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Valorization of Residual Streams from Pulp and Paper Mills: Pretreatment and Bioconversion of Primary Sludge to Bioethanol

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ABSTRACT: Primary sludge is a lignocellulosic residue from pulp and paper mills consisting of cellulosic fibers and ash. However, the high ash content (35%, mainly $CaCO_3$) and pH value affect the enzymatic hydrolysis of cellulosic fibers. Several pretreatments were used to reduce the $CaCO_3$ content and to adjust the pH. Enzymatic hydrolysis of primary sludge was enhanced when it was pretreated with HCl or spent acid (another residual stream of the same plant). Cellulosic fibers were converted to monomeric sugars by Cellic CTec2 cellulase with a dosage of 35 FPU $g_{CH}{}^{-1}$ for a carbohydrate concentration of 46 g L^{-1}. This conversion was enhanced from 20% to 88% for primary sludge pretreated with HCl and to 72% for samples pretreated with spent acid. The fermentation of 27 g L^{-1} of available sugars with *Pichia stipitis* led to ethanol concentrations of up to 10.5 g L^{-1} with a yield of 0.39 $g_{EtOH}\ g_{sug}{}^{-1}$.

1. INTRODUCTION

The production of energy and value-added chemicals and materials from renewable sources is gaining an increasingly prominent role in the future of the chemical industry. An efficient sustainability can be reached with the development of integrated processes that valorize the whole biomass, hence producing no or limited waste, or reusing this waste to produce value-added products.[1]

Eucalyptus globulus wood is commonly used as a raw material in the Portuguese pulp and paper industry. A large amount of solid waste containing short cellulosic fibers lost along the pulp and paper production line is generated: about 350 kt of primary sludge per year in Portugal.[2] This sludge is often incinerated, discharged in landfills, or used in composting. Finding an alternative solution to managing this heterogeneous solid material would decrease environmental and financial impacts and solve problems of acceptance in landfill sites or capacity limitations.[3,4] The bioconversion of this lignocellulosic waste, as well as of similar wastes (waste office paper or recycled paper), through saccharification and fermentation, is an environmentally viable alternative waste management and allows valorization of these materials. The feasible usage of these raw materials involves maximum conversion of cellulose and hemicelluloses polymers to fermentable monomeric sugars, which can be used as a carbon source to produce second-generation biofuels (e.g., bioethanol) and biochemicals (e.g., organic acids, biodegradable plastics).[3−9] Nevertheless, lignocellulose bioconversion is challenging because of the resistant nature of this type of biomass, which affects the hydrolysis step and selection of an appropriate microorganism capable of fermenting a variety of different sugars.[10]

Portuguese pulp and paper mills generate primary sludge with varied composition: 45−60% of carbohydrates (cellulose and hemicelluloses, mainly xylan), 5−20% of lignin, and 35−50% of inorganic matter (ash) in a dry basis.[7] The high ash content [mostly calcium carbonate ($CaCO_3$), from lime kiln sludge stream or paper production waste stream] makes the production of fermentable monosaccharides from the cellulosic fraction difficult. $CaCO_3$ is responsible for the alkaline pH of primary sludge (7−10), and it may also limit the solid loading capacity in the bioreactor, thus affecting the enzymatic hydrolysis pH and yield.[4,11] To enhance the enzymatic digestibility of cellulosic biomass and ensure good yields of sugars from both cellulose and hemicellulose, a pretreatment is necessary. The ideal pretreatment should also avoid further sugar degradation into potential inhibitors for enzymes or fermenting microorganisms, minimize energy demand and process costs, and consume little or no chemical agent.[11,12] When a treatment is previously applied over primary sludge, hydrolysis sugar yields may exceed 90%, compared to yields lower than 20% in the absence of a pretreatment.[1] Continuous or intermittent ultrasound, supercritical carbon dioxide (CO_2), nonionic surfactants, ozone, and inorganic acids (like sulfuric, phosphoric, or hydrochloric acids) have been used to pretreat lignocellulosic wastes (primary sludge, recycled paper, and waste paper) to enhance their digestibility.[4,6,7,11,13,14] Hydrolysis of pretreated lignocellulosic materials to soluble fermentable sugars is generally carried out with sulfuric acid or cellulolytic enzymes. When hydrolysis is carried out with acid catalysts, possible toxic compounds and sugar degradation products are formed that can inhibit fermentation and reduce yield. In the case of primary sludge from a pulp and paper mill, the acid is consumed both in the reaction with $CaCO_3$ and in the carbohydrate hydrolysis.

Enzymatic hydrolysis with cellulases and hemicellulases is a highly substrate-specific process. It requires less energy, and no inhibitors are produced during the reaction because it occurs under milder conditions with no use of chemical compounds. Sugar yields of 75−95% can be attained when this environmentally friendly hydrolysis process is used.[1,15]

The hydrolyzates of hardwood wastes, such as the case of Portuguese pulp and paper mill primary sludge, consist mainly

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of a mixture of glucose and xylose. The efficient use of both sugars in an ethanolic fermentation process depends on the selection of the appropriate microorganism. *Saccharomyces cerevisiae* yeast is widely known and used for its capability to ferment glucose into bioethanol with high levels of performance and efficiency. However, this microorganism lacks the ability to convert xylose to ethanol that can be overcome using a genetically modified *Saccharomyces* strain. Natural xylose-fermenting microorganisms include *Pichia stipitis* and *Candida shehatae* yeasts. *P. stipitis* coferments pentoses and hexoses and produces bioethanol with a high fermentation performance.[16−18]

