

Etanol from water hyacinth: A preliminary study of chemical pretreatment and enzymatic hydrolysis**Etanol de aguapé: um estudo preliminar de pré-tratamento químico e hidrólise enzimática**

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ABSTRACT

Considered a perennial plant, abundantly available, biodegradable and without competing with agricultural crops, the water hyacinth fulfills criteria considered necessary for biofuels production. The feasibility of producing ethanol from different pretreatments, in the liquor and in its broth after enzymatic hydrolysis, is investigated in this work. Only the liquor from the acidic pretreatment and after detoxification fermented well. An efficiently enzymatic hydrolysis occurred when the acidic and alkaline pretreatment were used, showing the action of these processes in the disruption of lignocellulose matrix, reaching higher sugars solubilization and, consequently, higher fermentation capacity.

Keywords: ethanol, pretreatment, biomass, fermentation capacity

RESUMO

Considerado uma planta perene, abundantemente disponível, biodegradável e sem competir com as culturas agrícolas, o jacinto-d'água preenche os critérios considerados necessários para a produção de biocombustíveis. A viabilidade de produzir etanol a partir de diferentes pré-tratamentos, no licor e no caldo após a hidrólise enzimática, é investigada neste trabalho.

Apenas o licor do pré-tratamento ácido e após a desintoxicação fermentou bem. Ocorreu hidrólise enzimática eficiente quando foram utilizados os pré-tratamentos ácidos e alcalinos, mostrando a ação desses processos na ruptura da matriz da lignocelulose, atingindo maior solubilização dos açúcares e, conseqüentemente, maior capacidade de fermentação.

Palavras-chave: etanol, pré-tratamento, biomassa, capacidade de fermentação

1 INTRODUCTION

Water hyacinth (*Eichhornia crassipes*) is a free-floating perennial plant, with dark green leaves circular to elliptical, attached to an inflated spongy petiole and a thick root, heavily branched, and dark fibrous which is present underneath the water. Due to its efficiency in the use of aquatic nutrients and solar energy for biomass production, it is considered the worst invasive aquatic plant in the world, leading to serious social, economic, and environmental problems. Excessive growth can result in complete coverage of aquatic surfaces, degrading natural habitats in several ways, causing the proliferation of insects, larvae and mollusks that transmit diseases, affecting the development of animal species, reducing the water flow in canals, dams and rivers, as well as difficulty in navigation and fishing (Pompêo, 1999; Martins & Pitelli, 2005; Zhang et al., 2010; Holanda et al., 2015).

Singh et al. (1984) reported a daily average productivity of water hyacinth of 0.26 ton of dry biomass per hectare in all seasons, whose seeds can remain viable for 20 years or longer. The enormous reproductive capacity of this perennial plant, biodegradability, without competing with agricultural crops, rich in cellulose (18,4%) and hemicellulose (49,2%), with a very low amount of lignin (3,6%), fulfills criteria considered necessary for the production of biofuels (Kumar et al., 2009). Favaro & Miranda (2013) estimated a cellulosic ethanol production potential equivalent to 104 liters per ton of dry biomass, with the hemicellulosic fraction being also used.

According to Hronnich et al. (2008), it is necessary to evaluate the viability of the water hyacinth as an energy crop through the collection, concentration, pretreatment and hydrolysis methods, finding the parameters that most influence the costs of the process.

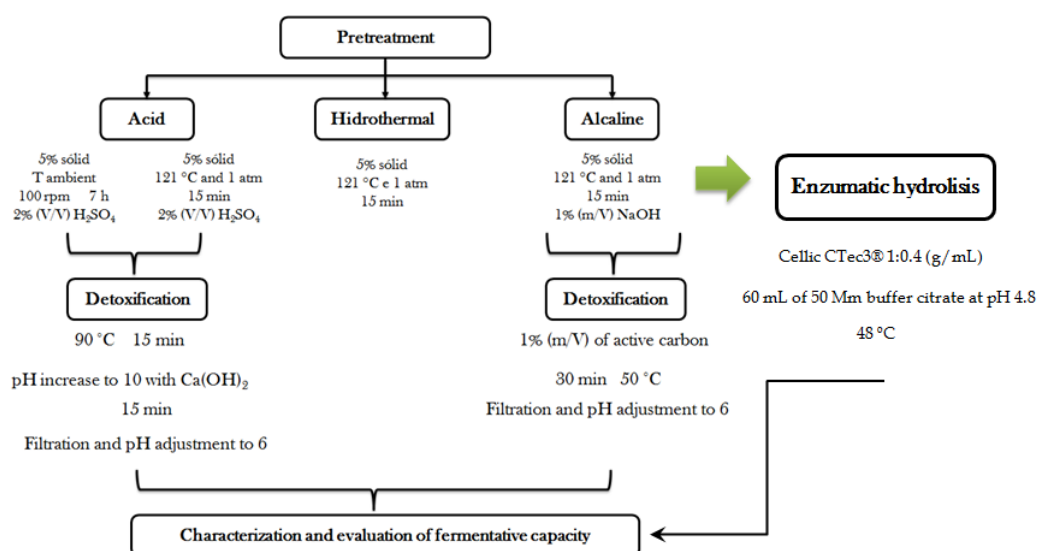
Considering the above mentioned, this work evaluates the influence of different pretreatments of dry and ground biomass on sugar release and ethanol fermentation capacity, in the pretreated broth as well in the residue submitted to enzymatic hydrolysis, aiming at an ethanol 2G perspective (lignocellulosic ethanol).

