ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 10, Issue, 01, pp. 33266-33273, January, 2020

RESEARCH ARTICLE OPEN ACCESS

SECOND GENERATION ETHANOL PRODUCTION FROM AGUAPÉ (*Eichhornia crassipes*)

Daniel de Azevedo Teixeira*[1], Philipe Luan, Brito[2], Andreia Teixeira de Oliveira Santos[1], Ciro Meneses Santos*[1], Lílian de Araújo Pantoja[2], Alexandre Sylvio Vieira da Costa[1] and Alexandre Soares dos Santos[2]

[1]Federal University of Jequitinhonha and Mucuri Valleys, Campus Mucuri, Teófilo Otoni, MG, Brazil
[2]Federal University of Jequitinhonha and Mucuri Valleys, Campus JK, Diamantina, MG, Brazil

ARTICLE INFO

Article History:
Received 17th October, 2019
Received in revised form
24th November, 2019
Accepted 09th December, 2019
Published online 31st January, 2020

Key Words:
Lignocellulosic Biomass,
Bioethanol, Aguapé,
Pretreatment.

ABSTRACT

The water hyacinth is an aquatic macrophyte plant considered as lignocellulosic biomass. The chemical composition of the water hyacinth is presented in considerable contents of cellulose and hemicellulose, and low levels of lignin, which constitutes a potential substrate for the production of ethanol. The present study aims to develop a technology for the production of ethanol of the second generation from the biomass of aguapé. To reach the proposed objectives, the chemical characterization of the aguapé biomass was carried out afterward, acid and alkaline pretreatment stages, optimization of the pretreatment acid saccharification, fermentation, and distillation. Initially, the centesimal composition of the aguapé was determined, indicating the presence of 32.50% cellulose, 28.61% hemicellulose and 7.46% lignin. Among the different forms of pre-treatment analyzed, the acid pretreatment presented a better performance in the saccharification process, presenting approximately 46.16% yield in the conversion of reducing sugars. The optimization of the acid pretreatment was applied a Factorial Planning through a central rotational compound design. The STATISTICA Version 8.0 program (Statsoft Inc., Tulsa,) was used for data analysis. Pretreatment with dilute sulfuric acid resulted in the removal of at least 81% of the hemicellulose present in the water and 87% of the conversion of reducing sugars. The Saccharification and Separate Fermentation showed higher efficiency compared to the Saccharification and Simultaneous Fermentation process, presenting better conversion of sugars using the Cellic CTec2® enzymatic cocktail. The enzymatic hydrolyzate submitted to Saccharification and Separated Fermentation with Saccharomyces cerevisiae presented a YP/S of 0.50 with 2.62 g/L of alcohol in the fermented mash. The obtained data indicated that the evaluated aguapé presents a high potential for the production of second-generation ethanol.

Citation: ***Daniel de Azevedo Teixeira, Philipe Luan, Brito, Andreia Teixeira de Oliveira Santos, Ciro Meneses Santos et al.*** **2020.** "Second generation ethanol production from aguapé (eichhornia crassipes)", *International Journal of Development Research*, 10, (01), 33266-33273.

INTRODUCTION

Consumption of fossil fuels, such as oil, is booming worldwide. However, environmental preservation policies have prioritized reducing the consumption of these fuels to avoid the emergence of negative consequences such as climate change, global warming and other environmental damage (TASNIM *et al*., 2017). Thus, technological and industrial development contributes to the expansion of fuel supply but also concerns about sustainability and the environment (ZHUANG *et al*., 2016; NEGAHDAR *et al*., 2016). The global scenario facing the adverse employment of fossil fuels presents the need to stimulate the development of new technologies to obtain alternative sources of fuels. Ethanol is one of the best alternatives for reducing the use of petroleum-based fuels and, in this context, Brazil stands out worldwide for its pioneering production of fuel ethanol from sugarcane juice (BAEYENS *et al* 2016). To ensure energy supply through ethanol, it is necessary to implement alternatives such as increasing the planting area, improving the existing production process, genetic improvement of sugarcane and fermenting microorganisms, and also by developing conversion. of

Corresponding author: **Daniel de Azevedo Teixeira,**
Federal University of Jequitinhonha and Mucuri Valleys, Campus Mucuri, Teófilo Otoni, MG, Brazil.

lignocellulosic agro-industrial waste in fermentable sugar sources (ZHOU *et al*., 2016). The need for large areas for sugarcane cultivation and the problems caused by the destination of their products have made agricultural residues and agro-industrial by-products an alternative of great interest for their potential as energy biomass. Thus, the technology for the production of second-generation ethanol was developed, in which the cellulose present in the structure of these materials is hydrolyzed to fermentable sugars to produce ethanol (NEVES *et al*., 2016). Lignocellulosic biomass, the main raw material for the production of second-generation ethanol, is composed of cellulose, hemicellulose, and lignin, as well as other components in smaller quantities. Sugarcane bagasse, an example of lignocellulosic biomass, is strongly recalcitrant due to the strong link between hemicellulose cellulose and lignin. pretreatment, hydrolysis, fermentation, and distillation. Pretreatment processes for lignocellulosic materials can be thermal, chemical, physical, biological or a combination of all, which will depend on the degree of separation required and the proposed end (VERARDI *et al*., 2016).

