African Journal of Biotechnology Vol. 11(4), pp. 843-851, 12 January, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.3032
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Oxalic acid pretreatment, fungal enzymatic saccharification and fermentation of maize residues to ethanol

Godliving Y. S. Mtui

Department of Molecular Biology and Biotechnology, University of Dar es Salaam, P.O. Box 35179, Dar es Salaam, Tanzania. E-mail: gmtui@hotmail.com.

Accepted 7 December, 2011

Dilute oxalic acid pretreatment was investigated as a feasible method for conversion of maize residues into sugars and subsequent production of bioethanol. Oxalic acid concentration of 200 mM for samples treated at 160°C for 10 min was found to be the best pretreatment conditions. Cellulases, mainly cellobiohydrolases from *Trichoderma reesei*, recorded activities of up to 116 U/ml during saccharification of the cellulosic substrates. The optimum saccharification conditions using 0.2% (v/v) enzyme concentrate were the incubation temperature of 45°C for 48 h. Up to 46% (w/w) glucose and 28% (w/w) xylose were obtained from the pretreated maize residues (stalk, leaf, husk and cobs). Furthermore, a native *Saccharomyces cerevisiae* (strain *KB*) was able to convert 78% (w/w) of glucose (and other fermentable sugars) to ethanol after 60 h of incubation at 32°C, under stationary culture conditions. The challenges encountered in chemical and biological conversions included incomplete hydrolysis and fermentation due to substrate recalcitrance and the inability of the yeast cells to utilize 5-carbon sugars such as xylose. This study therefore provided baseline research data and information that could be used for more elaborate and scaled-up studies for possible industrial-scale conversion of the readily available crop residues into bioenergy.

Key words: Bioethanol, cellulases, fermentation, hydrolysates, maize residues, oxalic acid, pretreatment, saccharification.

INTRODUCTION

Maize (*Zea mays*) is the main cereal crop grown all over the world mainly for human consumption. In 2009, over 159 million hectares of maize were planted worldwide, with a yield of over 5 tons/hectare (FAO, 2009). It generates more than 50% of its yield as residues mainly straw, husks, skins and trimmings, cobs and bran. In the sub-Saharan Africa, maize is the main staple food crop, generating substantial amounts of residues (Denning et al., 2009). In Tanzania, white maize represents over 75% of total cereals, with an average annual production of over 2.4 million tons (Nyange and Wobst, 2005). These residues are often burned, ploughed into the soil, or fed to animals (Adebowale, 2011). The wide availability and abundance of maize residues make them an attractive source of biofuel ethanol due to their large cellulose and hemicellulose reserves (Leathers, 2003; Lee et al., 2010). However, due to their rigid nature, pretreatments steps are necessary in order to expose the cellulose and hemicellulose chains to enzymatic hydrolysis and subsequent microbial mediated fermentation.

The maize residues are mainly composed of three components: Cellulose, hemicellulose and lignin. Cellulose is a polymer of D-glucose units with *beta*-1,4 linkages between each of about 500 to 10,000 glucose units, while hemicellulose is a polymer of sugars, primarily D-xylose with other pentoses and some hexoses with *beta*-1,4 linkages (Mtui and Nakamura, 2005; Lee et al., 2009). Lignin is a complex random phenolic polymer forming a sheath around the cellulose and hemicellulose polymers. The ratio of these three components varies depending on

the type of biomass, but in maize it is estimated at 35 to 40% cellulose, 25 to 35% hemicellulose and 15 to 20% lignin (Sun and Cheng, 2002; Koundinya, 2009; SDSU, 2010). Therefore, maize residues represent a cheap and readily available substrate for production of sugars that may be used alone or microbiologically fermented to produce renewable energy such as alcohols, biogas and other industrial chemicals.

A biomimetic approach where dicarboxylic acids mimic natural enzymes structurally has recently become an interesting area of research. Oxalic acid pretreatment at high temperature has become an essential step towards enzymatic hydrolysis of lignocellulosic biomass. Under mild conditions, oxalic acid is selective enough to avoid extensive cellulose and glucose degradation (Mosier et al., 2001, 2002). Therefore, oxalic acid is suitable as a pretreatment step in order to release fermentable hemicellulosic sugars without extensive formation of degradation products (Lu and Mosier, 2007; Lee et al., 2010). On the other hand, xylan, which forms a barrier that restricts cellulases access to cellulose, can be selectively removed by using mild oxalic acid pretreatment (Berlin et al., 2007; Lee et al., 2009, 2010). Furthermore, since agricultural residues are less recalcitrant than wood, a mild acid treatment which will not cause extensive degradation of arabinose and xylose while still preparing the cellulose for enzymatic saccharification would be desirable (Lee et al., 2009).

Enzymatic saccharification of cellulosic substrates is achieved by using hydrolytic enzymes, mainly cellulases and hemicellulases. Such enzymes include endoglucanase, cellobiohydrolase, *beta*-glucosidase and xylanases. The synergistic actions of these enzymes result in complete hydrolysis of cellulose and hemicellulose to glucose and xylose, respectively (Margeot et al., 2009). *Trichoderma reesei* is a cellulolytic mesophilic and filamentous fungus that is potentially important in producing both cellulases and hemicellulases, which are able to produce reducing sugars from lignocellulosic substrates, including corn stover (Juhasz et al., 2005). Temperature, pH, residence time and enzyme loading rate are important optimisation parameters in saccharification processes (Saha et al., 2005). Moreover, yeast fermentation of dilute acid-pretreated lignocellulosic wastes has achieved a great success in optimal production of ethanol depending on the strain used. However, in order to overcome recalcitrance challenges, it is necessary to bioprospect for adaptive *Saccharomyces cerevisiae* that would suit specific hydrolysates (Mtui and Nakamura, 2005; Talebnia and Taherzadeh, 2006).

