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Production of Enzymes from Agricultural Wastes

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Abstract

Waste obtained from agriculture field is highly nutritious which helps in the growth of microbes. Agricultural wastes predominantly comprise of lingo-cellulose large fraction of which contains polysaccharides. Therefore, these agricultural residues are potential sources of valuable products like industrial enzymes. The agricultural wastes like corn cob, rice bran and sugarcane bagasse have already been extensively used in various fermentation processes to synthesise enzymes. Agricultural wastes are often pre-treated before using them for fermentation so as to improve the yield of enzymes. This article intends to outline how the agricultural residues can be utilized as a raw material to synthesize enzymes of industrial importance. Furthermore, it will give an overview of the different types of enzymes obtained from these agricultural wastes.

Keywords: Agricultural Waste, Enzymes, Fermentation, Microbes

Introduction

Currently, agricultural industry is exponentially expanding. The improving economical growth and rapid increase in population has gained the interest of investors in agricultural industry, worth $3.2 trillion as per estimate of world bank. With urbanization and industrialization in the field of agricultural sector, the generation of waste has also increased and is now imposing several environmental challenges. From agriculture, approximately 5,000,000 metric tonnes of biomass is generated every year. This waste generated by agricultural industries serve as the breeding ground for pathogenic microbes, if not treated adequately and left unprocessed. Remarkably, these agricultural residues can be used as substrates for synthesizing valuable products or can act as a raw material for production of renewable energy. The concept of 'Bio-economy' and 'Bio-refinery' introduced by European

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Union has provided new direction, where the waste generated by one industry can act as potential substrate for another (Ravindran et al., 2018).

Most of waste generated in agricultural sector are lingo cellulosic in nature which encompass polysaccharides like hemi-cellulose, cellulose and lignin along with few other nutrients like pectin and proteins. Industries demand a persistent source of economical raw material for effective and sustainable operation. Therefore, connecting waste streams of specific industries with agriculture industries for successful valorisation will solve the challenge of waste accumulation (Donato et al., 2015).

Overview of Wastes generated by Agricultural industries and its Valorisation

Approximately, 1/3rd of food produced for consumption is wasted every year. Vegetables and fruits, along with tuber and roots account for the highest wastage rate, which represents 89 million tons from the total agricultural residue of 367 million tons per year. A part of this is utilized by farmers for animal bedding, animal fodder and different horticultural processes. Over the past decade, various studies have been conducted to employ agricultural wastes as substrates for generating valuable products (Bhuvaneshwari *et al.*, 2019). Presently, production of bio-ethanol and other bio-fuels are the industrial processes that use agricultural residues as raw material. The process involves the pre-treatment of waste followed by saccharification and fermentation. However, researchers are now exploring for viable strategies to convert lingo-cellulosic waste generated by different agricultural industries into suitable substrate for the synthesis of different enzymes (Sadh *et al.*, 2018).

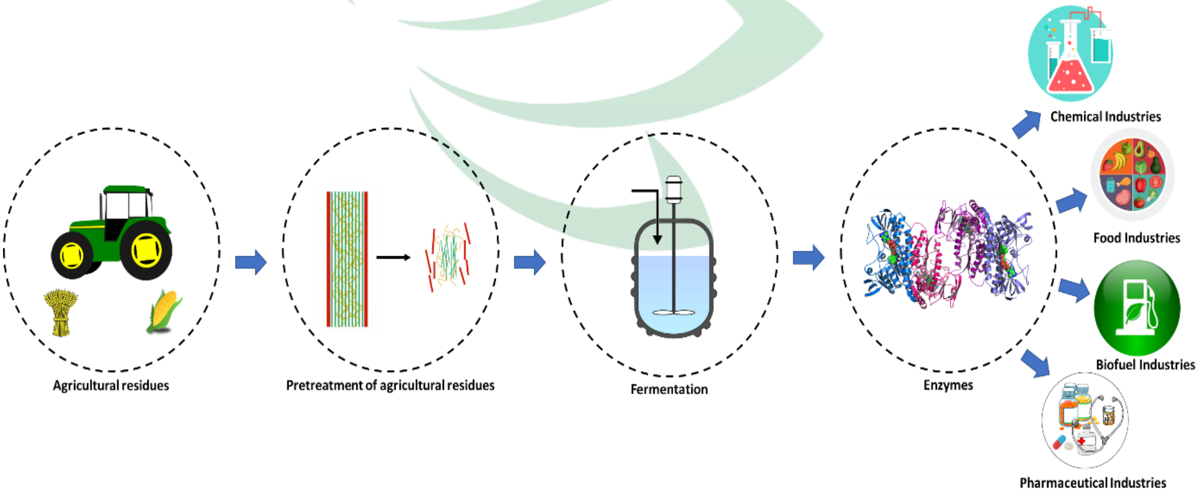

Figure1: Industrial production of enzyme utilizing agricultural residues

Industrial Enzymes

Enzymes are natural biocatalysts that have application in various industries like brewing, baking, detergent, paper and pulp industries. Owing to their high substrate specificity and rapid operative parameters, they are favoured over chemical catalysts. Enzymes are also synthesized by living organisms to meet metabolic demands but in low quantities. However, progress in molecular biology has allowed researchers to clone and synthesize microbial origin enzymes at massive scale according to the demand of different industries (Bharathiraja *et al.*, 2017).

