International Journal of Innovative Mathematics, Statistics & Energy Policies 4(1):1-9, Jan-Mar. 2016

© SEAHI PUBLICATIONS, 2016 **www.seahipaj.org** **ISSN: 2467-852X**

Production of Bioethanol from Elephant Grass (*Pennisetum purpureum)* Stem

M..O. Aiyejagbara[1], B..O. Aderemi[2], A.O. Ameh[2], E. Ishidi[1], E..F. Aiyejagbara[3] U. Ibeneme[1], M. S. Olakunle[2]

[1]Directorate of Polymer and Environmental Technology, Nigeria Institute of Leather and Science Technology, Zaria, Nigeria

[2]Department of Chemical Engineering, Ahmadu Bello University, Zaria

[3]Samaru College of Agriculture, Division of Agricultural College Ahmadu Bello University, Zaria, Nigeria

Corresponding author: M. O. Aiyejagbara

aiyemosun@gmail.com, ogbosoedith@gmail.com 08062091630/08034518844

ABSTRACT

The production of bio-ethanol from Elephant grass (*Pennisetun purpureum)* stem was carried out using elephant grass stem as a feedstock, combination of *Aspergillus niger* and *Saccharomyces cerevisiae* (brewer's yeast) as cells by simultaneous saccharification and fermentation (SSF). For this study, pre-treated and untreated elephant grass were considered for ethanol production. Alkaline pretreatment was done by soaking the sample in 1M NaOH for 2-10hrs at 1:10 solid/liquid ratio. The effects of temperature at 25ºC, 30ºC, 35ºC, 40ºC and 45ºC; pH values of 3.5, 4.0, 4.5, 5.0, 5.5, 6.0 and 6.5; substrate concentration values of 1%(w/v), 2%(w/v), 3%(w/v), 4%(w/v) and 5%(w/v); particle size range of 53-106µm, 106-150µm, 150-250µm, 250-300µm and 300-425µm; and cell loading combination of *Aspergillus niger* at 0.2%(w/v) 0.4%(w/v), 0.6%(w/v), 0.8%(w/v), 1%(w/v) concentrations and *Saccharomyces cerevisiae* (brewer's yeast) at 0.5% (w/v), 1%(w/v), 1.5%(w/v), 2%(w/v), 2.5%(w/v) on the hydrolysis and fermentation process were investigated to obtain optimum conditions of fermentation. The optimum conditions of fermentation were obtained at temperature of 35^0C, pH value of 5.5, substrate concentration of 30g/l, particle size range of 250-300µm and *Aspergillus niger* to yeast ratio of 0.6/1.5 after 72 hours of fermentation time. Also substrate concentration of 30g/l, gave highest ethanol concentration of 23.4g/l and a yield of 78%.

Keywords: Elephant grass, A. *niger*, S. *cerevisae*, glucose, ethanol, System A (treated sample) System B (Untreated)

INTRODUCTION

Energy availability, supply and use plays a central role in the way societies organize themselves, from individual welfare to social and industrial development. By extension, energy accessibility and cost is a determining factor for the economic, political and social interrelations among nations. Considering energy sources, human society has dramatically increased the use of fossil fuels in the past 50 years in a way that the most successful economies are large consumers of oil. However, geopolitical factors related to security of oil supply, high oil prices and serious environmental concerns, prompted by global warming, the use of petrol for transportation which accounts for one-third of greenhouse gas emissions [1], have led to a push towards decrease in its consumption. Indeed, the world's strongest economies are committed to the development of technologies aiming at the use of renewable sources of energy such as the substitution of liquid fuel gasoline by renewable ethanol [2]

Currently, established technologies in fuel-ethanol industry are primarily based on the fermentation of sugars derived from starch and sugar crops like sugarcane, sugar beet, sweet sorghum, corn and cassava [3; 4; 5; 6; 7; 8] of which, these are food crops and tend to increase the cost of production [9]. Therefore, production of bio-ethanol from lignocellulosic biomass becomes a necessity. A large amount of lignocellulosic wastes are generated through agricultural practices, paper-pulp industries, yard wastes, animal and human waste and many other agro-industries which are often disposed-off by burning [10].