This study investigated high temperature dilute oxalic acid pretreatment of maize residues followed by saccharification by using a crude cellulase extract from *T. reesei* and fermentation by a native *S. cerevisiae* (strain *KB*) isolated from a decomposing lignocellulosic heap. The objective of the study was to elucidate optimal conditions for efficient pretreatment, saccharification and fermentation of local maize residues using native enzmes and microorganisms.

MATERIALS AND METHODS

Sampling and sample handling

Maize stovers (stalks, leaves, husks and cobs) were collected in a maize farm at Kibaha, a coastal region in Eastern Tanzania, after a seasonal harvest. The samples were chopped to 1 cm length, followed by drying in the open sun for one week. In the laboratory, the chopped residues were mixed and crushed by a grinding miller to powder form to pass in a mesh size of 1 mm. The samples were oven-dried at 105 ± 3°C to constant weight and stored in a desiccator.

Oxalic acid pretreatment

Dry powdered samples were pooled and mixed thoroughly, and then 1 g of ground and oven-dried maize residue samples were put into pressure resistant Pyrex tubes and mixed with 100 ml of various (100, 200 or 300 mM) concentrations of oxalic acid. The tubes were tightly stoppered and incubated in an oven at different temperatures (150, 160 and 170°C) for 5, 10, and 15 min. After heating, samples were left to cool at room temperature.

Saccharification

Saccharification reaction was carried out by using 0.1, 0.2 and 0.3% (v/v) crude enzyme extract from *T. reesei*. The enzyme concentrate was obtained from Uppsala University, Sweden. 50 mM sodium acetate buffer was used to maintain the reaction mixture at pH 5.0. The pretreated samples in 125 ml Erlenmeyer flasks were incubated in a water bath at 40, 45, 50, 55 and 60°C, and sampling for analysis was done after incubation for 12, 24, 36, 48, 60 and 72 h at stationary incubation condition. The shake-flask samples (those shaken at 100 rpm using a rotary shaker) were incubated at the same temperatures. The hydrolysate (pretreated sample that has undergone enzymatic saccharification) was then subjected to various analyses.

Assays

Cellulase activity was calculated using 3,5-dinitrosalicyilic acid (DNS) method for the determination of reducing sugars concentration (Sinegani and Emtiazi, 2006). The DNS reaction was carried out at 100°C in a water bath. After cooling, absorbance was determined at 540 nm using a UV-Visible spectrophotometer. All fermentable sugars (excluding xylose) were reported as glucose concentration using DNS glucose assay (Wang, 2011). Glucose concentration was calculated based on glucose standard curves. Xylose concentration was determined by HPLC utilizing HPX-87H column (Bio-Rad Labs Inc., CA) and 5 mM H_2SO_4 as a mobile phase in deionized water. The HPLC column was run at 60°C with a mobile phase flow rate of 0.5 ml/min. standard curves were obtained by dissolving pure xylose in the mobile phase. Total reducing sugars yield was calculated as the sum of the yields of the main monosaccharides, namely glucose (including other undetermined reducing sugars such as cellobiose, mannose and galactose) and xylose.

Cellobiohydrolase (CBH) assay was carried out according to Wu et al. (2006) by measuring the activity of CBH on *para*-nitrophenyl *beta*-D-cellobioside (PNPC) substrate to form *para*-nitrophenol (PNP). At the end of the reaction, 10% Na_2CO_3 was added to maintain alkaline environment and enable PNP to fully develop a

Table 1. Xylose (% w/w) produced at various incubation temperature, oxalic acid concentration and reaction time during high temperature pretreatment of maize residues with oxalic acid.

Experiment number	Temperature (°C)	Oxalic acid concentration (mM)	Reaction time (min)	Maximum xylose yield (% w/w)
		100	15	22 ± 1.2
1.1	150	200	15	25 ± 2.0
		300	15	24 ± 1.7
		100	10	24 ± 3.5
1.2	160	200	10	28 ± 2.5
		300	10	18 ± 3.5
		100	5	20 ± 2.6
1.3	170	200	5	22 ± 1.8
		300	5	22 ± 3.2

Xylose yields were calculated based on the amount (g) of xylose produced per the dry weight (g) of the substrate.

yellow colour. The absorbance was measured immediately at 410 nm in one-minute intervals to assess the formation of PNP. One unit (U) of CBH activity was defined as activity of an enzyme that catalyzes the conversion of one micro mole of PNPC per minute.

Fermentation

A native strain of *S. cerevisiae* (strain *KB*) was isolated from decomposing cellulosic straw compost and maintained in malt extract agar at 4°C. The strain is currently being characterized and will be deposited in a culture collection facility. The enzyme-treated hydrolysate reaction mixture containing reducing sugars was subjected to fermentation by using precultured *S. cerevisiae* (strain *KB)* in 125 ml Erlenmeyer flasks. The medium, autoclaved at 121°C for 15 min, contained 50 mM sodium acetate buffer (pH 6), 5 g/L yeast extract, 5 g/L urea, and 0.05 g/L $MgSO_4.7H_20$. Incubation temperature was maintained at 30, 32 and 35°C in both stationary culture and shake-flask (100 rpm) culture conditions. Sampling was done after 12, 24, 36, 48, 60 and 72 h of incubation. Ethanol concentration was determined by gas chromatography (GC) using 120/80 6.6% carbowax column. The GC was set at 90°C oven temperature, 170°C injection and 175°C detection temperatures.

