



The Role of Lactic Acid Bacteria in Bioplastic Production Using Low-Cost Substrates

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Abstract

Many researchers have been focussed to explore technical solutions for creating biodegradable plastics. Today, microorganisms have become promising sources for producing bioplastics, which have various uses, including packaging and other applications. Furthermore, the ongoing increase in food and agricultural waste, along with its management strategies, has attracted global attention due to its significant environmental impacts on air, soil, and water contamination. Lactic acid bacteria (LAB) are well-studied for producing two common bioplastics, polylactic acid (PLA) and polyhydroxyalkanoates (PHA), using food and agricultural waste. This review focuses on producing PHA and PLA from low-cost substrates with LAB. It highlights the types of lactic acid and PHA made by LAB and their applications. After briefly explaining what LAB strains can produce bioplastics, the biosynthesis of PLA and PHA by LAB is described. The review also explains the definitions and pretreatment methods of cheap substrates for bioplastic production. Finally, it discusses factors that influence the bioplastic production process. Overall, efficient methods for turning waste into bioplastics with LAB offer a promising step toward sustainable waste management and reducing the harm caused by traditional plastics.

What is “already known”:

- LAB as promising microorganisms for producing lactic acid (monomer of PLA) and PHA, using low-cost food and agricultural waste substrate.
- Current bioplastic production is usually costly
- This ability of LAB presents a sustainable solution for waste management while simultaneously addressing the environmental threats posed by traditional plastics.

What this article adds:

- Substrate selection, fermentation conditions, and microbial strain characteristics are important in optimizing bioplastics production from waste.
- Pretreatment methods required to enhance the physicochemical properties of waste substrates to convert complex organic components into fermentable sugars effectively.
- The synthesis processes for production of biopolymers using LAB are described, along with their specific applications in packaging and other industries.
- The use of food and agricultural byproducts offers a pathway to making bioplastics more economically viable and environmentally friendly
- High enzyme stability at acidic pH and moderate temperatures for animal feed use.
- Potential applications in gut microbiome engineering and nutraceutical production

1. Introduction

The predominant method of synthesizing current plastics involves the use of fossil fuels. It has been established that greenhouse gas emissions accompany the production, use, recycling, and burning of plastic. Consequently, plastic pollution has emerged as a preeminent environmental concern [1]. In 1950, global plastic production was about 2 million tons. By 2022, this number had grown to 400.3 million tons. Plastic waste, when thrown away, can take anywhere from 10 to 1000 years to break down because of its durability and limited ability to degrade [2]. Additionally, only about 9% of all plastics produced are subjected to recycling, approximately 12% is incinerated, and the remaining 79% of the plastics are either disposed of in a landfill or discarded [3]. So, the development of an alternative method, such as creating "Biodegradable Plastic" or "Bioplastics," is imperative.

Bioplastics refer to a group of polymeric materials which are derived from biological resources or biologically synthesized by living organisms, such as bacteria [4]. The worldwide demand for bioplastics has exhibited an upward trend, attributable to their non-toxic characteristics, biocompatibility, and rapid degradation without environmental harm [5]. Bioplastics encompass a wide range of materials that can be classified as either biobased or biodegradable. 'Biobased' denotes materials sourced from biomass, whereas "biodegradability" describes a biochemical process where microorganisms break down these materials into water, carbon dioxide, and other substances. The biobased and biodegradable bioplastic group encompasses biobased polyesters, such as polylactic acid (PLA) and polyhydroxyalkanoates (PHA) [6]. The synthesis of bioplastics by microorganisms has garnered significant attention in recent times due to its potential applications in production and biodegradability [7]. In addition to their role in addressing plastic pollution through the degradation of plastic in natural ecosystems, microorganisms act as

the fundamental biosynthetic mechanisms for the production of bioplastics. The procurement of bioplastics is contingent upon the utilization of renewable resources [8].

PHA and PLA have been identified as primary catalysts for the proliferation of biodegradable plastics, with production expected to rise from 880,000 tons in 2017 to 5,330,000 tons in 2026 [9]. Commercializing bioplastics is challenging, which is reflected in their price—one kilogram of bioplastics costs at least three times more than one kilogram of traditional plastics. Production costs for bioplastics can be lowered by using cheap, widely available, and affordable substrates [10]. In fact, as indicated in the research by Longo et al. [11], the final cost of PHA and PLA production can be reduced primarily through the appropriate selection of microorganisms and carbon substrates. Food and agricultural byproducts represent a promising source of inexpensive nutrients, including carbon, nitrogen, and phosphorus, which are essential for the growth of microorganisms and the production of PHA and PLA [12]. The economic benefits of microbial fermentation include utilizing low-cost substrates and optimizing bioprocesses to increase efficiency and reduce costs [13]. As demand for eco-friendly alternatives to conventional plastics grows, using food and agricultural residues for bioplastics offers a dual benefit: reducing waste and lowering reliance on fossil-based plastics [14]. Producing bioplastics from food and agricultural waste can be a renewable, sustainable process and a practical waste disposal strategy, helping to avoid issues like emissions of greenhouse gases and contamination of underground water sources caused by landfilling. Therefore, valorizing food and agricultural wastes through the production of value-added bioplastics can be an ideal and feasible end use [15].

Lactic acid bacteria (LAB) have been recognized since antiquity as agents of uncontrolled fermentation, a process that can lead to the deterioration of food

products. LAB are considered promising microorganisms for biorefineries that transform waste biomasses into high-value bioplastics, based on their traits and innovative technological approaches [16]. Some recent research regarding lactic acid (as monomer of PLA) and PHA production by LAB using cheap substrates is illustrated in Table 1.

The objective of this review is to offer a comprehensive overview of LAB strains and their capacity to contribute to the production of biopolymers using cheap substrates. To achieve this, a meticulous examination of extant publications was conducted by utilizing Google Scholar, Web of Science, and PubMed bibliographic databases. Keywords included Bioplastics, Lactic acid bacteria, Food waste, Agricultural waste, fermentation, Polyhydroxyalkanoates, Polylactic acid, Biodegradable. Only publications in English language were considered, with titles and abstracts screened for relevance. Selected articles were thoroughly examined to assess the role of LAB in bioplastic production using cheap substrates.

