Extraction of Polyhydroxyalkanoate from Waste Water- A Review

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***Abstract*— The recovery of polyhydroxyalkanoate (PHA) from waste water using Bacteria, that is, from bacterial cells, is one of the major obstacles in the industrial production of these polyesters. The production of PHAs by submerged fermentation processes has been intensively studied over the last 30 years. In recent years, alternative strategies have been proposed, such as the use of fermentation or the production of PHAs. In This paper gives brief information about various papers in the same field.**

***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[1]. 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[2]) 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. Global bio economy concepts often neglect the biomaterials in the policy outlines.

1) 1 kg raw PHA pellets can save 2.9 kg CO2 equivalents compared to HDPE
2) 1 kg raw PHA pellets can save and up to 15 kg CO2 compared to polystyrene[3]

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[4].

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[5]

II. LITERATURE SURVEY

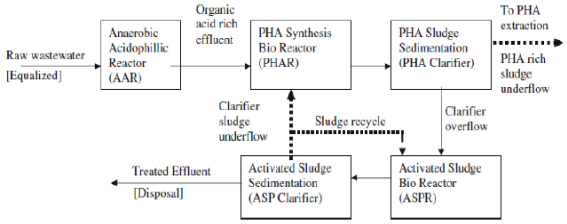
Ranjana Rai et al. (2011) [6] successfully performed experiments on extraction of biomass which was grown in waste water using Pseudomonas bacteria. Initially, the organism was grown under specific conditions at 30°C and 200 rev per min with mineral salt medium. After bacteria growth and its aged time, biomass was separated from water. Again the biomass was used to extract PHA from bacteria cell. PHA was recovered using various methods like dispersion of chloroform and Sodium Hypochlorite, Extraction using Hexane, Extraction using Acetone, Chloroform extraction, Soxhlet Extraction. PHA was successfully extracted from biomass. A detailed downstream processing study the effects of an extraction method on the yield, molecular weight, thermal properties of the polymer. Here, Using dispersion of Chloroform and Sodium Hypochlorite, give highest percentage yield of 31.38%. Another method Extraction using Chloroform give 23.04%, Using Acetone extraction it was 21.40%. The lowest yield was 12.64% using Soxhlet extraction. Therefore, Dispersion using chloroform and Sodium Hypochlorite had showed excellent performance for PHA extraction.

Here, characterization tests of PHA have done using structural characterization. The structural characterization of the kind of PHA monomer accumulated by the organism was carried out by performing nuclear magnetic resonance spectroscopy (NMR), attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy and gas chromatography mass spectroscopy (GC-MS).

They have also concluded that Mechanical, thermal, chemical, and surface study of the polymer revealed that the polymer is flexible, elastomeric, and semicrystalline, with moderate hydrophobicity.

Results of the analysis carried out on the polymer proves without a doubt that P(3HO) is a promising new biomaterial with great potential for soft tissue engineering, such as cardiovascular, skin, and nerve tissue engineering and drug delivery.

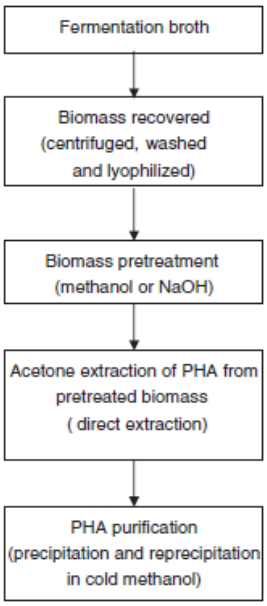
Parth Chakravarty et al. (2009) [7] have studied on PHA production in pilot scale continuous mode waste water treatment system. At the initial start-up period, the activated bio-sludge for anaerobic acidogenic reactor (AAR) was inoculated from the existing up-flow anaerobic sludge blanket (UASB) reactor; while the PHA synthesis reactor (PHAR) and activated sludge production reactor (ASPR) were inoculated from the aeration tank of the existing ETP. Here, PHA rich biomass was finally harvested at the clarifier which is made from milk and dairy waste water using Pseudomonas bacteria culture.



2.1 Flowsheet of process for PHA

PHA rich sludge from PHA clarifier was palletized in a cooling centrifuge followed by acetone wash and then lysis of sludge biomass with 5% sodium hypo chloride (NaOCl). The palette was further subjected to few acetone washes and finally PHA was extracted into hot (at 60°C) chloroform. Here, we are only focusing on extraction process for PHA, not focusing on sludge formation process. Using this separation process yield of PHA was 33.8%.

Xuag Jiangn et al. (2006) [8] show that a methodology was developed for the extraction of medium chain length PHA (mcl- PHA) from Pseudomonas. Here they have performed experiments based on Extraction using Acetone and pretreatment using NaOH or Methanol.



