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Research Article

Enzymatic hydrolysis and fermentation of ultradispersed wood particles after ultrasonic pretreatment

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ABSTRACT

Background: A study of the correlation between the particle size of lignocellulosic substrates and ultrasound pretreatment on the efficiency of further enzymatic hydrolysis and fermentation to ethanol.
Results: The maximum concentrations of glucose and, to a lesser extent, di- and trisaccharides were obtained in a series of experiments with 48-h enzymatic hydrolysis of pine raw materials ground at 380–400 rpm for 30 min. The highest glucose yield was observed at the end of the hydrolysis with a cellulase dosage of 10 mg of protein (204 ± 21 units CMCase per g of sawdust).
The greatest enzymatic hydrolysis efficiency was observed in a sample that combined two-stage grinding at 400 rpm with ultrasonic treatment for 5–10 min at a power of 10 W per kg of sawdust. The glucose yield in this case (35.5 g glucose l^{-1}) increased twofold compared to ground substrate without further preparation.
Conclusions: Using a mechanical two-stage grinding of lignocellulosic raw materials with ultrasonication increases the efficiency of subsequent enzymatic hydrolysis and fermentation.

1. Introduction

Modern economic development is closely associated with bioenergy as fossil fuel resources are depleted, leading to constant increases in fossil fuel prices. One way to solve this problem is to produce biofuels from renewable raw materials [1,2]. Bioethanol is traditionally produced from substrates containing both starch and lignocellulose [3,4,5]. While technologies based on starchy raw materials are well-developed, implemented and widely used, commercial cellulosic ethanol technology is still in its infancy. The conversion of lignocellulosic biomass to ethanol involves three processes: (1) a pretreatment process to increase the digestibility of cellulose and hemicellulose in the feedstock; (2) an enzymatic hydrolysis process to recover fermentable sugars from the pretreated material; and (3) a fermentation process to convert the obtained sugars into ethanol [6]. The main reason for this slow adoption is the recalcitrance of cellulose associated with lignin in the wood. Pretreatment technologies are aimed to increase enzyme accessibility to biomass and yields of fermentable sugars. Each pretreatment has a specific effect on the cellulose, hemicellulose and lignin fraction thus; different pretreatment methods and conditions should be chosen according to the process configuration selected for the subsequent hydrolysis and fermentation steps. In general, pretreatment methods fall into four different categories including physical, chemical, physico-chemical, and biological [4,7,8]. The main routes to produce ethanol from cellulose involve enzymes.

A major obstacle to complete substrate hydrolysis is the low availability of cellulose resulting from “shielding” by lignin [9,10]. Therefore, new methods and approaches are necessary to increase the availability of cellulose for enzymatic action. One approach is to break wood into ultrafine particles [11]. These technologies are limited by the need to increase the accessibility of fibers to enzymatic action. This limitation can be solved by mechanical treatment of raw materials to produce micron-sized particles [11,12,13] via dry grinding with mills of various designs (including ball mill). Other novel types of pretreatment such as microwaves, gamma radiation, and ultrasonication have been considered [14,15].

The aim of this study was to investigate the method of lignocellulose conversion in ethanol by enzymatic hydrolysis and subsequent fermentation with preliminary ultrasonic pretreatment.

2. Experimental

2.1. Materials

The object of this study was deresined *Pinus sylvestris* wood (Scots pine) with bark impurities no more than 5%, initial moisture content of 10.32% ± 0.37%, and no mechanical impurities. The corresponding air-dried wood was also studied. During the processing of lignocellulosic raw materials, knots and large splints were removed

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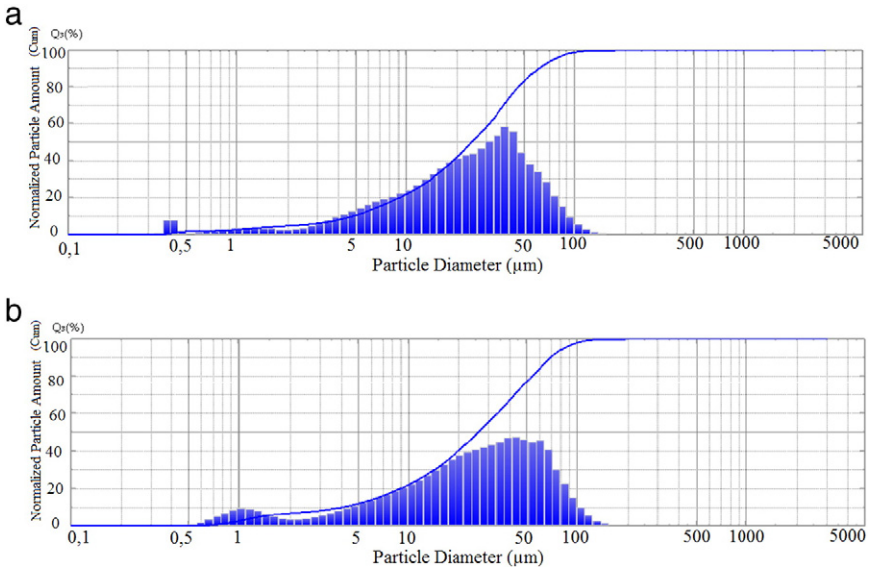