Standards were prepared using 1 g methanol, 5 g ethanol, 1 g propanol, 1 g butanol and 2 g iso-butyl alcohol. Distilled water was added into 120 ml serum bottle to make up a total of 100 g. Ethanol concentration was calculated based on the peak areas of the samples and standard solutions (Mtui and Nakamura, 2005). Ethanol yield (% w/w) was determined as the ratio of the ethanol produced to the amount of fermentable sugars (mainly glucose) consumed. All the experiments were carried out in triplicate.

RESULTS

The pretreatment of maize residues by dilute oxalic acid at 150 to 170°C resulted to partial hydrolysis of mainly the hemicellulose component to reducing sugars. Table 1 shows the amounts of xylose produced from samples treated by 100 to 300 mM oxalic acid at various (5 to 15 min) reaction times. It was observed that 200 mM oxalic acid concentration was the best dilution for maximum production of reducing sugars after 10 min of oven incubation at 160°C. It can be deduced from the results that higher temperatures and lower reaction times gave more-or-less the same effect. Based on that observation, the pretreatment of the subsequent experiments was set at the best conditions (200 mM oxalic acid for 10 min at 160°C). Furthermore, a summary of enzymatic saccharification results is shown in Table 2. Crude enzyme concentrates of varying concentrations ranging from 0.1 to 0.3% (v/v) at incubation temperature range of 40 to 60°C were tested for their ability to produce reducing sugars (mainly glucose) from maize residue hydrolysates pretreated at 200 mM acid for 10 min at 160°C. It was observed that the cellulolytic enzymes worked best at 45°C, although the enzymes were still stable at 60°C. The best enzyme concentration was 0.2%, although 0.1 and 0.3% concentrations gave comparable results. The average of the total reducing sugars yield (by weight) was about 72%, the maximum being 74% (of which glucose was 46% and xylose was 28%) at 0.2% enzyme concentration incubated at 45°C for 48 h.

The time course profiles during saccharification of maize residues for samples pretreated at 160°C at various oxalic acid concentration is shown in Figure 1. The enzyme concentration was 0.2% (v/v) and the incubation was increased exponentially and peaked after 45 h of incubation, after which no further production of reducing sugars was observed. The best reducing sugars yield was obtained for samples pretreated with 200 mM oxalic acid, while 100 and 300 mM oxalic acid pretreatments gave slightly lower values. In addition, during saccharification, the enzymatic actions were monitored by measuring the cellobiohydrolase (CBH, 0.2%, v/v) activity profiles from 40 to 60°C (Figure 2). The average initial enzyme activity was 116 U/ml. As saccharification proceeded in all the tested incubation temperatures, the CBH activities decreased and reached minimum values

Table 2. Maximum glucose production at various enzyme concentration and saccharification temperature for samples pretreated by 200 mM oxalic acid at 160°C for 10 minutes.

Experiment number	Amount of enzyme concentrate (% v/v)	Saccharification Temperature (°C)	Glucose concentration (g/L)	Total reducing sugars yield (% w/w)
		40	4.2 ± 0.1	70
		45	4.4 ± 0.3	72
2.1	0.1	50	4.3 ± 0.2	71
		55	4.2 ± 0.3	70
		60	4.2 ± 0.1	70
		40	4.3 ± 0.2	71
		45	4.6 ± 0.3	74
2.2	0.2	50	4.4 ± 0.2	72
		55	4.2 ± 0.3	70
		60	4.2 ± 0.2	70
		40	4.3 ± 0.2	71
		45	4.5 ± 0.3	73
2.3	0.3	50	4.4 ± 0.6	72
		55	4.3 ± 0.2	73
		60	4.2 ± 0.1	70

The enzyme loading was based on experimental observations that showed that below 0.1% (v/v) the hydrolysis was incomplete, while above 0.3% (v/v) there was no significant effect on the total reducing sugars produced.