The aim of this research is to study the batch production of ethanol by the bioconversion of primary sludge produced in a Portuguese pulp and paper mill. The stages studied included primary sludge pretreatment (to reduce or remove $CaCO_3$), enzymatic hydrolysis of the pretreated primary sludge, and ethanolic fermentation of the monomeric sugars produced. Pretreatment methods comprised the use of commercial acids, such as acetic acid (CH_3COOH), nitric acid (HNO_3), hydrochloric acid (HCl), and sulfuric acid (H_2SO_4) as well as another residual stream (spent acid) from the pulp and paper industry. The spent acid is a sodium sulfate−sulfuric acid solution generated in the production of chlorine dioxide (used for pulp bleaching) as a residual stream.[19] To overcome the disadvantage of CO_2 being released during the acid pretreatment, other alternatives were studied. Commercial CO_2 was also tested to decrease the pH of primary sludge suspensions, in order to study the viability of using the gaseous residual streams of pulp and paper industries.

2. EXPERIMENTAL SECTION

2.1. Lignocellulosic Material. Primary sludge originated from the primary treatment of the effluents of a local Portuguese pulp mill, which produces kraft pulp from *E. globulus*. The first stage of the wastewater treatment removes the suspended solids from the residual effluents by a sedimentation process, carried out in the primary clarifier unit, and then they are pressed to form primary sludge. Primary sludge was analyzed for moisture, ash, and total lignin contents according to the National Renewable Energy Laboratory (NREL) standard procedures.[20] Calcium carbonate ($CaCO_3$) was calculated from the total ash content, and the results were obtained by igniting a dry sample at 900 °C.

Primary sludge had a moisture content of 80%. On a dry weight basis, it consisted of 34.8% total ash, 3.8% total lignin, and 61.4% total carbohydrates (CH, calculated by difference). The $CaCO_3$ content was 26.7%. The pH value of primary sludge suspended in citrate buffer was 7.2.

2.2. Hydrolytic Enzymes and Fermentative Microorganisms. Cellulase Cellic CTec2, from Novozymes (Copenhagen, Denmark), and Accellerase 1500, from Genencor (Palo Alto, CA), were the enzymes tested to hydrolyze the carbohydrates of pretreated primary sludge into fermentable sugars. The cellulase activity (filter paper assay) was determined by the NREL standard procedure, designed to measure the cellulase activity in terms of filter paper units (FPU) per milliliter of undiluted enzyme solution.[20] Cellic CTec2 consists of a blend of cellulases, β-glucosidases, and hemicellulases, with a cellulase activity of 200 FPU mL^{-1}. The cellulase activity is optimal at 50 °C and pH 5.0. Accellerase 1500 is composed of exoglucanase, endoglucanase, β-glucosidases, and hemicellulases. It has a cellulase activity of 51.5 FPU mL^{-1} (optimal at 50 °C and pH 4.8).

P. stipitis DSM 3651 and *S. cerevisiae* (baker yeast) were the microorganisms used in ethanolic fermentation assays. The yeast strains were grown in liquid media consisting of 10 g L^{-1} glucose (Riedel de-Haën), 5 g L^{-1} peptone (Fluka), 3 g L^{-1} malt extract (Fluka), and 3 g L^{-1} yeast extract (Fluka) and transferred to agar slants with the same composition, kept at 4 °C.

2.3. Primary Sludge Pretreatments. $CaCO_3$ neutralization was carried out with different inorganic or organic acids: H_2SO_4 (Fischer), HCl (Fischer), HNO_3 (Panreac), CH_3COOH (Fluka), and spent acid from the mill. The spent acid concentration was calculated to be equivalent to a 50% H_2SO_4 solution, determined by titration with 1 M sodium hydroxide. Ethylenediaminetetraacetic acid (EDTA; BDH Chemicals, Radnor, PA) was also tested to decrease the $CaCO_3$ content. The chemical agent was diluted or dissolved in distilled water, performing a suspension of 250 mL containing 5 g (dry weight) of sludge. The amount of chemical agent was determined according to the stoichiometric reaction with $CaCO_3$. The suspension was mixed for a few minutes and filtered with a metal mesh screen of 60 mesh and 12.5 cm diameter. The retained solids were washed with distilled water and filtered again.

The other set of assays consisted of washing 5 g (dry weight) of primary sludge with 250 mL of distilled water, with no additional chemical compounds. The suspension was agitated and filtered with the same metal mesh screen. The method described corresponds to one washing cycle. Tests of 1, 2, 3, 6, 12, and 24 washing cycles were carried out.

Carbonated water was also used to remove $CaCO_3$ from primary sludge. A sample of 5 g (dry weight) of primary sludge suspended in 250 mL of distilled water was maintained under constant CO_2 bubbling for 10, 30, and 60 min.

After each pretreatment, the ash and $CaCO_3$ contents in pretreated primary sludge were analyzed according to the NREL standard procedures.[20] Pretreated primary sludge was suspended in citrate buffer, and the pH value was registered. The primary sludge samples pretreated with the most feasible procedures were tested for analyzing the following enzymatic hydrolysis efficiency.

