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Wax worm (*Galleria mellonella*) oxidative enzymes as a tool for plastics bio-recyling: A review

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Abstract

Plastic pollution has become a pressing global challenge, with the widespread accumulation of plastic waste in landfills, oceans, and ecosystems. Conventional plastic recycling methods face limitations and challenges, including sorting difficulties, contamination issues, and down-cycling of plastic materials. In response, the concept of bio-recycling, which involves the use of biological agents to break down plastic materials, has emerged as a potential solution. Wax worm bio-recycling offers significant environmental benefits by reducing plastic waste and minimizing pollution. It has the potential to divert plastic waste from landfills and incineration facilities, thereby reducing the environmental burden associated with plastic disposal. By breaking down plastic materials into biodegradable fragments, wax worm bio-recycling helps mitigate plastic pollution in landfills, oceans, and ecosystems. Furthermore, it aligns with the principles of a circular economy, promoting the effective recycling and reuse of plastics. The economic viability and market opportunities for wax worm bio-recycling technologies are promising. Ongoing research focuses on optimizing enzyme performance, improving scalability, and developing industrial applications. Cost-effectiveness, sustainability, and regulatory aspects are crucial considerations for large-scale production and application of wax worm oxidative enzymes. Public perception and awareness play a vital role in the adoption of this innovative approach, emphasizing the need for collaboration among stakeholders, including scientists, policymakers, and the general public.

Keywords: Bio-recycling, Economic viability, Environmental impact, Plastic pollution, Wax worm.

1. Introduction

Humans and industrial activities have led to great environmental pollution and the quest for remedial strategies in our society [1]. Plastic pollution has emerged as a pressing global environmental challenge, with significant implications for ecosystems, wildlife, and human health [2]. The durability and widespread use of plastic materials, coupled with inefficient waste management systems, have led to the accumulation of plastic waste in various environments. Plastics are found in landfills, oceans, rivers, and even remote areas such as polar regions and deep-sea ecosystems [3-5]. These materials persist in the environment for centuries, releasing harmful chemicals and causing physical harm to wildlife [6]. The fragmentation of larger plastic items into microplastics further exacerbates the problem, as these tiny particles can enter food chains and bioaccumulate in organisms [7]. The accumulation of plastic waste in landfills, oceans, and ecosystems has far-reaching environmental consequences. Plastic debris poses risks to marine life through entanglement, ingestion, and habitat disruption [8]. For example, sea turtles, seabirds, and marine mammals are particularly vulnerable to plastic ingestion, leading to intestinal blockages, starvation, and death [9].

Recognizing the urgent need to address plastic pollution, plastic recycling has gained prominence as a crucial strategy for waste management and environmental protection. The concept of plastic recycling involves collecting, sorting, processing, and reprocessing plastic waste into new products or materials [10, 11]. Bio-recycling, also known as biological recycling, is a concept that involves the utilization of biological agents, such as enzymes or microorganisms, to break down plastic materials and facilitate their recycling or degradation into environmentally friendly byproducts [12]. The process of bio-recycling harnesses the natural enzymatic activities of microorganisms or the application of specific plastic-degrading enzymes to initiate the breakdown of plastic polymers. These biological agents produce enzymes that can cleave the polymer chains of plastics, leading to the fragmentation of the material into smaller molecules or monomers [13, 14]. Enzymes play a crucial role in the bio-recycling process. Different enzymes target specific types of plastics, such as polyethylene terephthalate (PET), polyethylene (PE), or polypropylene (PP), and catalyze their depolymerization into simpler compounds. For instance, PETases are enzymes capable of breaking down PET, while esterases can degrade polyesters and lipases can act on various types of plastics [15].

