

Low-cost Fermentation of Polyhydroxyfatty Acid Esters

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Abstract: With the depletion of traditional fossil energy and environmental problems, there is an urgent need for alternative materials. Biosynthesis not only achieves low-carbon green economy, but also reduces energy consumption. Polyhydroxyfatty acid ester is a kind of material with excellent performance, but the production cost is high, mainly due to the high cost of fermentation carbon source, fermentation process and other factors. Using waste as substrate not only reduces fermentation costs but also solves environmental problems. On the other hand, mixed strains can be used simultaneously to reduce the cost of fermentation.

Keywords: Polyhydroxyfatty Acid Ester; Biosynthesis; Biomass Waste.

1. Overview of Polyhydroxyfatty Acid Ester (PHA)

1.1. Structure Classification of Polyhydroxyfatty Acid Esters (PHA)

In 1926, PHB were first identified in microorganisms, the first PHA to be identified. Fifty years later, new members of the PHA family are being synthesized in different microbial bodies [1]. Polyhydroxy-fatty acid ester (PHA) is a kind of macromolecular biolyester, which is an ester polymer in chemical nature. Under the action of microbial polymerase (PHAC), hydroxy-fatty acids with a certain length of carbon chain are interlinked to form ester bonds to form various types of PHA polyesters [2].

According to the different length of monomer carbon chain, PHA can be divided into short chain PHA and medium chain PHA. The length of monomer carbon chain of short chain PHA is generally 3 to 5, while the length of monomer carbon chain of medium and long chain PHA is between 6 and 14. According to different arrangement of monomers, they can be divided into homopolymers, random copolymers and block copolymers [3].

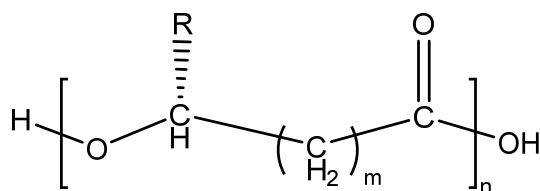


Figure 1. General structure of PHA

1.2. Material Properties of Polyhydroxyfatty Acid Ester (PHA)

The diversity of PHA material structure is due to the diversity of PHA monomer type, polymerization proportion, polymerization form, molecularweight of polymerization, resulting in the diversity of material properties, such as thermodynamic properties and biodegradability, which has a broad application prospect [4]. It is also biodegradable and biocompatible, and can be degraded into water and carbon dioxide by many microorganisms in the natural environment [5]. Different PHAs composed of a single monomer have

completely different thermal properties. The thermal properties of PHA composed of different kinds of short chain PHA monomers are also different. When the temperature exceeds the melting temperature, the middle and long chain PHA will exhibit viscosity [6]. Adding other polymers to the medium-long chain PHA can increase the melting point temperature and speed up the crystallization rate, which is helpful for polymer processing. The copolymer formed when a small amount of long chain PHA monomer is added to the PHA shows better flexibility and thermal stability in conventional thermoplastic processing [7].

2. Reduce Fermentation Cost through Substrate

At present, the main way to study the production of pha is through single microbial fermentation. Although the yield is high, the cost of fermentation substrate is relatively high. Meanwhile, single microbial fermentation requires strict aseptic environment in the fermentation process, which makes its production cost three times higher than that of traditional plastics and loses its competitiveness in the market [8]. Among the substrates required for PHA production, the cost of carbon source is the most expensive, accounting for 30% of the total cost, which is the main factor causing the high production cost of pha and limiting the large-scale application of PHA [9]. To be a stable carbon source, the quantity supply should be ensured and the quality should be relatively stable. Biomass waste is the waste generated by human production and consumption in the process of utilizing biomass. If it can be used as a carbon source, it is expected to reduce the cost of large-scale production of PHA. The use of biomass waste as a carbon source can not only reduce the expensive cost of microbial fermentation carbon source, but also make better use of the waste. The available carbon is mainly the following types: food processing waste, kitchen waste oil, lignin, etc.