Fig. 1. Size dispersion of particles obtained by grinding of pine wood on a ball mill PM400 at 380 rpm for 30 min: (a) without treatment (initial moisture 18%), (b) after drying (initial moisture 10%).

by excluding particles with sizes greater than 10 ∗ 2 mm. The wet raw material was dried via convection heating in ventilated drying ovens at 50°C until 10% of the initial moisture remained.

2.2. Raw materials grinding

Grinding was performed using a one- or two-stage scheme. In the one-stage, raw material destruction is performed on a knife mill LZM-1 M at 15,000 rpm for 5 min. In the two-stage, first raw material destruction is performed on a knife mill LZM-1 M at 15,000 rpm for 5 min and then on vario-planetary ball mill PM400 set at 380–400 rpm for 20–30 min. Each loading of chips was no more than 70 g in mass. This loading corresponded to 45–55% of the grinding chamber volume, accounting for the volume of the grinding elements. The grinding period consisted of 2 min of operation followed by 2 min of cooling and 1 min of operation followed by 2 min of cooling.

2.3. Raw material pretreatment

For ultradispersed chip particles, ultrasonication pretreatment was performed using a UZG 2–4 M unit (ultrasonic generator, manufactured in Russia) with an output power of up to 6 kW and a resonance frequency of 16.8–9.2/20.5–23.5 kHz. Each treatment lasted up to 25 min. As a control, we used ultradispersed chips that were not pretreated.

2.4. Enzyme hydrolysis

For enzyme hydrolysis, highly active preparations (EP) were obtained from recombinant strains of *Penicillium verruculosum* 221–151. Preparation contained 834 ± 33 mg protein g. Enzyme activities in the preparation were: CMCase — 14.9 ± 0.6 U/mg, β-glucanase — 19.9 ± 0.8 U/mg, against MCC — 0.54 ± 0.02 U/mg, xylanase — 15.0 ± 0.6 U/mg, and cellobiase — 0.73 ± 0.03 U/mg, against n-NPG — 1.25 ± 0.05 U/mg. Preparations were also obtained from *P. verruculosum* F10. Preparation contained 738 ± 30 mg protein g. Preparation had activities of CMCase — 4.9 ± 0.2 U/mg, β-glucanase — 10.4 ± 0.4 U/mg, against MCC — 0.2 ± 0.01 U/mg, xylanase — 3.2 ± 0.2 U/mg, cellobiase — 111.4 ± 5 U/mg, against n-NPG — 55.3 ± 2.2 U/mg. The following EP dosages were used: cellulase — 2, 5 or 10 mg of protein per g of substrate, cellobiase — 5 mg of protein per g of substrate (or for cellulases — up