Research has shown that wax worms can effectively degrade PE and PET, two widely used plastic polymers. Studies have shown that the digestive system of wax worms (*Galleria mellonella*) produces specific enzymes that can break down the chemical bonds of these plastics, resulting in their degradation into smaller fragments [16, 17]. The enzymes they produce interact with the plastic polymers, leading to their degradation and conversion into smaller molecules that can be utilized by the microorganisms as a source of energy and nutrients [18]. The bio-recycling approach offers several advantages in plastic waste management. Firstly, it provides a potential solution for the recycling of plastics that are challenging to degrade through conventional methods. Plastics like PET and PE, which are commonly used in packaging and single-use products, can be targeted and broken down by specific enzymes or microorganisms, allowing for their recycling and utilization as feedstocks for the production of new plastic materials [19]. Secondly, bio-recycling offers the possibility of reducing the environmental impact of plastic waste by minimizing its accumulation in landfills and ecosystems. By utilizing biological agents, the bio-recycling process can transform plastic waste into biodegradable byproducts that can be assimilated back into the natural carbon cycle, contributing to the reduction of plastic pollution [20].

2. Conventional Methods of Recycling Plastics

Conventional recycling methods for plastic involve two primary approaches: mechanical recycling and chemical recycling. Each method has its processes, advantages, and limitations.

2.1 Mechanical Recycling

Mechanical recycling involves physically processing plastic waste into new products without altering the chemical structure of the material. The process generally includes:

Collection and Sorting: Plastic waste is collected and sorted by type and color. This step is crucial to avoid contamination and ensure the quality of the recycled material.

Shredding and Washing: The sorted plastics are shredded into smaller pieces and washed to remove impurities like labels, dirt, and residues.

Melting and Reformation: The clean plastic pieces are melted and reformed into new products, such as pellets, fibers, or new plastic items. This method is most effective for thermoplastics, which can be remelted and reformed multiple times without significant degradation.

Mechanical recycling is simple and cost effective, but the recycled product is not always of the same quality as the virgin plastic [21]. As a typical case study, the European Union (EU) has implemented extensive mechanical recycling programs. According to Plastics Europe, in 2020, the EU recycled 9.2 million tonnes of plastic waste mechanically, representing a recycling rate of 32.5% for plastic packaging [22]. The EU's focus on improving sorting technologies and collection systems has been key to this success.

2.2 Chemical Recycling

Chemical recycling, also known as feedstock recycling, involves breaking down plastic waste into its basic chemical components through various chemical processes. This method can handle mixed and contaminated plastics that are unsuitable for mechanical recycling. Key processes include:

Pyrolysis: This process involves heating plastic waste in the absence of oxygen to break it down into smaller molecules, producing liquid or gaseous fuels and other valuable chemicals.

Gasification: Plastic waste is converted into syngas (a mixture of hydrogen and carbon monoxide) through high-temperature reactions with controlled oxygen. Syngas can be used to produce chemicals and fuels.

Hydrolysis and Depolymerization: These processes involve breaking down polymers into monomers or oligomers using chemical agents or enzymes, which can then be repolymerized into new plastics.

Chemical recycling method is more complex and costly but can produce high quality plastic that is the same as the virgin plastic. Eastman Chemical Company has developed a chemical recycling process known as Carbon Renewal Technology (CRT). This method uses a combination of chemical processes to convert mixed plastic waste into basic molecular building blocks [23]. These can then be used to produce high-quality plastics, including those suitable for food contact applications. Eastman's facility in Kingsport, Tennessee, aims to process up to 50,000 metric tons of plastic waste annually using this technology.

3. Challenges of Traditional Plastic Recycling

3.1 Sorting

Conventional plastic recycling methods face several limitations and challenges that hinder their efficiency and effectiveness. One major challenge is the complexity of plastic waste streams. Plastics come in a wide variety of types, compositions, and forms, making it challenging to sort and process them effectively. Different types of plastics require different recycling processes, and improper sorting can lead to contamination and reduced recycling rates. Sorting plastic waste poses a significant challenge. Manual sorting is time-consuming and prone to errors, leading to inefficiencies in the recycling process. Automated sorting technologies, such as near-infrared (NIR) spectroscopy, have been developed but struggle to accurately identify and separate different plastic types due to variations in color, additives, and polymer blends.

