BIOCHEMICAL UTILIZATION OF AGRO-INDUSTRIAL LIGNOCELLULOSES RICH WASTE FOR CELLULASE PRODUCTION

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***Abstracts*:** *Cellulose is the most abundant renewable natural biological resource, and the production of bio-based products and bioenergy from less costly renewable lignocelluloses materials in form of agro-industrial biological waste. The cost of enzyme production can be significantly reduced if low value biological substrates like fruit processing waste. The cost of lignocelluloses raw material influences the economy of many fermentation processes, hence the cost play a decisive role in future and scope of industries employing fermentation processes. Discarded plant parts like peels of fruits and agro harvest, paper huge waste in the civil area and generate obnoxious odour, can be effectively utilized for the production of cellulase enzymes. The recognition that environmental pollution is a worldwide threat to public health has given rise to a new massive industry for environmental restoration. Submerged and solid tate fermentation systems have been used to produce compounds of industrial interest from lignocellulose, as an alternative for valorization of these wastes and also to solve environmental problems caused by their disposal. When submerged fermentation systems are used, a previous stage of hydrolysis for separation of the lignocellulose constituents is required. The major constituents of lignocellulose are cellulose, hemicellulose, and lignin, polymers that are closely associated with each other constituting the cellular complex of the vegetal biomass It can be degraded by cellulase enzyme complex and is deceptively simple chemically, a polymer consisting only of glucose linked only by β1, 4 bonds. Cellulase refers to a class of enzymes produced chiefly by fungi, bacteria, and protozoans that catalyze cellulolysis (i.e. he hydrolysis of cellulose). So with help of valuable microbe agro industrial waste which is rich in lignocelluloses product could be used as media for enzyme production. The review discuss current knowledge on cellulase production by microorganism, and it discusss the industrial application of cellulase and up coming challenge in cellulase research especially in the direction of improving the process of economies of enzyme production.*

***Key words*:** *Cellulose, Lignocellulosic,cellulase, Ago-Industrial waste, enzyme*

INTRODUCTION

Cellulases are widely used for beverage, juice and paper industry. Cellulase is a synergistic enzyme that is used to break up cellulose into glucose or other oligosaccharide compounds (CHELLAPANDI and JANI 2008). The cellulase system in fungi is considered to comprise three hydrolytic enzymes: endo-(1,4)-D-glucanase (endoglucanase,endocellulase, CMCase [EC 3.2.1.4]), which cleaves linkages at random, commonly in the amorphous parts of cellulose, exo-(1,4)-D-glucanase (cellobiohydrolase, exocellulase, microcrystalline cellulase, avicelase [EC 3.2.1.91]), which releases cellobiose from non reducing or reducing end, generally from the crystalline parts of cellulose and β-glucosidase (cellobiase [EC 3.2.1.21]), Cellulase degrade cellulose to yield glucose and other soluble sugars which can be used either in juice liquefaction or as fuel. Higher saccharification efficiency, mild operating conditions with respect to pH and temperature, absence of by products and avoidance of pollution makes enzymatic hydrolysis superior over chemical processes in industry. However production of cellulase enzyme has been widely studied in submerged condition. Cost of enzyme production has restricted its application in large scale processes. Therefore, using cellulosic waste as substrate rather than expensive pure cellulase is better economically viable strategy. Cellulases are capable of the extensive solubilization of highly ordered form of cellulose and are reported to be produced from well known microbial sources such as aerobic and anaerobic fungi (HANG and WOODAMS, 1994; ONSORI et al. 2005) and anaerobic fungus (BARICHIEVIC and CALZA, 1990). The cost of enzyme production can be significantly reduced if low value biological substrates like fruit processing waste (KOIJAM et al. 2000; HANG and WOODAMS, 1994) are used.

There are reports of using various agriculture waste including rice bran (LEE et al. 2010), wheat straw (SINGH et al. 2009), cassava (POTHIRAJ et al. 2006) banana waste (BAIG, 2005) and food process waste like oil palm (ALAM et al.2005) and apple waste (HANG and WOODAMS, 1994) as substrate for cellulase production. Banana waste which is cellulose is use by different research but activity is very low and it was found because pH is alkaline at normal condition (BAIG et al. 2005). In India most of the enzymes used by the food processing industry are imported. Hence there is an immediate need to produce these enzymes in our country itself. We have conducted some basic experiments on enzyme production from mango peel and have got encouraging results. The objective of the proposed investigation is production of cellulases using mango peel as substrate. The fungus enriched peel left after enzyme extraction could be used as protein enriched animal feed supplement, thus reducing the environmental pollution problem to some extent.

