

Agricultural and Food Waste Treatment Using Microbial Enzyme Extracted from Thermophiles

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Abstract

Agricultural and food waste comprises the waste that produces throughout the food production and supply cycle. The problem with agricultural waste and food waste has been profound in many parts of the world and it poses an increasing risk to the natural ecosystem at an alarming scale. Researches have shown how utterly important it is to start naturally treating and managing these wastes as soon as possible to minimize and mitigate the impact on nature. The answer to this question lies in naturally occurring thermophiles. These are the microorganisms which grow optimally at higher temperatures between 60 to 80 degree Celsius. This group of microorganisms produces a variety of enzymes depending on their types. Many enzymes produce by thermophiles have been proven to be effective against treating waste related to agriculture and food. Thermophilic enzymes including cellulase and xylanase digest the Agri-food waste at optimal temperature, producing useful byproducts. Efficient use of thermophilic enzymes in waste management is not only less costly but also eco-friendly and beneficial to humankind. Manipulating thermophilic enzymes with sequencing of existing and new genomes will allow known and new enzymes with possibility of various application capacities.

Keywords: Agricultural and Food Waste Management; Enzyme Extraction; Microbial Enzyme; Thermophiles

Introduction

Agricultural and food waste is increasingly becoming a major global problem costing billion each year for its management. In Australia alone, government spends \$20 billion per year for about 7.3 million tonnes of food waste management [1]. Agricultural waste is another significant aspect of environment related waste posing similar hindrance in ecological and economic wellbeing. In total, both agricultural and food waste contribute to about 5% of Australian greenhouse emission [1]. The immensity of environmental pollution due to food and agricultural waste is no longer taken as an inconsequential or minor issue. Intensive agricultural practices and crop production is demanding more portion of natural resources than ever before. Efficiency of Agri-production and current environmental health seems to be inescapably intertwined and mostly emerges to be diametrically divergent [2].

Through the course of evolution of organisms, some exceptional microorganisms possess the unique ability to survive in extreme surrounding such as higher or lower Ph in acidic or saline environment, extreme high temperature, environment with or without the presence of Oxygen (aerobes and anaerobes), ability to utilize different sources of nutrient concentration, electromagnetic field and exposure to ionizing radiation, hydrostatic pressure [3]. Some of these extremities in natural setting give these microorganisms a unique quality different from other. One of such many organisms is thermophile.

Thermophiles and thermophilic enzyme

The microorganisms that survive at extreme temperature are generally referred as thermophiles. Many studies have shown that thermophilic organisms are particularly valuable in treatment of environmental pollution generated from food and Agri-waste.

These microorganisms thriving in temperature elevated condition possess the ability to exploit a range of cellulolytic carbohydrates. The nutrient residue present in lignocellulosic biomass at the higher temperature is utilized by thermophiles in the conversion process to bioenergy [4]. The degradation of biomass and substrate is optimal temperature dependent on thermophile biotype. Their ability to utilize α - and β -linked glucans found in starch of barley, chitin and laminarin, hemicellulose, xylans and mannans depends on optimal temperature of thermophilic biotype. Study suggests that optimum temperature ($T_{opt} \geq 80^\circ\text{C}$) helps to utilize crystalline cellulose substrate to some extent with free primary cellulases in microorganisms with temperature limiting the growth at 75°C [4]. The process of biomass deconstruction in thermophiles differs in temperature with ability to use either free cellulases or multi-enzyme cellulases complex such as cellulosomes. The capability of thermophiles to utilize food and agricultural waste is mainly based on the ability to use thermophilic primary cellulases, secondary cellulases such as endoglucanases and enzyme-complex such as cellulosomes.

Cellulose in the form of polysaccharides is found to be a major nutritional source prevalent in food and agricultural waste for

microbial growth and enzyme production. The waste material with cellulosic composition is utilized by mesophiles and thermophiles to convert into glucose and soluble sugars in the presence of cellulase enzyme [5]. The multi-enzyme complex in cellulase is composed of different soluble enzymes in extracellular space, namely, 1, 4- β endoglucanase, 1, 4- β -exoglucanase, and β -glucosidase (β -D-glucoside glucohydrolase or cellobiase) [6]. The widely explored and vital biotype responsible for cellulose deconstruction are known to be soil microorganisms. Researchers suggest that, *Bacillus* sp, *Cellulomonas*, *Thermoactinomyces* and *Pseudomonas* are the predominant cellulose digesting bacteria [7].

The role of thermophiles as biocatalyst to deconstruct lignocellulose is promising. However, more research and scientific investment is needed to optimize the output for waste treatment. This proposal aims to explore the new and existing possibilities of manipulating thermophiles and enzymes to exploit them in efficient and effective food and agricultural waste management.

Enzyme extraction process and application

Figure 1 shown above shows the simple representation of thermophilic enzyme production process and the application.

Figure 1: Thermophilic enzyme production and application (Zhu., *et al.* 2020).

When processed agriculture and food waste around 50°C for 72 hours, subject it to microbial digestion there is a higher chance of isolating thermophiles with cellulose decomposing enzymes. Cellulase produced can be accumulated from Luria Bertani (LB) agar plate [8]. A clear bacterial cellulose digestion activity on LB plates indicate the presence of cellulase and digestion of lignocellulose complex from waste materials. The same LB plates can be used to screen microbial enzyme activities when subjected to different reagents. The isolates with higher diameter of a clear zone can be morphologically and biochemically identified using API kit and also using Bergey's manual for systematically identifying bacteria.

In recent experiment conducted by Acharya, *et al.* [9] bacterial is the most prevalent microorganisms responsible for cellulose degradation at higher temperature. He concluded that *Bacillus spp.* are commonly and actively involved in cellulase production. Another experiment led by Ray, *et al.* [10] in cellulose basal growth media to examine the capability of *Bacillus subtilis* to digest lignocellulose with vigorous production of cellulase supports the hypothesis of predominant presence of thermophilic bacteria at higher temperature with abundant source of cellulose. The exceptional outcome of 30mm cellulolytic digestion zone was generated by thermophiles in his experiment. On separation and screening experiment steered by Verma, *et al.* [11] on sample collected from agricultural soil, the ability of optimal enzyme production in bacteria is achieved at 45°C, with 48 hours of incubation of *Bacillus subtilis* in a media containing 1.5% of carboxymethylcellulose (CMC). Among the 59 microbial isolates isolated by Abo-State, *et al.* [12] from agricultural and food waste, 37 isolates showed the active cellulolytic enzyme production were *Bacillus sp.* But a journal published by animal science and technology has identified another species of *Bacillus licheniformis* DK42 can produce xylanase and cellulase to decompose xylan and cellulose respectively [13].

Conclusion

When the most active enzyme producing microbial colonies is isolated for complex polysaccharides digestion it can optimally digest agricultural and food waste. The identification of individual thermophile species with effective enzyme production ability can be done using morphological characteristics, biochemistry and API kit. Detail analysis of thermophilic enzyme, quality and quantity

is needed to be carried out for effective condition for enzymes expression in thermophiles for optimal breakdown and treatment of agricultural and food waste. Provided the specific enzymes for specific polysaccharides digestion, soon we will be able to reuse and recycle agricultural and food waste for the welfare of nature and humankind as a whole.

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