INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
12–636/2013/15–1–140–144
http://www.fspublishers.org

Full Length Article

Production and Characterization of a Novel β-Glucosidase from *Fusarium solani*

Haq Nawaz Bhatti[1]*, Samia Batool[1] and Nosheen Afzal[2]
[1]*Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad-38040, Pakistan*
[2]*Department of Chemistry, Govt College University Faisalabad, Pakistan*
*For correspondence: hnbhatti2005@yahoo.com

Abstract

β-glucosidase was produced from *Fusarium solani* using different agricultural wastes under solid state fermentation (SSF). Optimum production of β-glucosidase was achieved with rice husk at pH 5, moisture level 60%, and fermentation period of 72 h. Higher yields of β-glucosidase (3206 U/gds) were obtained from *F. solani* when the medium was supplemented with lactose. The enzyme was partially purified with ammonium sulfate precipitation yielding a specific activity of 97.5 U/mg. β-glucosidase was subjected to various kinetic and thermodynamic parameters to seek the nature of the enzyme. Optimum pH and temperature were found to be 4.5 and 65°C, respectively. The enzyme possessed K_m 1 mM and V_{max} 55.6 µmol min^{-1}. It was also observed that β-glucosidase was thermally stable up to 65°C. Mg^{2+} ions enhanced the enzyme activity. These characteristics suggest that β-glucosidase isolated from *F. solani* could be used for hydrolysis of carbohydrates and cellulosic materials used in textile, paper, starch processing, animal feeds and fruits industries.

Keywords: β-glucosidase; Solid state fermentation; *Fusarium solani*; Rice husk

Introduction

Cellulases are an important class of enzymes which are used for the hydrolysis of cellulosic materials for the production of glucose, alcohol, cellulose acetate oligosaccharides etc. They have applications in various industries like fuel, textile, paper, starch processing, feeds, fruits, vegetables, etc. (Zaldivar *et al.*, 2001). These enzymes are also used for non-specific cleavage of chitosan to form low molar mass oligosaccharides (Xia *et al*., 2008). Now-a-days lignocellulosic materials are under intensive research due to depletion of fossil fuels and production of ecofriendly biofuels.

Lignocellulosic materials mainly consist of cellulose which is available abundantly in nature. Hydrolysis of lignocellulosic wastes is of prime importance for its conversion into important industrial products. β-glucosidase (EC 3.2.1.21) is an important class of cellulolytic complex that completely breaks down various ligocellulosic wastes/materials by cleaving the β-1, 4-glycosidic bond (Wallecha and Saroj, 2003). Lignocellulosic biomass has been reported a promising feedstock, because of its wide abundance and low cost (Pandey *et al*., 1999). It has been reported that cellobiose and glucose inhibit the activity of β-glucosidase during cellulose hydrolysis. Besides microorganisms, glucosidases are found in plants and animals (Haki and Rakshit, 2003). Fermentation of agro-industrial wastes would not only reduce the emission of various pollutants but also serve as a vital source of energy for the production of cellulases and protein biomass. It is considered as a route towards its conversion into food, feed, fuel like bioethanol, enzymes and various chemicals like glucose, modified starches, cellulose acetate, terpenol etc.

Recently, attention has been given to various β-glucosidases due to their ability to control trans-glycosylation reactions, which have great importance in the wine industry due to its capacity to improve the aroma. In addition, β-glucosidases are more efficient and specific in their actions as compared to acid hydrolysis to yield terpenol from terpenyl-glucoside. These compounds have applications in the cosmetic, tobacco and food industries (Yejun and Hongzhang, 2008).

Various organisms are known to produce extracellular cellulases, however, fungi are often preferred due to secretion of large amount of extracellular cellulases which are easily extracted and purified (Pandey *et al*., 1999). This research was carried out to explore the potential of *F. solani* to produce β-glucosidase under solid state fermentation (SSF) using agro-industrial wastes, which can be utilized for industrial applications as described in previous section.

Materials and Methods

Microorganism

Pure culture of *F. solani* was obtained from the Department

To cite this paper: Bhatti, H.N., S. Batool and N. Afzal, 2013. Production and characterization of a novel β-glucosidase from *Fusarium solani. Int. J. Agric. Biol*., 15: 140–144

of Plant Pathology, University of Agriculture, Faisalabad, and maintained on potato-dextrose-agar slants. Vogel's medium containing the spores of *F. solani* was incubated on orbital shaker operating at 140 rpm for 48-72 h at 30±1°C, and was used as inoculum in the fermentation media to produce β-glucosidase (Bhatti *et al*., 2007).