1. Types and applications of bioplastics produced by LAB

1.1. PHA

PHAs represent a novel category of biologically-derived, aliphatic polyesters that are both bio-based and biodegradable, and are synthesized via bacterial fermentation. This group includes poly(3-hydroxybutyrate), poly(3-hydroxyvalerate), and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHB, PHV, and PHBV) [27]. The classification of PHAs is based on their chain length, with three distinct groups identified: short-chain PHAs (comprising 3–5 carbon atoms per monomer); medium-chain PHAs (with 6–14 carbon atoms); and long-chain PHAs, which include 15 or more carbon atoms [31]. The PHA molecule is composed of 600 to 35,000 repeat units of (R)-hydroxy fatty acid monomers, with each unit possessing an R-group side chain. It has been determined that a

minimum of 150 hydroxy alkanoic acids, as well as over 90 bacterial taxonomic groups, are capable of producing these polyester-based materials [32].

The fundamental properties of PHA can be outlined as follows [33]:

- Insolubility in water and notable resistance to hydrolytic degradation
- Resistance to ultraviolet light, but susceptibility to acids and bases
- High solubility in chloroform and other hydrocarbons that contain chlorine
- Suitable for medical applications
- Facilitates anaerobic biodegradation in sediments by sinking in water
- Non-toxic and non-sticky when melted, in contrast to conventional polymers

These materials are well-suited for a broad spectrum of packaging applications, which encompass hot and cold cups, the lids of cups, containers for yogurt, as well as tubs, various types of trays, and packaging for single servings of foodstuffs. The hydrophobic nature of polyesters is the reason for the high water vapor barrier properties exhibited by PHA films, which approach those of low density polyethylene [34]. PHA appears to be both fully degradable and capable of undergoing composting, independent of moisture or temperature, while PHA-based materials are reported as antibacterial agents [35]. Despite the compelling motivations for incorporating PHA in numerous bioplastics, the current capacity for large-scale production is inadequate due to the substantial cost differences. Specifically, the production costs of PHA are up to ten times higher than those of conventional plastics. In order to address the issue of excessive production costs and to develop cheap PHA, global PHA production must increase from its current low capacities [36].

Table 1. Bioplastic (polyhydroxyalkanoates, PHA; polylactic acid, PLA) production by lactic acid bacteria (LAB) from low-cost substrates

Substrate	LAB strain	Bioplastic	Yield (%)	Conditions	Reference
Sludge from cardboard industry	<i>Enterococcus</i> sp. NAP11	PHA	79.27		[17]
Food waste	<i>Lactococcus lactis</i> NZ9000	PLA	NR	Strategy: mutation of key proteins and hosts	[18]
Cotton stalk	<i>Lactococcus cremoris</i>	PLA	lactic acid production: 51.4 g/L	Acid pretreatment with HCl	[19]
Whey waste	<i>Lactobacillus delbrueckii</i> ssp. <i>lactis</i> MTCC 911	PLA	14.2	lactic acid production: 6.75 g/L	[20]
Food waste	<i>Lactobacillus casei</i>	PLA	NR	Enzyme hydrolysis, fermentation, purification, separation, and polymerization	[21]
Food waste	<i>Lactobacillus manihotivorans</i> DSM 13343	PLA	lactic acid production: 18.69 g/L	Recruited for simultaneous saccharification and fermentation of the substrate	[22]
Food waste	<i>Lactobacillus casei</i>	PLA	80	<i>Aspergillus</i> spp. hydrolysis	[23]
Banana peels	<i>Enterococcus durans</i> BP130	PLA	6.7		[24]
Cassava bagasse	<i>Bacillus coagulans</i> and <i>Lactobacillus rhamnosus</i>	PLA	lactic acid production: 112.5 g/L	Simultaneous saccharification and co-fermentation was performed	[25]
Rice straw	<i>Lactobacillus lactis</i> 2369	PLA	NR	lactic acid production: 82.2 g/L	[26]
Modified Khardhenavis synthetic medium	<i>Lactobacillus mesenteroides</i>	PHA	36	Inoculum 10% v/v, temperature 30 °C, duration 24 h	[27]
Starch and yeast extract	<i>Lactobacillus plantarum</i> CW10, <i>Lactobacillus casei</i> WWD3	PHA	25.3	PHA extraction with chloroform	[28]
Harvesting residues from food waste, sugarcane crops, bagasse, molasses, and corn stover	<i>Lactobacillus pentosus</i> and <i>Bacillus subtilis</i>	PLA	NR	PLA properties: excellent twist retention characteristics and flexible	[29]
Molasses, corn syrup, whey, dextrose and cane or beet sugar	<i>Lactobacillus delbrueckii</i> , <i>L. amylophilus</i> , <i>L. bulgaricus</i> , <i>L. leichmanii</i> , <i>L. rhamnosus</i>	PLA	NR	PLA properties: thermoplastic; renewable packaging material	[30]
Corn steep liquor, whey, molasses, starchy materials, and wheat bran	<i>Lactobacillus helveticus</i> , <i>L. delbrueckii</i> subsp. <i>bulgaricus</i> , <i>L. acidophilus</i> , <i>L. casei</i>	PLA	NR	PLA properties: thermoplastic and high tensile strength	[16]

NR: Not reported

To enhance the properties of PHA, a variety of strategies have been employed, including the utilization of plasticizers, as well as copolymerization and the mixing of PHA with other polymers, notably PLA. Additionally, the incorporation of inorganic or organic fillers has been investigated as a means to optimize the properties of PHA [37].

1.2. PLA

PLA is an aliphatic polyester produced through the process of condensation copolymerization of lactic acid [38]. It is noteworthy that the latter constitutes approx.-imately 31% of the overall bioplastic

production [39]. PLA-based plastics represent the most prevalent category of commercial bioplastics available on the market. The commercial synthesis of lactic acid is predominantly reliant upon the fermenting sugars using homofermentative lactic acid-producing bacteria [37]. PLA production is characterized by its high scalability and cost-effectiveness, which are primarily attributable to the prevalence of carbohydrate feedstocks and the existence of well-established industrial processes. This attribute positions PLA as a preferred material for applications such as packaging and textiles [40]. PLA is being explored as a material suitable for packaging due to its versatility in processing using a variety of

methodologies, encompassing extrusion, injection molding, the blow molding technique, overcast film extrusion, and thermo-forming [41]. Polymeric materials such as PLA are also marketed for applications in which the material is intended for single-use and subsequent disposal. Such applications include bottles, beverage containers with integrated cooling trays and lids, as well as blister packaging, over-wrapped packaging, and stretch films [42]. There is a demonstrable correlation between the physical properties of PLA and its enantiomer content. Furthermore, the morphological features of PLA are influenced by varying amounts of lactic acid monomers or oligomers [43]. As a bioplastic, PLA shares characteristics with traditional plastics such as nylon, polypropylene, and poly(ethylene terephthalate) (PET), which contributes to its widespread use [44]. It is appreciated for its low density (around 1.25 g/cm³), transparency, and strong mechanical properties, including a tensile strength of 50-70 MPa and a tensile modulus ranging from 3.50 to 16 GPa. In general, tensile strengths ranging from 20 to 40 megapascals (MPa) are considered adequate for industrial and end-user applications. Polymeric material known as PLA is a biodegradable substance with glass transition and melting temperatures of 55-60 °C and 150-180°C, respectively [42]. When exposed to environmental conditions, PLA undergoes a process of biodegradation that results in the formation of lactic acid, thereby providing an eco-friendly alternative to conventional plastics. In controlled industrial composting conditions, PLA has been observed to require between 90 and 120 days to fully biodegrade. Despite its moderate thermal stability, which can be enhanced by blending with other polymers or adding plasticizers, PLA has found widespread application in packaging, biomedical devices, and 3D printing due to its ease of production and strong mechanical properties [45].

