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OPTIMIZATION OF ETHANOL PRODUCTION FROM SWITCHGRASS (*Panicum virgatum* L.) GROWN IN SOKOTO GEOECOLOGICAL ZONE OF NIGERIA

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ABSTRACT

The effect of acid concentration, temperature and heating time on fermentation of switchgrass (*Panicum virgatum* L.) fedstock grown during 2008/2009 and 2009/2010 seasons Sokoto, Nigeria in the production of ethanol was investigated. The hydrolysates was pretreated with 3, 4 and 5% hydrochloric acid concentrations at 30,40 and 50^0C for 20, 25 and 30 minutes for five days. The hydrolysate was fermented using *S. cerevisiae*, *Z. mobilis* and combination of the two organisms. The highest yield of reducing sugar was obtained from hydrolysates treated with 50 and 75 kg N/ha at all the temperature regimes used at 4% acid concentrations and at the treatment time of 20, 25 and 30 minutes. The control had the lowest quantity of reducing sugar at all the treatments and locations. The combination of *S. cerevisiae* and *Z. mobilis* gave the highest concentration of bioethanol compared with when single organism is used in the fermentation. Result of this study suggests that the combination of *S. cerevisiae* and *Z. mobilis* produced the highest bioethernol content and reinforced its potential as a source of bioethernol. This indicates the potentials of the hydrolyzing organisms, the fermentative organism and the switchgrass feedstock for large scale bioethanol production is emphasized.

Keywords: Bioethanol, Switchgrass, Bioenergy, Hydrolysis, Fermentation.

INTRODUCTION

The fluctuation costs of oil and gas, the finite nature of fossil fuels, and growing environmental, health and safety considerations, have necessitated world's wide interest in alternative source of energy [1]. Lignocellulosic biomass is a compelling candidate for alternative fuel production because it is readily available, and has the potential of having a relatively small environmental impact [2]. Switchgrass is one of the crops which proves to be advantageous, highly adaptable and can grow in many different regions including regions with less ideal soil quality [3]. It poses good tolerance to cold, disease and insects [4] and can readily be integrated into existing farming practices. This is because it is inexpensive to seed and establishes itself fairly quickly. The use of switchgrass relative to other annual row crops, leads to a 95% reduction in soil erosion and a 90%

in pesticide usage. Switchgrass also helps to improve soil quality and carbon sequestration due to its extensive roots system that increases carbon storage in the soil [5]. Fuel ethanol from lignocelluloses may also open new employment opportunities in rural areas and thus make a positive socio-economic impact [6]. Similarly, energy production from perennial cropping system, would help lower national dependant on fossil fuels and reduce emissions of green house gases (GHG), hence reducing environmental impact.

In order to convert biomass to fuels, there are generally two basic methodologies that could be employed; these are biological platform and thermo-chemical technologies. The first approach involves converting biomass to ethanol or related liquid fuels by a saccharification and fermentation process. This involves deconstructing polysaccharides to monosaccharide followed by fermentation to 2^{nd} or 3^{rd} generation biofuels. One of the most important considerations in overall biofuel production is the content of cellulose and hemicellulose in the cell walls of the biomass. This process requires high temperatures in an oxygen free atmosphere for short times to volatilize low molecular weight compounds which are then condensed rapidly to a liquid bio-crude [7]. The challenges of overcoming the recalcitrance of cellulosic biomass are an impediment to converting them into reactive intermediates and useful products [8]. Therefore, the utilization of cellulosic biomass for bioethanol production involved the hydrolysis of carbohydrate polymers using either dilute or concentrated acid into readily fermentable sugars [9]. The objective of the study is to optimize the potential of switchgrass for bioethanol production using dilute hydrochloric acid (HCl) hydrolysis and fermentation.

MATERIALS AND METHODS

Source and Preparation of Plant Sample

Switchgrass was fresh harvested from the net plot in the field and were thoroughly washed with tap water, chopped into small pieces, mashed mechanically and dried in a hot air oven at 65^0C for 6 hour. The dried material were pulverized into powder and stored at room temperature until when required.

Source and isolation of fermenting organisms

Zymomonas mobilis was isolated from rotten orange according to the method of [10]. The rotten orange samples were washed and then squeezed to obtain the juice which was serially diluted from tube 1 (10) to tube 5 (10) and 0.1ml aliquot from the 10^5 tube was plated onto the malt yeast peptone glucose agar (MYPGA), Yeast glucose agar (YGA) and peptone glucose agar (PGA) media using spread plate techniques. Each medium was treated with actidone (cycloheximide) to inhibit yeast growth. The plates were incubated in an anaerobic jar in which Gas Pak Sachet was placed to exhaust the oxygen in the jar and incubated at 30^oC for 2 days. Colonies suspected to be those of *Zymomonas* were isolated from the plates and purified by streaking on fleshy prepared media and incubated for 2 days at 30^oC in an anaerobic jar.

