Article Type: Review
J Name: Modern Phytomorphology
Short name: MP
ISSN: ISSN 2226-3063/ eISSN 2227-9555
Year: 2024
Volume: 18
Page numbers: 41-48
DOI: 10.5281/zenodo.200121
(10.5281/zenodo.2024-18)
Short Title: An analytical study on fermentative production of Polyhydroxyalkanoate (PHA) using *Bacillus sp.* isolated from whey protein

REVIEW ARTICLE

An analytical study on fermentative production of Polyhydroxyalkanoate (PHA) using *Bacillus sp.* isolated from whey protein

Srividya Shivakumar*
Department of Microbiology, School of Sciences, Jain (Deemed-to-Be-University), Sudhama Nagar, Bangalore, India

***Corresponding author:** Srividya Shivakumar, Department of Microbiology, School of Sciences, Jain (Deemed-to-be-University), Sudhama Nagar, Bangalore, India
Email: sk.srivdya@jainuniversity.ac.in

Received: 29.10.2024, Manuscript No: mp-24-151366| **Editor Assigned:** 01.11.2024, Pre-QC No. mp-24-151366 (PQ) | **Reviewed:** 16.11.2024, QC No. mp-24-151366 (Q) | **Revised:** 22.11.2024, Manuscript No. mp-24-151366 (R) | **Accepted:** 28.11.2024| **Published:** 04.12.2024

Abstract

A great carbon source for bacteria that make Polyhydroxyalkanoates (PHA) is whey. There are no reports employing raw whey, which has a relatively low amount of lactose, despite the fact that the majority of research have employed whey, which has considerable quantities of lactose. Therefore, the two-stage method used in the current investigation to evaluate PHA synthesis used whey with less lactose. Using Bacillus sp. which would have been isolated from food waste, the carbon source present in whey was first transformed into acetic acid. Using the bacterium Bacillus sp. CYR-1, the acetic acid generated in the first step was transformed into PHA in the second stage. Without first treating the whey, acetic acid derived from the whey was diluted many times before being utilized to make PHA. These studies led us to the conclusion that the synthesis of PHB utilizing whey containing little lactose may be accomplished using a two-stage procedure including Bacillus sp. CYR-1. The main objective of this paper is to learn more about the Isolation and screening of Bacillus sp. for whey-based Polyhydroxyalkanoate (PHA) Production. In the future, this study will aware people about the various application of Polyhydroxyalkanoate (PHA) Production.

Keywords: Biodegradable, Hydroxy alkanoic, Polyhydroxyalkanoate, Polymers, Whey

Introduction

All modern economies depend on plastics, which are produced primarily from fossil feed stocks with significant environmental consequences. Additionally, it is expected that the global manufacturing of plastics would continue to rise. Because plastics are difficult to degrade and accumulate in a variety of situations, their production has raised environmental concerns. Furthermore, just 31.1% of plastics were recycled in Europe in 2016 despite impressive efforts to raise recycling rates (recycling rates climbed by 79% from 2006 to 2016). The remainder of the plastic is either dumped in landfills (27.3%), utilized to generate power through energy recovery, or used as fuel for industrial activities. Therefore, bioplastics also known as biologically generated and/or biodegradable plastics need to be quickly substituted for these conventional polymers (Koller 2017; Koller 2020). However, even though it is believed that bioplastics may replace around 85% of plastic goods, only about 1% of the plastics produced worldwide are bioplastics. In 2022, 2.44 million tons of bioplastics are expected to be produced, of which only 1.086 million tons will be biodegradable, according to predictions.

This anticipated growth, meanwhile, still falls short of the world's expanding need for plastic. The biologically manufactured polyesters known as Polyhydroxyalkanoates (PHAs) have long been regarded as very advantageous substitutes for the widely used petroleum-derived plastics (Al Battashi et al., 2021; Amaro et al., 2019).

