ENVIRONMENTAL SCIENCE & TECHNOLOGY INNOVATION

# Avaliação da produção de etanol e ácido glucônico usando Sorghum bicolor L. Moench – híbrido Palo Alto 1009® como matéria-prima

***Evaluation of ethanol and gluconic acid production using Sorghum bicolor L. Moench – hybrid Palo Alto 1009® as a raw material***

*Emerson Leandro da Silva[1]; Gabriel Luiz Ferreira[2]; Beatriz Antoniassi Tavares[2]; Alcides Lopes Leão[3]; Raul Andres Uribes Martinez[4]; Marcelo Telascrea[2]; Marcia Rodrigues de Morais Chaves[2]*

[1]*Department of Post-Graduation in Materials Science and Technology, São Paulo State University -UNESP, Bauru, São Paulo, Brazil.*

[2]*Department of Chemical Engineering. Centro Universitário Sagrado Coração (UNISAGRADO), Bauru, São Paulo, Brazil.*

[3]*Department of Bioprocess and Biotechnology, FCA, São Paulo State University - UNESP, Botucatu, São Paulo, Brazil.*

[4]*Faculty of Science and Engineering, São Paulo State University - UNESP, Tupã, São Paulo, Brazil.*

E-mail do autor correspondente: *marcia_morais2004@yahoo.com.br*

## RESUMO

O aquecimento global e seus efeitos sobre o clima do planeta fizeram crescer a demanda por energia de baixa emissão de carbono e materiais de origem não fóssil para a produção de bens de consumo. Nesse contexto, a produção de etanol é muito importante, uma vez que ele pode ser utilizado como combustível e como matéria-prima nas indústrias baseadas em alcoolquímica. A utilização de biomassa celulósica é uma alternativa promissora para maximizar a oferta de etanol. Assim, este estudo avaliou a produção de etanol e ácido glucônico, utilizando o sorgo de alta biomassa (Sorghum bicolor L. Moench - híbrido Palo Alto 1009®) como matéria-prima. O processo incluiu pré-tratamento alcalino associado à explosão a vapor, hidrólise enzimática e, em seguida, por fermentação com *Saccharomyces cerevisiae* e *Aspergillus niger*. Verificou-se que o sorgo de alta biomassa pode ser utilizado como matéria-prima na obtenção de etanol e ácido glucônico. Apresentou facilidade de manuseio, preparo, hidrólise e fermentação, além de excelente rendimento (0,43 g etanol / g ART e 0,32 g AG / g biomassa).

Palavras-chave: Biorrefinaria; sustentabilidade, bioenergia.

***ABSTRACT***

*The global warming and its effects on the planet's climate have increased the demand for low carbon emission energy and non-fossil materials to produce consumer goods. In this context, the production of ethanol is very important, since it can be used as fuel and raw material in industries based on alcohol chemistry. The use of cellulosic biomass is a promising alternative to maximize the ethanol supply. Thus, this study evaluated the production of ethanol and gluconic acid by using the high-biomass sorghum (Sorghum bicolor L. Moench – hybrid Palo Alto 1009®) as raw material. The process included alkaline pre-treatment associated with the steam explosion, enzymatic hydrolysis, and then, by fermentation using Saccharomyces cerevisiae and Aspergillus niger. It was found that high-biomass sorghum could be used as raw material in obtaining ethanol and gluconic acid. It showed ease of handling, preparation, hydrolysis, and fermentation, as well as an excellent yield (0.43 g ethanol/g TRS and 0.32 g GA/g biomass).*

*Keywords: Biorefinery; sustainability, bioenergy.*

## 1. INTRODUCTION

Lignocellulosic biomass has an strategic importance as a renewable source of raw material for bioenergy and chemicals production. To meet the growing demand of ethanol in recent years, new technologies are under consideration, based on hydrolysis of biomass polysaccharides. Despite the high-biomass sorghum being specific to generate heat and electricity, it is also suitable for chemicals production due to its high cellulose amount. However, studies are needed to evaluate its properties as a raw material for chemicals production in bioenergy integrated system. Other chemicals, such as gluconic acid (GA), can be produced by fermentation of sugars derived from biomass. Its industrial preparation process includes a chemical route associated with high energy consumption, which justify the search for sustainable production alternatives.

The biorefinery development has been identified as an important alternative for the partial replacement of energy and chemicals from non-renewable sources (Cherubini and Strømman, 2011). The change in the characteristics of raw material sources is important to reduce the deleterious effects on the environment, such as $CO_2$ emission, associated with the greenhouse effect and climate change (Cherubini, 2010; Righelato and Spracklen 2007).