2.4. Enzymatic Hydrolysis. Enzymatic hydrolysis was performed in 250 mL Erlenmeyer flasks with a working volume of 100 mL. Initial concentrations of 25.7 and 46 g L^{-1} of carbohydrates from primary sludge (calculated in their monomeric form, i.e. the potential sugar concentration in the final enzymatic hydrolyzate), pretreated with HCl or spent acid, were used. An enzymatic dosage of 35 FPU per gram of carbohydrate (FPU $g_{CH}{}^{-1}$) of each enzyme was separately added. The reaction mixtures were incubated at 50 °C at 200 rpm for 24 h in an orbital incubator (Stuart S150, Lab Merchant Ltd., London, U.K.). Samples of 1.5 mL were withdrawn and centrifuged at 3500 rpm for 5 min (Hettich, Germany) before analysis.

2.5. Ethanolic Fermentation. Fermentation experiments took place in 250 mL Erlenmeyer flasks containing a total working volume of 100 mL. Steam-autoclaved (121 °C, 1 atm gauge for 15 min) liquid extracts obtained from the enzymatic hydrolysis (with Cellic CTec2) of 46 g L^{-1} of carbohydrates of primary sludge pretreated with HCl or spent acid were used as media, supplemented with 5 g L^{-1} peptone, 3 g L^{-1} malt extract, and 3 g L^{-1} yeast extract, from Fluka, to provide nutrients and nitrogen to the yeasts. A 10% v/v of total working volume of yeast inoculum was added to the fresh medium. The inoculum was prepared in a 100 mL Erlenmeyer flask with 50 mL of the medium as described in section 2.2, and yeast cells were transferred from the agar slants. It was held at 30 °C and 150 rpm for 12 h in the orbital shaker, before inoculation, to guarantee that the yeast was in its exponential growth phase. Fermentations were carried out in the same orbital shaker at 30 °C and 150 rpm.

Samples were collected from the fermentation broth and centrifuged at 3500 rpm for 5 min (Hettich, Germany) before analysis.

2.6. Analytical Methods for Hydrolyzates and Fermentation Broths. During the enzymatic hydrolysis of pretreated primary sludge, the production of glucose and xylose was evaluated by high-performance liquid chromatography (HPLC), after sample filtration with a 0.2 μm filter membrane (Whatman). A Knauer model K-301 HPLC with a refractive index detector was used. The sugars were analyzed with a PL Hi-Plex Ca 8 μm, 300 mm column (Varian) maintained at 80−85 °C. The eluent used was ultrapure water at a flow rate of 0.6 mL min^{-1}, previously filtered with a 0.2 μm filter membrane and degassed for 15 min. The hydrolysis efficiency was determined based in eq 1, where f is the carbohydrate fraction (in its polymeric form) in pretreated primary sludge. Because the carbohydrate content (cellulose and hemicellulose) was calculated by difference, a global mass conversion factor of 1.1 was applied to convert the polymeric form to the monomeric form.[20,21]

During ethanolic fermentation, yeast growth was measured by UV−vis spectrophotometry at 540 nm. Glucose, xylose, and ethanol were analyzed by HPLC as described above, during 30 min injection runs.

The sugar-to-ethanol conversion yield, based on the initial total sugar concentration, and ethanol productivity were determined by eqs 2 and 3, respectively.[20−22]

$$\text{hydrolysis yield, }\% = \frac{[\text{monomeric sugar}]\ (\text{g L}^{-1})}{1.1f[\text{biomass}]\ (\text{g L}^{-1})} \times 100 \tag{1}$$

$$\text{EtOH yield, } \text{g}_{\text{EtOH}}\ \text{g}_{\text{sug}}^{-1} = \frac{[\text{EtOH}]\ (\text{g L}^{-1})}{[\text{initial monomeric sugars}]\ (\text{g L}^{-1})} \tag{2}$$

$$\text{EtOH productivity, } \text{g L}^{-1}\ \text{h}^{-1} = \frac{[\text{EtOH}]\ (\text{g L}^{-1})}{\text{fermentation time (h)}} \tag{3}$$

3. RESULTS AND DISCUSSION

3.1. Pretreatment of Primary Sludge. The separated hydrolysis and fermentation of primary sludge is only viable if a previous and appropriate treatment is applied to convert or remove $CaCO_3$ because this compound affects, at least, the optimal pH for the enzymatic hydrolysis. Several chemical compounds and strategies were tested for this purpose, in order to find a pretreatment that consumes little or no chemicals and minimizes the energy demand, as well as process costs. The ash and $CaCO_3$ contents were measured in pretreated and dried primary sludge samples. The pH value of pretreated primary sludge, suspended in citrate buffer (0.5 M, pH 5.0), was registered. Table 1 compiles the results for each pretreatment applied.

