Review

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Growing long-chain polymers *in vitro* using engineered bacteria in synthetic biology and precision fermentation space

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Abstract: The synthesis of long-chain polymers has a conventional method of utilizing energy-intensive chemical procedures, which are normally not eco-friendly. Though synthesizing polymers using synthetic biology and precision fermentation is not possible in vitro, both methods, when integrated, provide a biological solution to synthesize polymers in vitro. In light of this, this article examines the means of engineering bacteria, including Escherichia coli and Pseudomonas, that are used to produce controlled synthesis of highvalue polymers like PHAs and PLA. New technologies and methodologies of synthetic biology, including the CRISPR-Cas9 system, have brought bacterial metabolic engineering to new levels, requiring higher polymer yield, longer chains, and improved functionality. Supporting these advancements, the precision fermentation method creates a controlled production environment for polymer growth and enhances the reproducible scalability of a complex industrial process. Nonetheless, certain critical tasks still exist to ensure that the metabolic load on the engineered bacteria is optimized and that the polymers are not degraded during the manufacturing process. This is apparent given the fact that the use of these biologically derived polymers in areas like bioplastics, medicine, textiles, and many other economies shows the capability to substitute for a petrochemical-based economy, hence enhancing sustainability. This review provides the current introductory state, where the prospects and difficulties detected about sustainable long-polymer synthesis using synthetic biology and precision fermentation are discussed.

Keywords: synthetic biology; long-chain polymers; engineered bacteria; metabolic engineering; biopolymer synthesis; biodegradable polymers; microbial fermentation;

1. Introduction

Polymers, which are enormously large molecular structures composed of repeating units or monomers, are the basis of thousands of products that range from food packaging to clothing, to electrical wiring and medical equipment. Conventional methods of producing these materials involve use of petroleum products and have been widely used in the development of goods such as plastics, synthetic fibers and rubbers. However, the restraints of synthetic processes including the use of fossil fuel and formation of nondegradable waste have led to a search for environmentfriendly polymer production processes [1]. Conventional methods currently used in the industry are however not sustainable; biologically derived polymers made by microorganisms via natural or genetically modified metabolic processes are however a more sustainable proposition [2].



Dr. Pranav Bhaskar Department of Pharmacology, University of Virginia School of Medicine, Charlottesville, Virginia – 22903, USA E-mail: pranavbhaskar@live.com Polymers are known to be widely used in today's industries, where they are used as precursors for production of goods that define today's day-to-day lives. In packaging, plastics derived from polymers mean that the packaging is lightweight, durable and flexibly applicable to meet the needs of preserving the items and cutting the cost of transportation [3]. This staple of the apparel business counts polyester and nylon as large components; both polymers known for their strength, flexibility, and durability.

In healthcare, polymers are used to create medical devices, drug delivery systems, and tissue engineering scaffolds because of their able biocompatibility and designable physical characteristics [4-6]. In industrial uses of polymers include lubricants, adhesives, and coatings due to the great mechanical and chemical properties they possess. Although these polymers are commonly used in many applications, most of them are produced using fossil fuel feedstocks and the environmental impacts of such production and eventual disposal are still unknown [7]. Consequently, the need for products that are more eco-friendly, bio-degradable products has never been this high.

More recently the field of synthetic biology has provided opportunities for synthesizing polymers through microbial systems, a huge departure from conventional chemical synthesis [8]. The syntheses of polymers by bacteria, yeast

Table 1. A comparison of traditional	versus biological methods	for polymer production.
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Aspect	Traditional Methods	Biological Methods	References
Raw materials	Primarily petrochemical-based	Often renewable, bio-based feedstocks	[<u>9</u> , <u>10]</u>
Production process and energy requirements	Chemical synthesis; generally high, especially for heating and pressure	Microbial fermentation; typically lower, as biological processes occur at ambient conditions	[<u>11</u>]
Environmental impact	High carbon footprint and pollution; with potential emissions and waste	Generally, lower carbon footprint; potential for circular economy; with reduced emissions and biodegradable products	[<u>12</u> , <u>13</u>]
Scale	Well-established for large-scale production	Often limited to smaller scales, but scaling up is an active area of research	[<u>14]</u>
Yield efficiency	Often lower yields due to complex reactions	Potentially higher yields through optimized microbial pathways	[<u>11</u>]
Polymer range	Limited to synthetic polymers	Diverse range of biopolymers, including biodegradable options	[<u>11</u>]
Process control	Highly controllable, with predictable outcomes	May have more variability, but improving with advanced biotechnology	[<u>15]</u>
Production time	Longer production cycles	Shorter production times with continuous fermentation processes	[9]
Sustainability	Often non-sustainable; depletes natural resources	Sustainable; utilizes renewable resources and reduces waste	[<u>10</u>]

