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Microbial Production of Polyhydroxyalkanoates (Biodegradable Plastics)

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ABSTRACT: Over the past two decades, the use of synthetic plastics has soared throughout the capitalist economy. Frequently, polymers that have been used as raw materials are returned to the environment in a severely degraded state before being hastily discarded. Researchers are becoming increasingly concerned about the potentially harmful effects of synthetic polymers and the products made with them. As a direct result, both the scientific community's interest in developing technical solutions to this issue and the general public's awareness of the importance of waste reduction have increased. Under a variety of environmental conditions, microorganisms, or a subset of them, can produce chemically unrelated substances known as "biomaterials." A significant subcategory of biomaterials is bioplastics. PHAs, also known as polyhydroxyalkanoates, are biodegradable substances that act as carbon and energy reservoirs in a variety of microorganisms. These materials are also referred to as "bioplastics. They have a wide range of applications in a variety of industries, including tissue engineering, bio-implant patches, drug delivery, surgery, and wound dressing, and are considered an antiticing alternative to petroleum-derived plastics.This review examined the most recent scientific publications on the isolation of bioplastic-producing bacteria from various geographic regions, the production of bioplastic from inexpensive carbon sources, and the most important production-related factors.In addition, the primary applications of these biopolymers in the field of biotechnology were considered.

Keywords: Bioplastic, Polyhydroxyalkanoate (PHA), waste cooking oil (WCO), and bioreactor

INTRODUCTION

Plastics are synthetic polymers composed of repeating molecular units in long chains known as monomers. The petrochemical industry produces monomers such as acrylonitrile, styrene, and vinyl chloride by distilling crude oil in refineries. Monomers are the constituents of polymers, which are the constituents of the petrochemical industry. Plastics are extremely common in human society and the natural world, with diverse physical properties, chemical compositions, and potential applications [1]. Synthetic plastics have been developed to become a major industry and one of mankind's greatest inventions and because they are an essential part of everyday life they are designed in such a way that they are inert to chemical and natural breakdown and suitable for long lasting and consistent [2]. According to [3], annual production surpassed 359 million tons in 2018, resulting in trillions of dollars in global economic gains. Actually, 93% of the world's 34 million tons of plastic waste is dumped in landfills and oceans yearly. People sometimes get rid of plastic waste by burning it, this combustion releases toxic smoke that contains some compounds cause cancer, liver cirrhosis, hepatitis, nerve disorder, nerve disorder, triggering depression and a wide range of diseases that can infect humans in the event of inhaling these releasing compounds. The burning of plastics also produces large amounts of vinyl chloride compounds, causing environmental pollution.

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In addition, plastic waste, especially plastic bags from supermarket, can clog drainage channels and rivers if they are discarded carelessly [4]. Plastic's petrochemical sources are depleted and will not be replenished for millions of years. According to a growing body of evidence, plastic could persist in the environment for hundreds of years as dangerous microscopic particles that endanger the environment, particularly marine ecosystems [5].The stability of these industrially produced polymers has a significant environmental impact, although they are generally inexpensive. Due to the environmental impact associated with these products, the rising concern about oil prices and the imminent fossil fuel crisis, the search for alternatives to reduce humanity's dependence on non-renewable resources is essential. Because they are inherently "eco-friendly," biodegradable plastics are the most efficient way to protect the environment from the risks posed by traditional petroleum-based plastics. This is due to environmental microorganisms' ability to degrade biodegradable plastics into less complex ones. Instead of using petroleum-based products, developing and using biodegradable plastics that degrade naturally over time is a promising alternative. Bioplastics such as polyhydroxyalkanoates (PHAs) and comparable substances can help to achieve sustainable development. PHA is a biodegradable and biocompatible polyester used in many prokaryotic organisms' cells to store carbon and energy. Various polymers can be produced depending on the carbon source used during the fermentation process and the bacterial species chosen. Although there are many types of plastic that are biodegradable in varying degrees, only polyhydroxybutyrate (PHBs) are 100% [6,7]. Despite PHA's excellent properties and environmental friendliness, its widespread use has been hampered because the fermentation process requires using expensive carbon sources, which accounts for roughly 40% of total PHA production costs. This is unfortunate because PHA materials offer a lot. It has been proposed that using waste products as carbon sources for producing PHA from microorganisms could reduce the costs of both PHA production and waste disposal simultaneously[8].

1. Synthetic plastics

Plastic waste pollution in ecosystems, particularly aquatic ecosystems, is recognized as a significant environmental burden due to the long time it takes for plastic to biophysically degrade and the negative effects it has on wildlife which is typically filled with municipal solid waste (MSW) at the end of life of these plastics. Petrochemical-based polymers are a major concern because of the long time it takes for the plastic to biophysically degrade. In addition, it's pose a significant environmental risk because they contain a number of potentially hazardous components. Phthalates, brominated flame retardants, bisphenol A (BPA), antimony trioxide, and polyfluorocarbons are among them (PFCs). These substances could contaminate the environment and endanger public health [9]. This has stimulated scientific and financial efforts to replace conventional polymers with biopolymers, ensuring similar properties as an alternative solution. These biodegradable polymers can be used in various industries, including biomedicine, agriculture, active packaging, aerospace, and cosmetics while reducing human activity's negative environmental effects [10,9].

2. Biodegradable plastics.

The fact that the vast majority of bioplastics are completely biodegradable contributes to their low overall environmental impact. Biopolymers, which are used to make bioplastics, are derived from organic materials that are abundant. PHAs, or polyhydroxyalkanoates, are a class of substances with a wide range of mechanical and thermal properties. These compounds are currently generating much interest due to their adaptability. PHAs are polyesters with a linear structure. In addition to chain length, the characteristics of the monomer used in synthesis include x, the side chain R, and the length of the alkyl chain n. These properties allow the monomer's properties to be altered. PHAs, also known as polyhydroxyalkanoates, are particularly intriguing biodegradable biopolymers because bacteria can produce them from a wide range of complex organic substrates. As a result, PHAs are among the biopolymers with the highest biodegradability [11,10].

