

SCREENING OF FACTORS RESPONSIBLE FOR CONVERSION OF MAIZE STRAW INTO BIOETHANOL

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ABSTRACT

Maize straw is a lignocellulosic waste that is annually added to the environment as pollutant because its accumulation constitutes a nuisance and in addition, its reducing sugar is not readily released. Pretreatment of maize straw makes its reducing sugar available for fermentation into bio-products such as bioethanol production under optimized conditions. This work aimed at determining the effect of different parameters on ethanol production by *Saccharomyces cerevisiae* from pretreated maize straw. Effects of pH, temperature, sugar concentration, nitrogen source, and inoculum load/sizes on ethanol yield of pretreated maize straw by *S. cerevisiae* SA01 and *S. cerevisiae* SA02 for optimum ethanol production were determined using standard methods. Ethanol content was estimated using a gravimetric method. The optimum ethanol production (1.97 g/L) was obtained at pH 5.5 while highest ethanol content (2.76 and 2.37 g/L) was at 30 °C by *S. cerevisiae* SA01 and *S. cerevisiae* SA02, respectively. The glucose concentration of 2 % supported the highest ethanol production (3.95 g/L) by *S. cerevisiae* SA02. Corn steep liquor was the best among the nitrogen sources used and increased the ethanol yield of *S. cerevisiae* SA01 and *S. cerevisiae* SA02 by 300 and 661 %, respectively. One percent of 1.0 MacFarland standard of both yeasts supported the highest ethanol production (14.99 g/L) from pretreated maize straw. Bio-conversion of maize straw to substrate for bioethanol production at optimized conditions will reduce environmental pollution, production cost and increase energy sources.

Keywords: Bioethanol; Maize straw; *Saccharomyces cerevisiae*; Corn steep liquor; Pollution

INTRODUCTION

Maize straw is one of the wastes that are annually released to the environment which add up to environmental pollution. Maize straw is the part that is left in the farmland after harvest of corn which contains the stems and leaves; and is also referred to as corn straw, corn stover, and maize stover (Heuze *et al.*, 2019). Maize straw is a lignocellulosic substrate whose reducing sugar is not readily available for fermentation. Pretreatment of lignocellulose makes reducing sugar easily available for fermentation to other useful products (Fasiku and Wakil, 2021).

Biological, chemical, and mechanical pretreatment techniques are various types of pretreatment that break down lignocellulose into three main components namely: cellulose, hemicellulose, and lignin (Kumar and Sharma, 2017; Nguyen *et al.*, 2021). Cellulose contains hexoses while hemicellulose has both pentoses and hexoses whereas lignin binds all components of lignocellulose together. *Lentinus squarrosulus*, *Pleurotus ostreatus*, and *Phanerochaete chrysosporium* are among organisms that have been used for biological pretreatment while mild alkali, dilute acid, ozonolysis, organosolv and ionic liquids are examples of the chemical pretreatment that have been reported (Kumar and Sharma, 2017; Fasiku and Wakil, 2021). Among mechanical methods of pretreatment that have been utilized are pyrolysis, milling, mechanical extrusion, and pulsed-electric field. Optimum conditions for the production of ethanol are important to improve ethanol production by the organism. Some of the conditions that affect ethanol production are pH, temperature, sugar concentration, source of nitrogen, and inoculum load/size (Martha *et al.*, 2020; Chatterjee and Mohan, 2021).

Nigeria is the highest producer of maize in Africa (Ogbeh, 2018) and, part of the maize plant that is annually left in the farmland polluting the environment (maize straw) in large quantity could be used for bioethanol production. Some wastes in the environment can also be utilized as a source of nitrogen such as corn steep liquor, soybean meal, groundnut cake, fish meal, blood meal, and others. The utilization of these wastes for the production of value-added products is turning waste into wealth which could also have a positive influence on the economic status of the country. This work aimed at screening factors responsible for conversion of maize straw into bioethanol.

