Microbial Engineering for PHB Production and E-Waste Biomining: A Sustainable Approach

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ABSTRACT

This review delves into the potential of microbial biotechnologies in tackling two major environmental issues that face modern society: the manufacturing of petroleum-based plastics and the management of electronic waste (E-waste). E-waste contains valuable and critical metals that are conventionally extracted using methods that pose a threat to the environment (Gopikrishnan et al., 2020). Through the novel process of biomining, microorganisms have been found to offer a more sustainable way of extracting these metals. Similarly, the production of ecologically-damaging petroleum-based plastics can be mitigated by biologically modifying microorganisms that are naturally capable of producing bioplastics, like polyhydroxybutyrate (PHB), intracellularly (Tsang et al., 2019). This study addresses the current state of these microbial technologies as well as their drawbacks, ultimately culminating in a set of solutions that provide ways for these processes to be scaled up to an industrial level.

Introduction

Due to the increasing reliance on technology in today's world, we have seen a boom in electronic waste (E-waste) production. Globally, about 20-50 million tons of E-waste are generated annually, with only 10% being properly recycled or disposed of. Conventional approaches towards managing E-waste, including landfilling, incineration, and unauthorized recycling yards, have not been properly regulated and are consequently linked to severe air, water, and soil pollution . Moreover, the extraction of valuable metals from E-waste through traditional hydrometallurgical and pyrometallurgical approaches often requires a large amount of chemicals and results in high levels of environmental pollution due to the emission of toxic fumes and greenhouse gasses. (Gopikrishnan et al., 2020).

At the same time, the world is dealing with the environmental burden posed by petroleum-based plastics, which are non-biodegradable and can stay in the environment for hundreds of years. These synthetic plastics are especially dangerous in marine environments, where they are projected to cause $13 billion in ecosystem service degradation each year. They frequently wind up in landfills, where harmful substances percolate into nearby groundwater sources and contribute to pollution. Additionally, the combustion of fossil fuels necessary to produce these plastics causes large quantities of carbon dioxide to seep into the atmosphere, which in turn exacerbates global warming (Naser et al., 2021). Moreover, petroleum-based plastics are recalcitrant to biodegradation as microorganisms have not yet evolved to efficiently degrade them. The need for sustainable and biodegradable alternatives has led to the exploration of bioplastics such as polyhydroxyalkanoates (PHA), which can be produced and degraded by various microorganisms (Tsang et al., 2019).

Microorganisms, such as bacteria, fungi, and archaea, offer a promising solution to these problems due to their diverse metabolic capabilities. Through the process of bioleaching, they can extract precious metals from E-waste by excreting acid that dissolves these metals into an extracellular solution from which they can be recovered. Additionally, microorganisms can facilitate bioaccumulation, a process in which they absorb metals at a rate faster than they are lost, thus establishing a high concentration of metals within the cell which can then be extracted. These biological methods offer a more environmentally friendly alternative to conventional metal recovery techniques (Gopikrishnan et al., 2020). Furthermore, certain microbes are capable of producing bioplastics like polyhydroxybutyrate (PHB), a biodegradable polymer in the PHA family, providing a sustainable substitute to petroleum-based plastics (Tsang et al., 2019).

In this review, we will initially explore the realm of microbial production of bioplastics, specifically focusing on the PHA family. Following that, we will delve into a comprehensive examination of E-waste management in today's world and uncover the significant role bacteria play in metal bioleaching and bioaccumulation processes. The scope of this review extends beyond mere exploration as it seeks to connect these seemingly disparate subjects through the lens of bioengineering sustainable practices. What makes this analysis unique is its distinct approach towards not only elucidating the underlying biological principles but also highlighting the immense potential for practical applications by scaling up these microbial techniques. By doing so, we aim to shed light on how these innovative technologies can contribute to sustainability efforts and foster circular economies.

Discussion

PHB and its Synthesis by Microorganisms

PHB is a type of biodegradable plastic belonging to the family of PHAs, which are synthesized by various bacteria as intracellular carbon and energy storage compounds under nutrient-limiting conditions (where essential nutrients like nitrogen, phosphorous, and sulfur required for the growth and reproduction of the organism are in short supply) with excess carbon. The stored PHAs are later used by the microorganisms when other energy sources are not available.