The water hyacinth aquatic plant is considered a promising source of energy biomass mainly due to its rapid growth. The water hyacinth is an aquatic macrophyte plant, originally from South America, well known for its rapid growth and the environmental problems that it causes. Water hyacinth growth occurs in water rich in nutrients, temperature, and pH. Its growth promotes the development of dense carpets making it difficult for light to pass to plants, reducing oxygen in the water and making ecosystems less fertile (DAS *et al*., 2016).
The chemical composition of water hyacinth biomass very favorable to the production of biofuels.

The water hyacinth, being a source of lignocellulosic biomass, is composed mainly of cellulose, hemicellulose, and lignin. Another characteristic feature of this plant is its high hemicellulose and cellulose content and low lignin content, which is an excellent raw material for the production of second-generation ethanol (ZHANG *et al*., 2018). Enzymatic saccharification or hydrolysis of lignocellulosic material is a fundamental step in the ethanol production process. The main objective of this process is to enable the conversion of cellulose and hemicellulose into fermentable sugars that can be converted to ethanol by microbiological action. The presence of hemicellulose and lignin hinders the access of hydrolysis reagents to cellulose, reducing the efficiency of this step. The objective of this research was to evaluate the use of water hyacinth, a highly polluting material that, through saccharification and fermentation, produces bioethanol, low-cost energy.

MATERIALS AND METHODS

Acquisition of raw material and characterization: Water hyacinth samples were collected from the Baguari Hydroelectric Power Plant reservoir, located on the Rio Doce riverbed, in the municipality of Governador Valadares-MG (19º 01'04.34 ”S; 42º 07'22.36” O; Elev 179m) (Figure 1) . After collection, they were ground in an electric shredder (John Deere-7350) to standardize the particles in an average size of 0.5 cm. Then the material was oven-dried at 60ºC for 48 hours until a constant weight was obtained. Subsequently, the dried sample was subjected to hammer milling (Quimis-340) to increase the contact surface to improve the efficiency of the analytical tests.

Ethanol Production: The fermentative processes were conducted using the hydrolyzate from enzymatic saccharification as a fermentative medium. Two distinct methodologies were used for the enzymatic hydrolysis and alcoholic fermentation process: simultaneous and separate. In both processes, Saccharomyces cerevisiae (brand FLEISCHMANN®) was used in the dehydrated form normally sold as dry and instant biological yeast. For the beginning of the fermentation process, yeast was inoculated in a proportion of 2% of the medium, followed by incubation at 28ºC under constant agitation of 36 G. The fermentation process was monitored for 25 hours in a fermentometer which consists of a system consisting of a conical Falcon® tube (50 mL) coupled to a device that allows the release of CO2 and prevents the entry of O2. This system makes it possible to monitor the fermentation process by successive weighing at regular time intervals up to constant weight. The detached CO2 mass is used to estimate the ethanol produced and consequently the concentration of fermentable sugars in the biomass (PANTOJA, 2006). The system was weighed every hour and the values obtained by the system mass loss (CO2) were used to determine the end of the fermentation. Fermentation was established within 25 hours due to the yeast growth profile, avoiding the phase of microorganism decline (DONG, *et al*, 2015). At the beginning and end of the fermentation process, 2 mL aliquots were removed for further analysis of reducing sugars (AR) according to item 4.14, and 2 mL at the end of the process to evaluate the ethanol content by distillation.

Saccharification and Simultaneous Fermentation (SSF): The simultaneous saccharification and fermentation (SSF) process used Novozymes®'s Cellic CTec2® commercial enzyme at 7 FPU / g dry mass. Weighed 1.5g of water hyacinth dry mass after acid optimized pretreatment and conditioned the sample into 50 ml Falcon® tubes coupled with airlock system (fermentometer) at 20% solid/liquid ratio in a buffer solution pH of 4.8. The hydrolysis process was initiated after the initial weighing of the fermentometers and then subjected to constant stirring in Shaker Ethiktechnology® for 36 G at 50 ° C.