2.1. Food Processing Waste:

Food processing waste is a kind of waste rich in nutrients in life. In most Chinese cities, its treatment not only does not make full use of energy, but also brings additional pressure to waste disposal. With the increase of people's awareness of environmental protection, the harmless treatment of waste

becomes very important, and the research of resource utilization is being carried out constantly. Anaerobic digestion can pretreat the waste under the condition of high solid content and high-water content. The requirement on the concentration of organic matter is not very strict, but the operation control is more complicated. If the proportion of oil in food processing waste is high, it will be treated with oil removal before anaerobic fermentation, but the oil component is difficult to be removed. In the process of anaerobic fermentation, long-chain fatty acids such as oil are organic matter that are difficult to degrade. In the process of fermentation, calcium fatty acid solids are easy to be produced, which will form massive precipitation in the fermentation and affect the fermentation effect. The solids may also clog the pipes in the fermentation system. In addition, the salt content of kitchen waste is generally relatively high, and the microorganisms that need to be enriched can synthesize PHA by using the hydrolytic acid liquid of kitchen waste containing certain salt as carbon source, and the salt-tolerant bacteria should be screened for synthesis under the condition of high salt content of garbage leachate. The byproduct of fermentation in this system, biogas residue, has comprehensive nutrition and rich nutrition. After the next step can be further processed into fertilizer. Therefore, it is urgent to develop the corresponding resource utilization of kitchen waste. After environmental protection treatment, kitchen waste can obtain volatile fatty acids through anaerobic fermentation, hydrolysis and acidification and other procedures. These products can be used as carbon sources for bacteria to synthesize PHA, which not only treats kitchen waste but also reduces the production cost of pha. Rathika R [10] took diluted sugarcane molasses as carbon source, and after 48 h fermentation by *Bacillus subtilis*, the biomass reached 9.5g /L, and the PHAs content reached 70.5% of the biomass. WenQX [11] used food fermentation broth as substrate to produce PHA with a yield of 44.8% of dry weight.

2.2. Kitchen Waste Oil

Oil is an indispensable component in people's normal life diet. It not only provides people with calories, but also contains fatty acids and various fat-soluble vitamins that the human body cannot synthesize but needs very much. Our country consumes a huge amount of edible oil every year, while the waste oil quantity is about 6 million t. The figure is also expanding constantly. At present, oils and fats can be further processed into surfactants and biodiesel in the industry. In the production process, a large amount of waste oils and other by-products will be discarded, and the continuous accumulation will bring great pressure to the environment and human beings. If a large number of oils and fats are not handled properly, the harmful substances will enter our living environment, which will be harmful to the environment and human body to a certain extent. Fats contain fatty acids and phytosterols. Fats and oils are hydrocarbons that can be used as carbon sources by microorganisms. Biosynthesis of biodegradable plastic-PHA from waste oil can solve the problem of environmental pollution caused by improper disposal of waste oil on the one hand, and realize the recycling of waste resources. Palm oil in edible vegetable oil is a kind of oil that people produce and consume a lot at present. However, after the production of palm oil, the wastewater still contains a lot of low-quality palm oil sludge spo, which is rich in nutrients. SPO mainly contains long-chain fatty acids, which is an ideal substrate for pha synthesis. The pha

synthesis efficiency of long-chain fatty acids obtained from oil treatment is higher than that of other carbon sources, and more than 1gpha can be synthesized per gram of vegetable oil. This is because the long-carbon fatty acids contained in oil are easily decomposed into many short-chain fatty acids, which are used through β -oxidation pathway to directly generate acyl coA, the precursor of PHA. However, many PHAs are synthesized by the dehydrogenation of acyl coenzyme A to enyl-coenzyme A, which is then processed by enyl-coenzyme A hydratase to generate hydroxyalkyl coenzyme A, and then further catalyzed to synthesize pha [12]. Ward PG [13] found that the yield of PHA produced by *Pseudomonas* using spo was similar to that by using fatty acids, avoiding the use of delicate carbon sources and greatly reducing the cost of substrate. Mohamad AH [14] used waste glycerol production as substrate to produce PHAs that reached 80% of dry weight.

2.3. Lignocellulose:

There are about 900 million tons of agricultural and forestry wastes produced in our country every year. The incineration of agricultural and forestry wastes is an important factor in the formation of haze. It not only wastes part of the energy that can be used, but also has a great impact on our environment. Therefore, it is of great significance to properly utilize agricultural and forestry wastes and develop clean utilization technology. The lignocellulose in agricultural and forestry wastes is wood cutting residues and planting wastes in industrial processing. The cheap lignocellulose in wastes cannot be effectively used because there is no suitable way for a long time. However, the compact structure of most lignocellulose prevents enzymes from breaking it down, so it is important to use it for good pretreatment. A lignocellulosic polymer containing oxypropanol that is depolymerized and deoxidized into monomers before it can be used by microorganisms for normal metabolic activities. In the process of lignin biodepolymerization, wood rot fungi and other microorganisms were found to be efficient in lignin biodepolymerization. White rot fungus in wood rot fungi mainly produces Laccase, Lignin peroxidase, and a variety of extracellular oxidoreductases as well as its synthesized free radical auxiliary enzyme system to depolymerize lignin [15]. After the above reaction, wood rot fungi completed the biodepolymerization of lignin, in which carbohydrate complexes, lignin side chains and aromatic ring structures were cracked. The biodepolymerization process is a REDOX reaction using lignin degrading enzyme system. The degradation rate of lignocellulose is very important when using microorganisms such as wood rot fungi. On the other hand, the cellulose structure after degradation also plays a crucial role in the later utilization. Li D [16] used fermented wood fermentation broth as carbon source to produce PHAs up to 50.3% of dry weight. Kumar [17] used lignocellulose after microbial fermentation as substrate for fermentation, and the final yield of PHA reached 11.1g/L.

