Chapter 6
Bioplastic Production Using Whey (Polyhydroxyalkanoates and Polyhydroxybutyrates)

Ananya Rana, Vikram Kumar, Tejpal Dhewa, and Neetu Kumra Taneja

Abstract It has been discovered that bio-based plastics, which are derived from organic and renewable resources, are effective substitutes for polymers derived from petroleum. Unfortunately, few of them, especially those made from whey, have been evaluated economically. The world needs alternatives to plastic since its production and buildup have adverse effects on the environment. Polyhydroxyalkanoates (PHAs) and polyhydroxybutyrates (PHBs) act as viable alternatives to conventional plastics given their biodegradability, biocompatibility, and potential for biological synthesis. PHAs are produced by bacteria, and one of the primary costs related to the production of plastic associated is the carbon supply that the bacteria use to ferment food. As a result, a number of industrial waste streams, such as whey, an effluent from the dairy sector, have been explored as potential carbon sources for bacterial growth and production of PHA and PHB. When whey is utilized to produce PHA, the process may become less expensive and better for the environment. Whey pre-treatments and the selection of a PHA or PHB-producing strain are just two issues that continue to impede the use of whey as a carbon source for PHA and PHB synthesis. In this chapter, the current state of knowledge on the use of whey for the production of PHA and PHB was reviewed, and creative solutions to the challenges faced throughout this manufacturing process were proposed.

Keywords Bioplastic · Renewable · PHA · PHB · Biodegradability · Biocompatibility

A. Rana · V. Kumar
Department of Basic and Applied Sciences, NIFTEM, Sonepat, India

T. Dhewa
Department of Nutritional Biology, Central University of Haryana, Jant/Pali, India

N. K. Taneja (✉)
Department of Basic and Applied Sciences, NIFTEM, Sonepat, India

Centre for Advanced Translational Research in Food Nanobiotechnology (CATR-FNB), NIFTEM, Sonepat, India

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6.1 Introduction

The majority of the manufacture of plastics, that is indispensable to all market capitalism, is accomplished through the use of fossil feed stocks with severe environmental consequences. However, the global output of plastics is soaring (204 million tonnes in the year 2002, up to 335 million tonnes in the year) and is expected to regular to rise. Because the plastic is not easily biodegradable and accumulate in a variety of situations, their production has led to environmental problems (European Commission, 2019). Nevertheless, despite impressive efforts to enhance recycling rates (recycling grew by 79% in Europe between 2006 and 2016); in 2016, 31.1% of European plastics were recycled. The rest of the plastics are dumped inside landfills (27.3%) or can be used for energy rehabilitation (41.6% European Bioplastic) (European Commission, 2019). Therefore, it is imperative that these "conventional" plastics be replaced with bioplastic, which are plastics that are organically generated and/or biodegradable. Despite the fact that it is believed that around 85% of total plastic wares might replaced by bioplastic, only about 1% of the total plastics manufactured worldwide are bioplastic (European Bioplastic) (Rosenboom et al., 2022). The output of bioplastic is anticipated to increase, reaching 2.44 million tonnes in 2022, with only 1.086 million tonnes being biodegradable (European Bioplastic). Unfortunately, this expected growth falls short of the ever-growing worldwide demand for plastics. Polyhydroxyalkanoates (PHAs) are biosynthesized polyesters came to be regarded as highly advantageous alternatives to conventional polymers manufactured from petroleum. It has been found that these molecules serve as carbon storage and reducing equivalents, and that they do so by aggregating in granules within bacterial cells. PHAs are biocompatible and biodegradable and have properties similar to those of conventional polymers (Geyer et al., 2017). As a result, PHAs are now manufactured commercially and used in many different kinds of products, such as packaging and medical equipment. Despite their superior qualities and environmental benefits, the high production costs of PHAs prevent their widespread application. The use of costly fermentation carbon sources, which account for about 40% of total PHA production costs, is a major factor in these high prices (Chaharsooghi et al., 2011). In order to reduce the cost of both PHA synthesis and garbage disposal, it has been suggested that waste products be used as carbon sources for PHA production by microorganisms. PHAs have been successfully produced using a wide variety of waste materials (Castilho et al., 2009). Wastewater from homes, food scraps, molasses, olive oil mill effluents, palm oil mill effluents, tomato cannery water, lignocellulosic biomass, coffee waste, starch, waste from the biodiesel industry, used cooking oil, pea shells, wastewater from paper mills, bio-oil from the fast pyrolysis of chicken beds, and cheese whey are some of the materials that can be used. The main byproduct of the dairy industry is whey, which is derived by precipitating and removing casein from milk during cheese-making (Nielsen et al., 2017). Over 120 million tonnes of whey are released from industries per year globally, but only 50% of it is used for human and animal feed. Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) of whey

