Evaluation of a pilot-scaled paddle dryer for the production of ethanol from lignocellulose including inhibitor removal and high-solids enzymatic hydrolysis

Egidio Violaab,*, Giuseppe Arcieria, Francesco Zimbardia, Vito Valerioa, Nadia Ceronea,c, Ugo De Coratoab

* Italian National Agency for New Technologies, Energy and Sustainable Economic Development (ENEA), Trisaia Research Centre, S.S. 106 Jonica, km 419 + 500, 75026 Rotondella, MT, Italy
b Italian National Agency for New Technologies, Energy and Sustainable Economic Development (ENEA), Territorial Office of Bari—Uni. Versus Consortium, Viale Japiga 188, Bari 70126, Italy
c University of Naples Federico II, Department of Chemical Engineering, Materials and Industrial Production, P.le Tecchio, 80125 Napoli, Italy

Abstract

The advantage of using paddle dryers (PD) in the production of sugars and 2nd generation ethanol from pretreated wheat straw was investigated. This machinery was employed in order to detoxify steam-exploded substrates and to mix different slurries in the hydrolysis step. The obtained hydrolysate was fermented by the yeast Saccharomyces cerevisiae. Acetic acid and furfural were reduced up to 11 and 26 fold respectively in the detoxified substrate. When fermentation was carried out at low solid suspension, the use of PD was as effective as water extraction in detoxifying exploded biomass, giving ethanol yields of 90% at 0.05 solid/liquid ratio (S/L) and 80% at 0.10 S/L. Moreover, by using PD the cellulose conversion yield was significantly improved in the hydrolysis step: when operating at higher S/L (0.4), the hydrolysis efficiency was twice the one achieved by using a bioreactor with a Rushton stirrer.

© 2016 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

The production of bioethanol from lignocellulosic biomass is widely reported and various near-commercial phase processes have been proposed [10]. However, research and development activities are still required to improve operations, efficiency and economics [4]. The transformation of sugars in byproducts during the pretreatment step is one of the most challenging issues. Besides lowering the yields of the desired products, these byproducts can completely inhibit the involved microorganisms. Bellido et al. [5] showed that an increase in acetic acid concentration led to a reduction in ethanol productivity, with complete inhibition observed at 3.5 g/L. On the other hand, the addition of furfural produced a delay on sugar consumption rates.

The water washing is a good method to remove inhibitors, but with this procedure the soluble sugars are separated from the solids [9]; these soluble carbohydrates, both free sugars and oligomers, are diluted and still mixed with inhibitors in the resulting aqueous stream, so their exploitation could be difficult and not economic. Other detoxification procedures are based on the use of calcium hydroxide, sodium sulfite, activated carbon, laccase, or extraction with organic solvents; however, the use of chemicals implies increase of economic costs and environmental burdens [12,14,11,23].

Another important issue is the ethanol concentration in the final fermentation broth, which has to be higher than 4–5% to reduce the cost of energy required for ethanol distillation [17,33]. In turn, this implies a load of 200–300 kg m⁻³ of lignocellulosics in the bioreactor, i.e., working with slurry at high solid/liquid ratio. Such slurries are difficult to mix because of the swelling properties of cellulose fibers, so negative effects on the yields of saccharification and fermentation have been highlighted [30].

Existing machineries (already used in agroindustry, pulping, biotech industry, etc.) can be utilized to deal with lignocellulosic biomass in the biorefinery field.

A paddle dryer (PD) is a low speed stirrer with fan-shaped hollow paddles, in which a hot fluid is circulating. It is currently used to dry sludge and granular materials. The performances of this machinery are based on the high rate of heat transfer that allows short drying time coupled with the homogeneous quality of the product (www.andrittzgouda.com).
In this work PD was tested to detoxify the exploded substrate (removing the volatile inhibitors by evaporation), and to obtain an efficient enzymatic hydrolysis of the substrate in high S/L suspensions. The condensate stream of water and organic molecules can be treated as liquid waste in an anaerobic digester. Alternatively, a separation step can be considered to recover the chemicals that could have an economic value as building blocks.

The achievement of positive results by using a modified PD (commercially available in its base form) may provide significant advantages in fulfilling economics and technical targets, i.e., by simplifying the plant/process and by increasing yields.