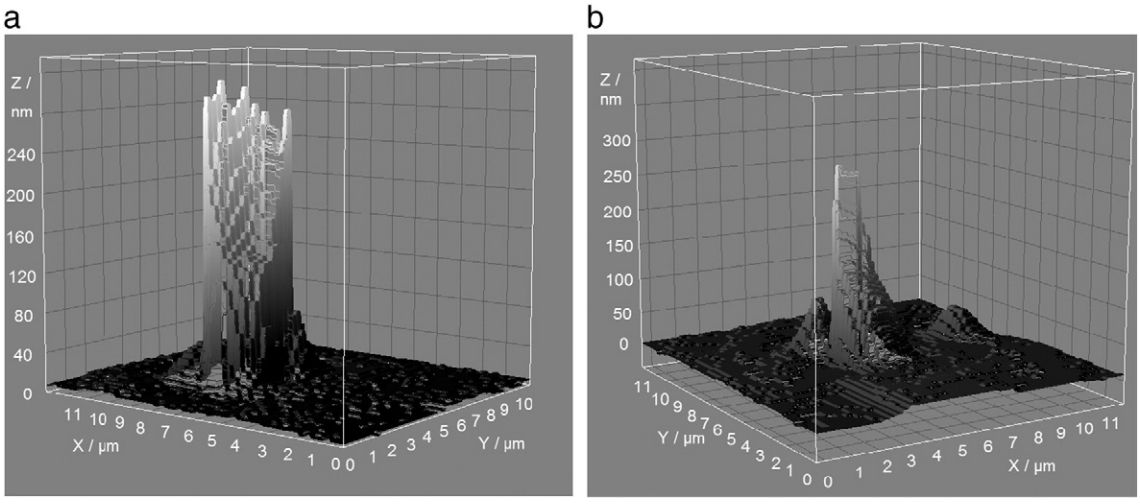
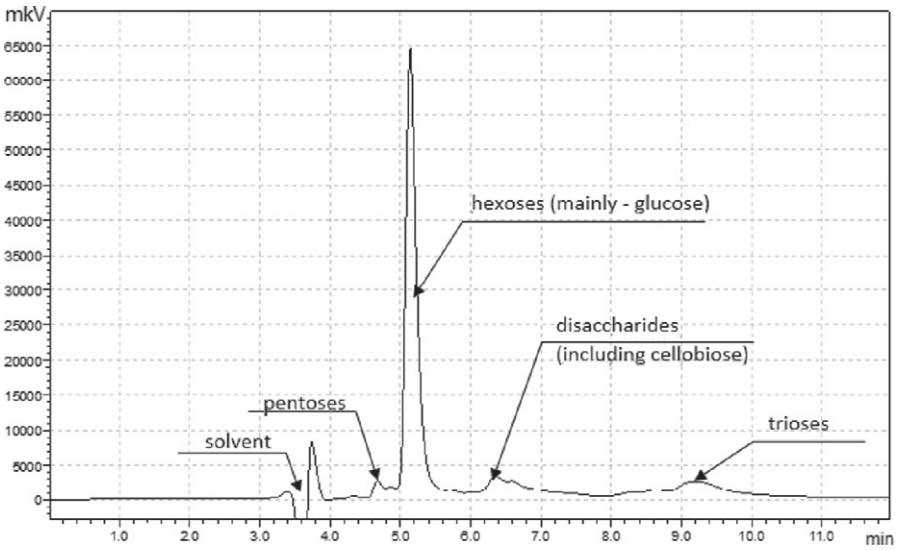


Fig. 2. 3-dimensional models of pine wood UDP obtained by grinding on PM400 for 30 min at 380 rpm: (a) without preliminary drying (initial moisture 18%); (b) after thermal drying at 75°C for 6 h (initial moisture 10%).



Type of wood	Grinding time at 400 rpm	Pentoses, g l^{-1}	Hexoses, g l^{-1}	Disaccharides, g l^{-1}	Trisaccharides, g l^{-1}
Scotch pine	30 min	1.10 ± 0.07	23.93 ± 0.23	2.18 ± 0.12	3.42 ± 0.14

Fig. 3. Comparison of chromatograms of enzymatic hydrolysates (48 h of hydrolysis, 10 mg of cellulase protein per 1 g of substrate) of the pine wood UDP grinded at 380 rpm for 30 min.

to 225 U per g of raw materials, cellobiases — 100 U per g of raw materials). The duration of hydrolysis was 48 h.

2.5. Fermentation and alcohol distillation

Hydrolysates were fermented using *Ethanol Red* (Fermentis, France). Yeasts were added at 0.1% of the mash volume. The fermentation temperature was 32°C. Hydrolysates were supplemented with ammonium nitrate for *Ethanol Red* at 0.01% (v/v). Fermentations were performed for 72–96 h.