MATERIAL AND METHODS

Collection of organisms

Pleurotus ostreatus was obtained from the Department of Botany, University of Ibadan, Ibadan, and was inoculated on potato dextrose agar, incubated at 28 °C for 7 days. Confirmed and identified yeast strains (*Saccharomyces cerevisiae* SA01 and *S. cerevisiae* SA02) from previous work stored on yeast extract agar at 4 °C were used for ethanol production.

Pretreatment of maize straw

Maize straw was pretreated with sodium hydroxide (alkali pretreatment) before treatment with *Pleurotus ostreatus* (biological pretreatment). About 2.5 % of sodium hydroxide solution was added to maize straw (10 %) for 1 hour which was thereafter autoclaved at 121 °C for 30 minutes (Nadeem *et al.*, 2015). The autoclaved sample was filtered using a muslin bag and washed many times with distilled water until the pH of the filtrate was about 7. The residue was dried in an oven at 105 °C until a constant weight was obtained. About 200 g of alkali-pretreated maize straw was mixed with 600 mL of distilled water and packed in a polythene bag. It was sterilized at 121 °C for 15 minutes and allowed to cool. It was inoculated with a full plate cut aseptically into discs using a sterile cork borer from the 7-day old culture of *Pleurotus ostreatus* and incubated at 28±2 °C for 21 days (Fasiku and Wakil, 2021). Pretreated maize straw samples were used for further studies.

Effect of pH of extraction buffer on ethanol production

The effect of pH of extraction buffer on ethanol production from pretreated maize straw was determined using about 100 mL each of different pH (4.0, 4.5, 5.0, and 5.5) of 0.1 M of acetate buffer to extract reducing sugar from pretreated maize straw. Filtrates were sterilized, allowed to cool, and inoculated with 2 % of 1.0 MacFarland standard of 2 strains of *S. cerevisiae* separately for 3 days. The reducing sugar concentration and ethanol content were determined after the fermentation period. The best pH for the highest ethanol production was selected for further work.

Effect of temperature on ethanol production

Acetate buffer at optimum pH of 5.5 was used to determine the optimum temperature for ethanol production. Acetate buffer (0.1 M, pH 5.5) was used to extract reducing sugar from pretreated maize straw and about 100 mL of filtrate was dispensed into different fermentation bottles sterilized at 121 °C for 15 minutes and allowed to cool. Each filtrate was inoculated with 2 % of 1.0 MacFarland standard of *S. cerevisiae* (*S. cerevisiae* SA01 and *S. cerevisiae* SA02) separately and incubated at varying temperatures (30, 35, 40, and 45) °C for 3 days using incubator. Reducing sugar contents and ethanol concentrations were determined at the end of the fermentation and the temperature that gave the highest ethanol content was selected for further studies.

Effect of glucose and fructose supplementations on ethanol production

The filtrate from the above was supplemented with different concentrations of glucose (0, 1, 2, 3, 4, and 5 %) and fructose (0, 1, 2, 3, 4, and 5 %) separately. The supplemented filtrates were sterilized at 121 °C for 15 minutes, allowed to cool, and then inoculated separately with 2 % of 1.0 MacFarland standard of *S. cerevisiae* SA01 and *S. cerevisiae* SA02. All treatments were fermented at 30 °C for 3 days. Ethanol contents were determined after fermentation. The sugar concentration where optimum ethanol production was obtained was selected for further studies.

Effect of nitrogen sources on ethanol production

The extracted filtrates were supplemented separately with 1 % of the following nitrogen sources: groundnut cake, soya meal, fish meal, and blood meal. For corn steep liquor as nitrogen source, corn steep liquor was mixed with 0.1 M acetate buffer (pH 5.5) in ratio 50:50 and 5 % of maize straw was boiled in the mixture, filtered with a muslin bag, and the filtrate was used as maize straw supplemented with corn steep liquor. Two percent (2 %) of glucose (optimum sugar concentration) was added to all the filtrates and sterilized at 121 °C for 15 minutes cooled and inoculated with 2 % of 1.0 MacFarland standard of *S. cerevisiae* SA01 and *S. cerevisiae* SA02. The inoculated filtrates were incubated at 30 °C for 3 days. Ethanol content was determined at the end of the fermentation and the best nitrogen source was selected for further work.