The industrial enzymes global market has been predicted to rise from 4.5 billion USD (2016) to $6.2 billion USD (2021). Enzymes are cost-intensive which add additional cost to operational processes. Furthermore, detailed analysis of economics of enzyme bioprocess states that 50% of the production cost is related to capital investment, whereas raw material cost only one-third of the total cost. Therefore, substitution of feed stocks with agricultural waste can substantially increase return on investment (de Castro *et al.*, 2014). Below mentioned is an overview of some enzymes produced using agricultural waste at industrial scale.

α-amylase

Alpha amylases belong to that class of enzymes that are known to breakdown α-1, 4 bonds present between two glucose units in a polysaccharide chain. The action of α-amylase result in the generation of α-limit dextrins and short chain oligomers. α-amylases are widely used for various purposes in different industries brewery, baking, paper and pulp, detergent, pharmaceutical and textile industry. For industrial use, α-amylases are mostly produced via a process known as submerged fermentation, often utilizing genetically improved strains of bacteria (Bacillus sp.) and fungi (Aspergillus sp.). Till date, a number of α-amylases have been obtained from different microorganisms that exhibit an array of properties like tolerance in saline environment, stability at high temperatures and towards alkaline solutions.

Cellulase

Cellulases are a group of enzymes belonging to a family of enzymes known as of glycoside hydrolases and are of great importance not only to industries but to natural world as well.

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Cellulases disintegrate the complex and crystalline cellulose fibres present in the lignocellulosic biomass (LCB) to release simpler sugars. Endoglucanases, exoglucanases and β-glucosidases are the main enzymes that consolidate cellulases which hydrolyse cellulose by breaking down glycosidic bonds between glucose subunits in the cellulose. Our planet has abundant lignocellulosic biomass generated from dead plants and agricultural residues. Cellulases aid in the destruction of cellulose present in LCB in the environment. From industrial perspective, they find a range of applications biorefinery, brewing, baking, detergents, textile, paper and pulp industry. Cellulases are mostly produced from Trichoderma reesei or its improved strains for industrial use. Apart from this, a number of microorganisms have been explored that produce cellulases such as Clostridium thermocellum Schizophyllum commune, Bacillus circulans Melanocarpus sp., Proteus vulgaris Aspergillus sp., Klebsiella pneumonia, Penicillium sp., Escherichia coli, Fusarium sp. and Cellulomonas sp (Jayasekara & Ratnayake, 2019).

Xylanase

Plant polysaccharides contain xylans as complex integral heteropolymers which are degraded through the action of enzymes xylanases. Due to heteropolymeric structure, xylan requires a group of enzymes for its hydrolysis such as p-coumaric acid esterase, endoxylanases, ferulic acid esterase, β-xylosidases, α-glucuronidase and acetylxylan esterase. Among these, most of the research has been carried out on β-xylosidases and endoxylanases. Xylanases are used in industries like biomedical, bioethanol, animal feed and food industry amid which they are majorly used in biorefinery where they are required to disintegrate the xylan present in LCB so as to further process the biomass for production of biofuels or other value-added products. Xylanase are not commonly produced by microbes. In contrast to bacteria, fungi have been majorly explored for xylanases as they produce high quantities of extracellular xylanase (Polizeli *et al*., 2005).

Hemicellulases

LCB contain a matrix of heteropolymeric hemicellulose fibres which are hydrolysed by a group of enzymes known as hemicellulases. They breakdown the glycosidic linkages present in the hemicellulose structure which not only contains glucose but other sugars as well like galactose, xylose, arabinose, mannose etc. In addition to breaking down glycosidic linkages, they are also equipped to attack esterified side chain groups present in the structure. α-arabinofuranosidases, α -glucuronidases, mannanases and α-d-galactosidases are among the most common hemicellulases that have been studied (Shah *et al*., 2015).

Control of Fermentation Processes

With the advancing fermentation technology, numerous studies are being conducted to assess the efficacy of enzyme production by different microbial strains using agricultural waste at lab or pilot scale. These extensive studies have rejuvenated the interest of researchers in solid-state fermentation for synthesis of diverse enzymes. Various residues of agricultural industries available are found to be suitable in terms of sustainability (Lizardi-Jiménez & Hernández-Martínez, 2017). Recently, researchers have introduced a new term mechanical index (Imp), which is used to categorized the physical properties of media and aids in implementation of high level of process control during fermentation. Furthermore, Imp can also positively or negatively correlate with biomass content, water retention, thermal conductivity, thermal diffusivity and gas permeability, as all of these parameters are involved in fermentation process. Hence, Imp can be used for effective determination of physical properties of medium and controlling the different parameters in solid-state fermentation process (Zhang *et al.*, 2017).

Conclusion

Ligno-cellulosic waste is readily available as cheap form of carbohydrate source for valorisation and generation of value-added products. Various microbes can be employed for the synthesis of different enzymes utilizing agricultural residues. Additionally, the pre-treatment of these wastes has also improved its saccharification rate even at low loading of enzyme. Literature evidences that agricultural waste can be potentially used as low-cost source for enzyme synthesis Moreover, extensive research is required in this direction to scale up the process.

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