Biopolymer- A beginning towards back to nature

S Gautam¹² and A Gautam¹
¹Department of Chemical Engineering, Shroff S R Rotary Institute of Chemical Technology, Ankleshwar, Gujarat, 393135 India
E-mail: shinaiitd@gmail.com

Abstract. Biopolymer is regarded as a polymer which can be biodegradable. Polyhydroxyalkanoates (PHAs) is one of the biopolymer which can be recovered from biomass. PHAs are naturally conserved in the cytoplasm of the bacterial cell during the growth. Bacteria/microbes store their energy from carbon sources in the form of hydrocarbons. Intracellular stored compounds are tightly linked with entire cell resulting difficulty of separation. The work aims to extract PHAs from biomass effectively. Chemical and mechanical separation of PHA can be done from biomass. A pretreatment of cells before chemical and mechanical separation is also effective for separation of PHA and has been carried out. Chemical extraction of PHA includes digestion of cell wall in acidic or alkaline medium and releasing PHA in broth, later sedimentation recovers PHA. In recent work different chemical methods were carried out to extract PHA of medium chain length. In one of these, sodium hypochlorite was used to denature the protein and chloroform was used for extraction of purified PHA. A recovery upto 96.6%, PHA by dried weight of cell, was obtained which is quite high comparing to reported literature. Other chemical disruption by sodium chloride, sodium hydroxide and hydrogen peroxide with and without pretreatment have also been carried out.

1. Introduction
Plastics are derived from petrochemical reserves. Common definitions of the term “biopolymer” also include biodegradable plastics from fossil fuels and non-biodegradable plastics from renewable resources.

Biopolymers are made from renewable resources and/or biodegradable waste materials (e.g., waste water, sewage sludge, organic waste) and are fully biodegradable by naturally occurring microorganisms. Typical molar masses of polyhydroxyalkanoate (PHA) vary, depending on the microbial production strain, fermentation conditions, substrate, and isolation method from typically 200 to 2000. Direct extraction of PHA from biomass is highly dependent on polymer structure and chain length of polymer. There have been many pretreatment methods which weakens cell wall of microbe. Later some solvents which oxidize cell wall can be utilize for final PHA extraction.

Biopolymer production is gaining a lot of attention due to biodegradability. The applications of biopolymer are widely in medical sectors. However, the cost of production is limiting its use every common human beings. The cost of production is highly dependent on carbon source which is pure substance for medical applications. Further washing and purification increase cost of production. Another application of biopolymer which is getting focus is, packaging industry [1]. The cost of biodegradable packaging is again not so affordable to medium scale or small scale industries. Therefore, it becomes almost necessary to find cost effective carbon source as well as reduce the use
of non-biodegradable polymer. One of such sources can be wastewater from sewage treatment plant, food industry wastewater, process industry wastewater and so forth.

Several pretreatment methods: physical, chemical or biological are applicable to disrupt the microbial cell. Application of heat is one of the pretreatment step, it affects the firmness of cell by denaturation of cell proteins and by destabilization of outer membranes. This can also be done by freeze drying (lyophilization) of biomass [2,3].

A number of methods to release PHA are available in literature, in brief can be divided in solvent extraction, chemical, enzymatic, mechanical operation, supercritical fluids, air classification [4]. Solvent extraction methods are based on that, PHAs are insoluble in water, however, soluble in limited number of solvents. Solvents containing chlorine such a chloroform, 1-2 dichloroethane, methylene chloride and some cyclic carbonates such as propylene and ethylene carbonates are widely used to recover PHAs from biomass. While purifying PHA through solvent extraction carbon chain length may be shorten due to dissolution of hydrocarbon in halogenated solvents. Chemical disruption of cells by sodium hypochlorite is widely used as it has several advantages over others. One of the advantages is the cells need not to be dried and can be used in wet condition. The polymer is not dissolved in hypo solution and remains solid which can be recovered by filtration or centrifugation.

Apart from hypochlorite, disruption of cell can also be done by surfactants. Non-PHA material can be solubilize in surfactant, however, some polymer can also be solubilize in surfactant and results in reduced and short chain length recovery of PHA.

In present work biomass was obtained from common process industry wastewater treatment plant. Biomass was generated in secondary treatment and discarded as waste material. A comprehensive comparison of different PHA recovery methods had been done and several trial and error methods had been approached. The disruption of cells is adapted as per literature. However, substrate and culture used in present work to generate biomass was not found in literature. It is reported here first time.

2. Materials and methods
To extract PHA, biomass was obtained from a local common effluent treatment plant. The wastewater was collected from different process industries like pharmaceuticals, dyes and pigments and specialty chemicals. The wastewater undergoes for treatment by primary, secondary and tertiary methods. In primary treatment physical removal of contamination was done by filtration, coagulation and sedimentation. After primary treatment wastewater was aerated for secondary treatment, where mixed culture consortia were prepared to disintegrate complex hydrocarbons with microbial digestion. After secondary treatment by microbes, sludges were generated which were discarded as solid waste and sent for landfilling (biomass). In the present work this sludge was collected and used to recover PHA.

