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Production of biodegradable food packaging from mango peel via enzymatic hydrolysis and polyhydroxyalkanoates synthesis: A review on microbial intervention

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ABSTRACT

The rising environmental problem of plastic packaging waste has led to the development of sustainable alternatives, particularly for food packaging. Polyhydroxyalkanoates (PHAs) are biodegradable, thermoplastic polyesters. They are employed in the production of various products, including packaging films. The bio-based nature and appropriate features of PHAs, similar to conventional synthetic plastics, have garnered significant attention from researchers and industries. The current study aimed to produce biodegradable food packaging using mango peel (a major agricultural waste) with enzymatic hydrolysis and PHAs synthesis. Mango peel is the hub for macro-and micronutrients, including phytochemicals. The process includes an enzymatic hydrolysis step that converts complex carbohydrates into simple sugars using mango peel as a substrate. The produced sugars are used as raw materials for bacteria to synthesize PHAs, which are a class of biodegradable polymers produced by these microorganisms that can serve as packaging materials in the food industry. To solve environmental problems and increase the utilization of agricultural by-products, this review presents a practical method for producing food packaging that is environmentally friendly.

1. Introduction

Global concerns about environmental pollution and sustainability have prompted a revaluation of conventional food packaging methods. Conventional packaging materials, primarily plastics derived from petroleum, contribute significantly to environmental degradation, including damage to land and marine environments, greenhouse gas emissions, and negative effects on species ([Gheorghita et al., 2020](#page-10-0)). Creating environmentally safe and sustainable food packaging is a good way to address these issues and maintain a standard of living. Although plastic packaging is practical and cost-effective, it presents a massive environmental concern owing to its persistence in the environment. Plastics can persist for hundreds of years without biodegradation, leading to widespread environmental contamination, particularly in oceans, where marine life is at risk of ingestion or entanglement in plastic waste. Furthermore, as plastics break down into smaller fragments, known as microplastics, there is growing concern that these particles contaminate food systems and potentially threaten human health. The production, use, and disposal of plastics derived from fossil fuels significantly contribute to climate change [\(Mendes and Pedersen](#page-10-0) [et al., 2021](#page-10-0)). Moreover, the production of paper, another common packaging material, is an energy- and carbon-intensive process driven largely by the burning of fossil fuels, which further exacerbates climate change. The burning of fossil fuels releases harmful chemicals into the environment, and the disposal of plastic waste contributes to air pollution by releasing toxic elements into the atmosphere. These environmental and public health threats underscore the current unsustainability of conventional plastic packaging systems in the current scenario

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(Lipiäinen [et al., 2022](#page-10-0)).

Biodegradable packaging materials offer significant environmental and economic benefits. These materials are designed to break down organically under the influence of microbes, thereby reducing environmental pollution. Compared to petroleum-based plastics, biodegradable materials are often made from renewable sources such as plants, making them more sustainable over their lifecycle. Natural packaging materials decompose naturally, thereby preventing the accumulation of waste in landfills and oceans. Biodegradable materials are also typically produced with lower energy consumption and greenhouse gas emissions than conventional plastics, helping to reduce the overall carbon footprint of the packaging industry ([Harvey et al., 2020](#page-10-0)). This not only provides an additional source of income for the agricultural sector but also promotes sustainable resource management. Among these biopolymers, those based on polylactic acid (PLA), polyhydroxyalkanoates (PHAs), and starch are particularly promising. These materials have functional properties similar to those of traditional plastics (e.g., strength and barrier properties) but are not derived from fossil resources, thus avoiding the environmental issues associated with conventional plastics [\(Naser et al., 2021\)](#page-10-0).

Mango (*Mangifera indica*) is a popular dietetic fruit consumed worldwide and is known as the "king of fruits." The mango industry is significant in many countries, consuming substantial resources for both fruit production and construction of new cultivation infrastructure ([Hussain et al., 2021](#page-10-0)). Given the large volume of mango peel generated as agro-industrial waste, it is important to explore the effective utilization of mango peel to maximize its environmental benefits and promote its acceptance as green biomass ([Amran et al., 2021](#page-9-0)). The major mango-producing countries include India, China, Thailand, Indonesia, and Mexico. With almost 40 % of global mango output, India leads the chart on the production front. During processing into derivatives, such as juices, purees, and dried fruits, up to 35–60 % of the mango's weight is recognized as waste, primarily consisting of peels, seeds, and stones ([Wardhan et al., 2022\)](#page-11-0). The peel comprises 15–20 % of the total weight of the fruit, and millions of tons of peel waste are generated annually. The nutritional profile and various bioactive compounds in mango peel make it desirable for value-added applications. Mango peels are rich in dietary fibers, pectin, polyphenols, carotenoids, and vitamins (A, C, and E), and contain essential minerals such as potassium, magnesium, and calcium. These components provide mango peel with excellent antioxidant, antimicrobial, and anti-inflammatory properties ([Lebaka et al.,](#page-10-0) [2021\)](#page-10-0).

Inappropriate disposal of mango peel waste poses environmental issues because organic waste in landfills undergoes anaerobic decomposition, producing methane, a greenhouse gas that is 86 times more potent than CO2. While incineration destroys waste, it also releases harmful pollutants into the air. Although composting and related emerging techniques such as liming addition are environmentally sustainable, they do not fully exploit the composite bioactivity potential of the peel [\(Kaur et al., 2023\)](#page-10-0). Mango peel, which is enriched with antioxidants and dietary fibers, can be used to manufacture nutraceuticals and functional foods. Mango peel extract can be added to beverages, snacks, and supplements to improve digestion and boost immunity. Owing to their high polyphenol and vitamin content, mango peel extracts have significant potential in cosmetics for anti-aging and skin-protective applications. Additionally, mango peel can be used in animal feed processing because of its high fiber and nutritional content, helping to meet the nutritional requirements of livestock and reducing the demand for conventional feed sources (Zuñiga-Martínez et al., [2022\)](#page-11-0). One of the newer applications of mango peel is in the production of biodegradable packaging materials. By processing mango peel waste into bioplastics using enzymatic hydrolysis and fermentation, these bioplastics can serve as environmentally friendly alternatives to conventional plastics. Additionally, mango peel waste can be utilized in bioenergy production as feedstock through anaerobic digestion or fermentation processes [\(Coppola et al., 2021](#page-10-0)). The aim of this review

article is to focus on the microbiological aspects of enzymatic hydrolysis, by which mango peel can be utilized as biodegradable food packaging for sustainable development.