Elephant grass (*pennisetum purpureum*) is a lignocellulosic material. Its high productivity and availability has been realized by researchers [11]. Although it is used as cattle feed but could be easily and cheaply cultivated for other purposes. Elephant grass has a fast growing rate and is harvested approximately four times annually [12]. In addition, the composition of elephant grass, which is approximately 30-40% cellulose and 25-30% hemicecullose [13], makes it a strong potential source of carbohydrates.

Therefore, this paper will discusse the production of bio-ethanol from the elephant grass stem using a combination of *Aspergillus niger* and *Saccharomyces cerevisae* as cells to produce enzymes for the hydrolysis and fermentation process.

MATERIALS AND METHODS

Source of Organism

A. niger cells was isolated from maize grains as a source of crude enzymes while *S. cerevisiae* (brewer's yeast) was obtained from Nigerian Breweries Plc, Kaduna.

Sample Preparation

The elephant grass (*Pennisetum purpureum*) stem was obtained from National Animal Production Research Institute (NAPRI) ABU Zaria. It was first dried to a constant weight in an oven (Gallenkamp model) at a temperature of 105°C for 48 hours, it was then milled using a Laboratory mill (Thomas wite model 4). A screen analysis was carried out with different sieve sizes to obtain desired particle sizes. Alkaline pretreatment was done by soaking the sample in 1M NaOH for 2-10 hrs at 1:10 solid/liquid ratio. After pretreatment, the samples were filtered and washed repeatedly with distilled water until pH of 7 was attained. The elephant grass was then dried in an oven at 50^{O}C to a constant dry weight for 48hours.

Substrate Analysis

The substrate analysis of the various pretreated and the untreated sample were conducted using Van Soest System of feed analysis [14].

Simultaneous Saccharification and Fermentation (SSF) of Elephant Grass

In a typical run, 100ml of 0.1M sodium acetate buffer solution was poured into 250ml Erlenmeyer flask, 0.2g of *Aspergillus niger* (crude enzyme), 0.5g of brewer's yeast (*Saccharomyces cerevisiae*), 2g of treated elephant grass was added and 0.1g of $MgS0_4$, 0.2g of $(NH4)H_2PO_4$ were added as nutrient. The flask was corked properly, sealed with aluminum foil paper and incubated at 30^{O}C for 5days in an incubator. This procedure was also carried out for the untreated elephant grass. All the experiments were performed in triplicate. Samples were withdrawn daily for analysis. The ethanol concentration was determined using an Anton par beer analyzer (Alcohol meter). In separate runs, the effect of pretreatment, substrates concentration, particle size, cell loading, reaction temperature reaction time and medium pH on the extent of fermentation were studied as stated below:

Effect of substrate concentration

This was investigated by carrying out the simultaneous saccharification and fermentation at 10g/l, 20g/l, 30g/l, 40g/l and 50g/l substrate concentration at constant temperature, pH and particle size of 30^{0}C, 5.0 and 150-250µm respectively and *A.niger*/yeast ratio 0.6/1.5% (w/v), [4]

Effect of temperature

The experiment was carried out at different temperatures of 25ºC, 30ºC, 35ºC, 40ºC and 45ºC. Other fermentation parameters - pH 5.5, 30g/l w/v substrate concentration, 150-250µm particle size and *A.niger*/yeast ratio 0.6/1.5%w/v were kept constant [15]

Effect of substrate particle size

The particle sizes of the sample were selected by passing it through different sizes of standard sieves. Fractions of particle sizes of 53-106µm, 106-150µm, 150-250µm, 250-300µm and 300-425µm were then fermented and analyzed for ethanol concentration.