and other microorganisms have been used through manipulating the metabolic processes of the same [2]. This biology-based approach has some added advantages, such as utilization of totally renewable feedstocks, lower energy input and possibly biodegradability, which offsets some of the issues arising from use of petroleum-based plastics [16, 17] (Table 1).

Some metabolic engineering successes especially in microorganisms for polymer production has involved improving the synthesis of the following polymers: polyhydroxyalkanoates (PHAs), polylactic acid (PLA), and other biopolymers for use as substitutes to conventional plastics. The engineered microbes can synthesize polymers *in vitro* to achieve the desired properties of polymers through the control of chain length, molecular weight and functionality [8]. The degree of control offered coupled with innovations in the field of precision fermentation technology make these biologically derived polymers a probable substitute for their petrochemical based counterparts in several industries [15].

Furthermore, new trends in genetic engineering such as CRISPR/Cas9 have enabled researchers to engineer bacteria and select specific strains to synthesize long-chain polymers to possess desirable characteristics [18]. Since

synthetic biology together with precision fermentation will slowly develop, there is a promising future for large scale and sustainable polymer manufacturing. It illustrates not only a technological evolution but also a stage which is critical for improving the ecological footprint of polymer production [19].

2. Engineering bacteria for polymer production

Synthetic biology, at its most basic, uses genetic engineering to reprogram the metabolism of microbes, including polymers, for their production. The aim is to fashion "living factories" that will parlay simple substrates into complex products. In the context of polymer production, synthetic biology has provided the ability to engineer microbial factories for the synthesis of complex, high value polymers with stringent control over composition, molecular structure and functionality [8]. For example, in polymer synthesis, particular biosynthetic pathways are engineered in bacteria, yeast, or other microorganisms to drive the production of polymer precursors and their subsequent polymerization into long chain polymers [20]. Synthetic biology for polymer production includes:

2.1 Gene editing and metabolic engineering

Gene editing tools such as CRISPR/Cas9, TALENs, and zinc finger nucleases enable scientists to precisely alter genetic material (introduce, delete or modify) in microbial genomes [21]. The use of these tools reprograms metabolic pathways to efficiently convert simple carbon sources such as glucose into polymer monomers. In metabolic engineering, the flux of metabolites is rerouted to produce desired polymers with increased yield and quality of the end product [22].

2.2 Chassis organisms

Escherichia coli and *Pseudomonas putida* are frequently used as the host chassis due to the fact they are genetically amenable, or model organisms, and or able to grow under several conditions [2]. These bacteria are then utilized as vehicles to produce synthetic gene networks and biosynthetic routes linked to polymer production. Depending on the tolerance to metabolic stress, the growth rate, as well as the ability to grow on different carbon sources, the chassis must be selected [8].

2.3 Pathway optimization

A critical issue in converting bacteria into factories to produce polymers is coping with the problem resulting from the additional new pathways: these pathways may have negative effects on the host organism. These pathways are optimized by controlling the levels of precursors, the activity of the enzymes involved, as well as the metabolic and energy costs needed for efficient synthesis of the copolymers with high cell density and viability rates [22].

2.4 Protein engineering

Under certain circumstances, enzymes used for biosynthesis require alteration in activity, stability or selectivity to synthesize polymers. These enzymes are engineered using protein engineering approaches such as directed evolution and rational design to enable efficient transformation of the intermediates to chains of polymers [23].