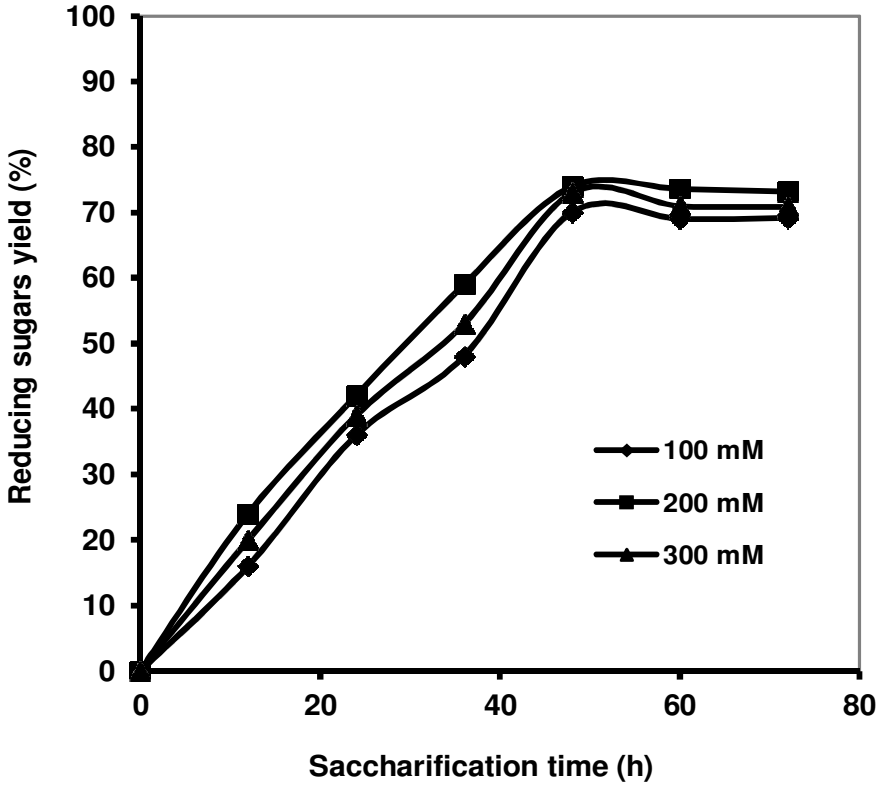

Figure 1. Time course profiles of enzymatic saccharification of maize residues pretreated at different oxalic acid (100 to 300 mM) concentrations at 160°C for 10 min. Values are averages of triplicate samples.

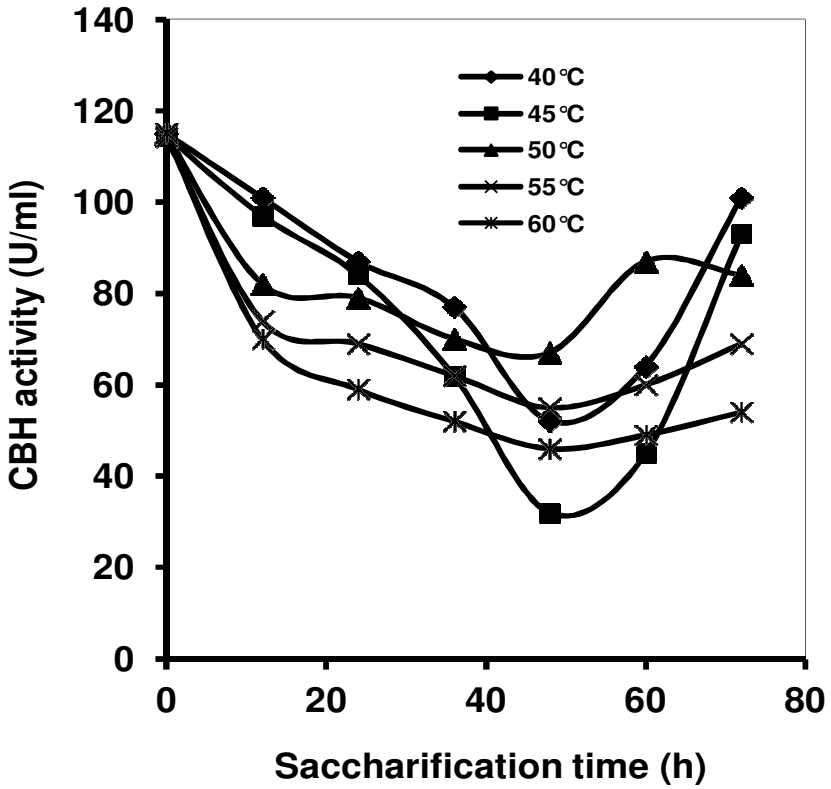

Figure 2. Enzyme activities of cellobiohydrolase (CBH) during saccharification of maize residues at various (40 to 60℃) incubation temperatures. Values are averages of triplicate samples.

Table 3. Maximum ethanol production from maize residue hydrolysate pre- saccharified at 45℃ for 48h using 0.2% (v/v) enzyme concentrate.

Experiment number	Culturing method	Incubation temperature (℃)	Ethanol concentration (g/L)	Ethanol yield (% w/w)
3.1	Static culture	30	3.3 ± 0.2	71.7
		32	3.6 ± 0.3	78.2
		35	3.4 ± 0.1	73.9
3.2	Shake-flask culture	30	2.7 ± 0.2	58.7
		32	2.9 ± 0.3	63.0
		35	2.8 ± 0.4	60.9

Fermentation was carried out by *S. cerevisiae* (strain *KB*) at pH 6.0. Shake-flask cultures were incubated in a rotary shaker set at 100 rpm.

after 48 h of incubation before rising again steadily, thus showing that at low activity values, the enzymes were mostly bound to the cellulosic substrates during catalysis. It is noteworthy that the 45℃ incubation condition gave more dramatic variations of CBH activity compared to other incubation temperatures. Following the observation made in Figure 1 and Table 2, the subsequent fermentation experiments were carried out using hydrolysates saccharified at the best conditions, which was 0.2 % (v/v) enzyme concentration at incubation temperature of 45℃ and incubation time of 48 h.

Furthermore, bioethanol fermentation by using a native *S. cerevisiae* (strain *KB*) isolated from a lignocellulosic compost heap was attempted both at static and shake-flask (100 rpm) culture conditions at 30 to 35℃ (Table 3). Overall, the results showed that fermentation at stationary culture condition resulted to more ethanol production compared to the fermentation experiments carried out at shake-flask culture conditions. The maximum ethanol production was achieved at the incubation temperature of

