Production of Bioplastic Compound - Polyhydroxy Alkanoates

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Bioplastics

- Bioplastics have numerous advantages over conventional, petrochemical-plastics (petro-plastics) due to their inherent biodegradability, sustainability, and environmentally friendly properties.
- These fascinating biopolymers represent a possible eco-friendly alternative to petro-plastics, helping in the preservation of limited fossil fuel resources and also in the reduction of greenhouse gas emissions.
- Bioplastics are made partly or wholly from biomaterials such as sugar cane, polylactides, polyglycolic acids, polyhydroxyalkanoates (PHAs), aliphatic polyesters, and polysaccharides.
- PHAs are one of the most investigated class of bioplastics expected to replace some of the today's petro-plastics, due to their biodegradable, thermoplastic, and mechanical properties (e.g., versatility, elasticity, flexibility, etc.).
- The PHA Market Research Report stated that the market opportunity for PHA is expected to reach almost USD 98 million by the year 2024.

Polyhydroxyalkanoates (PHAs)

- PHAs are a family of biologically synthesized carbon-storage polymers in the form of polyesters, having similar mechanical properties to those of petrochemical-based plastics, with the additional advantage of being completely biodegradable.
- These renewable polyesters can be produced by various microorganisms in response to various stress conditions (for example, excess carbon or limited phosphate, nitrogen, sulfur, or oxygen) and provide protection from nutrient starvation and extreme conditions.
- A number of Gram-negative and Gram-positive bacteria (more than 55 genera) have been employed for the production of PHAs.
- Among the various types of PHA-producing bacteria, *Cupriavidus necator* (formerly known as *Wautersia eutropha, Ralstonia eutropha, Alcaligenes eutrophus*, or *Hydrogenomonas eutropha*) has gained the most attention due to its ability to grow on various carbon substrate and ability to be cultivated to high cell density.

Structure and Metabolic Pathway

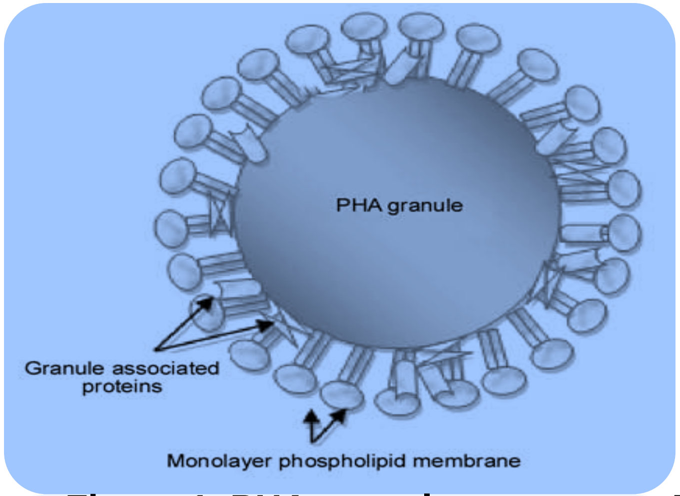
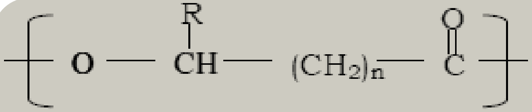


Figure 1. PHA granule: PHA are such linear polyesters surrounded by monolayer of phospholipis membrane and associated proteins



$$\left[O - \overset{R}{\overset{|}{CH}} - (CH_2)_n - \overset{O}{\overset{\|}{C}} \right]$$

n varies from 600 to 35000

R = hydrogen	Poly (3-hydroxypropionate)
R = methyl	Poly (3-hydroxybutyrate)
R = ethyl	Poly (3-hydroxyvalerate)
R = profile	Poly (3-hydroxyhexanoate)
R = pentyl	Poly (3-hydroxyoctanoate)
R = nonyl	Poly (3-hydroxydodecanpate)

Figure 2. PHA Chemical structure
SCL-PHA (3-5 C-length monomers)
MCL-PHA (6-14 C-length monomers)