Bacteria involved in the synthesis of polymers have their gene and metabolic pathways modified in synthetic biology to produce polymers of predetermined characteristics. This approach enables us to synthesize biopolymers with differently defined physical, chemical and functional properties for differed industrial uses. A few key polymers have been synthesized by engineered bacteria using synthetic biology tools which have the added advantages of biodegradability and renewability as against conventional petroleum derived plastics. For instance,

Polyhydroxyalkanoates (PHA) of are а group are biodegradable thermoplastic polymers which synthesized by bacteria as intercellular storage of energy and carbon. More constructively, synthetic biology applied to bioengineering of bacterial strains and optimizing the metabolic pathways (Figure 1) involved in the synthesis of PHA monomers from sugars of fatty acid in bacterial strain like *Pseudomonas putida* and *P. entomophila* [24], Burkholderia sacchari [25] and Escherichia coli [26] has exhibited improvement in PHA production. The two key classes of PHAs are polyhydroxybutyrate (PHB) and polyhydroxy valerate (PHV), which are known to have thermoplasticity. The interest in PHAs is increasing as environment-friendly solutions to conventional plastics characterized by recyclability and biodegradation as well as biocompatibility for packing, medical uses, and agriculture.

Polylactic acid (PLA) is a biodegradable polyester that is widely used in its bioplastic form. Although it is mainly made by chemical polymerization approaches from lactic acid, synthetic biology can also be accomplished where the bacteria produce lactic acid and convert it into PLA by fermenting renewable resources such as glucose or glycerol [27]. Technological improvement in the recent past has made it possible for bacterial strains to synthesize PLA from lactic acids, eliminating long processes involved and chemical components. PLA is used in packaging industry uses disposable products and biomedical engineering application in tissue engineering and drug delivery systems.

Cellulose is by far the most widespread biopolymer in nature and is normally obtained from plant origin. However, synthetic biology, bacteria like by Komagataeibacter xylinum (formerly known as Gluconacetobacter xylinus) has been modified to produce bacterial cellulose which is a highly pure and strong type of cellulose used in wound dressing, biomedical implants and flexible electronics [28]. Researchers have improved mechanical properties of cellulose and were able to produce cellulose derivatives with desired functionalities by engineering metabolic pathways of cellulose synthesis process.

Xanthan gum and *alginate* are considered biomedical polymers derived from *Xanthomonas campestris* and *Pseudomonas aeruginosa*, respectively [29]. Some of these biopolymers are employed in food products, pharmaceuticals and industries due to their thickening, gelling and stabilizing effects. Xanthan and alginate biosynthesis has been enhanced and their physicochemical attributes altered through the manipulation of metabolic pathways and their control in the producer bacteria.

3. Genetic circuit design and control mechanisms

One of the major achievements in the field of synthetic biology is the general capacity to engineer synchronized modules pivotal for the regulation of spatial and temporal behaviors as well as the levels of genetic transcription in engineered bacteria. These circuits imitate electronic circuits with the help of bio-elements like DNA, RNA, protein, etc., to manage cell activity. In polymer production genetic circuits enable production and regulation of polymers to be well controlled, ensuring high yields, quality and efficiency.

3.1 Inducible promoters

These genetic circuits can incorporate inducible promoters whose function is to regulate a specific gene that is crucial to polymer synthesis. These promoters are made to respond to different signals such as temperature, light or certain chemicals and therefore allow investigators to regulate when the synthesis of the polymer occurs or in what ratios [<u>30</u>]. For instance, an inducible promoter can only synthesize polymer once the density of the bacterium has



Figure 1. Metabolic pathways for polyhydroxyalkanoates (PHA) biosynthesis catalyzed by various enzymes: **PhaA** – 3-ketothiolase; **PhaB** – NADPH dependent acetoacetyl-CoA reductase; **PhaC** – PHA synthase; **PhaG** – 3hydroxyacyl-ACP-CoA transferase; **PhaJ** – (R)-specific enoyl-CoA hydratase; **FabG** – 3-ketoacyl-ACP reductase.

reached a threshold, thus promoting cell growth to the optimum level before it begins to synthesize the polymer [31].