3. History

Although these polymers were discovered in 1888, little is known about their composition and how they function. In 1926, a French scientist isolated poly-3-hydroxybutyric acid (PH3B) from Bacillus megaterium. The ability of this acid to store carbon and energy was discovered in 1959, and after one year the commercialization of PHA started. PHA was typically produced through a microbiological process for commercial use. On the other hand, extensive research has been conducted on enzymatic and chemical processes as potential means of improving control over a product's chemical and physical properties [10]. PHB's commercialization process began in 1963, when Chemistry and Engineering News published a story about the development of a fermentation-grown thermoplastic biopolymer at W. R. Grace & Co. The article discussed how the specific biopolymer evolved. According to the article, the polymer was drawn out of bacterial cells as PHB granules smaller than a micron grew. This occurred during the PHB's expansion. Bacteria produce a polymer that accounts for 70-80% or more of their dry weight after acetone treatment and chloroform extraction. Dr. Jim Baptiste was hired as Grace's first biochemist after his superiors recommended him. Because the company had a sizable business in packaging fruit with polyvinyl film, it was expected that "Grace Chemical" would hire a biochemist to understand how fruit ripens

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while contained in packaging. Dr. Jim Baptist eventually persuaded Werber that it was time to use the bacteria's holographic PHB and isolate it similarly to grains of starch, corn, and wheat. PHB had the advantage of being biodegradable and hydrophobic, biocompatible, and film-forming [12].

4. PHAs Classification

PHA can accumulate in up to 80% of the dry biomass. PHA will accumulate in environments rich in carbon and energy sources, but its growth will be hampered by a lack of nutrients required for biomass syntheses, such as oxygen, phosphorus, and nitrogen. The most important polymer of PHA is poly-3-hydroxybutyrate (PHB), which has the following monomer formula. CO, CH2, and OCH (CH3). Another important PHA polymer is polyhydroxy (PHV), and its monomer formula is as follows: CH2, OCH (CH2CH3), and C(O)- [13]. Polyhydroxybutyrate (PHB), the most common type of PHA, is produced by microorganisms. *Cupriavidus necator* has a remarkable ability to accumulate polyhydroxybutyrate (PHB) below phosphorous or nitrogen limitation, which can be up to 90% of its dry cell weight. Bioplastics have recently gained popularity as an alternative to conventional plastics derived from fossil fuels due to their ability to reduce petroleum consumption and the environmental impact of waste plastic (such as polyol). Low-impact bioplastics have enormous potential in the medical, agricultural, and packaging industries [5].

5. Bacterial strains have high PHAs producing potential

Numerous bacterial species, including *Ralstonia eutropha* and *Azotobacter* sp. can accumulate PHA as granules within their cells. In addition, *Bacillus* sp. and *Pseudomonas* sp. have been identified as influential PHA producers, and both can be isolated from oily soils, roots, activated sludge, and highly salty environments [14].

6. Sites of isolation

6.1. Rhizosphere environment

6.1.1 Groundnut plant rhizosphere environment

While many studies have been conducted on isolating PHB-producing microorganisms from various ecological environments, very few have been conducted on isolating PHB-producing microorganisms from the groundnut plant's rhizosphere. PHB-producing microorganisms can be isolated from the Arachis hypogaea plant's environment. [15] discovered that bacterial strains isolated from the rhizosphere of peanut plants have a high capacity for producing PHB. In order to develop bioplastics with improved properties for potential industrial applications, additional environments must be investigated in order to isolate microorganisms with a high PHB-producing capacity. This will hasten the production of bioplastics.

6.1.2. Clay soil rhizosphere of maize, wheat and trefoil

Microorganisms can produce significant amounts of PHB under ideal conditions, which include biochemical conditions, physical conditions (pH, temperature, and incubation times), and nutritional conditions (carbon sources, nitrogen sources, and C/N ratio). The complete and natural degradation of PHB to water and carbon dioxide by microbes in their natural environments is an ecological benefit associated with PHB's biological origins [16]. In a study conducted by [16], PHB-producing strains were successfully isolated from the rhizospheres of maize, wheat, and trefoil growing in clay soil. Furthermore, it has been discovered that clay soils have the highest PHB yields of any soil type.

6.1.3 Himalaya

The Himalayan microorganism ecology is one of the most distinctive. In a study conducted near the Pangi-Chamba altitude gradient in the Himalayas, 411 bacteria were isolated, and 70 of them were molecularly identified as potential polyhydroxyalkanoates (PHA) producers. The study's goal was to learn more about the bacterial communities responsible for bioplastic production. Proteobacteria were the most common phylum for PHA synthesis, accounting for 73% of all occurrences. The next three groups were Actinomycetes (11%), Firmicutes (10%), and Bacteroidetes (6%), with the most common genera being Pseudomonas and Janthinobacterium. For the first time, the ability of some genera, including *Collimonas*, *Pseudarthrobacter*, and *Paenarthrobacter*, to produce PHA on their own has also been reported. PHA-positive bacteria were found to be more prevalent at higher altitudes in the Himalayas, demonstrating that PHA is required for bacteria to survive and adapt. These findings revealed a diverse bacterial community as well as the genetic machinery for PHA synthesis, which could be used in commercial applications [17].

6.2. Extreme environment

6.2.1 Soil at the top of Volcano Mountain

Ten bacteria that produce bioplastic, specifically P(3-hydroxybutyrate) [P(3HB), were discovered in a study conducted by [4] for the isolation of bacteria from the soil of the upper portion of the Mount Marabi volcano in West Sumatra, Indonesia.