Effect of different concentration of corn steep liquor on ethanol production

The Effect of different concentrations of corn steep liquor, being the best nitrogen used, on ethanol production was studied. Different ratios (90:10, 80:20, 70:30, 60:40, and 50:50) of acetate buffer (0.1 M, pH 5.5) to corn steep liquor were prepared. These were used to extract sugar from pretreated maize straw. About 5 % of pretreated maize straw was boiled with different concentrations (ratio of acetate buffer to corn steep liquor) of corn steep liquor. The extracts were filtered with a muslin bag and supplemented with 2 % glucose. The supplemented filtrate was subjected to two different treatments. Firstly, pH was adjusted to 5.5 after supplementation with glucose while the other set's pH was not adjusted. Both treatments were sterilized at 121 °C for 15 minutes, cooled, inoculated with 2 % of 1.0 MacFarland standard of *S. cerevisiae* SA01 and *S. cerevisiae* SA02 separately, and were incubated at 30 °C for 3 days. Ethanol content was determined after fermentation.

Effect of different inoculum sizes and loads on ethanol production

Filtrates extracted with acetate buffer (0.1 M, pH 5.5) and corn steep liquor in ratio 60 to 40, supplemented with 2 % glucose without adjusting pH as explained above was dispensed into different fermentation bottles and sterilized at 121 °C for 15 minutes. Different inoculum sizes (1.0, 1.5, 2.0, 2.5, and 3.0 %) of 1.0 MacFarland standard of *S. cerevisiae* SA01 and *S. cerevisiae* SA02 were used to inoculate cooled filtrates separately. Two percent of different inoculum loads (0.5, 1, 2, and 3 MacFarland standard with their respective equivalent approximate CFU/ mL of 1.5×10^8 , 3.0×10^8 , 6.0×10^8 and 9.0×10^8) of *S. cerevisiae* SA01 and *S. cerevisiae* SA02 were also inoculated into another set. Inoculated filtrates were incubated at 30 °C for 3 days and ethanol concentrations were determined after fermentation.

Analytical Methods

Ethanol content was determined by a gravimetric method in reference to Fasiku and Wakil (2021). The specific gravity was determined by dividing the weight of distillate by the weight of distilled water and was used to determine the ethanol content with reference to ethyl alcohol conversion table.

Reducing sugar was determined using the dinitrosalicylic acid (DNS) method as described by Miller (1959).

The obtained experimental data were analyzed using Analysis of Variance to determine the means with SPSS version 23 and the level of significance was set at $P < 0.05$.

RESULTS

Effect of pH of extraction buffer on ethanol production

Effect of pH of buffer used to extract fermentable sugar from pretreated maize straw on ethanol content and consumed reducing sugar content is as shown in Figure 1. There was increase in reducing sugar consumed from 0.02 mg/g (pH 4.0) to 0.38 mg/g (pH 5.0) when fermented by *S. cerevisiae* SA01 and 0.19 mg/g (pH 4.0) to 0.53 mg/g (pH 5.5) when fermented with *S. cerevisiae* SA02. Though ethanol was not produced at pH 4.0 and 4.5 there was an increase in the concentration of ethanol produced by the two strains of *S. cerevisiae* with the increase in the pH from pH 5.0. An equal and highest concentration of ethanol (1.97 g/L) was attained by the two strains of *Saccharomyces cerevisiae* at pH 5.5. Optimum ethanol was produced when the pH of extracting buffer was 5.5 and was therefore selected for further studies.