The utilization of PLA-based bioplastics in food service items is experiencing a marked increase, such

as containers, drinking cups, salad containers, overwrap and lamination films, blister packs, and disposable coffee cups [39]. PLA's production has many advantages. It is recyclable and compostable. PLA has been demonstrated to exhibit superior thermal processability in comparison to other bioplastics [46]. However, the use of PLA is mostly limited because of its elevated fragility, as evidenced by a tensile strength less than 10% of the material's breaking strength. Additionally, PLA displays a deficiency in resilience, exhibits hydrophobic properties, and a slow rate of biodegradation that limit its versatility in various applications. However, PLA does possess certain drawbacks, including its comparatively high cost and its low thermal stability. These drawbacks stem from its substandard melting strength and poor stability, which consequently result in a relatively restricted manufacturing window. The poor melting properties of pure PLA pose a significant barrier to its utilization in the production of stretchable packaging films, particularly in processes that involve elongation or orientation, including film extrusion techniques such as blown and cast [37].

The incorporation of antimicrobial or antioxidant additives into bioplastics has been demonstrated to result in the acquisition of the desirable traits of these components. The advancement of active packaging, which incorporates antimicrobial and antioxidant properties, is particularly significant for guaranteeing food safety and extending product shelf life. In the field of medicine, the utilization of bioplastics encompasses applications such as medical capsule packaging and surgical wound dressing. These applications are of paramount importance in preventing contamination and promoting wound healing [6].

2. LAB for bioplastic production

LAB have been used as starter cultures to produce fermented foods and beverages, and also for preservation, since ancient times. In light of the demonstrated safety record of certain LAB strains over

extended periods of time, some of these strains have been designated as Generally Recognized as Safe (GRAS) by the Food and Drug Administration (FDA) [47]. LAB are a heterogeneous group of bacterial genera, which includes *Lactobacillus* (L.), *Streptococcus*, *Lactococcus*, *Pediococcus*, *Leuconostoc*, *Enterococcus*, and *Weissella*. These genera are known for their wide industrial applications. Also, the LAB group comprises the following genera: *Aerococcus*, *Alloiococcus*, *Carnobacterium*, *Dolosigranulum*, *Oenococcus*, *Tetragenococcus*, and *Vagococcus* [48].

LAB are classified into distinct categories according to their biochemical characteristics. These categories include Gram-positive, tolerant, anaerobic, catalase-negative, cytochrome-deprived, and non-spore-forming bacteria. These bacteria typically exhibit a rod-shaped morphology, though some may manifest as cocci. Notably, LAB demonstrate a remarkable degree of tolerance to low pH conditions. It is imperative to note that all LAB possess essential physiological properties, including the capability to ferment carbohydrates primarily into lactic acid by means of homofermentative or heterofermentative metabolism [49]. The optimal growth conditions for LAB are typically at pH levels ranging from 5.5 to 5.8; however, these organisms have been observed to demonstrate a degree of resilience and adaptability in environments with pH values as low as 5. The range of optimal growth temperatures for these bacteria varies depending on the specific genus and strain. The optimal temperature range for LAB growth typically extends from 20°C to 45°C. The presence of LAB has been documented in a variety of foodstuffs, including vegetables, dairy and meat products, beverages, soil, and sewage. Furthermore, the presence of LAB has also been documented in the gastrointestinal and gynecological tracts of humans [50].

LAB are capable of producing organic acids (primarily lactic acid), ethanol, bacteriocins, vitamins, enzymes, and aromatic compounds when they follow

certain metabolic pathways. The utilization of LAB in diverse industrial applications is contingent upon the profile of metabolites produced [49]. The classification of LAB is based on several criteria, including cell morphology, the manner of glucose fermentation, patterns of sugar metabolism, and the temperature range at which they grow. Figure 1 presents the LAB classification, with an emphasis on the strains that have been identified as bioplastic producers.

Lactobacilli are classified as Gram-positive, non-spore-forming, bacilli or coccobacilli, and are considered to be anaerobic. Lactobacilli have been identified in a variety of environments that provide abundant carbohydrates, including food, plants, and wastewater. These organisms have also been found in the oral and gastrointestinal tracts of both human and animal subjects [51]. A contemporary reevaluation of the taxonomy of *Lactobacillus* strains has been necessitated by the development of the field of microbiology. In the 2020 study by Zheng et al., a significant reorganization of the taxonomy of *Lactobacillaceae* and *Leuconostocaceae* was conducted, resulting in the delineation of 23 novel genera [52]. Members of the Lactobacilli are distinguished by their exclusive fermentative characteristics. Consequently, when glucose is the source of carbon, lactobacilli can be classified as either homofermentative or heterofermentative. In the homofermentative case, the predominant product is lactic acid, whereas in the heterofermentative case, lactic acid is produced along with acetic acid, ethanol, and CO₂ [49]. In a recent study, it was observed that *L. delbrueckii* and *L. bulgaricus* have been documented to exhibit homofermentative characteristics, leading to the production of D-lactic acid. A study by Bustamante et al. [53] examined the production of D-lactic acid, an essential monomer of PLA, from orange peel waste by six strains of *L. delbrueckii* ssp. *bulgaricus*. The study found that the yield of D-lactic acid ranged from 84% to 95%, highlighting the potential for efficient

utilization of waste materials in biomanufacturing processes. Furthermore, an engineered *Lactococcus lactis* strain was utilized to achieve a substantial productivity of D-lactic acid through the utilization of lactose or whey-derived lactose as a substrate [51].