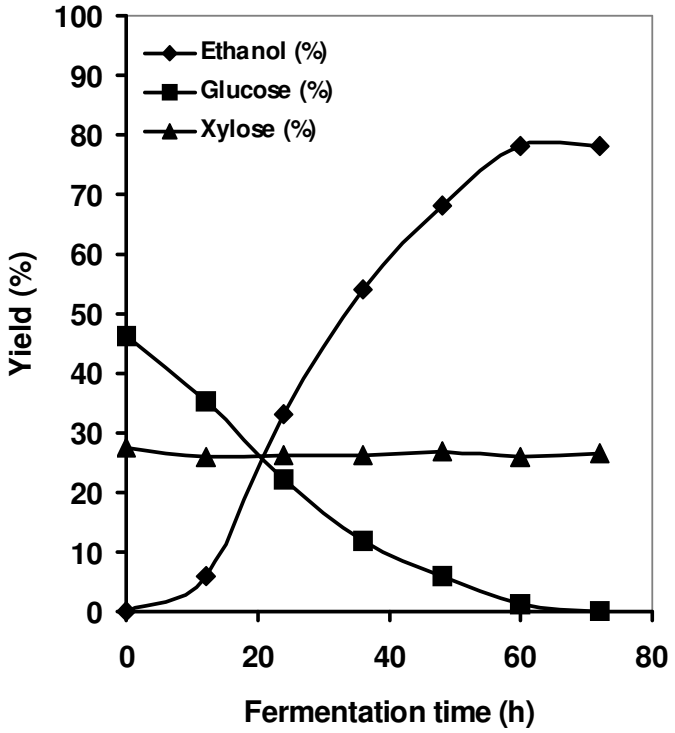

Figure 3. Fermentation of maize residue hydrolysate by a native yeast strain (*Saccharomyces cerevisiae* KB) at 32°C, pH 6.0 under static culture condition. The yield was calculated based on the ratio of the weight (in grams) of the product to the dry weight of the pretreated maize residues.

32°C, whereas the ethanol yield determined as the ratio of the ethanol produced to the glucose consumed was found to be 78.2% (w/w). Shake-flask culture fermentations resulted to comparatively lower ethanol yields in a range of 58 to 60% (w/w), depending on the fermentation temperature.

The results on time course profiles of fermentation of reducing sugars from pretreated maize residue hydrolysate samples are given in Figure 3. Glucose was consumed completely after 60 h of incubation. On the other hand, ethanol production increased exponentially up to 48 h of incubation, and reached its peak at 60 h. The ethanol yield (in relation to fermentable sugars, mainly glucose consumed) was attained maximally at 78.2% (w/w). It was observed that the native *S. cerevisiae* (strain *KB*) was unable to ferment five carbon sugars, and this was evidenced by the fact that the concentration of xylose remained unchanged even after 72 h of fermentation.

DISCUSSION

Pretreatment studies on lignocellulosic substrates by strong or dilute inorganic acids such as H_2SO_4, HCl or H_3PO_4 have resulted to hydrolytic products (Okafoagu and Nzelibe, 2002, Iranmahboob et al., 2002; Binder and Raines, 2010; Hsu et al., 2010; Canilha et al., 2011; Palkovits et al., 2011; Tian et al., 2011). However, hydrolyses carried out by these acids are non selective and their by-products are harmful to the environment. Therefore, their applications are currently being discouraged in large-scale applications (Lu and Mosier, 2007; Lee et al., 2009). On the other hand, applications of dilute dicarboxylic acids including acetic acid, oxalic acid, maleic acid and other organic solvents that mimic the natural catalytic processes provide a feasible avenue for pretreatment and hydrolysis of lignocellulosic substrates because they are selective, affordable and environmentally friendly (Viola et al., 2008; Lee et al., 2009; Zhao et al., 2009; Tan et al., 2010). This study has shown that dilute oxalic acid at high temperature is well suited for that purpose. It is noteworthy that the difference in temperatures between 150 and 170°C did not have much effect on the amount of sugars produced, neither were the differences in reaction times (5 to 15 min) and oxalic acid concentrations (100 to 300 mM). Higher temperatures and short reaction times seem to be the most preferable treatment methods for hydrolytic conversions. These observations were in line with the hydrothermal hydrolysis results for lignocellulosic substrates observed by Asada et al. (2005), Yu et al. (2008), Cybulska et al. (2010), Matsushita et al. (2010), Teng et al. (2010) and Ingram et al. (2011). One of the challenges facing biomimetric dicarboxylic acids catalysis, however, is the presence of lignin which tends to inhibit the accessibility to the cellulose and hemicellulose fibres. This problem could be solved through alkali delignification treatments prior to acid hydrolysis (Asada et al., 2005; Grabber et al., 2008).

Fungi with potential for hydrolysis of cellulosic substrates provide a feasible solution to biotechnological approaches towards industrial productions. Fungal enzymatic hydrolysis of agricultural residues has been widely investigated. These residues include corn, wheat, barley, rice, rye and pea (Sinegani et al., 2005; Valascova and Baldian, 2006; Hsu et al., 2010; Ingram et al., 2011; Liu et al., 2011). This study utilized maize residues as feedstocks and crude enzymes from *T. reesei*, a mesophilic filamentous fungus capable of producing a variety of cellulases. *T. reesei* proved to be a good producer of both endo- and exocellulases (data not shown). The enzymatic assays showed that *T. reesei* produces good performing cellobiohydrolases (CBH) with activities as high as 117 U/ml. The enzymes were responsible for the hydrolysis of maize residues to reducing sugars at a rate that could be applied in an industrial setting. The favourable conditions for maximum hydrolytic reactions (incubation temperature of 45°C for 48 h) observed in this study were in agreement with results reported by Taherzadeh and Kirimi (2007) that cellulases carry out hydrolysis of cellulose to glucose at optimal temperature range of 40 to 50°C and pH range of 4.5 to 5.0. Bioprospecting for lignocellulosic fungi with exclusive xylanolytic potential will therefore

solve the problem of selective xylan removal, which would facilitate direct access of cellulases to the cellulose chains (Pandey and Pandey, 2002; Garcia-Aparicio et al., 2007). The challenge encountered in enzymatic hydrolysis was the partial convertibility of substrates, whereby some of the hydrolysable components of the maize stovers were not degraded by CBH. This could be attributed to the presence of enzyme-inhibitory products such as lignin and other phenolic compounds in the hydrolysate (Mtui and Nakamura, 2005, 2007, 2008). However, the presence of inhibitory substances and low degradeability challenges can be met by the use of recombinant strains or enzymes that have been shown to be more effective than the natural ones (Alvira et al., 2011; Park et al., 2011).