The sugarcane industry is an example of well-established biorefinery (Mariano et al., 2013). Thus, it is a starting point for further research on the production of bioenergy and biofuels, especially ethanol. Studies have been devoted to the development of raw materials and processing to enhance the production of ethanol due to its economic and environmental importance (Wenger et al., 2012; Ho et al., 2015; Ko et al., 2015).

Ethanol is currently the only biofuel produced on a large scale. World production is about 98,65 billion liters, of which 84% (82.9 billion liters) are produced in Brazil and in the United States (Alternative Fuels Data Center, 2021). This fuel is easily used on vehicles with internal combustion engine.

Although 90% of the products from industries of organic process are petroleum-based, and research on obtaining these products from biomass is still very incipient (Deutschmann and Dekker, 2012; Pinazo et al., 2015), the low price of biofuels makes the bio-based production a key factor to cover the investments needed in the biorefinery industry (Bozell and Petersen, 2010)the choice of appropriate products for addition to the biorefinery\u2019s portfolio is challenged by a lack of broad-based conversion technology coupled with a plethora of potential targets. In 2004, the US Department of Energy (DOE. This shows the importance and the need for further research in this area.

The type and availability of raw materials are among the limiting factors of the biorefinery development (Fava et al., 2015)being food sector the first manufacture in Europe. Anyway they need to be further tested and validated and then transferred at the larger scale. In particular, they also need to become integrated, combining biomass pretreatments and recovery of bio-genic chemicals with bioconversion processes in order to obtain a large class of chemicals. This will help to (a. Regarding the raw material's availability, the production of a very large volume is needed to meet the industrial demand. Thus, the use of soil and water to the biomass production is the central point in the environmental and economic issue, once it establishes a competition with food production (Ravindranath et al., 2011).

Despite the potential use of agricultural residues and other lignocellulosic materials, bio-mass cultivation is necessary to ensure the raw material supply for the industry (Ekman et al., 2013; Forster-carneiro et al., 2013). Thus, the choice of biomass is crucial to equate the mentio-ned issue. The raw materials must have characteristics such as have rapid growth; be adapted to different types of soil and climate; be easy planting and harvesting; furthermore, be easy for industrial processing. Different lignocellulosic biomasses have been evaluated as a raw material to produce biofuel and bioproducts. Among them, miscanthus, switchgrass, elephant grass, and giant reed showed great versatility (Aresta et al., 2012; Menezes et al., 2016).

In the context of biorefinery supported by the sugarcane industry model, sorghum varieties have shown strategic importance. They can provide raw material without significant changes in the configuration of well-established industry for the production of heat, power, and ethanol (Kim et al., 2012; Nghiem et al., 2016). Sorghum is a tropical grass grown primarily in semi-arid and dry regions in the world, especially in areas where traditional plant breeding is not possible (Oikawa et al., 2015).

High-biomass sorghum has attracted the interest of researchers because of its high yield in lignocellulosic biomass, and it is specific for bioenergy production (Stefaniak et al., 2012). However, despite its characteristics of being specific to generate electricity, its potential for cellulosic ethanol and chemicals production is pointed due to its high amount of cellulose (Mullet et al., 2014). Sorghum biomass has the explosive growth cycle, reaching the point of harvest and efficient burning in boilers between 120 and 130 days. It has moisture levels around 50% that allow its direct burning after harvest. It requires less water and offers more resistance to heat than the other species used as energy biomass. In this context, high biomass sorghum has ideal characteristics to be used as a biomass energy production, biofuels, and chemicals of industrial interest (Silva et al., 2016).

The main purpose of this study is to evaluate the high-biomass sorghum (Sorghum bicolor L. Moench) – hybrid Palo Alto 1009® as raw material to produce bio-based chemicals. It was verified that the high-biomass sorghum, designed to produce energy by cogeneration process, is also able to produce ethanol and gluconic acid with a good yield and easy processing, turning into a key biomass to the sugarcane biorefinery-based industry.

## 2. MATERIALS AND METHODS

To evaluate the potential of high-biomass sorghum as raw material for gluconic acid and ethanol production, the biomass was first pretreated, followed by enzymatic hydrolysis. The obtained broth was fermented using *Aspergillus niger (A. niger)* to produce gluconic acid and *Saccharomyces cerevisiae (S. cerevisiae)* to produce ethanol. All reagents used were of analytical grade. The enzymatic complex used to hydrolyze the biomass was Cellulase Cellic CTec 3, Novozyme. The *A. niger* was from a mycology collection donated by the Lauro de Souza Lima Institute, Bauru, São Paulo, Brazil. The *S. cerevisiae* used was the industrial strain Y904.

### 2.1 Biomass preparation

The high-biomass sorghum preparation involved the harvesting, grinding, drying at 65°C during five days, and storage in sealed plastic bags until its use in this study.