Effect of pH

This was investigated by carrying out the fermentation process at the initial pH adjusted to 3.5, 4.0, 4.5, 5.0, 5.5, 6.0 and 6.5 using Sodium acetate buffer [15]. 30^0C temperature, 30g/l w/v substrate concentration, 106-150µm particle size and *A.niger*/yeast ratio 0.6/1.5% w/v, were maintained for pH study.

Effect of cell loading

Two cells *Aspergillus niger* (crude enzyme) and *saccharomyces cerevisiae* (brewer's yeast) were used in the fermentation. To investigate the effect of cell loading, *Aspergillus niger* concentration was kept constant while the yeast concentration was varied. Similarly yeast concentration was kept constant while the *Aspergillus niger* concentration was varied, this was done to get the optimum *Aspergillus niger*/yeast ratio.

For *Aspergillus niger* concentration, fermentation was carried out at different *A.niger* concentration of 0.2%(w/v), 0.4%(w/v), 0.6%(w/v), 0.8%(w/v) and 1.0%(w/v) at constant substrates concentration and constant yeast concentration [4]. Other conditions were identical to the normal fermentation conditions. Ethanol concentrations were measured with time.

For yeast concentration, fermentation was carried out at different yeast concentration of 0.5%(w/v), 1.0%(w/v), 1.5%(w/v), 2.0%(w/v) and 2.5%(w/v) at constant *Aspergillus niger* concentration. Other conditions were identical to the normal fermentation conditions. Ethanol concentration was measured with time.

RESULT AND DISCUSSION

Substrate Analysis of treated and untreated sample

Figure 1 below showed the result of the substrate analysis conducted on elephant grass treated with 1M NaOH at 2-10hrs and untreated elephant grass.

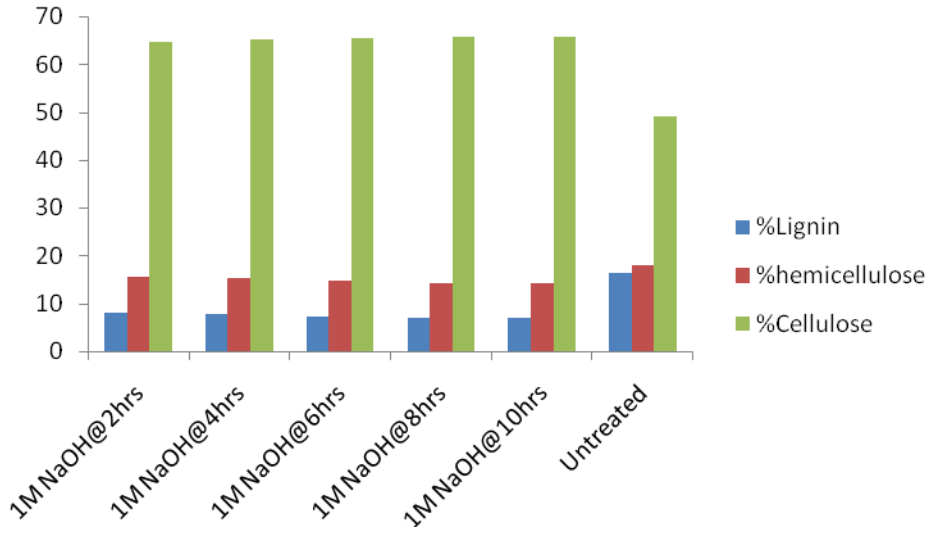

Figure 1: Substrate Analysis of Treated and Untreated Samples

The result obtained showed that the %lignin and %hemicellulose content of the elephant grass decrease as its residence time in 1M NaOH, increases from 2-8hrs and remain constant thereafter. While %cellulose content increases from 64.69-65.80% with increase in residence time. Whereas the untreated elephant grass gave %lignin, %hemicellulose and %cellulose of 16.47%, 17.92% and 49.12% respectively. This implies that the treated substrate has sufficient cellulose to be hydrolyzed to glucose and subsequently to ethanol. However when simultaneous saccharification and fermentation was carried out on both the sodium hydroxide pretreated and untreated sample, the untreated gave a higher yield of 83% while the pretreated sample gave a yield of 42% as shown in Figure 2. This could be due to loss of fermentable sugar during the pretreatment washing process, [11] reported a similar case.