Yeast fermentation of cellulosic hydrolyzate has been a subject of interest in research (Mtui and Nakamura, 2005; Talebnia and Jaherzadeh, 2006). In the current study, a native *S. cerevisiae* (*strain KB*) adapted to local conditions was used in the fermentation of the maize residue hydrolysates. Overall, the fermentation experiments achieved a maximum ethanol yield of 78.2% (w/w) from the produced fermentable sugars. These values were experimentally (not theoretically) calculated. The reason why the values exceeded the stoichiometrically possible theoretical ethanol yield for normal yeast could be due to the possibility that cellulose hydrolysis continued during the fermentation step. The stationary culture was superior to the shake-flask (100 rpm) culture condition as regards the amount of ethanol produced from hydrolysates. This could be attributed to the tendency of some microorganisms to work better under static conditions due to reduced stress caused by shaking, and also they may prefer a facultative anaerobic condition which is achievable in a static state (Mtui and Nakamura 2007, 2008). The inability of *S. cerevisiae* (strain *KB*) to ferment xylose sugars poses a challenge that needs a technological solution. Fermentation of 5-carbon sugars has continued to be a hurdle when it comes to conventional microbial fermenting organisms. This problem has been partially solved when mutants or genetically engineered microbes such as recombinant *Klebsiella oxytoca* strain P2 (Silva et al., 2005); *S. cerevisiae* (Lu and Mosier, 2007); and *Escherichia coli* KO11 (Rao et al., 2007; Trinh et al., 2010), are used for fermentation.

This study has shed some light on the potential of using the readily available crop wastes to address the challenges of producing renewable biofuel such as bioethanol through conventional biotechnology. Nevertheless, further research work is still needed to optimize production conditions and scale-up the processes for large-scale production. Gaining full understanding of the specific enzyme mechanisms on fungal cellulose degradation will be a useful tool for a better choice of fungi, enzyme or reaction parameters for improved performance (Petersen et al., 2009). Future research in this area will be directed towards increasing the substrate ratio, digestibility and compositional analysis, and application of mutants or transgenic microorganisms to improve enzymatic saccharification and fermentation.

Conclusion

Maize residues are abundant resources for bioenergy production. Oxalic acid concentration of 200 mM for samples treated at 160°C for 10 min was found to be the optimal pretreatment conditions for partial hydrolysis of the cellulosic components. Cellulases, mainly cellobiohydrolase concentrate from *T. reesei*, which recorded an activity of 116 U/ml, was the most suitable for saccharification of the substrate. For optimum saccharification reactions, incubation temperature of 45°C for 48 h gave up to 46% (w/w) glucose (including other fermentable monosaccharides) and 28% (w/w) xylose yields. In addition, a native *S. cerevisiae (*strain *KB*) was able to convert 78% (w/w) of the fermentable sugars to ethanol after 60 h of incubation at 32°C under stationary culture conditions. The challenges encountered in chemical and biological catalytic conversions of maize residues to bioethanol were inhibitory effects caused by recalcitrant lignolytic compounds. At the same time, the native *S. cerevisiae* (strain *KB*) could not ferment the five-carbon sugars such as xylose. To overcome such challenges therefore, future research outlook points at bioprospecting for 5-carbon degraders, using mutants and molecular biology techniques such as recombinant enzymes and microorganisms. The study provides useful baseline data and information for a more elaborate research on fungal cellulosic enzymes hydrolysis and subsequent large-scale microbial mediated fermentation of hydrolysates to bioethanol.

REFERENCES

Adebowale EA (2011). Maize residues as ruminant feed resources in Nigeria. FAO Documents Repository. 5pp. (http://www.fao.org/docrep. Cited: August 2011.

Alvira P, Negro MJ, Ballesteros M (2011). Effect of endoxylanase and *alpha*-L-arabinofuranosidase supplementation on the enzymatic hydrolysis of steam exploded wheat straw. Biores. Technol. 102: 4552-4558.

Asada C, Nakamura Y, Kobayashi F (2005). Chemical characteristics and ethanol fermentation of the cellulose component in autohydrolyzed bagasse. Biotechnol. Proc. Eng. 10: 346-352.

Berlin A, Maximenko V, Gilkes N, Saddler J (2007). Optimisation of enzyme complexes for lignocellulose hydrolysis. Biotechnol. Bioeng. 97: 287-296.

Binder JB, Raines RT (2010). Fermentable sugars by chemical hydrolysis of biomass. Proc. Natl. Acad. Sci. USA. 107(10): 4516-4521.

Canilha L, Santos VTO, Rocha GJM, Ameida de Silva JB, Giulietti M, Silva SS, Felipe MGA, Ferraz A, Milagres AMF, Carvalho W (2011). A study on pretreatment of sugarcane bagasse sample with dilute sulphuric acid. J. Ind. Microbiol. Biotechnol. 38: 1467-1475.