PHB has recently garnered significant attention due to its potential to be an eco-friendly substitute to petroleum-based plastics. Its physical properties, including its high melting point (~180°C) and rigidity (glass transition temperature ~5 °C), are often compared to those of the widely-used plastic polypropylene (Czerniecka-Kubicka et al., 2017; Holmes, 1985). More importantly, PHB is completely biodegradable in various types of environments (such as soil, compost, freshwater, and seawater) and is completely harmless to living organisms.

Microorganisms play a crucial role in the production of PHB. The production of PHB in microorganisms is a complex process involving several enzymes, with the key ones being PHA synthase (PhaC), β-ketothiolase (PhaA), and acetoacetyl-CoA reductase (PhaB). The process begins when PhaA combines two molecules of acetyl-CoA to form acetoacetyl-CoA. PhaB then reduces this compound to form (R)-3-hydroxybutyryl-CoA, which is finally polymerized by PHA synthase to form PHB (Koch & Forchhammer, 2021).

Biological Modification in Enhancing PHB Production

Given that the synthesis of PHB is driven by microbes, it can be postulated that, through metabolic engineering, microbes can be genetically modified to give higher PHB yields. Various studies have shown that microbes have the capability to significantly enhance PHB production when engineered.

A study conducted by researchers at Utah State University demonstrated one of the ways in which synthetic biological engineering can be used to enhance PHB production. The researchers genetically modified an *E. coli* strain to produce a protein called phasin that binds to PHB and reduces its granule size. Phasins have previously been utilized to stimulate PHA synthesis in cells by increasing the surface area to volume ratio (thereby decreasing the size) of the granules (York et al., 2001). The binding of phasin to PHB allowed alphahemolysin (HlyA) signal peptides (which are commonly used in Gram-negative bacteria like *E. coli* to direct proteins to the periplasmic space) to guide the PHB-phasin complex to the cell membrane, where it could be secreted into the surrounding medium via a type I secretion system. The engineered and wild-type *E. coli* strains were then cultured under controlled laboratory conditions, and the internal and secreted fractions of the PHB were measured after 48 hours. For the modified strain, the researchers measured that 36% of the total PHB was secreted into the culture, while the remaining 64% stayed in the cell. Meanwhile, in the non-engineered strain, 0% of the total PHB was secreted into the culture. Scanning electron microscopy images were then used to visualize and validate their results (Rahman et al., 2013). Cell lysis is a bottleneck in PHB recovery and this method provides a solution to that issue because PHB is being secreted.

Building on this concept of biological engineering for bioplastic production, another noteworthy study conducted at the Korea Institute of Science and Technology took a slightly different approach. The researchers modified a different Gram-negative bacteria, *Cupriavidus necator* H16, for the production of PHB under litho-autotrophic conditions (with hydrogen, carbon dioxide, and oxygen as substrates). By overexpressing genes involved in the process of PHB production, *phaC1* and *phaA*, they were able to increase PHB production in the experimental strain by a statistically significant amount. Using gas chromatography and mass spectrometry, it was determined that the PHB content of the experimental *C. necator* H16 strain was approximately 76.4% of the cell dry weight, as opposed to the wild-type strain, in which the PHB content was only 58.3% of the cell dry weight (Kim et al., 2022).

The findings from these investigations confirm the idea that genetically engineered organisms, which possess the ability to generate PHB via synthesis, offer a promising prospect for augmenting the production of this eco-friendly bioplastic material. If effectively executed on a grand scale in industrial settings, this approach holds the potential to transform the plastics sector. By offering a sustainable substitute for petroleum-derived plastics, it carries vast possibilities in redefining existing practices. However, there exist numerous shortcomings that need to be addressed before this process can be scaled up.

Challenges and Future Directions in Scaling Up PHB Production

The primary factor inhibiting the mass production of PHB is its production cost when compared to plastics based on petrochemicals (Mokhtari-Hosseini et al., 2009). Multiple strategies are being developed to help resolve this issue. For instance, the utilization of food waste (a readily available and inexpensive resource) as a substrate for the microbial production of PHB as opposed to traditional, expensive substrates like glucose is being explored (Tsang et al., 2019). Furthermore, another study identified a way for glycerol to be preferentially used as a carbon source in PHB-producing bacteria, which would lead to a reduction in production costs as glycerol is a byproduct in the production of biodiesel and is therefore relatively cheap and abundant (Ramachander et al., 2002).

