ENZYMATIC HYDROLYSIS OF WASTE OFFICE PAPER FOR ETHANOL PRODUCTION BY *SPATHASPORA PASSALIDARUM*

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Waste office paper was used as a feedstock for the production of bioethanol. Saccharification ofwaste office paper by sequential acid and enzyme hydrolysis produced a high quality hydrolysate that ensured its fermentability to produce ethanol. The optimal saccharification process resulted in total 42 g/L of glucose (63% cellulose conversion). *Spathaspora passalidarum* HMD 14.2 was able to coferment with glucose (98%), xylose (74%) and cellobiose (45%) released in the hydrolysate from waste office paper. There was an ethanol production of13 g/L. An ethanol yield of0.42 g/g, fermentation efficiency of 82% and volumetric ethanol productivity of 0.57 g/L/h were achieved. In our study, ethanol production was about 300% higher than that using acid hydrolysis and *Spathaspora passalidarum* HMD 14.2.

Keywords: fermentation, cellulose, lignocellulosic, bioethanol, waste paper

INTRODUCTION

Cellulosic biomass, such as waste newsprint, office paper, and municipal solid waste, is a relatively cheap and abundant alternative substrate for ethanol production.[1,2] Acid or enzymatic hydrolysis of these materials produces glucose and xylose.[3] However, the inability to conferment glucose and xylose is a major challenge to the economical use of lignocelluloses as a feedstock.[4]

Microorganisms suitable for the fermentation of paper enzymatic hydrolysis product should be able to ferment a wide range of saccharides, because other saccharides, like glucose, e.g. xylose and cellobiose, are also formed, to a smaller extent, by the hydrolysis of waste paper.[5]

Spathaspora passalidarum NN245[6] coassimilates xylose and glucose aerobically, uses xylose faster than glucose when the sugars are presented individually, and coferments glucose, xylose, and cellobiose from mixtures of pure sugars or hydrolysates under oxygen-limiting conditions.[7] Lima *et al*.[8] reported a higher ethanol production by *S. passalidarum* HMD 14.2 than by industrial strains of *Saccharomyces cerevisiae* when acid hydrolysate from waste office paper was used. Simultaneous glucose and xylose consumption was observed.

The results indicated that *S. passalidarum* HMD 14.2 produces ethanol not only from glucose, but also from xylose.

The current work aims to compare ethanol production from waste office paper using enzymatic hydrolysis with and without acid pretreatment. Separate hydrolysis fermentations with *S. passalidarum* HMD 14.2 were carried out with the enzymatic hydrolysate obtained after acid pretreatment, containing higher carbohydrate concentration (42 g/L glucose and 8 g/L xylose).

EXPERIMENTAL

Enzymatic hydrolysis

Waste office paper collected at the Laboratory of Bioprocess and Bioproducts from the Federal University of Pernambuco, Brazil, was used. For acid pretreatment, paper (2, 4, 8 and 10 g) was cut into pieces of 0.5 cm and mixed with 100 mL of1% V/V sulfuric acid solution in 500mL Erlenmeyer flasks. Acid pretreatment was carried out in a rotary incubator (TECNAL TE-421) at 50 ºC and 150 rpm, for 3 h. Pretreated waste office paper was recovered by filtration in qualitative filter paper. The solid fraction was used for enzymatic hydrolysis, which was performed in 500mL Erlenmeyer flasks, in the rotary shaker at 50 ºC and 150 rpm, during72 h, after addition of 100 mL sodium citrate buffer (pH 4.8). Enzymatic hydrolysis without acid pretreatment was also

performed. Celluclast 1.5L (68 FPU/mL) and a β-glycosidase (1340 CBU/mL) preparation (Novozym 188) were used. Enzyme loadings between 20 and 71FPU/g of waste office paper and 50% V/V (of the volumetric Celluclast 1.5L addition) β-glucosidase were used.

Microorganism and fermentations

The strain of *S. passalidarum* (HMD 14.2) was maintained in a solid medium containing 20 g/L glucose, 10 g/L yeast extract, 20 g/L peptone and 15 g/L agar, at pH 7.0.For the preparation of precultures, a loopful of cells was transferred from the agar slants to250mL flasks containing 50 mL of culture medium (similar to that used above, but without addition of agar), and incubated at 30 ºC for 7 h. Cells were harvested by filtration (0.45 µm filter), suspended in sterilized distilled water and used to inoculate the fermentation medium containing: $(NH_4)_2SO_4$(2 g/L); KH_2PO_4(2 g/L); $MgSO_4.7H_2O$ (0.75 g/L); yeast extract (4 g/L). These nutrients were dissolved in the enzymatic hydrolysate. Duplicate fermentations were carried out at 30 ºC, 80 rpm for 24 h, using 0.5 g/L of the initial biomass concentration in 250mL flasks with working volumes of 100 mL.