Saccharification and Separate Fermentation (SHF): The enzymatic hydrolysis and separate fermentation processes presented identical conditions to simultaneous hydrolysis and fermentation. Novozymes® Cellic CTec2® commercial enzyme was used at 7 FPU / g in 1.5g dry mass of water hyacinth, the solid/liquid ratio was 20% in pH 4.8 buffer solution. The hydrolysis process was started after weighing the fermentometers and constantly shaken in Shaker Ethiktechnology® at 36 G at 50°C. The alcoholic fermentation process used the same Saccharomyces cerevisiae yeast, and the inoculum occurred after 16 hours of hydrolysis at a concentration of 2% of the total hydrolyzate volume. The fermentometer after yeast inoculation was submitted to 36 G orbital agitation at 28 ºC for 25 hours and monitoring of detachment of (CO2) followed the same criteria as (SHF).

Statistical analysis: The experiments were elaborated in triplicate and their results are presented as mean ± standard deviation.

RESULTS AND DISCUSSION

Physicochemical characterization of waste from the paper industry: The centesimal characterization of water hyacinth biomass showed a high moisture content (83.05%), allowing

Table 1. Percentage of cellulose, hemicellulose and lignin fractions in different studies

Componentes	Nigam, (2002)	Gunnarsson e Petersen, (2007)	Reales-Alfaro *et al.*, (2013)	Dados do estudo
Celulose (%)	18,20	19,50	31,67	32,50
Hemicelulose (%)	48,70	33,40	27,33	28,61
Lignina (%)	3,50	9,30	4,40	7,46

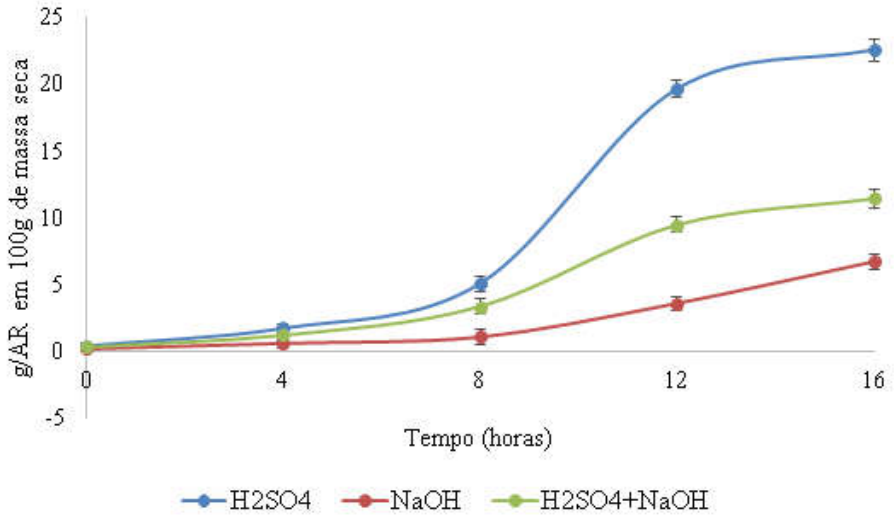

Figure 2. Percentage of fermentable sugars released in Celluclast® enzyme hydrolysis over 16 hours using water hyacinth after different types of pretreatment (acid, basic and acid + basic).

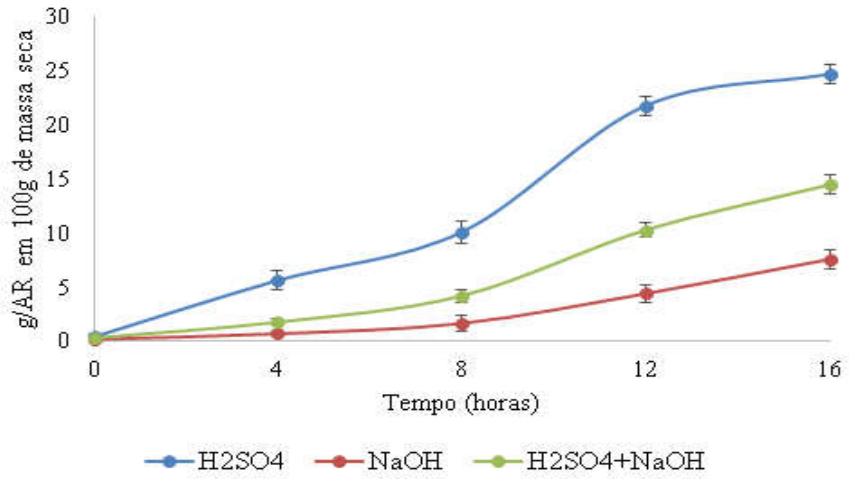

Figure 3. Percentage of fermentable sugars released in Cellic CTec2® enzyme hydrolysis over 16 hours using water hyacinth after different types of pretreatment (acid, base and acid + base)

Table 2. Type 2³ Rotational Central Composite Design with two levels of variation, 3 factors (H2SO4 concentration, S / L ratio and hydrolysis time), 6 axial points and 4 central points (15C, 16C, 17C, 18C) , autoclaved at 1 atm at 120 ° C, with the respective response factors for acid pretreatment of the dry mass of water hyacinth.