Cybulska I, Lei H, Julson J (2010). Hydrothermal pretreatment and enzymatic hydrolysis of prairie cord grass. Energy Fuels 24: 718-727.

Denning G, Kabambe P, Sanchez P, Malik A, Flor R, Harawa R, Nkhoma P, Zamba C, Banda C, Magombo C, Keating M, Wangila J,

Sachs J (2009). Input subsidies to improve smallholder maize productivity in Malawi: Toward an African Green Revolution. PLoS Biol 7(1): e1000023. doi:10.1371/journal.pbio.1000023. Cited: August 2011.
FAO (2009). Food and Agriculture Organization of the United Nations, Statistics Division (2009). "Maize, rice and wheat: Area harvested, production quantity, yield". http://faostat.fao.org/site/567/. Cited: November 2011.
Garcia-Aparicio MP, Ballesteros M, Manzanares P, Ballesteros I, Gonzalez A, Negro MJ (2007). Xylanase contribution to the efficiency of cellulose enzymatic hydrolysis of barley straw. Appl. Biochem. Biotechnol. 137: 353-365.
Grabber JH, Hatfield RD, Lu F, Ralph J (2008). Coniferyl ferulate incorporation into lignin enhances the alkaline delignification and enzymatic degradation of cell walls. Biomacromolecules, 9(9): 2510-2516.
Hsu TC, Guo GL, Chen WH, Hwang WS (2010). Effect of dilute acid pretreatment of rice straw on structural properties and enzymatic hydrolysis. Biores. Technol. 101: 4907-4913.
Ingram T, Wormeyer K, Lima JCI, Bockemuhl V, Brunner AG, Smirnova I (2011). Comparison of different treatment methods for lignocellulosic materials. Part I: Conversion of rye straw to valuable products. Biores. Technol. 102: 5221-5228.
Iranmahboob J, Nadim F, Monemi S (2002). Optimizing acid-hydrolysis: A critical step for production of ethanol from mixed wood chips. Biomass and Bioenergy 22(5): 401-404.
Juhasz T, Szengyel Z, Reczey K, Siika-Aho M, Viikari L (2005). Characterization of cellulases and hemicellulases produced by *Trichoderma reesei* on various carbon sources. Proc. Biochem. 40(11): 3519-3525.
Koundinya V (2009). Corn stover. In: Renewable Energy. National Information Resource for value-added Agriculture. Ag. Marketing Resource Center. (http://www.agmrc.org/renewable_energy/corn_stover.cfm). Cited: November 2011.
Leathers TD (2003). Bioconversion of maize residues to value added co-products by yeast-like fungi. FEMS Yeast Res. 3(2): 133-140.
Lee JW, Rita CLBR, Jeffries TW (2009). Simultaneous saccharification and ethanol fermentation of oxalic acid pretreated corncob assessed with response surface methodology. Biores. Technol. 100(24): 6307-6311.
Lee JW, Rodriguez RCLB, Kim HJ, Choi IG, Jeffries TW (2010). The roles of xylan and oxalic acid pretreated corncob during separate enzymatic hydrolysis and ethanol fermentation. Biores. Technol. 101: 4379-4385.
Liu D, Zhang R, Yang X. Wu H, Xu D, Tang Z, Shen Q (2011). Thermostable cellulose production of *Aspergillus fumigatus* Z5 under solid state fermentation and its application in degradation of agricultural wastes. Int. Biodegrad. Biodeterio. 65: 717-725.
Lu Y, Mosier NS (2007). Biomimetric catalysis for hemicellulose hydrolysis in corn stover. Biotechnol. Prog. 23: 116-123.
Margeot A, Hahn-Hagerdal B, Edlund M, Slade R, Monot F (2009). New improvements for lignocellulosic ethanol. Curr. Op. Biotechnol. 20(3): 372-380.
Matsushita Y, Yamauchi K, Takabe K, Awano T, Yoshinaga A, Kato M, Kobayashi T, Asada T, Furujyo A, Fukushima K (2010). Enzymatic saccharification of Eucalyptus bark using hydrothermal pretreatment with carbon dioxide. Biores. Technol. 101: 4936-4939.
Mosier NS, Sarikaya A, Ladisch CM, Ladisch MR (2001). Characterization of dicarboxylic acids for cellulose hydrolysis. Biotechnol. Prog. 17(3): 474-480.
Mosier NS, Ladisch CM, Ladisch MR (2002). Characterization of acid catalytic domains for cellulose hydrolysis and glucose degradation. Biotechnol. Bioeng. 79(6): 610-618.
Mtui G, Nakamura Y (2005). Bioconversion of lignocellulosic waste from selected dumping sites in Dar es Salaam, Tanzania. Biodegradation, 16: 493-499.
Mtui G, Nakamura Y (2007). Characterization of lignocellulosic enzymes from white-rot fungus *Phlebia chrysocreus* isolated from a Marine habitat. J. Eng. Appl. Sci. 2(10): 1501-1508.
Mtui G, Nakamura Y (2008). Lignocellulosic enzymes from *Flavodon flavus*, a fungus isolated from Western Indian Ocean off the Coast of Dar es Salaam, Tanzania. Afr. J. Biotechnol. 7(17): 3066-3072.
Nyange D, Wobst P (2005). Regional production on maize price volatility in Tanzania: An ARCH Model analysis. J. Agric. Econ. Dev. Policy Analysis for Sustainable Agriculture Development (PASAD). http://www.pasad.uni-bonn.de. Cited: November 2011.