In addition to utilizing less expensive compounds to help facilitate PHB production in microorganisms, developing a simplified method of extracting the PHB would aid in cost reduction as well. Most methods of recovering PHB involve extracting it from cells through the use of solvents such as methylene chloride, propylene carbonate, dichloroethane, or chloroform (Valappil et al., 2007). These solvents are not amenable to use on an industrial scale as they are costly and hazardous. This presents a significant challenge as cost-effective biomass recovery is necessary for large-scale production (Yashavanth P R., Meenakshi Das, Soumen K. Maiti, 2021). Further developments in methods such as those discovered by Rahman et. al in 2013, where the researchers found a way to discharge PHB granules in the surrounding growth medium, would allow for more efficient recovery.

Biomining and its Role in E-Waste Management

Similarly, microorganisms are being leveraged in the world of mining through the biomining of E-waste. Biomining is a biotechnological process in which microbes are utilized to extract valuable metals from ores and other solid materials, including E-waste (Johnson, 2014). This process is gaining significant attention due to its potential to address the growing problem of E-waste management. E-waste, which includes discarded electronic devices such as computers, smartphones, and televisions, contains a variety of precious metals and rare earth elements (REEs). REEs are a class of metals comprising the entire lanthanide series as well as Sc and Y. REEs are only becoming more critical due to their increasing use in green technologies and their supply chain volatility (Zhuang et al., 2015). However, traditional methods of extracting these metals are often environmentally damaging and energy-intensive as they require large quantities of toxic compounds to solubilize the metals (Bindschedler et al., 2017). Biomining offers a more sustainable and potentially cost-effective alternative to traditional mining, leveraging the natural abilities of microbes to leach and accumulate metals from various different sources.

Potential of Microorganisms in Biomining

The most precious element found in E-waste is gold (Au), which is used by our society at a rate of approximately 263.3 metric tons per year (Rao et al., 2020). It has a variety of applications in the automobile, medical, and jewelry industries (Corti & Holliday, 2004). Researchers at the University of Neuchatel in Switzerland investigated a way for Au to be extracted from E-waste through biomining. Certain fungi are capable of producing hydrogen cyanide (HCN) as a part of their natural metabolic processes. The HCN then reacts with gold in the E-waste to form a soluble gold cyanide complex, [Au(CN)2]. This process is known as cyanidation and it is commonly used in the hydrometallurgical recovery of gold. Once the gold is in the form of a cyanide complex, cyanotrophic fungi (which are capable of using HCN as a carbon and nitrogen source) such as *Fusarium solani* and *Trichoderma polysporum* degrade the complex which in turn releases gold ions into an extracellular solution. In addition, some fungal species are also capable of transforming the complexed gold directly into gold nanoparticles, which was confirmed using transmission electron microscopy and UV-vis spectroscopy (Bindschedler et al., 2017). This provides a significant advantage because it eliminates the need for the gold to be processed further as it is already in a solid, recoverable form.

Fungi are not the only microorganisms that are capable of recovering critical metals from electronic waste. In another study, researchers utilized *E. coli* strains to develop a novel biosorption-based flow-through process for the selective recovery of REEs from electronic waste. The strains were previously engineered to display lanthanide binding tags on their surfaces through the introduction of a plasmid. The researchers then encapsulated these bacteria into a permeable, environmentally-friendly material called polyethylene glycol diacrylate (PEDGA) hydrogel to create microbe beads. These beads were packed into columns, and a solution containing E-waste was passed through them. Due to the permeable nature of the hydrogel, the bacteria in the beads were able to interact with the surrounding environment and selectively bind to REEs in the solution, effectively extracting them. The researchers noted that the system remained efficacious after several uses, with the microbe beads retaining 85% of their original capacity to extract REEs after nine cycles of use, thus further promoting sustainability. When the system was tested with a real-world sample, a NdFeB magnet leachate, 97% of the captured material was pure REEs, indicating effective REE extraction (Brewer et al., 2019).

Both of these studies demonstrate the potential of microorganisms to pave the way for a sustainable biotechnology-based REE and precious metal recovery system. However, analogous to the case with bioplastics, there are multiple flaws that must be addressed before the use of microorganisms in this application can be ramped up to a larger scale.