Solid State Fermentation

Abundantly found agricultural waste products including rice husk, gram husk, wheat bran and wheat straw were used as substrate for maximum yield of β-glucosidase through SSF. SSF was carried out in 250 mL Erlenmeyer flasks containing 10 g of agricultural waste mixed with mineral solution containing 5.0, 2.0, 4.0, 0.2, 2.0, 2.5, and 1.0 g L^{-1} of KH_2PO_4, NH_4NO_3, $(NH_4)_2SO_4$, $MgSO_4.7H_2O$, peptone, trisodium citrate and yeast extract (pH 5), respectively. The factors affecting enzyme production were optimized by classical methods as described by Ellaiah *et al*. (2002). After appropriate fermentation period, 100 mL of distilled water was added to the flask containing fermented biomass and kept for 30 min on shaking at 120 rpm. The fermented extract was filtered thorough muslin cloth and the resultant extract was used for assay of β-glucosidase activity.

β-glucosidase Assay

β -glucosidase activity was determined with *p*-nitrophenyl-β-D-glucopyranoside (*p*NPG). *p*NPG (10 mM) was prepared in 50 mM acetate buffer (pH 5). The reaction was initiated by mixing 1 mL of 10 mM *p*NPG with an appropriately diluted enzyme extract (100 µL) for 10 min at 30°C. The reaction was quenched by the addition of 2 mL of 1 M Na_2CO_3 solution. The total volume of reaction mixture was adjusted to 10 mL with distilled water and absorbance was taken at 400 nm wavelength (Wood and Bhat, 1998). Proteins of the extract were determined by Bradford (1976) micro method.

Partial Purification and Characterization of B β-glucosidase

Solid ammonium sulfate [$(NH_4)_2SO_4$; 142 g/200 mL crude extract)] was adjusted to 95% saturation that was placed overnight at 4°C on magnetic stirrer. The supernatant was separated from cellular sediment debris obtained after centrifugation for 15 min at 10,000 rpm and 4°C. The residues after 95% $(NH_4)_2SO_4$ precipitation were re-dissolved in buffer and dialyzed against distilled water. This dialyzed extract was used for characterization of β-glucosidase (Leite *et al.,* 2007).

The activity of partially purified β-glucosidase was determined in a pH range of 2-10 in different buffers (acetate, phosphate and Tris/ HCl) using 10 mM *p*NPG as substrate. Temperature for activity of β-glucosidase was optimized by incubating the enzyme with 10 mM *p*NPG in a temperature range of 30-75°C (Rashid and Siddiqui, 1998).

The Michaelis-Menten kinetic constants (V_{max} and K_m) were determined with different substrate concentrations. Lineweaver-Burk plot was plotted between inverse of substrate concentration in mM and inverse of velocity, taking [1/S] along x-axis and [1/V] along y-axis. From the graph the values of V_{max} and K_m (Michaelis-Menton constant) were calculated **(**Rashid and Siddiqui, 1998).

Thermal inactivation studies of β-glucosidase were carried out by heating enzyme suspension at different temperature (65, 70, 75, 80°C). Time course extracts were taken, cooled in ice for 30-45 min and analyzed for β-glucosidase activity at 30°C. The kinetics for irreversible thermal denaturation (K_d) of β-glucosidase was determined and Arrhenius plot was applied to determine the activation energy for denaturation (*E*a) as described by Montes *et al.* (1995). Kinetic and thermal parameters for thermal inactivation of the enzyme were calculated from absolute rate equation (Bhatti *et al.* (2007). The free energy change (ΔG), enthalpy change (ΔH) and entropy change (ΔS) were calculated according to following equations:

$$t_{1/2} = 0.693/K_d \text{---------- (1)}$$

$$\Delta H^* = E\text{a} - \text{RT} \text{---------- (2)}$$

$$\Delta G^* = -RT \ln (K_d . h/K_b . T) \text{ --------- (3)}$$

$$\Delta S^* = (\Delta H^* - \Delta G^*)/T \text{---------- (4)}$$

Statistical Analysis

All the experiments were conducted in triplicate and results are reported as mean±SD (Steel *et al*., 1996).