Amostra	Pré-tratamento ácido Ácido Sulfúrico (%) (v/v)	Razão (S/L) (%)	Tempo (minutos)	Açúcar redutores (hidrolisado) %
1	2,00	10,00	15,00	20,50
2	2,00	10,00	55,00	14,85
3	2,00	30,00	15,00	5,42
4	2,00	30,00	55,00	0,81
5	8,00	10,00	15,00	9,50
6	8,00	10,00	55,00	3,41
7	8,00	30,00	15,00	20,32
8	8,00	30,00	55,00	14,93
9	0,75	20,00	35,00	1,31
10	9,24	20,0,	35,00	21,29
11	5,00	5,85	35,00	21,56
12	5,00	34,14	35,00	12,35
13	5,00	20,00	6,71	20,03
14	5,00	20,00	63,28	17,71
15C	5,00	20,00	35,00	22,94
16C	5,00	20,00	35,00	23,25
17C	5,00	20,00	35,00	23,18
18C	5,00	20,00	35,00	22,87

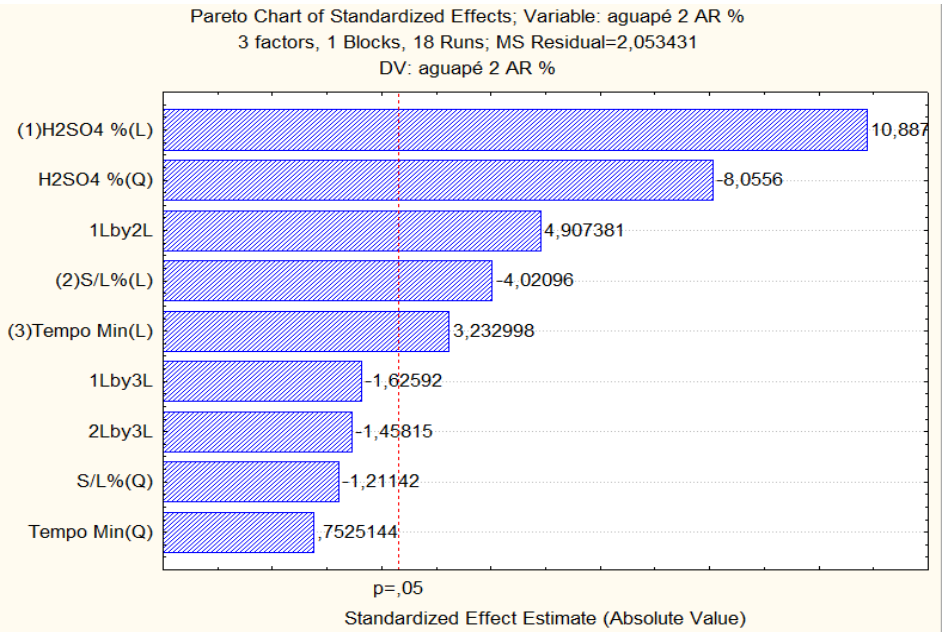

Figure 4. Pareto graph showing the effects of response factors in percentage of reducing sugars on the water hyacinth acid hydrolysis process