Determination of substrates and products

Samples were withdrawn, filtered (0.45 µm filter), and submitted to high performance liquid chromatography (Agilent HP 1100, Germany) analysis. Cellobiose, glucose, xylose, carboxylic acids, furfural, hydroxymethylfurfural (5-HMF), glycerol and ethanol were quantified in an Aminex HPX-87H$^+$ (Bio-Rad, Hercules, CA, USA) column at 60 ºC, using 5 mM H_2SO_4 at a flow rate of 0.6 mL/min as mobile phase, and detected using an RI-detector.

Glucose and ethanol yields, volumetric productivity and efficiency

The glucose yields (*Y*) from waster office paper, with and without acid pretreatment, obtained in enzymatic hydrolysis, were calculated by Equation 1.Ethanol yields ($Y_{E/G}$), obtained in the fermentations, were calculated by Equation 2. Volumetric productivity (Q_P) and fermentation efficiency (E_f) were calculated using Equations 3 and 4, respectively.

$$Y = (G)\frac{V}{m} * 100 \quad (1)$$

$$Y_{\frac{E}{G}} = \frac{\Delta E}{\Delta G} \quad (2)$$

$$Q_P = \frac{\Delta E}{\Delta t} \quad (3)$$

$$E_f = \frac{Y_{\frac{E}{G}}}{0.511} * 100 \quad (4)$$

where *G* (g/L) – glucose concentration; *V* (L) –volume of mixture (waste office paper and buffer); *m* (g) – mass of waste office paper (with or without acid pretreatment); ΔE (g/L) – variation of ethanol concentration; ΔG (g/L) – variation of glucose concentration; Δt (h)variation of fermentation time; 0.511 g/g (theoretical yield, determined by stoichiometric reaction of ethanol production from glucose).

RESULTS AND DISCUSSION

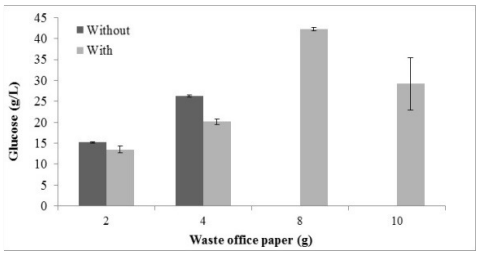
Carbohydrates, organic acids, furfural and 5-HMF were not detected in the liquid fraction after acid pretreatment, probably due to the moderate conditions in this step (50 ºC, 150 rpm, duration 3 h). After 96 hours of enzymatic hydrolysis, glucose and xylose concentrations were 15 g/L (Figure 1a) and 2.5g/L (Figure 1b), respectively, in enzymatic hydrolysis without acid pretreatment, using 2 g of waste office paper. On the other hand, glucose and xylose concentrations for 4 g of waste office paper were 26 g/L and 4 g/L, respectively. Enzymatic hydrolysis with 8 or 10 g of paper was not possible without acid pretreatment, since the material remained intact during all the evaluated period.

The highest glucose (Figure 1a) and xylose (Figure 1b) concentrations were obtained during enzymatic hydrolysis with acid pretreatment, using 8 g of waste office paper. Lower carbohydrate concentrations were found for the hydrolysis with 10 g of paper than for the hydrolysis with 8 g of paper. This was probably due to hard mixing and lower enzyme load.

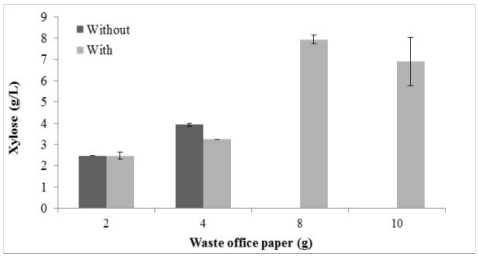
Figure 2 shows the glucose yields (*Y*) obtained during enzymatic hydrolysis of waste office paper with and without acid pretreatment. The results were significantly different for the assays with 2 and 4 g of waste paper, which led to lower yields using acid pretreatment. The yields showed a similar profile of glucose and xylose concentrations (Figures 1a and 1b). The highest glucose yield (*Y*) was obtained with 2 g of waste paper, without pretreatment (about 80%).

The pretreatment with dilute sulfuric acid in the rotary incubator was essential for enzymatic hydrolysis with masses of 8 and 10 g of paper. However, the enzymatic hydrolysate obtained with 8 g was selected for fermentation, since it allowed the highest glucose concentration (42 g/L). Wu *et al*.[9] obtained about 12 g/L of fermentable sugars (glucose, galactose and mannose) from waste newspaper, after pretreatment with 0.1N NaOH for 12 h and sequential enzyme hydrolysis.

