

Agro-Industrial Food Waste for Production of Industrial Enzymes

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The grave environmental, social, and economic concerns over the unprecedented exploitation of non-renewable energy resources have drawn the attention of policy makers and research organizations towards the sustainable use of agro-industrial food and crop wastes. Enzymes are versatile biocatalysts with immense potential to transform the food industry and lignocellulosic biorefineries. Microbial enzymes offer cleaner and greener solutions to produce fine chemicals and compounds. The production of industrially important enzymes from abundantly present agro-industrial food waste offers economic solutions for the commercial production of value-added chemicals. The recent developments in biocatalytic systems are designed to either increase the catalytic capability of the commercial enzymes or create new enzymes with distinctive properties. The limitations of low catalytic efficiency and enzyme denaturation in ambient conditions can be mitigated by employing diverse and inexpensive immobilization carriers, such as agro-food based materials, biopolymers, and nanomaterials. Moreover, revolutionary protein engineering tools help in designing and constructing tailored enzymes with improved substrate specificity, catalytic activity, stability, and reaction product inhibition. This review discusses the recent developments in the production of essential industrial enzymes from agro-industrial food trash and the application of low-cost immobilization and enzyme engineering approaches for sustainable development.

Keywords: enzymes ; agro-industrial food waste ; biocatalysis ; immobilization ; enzyme engineering

As global concern over food and agricultural sustainability, environmental resilience, and food safety has grown over the years, the food industry has been exploring more environmentally friendly ways to produce food and nutritional supplements. Enzyme biocatalysis, which operates at the nexus of microbiology, molecular biology, biochemistry, and organic chemistry is an illustration of a sustainable multidisciplinary technology. Biocatalysis has gained enormous industrial potential owing to its influential and eco-friendly prospects with effective kinetics and commercial benefits [1]. Enzymes are macromolecular biocatalysts with extensive applications due to their ability to operate in milder reaction conditions, high catalytic efficiency, superior product selectivity, and their negligible toxicity to the environment and the body [2,3]. They have been the subject of numerous research projects around the world in order to generate novel significant industrial processes. A variety of microbial species (bacteria, fungi) have been traditionally employed in the production of diverse products of commercial importance from different organic substrates converting them into simpler forms through enzymes [4]. Many microbes that are typically utilized as a source of enzymes have had their microbial endogenous and exogenous enzymes thoroughly researched [5]. A significant portion of microbial enzymes are utilized in a variety of industrial processes, including those for food processing, animal feed, biofuels, paper and pulp industries, pharmaceutical industries, textiles, polymer synthesis, and detergent industries [6]. Enzymes such as cellulases, xylanases, amylases, lipases, proteases, and pectinases have been commercially utilized in a wide range of industrial processes, especially in the food industry.

In recent times, the idea of 'circular economy', which refers to the use of organic waste from one industry as a source of raw materials for another, has gained much popularity [7]. It is based on the principle of sustainable development known as the '5Rs' (reduce, recycle, reuse, recovery, and restore) and replaces the traditional linear economic model (make–use–throw) with a more efficient circular one [8]. The food and agro-industrial sectors have been revolutionized owing to modernization and industrialization, which has dramatically increased the production of huge amounts of agro-industrial food waste [9]. The United Nations' Food and Agriculture Organization (FAO) estimates that every year, about 1.3 billion tons of food, which is one-third of global production, is wasted [10]. In addition to the food waste, various agro-industrial residues and crop waste in the form of lignocellulosic biomass (LCB) is generated annually around the globe [11–16]. Most of this plant-based waste is either landfilled or burned alongside other municipal combustible trash in an effort to recover energy [17]. Apparently, this organic refuse, which is a rich source of carbohydrates, proteins, lipids, organic acids, and other necessary minerals, can be channelized towards its value addition [9,18]. It could serve as an inexpensive fermentation source for microbes in the food industries, which digest it via enzymes into key components of

circular economies. The industrial applications of enzymes have significantly risen in the last decade, primarily in the food modification, biofuels production, biomedical and pharmaceutical research, and the transformation of agro-industrial waste [18–20].

Even though enzymes offer many more benefits over traditional chemical catalysts to valorize organic waste, a major bottleneck in their commercial viability is their non-reusability, high sensitivity, and poor catalytic activity and stability in extreme environmental conditions of temperature and pH [18]. These challenges therefore need to be critically removed through the development of stable biocatalytic systems. Enzyme immobilization has received significant attention in the past few years as an important engineering approach to customize and enhance a wide range of catalytic features of enzymes, including their activity, specificity, selectivity, and tolerance to inhibitors [12,19]. The development of flexible carriers, including agro-food and crop-based materials, metal organic frameworks, and nanomaterials, allows for the cost-economic immobilization of enzymes with better enzymological properties, enabling catalytic reactions to be carried out under rigorous processing environments [21].

