FUEL ETHANOL PRODUCTION FROM SWEET SORGHUM BAGASSE USING MICROWAVE IRRADIATION

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ABSTRACT: Sweet sorghum is a hardy crop that can be grown on marginal land and can provide both food and energy in an integrated food and energy system. Lignocellulose rich sweet sorghum bagasse (solid left over after starch and juice extraction) can be converted to bioethanol using a variety of technologies. The largest barrier to commercial production of fuel ethanol from lignocellulosic material remains the high processing costs associated with enzymatic hydrolysis and the use of acids and bases in the pretreatment step. In this paper, sweet sorghum bagasse was pretreated and hydrolysed in a single step using microwave irradiation. A conversion efficiency of 96% (0.82 g sugar/g bagasse) was obtained in a 5wt% sulphuric acid solution under 20 minutes of 180W microwave irradiation. An ethanol conversion efficiency of 98% (0.5 g ethanol/g sugar) was obtained after 24 hours of fermentation using a mixed culture of organisms to convert both hexose and pentose sugar in the broth. These results show the potential of producing 13 000 L/ha which is high enough to make the process economically attractive.
Keywords: sorghum *bicolor L. Moench*, bagasse, lignocellulose, pretreatment

1 INTRODUCTION

Utilization of fossil fuels to generate energy currently contributes to 84% of the global energy demand. As a result of projections that indicated the depletion time of fossil fuel resources to be roughly 110 years, worldwide research is being conducted concerning the development of alternative, sustainable energy sources [1].

Biofuels have drawn remarkable attention from many researchers from all the corners of the earth. There has been extensive research to develop or improve methods for producing biofuels as an alternative to petroleum-based transportation fuel [2]. Bioethanol accounts approximately 94% of global biofuels production [3]. The major bioethanol producing countries, the USA and Brazil, produce bioethanol from maize and sugarcane, while China and India use mainly sweet sorghum as a feedstock for producing bioethanol [4].

According to the South African biofuel industrial strategy published in 2007 [5], sugar beet and sugarcane are the main feedstocks that can be used for producing bioethanol in South Africa. Maize was excluded due to the rising concerns of food security, since maize is a staple food in South Africa.

1.1Bioethanol as biofuel

Bioethanol can be produced from many different feedstocks [2]. Bioethanol is an oxygenated fuel that contains 35% oxygen which reduces particulate and NOx emissions from combustion [6]. Due to the higher octane number, higher compression ratio and a shorter burn time, as compared to normal petrol, bioethanol has a theoretical efficiency advantage in an internal combustion engine. There are some disadvantages concerning the bioethanol properties, including a lower energy density than petrol, low flame luminosity, lower vapor pressure, miscibility with water, increase in exhaust emissions of acetaldehyde and vapor pressure increase when blended with petrol [7].

The ideal feedstock for bioethanol production should ensure food security, as well as sustainability in terms of water consumption, since South Africa is the 30th driest country in the world. Bioethanol feedstocks are classified into three categories [8]: sucrose-containing feedstock (*e.g.* sugar beet, sweet sorghum and sugar cane), starchy materials (*e.g.* wheat, maize and barley), and lignocellulosic biomass (*e.g.* wood, straw, bagasse and grasses). In the recent years, lignocellulosic feedstocks have been primarily considered for producing bioethanol, because lignocellulose does not compete with food crops [9].

1.2 Sweet sorghum bagasse as bioethanol feedstock

Sweet sorghum is a C4 crop in the grass family of genus Sorghum *bicolor L. Moench*. Sweet sorghum has been reported to be a promising alternative crop for fuel bioethanol, because it has a high photosynthetic activity and can produce non-edible biomass, food, as well as fermentable sugar syrup. Sweet sorghum is also known to be resistant to drought and is of particular interest as a potential crop for large volume bioethanol production [10].

There exists two possible routes to produce bioethanol from sweet sorghum. First of all, the juice obtained from the feedstock is used for bioethanol production, since it consists of sugars. Secondly, the bagasse after juice extraction is pretreated to obtain fermentable sugar, which can also be fermented to produce bioethanol [11].

The initial estimated world surface area for sorghum cultivation in 1972 was 40 Mha, with the largest areas being in India (16 Mha) and Africa (10.3 Mha), but by the 1980s, sorghum production had spread across the world. Japan and Europe use sweet sorghum for stock feed, while India and Africa use sorghum for human consumption and beer production.

The sweet sorghum plant consists of a kernel (7%), stalks (75%), which mostly contains sucrose and cellulose, leaves (10-15%), and the roots (10%). The grain kernel contains approximately 60-65% starch [12].

Bagasse is considered to be a good feedstock for bioethanol production, because it is abundant and cheaper than conventional agricultural feedstock [13].