Properties

There are three distinct categories of PHAs: MCL-PHAs, LCL-PHAs, and scl-PHAs. PHAs with medium-length chains exhibit low tensile strength, poor crystallinity, or high elongation-to-break ratios. PHAs are not readily hydrolyzed and are soluble in chloroform but insoluble in water. Although they are UV resistant, they do not have the best acid and basic resistance. These are exceedingly biocompatible and non-toxic, making them perfect for medicinal purposes. PHAs have a strong tensile strength and a high melting point of 175°C. They are biogenic polyesters that build up in microbial cells spontaneously (Kourmentza 2017; Jiang et al., 2018). Although PHA manufacture is an expensive procedure, it may be made from a variety of locally accessible materials, including agricultural waste, dairy waste, maize, etc. One of the primary uses for polymers is the packaging. Plastic waste is dumped in landfills, where it remains for more than a century. Polythene bags and bottles that are thrown away block sewers, pollute streets and beaches and are particularly challenging to collect. Thus, biodegradable polymers like PHAs aid in the resolution of such problems. Other uses include things like disposable medical equipment and catering supplies. PHAs are employed as biodegradable carriers for long-term dosages of pharmaceuticals, medications, hormones, insecticides, and herbicides. They can also function as stereo regular compounds (Morgan-Sagastume 2020; Morgan-Sagastume 2016).

Biodegradability

PHA biodegradability has been studied in a variety of natural conditions, and 700 different strains of microbes have been found. Chowdhury discovered de-polymerase enzymes from Pseudomonas strains for the first time in 1963. These microbes use nutrients from degraded substances. These microbes release PHA depolymerase, which hydrolyzes the polymer extracellularly to produce water-soluble compounds. These substances were used by the bacteria as sources of carbon and energy. Some microbes, including *Pseudomonas lemoignei, P. stutzeri, Comamonas testosteroni, Alcaligenes faecalis, and C. acidovorans*, have also produced several PHA depolymerizes that have been purified. The PHA depolymerase reaction conditions, PHA concentration, and PHA properties all significantly affect how quickly PHA is broken down by the enzyme. PHA biodegradation generates water and carbon dioxide under aerobic circumstances, whereas methane is produced under anaerobic ones (Morgan-Sagastume 2015; Gowda et al., 2014). The rate of PHA breakdown is fastest in anaerobic sewage and slowest in seawater. According to the author, the chemical completely dissolved after 6 weeks in anaerobic sewage, 350 weeks in sea water and 75 weeks in soil.

PHB

Poly-3-Hydroxybutyrate is the PHA structure that is the most well-known and understood (PHB). PHB is a sturdy, extremely hydrophobic polymer that is completely biodegradable, making it the greatest substitute for conventional plastics. PHB shares many of the same physical and chemical characteristics as polypropylene. Due to PHB's high melting point of 177°C, processing the polymer is challenging, as shown in tab. 1.

Table 1. Comparison between PHB and polypropylene properties.

Properties	PHB	Polypropylene
Melting temperature (C)	177	176
Glass transition temperature(C)	2	-10
Crystallinity (%)	60	50-70
Tensile strength (MPa)	43	38-14
Extension to break (%)	5	400

PHB may be produced in two different methods, the first of which is in-vitro, using a system devoid of cells. This happens from monomers like lactones, hydroxyalkanoic acids, or thioesters that are not produced naturally. The second strategy is in vivo manufacturing, which involves genetically altering plants or fermenting substrate through microbes to further promote PHB production metabolic pathways, as shown in fig. 1.