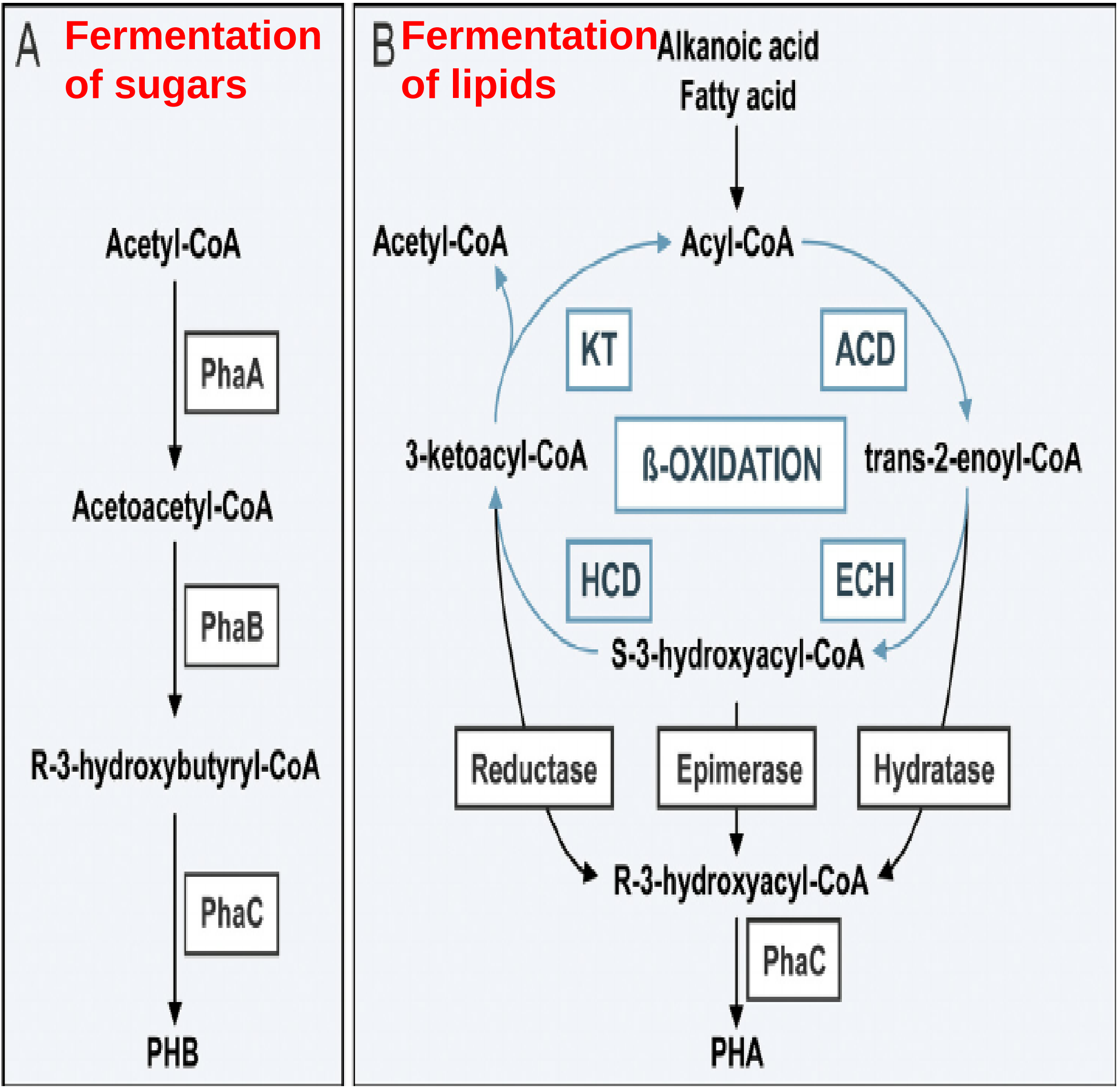


Figure 3. PHA Metabolic pathway

…Polyhydroxyalkanoates (PHAs)

