BIOETHANOL PRODUCTION FROM CELLULOSIC FIBERS: COMPARISON BETWEEN BATCH AND FED-BATCH SACCHARIFICATION

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Unbleached pulp from the pulp and paper industry was used as a cellulosic material source to produce bioethanol in a separate hydrolysis and fermentation process. In the batch enzymatic saccharification, three cellulolytic enzymes and different initial carbohydrate (CH) contents were tested. Cellic® CTec2 was shown to be the most efficient enzyme with the lowest dosage used: 35 FPU g_{CH}^{-1}. Sugar production increased when the initial carbohydrate content increased from 58 to 116 g L^{-1}, while the hydrolysis efficiency decreased. Enzymatic hydrolyzates from the batch and fed-batch operations were further used in ethanolic fermentation with *Saccharomyces cerevisiae* ATCC 26602. An ethanol concentration of up to 28 g L^{-1} was achieved from 80 g L^{-1} of reducing sugars. A sugar-to-ethanol conversion of up to 69% and an ethanol productivity of 0.39 g L^{-1} h^{-1} were obtained with batch hydrolyzates. Nevertheless, the fed-batch mode led to higher bioethanol production per gram of carbohydrate and per enzyme unit.

Keywords: cellulosic fibers, SHF, bioethanol, batch, fed-batch

INTRODUCTION

The use of renewable sources to produce energy and value-added products is gaining an increasingly prominent role in the chemical industry. The finite nature of fossil fuels, the unstable price of petroleum and environmental, health and safety considerations are forcing the search for new energy sources and alternative routes to fossil fuel based products. Cellulosic materials from many agricultural, municipal and forestry activities are abundant, available and renewable carbohydrate sources that can be valorized by biological pathways to produce bioethanol.[1,2] This so-called second generation bioethanol has the advantage of the use of feedstocks that do not compete with food, the higher reduction of greenhouse gas emission and the consumption of waste residues.[3] The chemical composition differences between various types of lignocellulosic materials are one of the key factors affecting the efficiency of bioethanol production.[1,2] Lignocellulosic biomass mainly consists of cellulose, hemicelluloses and lignin. Cellulose and hemicelluloses are polysaccharides that can be hydrolyzed to sugars and then fermented to bioethanol, whilst lignin cannot be used for bioethanol production.[4] The bioconversion of lignocellulosic biomass to bioethanol generally consists of: i) treatment to change the lignocellulosic structure and/or remove lignin, ii) enzymatic hydrolysis to degrade polysaccharides into fermentable monosaccharides, iii) fermentation of monosaccharides to bioethanol and iv) ethanol recovery from the fermentation process.[5] Two main strategies are usually performed: separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF). Nevertheless, large scale commercialization of lignocellulose bioconversion is still hindered by many factors, mainly the high costs of lignocellulose treatment and enzymes required, the lack of microorganisms capable of fermenting both hexoses and pentoses and bioethanol distillation costs.[6,7] Strategies to overcome these drawbacks have been studied. Xie *et al.*[6] reported the feasibility of cellulase recovery and recycling to decrease the enzyme costs. The search of a robust ethanologenic organism capable of efficiently fermenting both hexoses and pentoses, and simultaneously yielding high ethanol concentration and productivity, being tolerant to ethanol and to the

lignocellulose-derived inhibitors is also an important challenge for the production of sustainable lignocellulosic bioethanol.[3,7] *Saccharomyces cerevisiae* is commonly used, but it is unable to ferment pentoses. Moreover, ethanol concentration must exceed 40 g L^{-1} before the distillation step in order to make the bioconversion process economically feasible. Therefore, the enzymatic hydrolysis and ethanolic fermentation should be improved to increase ethanol concentration and productivity.[7] In the present work, the fed-batch operation was used in the enzymatic hydrolysis step to facilitate the suspension mixture in the bioreactor and to enable a reduction of the effective enzyme dosage.