3.2 Downcycling

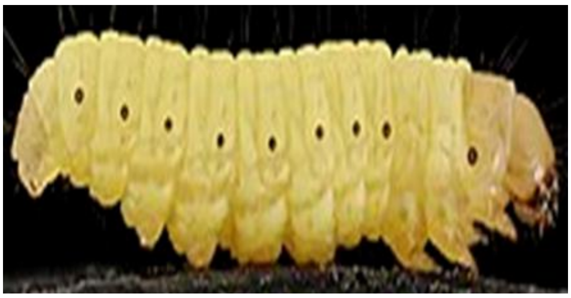
This is another challenge associated with traditional plastic recycling methods. It refers to the process of converting plastic waste into lower-value products that are of lower quality or functionality than the original material [24]. This occurs when plastic materials undergo degradation during recycling or when their properties degrade with each recycling cycle. Downcycling limits the potential for multiple recycling loops and reduces the overall value and sustainability of the recycling process. Downcycling is a common outcome of traditional plastic recycling processes. This occurs due to the degradation of polymer properties during recycling, resulting in a limited number of recycling cycles before the material becomes unsuitable for further processing.

4. Wax Worms (*Galleria mellonella*)

Wax worms are the caterpillar larvae of the greater wax moth, a species that belongs to the Pyralidae family. Another closely related specie of greater wax moth known as lesser wax moth (*Achroia grisella*) exists. The wax worm caterpillar larvae have a creamy-white appearance with dark markings on their body segments and measure approximately 2.5 centimeters in length [25]. These organisms are typically found in environments rich in organic matter, such as beehives and compost piles, where they feed on beeswax and other natural materials [26]. A typical wax worm caterpillar larvae and greater wax moth are depicted in Figure 1.

They have gained significant attention in recent years as a potential bio-recycling tool for plastic waste due to their unique ability to feed on and break down certain types of plastic materials [27, 28]. The potential applications of wax worms in plastic bio-recycling are significant. They can also be used in various settings, including waste treatment facilities, landfills, or specific plastic waste processing centers. Wax worms have shown promise in degrading plastic films, packaging materials, and even expanding polystyrene (EPS) commonly used in packaging and insulation [29].

(A)



(B)

Figure 1 (A) *Galleria mellonella* larva (B) Greater wax moth [25]

The life cycle of the wax worm as shown in Figure 2 consists of several distinct stages, each characterized by specific biological and behavioral characteristics [30]. Understanding the life cycle of wax worms is essential for their cultivation and utilization as a bio-recycling tool for plastic waste.

*Egg Stage***:** The life cycle of wax worms begins with the egg stage. Female wax moths lay their eggs in suitable environments, such as beehives or stored honeycombs. The eggs are small, oval-shaped, and typically whitish in color. The duration of the egg stage varies depending on environmental conditions but generally ranges from 4 to 21 days [31].

Larval Stage: After the eggs hatch, wax worms enter the larval stage. Larvae are the primary stage of interest in plastic bio-recycling due to their plastic degradation abilities. Wax worm larvae are whitish or cream-colored with a soft, cylindrical body and distinct head capsules. They have chewing mouthparts and feed voraciously on beeswax, honeycomb, and potentially certain types of plastic materials [32]. The larval stage of wax worms can last anywhere from 20 to 45 days, depending on various factors such as temperature and food availability. During this stage, the larvae undergo several molting phases, shedding their old exoskeletons and growing larger in size. They accumulate energy reserves and nutrients necessary for pupation [33].