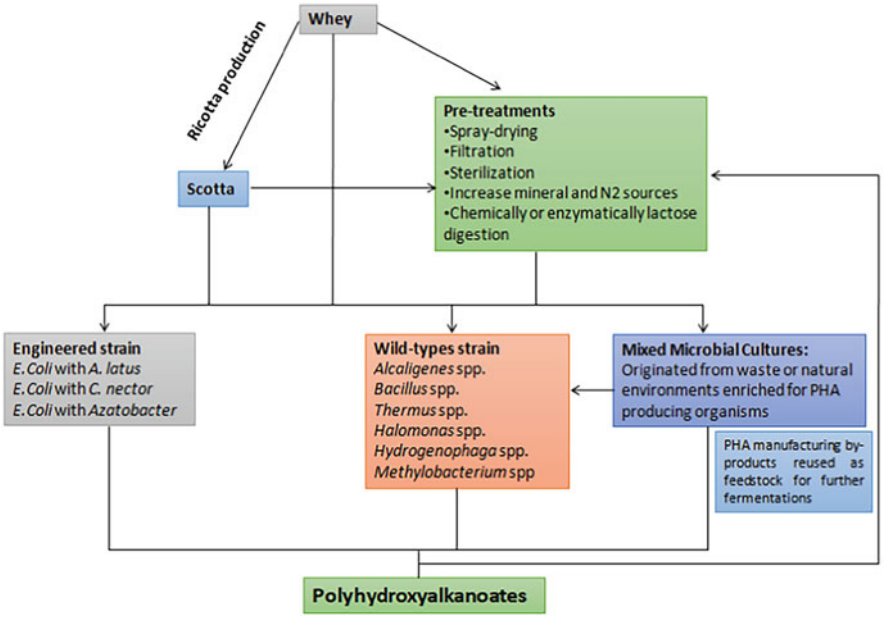

Fig. 6.1 Diagram showing probable whey-based polyhydroxyalkanoates routes. PHA fermentations use whole whey. Nevertheless, pre-treatments usually increase PHA production. Ricotta cheese whey, or scotta, is produced from whey. Scotta can produce PHA directly or after pre-treatments

are very high, e.g. acid whey has a BOD value of 35,000–45,000 mg per litre and COD values of 55,000–70,000 mg per litre (Poonia, 2020). The disposal of the residual whey creates environmental problems resulting from the relatively large organic load it carries, as whey is constituted primarily of lactose (39–60 g/L), lipids (0.99–10.58 g/L), proteins (27–60 g/L), and mineral salts (4.6–8 g/L). Lactose accounts for the majority of cheese whey's biochemical oxygen requirement; consequently, it is crucial to develop a biotechnological application for lactose. Whey lactose would significantly lower PHA production costs without affecting food production or the environment. This chapter summarizes current understanding regarding the production of PHA and PHB using cheese whey (Fig. 6.1). In addition, the challenges and potential of employing the industrial production of PHAs uses whey.

6.2 Microorganisms Used to Produce PHAs and PHBs from Whey

Whey is a nutrient-dense medium ideally suited for microbial growth. Yet, it has been demonstrated that locating bacteria capable of producing PHAs efficiently while growing on whey is incredibly tough. Sadly, it has been demonstrated that several well-explained PHA-producing microbial species are incapable of synthesizing PHAs directly from whey (Amaro et al., 2019). *Cupriavidus necator*, formerly known as *Ralstonia eutropha*, *Wautersia eutropha*, and *Alcaligenes eutrophus*, is capable of collecting PHAs around 80% of total dry weight while growing in the presence of glucose, but is incapable of efficiently growing and producing PHAs in presence of lactose, the primary carbon source of whey. Another instance is the halophilic archaeal bacteria *Haloferax mediterranei,* which has the capacity for PHAs accumulation to the tune of nearly 60% of its dry weight (Sharma et al., 2016). Despite the fact that *H. mediterranei* can consume lactose, but it does not used as an efficient source for growth and PHA production. *Alcaligenes latus* stands out as an exception because it is a commercial PHA synthesizer and can extract PHAs from up to 70% of its dry weight from a wide range of sugar sources. *Alcaligenes latus* may produce whey lactose to PHA (0.11 $g\,L^{-1}\,h^{-1}$), according to a recent study (Amaro et al., 2019). *Alcaligenes latus* can manufacture PHAs from whey, but more research is needed to maximize its potential. In certain cases, pre-fermenting whey lactose into glucose and galactose helped highly PHA-producing bacteria make PHA from it (Surendran et al., 2020). Utilizing this hydrolyzed whey lactose, it was demonstrated that high PHA producers, such as *C. necator* and *H. mediterranei*, may manufacture PHAs. Yet, from an industrial standpoint, it is preferable to minimize the additional cost of PHAs that result from transforming whey lactose to glucose and galactose before to fermentation. Through the use of genetic engineering techniques, these conversion steps have already been omitted. For example, lactose-consuming *Escherichia coli* cells were engineered to express genes involved in PHA biosynthesis pathway from bacteria with a high PHA production rate (Bosco et al., 2021).