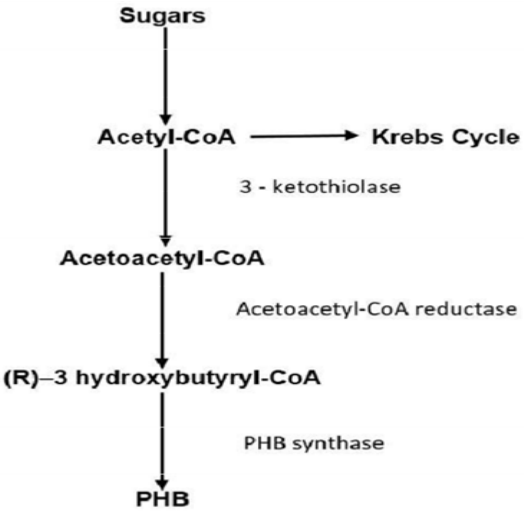

Figure 1. **Metabolic pathway for the PHB synthesis.**

More than 300 microorganisms have been reported to collect PHB, although not all of them can do so in quantities sufficient for industrial production. The bacteria *C. necator, Pseudomonas oleovorans, Methylobacterium extorquens, Azohydromonas, Pseudomonas putida, Bacillus spp., Aeromonas hydrophila, Paracoccus denitrificans, Azotobacter vinelandii,* and *recombinant E. coli* are capable of generating and accumulating bacteria on.

Properties by PHB

PHB is a linear polymer with an ester linking group and a methyl functional group (-CH3) (-COOR). These functional groups account for PHB's thermos plasticity, hydrophobicity, brittleness, and high crystallinity. It crystallizes quickly between 80°C and 100°C and slowly at temperatures below 60°C or above 130°C because of its high alkalinity. PHB's application is constrained by its low rate of degradation and relative brittleness as a result of its crystallinity (Nahar et al., 2019; Kanavaki et al., 2021). When PHB is kept at room temperature for a longer time, it becomes more fragile. PHB has a relatively low melting point during processing because of its low molecular weight. PHB is not soluble in water, however, it is 100% biodegradable. PHB doesn't contain any catalyst residue, unlike other synthetic polymers. It does not contain chain branching and is isotactic. PHB is relatively impermeable to oxygen, carbon dioxide, and water.

PHB manufacturing from dairy waste

Even if these microorganisms have great qualities and are good for the environment, using microbial polymers excessively still raises the cost of production. The primary drivers of these expenses are the high costs of downstream processing and substrate used as raw materials, which contribute to at least 40%-50% of production costs and 70%-80% of overall costs, respectively. Due to these difficulties, a variety of wastes and byproducts from the agricultural and food industries have been investigated as prospective alternatives for carbon and nitrogen sources for the growth of microorganisms as well as the synthesis of polymers. It has been discovered that wastes and byproducts from the oleo chemical (glycerol), dairy (cheese and whey), distillery (dregs, thin stillage), sugar cane or sugar beet molasses, extracts or hydrolysates from organic crops, etc., are useful for this. Because it is available in endless amounts and is a cheap, carbon-rich raw material, cheese whey is commonly employed (Colombo 2017; Vermeer et al., 2021).

Whey is the primary by-product of the manufacture of cheese or casein since it makes up between 80% and 90% of processed milk and provides 55% of the nutrients. According to estimates, when 1 kilogram-2 kilogram of cheese is made from 10 kg of cow's milk, 8 kg to 9 kg of milk whey can be obtained. Milk whey typically contains 70% crude proteins, including lacto globulin, lacto globulin, and immunoglobulin, as well as proteases, peptones, and native enzymes still present in the serum (Aljuraifani et al., 2019; Estévez-Alonso et al., 2021). The global dairy sector produces around 120 million tons of whey per year, which results in high BOD (27 kg/m3 to 60 kg/m3) and COD levels (50 kg/m3 to 102 kg/m3). Since high lactose content causes high BOD in whey, it is essential to discover a suitable biotechnological application for lactose. The use of whey in PHB production is crucial since it would significantly reduce PHB production costs without interfering with the human food supply and address environmental issues at the same time.

Literature Review

Sachin Kumar et al. studied Polyhydroxyalkalonates (PHAs) are employed in a wide range of industrial and medicinal applications, including scaffolds, sutures, heart valves, and drug administration. The pH and trace elements, ammonium chloride and ammonium sulphate, were optimized together with the organic waste source, whey, soy extract, molasses, distillery waste liquor, or maize extract, peptone, nitrogen source beef extract, yeast extract, or any of these. The results of this investigation show that the isolate is one of the microorganisms that produce a lot of PHB and uses whey as a carbon source (Kumar et al., 2022).