Sacharomyces cerevisiae was isolated from palm wine in accordance with the method described by [11] as follows: The palm wine samples were serially diluted to 10^5 tubes. Then 0.1ml aliquot from the 10^5 tube was inoculated onto malt extract agar and saboraud dextrose agar plates. The plates were incubated at ambient laboratory temperature (35 ± 2^oC) for 72 hours. Colonies that developed after the incubation periods were cultured repeatedly to obtain pure cultures which were maintained on agar slants for further characterization and identification. The bacterial isolates were characterized and identified in accordance with the method described by [12, 13 and14].

Acid Hydrolysis of Pulverized Switchgrass

This was carried out according to the method described by [15, 16 and 17] as follows: Forty grams (40g) of switchgrass treated with 0 kg N/ ha^{-1} was weighed into 2 litre capacity conical flasks. Then 1 litre of varying dilute hydrochloric acid (HCl) concentrations of 3%, 4% and 5% were added into the flasks. The flasks were covered with cotton wool, wrapped in aluminum foil, heated in a digital thermostatic water bath (HH-S) at for 20, 25 and 30 minutes at 30^{o}C, 40^{o}C and 50^{o}C and autoclaved at 121^{o}C for 15 minutes. The flasks were allowed to cool, filtered through No.1 Whatman filter paper and the pH was adjusted to 4.5 with 0.4M sodium hydroxide (NaOH). The same procedure was repeated for 25, 50 and 75 kgN/ ha^{-1} treated materials.

Determination of Reducing Sugar Content of hydrolysed Switchgrass

The reducing sugar content of hydrolysed feedstock was determined using the [18]. The reducing sugar content of the switchgrass was assayed by pipette out standard glucose sodium 0 to 1ml, range and make up the sodium to 2ml with distilled water. Two (2) ml of alkaline cu-reagent was added to all the test tube. The content was mixed and kept in a boiling water bath for 8 minutes. Cool under running water and 2 ml of phosphomolybdic acid reagent was added to all the test tubes. They were allowed for 10 minutes before mixing the content and the volume was made to 25 ml with distilled water. The absorbance of the samples was measured at 381.4 nm using UV-VIS Spectrophotometer. The reducing sugar content was subsequently determined by making reference to a standard curve of known glucose concentrations.

Fermentation of Hydrolysed Switchgras

The fermentation of the hydrolysed samples was carried out in accordance with the methods described by [19 and 20] as follows: Fifty mililiters (50 ml) of the switchgrass (SGNO) hydrolysates was dispensed into three different 500ml capacity conical flask. The flasks were covered with cotton wool, wrapped in aluminum foil and autoclaved at 121^{o}C for 15 minutes. The tubes were allowed to cool at room temperature and aseptically inoculated with the fermentative organisms as follows:

A: Inoculated with *Saccharomyces cerevisiae*

B: Inoculated with *Zymomonas mobilis*

C: Inoculated with *Saccharomyces cerevisiae and Zymomonas mobilis*

All the flasks were incubated anaerobically at 30^{o}C for 5 days. The same procedure was repeated all the hydrolysates.

Fractional Distillation of Fermented Broth

This was carried out according to the method described by [20] as follows: The fermented broth was dispensed into round-bottom flasks fixed to a distillation column enclosed in running tap water. A conical flask was fixed to the end of the condenser to collect the distillate. A heating mantle with the thermostat adjusted to 78^{o}C was used to heat the round-bottom flask containing the fermented broth.

Determination of ethanol content

This was carried out using UV-VIS quantitative analysis of alcohols using chromium VI reagent according to the method described by [20 and 21] as follows: One (1) ml of standard ethanol was diluted with 100ml distilled water to give a concentration of 1%. Then each of 0,2,4,6 and 8mls of the 1% ethanol was diluted to 10mls with distilled water to produced 0, 0.2, 0.4, 0.6, and 0.8% of the ethanol. To each of the varying ethanol concentration, 2 mls of chromium reagent was added and allowed to stand for an hour for colour development. The absorbance of each concentration was

measured at 381.4 nm using UV-VIS spectrophometer and the reading was used to develop standard ethanol curve. A portion (4 mls) of each bioethanol samples were put in test tubes and treated with 2 mls of the chromium reagent. The mixture was allowed to stand for an hour and the absorbance measured at 381.4 nm using the UV-VIS spectrophotometer.

RESULTS

Effect of hydrolysis on reducing sugar content of switchgrass

The results of reducing sugar content of hydrolysates obtained from switchgrass after hydrolysis with 3, 4 and 5% HCl at 30, 40 and 50^0C for 20, 25 and 30 minutes at lowland in 2008/2009 season is presented in (Table 1). Reducing sugar yield did not differ significantly ($p<0.05$) with acid concentrations, temperature regimes and heating time in 2008/2009 (Table 1). At lowland, temperature significantly affected the reducing sugar on hydrolysates treated with 3% HCl at 40 and 50^0C in 2009/2010 season show a significant ($P<0.05$) effect of temperature. The highest reducing sugar yield of 6.53% was obtained from the sample treated with 50 kg N ha^{-1} at 40^0C (Table 2) and treatment with 4% HCl at 40^0C. The lowest yield of 4.85% was obtained from the control which was not significantly different from hydrolysates treated with 25 and 75 kg N ha^{-1} but significantly different from hydrolysate treated with 50 kg N ha^{-1}. At 30^0C and 50^0C there was no significant effect of temperature on reducing sugar yield at different nitrogen rates. The effect of temperature regimes on reducing sugar yield of hydrolysates treated after hydrolysis with 5% HCl was significantly affected (Table 2). The control had the lowest reducing sugar yield which was however, not significantly different from hydrolysates treated with 25 kg N ha^{-1}.