Instead, lactose degrading genes have been introduced into high-producing bacteria. While, Genetic engineering can improve whey PHA production, but it requires more carefully regulated production facilities (Mitra et al., 2020). Genetically modified microorganisms (GMMs) are widely used in scientific research facilities and the biotechnology industry, raising worries about unintentional release into the environment, as well as the transfer of genes to other types of microbes/non-microbes hosts. In light of this, biosafety legislation of particular nations has deemed it important to limit their use in order to prevent any potential problems they may offer. The order mandates the notification, risk assessment, and permission of work involving GMOs, as well as the approval of laboratories and facilities where such work will occur (Mitra et al., 2020). There are four classes of laboratories for working with GMMs, each with its own set of regulations regarding containment, association of the laboratories and area nearby laboratories, work execution plans,

technical facilities, internal supervision, and control (Council Directive 98/81/EC of 26 October 1998 amending Directive 90/219/EEC on the contained use of genetically modified micro-organisms) (Amaro et al., 2019). Thus, biotechnology laboratories and biosafety precautions are necessary for businesses to protect their employees, the public, and the environment against hazardous bacteria and substances, as well as qualified personnel, which raises plant management expenses and PHA manufacturing prices. Whey-based PHA production using mixed microbial cultures (MMCs) has recently gained attention (Amaro et al., 2019). MMCs do not need sterile conditions and can adapt to altering industrial effluent, although they produce less PHA. MMCs directly generate PHAs from cheese whey lactose or after lactose digestion by another MMC. MMCs are typically collected from waste sources and increased for PHA synthesis via feast and famine cycles to develop strains that can produce PHA-based carbon storage (Valentino et al., 2015). The utilization of an unknown microbial population to manufacture PHAs, in medical applications, PHAs' production efficiency, polymer characteristics, and biocompatibility pose issues, despite their remarkable versatility and low cost. To the best of our knowledge, a study isolating single organisms for PHA synthesis from whey has not yet been conducted. Another approach involves using MMCs to break down the lactose into organic acids, which are then used as a substrate for a high-PHA strain. But still, PHAs from whey have not been produced using this method (McAdam et al., 2020).

6.3 Pre-treatment of Whey

For direct PHA synthesis, whey poses various challenges as a substrate. First, it has been reported that whey has a low C-N ratio, which significantly impedes PHA formation. In addition, lactose sugar is not a good carbon source for PHA-producing strains, as was previously mentioned. However, as whey is a complicated, non-sterile, and frequently changeable by-product, its immediate application in research laboratories and manufacturing processes leads to (Amaro et al., 2019). Consequently, most research on PHA production from whey has used whey derivatives that have been pretreated (Fig. 6.1 and Table 6.1). To adjust lactose concentration, whey powder is diluted with water before fermentation. Spray-dried powdered whey may be stored longer and doesn't vary seasonally. Spray-drying a PHA production pipeline will increase costs (Chang et al., 2021). Based on the reasoning for making use of whey powder in screening and enhancing PHA production, fermentations with whey may be comparable to that using whey powder. Ultrafiltration removes most of whey's proteins and other particles, leaving a lactose-rich permeate (Amaro et al., 2019). To remove protein, whey is ultra filtered, acidified to a pH close to 4, heat treated, centrifuged, and filtered to produce a supernatant. Whey permeates or supernatants simplify production but cost more. *Methylobacterium* sp. ZP24 used whole whey and whey supernatant to grow biomass and PHA on a complex mineral medium. (Pantazaki et al., 2009). When

Table 6.1 A summary of the research aiming at manufacturing PHAs from whey, broken down by the type of whey pretreatment