The raw biomass was placed in an aqueous solution of sodium hydroxide (4% wt) and sodium hypochlorite (4% v/v), with biomass/alkaline solution at a ratio of 1:20 (w/v). It was transferred to an autoclave (1.0 $kgf.cm^{-2}$, 121°C) for 30 minutes. After this time, the autoclave was rapidly depressurized through the full opening of the valve. This process is similar to the well-known steam-explosion process. The biomass after this procedure was washed in tap water, and a sample called by the first explosion of biomass (1st. pretreatment) was taken. This procedure was repeated, obtaining the second explosion of biomass (2nd. pretreatment).

The biomass characterization was conducted following the TAPPI standards (T 222 om 11; T9 wd-75; T5 wd -73, T12 wd-82, and T211 om-12), by determining the moisture, ash, extractives soluble in ethanol-toluene, lignin, and holocellulose (cellulose + hemicellulose) contents.

### 2.2 Biomass hydrolysis

Enzymatic hydrolysis was performed using 41.0 g (dry basis) of the second pretreated biomass and 500 mL of distilled water added to the glass reactor. Citric acid was used to adjust the pH to 4.8 once it is the optimal pH for carrying out the enzymatic hydrolysis (Weiss et al., 2013). Afterwards, 15 mL of the enzyme complex was transferred to the reactor. The reactor was placed in an environmental chamber at 50°C, in a constant magnetic stirring. Samples were collected in 2, 4, 6, 8, 10, 24, and 48 h of the hydrolysis process. At the end of this process, the hydrolyzed material was filtered under a negative pressure system using a 3 µm paper filter, followed by the filtration with a 0.45 µm cellulose acetate membrane. The material retained on the filter was dried and weighed, being considered as non-hydrolyzed fraction. The percentage of hydrolyzed material was obtained by the difference between the amount of initial biomass and the final material (non-hydrolyzed). The clarified liquid was sterilized in an autoclave (1.0 $kgf.cm^{-2}$, 121°C) for 20 minutes and stored in the refrigerator until the fermentation process.

The glucose content determination in the hydrolyzed was performed by dinitro salicylic acid (DNS) test, using ultraviolet-visible spectrophotometry (UV-Vis) and a standard glucose curve, as described by Marsden and co-workers (Marsden et al., 1982). The dilutions of the sample were considered in the calculations, multiplying the result by this factor.

### 2.3 Gluconic acid preparation

For gluconic acid preparation, two experiments were conducted, and the sources of sugar were a commercial broth (glucose) and a hydrolysate broth (glucose from hydrolyzed biomass). The commercial broth was prepared with the following components: glucose 150 $g.L^{-1}$, peptone 0.02 $g.L^{-1}$, $(NH_4)_2SO_4$ 0.59 $g.L^{-1}$, KCl 0.25 $g.L^{-1}$, $KH_2PO_4$ 0.25 $g.L^{-1}$, $MgSO_4.7H_2O$ 0.25 $g.L^{-1}$. The hydrolysate broth was prepared with the following components: glucose from hydrolyzed biomass 91.99 $g.L^{-1}$, peptone 0.02 $g.L^{-1}$, $(NH_4)_2SO_4$ 0.59 $g.L^{-1}$, KCl 0.25 $g.L^{-1}$, $KH_2PO_4$ 0.25 $g.L^{-1}$, $MgSO_4.7H_2O$ 0.25 $g.L^{-1}$.

The broths were sterilized in an autoclave (1.0 $kgf.cm^{-2}$, 121°C) for 20 minutes. The fermentation process of the commercial and the hydrolysate broths was conducted for 24 h. The reactor was composed by a glass bottle with a lid containing an aeration inlet tube connected to the bacteriostatic filter; a glass tube for air outlet; a glass tube for introduction of solution for pH correction; and a pH sensor. The reactor, its connections (except the pH sensor), and all solutions used during the fermentation process were previously sterilized in autoclave (1.0 $kgf.cm^{-2}$, 121°C).

Within the laminar flow hood, the broth and the *Aspergillus niger* ($10^7$ UFC) were added to the reactor. The reactor was placed in an environmental chamber at 30°C, under magnetic stirring at 200 rpm, during 24 h. The pH in the first 12 h was maintained at 6.5; after that, it was adjusted to 5.5 (pH adjusted with NaOH 4% and 10% acetic acid when needed). Aeration was maintained through 90 bubbles per minute.

The gluconic acid determination was performed from the measurement of glucose consumed by the *A. niger* during the broth fermentation process. Glucose was quantified by the DNS method, as described above. The *A. niger* oxidizes and converts glucose into gluconic acid spontaneously under the fermentation experimental conditions (Ramachandran et al., 2006). Thus, the glucose consumed was considered as produced gluconic acid.