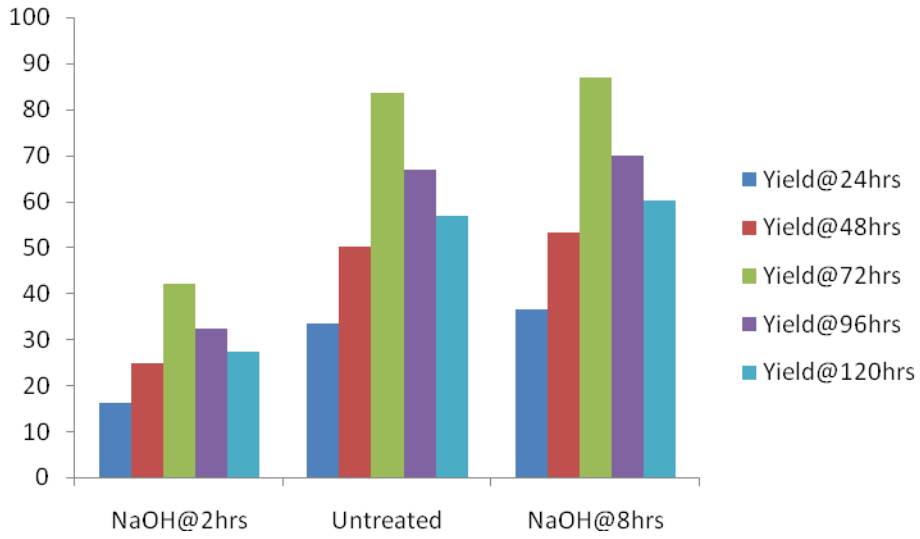

Figure 2: Substrate Analysis of Treated and Untreated Samples

However when the residence time of pretreatment was increased from 2hrs to 8hrs, the sodium hydroxide pretreated sample gave a higher ethanol yield of 86% which could be as a result of increase in cellulose content with increase in residence time as shown in Figure 1.

Effect of substrate concentration on % yield of ethanol.

Figure 3 showed the result of the effect of substrate concentration on ethanol yield from untreated elephant grass. The result showed that as substrate concentration increases from 10g/l to 30g/l, ethanol yield increased to 78.1% after 72 hours of fermentation. Further increase in substrate concentration up to 50g/l resulted in low ethanol yield. The decrease in ethanol yield beyond the optimum concentration of 30g/l could be as a result of product inhibition. This implies that the ethanol produced inhibits the activity of the yeast, hence the drop in ethanol yield.

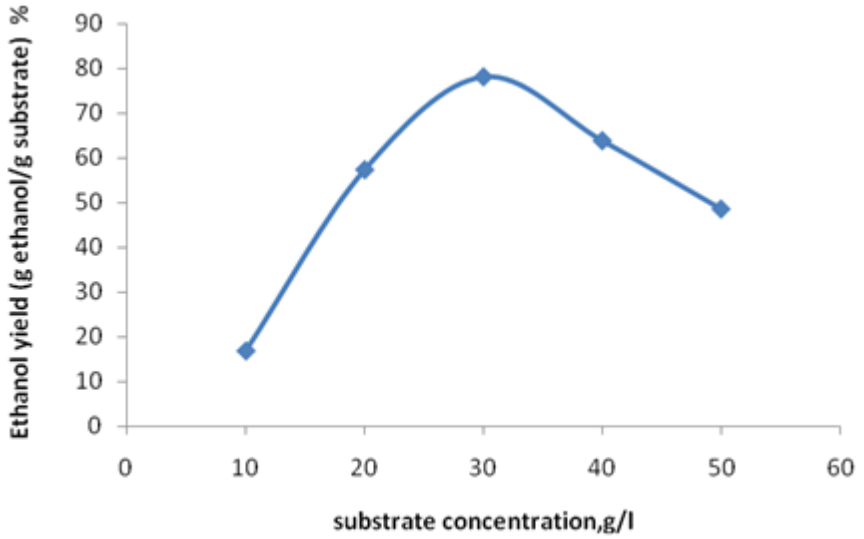

Figure 3: Effect of substrate concentration on ethanol yield

Effect of temperature on % yield of ethanol.