Table 1. Chemical Composition and pH Value of Untreated and Pretreated Primary Sludge

	content in dry weight basis (% w/w)			
type of pretreatment	organic	ash	$CaCO_3$	pH
none (untreated)	65	35	27	7.2
chemical agent				
EDTA	97.4	2.6	2.6	5.7
CH_3COOH	97.9	2.1	1.9	5.3
HNO_3	98.1	1.9	1.9	5.3
HCl	98.2	1.8	0.5	5.2
H_2SO_4	89.2	10.8	0.7	5.4
spent acid	78.5	21.5	1.1	5.0
water (no. of washing cycles)				
1	77.5	22.5	11	6.4
2	86.5	13.5	6.9	6.5
3	86.4	13.6	8.8	6.7
6	95.9	4.1	0.5	6.3
6 (recirculated water)	91.6	8.4	0.6	6.3
12	96.2	3.8	0.5	5.4
24	98.6	1.4	0.5	5.0
carbonated water (with CO_2 bubbling, min)				
10	69.8	30.2	18.2	6.9
30	72.2	27.8	15.4	6.6
60	78.0	22.0	15.4	6.5

A pretreatment consisting of water washing is presented in this work as an alternative approach without the addition of chemicals. When suspended in water, fibers and $CaCO_3$ particles are dispersed. During the filtration process of the suspended primary sludge, $CaCO_3$ particles may be dragged by water and eliminated through the fine metal mesh screen with the appropriate porosity. The elimination of $CaCO_3$ by a dissolution effect is not very probable because its solubility in water is very low (up to 0.02 mg mL^{-1}).[23] As shown in Table 1, when primary sludge was washed once with water, the ash and $CaCO_3$ contents decreased to 22.5% and 11%, respectively. The sequence of 2 or 3 washes enabled a decrease of the ash and $CaCO_3$ from 35% to 14% and from 27% to 7−9%, respectively. As the sequential washes increased, these water-based techniques became more effective on ash and $CaCO_3$ removal. For 6 or more sequential washes with water, $CaCO_3$ was practically all removed. The pH value of primary sludge pretreated in sequences of 12 and 24 washes, suspended in a citrate buffer, was very close to the adequate value for the enzymatic hydrolysis reaction. Nevertheless, a high number of washing cycles may be impracticable on an industrial scale, where water utilization must be minimized. Taking into account that $CaCO_3$ removal is essentially by dragging, the substitution of fresh water by recirculated water can be a feasible alternative to reduce fresh water demand.

Acid neutralization with HCl or H_2SO_4 is the most common method used in several works to treat paper sludge.[7,9] The present work also studies HNO_3 and CH_3COOH addition. Furthermore, alternative methods, such as neutralization with spent acid, were also carried out. According to Table 1, when chemical agents were used, $CaCO_3$ diminished significantly in pretreated primary sludge and the pH value decreased. Acid neutralization was more efficient with HCl and less with CH_3COOH. Both H_2SO_4 and HCl were effective acids, almost promoting complete removal of $CaCO_3$. However, primary sludge pretreated with H_2SO_4 still presented 11% of ash, while this fraction was insignificant with the other acids. This ash was attributed to $CaSO_4$ salts, which remained in the pretreated sludge and were not dissolved (low solubility) or dragged in the following washing.[11] Primary sludge pretreated with spent acid had 1.1% of residual $CaCO_3$ and 21.5% of ash. The high amount of ash in the pretreated primary sludge is due to the presence of $CaSO_4$ (produced in the reaction of H_2SO_4 and $CaCO_3$) and Na_2SO_4 (originally in the spent acid composition). Nevertheless, the organic fraction in the pretreated primary sludge increased to 78.5%. This pretreatment can be advantageous because other residual streams from pulp mills are being reutilized.

The use of CO_2 to decrease the pH was also considered. The increment in the bubbling time enhanced the decrease in the $CaCO_3$ amount. Nevertheless, after 60 min the $CaCO_3$ amount was only reduced to 15.4% in pretreated primary sludge. The pH value of pretreated primary sludge still remained high (6.5), and additional acid is needed before the hydrolysis step.

3.2. Enzymatic Hydrolysis of Pretreated Primary Sludge.

3.2.1. Primary Sludge Pretreated with HCl. In the enzymatic hydrolysis of untreated primary sludge, conversion yields lower than 20% were evaluated after 24 h of hydrolysis (data not shown). These previous results showed that a pretreatment is necessary to increase the digestibility of primary sludge.

Samples of primary sludge previously treated with HCl and spent acid were further used in enzymatic hydrolysis assays. An enzyme dosage of 35 FPU g_{CH}^{-1} of Cellic CTec2 (Novozymes) or Accellerase 1500 (Genencor) was used to hydrolyze an initial concentration of 25.7 or 46 g L^{-1} of carbohydrates of pretreated primary sludge.

The yield and sugar concentration (glucose and xylose) obtained after enzymatic hydrolysis of 25.7 g L^{-1} of carbohydrates from primary sludge previously treated with HCl are shown in parts a and c of Figure 1.

A yield of 79% was obtained with Cellic CTec2 at 24 h (Figure 1a). The corresponding hydrolyzate contained 16.9 g L^{-1} of glucose and 3.4 g L^{-1} of xylose (Figure 1c). For the same time, a conversion yield of 74% was achieved when Accellerase 1500 was used (Figure 1a). Concentrations of 16.3 g L^{-1} of glucose and 2.8 g L^{-1} of xylose were obtained (Figure 1c). When the initial carbohydrate concentration was increased to 46 g L^{-1}, both the hydrolysis yield (Figure 1b) and total amount of sugar (Figure 1d) increased, regardless of the enzyme used. Cellic CTec2 converted 88% of carbohydrates (Figure 1b), producing 33.1 g L^{-1} of glucose and 7.3 g L^{-1} of xylose (Figure 1d). A hydrolysis yield of 77% was determined in the case of Accellerase 1500 (Figure 1b), leading to a glucose concentration of 30.2 g L^{-1} and a xylose concentration of 5.4 g L^{-1} (Figure 1d).