2.6. Analysis methods

Grinding efficiency was assessed by passage through a sieve of 0.1 mm. In the experiment, only samples with a 100% passage. After that, grinding efficiency also was determined sequentially using a number of techniques: laser interference microscopy (LIM) [16] with a MII-4 M at a wavelength of 473 nm and laser diffraction analysis using a SALD-3101 (Shimadzu, Japan) [17]. The biochemical composition of the raw materials was determined according to the procedure described by Yanagisawa et al. [18]. After hydrolysis, we determined glucose concentrations using the glucose oxidase-peroxidase method [19] using a Photoglucose and Glucose-FS kit. Reducing sugar content was determined as described by Nelson [20] and Somogyi [21]. For more quantitative analysis of the carbohydrates, HPLC analysis was performed using an LC-20 Prominence (Shimadzu, Japan) HPLC with a SupelcoGel LC-NH2 column (mobile phase: ACN:H_2O 72:25) and a refractive index detector (RID-10A). The alcohol content in the hydrolysate after fermentation was determined using a pycnometer [22].

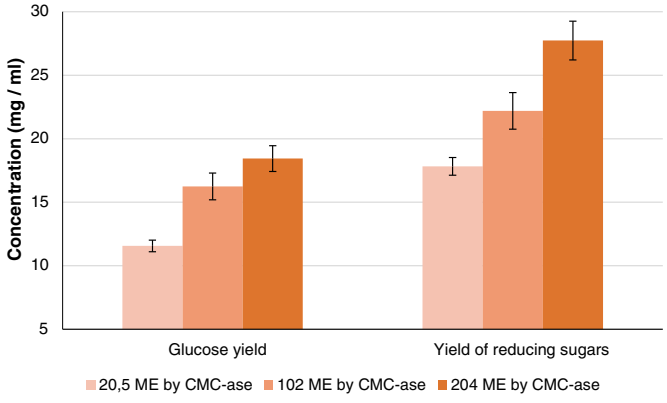


Fig. 4. Change in glucose and reducing sugar content in the hydrolysates of pine substrate UDP depending on the amount of introduced cellulases. Amount of enzyme (by the number of units of CMCase activity per gram of substrate).

2.7. Results processing

The experimental data obtained were subjected to statistical analysis using Microsoft Excel 2013 and the software package STATPLUS. Comparisons of experiments were carried out at the 5% significance level with Student t-tests.

3. Results and discussion

In the first series of experiments, we obtained pine wood ultradispersed particles (UDPs) using various schemes of raw material grinding and pre-drying. The experimental data showed that the optimal method for obtaining UDP was two-stage grinding. In the first stage, raw material destruction is performed on a knife mill LZM-1 M at 15,000 rpm for 5 min. In the second stage, a vario-planetary ball mill PM400 set at 380–400 rpm is used for 20–30 min. The obtained UDP differed in size (Fig. 1) and shape (Fig. 2).

Fig. 1 and Fig. 2 show that, regardless of initial wood moisture content, the grinding efficiency, particle size distribution, and geometries are similar. Predominantly sharp-edged particles with sizes ranging from 1 to 50 μm were detected during grinding.

In classic lignocellulosic material production, acid hydrolysis consists of a two-stage percolation process at 175–190°C. Sulfuric acid at concentrations of 0.5–0.6% is used as a catalyst. Acid hydrolysis is followed by neutralization, air blowing and the addition of nutrient salts containing nitrogen and phosphorus. In addition to producing

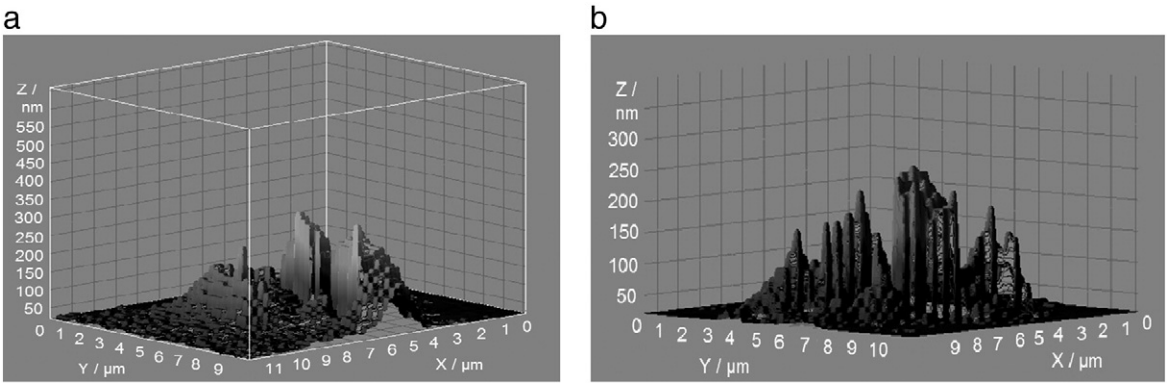


Fig. 5. 3-dimensional models of pine wood UDP obtained by grinding on PM400 ball milling for 30 min at about 380 rpm and ultrasonicated (10 W per kg of sawdust) for 0 (a) and 5 (b) min.

sugar hydrolysates, this process produces high quantities of highly toxic products, which cause environmental problems. The final ethanol concentration in the mash was 1.5–1.8% [7,8].

