Thermophiles: A Bio-Gadget towards Waste Reclamation through Cellulase Production

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***Abstract*—Lignocellulose wastes dominate our earth being considered as a serious cause for environmental pollution issues. However the lignocellulosic biomass can be converted to higher value-added products such as biofuel, enzymes etc. usually requiring a multi-step processing such as pretreatment, hydrolysis, fermentation etc. Cellulose is major polysaccharides in lignocellulosic biomass wastes being converted into simple sugars usually requiring synergistic action of multiple cellulolytic enzymes from different bacteria. Thermophiles act as a better source towards better lignocellulose biomass utilization for fermentation for various value-added product as well as biofuel production. Several researches were conducted on cellulolytic activity of thermophilic bacteria using various substrates. However the present study is mainly based on waste utilization containing cellulose as major component into different byproducts through cellulase enzymes from thermophiles which would enlighten the present market towards waste utilization ultimately combating the environmental pollution.**

***Index Terms*—Lignocellulose biomass, enzymes, thermophiles, cellulase.**

I. Introduction

The greatest challenges of twenty-first century is to meet the growing demand of energy for transportation, heating and industrial processes, and to provide raw materials for chemical industries in sustainable ways Wastes are present in many forms all around our environments which can be better utilized using microbes for various activities finally converting them into biodegradable form before releasing to the environment ultimately reducing the pollution problem. Lignocellulosic materials act as a major component of environmental wastes because of rising urbanization which are essential to the functioning of modern industrial societies. Recently lignocellulosic biomasses have gained increasing research interests and special importance because of their renewable nature [1], [2]. Therefore, the huge amounts of lignocellulosic biomass can potentially be converted into different high value products including bio-fuels, value added fine chemicals, and cheap energy sources for microbial fermentation and enzyme production [1], [3], [4], etc.

II. Biotechnological Importance of Lignocellulosic Biomass

From the biotechnological point of view a wide variety of lignocellulosic biomass resources are available as potential candidate that are also convertible into high value bi-products like bio-ethanol/bio-fuels, enzymes etc. There is a vast improvement of green biotechnology occurred related to lignocellulose biomass in the current scenario. A considerable amount of such materials as waste byproducts are being generated through agricultural, domestic, municipal and industrial practices. Sadly, much of the lignocellulosic biomass is often disposed of by burning, which is not restricted to developing countries alone. Recently lignocellulosic biomasses have gained increasing research interests and special importance because of their renewable nature. The ever increasing costs of fossil fuels and their greenhouse effects are creating a core demand to explore alternative cheaper and eco-friendly bio-fuels resources as a strategy for reducing global warming [1]-[3]. Ligninolytic, cellulases and hemicellulases are important industrial enzymes having numerous applications and biotechnological potential for various industries including chemicals, fuel, food, brewery and wine, animal feed, textile and laundry, pulp and paper and agriculture [5], [6].

III. Thermophilic Bacteria

Recent research is mainly focused on thermophiles because of their better industrial utilization. Tolerance to high temperatures is one of the major covetable properties for cellulase in industrial activities [7]. For example; a) in textile industry, during biopolishing of cotton, the required enzyme would be highly stable at temperature up to 100 ℃ [8]; (b) in food industry, unsatisfactory acidic depolymerization of cellulose leads to the increased demand of thermostable cellulases [9]. There are various earlier reports available on thermophilic bacteria having cellulolytic properties from various natural environments. For eg. *Bacillus subtilis* [10], *Bacillus licheniformis* MVS1 [11], *Caldicellulosiruptor saccharolyticus* [12], *Clostridium thermocellum*, *Paenibacillus* sp., *Pyrococcus horikoshi* etc. [10]–[12]. Many thermophiles having optimum temperature range between 65-70 ℃ may include neutral to alkalithermophilic (pH 6-10), acidothermophilic (pH 2-5) as per the pH requirement.