Table 1: Morphological and biochemical characterization of isolates from rotten orange

Isolates	Gram Reaction	Motility	Catalase	Glucose	Fructose	Maltose	Arabinose	Urease	Oxidase	Lactose	Organism
1	- Rod	+	+	+	+	-	-	-	-	-	*Zymomonas mobilis*

Key: +: fermentation; -: no fermentation/negative

Table 2: Morphological and biochemical characterization of isolates from palm wine

Isolates	Colonial characteristics	Cell shape	Man	Glu	Fru	Suc	Mal	Ara	Lac	Gal	Cat	Organism
1	Smooth creamish	Spherical	-	AG	AG	AG	AG	-	-	AG	+	*Saccharomyces cerevisiae*

Key: Man: minnitol Glu: glucose; Fru: fructose; Suc: sucrose; Mal: maltose; Ara: arabinose; Lac: lactose; Gal: galactose; Cat: catalase; AG: acid/gas production, -: no fermentation; +: positive

The results on the effects of acid concentration on the reducing sugar content of hydrolysates obtained from the upland sample hydrolysates; hydrolysed with varied acid concentration in 2008/2009 and 2009/2010 are presented in Tables 3 and 4. The result revealed that in 2008/ 20009 the highest reducing sugar yield of 5.42% was obtained from hydrolysates treated with 75 kg N ha^{-1} at 40^0C and the lower of 1.93% was obtained from hydrolysates not treated with nitrogen at 50^0C. The hydrolysates treated with 4% HCl had the highest reducing sugar yield of 5.86% was obtained from hydrolysates treated with 75 kg N ha^{-1} at 40^0C. This was followed by a yield of 5.50% from the same hydrolysate at 30^0C and the minimum yield of 1.20% was obtained from the control

treatment at 50^0C. Similarly, hydrolysates hydrolyzed with 5% HCl at different temperature regimes produced the highest yield of 4.5% obtained from hydrolysates treated with 75 kg N ha^{-1} at 30^0C.

In 2009/2010, the results of reducing sugar yield obtained from the hydrolysates after hydrolysis with varied acid concentation and temperature regimes at 30^0C, 40^0C and 50^0C at upland is presented in Table 4. The concentation of reducing sugar in 3% HCl, differs significantly and the highest (5.86%) yield was obtained at 40^0C from hydrolysates treated with 75 kg N ha^{-1}. The lowest yield (2.19%) was obtained in hydrolysate treated without application of nitrogen. At 30^0C and 50^0C, hydrolysate treated with different nitrogen rates produced statistically similar reducing sugar yield. The data on reducing sugar yield with 4% HCl indicated that at 30^0C and 40^0C there was significant ($P<0.05$) difference in reducing sugar concentration. The lowest (3.34% and 1.61%) yields were obtained from the control and highest yield (5.60% and 5.50%) were obtained from hydrolysates treated with 75 kg N ha^{-1} (Table 4).

The effect of temperature on reducing sugar yield of hydrolysates treated with 5% HCl revealed that at 30^0C there was a significant difference on reducing sugar yield. The lowest (2.28%) yield was obtained from the control which was not significantly different from hydrolysates treated with 25 and 75 kg N ha^{-1} and the highest (4.93%) was obtained from hydrolysates treated with 50 kg N ha^{-1}. At 40^0C and 50^0C there was no significant difference on reducing sugar yield at different rates of nitrogen application as they produced statistically similar results (Table 4).

Table 3: Effect of temperature on the reducing sugar produced by switchgrass treated with different levels of nitrogen (lowland, 2008/2009)

		Temperature (^{o}C)		
Conc. (%)	**Samples**	30	40	50
3%	N_0	3.65 ± 0.32	4.29 ± 0.57	3.38 ± 0.62
	N_{25}	4.20 ± 0.86	4.55 ± 0.87	3.88 ± 0.67
	N_{50}	4.92 ± 0.37	5.31 ± 0.23	4.57 ± 0.45
	N_{75}	4.18 ± 1.09	4.80 ± 1.07	4.11 ± 0.96
		Temperature (^{o}C)		
		30	40	50
4%	N_0	4.93 ± 0.87	4.89 ± 0.35	2.97 ± 0.20
	N_{25}	5.70 ± 0.14	5.33 ± 0.44	3.62 ± 0.20
	N_{50}	5.71 ± 0.03	5.74 ± 0.11	4.57 ± 1.08
	N_{75}	5.05^a ± 0.66	5.72 ± 0.12	4.45 ± 1.19
		Temperature (^{o}C)		
		30	40	50
5%	N_0	4.95 ± 0.97	2.67 ± 0.17	2.75 ± 0.20
	N_{25}	4.28 ± 0.75	3.08 ± 1.02	3.50 ± 1.22
	N_{50}	5.54 ± 0.22	3.85 ± 0.92	4.36 ± 1.14
	N_{75}	4.65 ± 0.22	3.75 ± 0.67	3.92 ± 0.46

