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Temperature Optimization for Bioethanol Production from Corn Cobs Using Mixed Yeast Strains

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Abstract: Problem statement: Dilute sulphuric acid and enzymatic hydrolysis methods were used for sugar extraction. Xylose and glucose sugars were obtained from corn cobs. **Approach:** Acid hydrolysis of corn cobs gave higher amount of sugars than enzymatic hydrolysis. **Results:** The results showed that optimal temperature and time for sugar fermentation were approximately 25°C and 50 h by two yeast strains (*S. cerevisiae* and *P. Stipitis*) respectively. At 20 and 40°C, less bioethanol was produced. Bioethanol produced at 25°C was 11.99 mg mL^{-1}, while at 40 and 20°C were 2.50 and 6.40 mg mL^{-1} respectively. **Conclusion/Recommendations:** Data obtained revealed that xylose level decreased from 27.87-3.92 mg mL^{-1} during the first 50 h of fermentation and complete metabolism of glucose was observed during this time. Xylose and bioethanol levels remained constant after 50 h. Varying the temperature of the fermentation process improves the effective utilization of corn cobs sugars for bioethanol production can be achieved.

Key words: Bioethanol, corn cobs, optimization, fermentation, hydrolysis

INTRODUCTION

In an attempt to maximize waste product into useful material, this article seeks to determine the optimal temperature for large scale bioethanol production from corn cobs. Corn cob, a waste product of corn contains large amount of sugars that can be further utilized to produce various compounds (Cao *et al*., 1996; Adesanya and Raheem, 2009). The bioconversion of lignocellulosics to biofuel from cheap non-edible materials such as corn cob for renewal energy is imperative. Thus, by varying temperature conditions during the fermentation process, maximum productivity of biofuel on an industrial scale can be optimized.

In the brewing industry, production of biofuel is carried out by the fermentation of starchy materials, in which case, sugars are converted into bioethanol with carbon dioxide and water (Hongguang, 2006) as by-products. For waste plant materials to be valuable, it must be converted to fuel as a sustainable substitute to fossil fuel. Therefore, there is a need for renewable energy resources from non-edible agricultural sources such as corn cob to replace fossil forms. This is because gas emissions from plant feedstock fuel are less than those emitted by fossil forms and thus beneficial to the environment and global warming (Demirbas, 2005; Hongguang, 2006). Bioethanol produced from corn uses only a small part of the plant material, whereby only the starch from the kernel is transformed into bioethanol (Cao *et al*., 1996). Several research studies have been carried out on the production of bioethanol from corn cobs through simultaneous saccharification and fermentation of lignocellulosic agricultural wastes by Kluyveromyces marxianus 6556 (Zhang *et al*., 2009), using *Aspergillus niger* and *Saccharomyces cerevisae* in simultaneous saccharification and fermentation (Zakpaa *et al*., 2009) and from Lignocellulosic Biomass (Kumar *et al*., 2009).

Corn however, is a main staple food in South Africa with an annual production of 8.04 million tons (Adesanya and Raheem, 2009). The cobs produced from corn are mainly used as manure for agricultural production. According to the report of Latif and Rajoka (2001), modern biotechnology allows the use of such lignocellulosic substrates as corn cobs in the production of chemicals and fuels, utilizing microorganisms. It has been shown that when corn is used for bioethanol

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production at higher temperatures, yeast cells die resulting in a decrease in alcohol yield when the pulp is concentrated, while optimal temperature for maximum productivity occurs at 32°C (Araque *et al*., 2008). It is therefore, necessary to select the optimum temperature at which yeast strains can ferment the sugars from lignocellulosic material.

The Simultaneous Saccharification and Fermentation (SSF) process has been identified as economically viable for the conversion of these substrates to fermentation products (Cao *et al*., 1996). Conversion of glucose and xylose to ethanol by co-yeast strains has been successfully obtained by Taniguchi *et al*. (1997) using a respiratory deficient mutant of *Saccharomyces cerevisiae* and *Pitchia stipitis. Pichia stipitis* strains ferment xylose at a high capacity of 57 g L^{-1} than any other yeast, provided the pH is maintained at between 4.5 and 6 and temperature of 25-26°C (Jeffries *et al*., 2007). According to Jeffries *et al*. (2007), maximum yield of ethanol is obtained when a mixture of *S. cerevisiae* and *P. stipitis* are introduced into a medium containing both glucose and xylose. The amount of bioethanol produced therefore, depends on the optimal temperature which, invariably influence sugar utilization by yeast cells (Mwesigye and Barford, 1996).