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IV. Cellulase Enzyme from Thermophiles

Among the diverse group of microbes, bacteria act as a major environmental source due to their distinguishing characteristics such as adoptability at any environmental conditions, short generation time, better expression system and genetic engineering, have the extreme capacity to produce highly stable enzyme and serve as highly potent sources of individually important enzymes [13], [14]. Cellulolytic bacteria are mostly including aerobic, anaerobic, mesophilic, thermophilic, psychrophiles, halophiles etc. For enhanced thermostable cellulase enzyme production, a number of formulated production media as well as different substrate need to be optimized. Recent research is mainly focused on cellulases from thermophiles because of their better industrial utilization. There are various earlier reports available on thermophilic bacteria having cellulolytic properties from various natural environments. Many thermophiles having optimum temperature range between 65-70 ℃ may include neutral to alkalithermophilic (pH 6-10), acidothermophilic (pH 2-5) as per the pH requirement. Many thermophiles having optimum temperature range between 65-70 ℃ may include neutral to alkalithermophilic (pH 6-10), acidothermophilic (pH 2-5) as per the pH requirement. Tolerance to high temperatures is one of the major covetable properties for cellulase in industrial activities. For example; a) in textile industry, during biopolishing of cotton, the required enzyme would be highly stable at temperature up to 100 ℃; (b) in food industry, unsatisfactory acidic depolymerization of cellulose leads to the increased demand of thermostable cellulases.

V. Cellulase Production Using Lignocellulosic Wastes

Lignocellulosic wastes have become a serious cause for environmental pollution. Waste biomass is a heterogeneous matrix of three interlinked polymers of cellulose–hemicellulose–lignin. Polymers such as cellulose and hemicellulose need to be fractioned and converted into monomers using enzyme-catalyzed process for the production of value-added products. Enzymatic conversion of cellulose to simple sugars (for the production of platform chemicals) represents major costs in overall process of biomass to value-added product generation. So, extensive studies have been conducted for cellulase production from bacteria and fungi along with accessory proteins that act synergistically and even in adverse conditions like in the presence of inhibitory components released during chemical and thermal pretreatment of biomass [15]. So there is a need towards searching of novel bacterial strains from extremophiles like thermophiles from different thermal environment. There are different methodologies for cellulase enzyme production from various wastes were listed in Fig. 1.

A. Research Activities on Lignocellulose Decomposition

Isolation of new thermostable bacterial strain from different wastes as substrates like domestic, municipal, industrial etc. is also another major aspect towards waste utilization as well as enhanced cellulase enzyme production. For the bioconversion of cellulose substrates for example, pure commercial substrates like CMC or avicel, agro-wastes, vegetable peel wastes, lignocellulosic plant biomass, fruit wastes etc., bacterial cellulases act as inducible enzymes. The new bacteria could be isolated from the raw substrates/wastes and could be optimized at high temperature and also thermostability could be studied for better cellulase activity which may lead towards search for novel new strains for waste management and various industrial product formation etc. Various bacterial strains involved in waste utilization for cellulase production are described in Table I.

TABLE I: Waste Utilization by Different Microorganisms for Cellulase Production

Sl. No.	Type of wastes	Bacterial strains	References
1	Municipal solid waste compost	*Thermomonospora curvata*	[22]
2	Banana waste	Consortia	[23]
3	Coffee pulp waste (CPW) and pineapple waste (PW) residues	*Acinetobacter* sp. TSK-MASC	[24]
4	Fruit Waste	*Streptomyces* sp.	[25]
5	Palm oil industrial solid waste	Actinomycetes Isolate 12.3.A	[26]
6	Cassava bagasse, pine leaves, wheat bran and rice bran	*Cellulomonas cellulans*	[27]
7	Agro-Industrial Wastes	*Bacillus licheniformis* MTCC 429	-
8	Wastes dumpsites in Lagos, southwest Nigeria	*Bacillus licheniformis*	-
9	Coir waste and saw dust as substrate	*Bacillus* sp., *two Pseudomonas* sp. and *Proteus* sp.	-