Okafoagu CU, Nzelibe HC (2002). Effect of acid hydrolysis of *Garcinia kola* (bitter kola) pulp waste on the production of CM-cellulase and glucosidase using *Aspergillus niger*. Afr. J. Biotechnol. 5(10): 919-922.
Palkovits R, Tajvidi K, Rupper AM, Procelewska J (2011). Heteropoly acids as efficient acid catalysts in the one-step conversion of cellulose to sugar alcohols. Chem. Comm. 47: 576-578.
Pandey P, Pandey AK (2002): Production of cellulose-free thermostable xylanases by an isolated strain of *Aspergillus niger* PPI, utilizing various lignocellulosic wastes. World J. Microbiol. Biotechnol. 18: 281-283.
Park JI, Kent MS, Datta S, Holmes BM, Huang Z, SimMons BA, Sale KL, Sapra R (2011). Enzymatic hydrolysis of cellulose by cellobiohydrolase domain of CelB from hyperthermophylic bacterium *Caldicellulosiruptor saccharolyticus*. Biores. Technol. 102: 5988-5994.
Petersen L, Ardevol A, Rovira C, Reilly, PJ (2009). Mechanism of cellulose hydrolysis by inverting GH8 endoglucanases: A QM/MM metadynamics study. J. Phys. Chem. 113(B): 7331-7339.
Rao K, Chaudhari V, Varanasi S, Kim DS (2007). "Enhanced ethanol fermentation of brewery wastewater using genetically modified strain *E. coli* KO11". Appl. Microbiol. Biotechnol. 74: 50-60.
Saha BC, Iten LB, Cotta MA, Wu YV (2005). Dilute acid pretreatment, enzymatic saccharification and fermentation of rice hulls to ethanol. Biotechnol. Prog. 21(3): 816-822.
SDSU (2010). Studies on corn stover pretreatment. San Diego State University (SDSU). (http://www.bioenergy3.org/2010/04/corn-stover-testing/). Cited: November 2011.
Silva GP, Araujo EF, Silva DO, Guimaraes WV (2005). Ethanolic fermentation of sucrose, sugarcane juice and molasses by *Escherichia coli* strain KO11 and *Klebsiella oxytoca* strain P2. Braz. J. Microbiol. 36: 395-404.
Sinegani AAS, Emtiazi G, Hajrasuliha S, Shariatmadari H (2005). Biodegradation of some agricultural residues by fungi in agitated submerged cultures. Afr. J. Biotechnol. 4(10): 1085-1061.
Sinegani AAS, Emtiazi G (2006). The relative effects of some elements on the DNS method in cellulase assay. J. Appl. Sci. Environ. Manage. 10(3): 93-96.
Sun Y, Cheng J (2002). Hydrolysis of lignocellulosic materials for ethanol production: A review. Biores. Technol. 83: 1-11.
Taherzadeh M, Kirimi K (2007). Enzyme-based hydrolysis processes for ethanol from lignocellulosic materials: A review. Bioresources, 2(4): 707-738.
Talebnia F, Jaherzadeh MJ (2006). *In situ* detoxification and continuous cultivation of dilute-acid hydrolysate to ethanol by encapsulated *S. cerevisiae*. J. Biotechnol. 125(3): 377-384.
Tan H, Yang R, Sun W, Wang S (2010). Peroxidase-acetic acid pretreatment to remove bagasse lignin prior to enzymatic hydrolysis. Ind. Eng. Chem. Res. 49: 1473-1479.
Teng C, Yan Q, Jiang Z, Fan G, Shi B (2010). Production of xylooligosaccharides from the steam explosion liquor of corncobs coupled with enzymatic hydrolysis using a thermostable xylanase. Biores. Technol. 101: 7679-7682.
Tian S, Zhu W, Gleisner R, Pan XJ, Xhu JY (2011). Comparison of SPORL and dilute acid pretreatment for sugar and ethanol productions from Aspen. Biotechnol Prog. 27(2): 419-427.
Trinh CT, Huffer S, Clark ME, Blanch HW Clark DS (2010). Elucidating mechanisms of solvent toxicity in ethanologenic *Escherichia coli*. Biotechnol. Bioeng. 106(5): 721-730.
Valascova V, Baldian P (2006). Degradation of cellulose by the brown rot fungus *Piptoporus betulinus* – production of extracellular enzymes and characterization of the major cellulases. Microbiol. 152: 3613-3622.
Viola E, Cardinale M, Santarcangelo R, Villone A, Zimbardi F (2008). Ethanol from eel grass via steam explosion and enzymatic hydrolysis. Biomass and Bioenergy, 32(7):613-618.
Wang NS (2011). Glucose Assay by dinitrosalycylic colorimetric method. Departmen t of Chemical & Biomolecular Engineering,

University of Maryland, College Park, MD 20742-211. http://www.eng.umd.edu/~nsw/ench485/lab4a.htm. Cited: November, 2011.

Wu B, Zhao Y, Gao PJ (2006). Estimation of cellobiohydrolase I activity by numerical differentiation of dynamic ultraviolet spectroscopy. Acta Biochimica Sinica, 38(6): 372-378.

Yu Y, Lou X, Wu H (2008). Some recent advances in hydrolysis of biomass in hot-compressed water and its comparisons with other hydrolysis methods. Energy and Fuels, 22: 46-60.

Zhao X, Chen K, Liu D (2009). Organosolv pretreatment of lignocellulosic biomass for enzymatic hydrolysis. Appl. Microbial. Biotechnol. 82: 815-827.