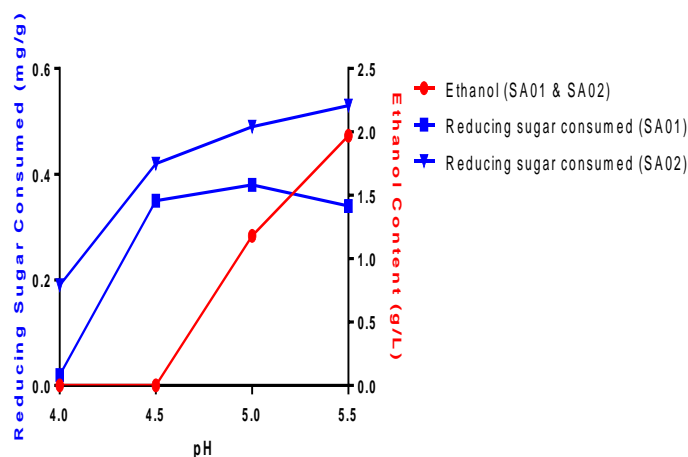


Figure 1 Effect of pH of Extraction Buffer on Ethanol Production and Reducing Sugar Consumed by Two Strains of *Saccharomyces cerevisiae*. Where SA01 is *S. cerevisiae* SA01 and SA02 is *S. cerevisiae* SA02

Effect of temperature on ethanol production

Figure 2 shows the effect of temperature on utilized reducing sugar and ethanol production by *S. cerevisiae* SA01 and *S. cerevisiae* SA02. The highest reducing sugar was used at 45 °C by *S. cerevisiae* SA02 (0.68 g/L) while *S. cerevisiae* SA01 utilized the highest reducing sugar (0.50 g/L) at 40 °C. A decrease in ethanol content from 2.76 g/L to 1.58 g/L and 2.37 g/L to 1.58 g/L by *S. cerevisiae* SA01 and *S. cerevisiae* SA02, respectively was recorded with the increase in incubation temperature from 30 to 45 °C. At varying temperatures, the highest ethanol content was obtained by *S. cerevisiae* SA01 (2.76 g/L) and *S. cerevisiae* SA02 (2.37 g/L) at 30 °C, and this temperature was used for further studies.

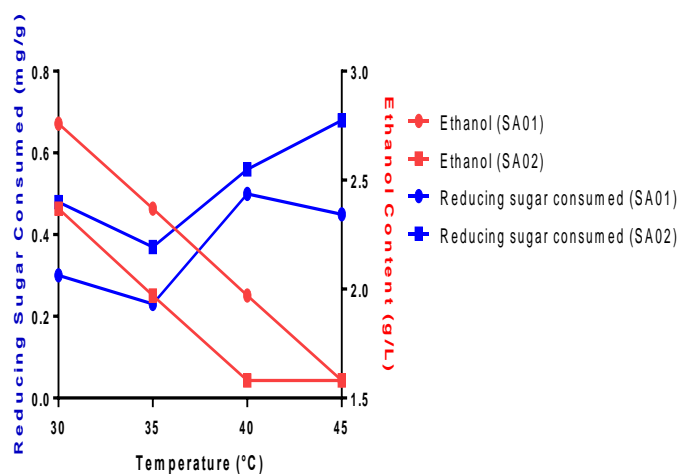


Figure 2 Effect of incubation temperature on ethanol production by *Saccharomyces cerevisiae*. Where SA01 is *S. cerevisiae* SA01 and SA02 is *S. cerevisiae* SA02

Effect of glucose and fructose supplementations on ethanol production

The effect of different concentrations of supplemented glucose and fructose on bioethanol production by *Saccharomyces cerevisiae* SA01 and *S. cerevisiae* SA02 is as presented in Figure 3. Ethanol yield of *S. cerevisiae* SA01 in the filtrate supplemented with glucose ranged from 2.37 g/L to 2.76 g/L with the highest content at 2 and 3 % of glucose while that of *S. cerevisiae* SA02 ranged from 2.37 to 3.95 g/L with the highest at 2 % glucose. Ethanol yield of *S. cerevisiae* SA01 and *S. cerevisiae* SA02 with different concentrations of fructose ranged from 1.97 to 2.76 g/L. The concentration of glucose (2 %) where the highest yield of ethanol (3.95 and 2.76 g/L) was obtained by *S. cerevisiae* SA01 and *S. cerevisiae* SA02, respectively was selected for further studies.