Swati Mohapatra et al. studied opportunities and difficulties with bacillus and biopolymer. By taking the place of the current non-degradable, petrochemical-based polymer, the microbially produced Polyhydroxyalkanoates biopolymers may influence the scenario of the global climate. On the other hand, Bacillus species are superior to other bacteria because they are abundant even under harsh ecological situations, have greater growth rates despite using inexpensive substrates, higher capacity for producing PHAs, and have simplicity in PHA extraction. Hydrolytic enzymes found in Bacillus species can be used to produce PHAs at a low cost. On the other hand, Bacillus species are superior to other bacteria due to their abundance even under harsh ecological situations, greater growth rates despite using inexpensive substrates, higher capacity for producing PHAs, and simplicity in PHA extraction. Hydrolytic enzymes from Bacillus species can be used to produce PHAs at a low cost (Mohapatra 2017).

Sasikala Sadasivam et al. studied Recent research that has identified Polyhydroxyalkanoates as a viable substitute for traditional petrochemical plastics due to their comparable material characteristics and total degradability. These are microbial polyesters produced intracellularly by various bacteria as a source of carbon and energy. Furthermore, PHA synthase was partially precipitated using ammonium sulfate and dialysis. An ion-exchange chromatogram showed PHA synthase to be present as peaks at 200 nm. The present effort is focused on scaling up the medium for PHA produced by Bacillus sp. in lab conditions for large-scale manufacturing (Mohapatra 2017).

Discussion

Applications

PHAs have been utilized in the realm of medicine since the early 1970s. Since biopolymers readily breakdown and replicate the characteristics of traditional polymers, they can be utilized to address issues brought on by plastics. Based on how they are made, biopolymers may be divided into three categories: those that are taken from biomass, those that are made from bio-derived monomers, and those that are created by microbes. There are a variety of PHA-based products that are either already on the market or are being developed, such as biodegradable surgical staples, vascular prosthetic implants, screws, and cords, bioresorbable suture material, surgical mesh endoprostheses, and skin staples, wound or burn dressings, plates, pins, patches for surgical repair of intestinal and pericardial defects, mesh plugs for coloproctological applications or hernioplasty, or coronary stent (Fig.2).

Figure 2. PHA-based medical devices that are currently being researched or utilized in medical practice.

PHAs (which can be micro- or nanoparticles) can contain hydrophobic medicines since they are hydrophobic. PHA nanoparticles were used to construct a unique medication delivery method. This method of targeting malignant cells is efficient both in vitro and in vivo. The PHBPhaC-GFP-A33scFv nanoparticle preferentially targets the colon cancer cell lines SW1222 (A33^{+}) or HT29 (A33^{-}). This was demonstrated when the PHB nanoparticles were coupled with an engineered PHA synthase conjugated with green fluorescent protein and a single chain variable fragment antibody (A22scFv) specific to colon cancer (GFP).

Applications for PHB include items like disposable plates, cups, cutlery, trays, and food containers. Other (possible) uses include the usage of garbage and shopping bags, soil retention sheathing, and other agricultural films, as well as ordinary packaging. Additionally, PHB may be spun into fibers that can be utilized to create woven and non-woven biodegradable one-use fabric items, such as medical sutures.

Polyhydroxybutyrate (PHBS)

Bacteria produce macromolecules called PHBs. They are inclusion bodies that the bacteria store as reserve resources as they develop under various stress circumstances. PHB is a polymer known as Polyhydroxyalkanoate (PHA), which is a kind of polyester. Although Polyhydroxyalkanoates are produced by a variety of organisms, poly-3-hydroxybutyrate (PHB) is likely the most prevalent type. Other polymers in this class include Poly-4-hydroxybutyrate (PHB), Polyhydroxyvalerate (PHV), Polyhydroxyalkanoate (PHO), Polyhydroxyalkanoate (PHH), and their copolymers.