The result of the effect of temperature on % yield of ethanol is shown in Figure 4. A maximum ethanol yield of 77.6% was obtained at temperature of 35^{O}C after 72hours of fermentation and ethanol yield of 28.1% when the temperature was increased to 45^{O}C. The optimum temperature of 35^{O}C as observed might be due to the fact that enzyme activity and other chemical reaction in the cells were favored at this temperature. Badal et al 2005, reported a similar result on the effect of temperature on *E.coli* activity on wheat straw [16] .

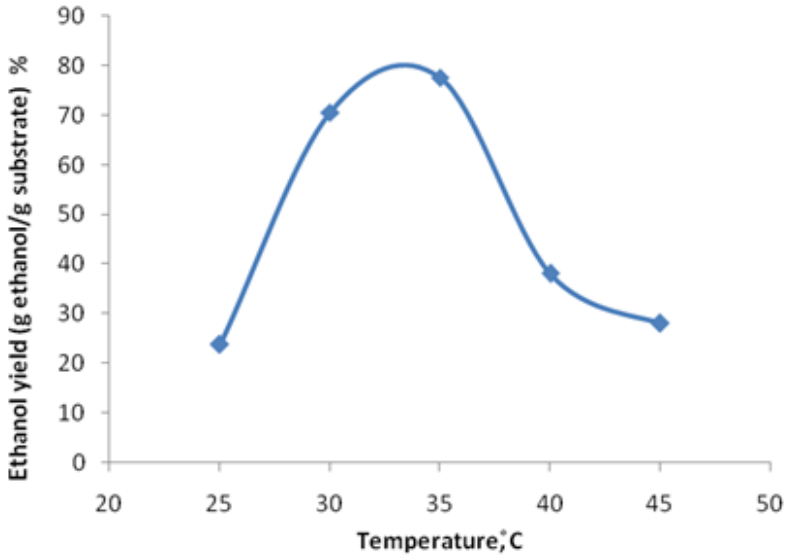

Figure 4: Effect of temperature on ethanol yield

Effect of particle size on % yield of ethanol

The result of particle size on fermentation of elephant grass at pH of 5.0, temperature of 35^{0}C, substrate concentration of 3% w/v and *A.niger*/yeast ratio 0.6/1.5%w/v was presented in Figure 4. The result

showed that as the particle size increases from 53µm to 300µm ethanol yield increases from 24.9% to 80.1%. Further increase in particle size to 425µm, resulted to a decrease in ethanol yield. This implies that as the particle size increases beyond 300µm surface area available for enzymatic attack decreases. The low yield recorded between 53µm to 250µm is contrary to expectation, and could be that the cellulose content of the smaller particle size was lost to the larger particle size i.e.300µm - 425µm during the screen analysis.