Values are mean ± standard error Means in a column with different superscripts are significantly different ($P<0.05$)

Table 4: Effect of temperature on the reducing sugar concentration from hydrolysate treated with different concentrations of HCl (lowland, 2009/2010)

Conc. (%)	Samples	Temperature (^oC) 30	40	50
3%	N_0	4.48 ± 0.40	4.26^b ± 0.59	2.81^b ± 0.27
	N_{25}	5.24 ± 0.21	4.76^{ab} ± 0.37	4.74^a ± 0.50
	N_{50}	5.49 ± 0.23	5.93^a ± 0.56	5.40^a ± 0.40
	N_{75}	5.60 ± 0.17	5.48^a ±0.10	5.18^a ± 0.22
		Temperature (^oC)		
		30	40	50
4%	N_0	4.85^b ± 0.08	3.73± 0.87	4.14 ± 0.66
	N_{25}	5.28^{ab} ± 0.17	4.11 ± 0.75	4.37± 0.12
	N_{50}	6.26^a ± 0.52	6.53 ± 0.21	5.75 ± 0.69
	N_{75}	5.62^{ab} ± 0.54	5.92 ± 0.63	4.77 ± 0.63
		Temperature (^oC)		
		30	40	50
5%	N_0	4.09 ± 0.58	3.41^b ± 0.53	2.81^b ± 0.43
	N_{25}	4.69 ± 1.15	4.79^{ab} ± 0.38	4.15^{ab} ± 0.92
	N_{50}	5.24 ± 0.56	5.73^a ± 0.53	5.55^a ± 0.32
	N_{75}	5.18 ± 0.22	5.54^a ± 0.12	5.42^a ± 0.52

Values are mean ± standard error. Means in a column with different superscripts are significantly different (P<0.05)

Effect of Heating Time on the Reducing Sugar Concentration

The results of reducing sugar yield obtained from the hydrolysates after hydrolysis with 3, 4 and 5% HCl for 20, 25 and 30 minutes at lowland in 2008/2009 season is presented in Table 36. Results revealed that there is no significant ($p<0.05$) differences in reducing sugar yield with varied acid concentrations and heating time (Table 5). At lowland in 2009/2010 season, samples treated with 3% HCl at 30 minutes results indicated that there was no significant difference on reducing sugar yield at the different nitrogen rates, but at 20 and 25 minutes of heating there were significant ($P<0.05$) difference in reducing sugar concentration. The highest concentration (5.80^0%) was obtained with 50 kg N ha^{-1} at 20 minutes followed by a yield of 5.40 at 25 minutes of the same hydrolysate (Table 6).

The results of reducing sugar yield from hydrolysates after hydrolysis with 4% HCl for 20 minutes revealed that there was no significant effect of heating on the reducing sugar yield. At both 25^0C and 30^0C the highest (5.84% and 6.16%) yield was obtained from hydrolysates treated with 50 kg N ha^{-1} and the lowest yield was obtained from hydrolysates treated with zero (0) nitrogen. However, treatment with 5% HCl for 20 minutea showed a significant increase in reducing sugar yield. The highest yield of 6.50% was obtained with the hydrolysates treated with 75 kg N/ha and the control had the lowest yield of 4.98%. However, for the hydrolysates treated for 25 and 30 minutes there were no significant difference in reducing sugar yield at the various heating times applied (Table 6). The result of reducing sugar yield obtained from upland hydrolysates after hydrolysis with 3% HCl indicated that there were no significant difference in reducing sugar yield at various heating times applied (Table 7). The results of reducing sugar yield with 4% HCl indicated that the highest reducing sugar yield of 4.62% was obtained from hydrolysates treated with 75 kg N ha^{-1} at 30 minutes followed by a yield of 4.15% from the same hydrolysates heated for 20 minutes and the least yield of 1.34% was obtained from hydrolysates treated with zero nitrogen

at 25 minutes (Table 7). The treatment hydrolysed with 5% HCl had the highest reducing sugar yield of 2.94% was obtained from hydrolysates obtained from a plot treated with 75 kg N ha^{-1} at 30^0C and the lowest yield of 1.61% was obtained from the control treatment at 25 minutes (Table 7).