Results and Discussion

Production of β-glucosidase from *F. solani*

The results revealed that rice husk had the maximum potential among other substrates, for the production of β-glucosidase (Fig. 1a). Maximum enzyme activity (1226 U g^{-1} dry substrate, U g^{-1}ds) was observed for rice husk and minimum (836 U g^{-1}ds) was recorded for wheat straw. Selection of a particular substrate is important parameter in case of SSF. So rice husk was selected in subsequent experiments.

It was found that maximum production of β-glucosidase (1562 U g^{-1}ds) was obtained in flasks having 60% moisture level (Fig. 1b) and an increase and/or decrease of moisture levels decreased the enzyme biosynthesis. Optimum moisture level is necessary to carry out SSF as it influences substrate porosity and hence oxygen transfer rate. The metabolic activities of the microorganism are very sensitive to changes in pH (Ellaiah *et al*., 2002). Maximum β-glucosidase activity (2105 U g^{-1}ds) was observed at pH of 5 (Fig. 1c). The β-glucosidase production by *F. solani* was affected when pH level was higher or lower than the optimum value the β-glucosidase production.

Effect of incubation period on the production of β-

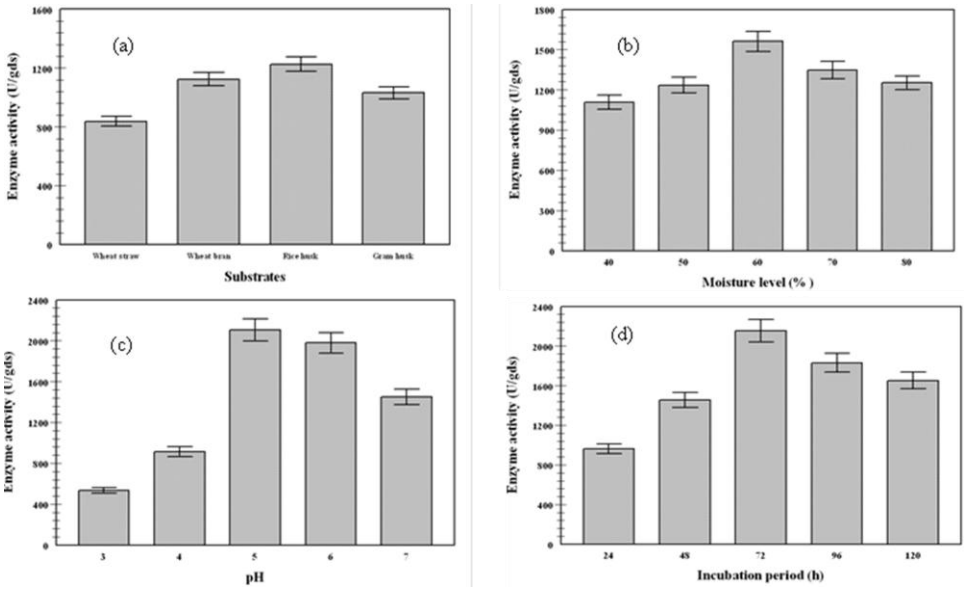

Fig. 1: Effect of **(a)** substrate **(b)** moisture level **(c)** pH of medium **(d)** incubation period on the production of β-glucosidase by *F. solani*

