FERMENTATION OF LIGNOCELLULOSIC BIOMASS USING ULTRASONIC PRETREATMENT

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ABSTRACT: The potential fermentation of the sugars derived from water hyacinth, sweet sorghum bagasse and amaranth using indirect ultrasonic pretreatment, was investigated. The optimum sugar and ethanol yield for the ultrasonic bath pretreatment with 7% NaOH was found to be 289 g ethanol/kg biomass, 231 g ethanol/kg biomass, 189 g ethanol/kg biomass, for the sweet sorghum bagasse, amaranth and water hyacinth. The high energy input required of 486 kJ/g biomass implies that ethanol production from these feedstock without further optimisation is not economically feasible, but considering the high energy potential of sweet sorghum bagasse (1,127,100 MJ/ha), further optimisation should be considered.
Keywords: bioethanol, energy, lignocellulosic sources, pretreatment, sorghum *bicolor L. Moench*

1 INTRODUCTION

Fossil fuels account for approximately 86% of the primary global energy demand, with renewable sources (solar, wind, geothermal, biomass and hydroelectricity) and nuclear power making up the remaining 14% [1]. The world's energy supply is depleting rapidly whilst the global population is growing, making the need for alternative fuel increasingly urgent [2], [3], [4].

Another major concern associated with fossil fuels is pollution. In the EU the transportation sector alone produces 27.4% of all the greenhouse gas emissions [5]. Considering that biofuels emit only a fifth of the CO_2 gasses released by fossil-based engine fuels the conversion from biomass to liquid fuel seems like an attractive alternative to petrochemical production [5], [6].

Schill [7] states that by the end of 2013 biomass accounted for approximately 10% of the global energy supply, and that 2% of the fuel used in the transportation sector was derived from biomass in the form of bioethanol and biodiesel. The use of bioethanol and biodiesel is expected to increase within the transportation sector, assuming the rest of the world will follow the USA and Brazil's example with regards to the commercialization of bio-fuel production [3], [4], [7].

Converting biomass to bioethanol as an alternative fuel source is a global controversial issue. It is asked whether the use of edible feedstock to produce biofuel is ethical in a world where 1 out of every 8 people suffer from chronic malnutrition, specifically in developing countries [8].

Advanced technologies within the bio-energy sector have been developed over the last few years which utilize non-edible lignocellulosic biomass as feed to the bioethanol production process [2], [7], [9], but further advances are required to fully commercialize the production of bioethanol from non-edible lignocellulosic biomass.

Second generation bioethanol is produced from non-edible lignocellulosic biomass [2], [10]. The main steps required in the production process of converting lignocellulosic biomass into bioethanol include pretreatment of lignocellulosic biomass, hydrolysis of cellulose and hemicellulose, sugar fermentation (using yeast like *Saccharomyces cerevisiae* in combination with bacteria like *Zymomonas mobilis*, separation of lignin residue and recovery and purification of ethanol to meet fuel standards [10], [11].

The major differences between first and second generation bioethanol production entails the pretreatment step which is not required in the former and the fermentation process that needs to be altered in order to ferment unusual sugars present in lignocellulosic biomass [10], [12]. Pretreatment methods break the lignin structure apart and allow access to the cellulose and hemicellulose, which enables hydrolysis to take place and convert cellulose into predominantly glucose and hemicellulose into sugars like glucose, galactose and xylose.

In order to commercialize the utilization of lignocellulosic biomass as feedstock to bioethanol production, the pretreatment step of the process needs to be optimized [14]. One of the available pretreatment methods is ultrasonication. During ultrasonication the biomass structure is altered by inducing cavitations and size reduction due to the collision of particles. In a previous pretreatment study a sugar yield of 132.96 mg sugar/g dry matter was reported using ultrasound (100% power, 20 minutes, 10 w/v% biomass loading, direct probe) [15].