### 2.4 Ethanol preparation

For the ethanol production was used a hydrolyzed broth prepared by adding the following nutrients: $KH_2PO_4$ 5,0 $g.L^{-1}$; $(NH_4)Cl$ 1,5 $g.L^{-1}$; $MgSO_4.7H_2O$ 1,0 $g.L^{-1}$; KCl 1,0 $g.L^{-1}$; 6.0 $g.L^{-}1$ yeast extract, and glucose obtained from biomass hydrolysis as the only source of sugar. The *S. cerevisiae* yeast 2% (w/v) was added to the hydrolyzed broth for fermentation.

The glass reactor was placed in an environmental chamber at 30°C, under magnetic stirring at 200 rpm, during 25 h, 50 h, and 100 h. After fermentation, the ethanol was separated from the mixture by fractional distillation.

The ethanol was quantified using the oxidation method to acetic acid by reaction with potassium dichromate in an acidic medium, and detection by UV-Vis at 580 nm, according Sumbhate and Nayak (Sumbhate and Nayak, 2012).

## 3. RESULTS AND DISCUSSION

### *3.1 Sorghum pretreatment and hydrolysis*

The fresh matter yield of the biomass sorghum used in this study is 92.36 Mg. $ha^{-1}$ and dry matter 38.35 Mg. $ha^{-1}$. High biomass sorghum samples were analyzed before and after pretreatments, and the results are shown on Table 1. The moisture content of the raw sample is higher due to the presence of extractives in its fibers that are water holders. Samples with pretreatment have lower moisture content due to lower rehydration capacity of its fibers caused by the extractive's removal. As the number of pretreatment increases, it was observed that the extractives and moisture contents decreased. The pretreatment decreased the percentage of ash present in the biomass, since the samples were washed after each treatment, removing the inert materials.

**Table 1.** Lignin, holocellulose, ash, and extractives of biomass samples before and after pretreatment.

| Sorghum | Lignin | Holocellulose (cellulose + Hemi-cellulose) | Ash | Extractives |
|---|---|---|---|---|
| **Untreated** | 11.50[a] | 55.27[c] | 6.989[a] | 25.67[a] |
| **1st. treatment** | 1.81[b] | 88.47[b] | 1.921[b] | 7.84[b] |
| **2nd. treatment** | 1.65[b] | 95.87[a] | 1.463[c] | 0.95[c] |
| **F** | 21156.727 | 1252.393 | 552082.046 | 2583.437 |
| **MDS** | 0.16829 | 2.65178 | 0.01791 | 1.08901 |
| **GA** | 4.99 | 79.87 | 3.46 | 11.49 |
| **VC (%)** | 1.35 | 1.33 | 0.21 | 3.78 |

GA (general average); VC (variation coefficient); MDS (Minimum deviation of significance). The average with same letter (in row) is not statistically different on Tukey's test at 5% of probability ($P < 0.01$).

The composition of high-biomass sorghum (untreated) shows its ideal characteristics as combustion material. The lignin content (11.5%) is higher than the other high-biomass sorghum hybrids reported in the literature (6.6% to 8.1%) (Nagaiah et al., 2012), which reflects on the biomass calorific power. The holocellulose content (55.9%) is slightly less than the reported hybrids (48% to 69%), but it is suitable for the hydrolysis and fermentation process.

In samples submitted to pretreatment, lignin decreased considerably and left the holocellulose in greater quantity. Despite the high lignin content, the sorghum delignification process was easy, when only two steps were needed to decrease the lignin content in 86% and increase the holocellulose content in 71.5%. Thus, biomass with two pretreatments is the best process for enzymatic hydrolysis and fermentation.

Enzymatic hydrolysis of biomass subjected to two pretreatments was performed, yielding the hydrolysate broth. Samples collected at different hydrolysis times showed the behavior as described in the Fig. 1. Enzymatic hydrolysis was satisfactory, and the high rate of biomass hydrolysis occurred in the first 4 hours. Approximately 94% of the pretreated biomass was hydrolyzed in 8 h. The process tends to stabilize from 10 hours of hydrolysis, thus a longer time to achieve higher content of sugar hydrolysate is not necessary.