The fermentation of sweet sorghum stem juice, which contained most of the soluble carbohydrates, using immobilized yeast cells, was studied [14]. The residual bagasse was hydrolyzed with acid or enzyme to soluble oligosaccharides and then fermented to bioethanol. This approach achieved 68.6% of the theoretical yield based on total polysaccharides and exceeded that based on oligosaccharides of sorghum stem by 53.7%.

Another study [15] demonstrated the production of bioethanol from sweet sorghum bagasse. It was found that the bagasse from sweet sorghum bagasse could be

efficiently converted to fermentable sugars, *i.e* glucose and pentose sugars, through pretreatment and hydrolysis. Glucose is the easiest sugar to convert to bioethanol using yeast (*S. cerevisiae*), while the pentose sugars have relied on a bacterial strain (*Z. mobilis*).

2 MATERIALS AND METHODS

2.1 Materials

Sweet sorghum bagasse (Hunni green) was obtained from sweet sorghum harvested at six months by the Agricol Research Company in Potchefstroom, North West Province, South Africa. The bagasse was obtained after the juice had been pressed from the plants. The bagasse was dried to 10% moisture content and thereafter, milled and screened to a particle size of ±1.5mm. The milled bagasse was packed in air-tight bags and then stored at room temperature for further use.

2.2 Microorganisms and media

Z. mobilis ATCC 31821 was obtained from the American Type Culture Collection (ATCC) and maintained as a freeze dried pellet at -80°C till further use. The freeze dried organism was rehydrated with sterile water and inoculated on sucrose broth medium and was grown for two days at 30°C, 120 rpm. Stock cultures were either made up in 15% glycerol for long term storage at 4°C; as it was subcultured on nutrient agar plates for 72 hrs at 32°C, from which an inoculum was prepared.

Commercial S. cerevisiae was used for fermentation. Firstly the dried cells of yeast were revived from the inactive state by using a broth containing yeast extract, peptone, $(NH_4)_2SO_4$, K_2HPO_4 and $MgSO_4.7H_2O$. Prior to fermentation, the broth was incubated at 32°C for 24 hrs. This was done to reduce lag time of the yeast.

2.3 Experimental methods

2.3.1 Microwave assisted pretreatment methods

The first set of pretreatment experiments was carried out at a power level of 300 W. This power level was chosen since it allowed the required length of pretreatment time without any volumetric losses of the liquid, which was also required for further use. The experiments were done in triplicate.

For the microwave pretreatment in an acid environment, bagasse samples of 5 g were weighed into 100 mL of sulphuric acid solution, either 1, 3, 5 or 7 wt%, in a 500 mL Duran bottle. Bottles were placed in a microwave maintained at different powers for 20 min. Three microwave treatment wattages were selected: 100, 180, or 300 W. Samples (2mL) were taken out of the mixture at time intervals of 5 min for analysis of the reducing sugars. After pretreatment, the remaining samples were neutralised with sodium hydroxide and filtered. The filtrate were used for fermentation and available sugars were quantified using High Performance Liquid Chromatography (HPLC). The insoluble residues were dried at 50°C for 24 hrs for Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR).

For the microwave pretreatment in a base environment, bagasse samples of 5 g were mixed with 100 mL, of either 1, 3, 5 or 7 wt% sodium hydroxide solutions, in Duran bottles. The bottle was placed in a microwave oven at varying wattage (100, 180 and 300 W) for a time period of 20 minutes. Samples (2 mL) were taken from the mixture at time intervals of 5 min for analysis of the reducing sugars. Sugar monomers including glucose, arabinose, galactose, mannose and xylose present in the filtrate were quantified using HPLC. After treatment the liquids to be used in fermentation were collected by filtration, and neutralized with sulphuric acid. The residues were washed with deionised water until a pH of 6 was reached for SEM and FTIR analyses.

2.3.2 Fermentation

The microwave pretreated sweet sorghum bagasse samples with optimal sugars were fermented. Fermentation was carried out in 250 mL Duran bottles. The amount dispersed into the bottles varied, due to liquid loss during microwave pretreatment. The pH was kept constant at 4.8. *Z. mobilis* and *S. cerevisiae*; 1 and 3% v/v, 3 and 5% v/v, and 5 and 10% v/v of batch, respectively were added and fermentation was carried out in a shaker incubator at 32°C for 24 hrs at a shaking speed of 120 rpm. Samples were collected and analysed for cell growth, sugar and ethanol content at set time intervals.

3 RESULTS AND DISCUSSION

3.1 Compositional analysis of sweet sorghum bagasse

Compositional analysis of untreated raw sweet sorghum bagasse (SSB) was done by the Agricultural Research Council of South Africa. Table I shows the initial composition of sweet sorghum bagasse harvested at 6 months in the Potchefstroom area which contained cellulose (36.6%), hemicellulose (22.96%), and lignin (5.90%).