Pupal Stage: When the larval stage is complete, wax worms enter the pupal stage. Pupation involves the transformation of the larva into a pupa, which is an intermediate stage between the larva and the adult moth. The pupal stage is characterized by significant morphological changes as the larval tissues undergo reorganization and development into adult structures [34]. During pupation, the wax worm constructs a cocoon made of silk and other materials, which provides protection and support during the metamorphosis process. Inside the cocoon, the wax worm undergoes a series of physiological changes, such as the development of wings, antennae, and reproductive structures [34]. The duration of the pupal stage varies depending on environmental conditions but typically ranges from 10 to 30 days. It is important to note that during this stage, the pupa is relatively inactive and does not feed [35].

Adult Stage: After the pupal stage is complete, the adult wax moth emerges from the cocoon. The adult moth has a wingspan of approximately 2 to 3 centimeters, with a gray or brown coloration. The primary purpose of the adult stage is reproduction. Adult female moths release pheromones to attract males for mating, and after successful reproduction, they lay eggs to initiate the next generation. The adult stage of wax worms is relatively short, typically lasting for a few weeks. During this time, the adult moths focus on mating, egg-laying, and ensuring the survival of their offspring [36].

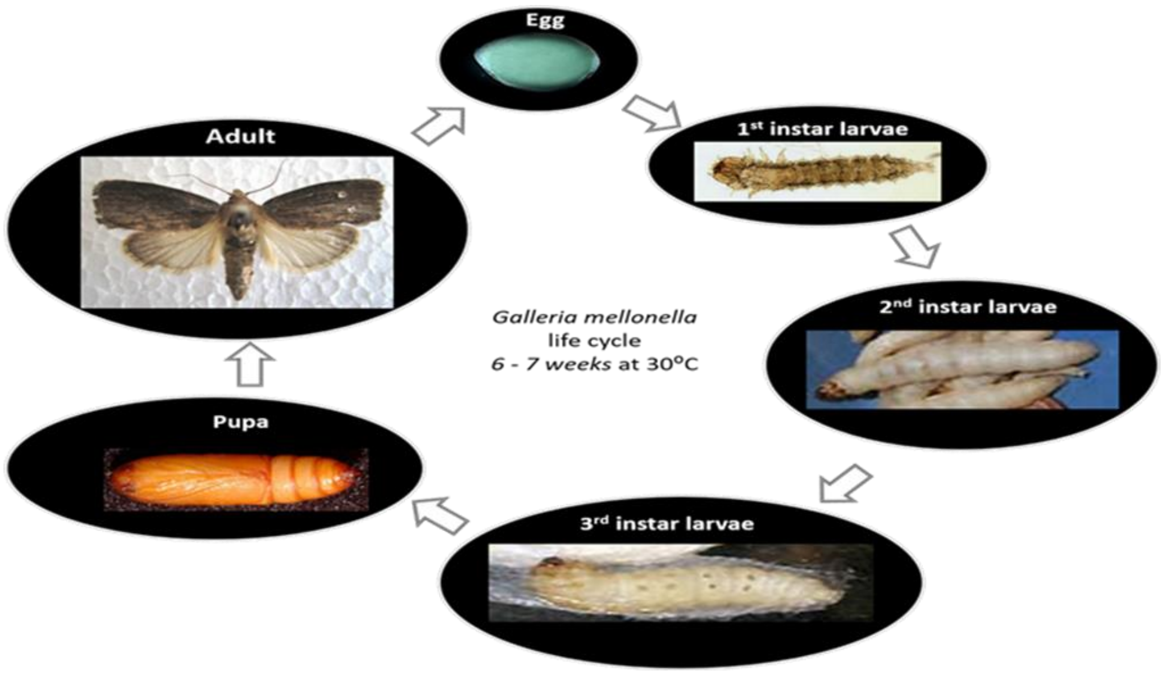


Figure 2 Life cycle of *Galleria mellonella* [30]