3.2.2. Primary Sludge Pretreated with Spent Acid. Figure 2 shows the hydrolysis yield and sugar concentration obtained in the enzymatic hydrolysis of primary sludge previously treated with spent acid, as an alternative to HCl. Slightly lower hydrolysis yields were obtained: 78 and 69% with Cellic CTec2 and Accellerase 1500, respectively, after 24 h of enzymatic hydrolysis of 25.7 g L^{-1} of carbohydrates of pretreated primary sludge. The corresponding concentrations of glucose and xylose are also slightly lower in comparison with the use of HCl-pretreated sludge. In contrast to the increased hydrolysis yield observed for the HCl-pretreated sludge, when increasing initial carbohydrate concentration to 46 g L^{-1}, the hydrolysis yield decreased: Cellic CTec2 and Accellerase 1500 converted respectively 72 and 68% of the carbohydrate fraction from spent acid pretreated sludge. This decrease in the hydrolysis yield for higher concentrations of initial carbohydrates may be explained by the presence of a significant residual ash in primary sludge pretreated with spent acid (21.5%; Table 1). Despite decreasing $CaCO_3$ content and pH, the amount of ash remained high, and its presence may have hindered the enzyme accessibility to the carbohydrate fraction. As a solution to increasing the enzymatic digestibility of primary sludge

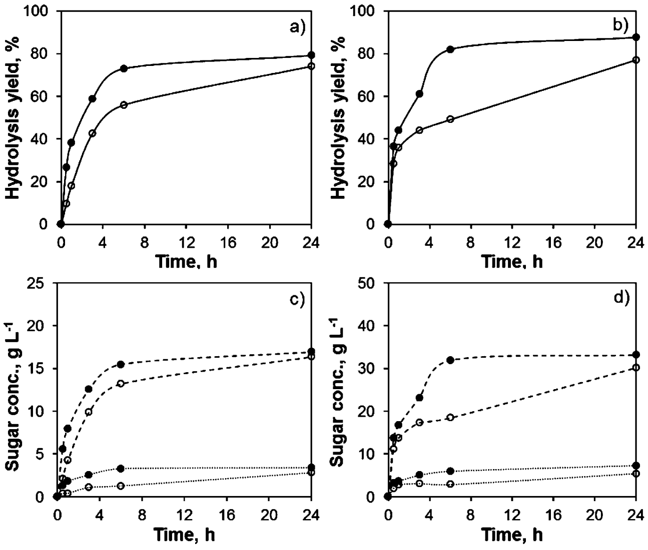

Figure 1. Enzymatic hydrolysis of (a and c) 25.7 and (b and d) 46 g L^{-1} of carbohydrates of primary sludge pretreated with HCl using 35 FPU g_{CH}^{-1} of Cellic CTec2 (●) or Accellerase 1500 (○). The hydrolysis yield (solid line), glucose concentration (dashed line), and xylose concentration (round-dotted line) are shown.

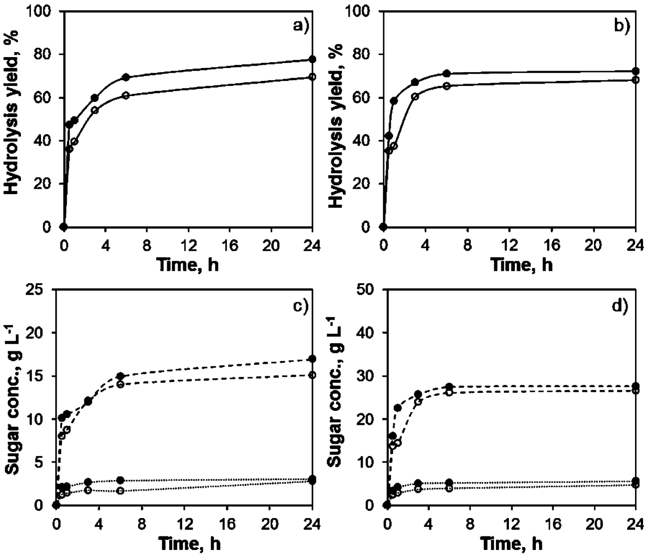

Figure 2. Enzymatic hydrolysis of (a and c) 25.7 and (b and d) 46 g L^{-1} of carbohydrates of primary sludge pretreated with spent acid using 35 FPU g_{CH}^{-1} of Cellic CTec2 (●) or Accellerase 1500 (○). The hydrolysis yield (solid line), glucose concentration (dashed line), and xylose concentration (round-dotted line) are shown.

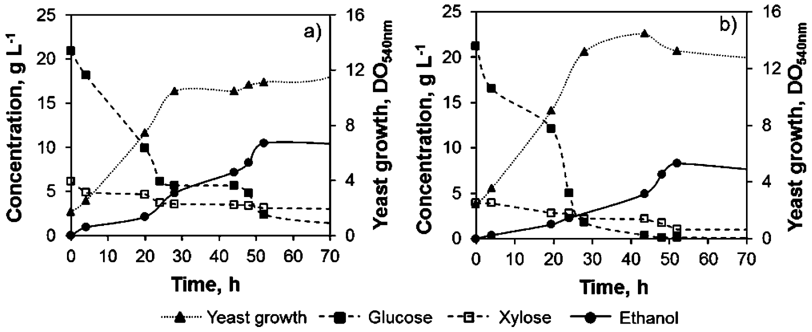

Figure 3. Ethanolic fermentation of the hydrolyzates obtained from the enzymatic hydrolysis of 46 g L^{-1} of carbohydrates of primary sludge pretreated with HCl, with (a) *P. stipitis* DSM 3651 and (b) *S. cerevisiae*.

pretreated with spent acid, the removal of Na_2SO_4 salts from spent acid before its use to neutralize $CaCO_3$ is suggested.