2. Materials and methods

Steam Explosion (SE) pretreatment was chosen to improve cellulose digestibility, because of its efficiency and low environmental burden [13,7,2]. Wheat straw was used as feedstock (representative lignocellulosic biomass), due to its abundance as agricultural residue [28]. The pretreatment at 210 °C for 6 min was previously assessed as optimal for the bioconversion goal [31]. The schemes of the operations are reported in Fig. 1. In Path A the exploded straw was extracted with water at 60 °C, the slurry was then filtered to produce the detoxified substrate, used for benchmark tests of bioconversion. In Path B the detoxification was carried out in the PD system and the hydrolysis was done in the same apparatus by introducing water, buffer and enzymes (see below).

2.1. Materials

As raw material, locally harvested wheat straw was utilized. Before the steam explosion treatment, the straw was grinded with a common straw chopper, equipped with sieve having holes with a diameter of 1 in.; the average particle size of the grinded straw was 1 cm length, 1 mm thick.

The commercial product Celiac® Ctec2 was used as enzymes (from Novozyme A/S, Denmark). The yeast (Saccharomyces cerevisiae, YSC-2, dry solid form, stored at 4 °C) and other chemicals were purchased from Sigma–Aldrich.

2.2. Steam explosion treatment and water extraction

The SE was carried out with a continuous reactor (Stake Tech II CO-AX Feeder Digester) able to process 150–300 kg/h of freshly harvested or dried biomass. The reactor is a tubular steel cylinder, having inside a screw that, continuously, moves the cooked biomass toward a blow valve. The treatment consisted in exposing the biomass (previously humidified in order to have DM around 50%) to saturated steam at 210 °C for a residence time of 6 min, then suddenly decompressing the slurry through the blow valve. A

![Flowchart](image)

Fig. 1. Pretreatment, detoxification and bioconversion of wheat straw. Path A: typical route to produce 2nd Generation ethanol; Path B: the new concept of using the PD as detoxifier and hydrolyser.
batch of 400 kg of exploded straw (brown pulp, pH 4.2, DM 55–60%, produced in 2 h of plant exercise) was recovered. It was loaded in the PD without any other treatment (path B), or it was detoxified by water extraction (path A). In the latter case, 200 kg of exploded straw was extracted with 2000 l of water at 60 °C in a 2 m³ tank; the slurry was filtered on a counter current multistage belt filter (Koline-Sanderson K-S ADPEC Horizontal Vacuum Filter) to separate the soluble substances (mainly hemicellulose and inhibitors) from the cellulose and lignin; then the insoluble matter (IM) was recovered and stored at 4 °C.

2.3. Paddle dryer detoxification

A PD with internal working volume of 25 l was used (from MFG-Dutch Gouda, Netherlands). About 6 kg of exploded biomass were treated in each test; the rotation speed of the paddles was set at the optimized value of 40 rpm (minimum value that guaranteed a good mixing without loss of efficiency). The paddles and the external jacket were heated by hot water, using a recirculation system. The temperature inside the PD was regulated by a PID (proportional–integral–derivative) controller and kept at 65 ± 5 °C (Fig. 2). The machinery was equipped with a removable top cover connected to a vacuum line for conveying the volatiles in a condensation system, thus avoiding the pollution of inhibitors in the air.

The PD was employed by using two configurations or methods: the first was the conventional way for which the machinery was built; the second was planned during this work to obtain a moist substrate. More specifically the two methods can be described as follows: method (1) complete biomass drying, producing a detoxified dry material (DDM method); method (2) incomplete drying, producing a detoxified moist material (DMM method); in this method, the moisture that leaves the substrate as vapor was partially balanced by spraying demineralized water (about 50 ml/min) on the biomass during the treatment, in order to maintain the DM at 40–50%.

