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TEMPERATURE OPTIMIZATION FOR BIOETHANOL PRODUCTION FROM CORN COBS

*Clarence S Yah, Sunny E Iyuke, Emmanuel I Unuabonah¸ Odelia Pillay, Chetty Vishant and Samuel M Tessa
School of Chemical and Metallurgical Engineering, Faculty of Engineering and the Built Environment
University of the Witwatersrand, Wits 2050 Johannesburg, South Africa

ABSTRACT

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

Keywords: 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*., 1986; 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*., 1986). 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 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

Corresponding author email: sunny.iyuke@wits.ac.za

(Cao *et al*., 1986). 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 57g/l 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). Presently, there is no report on the combined use of *P. stipitis* yeast to *S. cerevisiae* in the production of bioethanol from corn cobs. This study, therefore, seeks to optimize temperature for bioethanol production using corn cobs using two yeast strains.

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*. (1986). 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.

Ammonia steeping: Twenty grams of milled corn cobs of particle size of 2mm was mixed with 100ml 2.9 M NH_4OH solution in a 250ml Erlenmeyer flask. The mixture was then incubated in a shaker for 24h at 30^0C. The content was then filtered using a 2µm filter paper into 250ml Erlenmeyer flask. It was further rinsed twice using distilled water. The corn cobs were then dried at 30^0C in an oven overnight.

Dilute acid hydrolysis: The dried corn cobs were then delignified by treating with 0.3 M HCl solution at 121^0C for 1h. 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 250ml flask, 50ml of water and 300µl of cellulose was added to the cellulosic residue to convert cellulose to fermentable sugars at 50^0C for 48 h (Sun and Cheng, 2002).

Yeast Culture: Each yeast strain was grown in cooled 25ml broth yeast potato dextrose (YPD) medium prepared by adding 1 g of yeast extract, 2g of peptone powder and 2g of glucose powder to 25ml of distilled water and autoclaved at121^0C for 15minutes. The cultured medium was then placed in an incubator shaker at 220rpm for 18h.

Bioethanol fermentation: Twenty five ml each of hemicellulose hydrolyzate and cellulose hydrolyzate were mixed, inoculated in 500µl 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 180rpm. 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 15^0C to 40^0C. The fermentation process was carried out according to Cao *et al*. (1986).

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 1.6mg/ml and 30.23mg/ml of glucose and xylose respectively while the enzymatic hydrolysis 0.12mg/ml and 5.7mg/ml of glucose and xylose respectively. This indicates that enzymatic hydrolysis produces fewer sugars than acid hydrolysis (Fig.1). The fermentation process was repeated for the temperatures 20°C, 25°C, 30°C and 40°C. During the fermentation process, the levels of glucose, xylose and bioethanol were measured after every 5h.

The result in figure 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 25^0C than at 20^0C, 30^0C and 40^0C. It took 25h for the glucose to be completely metabolized at 25^0Cwhile 50h at 20^0C and 30^0C respectively. It also took 63h for the glucose to be metabolized by the yeast strains at 40^0C (Fig. 2).The glucose concentrations for the temperatures 20°C, 25°C, 30°C and 40°C all dropped from 0.74mg/ml to 0mg/ml at time 25h (25°C), 50h (20 and 30°C) and 63h(40°C) (Fig. 2).

The results of xylose fermentation at varying temperatures are shown in figure 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°C, 30°C and 40°C (Fig. 3). The xylose concentrations for the temperatures 20°C, 25°C, 30°C and 40°C all dropped from 29.77 mg/ml to 11.99 mg/ml (20°C), 3.92mg/ml (25°C), 5.80mg/ml (30°C) and 15.01mg/ml (40°C) respectively at time 50h (Fig. 3).