Types of whey	Type of culture	Microorganisms type	Type of PHA
Permeate of fermented whey powder	MMC	Not define	P-3(HB-co-HV)
Fermented whey supernatant	MMC	Not define	PHB/P-3 (HB-co-HV)
Supernatant of whey + additives	Pure cultures	*Bacillus megaterium* *Thermus thermophilus HBB* *Methylobacterium* sp. *ZP24*	PHB Diverse PHB
Fermented whey powder	MMC	Not define	P-3(HB-co-HV)
Hydrolyzed whey + additives	Pure cultures	*Halomonas halophila*	PHB
Hydrolyzed whey powder	Pure cultures	*Haloferax mediterranei*	P (3HB-co-3HV)
Fermented whey powder	MMC	Not define	P (3HB-co-3HV)
Supernatant of whey powder + additives	Pure cultures	*Alcaligenes latus*	PHB
	Formulated cultures	Engineered *E. coli* with *Alcaligenes latus* PHA biosynthesis genes	PHB
		Engineered *E. coli* with *Azatobacter* spp. PHA biosynthesis genes	PHB

extrapolating from using whey concentrates or supernatants to whole whey, care should be used.

Minerals and nitrogen sources are other sorts of additives that are commonly employed in PHA synthesis research including whey (Table 6.1). Instead of permeates or supernatants, whole whey might eliminate these additives. In other studies, it is unclear if the microorganisms can It would be excellent for industrial production if large quantities of PHAs could be accumulated utilising whey without additions. The addition of salts ((NH4)2SO4, 5 g L^{-1}; Na2HPO4, 2.5 g L^{-1}; KH2PO4, 2.5 g L^{-1}; MgSO4, 0.2 g L^{-1}; and MnSO4, 0.01 g L^{-1}) to whey supernatant nearly doubled *Bacillus megaterium's* biomass and PHA production by tenfold (Koller et al., 2008). Thus, it would be worthwhile to over look the use of other affordable waste materials as whey additions if whey were to be supplemented in order to increase PHA synthesis; this subject will be addressed later in the chapter.

The manufacture of PHA should make greater use of whole whey as a research tool. However, using entire whey presents significant difficulties in the experimentation process, one of which is sterility concerns. When employing MMCs, these concerns are less important; nonetheless, sanitation is absolutely necessary when working with single cultures. It is impossible to sterilize complete whey by heating it to high degrees because the total whey protein will precipitate at those temperatures

(Bosco & Chiampo, 2010). Moreover, filtering of whey is problematic because of the large quantity of suspended particulates that it contains. It is possible to apply a procedure called low-temperature pasteurization, but doing so typically requires multiple cycles of heat and cold treatments, which are operations that are both time-consuming and expensive. In addition, the method of pasteurization does not guarantee complete sterilization, which may result in sterility issues during the fermentation phase of the process (Webb & Whittier, 1948).

Hence, UV radiation or high-temperature-short-time pasteurization may be utilised prior to fermentation with pure strains in entire whey. These procedures could kill any whey bacteria (Shabbir et al., 2021). In addition to that, the utilization of antibiotics as a potential solution has also been investigated. *Vancomycin* was proven to minimize *Bacillus cereus* contamination in *Hydrogenophaga pseudoflava* PHA synthesis using whey without considerable compromising the production efficiency of PHA. This was accomplished by inhibiting the growth of the *Bacillus cereus*. The utilization of untreated entire whey results in a high lactose concentration, which is another issue that occurs (Koller et al., 2008). It was demonstrated that the content of lactose in the whey was extremely important for the synthesis of biomass and PHA by *B. megaterium* CCM 2037. When grown in 20 g/L diluted whey supernatant, biomass and PHAs increased to 2.51 g/L and 0.79 g/L, respectively (Amaro et al., 2019). This strain achieved a biomass of about 1.5 g/L when cultured in 40 g/L lactose-containing whey supernatant and synthesized <0.5 g/L PHAs. Whey lactose must be converted into galactose and glucose before fermentation, in addition to the aforementioned pre-treatments (Bosco & Chiampo, 2010). This is necessary for the growth of certain bacteria (Table 6.1). A few of the authors have circumvented the need for lactose hydrolysis by employing a medium that imitates hydrolyzed whey and contains galactose and glucose as carbon sources.

6.4 Alternative Strategies to Enhance PHAs Production from Whey

This chapter focuses on two essential elements that must be considered in order to achieve commercially viable PHA generation from cheese whey: microbe selection and whey pre-treatment needs. Despite the fact that not all parameters may be discussed in this article, it is necessary to note that a number of additional crucial factors must be considered for the efficient generation of PHAs from whey.

