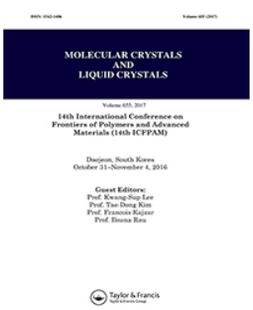

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Production of second-generation ethanol from saccharine sorghum bagasse

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ABSTRACT
The sweet sorghum is considered an alternative to complement the sugar cane crop. This study aimed to evaluate the production of 2G ethanol using enzymatic hydrolysis followed by simultaneous fermentation (SSF) compared with the simple fermentation (SHF). The enzymatic hydrolysis used the enzyme complex Accellerase 1500 ® cellulose and for the fermentation was used the yeast *Saccharomyces cerevisiae*. The obtained yield of ethanol was 96%, where a simple fermentation resulted in lower ethanol content as compared to simultaneous fermentation, 26.8 g/L and 46.6 g/L respectively. Therefore, the sweet sorghum is very promising for 2G ethanol using simultaneous fermentation.

KEYWORDS
Agriwastes; biomass; enzymatic hydrolysis; biofuel; saccharine sorghum; second-generation ethanol

1. Introduction

The oil crisis, along with a growing concern with the environment and a higher demand of biofuel, raised the search for alternative energy sources not only in Brazil, but also around the world. Currently many researches focus on the development of new renewable elements to substitute oil-derived energy, which highlights biomass, as it is a widely available, renewable, and low-cost material. Furthermore, when we use vegetal biomass as raw material to produce ethanol, it contributes to reduce carbon dioxide emission and decreases our dependency on non-renewable resources [1].

Vegetal biomass is a renewable resource composed of lignin, hemicellulose, cellulose, and extractives. Cellulose is the most common polymer on Earth, made of glucose chains, which makes it essential to the fermentation process, in which cellulose is broken down into simple glucose molecules. There are different types of biomass to produce second-generation ethanol, such as sugarcane bagasse, rice starch, corn, wheat, and saccharine sorghum. The difference lies on their chemical composition: sugarcane and sorghum have 35–50% of cellulose, followed by 20–35% hemicellulose, 10–25% of lignin, and a small amount of ash and extractives. Cardoso et al., ([2013) reported the following values for sweet sorghum straw: cellulose, 35.87; hemicellulose, 26.04; and lignin 7.52 [2]. Reis et al., ([2016) reported value of: cellulose 42.46; hemicellulose, 38.0; lignin 4.0; ash, 1.0; and extractives 11.72 [3].

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Sweet sorghum is a sugar crop, similar to sugar cane and sugar beets, that may show promise as a source of sugar for ethanol fermentation. Many studies have been published using the sweet sorghum juice [4]. The present study was developed using the bagasse, after the juice extraction. Usually bagasse, either from sugar cane or any other biomass source, is the raw material used to produce second-generation ethanol, is highly recalcitrant due to the bonds between cellulose, hemicellulose, and lignin. Thus, before the final production of ethanol, we first need to put this bagasse trough several steps: pre-treatment, hydrolysis, fermentation, and distillation [5]. Pre-treatment is one of the most important steps and its purpose is to remove structural and compositional barriers of lignocellulosic materials in order to increase the yield of fermentable sugar from cellulose and hemicellulose; it also intends to offer a higher hydrolysis percentage [5, 6].

The optimal pretreatment conditions of corresponding methods are also an important variable to improve the enzymatic hydrolysis and ethanol yield. Recently, various pretreatment on SSB for ethanol production, such as AFEX, lime and H_2SO_3-steam have been reported [7].

There are many challenges to produce second generation ethanol cost competitive and one important example is to determine the best process to obtain monosaccharides to hydrolyze. There are two types of hydrolysis: acid and enzymatic. The present studied process intends to hydrolyze hemicellulose and polysaccharides into xylose and glucose, respectively [8]. Therefore, an ideal hydrolysis process should be economically viable concerning glucoside yield, global cost, and hydrolyzed fermentation.