2. Mango peel composition

Despite being discarded as waste, mango peel is a rich source of nutrition and contains various bioactive compounds with multiple potential applications. It contains macronutrients, micronutrients, polyphenols, flavonoids, carotenoids, tannins, and other beneficial compounds, all of which contribute to its potent antioxidant, antimicrobial, anti-inflammatory, and anticancer properties. Given its rich chemical composition, mango peel has significant potential for use in the development of novel, sustainable functional foods, medications, cosmetics, and packaging materials ([Mwaurah et al., 2020](#page-10-0)). This section of the article delves deeper into the composition of mango peel and explores its potential uses in various fields.

2.1. Macronutrients

Mango peel contains carbohydrates, mainly dietary fibers. A large component of the peel weight when dried is fiber, including both soluble and insoluble fiber types. These soluble fibers are important because they help the peel gel, a key factor in having a successful jam and helping the gut [\(Ajila and Rao, 2013](#page-9-0)). A high-fiber diet benefits bowel health and prevents constipation owing to its insoluble fibers [\(Lebaka et al.,](#page-10-0) [2021\)](#page-10-0). Mango peel is less abundant in proteins than in carbohydrates. These proteins are required for certain bodily functions and contain essential amino acids. In mango peel, the enzyme levels of polyphenol oxidase and peroxidase also reflect the high protein content, which is in turn associated with biochemical properties. Mango peel contains low-level lipids rich in unsaturated fatty acids. This quality of lipids adds to the nutritional value of the peel and could also explain some of its beneficial health effects ([Assoi et al., 2024\)](#page-9-0).

2.2. Micronutrients

It has abundant sources of vitamins, especially vitamins A, C, and E, which are rich in carotenoids and are important in vision, immunity, and skin health. Vitamin C, also known as ascorbic acid, is a powerful antioxidant that supports immune functions, skin health, and collagen synthesis of collagen ([Jafari et al., 2019](#page-10-0)). Owing to its powerful antioxidant properties, vitamin E prevents oxidative stress in cells and promotes skin health [\(Michalak et al., 2021\)](#page-10-0). Mango peel contains high levels of minerals necessary for function, such as potassium, magnesium, calcium, and phosphorus. Ensuring fluid balance, nerve function, and precise muscle contractions are only a few reasons why potassium is necessary. In fact, magnesium is involved in *>*300 enzymatic reactions in the human body, such as protein synthesis and muscle and nerve function. Calcium is vital for bone health, muscle function, and nerve signalling. It is essential for bone and tooth formation, energy production, and cell membrane functions [\(Lebaka et al., 2021\)](#page-10-0).

2.3. Phytochemicals

Among the different polyphenols, mango peel is the most abundant, making it a powerful antioxidant. Polyphenols in mango peels [\(Suleria](#page-10-0) [et al., 2020](#page-10-0)). Mangiferin is a xanthonoid with potent antioxidant, anti-inflammatory, and anti-cancer properties [\(Dutta et al., 2023](#page-10-0)). Quercetin is a flavonoid with anti-inflammatory and antioxidant properties [\(Tian et al., 2021](#page-11-0)). Gallic Acid is an organic acid with antimicrobial and antioxidant properties ([Wianowska and Olszowy-Tomczyk,](#page-11-0) [2023\)](#page-11-0). Mango peel contains antioxidants in the form of flavonoids and has both anti-inflammatory and cancer-preventing properties [\(Mirza](#page-10-0) [et al., 2021\)](#page-10-0). Rutin provides powerful antioxidants and vascular health benefits [\(Semwal et al., 2021\)](#page-10-0). Carotenoids, found in abundance in mangoes, are excellent antioxidants that promote healthy vision and immune function and give the peel its typical yellow-orange hue ([Yahia](#page-11-0) [et al., 2023\)](#page-11-0). Tannins are known to have astringent properties and contribute to the antimicrobial and antioxidant activities of mango peels ([Yadav et al., 2022](#page-11-0)).

2.4. Other bioactive compounds

Triterpenes have anti-inflammatory, antibacterial, and anticancer properties among other biological activities. When present in trace amounts, phytosterols can promote cardiovascular health by lowering the cholesterol levels. Polyphenol oxidase and peroxidase are two enzymes found in mango peel that contribute to its antioxidant and browning responses ([Adeseko et al., 2022\)](#page-9-0).

2.5. Mango peel components enhancing PHA production

The major constituents of mango peel are high-value carbohydrates. Mango peel contains soluble and insoluble polysaccharides, such as cellulose, hemicelluloses, and pectin. These polymeric sugars can be degraded by enzymatic hydrolysis or microbial fermentation to simple sugars (e.g., glucose and fructose) and used as carbon sources for the biosynthesis of PHA-producing bacteria, such as *Cupriavidus necator* and *Pseudomonas putida.* Carbohydrates are important in the PHA biosynthetic pathway because they represent the principal carbon sources necessary for all metabolic pathways that lead to their production. When metabolized under limiting nitrogen (N), phosphate (P), or oxygen conditions, these sugars induce bacteria to reroute carbon fluxes toward PHA synthesis for energy storage ([Jiang et al., 2016;](#page-10-0) [Zheng et al., 2020](#page-11-0)).