From the results presented in Figures 1 and 2, Cellic CTec2 is more adequate to hydrolyze primary sludge than Accellerase 1500, independent of the pretreatment applied or the initial carbohydrate content used. The enzymatic hydrolysis of primary sludge is strongly favored when a pretreatment is applied. $CaCO_3$ neutralization with HCl enabled better results in the enzymatic hydrolysis than $CaCO_3$ neutralization with spent acid. Nevertheless, spent acid remains a good alternative for primary sludge pretreatment because it is a low-value residual stream from the pulp mill. The enzymatic hydrolyzates used in the fermentation step contained 82−85% of glucose and 15−18% of xylose.

The outcomes of this work for sludge hydrolysis are in the range of some results in the literature, achieved with different lignocellulosic materials (and composition), pretreatments, enzymes, and operation conditions. Chen et al.[24] enhanced the enzymatic hydrolysis yield of recovered office printing paper (with 12% of ash) when a pretreatment was applied. The recovery sugar was nearly 70 and 85% respectively after acidification or filler removal. The authors reported that even an ash content of 4% may still interfere with the enzymatic hydrolysis, even after pH adjustment. This interference was explained by direct interactions between the enzyme and $CaCO_3$ because ash adsorbs enzymes with a greater affinity than fibers, decreasing the enzymatic hydrolysis efficiency.[24] Marques et al.[7] reported enzymatic hydrolysis yields of 45−100% in the bioconversion of recycled paper sludge neutralized with HCl. Peng and Chen[9] determined a maximum degree of saccharification of 82% in the enzymatic hydrolysis of paper sludge after 82.7 h of reaction.

3.3. Ethanolic Fermentation of Primary Sludge Hydrolyzates.

The hydrolyzates, obtained after the enzymatic hydrolysis, with Cellic CTec2, of 46 g L^{-1} of carbohydrates from primary sludge pretreated with HCl or spent acid, were autoclaved at 121 °C for 15 min prior to the ethanolic fermentation stage. It was observed that glucose and xylose were significantly lost during the sterilization process. The exclusion of sterilization or finding alternative sterilization methods is under study. Nevertheless, for the following fermentation step, glucose and xylose remained after sterilization were measured and were used to follow fermentation and to evaluate the ethanol yield and productivity. The fermentation performance of the enzymatic hydrolyzates, obtained from primary sludge pretreated with HCl, with *P. stipitis* DSM 3651 and *S. cerevisiae*, is shown in parts a and b of Figure 3, respectively. The ethanol yield and productivity are shown in Table 2.

Table 2. Ethanolic Fermentation, with *P. stipitis* DSM 3651 or *S. cerevisiae*, of Enzymatic Hydrolyzate Primary Sludge, Pretreated with HCl or Spent Acid

	primary sludge pretreatment			
	HCl		spent acid	
	P. stipitis	*S. cerevisiae*	*P. stipitis*	*S. cerevisiae*
available sugars, g L^{-1}	27.0	25.2	22.1	21.8
[EtOH], g L^{-1}	10.5	8.3	8.5	7.1
time, h	52	52	52	52
EtOH productivity, g L^{-1} h^{-1}	0.20	0.16	0.16	0.14
EtOH yield,[a] g_{EtOH} g_{sug}^{-1}	0.39	0.33	0.39	0.32

[a] The theoretical yield is 0.51 g_{EtOH} g_{sug}^{-1}.

P. stipitis DSM 3651 produced 10.5 g L^{-1} of ethanol after 52 h of fermentation. At the end, a glucose concentration of 2.4 g L^{-1} and a xylose concentration of 3.1 g L^{-1} of xylose still remained. In the fermentation carried out with *S. cerevisiae*, all glucose was used. The ethanol produced by *S. cerevisiae* (8.3 g L^{-1}) was lower than that with *P. stipitis* DSM 3651, but a higher cell density was registered. Therefore, glucose consumed led to the production of new cellular material rather than generating ethanol.

The fermentation performance of the enzymatic hydrolyzates, obtained from primary sludge pretreated with spent acid, with *P. stipitis* DSM 3651 and *S. cerevisiae*, is shown in parts a and b of Figure 4, respectively. Table 2 shows the fermentation parameters obtained. Glucose was fully consumed, and the xylose content remained practically constant during fermentation of primary sludge pretreated with spent acid. Ethanol concentrations of 8.5 and 7.1 g L^{-1} were produced by *P. stipitis* DSM 3651 and *S. cerevisiae*, respectively.