Problem statement: From the above it is obvious several microorganisms have used in the production of bioethanol but non has utilized a combination of *S. cerevisiae* and *P. stipitis* in the production of bioethanol from corn cobs. This study, therefore, utilized an agricultural waste material (corn cobs) in the production of bioethanol as a cheap but effective alternative fuel source to power automobile. Furthermore, time and temperature in the bioethanol production process using the two yeast strains (*S. cerevisiae* and *P. stipitis*) were optimized.

MATERIALS AND METHODS

The chemicals and reagents used in the study were of analytical grade. The sugar extraction process from the corn cobs was according to Cao *et al*. (1996). The sugar analyses were determined using the HPLC (Agilent Technologies, Waldbronn, Germany). Two strains of yeast: *S. cerevisiae* and *P. stipitis* were used for the fermentation experiment and were obtained from the School of Molecular Biology, University of the Witwatersrand.

Approach: Methods used in the production of bioethanol in this study were the acid hydrolysis and the enzyme hydrolysis methods after the corn cob were steeped in ammonia hydroxide solution to release lignin from the cob. Both methods were compared to determine which gives better yield of fermentable sugars. The fermentable sugars were then treated with the yeast strains at different temperatures and time. This is to optimize the temperature and time in the use of both yeast strains in the production of bioethanol from corn cob.

Ammonia steeping: Twenty grams of milled corn cobs of particle size of 2 mm was mixed with 100 mL 2.9 M NH_4OH solution in a 250 mL Erlenmeyer flask. The mixture was then incubated in a shaker for 24 h at 30°C. The content was then filtered using a 2 µm filter paper into 250 mL Erlenmeyer flask. It was further rinsed twice using distilled water. The corn cobs were then dried at 30°C in an oven overnight.

Dilute acid hydrolysis: The dried corn cobs were then delignified by treating with 0.3 M HCl solution at 121°C for 1 h. The amount of HCl added to dry biomass weight is in the ratio of 1:10 w/v. 0.5 M NaOH was then used to neutralize the acidic hemicellulose hydrolyzate. The pre-treated cellulosic residue was then washed with distilled water to remove residual acid.

Enzymatic hydrolysis: In a 250 mL flask, 50 mL of water and 300 µL of cellulase was added to the cellulosic residue to convert cellulose to fermentable sugars at 50°C for 48 h (Sun and Cheng, 2002).

Yeast culture: Each yeast strain was grown in cooled 25 mL broth Yeast Potato Dextrose (YPD) medium prepared by adding 1 g of yeast extract, 2 g of peptone powder and 2 g of glucose powder to 25 mL of distilled water and autoclaved at121°C for 15 min. The cultured medium was then placed in an incubator shaker at 220 rpm for 18 h.

Bioethanol fermentation: Twenty five ml each of hemicellulose hydrolyzate and cellulose hydrolyzate were mixed, inoculated in 500 µL each of yeast medium and covered with cheese cloth to allow for proper gaseous exchange. The samples were then put into incubator shakers at different temperatures and shaken for 180 rpm. The sugar concentrations were then analysed with HPLC according to the method described by Duke and Henson (2008). In order to remove the yeast cells from the fermentation products, the cultured broth were sterilely filtered. The temperature was varied from 15-40°C. The fermentation process was carried out according to Cao *et al*. (1996).

RESULTS

In order to investigate the optimum temperature the acid and enzymatic hydrolysis were used to determine the amount of sugars produced. There was a significant difference ($p<0.001$) of the sugars obtained from acid and enzymatic hydrolysis. The results showed that the acid hydrolysis produced 1.6 and 30.23 mg mL^{-1} of glucose and xylose sugars respectively while the enzymatic hydrolysis gave 0.12 and 5.7 mg mL^{-1} of glucose and xylose sugars respectively. This indicates that enzymatic hydrolysis produces fewer sugars than acid hydrolysis (Fig. 1). The fermentation process was repeated for the temperatures 20, 25, 30 and 40°C. During the fermentation process, the levels of glucose, xylose and bioethanol were measured after every 5 h.

