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Production of Cellulase by *Trichoderma* sp. by Utilization of Various Agro-food Residues

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Abstract
This mini-review has been written from the standpoint of the potential of lignocellulose bioconversion in meeting the ever-growing demands of the world for various products. The mini-review is divided into three sections - uses of cellulose, possible sources and submerged fermentation and global environmental perspectives; solid state fermentation-details and measurement protocols and; process parameters – submerged and solid-state fermentation (SSF).

Keywords
cellulases, solid-state fermentation, nutrients.

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Introduction

The recyclable bio-based catalyst plays a crucial role in the conversion of the substrates into a valuable product. These catalysts are named as enzymes produced from the biological resources such as micro-organisms especially fungi. Cellulases are highly recognized enzymes for their production at industrial level. The demand for the utilization of renewable energy resources directly meets the demand of the cellulases for the conversion of pretreated cellulose into monomeric sugars. The recent problem concerned with the non-renewable fossil fuel resources depletion in the upcoming decade shifts the technology towards the bio-based resources to be used. Bioresources can be agricultural crops residue (like wheat, rice straw) (Wyman and Goodman, 1993), and residential waste (waste paper, food) (Yu, 1996) that can be used as a substrate for energy generation products. Cellulose is the major and most abundant bioresource in the world to be used for the production of cellulases. Each plant cell wall consists of around 58-60% of cellulose. It is made up of a continuous chain of monomeric glucose units joined together by the 1,4-β-glucosidic bond. Although many physicochemical methods have been already utilized for decomposing such complex organic matter, still they lag behind because of low efficiency and high cost (Howard et al., 2003). At this point, enzyme systems can offer a viable approach to solving this puzzle.

Cellulases

The enzyme cellulases are a combination of three main enzymes categorized for the cellulose hydrolysis into sugar in a series of conversion (Mukataka *et al*., 1998). The first enzyme class cellobiohydrolases cause the exoglucanase activity to break the external or inter-cellulose bonds to make 4-8 oligomeric units of the glucose, while the second class called endo-1,4-β-gluconases hydrolyze the long chains of cellulose sugar into dimeric units as cellobiose. The third and most important class of the cellulases. 1,4-β-D-glucosidase reduces the dimeric glucose in the monomeric glucose units, which are free to use further for the fermentation or other applications (Howard et al, 2003). A variety of microbes has been studied for the cellulase enzyme production potentials as shown in the Annexure I. Out of these, the most suitable and industrially commercialized is the fungi *Trichoderma reesei* (Kubicek *et al*., 1990). Tolan and Foody, mentioned the applications of the cellulases in use for the animal feed digestive enhance, beverages, hydrolysis of cellulose for bioethanol production, pulp industry and fruit juices processing (Tolan and Foody, 1999).

Despite this, it has many limiting factors that further augment the cost of production.

Caveat and Options

The cellulase enzyme production recites with the carbon sources cost to fulfill the market demand (Ruy and Mandels, 1980). The cost was expensive for the cellulase production because of high purity and production process -submerged and solid state. However, the enzyme production from different substrates (mostly lignocellulosic), for example wheat (Doppelbauer *et al*., 1987; Thygesen *et al*., 2003), bagasse from sugar cane (Kawamori *et al*., 1986; Amany *et*

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al., 1999),waste paper sludge (Wyk and Mohulatsi, 2003; Maheshwari *et al*., 1994), the waste coming as newsprint (Chen and Wayman, 1991), corn fibre and cobs (Vlaev *et al*., 1997; Xia and Xueling, 2004), few as aspen wood (San Martin *et al*.,1986), and willow (Reczey *et al*., 1996) is cost effective. Lately, even wastewater sludge has been used for the same purpose. This is another efficient alternative of bioconversion of sludges resulting in their sustainable management and utilization (Molla *et al*., 2004).

Submerged Fermentation hindrances

The submerged fermentation is widely studied from lab scale to small scale level (Haltrich *et al*., 1996; Kim *et al*., 1997; Xia and Cen, 1999). But the activity loss and low nutrient consumption rate made this process very slow and less productive. So that, the stirred tank reactors used further to improvise it, but here also the sheer force problem arose. As the fungal cell grows and mycelia develops, they get damaged by the stirrer of the reactor and get non- uniformly distributed which causes the depletion in enzyme yield and activity. Then, the Airlift and bubble column reactors were recommended for the growth of the cells in which shear force is less and air consumption is higher than stirred tank reactor (Kim *et al*, 1997). Xia and Len (1999) concluded that the submerged process hindered its industrial uses because of its high cost of production.