3.2 Riboswitches and small RNA regulators

Riboswitches are RNA based molecular appliances that act as sensors of small molecules or changes in metabolites concentrations as well as a players of gene switch [32]. In relation to polymers, riboswitches offer the possibility of detecting the concentration of the precursor molecules or the molecules intermediate in polymer synthesis so that expression of additional genes can produce them to restore the polymer equilibrium [33]. Likewise, small RNA molecules can simultaneously control several genes thereby offered another layer of dicta tiple biosynthesis of polymers [34].

3.3 Metabolic flux control and feedback loops

It is important to have the relative amounts of polymer, cell, and fresh medium right to get great yields. Circuits can contain feedback mechanisms which watch over metabolic flow and change the rate of selected enzymes responsible for polymer synthesis [35]. For instance, if there is an accumulation of a particular polymer precursor in the cell, then there can be negative feedback to reduce uptake of the precursors because they can be toxic and to allow more important processes to take place.

3.4 Compartmentalization and synthetic organelles

Another new trend complementing the genetic circuits is functionality separation of polymer synthesis routes in synthetic organelles [36]. Since the researchers know which reactions are to be carried out by the enzymes located in which part of the cell, they can eliminate metabolites from

affecting other reactions, get a higher polymer formation, and save the cell from poisonous intermediates. This concept is being developed using bacterial and yeast system to enhance the process of polymer formation.

Synthetic biology has achieved exacting manipulation of microbial polymer biosynthesis apparatus through genetic circuits allowing the construction of preprogrammed bacteria that will respond to environmental triggers and self-govern production processes. This level of control is important particularly in maximizing efficiencies in terms of polymer yield and quality and in standardizing the method for large-scale production.

4. Engineering the fermentation environment

Precision fermentation is a modern biotechnological platform that uses sophisticated control of microbial fermentation for the purpose of delivering predetermined molecules, including long-chain polymers. By optimizing the fermentation environment, scientists can enhance the efficiency and yield of biologically driven polymer synthesis [16]. Precision fermentation is defined as the use of bacteria, yeast, or fungi to manufacture specific compounds with good precision and quality. In contrast to classical fermentation which involves large scale production in which little control over the specific pathways could be taken, precision fermentation is exactly the opposite where the key idea is to secure the best possible conditions for production of the compounds of interest.

With reference to polymer manufacture, precision fermentation utilizes synthetic biology to blend with complex fermentation processes to enable engineered microorganisms to develop long-chain polymers which include PHAs, PLA, and other biopolymers [37, 38].

Precision fermentation operates with the control of different parameters such as nutrient growth factors, environment pH, oxygen available, temperature, and fermentation time to guide the pathway of metabolic activities of microbes toward biosynthesis of polymers with desired properties [14]. Precise fermentation methods produce valuable outcomes including product-specific manufacturing and improved production levels and environmental friendliness. The deliberate modification of microorganisms and their fermentation conditions through precision fermentation enables the generation of highly valuable polymers that can be designed for different industrial purposes from medical equipment to packaging products to textile manufacturing.

4.1 Bioprocess optimization for long polymer synthesis

Researchers need to optimize fermentation parameters during long-chain polymer synthesis of PHAs and PLA in order to maximize molecular weight and polymer output rates. Several important bioprocess parameters determine the optimization process which biosynthesizes long polymers that meet desired physical and chemical requirements.

4.1.1 Nutrient management

The production of microbial polymers and its subsequent microbial growth requires adequate carbon as well as nitrogen and phosphorus resources [39]. Carbon sources such as glucose, fructose, and glycerol serve as precursors for the synthesis of polymer monomers. Under constrained nitrogen and controlled phosphorus conditions bacteria reorient their metabolism to produce polymer granules by using their metabolic materials for polymer synthesis. The optimal maintenance of these nutrients through balanced control promotes higher polymer production without harming microorganisms.

4.1.2 Oxygen supply and aeration

The production of polymers requires oxygen as a vital element in aerobic fermentation procedures [40]. Aeration control methods need to preserve proper oxygen levels because they protect both metabolic processes and biosynthesis efficiency [11, 41]. The reduction of enzyme activity occurs when oxygen levels decrease thus leading to decreased polymer molecular weights. Real-time dissolved oxygen measurement alongside rate adjustments of aeration systems is essential to optimize a polymer synthesis process.