Challenges and Future Directions in Scaling Up the Biomining Process

One of the main issues in optimizing the efficiency of the biomining process lies in the environment in which the microorganisms are placed. Parameters such as pH, temperature, pulp density (the solid-liquid ratio in the leaching environment), and the concentration of the leaching agent can all affect how effectively a microorganism is able to extract metals. These factors must be altered based on the type of microorganism being utilized for leaching; for instance, acidophilic bacteria (which are frequently used for bioleaching) thrive in acidic conditions (Arya & Kumar, 2020). However, maintaining optimal conditions for a certain microorganism would be highly difficult on a large scale simply because there are so many parameters (such as carbon sources, aeration, pH, and temperature) that need to be considered. Since biomining is already a much slower process compared to traditional physical and chemical processes, not being able to create the most conducive environment possible for a singular microorganism would prevent biomining from being feasible at an industrial level (Han et al., 2022).

A method that can potentially address this flaw is the use of mixed cultures of microorganisms. For instance, Biswal and Balasubramanian (2023) achieved recovery efficiencies of 99.7% (nickel), 99.9% (copper), and 84% (lithium) during the bioleaching of metals from lithium-ion batteries in the presence of a consortium of *Ferroplasma sp.*, *Sulfobacillus sp.*, *Leptospirillum ferriphilum*, and *Acidithiobacillus caldus* at 45 °C. The use of a microbial consortia allowed the culture to have a broader range of tolerance, thereby providing it with more resilience to environmental changes. Although the culture still achieved extremely high recovery rates, less factors needed to be monitored and kept constant, which would make this method much more feasible on an industrial level.

In a similar study, researchers used a consortium of bacteria from the *Frankia* genus to recover high-value metal resources from E-waste. However, these researchers investigated a two-step bioleaching process. The first step allowed the bacterium to grow and acclimate to the E-waste environment, and the second step was where the actual bioleaching took place. The addition of an initial phase allowed the microorganisms to synthesize secondary metabolites, phosphatase enzymes, and organic acids, all of which aid in the solubilization of metals within E-waste. Consequently, the two-step leaching process yielded comparatively higher metal recovery rates (Narayanasamy et al., 2022).

Since the use of microbial consortia has the potential to elevate biomining to an industrial level, it is clear that further research in this field should delve deeper into the complex dynamics of these microbial communities. For instance, understanding how microorganisms in a consortium interact to produce biochemical compounds that can enhance the leaching process (such as secondary metabolites, phosphatase enzymes, and organic acids) is paramount. With this information, researchers can modify the metabolic pathways of these organisms through gene manipulation, leading to an increase in production of these beneficial compounds.

Moreover, another major challenge in transitioning to industrial-scale biomining lies in the diverse nature of electronic waste. Most laboratory-based studies involve a uniform source of E-waste, which does not reflect the wide variety of materials found in real-world E-waste. The viability of microorganisms to biomine will likely vary from batch to batch, which would inhibit consistent and effective metal recovery (Han et al., 2022). Once again, the use of microbial consortia offers a promising solution to this problem. Engineering a culture with a wide range of microorganisms (that have differing metabolic capabilities) would allow researchers to ensure consistent processing of E-waste even in the face of variable compositions (as certain microorganisms in the culture may be able to process materials that others cannot).

Conclusion

In the face of mounting environmental challenges, it is clear that microbes have the potential to transform the fields of plastic production and E-waste management. Through the lens of bioengineering, this review has explored the ways in which microorganisms can be utilized to promote sustainable practices within these domains.

As discussed, genetic modifications to PHB-producing bacteria can give them the capability to generate significantly higher yields of this bioplastic. However, given the elevated production cost of PHB when compared to traditional petroleum-based plastics, strategies such as the use of relatively inexpensive substrates (like glycerol and food waste) and the development of simplified extraction systems should continue to be explored.

Similarly, biomining has been proven to provide a more environmentally-friendly way of extracting metals from E-waste than conventional hydrometallurgical and pyrometallurgical approaches. It allows this process to be completed without the use of toxic compounds that pollute the environment. However, considering how susceptible certain microorganisms are to variability (whether it is the type of E-waste being used or the surrounding conditions), it is evident that future research should explore the intricacies of microbial consortia and how different microorganisms can work synergistically to enhance the efficiency of the biomining process.

As our society continues to grapple with growing environmental concerns, the potential of microorganisms to create effective alternatives to traditional methods of plastic production and E-waste management cannot be overlooked. This review has highlighted the future directions for scaling up these biotechnological processes to an industrial level, and it is hoped that it can serve as a valuable tool to researchers and practitioners in the field.

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