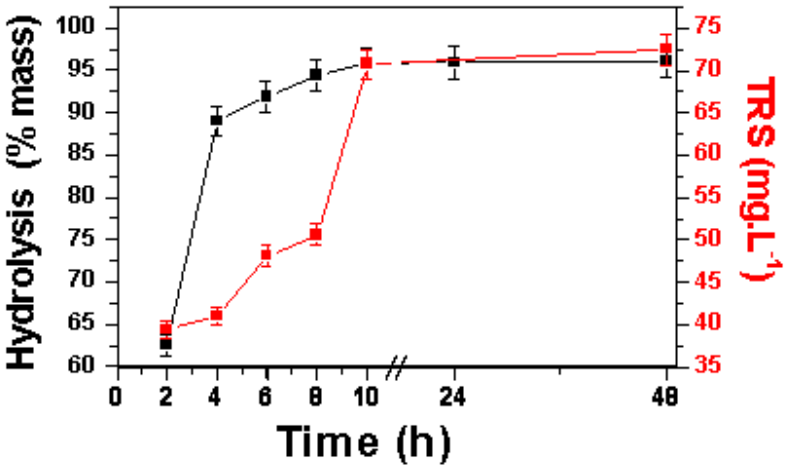



**Fig 1.** High biomass sorghum hydrolysis as a time function.

The maximum enzyme efficiency occurred within 10 h (Fig. 1a, red line), when approximately 70.8 $g.L^{-1}$ of glucose were formed. During the entire process, it was formed 72.5 $g.L^{-1}$ of glucose. This result demonstrates that the optimal hydrolysis time is 10 h, compatible with processing time in industrial plants.

In terms of hydrolysis, this material shows high potential to be used as biomass for cellulosic ethanol production in industrial units that operate with traditional materials such as sugarcane bagasse. It is important to note that the enzymatic complex used in this process is the same used to hydrolyze sugar cane bagasse in the cellulosic ethanol industry. Thus, high-biomass sorghum can substitute the sugar cane bagasse in these industrial unities.

### 3.4 Sorghum to gluconic acid

Glucose fermentation to gluconic acid produced from commercial and hydrolyzed broths were performed as described in the materials and methods section, and the results are presented on Table 2. The glucose content was greater than the hydrolyzed material due to the concentration process during the broth preparation.The glucose fermentation by A. niger leads to the formation of gluconic acid under the experimental conditions used in this study (Ramachandran et al., 2006). The production of gluconic acid was 101.0 $g.L^{-1}$ for commercial broth, and 26.15 $g.L^{-1}$ in the hydrolysate broth. Whereas the volume of the hydrolysate broth (500 ml) obtained in processing sorghum 40.0 g with two pretreatments, the production of gluconic acid was 0.319 g $GA.g^{-1}$ biomass (319 $kg.ton^{-1}$ biomass).

**Table 2**. Gluconic acid fermentation using commercial and hydrolyzed broths by *A. niger*.

| | Commercial broth | Hydrolyzed broth |
|---|---|---|
| Initial Glucose ($g.L^{-1}$) | 150.7 | 91.99 |
| Final Glucose ($g.L^{-1}$) | 57.44 | 67.97 |
| Gluconic acid production ($g.L^{-1}$) | **101.0** | **26.15** |
| Yield (GA/Glucose fed) ($g.g^{-1}$) | **0.67** | **0.28** |

Fermentation for 24 hours. GA: gluconic acid.

The gluconic acid yield from commercial and hydrolyzed broths were 61.5% and 25.7% of the theoretical yield (1.089 g. $g^{-1}$), respectively (Ramachandran et al., 2006). These values are smaller than the ones described in the literature (Lu et al., 2015). The yields are addressed to the dispersed fermentation and initial glucose concentration, being reported as 330 $g.L^{-1}$ and 450 $g.L^{-1}$ to produce 98% of the theoretical yield (Singh and Kumar, 2007). Glucose concentration in the hydrolyzed broth is 38.45% lower than the commercial broth. This resulted in a 57.82% decrease of the conversion of glucose into gluconic acid, in relation to the commercial broth. Thus, the results suggest that the initial glucose concentration is crucial in the fermentation efficiency.

Despite the high yield of hydrolysis process, the fermentation process was not in the best conditions for gluconic acid production. Results pointed out the feasibility of high biomass sorghum as raw material to GA production; however, further research is needed to improve its production, considering the biorefinery industry establishment.