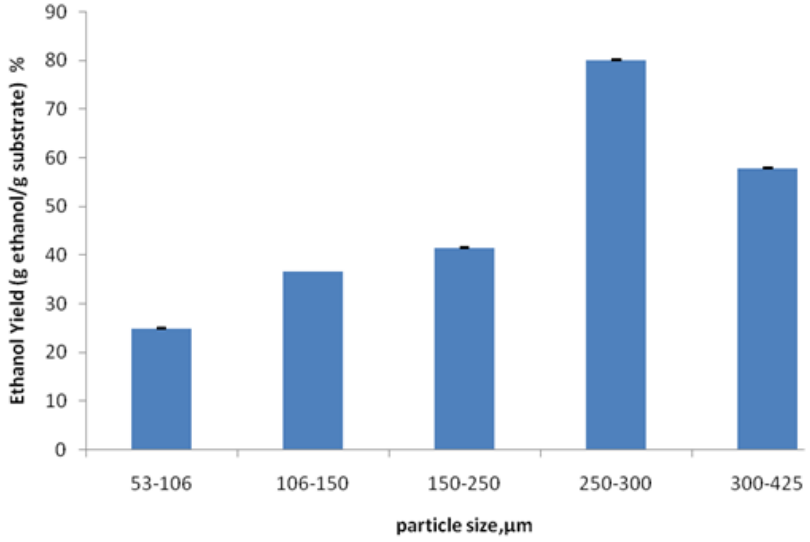

Figure 5: Effect of particle size on ethanol yield

Effect of pH on % yield of ethanol

Results obtained in Figure 6 showed the effect of pH on % yield of ethanol. The result showed that, as the pH increased from 3.5 to 5.5, the ethanol yield increased to a maximum of 79.8%. Further increase in pH to 6.5 resulted in decrease in the yield of ethanol. The decrease in ethanol yield may be due to the fact that enzymes are more active in mildly acidic medium (pH of 4.5-5.0 for *A. niger* cells and pH of 5.0-5.5 for yeast cells). The reported pH tolerance range for yeast is 3.5-6.5 while that of *A. niger* is 3.5-8.0 [17]. Similar result was reported by Ohgren et al 2006, in simultaneous sacharification and fermentation of corn Stover in which the highest ethanol concentration was obtained at pH of 5.5

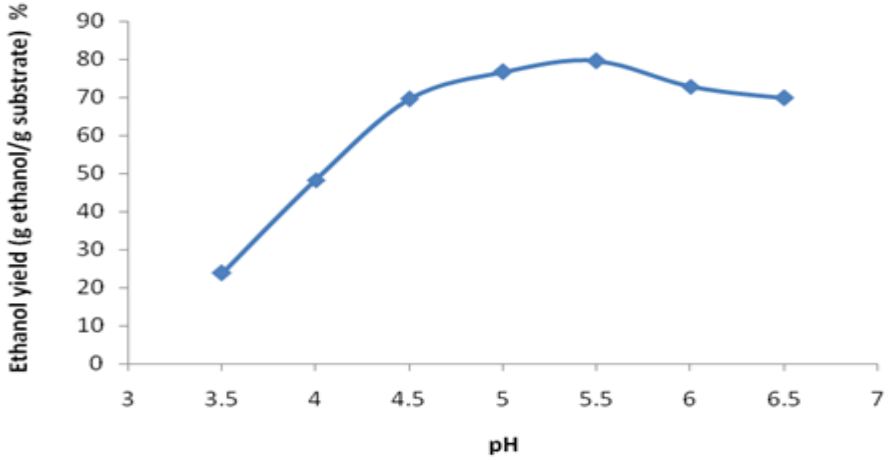

Figure 6: Effect of pH on ethanol yield

Effect of *Aspergillus niger* concentration on % yield of ethanol.

At fixed yeast concentration and other fermentation conditions kept constant, the effect of *Aspergillus niger* concentration on ethanol yield was investigated. The result obtained in Figure 7 showed that as *A. niger* concentration increases from 0.2% w/v to 0.6% w/v ethanol yield increases from 66.9% to 82.4% after 72hours of fermentation. Subsequent increase in *A. niger* concentration beyond 0.6% w/v resulted in decrease of ethanol yield. This may be due to the fact that as more cells were introduced and substrate concentration was not increased proportionally, the glucose produced was being used up by the cells for survival, hence resulting in low ethanol yield.