- Polyhydroxyalkanoates (PHAs) are a family of bacterially synthesized biopolyesters with biodegradability, biocompatibility, thermoprocessibilty, and flexible strengths.
- PHAs are classified into two main groups, namely:
  - short chain length PHAs (scl) consist of 3–5 carbon atoms eg. PHB
  - Medium chain length (mcl)-PHAs consist of monomers with 6–14 carbon atoms, which are mostly biosynthesized by *Pseudomonas* spp.
- Poly-(*R*)-3-hydroxybutyrate (PHB) was the first PHA discovered and has been the best-studied PHA member.
- PHAs have found applications in the form of packaging materials including films, boxes, coating, fibers and foam materials, biofuels, medical implants, and drug delivery carriers.
- For large-scale applications such as biodegradable packaging materials, the cost and properties of PHAs are very important.
- Over the past years, process development and metabolic engineering approaches have been adopted:
  - to develop recombinant PHA production strains for improving the strains’ ability to produce PHA,
  - changing the PHA structures to obtain better thermal and mechanical properties.

Biosynthesis of PHA

- PHA synthase is the key enzyme in polymerizing PHA using the (*R*)-3-hydroxyacyl-CoA as a substrate.
- Depending on the subunits, amino acid sequence, and substrate specificity, this synthase can be classified into four groups (class I–IV).
- PHA synthases under class I, III, and IV were found to polymerize scl monomers, while class II polymerizes mcl monomers.
- One of the major restrictions for the wide commercialization and industrialization of PHAs is the high cost of production.

...Biosynthesis of PHA

- One of the major reasons for their high cost is the price of the carbon substrate used in the microbial cultivation process, which can account for 45 to 50% of the total production cost.
- In response to this issue, much research has been conducted into the possibility of using industrial waste streams for fermentation processes to make PHAs economically favorable.
- Plant and animal oils have been demonstrated to be excellent carbon sources for high yield production of PHAs.
- Important examples are the utilization of waste plant oils, molasses from the sugar industry, lignocellulosic materials, oil palm shell, pressed fruit fiber, biodiesel waste, and waste animal oil.
- So far, PHA production using waste plant oils (e.g., palm kernel oil, crude palm oil, palm oil, jatropha oil, sludge palm oil, soybean oil) as inexpensive carbon sources has been taken into consideration owing to its widespread availability and renewability.