Table 5: Effect of temperature on the reducing sugar concentration produced from hydrolysates treated with different concentrations of HCl (upland, 2008/2009)

		Temperature (^{o}C)		
Conc. (%)	**Samples**	30	40	50
3%	N_0	$2.24^{b} \pm 0.54$	$2.46^{b} \pm 0.23$	$1.93^{b} \pm 0.21$
	N_{25}	$4.74^{a} \pm 0.97$	$4.62^{ab} \pm 0.88$	$3.42^{ab} \pm 0.87$
	N_{50}	$4.57^{a} \pm 0.55$	$4.70^{ab} \pm 1.02$	$4.71^{a} \pm 0.54$
	N_{75}	$4.83^{a} \pm 0.34$	$5.42^{a} \pm 0.12$	$3.75^{ab} \pm 0.79$
		Temperature (^{o}C)		
		30	40	50
4%	N_0	3.01 ± 0.94	$1.27^{d} \pm 0.17$	1.20 ± 0.70
	N_{25}	4.32 ± 0.92	$2.23^{c} \pm 0.14$	3.81 ± 0.59
	N_{50}	3.64 ± 0.78	$4.12^{b} \pm 0.53$	2.96 ± 0.14
	N_{75}	5.50 ± 0.28	$5.86^{a} \pm 0.04$	3.86 ± 0.77
		Temperature (^{o}C)		
		30	40	50
5%	N_0	$1.89^{b} \pm 0.25$	$1.78^{b} \pm 0.31$	2.33 ± 0.29
	N_{25}	$3.16^{ab} \pm 0.27$	$3.11^{ab} \pm 0.36$	2.52 ± 0.49
	N_{50}	$3.36^{ab} \pm 0.21$	$4.17^{a} \pm 0.67$	3.92 ± 0.78
	N_{75}	$4.50^{a} \pm 0.93$	$3.60^{ab} \pm 0.94$	2.57 ± 0.17

Values are mean ± standard error Means in a column with different superscripts are significantly different (P<0.05)

Table 6: Effect of temperature on the reducing sugar concentration from hydrolysates treated with different HCl concentrations at upland in 2009/2010.

		Temperature (^{o}C)		
Conc. (%)	**Samples**	30	40	50
3%	N_0	$2.19^{a} \pm 0.75$	$1.94^{c} \pm 0.17$	$2.90^{a} \pm 0.70$
	N_{25}	$4.37^{a} \pm 1.00$	$3.83^{b} \pm 0.78$	$3.43^{a} \pm 0.86$
	N_{50}	$4.13^{a} \pm 0.76$	$5.51^{a} \pm 0.25$	$3.86^{a} \pm 0.77$
	N_{75}	$4.74^{a} \pm 0.97$	$5.86^{a} \pm 0.04$	$5.19^{a} \pm 0.41$
		Temperature (^{o}C)		
		30	40	50
4%	N_0	$3.34^{b} \pm 0.62$	$1.61^{b} \pm 0.03$	1.78 ± 0.29
	N_{25}	$5.40^{a} \pm 0.19$	$2.60^{b} \pm 0.31$	3.83 ± 0.58
	N_{50}	$5.50^{a} \pm 0.28$	$4.67^{a} \pm 0.80$	3.75 ± 0.79
	N_{75}	$5.60^{a} \pm 0.30$	$5.50^{a} \pm 0.23$	4.04 ± 0.96

		Temperature (^oC)		
		30	40	50
5%	N_0	$2.28^b \pm 0.26$	1.59 ± 0.19	2.06 ± 0.36
	N_{25}	$3.70^{ab} \pm 0.81$	2.57 ± 0.17	3.83 ± 0.83
	N_{50}	$4.93^a \pm 0.87$	3.89 ± 0.79	3.59 ± 0.94
	N_{75}	$4.56^{ab} \pm 0.87$	3.51 ± 1.07	2.97 ± 0.20

Values are mean ± standard error. Means in a column with different superscripts are significantly different (P<0.05)

Table 7: Effect of heating time on the reducing sugar produced by switchgrass treated with different levels of nitrogen (lowland, 2008/2009)

Conc. (%)		**Heating time (minutes)**		
	Samples	20	25	30
3%	N_0	3.90 ± 0.76	$3.58^a \pm 0.64$	$2.81^a \pm 0.42$
	N_{25}	4.62 ± 0.53	$4.34^a \pm 0.57$	$3.21^a \pm 0.25$
	N_{50}	4.94 ± 0.46	$4.92^a \pm 0.48$	$4.03^a \pm 0.51$
	N_{75}	4.70 ± 0.58	$4.65^a \pm 0.50$	$3.41^a \pm 0.58$
		Heating time (minutes)		
		20	25	30
4%	N_0	3.51 ± 1.07	3.85 ± 0.98	2.50 ± 0.12
	N_{25}	4.41 ± 0.83	3.92 ± 0.12	3.39 ± 0.75
	N_{50}	5.65 ± 0.06	5.55 ± 0.08	4.16 ± 1.06
	N_{75}	5.16 ± 0.16	5.19 ± 0.13	3.35 ± 0.17
		Heating time (minutes)		
		20	25	20
5%	N_0	2.81 ± 0.35	2.45 ± 0.17	3.86 ± 0.36
	N_{25}	3.61 ± 0.77	2.94 ± 0.88	4.04 ± 0.96
	N_{50}	4.01 ± 0.99	3.65 ± 1.03	5.06 ± 0.41
	N_{75}	3.77 ± 0.37	3.10 ± 0.07	4.94 ± 0.50