In our experiments, we decided to replace the acid hydrolysis step with an enzymatic step, allowing for the simultaneous reduction of material and energy consumption as well as toxic waste formation. Earlier, we conducted similar studies for ultradispersed grain raw materials. These studies found that ultradispersed grains allowed the cooking process to be eliminated and allowed enzymatic hydrolysis to be performed at temperatures below 60°C [23]. While analyzing the effect of particle size on the efficiency of enzymatic hydrolysis, we found that all of the experimental conditions studied resulted in the accumulation of mainly glucose and, to a lesser extent, di- and trisaccharides (Fig. 3). Maximum accumulation of these compounds was obtained in the series of experiments consisting of a 48-h enzymatic hydrolysis of pine raw materials ground at 380–400 rpm for 30 min. An increase of grinding time to 40 min did not significantly affect the yield of reducing substances. The highest glucose yield was observed at the end of the hydrolysis with a cellulase dosage of 10 mg of protein (204 ± 21 units CMCase per g of sawdust, Fig. 4). The initial content of cellulose in the pine wood UDP was 43.5%.

Thus, the "ultragrinding" of wood allows for the enzymatic hydrolysis of cellulose, allowing the acid hydrolysis step to be eliminated and reducing the cost of the final product. However, the hydrolysis process proceeds rather slowly, and the amount of sugar accumulated during hydrolysis is insufficient for fermentation.

It is known that enzymatic hydrolysis of glycosidic linkages is not the limiting step of this rather slow process. Most likely, this process is limited by cellulose hydration, a step required for enzyme action. Hydration can take place in several stages [24].

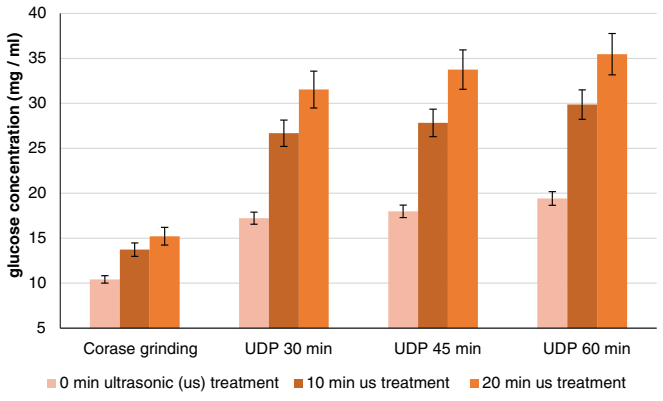


Fig. 6. Change in glucose content in pine wood UDP hydrolysate depending on the dispersion degree of raw materials and ultrasonic treatment duration. Duration of ultrasonic treatment with an intensity of 10 W per kg of sawdust.

The digestion of insoluble cellulose involves a number of discrete steps. The first step creates a higher degree of hydration in the surface layer molecules due to non-catalytic dispersion of microfibril bundles. Subsequent steps are associated with the coherent action of main structural elements within the enzyme: the cellulose binding domain (CBD), the catalytic domain (CD) and the linker. The enzyme must first bind to the substrate, usually via the CBD. The enzyme then must find an accessible site on the substrate and transfer an individual cellulose chain into its active site. Finally, the enzyme hydrolyzes the β-1,4 glycosidic bond, releases the product, and either translates along the chain or releases it. Exocellulases translate along the chain, cleaving off G2 units multiple times before release. Once the cellulose chain is released from the active site, the CD may rebind to the same or neighboring chain. The enzyme may also dissociate and rebind elsewhere. Given that the cellulase hydrolysis rates of soluble substrates are much greater than those of insoluble substrates, it is generally believed that substrate access is the rate limiting step in cellulose digestion [25].

By increasing substrate specific surface areas, ultradispersion increases the rate of enzymatic hydrolysis by increasing the number of access points available for the formation of enzyme-substrate complexes. However, in our experiments, the degree of cellulose hydrolysis did not exceed 25–30%.