The feasibility and sustainability of bioethanol production from cellulose have been demonstrated using commercial products, such as Avicel or Sigmacel.[8] Unbleached pulp (used in the present work) is a more reliable feedstock to mimic cellulose in natural lignocellulosic materials after the pretreatment stage. Unbleached pulp, the main product from the pulp and paper industry, results from the wood cooking process (generally kraft cooking), where the lignin is mostly degraded and dissolved in the cooking liquor. Therefore, it consists in cellulosic fibers more accessible to cellulolytic enzymes.[9-11] Pulp and paper mills also produce primary sludge, a solid waste generated in the wastewater treatment plant, consisting of cellulosic fibers lost along the pulp production line.[12] However, this lignocellulosic residue contains up to 35% (w/w) of ash (mainly calcium carbonate, 27%).[13] $CaCO_3$ is responsible for the alkaline pH of primary sludge (7-10), which differs significantly from the optimum enzymatic hydrolysis pH (4.5-5.5). Therefore, $CaCO_3$ can hinder the enzymatic hydrolysis process and limit the solid loading capacity in the bioreactor.[12,14]

The aim of this work was to study bioethanol production from unbleached pulp, using a separate hydrolysis and fermentation (SHF) strategy, in order to further apply similar conditions upon primary sludge. Different commercial cellulolytic enzymes were tested in the enzymatic hydrolysis of unbleached pulp cellulosic fibers, where batch or fed-batch operation was tested. *Saccharomyces cerevisiae* ATCC 26602 was the microorganism selected to study the evaluation of the kinetics and yield of the fermentation of the monosaccharides in bioethanol.

EXPERIMENTAL

Cellulosic fibers

Unbleached pulp, provided by a Portuguese pulp mill that uses *Eucalyptus globulus* as raw material, was analyzed for moisture, ash and total lignin according to NREL standard procedures.[15] Carbohydrates (CH) content was calculated by difference. This lignocellulosic material contained 96.1% of carbohydrates, 2.8% of total lignin and 1.1% of ash, on a dry weight basis.

Enzymatic activity and enzyme selection

Three commercial cellulolytic enzymes were tested to convert the carbohydrates of unbleached pulp into fermentable sugars. The protein content was determined according to the Bradford method.[16] Cellulase activity (filter paper assay) was determined by the NREL standard procedure, designed to measure cellulase activity in terms of filter paper units (FPU) per milliliter of undiluted enzyme solution.[15] Cellic® CTec2 (liquid), from Novozymes (Denmark), consists of a blend of cellulases, β-glucosidases and hemicellulases. It contained 61.2 mg_{BSA} mL^{-1} of protein and a specific cellulase activity of 3.28 FPU mg_{BSA}^{-1} (200.7 FPU mL^{-1}). The cellulase activity is optimal at pH 5.0 and 50 ºC. Accellerase® 1500 complex (liquid), provided by Genencor (USA), is mainly composed of exoglucanase, endoglucanase, β-glucosidases and hemicellulases. It had a protein content of 30.5 mg_{BSA} mL^{-1} and a specific cellulase activity 1.69 FPU mg_{BSA}^{-1} (51.5 FPU mL^{-1}), optimal at pH 4.8 and 50 ºC. Dyadic® Cellulase CP Conc (Dyadic, USA), powder, consists of cellulases and β-1,3-1,4-glucanases. The protein content was 0.17 mg_{BSA} mg^{-1} and the specific cellulase activity was 1.47 FPU mg_{BSA}^{-1} (0.25 FPU mg^{-1}), optimal at pH 4.8 and 50 ºC.

Each enzyme was tested separately, using different dosage, to evaluate its hydrolysis efficiency upon unbleached pulp. Enzyme dosages from 35 to 140 FPU per gram of carbohydrate (FPU g_{CH}^{-1}) were added to 25 g L^{-1} of carbohydrates from unbleached pulp in a total working volume of 50 mL, placed in 100 mL Erlenmeyer flasks. The Erlenmeyer flasks were kept in an orbital shaker at 50 ºC and 200 rpm for 24 h.

Enzymatic saccharification

Initial carbohydrate contents of *c.a.* 29 to 116 g L^{-1}, from unbleached pulp, were loaded in 250 mL Erlenmeyer flasks and used in batch enzymatic hydrolysis assays. For a working volume of 100 mL, a maximum dry mass of unbleached pulp of 3-12 g was added, thus corresponding to a solids consistency of *c.a.* 3-12% (w/w). Citrate buffer (0.05 M, pH 5.0) was added. Cellic® CTec2 was selected due to its high enzymatic activity and conversion efficiency. The enzymatic hydrolysis assays were mostly carried out with an initial enzyme dosage adjusted to *c.a.* 35 FPU g_{CH}^{-1} and held at 50 ºC and 200 rpm in an orbital

incubator. In further assays, enzymatic hydrolysis was performed under fed-batch operation conditions, in which 29 g L^{-1} of carbohydrates was initially loaded. During the hydrolysis process, the equivalent of 15 g L^{-1} or 29 g L^{-1} of carbohydrates was periodically added per hour (assay Fb1H) or each 2 hours (assay Fb2H), respectively, during the initial 6 h of hydrolysis, till a final sum-up content of *c.a.*116 g L^{-1} was reached. The resulting enzymatic hydrolyzates, after filtration, were used as culture media in the fermentation process.