The genus *Pediococcus* is characterized by a coccus-shaped morphology, a Gram-positive cell wall composition, homofermentative metabolic characteristics, and the absence of catalase activity. Eleven species of pediococci have been identified, with several strains being separated from diverse environmental sources, including fermented meat and vegetables [54]. In their seminal work, Qiu et al. [55] pioneered the application of metabolic engineering strategies in *Pediococcus acidilactici*, thereby developing a strain capable of rapidly fermenting D-lactic acid from lignocellulosic substrates.

The genus *Weissella* is classified within the *Leuconostocaceae* family. Members of this genus are characterized by their Gram-positive bacterial structure, absence of catalase, and facultative

anaerobic nature. The microorganisms in question are classified as heterofermentative, a category that encompasses the production of lactic acid, acetic acid, CO₂, and ethanol [56].

Lactococci are classified as Gram-positive, aerobic cocci, catalase-negative, and are prevalent in the dairy industry's cheese-making process. It has been established that the glycolytic pathway is characteristic of lactococci; the fermentation of glucose by these organisms predominantly results in the production of L-lactic acid [57].

Streptococcus is a spherical, Gram-positive bacterium that is classified within the *Streptococcaceae* family. It has been determined that certain *Streptococcus* species play a pivotal role as a starter culture in specific kinds of fermented dairy products, owing to their rapid propensity to induce acidification during the fermentation process. These organisms possess sophisticated nutritional requirements and generate lactic acid and other organic

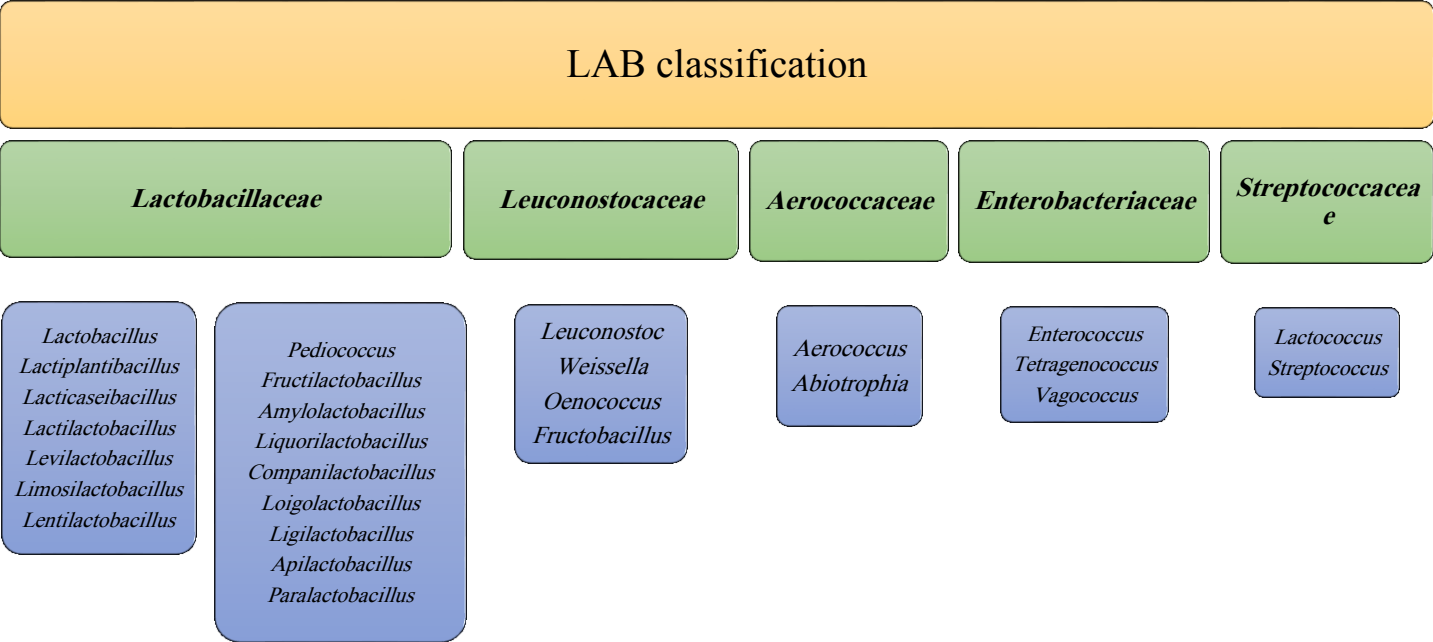


Figure 1. Lactic acid bacteria (LAB) classification

acids through the process of carbohydrate fermentation [45].

The genus *Enterococcus* comprises Gram-positive cocci that manifest as solitary cells, pairs, or short chains. Enterococci are classified as facultative anaerobes and are catalase-negative. It has been established that these organisms are classified as homofermentative, which means they are capable of producing L-lactic acid through a process known as glycolysis [58].

According to the findings of recent studies, the utilization of mixed microbial cultures (MMC) has been shown to yield greater benefits when compared with the use of pure cultures in the synthesis of bioplastics. Mixed cultures have been demonstrated to possess the capacity to expeditiously transform complex substrates derived from industrial wastewaters into bioplastics [59].

3. Bioplastics production by LAB

3.1. PHAs biosynthesis

PHA is a secondary metabolic product which is synthesized not only by various bacteria but also many archaea. PHA is a group of polyesters synthesized by various bacteria. It is composed of oxygen, hydrogen, and carbon. The substance is stored within the cytoplasm of cells, where it functions as an energy storage medium and as a constituent of intracellular carbon molecules [60]. The insolubility of PHAs in water results in their accumulation in the form of granules within the cellular cytoplasm of bacteria. The osmotic state of a cell is sustained by two processes: the formation of soluble intermediates and the subsequent polymerization of these intermediates, leading to the formation of insoluble compounds. This process prevents the leakage of polymerized molecules outside of the cell. As a consequence, energy reserves are maintained in harsh conditions [59]. The PHA biosynthesis pathway has been demonstrated to be associated with a variety of significant metabolic

processes in bacteria. These include amino acid degradation, as well as glycolysis, the Calvin cycle, the Krebs cycle, the serine pathway, the β -oxidation process, and de novo fatty acid synthesis [32].