2.4. Hydrolysis and fermentation (bioconversion)

In path A, for the pre-hydrolysis step, the bioconversion of the detoxified substrate was carried out in a conventional bioreactor (21 glass vessel, equipped with: engine, Rushton stirrer, controller of temperature and rpm; model Biostat B of B. Braun Biotech); the sequential SSF was carried out in shaken flasks (200 ml, orbital shaker at 150 rpm). The first step of hydrolysis was carried at 50 °C, 50 rpm, for 24 h, and then the process was continued at 35 °C for 72 h with the addition of the yeast. As overall, the procedure was a separate pre-hydrolysis step (SH) followed by simultaneous saccharification and fermentation (SSF). This sequence exploits the higher activity of the enzymes at 50 °C to reach a high sugar concentration level in the short term, and avoids enzyme inhibition (by product) in the long term because the yeast continuously metabolizes it during the SSF. The mix of enzymes CteC2, composed by cellulases (endo- and exo-) and cellobiases, (6 g of commercial solution per 100 gDM of substrate; protein content in solution: 73 mg/ml; activity: 151 FPU/ml; specific gravity 1.2 g/ml) was used. The enzymatic hydrolysis was carried out at different solid-to-liquid consistencies (S/L, where S is the weight of dry solid phase, and L is the weight of the total liquid phase): S/L 0.05; 0.10; 0.20; 0.40, in a medium containing 0.05 M sodium acetate buffer (pH 5). After the pre-hydrolysis step, the suspension was cooled at 35 °C for the SSF step, supplemented with yeast (S. cerevisiae) and nutrients to obtain a medium containing 3 g/l of yeast, 2.5 g/l of yeast extract, 0.25 g/l of (NH4)2HPO4, 0.025 g/l of MgSO4·H2O.

In Path B, the SH was carried out in the PD system at 50 °C for 24 h (after the detoxification step), at the same conditions of enzyme dosage and S/L ratio as in Path A. The SSF step was carried
out in flasks by using the obtained hydrolysate and introducing the yeast and supplements as described above.

All experiments were performed in duplicate and the analytical determinations in triplicate. The yields were reported as percentage ratios between the obtained products and the stoichiometric values expected from the complete conversion of the glucan (contained in the substrate submitted to the bioconversion) to glucose and of the glucose to ethanol.

2.5. Samples preparation and analyses

The wheat straws and the dried exploded materials were ground with a mill equipped with a 50 mesh sieve and dried overnight at 60°C. The extractives were determined by Soxhlet extraction using a mixture of toluene and ethanol (2:1) for 6 hours (CPIA G-13 method) [8]. The lignin and carbohydrate contents were determined by the Klason procedure (TAPPI T13 m-54) [29]. The sugar analysis was carried out on the hydrolysates by HPLC (High Performance Ion Chromatography, DIONEX DX300) with Carbopac PA1 column, using 2 mM NaOH as eluent (flow rate 1.0 ml/min, at 28°C) and a PED (pulsed electrochemical detector). Soluble lignin in the Klason filtrate was determined by UV spectrophotometry (HITACHI Co. V2000) at 205 nm (TAPPI Useful Methods 25D). The ash content was determined by combustion at 600°C (ASTM-1102, modified) [3].

Acetic acid, furfural and hydroxymethylfurfural were determined in the aqueous phase by HPLC (hp1100 series) equipped with diode array UV detector, column Phenomenex Synergi Fusion-RP 80, and using as eluent a mix of acetonitrile and water, solvent gradient 3–50%.

The concentration of the ethanol in the broth was determined by using a HPIC (Dionex LC30), equipped with an AS50 automatic injector, column Nucleogel Ion 300 OA, refractive index ED50 as detector, and using as eluent H₂SO₄ 0.05 M (40°C, 0.4 ml/min).

3. Results and discussion

3.1. Materials characterization

The composition of the straw is reported in Table 1. The inorganic matter was used as a tracer to assess the mass balance through the SE and drying steps. The ash content in the products was compared to its value in the raw material to obtain the actual mass; the recovery of each component was calculated from the percentage composition obtained by the chemical analysis and the actual mass:

Solid produced from 100 g of straw = 100g × (ash in the straw) / (ash in the product).

Recovery of the component i from 100 g of straw = % (i) × solid produced

This method was previously used to assess the loss of organic mass after the steam explosion treatment [32].

Table 1 shows the recoveries after each step. On this basis, it was also possible to evaluate the mass loss as volatile matter, both as H₂O from dehydration reactions and small organic molecules. During the SE, 65% of the hemicellulose (xylan, arabinan, galactan) and 10% of the glucan was degraded by hydrothermal reactions. Using the ash tracer method it was calculated that 15.1% of the starting mass was transformed in volatile matter, removed with the steam during the explosion flash and the drying. After SE treatment, 24.2% of the straw was solubilized by water extraction, 72% of the hemicellulose contained in the exploded product was solubilized, while 95.4% of the glucan remained in the insoluble material, constituting more than half of the residue. Most of the inorganics (58%) were extracted by water after the SE. The treatment of the exploded straw with PD has slightly affected the composition: glucan and xylan contents were reduced, while mass loss, acid insoluble residue (Klason lignin) and other undetermined matter were increased.