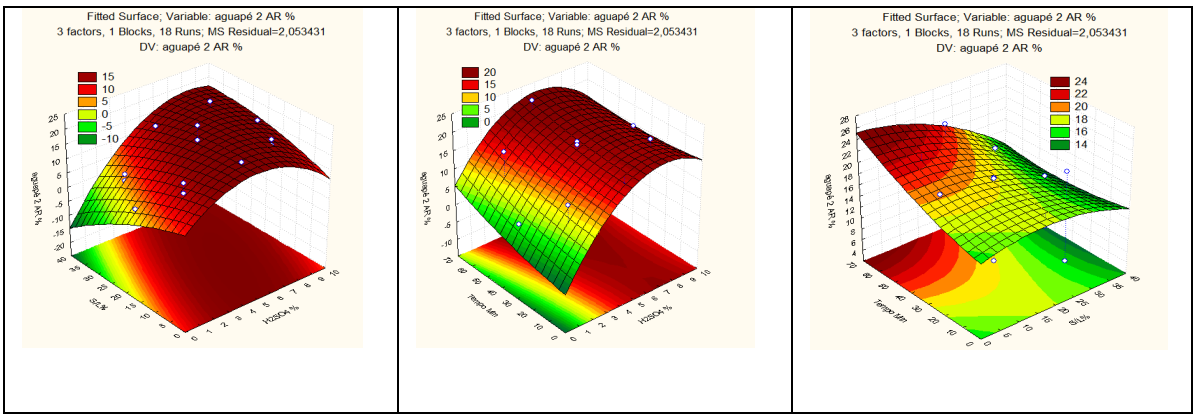

Figure 5. Response surface graphs for the percentage of reducing sugars (AR%) removed from palm oil as a function of the combined effects of time, H2SO4 concentration, and S / L ratio (solid-liquid ratio)

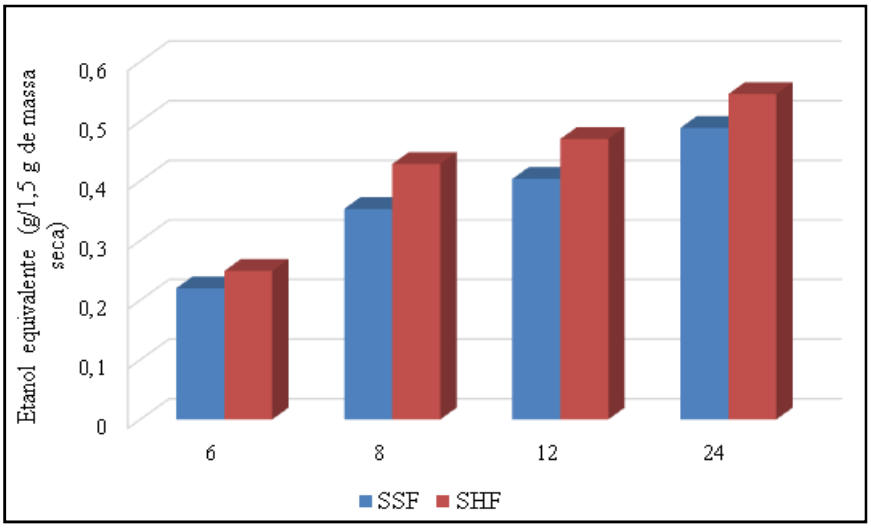

Figure 6. Ethanol production obtained from the release of carbon dioxide (CO2) during fermentation processes (SSF and SHF) from media from pretreated hydrolysate

Table 3. Comparison of carbohydrate and lignin fractions of water hyacinth (Eichhornia crassipes) before and after acid pretreatment

Frações	Antes do pré-tratamento ácido %	Após do pré-tratamento ácido %
Celulose	32,54 ±0,86	43,35 ±0,46
Hemicelulose	28,61 ±1,16	5,52 ±0,17
Lignina	7,46 ±0,24	6,73 ±0,36

Table 4. Variables of fermentative processes (SSF and SHF) using as fermentation agent S. cerevisiae yeast after acid optimized pretreatments and subsequent enzymatic saccharification

Processo Fermentativo	G_i g/L	G_f g/L	Tempo (hs)	$EtOH_f$ g/L	Yp/s (g_p/g_s)	Q_p (g/L/h)	E_f (%)
SHF	5,93	0,53	25	2,62	0,50	0,109	98
SSF	3,40	0,48	25	1,51	0,49	0,062	96

Yp/s = rendimento de produção em etanol; Qp= produtividade volumétrica e *Ef*= eficiência fermentativa Gi=glicose inicial Gf= glicose final