The biomass received was in the form of slurry. The slurry was washed with distilled water and decanted to recover solids. The process was repeated 4-5 times until supernatant liquid was clear. The sample was measured in volume and the dry weight of that specific volume was carried out in hot air oven at 60°C until biomass is completely dried. To recover PHA, wet biomass was used in all experiments, whilst hypochlorite and chloroform were used to extract PHA. Hypochlorite (30% v/v) with biomass was stirred at 23°C for 2 h. This step weakens walls of cell and release PHA. Cell walls were oxidized and remained in dissolved form in hypochlorite solution then in separating funnel hypochlorite was removed. To increase purity of PHA, chloroform was added in equal volumes to the remaining biomass and stir it for another 1 h. A distinguished layer of PHA was observed in the mixture of biomass, chloroform which was recovered successfully. The recovered PHA was washed with distilled water for 2-3 times to remove sodium hypochlorite or chloroform. Hypochlorite (10% V/V), sodium chloride (99.8%) and chloroform (35%) were procured from Merck, India and were of analytical quality.

3. Results and discussion
Current work demonstrates preliminary findings of PHA recovery from biomass with several methods optimized in literature. The methods applied here were used in literature for different cultures and
substrates. As discussed in section 2 substrate used in this work was wastewater from industries. To produce biomass or in other terms to reduce wastewater parameters like total Total Kjeldal Nitrogen, Chemical oxygen demand and total phosphorus, mixed culture consortia was used for secondary treatment.

Results of PHA extracted from biomass with different methods are shown in table 1. In first method sodium hypochlorite was used to disrupt the microbial cell, it dissolves the cell wall and PHA remains undissolved and recovered as a precipitate. The procedure was followed as per Hahn et al., 1994. The recovery of PHA was carried out at dry cell weight basis. The recovery of PHA and purity can be increased by varying sodium hypochlorite solution concentration.

| S.N. | Method                              | Physical appearance | Recovery of PHA based on cell dry weight (%) |
|------|------------------------------------|---------------------|---------------------------------------------|
| 1    | Disruption by sodium hypochlorite  | Clear white         | 71.8                                        |
| 2    | Disruption by hydrogen peroxide treatment | Clear white     | 49.1                                        |
| 3    | Denaturation with Sodium chloride  | Dark brown          | 82.3                                        |
| 4    | Denaturation with Sodium hydroxide | Dark brown          | 96.6                                        |
| 5    | Weakening by heating and disruption by hydrogen peroxide | Clear white | 31.3                                        |
| 6    | Weakening by heating and disruption by sodium chloride | Dark brown       | 40.6                                        |

High purity P (3HB-co-3HV) was recovered by Eutropha R [5]. The procedure includes pretreatment of biomass at 120°C for 60 sec and hydrogen peroxide was added. Recovery of PHA with pretreatment by heat and without heat was observed. Recovery was found to be less with heat treatment. However, purity may have increased.

Similar heat pretreatment was also applied in other experiment and sodium chloride was used for denaturing the cell wall. In these conditions again heat pretreatment depicts reduced recovery of PHA. Sodium hydroxide was used to remove cell walls from biomass and recovery of PHA was more than 96%.

![Figure 1](image1.jpg)

**Figure 1.** Images of PHA extracted by two different methods (a) sodium hydroxide, (b) Sodium hypochlorite.
The biomass generated from wastewater treatment was of dark brown color, it was same as of wastewater. Treatment with sodium hypochlorite and hydrogen peroxide oxidized the aromatic compounds which prevails color to the biomass (figure 1). The PHA recovered by this method is white in color. In other cases where oxidizing agent was not used, PHA was brown in color same as the biomass received. The recovery of PHA was calculated by dry weight basis.

The observations and experiments were performed on real effluent treatment plant biomass and represent the first observation in literature for extraction of PHA from biomass of process industries effluent treatment plant. The extraction of PHA from sodium hypochlorite as reported in literature is upto 91%. PHA was produced from glucose and contains a single source of carbon [6]. However, PHA recovered is from heterogeneous source of wastewater from process industries. A common source was the sewage wastewater or any food industry wastewater which contained high cellulose, protein and glucose. The PHA recovery in current work is 71%, the reduced recovery may be the result of reduced sodium hypochlorite concentration. As well as aromatic and cross linked substances present in wastewater which are difficult to disintegrate by mixed culture and may contain less amount of PHA within cell. Hahn et al in 1994 utilized single strain culture that is, *Alcaligen eutrophos* which resulted in more purified and good recovery of PHA. PHA recovery can be enhanced with increasing concentration of hypochlorite.

A sewage wastewater treatment plant was used to recover PHA at pilot scale with mixed culture consortia to remove biological nitrogen and reduce chemical oxygen demand. PHA accumulated by microbes was 49% [7]. In other study of glycerol from biodiesel, a side product as a substrate was taken for PHA production. The recovery of PHA was obtained only 24% [8]. Optimization of the process is in process. The heat application as a pretreatment step can reduce the molecular weight of the polymer as well as solubilization of polymer in second step of the chemical treatment. The characterization of biopolymer produced for functional groups and molecular weight is undergoing.

### 4. Conclusion

It presents a brief explanation of PHA recovered by different methods. The chemical disruption of microbe cells with sodium hypochlorite, sodium hydroxide, sodium chloride and hydrogen peroxide. The application of heat was done to increase the kinetics of the process however resulted in reverse. As per literature available physico-chemical method can be a good combination to extract PHA. It will retain molecular weight of polymer intact as well as it will result in higher yield. In physical separation dissolved air flotation can be a good unit operation where polymer can be separated as hydrophobic particles with bubbles. Ultimate purpose of the work is to optimize the extraction process of PHA from cost effective source so that the generated biopolymer can be used for everyday commodities.

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