Solid state fermentation (SSF)

For the SSF, various complex substrates have been used (Yang *et al*., 2004; Xia and Cen, 1999). For instance, *Trichoderma* spp. was grown on rice chaff by Yang *et al*., (2004) in tray fermenter. They pretreated the solid-state medium (rice chaff) and the acid mineral solution was supplemented. The sterilization condition was 121°C for 40 min and for inoculation, spore suspension was used. The fermentation period was 8-10 days at 30°C and 85% relative humidity. The final enzyme activity was reported as 5.64 U/ g rice chaff.

At this juncture, solid state fermentation could serve as low capital, low cost, less operating and maintenance expenses process (Deschamps *et al*., 1985; Chahal, 1991). In prior publications, the cheaper and abundant agricultural residues were also used for enzyme production but here also the production cost is still considerable. (Rao *et al*., 1983; Chahal *et al*., 1996).

Analytical methods

Enzyme activity

The IUPAC has set the standards for estimation of enzyme activity in terms of FPU (filter paper units) per ml of the sample in undiluted form. 1 FPU of that enzyme corresponds to when 50 mg of the cellulose filter paper releases 2.0 mg of the reducing sugar as glucose in an hour.

Filter-paper activity can be determined as follows: the supernatant of the sample is added to 0.05 M citrate buffer with pH 4.8 and cellulose filter paper is added as 50 mg weight and rolled. This is followed by incubation at 50°C for exactly 60 min. Then, the leased sugar is calculated with the blank enzyme without filter paper by DNS (di- nitrosalicylic acid) method by multiplying the concentration with 0.18 for undiluted samples and for diluted samples the Ghosh protocol is followed (Ghose, 1987; Adney and Baker, 1996; Yang *et al*., 2004).

Operational Parameters

The operational parameters play a role in producing the cellulase enzymes economically. Based on the recent literature cited, the parameters assorted as significant for the production of cellulases from *Trichoderma* spp. are as follows:

Physical

Substrate Water activity

In the study done by Xia *at al*., the experiments with different water percentages (50, 60, 70 and 80%) of the substrate (corncob residue) were carried out in shallow tray fermenters using *Trichoderma reesei*. After 6 days of cultivation, 70% of water content was found optimum and the cellulase enzyme activity was ~300 FPU/gram of cellulose and 126 FPU/g of koji obtained (Xia *et al*., 1999). The relative enzyme production has been found to be reduced significantly in the case of submerged fermentation (Howard *et al*. 2003).

Solids concentration

Xia and Cen (1999) have studied SSF for cellulases by giving different dosages of wheat bran (20, 30, 40 and 50%) at70% water content of substrate (28–30°C). The solid loading doses were optimum as 30% of wheat bran after the results of 6 days incubation. Lower and higher doses may reduce the enzyme productions since lower doses lessen enzyme productivity, while higher dosage causes the fungal growth to be very extensive and inhibits cellulase enzyme production. In case of submerged fermentation, the relatively moderate concentration of substrate (15.0 g/l, Steam-pretreated willow) was found to have a higher yield of cellulase compared to (45.0 g/l Steam-pretreated willow) (Reczey *et al*. 1996). Corn cob residue as the substrate also provided 40 g/l as optimal solids concentration for *Trichoderma reesei* ZU-02. Higher substrate concentrations resulted in increased mass transfer resistance and hence poor cellulase productivity. (Table1).

Table 1: Effect of substrate and culture times on cellulose production (Modified from Xia and Xueliang 2004).

Substrate g/l	Cellulose g/l	Culture time h	Cellulase IU per ml	Yield IU per gram cellulose	Productivity IU perliter per hour
80	49.20	360	9.38	190.7	24.4
50	30.75	240	6.62	215.3	27.6
40	24.60	168	5.25	213.4	31.3
30	18.45	120	2.16	117.0	18.0

C/N ratio

Xia and Xueliang (2004) found that with different C/N ratios (6, 7, 8 and 9), ratio 8 was the optimal for maximum cellulase enzyme activity.