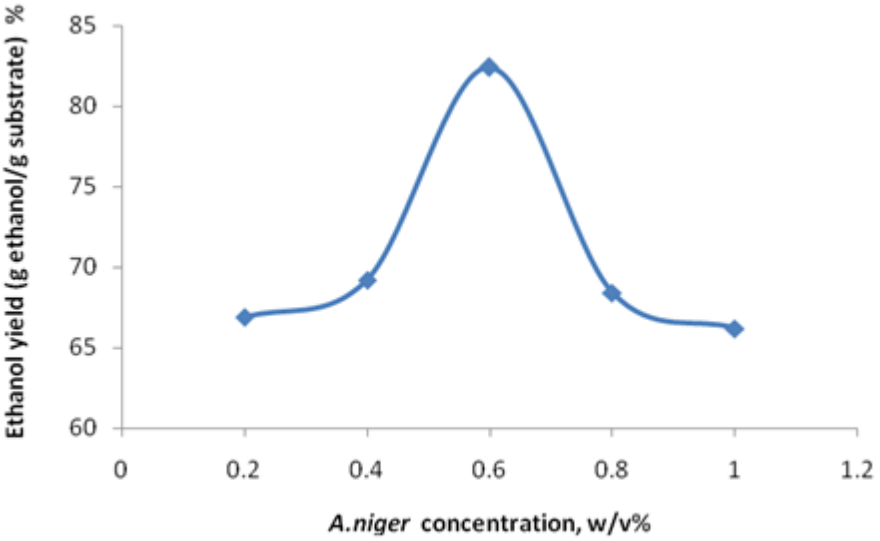

Figure 7: Effect of *A. niger* concentration on ethanol yield

Effect of yeast concentration on % yield of ethanol

The effect of yeast concentration on ethanol yield was also investigated at fixed concentration of *A. niger* and other fermentation conditions kept constant. The result obtained in Figure 8 showed that as yeast concentration increases from 0.5%w/v to 1.5%w/v % yield of ethanol increased from 36.6% to 83.6% after 72hours of fermentation. Further increase in yeast concentration beyond 1.5%w/v resulted in decrease in % yield of ethanol. This may be as a result of the presence of more yeast consuming the limited glucose for self-sustenance, thereby resulting in low yield of ethanol.

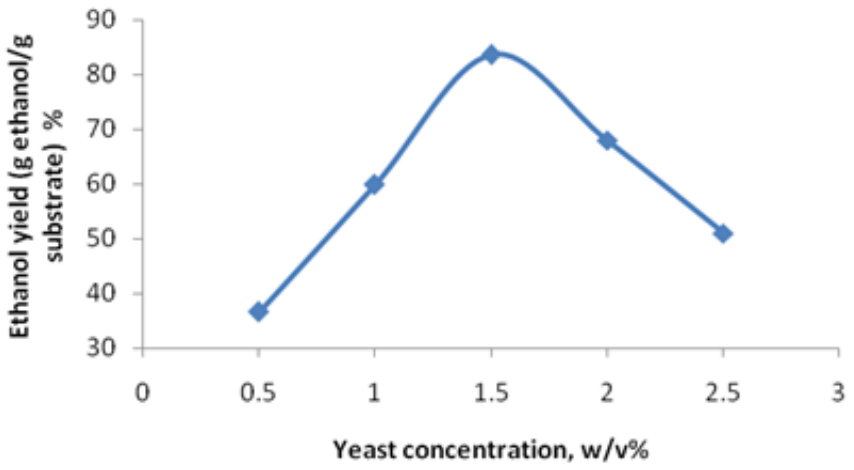

Figure 8: Effect of yeast concentration on % yield of ethanol

CONCLUSIONS

From the results obtained from this investigation, the following conclusions can be drawn;

i. optimum fermentation conditions of 35°C, substrate concentration 30g/l, particle size 250-300µm, pH 5.5, A. *niger/yeast* ratio 0.6/1.5% w/v and fermentation time of 72 hours are recommended.

ii. It is therefore recommended that; the grass should not be treated with NaOH to avoid destruction of the solid sugar content of the elephant grass.

iii. Elephant grass is a viable source of bio-ethanol.