The bacteria utilized for PHA production can be categorized into two distinct groups, contingent upon the stress conditions imperative for PHA synthesis. The initial group necessitates the restriction of nutrients such as nitrogen, phosphorus, sulfur, or magnesium, in conjunction with an abundance of carbon sources. In contrast, the other group does not need nutrient limitation for PHA synthesis [61]. *Leuconostoc mesenteroides* [62], *L. plantarum* [28], and *L. bulgaricus* are PHA-producing LAB. It has been documented that certain genera of LAB, including *Lactococcus*, *Lactobacillus*, *Pediococcus*, and *Streptococcus*, when cultivated on MRS broth, have been identified as potential producers of PHB [51]. PHA and related metabolic pathways share several common intermediates, with acetyl-CoA being the most prominent. In many bacteria that produce PHA, the conversion from acetyl-CoA to PHA heavily depends on nutritional conditions [63]. In the context of nutrient-rich environments, the elevated production of Co-A from the Krebs cycle exerts a regulatory effect on PHA production. Specifically, it functions as an inhibitor by suppressing the activity of 3-ketothiolase, a pivotal enzyme in the PHA production pathway. This regulatory mechanism enables acetyl-CoA to be channeled into the Krebs cycle, facilitating the process of energy generation and cell development. Conversely, under conditions of nutrient insufficiency (i.e., where a critical nutrient, like nitrogen or phosphorus, is scarce in the presence of a plentiful carbon source), the concentrations of Co-A do not demonstrate inhibitory characteristics, thereby enabling acetyl-CoA to be induced towards PHA synthesis pathways for PHA formation [2]. This regulatory method allows for the optimization of nutrient sources by PHA-accumulating bacteria, enabling them to adjust to environmental

fluctuations. A schematic of PHA production by LAB and the PHA synthesis pathway is demonstrated in [Figure 2](#).

It is well established that a multitude of bacterial strains possess the capacity to convert sugars and fatty acids into PHA via three distinct metabolic pathways. These pathways use acetyl-CoA as an intermediary. The biosynthesis of PHAs begins when two acetyl-CoA molecules are combined by 3-ketothiolase (PhaA), forming acetoacetyl-CoA. The PhaB enzyme (acetoacetyl-CoA reductase) is NADH-dependent and catalyzes the transformation of acetoacetyl-CoA into 3-hydroxybutyryl-CoA. Eventually, the process of polymerization of 3-hydroxybutyryl-CoA monomers into PHA is initiated by the enzyme poly-3-hydroxybutyrate polymerase (PhaC) [64].

PHA can be extracted and purified from its microbial hosts through various methods. Recently, bio-based techniques have been introduced, involving

the digestion of bacterial biomass within animal guts. However, a preponderance of evidence suggests that organic solvents, both halogenated and non-halogenated, exhibit the most efficacious recovery rate and highest yield and purity with a minimum of alteration to the PHA structure [65].

3.2. PLA biosynthesis

The production of PLA involves the fermentation of raw materials to produce monomers, which are subsequently polymerized. Carbohydrates from sources such as food and agricultural byproducts are converted into dextrose and subsequently fermented into lactic acid. In the commercial context, microorganisms that are particularly well-suited for the production of lactic acid include *Rhizopus oryzae*, *Lactobacillus*, and genetically engineered *Escherichia coli*. These microorganisms are preferred for their

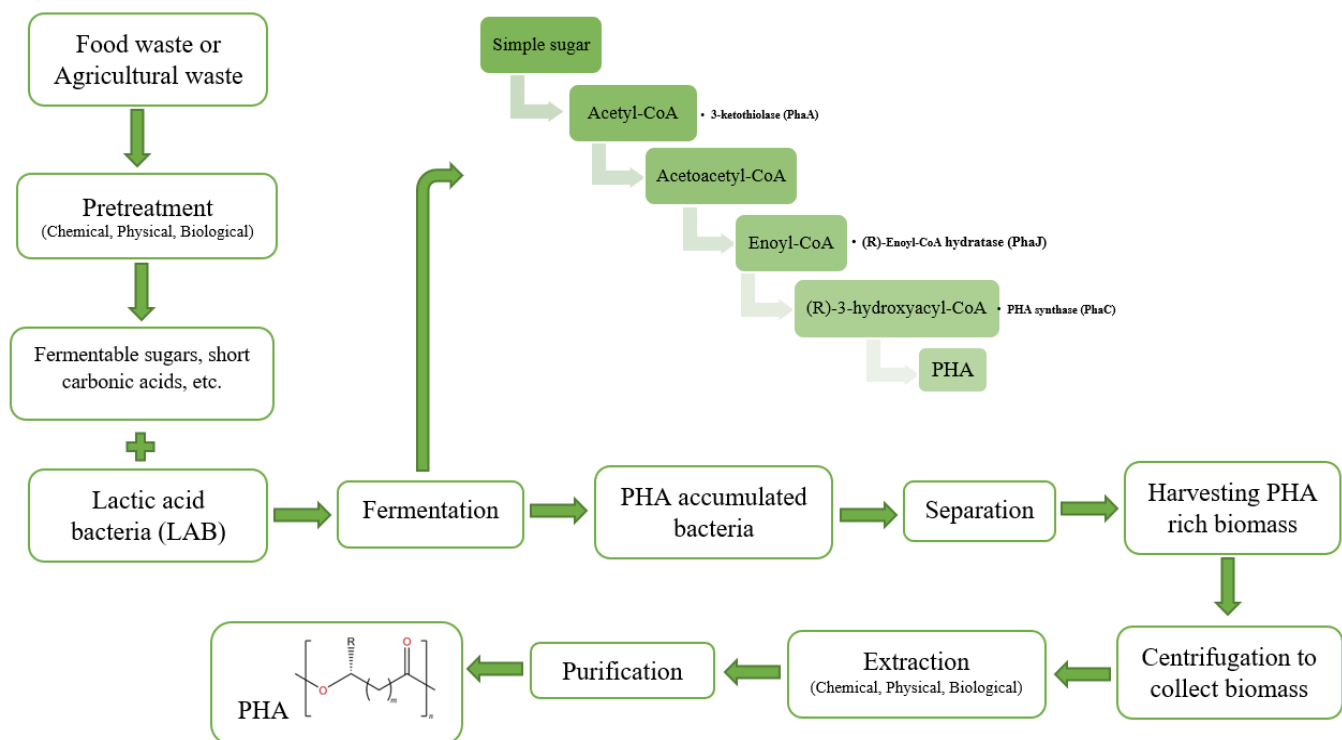


Figure 2. Schematic of Polyhydroxyalkanoates (PHA) production by lactic acid bacteria (LAB)

robust characteristics and the high yield they produce [66]. A schematic of PLA production by LAB and the PLA synthesis pathway is illustrated in Figure 3. The utilization of metabolic pathways by LAB strains to catabolize sugars is classified as either homofermentative or heterofermentative. The process of homofermentation is catalyzed by the Embden–Meyerhof pathway, which results in the exclusive production of lactic acid in significant quantities [51]. The subsequent breakdown of carbohydrates occurs via the glycolytic pathway, ultimately yielding pyruvate. The heterofermentative process, unique to certain LAB, is characterized by the phosphogluconate and phosphoketolase pathway, leading to the production of various products, including lactic acid, ethanol, acetic acid, and carbon dioxide [53]. It has been established that the homofermentative polymers of PLA are semi-crystalline, while the heteropolymers are considered amorphous. This method utilizes *Lactobacillus* species, including *L. bulgaricus* and *L. delbrueckii* [67]. Beside the homo- and heterofermentative pathway, the third important pathway for lactic acid production is the Bifidum-pathway (fructose-6-phosphate shunt).