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6.2.2. Soil contaminated with organic waste

Soil and organic residue samples were collected from various areas near Al-Mustansiriya University and Al-Zahra in Baghdad for a study that examined the capacity of bacteria to produce PHB, characterized by FTIR and GC-MS, and characterized the bacteria that produced it. Baghdad is home to Al-Mustansiriya University and Al-Zahra University. There were 28 different bacterial isolates discovered in total (6 from soil and 22 from organic waste). According to the screening results for PHB production using Sudan Black staining, five isolates (three from organic waste designated W1, W2, and W3, and two from soil samples designated S1 and S2) were highly effective at producing PHB granules. These isolating agents were obtained from three different sources: soil samples (S1 and S2), organic waste (W1, W2, and W3), and organic waste (W4 and W5) (W3) [18].

6.3. Marine sources

6.3.1 Beaches

PHB is biodegradable, water-resistant, and can be processed using a thermoplastic technique, similar to polypropylene. It is possible to produce bioplastics and biopolymers by separating microorganisms from renewable sources (polysaccharides). Many prokaryotic organisms can synthesize intracellular storage compounds when grown in environments where growth is constrained due to nutrient depletion, such as phosphorus or nitrogen, but the carbon substrate is still available. Because halophilic microorganisms are cost-effective and PHB recovery is much easier in halophilic organisms, it can be of much use in the production [19]. [19] isolated PHB-producing halotolerant bacterial organisms from marine sources during their research. PHB was produced in large quantities on a large scale using wastewater as the substrate and a productive bacterial strain.

6.3.2 Mangroves

The manufacture of bioplastics from marine organisms are widely used in biotechnology applications for long-term environmental management. A study conducted in Saudi Arabia discovered effective marine bacterial isolates such as Halomonas halophila, Tamlana crossing, Erythrobacter aquimaris, and Bacillus aquimaris. This study aimed to find new PHB-producing bacterial strains in mangrove forest roots in Saudi Arabia's Red Sea region. This study, the first to describe E. aquimaris as a PHB product, supported the theory that marine rhizospheric bacteria represent a novel area of research for producing economically valuable biopolymers. According to the hypothesis, producing PHB by marine rhizospheric bacteria is a novel study area [20].

7. The use of waste materials as carbon sources in the production of PHA.

Plastics are now used to make a wide range of industrial products, from automobiles to medical equipment. Plastics, on the other hand, pose serious environmental challenges due to their resistance to microbial breakdown. Using biodegradable polymers, such as copolymers and polyhydroxyalkanoates (PHAs), is thus one of the most important solutions to the problem of polymer waste accumulation in nature. Unfortunately, the high cost of substrate, isolation, and maintenance of microorganisms and low polymer production have all hampered the industrial-scale production of biodegradable polymers. Another study discovered that producing biodegradable polymers in pure culture is roughly 20 times more expensive than producing synthetic plastics. Many microorganisms produce PHB, a type of polyhydroxyalkane, which they store as granules and produce them under persistent adverse nutritional conditions in order to survive as they decompose and provide the carbon and energy resources they need. In fact, the cell does not produce these compounds under any adverse conditions, but if more carbon sources are available than the bacterial need in addition to restricting elements such as phosphorous, magnesium and nitrogen, the central metabolism of the cell towards the production of this polymer precedes. Under any unfavorable conditions, the cell will not produce these compounds. Bacteria are skilled at efficiently making the most of their available space and storing resources. These polymers are similar to synthetic (petrochemical) plastics in many ways. Because of their ability to degrade, these biopolymers can be considered natural, safe, and environmentally friendly, which is one of their main advantages over synthetic plastics. Another significant difference is the inability of synthetic plastics to degrade. Using renewable resources also allows for producing non-toxic, biodegradable, and environmentally friendly polyhydroxybutyrate [21]. PHAs have excellent and environmentally friendly properties, but they are not widely used due to the high cost of production. The use of expensive carbon sources for fermentation, which accounts for approximately 40% of the total costs associated with PHA production, has been identified as a significant contributor to those costs. It has been proposed that microbes could use waste materials as carbon sources to produce PHA at a lower cost. One of the major costs involved in producing PHA, a bacterial product, is the carbon source used by the bacteria during the fermentation process [8].

7.1.Whey

As a result, numerous industrial wastes produced during the bacterial PHA production process have been investigated as potential carbon sources. One of these wastes is whey, a contaminant in the environment and a byproduct of the dairy industry. The process can be made more affordable and environmentally friendly by using

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whey instead of other ingredients to make PHA. Two challenges remain when using whey as a carbon source: whey pre-treatment and strain selection for PHA production.[8] summarizes the current state of knowledge as well as novel approaches to overcoming production-related challenges (Table 1).

Table 1. Summary of the studies aimed at producing PHAs from whey divided by the whey pre-treatment type.