Values are mean ± standard error Means in a column with different superscripts are significantly different (P<0.05)

The results of reducing sugar yield of upland samples after hydrolysis with 3% HCl for 2009/2010 season showed that at different heating times there was significant (P<0.05) difference in reducing sugar yield. The low levels of reducing sugar yield (2.33%, 2.16% and 1.53%) were obtained from hydrolysates treated without application of nitrogen while the high levels (4.83%, 5.47% and 4.00%) were obtained from hydrolysates treated with 75 kg N ha^{-1} (Table 8). Hydrolysis of sample treated with 4% HCl indicate a significant (P<0.05) effect on reducing sugar yield. The highest reducing sugar yield was obtained from the hydrolysates treated with 50 kg N ha^{-1} and the control had the lowest.

The result on heating time indicate that treatment with 5% HCl showed a significant effect (P<0.05) on reducing sugar yield at 20 minutes. The highest yield of 2.50% was obtained from hydrolysates treated with 75 kg N ha^{-1} the control had the lowest 1.30 %.

Table 8: Effect of heating time on the reducing sugar produced by switchgrass treated with different levels of nitrogen (lowland, 2009/2010)

Conc. (%)		Heating time (minutes)		
	Samples	20	25	30
3%	N_0	$4.65^{b} \pm 0.57$	$3.90^{b} \pm 0.84$	3.79 ± 0.54
	N_{25}	$5.21^{a} \pm 0.37$	$4.74^{ab} \pm 0.37$	4.03 ± 0.70
	N_{50}	$5.80^{a} \pm 0.17$	$4.92^{ab} \pm 0.34$	4.34 ± 0.32
	N_{75}	$5.70^{a} \pm 0.03$	$5.40^{a} \pm 0.17$	5.01 ± 0.25
		Heating time (minutes)		
		20	25	30
4%	N_0	2.52 ± 0.21	$4.23^{b} \pm 0.55$	$3.63^{b} \pm 0.77$
	N_{25}	4.21 ± 1.29	$4.51^{ab} \pm 0.22$	$5.02^{ab} \pm 0.19$
	N_{50}	4.92 ± 0.55	$5.19^{ab} \pm 0.51$	$6.16^{a} \pm 0.14$
	N_{75}	4.59 ± 0.11	$5.84^{a} \pm 0.34$	$5.22^{ab} \pm 0.80$
		Heating time (minutes)		
		20	25	20
5%	N_0	$4.98^{b} \pm 0.66$	4.38 ± 0.98	2.75 ± 0.26
	N_{25}	$5.05^{b} \pm 0.45$	3.85 ± 0.15	3.87 ± 0.76
	N_{50}	$5.85^{ab} \pm 0.17$	5.31 ± 0.35	4.77 ± 0.24
	N_{75}	$6.50^{a} \pm 0.16$	5.13 ± 0.07	4.17 ± 0.93

Values are mean ± standard error Means in a column with different superscripts are significantly different ($P<0.05$)

Concentration of Bioethanol produced from distillated Switchgrass broth

The ethanol content produced from Switchgrass treated with different levels of nitrogen using *Saccharomyces cerevisiae*, *Zymomonas mobilis* and combination of *Saccharomyces cerevisiae* and *Zymononas mobilis* are presented in Tables 9 and 10**.** The results revealed that high concentrations of bioethanol of 0.35 and 0.36 was produced using *S. cerevisiae* and *Z. mobilis* respectively form hydrolysate treated with 50kg N ha^{-1}. In contrast, relatively low concentration of 0.22 and 0.24 were obtained for both seasons when *Z. mobilis* was used on hydrolysates treated with no nitrogen. The combination of *S. cerevisiae* and *Z. mobilis* had the highest ethanol content as compared to the yields obtained from the samples treated with the individual organisms at lowland in both seasons (Table 9). The trend was consistent at upland and at all levels of nitrogen application in both seasons. The highest ethanol content of 0.28 and 0.29 were obtained during 2008/2009 and 2009/2010 using *S. cerevisiae* and *Z. mobilis* respectively from switchgrass treated with 50 kg N ha^{-1} and low concentration of 0.18 and 0.19 for both years, were obtained when *Z. mobilis* was used on hydrolysate treated with no nitrogen (Table 11 & Table 12). Generaly, it was observed that the combination of two organisms produced the highest yield of bioethanol at upland in both seasons.