Temperature

As cellulase production is a function of growth (equation 1; Velkovska *et al*. 1997), the temperature has been found to be a less important parameter for enhancing cellulase production. This can be easily inferred from Table 2.

Table 2: Effect of temperature on cellulose activity (Modified from Kansoh *et al*. 1999)

Temperature (°C)	Cellulase activity (U/ml)	
	Sample A	Sample B
25	6.5	5.1
30	7.8	6.2
35	5.7	4.1
40	1.8	1.5

$$\frac{dE_t}{dt} = k_2 X - K_3 E_t$$

where *Et* is total enzyme activity, χ_2 is secondary mycelium concentration, χ_2 and K_3 is are the rate constants for synthesis and decayrespectively.

Supply and availability (aeration and agitation)

The O_2 supply is the major concern for higher growth of cellulase enzyme production as it was investigated by Rakshit and Sahai, that the DO level below 15% influences the cellulase enzyme production (Rakshit and Sahai (1991).However, different air pressures were optimized and studied for the growth of *Trichoderma viride*-SL1 species, in which 1400 IU/g was obtained at optimum pressure amplitude and 450 IU/g in case of tray fermenter (Tao *et al*. 1999).

Rheology (particle size, viscosity, density, surface tension)

In the case of submerged fermentation, the most recommended particle size is 375 µm, which results in maximum enzyme production rate. (Muniswaran and Charyulu 1994). The viscosity of fermentation broth has also been reported to reach as high as 1500 cP during the course of fermentation. Also, density and surface-tension are important parameters but are less studied (Domingues *et al*. 2000).

Light activity (effect of radiation/ illumination)

Radiation/ illumination is an important factor for inducing certain metabolic reactions responsible for cellulase synthesis (Pelczar *et al*. 1993).

Hydrostatic pressure

In one of the studies carried out in fermenters at atmospheric pressure of 1.7 bar with DO levels varied. It showed an increase in productivity from 16.7 to 12.2 FPU/1/h. The production rate maybe increased due to the higher levels of the DO Supplied. (Reczey *et al*. 1996).

Chemical

pH effect

Cellulase activity for the submerged culture experiments executed with varying initial pH is listed in Table 3. As cellulase production is proportional to mycelial growth, pH becomes an indirect parameter (similar to temperature) for enhancing cellulase production.

Table 3: The effect of pH on cellulose activity (Kansoh *et al*. 1999).

Initial pH	Cellulase activity (U/ml)	
	Sample A	Sample B
4.0	1.4	1.1
5.0	7.0	5.8
6.0	7.4	6.6
7.0	6.8	4.8
8.0	0.9	0.8

Type of substrate (simple, moderate and complex)
As per given subject, we will restrict our discussion to complex substrates only (different agro-industry residues).

- **Bagasse** – It refers mainly to the fiber remaining after the extraction of the sugar-bearing juice from sugarcane. Studies carried out on alkaline pulping of the bagasse with H_2O^2 using *Trichoderma reesei* as microorganism produced a maximum of 7.8 U/ml of cellulase activity at 30 ^{0}C and an initial pH of 6.0 (Kansoh *et al*. 1999). Table 1 and 2 suggest that cellulase production is maximum at culture's optimal temperature and pH (30 °C and 6.0). Therefore, type of substrate influences cellulase production but are not capable of altering optimum temperature and pH.

- **Sugar beet pulp** –The enzyme activity when sugar beet pulp was taken as substrate obtained as 0.82 U per ml by using *T. reesei* QM9414 as compared to the substrate used citrus pectin with 0.12 U/ml (Olsson *et al*. 2003).

- **Corn cob residue** – When corn cob residue substrate was used the cellulase enzyme activity was found around 5.25 IU/ml i.e. 213.4 IU/g of cellulose calculated. This was achieved with the micro-organism grown as *T. reesei* ZU-02 strain for 7 days in corn cob substrate. (Xia *et al*.2004).

- **Coconut coir pith** – Coconut coir pit is an alternative source, which is also abundant in the countries falling under the tropical region category. The *T. reesei* strain number NCIM 1051 was used on this substrate and the activity was found 4.27 IU/g cellulose. After 7 days of incubation, cellobiase activity was also obtained as 1.8 IU/g of cellobiose.