Substrate	Type of culture	Microorganism	Culture method	Productivity1	Type of PHA	Reference
Whey powder supernatant+ additives	Pure cultures	*Alcaligenes latus*	Batch culture	0.11g/L/H	PHB	Berwig et al., 2016
	Engineered cultures	Engineered *Escherichia coli* with *Alcaligenes latus* PHA biosynthesis genes	Fed-batch culture	2.57 g/L/h; 4.6 g/L/h; 1.35 g/L/h	PHB	Ahn et al., 2000, 2001; Park et al., 2002
		Engineered *Escherichia coli* with *Cupriavidus necator* PHA biosynthesis genes	Batch culture; Fed-batch culture; Fed-batch culture; Fed-batch culture; Fed-batch culture	5.2 g/L/h; 1.408 g/L/h; 0.90 g/L/h; 0.57 g/L/h; 0.33 g/L/h	PHB	Lee et al., 1997; Wong and Lee, 1998; Kim, 2000; Farinha, 2009; Pais et al., 2014
		Engineered *Escherichia coli* with *Azotobacter* sp. PHA biosynthesis genes	Fed-batch culture	2.13 g/L/h	PHB	Nikel et al., 2006
	MMC	Undefined	Batch culture	0.0035 g/L/h; 0.0226 g/L/h	Not specified; PHB	Bosco and Chiampo, 2010; Carletto, 2014
Whey permeate+ additives	Pure cultures	*Sinorhizobium meliloti; Bacillus megaterium; Bacillus* spp.; *Sinorhizobium* spp.	Batch culture	0.005 g/L/h	PHB	Povolo and Casella, 2003
		Hydrogenophaga pseudoflava	Batch culture	0.0039 g/L/h; 0.05 g/L/h	PHB;P-3(HB-co-HV)	Povolo and Casella, 2003; Koller et al., 2007b
		Methylobacterium sp. ZP24	Batch culture	0.02 g/L/h	PHB	Yellore and Desai, 1998
	Engineered cultures	Engineered *Cupriavidus necator* with *Escherichia coli* lactose degradation genes	Batch culture	0.02 g/L/h	PHB	Povolo et al., 2010
	MMC	Undefined	Batch culture	0.018 g/L/h	PHB	Carletto, 2014
Hydrolyzed whey permeate+ additives	Pure cultures	*Pseudomonas hydrogenovora*	Fed-batch culture; Batch culture; Fed-batch culture	NS;0.03 g/L/h; 0.04 g/L/h	PHB;PHB;P-3(HB-coHV);	Koller et al., 2006, 2007b, 2008
		Haloferax mediterranei	Fed-batch culture; Fed-batch culture; Batch culture; Fed-batch culture	NS; 0.14 g/L/h; 0.05 g/L/h; 0.148 g/L/h	P(3HB-co-3HV-co4HB);P-(3HB-co3HV)/P(3HB-co-3HVco-4HB);P-3(HB-coHV);P-(3HB-co-3HV)	Koller et al., 2006, 2007a,b; Koller, 2015
		Cupriavidus necator	Fed-batch culture	0.17 g/L/h	P-(3HB-co-3HV)	Marangoni et al., 2002
		Hydrogenophaga pseudoflava	Batch culture	0.012 g/L/h	P-3(HB-co-HV)	Koller et al., 2007b
	Engineered cultures	Engineered *Cupriavidus necator* with *Escherichia coli* lactose degradation genes	Batch culture	0.7 g/L/h	PHB	Povolo et al., 2010
Whey+additives	Pure cultures	*Bacillus cereus PS-10*	Batch culture	NS	PHB	Sharma and Bajaj, 2015
		Pseudomonas aeruginosa	Batch culture	0.005 g/L/h	Diverse	Singh and Mallick, 2009
Fermented whey powder permeate	MMC	Undefined	Fed-batch culture; Fed-batch culture; Batch culture; Fed-Batch culture	0.56 g/L/h; 0.25 g/L/h; 0.80 Cmol/Cmol; 0.204 g/L/h	P-3(HB-co-HV)	Duque et al., 2014; Oliveira et al., 2017; Carvalho et al., 2018; Domingos et al., 2018
Whey supernatant +additives	Pure cultures	*Bacillus megaterium*	Batch culture	0.06 g/L/h	PHB	Obruca et al., 2011
		Thermus thermophilus HB8	Batch culture	0.023 g/L/h	Diverse	Pantazaki et al., 2009

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		Methylobacterium sp. *ZP24*	Batch culture	0.12 g/L/h	PHB	Yellore and Desai, 1998
Fermented whey supernatant	MMC	Undefined	Fed-batch culture	0.42 mg CODPHA mg/CODSS	PHB/ P-3(HB-co-HV)	Colombo et al., 2016
Fermented whey powder	MMC	Undefined	Fed-batch culture	0.68 gCOD/gCOD; 0.6 Cmol PHA/Cmol Substrate	P-3(HB-co-HV)	Gouveia et al., 2017; Fradinho et al., 2019
Fermented whey permeate+ additives	MMC	Undefined	Fed-batch culture	0.59 gCODPHA/gCO DSubstrate	P-3(HB-co-HV)	Janarthanan et al., 2016
Hydrolyzed whey+ additives	Pure cultures	*Halomonas halophile*	Batch cultures	0.05 g/L/h	PHB	Kucera et al., 2018
Whey/whey supernatant	Pure cultures	*Methylobacterium* sp. *ZP24*	Fed-batch culture	0.08 g/L/h	PHB	Nath et al., 2008
Hydrolyzed whey powder supernatant+ additives	Pure cultures	*Haloferax mediterranei*	Batch culture	0.16 g/L/h	P(3HB-co-3HV)	Pais et al., 2016
Fermented whey powder supernatant+ additives	MMC	Undefined	Batch culture	0.46 CODPHA/gCOD Substrat	P(3HB-co-3HV)	Valentino et al., 2015
Whey powder+ additives	Engineered cultures	Engineered *Escherichia coli* with *Cupriavidus necator* PHA biosynthesis genes	Fed-batch culture	1.4 g/L/h -	PHB	Wong and Lee, 1998

1Values refer to the best yields obtained for PHA production from whey in each article. To facilitate comparison, when necessary, calculations were performed to arrive at values in g/L/h. Otherwise, we report the values as presented in the cited work. NS, not specified.

7.2. Camelina oil

Pseudomonas sp., which produces medium chain length polyhydroxyalkanoates, demonstrated high yields on oils and fats. Camelina sativa seeds are a competitive crop for the non-food chain because they contain approximately 43% (w w-1) oil in dry matter and approximately 90% (w W-1) unsaturated fatty acids. Camelina oil was used for the first time in a study to produce medium-length polyhydroxyalkanoates by different Pseudomonas strains [6]. Polyhydroxyalkanoate production was evaluated in a medium containing a trace amount of nitrogen and supplemented with crude Camelina oil or saponified oil to compare the ability of strains of *Pseudomonas* sp. To improve production, batch-feeding assays were duplicated using a phosphate-limited medium (Figure 1,2).