us to consider that each ton of water hyacinth is obtained about 200 kg of dry mass. Ash content (16.68%), Lipid (3.5%), Protein (12.74%) and Crude Fiber (67.02%) presented similar results to the study by REZANIA *et al*., 2015; GAO *et al*., 2016, HENRY-SILVA and CAMARGO, 2006, NIGAM, 2002 describing results referring to Eichhornia crassipes dry mass with 70% Crude Fiber, 4.7% Lipids, 25% Ash and Protein 13% . The ash content is high compared to other vegetables because of the plant's ability to remove inorganic substances from water bodies such as heavy metals. Water hyacinth, like most other lignocellulosic biomasses, varies in their chemical composition, which is related to various factors, such as harvesting phase, source reservoir, climatic factors, and water reservoir composition. The contents of cellulose (32.50%), hemicellulose (28.61%) and lignin (7.46%) found in this study, listed in Table 1, are similar to the data described by (Reales-Alfaro, 2013) which proposed the production of ethanol from from water hyacinth by pretreatment with sulfuric acid (2% v/v) and described considerable fraction of hemicellulose (21.33%) with low lignin content (4.40%), lignin presented by water hyacinth contributes to the lower crystallinity and recalcitrance of the biomass, which directly reflects the better efficiency of the hydrolysis process and the conversion of sugars to ethanol. Another interesting aspect is the concentration of available carbohydrates that comprise the sum of the contents of Cellulose (32.50%) + Hemicellulose (11.13%) + Starch (2.62%) and total soluble sugars (1.98%).) making a total of 48.20% carbohydrates. These data reflect a stoichiometric projection of 166L of ethanol per tonne of dry mass, which makes this residue a raw material with interesting potential for ethanol production.

The results obtained by enzymatic hydrolysis showed the maximum peak sugar conversion in 16 hours when using both Celluclast® and Cellic CTec2® enzymes. The comparative analysis between the different types of pretreatment highlights the treatment with sulfuric acid which presented 22.60g reducing sugars using Celluclast® (Figure 2 and Figure 3) and 24.74g using the Cellic CTec2® enzymatic cocktail. Given the results obtained in biomass hydrolysis after acid treatment and comparing the different enzymatic compounds, a better yield was observed using the Cellic CTec2® * enzyme ($p < 0.05$).

DCCR Experimental Design for Optimizing Diluted Sulfuric Acid Pretreatment: To establish the best condition for dilute acid pretreatment, an experimental DCCR design was proposed for the optimization of acid pretreatment. The conditions established by the experimental design as well as the results presented are shown in Table 2. The results showed on Table 2, that the maximum release of reducing sugars present in the hydrolyzate (23.25%) occurred in the test referring to the condition used for the central point (15C, 16C, 17C, and 18C). Thus, the condition established for the optimization of acid pretreatment was: 5% H2SO4 concentration, 20% solid-liquid ratio and 35 minutes reaction.

Table 2: Type 2^3 Rotational Central Composite Design with two levels of variation, 3 factors (H2SO4 concentration, S / L ratio and hydrolysis time), 6 axial points and 4 central points (15C, 16C, 17C, 18C) , autoclaved at 1 atm at 120 ° C, with the respective response factors for acid pretreatment of the dry mass of water hyacinth. Importantly, the content of reducing sugars released in the central point condition (23.2%) indicates that acid pretreatment promoted approximately 82% of the conversion of hemicellulose fractions (28.17%). Optimization of acid pretreatment may contribute to greater efficiency in removing hemicellulose fractions and preserving cellulose concentrations, which may be significant for better saccharification process efficiency (NANDA, *et al*., 2013). The estimated effects of the studied variables and their interactions are shown below in Figure 4, and highlighted in red are the significant effects at $p < 0.05$. The analysis of the Pareto graph represented in Figure 4, also makes it possible to observe the estimated values of the effects of each variable, as well as to verify whether or not they were statistically significant at $p < 0.05$. The results indicate that the most relevant factor, at a significance level of 95% ($p < 0.05$), was the acid concentration, which presented a significant and positive linear effect and a significant and negative quadratic effect. for the removal of hemicellulose, represented by the release of reducing sugars. The positive linear effect indicates that increased H2SO4 concentration led to a greater release of AR into the medium (Figure 4). Regarding the solid-liquid ratio, there was a significant negative linear effect for AR, which is probably due to the limitation of mass and heat transfer due to the increase of the solid fraction over the liquid phase, water, solvent and process reagent, where the catalyst (H2SO4) is present. The time had a positive and significant linear effect on the acid hydrolysis process, a predictable consequence of the sugar degradation kinetics by acid hydrolysis. To better analyze the combined effects exerted by each factor on the release of RA, response surface graphs were generated (Figure 5). The graphs presented showed that the maximum release of RA occurred within a range of 3 to 7% H2SO4 concentration, 20 to 35 minutes of pretreatment and a solid to liquid ratio of 15 to 20%. The centesimal chemical characterization of solid residue after optimized acid pretreatment showed changes in the chemical composition of water hyacinth (Table 3). The cellulose concentration was increased to 43.35% in the pretreated solid, hemicellulose concentration was reduced to 5.52% and lignin obtained a small reduction to 6.73%, improving the efficiency of the enzymatic saccharification process. Lignin fractions present in lignocellulosic biomass represent conditions harmful to the action of microorganisms, as well as enzymatic activity. Therefore, substrates with low lignin concentration have higher enzymatic activity (NANDA *et al*., 2013).