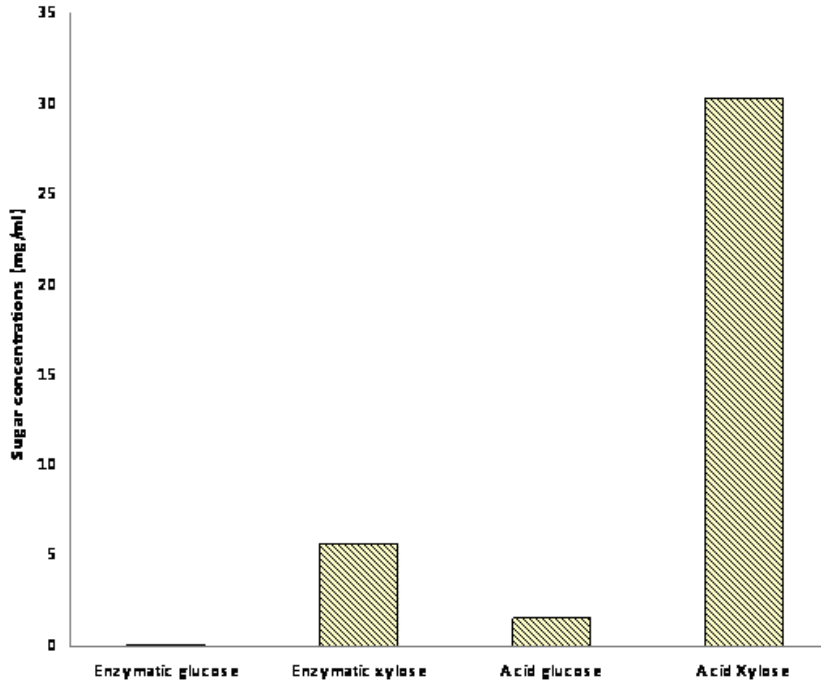

Fig. 1. The concentration of sugars produced from corn cobs using both acid and enzymatic hydrolysis.

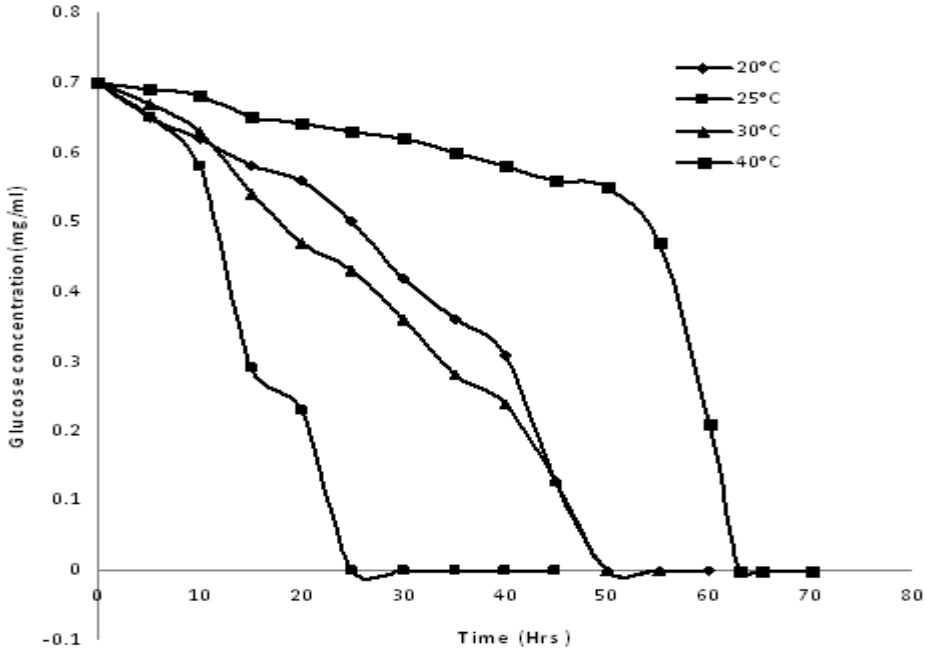

Fig. 2. The amount of Glucose fermentation from corn cob by *S. cerevisiae* and *P. Stipitis.*

The result of the bioethanol concentration at the various temperatures is shown in figure 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 11.99mg/ml at 25°C. The lowest concentration of bioethanol produced was 2.47 mg/ml at a temperature of 40°C. At temperatures of 20°C and 30°C,

the concentrations of bioethanol were found to be 6.40mg/ml and 11.08mg/ml respectively (Fig. 4).

Figure 5 shows the production of bioethanol at 25^0C. 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 12.5h), while the xylose takes approximately 48h 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.