Another important component of mango peel is reducing sugars such as glucose and fructose. As such, many PHA-producing bacteria do not need to convert these monosaccharides before they can be used as inputs of metabolic energy for making bioplastics. Microorganisms, however, can take up and utilize these reducing sugars without extensive enzymatic degradation prior to incorporation into their metabolic pathways. Rather, research has demonstrated the potential to increase PHA production yields by utilizing sugar-rich agricultural residues, such as mango peel, in preparation for industrial bioplastic mass production ([Andler et al., 2021](#page-9-0)). Mango peel is a treasure of polyphenols (antioxidants), vitamins, and minerals, which may contribute to microbial metabolism or PHA synthesis. Mangiferin and quercetin are members of the polyphenol group, possessing the ability to scavenge a large number of free radicals that may also be present during downstream use by improving antioxidative defenses when bacterial cells undergo fermentation. These reduced species aid in the internal redox state of bacteria, promoting their metabolism, including more efficient synthesis of PHAs (Borrero-de Acuña et al., 2023).

Other minerals, such as potassium, magnesium, and calcium, are present in mango peel, which might affect microbial metabolism. Potassium is important for the maintenance of osmotic balance and can increase enzyme activity within a bacterial cell, whereas magnesium acts as a cofactor for many enzymes, including ATP, which is actively involved in energy transfer during PHA biosynthesis. Calcium may participate in bacterial cell membrane stabilization, promoting better growth and greater PHA accumulation (Estévez-Alonso et al., 2023). The fatty acid and lipid content in mango peel is another attractive aspect of PHA production. For example, certain bacteria (e.g., *Pseudomonas*) can utilize fatty acids as substrates for PHA biosynthesis. Lipids can generate medium-chain-length PHAs (mcl-PHAs), which are known for their unusual polymer mechanical properties that are relevant to diverse industrial applications. Accordingly, the use of mango peel lipids could vary in PHAs structure, which would broaden their applicability and economic value [\(Teshome et al., 2023](#page-10-0)).

3. Different methods of producing PHAs from mango peels

Various methods have been employed to produce PHAs from mango peel, and each process has its own advantages and drawbacks. The key steps are the pretreatment of mango peels, hydrolysis for releasing fermentable sugars followed by microbial fermentation, and PHAs extraction. Acid hydrolysis uses strong acids, such as sulfuric acid, to degrade complex carbohydrates into sugars, and in a study by Muigano et al. (2024), the bacterium *Halomonas alkalicola* successfully used fruit peel hydrolysates for sustainable polyhydroxyalkanoate production. Pretreatment with dilute sulfuric acid allowed optimal release of reducing sugars, resulting in 16.92 % PHA content. *H. alkalicola* also showed potential for evaluating mango, banana, and pineapple peels for PHA production. However, low yields may be achieved because of the slow growth rate. These processes are highly efficient, but some produce toxic byproducts and cause sugar degradation, for which chemicals sometimes require costly post-treatment steps to be neutralized into other compounds. Furthermore, a corrosive chemical environment produces a lower total PHAs yield (Kag et al., 2024).

Fruit peel extracts can be used directly in liquid cultures as a carbon source for submerged fermentation processes using microbial fermentation techniques suitable for banana, orange, and apple peels (Umesh et al., 2021; Rayasam et al., 2020). A study conducted by Pervaiz et al. (2024) explored *Bacillus* sp. MB353 is used in sequencing batch reactors to produce PHB, demonstrating the economic viability and ecological impact of using synthetic wastewater for submerged fermentation and bioplastic production. Solid-state fermentation (SSF) is a process in which fruit peels (often citrus/apple peels) are fermented without a freeflowing liquid, as reviewed by [Zytner et al. \(2023\)](#page-11-0). A comparative analysis of the different methods for producing PHA is presented in [Table 1](#page-3-0).

4. Enzymatic hydrolysis of mango peel

The conversion of mango peel, an agro-industrial waste, into valuable bioplastics, such as PHAs, is an innovative and sustainable process that addresses environmental concerns and adds value to waste products. This comprehensive process involves several stages, from the initial collection and preparation of the mango peel to the final extraction and application of PHAs [\(Avgoulas, et al., 2023](#page-9-0)).

4.1. Collection and preparation of mango peel

The first step in this process involves the collection of mango peel from the mango processing industries. Typically discarded as waste, mango peel is rich in cellulose, hemicellulose, pectin, and various other bioactive compounds. Before enzymatic hydrolysis, the peel must be thoroughly cleaned to remove any dirt or impurities. To enhance the efficiency of the subsequent enzymatic reactions, the peel is then sizereduced through milling or grinding, which increases the surface area available for enzyme action ([Jeevitha, et al., 2023](#page-10-0)).

4.2. Enzymatic hydrolysis of mango peel

Enzymatic hydrolysis is a key step in the breakdown of complex carbohydrates in mango peel into simpler sugars. [Table 2](#page-4-0). explains the few enzymes that might be involved in the hydrolysis of mango peel. Enzymatic hydrolysis involves several steps, as explained in the following section.

4.2.1. Selection of enzymes

Enzymatic hydrolysis of mango peel includes the selective use of enzymes to convert complex carbohydrates to more accessible sugars, which represents a very important process in mango peel processing. Identification of the appropriate enzymes is essential for the efficient performance of this process ([Vilas-Franquesa, et al., 2024\)](#page-11-0). Cellulases

Table 1

Comparative analysis of different extraction methods with respect to various factors.