Table 9: Effect of heating time on the reducing sugar produced by switchgrass treated with different levels of nitrogen (upland, 2008/2009)

Conc. (%)		Heating time (minutes)		
	Samples	20	25	30
3%	N_0	2.28 ± 0.37	2.51 ± 0.30	1.48^{a} ± 0.15
	N_{25}	2.44 ± 0.11	3.83 ± 1.02	3.54^{a} ± 0.79
	N_{50}	3.67 ± 1.02	3.60 ± 0.60	3.42^{a} ± 0.58
	N_{75}	3.82 ± 0.78	3.55 ± 0.76	3.28^{a} ± 0.49
		Heating time (minutes)		
		20	25	30
4%	N_0	2.20 ± 0.76	1.34^{b} ± 0.29	2.02^{b} ± 0.27
	N_{25}	2.21 ± 0.19	2.80^{ab} ± 0.19	3.04^{b} ± 0.47
	N_{50}	4.13 ± 0.69	2.71^{ab} ± 0.45	2.94^{b} ± 0.45
	N_{75}	4.15 ± 0.76	3.73^{a} ± 1.05	4.62^{a} ± 0.63
		Heating time (minutes)		
		20	25	20
5%	N_0	1.91 ± 0.24	1.61^{b} ± 0.03	1.91 ± 0.45
	N_{25}	2.22 ± 1.28	2.15^{ab} ± 0.43	2.51 ± 0.22
	N_{50}	2.46 ± 0.18	2.39^{ab} ± 0.13	2.54 ± 0.44
	N_{75}	2.30 ± 0.10	2.72^{a} ± 0.22	2.94 ± 0.36

Values are mean ± standard error Means in a column with different superscripts are significantly different (P<0.05)

Table 10: Effect of heating time on the reducing sugar produced by switchgrass treated with different levels of nitrogen (upland, 2009/2010)

Conc. (%)		Heating time (minutes)		
	Samples	20	25	30
3%	N_0	2.33^{b} ± 0.29	2.16^{b} ± 0.19	1.53^{b} ± 0.25
	N_{25}	2.67^{b} ± 0.17	4.00^{a} ± 0.85	2.60^{ab} ± 0.15
	N_{50}	3.13^{b} ± 0.33	4.62^{a} ± 0.63	3.73^{ab} ± 1.05
	N_{75}	4.83^{a} ± 0.34	5.47^{a} ± 0.11	4.00^{a} ± 0.82
		Heating time (minutes)		
		20	25	30
4%	N_0	1.88^{b} ± 0.25	1.57^{b} ± 0.13	2.27^{b} ± 0.33
	N_{25}	3.09^{ab} ± 0.30	2.56^{ab} ± 0.27	3.91^{ab} ± 0.54
	N_{50}	4.55^{a} ± 0.55	3.45^{a} ± 0.85	5.43^{a} ± 0.12
	N_{75}	3.81^{a} ± 0.85	3.28^{ab} ± 0.49	4.95^{a} ± 0.97
		Heating time (minutes)		
		20	25	20
5%	N_0	1.30^{b} ± 0.18	1.67^{b} ± 0.06	1.60^{b} ± 0.33
	N_{25}	2.30^{a} ± 0.10	2.90^{ab} ± 0.40	2.51^{b} ± 0.22
	N_{50}	2.46^{a} ± 0.18	3.03^{ab} ± 0.23	2.56^{b} ± 0.17
	N_{75}	2.50^{a} ± 0.12	3.85^{a} ± 0.98	4.80^{a} ± 1.07

Values are mean ± standard error Means in a column with different superscripts are significantly different (P<0.05)

Table 11: Concentration of Bioethanol produced from Switchgrass treated with different levels of nitrogen using fermentative organisms at lowland in 2008/2009 and 2009/2010 seasons

Samples	Fermentative Organism	Concentration of Bioethanol Yield (%) 2008/2009	2009/2010
SG NO	*Saccharomyces cerevisiae*	0.20 ± 0.03	0.22^{b} ± 0.06
	Zymononas mobilis	0.18 ± 0.02	0.19^{b} ± 0.02
	S. cerevisiae + Z. mobilis	0.23 ± 008	0.24^{a} ± 0.4
SG N25	*Saccharomyces cerevisiae*	0.23^{a} ± 0.18	0.20^{b} ± 0.05
	Zymononas mobilis	0.19^{b} ± 0.02	0.20^{b} ± 0.03
	S. cerevisiae + Z. mobilis	0.25^{a} ± 0.01	0.25^{a} ± 0.04
SG N50	*Saccharomyces cerevisiae*	0.23^{b} ± 0.18	0.25^{a} ± 0.01
	Zymononas mobilis	0.20^{b} ± 0.05	0.22^{b} ± 0.06
	S. cerevisiae + Z. mobilis	0.28^{a} ± 0.13	0.29^{a} ± 0.02
SG N75	*Saccharomyces cerevisiae*	0.22^{b} ± 0.06	0.24^{b} ± 0.04
	Zymononas mobilis	0.20^{b} ± 0.03	0.20^{b} ± 0.05
	S. cerevisiae + Z. mobilis	0.25^{a} ± 0.04	0.28^{a} ± 0.13

Values are mean standards error Mean in a column with different superscripts are significantly different (P<0.05)

Table 12: Concentration of Bioethanol produced from Switchgrass treated with different levels of nitrogen using fermentative organism at upland in 2008/2009 and 2009/2010 seasons