PHB is reportedly created by microbes in reaction to physiological stress, particularly when nutrients are scarce (e.g., *Cupriavidus necator, Methylobacterium rhodesianum,* or *Bacillus megaterium*). Microorganisms use the polymer, which is predominantly a byproduct of carbon absorption as a kind of energy storage molecule that may be digested in the absence of other readily available energy sources. The condensation of two acetyl-CoA molecules to form acetoacetyl-CoA, which is then reduced to hydroxybutyrate-CoA, is the initial step in the microbial synthesis of PHB. Then, to create PHB, this later chemical is employed as a monomer. Then, the cells are broken apart to retrieve the PHA granules.

Natural polymers like "Polyhydroxy butyrate" (PHB) provide a useful and environmentally friendly option for the creation of various biodegradable plastics. One of these polymers' most important characteristics is that they may be produced from renewable carbon sources, including milk whey, an agro-industrial waste product. PHB, a polyester that is a member of the Polyhydroxyalkanoates family and may be utilized as a carbon source, is produced by several bacterial strains when they are grown in demanding culture conditions, such as nutrient depletion and an abundance of carbon supply. In these conditions, PHB is stored intracellularly by bacterial cells as granules as a carbon and energy reserve (Zheng 2021).

PHB is regarded as one of the primary replacements for conventional plastics due to its advantageous physicochemical properties, which include being a robust, totally biodegradable, and extremely hydrophobic substance. PHB shares many of the same physical and chemical characteristics as polypropylene, however manufacturing this polymer is challenging due to its high melting point of 177°C. Intracellular enzymes polymerize the hydroxyalkanoic acids by forming an ester bond between the carboxylic acid groups of one monomer as well as the hydroxyl group of the next. Polyhydroxyalkanoates can be produced in two different ways: first, in vitro, using monomers such as lactones, hydroxyalkanoic acids, or thioesters that cannot be produced naturally; second, in vivo, using genetically modified plants or microorganisms that ferment substrates to activate Polyhydroxyalkanoates' metabolic pathways. The biopolymers' monomeric composition depends on the metabolic pathways utilized for their production and the external carbon source used as a raw material. The three metabolic activities that create PHAs and yield Acetyl-CoA are fatty acid breakdown, fatty acid biosynthesis, or sugar degradation. This demonstrates how the enzymes PHB synthetase and "acetoacetyl-CoA reductase catalyzes" the polymerization of hydroxybutyrate molecules, the conversion of "acetoacetyl-CoA molecules" into three "hydroxybutyric-CoA molecules", and the reversible addition of an acetyl group to an "Acetyl-CoA molecule".

PHB can be produced by more than 300 distinct microorganisms, although not all of them do so in big enough numbers to be employed in industrial manufacturing. The most productive bacteria that can accomplish this include *Methylobacterium* extort, *Aeromonas hydrophila, Paracoccus denitrificans, Pseudomonas putida, Pseudomonas oleovorans,* and *C. necator*. When selecting the right strain, it is important to take into account the rapid replication rate, high PHB synthesis yield, consumption of a cheap carbon source, or quality of the PHB produced. As a consequence, the species *Bacillus megatherium*, a sporulated, aerobic, but also Gram-positive bacteria, demonstrates all the characteristics mentioned above. Large, convex colonies with uniform borders that are wet and non-hemolytic are among this species' macroscopic characteristics. Additionally, this bacterial species generates PHB by the fermentation of raw materials such as agricultural or industrial wastes, especially milk whey, the liquid collected after the coagulation, and the casein proteins that are separated from milk to form cheese. Whey, which retains 55% of the total milk's components, has a high lactose content of about 5%, or 94 g, as well as being a nutrient-rich source of proteins, minerals, and lipids. One to two kilos of cheese may be produced from every 10 liters of cow's milk, but this industrial process also produces 8 kg to 9 kg of milk whey. This makes the product the most useful option to using as a substrate for the synthesis of PHB. The amount of beta, alpha, or immunoglobulin crude proteins, protease-peptones, or native enzymes that are left in milk depends on where the milk comes from. Therefore, the bacterial synthesis of PHB from milk whey is a possible substitute for petroleum-based polymers like propylene and polypropylene.