Application cellulase enzyme was study in mango and guava juice for enzyme clarification and yield improvement.

Cellulase and its applications

Cellulases, a class of hydrolases, fall under synergistic enzyme category. It is involved in the break down of polysaccharide cellulose into glucose or other oligosaccharide compounds (CHELLAPANDI and JANI 2008). The cellulase system in fungi comprises of three hydrolytic enzymes: endo-(1,4)-D-glucanase (endoglucanase, endocellulase, CMCase [EC 3.2.1.4]), which cleaves linkages at random, commonly in the amorphous parts of cellulose, exo-(1,4)-D-glucanase (cellobiohydrolase, exocellulase, microcrystalline cellulase, avicelase [EC 3.2.1.91]), which releases cellobiose from non reducing or reducing end, generally from the crystalline parts of cellulose and β-glucosidase (cellobiase [EC 3.2.1.21]), Extracellular cellulases have many industrial applications from the generation of juice clarification bioethanol, a realistic long-term energy source, to the finishing of textiles. These industrial processes require cellulolytic activity under a range of pH, temperature and there stability for long time storage.

Cellulose is the primary product of photosynthesis in terrestrial environments, and the most abundant renewable bioresource produced in the biosphere (100billion dry tons/year). Cellulose biodegradation by cellulases and cellulosomes, produced by numerous microorganisms, represents a major carbon flow from fixed carbon sinks to atmospheric CO_2. It is very important in several agricultural and waste treatment processes and widely used in production of sustainable biobased products and bioenergy replacing fossil fuels. Cellulases find profound applications in converting low value agricultural and forestry residues to fermentable sugars deinking, drainage improvement, and fiber modification.

Historically, enzyme demand has been concentrated on the more developed economies due to the high value-added nature of enzymes, and the significant technical resources needed for their development, production and application. This market scenario represents an increase of 33% in the total US industrial enzyme market. The large market potential and the important role that cellulases play in the emerging bioenergy and bio-based products industries provide a great motivation to develop better cellulase preparations for plant cell wall cellulose hydrolysis. These improved cellulases must also have characteristics necessary for biorefineries, such as higher catalytic efficiency on insoluble cellulosic substrates, increased stability at elevated temperature and at a certain pH, and higher tolerance to end-product inhibition. The cornerstone of enzyme engineering is to understand the biochemical basis of the enzyme and with these facts in view, the present study was formulated and the critical review of the research priorities in these lines are discussed in this chapter.

Microorganisms producing cellulases.

Primarily carbohydrate degraders are cellulolytic microbes and are generally unable to use proteins or lipids as energy sources for growth (LYND et al. 2002). Cellulolytic microbes notably the bacteria, *Cellulomonas* and *Cytophaga* and most fungi can utilize any carbohydrates including cellulose (POULSEN et al. 1988; RAJOKA et al. 1997), while the anaerobic celluloytic species have a restricted carbohydrate range, to cellulose and or its hydrolytic products. The ability to secrete large amounts of extracellular protein is characteristic of certain fungi and such strains are most suited for production of higher levels of extracellular cellulases. One of the most extensively studied fungi is *Aspergillus niger*, which converts native as well as derived cellulose to glucose (NARASIMHA et al. 2006).

Most commonly studied cellulolytic organisms include: Fungal species- *Aspergillus* (MILALA et al. 2005; OSHOMA et al. 2005; JUWAIED et al. 2010; NORMA et al. 2010), *Trichoderma* (DOMATO et al. 2010; VINTILA et al. 2010; JUWAIED et al. 2010; JAMAL et al. 2010), *Fusarium (*RAMANATHAN et al. 2010), Bacteria-*Bacilli* (FEMI-OLA et al. 2008; SHABEB et al. 2010), *Pseudomonads* (BAKARE et al. 2005), *Acinetobacter* (EKPERIGIN (2007) and Actinomycetes- *Streptomyces, Actinomucor,* and *Thermoactinomyces* (ABOUL-ENEIN et al. 2010). A wide variety of gram positive and negative species are reported to produce cellulose which includes: actinomycetes such as *Streptomyces spp, Thermoactinomycetes* spp *and Thermonospora curvata* (EMERT et.al. 1974).While several fungi can metabolize cellulose as an energy source, only few strains are capable of secreting a complex of cellulase enzymes, which could have practical application in the enzymatic hydrolysis of cellulose. Besides *T. reesei,* other fungi like *Humicola, Penicillium* and *Aspergillus* have the ability 35-40 to yield high levels of extracellular cellulases. Aerobic bacteria such as *Cellulomonas, Cellovibrio* and *Cytophaga* are capable of cellulose degradation in pure cultures (LYND et al. 2002; POULSEN et al. 1988; RAJOKA et al. 1997).