Samples	Fermentative Organism	Concentration of Bioethanol Yield (%) 2008/2009	2009/ 2010
SG N_O	*Saccharomyces cerevisiae*	0.28^{a} ± 0.13	0.30^{a} ± 0.01
	Zymononas mobilis	0.22^{b} ± 0.06	0.24^{b} ± 0.04
	S. cerevisiae + Z. mobilis	0.30^{a} ± 003	0.30^{a} ± 0.03
SG N25	*Saccharomyces cerevisiae*	0.29^{a} ± 0.02	0.30^{a} ± 0.02
	Zymononas mobilis	0.23^{b} ± 0.08	0.25^{b} ± 0.01
	S. cerevisiae + Z. mobilis	0.31^{a} ± 0.02	0.32^{a} ± 0.02
SG N50	*Saccharomyces cerevisiae*	0.31^{a} ± 0.20	0.29^{b} ± 0.02
	Zymononas mobilis	0.26^{b} ± 0.04	0.25^{b} ± 0.02
	S. cerevisiae + Z. mobilis	0.35^{a} ± 0.19	0.36^{a} ± 0.02
SG N75	*Saccharomyces cerevisiae*	0.31^{a} ± 0.02	0.30 ± 0.01
	Zymononas mobilis	0.27^{b} ± 0.01	0.28 ± 0.01
	S. cerevisiae + Z. mobilis	0.32^{a} ± 0.02	0.32 ± 0.01

Values are mean standards error Mean in a column with different superscripts are significantly different (P<0.05)

DISCUSSION

Zymomonas mobilis and saccharomyses cerevisiae were isolated from rotten orange and palm wine respectively. These organism been sugar –loving are found to thrive in such substrates as they receive suitable juices that they utilized as their growth factor.

High yields obtained from hydrolysates treated with 4 and 5% HCl at 30, 40 and 50^0C for 30, 20 and 25 minutes respectively indicate that acid concentration of 4-5% and temperature range of 20-50^0C are suitable for hydrolysis of the switchgrass hydrolysates. The low level of reducing sugar was obtained from all the hydrolysates of the switchgrass could be due to complex structural and chemical mechanisms for resisting assault on its structural sugars from the microbial and animal kingdoms. Natural factors believed to contribute to the recalcitrance of lignocellulosic feedstock to chemicals or enzymes include (i) the epidermal tissue of the plant body, particularly the cuticle and cuticular waxes; (ii) the arrangement and density of the vascular bundles; (iii) the relative amount of sclerenchmymatous (thick wall) tissue, (iv) the degree of liginification; (v) the structural heterogeneity and complexity of cell-wall constituents such as microfibrils and matrix polymers; (vi) the challenges for enzymes acting on an insoluble substrate; and (vii) the inhibitors to subsequent fermentation that exist naturally in cell walls or are generated during conversion process.

At the molecular level, the crystalline cellulose core of cell-wall microfibrils is highly resistant to chemical and biological hydrolysis because of its structure, in which chains of cellodevtrins are precisely arranged. The chain conformation of the glucose residues in cellulose forces the hydroxyl groups into radial (equatorial) orientation and the aliphatic hydrogen atoms into axial positions. As a result, there is strong interaction with hydrogen bonding between adjacent chains in a cellulose sheet and weaker hydrophobic face of cellulose sheets makes crystalline cellulose resistant to acid hydrolysis because it contributes to the formation of a dense layer of water near the hydrated cellulose surface. The strong interchain hydrogen-bonding network makes crystalline cellulose resistant to enzymatic hydrolysis, whereas hemicelluloses and amorphous cellulose are readily digestible. Higher-order structures in plants also contribute to biomass recalcitrance. For example, access to the crystalline cellulose core of microfibrils is restricted by a coating of amorphous cellulose and hemicelluloses. Also at microscopic and macroscopic scale, the complex heterogeneous nature of biomass creates mass-transport limitations for delivery of chemical or biochemical catalysts.

The relatively high bioethanol yield obtained when the combination of *Saccharomyces cerevisiae* and *Zymomonas mobilis* was used to ferment the hydrolysates was not surprising as it was reported that *Z. mobilis* tend to produce high ethanol yield when used synergistically with other organism than when used alone. The general low bioethernol production may be attributed to the fact that as the organism fermented the broth, there was the production and accumulation of intermediate co-produces that have a detrimental effect on the fermentative organism and tend to inhibit or slow down their metabolic activity during the fermentation period. Coupled with this, *Zymomonas mobilis* was reported to ferment only glucose, fructose and sucrose but not pentose sugars, this render them less suitable for the fermentation of cellulosic materials. The findings in this experiment are in conformity with the reported study on ethanol volume as low as 0.06 ml/g from apple and grass juices. However, the findings were not in agreement with the reported ethanol value of 24.53 g/l from dilute ammonia treated sorghum.

CONCLUSION

The study revealed that switchgrass could be hydrolyzed using dilute hydrochloric acid with resultant high yields of reducing sugar. Also it revealed that *S.cereviae* and *Z. mobilis* isolates could serve as fermentative organisms for the production of ethanol. However, there is the need to optimize their usage in the bioethanol production so as to free more of their reducing sugar content for fermentation. Further work will focus on the use of enzymatic hydrolysis to release hydrolysates to optimize the bioethanol production.

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