Whey as a substrate: Dairy industry waste

Since whey has a high biological and chemical oxygen requirement, it is regarded as a major contaminant in the cheese business. Whey, which is sometimes viewed as waste, really has great nutritional content and may be utilized to create goods with value added, however some of them need costly enzymatic production. Through bacterial or yeast fermentations and buildup during algal development, whey may be economically converted into useful products. Fermentation techniques can be used to create specific molecules or create novel meals and drinks. In the first instance, much research has been done to find biofuels that can take the place of those made from gasoline. Additionally, it has been investigated if biodegradable polymers produced during the bacterial fermentation of whey may replace plastics made from petroleum. In addition, some organisms have the capacity to create metabolites widely utilized in the food and pharmaceutical sectors (such as lacto bionic acid, polysaccharides, lactic acid etc.) utilizing whey as a growth substrate. Nevertheless, new low cost functional whey based foods and beverages have been created, highlighting the benefits of fermented whey derived products for health and using whey's superior nutritional value. the many uses of whey as a sustainable raw resource for the production of certain chemicals, foods, and beverages via microbial fermentation. The first publication to summarize how whey is converted by bacteria into a variety of foods and products that are helpful for industry.

Whey is a substance that is intriguing microbiologically. Historically, the microbiological quality of whey was not a major issue because it is a waste product in the dairy industry and a byproduct of the production of casein and cheese. Whey's protein & lactose contents are increasingly acknowledged as a significant resource, so products made from whey after reverse osmosis and ultrafiltration are essential to the dairy sector. It is difficult to maintain the microbiological quality of these whey products to fulfil consumer requirements. The starter cultures used to make these products are the source of many of the bacteria discovered in the raw whey leaving the casein or cheese making plant. Changes in whey handling have resulted in an improvement in the quality of products manufactured from whey. However, because the processing of whey calls for large filtering membranes where biofilm can form and contaminate the finished product, there are additional challenges.

Manufacturing of whey

One of the largest sources of dietary protein now accessible is whey, the liquid byproduct of the production of cheese, casein, and yoghurt. A significant source of carbohydrates for the globe, 8.6 million tons of lactose and 1.5 million tons of progressively high value protein may be found in the 180 million tons of whey produced globally in 2013. Recent studies indicate that whey protein is perhaps the most nutritionally useful protein now accessible. It is therefore not surprising that nutritional industries including sports, clinical, and newborn nutrition are driving dairy production to record levels of investment. Due to its abundance of natural goodies, such as high gelling b-lacto globulin, lactoferrin, the mother's milk equivalent protein a-lactalbumin, and immunoglobulin, as well as its function as a precursor to the probiotic galacto oligo saccharides, whey is proving to be one of the most intriguing food sources available at the moment (GOS). 80%-90% of the entire volume of milk entering the processing is made up of whey, which has 25 minerals, vitamins, soluble protein, and minerals as well as around 50% of the nutritious content of the original milk. The pH range for sweet whey, a byproduct of making hard, semi-hard, or soft cheese and rennet casein, is shown in tab. 2 as 5.9 to 6.6. Acid whey, a last byproduct of mineral acid precipitated casein, with a pH range of 4.3 to 4.6.

Table 2: Whey composition.

S.NO	Constituent	Sweet Whey	Acid whey
1	Water %	93-94	94-95
2	Dry Matter %	6-6.5	5-6
3	Lactose %	4.5-5	3.5-4.3
4	Lactic Acid %	Traces	up to 0.8
5	Total Protein %	0.8-1.0	0.8-1.0
6	Whey Protein %	0.6-0.65	0.6-0.65
7	Citric Acid %	0.1	0.1
8	Minerals %	0.5-0.7	0.5-0.7
9	pH	6.4-6.2	5.0-4.6

Filtering the curd particles still present in the whey is the first step in the numerous procedures used to handle the whey and its final products. Following that, casein fines, as well as fat, are removed, in part to increase the economic output or in part because these substances obstruct subsequent processing. Producing whey powder, delicious whey, or lactose has historically been the main focus of whey solids processing. Due to the increased demand for whey proteins, “Whey Protein Isolate” (WPI), the associated products WPC35-80, lactose, or permeate get around 40% of the processed whey solids. Whey is no longer seen as an unwelcome by-product but as a very beneficial source of nutrients.