The production of engineered enzymes by promising protein engineering tools such as directed evolution, rational design, and computational methods, aids in improving the enzymological properties with increased purity, catalytic efficiency, specificity, and expression yield, owing to the altered amino acid sequence [22]. The application of tailored enzymes for food processing enables their cost-effective production to achieve sustainable development. Figure 1 shows schematic representation of enhancing the value of enzymes by immobilization and protein engineering approaches through valorization of agro-industrial food/crop waste.

This review encompasses the valorization possibilities of agro-industrial food waste through microbial enzymes. The review also highlights the novel strategies for food enzyme immobilization and their potential applications in the food industry. Moreover, the deeper insights on development of engineered enzymes for sustainable and green processing of the waste biomass into diverse bioproducts are highlighted.

Traditionally, the microbial enzymes have been of much importance in food preparation techniques. The state-of-the-art developments in enzyme technology over the last few years have led to the creation of novel enzymes with a broad range of applications in several industries. These industries are majorly associated with biofuel production, food modification, agro-industrial waste transformation, laundry, and pharmaceutical and biomedical research [18]. Microorganisms including fungi, yeast, and bacteria, as well as their enzymes, are frequently utilized in a variety of food preparations to enhance flavor and texture, and they also provide enormous economic advantages to various enterprises [40]. The majority of the world's enzyme application is categorized into two categories as special enzymes used in research, therapeutics and diagnostics, and industrial enzymes for food and animal feed industries. The market for these enzymes is estimated to reach at about \$7 billion USD by the year 2023 and is expected to increase at a 7.1% compound annual growth rate from 2020 to 2027 [41,42]. The valorization of agro-industrial food wastes by the production of low-cost enzymes under solid-state fermentation is a promising and extensively explored method [17]. Different sets of enzymes such as amylases, proteases, lipases, laccases, cellulases, xylanases, and pectinases, among other enzymes, have been produced by SSF using inexpensive food wastes. SSF offers a number of benefits, including lower cost, higher yield, less waste, and simpler equipment and culture media derived from organic, solid agricultural products or waste.

Low-Cost Enzyme Immobilization Strategies

The bioconversion of agro-industrial food and crop waste into valuable fuels and bioproducts often depends on the cost of the method, equipment, and infrastructure, as well as the market value of the finished products. Enzymes offer an array of advantages in the valorization of abundantly generated agro-food wastes [19,21,41,42,69]. They perform biochemical catalytic reactions with exceptional specificity and decent stereo-selectivity, and, under very mild reaction conditions, enable the development of more environmentally friendly, green, and sustainable biochemical processes [21]. The operating range of enzymes is, however, rather constrained, and enzymes originating from natural sources are particularly effective only under their optimal conditions [18]. Moreover, a lack of maintenance of their stability, catalytic activity, and recovery under variable industrial bioprocessing conditions of pH, temperature, water activity, and solvent properties is a major hurdle [71]. Therefore, the development of steady biocatalytic systems involving enzymes is crucial for expanding their commercial applications. In the last few years, enzyme immobilization has received significant attention as an important bio-engineering approach to customize and enhance a wide range of enzyme catalytic characteristics, including activity, physicochemical stability, specificity, selectivity, and tolerance of inhibitors [12,72,73]. The overall cost of the enzymatic production process can be reduced by immobilizing the industrially significant enzyme support matrixes [74]. To create reusable, long-standing, and stable immobilized biocatalytic systems, the choice of the supporting matrix and the immobilization technique is essential [4]. A number of emerging support materials have been recently used for enzyme

immobilization, such as magnetic nanoparticles, graphene oxide, polyurethane foam, or chitosan [75]. The inexpensive immobilization matrixes possess several advantageous properties that help in developing highly efficient biocatalysts (Figure 4).

Enzyme Engineering Approaches

Though enzymes catalyze a wide variety of biochemical reactions, yet they are not suited for many essential catalytic bioprocesses or other industrially relevant substrates that are beyond their natural cellular micro-environments. The desired attributes of diverse industries can be satisfied by using tailored enzymes in novel, cutting-edge enzyme engineering and stabilization techniques, opening up new opportunities for their use in biocatalysis. In the present scenario, the high value of protein engineering has been well recognized in industrial-level biotransformation [100]. Protein engineering, with assistance from molecular approaches or directed evolution, rational design, or computational methods, enables the accelerated designing of biocatalysts that are ideal for any desired bioprocess with commercial applications [101]. Figure 5 explains the different strategies of enzyme engineering for improved biocatalysts.