Many variables are involved in the ethanol yield, including those related to agronomical cycle. During crop cycle the amount of cellulose, hemicellulose and lignin can change and my cause a decrease of 2G ethanol amount. Biomass physical and chemical properties involved a lower glucose yield and concentration at the end of enzymatic hydrolysis and, consequently, a lower 2G ethanol concentration at the end of simultaneous saccharification and fermentation, proving that there is strong relationship between structure, chemical composition, and fermentable sugar yield [9].

Acid hydrolysis uses strong acids in order to attack glycoside bonds between monosaccharides of a polysaccharide. These acids are normally used in laboratory to obtain hydrolysis; the most common are trifluoroacetic (TFA), hydrochloric, and sulfuric acid in low temperatures. However, the acid hydrolysis method could be greatly disadvantageous, as it requires equipment that is highly resistant to corrosion, which increases the costs to obtain ethanol, the final product. Furthermore, it is necessary to recover the acid used in the process for economic reasons and to avoid environmental problems [10].

Unlike acid hydrolysis, the enzymatic process offers fermentation simultaneous to hydrolysis (Simultaneous Saccharification and Fermentation – SSF). This allows better yields, has low maintenance costs, reduces cellulase inhibition by hydrolysis products, and minimizes contamination risks, among other advantages [11]. Enzymes from the class of cellulase catalyze this type of hydrolysis. Most of them show great efficiency under mild conditions, that is to say, with pH between 4 and 5 and temperature between 45 and 50°C [12, 13].

There are several types of raw material to produce this type of ethanol, one of which is the biomass of sweet sorghum or saccharine. According to the Brazilian Agricultural Research Corporation (EMBRAPA), this material presents a proportion of lignin lower than 12%, whereas the sugarcane bagasse has 20 to 22%. This difference is important to produce second-generation ethanol, as the lower the lignin content makes easier the process to obtain ethanol, therefore resulting in higher yield, with a more efficient energy and mass balance [14].

Sweet sorghum shows several advantages in comparison to sugarcane, e.g. it has seeds, presents a shorter vegetative cycle (90–140 days), uses less water and fertilizer, has sugar peaks

in different times of the year, can be planted among other cultivations, and it probably can be seeded in non-cultivated areas and in places where sugarcane does not adapt well. This flexibility results from the fact that sweet sorghum is a plant of strong natural characteristics and rapid growth. Also, the ethanol yield from sorghum grains is approximately five fold higher than the ethanol yield from sugar cane (sugar cane: 80 L/ton.; sorghum grains: 450 L/ton.) [15]. Depending on the planted varieties the yield can change substantially. In the literature is reported values for ethanol produced from bagasse using an average value of 71% moisture content. The resultant ethanol produced from the bagasse was reported 300 L per dry ton. [16].

Therefore, considering all that was presented, the main objective of this paper is to verify the potential of cellulosic ethanol production from saccharine sorghum bagasse using enzymatic hydrolysis and verifying two types of fermentation: simple and simultaneous.

2. Materials and methods

2.1. Materials

Saccharine sorghum biomass was used as raw material; the enzyme was Accellerase® 1500 cellulase, which is an enzymatic complex used to lignocellulosic biomass [17]. Finally, the chosen yeast was *Saccharomyces cerevisiae*, from Fleischmann®, as it is widely used in industrial processes such as ethanol production.

2.2. Methods

2.2.1. Moisture content

With a fresh biomass, it was necessary to determine its moisture content in order to determine the hydrolysis yield in a dry basis. The moisture content of the sample was determined by weight difference before and after oven drying the sample at 105°C. The calculation used to determine the moisture content is described in the following equation: $U\% = (P_u - P_s / P_u) \times 100\%$

2.2.2. Preparation of raw material

Most of the publication about sweet sorghum ethanol deals with juice fermentation and the use of the bagasse is a recent alternative under consideration. An energy balance was done by Worley ([1992), but at that time the bagasse was used as animal feed [18].

To prepare the raw material, firstly was performed the grinding of the saccharine sorghum in a knives mill. Then the material was oven dried at 60°C for 24 hours and stored it for posterior use.

The chemical pretreatment is one of the most important in a process of second-generation ethanol. In this step that the vegetable fibers open, exposing the cellulose microfiber, with a partial loss of lignin and hemicellulose from the biomass. During the sample opening stage, it was performed a basic treatment associated with steam explosion. Steam explosion is a very common pre-treatment aiming to expose the biomass components, such as the microfibrils of cellulose or to remove more complex components such as lignin.

