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 CaCO₃) and pH value affect the enzymatic hydrolysis of cellulosic fibers. Several pretreatments were used to reduce the CaCO₃ 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 gCH⁻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 gEtOH gₕₛug⁻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. 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 and 750 kt of paper production waste stream makes the content mostly calcium carbonate (CaCO₃), from lime kiln sludge, or used in composting. Finding an alternative solution to managing this heterogeneous solid waste paper) to enhance their digestibility. Hydrol

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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 CaCO₃), 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 (CH₃COOH), nitric acid (HNO₃), hydrochloric acid (HCl), and sulfuric acid (H₂SO₄) 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 CO₂ being released during the acid pretreatment, other alternatives were studied. Commercial CO₂ 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 (CaCO₃) 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 CaCO₃ 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 hemi-cellulases. 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. CaCO₃ neutralization was carried out with different inorganic or organic acids: H₂SO₄ (Fischer), HCl (Fischer), HNO₃ (Panreac), CH₃COOH (Fluka), and spent acid from the mill. The spent acid concentration was calculated to be equivalent to a 50% H₂SO₄ solution, determined by titration with 1 M sodium hydroxide. Ethylenediaminetetraacetic acid (EDTA; BDH Chemicals, Radnor, PA) was also tested to decrease the CaCO₃ 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 CaCO₃. 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 CaCO₃ from primary sludge. A sample of 5 g (dry weight) of primary sludge suspended in 250 mL of distilled water was maintained under constant CO₂ bubbling for 10, 30, and 60 min.

After each pretreatment, the ash and CaCO₃ 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⁻¹ CH) 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 3800 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 on 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.

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.

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

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

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

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 CaCO₃ 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 CaCO₃ 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

<table>
<thead>
<tr>
<th>type of pretreatment</th>
<th>organic content (% w/w)</th>
<th>ash content (% w/w)</th>
<th>CaCO₃ content (% w/w)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>none (untreated)</td>
<td>65</td>
<td>35</td>
<td>27</td>
<td>7.2</td>
</tr>
<tr>
<td>chemical agent</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EDTA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH₃COOH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HNO₃</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>HCl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H₂SO₄</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>spent acid</td>
<td>78.5</td>
<td>21.5</td>
<td>1.1</td>
<td>5.0</td>
</tr>
<tr>
<td>water (no. of washing cycles)</td>
<td>77.5</td>
<td>22.5</td>
<td>11</td>
<td>6.4</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
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<tr>
<td>3</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 (recirculated water)</td>
<td>91.6</td>
<td>8.4</td>
<td>0.6</td>
<td>6.3</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>carbonated water (with CO₂ bubbling, min)</td>
<td>69.8</td>
<td>30.2</td>
<td>18.2</td>
<td>6.9</td>
</tr>
<tr>
<td>10</td>
<td></td>
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<tr>
<td>30</td>
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<tr>
<td>60</td>
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</table>

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 CaCO₃ particles are dispersed. During the filtration process of the suspended primary sludge, CaCO₃ particles may be dragged by water and eliminated through the fine metal mesh screen with the appropriate porosity. The elimination of CaCO₃ by a dissolution effect is not very probable because its solubility in water is very low (up to 0.02 mg mL⁻¹). As shown in Table 1, when primary sludge was washed once with water, the ash and CaCO₃ contents decreased to 22.5% and 11%, respectively. The sequence of 2 or 3 washes enabled a decrease of the ash and CaCO₃ 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 CaCO₃ removal. For 6 or more sequential washes with water, CaCO₃ 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 CaCO₃ 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 H₂SO₄ is the most common method used in several works to treat paper sludge. The present work also studies HNO₃ and CH₃COOH addition. Furthermore, alternative methods, such as neutralization with spent acid, were also carried out. According to Table 1, when chemical agents were used, CaCO₃ diminished significantly in pretreated primary sludge and the pH value decreased. Acid