However, the microbes commercially exploited for cellulase preparations are mostly limited to *T. reesei, H. insolens, A. niger, Thermomonospora fusca, Bacillus sp*, and a few other organisms. A detailed list of the cellulolytic microorganisms is presented in (Table 1).

Utilization of Agro-horticulture wastes for cellulase production

Agro-horticulture and industrial by product which are rich in lignocelluloses has enormous potentials for enzyme production. Their conversion into useful products may ameliorate the environmental problems. Some agro-wastes like cereal straw, leaves corncobs etc (SHAFIQUE et al. 2004; MILALA et al 2005; SHABAB et al 2010) and horticulture food process waste (DAMATO et al 2010; SUN et al. 2010; RASHAD et al 2009) and other cellulose rich waste like paper, coir and wood extract waste (SIHAM et al. 2007; SAMUEL et al. 2010; JUWAIED et al. 2010) (Table 2). In most parts of the country, these materials are mainly used as animal feeds or cause environment pollution. A large quantity is left on farmlands to be decomposed by microorganism such as bacteria (CHEN et al. 2004; FEMI-OLA et al. 2008; MOHAMED et al. 2010) and fungi (MILALA et al. 2005; NARASIMHA et al. 2006; JUWAIED *et al.*2010; NORMA et al 2010).

Economically, the most important industrial material other than foodstuffs affected by microorganisms are cellulose and wood products including the wood itself (SIHAM et al. 2007; OJUMU et al. 2003; FEMI-OLA et al. 2008). Production of wood products such as pulp, paper, textiles from natural fibers such as cotton flax and jutes are enhanced by microorganisms specifically fungi. Cellulose which forms about 40-50% of plants composition is the most abundant organic matter on earth. Proper utilization of these wastes in the environment will eliminate pollution and convert them into useful by-products is a matter of concern.

Table 1

Cellulase producing microbe in solid state production (SSF) and Submerged fermentation (SmF) media

Higher cellulase producing microbe			
Serial number	Genus	Species	Referance
Fungi	*Aspergillus*	*Aspergillus niger*	NARASIMHA et al.2006, MILALA et al. 2005, JUWAIED et al.2010, NORMA et al.2010
		Aspergillus flavus	AJAYI et al.2007
		Aspergillus. nidulans	OSHAMA et al.2005
		Aspergillus terreus	POTHIRAJ et al.2006
		Aspergillus aculeatus	TAKADA et al.1998
	Rhizopus	*Rhizopus stolonifer*	POTHIRAJ et al.2006
	Trichoderma	*Trichoderma reesei*	DOMATO et al.2010
		Trichoderma viride	VINTILA et al.2010, JUWAIED et al.2010
		Trichoderma harzianum	JAMAL et al.2010
	Fusarium	*Fusarium oxysporum*	RAMANATHAN et al.2010
Bacteria	*Pseudomonas*	*Pseudomonas fluorescens*	BAKARE et al.2005
	Bacillus	*Bacillus subtilis*	FEMI-OLA et al.2008; SHABEB et al.2010
		Bacillus pumilus	KOTCHONI et al.2006, ARIFFIN et al.2006
	Acinetobacter	*Acinetobacter anitratus*	EKPERIGIN 2007
	Sinorhizobium	*Sinorhizobium fredii* CCRC	CHEN et al.2004
Actinomycetes	*Cellulomonas*	*Cellulomonas. fimi* *Cellulomonas.bioazotea*	POULSEN et al.1988
	Streptomyces sp	*Streptomyces. drozdowiczii* *Streptomyces. lividans*	RAJOKA et al.1997
	Thermoactinomyces	*Thermoactinomyces fusca*	EMERT et. al. 1974
		Thermomonospora curvata	

Cellulase (a complex multienzyme system) acts collectively to hydrolyze cellulose from agricultural wastes to produce simple glucose units. Cellulases are synthesized by cellulolytic fungi such as the *Chaetomium, Funsarium Myrothecium and Trichoderma* *species*. Other species include the *Penicillium* and *Aspergillus* species Cellulase with its immense importance is being imported for use at very high cost.