In a beaker, was added 100 g of bagasse in a 2 L solution of NaOH at 2% (m/v). The mixture was put in a vertical autoclave for 30 minutes at a 133°C temperature and 2 atm pressure. After that, the pressure was rapidly released in a "steam explosion." Finally, samples were removed, washed with distilled water, and sifted manually removing the excess of water from the fibers.

The last stage of the pre-treatment is bleaching, in which the sample that went through the process of having its fibers opened is put into a NaOH 2% and NaClO 2% (v/v) solution. The material was put into a vertical autoclave for 30 minutes at a 133°C temperature and 2 atm pressure. After that, the pressure was rapidly released in a "steam explosion." Finally, samples were removed, washed, and sifted, manually removing the excess of water from the fibers. The process was then repeated once more. After that stage, samples were filtered in a vacuum-filter, put into closed flasks, and stored in a freezer until being used for hydrolysis.

2.2.3. Enzymatic hydrolysis

For the enzymatic hydrolysis, approximately 14 g (dry matter) of pretreated biomass were added to 500 mL of distilled water. Using citric acid, the pH was adjusted to 4.8. This adjustment was needed so that the mixture presented ideal pH to perform the enzymatic hydrolysis. Then, it was added 3.5 mL of Accellerase 1500 enzyme to each flask; closed the flasks, and incubated them at a 50°C under constant magnetic stirring during the desired time. The enzymatic hydrolysis was analyzed after three periods: 25 hours, 50 hours, and 100 hours.

2.2.4. Fermentation

It was used two methods for fermentation: Separated Hydrolysis and Fermentation (SHF) and Simultaneous Saccharification and Fermentation (SSF). The SHF happened from the solution of hydrolyzed biomass obtained in 2.2.3. It was added 2% (m/v) of *Saccharomyces cerevisiae* yeast to the hydrolyzed material. Then, the flasks were sealed and incubated at 50°C, at constant magnetic agitation during the desired time. SHF was performed during 25 hours, 50 hours, and 100 hours. After fermentation, the ethanol was separated from the mixture through fractional distillation.

The SSF, i.e. hydrolysis happening at the same time as fermentation, was performed exactly as SHF, but it was added 2% (m/V) of *Saccharomyces cerevisiae* yeast and 3.5 mL of Accellerase® 1500 enzyme to the hydrolyzed material. Then, samples were taken to the incubator at 38°C during 25 hours, 50 hours, and 100 hours.

2.2.5. Distillation of ethanol

Ethanol was separated from the mixture through fractional distillation at around 95°C for all samples.

2.2.6. Total sugar in the mixture of hydrolyzed biomass

The amount of reducing sugar in the hydrolyzed sample was determined using the Eynon-Lane method.

2.2.7. Ethanol determination

The ethanol obtained through distillation was quantified through the oxidation of potassium dichromate and post titration with sodium thiosulfate solution.

Titration was performed in a Metrohm Titrando 852 automatic titrator, using the software Tiamo to obtain a direct determination of the ethanol concentration on each sample.

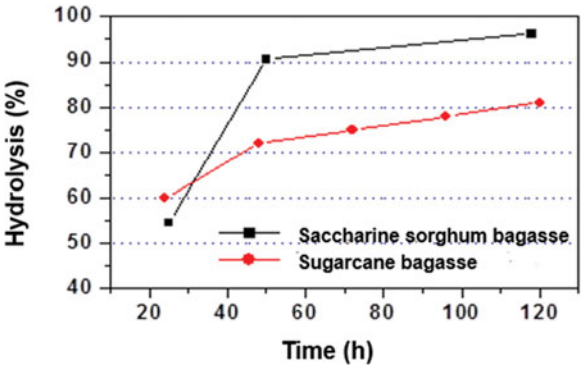

Figure 1. Sorghum hydrolysis in comparison with sugarcane hydrolysis.

3 Results and discussion

3.1. Biomass pre-treatment

The production of second-generation ethanol is more complex than the common ethanol, as the cellulosic material needs to be hydrolyzed, which is indispensable to obtain fermentable sugars. Pre-treatment is of crucial importance in the hydrolysis process, since cellulose is associated with other substances (e.g. hemicellulose, lignin, extractives, and ashes) in the vegetable biomass. Thus, some processes are necessary to expose the cellulose to the enzyme. To produce ethanol from enzymatic hydrolysis, the most used pre-treatments are acid and steam explosion at high pressure and temperature without the addition of chemical reagents.