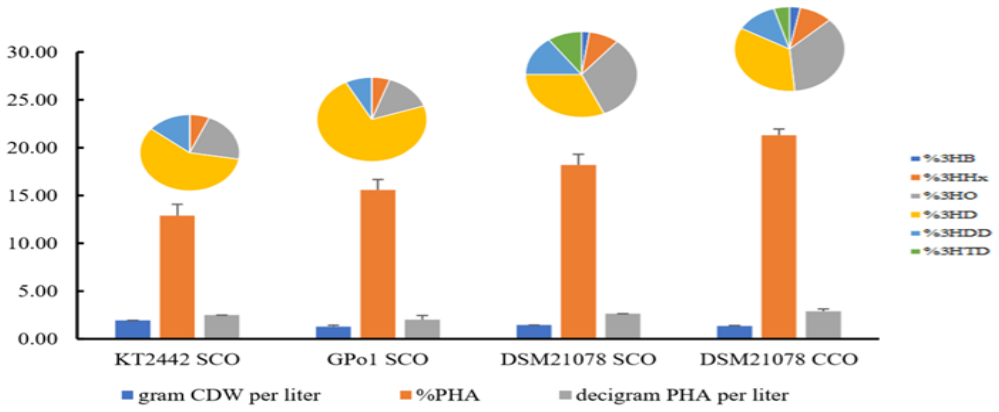


Figure 1. Production of mcl-PHA (medium-chain-length polyhydroxyalkanoates) by bacterial isolates using crude Camelina oil (CCO) or saponified oil (SCO).

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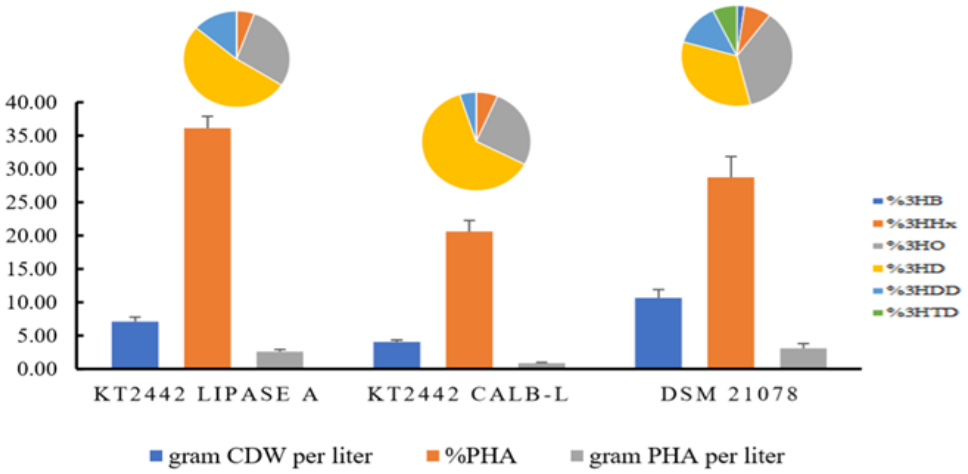


Figure 2. Production of MCL-PHA (medium-chain-length polyhydroxyalkanoates) ith R-Modified culture medium and Camelina oil.

Camelina oil has been shown to be an appropriate substrate for producing medium chain-length polyhydroxyalkanoates. This is because this non-food vegetable oil produced positive results for *Pseudomonas* resinovorans DSM 21078 without any pre-treatment.

7.3. Waste cooking oil

[22] described a method for using waste cooking oil (WCO) as a substrate for Bacillus thermoamylovorans to produce PHA. PHA was used as a biofuel, which is 3-hydroxyalkanoate methyl ester (3HAME). The biopolymer product was compared against PHA produced using both fresh palm oil (FPO) and coconut oil (CO) as the carbon source, The results yielded the most biomass (4,000.1 g/L) and most PHA (3.50.1 g/L, which corresponds to 87.5% of the cell dry mass (CDM)) when waste cooking oil (WCO) was used as a carbon source for 48 hours of batch cultivation at 45 °C and 150 rpm.

7.4. CO2

Carbon capture and utilization, or CCU, is recognizing and utilizing carbon dioxide as a valuable resource for producing carbon-based chemicals to meet current and future demand. This capture has the potential to add much-needed capacity as the world transitions to a low-carbon economy. Includes the production of bioplastics Carbon dioxide, rather than oil, is an intriguing alternative fuel source for the CCU. When nutrients are scarce, a biphasic fermentation process results in the accumulation of PHA first, followed by the growth of biomass. A wide range of prokaryotes can produce PHAs, but *Cupriavidus necator* has received the most research attention. This bacterium can grow and produce autotrophic PHA by using carbon dioxide as its sole carbon source and hydrogen as its energy source (*Ralstonia eutropha* and *Wautersia eutrophus* and *Alcaligenes eutropha*). Until now, the majority of research aimed at improving the efficiency of the homopolymer poly(3-hydroxybutyrate) (PHB) has focused on producing PHA using CO2. The production of PHA from *C. necator* can result in the production of bioplastics and CCU bridges, in addition to the production of these. Researchers sought to understand microbial PHBV production in mixed feeding conditions and control of monomer distribution in the polymer in a 2018 study. The researchers developed a microbial production process that converts carbon dioxide and valeric acid into the bioplastic known as 3-hydroxybutyrate-co-3-hydroxy valerate as part of their research (PHBV). Continuous CO2 sparing with pulse feeding and valeric acid pH were tested to produce a PHBV co-polyester with predetermined properties (Figure 3) [23].