- **Paper mill waste**– In some studies, the *Aspergillus niger* and *T.reesei* were simultaneously used for the cellulase enzyme activity in the paper mill sludge waste with the values optimum of 0.8 mg/ml (Maheshwari *et al*. 1994). The mixed culture fermentation was with nutrient-limited conditions was also tested to form the cheaper substrate medium (Gutierrez-Correa *et al*. 1999).

- **Saccharified sunflower stalks** – Sharma *et al*. (2002) discussed steam explosion pretreated sunflower stalks with the method used of depressurization at 15psi for 90 minutes. The enzyme yield was 66% by using *T. reesei* RUT-C30. The maximum FPU was 1.05 at 28^{0}C and 8 days incubation period.

Inducer or inhibitor substances
Certain inducers (e.g. vitamins, coenzymes) or inhibitor chemical substances (e.g. antibiotics, metal ions) in trace amounts may be helpful in enhancing cellulase production. These substances could be added either along with the substrate or during the fermentation period.

Biological
Culture efficacy (new strains)
Total fermentation time and product yield are mainly dependent upon the type of strain. For example, with complex substrate wheat straw, *Trichoderma* reesei RUT-C30 produced insignificant cellulase activity (Thygesen *et al*. 2003) but with another similar substrate sugarcane bagasse, *Trichoderma reesei* NRRL 3653 showed very impressive cellulase activity (Kansoh *et al*. 1999). In the above case, similar substrates gave a wide difference of cellulase production by the two strains suggesting the influence of culture type. A mutant of *Trichoderma reesei* QM 9414 has also been found to show a 50-90% increase in cellulase activity for acid swollen cellulose (Gadgil *et al*. 1995). Unfortunately, strain degeneration or instability of mutants limits their use (Pelczar *et al*. 1993).

Inoculation
a. Mycelial, sporulated or combination - Mycelial mass as inoculation can be helpful in some cases, probably for achieving product formation faster. However, spores are more effective because of their high density in terms of numbers per unit volume. This makes spores sometimes more desirable compared to mycelia.

The effect of the inoculum size was investigated by using a range of spore suspension between 10^5 and 10^7 spores/ml. When inoculum is high, mycelia are formed but pellets are not formed in higher number. Similarly, at low spore suspension, higher pellets are formed. This means pellet size is inversely proportional to the spore suspension concentration (Domingues *et al*. 2000).

b. Volume/ quantity- In general, 5-10% v/v inoculum is used for *Trichoderma* spp. (Muniswaran and Charyulu 1994).

c. Age - For the production of cellulase, 48 hours old culture has been used in many studies (Kansoh *et al*. 1999; Domingues *et al*. 2000; Gutierrez-Correa *et al*. 1999).

Sterility
Strict aseptic conditions during start-up of fermentation and incorporation of certain antibiotics in the fermentation medium can help in maintaining aseptic conditions.

Global Environmental Perspective
The production of cellulases from agro-based wastes can play a pivotal role in environmental protection by reducing GHG emissions from indeterminate storage of these residues. Consider, an example of the production of ethanol. On the basis of ethanol production, cellulase enzyme costs 0.1 to 2 USD/liter of ethanol produced (Hettenhaus and Glassner, 1997), but biotechnological advancements in the cellulase enzyme activity enhancement reduce the cost to less than 0.01USD. This will eventually put less load on the fossil fuel utilization and hence a clean environment. In toto, cellulase production from agriculture waste hastridentate

global benefits - sustainable management of residues; production of value-added products and mitigation of climate change.

Conclusion

Lignocellulose biotechnology accrues benefits from a capital costs investment perspective since its biodegradation could be carried out in submerged or solid-state fermentation with each technology backing set of advantages and limitations (mainly production scale). From different articles reviewed, it was found that the most important factor that can solely influence cellulase productivity is the "substrate". Further, cellulase production can play a vital role in achieving higher rates of bioconversion of agricultural residues and if they are produced from cheaper raw material, this could give a holistic approach to the "Biorevolution".

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References

Adney B. and Baker J. Measurement of cellulaseactivities, Chemical analysis and testing task laboratory analytical procedure. *National Renewable Energy Laboratory*,1996.

Chahal DS. Production of Trichoderma reesei cellulase system with high hydrolytic potential by solid-state fermentation. Enzymes in biomass conversion. *ACS Symp Ser*, 1991, 460: 111-22.