4

Table 2

List of few enzymes used in the enzymatic hydrolysis of mango peel.

are enzymes that break down cellulose, a major constituent of mango peels. Cellulose is a complex polysaccharide composed of long chains of glucose molecules that are connected by $\beta - 1$,4-glycosidic bonds. These bonds are hydrolyzed by cellulases, producing glucose and cellobiose, which can then be further broken down into glucose. This glucose can be converted to useful bioproducts, such as PHAs, during successive fermentation processes [\(Shokri, et al., 2022](#page-10-0)). Mango peel contains a significant amount of pectin, a heteropolysaccharide found in the cell walls of plants, particularly in the middle lamella or the 'cement layer' of plant cells. Pectinases, the enzymes responsible for breaking down pectin, include polygalacturonases (PGs), pectin lyases (PLs), and pectin esterases (PEs). These enzymes degrade pectin into monomeric components, primarily producing galacturonic acid. Additionally, pectinases release other monomeric sugars, thereby increasing the total pool of fermentable sugars in mango peel mass [\(Ezugwu et al., 2023\)](#page-10-0). Hemicellulases are enzymes that hydrolyze hemicellulose, a heterogeneous group of non-cellulosic polysaccharides that includes a range of sugars, such as xylans, mannans, galactans, and glucans. The hemicellulose structure with system xylanases, mannases, and arabinofuranosidases ulently selects hemicellulose into individual monosaccharides. Microbial fermentation can easily convert these simple sugars into value-added products ([Borker et al., 2021](#page-9-0)).

Enzymatic hydrolysis breaks down lignocellulosic materials into sugars, making more of the substrate available for bacterial fermentation. Enzyme-bacterial strain coupling generates a channelled flow path from organic waste to PHA production [\(Chavan et al., 2022\)](#page-10-0). Efficacy depends on the compatibility of the enzymatic action on the substrate with the metabolic potentials existing in each strain. The pH, temperature, and enzyme concentration also affect the efficiency of enzymatic hydrolysis, resulting in varying degrees of utilization by the bacterial strain. The synergistic effects of the combination help reach an optimal PHA yield and a more sustainable production process with efficient conversion rates between enzymes and bacteria [\(Saravanan et al.,](#page-10-0) [2022\)](#page-10-0).

4.2.2. Pretreatment

To improve the efficacy of enzymatic hydrolysis of mango peel, it is necessary to increase enzyme accessibility to the polysaccharides in the peel. This is accomplished using several pretreatment techniques that upset the intricate structure of mango peel and increase its susceptibility to enzymatic assault. The most common methods for reducing the size of mango peel particles are physical methods such as milling and grinding.

By further breaking the peel components into smaller pieces, they increase the accessible surface area in contact with the enzyme, thereby increasing the rate of hydrolysis ([Wongkaew, et al., 2021](#page-11-0)). Therefore, the larger surface area gives enzymes more room for interaction with the cellulose, hemicellulose, and pectin of the peel. Chemical modification of the peel structure by treatment with a moderate acid or alkali. Acid pretreatment, which often entails diluted sulfuric acid, breaks down the lignin structure, converts hemicellulose to xylose, and increases the accessibility of cellulose. Hemicellulose and lignin are often dissolved via alkali treatment, which involves boiling sodium hydroxide or calcium hydroxide. The dispersion of various chemicals in the peel matrix permits enzyme penetration and acts on polysaccharides to readily undergo degradation ([Monica et al., 2022](#page-10-0)).

4.2.3. Hydrolysis process

Once the mango peel is pretreated, complex polysaccharides are enzymatically hydrolyzed into simple sugars. This process typically occurs in a bioreactor, where conditions such as temperature and pH are carefully monitored and adjusted to optimize enzyme activity. Enzymatic hydrolysis generally occurs at temperatures between 40 ◦C and 50 ◦C, the range in which enzymes exhibit the highest catalytic activity, leading to a rapid and efficient breakdown of polysaccharides ([Sharif](#page-10-0) [et al., 2023\)](#page-10-0). It is important to maintain the right temperature because deviations can reduce enzyme productivity or denature the enzyme, and do not achieve the hydrolysis process. It also maintained the pH level preferentially around a neutral, slightly acidic level between 4.5 6.0. This pH range was set to maintain the structure and catalysis of the enzymes. Specific pH optima for different enzymes, such as cellulases and pectinases, are optimal under slightly acidic conditions. Balanced pH conditions in the bioreactor lead to the optimal performance of all enzymes responsible for hydrolysis. Enzymatic hydrolysis can last from a few hours to several days depending on the enzyme activity, concentration of the substrate, and degree of pretreatment [\(Sharif et al., 2023](#page-10-0)). Under the action of enzymes, cellulose, hemicellulose, and pectin in mango peel are broken down into their respective simple sugars. Cellulases break down cellulose into glucose, pectinases convert pectin into galacturonic acid and other monosaccharides, and hemicellulases hydrolyze hemicellulose into sugars, such as xylose and mannose ([Wongkaew et al., 2021](#page-11-0)).

4.2.4. Separation of hydrolysates

The solutions obtained after enzymatic hydrolysis are

monosaccharides such as glucose, galacturonic acid, and other simple sugars. These sugars are crucial for subsequent biotechnological processes, for example, fermentation to produce PHAs. However, the hydrolysis process leaves some residual solid material consisting of an undigested portion of fibers and residues of the enzyme used. The solids must be separated from the liquid that contains valuable sugars; otherwise, the hydrolysates will have coloration. Separation is usually implemented using filtering and centrifugal methods. Filtration is a simple and well-established process in which a liquid-particle suspension flows through a porous medium that selectively retains solid particles. The hydrolysis present in this process was filtered using filters with different pore sizes that captured the solid residues and allowed only the liquid containing simple sugars to pass through. Filtration can be performed through different assemblies using gravity filtration, vacuum filtration, or pressure filtration, according to the scale and need of the process. The centrifuge separation of solids in a liquid is based on their density. In this method, the hydrolyzed cocktail is vortexed, divided into centrifuge tubes, and spun at high speed. The heaviest sediment is the solid particles, which form a pellet at the bottom of the tubes, while the lighter liquid containing the soluble sugars remains at the top as a clear supernatant. These processes under the above separation systems lead to a clear solution of simple sugars without any solid impurities in the hydrolysates. This solution must remain clear for the next stage because any impurity can affect the next stage of processing. For example, regarding microbial fermentation for PHA biosynthesis, a transparent sugar solution is critical for maximum microbial growth and PHA synthesis, which translates into higher product yield and purity ([Xue et al., 2021; Vilas-Franquesa et al., 2024\)](#page-11-0).