3.2. Detoxification

The dry matter of exploded straw was 58%; the complement 42% contained the moisture and the organic molecules that evaporated overnight at 65°C. The concentration of catechol, hydroxybenzaldehyde and formic acid in the exploded straw were below 50 ppm, so these molecules were not considered in the analysis. The drying in the PD was followed by determining the DM of samples picked up during the trial (in the case of DDM method). After 40 minutes the substrate had a 99% DM; in order to guarantee efficient removal of inhibitors, the trials were prolonged up to one hour. In Fig. 3 the concentrations of these molecules in the substrate along the PD treatments are reported. By using the dry material method or moist material method, the content of acetic acid was reduced by 7 and 11 fold, the furfural content by 13 and 26 fold, respectively. With the water spraying (DDM method), the biomass keeps moisture, but the volatile inhibitors were removed more efficiently, thanks to the stripping effect of the vapor flow continuously leaving the fibers.

The inhibitors reduction with the paddle dryer offers the following advantages compared to the detoxification made by water extraction: (1) no hemicellulose was removed, allowing the fermentation of C5 sugars together with C6; (2) no filtration is necessary; (3) the water consumption is reduced (1 l/kg with PD vs 10 l/kg with water extraction).

Table 1
Straw composition and mass recovery of the constituents after treatments.

| Component   | Exploded straw | Insoluble matter | Soluble matter | Exploded straw after PD |
|-------------|---------------|------------------|----------------|-------------------------|
| Inorganic   | 6.9           | 6.9              | 2.9            | 4.0                     | 6.9                   |
| Lignin (Klason) | 20.0          | 27.5             | 20.0           | 7.5                     | 29.3                  |
| soluble Klason lignin | 2.0     | 2.9              | 1.6            | 1.3                     | 3.1                   |
| Extractives | 8.0           | –                | –              | –                       | –                     |
| Arabinan    | 2.3           | 0.2              | 0.0            | 0.2                     | 0.3                   |
| Galactan    | 0.7           | 0.5              | 0.0            | 0.5                     | 0.5                   |
| Glucan      | 38.0          | 34.2             | 32.6           | 1.6                     | 31.3                  |
| Xylan       | 19.4          | 7.2              | 2.2            | 5.0                     | 4.8                   |
| nd          | 2.6           | 5.5              | 1.4            | 4.1                     | 8.4                   |
| Balance     | 100           | 84.9             | 60.7           | 24.2                    | 84.6                  |
| Mass loss   | 15.1          |                  |                |                         | 15.4                  |

* Determined as ashes; extractives not determined in the treated material.
3.3. Enzymatic hydrolysis

The stirring methods currently used in bioreactors are not efficient with high dry matter, so specifically designed reactors have to be used [19,34]. In the case of the lignocellulosic slurries, loads higher than 10% in mass involve bulk dragging at the expenses of microcirculation and of hydrolysis yield. The first step of the enzymatic action is the most difficult to deal with as these concentrated biomass slurries are highly viscous with non-Newtonian behaviors that pose several technical challenges to the conversion process [27]. Conventional bioreactors can be used only when the slurry becomes more fluid and homogeneous. Starting from a DM of 20% Roche et al. [24] found that the saccharified corn stover liquefied to the point of being pourable at biomass conversion of about 40%, after roughly 2 days. The fed batch procedure has been used to reach high final concentration of solid in SSF experiments from pretreated spruce [26], without highlighting major differences in overall performance between batch and fed-batch; however, the ethanol productivity during the first 24 h was higher in the fed-batch SSF experiments. Zhang et al. [34] have obtained ethanol concentrations of up to 84.7 g/l from a DM content of 25% by adding pretreated corncobs every 4 h during

![Fig. 3.](image-url) Removal of the volatile inhibitors during 1 h of PD treatment, using the detoxified dry material (DDM) method, and the detoxified moist material (DMM) method. In the plots are reported the concentrations of the inhibitors as wt% of the exploded straw (DM).