The result in Fig. 2 shows the concentration of glucose during the fermentation period. It was found that the level of sugar utilization by the yeast strains was faster at 25°C than at 20, 30 and 40°C. It took 25 h for the glucose to be completely metabolized at 25°C, 50 h at 20 and 30°C respectively. It also took 63 h for the glucose to be metabolized by the yeast strains at 40°C (Fig. 2). The glucose concentrations for the temperatures 20, 25, 30 and 40°C all dropped from 0.74-0 mg mL^{-1} at time 25 h (25°C), 50 h (20 and 30°C) and 63 h (40°C) (Fig. 2).

The results of xylose fermentation at varying temperatures are shown in Fig. 3. The results indicated that at 25°C, the yeast strains utilize the xylose faster than at any other temperature. The utilization was poor at 20, 30 and 40°C (Fig. 3). The xylose concentrations for the temperatures 20, 25, 30 and 40°C all dropped from 29.77-11.99 mg mL^{-1} (20°C), 3.92 mg mL^{-1} (25°C), 5.80 mg mL^{-1} (30°C) and 15.01 mg mL^{-1} (40°C) respectively at time 50 h (Fig. 3).

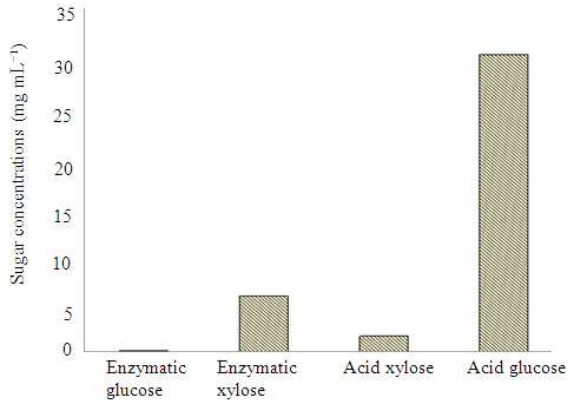

Fig. 1: The concentration of sugars produced from corn cobs using both acid and enzymatic hydrolysis

The result of the bioethanol concentration at the various temperatures is shown in Fig. 4. The two yeast cells were able to ferment the sugars at optimum temperature (Fig. 4).

The highest concentration of bioethanol produced from both sugars was 11.99 mg mL^{-1} at 25°C. The lowest concentration of bioethanol produced was 2.47 mg mL^{-1} at a temperature of 40°C. At temperatures of 20 and 30°C, the concentrations of bioethanol were found to be 6.40 and 11.08 mg mL^{-1} respectively (Fig. 4).

Figure 5 shows the production of bioethanol at 25°C. The results showed that the concentrations of the sugars decreased while the concentration of bioethanol increased with respect to time. According to Jeffries *et al.* (2007) by using *S. cerevisiae* only, the glucose gets converted quickly (after about 12.5 h), while the xylose takes approximately 48 h to be converted to bioethanol and other products. Therefore, the addition of *P. stipitis* yeast to *S. cerevisiae* enhanced the conversion rate of the sugars into bioethanol.

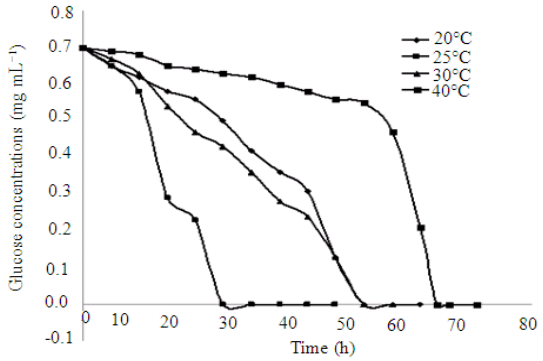

Fig. 2: The amount of Glucose fermentation from corn cob by *S. cerevisiae* and *P. Stipitis*