… Biosynthesis of PHA

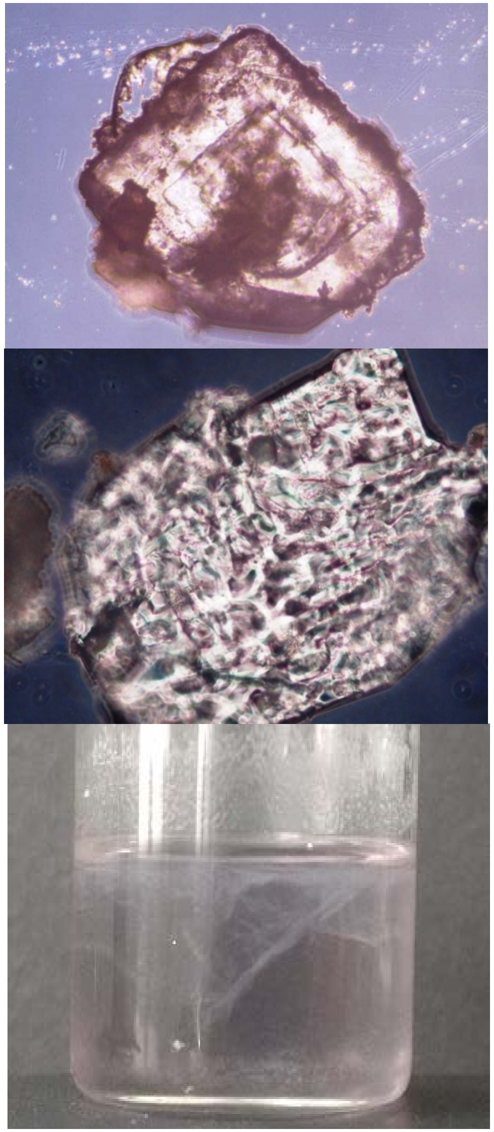
- In a study by Mozejko and Ciesielski, saponified waste palm oil (SWPO) was used as the sole carbon source for the production of mcl-PHA using *Pseudomonas* sp. strain G101. It was found that under fed-batch fermentation conditions with a total SWPO fed at 15 g/L, the bacterial strain in the study could produce up to 43% of mcl-PHA by dry cell weight.
- *P. oleovorans* NRRL B-14683, *P. resinovorans* NRRL B-2649, *P. corrugata* 388, and *P. putida* KT2442 synthesized mcl-PHA from crude Pollock oil (a by-product of the Alaskan fishing industry) ranging from 6 to 53% PHA content.
- *Salinivibrio* sp. M318, a halophilic bacterium isolated from fermenting shrimp paste, was recently reported to be capable of producing P(3HB) using mixtures of waste fish oil and glycerol as sources of carbon together with fish sauce as a nitrogen source (Van Thuoc et al., 2019).
- A production yield of up to 10 g/L of DCW and 51.7 wt% of P(3HB) were achieved during growth phase after 48 h of shake flask batch cultivation, and reached up to 69.1 g/L of DCW and 51.5 wt% of P(3HB) content after 78 h of fed-batch cultivation.
- This approach not only reduces the production cost for PHAs, but also makes a significant contribution toward the reduction of environmental pollution caused by the used oil.
- Advancements in the genetic and metabolic engineering of bacterial strains have enabled a more efficient utilization of various carbon sources, in achieving high PHA yields with specified monomer compositions

TABLE 1 | Summary of bacterial strains along with the waste oil carbon feedstocks that are utilized to produce various types of PHAs.

Bacterial strain	Waste carbon feedstock	Type of PHA produced	Reference
PLANT OILS			
Pseudomonas sp. DR2	Waste vegetable oil	mcl-PHAs (C6-C16)	Song et al., 2008
Pseudomonas sp. G101 and G106	Waste rapeseed oil	mcl-PHAs	Mozejko et al., 2011
Pseudomonas sp. G101	Saponified waste palm oil	mcl-PHAs	Mozejko and Ciesielski, 2013
P. putida KT2440	Hydrolyzed waste cooking oil	mcl-PHAs	Ruiz et al., 2019
P. putida S12	Sludge palm oil	Elastomeric mcl-PHAs	Kang et al., 2017
P. oleovorans ATCC 29347	Saponified jatropha oil	P(3HB-co-3HV)	Allen et al., 2010
C. necator H16	Spent palm oil + 1,4-butanediol	P(3HB-co-4HB)	Rao et al., 2010
C. necator H16	Waste frying oil (rapeseed)	P(3HB)	Verlinden et al., 2011
C. necator H16	Waste cooking oil (palm oil)	P(3HB)	Kamilah et al., 2013
C. necator Re2058/pCB113	Waste cooking oil (palm oil)	P(3HB-co-3HHx)	Kamilah et al., 2018
C. necator H16	Jatropha oil	P(3HB)	Ng et al., 2010
C. necator H16	African elemi oil, bitter apple oil, desert date oil, and *Amygdalus pedunculata* oil	P(3HB)	Zainab-L et al., 2018
C. necator Re2058/pCB113	African elemi oil, bitter apple oil, desert date oil, and *Amygdalus pedunculata* oil	P(3HB-co-3HHx)	Zainab-L et al., 2018
C. necator Re2058/pCB113	Sludge palm oil	P(3HB-co-3HHx)	Thinagaran and Sudesh, 2019
B. thailandensis	Used cooking oil	P(3HB), rhamnolipids	Kourmentza et al., 2018
ANIMAL OILS			
C. necator Re2058/pCB113	Low-quality waste animal fat	P(3HB-co-3HHx)	Riedel et al., 2015
C. necator H16	Low-quality waste animal fat	P(3HB)	Riedel et al., 2015
C. necator H16	Tallow	P(3HB-co-3HV)	Taniguchi et al., 2003
C. necator H16	Emulsified waste fish oil	P(3HB)	Kaesavan, 2014
C. necator H16	Emulsified waste fish oil with γ-butyrolactone	P(3HB-co-4HB)	Kaesavan, 2014
C. necator H16	Emulsified waste fish oil with sodium valerate	P(3HB-co-3HV)	Kaesavan, 2014
P. oleovorans NRRL B-14683	Crude Pollock oil	mcl-PHA	Ashby and Solaiman, 2008
P. resinovorans NRRL B-2649	Crude Pollock oil	mcl-PHA	Ashby and Solaiman, 2008
P. corrugata 388	Crude Pollock oil	mcl-PHA	Ashby and Solaiman, 2008
P. putida KT2442	Crude Pollock oil	mcl-PHA	Ashby and Solaiman, 2008
P. oleovorans NRRL B-778	Crude Pollock oil	mcl-PHA	Ashby and Solaiman, 2008
P. oleovorans NRRL B-14682	Crude Pollock oil	mcl-PHA	Ashby and Solaiman, 2008