![Fig. 4.](image-url) The hydrolysis yields (cellulose conversion) with path A and path B (using PD with detoxified dry and moist material methods, DDM and DMM, respectively) at different solid to liquid ratio.
the first 24 h of SSF. The invention of new machinery and processes able to deal with concentrated slurries after the pretreatment, eventually in conjunction with hydrolysis and fermentation, is actively pursued and object of several patents [25,16]. In this work, we tested the PD to liquefy and hydrolyze slurries of SE treated straw, after the detoxification steps (DDM and DMM methods). In Fig. 4 the results obtained with these methods compared with those achieved with the water extracted substrate (Path A) are reported. In the case of low S/L and moist material method, cellulose hydrolysis was almost complete after the bioconversion process, but the yield was slightly lower in PD than that obtained in a conventional stirred reactor at 150 rpm. Significant lower yield was obtained in the case of the dried substrate, according to the hornification effect that caused an average loss of efficiency of 12% [20]. In general, the yields decreased with increasing S/L. The PD with the moist material method has given similar results obtained with stirred reactor at S/L 0.1. From that point ahead the PD showed superior performances. The decreasing of conversion with increasing solids concentrations was previously published [18]. Various factors could be suspected to play a role, such as insufficient mixing, high lignin content, inhibitors derived from hemicellulose, product inhibition (cellobiose or ethanol), [15] have found that the adsorption of cellulases decreases with increasing solids, thereby depressing its activity.

At S/L 0.4 the cellulose hydrolysis yield achieved with PD was 52% versus 28% in conventionally stirred reactors, clearly showing the advantage of using this machinery to carry out a pre-hydrolysis step (liquefaction) before the fermentation.

**Fig. 5.** Ethanol yields obtained through Path A and Path B (using PD with detoxified dry and moist material methods, DDM and DMM, respectively).

**Fig. 6.** Residual sugars detected in the fermentation broth at the end of the processes schematized in Fig. 1: Path A and Path B (using PD with detoxified dry and moist material methods, DDM and DMM, respectively).
3.4. Fermentation

Preliminary tests have shown that the exploded straw did not produce ethanol without detoxification. In fact, even if the enzymes efficiently hydrolyzed the cellulose, the subsequent conversion of glucose into ethanol was completely inhibited. In Figs. 5 and 6 the obtained results are shown. The concentrations (reported on the bars of Fig. 5) and the yields do not directly correlate, because the substrates have a different glucose/saccharo ratio (see Table 1).

At S/L 0.05 the cellulose was converted into glucose and this sugar was quantitatively metabolized into ethanol and other byproduct, like glycerol, acetic acid and lactic acid. A lower ethanol production was observed using PD and dry material method (Fig. 5); this can be attributed to the hornification that occurs by drying the substrate, which reduces the enzymatic hydrolysis efficiency. The xylose was obtained from the hydrolysis of hemicellulose and its amount is congruent with the complete hydrolysis of the xylan. Low amounts (< 1 g/l) of acetic acid, lactic acid and glycerol were also detected, due to the residual bacterial interference; their concentration did not seem to be linked to the different methods, since their production appeared random, as observed in the different S/L cases.

At S/L 0.10 the fermentation occurred both in Path A and Path B, but in the latter case only by using the detoxified moist sample. In fact, by using the dried substrate, the glucose was not fermented; this could be due to the fact that the concentration of inhibitors in the slurry had reached the threshold of toxicity, or, probably, new toxic compounds were formed, as the material was drying, which contributed to the inhibition. Through Path A and Path B (DDM method), the ethanol yields were 82 ± 3% and 79 ± 3%, respectively. By drying with PD, the biomass hornification increased, and, as consequence, the hydrolysis efficiency decreased; it follows that the DDM method is more suitable.

At S/L 0.2 the PD increased the enzymatic hydrolysis yield, but ethanol was not produced; the inhibitor concentration overcomes the toxicity threshold also in the moist material. Only in Path A the fermentation occurred, but with low yield (51%).

At S/L 0.4 the fermentation did not start at all, but, as reported in Fig. 4, the hydrolysis yield was significantly enhanced with the use of PD. In this case the method could be coupled with the use of tolerant strains, to obtain higher concentrate ethanol broths.

Overall, the PD resulted in an efficient system to enhance the enzymatic hydrolysis at high S/L because the slurry at high density can be efficiently mixed. A significant advantage of using PD compared to the water extraction is the higher availability of xylene in the hydrolysate; indeed, in Path A, the soluble xylan is taken away together with the inhibitors. The availability of microbial strains able to metabolize CS sugars makes the use of PD highly interesting [22].