3. Reduce the Cost of Fermentation through Strains Mixed Flora Fermentation:

Compared with the energy-consuming production method of chemical synthesis of PHA, PHA synthesis by microbial fermentation has mild conditions, simple operation and wide application, which is more in line with the green development

concept of low energy consumption. Microbial synthesis of biodegradable plastics has become a hot research direction and has a good development prospect and broad application market. A variety of PHA-synthesizing bacteria can be isolated in a variety of environments, and they can use different carbon sources for efficient fermentation, such as: *Pseudomonas*, *Alcaligenes*, et al [18]. *Rhodospirillum rubrum* is a purple non-sulfur bacterium, belonging to the class of α -protein bacteria, which is known for its metabolic diversity and can undergo autotrophic or heterotrophic metabolism. *rubrum* is able to grow using aerobic respiration as well as anaerobic photocooperation using light as an energy source. Often, excess carbon is supplied with nitrogen, phosphorus, oxygen, potassium, or other essential nutrients, this unbalanced growth condition is conducive to the accumulation of PHA in *rubrum* [19]. But at present, the high production cost of PHA limits the large-scale application of PHA [20].

A variety of bacteria in nature have the ability to synthesize PHA, so the synthesis of PHA can also be carried out by using mixed bacteria community. Fermentation in open environment saves costly sterilization and aseptic fermentation environment, and mixed bacteria community reduces the production cost of PHA. To make the mixed bacteria produce PHA, first determine the fermentation substrate, using biomass waste can significantly reduce the cost of PHA production, and conduct the pretreatment of substrate. For the enrichment and screening of mixed strains, the treated substrate is used to enrich PHA synthetic bacteria. The enrichment process is usually carried out by the satiety and starvation method. Firstly, the carbon source is sufficient so that all microorganisms can grow rapidly, and then the carbon source is restricted so that the bacteria that have not accumulated PHA cannot continue to survive, so that the bacteria with the ability of PHA synthesis can be successfully enriched. The enrichment flora is used for the accumulation and production of PHAs, and the biomass of the flora will affect the production level of PHAs. Huang [21] proposed the method of extended culture for PHA production. By adding carbon sources and nutrient elements other than carbon sources into the substrate, the bacteria obtained carbon sources and accumulated PHAs in one fermentation container, and obtained other growth elements in another container, and grew with in vivo PHA. After repeated culture, the biomass of enriched bacteria was continuously improved, and the bacteria with high PHA synthesis ability was preserved. Under this method, microbial biomass was increased 52 times to 17.22 g/L cell concentration, and the PHA synthesis efficiency was 0.49 g/g (CDW).

4. Conclusion and Prospect

With the depletion of fossil fuels and environmental problems, it is important to seek reasonable solutions. Polyhydroxyfatty acid esters are widely studied for their good properties. More and more kinds of polyhydroxyl fatty acid esters with excellent properties have been developed and can be used more widely. But his mass production became a problem. A lot of research has been done on its high production costs. The use of cheap carbon sources, such as kitchen waste, kitchen waste oil and lignocellulose, can not only solve the problem of waste disposal, but also provide help for the production of polyhydroxyfatty acid ester, reducing the cost of production. On the other hand, genetic engineering technology uses strains to optimize and transform,

using mixed bacteria and halophilic bacteria, etc., to better synthesize more polyhydroxyfatty acid esters under non-sterilization conditions. PHA, as a bioplastic, will save petrochemical resources, reduce environmental pollution and achieve sustainable development of resources. In future research, polyhydroxyfatty acid ester materials with more comprehensive performance and lower cost will be developed to replace the existing materials with pollution or poor performance, and really play a role in our life.

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