Figure 5 shows that the concentrations of glucose and xylose decrease as the concentration of bioethanol

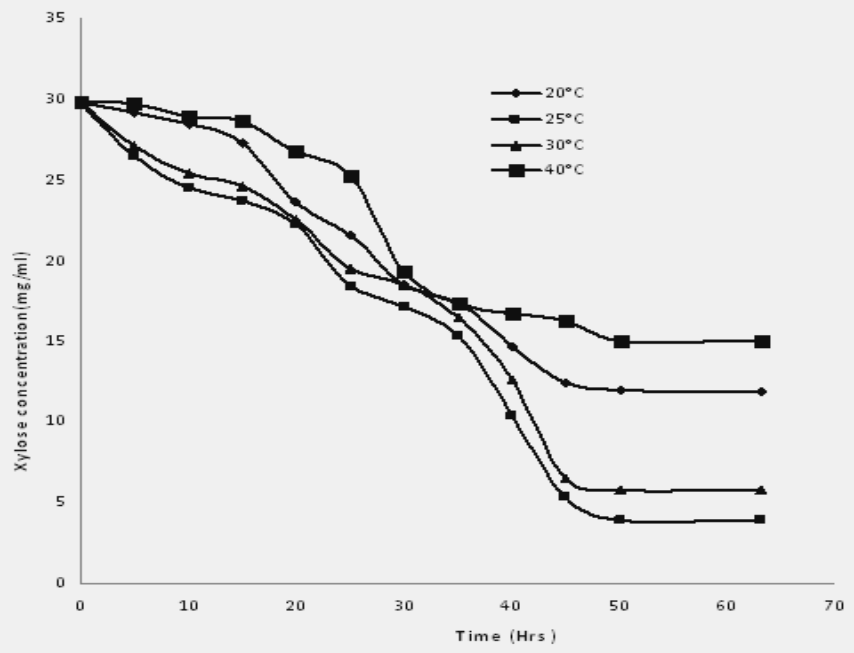

Fig. 3. The amount of Xylose fermentation from corn cob by *S. cerevisiae* and *P. Stipitis.*

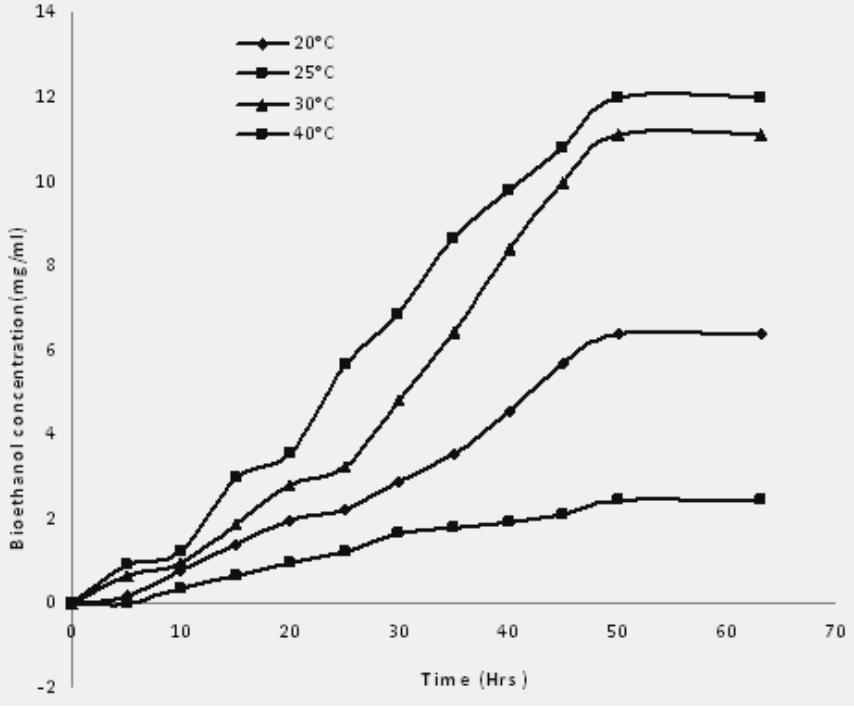

Fig. 4. The amount of bioethanol produced from glucose and xylose sugars.