4. Conclusions

Paddle dryer (PD) can be used in the process of ethanol production from lignocellulosics with minor modifications. It can be employed to remove the main volatile inhibitors produced during the steam explosion treatment. By working at 65 °C for 1 h, acetic acid in the substrate was reduced by 11 fold, while furfural by 26 fold. By using PD to produce a detoxified moist material, the ethanol obtained at lower S/L was comparable to that obtained with the detoxification by water washing. These results can contribute to simplify the process, reduce water consumption and save plant cost. By using PD to produce a dry material, the ethanol production yield decreased, but the advantage of water saving and the availability of more xylose in the substrate should be reconsidered. The mixing efficiency of PD can be exploited to carry out enzymatic hydrolysis at high solid loading. At S/L 0.4, the saccharification yield was twice that obtained by using a conventional stirred reactor.

Acknowledgement

The Misters E. Alvino, M. Carnevale, G. Pinto and A. Battafarano are acknowledged for the skilled technical assistance in the plant tests and general maintenance.

References

[2] V. Agbor, C. Carere, N. Cicik, R. Sparling, D. Levin, Biomass pretreatment for consolidated bioprocessing (CBP), Adv. Bioenineeris (2014) 234–258.
[3] A.S.T.M. Standard C33, Specification for Concrete Aggregates, ASTM, International, West Conshohocken, PA, 2003, doi: http://dx.doi.org/10.1520/ C0033-03ED1. www.astm.org.
[4] S. Banerjee, S. Mudliar, R. Sen, B. Giri, D. Sarpatwe, T. Chakrabarti, R.A. Pandey, Commercializing lignocellulosic bioethanol: technology bottlenecks and possible remedies, Biofuels Bioprod. Biorefin. 4 (2010) 77–93.
[5] C. Belillo, S. Bolado, M. Coca, S. Lucas, G. González-Benito, M.T. García-Cubero, Effect of inhibitors formed during wheat straw pretreatment on ethanol production by Pichia stipitis, Bioresour. Technol. 102 (2011) 10868–10874.
[6] J. Chen, W. Zhang, H. Zhang, Q. Zhang, H. Huang, Screw extrude steam explosion: a promising pretreatment of corn stover to enhance enzymatic hydrolysis, Bioresearch. Technol. 101 (2014) 230–235.
[7] CFPA, Canadian Pulp and Paper Association Standard Methods, Standard Testing Methods, technical section, Canadian Pulp and Paper Association, Montreal, Canada, 1970.
[8] J. De Bari, E. Viola, D. Barisano, M. Cardinale, F. Fannia, F. Zimbardi, et al, Ethanol production at flask and pilot scale from concentrated slurries of steam exploded aspen, Ind. Eng. Chem. Res. 41 (2002) 1745–1753.
[9] P. Fairley, Next generation biofuels, Nature 474 (2011) 2–5.
[10] P. Ge, B.-Y. Cai, H.-Z. Liu, B.-Z. Fang, G. Song, X.-F. Yang, W.-X. Ping, Comparison of different detoxification methods for corn cob hemicellulose hydrolysate to improve ethanol production by Candida shehatae ACC 20335, Afr. J. Microbiol. Res. 5 (2011) 1163–1168.
[11] L. Hongqiang, C. Hongzhang, Detoxification of steam-exploded corn straw produced by an industrial-scale reactor, Process Biochem. 43 (2008) 1447–1451.
[12] T. Ichii, C.I. Ishizawa, M.F. Davis, M.E. Himmel, W.S. Adney, D.K. Johnson, Cellulase digestibility of pretreated biomass is limited by cellulase accessibility, Biotechnol. Bioeng. 98 (2007) 112–122.
[13] M. Jurado, A. Prieto, A. Martínez-Alcalá, Á.T. Martínez, M.J. Martínez, Laccase detoxification of steam-exploded wheat straw for second generation ethanol, Bioresearch. Technol. 100 (2009) 6378–6384.
[14] B.R. Kristensen, C. Felby, J. Henning, Yield-determining factors in high-solids enzymatic hydrolysis of lignocellulosic, Biotech. Biofuels. 2 (2009) 11.