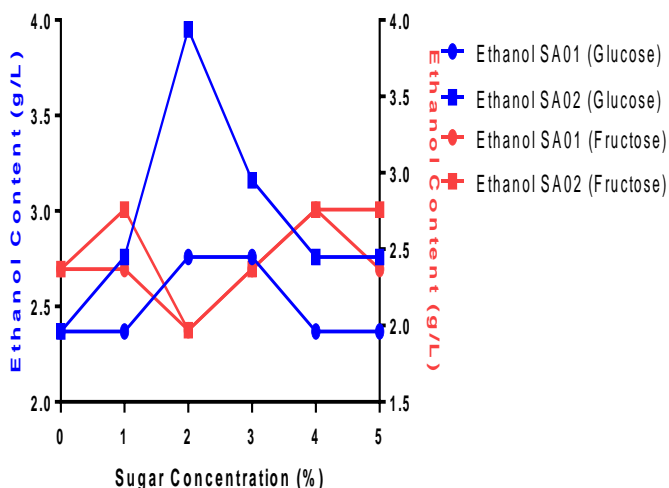


Figure 3 Effect of Glucose and Fructose supplementation on ethanol production by *Saccharomyces cerevisiae*

Effect of nitrogen sources on ethanol production

The effect of nitrogen sources on ethanol yield of the pretreated maize straw filtrate fermented with *S. cerevisiae* SA01 and *S. cerevisiae* SA02 is shown in Figure 4. Supplementation with different wastes as nitrogen sources (corn steep liquor, groundnut cake, soya meal, fish meal, and blood meal) resulted in high ethanol yield. In filtrate fermented with *Saccharomyces cerevisiae* SA01, the highest ethanol content (12.23 g/L) recorded for groundnut cake was not significantly different ($P>0.05$) from the ethanol yield observed for corn steep liquor (11.84 g/L) and Soya meal (11.84 g/L) while the least ethanol content (7.10 g/L) was recorded for blood meal. In filtrate fermented by *S. cerevisiae* SA02, the least ethanol content (9.47 g/L) was recorded in blood meal while the highest ethanol yield (13.02 g/L) was observed in corn steep liquor. *S. cerevisiae* SA02 had a higher ethanol yield than *S. cerevisiae* SA01 with all the nitrogen sources used. Corn steep liquor was selected as a nitrogen source for further work.