4.1.3 pH control

Maintaining a stable pH is essential for enzyme activity and microbial metabolism. The product of polymer biosynthesis becomes compromised when the pH deviates from its optimal range while unwanted byproducts form during this time [11]. The fermentation broth needs automated pH control systems to maintain its pH levels within the optimal range for polymer synthesis during precision fermentation processes [41].

4.1.4 Temperature optimization

The growth process and polymer production rates of different microorganisms require unique optimal

temperature ranges. The optimal temperature during fermentation enables bacterial enzymes to produce polymers best while protecting cellular well-being [11]. The stability of temperature controls long-chain polymer synthesis because temperature changes affect both the length and molecular weight of the polymers produced [42].

4.1.5 Fed-batch and continuous fermentation

In many cases, fed-batch fermentation, where nutrients are gradually added to the fermentation broth, is preferred for long polymer production [43]. This method prevents substrate inhibition and allows for better control over the fermentation process, leading to higher polymer yields. Continuous fermentation, where the fermentation broth is continuously replenished with fresh medium, can also be used to maintain stable growth conditions and prolonged polymer production, although it requires careful management to prevent contamination or metabolic shifts.

4.1.6 Metabolic flux analysis

The engineered microbial metabolic pathways receive metabolic flux analysis to monitor metabolite movement throughout their production process for optimizing long polymer bioprocesses [44]. The approach enables researchers to find system constraints in synthesizing polymer precursor molecules, which helps improve fermentation conditions for optimal yield and polymer length achievement.

The optimization of bioprocesses in precision fermentation technology both increases the production of long polymers alongside their quality while establishing economical and large-scale manufacturing capabilities for industrial implementations.

4.2 Scale-up challenges and industrial production

The scale-up needs of the industrial level precision fermentation application are also significant, but still, the technology provides significant advantages in a controlled polymer production. Commercial-scale production requires technical, logistical, and economical answers that go beyond the laboratory-scale fermentation.

4.2.1 Process consistency

Platform operation uniformity throughout the fermentation processes is the main barrier in the precision fermentation scale-up [45, 46]. It is difficult to duplicate the same optimized control that the laboratory fermentations have when fermentation is transferred to large bioreactors. As a result of scale-up process, the oxygen transfer and temperature gradients and nutrient distribution vary that greatly impacts polymer yield and quality [47].

4.2.2 Bioreactor design and mixing

The adoption of the fermentation scale of production determines the pre-eminence of bioreactor system development [45, 46]. The large-scale bioreactors design requirements include proper mixing as this is a basis for nutrients and oxygen-transfer functions in the fermentation broth. The absence of proper mixing of components of fermentation broth leads to dead areas where microbial cells

are not able to achieve proper nutrition of cells or oxygen, therefore, decreasing their polymer production. The appropriate development of bioreactors needs to have better mixing equipment and constant monitoring capabilities to deal with these challenges.

4.2.3 Metabolic burden on microbes

The increased production at higher volumes is in conjunction with the further metabolic pressures to engineered organisms [48]. Microbial cells that function in mass scale environments evoke stress due to issues associated with nutritional defect or metabolic overload and waste product accumulation [49]. The production of polymers can be adversely influenced when as a result of metabolic complications, cells die [50]. The researchers have the task of optimizing the parameters of fermentation so that the microbial cells are free from overburdening and the maximum output of polymer was obtained.

4.2.4 Economic viability

The scale of polymers' production at the industrial level requires cost-effective functioning to be profitable. The sustainable polymer production capacities of precision fermentation are confronted by high costs of microbial engineering and maintenance of fermentation conditions as well as acquisition of raw materials [51]. Three main aspects determine commercial viability of the process and these are the choice of cheaper raw materials for fermentation and a greater enhancement of microbial rates of production.

4.2.5 Product purification and downstream processing

Fermentation process stops when the process of purification of the polymer from the fermentation broth is needed. Companies handling the long-chain polymers must carry out numerous complex downstream processing that involves breaking the cell walls and extraction of the polymers while carrying out purification steps [52]. Upscaling for these processes gets complicated as a result of large volumes of solvent and energy expenditure resulting to cost increments [51]. For biologically produced polymers to succeed in the commercialization, there is need to enhance sustainable efficient downstream processing methods.