Downstream Processing of PHA

- Unlike upstream processing, reports on the downstream processing and purification of PHAs are scarce.
- PHAs are present as amorphous solid granules suspended in the cytoplasm of the cells with 5–10 wt% of water along with a protein component containing PHA synthase and PHA depolymerase that binds strongly to the PHA granule.
- The separation of PHA from non-PHA cell mass (NPCM) is technically challenging because both PHA and NPCM are in the solid phase.
- **Chemical Treatment:** PHA molecules are dissolved in appropriate solvents and later the PHA is precipitated.
- The most commonly used solvents are chlorinated hydrocarbons, namely, chloroform, 1,2-dichloroethane, methyl ethyl ketone (MEK), and methyl isobutyl ketone (MIBK), or some cyclic carbonates such as ethylene carbonate and 1,2- propylene.
- PHA is precipitated using non-solvents like methanol or ethanol.



...Chemical Treatment

- In chemical digestion method, the NPCM is digested by sodium hypochloride or surfactants like sodium dodecyl sulfate (SDS), Triton X-100, palmitoyl carnitine, or betaine.
- Sodium hypochloride has a strong non-selective oxidizing property that can digest the NPCM.
- Sequential treatment with sodium hypochloride and a mixture of surfactants promoted the recovery of PHA with 50% reduced cost when compared to solvent extraction.
- Even though chemical digestion has a low operating cost, it is considered unattractive due to complications in wastewater treatment and also the cost involved in the utilization of surfactants.
- The enzymatic degradation technique is highly efficient and yields up to 92% purity because the enzymes are specific in their action.
- However, the cost of enzymes and the complexity of the procedure make it unattractive for industry.

Mechanical Treatment

- Mechanical disruption is the most common method for the removal of intracellular protein.
- Bead milling and highpressure homogenization are the most commonly used mechanical disruption techniques for PHA recovery.
- Mechanical disruption is the most favored method for PHA recovery due to the minimal damage caused to the polymer during the recovery process.
- Additionally, mechanical disruption is cost-effective and environmentally friendly.

Biological Recovery

- The concept is based on utilizing animals, which are fed with dried bacterial cells containing PHA.
- The animals will digest the NPCM and defecate the PHA.
- Mealworms and rats are attractive animal models to be used for PHA purification.
- The purity of PHA recovered from mealworms was lower than that recovered by rats.
- However, washing the fecal pellets with water and 1% sodium dodecyl sulfate (SDS) was able to improve the purity up to 100%.
- Later, the 1% SDS was replaced with 0.1 M sodium hydroxide.
- PHA recovered by this method was close to its native morphology in the bacterial cells, while the molecular weights of PHA were not affected.
- **Currently, this biological recovery method is being scaled-up at a mealworm farm in Malaysia**.