[15] M.R. Ladjisch, M. Nathan, M.K. Young (2013), Biomass liquefaction processes, and uses of same. U.S. Patent Application, 13/754,352.
[16] K. Liu, X. Lin, J. Yue, X. Li, X. Fang, M. Zhu, J. Lin, Y. Qu, L. Xiao, High concentration ethanol production from corn cob residues by fed-batch strategy, Bioresearch. Technol. 101 (2010) 4952–4958.
[17] Y. Lu, Y. Wang, G. Xu, J. Chu, Y. Zhuang, S. Zhang, Influence of high solid concentration on enzymatic hydrolysis and fermentation of steam-exploded corn stover biomass, Appl. Biochem. Biotechnol. 160 (2010) 360–369.
[18] D. Ludwig, M. Buchmann, T. Hirth, S. Rupp, S. Zibek, High solids enzymatic hydrolysis of pretreated lignocellulosic materials with a powerful stirrer concept, Appl. Biochem. Biotechnol. 172 (2014) 1699–1713.
[19] X. Luo, J.Y. Zhu, Effects of dryin-induced fiber hornification on enzymatic saccharification of lignocellulose, Enzyme Microb. Technol. 48 (2011) 92–99.
[20] B. Palmqvist, G. Lidén, Combining the effects of process design and pH for improved xylene conversion in high solid ethanol production from Arundo donax, AMBI Express 4 (2014) 41.
[21] B. Palmqvist, B. Hahn-Hägerdal, Fermentation of lignocellulosic hydrolysates: I. inhibition and detoxification, Bioresearch. Technol. 74 (2000) 17–24.
[22] C.M. Roche, C.J. Dibble, J.S. Knutsen, J.J. Stickel, M.W. Liberatore, Particle concentration and yield stress of biomass slurries during enzymatic hydrolysis at high-solids loadings, Biotechnol. Bioeng. 104 (2009) 290–300.
[23] R.Romero, B. Stromberg (2011), Method and apparatus for mixing a lignocellulosic material with enzymes. U.S. Patent No. 8,617,355. 31.
[24] A. Rudolf, M. Akkasawel, G. Zach, G. Lidén, A comparison between batch and fed-batch simultaneous saccharification and fermentation of steam pretreated spruce, Enzyme Microbial Technol. 37 (2005) 195–204.
[25] J.J. Stickel, J.S. Knutsen, M.W. Liberatore, W. Luu, D.J. Bousfield, D.J. Klingenberg, C.T. Scott, T.W. Root, M.R. Ehnhard, T.O. Monz, Rheology measurements of a biomass slurry: an inter-laboratory study, Rheol. Acta 48 (2009) 1005–1015.
[26] F. Talebnia, D. Karakashev, I. Angelidaki, Production of bioethanol from wheat straw: an overview on pretreatment, hydrolysis and fermentation, Bioresearch. Technol. 101 (2010) 4744–4753.
[29] Technical Association of the Pulp and Paper Industry, T.A.P.P.I. Standards: Testing Methods, Recommended Practices, Specifications of the Technical Association of the Pulp and Paper Industry, Technical Association of the Pulp and Paper Industry, New York, 1950.

[30] S. Viamajala, J.D. McMillan, D.J. Schell, R.T. Elander, Rheology of corn stover slurries at high solids concentrations—effects of saccharification and particle size, Bioresour. Technol. 100 (2009) 925–934.

[31] E. Viola, F. Zimbardi, V. Valerio, F. Nanna, A. Battafarano, Use of a two-chamber reactor to improve enzymatic hydrolysis and fermentation of lignocellulosic materials, Appl. Energy 102 (2013) 198–203.

[32] E. Viola, F. Zimbardi, M. Cardinale, G. Cardinale, G. Braccio, E. Gambacorta, Processing cereal straws by steam explosion in a pilot plant to enhance digestibility in ruminants, Bioresour. Technol. 99 (2008) 681–689.

[33] A. Wingren, M. Galbe, G. Zacchi, Techno-economic evaluation of producing ethanol from softwood: comparison of SSF and SHF and identification of bottlenecks, Biotechnol. Prog. 19 (2003) 1109–1117.

[34] (a) J. Zhang, D. Chu, J. Huang, Z. Yu, G. Dai, J. Bao, Simultaneous saccharification and ethanol fermentation at high corn stover solids loading in a helical stirring bioreactor, Biotechnol. Bioeng. 105 (2010) 718–728.