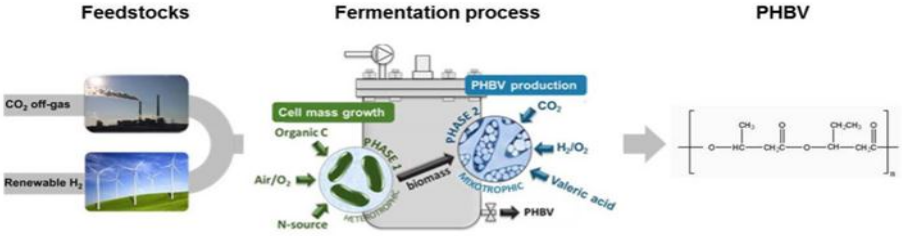


Figure 3. Simulation and modeling of PHB production by *Necator Cupriavidus* through the fermentation process using carbon dioxide and hydrogen.

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7.5. Sewage

Because transporting wastewater remains a major constraint, studies must continue to focus on acquiring low-cost raw materials and low transport costs. Using sewage to produce bioplastics is one viable option for lowering these costs. Because the sewage user is distinguished by the abundance of wastewater in large quantities without the need for transportation or the production of bioplastics on-site. A study conducted in 2019 evaluated polyhydroxybutyrate (PHB) production by several wastewaters and using the pure bacterial culture of Bacillus sp. CYR1, co-cultures of *Cupriavidus* sp. CY1-*Bacillus* sp. CYR1. Total organic carbon (TOC) and chemical oxygen (COD) demand analyzers were used in conjunction with mixed cultures to assess wastewater carbon removal. PHB production was also evaluated using pure bacterial culture for incorporating lower (0.1 g/L) and higher (107 g/L) COD-containing wastewater. PHB production from wastewater collected from the cheese whey factory (151 mg/L) was found to be higher than PHB production from the sewage treatment plant (145 mg/L). The results showed that waste could be used to produce valuable materials via microbial fermentation techniques (Table 2) [24].

Table 2. Initial characteristics and PHB production capacity of various wastewaters.

S. no.	Wastewater source	COD (mg/l)	Nitrogen (mg/l)	PHB (mg/l)
1	Muroran sewage treatment plant	100	25	2.6
2	Concentrated Muroran sewage	283	81	8.0
3	Noboribetsu cheese whey factory (no dilution)	107,250	1375	71
4	Noboribetsu cheese whey factory (15-fold dilution)	6680	113	151
5	Date sewage treatment plant[a]	146	52	145

[a] Co-culture of Bacillus CYR1 and Cupriavidus CY1 was used for the production of PHB from sewage collected from the Date sewage treatment plant.

PHA was developed in a 2020 study to replace non-biodegradable plastics for short-term usage. It is defined as a family of natural biopolymers that includes various structures such as short-chain (scl) and medium-chain-length (MCL) monomers and their polymers. The market for PHA has grown slowly since the 1980s due to its limited variety, good mechanical properties, and high production cost. The most recent strategies or approaches, such as synthetic biology, metabolic engineering, and morphology engineering, have been presented to improve PHA production, reduce production costs, and expand PHA diversity. Extremophilic Halomonas spp. was used to demonstrate the feasibility and challenges of producing PHA more competitively and developing next-generation industrial biotechnology (NGIB) (Table 3) [25].

Table 3. Strategies and approaches for engineering halophiles used for PHA production.

Purpose	Strategy/ Approach	How to do?	Strain	Reference
Improving PHA synthesis	Rapid genome editing tools for engineering	Construct and optimize CRISPR/Cas9	*Halomonas bluephagenesis, Halomonas campaniensis*	Qin et al.(2018); Elhadi et al.(2016)
	Promoter and RBS engineering, Pathwas optimizations	Saturation mutagenesis on core regions of promoters and RBS calculator, PHA synthesis flux enhancements		Shen et al.(2018) Chen et al.(2019)
	Overexpression on key proteins in PHA pathways, Better O2 uptakes, Adjusting reducing powers	T7-like promoter equipped to phaCAB, overexpression of Vitreoscilla hemoglobin in the periplasm, deletion on flavoprotein encoded by ETF to change NAD/NADH+	*H.bluephagenesis H.campaniensis*	Zhao et al.(2017) Ouyang et al.(2018) Ling et al.(2019)
Increasing PHA diversity	Designing new pathways, such as P3HB4HB	Introductions of 4HB and 3HV synthesis pathways to PHB producers, respectively.	*H.bluephagenesis Caldimonas taiwanensis*	Ye et al.(2018), Sheu et al.(2009)
	Regulating culture medium composition	Changing carbon sources to produce different PHA	*Haloferax mediterranei*	Pais et al.(2016)
Easy downstream separation	Morphology engineering on cell shapes and PHA granular sizes, self-flocculation for wastewaterless growth	Adjusting expressions of fission protein gene ftsZ and/or skeleton gene mreB,Overexpressing minCD to inhibit ftsZ combined with PhaP in enlarged cells, deleting etf operon encoding flavoprotein.	*H.campaniensis, H.bluephagenesis*	Jiang et al.(2017) Shen et al.(2019) Ling et al.(2019)

8. Methods for the production of PHAs

Bioplastics are manufactured in various methods on a large scale, and the best method is chosen based on the advantages and disadvantages of each method. The old methods are then updated, and their shortcomings are

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introduced into the new methods to make them more successful. International interest in creating and developing new production methods has increased to increase production while decreasing production costs. The old methods' disadvantages drive the search for new ways to produce PHA. Table 4 depicts some of these old methods and their flaws and advantages (Table 4) [9].

Table 4. Advantages and disadvantages of some commonly used PHA production methods.