Cost-effective production of PHB

The environment is currently under serious threat from non-degradable plastics and untreated industrial wastewater. Non-biodegradable plastic is a major environmental pollutant; it takes years for full decomposition. All kinds of life on earth are seriously threatened by the very poisonous chemicals employed in the production of plastic. In addition to harming the environment, these compounds have been linked to several other issues, including cancer, nervous system and immune system damage, and birth abnormalities. The majority of plastic items are made from polypropylene, a petroleum product. Petroleum products are becoming more and more costly as demand declines. Without a viable alternative energy source, petroleum is essential for contemporary existence. Because of their exceptional biodegradability, biopolymers—sustainable and environmentally friendly materials—have attracted a lot of attention as environmental consciousness and the price of oil have increased. Due to the present issues with solid waste management as well as the environment, there is also a lot of interest in the creation of biodegradable polymers. For instance, numerous different species of bacteria synthesize PHA as an internal fossil fuel and energy store material when resources are few. Poly-b-hydroxybutyrate (PHB) from Bacillus megaterium was discovered to be the first PHA. PHB is getting a lot of interest since it resembles hetero-plastic polypropylene in terms of properties. Up until recently, the industrial-scale manufacturing of commercial PHB utilized microbial isolates or purified substrates.

However, these technologies' high manufacturing costs, which are mostly caused by the cost of the carbon source and polymer recovery, have limited their potential for commercialization. The cost of raw materials accounts for around 40% of the total cost of making PHB. As a result, using a cheap carbon source is essential to lowering the high cost of PHB production. To address the cost-production problem, waste materials are being exploited as a source of nutrients. PHBs are difficult to develop and sell because of their high manufacturing costs when compared to plastics made from petrochemicals (Pradesh 2018). Recently, significant effort has been made to lower the cost of PHB manufacturing by employing techniques including creating effective bacterial strains, improving fermentation, and recovering waste. The majority of publications on the manufacturing of PHB indicated that the cost of the carbon substrate was a significant factor in the total cost of PHB production. As a result, choosing an effective carbon substrate is crucial since it confirms the eventual product's overall cost. The alternate strategy is to select carbon substrates that are readily accessible, economically viable, and renewable for both microbial growth and effective PHB synthesis. Consequently, the goal of the current study was to identify PHB-producing bacteria and analyze how they produce PHB from whey.

Conclusions

It is vital to generate bioplastics in an environmentally and economically sustainable way in order to solve the environmental concerns brought on by the production and buildup of conventional plastics. Due to their marketable qualities and biological production from cheap waste substrates, such as whey, PHAs might be a significant component of this bioplastic solution. To effectively produce PHAs from whey, however, there are still a lot of obstacles to be addressed. The study on the production of PHA from whey, with a focus on whey pre-treatments or the choice of producing microbes, is summarized in this article.

It took two steps and the utilization of Bacillus sp. CYR1 to convert CW with little lactose into PHA. With the help of CW, a newly discovered C1 bacterium successfully fermented acetic acid. To lower the nitrogen level and boost PHA production, the liquid that has undergone acetic acid fermentation must be diluted. Additionally, the PHA production was improved by pretreating the raw CW. The PHA generated by the two-stage CW technique was PHB.

References

Koller M. (2017). Advances in Polyhydroxyalkanoate (PHA) Production. *Bioengineering*. **4**:88.

Koller M. (2020). Advances in Polyhydroxyalkanoate (PHA) Production, Volume 2. *Bioengineering*. **7**:24.