Interactions of research in microbial engineering and bioreactor crafting and bioprocess refinement will propel precision fermentation as a way to revolutionize the industry of polymer foods with the sustainable polymers alternatives to existing technology.

5. Overcoming the challenges in long-chain polymer production

Although some key challenges for scaling up production of long-chain polymers to commercial applications still exist, significant progress was achieved in the developments of production procedures in synthetic biology and precision fermentation. The engineering of microbial cells to survive, in optimized product production through well-calibrated mechanisms, fulfils the micellar mixing process of making polymers in an efficient manner [49]. The stability of the polymer quality still is an important factor for all the stages of fermentation, from their production to extraction and processing [42]. Biologically produced polymers need to meet the industry standards without forgetting to ensure that it is safe for general use. This part outlines tactics of addressing the described challenges while working on metabolic load and polymer stability retention as well as regulatory compliance review.

5.1 Metabolic burden and cell viability

The prime barrier to synthesis of long-chain polymers by means of engineered bacteria is due to the high stress subjected to cells [39]. The manufacture process highly taxes cells metabolically when their metabolic chain is forced to make polymer pre-cursors then assemble into longer chain structures. Shifts in metabolic pathways of engineered bacteria lead to loss of cell growth and productivity and a decrease in levels of polymer production particularly with an increase in manufacturing to industrial production [53]. Measures of dealing with metabolic burden and enhancing cell viability are:

5.1.1 Balanced pathway engineering

Bacteria that have been engineered suffer from lessened metabolic burdens, as scientists are able to amend production levels of polymers in comparison with basic cellular functions. The optimization of gene expression that regulates polymer synthesis guides the cells away from overburdening with the precursor or energy demand [48]. Engineered cells that contain both inducible promoters or synthetic control elements initiate polymer synthesis only after their attainment of respectable growth levels which ensures their cell survival [54].

5.1.2 Modular metabolic engineering

In order to optimize the control and optimization process scientists divide the polymer biosynthesis pathways into individual units. A splitting of the operation of manufacturing polymer precursor and polymerization can keep the best operating conditions as diverse between the two processes [48]. A modular design is a strategy that limits the cellular metabolic calls while increases production output.

5.1.3 Co-culture systems

A co-culture system distributes polymer synthesis work among different microbial strains or species to handle distinct steps within the biosynthetic pathway. A multispecific bacterial culture involves two different processes, that is, one strain producing precursors which are converted into long chain polymers by the other strain [42, 55]. Biological stress is spread equally through the span of the manufacturing process by the division of labor between organisms; this increases the manufacturing efficiency of the system greatly.

5.1.4 Metabolite recycling

The genetically modified microbes, useful in the manufacture of polymers, accumulate the unwanted byproducts that are harmful to their growth performance and lowers the amount of polymers yield. By implementing

metabolite recycling strategies – such as pathway engineering to convert these byproducts into useful metabolites – can alleviate metabolic stress and cell viability during fermentation is improved [56, 57].

5.1.5 Stress tolerance engineering

Finally, such engineering of microbes as enhanced endurance against environmental and metabolic stresses, such as oxidative stress or nutrient limitation, may greatly increase viability of cells at large-scale fermentation [58]. The use of stress-resistant genes or changes in the regulatory network in a modified version assist bacteria to withstand the burden of polymer production resulting in more production of polymers and productivity [59].

5.2 Polymer degradation

It is difficult to preserve the integrity and quality of longchain polymers if degradation occurs during their production and processing. The biodegradable nature of biological polymers including PHAs) and polylactic acid (PLA) does not protect against premature degradation which occurs in fermentation reactions thereby shortening the polymer length and deteriorating mechanical strength [<u>60</u>]. The degradation of polymers becomes accelerated by extreme environmental conditions which negatively impacts the consistency of polymer quality [<u>61</u>]. Producers implement several methods to avoid polymer degradation.