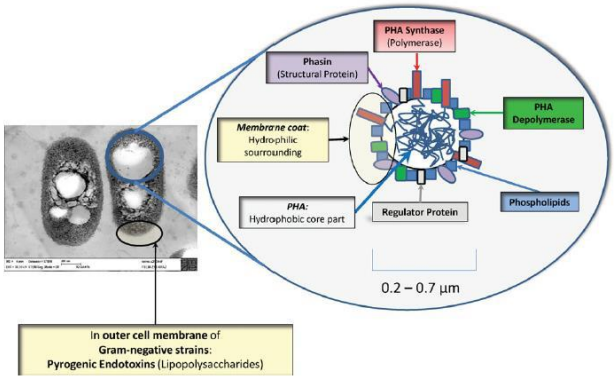
2.2 Schematic flowchart of the PHA recovery process

Solvent extraction is simpler in terms of the number of steps employed but there are still important choices to be made when designing a process. A PHA extraction process invariably involves three steps. These are biomass pretreatment, solvent extraction, and polymer purification. The pretreatment step may incorporate enzymes to degrade proteins and DNA, heating to denature these macromolecules, surfactants to remove lipids and/or solvents to remove water and polar lipids. Pretreatment is critical to the subsequent solvent extraction process as it affects the accessibility and the solubility of the PHA. Solvent extraction of scl-PHA most often involves chlorinated hydrocarbons such as chloroform but mcl-PHA is soluble in a much broader solvent range, so cheaper and less toxic solvents may be used. For pretreatment process they used 20 ml Methanol or 0.1 N NaOH or Water with 1g sample of Biomass for 30 min at 22°C. And then Extraction using Acetone was done to extract PHA from pre-treated biomass. They have found that % yield of PHA is 24% using Extraction using Acetone alone. But they have noted that % yield of PHA can be increase by Pretreatment of Biomass using Methanol or Water or NaOH. They have also described about lost PHA during recovery process. They have stated that a PHA balance of a recovery

process in which methanol pretreatment was followed by direct acetone extraction under ambient conditions and then precipitated in cold methanol was conducted to determine where PHA was lost during the process. For the biomass containing 66% PHA, the total amount of PHA detected from all steps of the separation process was 94% of the initial PHA but for the 10% PHA biomass the total amount detected was only approximately 74%. They have also described about lost PHA during recovery process. They have stated that a PHA balance of a recovery process in which methanol pretreatment was followed by direct acetone extraction under ambient conditions and then precipitated in cold methanol was conducted to determine where PHA was lost during the process. For the biomass containing 66% PHA, the total amount of PHA detected from all steps of the separation process was 94% of the initial PHA but for the 10% PHA biomass the total amount detected was only approximately 74%. This is likely due to the inaccuracy involved in working with a much smaller quantity of PHA.

Martin Koller et al. (2013)[9] reviewed strategies for recovery and purification of PHA biopolyesters from biomass.

- Paper shows the applicability of a PHA recovery method depends on the subsequent factors:
- The microbial production strain (different strains display different fragilities of the cell envelops)
- The type of PHA regarding the composition on the molecular level (scl-PHA, mcl- PHA)
- The intracellular load of PHA
- The required product purity that is determined by the final application of the biopolymer
- The eventual in house disposability of chemicals for PHA recovery, e.g., extraction solvents
- The acceptable impact of the isolation method on PHA final molar mass



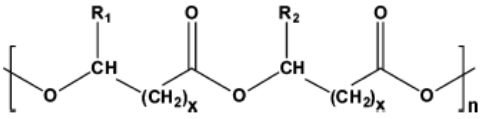
2.3 Bacteria with PHA granules in it

They showed various methods to recover PHA. In first method they discussed various methods of direct extraction of PHA from biomass. Here they discussed about pretreatment of Biomass and Solvent extraction using application of Halogenated Solvents and use of non-halogenated solvents. They stated that using of halogenated solvents like chloroform has harmful character so it is major drawback for this process. Also, solvents like chloroform and alcohol for precipitation are further discarded due to increasing cost using distillation apparatus. On laboratory scale, this problem can be overcome using Soxleth apparatus enabling the use of reduced solvent volumes under continuous process mode. But, this process is not feasible method for implantation on industrial scale.

Also, talked about application of Supercritical fluids. But here in case of supercritical fluid the operating condition is highly dangerous because of higher pressure in it so this method cannot be generally applied in practical work. Another methods are cell disruption by various chemicals, mechanical disruption of cells, disruption of osmophilic microbial cells,etc.

Mohammad Madkour et al. (2013) [10] also reviewed various methods for recovery of PHA from Biomass. The recovery of polyhydroxyalkanoates (PHAs) from biomass, that is, from bacterial cells, is one of the major obstacles in the industrial production of these polyesters. Since PHAs are naturally synthesized as intracellular storage compounds for carbon and energy and are for this deposited in the cytoplasm of the bacterial cell, PHAs are more or less tightly linked with the entire

biomass, and the polyesters must be released from the cells before their isolation and purification can be conducted. This additional step, that is, the release from the cells, is a major difference from most other biotechnological processes where the product occurs outside of the cells because it is secreted into the medium in a bioreactor or because it is synthesized in vitro in an enzyme reactor in a cell free system. This additional step contributes significantly to the overall costs of production. In this review they provided an overview about the different processes that result in the release of PHA from the cells, and we evaluate these processes with regard to the suitability at large scale in the industry.