4.3. Fermentation for PHA production

The simple sugars obtained from the hydrolysis of mango peel served as a carbon source for microbial fermentation to produce PHAs. This stage involves several key steps, as illustrated in Fig. 1.

4.3.1. Selection of microorganisms

They are subsequently used as substrates by specific types of PHAproducing bacteria in the biosynthesis of PHAs in the

biotransformation of natural sugars obtained from enzymatically hydrolyzed mango peel [\(Agboola, 2023](#page-9-0)). These microorganisms included *Cupriavidus necator, Ralstonia eutropha, Pseudomonas putida* etc. These bacteria are known to be the most efficient in the conversion of carbon substrates to intracellular PHA granules, and are therefore of interest for industrial biopolymer production. *Cupriavidus necator*, previously known as *Ralstonia eutropha*, is one of the most studied and utilized bacteria for industrial-scale PHA production. It can be metabolized efficiently using various carbon sources, such as glucose and other fermentable sugars from mango peel hydrolysates. When nutrients become limited, pHAs accumulate intracellularly and compete with biologically functional pathways of C. necator under conditions where no nutrition is available (NNA, e.g., nitrogen or phosphorus limiting conditions). The bacteria store and use these granules as an energy and carbon reserve to pull from when nutrients from the surrounding area are difficult to obtain. Another well-studied PHA-producing bacterium, *Ralstonia eutropha* is often referred to as *C. necator* in the literature because of its historical naming conventions. For instance, *C. necator* has a strong capacity to convert simple sugars into PHAs under nutrient limitation. The metabolic pathways of *R. eutropha* are well known, facilitating the engineering of its metabolism to improve and scale up PHA production processes on an industrial scale. Pseudomonas putida can metabolize a wide range of organic compounds and produce medium-chain-length PHAs (mcl-PHAs) with properties different from those of the short-chain-length PHAs (scl-PHAs) produced by *C. necator* and *R. eutropha (*[Volova, et al., 2020\)](#page-11-0). *P. putida* can use a variety of carbon sources, ranging from complex organic substrates to simple sugars. The unique material properties of mcl-PHAs indicate that they are of particular use to provide biopolymers with increased flexibility and toughness. These PHA-producing bacteria were cultivated in bioreactors under controlled conditions to optimize PHA synthesis. The process involves providing bacteria with a carbon-rich feedstock (such as the hydrolysates from mango peel) while limiting other essential nutrients to induce PHA accumulation. Bacteria convert simple sugars into PHAs, which are stored as intracellular granules. After fermentation, bacterial cells are harvested, and PHAs are extracted and purified for use in various applications, including biodegradable packaging, biomedical devices, and agricultural films [\(Zytner et al., 2023\)](#page-11-0).

Fig. 1. Process flow of Polyhydroxyalkanoates (PHA) production.

4.3.2. Criteria for selecting bacterial strains used in the PHA synthesis process

Proper selection of the bacteria used in the process is a key step towards achieving high production efficiency and sustainability of PHA. The criteria for the selection of PHA-producing bacteria are complex and involve various properties such as growth rate, substrate utilization capacity, genetic stability, environmental tolerance, ease of downstream processing, and regulatory aspects. The initial criterion is that the bacteria should have a high potential for PHA accumulation. One of the routine procedures usually performed to find a considerable number of PHA particles in strain screening is the Nile blue and Sudan black staining techniques. A well-known example might be *Cupriavidus necator* and *Ralstonia eutropha* owing to their high PHA accumulation. Growth rate and yield are also important for bacteria. It is desirable to have a bacterium that grows fast and reaches a high cell density capable of synthesizing large amounts of PHAs in a shorter time. *Bacillus megaterium* has been well documented as a fast-growing and highly PHAaccumulating microbe.

Another important criterion is the substrate utilization. A robust bacterial strain that can consume a large variety of low-cost and renewable carbon sources (e.g., agricultural waste and industrial byproducts) is desirable. One versatile strain is *Pseudomonas putida*, which is capable of using a plethora of substrates, such as glycerol and fatty acids. This criterion ensures the stable production of PHA over multiple generations. This stability, a result of their genetics seen in

bacteria such as *Halomonas boliviensis* means that they can be relied upon for ongoing production. Bacteria should be able to grow at different pH levels and temperatures and have access to more nutrients are considered adaptable. *Alcaligenes latus* is a species of Alphaproteobacteria that is known for its environmental versatility. Convenience of downstream processing was also considered. Those that lyse easily are of interest as they simplify the process into potentially few steps or lower costs, both due to fewer and simpler extractions. *Azotobacter vinelandii* can easily extract PHA. Finally, safety and regulatory issues are the major obstacles. The chosen bacteria must be non-pathogenic and safe to be introduced into an industrial process. Safety standards are met with regulatory approval for certain bacterial strains. In general, *Escherichia coli* is a widely studied and safe host for PHA production. Table 3. summarizes the different strain selections, including growth conditions, genetic characteristics, and PHA yield performance.

4.3.2. Fermentation process

The fermentation process for the production of polymers, viz. PHAs from simple sugars obtained from mango peel, is carried out using bioreactors under controlled conditions [\(Pattanaik et al., 2021\)](#page-10-0). The main aim here is to set conditions that allow microbes (through growth) to produce and accumulate PHA in cells. Maintaining the bioreactor at a temperature of 30–37 ◦C, at which the growth and metabolic activities of PHA-producing bacteria such as *Cupriavidus necator, Ralstonia eutropha*, and *Pseudomonas putida* are optimal, these temperatures are

Table 3

Details of various strain used in the production of PHA with different growth conditions and genetic characteristics.