Ethanolic fermentation

Fermentation assays were carried out with *Saccharomyces cerevisiae* ATCC 26602 (American Type Culture Collection, Virginia, USA) in 250 mL Erlenmeyer flasks with a total working volume of 100 mL, maintained at 30 ºC and 150 rpm in an orbital shaker. A 10% (v/v) of total working volume of yeast inoculum was added, previously incubated at least for 12 h at 30 ºC and 150 rpm, to guarantee that the yeast was in its exponential growth phase. Fermentation culture media consisted of the liquid extracts enriched with glucose and xylose obtained from the enzymatic hydrolysis of unbleached pulp, under batch or fed-batch operation conditions. Peptone (5 g L^{-1}), malt extract (3 g L^{-1}) and yeast extract (3 g L^{-1}) were added to provide nutrients to *S. cerevisiae* ATCC 26602.

Figure 1 shows the process scheme for SHF of unbleached pulp. Figure 1a illustrates the batch operation carried out in the enzymatic hydrolysis step, whilst the fed-batch operation is depicted in Figure 1b.

Analytical methods and calculations

Samples were collected during the enzymatic hydrolysis to evaluate the production of fermentable sugars and the efficiency of the saccharification step. Ethanol production, yeast growth and sugar consumption were determined in the samples taken from the fermentation broth. The sugar concentration was measured by the colorimetric DNS method. Yeast growth was measured by UV-Vis spectrophotometry at 540 nm. Ethanol concentration was evaluated by HPLC (KnauerSmartline), equipped with a PL Hi-PlexCa 8 µm, 300 mm column (Varian) at 80-85 ºC and an RI detector. The eluent used was water at a flow rate of 0.6 mL min^{-1}. Enzymatic hydrolysis yield was determined by Eq. 1, where *f* is the carbohydrate fraction of unbleached pulp in its polymeric form and 1.1 is the global mass conversion factor applied to convert carbohydrate polymers to monosaccharides. The percentage of the theoretical bioethanol yield was determined by Eq. 2 (based on initial reducing sugar concentration), where 0.51 is the mass conversion factor of glucose to ethanol. Ethanol productivity in the fermentative process was determined by Eq. 3.[15,17]

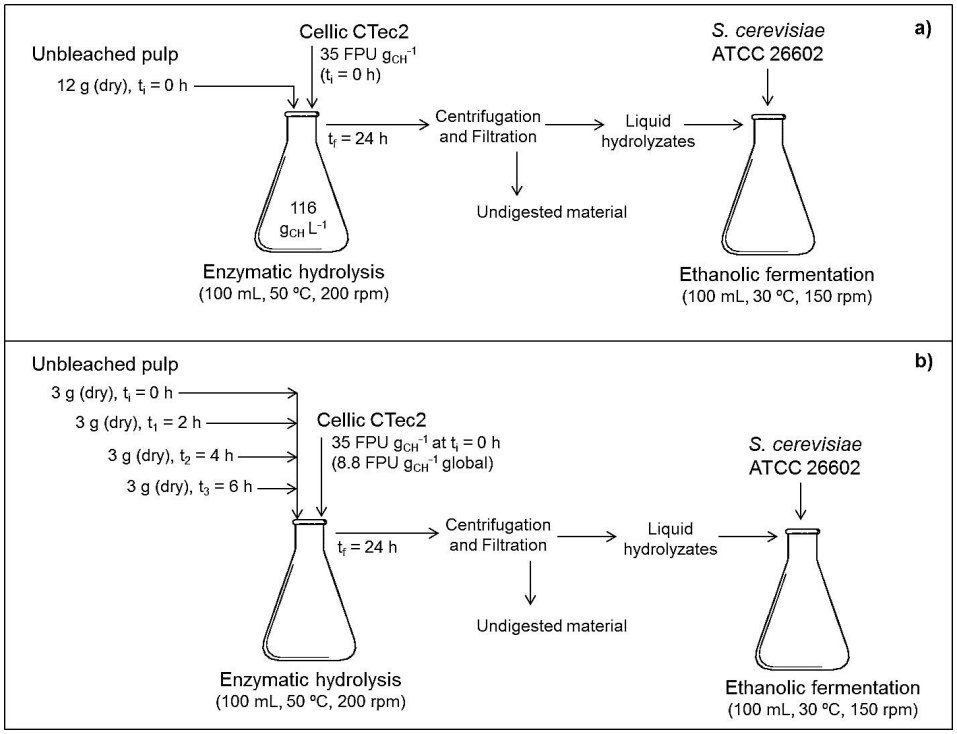

Figure 1: Process scheme for separate hydrolysis and fermentation of unbleached pulp: a) enzymatic hydrolysis in batch operation, b) enzymatic hydrolysis in fed-batch operation