neutralization was more efficient with HCl and less with CH3COOH. Both H2SO4 and HCl were effective acids, almost promoting complete removal of CaCO3. However, primary sludge pretreated with H2SO4 still presented 11% of ash, while this fraction was insignificant with the other acids. This ash was attributed to CaSO4 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 CaCO3 and 21.5% of ash. The high amount of ash in the pretreated primary sludge is due to the presence of CaSO4 (produced in the reaction of H2SO4 and CaCO3) and Na2SO4 (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 CO2 to decrease the pH was also considered. The increment in the bubbling time enhanced the decrease in the CaCO3 amount. Nevertheless, after 60 min the CaCO3 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 gCH−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 CaCO3 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
pretreated with spent acid, the removal of Na₂SO₄ salts from spent acid before its use to neutralize CaCO₃ is suggested.

From the results presented in Figures 1 and 2, Cetic CTec2 is more adequate to hydrolyze primary sludge than Accellerase 1500, independent of the pretreatment used or the initial carbohydrate content used. The enzymatic hydrolysis of primary sludge is strongly favored when a pretreatment is applied. CaCO₃ neutralization with HCl enabled better results in the enzymatic hydrolysis than CaCO₃ 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 CaCO₃ 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 Chen⁸ 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 Cetic 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 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.

![Figure 3](image_url)

**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*.

<table>
<thead>
<tr>
<th>primary sludge pretreatment</th>
<th>HCl</th>
<th>spent acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>available sugars, g L⁻¹</td>
<td>27.0</td>
<td>25.2</td>
</tr>
<tr>
<td>[EtOH], g L⁻¹</td>
<td>10.5</td>
<td>8.3</td>
</tr>
<tr>
<td>time, h</td>
<td>52</td>
<td>52</td>
</tr>
<tr>
<td>EtOH productivity, g L⁻¹ h⁻¹</td>
<td>0.20</td>
<td>0.16</td>
</tr>
<tr>
<td>EtOH yield, a(1) EtOH g⁻¹</td>
<td>0.39</td>
<td>0.33</td>
</tr>
</tbody>
</table>

*a* The theoretical yield is 0.51 gEtOH g⁻¹ of ethanol after 52 h of fermentation. 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 hydrolyzates from primary sludge pretreated with spent acid. Ethanol concentrations of 8.5 and 7.1 g L⁻¹ were produced by *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 hydrolyzates from 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 gEtOH g⁻¹; Table 2) were higher than the ones obtained with *S. cerevisiae* (up to 0.33 gEtOH g⁻¹). The highest ethanol productivity was observed in the fermentation of hydrolyzates from primary sludge pre-

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

<table>
<thead>
<tr>
<th>primary sludge pretreatment</th>
<th>HCl</th>
<th>spent acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>available sugars, g L⁻¹</td>
<td>27.0</td>
<td>25.2</td>
</tr>
<tr>
<td>[EtOH], g L⁻¹</td>
<td>10.5</td>
<td>8.3</td>
</tr>
<tr>
<td>time, h</td>
<td>52</td>
<td>52</td>
</tr>
<tr>
<td>EtOH productivity, g L⁻¹ h⁻¹</td>
<td>0.20</td>
<td>0.16</td>
</tr>
<tr>
<td>EtOH yield, a(1) EtOH g⁻¹</td>
<td>0.39</td>
<td>0.33</td>
</tr>
</tbody>
</table>

*a* The theoretical yield is 0.51 gEtOH g⁻¹ of ethanol after 52 h of fermentation. The ethanol 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 hydrolyzates.
Peng and Chen 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 gEtOH gsug$^{-1}$ was used, and a yield of 0.25 gEtOH gsug$^{-1}$ and a productivity of 0.33 g L$^{-1}$ h$^{-1}$ were achieved. 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 gCH$_{1}$ 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 gEtOH gsug$^{-1}$ and a productivity of 0.20 g L$^{-1}$ h$^{-1}$.

Acknowledgments

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References