Fermentation Processes: The fermentation process of Saccharification and Separated Fermentation presented higher concentrations of ethanol compared to Simultaneous Saccharification in all analyzed periods (6, 8, 12, 24 hours). The results, available in Figure 6, presented at 24 hours of fermentation showed a higher concentration of ethanol 0.54 g (SHF) and 0.49 g (SSF) * ($p < 0.05$). The results presented were superior to those found by Satyanagalakshmi, *et al*., 2011 who obtained approximately 0.29 g of ethanol using the water hyacinth after optimizing diluted H2SO4 pretreatment. The difference between the process results can be explained by the fact that SHF works under optimal pH and temperature conditions, which provides better cellulase enzymatic activity and better yeast metabolic activity (fermentation), the same does not occur in the process. SSF because it works in an intermediate range of the optimal pH and temperature values of the enzyme and the fermentable microorganism (SUN; CHENG, 2002). The determination of ethanol content was established by pre-distilled samples and subsequently quantified according to the method described by Pilone (1985), whereas the final hydrolytic efficiency (Ef) values were calculated according to equation 13. At the end of the fermentation process, it was possible to observe the

concentrations of glucose, ethanol and yield variables, these values are presented in Table 4. Based on the data presented, it can be observed that practically all glucose was converted to ethanol. However, at the end of the process, a small residual glucose concentration of 0.48 g / L (SSF) and 0.53g / L (SHF) was observed, indicating the presence of non-fermentable sugars. Ethanol concentrations obtained after the distillation process show better results through the SHF 2.62 g / L process when compared to SSF 1.51 g / L results. Ethanol concentrations obtained in both SSF and SHF processes were higher than the 1.40 g / L described by Zhang *et al*., 2018 using water hyacinth after acid optimized pretreatment. Ethanol yield and fermentative efficiency were considered important variables of the fermentative process. As described in the previous results, the SHF process showed higher yield in ethanol production SHF 0.50 Yp / s and fermentative efficiency 98% (Ef). The data of the process variables found in this study were superior to those described by Sornvoraweat, Kongkiattikajorn (2010) who found a fermentative efficiency (Ef) close to 96%, working with water saccharification and separate fermentation, with S.cerevesiae as a fermentative agent. The values obtained for Ef were also higher than those found by Das, *et al*. (2016) and Yan, *et al*. (2015), using water hyacinth as input for bioethanol production in different fermentation processes with the values of Ef of 83.14% and 86.22%, respectively.

Conclusão

A caracterização química da aguapé (*Eichhornia crassipes*) resultou na determinação do valor de 48,20% de carboidratos totais, que se convertidos a etanol resultaria em 312 L por tonelada de resíduo. O uso exclusivo da fração celulósica, 32,5%, permite projetar produção de 210 L de etanol por tonelada de massa seca de aguapé. Portanto, a biomassa de aguapé apresenta boas condições para a produção do etanol de segunda geração. Neste contexto, conclui-se que a melhor metodologia encontrada para a produção de etanol de segunda geração por meio de *Eichhornia crassipes* (aguapé) é através do pré-tratamento com ácido sulfúrico diluído. O processo de otimização do pré-tratamento ácido com o uso do planejamento DCCR demonstrou-se bem eficiente promovendo cerca de 85% da remoção de hemicelulose. O processo de sacarificação enzimática da aguapé após o pré-tratamentos ácido otimizado proporcionou conversão superior de 80% da celulose a glicose, onde a enzima Cellic CTec2® demonstrou melhor desempenho que o composto enzimático Celluclast®. A sacarificação e fermentação separada (SHF - *Separated Hydrolysis and Fermentation*) demonstrou melhor desempenho na produção alcoólica em relação a sacarificação e fermentação simultânea, produzindo cerca de 2,62 g/L de etanol. O uso da levedura de panificação *Saccharomyces cerevisiae* (Fleischmann®) mostrou-se adequada para a conversão dos açúcares derivados do pré-tratamento da aguapé, obtendo-se YP/S de 0,50 de rendimento de produção de etanol e 98% de eficiência fermentativa.

REFERÊNCIAS

Baeyens, J., Kang, Q., Appels, L., Dewil, R., LV, Y., Tan, T. Challenges and opportunities in improving the production of bio-ethanol. Progress in Energy and Combustion Science 47 60-88. 2016.

Das, S.P., Gupta, A., Das, D., Goyal, A., Enhanced bioethanol production from water hyacinth (Eichhornia crassipes) by statistical optimization of fermentation process parameters using Taguchi orthogonal array design. Int. Biodeterior. Biodegrad. 109, 174–184. 2016.