The results were lower than those obtained in the same fermentation time of the enzymatic hydrolyzates from primary sludge pretreated with HCl. However, the total available sugars at the beginning of the fermentation were here even lower than the ones reported in Figure 3. According to Table 2, similar ethanol yields (based on the initial available sugars) were obtained in the fermentation of primary sludge hydrolyzates, regardless of the pretreatment used. However, ethanol yields obtained with *P. stipitis* DSM 3651 (0.39 g_{EtOH} g_{sug}^{-1}; Table 2) were higher than the ones obtained with *S. cerevisiae* (up to 0.33 g_{EtOH} g_{sug}^{-1}). The highest ethanol productivity was observed in the fermentation of hydrolyzates from primary sludge pre-

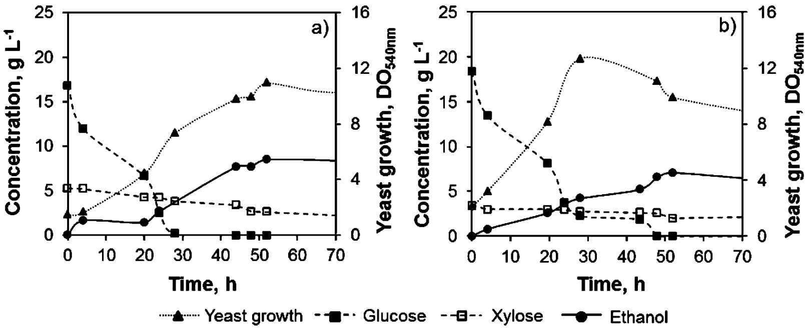

Figure 4. Ethanolic fermentation of the hydrolyzates obtained from the enzymatic hydrolysis of 46 g L^{-1} of carbohydrates of primary sludge pretreated with spent acid, with (a) *P. stipitis* DSM 3651 and (b) *S. cerevisiae.*

treated with HCl with *P. stipitis* DSM 3651 (0.20 g L^{-1} h^{-1}; Table 2).

Peng and Chen[9] obtained 9.5 g L^{-1} of ethanol in the fermentation of an enzymatic hydrolyzate from paper sludge, containing a total reducing sugar concentration of 27.8 g L^{-1}. An ethanol yield of 0.34 g_{EtOH} $g_{sug}{}^{-1}$ (based on total sugar) and a productivity of 0.59 g L^{-1} h^{-1} were achieved with *S. cerevisiae* GIM-2.[9] Marques et al.[7] produced an ethanol concentration of 19.6 g L^{-1} in the fermentation of enzymatic hydrolyzates from recycled paper sludge containing 56.2 g L^{-1} of glucose, 12.9 g L^{-1} of xylose, and 6.8 g L^{-1} of cellobiose. *P. stipitis* CBS 5773 was used, and a yield of 0.25 g_{EtOH} $g_{sug}{}^{-1}$ and a productivity of 0.33 g L^{-1} h^{-1} were achieved.[7] Therefore, the results obtained in the present work are within the range of results reported in the literature for similar lignocellulosic residues. This work gives evidence that biological concerns are overcome for valorization of primary sludge from pulp and paper mills.

4. CONCLUSIONS

Primary sludge, a residual solid waste from Portuguese pulp and paper mills, can be converted to bioethanol by separated enzymatic hydrolysis and fermentation. Using this biological process, a pretreatment should be applied to decrease the $CaCO_3$ amount and pH value of primary sludge. $CaCO_3$ neutralization with HCl or spent acid (a residual stream from the mill) is efficient but releases CO_2. Concerning alternative methods, a sequence of 12 or more washing cycles with reused water may be a good substitute. Higher sugar conversion yields (88%) were obtained in the enzymatic hydrolysis of primary sludge pretreated with HCl (carbohydrate concentration of 46 g L^{-1}), using 35 FPU $g_{CH}{}^{-1}$ of Cellic CTec2. Being the primary sludge collected in a pulp and paper mill that uses *E. globulus* as the raw material, the enzymatic hydrolysis releases 82–85% of glucose and 15–18% of xylose. In the following fermentation, no significant differences were registered between hydrolyzates produced after HCl or spent acid pretreatment or using the yeast strains *P. stipitis* DSM 3651 or *S. cerevisiae*. However, *P. stipitis* DSM 3651 showed a better performance, leading to ethanol concentrations of up to 10.5 g L^{-1} produced from 27 g L^{-1} of sugars, with a yield of 0.39 g_{EtOH} $g_{sug}{}^{-1}$ and a productivity of 0.20 g L^{-1} h^{-1}.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Balat, M. Production of Bioethanol from Lignocellulosic Materials via the Biochemical Pathway: A Review. *Energy Convers. Manage.* **2011**, *52*, 585.

(2) CELPA—Portuguese Paper Industry Association. Statistical Bolletin 2012. http://www.celpa.pt/FileGet.aspx?FileId=4435 (accessed Sept 2013).

(3) Kádár, Zs.; Szengyel, Zs.; Réczey, K. Simultaneous Saccharification and Fermentation (SSF) of Industrial Wastes for the Production of Ethanol. *Ind. Crop. Prod.* **2004**, *20*, 103.

(4) Kang, L.; Wang, W.; Lee, Y. Y. Bioconversion of Kraft Paper Mill Sludges to Ethanol by SSF and SSCF. *Appl. Biochem. Biotechnol.* **2010**, *161*, 53.

(5) Duff, S. J. B.; Murray, W. D. Bioconversion of Forest Products Industry Waste Cellulosics to Fuel Ethanol: A Review. *Bioresour. Technol.* **1995**, *55*, 1.