Geobacillus sp. HTA426, a cellulolytic hot spring isolate, was studied for utilization of wastes such as sugarcane bagasse (CMCase activity = 103.67 U/mL), rice straw (74.70 U/mL) and water hyacinth (51.10 U/mL) for cellulase enzyme production. The CMCase enzyme was thermostable over a wide temperature range of 50 ℃ - 70 ℃ after 5 h of incubation and ultimately proved to be a potential candidate for developing a more efficient and cost-effective process for converting lignocellulosic biomass into biofuel and other industrial process [16]. *Bacillus* sp. isolated from lignocellulosic waste materials such as raw & defatted Palm kernel cake (PKC) and also vegetable wastes showed highest cellulase activity i.e. 2.65 FPU/ml, 7.73 FPU/ml and 85.48 FPU/ml respectively at 50 ℃ [17]. *Bacillus vallismortis* RG-07, a thermos-solvent tolerant soil thermophile was reported maximum cellulase production i.e. 4105 U ml^{-1} at 65 ℃ using sugarcane bagasse as substrate [18]. A hotspring extreme thermophile, *Isoptericola variabilis* sp. IDAH9, showed CMCase i.e. endoglucanase activity using rice bran & CMC as substrate at 65 ℃ [19]. *Bacillus subtilis*, a faecal thermophile exhibited better cellulolytic activity i.e. 4 323 ± 0 065 U ml^{-1} at 60 ℃ using corn stover as substrate. The crude cellulase was found to possess a strong antibacterial potential against *Staphylococcus aureus*. The thermophilic strain could be used in cost-efficient cellulase production using agricultural residual biomass useful for biofuel production [20]. Cow dung were used as substrate for thermophilic *Bacillus* sp. having cellulase activity at 50 ℃ using NH_4NO_3 as nitrogen source [21]. Vegetable market, horse dung, municipal waste, mushroom compost, decomposing litter nests of birds, soils from furnace area, zoo dump, cattle dung and industrial wastes could be used as source for novel thermophilic cellulolytic bacteria. There is a large range of commercial cellulases available from microbial sources. These cellulases also showed activity at a temperature range between 55-60 ℃.

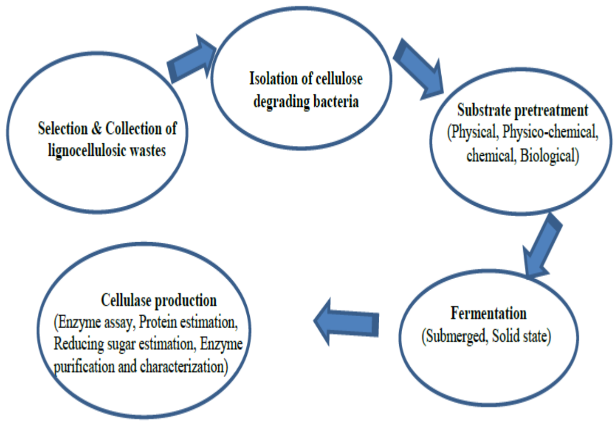

Fig. 1. Detail methodology for various wastes to enzyme production.

VI. Conclusions

Thermophiles represent a better competent for production of value-added products as far as global industrial enzyme market is concerned. The present paper is mainly focused on study of cellulase from thermophilic bacteria using lignocellulosic wastes. The lignocellulose waste biomass holds a significant replacement towards renewable energy demand in the current industrial market scenario. The cellulase enzymes from thermophiles play a major role in waste recycling as well as reclamation. Biotechnology as well as bio-engineering play major role towards waste management for improvement of soil health in place of addition of various toxic chemicals for waste recycling as well as products like ethanol, butanol, and other biofuels etc. from waste lignocellulosic biomass.

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