REFERENCES

1. Wyman, C. E. (1996). Handbook on Bioethanol: production and utilization. CRC. Press
2. Chum, H.L. and Overend, R.P. (2001). Biomass and renewable fuels. *Fuel Processing Technology*, 71(1): 187-195.
3. Abouzied, M.M and Reddy, C.A. (1996). Direct fermentation of potato starch to ethanol by cacultines of *Aspergillus niger* and *Saccharomyces cerevisiae*. *Applied and Environmental Microbiology*, 52: 1055-1059.
4. Highina, B.K., Aderemi, B.O. and Abu, E. (2006). The kinetics of glucose production from rice straw by Aspergillus niger . *African journal of Biotechnology*, 7(11): 1745-1752
5. Pandey, M., Soccol, C.R., Nigam, P., Brand, D., Mohan, R. and Roussoss, S. (2000). Biotechnology potential of agro – industry residues, part II, cassava bagasse. *Bioresource Technology*, 74: 81-87.
6. Shendong, Z., Yuanxin, W., Ziniu, Y., Xuan, Z., Cuwen, W., Faquan, Y. and Yongping, X. (2005). Simultaneous saccharification of microwave/alkali pre-treated rice straw to ethanol. *Biosystems Engineering*, 92(2): 229-235.
7. Ohgren, K. Bengtsson, O. Gorwa-Grauslund, M.F, Galbe, M. Hahn-Hagerdal, B. and Zacchi, G. (2006). Simultaneous saccharification and co-fermentation of glucose and xylose in steam-pretreated corn stover at high fiber content with Saccharomyces cerevisiae TMB3400. *Journal of Biotechnology* 126(4): 488–498.
8. Seeman, J., Patel,R. and Shobha, K.S. (2007). Study of ethanol production from fungal pretreated wheat and rice strain. *The Journal of Microbiology*. 4(1): 160-163.

9. Agbodike, T.C. Ado, S.A. and Abdullahi, I.O. (2013). Bio-ethanol production from elephant grass using co-cultures of Aspergillus niger and Saccharomyces crevisiae in simultaneous saccharification and fermentation. *South Asian Journal of Experimental Biology,* 3(4):152-157.
10. Cheng, S.W. and Anderson, B.C. (1997). Investigation of ethanol production from municipal primary waste water. *Bioresource Technology,* 59: 81-96.
11. Isaias, B.S., Olga, M.M., Mohand, B. and Cesar, A.M. (2011). Ethanol production by enzymatic hydrolysis of elephant grass. *Journal of Life Sciences,* 5: 157-161.
12. Inter Press Service (2007). Capim Elefante: Novo campeao de Biomassa no Brazil. http://www.mwglobal.org/ipsbrasil.ne/nota.php?idnews=3292.html
13. Santos E. A., Silva D. S. and Filho J. L. Q. (2001). Composicao Quimica do Capim-Elefante cv. Roxo Cortado em Diferentes Alturas, *Revista Brasileira de Zootecnia* 30. 18-23.
14. Galyean, M.L. (2010). Laboratory procedures in animal nutrition research pp. 13-65.
15. Omemu, A.M, Akpan, I., Bankole, M.O. and Teniola, O.D. (2005). Hydrolysis of raw tube starches by amylase of *Aspergillus niger* AMO 7 isolated from the soil. *African journal of Biotechnology,* 4(1): 199-205.
16. Badal, C.S., Loren B.I., Michael A.C. and Victor, Y.W. (2005). Dilute acid pretreatment, enzymatic saccharification and fermentation of wheat straw to ethanol. *Process Biochemistry,* 40:3693-3700.
17. Rezael, P.S., Najafpour,G.O., Shafaghati, H., and Mahjoub, S. (2009). Production of α- Amylase from starch using *Aspergillus niger*. *World applied sciences journal,* 7: 306-311.