Current Challenges and Future Prospects

Sustainable development based on the idea of a circular economy could possibly assist in achieving the targets of global waste minimization, valorization, and its recycling. The agro-industrial food waste which is an underutilized resource ideally fulfills the criteria for circular economy for conversion into useful bioproducts. Agro-food waste contains a significant amount of latent nutrients that can be efficiently extracted, recycled, repurposed, and used as substrates for enzyme production. Enzymes have been widely explored in the food industry and lignocellulosic biorefineries for producing numerous value-added biochemicals. However, the scaling up of enzyme production still faces a huge research gap to meet the industrial requirements. The significant challenges and barriers, including high production costs, low stability, and long reaction times, among others, still persist in the commercial applicability of enzymes. Moreover, the market cost of enzymes is quite high owing to the fact that expensive synthetic substrates and processes are used for their production. For enzyme prices to be competitive, they would need to be an average of \$0.10 per gallon [123]. The development of biocatalytic enzyme systems from low-cost agro-food wastes represent a distinctive technological approach for environmental and economical sustainability. Different strategies to improve enzyme production costs have been proposed through comprehensive research efforts over the past few decades. Additionally, the shortlisting of agro-food wastes as the carriers for enzyme immobilization with various operational requirements is challenging, but exciting in terms of further mitigating the cost-related issues of enzyme applicability at industrial levels.

There are certain challenges also related to enzyme immobilization practices. These include enzyme distortion during immobilization, steric hindrance of enzymes with substrate, rapid consumption of the substrate, etc. The distortion of the enzyme during immobilization occurs when the enzyme is being used under more severe conditions than normal conditions. However, stabilizing the enzyme during immobilization might allow for higher activity than the soluble enzyme. The steric interference of enzymes with the substrate may depend upon the enzyme loading on the immobilization support. If the surface of the support material does not completely block the active site, a reasonable activity against large substrates can be found with minimal enzyme load, resulting in enzyme molecules with free space around them to bind with the substrate. Apparently, the substrates with different molecular sizes and using different enzyme loadings could help in understanding if the issues are caused by steric hindrances or enzyme distortion. In certain cases, the enzyme is physically adsorbed on the surface of the immobilization matrix and may release from the support, resulting in lower efficiency. This issue may be discovered by measuring the activity in the washing solutions, particularly the initial ones. Therefore, a deeper understanding of the mechanisms of enzyme immobilization on the support matrixes is necessary to mitigate these inadequacies.

Looking forward, the improvements in the science and engineering knowledge for choice of microorganism, enzyme production under SSF and SmF systems, and maintenance of optimum chemical, physical, and biological parameters could develop the sustainable bioprocess. The development of stable biocatalytic systems by novel immobilization technologies using agro-food wastes as carriers could also elevate the industrial applications of enzymes. Over the last several years, the increase in the market demand for enzymes to establish new technological bioprocesses has substantially driven the need for engineered enzymes with unique biocatalytic and economic attributes. Research on engineering of the local enzyme environments and their catalytic regions using exciting computational and machine learning technologies is expected to further increase in coming years and involve multi-step reaction cascades, economizing the overall bioprocess. Moreover, powerful tools like life-cycle assessments and techno-economic analyses

could be used for the evaluation of the viable commercial-scale biocatalytic processes. Further understanding of enzymes can be more effectively used in a range of industrial processes, which will come from research studies of both known and yet-to-be discovered enzymes.

Conclusions

The management of food and agricultural trash is one of the most pressing issues for modern civilization. The proper repurposing of agro-food wastes utilizing green technology is critical to reduce the negative and destructive consequences of waste disposal that produce compounds with added value, aiding towards implementing circular economy. Microbial enzymes play a key role in the valorization of agro-industrial crop and food wastes compared to conventional chemical catalysts. The utilization of agro-food waste to produce commercially important enzymes by microorganisms offers great promise for efficient waste utilization and sufficient biocatalytic systems with high conversion efficiencies, thereby allowing achievement of the targets of sustainable development. Furthermore, using novel, inexpensive enzyme immobilization supports and engineered enzymes can exhibit improved catalytic performance when applying them to industrial food applications.

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