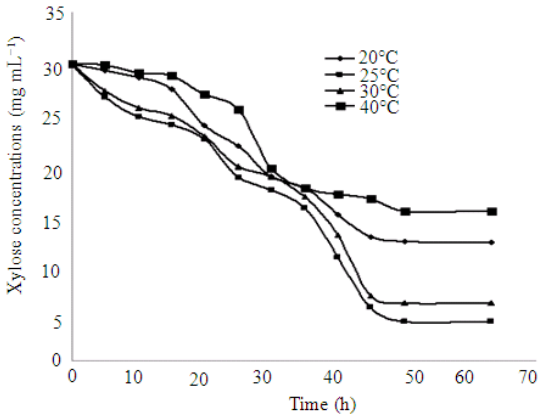

Fig. 3: The amount of Xylose fermentation from corn cob by *S. cerevisiae* and *P. Stipitis*

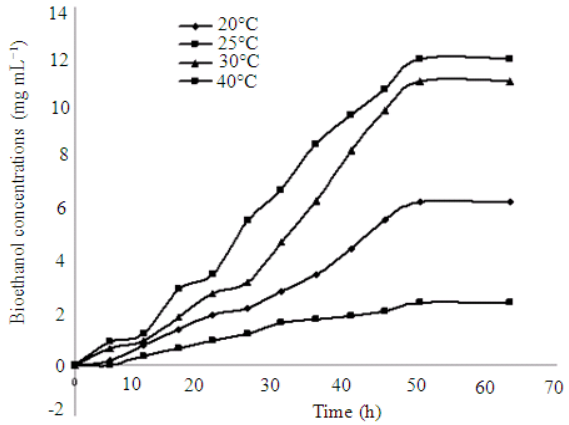

Fig. 4: The amount of bioethanol produced from glucose and xylose sugars

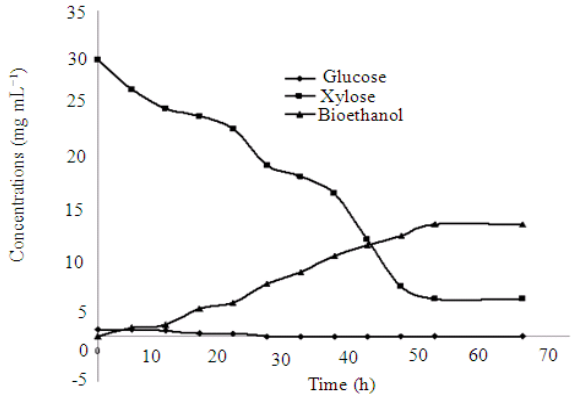

Fig. 5: Temperature optimization of bioethanol production from glucose and xylose sugars at 25°C

Figure 5 shows that the concentrations of glucose and xylose decrease as the concentration of bioethanol increased to a constant concentration of 11.99 mg mL^{-1} at 25°C. All of the glucose was used up. However, the final concentration of xylose was found to be 3.92 mg mL^{-1} after 50 h.

DISCUSSION

The high concentration of xylose present after acid hydrolysis (Fig. 1), could be due to the fact that very small amount of lignin was removed during ammonia steeping. Similar observation has been made by Cao *et al.* (1996) and Kumar *et al*. (2009) where they found very high amounts of xylose produced during acid hydrolysis from hemicellulosic material. The analytical studies reveal glucose level of 1.62 mg mL^{-1} during acid hydrolysis and enzymatic level of 0.12 mg mL^{-1}. The concentration of the sugar hydrolysates after acid hydrolysis was similar to previous reports by Latif and Rajoka (2001). The xylose fraction during acid hydrolysis was 30.23 mg mL^{-1} as compared to 5.70 mg mL^{-1} of enzymatic hydrolysis. This also follows similar findings by Deng *et al*. (2007) that cellulosic biomass can be easily be hydrolyzed with dilute acid to produce monomeric sugars. The high xylose production was due to the ammonia steeping process which stimulated the cellulosic materials to swell, therefore promoting the efficiency of the acid hydrolysis process. This finding confirm earlier reports by Cao *et al*. (1996) that after the ammonia steeping process the corn cob hemicellulosic fraction can easily be hydrolyzed by dilute acid as well as separated from the cellulosic fraction. Thus, acid hydrolysis of corn cobs after ammonia steeping gave better yield of fermentable sugar than the enzymatic method.