Residence times in excess of 20 minutes (high power) led to a decrease in sugar yields, while longer times were required for optimum sugar yield in the case of a low power setting. This trend is consistent with results obtained using other biomass such as palm oil and corncob [15]. The combined chemical and physical treatment systems allow the hemicellulose to dissolve and provide sufficient alteration of the lignin structure to improve access for the hydrolytic enzymes. The lignin is removed using either enzymatic, acid or alkali hydrolysis. The resulting 5- and 6-ring sugars are then fermented to ethanol [16].

1.1 Sweet Sorghum Bagasse

Sweet sorghum, *Sorghum bicolor (L.)*, is an African plant that produces grain for human consumption [17]. It forms part of the same species as grain sorghum and forage sorghum. There are high levels of sucrose present in the stem and that the sugar yields are similar to that of sugar cane. It is important to note that this plant has a high genetic variety which could result in large variations concerning sugar yields [17]. Sweet sorghum bagasse is the biomass which remains after juice extraction from the sweet sorghum plant's stem [12].

The problem associated with using sweet sorghum bagasse as the only feedstock is the continuous availability of the plant material. Additional crops will have to be planted in order to reach the new demand created by bioethanol production when refineries come into operation [18].

1.2 Water Hyacinth

Water hyacinth, *Eichhornia crassipes*, is a floating, freshwater aquatic plant typically found in lakes, rivers and swamps. The plant is native to northern South America regions [16]. It is, however, an invasive plant, specifically in South Africa, and causes various problems due to the fact that it depletes nutrients in water bodies and reduces oxygen levels.

Utilizing water hyacinth as feedstock for bioethanol production will assist in eradicating the plant in South Africa. Three case studies analysed the chemical analysis of water hyacinth and the cellulose was found to be between 18.4-28.9wt%, the hemicellulose between 30.8-49.2wt%, lignin between 3.6-10.0wt% and crude protein between 9.0-21.0wt% [19], [20], [21].

The greatest stumbling block in using water hyacinth as feedstock to bioethanol production is the high water content of the plant. Water hyacinths are classified as monocots (monocotyledons), more commonly referred to as flowering plants. They fall in the same class as true grains like rice, wheat and maize [22].

1.3 Amaranth

The amaranth, *Amaranthus L.*, is a seed plant which is divided into more than 60 different species. The African amaranth (*Amaranthus muricatus*) is recognised as a viable food source by the South African Department of Agriculture and Fisheries [23]. Although amaranth has been viewed as a weed, multiple people worldwide value amaranths as leaf vegetables [24].

This gives rise to the controversial issue of converting food crops to fuel resources, and establishes a major issue with regards to the utilization of amaranth as feedstock to bioethanol production as it is classified as a starchy feedstock [24]. The plant is very versatile and is able to germinate in soil temperatures of 18-25°C, the main amaranth producing regions is South Africa are listed as Limpopo, North West, Mpumalanga and KwaZulu-Natal [23]. It has also been reported that the African amaranth grain contains 12-17wt% protein and has high levels of amino acids, the stems and leaves yield an even higher (15-24 dry wt%) amount of protein.

2 MATERIALS AND METHODS

2.1 Materials

The feedstock chosen for the ultrasonication experiments included dried water hyacinth, dried sweet sorghum bagasse and dried amaranth.

2.2 Chemicals

Sodium hydroxide was used during the alkaline pretreatment. The pH of the various HPLC and FTIR samples were adjusted using H_2SO_4 and NaOH. The enzyme, Novozyme NS-22192, and the surfactant, Tween 80, were used during the enzymatic hydrolysis. Fermentation was performed using the yeast, *Saccharomyces cerevisiae*, and the bacterium, *Zymomonas mobilis*.

2.3 Experimental procedure

Ultrasonic pretreatment of water hyacinth biomass was done using indirect sonication in an ELMA® ultrasonic bath. The experimental procedure for all three feedstock was identical.

200 ml of NaOH (3, 5, 7 wt%) was placed in 250 ml Scott Duran® bottles. Dried feedstock (10g, 50 g/L) was added to the alkaline solution and shaken to ensure the biomass was thoroughly distributed in the solution. The bottles were then placed inside the water-filled ELMA® ultrasonic bath and the temperature controlled at 20°C. A frequency of 35 kHz was used, while the treatment times were set to 1, 2 and 3 hours. The ultrasonic bath allows three modes of operation namely sweep, degas and normal, of which only normal was tested. Power inputs of 150 W, 300 W and 450 W were used.