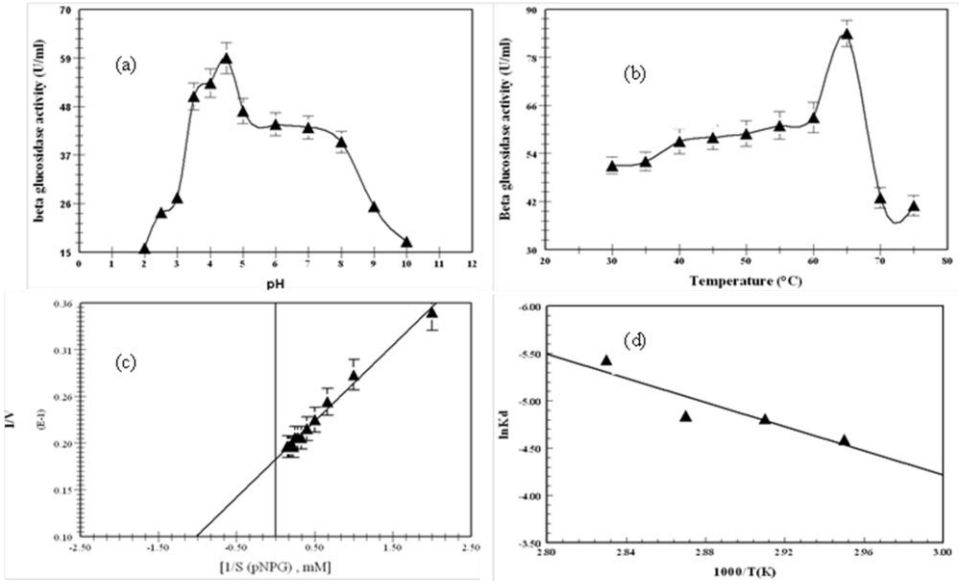

Fig. 2: Effect of (a) pH (b) Temperature (c) substrate concentration (d) heat treatments on the activity of β-glucosidase by *F. solani*

glucosidase by *F. solani* revealed that biosynthesis of the enzyme increases with incubation period (Fig. 1d). Maximum biosynthesis of the enzyme (2153 U g^{-1}ds) was observed after 72 h and the enzyme activity decreased thereafter, which might be due to repression of metabolites. Sabosh *et al.* (2007) also observed maximum production of glucosidase after 3 d of fermentation on wheat bran under SSF. Kang *et al.* (2004) recorded maximum activity (100 U g^{-1}ds) of β-glucosidase activity from *Aspergillus niger* after 5 d of fermentation when grown on rice straw during SSF. The effect of different carbon sources including maltose, fructose, lactose and starch (1%) on β-glucosidase production was also studied, which was found to be enhanced up to 3206 U g^{-1}ds by using lactose as carbon source.

Partial purification and characterization of β-glucosidase

The crude enzyme extract possessing 1.07 mg of total

Table 1: Kinetic and thermodynamic parameters of *F. solani* β-glucosidase

Temperature (K)	K_d (min^{-1})	$t_{1/2}$ (min^{-1})	ΔH^* ($kJmol^{-1}$)	ΔG^* ($kJmol^{-1}$)	ΔS^* ($Jmol^{-1}K^{-1}$)
338	0.004	159	50.49	110	-176
343	0.007	87	50.45	110	-170
348	0.008	85	50.41	111	-174
353	0.01	68.5	50.37	112	-174

Table 2: Effect of various metals on *F. solani* β-glucosidase activity

Metals (Conc. 1mM)	Relative activity (%)
Mg	488
Hg	100
Cd	101
Pb	98
K	93
Control	100

protein was partially purified with $(NH_4)_2SO_4$ and the resultant specific activity of the enzyme increased from 51.4 to 97.5 U mg^{-1}. The β-glucosidase isolated from *F. solani* was active within the pH range of 3.0-8.0. The maximum activity of the enzyme was (59 U mL^{-1}) observed at pH 4.5 (Fig. 2a), which was identified as optimum pH as well. It revealed that β-glucosidase was acidic in nature. Riuo *et al.* (1998) purified and characterized β-glucosidase that had a pH and temperature optima of 5 and 50°C, respectively. β-glucosidase was assayed at different temperatures (30-75°C) at pH 5 and maximum activity (84 U mL^{-1}) was obtained at 65°C that was also optimum temperature of the enzyme activity (Fig. 2b). Leite *et al.* (2007) reported that β-glucosidase s isolated from *Aureobasidium pullalans and Thermoascus auratiacus* have pH and temperature optima of (4-4.5 and 4.5) and 80 and 75°C, respectively. Rashid and Siddiqui (1998) isolated β-glucosidase from *Aspergillus niger*. The pH optimum was found to be 3.4. Riuo *et al.* (1998) characterized a novel β-glucosidase from *A. oryzae* using *p*NPG and it was observed that the enzyme showed optimum activity at pH 5.0 and at 50°C temperature. Jeya *et al.* (2009) isolated β-glucosidase from *Penicillium purpurogenum.* The optimum temperature and pH of the isolated enzyme was 32°C and 4, respectively.