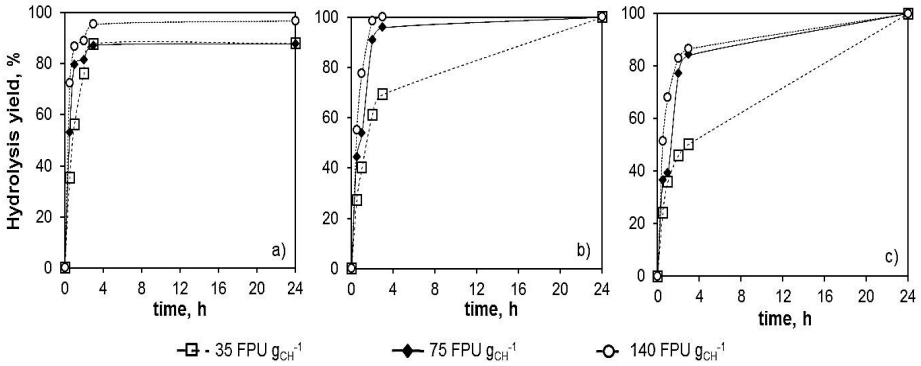

Figure 2: Enzymatic hydrolysis of unbleached pulp (equivalent to 25 g L^{-1} of carbohydrates) with commercial cellulolytic enzymes: a) Cellic® CTec2 from Novozymes; b) Accellerase® 1500 from Genencor; and c) Dyadic® Cellulase CP from Dyadic

The percentage of theoretical bioethanol yield, based on the total carbohydrates added, was also determined using Eq. 4.

$$\text{Hydrolysis yield, } Y_{Hidr}(\%) = \frac{[\text{Reducing sugars}]\ (g\ L^{-1})}{1.1 \times f \times [\text{Pulp}]\ (g\ L^{-1})} \times 100 \quad (1)$$

$$\text{Fermentation yield, } Y_{Ferm}(\%) = \frac{[\text{EtOH}]\ (g\ L^{-1})}{0.51 \times [\text{Reducing sugars}]\ (g\ L^{-1})} \times 100 \quad (2)$$

$$\text{Productivity, } P\ (g\ L^{-1} h^{-1}) = \frac{[\text{EtOH}]\ (g\ L^{-1})}{t\ (h)} \quad (3)$$

$$\text{Global yield, } Y_G(\%) = \frac{[\text{EtOH}]\ (g\ L^{-1})}{0.51 \times 1.1 \times f \times [\text{Pulp}]\ (g\ L^{-1})} \times 100 \quad (4)$$

A practical yield based on the amount of ethanol produced per gram of carbohydrate supplied and per enzyme unit consumed ($Y_{EtOH} = g_{EtOH}\ g_{CH}^{-1}\ FPU^{-1}$) was also calculated.

RESULTS AND DISCUSSION

Comparison of cellulolytic enzymes performance

Three different commercial cellulolytic enzymes were first tested to hydrolyze unbleached pulp, in order to select the adequate enzyme complex to efficiently convert carbohydrates from unbleached pulp to fermentable sugars. Cellic® CTec2 from Novozymes, Accellerase® 1500 from Genencor and Dyadic® Cellulase CP from Dyadic were used with enzymatic dosages of 35, 75 and 140 FPU g_{CH}^{-1}, as recommended by the enzyme suppliers.