Table I: Composition of sweet sorghum bagasse

Element	Composition (%)
Cellulose	36.60
Hemicellulose	22.96
ADF	42.50
NDF	65.46
ADL (Lignin)	5.90
Moisture	10.85
Ash	3.07

Table I it can be seen that a maximum theoretical total sugar yield of 59.56 g/100 g dry sweet sorghum bagasse can be obtained from the cellulose and hemicellulose contained in the bagasse used in this study.

The initial sugar yields from the juice contained in the sweet sorghum bagasse before pretreatment were quantified, as shown in Table II.

Table II: Initial sugar composition (g/g) from juice remaining in sweet sorghum bagasse

Sugar	Yield (g/g)
Sucrose	0.15
Glucose	0.06
Fructose	0.04

Sucrose, glucose and fructose were present in different concentrations in the juice that was still

remaining in the bagasse after extraction. The sucrose yield was very high in the initial biomass, but during pretreatment its concentration decreased as it was decomposed to glucose and fructose. The total theoretical sugar yield that can be obtained from the bagasse and the residual sugar monomer in the juice still present in the bagasse can be calculated to be 84.56 g/100 g bagasse solids.

3.2 Pretreatment results

Total sugar yields obtained through acid and alkali pretreatment of sweet sorghum bagasse (SSB) with microwave were compared at different irradiation power settings. The highest total sugar yield was 0.82 g/g with acid pretreatment at 180 W. The efficiency of microwave acid pretreatment was found to be 96%. The high total sugar yields under acid conditions prove that acid is an effective catalyst for microwave pretreatment of SSB.

The covalent bonds, hydrogen bonds and Van der Waals forces in the biomass structure can easily release sugars without requiring higher temperatures for microwave acid pretreatment. This explains the optimal yield at 180 W.

The yield realised with alkali pretreatment was only 32%. The alkali catalyst, on the other hand, acts by removing lignin-carbohydrate bonds. These bonds are much stronger and require additional energy to be broken. This explains the highest sugar yield for alkali microwave pretreatment at 300 W.

3.3 Fermentation

Fermentation was conducted only for optimal sugars obtained from microwave pretreatment with different microwave powers. The concentration of *S. cerevisiae* and *Z. mobilis* was varied to observe the acceleration of ethanol production within 24 hours of fermentation. The study revealed the highest ethanol yield of 0.5 g/g sweet sorghum bagasse that was pretreated using microwave-acid pretreatment, with a concentration of 10% v/v *S. cerevisiae* and 5% v/v *Z. mobilis* during fermentation.

An ethanol yield of approximately 0.18 g/g of substrate was obtained from sweet sorghum bagasse pretreated using microwave-alkali pretreatment, with concentration of 10% v/v *S. cerevisiae* and 5% v/v *Z. mobilis* during fermentation.

Zymomonas mobilis showed a potential in fermenting xylose sugars that were mainly obtained during microwave-alkali pretreatment. The results also show that *S. cerevisiae* and *Z. mobilis* are a good combination for producing ethanol from sweet sorghum bagasse. This also proves that sweet sorghum bagasse is a very good feedstock for producing bioethanol in South Africa that could have an impact in integrated food and energy systems.

4 CONCLUSIONS

The optimum reducing sugar yield for microwave pretreatment using dilute sulphuric acid was found to be 0.82 g/g of biomass at 180 W irradiation and a pretreatment time of 15 minutes. With alkaline microwave (calcium hydroxide) pretreatment an overall sugar yield of 0.27 g/g of biomass was obtained at 300 W irradiation and a pretreatment time of 10 minutes.

SEM analysis showed structural changes and biomass disruption after microwave pretreatment for both acid and alkali pretreatment, while the FTIR spectra showed the stretching of hydrogen bonds of pretreated sweet sorghum bagasse and also indicated structural changes after microwave treatment. The use of calcium hydroxide pretreatment resulted in the production of higher xylose yields than sulphuric acid pretreatment.

An ethanol yield of 0.5 and 0.32 g/g was achieved during fermentation of sweet sorghum bagasse using 5:10 and 1:3% v/v of *Z. mobilis* to *S. cerevisiae*, respectively for acid pretreated bagasse. An ethanol yield of 0.13 and 0.18 g/g was achieved during fermentation of sweet sorghum bagasse using 5:10 and 1:3% v/v of *Z. mobilis* to *S. cerevisiae*, respectively for alkali pretreated bagasse.

The results obtained from this study gave an indication that sweet sorghum bagasse can be used for bioethanol production through microwave-based pretreatment and fermentation with mixed cultures.

The next step would be to verify the effectiveness of microwave pretreatment using a laboratory scale continuous microwave system. This will be of much help in identifying the appropriate parameters that will be effective in the microwave-based pretreatment process.

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