5. Mechanism of Plastic Degradation by Wax Worms

The enzymatic degradation of plastic by wax worms involves a series of processes that occur within their digestive system. These processes, driven by the enzymes produced by both the wax worms and their gut microbiota contribute to the breakdown of plastic polymers. Wax worms possess enzymes such as lipases and esterases that play a crucial role in the degradation of plastic polymers. These enzymes catalyze the hydrolysis of the ester linkages present in plastic molecules, leading to the fragmentation of the polymer chains [37]. The gut microbiota of wax worms also contributes to the enzymatic degradation process. It consists of diverse microorganisms that produce enzymes capable of breaking down plastic polymers. These microorganisms secrete a range of enzymes, including cutinases and proteases, which can target and cleave specific chemical bonds within the plastic polymer structure. The plastic degradation process begins when wax worms consume plastic materials. Once ingested, the plastic enters the digestive system, where it encounters the enzymatic action of both the wax worm and its gut microbiota. The enzymes present in the gut help to initiate the breakdown of the plastic polymer into smaller fragments [38]. As the plastic is enzymatically degraded, it undergoes a series of chemical changes. The enzymes act on the polymer chains, breaking them down into shorter oligomers and eventually into monomers [38]. This enzymatic cleavage of the plastic polymer leads to the reduction in molecular weight and the fragmentation of the plastic material. The smaller plastic fragments resulting from enzymatic degradation can be further metabolized by the wax worms and their gut microbiota. These fragments are utilized as a source of energy and nutrients for the growth and development of the wax worms.

Overall, the enzymatic degradation of plastic by wax worms involves the action of specific enzymes, such as lipases, esterases, cutinases, and proteases, produced by both the wax worms themselves and their gut microbiota. These enzymes catalyze the hydrolysis of the plastic polymer, leading to the fragmentation of the plastic material into smaller oligomers and monomers.

Wax worms possess oxidative enzymes that play a significant role in facilitating the degradation of plastic materials. These enzymes contribute to the breakdown of plastic polymers and are essential for the plastic-degrading capabilities of wax worms.

5.1 Laccase

This is an oxidative enzyme found in wax worms that has been shown to participate in the degradation of plastic polymers. Laccases are copper-containing enzymes that catalyze the oxidation of a wide range of substrates, including phenolic compounds [39]. Research has demonstrated that wax worms produce laccase enzymes that can act on polyethylene, promoting its degradation [40]. Laccase enzymes are involved in the initial oxidative steps of plastic degradation, breaking down the polymer chains.

5.2 Peroxidases

Peroxidases are enzymes that catalyze redox reactions using hydrogen peroxide as a co-substrate. They play an important role in the degradation of various organic compounds, including plastics. Peroxidases are involved in oxidative reactions that contribute to the breakdown of plastic polymers. In the context of plastic degradation, peroxidases act as oxidative catalysts, initiating the oxidation of plastic molecules and facilitating their degradation. They generate reactive oxygen species that attack the polymer chains, leading to the fragmentation and degradation of the plastic material [41].

For example, peroxidases have been implicated in the degradation of polystyrene (PS) and PE. Research has shown that peroxidases produced by white rot fungi, such as *Phanerochaete chrysosporium*, can degrade PS by initiating the oxidation of the polymer chains [42]. Similarly, peroxidases have been found to participate in the degradation of PE, contributing to its breakdown [43].

5.3 Cytochrome P450

Cytochrome P450 enzymes are a diverse group of oxidative enzymes found in various organisms, including wax worms. These enzymes are involved in the oxidation of a wide range of compounds, including xenobiotics and pollutants. Cytochrome P450 enzymes have been identified in wax worms and are thought to contribute to the metabolism of plastic polymers [44]. Their activity may enhance the breakdown of plastic materials and promote the degradation process.

The presence of these oxidative enzymes in wax worms highlights their role in plastic degradation. Through their catalytic activity, laccases, peroxidases, and cytochrome P450 enzymes facilitate the oxidation and breakdown of plastic polymers, contributing to the overall degradation process.