Al Battashi H, Al-Kindi S, Gupta VK, Sivakumar N. (2021). Polyhydroxyalkanoate (PHA) Production Using Volatile Fatty Acids Derived from the Anaerobic Digestion of Waste Paper. *J Polym Environ*. **29**:250-259.

Amaro TMM, Rosa D, Comi G, Iacumin L. (2019). Prospects for the Use of Whey for Polyhydroxyalkanoate (PHA) Production. *Front Microbiol*. **10**:992.

Kourmentza C. (2017). Recent Advances and Challenges towards Sustainable Polyhydroxyalkanoate (PHA) Production. Bioengineering. **4**:55.

Jiang Y, Hebly M, Kleerebezem R, Muyzer G, van Loosdrecht MCM. (2011). Metabolic modeling of mixed substrate uptake for polyhydroxyalkanoate (PHA) production. *Water Res*. **45**:1309-1321.

Morgan-Sagastume F. (2020). Mixed-culture polyhydroxyalkanoate (PHA) production integrated into a food-industry effluent biological treatment: A pilot-scale evaluation. *J Environ Chem Eng*. **8**:104469.

Morgan-Sagastume F, Heimersson S, Laera G, Werker A, Svanström M. (2016). Techno-environmental assessment of integrating polyhydroxyalkanoate (PHA) production with services of municipal wastewater treatment. *J Clean Prod*. **137**:1368-1381.

Morgan-Sagastume F. (2015). Integrated production of polyhydroxyalkanoates (PHAs) with municipal wastewater and sludge treatment at pilot scale. *Bioresour Technol*. **181**:78-89.

Gowda V, Shivakumar S. (2014). Agrowaste-based Polyhydroxyalkanoate (PHA) production using hydrolytic potential of Bacillus thuringiensis IAM 12077. *Braz Arch Biol Technol*. **57**:55-61.

Nahar S, Jeong MH, Hur JS. (2019). Lichen-associated bacterium, a novel bioresource of Polyhydroxyalkanoate (PHA) production and simultaneous degradation of naphthalene and anthracene. *J Microbiol Biotechnol*. **29**:79-90.

Kanavaki I, Drakonaki A, Geladas ED, Spyros A, Xie H, Tsiotis G. (2021). Polyhydroxyalkanoate (PHA) production in Pseudomonas sp. PHDV1 strain grown on phenol as carbon sources. Microorganisms. **9**:1636.

Colombo B. (2017). Enhanced polyhydroxyalkanoate (PHA) production from the organic fraction of municipal solid waste by using mixed microbial culture. *Biotechnol Biofuels*. **10**.

Vermeer CM, Rossi E, Tamis J, Jonkers HM, Kleerebezem R. (2021). From waste to self-healing concrete: A proof-of-concept of a new application for polyhydroxyalkanoate. *Resour Conserv Recycl*. **164**:105206.

Aljuraifani AA, Berekaa MM, Ghazwani AA. (2019). Bacterial biopolymer (polyhydroxyalkanoate) production from low-cost sustainable sources. *Microbiologyopen*. **8**:00755.

Estévez-Alonso Á, van Loosdrecht MCM, Kleerebezem R, Werker A. (2021). Simultaneous nitrification and denitrification in microbial community-based polyhydroxyalkanoate production. *Bioresour Technol*. **337**:125420

Kumar S, Sharma MK, Sharma MK, Kumar G. (2022). Isolation of local strain of Bacillus sp. SM-11, producing PHB using different waste raw as substrate. *Res J Pharm Technol*. **15**:2053-2058.

Mohapatra S. (2017). Bacillus and biopolymer: Prospects and challenges. *Biochem Biophys Reports*. **12**:206-213.

Zheng Y. (2021). Effects of chemical oxygen demand concentration, pH and operation cycle on polyhydroxyalkanoates synthesis with waste sludge. *Environ Technol*. **42**:1922-1929.

Pradesh A. (2018). Polyhydroxyalkanoate (PHA) Production by Genetically Engineered Microalgae: A Review. *J New Biol Reports*. **7**:68-73.