The recovery and purification of lactic acid involves the application of distillation or membrane filtration techniques prior to polymerization through direct polycondensation or ring-opening of lactide [68]. The addition of a strong alkali (like calcium hydroxide) to the fermentation medium results in the conversion of lactic acid to its basic salt. Subsequent desorption of the salt occurs through the addition of a strong acid (such as sulfuric acid). In contrast, the process of reactive extraction can be employed to separate lactic acid from the culture medium. Subsequently, efforts were made to identify methodologies for the efficient and environmentally sound synthesis of lactic acid. Consequently, the conventional incorporation of calcium carbonate as a lactic acid neutralizer was superseded by the employment of sodium hydroxide,

thereby rendering the process ecologically sustainable [69]. The purification of lactic acid is achieved through the implementation of a two-step electrodialysis system. In summary, the process involves two stages. First, bacteria and proteins are removed from the fermentation broth by microfiltration. Then, nanofiltration is used to remove any remaining bacteria and proteins. The clarified fermentation broth is subsequently subjected to a process of electrodialysis, a process that involves the application of an electric current to a liquid medium. This process of electrolysis results in the concentration of the liquid and the subsequent transformation of the concentrate into lactic acid [43]. Rajendran and Han's [21] techno-economic analysis of sustainable PLA production from food waste using *L. casei* revealed that carbohydrate content exerts a substantial influence on production rates, underscoring the significance of substrate selection. Subsequently, the produced lactic acid was extracted from the culture medium through a separation technique integrated with a membrane. Thereafter, the process of PLA production involved the ring-opening reaction of the lactic acid monomers to create a polymer. The authors collected food waste samples from four countries—India, China, the United States, and Brazil—that vary in their protein, fat, carbohydrate, and water contents.

4. Cheap substrates for bioplastic production

4.1. Food waste

The global food sector is responsible for the generation of a substantial amount of food waste and by-products on an annual basis. The generation of food waste is an issue that pervades all stages of the food supply chain. The classification of food waste is typically divided into three categories: industrial, agricultural, and household [70]. Food waste generally falls into three main categories: (i) side streams from food processing industries like whey, molasses, starch, and lignocellulosic biomass; (ii) homogeneous waste streams that contain lipids in the form of oils and fats;

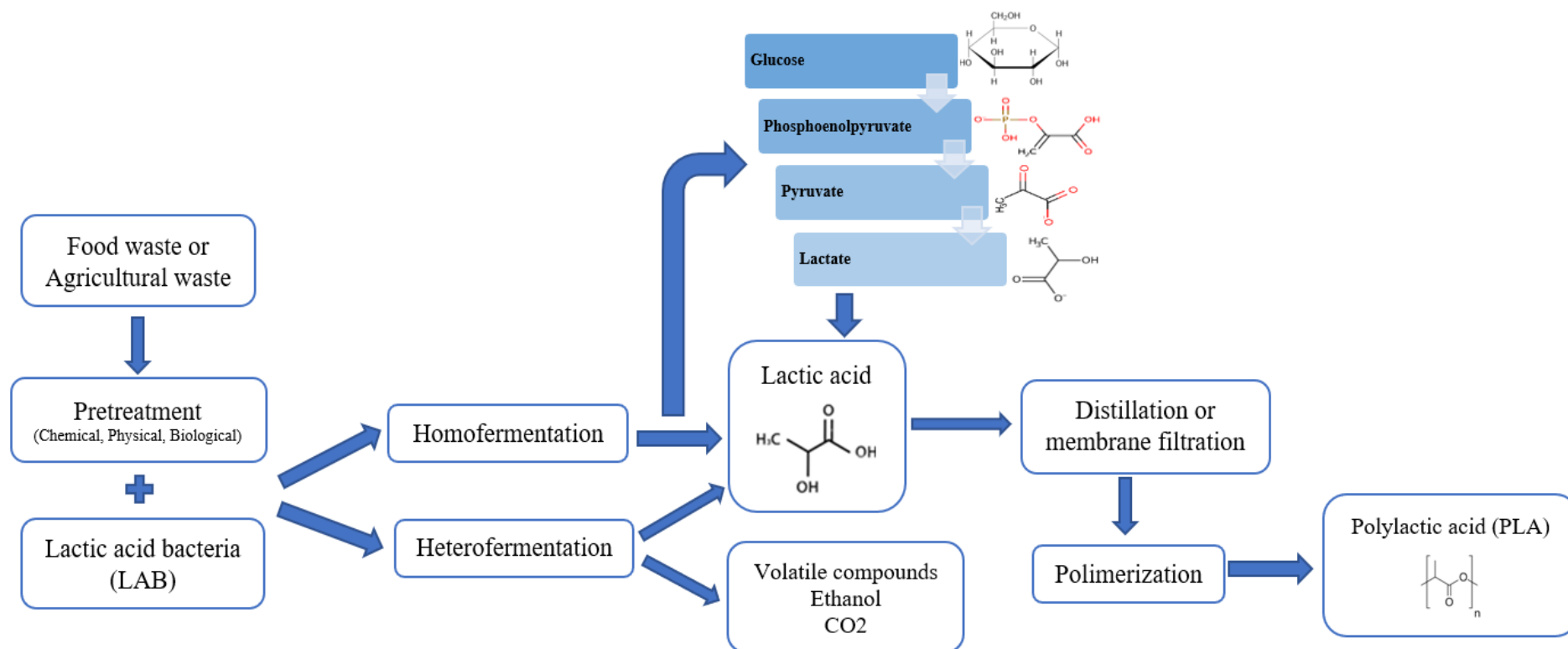


Figure 3. Schematic of Poly(lactic acid) (PLA) production by lactic acid bacteria (LAB)

PLA production is not necessarily a copolymerization; but enantiomerically pure D or L lactic acid can also be used. It should be mentioned that firstly the cyclic dilactide is produced from lactic acid, only afterwards, via Ring Opening Polymerization (ROP), PLA can be formed (ROP has not indicated in this Figure).
 beside the homo- and heterofermentative pathway, the third important pathway for lactic acid production: the Bifidum-pathway (= fructose-6-phosphate shunt).

and (iii) inhomogeneous food wastes, such as household refuse [65]. According to the Food and Agriculture Organization of the United Nations (FAO), food waste is described as the "loss of quality and quantity of food throughout the supply chain during the production, after-harvest, and manufacturing stages." On average, approximately 30% of food is converted into food waste, with a significant amount of this waste being produced globally. A variety of food waste types, including lignocellulosic residues, starch, and oils, are being examined for their capacity to be transformed into high-value bioplastics [15].