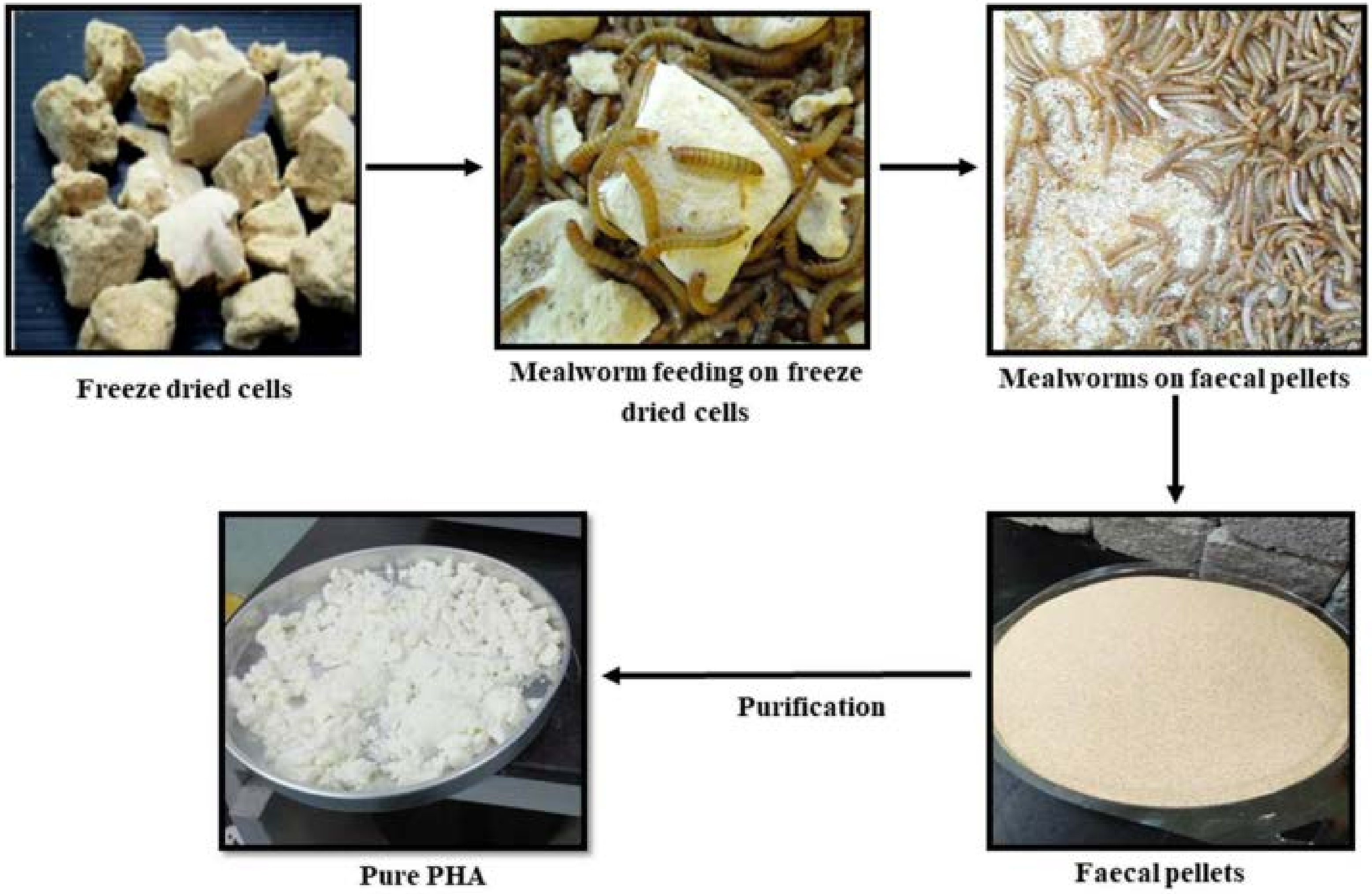


FIGURE 2 | Biological recovery process of PHA from bacterial cells by using mealworms.

THANKS