5.2.1 Inhibition of degradative enzymes

Many bacteria produce enzymes, such as depolymerases, that break down long-chain polymers either for intracellular energy storage or as part of natural degradation processes [62]. Expressing these enzymes during fermentation can have an unfavorable impact on both the amount and quality of polymers formed. A possible answer is to turn off or inactivate the genes that make these enzymes inside the engineered bacteria, so the polymers are not broken down early in the process [63]. Another way is to find synthetic biology tools that restrengthen these enzymes during polymerization, so that the degradation only happens when it is intended [64].

5.2.2 Stabilization of polymer structures

There are bacteria that produce depolymerases that function in either supplying the cell with energy or as a component of how other lifeforms naturally break down materials. Expressions of these degradative enzymes during fermentation bring about less production and lower quality of the formed polymers. It is necessary to either knock out or silence genes producing depolymerase enzymes in synthetic organisms to prevent polymers from breaking down wrongly while bacteria are made [63]. The new technology makes it possible for scientists to limit when enzymes are activated so they do not start breaking down the polymer until it should begin [65].

5.2.3 Control of fermentation parameters

Maintaining the stability of the polymer depends greatly on the environment used for the fermentation [$\underline{66}$]. There is little polymer degradation when the pH, temperature, and

oxygen in the fermentation are kept at the right level. Normally, constant monitoring using computers helps to ensure polymer synthesis takes place more than the degradation of the product.

5.2.4 Post-fermentation processing

After synthesizing polymers, their extraction and purification take place at high temperatures or with harsh chemicals, which in turn, makes these polymers more likely to degrade [66]. Mild extraction methods (e.g., solvent-free extraction techniques) and optimized drying or precipitation processes can protect the integrity of long-chain polymers [67]. Using polymer stabilizers in the manufacturing stage also helps reduce deterioration [68].

6. Applications of long polymers produced by engineered bacteria

Long polymer molecules from the bacteria used in microbial production have a wide range of applications. Made by synthetic biology and precision fermentation, long-chain polymers work well in place of common, petroleum-based plastics. Nowadays, industries use biologically derived polymers to meet their increasing demand for green materials in the production of bioplastics, medical devices, and other types of textiles [69, 70]. In this section, examples are mentioned of how different types of made-by-engineered bacteria polymer chains benefit the environment and help advance various sectors.

6.1 Bioplastics and environmental sustainability

Long-chain polymers produced by engineered bacteria, and explicitly polyhydroxyalkanoates (PHAs) and polylactic acid (PLA), find their main utility in bioplastics. They are not like simple plastics because they are renewable and will break down naturally. Key advantages of bioplastics are:

6.1.1 Biodegradability

The most beneficial thing about bioplastics is that they break down naturally over time [$\underline{60}$]. Thanks to bio-plastics made by engineered bacteria, the materials are able to breakdown and become harmless substances if the surrounding conditions are right. Because bioplastics degrade in an eco-friendly way, there is less plastic buildup in landfills and oceans, which improves the health of the environment [$\underline{71}$].

6.1.2 Reduced carbon footprint

Converting biomass into bioplastics causes less greenhouse gas pollution than when we use fossil fuels to create plastics [72]. The process of turning waste or plant sugars into PHAs completes the carbon cycle, stopping CO_2 emissions both during plant life and as PHAs decompose [73].

6.1.3 Circular economy

Using engineered bacteria in bioplastics production, waste streams can be turned into useful products, helping to support a circular economy [73]. Biowaste from agriculture or industries can be made into bioplastics, making it easier for industries to cut down on using raw materials. By using

this method, we are able to reduce wastes and also create value from items we were going to discard.

6.1.4 Diverse applications

Bioplastics can be modified to suit various uses such as in packaging, disposable spoons or forks, films used in agriculture, and biodegradable bags [70]. Since bioplastics come in many forms, companies are able to switch to eco-friendly solutions that reduce their use of traditional plastics. Some companies focus on making PHA plastics, such as Mango Materials [74] and Full Cycle Bioplastics, while others, like scientists, aim to enhance the properties and uses of plastic by working on different types of bacterial strains.