Figure 2 shows the conversion yield during the enzymatic hydrolysis of 25 g_{CH} L^{-1} of carbohydrates, which corresponds to nearly 26 g L^{-1} of unbleached pulp added. According to Figure 2, all enzymes have the ability to hydrolyze unbleached pulp. When the enzymatic dosage was increased from 35 to 140 FPU g_{CH}^{-1}, the hydrolysis reaction rate increased. This behavior was observed for all enzymes. After 3 h of hydrolysis, Cellic® CTec2 converted at least 90% of polysaccharides into monosaccharides for all the enzymatic dosages tested (Fig. 2a). A conversion yield of 97% was achieved for the highest enzymatic dosage (140 FPU g_{CH}^{-1}). Figure 2b shows that Accellerase® 1500 converted the total amount of carbohydrates after 3 h of hydrolysis, when the highest enzymatic loading was used (140 FPU g_{CH}^{-1}). For the same reaction time, only 69% of carbohydrates were hydrolyzed by Accellerase® 1500 at a dose of 35 FPU g_{CH}^{-1}. Regarding Dyadic® Cellulase CP, the conversion yield was lower than 90%, after 3 h of hydrolysis, for all enzymatic dosages (Fig. 2c). With the lowest enzyme dosage, only 50% of polysaccharides were converted to monosaccharides at 3 h of reaction. The high conversion of polysaccharides into monosaccharides showed the ability of the enzyme complexes to hydrolyze both cellulose and hemicelluloses. In general, in the hydrolytic assays, the enzymatic hydrolysis rate was higher with Cellic® CTec2. Accellerase® 1500 and Dyadic® Cellulase CP may be more sensitive to the presence of the existing lignin (2.8%), which decreases the enzymatic hydrolysis rate and yield.[18] Cellic® CTec2 showed a good enzymatic activity upon unbleached pulp, even with the lowest enzymatic dosage tested, which can be a positive factor for reducing costs. Concerning the high yields obtained and the enzyme savings using Cellic® CTec2, this enzymatic complex was selected for further enzymatic hydrolysis trials.

Enzymatic saccharification of unbleached pulp

Effect of solid concentration

In order to maximize the production of fermentable sugars, and consequently the bioethanol concentration in the fermentation step, the effect of using higher initial carbohydrate content on enzymatic hydrolysis yield was studied.

Figure 3 shows the reducing sugars concentration and the conversion yield obtained in the enzymatic hydrolysis of different initial carbohydrate contents from unbleached pulp, using an enzyme dosage of 35 FPU g_{CH}^{-1} of Cellic® CTec2. The higher rates of sugar production occurred in the first 6 h of enzymatic hydrolysis. After 24 h of reaction, concentrations of 27, 45, 68 and 79 g L^{-1} of reducing sugars were obtained for initial carbohydrate contents of 29 (B1), 58 (B2), 88 (B3) and 116 (B4) g L^{-1}, respectively. The corresponding substrate (unbleached pulp) consistencies were of 3.0, 5.7, 9.0 and 12% (w/w).

For the same time basis (24 h), hydrolysis yields were 95, 78, 77 and 68% when the initial carbohydrate content was 29, 58, 88 and 116 g L^{-1}, respectively (Fig. 3b). Therefore, the enzymatic hydrolysis efficiency decreases as the substrate consistency increases.

Increasing the initial carbohydrates concentration, the lignin amount in the reaction mixture also increases and the negative effect of this compound may become relevant. Increasing the solid loading in batch enzymatic hydrolysis also causes non-uniformity, mass and heat transfer problems because of the high viscosity of the unbleached pulp suspension. Inhibition effects of end-products also may occur because of high concentrations of glucose and cellobiose.[11,19,20] It is reported that 100 g L^{-1} of glucose in the enzymatic hydrolyzate can decrease the hydrolysis yield by 80%.[4] The effect of high consistency on the hydrolysis of unbleached hardwood pulp was studied by other authors, from 2 to 20% (w/w) substrate concentration, with an enzymatic dosage of 20 FPU per gram of cellulose in Erlenmeyer flasks. The increase in pulp consistency resulted in a decrease in the amount of free water in the substrate matrix and it took longer to liquefy the solid matrix (40 h to liquefy unbleached pulp at 20% consistency). On the other hand, the enzymatic hydrolysis of 2 and 5% (w/w) substrate consistency yielded nearly 17 and 40 g L^{-1} of glucose, with hydrolysis efficiencies of 100 and 95%, respectively.[11] The yields obtained in our work are lower, probably caused by the differences in the chemical composition of the substrates, mainly the lignin content (2.8%, in a dry weight basis, against 1.7% in the unbleached hardwood used by Zhang *et al.*[11]).