Michaelis-Menten kinetic constants (V_{max} and K_m) were determined using concentrations of *p*NPG ranging from 1-10 mM (Fig. 2c). K_m was recorded as 1 mM with V_{max} 55.6 µmoL $min^{-1,}$ and such lower K_m indicated that enzyme had greater tendency towards *p*NPG. Yejun and Hongzhang (2008) isolated a β-glucosidase from corn stover and observed a K_m and V_{max} of 2.3 mM and 18.6 µmoL min^{-1}, respectively.

Thermo-inactivation of β-glucosidase: Thermal inactivation of the β-glucosidase isolated from *F. solani* was studied at four temperatures (65, 70, 75 and 80°C) and its energy of inactivation was determined thereof (Fig. 2d). The results revealed that the β-glucosidase was very stable at 65°C and an increase in temperature up to 80°C resulted in progressive loss of its activity. The thermodynamics of thermo-inactivation of enzyme was also calculated (Table 1). The linear trend of residual activity was obtained against pre-incubation time, which indicated a first order plot (Fig. 2d). Rashid and Siddiqui (1998) also reported a first order kinetics for β-glucosidase obtained from *A. niger*. The slope was drawn using 1n of K_d ($\ln K_d$) against the reciprocal of the absolute temperature (Fig. 2d). Energy of activation (E_a) for denaturation was found to be 53.31 kJ mol^{-1}. Such a higher E_a for *F. solani* β-glucosidase indicated that the conformation of the enzyme was still stable at the temperature on which the enzyme has been assayed (Rodrigo *et al.*, 2007).

β-glucosidase presented a larger Gibbs free energy (ΔG^*) at all the temperatures. However, when the temperature was increased from 65-80°C there was reduction in ΔG^* (Table 1), indicating destabilization of the protein at this temperature (Rodrigo *et al.*, 2007). The values of change in Entropy (ΔS^*) did not reflect a significant change at varying temperatures. Increase in entropy by temperature is considered to be due to the exposure of hydrophobic residues during the unfolding of the enzyme at increasing temperature. An analysis of ΔH^* enzyme it can be concluded that the enthalpy change was independent of temperature, therefore, no change in enzyme heat capacity was observed (Cobos and Estrada, 2003), and hence, the enzyme under investigation is thermostable and quite suitable for textile, paper, food, and animal feed industries.

Effect of Metals on β-glucosidase Activity

Metal ions including K^+, Hg^{2+}, Cd^{2+}, Pb^{2+} and Mg^{2+} (1 mM) were examined for their effect on β-glucosidase activity. The data (Table 2) showed that Mg^{2+} ions enhanced the activity of β-glucosidase presumably by increasing the binding affinity of enzyme and stabilizing the conformation of the catalytic site (Mansfield *et al.*, 1998). Contrarily, K^+ ions suppressed the activity of β-glucosidase. Nakkharat and Haltrich (2006) reported that Na^+, K^+ and Mg^{2+} ions strongly triggered the activity of β-glucosidase enzyme and added to its stability.

Conclusions

Present work describes the isolation and characterization of a novel β-glucosidase from *F. solani* under SSF conditions using rice husk as substrate. Production of β-glucosidase with optimized conditions showed that maximum activity of the enzyme was attained after 96 h. Optimum pH and temperature of the enzyme were identified as 4.5 and 65°C, respectively. The thermal stability of β-glucosidase suggested its possible utilization in the hydrolysis of carbohydrates and cellulosic materials used in textile, paper, starch processing, animal feed and fruit industries.

References

Bhatti, H.N., G. Mustafa and M. Asgher, 2007. Production of glucoamylase by *Fusarium moniliformi* under solid-state fermentation. *J. Chem. Soc. Pak.*, 29: 99–102

Bradford, M.M., 1976. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Anal. Biochem*., 72: 248–254

Cobos, A. and P. Estrada, 2003. Effect of polyhydroxylic cosvolents on the thermostability and activity of xylansae from *Trichoderma reesei* QM9414. *Enz. Microb. Technol*., 33: 810–818

Ellaiah, P., K. Adinarayana, Y. Bhavani, P. Padmaja and B. Srinivasulu, 2002. Optimization of process parameters for glucoamylase production under solid state fermentation by a newly isolated *Aspergillus* species. *Proc. Biochem*., 38: 615–620