It is known that ultrasonication is an effective method for loosening substrate structure to increase substrate accessibility for enzymatic hydrolysis [14,26]. Therefore, in the following series of experiments, wood UDP was processed using ultrasonication. Studies have shown that low-intensity ultrasonication has practically no effect on particle size or geometry. However, more powerful intensities (up 10 W per g of sawdust) resulted in further UDP destruction and increases in surface excision (Fig. 5).

An analysis of data from the literature and the results obtained in this study showed that high power ultrasonication breaks cellulosic fibers and results in the formation of disordered ultra- and nanostructures [27].

It has also been shown that the ultrasonic processing of cellulosic substrates allows for selective dispersal of fungal CBD-containing enzymes [28]. Our research found that the greatest efficiency of enzymatic hydrolysis was obtained with a method that combined two-stage grinding at 400 rpm with ultrasonic treatment for 5–10 min at a power - 10 W per g of sawdust. With this method, glucose yield was increased twofold (35.5 g glucose l^{-1}, 61% of theoretical) compared to a ground substrate without further preparation [Fig. 6].

Similar results have been obtained by other authors in their studies of wood hydrolysis following intense mechanical or complex physical pretreatments [4,25,29,30,31].

Our experiments determined the optimal parameters for substrate pretreatment, increasing enzymatic hydrolysis efficiency and generating

Table 1
Effect of pine wood ultrasonic treatment and grinding duration on the alcohol accumulation during fermentation by yeast *S. cerevisiae* strain *Ethanol Red*.

Duration of ultrasonic treatment on sawdust UDP	Alcohol content depending on the actual grinding duration, vol. %		
	30 min	60 min	Coarse grinding
Ultrasonic 10 W per g sawdust 20 min	2.91 ± 0.15	3.11 ± 0.23	1.09 ± 0.10
Control without treatment	2.07 ± 0.04	2.18 ± 0.03	0.79 ± 0.08

hydrolysates with fermentable sugar contents high enough for fermentation.

Next we fermented hydrolysates obtained from pine sawdust UDP processed for 20 min with an ultrasonic intensity 10 W per kg of sawdust and subjected to enzymatic hydrolysis at the previously determined optimal conditions. Coarse-ground samples and untreated UDPs were used as controls. As shown in Table 1 preliminary ultragrinding and ultrasonic treatment increased the degree of hydrolysis, leading to increased alcohol yield in all variants. This increase in alcohol yield is likely due to both the higher concentration of fermentable sugars and lower content of substances that inhibit sugar metabolism [32]. The final ethanol content obtained was higher than that obtained with classical technologies based on acid hydrolysis [8].

In summary, by combining mechanical two-stage ultradispersion with sonication, the enzymatic hydrolysis and fermentation of lignocellulosic raw materials is plausible. This process reduces waste toxicity, increases the alcohol yield and reduces overall costs.

4. Conclusions

The suggested technological approaches for dispersing plant material allow for intensification of the subsequent enzymatic hydrolysis step. The optimal method of lignocellulose destruction consists of two steps: the first step involves treatment for 5 min in a knife milling machine at 15,000 rpm. The second step involves treatment with a vario-planetary ball mill for 60 min at 400 rpm for lignocellulosic feedstocks.

The enzymatic hydrolysis of lignocellulosic feedstocks proceeds most efficiently when crushed materials are subjected to pre-sonication for 20 min at a power of 10 W per kg of sawdust, followed by the introduction of a cellulase with an activity greater than 185 U per g raw materials. The glucose yield obtainable with this method is up to 60% of the theoretical limit.

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Conflict of interest statement

The authors declare that there are no conflict of interest.