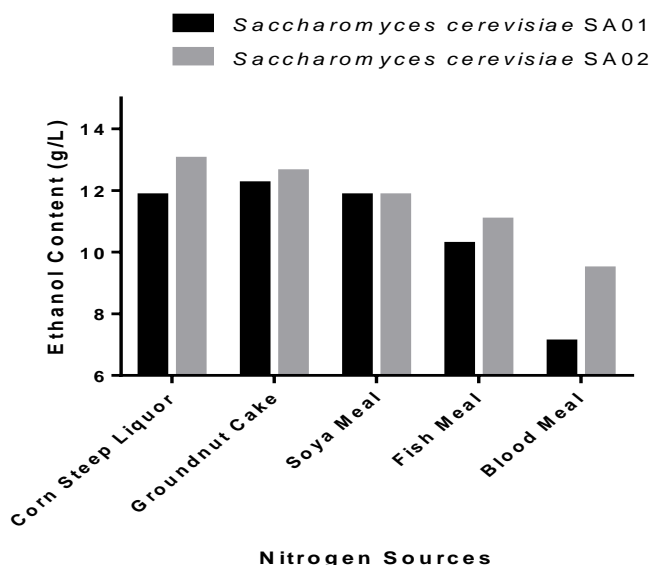


Figure 4 Effect of different nitrogen sources on ethanol production

Effect of different concentration of corn steep liquor on ethanol production

Effect of different concentrations of corn steep liquor without adjustment of pH after supplementation on ethanol yield is shown in Figure 5a. There was an increase in ethanol content with an increase in the concentration of corn steep liquor by the two strains of *S. cerevisiae*. The highest ethanol content (14.20 g/L) produced by *Saccharomyces cerevisiae* SA01 and *Saccharomyces cerevisiae* SA02 were recorded in the filtrate with a ratio of 60 mL acetate buffer to 40 mL corn steep liquor (60:40) and 50 mL acetate buffer to 50 mL corn steep liquor (50:50), respectively. There was no significant difference ($P\leq 0.05$) in ethanol content obtained with *Saccharomyces cerevisiae* SA02 at 60:40 and 50:50 acetate buffer to corn steep liquor. Ratio 60:40 acetate buffer to corn steep liquor was used for further work.

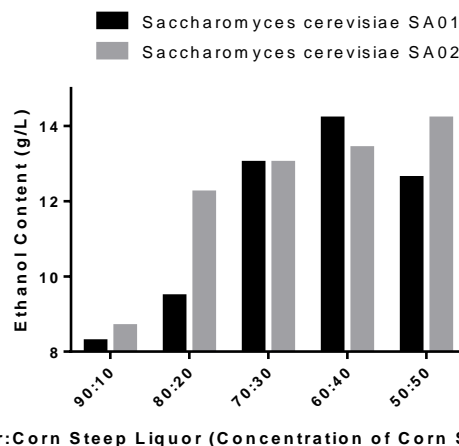


Figure 5a Effect of concentration of corn steep liquor on ethanol content without pH adjustment

The pH of filtrates extracted with different concentrations of corn steep liquor was adjusted to 5.5 and the effect on ethanol content was observed (Figure 5b). An increase in ethanol content was observed with an increase in the concentration of corn steep liquor. Higher ethanol content was recorded by *S. cerevisiae* SA02 than *S. cerevisiae* SA01 in all concentrations of corn steep liquor. The highest amounts of ethanol recorded by *Saccharomyces cerevisiae* SA01 (13.41 g/L) and *Saccharomyces cerevisiae* SA02 (13.81 g/L) were observed at ratio 70:30 and 50:50 concentrations of buffer to corn steep liquor respectively. These values were not as high as what was obtained when pH was not adjusted after supplementation. Use of 60:40 buffer to corn steep liquor without adjustment of pH after supplementation as presented in Figure 5a was used for further work.

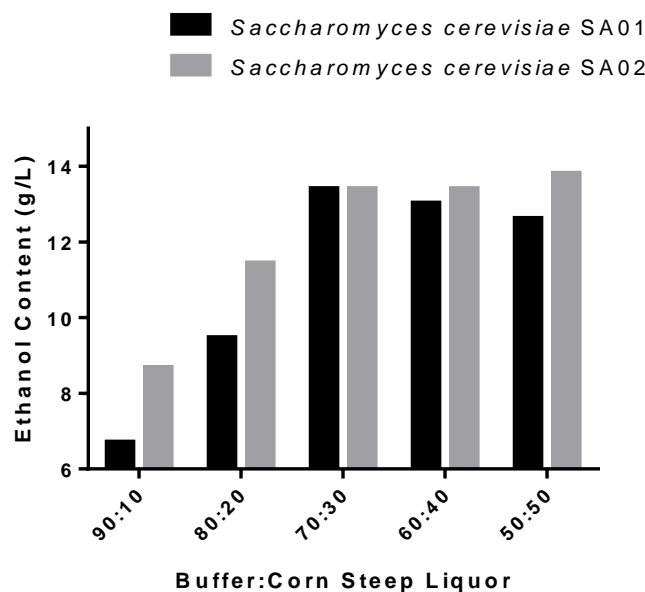


Figure 5b Effect of concentration of corn steep liquor on ethanol content with pH adjustment