(6) Lynd, L. R.; Lyford, K.; South, C. R.; van Walsum, P.; Levenson, K. Evaluation of Paper Sludges for Amenability to Enzymatic Hydrolysis and Conversion to Ethanol. *Tappi J.* **2001**, *84*, 50.

(7) Marques, S.; Alves, L.; Roseiro, J. C.; Gírio, F. M. Conversion of Recycled Paper Sludge to Ethanol by SHF and SSF using *Pichia stipitis*. *Biomass Bioenerg.* **2008**, *32*, 400.

(8) Park, I.; Kim, I.; Kang, K.; Sohn, H.; Rhee, I.; Jin, I.; Jang, H. Cellulose Ethanol Production from Waste Newsprint by Simultaneous Saccharification and Fermentation using *Saccharomyces cerevisiae* KNU5377. *Process Biochem.* **2010**, *45*, 487.

(9) Peng, L.; Chen, Y. Conversion of Paper Sludge to Ethanol by Separate Hydrolysis and Fermentation (SHF) using *Saccharomyces cerevisiae*. *Biomass Bioenerg.* **2011**, *35*, 1600.

(10) Fitzpatrick, M.; Champagne, P.; Cunningham, M. F.; Whitney, R. A. A Biorefinery Processing Perspective: Treatment of Lignocellulosic Materials for the Production of Value-Added Products. *Bioresour. Technol.* **2010**, *101*, 8915.

(11) Wang, X.; Song, A.; Li, L.; Li, X.; Zhang, R.; Bao, J. Effect of Calcium Carbonate in Waste Office Paper on Enzymatic Hydrolysis Efficiency and Enhancement Procedures. *Korean J. Chem. Eng.* **2011**, *28*, 550.

(12) Hendricks, A. T. W. M.; Zeeman, G. Pretreatments to Enhance the Digestibility of Lignocellulosic Biomass. *Bioresour. Technol.* **2009**, *100*, 10.

(13) Kojima, Y.; Yoon, S.-L. Improved Enzymatic Hydrolysis of Waste Paper by Ozone Pretreatment. *J. Mater. Cycles Waste* **2008**, *10*, 134.

(14) Taherzadeh, M. J.; Karimi, K. Pretreatment of Lignocellulosic Wastes to Improve Ethanol and Biogas Production: A Review. *Int. J. Mol. Sci.* **2008**, *9*, 1621.

(15) El-Zawawy, W. K.; Ibrahim, M. M.; Abdel-Fattah, Y. R.; Soliman, N. A.; Mahmoud, M. M. Acid and Enzyme Hydrolysis to Convert Pretreated Lignocellulosic Materials into Glucose for Ethanol Production. *Carbohydr. Polym.* **2011**, *84*, 865.

(16) Mendes, C. V. T.; Carvalho, M. G. V. S.; Baptista, C. M. S. G.; Rocha, J. M. S.; Soares, B. I. G.; Sousa, G. D. A. Valorisation of Hardwood Hemicelluloses in the Kraft Pulping Process by using an Integrated Biorefinery Concept. *Food Bioprod. Process.* **2009**, *87*, 197.

(17) Mendes, C. V. T.; Rocha, J. M. S.; Soares, B. I. G.; Sousa, G. D. A.; Carvalho, M. G. V. S. Extraction of Hemicelluloses prior to Kraft Cooking: A Step for an Integrated Biorefinery in the Pulp Mill. *O Papel* **2011**, *72*, 79.

(18) Nigam, J. N. Ethanol Production from Wheat Straw Hemicellulose Hydrolysate by *Pichia stipitis*. *J. Biotechnol.* **2001**, *87*, 17.

(19) Deshwal, B. R.; Lee, H. K. Manufacture of Chlorine Dioxide from Sodium Chlorate: State of the Art. *J. Ind. Eng. Chem.* **2005**, *11*, 330.

(20) *Chemical Analysis and Testing Laboratory Analytical Procedures (LAPs)*; National Renewable Energy Laboratory: Washington, DC, 2008.

(21) Faga, B. A.; Wilkins, M. R.; Banat, I. M. Ethanol Production through Simultaneous Saccharification and Fermentation of Switchgrass using *Saccharomyces cerevisiae* D5A and Thermotolerant *Kluyveromyces marxianus* IMB Strains. *Bioresour. Technol.* **2010**, *101*, 2273.

(22) Siqueira, P. F.; Karp, S. G.; Carvalho, J. C.; Sturm, W.; Rodríguez-Léon, J. A.; Tholozan, J.-L.; Singhania, R. R.; Pandey, A.; Soccol, C. R. Production of Bio-ethanol from Soybean Molasses by *Saccharomyces cerevisiae* at Laboratory Scale, Pilot and Industrial Scales. *Bioresour. Technol.* **2008**, *99*, 8156.

(23) Liley, P. E.; Thomson, G. H.; Daubert, T. E.; Buck, E. B. Physical and Chemical Data. In *Perry's Chemical Engineers' Handbook*; Perry, R. H., Green, D. W., Maloney, J. O., Eds.; MgGraw-Hill: New York, 1997; pp 2−9.

(24) Chen, H.; Venditti, R. A.; Jameel, H.; Park, S. Enzymatic Hydrolysis of Recovered Office Printing Paper with Low Enzyme Dosages to Produce Fermentable Sugars. *Appl. Biochem. Biotechnol.* **2012**, *166*, 1121.