6.2 Medical applications

Long-chain polymers produced by engineered bacteria hold significant promise in the medical field, where their biocompatibility, biodegradability, and functional properties make them suitable for various applications [<u>19</u>], such as:

6.2.1 Drug delivery systems

Biopolymers like PHAs and alginate can be modified so they can be used as drug delivery systems that slowly let out medicine when needed [70]. Since these materials are biodegradable, drugs can be released slowly, helping to make the treatment more effective and preventing harsh side effects [75, 76]. Such systems can be tailored to deliver cancer therapeutics, anti-inflammatory agents, or vaccines.

6.2.2 Tissue engineering

There is a growing demand for biocompatible scaffolds in tissue engineering, and engineered bacterial polymers look like they could be part of the solution [77]. For example, scaffolds made from PHA can support cell growth and still permit nutrients to reach the cells and waste to leave them [78]. The ability of these materials to break down allows the body to replace them naturally, reducing the likelihood of needing another surgery.

6.2.3 Wound healing

Hydrogels or films formulated from engineered-bacteriabased biopolymers can be used in wound healing treatments [79]. By using these materials, medical professionals can help keep moisture in the wound, protect it from infection, and give it the substances it needs to heal. These properties allow these polymers to be used in medical dressings and advanced wound care products.

6.2.4 Sutures and implants

Absorbable sutures and implants created with long-chain polymers mean that patients do not need surgery to remove them [80]. An example is when sutures made from PHA or PLA aid in the healing process and disappear harmlessly after healing, lowing the probability of side effects for the patient. Orthopedic surgeons are now considering PHAs for use as biomaterials in implants, scaffolds for bone repair, and pins and screws used for fixation [81].

Many research organizations and companies are currently working on producing medical devices from biopolymers and are considering using genetically modified bacteria for 3D printing of tissues and organs.

6.3 Specialty polymers for textile and industrial use

Besides bioplastics and use in medicine, engineered bacteria can create long-chain polymers that have many uses in both textiles and industry [<u>39</u>]. They can help boost the qualities and eco-friendliness of a wide range of items.

6.3.1 Eco-friendly textiles

With PHA, we can manufacture eco-friendly textile fibers that provide a better alternative to conventional synthetic fibers coming from petroleum [82]. The production of biodegradable fibers allows for the creation of fabrics for clothing and home textiles, and this also supports the increase in demand for environmental-friendly trends.

6.3.2 Coatings and adhesives

With engineered fermentation, long-chain polymers are developed and can be used to create eco-friendly coatings and adhesives in different industries [60, 69]. They can help protect against water, add antimicrobial effects, and are biodegradable, so they are good for use in packaging, building materials, and everyday items.

6.3.3 Functional additives

Engineered polymers produced by bacteria are useful in different industrial areas [69]. For example, they can enhance the properties of composites, improving mechanical strength, flexibility, and thermal stability. The ability to be used in many ways draws industries such as automotive, aerospace, and electronics to them.

6.3.4 Agricultural films

Engineered bacteria can make specialty polymers that can be used to make biodegradable agricultural films [69]. With these films, plastic use in farming can be reduced as the crops are still protected and the soil is kept healthy. By growing for a season and then decomposing, they do little damage to the environment.

7. Conclusion

Both engineered bacteria and precision fermentation may give the industry a rare opportunity to refresh the way polymers are manufactured. Through knowledge in synthetic biology and Bioprocess optimization, scientists are designing new microbial systems for producing longchain polymers economically and environmentally friendly. Encouraging this new way of thinking will cover the growing demand for ecological materials and may also redefine the future of bioplastic, specific polymers, and medical science that depend less on fossil fuels. Since these polymers are flexible and well-integrated, they are being searched for as more eco-friendly alternatives in industry.

Consequently, to reach an industrial standard for engineered bacteria production of polymers, the experts

have to solve some key problems. For these biopolymers to be considered sustainable in different fields, their effects on metabolism, ways they can be broken down, and adherence to bioregulatory standards must be analyzed. Because the above challenges are complex, colleges and companies must team up, together with modern advances in genetic engineering and fermentation. Building on this proactive research approach, the field gets the prospect of delivering on the promise of biologically derived polymers – and a more sustainable and responsible materials future.

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