Strain Name	Growth Condition	Genetic Characteristics	PHA Yield (g/L)	PHA Type Produced	Reference
Cupriavidus necator H16	30 °C, pH 7.0, aerobic, glucose as C source	Contains phaC gene for PHA synthase, 6-8 plasmids	$4.8 - 6.2$	Poly(3-hydroxybutyrate) (PHB)	(Averesch et al., 2023)
Pseudomonas putida	30 °C, pH 7.0, aeration with	Encodes mcl-PHA synthase genes	$0.9 - 1.3$	Medium-chain-length PHA	(Szacherska et al.,
KT2440	N-limited media	$phaCl$, $phaCl$		$(mcl-PHA)$	2022)
Hydrogenophaga	30 \degree C, pH 7.0, sucrose as C	phaC genes, known for high PHA	$2.0 - 2.8$	PHB	(Saratale et al., 2021)
pseudoflava	source	productivity			
Cupriavidus taiwanensis	30 °C, pH 7.0, fructose media	Carries phaC gene, related to Cupriavidus necator	$4.2 - 5.0$	PHB	(Guleria et al., 2022)
Pseudomonas stutzeri	30 °C, pH 7.0, glycerol as C	Encodes genes for mcl-PHA and PHB	$0.8 - 1.5$	PHB, mcl-PHA	(Sharma et al., 2020)
	source	production			
Synechocystis sp. PCC 6803	30 °C, light, CO2 as C source	Cyanobacterium, contains phaC genes for PHA production	$0.5 - 0.8$	PHB	(Tharasirivat et al., 2023)
Vibrio natriegens	37 °C, pH 7.2, glucose & N- limited media	Contains phaC genes, halophilic, rapid growth	$1.2 - 1.9$	PHB	(Mutyala and Kim et al., 2022)
Corynebacterium	30 °C, pH 7.0, glucose as C	phaC, phaA genes, can produce PHA	$0.9 - 1.8$	PHB	(Choi et al., 2022)
glutamicum	source	with N limitation			
Bacillus subtilis	37 °C, pH 7.0, glucose media	Engineered with phaCAB genes for	$2.0 - 3.2$	PHB	(Liu et al., 2022)
(recombinant)		enhanced PHA yield			
Methylobacterium extorquens	30 °C, methanol as C source	Carries phaABC genes for PHA biosynthesis	$0.7 - 1.1$	PHB	(Arenas et al., 2023)
Halomonas boliviensis	35 °C, high salinity, N-limited media	Salt-tolerant, phaC genes for PHB synthesis	$3.5 - 5.0$	PHB	(Celik et al., 2023)
Pseudomonas fluorescens	28 °C, pH 7.0, aeration, fatty	phaC1, phaC2 genes for mcl-PHA	$0.9 - 1.4$	mcl-PHA	(Silva et al., 2021)
	acids as C source	production			
Cupriavidus necator H16	30 °C, pH 7.0, aerobic, glucose	Contains phaC gene for PHA synthase,	$4.8 - 6.2$	Poly(3-hydroxybutyrate)	(Averesch et al., 2023)
	as C source	6-8 plasmids		(PHB)	
Aeromonas hydrophila 4AK4	37 °C, pH 7.2, glucose media	Carries phaC, phaJ genes for mcl-PHA production	$0.8 - 1.2$	mcl-PHA	(Reddy et al., 2022)
Burkholderia cepacia	30 °C, pH 7.2, glucose, and N- limited media	Encodes phaC gene, produces mcl-PHA	$0.6 - 1.5$	mcl-PHA	(Chin et al., 2022)
E. coli (recombinant)	37 °C, pH 7.0, glucose media	Engineered with phaCAB genes from Cupriavidus	$1.8 - 3.0$	PHB	(Meng et al., 2022)
Alcaligenes latus	30 °C, aeration with glucose $\&$ N-limited media	phaA, phaB genes, high PHA accumulation rate	$5.0 - 6.5$	PHB	(Zhou et al., 2022)
Azohydromonas lata	32 °C, pH 7.0, acetate as C source	<i>phaC</i> gene cluster for PHA synthase	$1.0 - 1.2$	PHB	(Koller and Obruča, 2022)
Bacillus megaterium	37 °C, pH 6.8, glucose & glycerol as C source	Contains phaC for PHA synthase	$0.7 - 1.8$	PHB	(Lim et al., 2023)
Ralstonia eutropha NCIMB	35 °C, N-limited media,	phaA, phaB, phaC operon for PHB	$2.5 - 4.0$	PHB	(Barde et al., 2021)
11,599	acetate as C source	production			
Pseudomonas putida	30 °C, pH 7.0, aeration with	Encodes mcl-PHA synthase genes	$0.9 - 1.3$	Medium-chain-length PHA	(Liu et al., 2021)
KT2440	N-limited media	(phaCl, phaCl)		$(mcl-PHA)$	

maintained to maintain the function of enzymes of the bacterial cells at rates where the conversion of simple sugars to PHAs occurs ([Lhamo](#page-10-0) [et al., 2021\)](#page-10-0). The pH of the fermentation medium was maintained at 7.0 (neutral). One of the reasons why the pH needs to be just right (neutral pH) is that extreme pH levels denature the enzymes and prevent bacterial growth and PHA production. During fermentation, the fermented mixture was continuously tested, and the pH was adjusted to keep it stable. Bacteria require abundant oxygen and nutrients within the bioreactor; therefore, proper aeration and agitation are key. To maintain a sufficient oxygen supply and nutrients, aeration is achieved by sparging air or oxygen into the bioreactor, and mechanical agitation (utilizing impellers or stirrers) ensures that oxygen and nutrients are evenly distributed. Good aeration and agitation also ensured that clumps or biofilms did not form, hindering the bacteria. PHA accumulation is induced by fermentation under nutrient-limited conditions. Bacteria are supplied with an excess amount of carbon sources of carbon source (simple sugars derived from mango peel hydrolysates) but only a limited amount of biological nutrients, either nitrogen or phosphorous. They synthesize PHA intracellularly and accumulate in the form of granules because of nutrient limitations to supply energy and carbon sources during starvation periods of scarcity [\(Keller et al., 2024](#page-10-0)).

4.3.3. Cell harvesting

Once the fermentation process was complete, the bacterial cells containing PHAs were harvested. This is usually performed through centrifugation or filtration to separate the cells from the fermentation broth [\(Mannina et al.,2020](#page-10-0)).