The pretreated sample was hydrolysed enzymatically at a pH of between 5.0 and 5.1. Novozyme NS-22192 (0.5 g/L) and Tween 80 (1.25 g/L) were added to the broth for hydrolysis, with a 20 vol% buffer solution. The citrate buffer solution was prepared using 20.1 g/L of citric acid and 8.0 g/L of sodium hydroxide. The pH of the buffer was adjusted to 5.0 with dilute hydrochloric acid.

The broth was placed in an oven for 48 hours at a temperature of 50°C. The hydrolysed broth was fermented using *Saccharomyces cerevisiae* for fermentation of the C-6 sugars. *Zymomonas mobilis* was added to assist with fermentation of the pentose sugars such as xylose and arabinose. The yeast and bacterium were added in a 10:5 v/v% ratio of yeast to bacterium. Fermentation was carried out for 24 hours in an oven at 32°C.

2.4 Analytical method

Following the alkaline pretreatment step, a sample was taken. A liquid sample was filtered using a 0.45 micron filter and the pH adjusted to between 6.95 and 7.05 to be analysed using HPLC. A solid sample was obtained using a Büchner filter and analysed by means of FTIR.

Liquid samples were also prepared in this fashion for HPLC analysis following the enzymatic hydrolysis and fermentation steps. An Agilent 1200 high-performance liquid chromatography (HPLC) system was used for all analyses. FTIR analysis was done on the untreated biomass, as well as the alkali pretreated water hyacinth using indirect sonication. A Shimadzu IRAffinity-1 Fourier Transform Infrared Spectrophotometer (FTIR) was used for these analyses.

3 RESULTS AND DISCUSSION

3.1 Theoretical yields

From the compositional analysis of the various feedstock the cellulose and hemicellulose content could be calculated using the following equations [26]:

$$\text{Cellulose} = \text{ADF} - \text{ADL} \qquad (3.1)$$

$$\text{Hemicellulose} = (\text{NDF} - \text{ADF}) \qquad (3.2)$$

The cellulose and hemicellulose content was used to determine what the theoretical maximum ethanol yield is, assuming cellulose to consist mainly of the 6-ring sugar, glucose, and the hemicellulose to consist mainly of the 5-ring sugar, xylose. The theoretical sugar content and ethanol yield are shown in Figure 1.

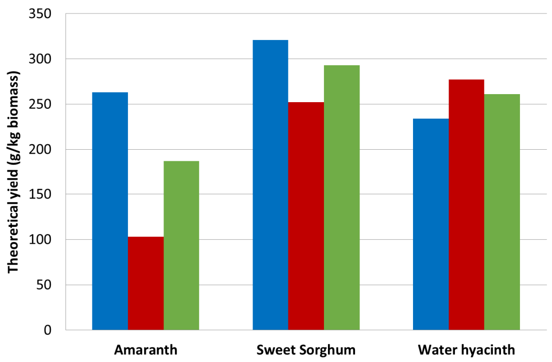

Figure 1: Theoretical sugar content of the various feedstock, as well as the theoretical ethanol yield based on these sugars (■ glucose; ■ xylose; ■ ethanol).

The sweet sorghum bagasse has the highest sugar content of 573 g/kg, followed by the water hyacinth (511 g/kg) and the amaranth (366 g/kg). The theoretical ethanol yields are calculated to be 293 g/kg, 261 g/kg and 187 g/kg for the sweet sorghum bagasse, water hyacinth and amaranth, respectively.

3.2 Energy input

The first parameter of importance during sonication is the energy input, as shown in Figure 3, 4 and 5. In the case of the amaranth (79%) and the water hyacinth (72%), the highest ethanol yield was obtained at an energy input of 300 W, while the highest ethanol yield (99%) for the sweet sorghum bagasse was found at an energy input of 450 W.