Haki, G.D. and S.K. Rakshit, 2003. Developments in industrially important thermostable enzymes: a review. *Bioresour. Technol*., 89: 17–34

Jeya, M., A.R. Joo, K.M. Lee, M.K. Tewari, K.M. Lee, S.H. Kim and J.K. Lee, 2009. Characterization of β-Glucosidase from a strain of *Penicillium purpurogenum* KJS506. *Appl. Microbiol. Biotechnol*., 108: 1187–1198

Kang, S.W., Y.S. Park, J.S. Lee, S.I. Hong and S.W. Kim, 2004. Production of cellulases and hemicellulases by *Aspergillus niger* KK2 from lignocellulosic biomass. *Bioresour. Technol*., 91: 153–156

Leite, R.S.R., E. Gomes and R. da-Silva, 2007. Characterization and comparison of thermostability of purifies β-glucosidase from a mesophilic *Aureobasidium pullalans* and a thermophilic *Thermoascus aurantiacus*. *Process Biochem*., 42: 1101–1106

Mansfield, S.D., J.N. Saddler and G.M. Gubitz, 1998. Characterization of endoglucanase from the brown rot fungi *Gloeophyllum sepiarium* and *Gloeophyllum traberum*. *Enz. Microbial. Technol*., 23: 133–140

Montes, F.J., E. Battaner, J. Catalan and M.A. Galan, 1995. Kinetics and heat inactivation mechanism of D-amino acid oxidase. *Process Biochem*., 30: 217–224

Nakkharat, P. and D. Haltrich, 2006. Purification and characterization of an intracellular enzyme with β-glucosidase and β-galactosidase activity from the thermophilic fungus *Talaromyces thermophilus* CBS 236.58. *J. Biotechnol*., 123: 304–313

Pandey, K.M., 1999. Characterization of a novel ginsenoside-hydrolyzing β-glucosidase from *Paecilomyces Bainier* sp. 229. *Biosci. Biotechnol. Biochem*., 72: 352–359

Rashid, M.H. and K.S. Siddiqui, 1998. Thermodynamic and kinetic study of stability of the native and chemically modified β-glucosidase from *Aspergillus niger*. *Process Biochem*., 33: 109–115

Riuo, C., J.M. Salmon, M.J. Vallier, Z. Gunata and P. Barre, 1998. Purification, characterization and substrate specificity of a novel highly glucose-tolerant β-glucosidase from *Aspergillus oryzae*. *Appl. Environ. Microbiol*., 64: 3607–3614

Rodrigo, S.R.L., G. Eleni and D.S. Roberto, 2007. Characterization and composition of thermostability of purified β-glucosidase from mesophilic *Aureobasidium pullulans* and a thermophilic *Thermoascus aurantiacus*. *Process Biochem*., 42: 1101–1106

Sabosh, C.M., V. Buddola and B.R. Rajesekhar, 2007. Cellulolytic enzymes on lignocellulosic substrates in solid state fermentation by *Aspergillus niger*. *Ind. J. Microb*., 47: 323–328

Steel, R.G.D., J.H. Torrie and D.A. Dickey, 1996. *Principles and Procedures of Statistics: A Biometrical Approach*, 3rd edition. McGraw Hill Book Company, Inc. New York, USA

Wallecha, A. and M. Saroj, 2003. Purification and characterization of two β-glucosidases from a thermo-tolerant yeast *Pichia etchellsii*. *Proteins Proteom*., 1649: 74–84

Wood, T.M. and K.M. Bhat, 1988. Methods for measuring the cellulase activities. *Meth. Enzymol*., 160: 87–112

Xia, W., P. Liu and L. Liu, 2008. Advances in chitosan hydrolysis by non-specific cellulases. *Bioresour. Technol*., 99: 6751–6762

Yejun, H. and C. Hongzhang, 2008. Characterization of composition of rice husk β-glucosidase from corn stover and its application in simultaneous saccharification and fermentation. *Bioresour. Technol*., 32: 632–637

Zaldivar, J., J. Nielsen, and L. Olsson, 2001. Fuel ethanol production from lignocellulose: a challenge for metabolic engineering and process integration. *Appl. Microbiol. Biotechnol*., 56: 17–34

(Received 08 August 2012; Accepted 24 October 2012)