### 3.5 Sorghum to ethanol

The hydrolyzed broth fermentation was performed according to the procedure described in 3.4. After fractional distillation, it was determined the ethanol production resulting. Then, the yield was calculated by the Equation 1. The yield of fermentation was determined for each of the periods of time 25 h, 50 h, and 100 h. It has been observed that increasing the fermentation time favors a higher yield of ethanol production.

$$Y_{Ethanol/Glucose} = \frac{C_{ethanol\,f}}{1.053(C_{sac\,0} - C_{sac\,f})} \qquad (1)$$

Where:

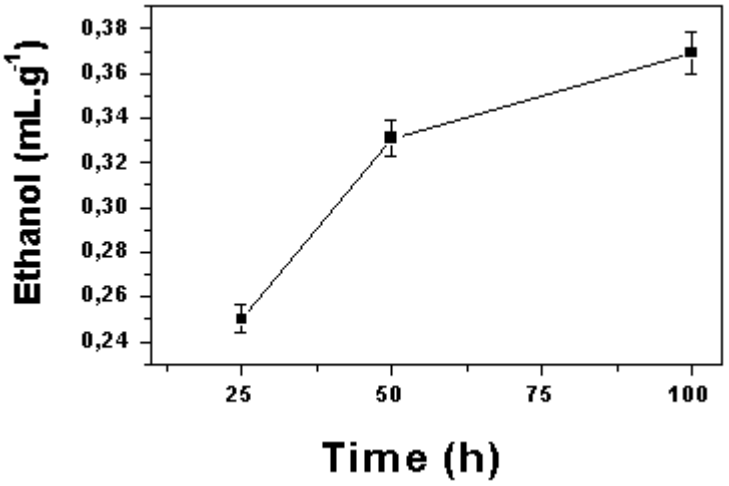
$Y_{Ethanol/Glucose}$ = Ethanol yield produced by glucose consumed

$C_{ethanol\,f}$ = Final ethanol concentration (g/L)

$C_{sac\,0}$ = Initial glucose concentration (g/L)

$C_{sac\,f}$ = final glucose concentration (g/L)

The fermentation yield of the 100h was 0.43 g ethanol/g TRS. This value is considered very good, compared to the theoretical value of 0.51 g ethanol/g TRS (Kumar et al., 2016). The yield for 25 h and 50 h were respectively 0.28 g ethanol/g TRS and 0.38 g ethanol/g TRS. The fermentation yield for 50h was 32.5% higher in relation to 25 h, but only 11.5% lower compared to the fermentation yield for 100 h. This fact is explained by the sharp decrease in sugar concentration in the medium, it is important to keep the ethanol production rate. The ethanol yield for 25 h fermentation process was 244.3 $mg.g^{-1}$ of pretreated biomass. This value is higher than the ethanol yield produced from some hybrids of biomass sorghum (116 $mg.g^{-1}$ biomass) reported by Dien and co-workers (Dien et al., 2009). Fig. 02 shows the yield of the fermentation process (volume of ethanol produced per mass of pretreated biomass) as the time function.




Considering the 25h ==fermentation process, it is possible to produce 309.0 L ethanol / ton pretreated biomass. The gravimetric yield of the biomass pretreatment process is 0.4 g/g raw biomass. Thus, it is possible to produce about 124 L ethanol/ton of dried raw biomass, which corresponds to the ethanol productivity of 2480 $L.ha^{-1}$. This result is in accordance with the results related in the literature (Prakasham et al., 2014), being comparable to the ethanol productivity of some hybrids of *Sorghum bicolor* L. (2129 - 6388 $L.ha^{-1}$), corn (3800 $L.ha^{-1}$), poplar (1500 - 3400 $L.ha^{-1}$) and cassava (4500 $L.ha^{-1}$). Thus, *Sorghum bicolor* L. Moench - Hybrid Palo Alto 1009® could be pointed out as a promising raw material to produce cellulosic ethanol.

## 4. CONCLUSIONS

This study enabled the evaluation of high-biomass sorghum (*Sorghum bicolor* L. Moench - Hybrid Palo Alto 1009®) as a feedstock for cellulosic ethanol and gluconic acid preparation. Results were very important, considering the strategic use of high-biomass sorghum, which can serve for the heat and power production in cogeneration process. Thus, this study contributed

to expand the possibilities of biomass use for the bioenergy and chemicals production in an integrated system.

Although the study was performed in laboratory scale, the process of obtaining ethanol from hydrolysis and fermentation of sorghum was considered successful with ethanol yield of glucose consumed 0.43 g/g TRS, compared to the theoretical yield 0.51 g/g TRS. However, for gluconic acid production, is is necessary to concentrate the hydrolyzed broth to increase the glucose concentration, and the yield of gluconic acid.

It is important to note that sorghum presented ease of preparation and hydrolysis process, indicating that it is an excellent raw material to produce cellulosic ethanol and gluconic acid.

## Acknowledgments

The authors are grateful to the Centro Universitário do Sagrado Coração – Unisagrado, which provided technical and financial resources for the development of this study.

## REFERENCES

Alternative Fuels Data Center (2021) World Ethanol Production. US Department of Energy. Available in: www.afdc.energy.gov/10331. July 28, 2021.

Aresta, M.; Angela, D.; Franck, D. **Biorefinery: From Biomass to Chemicals and Fuels**. Edited by Walter de Gruyter. Ilustrated. Berlin, 2012.