Chahal PS, Chahal DS, Le GBB. Production of cellulase in solid-state fermentation with *Trichoderma* reeseiMCG80 on wheat straw. *Appl Biochem Biotechnol*. 1996, 57:58:433–42.

Chaudhuri, BK and Sahai, V. Production of cellulose enzyme from lactose in batch and continuous cultures by a partially constitutive strain of *Trichoderma* reesei. *Enzyme Microb. Technol.* 1993,15: 513-518.

Chen, S and Wayman, M. Cellulase production induced by carbon sources derived from waste newsprint. *Process Biochem*. 1991, 26: 93-100.

Deschamps F, Giuliano C, Asther M, Huet MC, **Roussos S.** Cellulase production by *Trichoderma harzianumin* static and mixed solid-state fermentation reactors under non aseptic conditions. *Biotechnol Bioeng*.1985,27: 1385-8.

Domingues FC, Queiroz JA, Cabral JMS, Fonseca LP. The influence of culture conditions on mycelial structure and cellulase production by *Trichoderma reesei* Rut C-30. *Enzymeand Microbial Technology*. 2000, 26: 394-401

Doppelbauer, R., Esterbauer, H., Steiner, W., Lafferty, R. & Steinmuller, H. The use of cellulosic wastes for production of cellulases by *Trichoderma reesei*. *Appl. Microbiol. Biotechnol*. 1987,26: 485-494.

Gadgil NJ., HF Daginawala, T Chakrabarti, P Khanna. Enhanced cellulase production by a mutant of *Trichoderma reesei*. *Enzyme and Microbial Technology*. 1995, 17: 942-946.

Ghose, TK. Measurement of cellulase activities. *Pure & applied chem*. 1987,59:257-268.

Gutierrez-Correa M, Portal L, Moreno P, Tengerdy RP. Mixed culture solid substrate fermentation of *Trichoderma reesei* with Aspergillus niger on sugar cane bagasse. *Bioresource Technology*.1999,68: 173-178.

Haltrich D, Nidetzky B and Kulbe KD. Production of fungal xylanases. *Biores. Technol.* 1996, 58, 137-161.

Hettenhaus J and Glassner D. Ethanol Production from Biomass. Hydrolysis. 1997(Available at http://www. ceassist.com.)

Howard RL, Abotsi E, Jansen van Rensburg EL, Howard S. Lignocellulose biotechnology: issues of bioconversion and enzyme production. *African Journal of Biotechnology.* 2003, 2 (12): 602-619.

Kansoh AL, Essam SA, Zeinat AN. Biodegradation and utilization of bagasse with *Trichoderma reesie*. *Polymer Degradation and Stability*. 1999,63: 273-278.

Kawamori, M., Morikawa, Y., Ado, Y. & Takasawa, S. Production of ethanol from biomasses. Part IV. Production of cellulases from alkali-treated bagasse in *Trichoderma reesei. Appl. Microbiol. Biotechnol.* 1986, 24, 454-458.

Kim SW, Kang SW and Lee JS. Cellulase and xylanase production by *Aspergillus niger* KKS in various bioreactors. *Biores. Technol.*1997, 59, 63-67.

Kubicek CP, Eveleigh DE, Esterbauer H, Steiner W, Kubicek-Pranz EM. *Trichoderma cellulases*: biochemistry, physiology, genetics and applications. The Royal Society Chemistry, Cambridge, UK,1990.

Maheshwari DK, Gohade S, Paul J, Varma A. Paper mill sludge as a potential source for cellulase production by Trichoderma reesei QM 9123 and Aspergillus niger using mixed cultivation. *Carbohydrate Polymers*.1994,23: 161-163.

Molla, A.H., Fakhru'l-Razia, A., Hana, M.M. and Alam, M.Z. Optimization of process factors for solid-state bioconversion of domestic wastewater sludge. *International Biodeterioration & Biodegradation*. 2004,53: 49 – 55.

Mukataka S, Kobayashi N, Sato S, Takahashi J. Variation in cellulose constituting components from *Trichoderma reesei* with agitation intensity. *Biotechnol Bioeng*. 1998,32: 760 -3.

Muniswaran PKA. and Charyulu NCLN. Solid substrate fermentation of coconut coir pith for cellulase production. *Enzyme and Microbial Technology*.1994,16: 436-440.