Production method	Advantages	Disadvantages
Production by Pure Bacterial Cultures	These single isolates may have the ability to produce and bioremediation of PHA.	Need sterilization to guarantee the effective production
	Huge potential for reducing PHA production cost	Sometimes the production process may require pre-treatment or substrates such as physicochemical or biological hydrolysis
Production by substrate hydroxylation	Many substrates can be suitable for production	Usually required several steps and/ or solvents to prepare the substrate's hydrolysates
	It may be an effective method for the utilization of several substrates that are normally difficult to use	May be a time-consuming method as it requires a separate hydrolysis stage before production
	Cost-effective in many cases as the used substrates may be very cheap	

9. Influencing Factors in PHA Production

9.1. Carbon sources

Carbon sources can be divided into three categories in general. The three primary types used as growth substrates are sugars, hydrocarbons, and triglycerides. Other substrates such as methanol, methane, ethyl alcohol and compounds containing triglycerides such as glycerol, waste frying oil, plant oils and animal fats have also been reported. Various hydrocarbons, including alkene, alkyne, alkane, and a variety of other hydrocarbons, demonstrated the greatest affinity for the production of PHA. It has been reported that using salts, ashes, whey, and fats as carbon sources can reduce production costs by up to 50% while also increasing the amount of PHA that can be produced. The culture conditions and substrates used significantly impact the quantity and quality of PHA products produced [26].

9.2. Nitrogen sources

Numerous inorganic and organic nitrogen sources, including ammonium sulfate, ammonium chloride, ammonium carbonate, ammonium bicarbonate, ammonium nitrogen, ammonium nitrate, amides, urea, sodium nitrate, nitrate, poly-beta-glutamate (PGA), and polyamide, have been screened for their suitability and improvement PHAs yield. The organic and inorganic nitrogen sources showed that their amount had a linear relation with bacterial growth, which means that the increasing of the nitrogen content in the culture medium, leads to an increase in the number of bacterial cells. It also showed that the concentration of nitrogen source and PHA stored inside the bacterial cell had an opposite effect on cell biomass yield. . Lower nitrogen source concentrations resulted in better PHA accumulation within the bacterial cells, but the number of bacterial cells in the fermentation medium increased as the biocatalyst activity increased [26].

9.3. pH

The pH of the extracellular environment in which bacteria live significantly impacts their enzymatic activity. When compared to acidic or basic pH environments, fermentation media with a neutral pH produce more PHAs. When the neutral pH in pure culture media fluctuates, the fermentation process slows down and the cellular activity of the enzymes, specifically Pha Z and Pha C, is reduced. This eventually has an impact on cell biomass. In most cases, the nature of the medium determines how the pH level influences the amount of PHA produced. The pH fluctuation trend is generally visible when the fermentation medium is neutral. When the substrate is added to the fermentation medium early in the incubation process, the medium's pH rises and falls, according to train states [26].

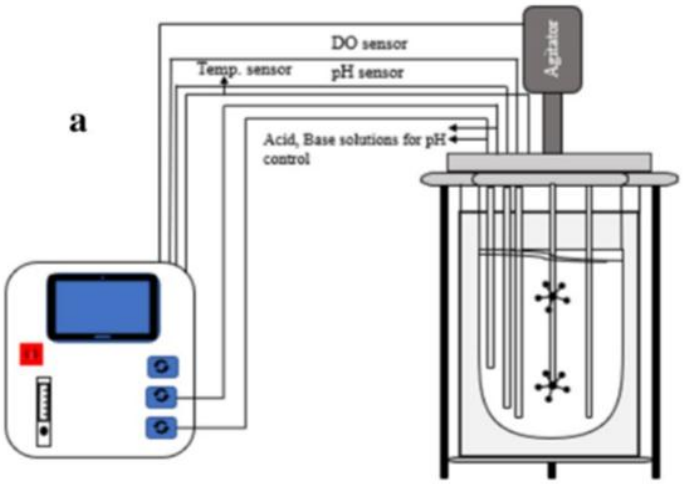
9.4. Temperature

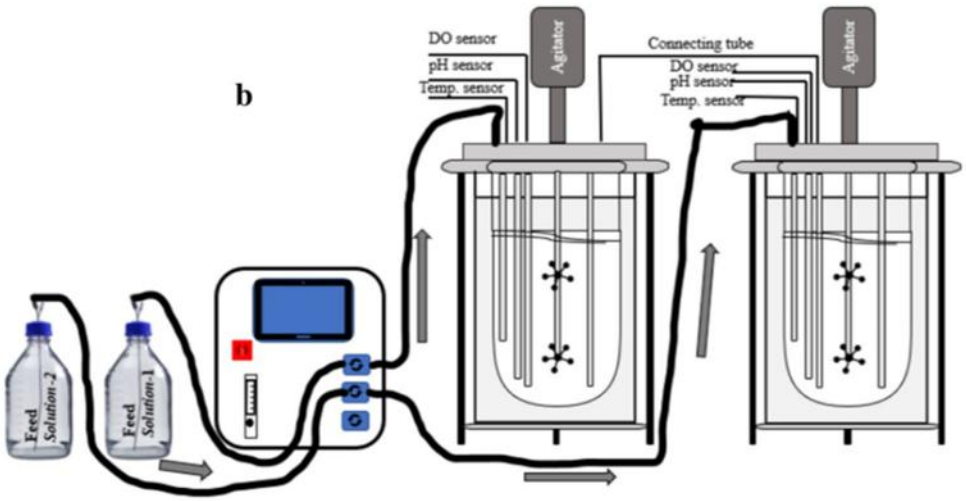
Temperature is a key factor for the integration of PHA production within an industrial process. When scaling-up, the energy consumption for temperature control can be an economic constraint. To date, and through a very limited number of publications, the effect of temperature in mixed microbial cultures (MMCs) has been evaluated. The first study into the effect of temperature (between 15 and 35 degrees Celsius) on the rate at which PHB accumulates in sequential batch reactors (SBRs) by activated sludge was conducted in 1999. More than 10 years later, two more publications were published with the aim of studying the effect of long- and short-term temperature on PHB-producing MMCs. In 2017 in a more recent study conducted to evaluate PHA production at 15-30°C, lower temperatures gave lower production rates but higher yields of PHA [27].