6.4.1 Recycling of Whey to PHAs Production

As stated in the preceding sections, the manufacture of PHAs from whey requires the addition of a number of chemicals. Thus, it would be quite intriguing if these

compounds could be derived from other waste sources. *H. mediterranei* was utilized to assess the viability of reusing organic material as substrate in future PHA biosynthesis pipelines by fermenting enzymatically digested whey lactose to produce PHA (Khatami et al., 2021). *H. mediterranei* is a severe halophile, and as a result, it's utilization has the benefits of decreasing disinfectant charges and facilitating differential osmotic pressure-based PHA extraction methods. Despite this, *H. mediterranei* fermentations for PHA generation generate huge quantities of very salty waste streams (Mitra et al., 2020). Thus, writers have evaluated the viability of incorporating these waste streams into succeeding fermentations. In spite of the modest yields achieved, the recycling of wastes enabled the manufacture of PHA, presenting intriguing opportunities for future research on the re-use of organic waste in PHA biosynthesis processes. Applying these flows to additional biotech manufacturing techniques are additional intriguing possibility that, to our knowledge, has not yet been detailed (Koller et al., 2008). Consequently, to add value through eco-friendly procedures, research on recycling waste streams during PHA synthesis from whey is crucial.

It has been demonstrated that additions, such as nitrogen sources, are necessary for optimizing the generation of PHA from whey. As mentioned, these additions from food waste might be valuable. Whey has been used to make PHA from waste frying oils, however this additive source for PHA production is largely investigated (Caldeira et al., 2020). It has been demonstrated that protease-hydrolyzed whey functions as a complicated nitrogen source to increase PHA synthesis from wastage of fried by roughly 40% utilizing *C. necator*. Thus, the blending of waste sources offers virtually limitless opportunities to improve the effectiveness of PHA synthesis without the addition of expensive chemicals. Additionally, to testing different waste streams for PHA production increases, cheapest additives could also be tested (Obruca et al., 2014). For instance, adding 1% ethanol as an external stress chemical to cheese whey increased *B. megaterium* PHA synthesis by nearly 40%. For maximizing PHA synthesis from cheese whey, the usage of ethanol or other stressors, like PHA production boosters, must unquestionably be considered.

6.4.2 Improvement in PHAs Purification Process

Another potential issue associated using entire whey for microbial PHA production extracts and purifies PHAs. PHA extraction from bacterial cells started with two approaches. The first method utilised PHA's solubility in chloroform and insolubility in methanol. Solvent-based PHA extraction yielded above 90% (Amaro et al., 2019). Unfortunately, the usage of solvents makes these methods both environmentally and economically undesirable. The second method involves cleaning with enzymes and detergents to remove biological entities while preserving PHAs. These solutions are more eco-friendly, but typically result in less PHA production and high prices when enzymes are employed (El-malek & Steinbüchel, 2022). Many new solvents and extraction methods, including creating PHA-secreting microbial strains, are being

evaluated as potential alternatives to the most commonly used extraction methods. Except for a modified *E. coli* study that produced partial PHA, this strategy has not been explored. For the generation of PHAs from whey (Tan et al., 2014), Yet, cheaper PHA extraction methods have not been thoroughly examined. One and only ecologically acceptable method for PHA extraction from whey uses a single microbe to break down *H. mediterranei* cells using their high osmotic pressure (Pagliano et al., 2021). However, it has to be determined if while using whole whey, PHA extraction yields and purity are equivalent, which is rich in proteins and lipids and adds an additional degree of complexity to PHA extraction operations (Amaro et al., 2019).

6.5 Conclusion

To address the environmental issues caused by the manufacture and accumulation of traditional plastics, it will be necessary to produce bioplastic in a sustainable and cost-effective manner. PHAs have qualities that are desirable in the market and can be biologically synthesized from inexpensive waste substrates like whey, suggesting that they could play a key part in this bioplastic solution. While effective generation of PHAs from whey is a desirable goal, there are still several obstacles that must be solved. We have compiled a summary of the literature on whey pre-treatments and generating microbe selection for PHA generation from whey. Based on what we know presently, it seems likely that PHA can be extracted from whey using a variety of methods. The lack of research on the use of whey for the industrial production of PHAs, taking into accounts the associated costs and environmental impacts, is another key finding of this review. Thus, this research may inspire and direct efforts to develop commercially feasible methods for extracting PHAs from whey.

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