5.4 Lipases

These are a class of enzymes that catalyze the hydrolysis of ester bonds in lipids and other ester-containing compounds. In the context of plastic degradation, lipases are involved in breaking down ester linkages present in various types of plastic polymers, including PET and polylactic acid (PLA). Lipases act on the ester bonds within these polymers, leading to their cleavage into smaller molecules [44].

For example, lipases have been found to facilitate the degradation of PET by breaking down its ester bonds, resulting in the production of mono (2-hydroxyethyl) terephthalic acid (MHET) and terephthalic acid (TPA). This enzymatic activity enables the conversion of PET into more manageable compounds that can be further metabolized by microorganisms or utilized as building blocks for the synthesis of new materials.

5.5 Esterases

They are enzymes that catalyze the hydrolysis of ester bonds, similar to lipases. However, esterases have a broader substrate specificity and can act on a wider range of ester-containing compounds. In the context of plastic degradation, esterases are involved in breaking down ester linkages present in various types of plastics, including PE and PP [45].

Studies have demonstrated the role of esterases in the degradation of PE and PP. For instance, esterases produced by bacteria such as Pseudomonas and Bacillus species have been shown to degrade polyethylene films, leading to surface erosion, pitting, and fragmentation [46]. Esterases act on the ester linkages present in these plastics, initiating their breakdown into smaller molecules. Various bacteria and fungi in wax worm that secrets some enzymes that are involved in plastic degradation are listed in (Table 1).

Table 1 Microbial enzymes in wax worm and the type of plastics they degrade

S/n	Microorganisms	Enzymes	Plastics type	References
	Bacteria			
1	*Enterobacter asburiae*	Peroxidases, Laccases	PS and PE, PE	[47]
2	*Citrobacter freundii*	Peroxidases	PS and PE	[47]
3	*Bacillus* spp.	Esterases, Peroxidases, Lipases Cytochromes P450, Laccases	PE and PP, PS and PE, PET and PLA PS and PE, PE	[44, 50]
4	*Acetinobacter* spp	Esterases, Peroxidases	PE and PP, PS and PE	[47]
5	*Massilia* sp	Lipase	PLA	[47]
6	*Serratia* spp	Lipases	PET	[47]
7	*Pseudomonas* spp.	Esterases, Peroxidases, Lipases	PE, PS and PE, PET and PLA	[38]
8	*Ideonella sakaiensis*	Esterases	PE and PP	[47]
9	*Streptomyces* spp.	Peroxidases	PS and PE	[47]
	Fungi			
10	*Fusarium* spp.	Cytochromes P450	PS and PE)	[47]
11	*Penicillium* spp.	Lipases	PET and PLA	[48]
12	*Aspergillus flavus*	Cytochromes P450, Laccases	PS and PE, PE	[48]

6. Advantages of Plastic Bio-recycling using wax worm

Specificity: One of the advantages of wax worm oxidative enzymes is their specificity in targeting plastic polymers. This specificity ensures that the enzymatic treatment is focused on the plastic waste, increasing the efficiency and reducing the potential negative impacts on the environment.

Eco-friendly: The use of wax worm oxidative enzymes in plastic waste treatment is environmentally friendly compared to conventional methods. Traditional plastic degradation approaches often involve the use of harsh chemicals or energy-intensive processes, which can have adverse effects on ecosystems and contribute to pollution. In contrast, wax worm enzymes offer a more sustainable and environmentally friendly alternative. They operate under mild conditions and utilize natural enzymatic processes, reducing the reliance on harmful chemicals and minimizing environmental impacts.