Das, A. Paul, T., Ghosh, P.Ghosh, U., Production of bioethanol as useful biofuel through the bioconversion of water hyacinth (Eichhornia crassipes). Biotech, v. 6, n. 1, p. 70, 2016.

Dong, S., YI, C.F., LI, H., Changes of Saccharomyces cerevisiae cell membrane components and promotion to ethanol tolerance during the bioethanol fermentation. *The International Journal of Biochemistry & Cell Biology* 69 196–203. 2015.

Henry-Silva, G.G., Camargo, A.F.M.. Composição química de macrófitas aquáticas flutuantes utilizadas no tratamento de efluentes de aqüicultura. Planta daninha, Viçosa , v. 24, n. 1, p. 21-28, 2006.

Negahdar, L., Delidovich, I., Palkovits, R. Aqueous-phase hydrolysis of cellulose and hemicelluloses over molecular acidic catalysts: Insights into the kinetics and reaction mechanism. Applied Catalysis B: Environmental 184 285–298. 2016.

Neves, P.V., Pitarelo, A.P., Ramos, L.P. Production of cellulosic ethanol from sugarcane bagasse by steam explosion: Effect of extractives content, acid catalysis and diferente fermentation Technologies. Bioresource Technology 208 184–194. 2016.

Nanda, S. Kozinski, J.A. Dalai, A.K. Biomass-an overview on classification, composition and characterization. Biomass Processing, Conversion and Biorefinery, Pages 1-35. 2013.

Nigam, J. N. Bioconversion of water-hyacinth (Eichhornia crassipes) hemicellulose acid hydrolysate to motor fuel ethanol by xylose-fermenting yeast. *J. Biotechnol.* 97(2), 107 116. (2002).

Pantoja L., Maeda, R.N., Carvalho, S.M.S., Aguiar, J. P.L., Monteiro, F.M., Lima, Q. A., Mendonça, F. G., Yuyama, L.K.O., Pereira, N.J. (2006) Aprovechaimento biotecnológico de la guanabana en la elaboración de bebidas alcohólicas fermentadas utilizando levadura inmovilizada em alginato. Brazilian Journal of of Food Technology , 96.

Pilone, G. J. Determination of ethanol in wine by titrimetric and spectrophotometric dichromate methods: collaborative study. *Journal - Association of Official Analytical Chemists*, v. 68, p. 188-190, 1985.

Rezania, S., Ponraj, M., Din, M.F.M., Songip, A.R., Sairan, F.M., Challiapan, S. The diverse applications of water hyacinth with main focus on sustainable energy and production for new era: An overview. Renewable and Sustainable Energy Reviews, v. 41, p. 943–954, 2015.

Sun, Z., Shupe, A., Liu, T., HU, R., Amidon, T.E., LIU, S. Particle properties of sugar maple hemicellulose hydrolysate and its influence on growth and metabolic behavior of Pichia stipitis. Bioresource Technology 102 2133–2136.2011.

Timung, R., Mohan, M., Chilukoti, B., Sasmal, S., Banerjee, T., , Goud, V.V. Optimization of dilute acid and hot water pretreatment of different lignocellulosic biomass: A comparative study. Biomass and Bioenergy 81 9-18.2015.

Verardi, A., Blasi, A., Molino, A., Albo, L., CALABRO. Improving the enzymatic hydrolysis of Saccharum officinarum L. bagasse by optimizing mixing in a stirred tank reactor: Quantitative analysis of biomass conversion. Fuel Processing Technology, v. 149, p. 15–22, 2016.

Yan, J., Wei, Z., Wang, Q., He, M., Li, S. and Irbis, C. Bioethanol production from sodium hydroxide-hydrogen

peroxide-pretreated newly isolated thermotolerant Kluyveromyces marxianu strain. *Bioresource technology*. 2015,

Zhang, P. Y-H, Himmel, M.E., Mielenz, J.R., Outlook for cellulase improvement: Screening and selection strategies. *Biotechnology Advances* 24 452–481.2018.

Zhou, Z., Xue, W., Lei, F., Cheng, Y., Jiang, J., Sun, D. Kraft GL-ethanol pretreatment on sugarcane bagasse for effective enzymatic hydrolysis. *Industrial Crops and Products,* v. 90, p. 100–109, 2016.

Zhuang, X., Wang, W., YU, Q., Wang, W.Q.Q., Tan, X., Zhou, G., Yuan, Z. Liquid hot water pretreatment of lignocellulosic biomass for bioethanol production accompanying with high valuable products. *Bioresource Technology* 199 68–75.2016.

****