Table 2

Lignocellulosic rich organic by product is use as substrate to produce cellulase production by fungi, bacteria in solid state production (SSF) and Submerged fermentation (SmF)

Lignocellulosic substrate use for celllulase production						
S.No	**Substrate use for cellulose production**	**Enzyme production**	**Enzyme producing microbial stain**	**Type of microbe**	**Fermentation type**	**Reference**
1	**Pineapple Waste**	Endoglucanase, Exoglucanase, β-glucosidase	*Bacillus pumilus EB3*	Fungi	SmF	OMOJASOLA, et al.2008
2	Paper, wood, litmus paper	β-glucosidase	*Fusarium oxsporum*	Fungi		SIHAM et al.2007
3	Olive processing residue	Endoglucanase, Exoglucanase, β-glucosidase	*Trichoderma reesi*	Fungi		DAMATO et al.2010
4	Agricultural wastes	Endoglucanase, Exoglucanase, β-glucosidase	*Aspergillus niger*	Fungi	SmF	MILALA et al.2005
5	Coir waste and saw dust as substrate	Endoglucanase,	*Bacillus spp, two Pseudomonas spp and Proteus spp*	Becteria	SmF	SAMUEL et al.2010
6	Coir waste and saw dust as substrate	Endoglucanase,	*Aspergillus niger and Aspergillus fumigatus*	Fungi	SmF	SAMUEL et al. 2010
7	Wheat bran	Endoglucanase, FP-ase, β-glucosidase	*Aspergillus niger and Trichoderma viride*	Fungi	SmF	JAVED et al.2006
8	Waste paper	Endoglucanase, Exoglucanase and FPase	*mixing of A. niger and T. viride*	Fungi	SSF	JUWAIED et al.2010
9	Saw dust, Bagasse were	Endoglucanase, Exoglucanase, β-glucosidase	*Aspergillus niger*	Fungi		GURUCHANDRAN 2010
10	Apple pomace	Endoglucanase, Exoglucanase, β-glucosidase	*Tricoderma species*	Fungi	SSF	SUN et al.2010
12	Woods extracts	Endoglucanase, Exoglucanase, β-glucosidase	*Bacillus subtilis*	Bacteria	SSF	FEMI-OLA et al.2008
13	Banana stalk	exoglucanase	*Bacillus subtilis*	Bacteria	SSF	SHAFIQUE et al.2004
14	Citrus Limonium and Carica papaya Wastes	Endoglucanase, Exoglucanase, β-glucosidase	*Pleurotus ostreatus*	Fungi	SSF	RASHAD et al.2009
15	Palm fruit	Endoglucanase, Exoglucanase, β-glucosidase	*Bacillus coagulans*	Bacteria	SSF	ODENIYI et al.2009
16	Banana agrowaste	Endoglucanase, Exoglucanase, β-glucosidase	*Trichoderma lignorum*	Fungi	SmF	BAIG .2004
17	Waste paper	FPase, Endoglucanase	*Aspergillus niger, Gliomastix murorum , Acremonium murorum, Stachybotrys chartarum*	Fungi	SSF	PEČIULYTĖ 2007
18	Molasses	Endoglucanase	*Bacillus subtilis*	Bacteria	SmF	SHABAB et al.2010
19	Cassava waste	Endoglucanase	*Aspergillus niger, Aspergillus terreus*	Fungi	SSF	POTHIRAJ et al.2006
20	Freshly ripe tomato fruits	Endoglucanase, Exoglucanase, β-glucosidase	*Aspergillus flavus Linn*	Fungi	SSF	AJAY et al.2010
21	Sawdust, bagasse and corncob	Endoglucanase, Exoglucanase, β-glucosidase	*Aspergillus flavus*	Fungi	SSF	OJUMU et al.2003

[1]Endoglucanase (EC 3.2.1.4), exoglucanaseEC (3.2.1.91) and β-glucosidase (EC 3.2.1.21)
[2] Solid state fermentation (SSF),
[3] Submerged fermentation (SmF)

CONCLUSION

In modern era cellulase enzyme is highly need full for industrial as well domestic importance for human being, with use of syntactic substrate the enzyme caste is increases. So there is come need how to increase enzyme production to less caste of substrate like agro-industrial waste which have highly fiber as well micronutrient. Also need to screen of highly lignocelluloses degrading microbe in adverse condition in temperature and salinity as well alkali residence.

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