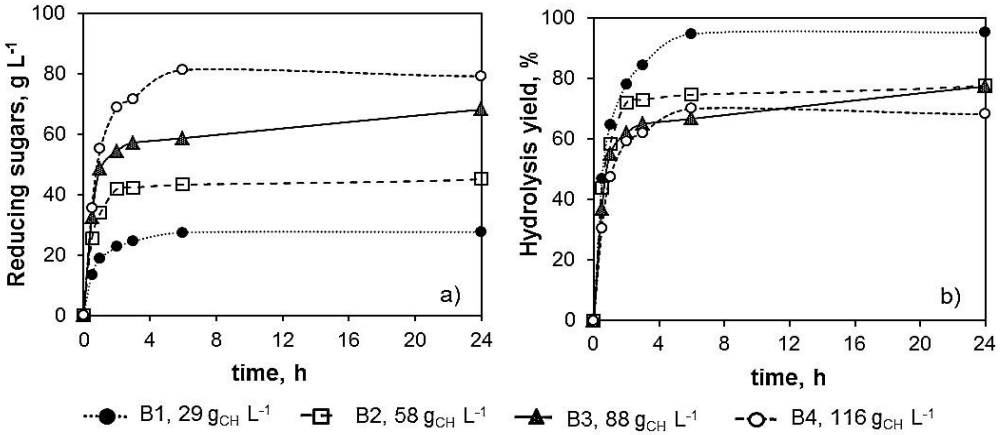

Figure 3: Enzymatic hydrolysis of unbleached pulp with Cellic® CTec2 (35 FPU g_{CH}^{-1}), for different initial carbohydrate concentrations (29-116 g_{CH} L^{-1}), under batch conditions: a) reducing sugars produced, b) hydrolysis yield

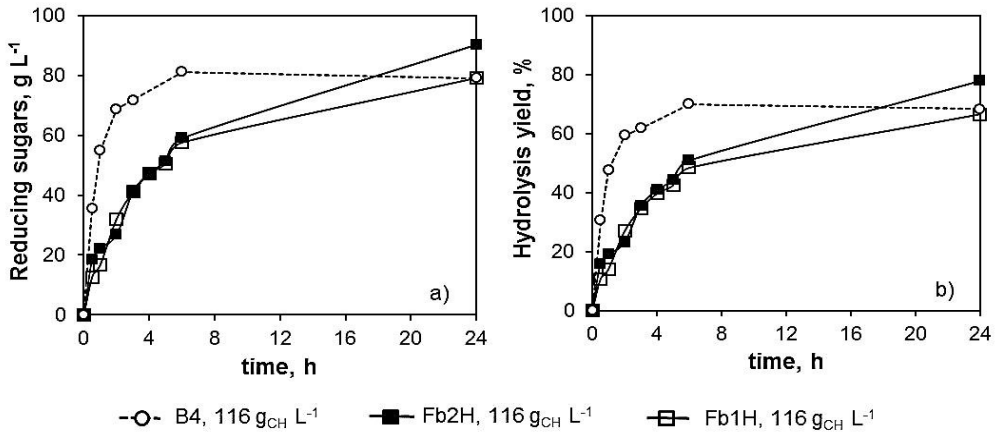

Figure 4: Enzymatic hydrolysis of unbleached pulp with Cellic® CTec2 (35 FPU g_{CH}^{-1}, at the start-up of the reaction) under batch (B4) and fed-batch (Fb) conditions with a carbohydrate concentration equivalent to 116 g L^{-1}: a) reducing sugars produced, b) hydrolysis yield

Different affinities from the enzymes to natural substrates may also explain the observed differences. Moreover, the same authors tested a peg mixer for batch hydrolysis, under the same conditions as the Erlenmeyer flasks. In the hydrolysis of unbleached pulp at 20% (w/w) consistency, it took only 1 h to liquefy the solid substrate, thus showing the importance of the stirring at high solids consistency.[11] Therefore, finding alternative strategies to orbital mixing when using high solid consistencies in Erlenmeyer flasks at laboratory scale is important, in order to obtain a good mixture of the solid (pulp) and liquid (enzyme solution) phases.