Effect of different inoculum sizes and loads on ethanol production

Table 1a shows the effect of inoculum load on ethanol content of pretreated maize straw filtrates fermented by two strains of *S. cerevisiae* separately for 72 hours. An increase in the amount of ethanol with an increase in inoculum load was recorded

by the two strains of *S. cerevisiae*. The highest concentration of ethanol (14.20 g/L) was attained at an inoculum load of 3.0 MacFarland standard by *S. cerevisiae* SA01 and 2.0 MacFarland standard by *Saccharomyces cerevisiae* SA02. The least ethanol content recorded by *S. cerevisiae* SA01 (13.02 g/L) and *S. cerevisiae* SA02 (13.41 g/L) at inoculum load of 0.5 MacFarland standard were significantly different ($P \leq 0.05$) from their highest ethanol content (14.20 g/L).

Table 1a Effect of inoculum load on ethanol content (g/L) by *Saccharomyces cerevisiae*

MacFarland Standard	<i>Saccharomyces cerevisiae</i> SA01	<i>Saccharomyces cerevisiae</i> SA02
0.5	13.02 ^a	13.41 ^a
1.0	13.41 ^{ab}	13.81 ^{ab}
2.0	13.81 ^{ab}	14.20 ^b
3.0	14.20 ^b	14.20 ^b

Mean values with different superscript letters along the column were significantly different ($p \leq 0.05$)

Effect of inoculum size on ethanol yield is as shown in Table 1b. Equal and highest ethanol yield (14.99 g/L) was observed by *S. cerevisiae* SA01 and *S. cerevisiae* SA02 at 1 % inoculum size. A decrease in ethanol content was recorded by the two strains of *S. cerevisiae* with an increase in inoculum size. Ethanol yield of 14.99 g/L with an inoculum size of 1.0 % of *S. cerevisiae* SA01 is significantly different ($P \leq 0.05$) from ethanol yield from other inoculum sizes.

Table 1b Effect of inoculum size on ethanol content (g/L) by *S. cerevisiae*

Inoculum Size (%)	<i>Saccharomyces cerevisiae</i> SA01	<i>Saccharomyces cerevisiae</i> SA02
1.0	14.99 ^b	14.99 ^b
1.5	13.41 ^a	14.99 ^b
2.0	13.02 ^a	13.81 ^a
2.5	13.02 ^a	13.81 ^a
3.0	12.62 ^a	14.20 ^{ab}

Mean values with different superscript letters along the column were significantly different ($p \leq 0.05$)

DISCUSSION

The observed increase in ethanol yield with an increase in the pH till optimum pH 5.5 has been reported by some researchers (Wakil *et al.*, 2013; Abo *et al.*, 2018; Adelabu *et al.*, 2018; Tasnim and Farasat, 2018). Adelabu *et al.* (2018) reported an increase in ethanol production from sorghum straw with an increase in pH till pH 5.5 thereafter a decrease in ethanol content. Acidity or alkalinity of fermentation medium has effects on the growth and production of metabolites, each microorganism has a pH range under which it can perform maximally and *Saccharomyces cerevisiae* has been known for the production of ethanol under slightly acidic conditions (pH value of between 5.0 and 6.0) (Nadeem *et al.*, 2015; Chatterjee and Mohan, 2021).

The influence of temperature observed on the ethanol yield by the yeasts could be due to the effect of temperature on growth, metabolism, survival of fermenting organisms, and fermentation (Tiwari *et al.*, 2015). The recorded optimum temperature (30 °C) for the production of ethanol in this study has been reported by some researchers (Nadeem *et al.*, 2015; Taiwo *et al.*, 2018; Tasnim and Farasat, 2018) however, Chatterjee and Mohan (2021) recorded highest ethanol production at 35 °C.