Generalized chemical structure of polyhydroxyalkanoates:

R1/R2, alkyl group; n: 100−30000; X: 0−4

Here, they have discussed about advantages and disadvantages of various methods like solvent extraction, supercritical fluids, etc. Detailed information based on Pretreatment of Biomass, release of PHA from cell and final purification of PHAs is discussed. In this paper, they have highly focused on downstream process for PHA recovery.

S. Follonier et al. (2015) [11] performed experiment to produce mcl- PHA using freezed biomass using methylene dichloride. Here biomass was produced using grape pomace.

The polymer was extracted and purified using method as follow:

The biopolymer was recovered from the freeze-dried biomass by solvent extraction using methylene chloride (CH_2Cl_2), and purified by precipitation in cold methanol. Briefly, the biomass was crushed into small pieces with a mortar and pestle, and added to CH_2Cl_2 (60 g of biomass for 1 L CH_2Cl_2). The residual cell biomass was separated from the mcl-PHA containing solvent phase by pressure filtration (1 L stainless steel filtration holder white ribbon filter papers. The filtrate was concentrated in a rotary and then added dropwise into ice-cold methanol under agitation (volumetric ratio methanol/mcl-PHA solution at least 3:1). The methanol phase was then discarded and the polymer recovered using CH_2Cl_2, which was subsequently evaporated in a vacuum dryer.

J.A. Ramsay et al. (1994) [12] performed experiments for extraction of PHA using chlorinated solvents with pretreatment with acetone and without pretreatment. They have done experiments using A. eutrophus biomass which was contained 50% PHB by dry weight.

Here, they have used three different solvents in solvent extraction method: chloroform, Methylene Chloride and 1,2 Dichloroethane. Contact time was 12 hrs for all three solvents.

For chloroform, without pretreatment with acetone the purity was highest at 94% and with pretreatment with acetone the purity was highest at 96%.

For Methylene Chloride, purity was 93% and 98% with pretreatment without acetone and pretreatment with acetone respectively.

For 1,2 Dichloroethane, purity was same as purity using methylene chloride.

T. Pittmann et al. (2016) [13] show that Biopolymers, which are made of renewable raw materials and/or biodegradable residual materials, present a possible alternative to common plastic. A potential analysis, based on experimental results in laboratory scale and detailed data from German waste water treatment plants, showed that the theoretically possible production of biopolymers in Germany amounts to more than 20% of the 2015 worldwide biopolymer production. In addition a profound estimation regarding all European Union member states showed that theoretically about 115% of the actual worldwide biopolymer production could be produced on European waste water treatment plants.

T. Pittmann et al. (2014) [14] reviewed that the production of polyhydroxyalkanoates (PHA) as a side stream process on a municipal waste water treatment plant (WWTP) at different operation conditions. Therefore various tests were conducted regarding a high PHA production and stable PHA composition. Influence of substrate concentration, temperature, pH and cycle time of an installed feast/famine-regime

were investigated. The results demonstrated a strong influence of the operating conditions on the PHA production. Lower substrate concentration, 20 °C, neutral pH-value and a 24 h cycle time are preferable for high PHA production up to 28.4 % of cell dry weight (CDW). PHA composition was influenced by cycle time only and a stable PHA composition was reached.

Emmanouela Korkakaki et al. (2016) [15] shows that Leachate from the source separated organic fraction of municipal solid waste (OFMSW) was evaluated as a substrate for polyhydroxyalkanoates (PHA) production. Initially, the enrichment step was conducted directly on leachate in a feast-famine regime. Maximization of the cellular PHA content of the enriched biomass yielded to low PHA content (29 wt%), suggesting that the selection for PHA-producers was unsuccessful. When the substrate for the enrichment was switched to a synthetic volatile fatty acid (VFA) mixture -resembling the VFA carbon composition of the leachate-the PHA-producers gained the competitive advantage and dominated. Subsequent accumulation with leachate in nutrient excess conditions resulted in a maximum PHA content of 78 wt%.

Leda R. Castilho et al. (2009) [16] described Polyhydroxyalkanoates are biodegradable polymers produced by prokaryotic organisms from renewable resources. In recent years, alternative strategies have been proposed, such as the use of solid-state fermentation or the production of PHAs in transgenic plants. This paper gives an overview of submerged and solid-state fermentation processes used to produce PHAs from waste materials and by-products. The use of these low-cost raw materials has the potential to reduce PHA production costs, because the raw material costs contribute a significant part of production costs in traditional PHA production processes

III. CONCLUSION

This paper concludes the brief information about various papers described here.

ACKNOWLEDGMENT

I express my sincere thanks to internal guide Dr. Shina Gautam (Associate Prof. of Chemical Department), for her motivational support and continuous flow of encouragement while working in the direction of preparing of the project. I would like to appreciate the guidance given the judging panel especially in our project presentation that has improved our presentation skills by their comment and tips.

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