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10. PHAs production bioreactor

PHA, which is used as a backup energy source, is stored as cellular inclusions within the cells of many microorganisms, and these microorganisms have been identified as PHA producers. PHA can be produced in shake flasks and bioreactors by subjecting the fermentation process to specific conditions and using cultures with the necessary physiological properties and nutrients. One of the most suitable techniques for industrial-scale production is the production of biopolymers on a bioreactor scale because it allows for a large workload while avoiding the constraints that can be encountered under shake flask conditions. This technique is one of the best suited for large-scale production. Because PHA biosynthesis is a multi-phase process, if you want to improve the operating conditions of the bioreactor, you must make changes to both the feeding strategy and the bioreactor's operating mode. For producing PHA at the industrial bioreactor scale, both continuous fed-batch feeding and fed-batch feeding strategies, as well as continuous fed-batch feeding, are viable options. A wide range of factors influence PHA production at the bioreactor scale, including nutrient supply, carbon source, dissolved oxygen level, pH, temperature, and production modes. PHA can be used for a wide range of applications in technology and science, most notably in medicine (Figure 4a,b,c) [26].





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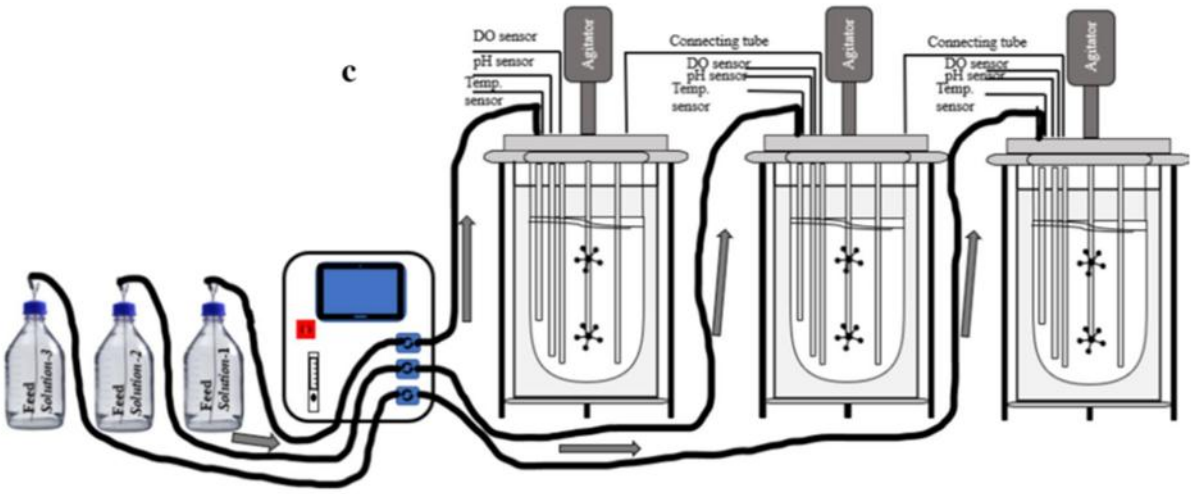


Figure 4. Schematic diagrams of continuous tank bioreactors a single stage, b two-stage, and c three-stage

11. PHAs applications

Due to their biocompatibility, biodegradability, and green credentials, PHAs polymers are widely used as biofuel via the methyl esterification method and in the synthesis of pharmaceutical and medical devices. In addition to surgical applications, drug delivery, bio-implant patches, and tissue engineering, PHAs polymers have many additional applications. Nanotechnology's ability to control the volume of matter on such a small scale has created new opportunities in the medical sciences. This is because nanotechnology is one of the most rapidly expanding scientific fields. Due to their compatibility and uniformity, as well as the fact that they can be used as primary chemicals rather than a large number of other end products, PHAs have found applications in a variety of nanotechnology subfields. As a blend with other polymers, they can also be used in medical applications, such as scaffold materials in tissue engineering and drug carriers. This is possible due to the materials' high biocompatibility. In addition, they have superior adhesion to lignocellulose fibers compared to conventional polyolefins, which enables their use in bio-composites for nanotechnology and drug delivery. PHAs can also be used to create thin films and nanogels for various applications. The development of protein chips employing PHAs for selective protein stabilization is one example of a nanobiotechnology application that advances the objective of specific detection of the hepatitis B virus [28].

Initially, the high cost of production was a hindrance to the production of PHA, which was a future trend. As a result, researchers have focused their efforts on discovering low-cost substrates to reduce the overall production cost. In recent years, research has been conducted on a wide range of low-cost carbon sources, including vegetable oils, whey, frying oils, organic substrates, and others, with the aim of reducing the overall production cost. Under optimal fermentation conditions, the application of synthetic biology and genetic engineering is anticipated to produce a PHAs strain with a high yield and the capacity for rapid, dense growth. Methods of purification that permit controlled decomposition can be used to reduce production costs and increase the accumulation of granules of large size. Additionally, low-cost substrates and continuous fermentation by MMC can be used to increase production yields. In addition, work must be done to produce desirable PHA congeners via controlled fermentation, and each congener's biological and physicochemical properties must be elucidated.

CONCLUSION

Petrochemical-based industrial plastics may one day be replaced by bioplastics, also known as polyhydroxyalkanoates (PHAs), which are environmentally friendly, recyclable, and biodegrade. In recent years, there has been an increase in research into the production of bioplastics using microorganisms; however, there are still production obstacles to overcome. The high cost of carbon and nitrogen sources is the most significant issue, which has prompted research into sources of carbon and nitrogen that are less expensive, such as waste oils و plants and others.

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