Bozell, J.; Gene, R.P. Cutting-Edge Research for a Greener Sustainable Future Technology Development for the Production of Biobased Products from Biorefinery Carbohydrates — the US Department of Energy S Top 10 Revisited. **Green Chemistry**, v. 12, p. 539–54, 2010.

Cherubini, F. The Biorefinery Concept : Using Biomass instead of Oil for Producing Energy and Chemicals. **Energy Conversion and Management** v.7, p.1412–21, 2010.

Cherubini, F.; Strømman, A.H. Chemicals from Lignocellulosic Biomass: Opportunities, Perspectives, and Potential of Biorefinery Systems. **Modeling and Analysis**, v.7491, p.548–61, 2011.

Deutschmann, R.; Dekker, R.F.H. From Plant Biomass to Bio-Based Chemicals: Latest Developments in Xylan Research. **Biotechnology Advances**, v. 30 (6), p. 1627–40, 2012.

Dien, B.S.; Pedersen, J.F.; Sattler, S.E.; Chen, H. Improved Sugar Conversion and Ethanol Yield for Forage Sorghum ( Sorghum Bicolor L . Moench ) Lines with Reduced Lignin Contents. **Bioenergy Research**, v. 2, p. 153–64, 2009.

Ekman, A.; Wallberg, O.; Joelsson, E.; Börjesson, P. Possibilities for Sustainable Biorefineries Based on Agricultural Residues – A Case Study of Potential Straw-Based Ethanol Production in Sweden. **Applied Energy**, v. 102, p. 299–308, 2013.

Fava, F.; Totaro, G.; Diels, L.; Reis, M.; Duarte, J.; Poggi-varaldo, M.; Ferreira, B.S.; Carioca, O.B. Biowaste Biorefinery in Europe: Opportunities and Research & Development Needs. **New Biotechnology**, v. 32 (1), p. 100–108, 2015.

Forster-carneiro, T.; Berni, M.D.; Dorileo, I.L.; Rostagno, M.A. Resources, Conservation and Recycling Biorefinery Study of Availability of Agriculture Residues and Wastes for Integrated Biorefineries in Brazil. **Resources, Conservation & Recycling**, v. 77, p. 78–88, 2013.

Ho, Y.K.; Doshi, P.; Yeoh, H.K.; Ngoh, G.C. Why Are Two Enzymes Better than One for the Efficient Simultaneous Saccharification and Fermentation (SSF) of Natural Polymers? Hints from Inside and Outside a Yeast. **Industrial & Engineering Chemistry Research**, v. 54 (42), p. 10228–44, 2015.

Kim, M.; Han, K.J.; Jeong, Y.; Day, D.F. Utilization of Whole Sweet Sorghum Containing Juice, Leaves, and Bagasse for Bio-Ethanol Production. **Food Science and Biotechnology**, v. 21 (4), p. 1075–80, 2012.

Ko, J.K.; Um, Y.; Park, Y.C.; Seo, J.H.; Kim, K.H. Compounds Inhibiting the Bioconversion of Hydrothermally Pretreated Lignocellulose. **Applied Microbiology and Biotechnology**, v. 99 (10), p. 4201–12, 2015.

Kumar, V.B.; Pulidindi, I.N.; Kinel-tahan, Y.; Yehoshua, Y.; Gedanken, A. Evaluation of the Potential of Chlorella Vulgaris for Bioethanol Production. **Energy Fuels**, v. 30 (4), p. 3161–66, 2016.

Lu, F.; Ping, K.; Wen, L.; Zhao, W.; Wang, Z.; Chu, J.; Zhuang, Y. Enhancing Gluconic Acid Production by Controlling the Morphology of Aspergillus Niger in Submerged Fermentation. **Process Biochemistry**, v. 50 (9), p. 1342–48, 2015.

Mariano, A.P.; Dias, M.O.S.; Junqueira, T.L.; Cunha, M.P.; Bonomi, A.; Filho, R.M. Butanol Production in a First-Generation Brazilian Sugarcane Biorefinery: Technical Aspects and Economics of Greenfield Projects. **Bioresource Technology**, v. 135, p. 316–23, 2013.

Marsden, W.L.; Gray, P.P.; Nippard, G.J.; Quinlan, M.R. Evaluation of the DNS Method for Analysing Lignocellulosic Hydrolysates. **Journal Chemical Technology Biotechnology**, v. 32, p. 1016–22, 1982.