Olsson Lisbeth, Tove MIE. Christensen, Kim P. Hansen,

Eva A. Palmqvis. Influence of the carbon source on production of cellulases, hemicellulases and pectinases by Trichoderma reesei Rut C-30. *Enzyme and Microbial Technology*. 2003, 33: 612–619.

Pelczar JR Michael J., E.C.S. Chan, Noel R. Krieg. Microbiology. Tata McGraw-Hill. 5th edition, 1993,ISBN 0- 07-049234-4: 239.

Pourquie, J. and Desmarquest, J. P. Scale-up of cellulase production by Trichoderma reesei. In Enzyme Systems: Lignocellulosic Degradation, ed. M. P. Coughlan. 1989, 283- 297.

Rakshit, SK. and Sahai, V. Optimal control strategy for the enhanced production of cellulase enzyme using the new mutant *Trichoderma reesei* E-12. Bioprocess Eng. 1991, 6: 101–107.

Rao MNA, Mithal BM, Thakkur RN, Sastry KSM. Solid- state fermentation for cellulase production by Pestalotiopsisversicolor. *Biotechnol Bioeng*. 1983, 25:869–72.

Reczey K., Zs. Szengyel, R. Eklund, G. Zacchi. Cellulase production by *T. reesei Bioresource Technology*.1996,57: 25- 30.

Ruy, D. and Mandels, M. Cellulases: biosynthesis and applications. *Enzyme Microb. Technol.*1980,2, 91-102.

San Martin, R., Blanche, W. H., Wilke, R. C. & Sciamanna, F. A. Production of cellulase enzymes and hydrolysis of steam-exploded wood. *Biotechnol. Bioengng.* 1986,28, 564-569.

Schafner, D. W. & Toledo, S. Cellulase production in continuous culture by *Trichoderma reesei on* xylose-based media. *Biotechnol. Bioengng*, 1992, 39, 865-869.

Sharma SK, Kalra KL, Grewal HS. Fermentation of enzymatically saccharified sunflower stalks for ethanol production and its scale up Bioresource Technology. 2002, 85: 31-33

Tao Sun, Beihui Liu, Zuohu Li, Deming Liu. Effects of air pressure amplitude on cellulase productivity by Trichoderma viride SL-l in periodic pressure solid state fermenter. *Process Biochemistry*.1999, 34:25-29.

Thygesen Anders, Anne Belinda Thomsena, Anette S. Schmidt, Henning Jørgensen, Birgitte K. Ahring, Lisbeth Olsson. Production of cellulose and hemicellulose-degrading enzymes by filamentous fungi cultivated on wet-oxidised wheat straw. *Enzyme and Microbial Technology*. 2003,32:606-615.

Tolan JS and Foody B. Cellulase from submerged fermentation. *Adv Biochem Eng Biotechnol*. 1999, 65: 41-67.

Vlaev, S. D., Djejeva, G., Raykovska, V. and K. Schugerl. Cellulase production by *Trichoderma* sp. Grown on corn fibre substrate. Process Biochemistry. 1997,32: 561-565.

Wase DAJ, McManamey WJ, Raymahasay S. and VaidAK. Comparison between cellulose production by *Aspergillus fumigatus* in agitated vessels and in an air-lift fermentor. *Biotechnol. Bioeng.* 1985,27: 1166-1172.

Wyk, J.P.H. and Mohulatsi, M. Biodegradation of wastepaper by cellulase from *Trichoderma viride*. *Bioresource Technology*. 2003,86: 21-23.

Wyman, C.E., Goodman, B.J. Biotechnology for production of fuels, chemicals and materials from biomass. **Appl. Biochem. Biotechnol.** 1993,39: 39-59.

Xia, L and Cen, P. Cellulase production by solid state fermentation on lignocellulosic waste from the xylose industry. **Process Biochemistry.** 1999,34: 909-912.

Xia, L and Xueliang, S. High-yield cellulase production by Trichoderma reesei ZU-02 on corn cob residue. Bioresource Technology.2004, 91: 259–262.

Yang YH, Wang BC Wang QH Xiang LJ Duan CR. Research on solid-state fermentation on rice chaff with a microbial consortium. Colloids and Surface B:Biointerfaces. 2004, 1-6.

Yu, P. Analysis of a municipal recyclable material recycling program. Resour. Conserv. 1996,Recy.