According to Fig. 2 and 3, the concentrations of xylose and glucose decreased with respect to and temperatures time for all temperatures (Cao *et al*., 1996). It can also be seen that between 25 and 30°C, the sugars were used up faster than at 20 and 40°C. It can be seen that at 25°C, the glucose concentration reached 0 mg mL^{-1} after 25 h and the concentration at 30°C reached 0 mg mL^{-1} after 50 h. The reason for this is because *S. cerevisiae* and *P. stipitis* are known to convert sugars into bioethanol at temperature range of 25 and 30°C (Van Vleet and Jeffries, 2009).

Figure 3 shows the concentration of xylose which also decreased with respect to time for all temperatures correlating with the reported by Cao *et al*. (1996). The xylose was converted faster at 25°C than at 30°C. At this temperature the xylose concentration was found to be approximately 3.92 mg mL^{-1} after 50 h. This could be due to the fact that *P. stipitis* converts xylose into bioethanol at an optimum temperature of 25°C (Jeffries *et al*., 2007). Theoretically, 100 g of glucose should produce approximately 50.4 g of bioethanol and 48.8 g of carbon dioxide. However, practically, microorganisms use up most of the glucose sugar for growth. Thus, the actual yield of bioethanol is less than 100 % (Araque *et al*., 2008). From literature it has been shown that the operating temperatures are less than expected because yeast cells performance may have been inhibited by other inherent components within in the fermentation process (Galitsky *et al*., 2003; Sinha *et al*., 2006; Deng *et al*., 2007).

In Fig. 4, the concentration of the bioethanol was found to increase with respect to time for all temperatures which supports results obtained in literature (Cao *et al* 1996; Demirbas, 2005). The highest amount

of bioethanol was produced at 25°C and was found to be 11.99 mg mL^{-1} at approximately 50 h of metabolism. The second highest concentration of bioethanol at 30°C was found to be approximately 11.08 mg mL^{-1} after 50 h. At 40°C, there was a poor conversion of sugars and therefore the bioethanol produced after 50 h was approximately 2.47 mg mL^{-1}. This suggests that 25°C and 50 h are the optimum temperature and time for the production of bioethanol using a combination of *S. cerevisiae* and *P. stipitis* yeast strains.

During fermentation at high temperatures, Araque *et al*. (2008) observed that some adaptable resistance factors from the yeast cells can be generated that can give rise to the difference in ethanol yield. Similar effects were reported previously by Abdel-Fattah *et al*. (2000). Initial rapid decrease of sugar observed in Fig. 4 was due to a rapid multiplication of yeast cells and the rapid conversion of the sugars to alcohol via the glucose metabolism (Gibson *et al*., 2008). Generally there was a positive correlation between the sugar reduction of the fermenting medium and a concomitant increase in the ethanol production (Fig. 5). Figure 5 shows the optimum temperature of bioethanol production from glucose and xylose at 25°C where the highest amount of ethanol was produced. Generally, during fermentation, monomeric sugars are metabolized faster than di-, tri- and polymeric sugars. There was a significant difference ($p<0.001$) in ethanol production when the fermentation process approached 50 h after that the concentrations of xylose and bioethanol remain constant. This is due to the yeast cells dying and hence after this point no fermentation was really successful.

CONCLUSION

Varying the temperature of the fermentation of corn cobs sugars has an impact on bioethanol production. It was observed that the concentration of sugars (glucose and xylose) after enzymatic hydrolysis was less than that of the acid hydrolysis. The results showed that the combination of ammonia steeping followed by dilute acid hydrolysis gave high amount of sugars. The glucose and xylose concentrations were found to decrease with respect to time whilst that of the bioethanol was found to increase with respect to time. The optimum time and temperature for bioethanol production *S. cerevisiae* and *P. stipitis* strains were found to be at 50 h and 25°C respectively.

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