Menezes, B.R.S.; Daher, R.F.; Gravina, G.A.; Pereira, A.V.; Pereira, M.G.; Tardin, F.D.; Silva, V.B.; Rodrigues, E.V.; Araújo, M.S.B.; Oliveira, M.L.F.; Almedia, B.O. Combining Ability in Elephant Grass (Pennisetum Purpureum Schum.) for Energy Biomass Production. **Australian Journal of Crop Science**, v. 10 (9), p. 1297–1305, 2016.

Mullet, J.; Morishige, D.; Mccormick, R.; Truong, S.; Hilley, J.; Mckinley, B.; Anderson, R.; Olson, S.; Rooney, W. Energy Sorghum — a Genetic Model for the Design of C 4 Grass Bioenergy Crops. **Journal of Experimental Botany**, v. 2007, p. 1–11, 2014.

Nagaiah, D.P.; Rao, S.; Prakasham, R.S.; Uma, A.; Radhika, K. High Biomass Sorghum as a Potential Raw Material for Biohydrogen Production: A Preliminary Evaluation. **Current Trends in Biotechnology and Pharmacy**, v. 6 (2), p. 183–89, 2012.

Nghiem, N.P.; Montanti, J.; Johnston, D.B. Sorghum as a Renewable Feedstock for Production of Fuels and Industrial Chemicals. **Bioengineering**, v. 3, p. 75–91, 2016.

Oikawa, P.Y.; Jenerette, G.D.; Grantz, D.A. Offsetting High Water Demands with High Productivity: Sorghum as a Biofuel Crop in a High Irradiance Arid Ecosystem. **Bioenergy**, v. 7, p. 974–83, 2015.

Pinazo, J.M.; Domine, M.E.; Parvulescu, V.; Petru, F. Sustainability Metrics for Succinic Acid Production : A Comparison between Biomass-Based and Petrochemical Routes. **Catalysis Today**, v. 239, p.17–24, 2015.

Prakasham, R.S.; Nagaiah, D.; Vinutha, K.S. Sorghum Biomass: A Novel Renewable Carbon Source for Industrial Bioproducts Sorghum Biomass: Biofuels, v. 5 (2), p. 159–74, 2014.

Ramachandran, S.; Fontanille, P.; Pandey, A.; Larroche, C. Gluconic Acid: Properties. Applications and Microbial Production. **Food Technology and Biotechnology**, v. 44 (2), p. 185–95, 2006.

Ravindranath, N.H.; Lakshmi, C.S.; Manuvie, R.; Balachandra, P.; Biofuel Production and Implications for Land Use, Food Production and Environment in India. **Energy Policy**, v 39 (10), p. 5737–45, 2011.

Righelato, R.; Spracklen, D.V. Carbon Mitigation by Biofuels or by Saving and Restoring Forests? **Science, v.** 317 (5840), p. 902, 2007.

Silva, F.A.S.; Azevedo, C.A.V. The Assistat Software Version 7.7 and its use in the analysis of experimental data. **African Journal of Agricultural Research**, v. 11(39), p. 3733-3740, 2016.

Silva, A.L.; Tardin, F.D.; Rotta, G.W.; Baldoni, A.B.; Barelli, M.A.A.; Silva, A.F.; Gonçalves, D.R.; Silva, F.B.; Ribeiro, J.R.; Parrella, R.A.C. Characterization of Biomass Sorghum Cultivars Aiming to Generate Energy for the North Region of Mato Grosso, Brazil. **Nativa**, v. 4 (3), p. 175–78, 2016.

Singh,O.V.; Kumar, R. Biotechnological Production of Gluconic Acid: Future Implications. **Applied Microbiology Biotechnology**, v. 75, p.713–22, 2007.

Stefaniak, T.R.; Dahlberg, J.A.; Bean, B.W.; Dighe, N.; Wolfrum, E.J.; Rooney, W.L. Variation in Biomass Composition Components among Forage, Biomass, Sorghum-Sudangrass, and Sweet Sorghum Types. **Crop Science Abstract - Forage & Grazinglands**, v. 52, p. 1949-1954, 2012.

Sumbhate, S.V.; Nayak, S. Colorimetric Method for the Estimation of Ethanol in Alcoholic-Drinks. **Journal of Analytical Techniques**, v.1, p. 1–6, 2012.

Weiss, N.; Börjesson, J.; Pedersen, L.S.; Meyer, A.S. Enzymatic Lignocellulose Hydrolysis: Improved Cellulase Productivity by Insoluble Solids Recycling. **Biotechnology for Biofuels**, v. 6 (5), p. 1–14, 2013.

Wenger, J.; Sommer, B.; Núñez, S.; Engelking, H.; Bartek, T.;Funke, M.; Eiting, M. Lonza's Successful Fermentation Scale-up Track Record. **New Biotechnology**, v. 29, p. S73–74, 2012.