Effect of fed-batch substrate feed

Fed-batch processes have largely solved the problems of stirring or substrate inhibition, by maintaining low substrate concentrations in the bioreactor. This is achieved by intermittent feeding of the substrate and leads to increased yields.[21] Two fed-batch strategies were tested, which started with an initial carbohydrate concentration of 29 g L^{-1}. The carbohydrates were hydrolyzed by 35 FPU g_{CH}^{-1} of Cellic® CTec2 initially loaded, without additional dosage of enzyme. The main differences between the two strategies were the frequency of carbohydrates addition and the amount of carbohydrates added. One experiment consisted in adding the same amount of carbohydrates initially loaded, equivalent to 29 g L^{-1}, which was added 3 times, every 2 h during the first 6 h of hydrolysis (Fb2H). In the second strategy, carbohydrates were fed per hour during the initial 6 h of hydrolysis, at lower loading equivalent to 15 g L^{-1} (Fb1H). The hydrolysis reactions were followed for 24 h. Figure 4 shows the production of reducing sugars and the hydrolysis yield during both fed-batch processes. The use of a fed-batch strategy improved final hydrolysis yield and sugar concentration when compared to the batch enzymatic hydrolysis of equal amount of total carbohydrates (supplied once at the beginning of the reaction). In the batch enzymatic hydrolysis of 116 g L^{-1} of carbohydrates, a conversion yield of 68% was determined after 24 h of reaction (B4), whereas the fed-batch enzymatic hydrolysis of 116 g L^{-1} of carbohydrates added periodically (Fb2H) converted till 78% of total carbohydrates after 24 h of reaction, as observed in Figure 4b. A higher amount of reducing sugars was produced in the same reaction time using fed-batch strategies. At 24 h of the hydrolysis process, nearly 90 g L^{-1} of reducing sugars were available for the fermentation step, as shown in Figure 4a (Fb2H), compared to 80 g L^{-1} in batch process (B4). During the first 6 h, the hydrolysis rate was lower in fed-batch hydrolysis than in batch hydrolysis. However, it is mandatory to highlight that the total enzyme dosage used in the fed-batch hydrolysis based on the total carbohydrate amount added was one quarter (8.8 FPU g_{CH}^{-1}) of the total enzyme dosage used in the batch hydrolysis (35 FPU g_{CH}^{-1}). Nevertheless, the enzyme remained active during 24 h of hydrolysis and the final conversion yield was higher.

Kuhad *et al.*[19] also compared the batch and the fed-batch operation modes in the enzymatic hydrolysis of deinked newspaper: the fed-batch mode improved the enzymatic saccharification and increased the amount of sugars produced.

Nevertheless, the comparison with other results found in literature is quite ambiguous, since authors use different substrates, enzymes and operation conditions.

Ethanolic fermentation of unbleached pulp hydrolyzates

Enzymatic hydrolyzates obtained in batch hydrolysis of 29 (B1) and 116 g L^{-1} (B4) of carbohydrates, as well as the one obtained in fed-batch hydrolysis of 116 g L^{-1} of carbohydrates (Fb2H assay), were further used in the ethanolic fermentation process. Figure 5 shows ethanol and sugar concentrations profiles, as well as cell density, registered along the fermentation of both enzymatic hydrolysis operations.

Table 1 compiles the most relevant parameters determined during ethanolic fermentation, such as the percentage of theoretical bioethanol yield and productivity. As observed in Figure 5b, *S. cerevisiae* ATCC 26602 produced an ethanol concentration of 9 g L^{-1} after 48 h of fermentation, from 27 g L^{-1} of fermentable sugars (obtained from B1 assay), with a production rate of 0.19 g L^{-1} h^{-1}. The conversion efficiency of fermentable sugars to bioethanol was 68%, based on the initial sugar concentration. An ethanol concentration of 28 g L^{-1} was obtained after 72 h in the fermentation of the B4 enzymatic hydrolyzates, which contained 80 g L^{-1} of reducing sugars, as also shown in Figure 5b. In this fermentative process, the bioethanol yield and productivity were of 69% and 0.39 g L^{-1} h^{-1}, respectively. The enzymatic hydrolyzates containing 90 g L^{-1} of reducing sugars (Fb2H assay) yielded an ethanol concentration of 25 g L^{-1} in the fermentative process with *S. cerevisiae* ATCC 26602 (Fig. 5b), obtained after 72 h of fermentation (a productivity of 0.35 g L^{-1} h^{-1}). A conversion yield of initial sugars into ethanol of 55% was determined, lower than the results observed in the fermentation of the hydrolyzates obtained from the batch enzymatic hydrolysis of 116 g L^{-1} of carbohydrates (Table 1). The consumption of sugars is quite similar for B4 and Fb2H assays, but the evolution of ethanol concentration differs, which was not expected.

The parameters that evaluate the global process, *i.e.* enzymatic hydrolysis plus ethanolic fermentation, are shown in Table 2. Because the global process cost depends significantly on the enzyme consumption and price, the amount of ethanol produced was calculated per total enzyme used in each assay. The same initial enzyme dosage was used (35 FPU g_{CH}^{-1}) in both assays, but in the fed-batch enzymatic hydrolysis the initial carbohydrates amount was lower (29 g L^{-1}) and it was periodically added until it reached a final amount of 116 g L^{-1} (29 + 3×29 g L^{-1}), without further addition of enzyme.