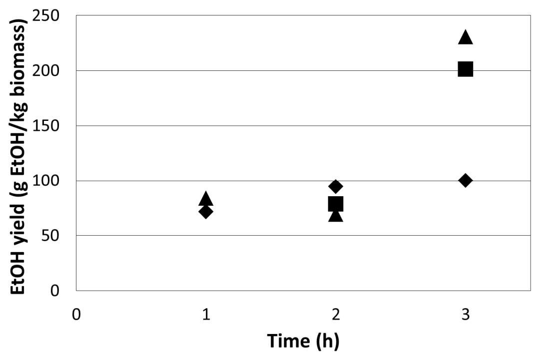

Figure 2: The effect of energy input on sugar yield using indirect sonication at 300 W on amaranth (◆ 3%; ● 5%; ▲ 7%)

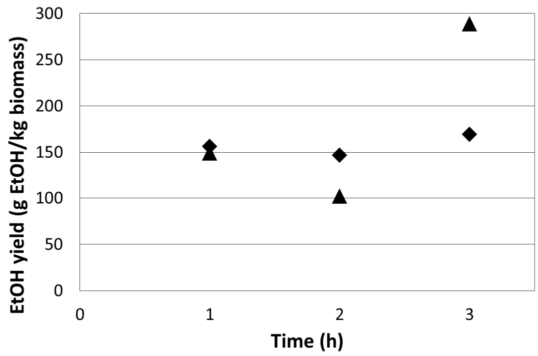

Figure 3: The effect of energy input on sugar yield using indirect sonication at 450 W on sweet sorghum bagasse (◆ 3%; ▲ 7%)

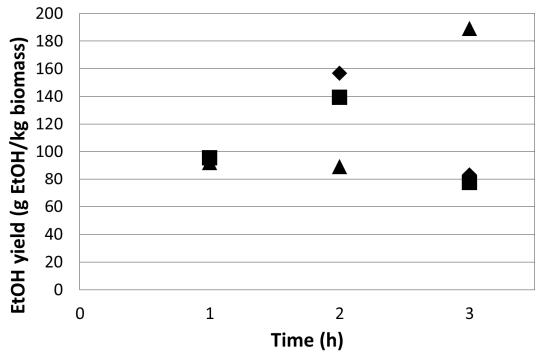

Figure 4: The effect of energy input on sugar yield using indirect sonication at 300 W on amaranth (◆ 3%; ● 5%; ▲ 7%)

A longer pretreatment time resulted in higher sugar and ethanol yields. The energy input and output for the various feedstock is shown in Table I.

Table I: Energy input and output using ultrasonic pretreatment

Feedstock	Energy input [kJ/g]	Energy output [kJ/g]
Amaranth	324	5.67
Sweet sorghum	486	8.67
Water hyacinth	324	6.93

The energy efficiency of fermentation using ultrasonic pretreatment ranges between 1.75% and 2.14%. The energy potential of these crops is shown in Table II.

Table II: Energy potential using ultrasonic pretreatment

Feedstock	Biomass [ton/ha]	Energy yield [MJ/ha]
Amaranth	90	510,300
Sweet sorghum	130	1,127,100
Water hyacinth	30	207,900

Both the amaranth and sweet sorghum bagasse show huge potential as energy crops, especially if these values are compared to maize (284,000 MJ/ha) and fodder beet (267,214 MJ/ha).

4 CONCLUSIONS

The highest ethanol yield for the indirect sonication pretreatment was found with a NaOH strength of 7% for all the feedstock. The maximum ethanol yield of 289 g/kg was obtained with the sweet sorghum bagasse, which corresponds to a 99% theoretical conversion of the available sugars.

The energy input of 486 kJ/g for the sweet sorghum bagasse, is however very high and further optimisation is required to increase the efficiency beyond the current 2%. Sweet sorghum has the highest energy potential as a crop, with a maximum energy yield of 1,127,100 MJ/ha, making it more attractive than maize and other energy crops.

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