The observed higher yield of ethanol from glucose than fructose by *S. cerevisiae* SA01 and *S. cerevisiae* SA02 was probably due to easy assimilation of glucose as a source of carbon and energy by the yeasts (Mori *et al.*, 2019). The significant effect of different concentrations of sugar recorded in this work has been reported by some researchers (Liang *et al.*, 2013; Nadeem *et al.*, 2015; Mori *et al.*, 2019). Liang *et al.* (2013) observed an increase in ethanol content with an increase in sugar concentration until a maximum ethanol content was obtained and any further increase in sugar concentration resulted in a decrease in ethanol content. Mori *et al.* (2019) studied the effect of initial glucose concentration on ethanol yield and observed that increase in ethanol content was observed with an increase in glucose concentration till 6 g/L thereafter, a decrease in ethanol content was recorded with an increase in glucose concentration. The highest ethanol content was recorded when pretreated maize straw was supplemented with 2 % glucose which is similar to the work of Nadeem *et al.* (2015) who supplemented fermentation medium with 2 % of glucose to improve the yield of ethanol.

Nitrogen sources has effects on ethanol yield, a result similar to what was reported by Martha *et al.* (2020). Corn steep liquor is a byproduct obtained during the production of *Ogi* and is usually poured away as waste with no positive economic value which could even add to the pollution of the environment. Corn steep liquor used as a nitrogen source in this work improved the ethanol yield of *S. cerevisiae* SA01 by 300 % and *S. cerevisiae* SA02 by 661 %. This could be attributed to corn steep liquor being rich in nutrients and being a good source of nitrogen which is important for the growth and metabolism of yeast (Taiwo *et al.*, 2018). Taiwo and others (2018) replaced yeast extract with corn steep liquor and observed rapid utilization of reducing sugar and good yield of ethanol. However, almost no

ethanol was produced because of quite low biomass concentration when Liu *et al.* (2016) replaced yeast extract with different concentrations of corn steep liquor as a source of nitrogen.

Vegetal by-products (corn steep liquor, groundnut cake, and soy meal) as nitrogen sources had better production of ethanol than animal by-products (fish meal and blood meal) as nitrogen sources by both *S. cerevisiae* SA01 and *S. cerevisiae* SA02 in this study. A similar result was observed when Yatmaz *et al.* (2016) used fish meal and soy meal as a nitrogen source for the production of β -Mannanase where soya meal as a nitrogen source had a better yield of β -Mannanase than a fish meal as a source of nitrogen.

Microbial load and size play important role in the yield of ethanol by *S. cerevisiae* SA01 and *S. cerevisiae* SA02. An increase in ethanol yield observed with an increase in inoculum load was possibly due to an increase in the number of cells with no increase in the volume making the cell metabolize reducing sugar quickly to bioethanol. The decrease in ethanol production observed with an increase in inoculum size of *S. cerevisiae* SA01 and *S. cerevisiae* SA02 could be due to limited nutrients that were present in the fermentation medium which might not be enough for high inoculum size. This was also observed by Adelabu *et al.* (2018) who reported a decrease in ethanol production from sorghum straw with an increase in inoculum size.

CONCLUSION

Temperature, pH, sugar concentration, nitrogen source, and inoculum loads/sizes have effects on ethanol production by *S. cerevisiae* SA01 and *S. cerevisiae* SA02. The highest ethanol content (14.99 g/L) by *S. cerevisiae* SA01 and *S. cerevisiae* SA02 was produced at pH 5.5, 30 °C when filtrate was supplemented with 2 % glucose, corn steep liquor as nitrogen source, at inoculum load of 1 % of 1.0 MacFarland standard and at 72 hours fermentation period. Bioconversion of maize straw to bioethanol under optimized conditions will reduce environmental pollution, impact positively on the economic status of the nation, and increase sources of energy.

Acknowledgments: Not applicable

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