2 MATERIALS AND METHODS

The water hyacinth was collected with the Petroleum and Energy from Biomass Research Group (PEB) at the Macela weir, in Itabaiana-SE, Brazil, suspending the entire clump of the plant to the surface and storing it in plastic bags. In the laboratory, the roots were removed and the stem and leaves were cut into small pieces and dehydrated in a dryer with forced air circulation at 50 °C until constant weight. The dry biomass was ground in a knife mill and stored at room temperature.

The analytical treatments performed in this work is detailed in Figure 1.

Figure 1. Analytical pretreatment and enzymatic hydrolysis procedure to characterize and evaluate the fermentation capacity



Acidic (H₂SO₄ 2% (V/V), alkaline (1% NaOH (m/V) and hydrothermal pretreatment were used with 5% (m/V) biomass, in autoclave at 121 °C for 15 min. After treatment, the samples were filtered and part of the hydrolyzed broth was subjected to detoxification with Ca(OH)₂ (Kumar et al., 2009). The filtration residue was washed to neutrality, dried at 50 °C and subjected to enzymatic hydrolysis with the enzyme Cellic CTec3®, in the ratio of 1:0.4 (g:mL) with 60 mL of 50 Mm buffer citrate at pH 4.8. This suspension was brought to a water bath at 48 °C, with occasional agitation, and samples were collected at 48 and 72 h.

The total reducing sugars (TRS) were determined by the DNS colorimetric method (Miller, 1959), after hydrolysis of the extract with H₂SO₄ 1.5 M and neutralization with NaOH 2 N (Silva et al., 2015). Total reducing sugars (% TRS) and mass yield (% MY) content released in the pretreatment liquor were calculated according Equations 1 and 2, respectively,

where C (g/L) is the TRS concentration obtained from a glucose standard curve, V (L) is the volume of the extracted liquor and Biomass (g) is the waste mass. The mass yield considers the biomass's initial and final weights (before and after the pretreatment).

$$\% \text{ TRS} = 100 \cdot \frac{C \text{ (g/L)} \cdot V \text{ (L)}}{\text{Biomass (g)}} \quad (1)$$

$$\% \text{ MY} = 100 \cdot \frac{m_{\text{final}} \text{ (g)}}{m_{\text{initial}} \text{ (g)}} \quad (2)$$

The fermentation capacities, in the pretreatment and enzymatic hydrolysis broths, were conducted in test tubes with screw cap and Durhan tube, with addition of 10% (V/V) of a suspension of *Pichia stipitis* NRRL Y-7124 yeast, gently donated by Embrapa Agroenergia. The efficiency of the fermentation was considered as higher CO₂ formation in the Durhan tube.

3 RESULTS AND DISCUSSION

The results of the TRS contents released and the evaluation of the fermentative capacity are presented in Table 1. For comparison, enzymatic hydrolysis was performed in the residue without pretreatment. After the acid pretreatment, the pH in the liquor was 1.0, while in the alkaline was above 14. In the hydrothermal, the pH was 6.0. One part of the liquor had the pH adjusted directly to 6.0, while another underwent detoxification and had its subsequent adjustment to this pH.

The acid and alkaline pretreatments generated lower mass yields and, as expected, good release of sugars. The hydrothermal treatment was not efficient, resulting in a lower amount of liberated total reducing sugars (% TRS). The liquor from acidic pretreatment, after detoxification, was the most promising in the release of sugars and showed a high fermentation capacity (qualitative). This is important because the acidic pretreatment is effective to remove hemicellulose from the matrix (Mosier et al., 2005) and *Pichia stipitis* is able to ferment xylose (principal hemicellulose component), mainly if no inhibition is verified (Silva et al., 2016).

Table 1. Evaluation of the total reducing sugar and fermentative capacity indifferent pretreatments

Treatment	MY (%)	Pretreatment broth			Enzymatic hydrolisis broth		
		Detoxification	TRS (%)	Fermentation capacity	Time (h)	TRS (%)	Fermentation capacity
H ₂ SO ₄ 2% (V/V)	29,96	No	13.06 ± 1.21	+	48	50.18 ± 1.24	++
		Yes	14.32 ± 0.78	++	72	62.93 ± 4.82	+++
NaOH (1% (m/V)	32,66	No	3.81 ± 0.07	-	48	85.33 ± 0.75	+++
		Yes	10.07 ± 0.36	-	72	61.27 ± 2.98	+++
Hydrothermal	66.86	No	2.11 ± 0.22	+	48	28.69 ± 1.10	+
		Yes	2.03 ± 0.07	+	72	29.72 ± 1.98	+
Water hyacinth	-	-	-	-	48	12.75 ± 3.81	+
		-	-	-	72	24.85 ± 0.19	+
- does not ferment		+ little fermentation		++ good fermentation		+++ excellent fermentation	

It was possible to observe the importance of the previous pretreatment to enzymatic hydrolysis, where the residues pretreated in acid and alkaline medium presented expressive values of released sugars (74.6 and 85.1% in 48 h and 60.5 and 59.4% in 72 h, respectively) in comparison with the hydrothermal and no treated conditions. These results were also reflected in an excellent fermentation capacity of these broths.

4 CONCLUSIONS

The liquor of the acidic pretreatment showed higher fermentation capacity, and more promising to the fraction. On the other hand, to the enzymatic hydrolysis, acidic and alkaline indicated an effective action and the most of sugars were solubilized and fermented.

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