In this paper, it was used the steam explosion at low pressure and temperature, associated with low concentrations (2%) of sodium hydroxide and sodium hypochlorite as pre-treatment for saccharine sorghum. This methodology is potentially advantageous, for it uses less energy than explosions at high pressure and resulted in biomass with a low amount of lignin. Lignin is known for having inhibiting properties in the enzymatic hydrolysis process. An undesirable consequence of the process is the production of chemical residues, which must be handled.

3.2. Enzymatic hydrolysis

The enzymatic hydrolysis was performed as described in the experimental procedure. The selection of the conditions (time, pH, and temperature) were based on results presented in the technical data sheet of Accellerase 1500® Duppont yeast relating to sugarcane, due to its similarities with saccharine sorghum.

Hydrolysis results are shown in Figure 1. Can be observed that the cellulose of saccharine sorghum hydrolyzed easily, offering a 96% yield of biomass after 118 hours.

The hydrolysis of sorghum bagasse was higher than that described for sugarcane bagasse. This fact is due to a difference in the pretreatment (acidic, for sugarcane; basic, for sorghum) and in the higher concentration of solids in the sugarcane hydrolysis, which reduces the yield in comparison with sorghum.

3.2.1. Total reducing sugar

After the enzymatic hydrolysis, it was picked an aliquot of each sample to determine the amount of total reducing sugar (TRS). Through the concentration of total reducing sugar it is

Table 1. Values of total reducing sugars after the hydrolysis of sorghum bagasse.

Hydrolysis Time / h	TRS / g
25	6.579
50	8.621
118	10.0

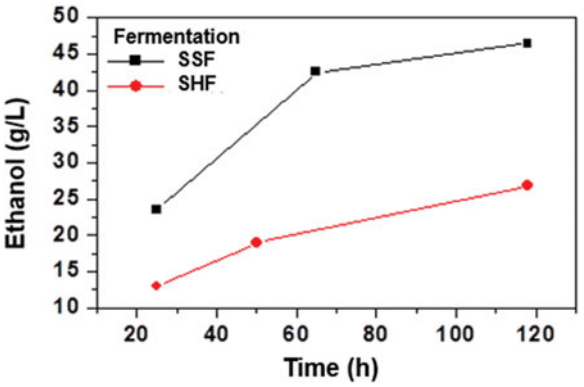

Figure 2. Comparison between SHF and SSF.

possible to evaluate the hydrolysis and the fermentation processes used to obtain ethanol. The concentration of total reducing sugars was determined according to Materials and Methods and are presented in Table 1.

3.3. Separated hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF)

After the hydrolysis of saccharine sorghum bagasse in different times, a SHF was performed. The SSF was performed to determine the synergy of the process of hydrolysis and fermentation as presented in Figure 2.

SHF in 100 hours resulted in a maximum yield of 26.8 g/L of ethanol, whereas SSF resulted in 46.6 g/L.

The fact that SSF has higher yields than SHF is due to the dynamics of the process itself: as cellulose is transformed in short-chain sugars by enzymes, these sugars are transformed in ethanol by the microorganism. Therefore, this process tends to increase the conversion of ethanol through the reduction of growth inhibition compounds of microorganisms. SSF still presents another important advantage for industrial production: it eliminates the use of two reactors that is, one for hydrolysis and another for fermentation, which reduces the costs.

4. Conclusions

Saccharine sorghum is a biomass with high potential to produce first and second-generation ethanol, due to its short-cycle cultivation and the fact that it is possible to produce fermentable sugars both from its juice and through the hydrolysis of the bagasse.

The pre-treatment with basic reagents and steam explosion in low pressure and temperature efficiently removed lignin, exposing the cellulose to enzymatic attack.

SSF proved the best option to obtain ethanol, according to the proposed methodology.

The method used to analyze the total reducing sugar was not advantageous in comparison with the one using specific equipment. This can be observed when to obtained results were compared in the hydrolysis graphic.

More detailed studies are needed to determine the economic viability of the production of ethanol from sorghum bagasse using the methodology proposed in this study.

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