloroform or Dichloromethane[3]. So this modified process gives the higher yield of PHA and low cost process. Final product is further characterized. The structural characterization of the kind of PHA monomer accumulated by the organism was carried out by performing Fourier transform infrared FTIR spectroscopy.



2. FTIR

FTIR shows various groups of chemical bonds and structures. On first region carboxylic acid and alkane groups are present and on fingerprint region unique groups are ester, alkane and little impurities of halo compounds.

Varying various concentration of Sodium Hypochlorite yield is also varying. According to experiments when concentration of Sodium Hypochlorite is 90ml with 10ml of water, it gives highest yield of Polyhydroxyalkanoate.

## 5. CONCLUSION

Now, It can be concluded that it is possible to produce PHA using sugar industry waste water. Based on performing experiments, Using dispersion method yield can achieve up to 62 % and for modified method it is 71 %.Cost saving is possible by eliminating solvents like Methanol, Chloroform, MDC using modified method. FTIR graph comparison shows polymeric groups are same for hence modified method is cost efficient and able to eliminate use of hazardous chemicals like chloroform, MDC, methanol.

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