4.4. Extraction and purification of PHAs

As stated above, PHAs are extracted and purified from microorganisms, mainly by microbial fermentation with bacteria using renewable substances for PHA synthesis. After the fermentation process, PHAs are recovered by solvent extraction, mechanical disruption, or enzymatic digestion to release PHAs from microbial cells. High-purity PHAs were then extracted using purification techniques including solvent precipitation, filtration, and drying. Fig. 2 summarizes the PHA pretreatment, extraction, and purification processes.

For the extraction of PHAs from bacterial cells, solvent extraction methods were used because they can efficiently dissolve PHAs away

from other cellular components [\(Fig. 3\)](#page-8-0). Obtaining high-purity PHAs for various applications ([Kurian et al., 2021\)](#page-10-0). The solvent selected for the extraction step was pivotal to the process. The most frequently used solvents for PHA purification are chloroform, methylene chloride, or a combination of them, with a mixture of chloroform and methanol being common. The solvents were chosen for their ability to solubilize PHAs without affecting other cellular materials. The high extraction efficiency and purity resulted from the ability of the solvent to solubilize PHAs. PHA extraction can be performed only after bacterial cells are lysed to release intracellular PHA granules [\(Mannina, et al., 2020\)](#page-10-0). There are multiple methods of lysis, which can be achieved mechanistically (bead milling, sonication), chemically (detergent, alkali), or enzymatically. The cells need to be cracked open, and the cell walls and membranes are torn so that the PHAs are released into the extraction medium. This mixture was further subjected to the desired solvent after lysis of bacterial cells. Both PHAs need to be organic solvents into the PHAs can dissolve (they are hydrophobic biopolymers). The remaining cellular debris, that is, proteins, lipids, and nucleic acids, are usually insoluble and separated from the PHA-solvent solution. This mixture was then separated and the solvent phase containing PHA was separated from the solid residues using separation techniques. This separation can be achieved by removing the solid debris, while retaining the clear PHA-solvent solution via filtration or centrifugation. Additional purification steps included washing the PHA solution with water or other solvents to remove any remaining impurities. The PHAs were then dissolved in a common solvent, followed by the precipitation of the solutes from the solvent to recover the PHAs. This is usually achieved by the addition of a nonsolvent (such as methanol or ethanol) to the PHA solution. One of the nonsolvents makes PHAs torsible and precipitates as a solid from the solution. The precipitated PHAs can now be harvested by filtration or centrifugation and then dried to remove any solvent traces ([Rahman et al., 2022\)](#page-10-0). 4. Production Process of Biodegradable Packaging

PHAs, which are eco-friendly packaging materials, are produced and processed using the same processing technology as that used for conventional plastics. PHAs are converted into usable packaging via extrusion, injection molding, and blow molding. These methods, such as those used in the plastic industry, can be applied to bioplastics with the necessary specifications of mechanical properties, flexibility, and thermal stability being satisfied in the final product. Essential to the

Fig. 2. Flow chart explaining different processes of pretreatment, extraction and purification of PHAs.

Fig. 3. A comprehensive technique that demonstrates the solvent-based method for recovering PHA from bacteria that are abundant in PHA. AS, anti-solvent; S, solvent; PHA, polyhydroxyalkanoate; PHA (s), polyhydroxyalkanoate in suspended solid form; ER (s), extraction residue as suspended solids. *If the extraction is conducted directly on moist microbial slurry. **If an anti-solvent is employed.

development of plastic profiles, such as films, sheets, and fibers, extrusion is the name of this technique. During extrusion, the PHA granules were loaded directly into an extruder and melted. The molten PHA is then extruded through a die to form a shape. Molten PHA is extruded via a flat die, cooled, and solidified to produce a film for subsequent processing [\(Roohi et al., 2024](#page-10-0)). The ability to produce the same products with uniform thickness and consistency lends itself to the production of packaging films via extrusion. Another popular method for producing intricate shapes with excellent functionality is injection molding. This process involves melting PHA granules, which are subsequently injected inside a mold cavity under high pressure. When the PHA was cooled and hardened, the mold was opened and the final part was ejected. By far the most versatile of all plastic processing methods, it can be used to produce larger packaging items, such as containers, or to manufacture smaller items, such as lids and caps. When combined with high-quality finishes and delicate designs, this process is ideal for creating packaging components that are intricate in nature and built to last. Hollow objects, including bottles, were formed by blow molding. The process begins with the extrusion or injection molding of a preform, a small, tubular piece of plastic. The preform was then heated and inserted into a bottle mold prior to blowing air through it to force it to expand and take the shape of the mold. The chamber was opened when the plastics cooled, and the final product was eliminated. This unique process is especially suited for making a wide range of lightweight plastic containers that are most commonly used in the packaging industry, ranging from water bottles to soft drink containers [\(Amaro et al., 2020\)](#page-9-0).

In fact, there are several additives which can be used during the processing of PHA-based materials to help improve their properties. Additives are used to control mechanical strength, flexibility, thermal stability, and other functional attributes of the final product. These include plasticizers, fillers, stabilizers, and impact modifiers. The addition of plasticizers improves the flexibility and flowability of PHA materials ([Kumar et al., 2021\)](#page-10-0). They decrease the intermolecular forces in the polymer matrix, which increases flexibility and can be processed more easily. This is even more vital for wraps and films, where packaging needs to be flexible. Calcium carbonate, talc, or natural fibers can be employed as fillers to improve the mechanical properties of PHAs. Depending on the specific packaging requirements, fillers can provide greater rigidity, strength, and additional thermal stability in some cases. In addition, they can lower their manufacturing costs by determining a part of the PHA with a more economical substance. These additives are used to enhance the thermal stability of PHA materials. Because PHAs undergo thermal degradation (especially during processing at high temperatures), stabilizers are used to prevent breakdown and maintain polymer integrity. This will ensure that the PHA material has the performance it needs throughout its lifecycle, both during and after manufacturing. Impact Modifiers are compounds used to boost the toughness and impact resistance performance of PHA-based materials. This is particularly critical in packaging applications, where the material must be able to withstand mechanical stresses, such as those associated with dropping or stacking. These impact modifiers improve the strength and lifespan of packaging, making it more realistic for real-world use in the real world ([Mehrpouya, et al., 2021\)](#page-10-0).