4.2. Agricultural waste

The most significant loss index, with a value exceeding 20%, was observed in Central and Southern Asia, which was followed by North America and Europe, which exhibited a loss index of more than 15%. It was determined that roots, tubers, and oleaginous crops exhibited the most significant post-harvest to transportation losses, with a magnitude exceeding 20%, while fruits and vegetables demonstrated the least substantial losses, with values below 10% [71]. A categorization of agricultural waste can be established through the classification of waste according to its origin, resulting in two distinct categories: residuals from the field and residuals from processing [72]. Field remnants are defined as agricultural harvesting and treatment by-products that linger on the field. Field remnants encompass a wide array of botanical components, including seed husks, leaf litter, stem detritus, fruit remnants, dairy products, vegetable remains, grain residue, meat and poultry remains, and crop residue. Conversely, process remnants persist subsequent to the processing of crops into additional utilizable resources [2]. Agricultural waste is a broad category encompassing diverse types of waste. The waste in question includes animal waste, which may consist of dead animals or excreta, as well as processing waste, such as cans of fertilizer and packaging materials. In addition, hazardous waste, such as

herbicides, pesticides, and insecticides, is also included [73]. Within the agricultural sector, an estimated 140 billion metric tons of waste are generated annually, with half of this waste ending up in landfills. This phenomenon not only imposes substantial economic burdens but also exacerbates environmental damage [74]. The composition of agricultural waste is typically dominated by cellulose (38–50%), followed by hemicellulose (22–31%) and lignin (16–26%). Hemicellulose and cellulose are defined as chain compounds of polysaccharides, whereas lignin is characterized as a natural phenolic polymer. Observations of lignin's interaction with hemicellulose and cellulose during natural occurring processes have indicated the formation of ester or ether bonds [75].

5. Pretreating low-cost substrates

In order to employ waste materials as an initial substrate for bioplastic production, it is necessary to subject some of the waste to pretreatment. The purpose of pretreatment is to enhance the physicochemical and biological properties of the waste, as well as to convert the compound organic components (cellulose, hemicellulose, and lignin) of the waste into simple sugars for fermentation. A variety of pretreatment technologies, encompassing physical, chemical, biological, and enzymatic hydrolysis, have been examined to ascertain their impact on bioplastic production [40].

Physical pretreatment methods like heating, microwaves, milling, and ultrasound are used to increase surface area and improve the conversion of substrates such as glucose, proteins, and starch into fermentable materials [76]. Acid pretreatment, a common procedure in the fields of agriculture and lignocellulosic waste management, has been shown to enhance enzyme accessibility. However, this treatment can also generate inhibitors, such as furan derivatives, phenolics, and carboxylic acids, which have the potential to influence the fermentation process [77].

Alkaline pretreatment, employing solutions such as NaOH or ammonia, has been demonstrated to disrupt the ester bonds between plant polysaccharides and lignin. This process facilitates the solubilization of lignin and enhances the breakdown of materials [78].

Biological pretreatment has been demonstrated to be a more energy-efficient approach, enhancing enzyme accessibility in comparison to chemical methods. Notwithstanding the challenges associated with slow reaction kinetics and polysaccharide degradation, biological pretreatment has proven to be a viable alternative. The biological conversion of waste materials to fermentable substrates frequently involves white rot fungi, which play a pivotal role in delignification and enhancing the efficiency of enzymatic saccharification processes [79]. Enzymatic hydrolysis of food waste is imperative for the conversion of polymers into monomers, thereby enhancing the breakdown of lignocellulosic materials, though lignin remains resistant [80]. The integration of methodologies, including the combination of physical and chemical treatments, has been demonstrated to enhance performance [40].

6. Factors affecting bioplastic production by LAB

A multitude of factors have been documented by numerous researchers as contributors to the variation in bioplastic production yield, including the C/N ratio, substrate concentration, inoculum size, nutrients, mode of fermentation (batch and fed batch), and operating parameters (pH and temperature) [61]. It is imperative to emphasize the pivotal role of strain selection in determining the yield of bioplastic precursors due to their impact on the type and percentage of these substances produced. A comprehensive analysis of the biochemical and physical characteristics of the bioplastic has been undertaken, revealing a significant dependence on the type of PHA accumulated by different strains [63].

Another significant factor that influences the potential of microorganisms to accumulate PHA is the composition of the nutrient medium. The absence or supplementation of essential nutrients has been demonstrated to exert an influence on PHA production, as indicated by the abundant presence of waste comprising carbon sources, amino acids, and fatty acids [81].

The large-scale industrial production of bioplastics necessitates the establishment of controlled conditions, which can be facilitated by the utilization of substantial fermenters that operate in diverse modes. The attainment of optimal results is contingent upon the implementation of meticulous adjustments to both the feeding strategy and the operational mode of the fermentor. The conditions under which fermentation occurs are contingent upon the specific microbial strain employed. The operating temperature is typically maintained within the range of 30 to 37°C, accompanied by low humidity and dissolved oxygen level, owing to the low stirring speed [59].

In order to effectively convert substrate into targeted products, it is imperative to enhance the synthetically efficient capabilities of microbial cell factories through a variety of strategies. Figure 4 provides a synopsis of the strategies for enhancing bioplastic synthesis efficiency [1]. The advent of synthetic biology has led to the advent of two primary stresses: osmotic stress and metabolite stress. These two stresses have arisen as a result of the accumulation of target products. Furthermore, acid or base changes have also been induced by these target products. These phenomena render microbial cells more susceptible to damage or inhibition, thereby impacting cell growth and production. It is imperative to improve the microbial ability to withstand environmental stress. The enhancement of tolerance in chassis cells can be achieved through three primary aspects: the enhancement of tolerance to (i) acid-base stress; (ii) osmotic stress; and (iii) metabolite stress [82].