Table 2 shows that the ethanol yield based on total carbohydrates is higher for the batch assay (47%) compared to the fed-batch assay (43%). Nevertheless, a lower amount of enzyme was used in the fed-batch assay (Fb2H), corresponding to an ethanol yield of 0.0021g_{EtOH} g_{CH}^{-1} FPU^{-1}, higher than the value determined for the batch assay (B4). The ethanol productivity per enzyme unit was also higher in the Fb2H assay, confirming that using fed-batch conditions in the enzymatic hydrolysis step reduces the use of enzyme and therefore the enzyme costs in the global process.

Table 1
Ethanolic fermentation of enzymatic hydrolyzates of unbleached pulp, produced under batch (B1 and B4) or fed-batch conditions (Fb2H)

Enzymatic hydrolyzate	Initial sugars (g_{sugar} L^{-1})	Sugars consumed (%)	[EtOH] (g L^{-1})	time (h)	Y_{Ferm} (%)	P (g L^{-1} h^{-1})
B1	27	91	9	48	68	0.19
B4	80	88	28	72	69	0.39
Fb2H	90	88	25	72	55	0.35

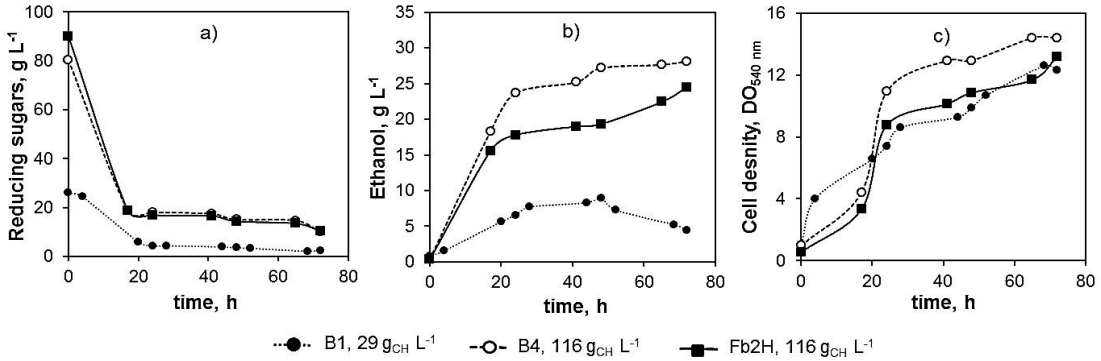

Figure 5: Ethanolic fermentation of enzymatic hydrolyzates of unbleached pulp (produced either in batch or in fed-batch enzymatic hydrolysis) with *Saccharomyces cerevisiae* ATCC 26602:
a) reducing sugars consumption, b) ethanol production, c) cell density

Table 2
Global yield (Y_G), including both enzymatic hydrolysis and ethanolic fermentation, as well as practical yield (Y_{EtOH}), based on the carbohydrate fraction of unbleached pulp and total enzyme units used

Enzymatic hydrolyzate	Total carbohydrates (g_{CH} L^{-1})	Enzyme units (FPU g_{CH}^{-1})	Y_G [(1)] (%)	$Y_{EtOH}\times10^3$ (g_{EtOH} g_{CH}^{-1} FPU^{-1})	P' $\times10^3$ [(2)] (g L^{-1} h^{-1} FPU^{-1})
B4	116	35.0	47	0.6	1.0
Fb2H	116	8.8	43	2.1	3.4

[(1)] – reported to the theoretical yield; [(2)] – P' is P per FPU

The knowledge achieved in this work with the bioconversion of unbleached pulp will be further applied in the bioconversion of primary sludge, which is a low-cost raw material and where the enzyme cost contribution should also be minimized.

CONCLUSION

From the studied enzymes, Cellic® CTec2 was selected due to its high enzymatic activity and conversion efficiency.

In batch enzymatic saccharification, the conversion yield of initial carbohydrates to fermentable sugars decreased when the initial solids concentration was increased. To overcome this bottleneck, a fed-batch strategy was employed. In fed-batch operation, stirring was facilitated and the enzymatic hydrolysis efficiency was improved. Higher fermentable sugars concentrations were available to the ethanolic fermentation, compared to the batch operation mode. However, the ethanolic fermentation of the hydrolyzates obtained from the fed-batch enzymatic hydrolysis led to a slightly lower ethanol concentration and lower values of yield and productivity. Nevertheless, considering the overall process (fed-batch enzymatic saccharification and fermentation), higher values were achieved for the global ethanol yield and productivity based on the total enzyme units added.

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