5. Applications of PHAs based packaging

PHA, biodegradable, biopolymer, bioplastic, and food packaging. Compared to petroleum-based plastics, food packaging made of PHAs is highly beneficial in terms of environmental problems and can satisfy the expanding requirement for food packaging using sustainable packaging solutions. The biodegradability of PHA-based food packaging is a major benefit ([Meereboer et al., 2020\)](#page-10-0). Unlike conventional plastics, which can last hundreds of years in the environment, PHAs biodegrade naturally into water, carbon dioxide, and biomass after digestion by microorganisms. This property decreases the degree to which plastic waste can end up in landfills or the ocean and contains environmental contamination, which is a hazard for wildlife and ecosystems. Being completely biodegradable by composting is one of the most cost-effective options for serving food in single-use applications, such as disposable cutlery, plates, cups, and food containers. These items, which are generally used in high numbers in the food service industry, are major contributors to the proliferation of plastic waste. Restaurants, cafés, and catering services can make a massive dent in their environmental impact and comply with new regulations that address plastic waste by choosing compostable PHA-based alternatives. PHAs have excellent moisture and gas barrier properties that are necessary for food packaging applications. Moisture and gas barriers allow food products to persist longer by isolating them from oxygen and humidity. PHAs can be used to produce films and coatings that help minimize the use of preservatives and extend the shelf life of items, such as fresh produce, meat, dairy, and baked goods ([Adeleye et al., 2020\)](#page-9-0). Because PHA are flexible and strong, PHA-based films can be adopted in numerous ways for food packaging.

As a film covering, they are used to pack fruits, vegetables, cheese, and other items that go bad quickly. Moreover, PHAs can be used as coatings in paper or cardboard packaging to improve the barrier properties of these materials. When PHAs is coated with paper, the materials become damp-resistant, which makes it a good option to pack fast-food products and snacks that are often oily or greasy. PHAs can also be processed to make thermoformed food containers that are used for takeaway, ready-to-eat meals, salads, and deli products. These containers are lightweight and robust, and can be quickly shaped during the forming step [\(Mannina et al., 2020\)](#page-10-0). PHAs are utilized to produce containers such that the packaging is biodegradable after its intended use. In addition to traditional packaging concepts, PHAs are considered for developing innovative packaging solutions. For instance, active packaging systems consisting of PHA materials can be manufactured to release antimicrobial agents that can keep food items safe for a longer period. The system helps to package fresh meat, poultry, and seafood products, which are the products most contaminated by microbes. The current trends in the demand for eco-friendly solutions on the market are promoting the growth of eco-friendly food packaging products worldwide. Demand is driven by an increase in consumers' awareness of environmental problems.,wadaysThe consumers buy products that are environmentally friendly [\(Shahid et al., 2021\)](#page-10-0).

6. Future directions and innovations

Bio-based PHAs are at the forefront of research and development and are driven by growing environmental concerns and regulatory pressures. As the interest in sustainable solutions intensifies, advancements are being made in production efficiency, material properties, and the development of new applications. The increasing availability of alternative biotechnological systems and cost optimization strategies is further enhancing the potential of PHAs to revolutionize the biopackaging industry. Advanced genetic engineering of strains and fermentation optimization have led to improved PHA yields from various renewable feedstocks such as agricultural wastes. Current improvements continue to reduce costs, making PHA-based packaging more competitive with traditional plastics. Functional additives may be incorporated during blending, whereas PHA blends with other biodegradable polymers may provide improved mechanical, thermal, and barrier properties towards PHA-based packaging. Plasticizers can be added to improve flexibility, and nanomaterials can be added to improve strength and barrier performance. These breakthroughs open an array of potential uses for PHA-based packaging applications, from flexible films to rigid containers. Upcoming innovations will see the advent of active and intelligent packaging systems to prolong shelf life and improve food safety. Antimicrobial compounds, oxygen scavengers, and moisture absorbers can be incorporated into active packaging to prevent food spoilage and contamination. Smart packaging perfective packaging with freshness, temperature, or pH indicators, for example, can communicate the state of packaged food in real time, boosting consumer trust and reducing food waste. Closed-loop systems in which PHA-based packaging is collected, composted into nutrients for the soil, and returned as valuable materials for new PHA packaging correspond to a sustainable concept in the sense of a circular economy. Moreover, ongoing research is investigating the possibility of producing PHA from other industrial wastes and by-products to decrease the environmental load.

7. Conclusion

This review suggests that enzymatic hydrolysis of mango peel and PHA synthesis might yield biodegradable food packaging, thereby mitigating the problems associated with plastic waste and agricultural by-products. This study has discovered that mango peel, which is mostly discarded as garbage, can be successfully transformed into useful biopolymers. The complex carbohydrates in the mango peel were hydrolyzed into fermentable sugars through enzymatic hydrolysis using selected enzymes such as cellulase, pectinase, and amylase under optimized conditions. These sugars are further used by a selected group of microbial strains to produce biodegradable PHA that are suitable for food packaging. PHA-based packaging materials were dimensionally stable with the required food-safety standard properties. By doing this, it would not only create another sustainable solution for conventional plastic packaging, but it could also turn agricultural waste into a valueadded product well stated for a circular economy. This study has implications for the scalable implementation and integration of existing agricultural and industrial processes.

CRediT authorship contribution statement

Vinay Kumar Pandey: Writing – original draft, Conceptualization, Project administration. **Zaryab Shafi:** Writing – review & editing. **Anjali Tripathi:** Visualization. **Gurmeet Singh:** Data curation. **Rahul Singh:** Supervision. **Sarvesh Rustagi:** Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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