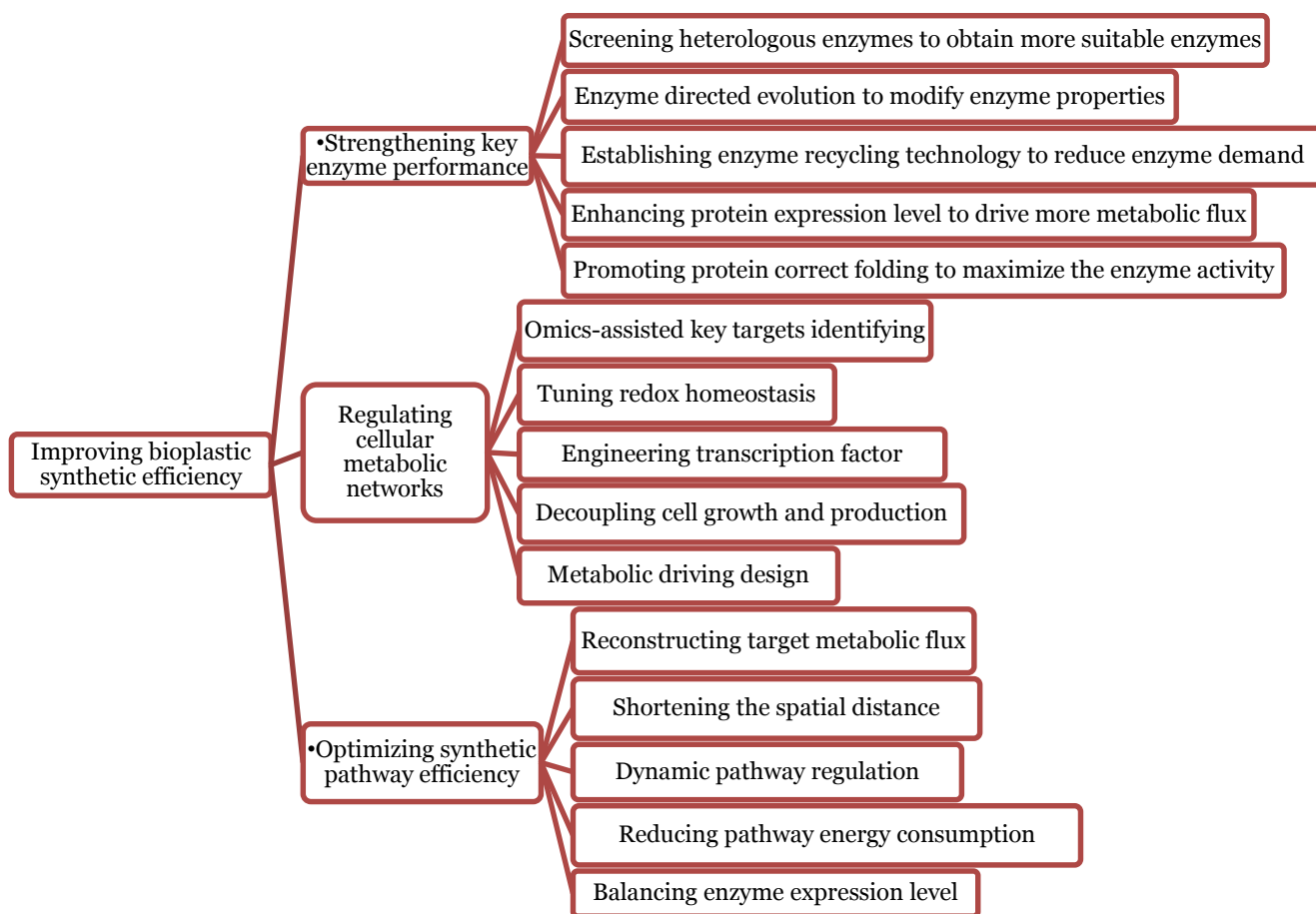


Figure 4. Strategies for improving bioplastic synthesis efficiency

It is imperative to consider microbial inhibitors as a parameter in the execution of biosynthesis. Impurities, including but not limited to methanol, fatty acids, hydroxide residues, and salts, have been observed to impede microbial activity [83]. A primary objective is to identify a strain capable of either tolerating or metabolizing the potential impurities present in the substrate. A variety of microbial inhibitors exist, including phenolic-based compounds, organic acids (e.g., formic, acetic, and levulinic acids), and the furfurals present in substrates derived from lignocellulose [41].

7. Conclusion

The plastic crisis is a pressing environmental issue that has yet to be adequately addressed on a global scale.

The utilization of bioplastics holds considerable promise in supplanting conventional plastics, which are recognized for their comparatively less environmentally friendly characteristics. The demand for bioplastics is increasing for two primary reasons. First, the cost of raw materials used in plastic production is rising. Second, there is a growing inclination at various levels to adhere to the principles of the circular bioeconomy. Bioplastic is a natural, polymeric material that has undergone significant development in recent decades, primarily due to its notable properties of biocompatibility, as well as biodegradability and material properties. The transformation of low-cost substrates, such as food and agricultural byproducts, into bioplastics, presents a promising solution to address critical environmental

challenges, including waste management, pollution control, and the demand for sustainable materials. Bioplastics have a broad range of utilization in various sectors, including the packaging, the cosmetics, pharmaceutical, the medicinal, and agricultural industries.

A considerable number of bacterial strains, including LAB, have been demonstrated to possess the capacity to synthesize bioplastics from food and agricultural waste with high productivity and efficiency. The utilization of LAB in fermentation processes has a longstanding history of being carried out safely. Consequently, lactic acid, the primary product of lactic acid fermentation, is regarded as an environmentally sustainable platform compound, extensively utilized in the production of PLA. Therefore, investing in such technology constitutes a pivotal step to a more resilient future, as the manufacturing of these bioplastics can concurrently assist with managing waste and alleviate the growing demand for conventional petrochemical-based plastics. Bioplastics are still in their nascent stage of development. Further research is necessary to enhance the properties of bioplastics, making them more competitive against conventional plastics. This research should also focus on reducing the cost of bioplastics. A significant market change can result from addressing waste and overuse of nonrenewable resources. Also, most of the literature searches are conducted in a lab-scale, and the process of scaling up laboratory-scale parameters to pilot-scale parameters frequently encounters failure, primarily due to the presence of supplementary factors associated with the nature of the pilot-scale plant. So, more investigation is needed in scaling up of bioplastic production by LAB.

Conflict of interest

The authors declare no conflict of interest.

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