Potential for large-scale implementation: The potential for large-scale implementation is an important consideration in plastic waste treatment. Wax worms can be easily cultured and mass-produced, providing a renewable and readily available source of enzymes. This scalability makes the utilization of wax worm oxidative enzymes feasible for industrial-scale plastic waste treatment facilities [48]. Their application can be integrated into existing recycling processes or developed as standalone treatment methods to enhance the overall efficiency of plastic waste management. The potential applications of wax worm oxidative enzymes in plastic waste treatment, their specificity in degrading different types of plastics, and their environmentally friendly nature make them promising tools in the fight against plastic pollution. Further research and development in this area can lead to innovative and sustainable solutions for plastic waste management, contributing to a more circular and environmentally conscious approach.

7. Challenges and Future Directions in Utilizing Wax Worm Oxidative Enzymes for Large Scale Plastic Biorecycling

While wax worm oxidative enzymes have shown promise in plastic bio-recycling, there are several challenges that need to be addressed for their large-scale implementation. One of the challenges is the efficiency of enzyme activity. Wax worm enzymes, such as the enzyme found in the gut of *Galleria mellonella*, have demonstrated the ability to degrade certain types of plastics, including PE and PP. However, the degradation rates are currently relatively low, and the process needs to be optimized for enhanced efficiency [48]. Another challenge is the optimization of the environmental conditions for enzyme activity. Factors such as temperature, pH, and substrate specificity can significantly affect the enzymatic performance. The screening, isolation and measurement of the activities of microorganisms require a suitable substrate or condition that will favour their growth and consequently high yield of enzymes [48]. The enzymes may have specific temperature and pH optima for optimal activity, and deviations from these conditions can reduce their efficiency. Additionally, the substrate specificity of the enzymes may limit their effectiveness on a wide range of plastic types [49].

To overcome the current challenges and advance the utilization of wax worm oxidative enzymes for large-scale plastic bio-recycling, further research is needed to optimize enzyme performance, improve scalability, and develop industrial applications. Firstly, research efforts should focus on enhancing the efficiency of wax worm oxidative enzymes. This can be achieved through enzyme engineering techniques, such as protein engineering or directed evolution, to modify and improve the enzymatic properties. By increasing the degradation rates and expanding the substrate specificity, the efficiency of plastic bio-recycling can be significantly enhanced.

Secondly, scalability is a critical factor for industrial applications. The production and purification of wax worm oxidative enzymes need to be optimized to meet the requirements of large-scale plastic bio-recycling processes. Cost-effective and sustainable production methods, such as recombinant protein expression systems or fermentation techniques, should be explored to ensure the availability of enzymes in sufficient quantities.

Lastly, the development of industrial applications for wax worm oxidative enzymes is essential. Research should focus on integrating these enzymes into practical and efficient plastic recycling systems. This includes the design of enzymatic reactors, process optimization, and the development of downstream processing techniques for the recovery and reuse of the degraded plastic materials. By addressing these research avenues, it is possible to optimize the performance of wax worm oxidative enzymes, improve scalability, and develop industrial applications for large-scale plastic bio-recycling.

8. Conclusion

The problem of plastic pollution has become a global challenge, with the accumulation of plastic waste in landfills, oceans, and ecosystems posing severe environmental threats. The concept of bio-recycling, which involves the use of biological agents to break down plastic materials has emerged as a potential solution. Wax worm oxidative enzymes have shown promise in plastic bio-recycling by degrading various types of plastics. However, there are challenges that need to be addressed. The efficiency of enzyme activity, affected by factors like temperature, pH, and substrate specificity, needs optimization for enhanced performance. Additionally, scaling up wax worm bio-recycling and integrating it into industrial applications require further research and development. Despite these challenges, wax worm bio-recycling offers several environmental and economic benefits. It helps reduce plastic waste, minimizes pollution, and supports the transition to a circular economy. The economic viability and market opportunities for wax worm-based bio-recycling technologies are promising, but considerations of cost-effectiveness, sustainability, and regulatory aspects are essential for successful implementation. Public perception and awareness play a significant role in promoting the adoption of these innovative recycling methods.

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