Fermentations were carried out using *S. passalidarum* HMD 14.2. Figure 3 shows cellobiose, glucose, xylose, glycerol and ethanol concentrations. Glucose demonstrated the highest consumption (98.2%), followed by xylose (74.8%) and cellobiose (45.3%), after 24 hours of fermentation. These values indicate the preference of *S. passalidarum* HMD 14.2 for glucose uptake. Simultaneous cofermentation of glucose, xylose, and cellobiose is problematic for most microorganisms because glucose represses the use of the other saccharides. Surprisingly, *S. passalidarum*, which ferments xylose and cellobiose natively, can also coferment these two sugars in the presence of 30 g/L of glucose.[4] Lima *et al*.[8] reported that in ethanol production by *S. passalidarum* HMD 14.2, using acid hydrolysis of waste office paper, the consumption of these carbohydrates could be observed.



(a)



(b)

Figure 1:Effects of waste office paper mass on the release of glucose (a) and xylose (b) measured after enzymatic hydrolysis during 96 hours, with and without acid pretreatment

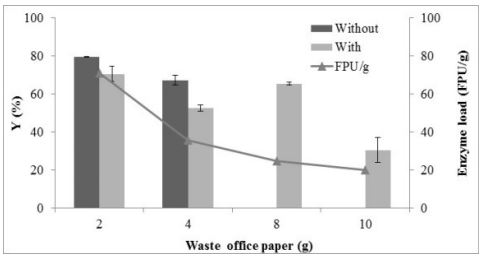


Figure 2: Effects of waste office paper mass and enzyme load on the glucose yield obtained from enzymatic hydrolysis during 96 hours, with and without acid pretreatment

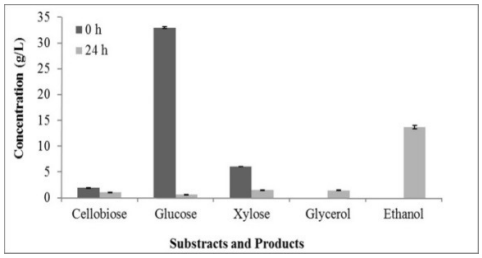


Figure 3: Substracts and product concentrations initially and after 24 hours of fermentation of the enzymatic hydrolyzate

Glycerol production was lower than 2 g/L and acetic acid was not detected. Ethanol production achieved 13 g/L after 24 hours (Figure 3). Volumetric productivity (Q_P), ethanol yield from glucose ($Y_{E/G}$) and fermentation efficiency (E_f) were of 0.57 g/L·h, 0.42 g/g and 82.8%, respectively. Thus, it is estimated that 1 g of waste office paper can be converted to 0.163 g of ethanol by the processes described here (13 g/L of ethanol and 80 g/L of waste office paper). On a large scale, based on the optimum conditions found in this study (8 g paper mass with acid pretreatment), it can estimated that an ethanol yield of 163 Kg/1000 kg of waste office paper can be obtained, which divided by the ethanol density of 0.789 Kg/L equates 206 L/1000 Kg. In fermentations using *S. passalidarum* HMD 14.2 and acid hydrolysis, 3.52 g/L ethanol (45 L/1000 Kg) was produced using 100 g/L of waste office paper.[8]

CONCLUSION

Acid pretreatment was essential in the enzymatic hydrolysis of 8 and 10 g of waste office paper. Thus, a hydrolyzate of adequate quality without fermentation inhibitors was produced. *S. passalidarum* HMD 14.2 contributed to high efficiency and yield, indicating a potential for ethanol production from hydrolysates containing glucose and xylose. Waste office paper was found to be a promising feedstock for ethanol production using *S. passalidarum* HMD 14.2after the pretreatment with dilute sulfuric acid.

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REFERENCES

[1] I. K. Park, K.Kyunghee, S. Hoyong, R. Inkoo, J. Ingnyol *et al*., *Process Biochem*., **45**, 4872 (2010).
[2]A. K. Dubey, P. K. Gupta, N. Garga, S.Naithani, *Carbohyd. Polym*., **88**, 825 (2012).
[3]L. Wang, M. Sharifzadeh, R.Templer, J. Murphy, *Appl. Energ*., **111**, 1172 (2013).
[4]T. M. Long, Y. K. Su, J. Headman, A. Higbee, L. B. Willis *et al*., *Curr. Opin. Chem. Biol*., **20**, 372 (2012).
[5] V. Brummer, T. Jurena, V. Hlavacek, J. Omelkova, L. Bebar *et al*., *Bioresour. Technol*., **152**, 543 (2014).
[6] X. Hou, *Appl. Microbiol. Biotechnol*., **94**, 205 (2012).
[7] N. H. Nguyen, S. O. Suh, C. J. Marshall, M. Blackwell, *Mycol. Res*., **110**, 1232 (2006).
[8] D. A. Lima, R. L. N. Luna, C. Martín, E. R. Gouveia, *Cellulose Chem. Technol*., **49**, 463(2015).
[9] F. C. Wu, S. S. Huang, I. L. Shih, *Bioresour. Technol*., **167**, 159 (2014).