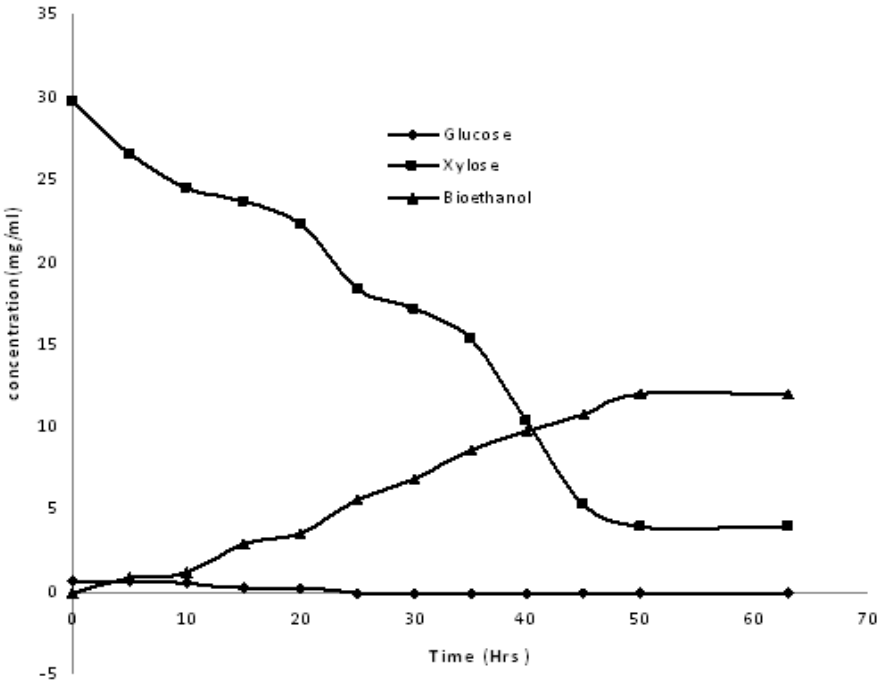

Fig. 5. Temperature optimization of bioethanol production from glucose and xylose sugars at 25^{o}C.

increased to a constant concentration of 11.99 mg/ml at 25°C. All of the glucose was used up. However, the final concentration of xylose was found to be 3.92mg/ml after 50h.

DISCUSSION

The high concentration of xylose present after acid hydrolysis (Fig.1) could be due to the fact that very little lignin was removed during ammonia steeping. Similar observation has been made by Cao *et al*. (1986) 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 1.62mg/ml during acid hydrolysis and enzymatic level of 0.12mg/ml. 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 30.23mg/ml as compared to 5.70 mg/ml 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.

According to Figure 2, the concentrations of glucose decreased with respect to time for all temperatures (Cao *et al*., 1986). It can also be seen that at 25°C and 30°C, the glucose was used up faster than at 20°C and 40°C. It can be seen that at 25°C, the glucose concentration reached 0mg/ml after 25h and the concentration at 30°C reached 0mg/ml after 50h. The reason for this is because *S. cerevisiae* and *P. stipitis* are known to convert sugars into bioethanol at temperature range of 25°C and 30°C (VanVleet and Jeffries, 2009).

Figure 3 shows the concentration of xylose which also decreased with respect to time for all temperatures correlating with those reported by Cao *et al*. (1986). The xylose was converted faster at 25°C than at 30°C. At this temperature the xylose concentration was found to be approximately 3.92mg/ml after 50h. This could be due to the fact that *P. stipitis* converts xylose into bioethanol at an optimum temperature of 25°C (Jeffries, 2007). Theoretically, 100g of glucose should produce approximately 50.4g of bioethanol and 48.8g of carbon dioxide. However, in practice, the microorganisms use most of the glucose for growth and the actual yield is less than 100% (Araque *et al*., 2008). From literature it has been shown that the operating temperatures are less than desired because yeast cells performance can be inhibited by other inherent components within in the fermentation process (Vollhals, 1994; Sinha *et al*., 2006; Deng *et al*., 2007).

In figure 4, the concentration of the bioethanol was found to increase with respect to time for all temperatures which also correlates to literature (Cao *et al*, 1986; Demirbas, 2005). The highest amount of bioethanol was produced at 25°C and was found to be 11.99 mg/ml approximately 50 h of metabolism. The second highest concentration of bioethanol was found at 30°C to be approximately 11.08mg/ml after 50h. At 40°C, there was a poor conversion of sugars and therefore the bioethanol produced after 50 h was approximately 2.47mg/ml. Araque *et al*. (2008) during fermentation at high temperatures, 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). From figure 4, the initial rapid decrease of sugar 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 sugars 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 50h 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 temperature for bioethanol production *S. cerevisiae* and *P. stipitis* strains was found at 25°C. It was also observed that after 50 h, the xylose and bioethanol concentrations remained constant while glucose was completely metabolized. This was as a result of yeast cells death and hence zeros conversion of sugars to bioethanol.

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