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References

[1] Reijnders L, Huijbregts M. A seed to wheel perspective. Biofuels for road transport. London: Springer-Verlag; 2008. http://dx.doi.org/10.1007/978-1-84882-138-5[Limited:].
[2] Visioli LJ, Stringhini FM, Salbego PRS, Chielle DP, Ribeiro GV, Gasparotto JM, et al. Use of agroindustrial residues for bioethanol production. In: Gupta VK, Tuohy MG, Kubicek CP, Saddler J, Xu F, editors. Bioenergy research: Advances and applications. 1st Ed.Chennai: Elsevier; 2014. p. 49–56. http://dx.doi.org/10.1016/B978-0-444-59561-4.00003-6.
[3] Alvira P, Tomás-Pejó E, Ballestros M, Negro MJ. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review. Bioresour Technol 2010;101:4851–61. http://dx.doi.org/10.1016/j.biortech.2009.11.093.
[4] Hallenbeck PC, editor. Microbial technologies in advanced biofuels production. Springer Science + Business Media; 2012. http://dx.doi.org/10.1007/978-1-4614-1208-3.
[5] Patni N, Pillai SG, Dwivedi AH. Wheat as a promising substitute of corn for bioethanol production. Procedia Eng 2013;51:355–62. http://dx.doi.org/10.1016/j.proeng.2013.01.049.
[6] Mosier N, Wyman CE, Dale B, Elander R, Lee YY, Holtzapple M, et al. Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresour Technol 2005;96:673–86. http://dx.doi.org/10.1016/j.biortech.2004.06.025.
[7] Taherzadeh MJ, Karimi K. Acid-based hydrolysis processes for ethanol from lignocellulosic materials. BioResources 2007;2:472–99.
[8] Mood SH, Golfeshan AH, Tabatabaei M, Jouzani GS, Najafi GH, Gholami M, et al. Lignocellulosic biomass to bioethanol, a comprehensive review with a focus on pretreatment. Renew Sustain Energy Rev 2013;27:77–93. http://dx.doi.org/10.1016/j.rser.2013.06.033.
[9] Berlin A, Gilkes N, Kilburn D, Bura R, Markov A, Skomarovsky A, et al. Evaluation of novel fungal cellulase preparations for ability to hydrolyze softwood substrates — Evidence for the role of accessory enzymes. Enzyme Microb Technol 2005;37:175–84. http://dx.doi.org/10.1016/j.enzmictec.2005.01.039.
[10] Harun R, Danquah MK. Enzymatic hydrolysis of microalgal biomass for bioethanol production. Chem Eng J 2011;168:1079–84. http://dx.doi.org/10.1016/j.cej.2011.01.088.
[11] Shutova VV, Yusipovich AI, Parshina EY, Zakharkin DO, Revin VV. Effect of particle size on the enzymatic hydrolysis of polysaccharides from ultrafine lignocellulose particles. Appl Biochem Microbiol 2012;48:312–7. http://dx.doi.org/10.1134/S000368381203012X.
[12] Inoue H, Yano S, Endo T, Sakaki T, Sawayama S. Combining hot-compressed water and ball milling pretreatments to improve the efficiency of the enzymatic hydrolysis of eucalyptus. Biotechnol Biofuels 2008;1:1–9. http://dx.doi.org/10.1186/1754-6834-1-2.
[13] Menon V, Rao M. Trends in bioconversion of lignocellulose: Biofuels, platform chemicals & biorefinery concept. Prog Energy Combust Sci 2012;38:522–50. http://dx.doi.org/10.1016/j.pecs.2012.02.002.
[14] Taherzadeh MJ, Karimi K. Pretreatment of lignocellulosic Wastes to improve ethanol and biogas production: A review. Int J Mol Sci 2008;9:1621–51. http://dx.doi.org/10.3390/ijms9091621.
[15] Yang F, Li L, Li Q, Tan W, Liu W, Xian M. Enhancement of enzymatic in situ saccharification of cellulose in aqueous-ionic liquid media by ultrasonic intensification. Carbohydr Polym 2010;81:311–6. http://dx.doi.org/10.1016/j.carbpol.2010.02.031.
[16] Brazhe AR, Brazhe NA, Maksimov GV, Ignatyev PS, Rubin AB, Mosekilde E, et al. Phase-modulation laser interference microscopy: An advance in cell imaging and dynamics study. J Biomed Opt 2008;13:034004. http://dx.doi.org/10.1117/1.2937213.
[17] Choi H, Lee W, Kim S, Jung WS, Kim JH. Optimum refractive index of poly-component particulate systems for measurement of particle size distribution by laser diffraction method analyzer. Mater Chem Phys 2009;117:18–22. http://dx.doi.org/10.1016/j.matchemphys.2009.05.035.
[18] Yanagisawa M, Nakamura K, Ariga O, Nakasaki K. Production of high concentrations of bioethanol from seaweeds that contain easily hydrolyzable polysaccharides. Process Biochem 2011;46:2111–6. http://dx.doi.org/10.1016/j.procbio.2011.08.001.
[19] Blaedel WJ, Uhl JM. Nature of materials in serum that interfere in the glucose-oxidase-peroxidase-0-dianisidine method for glucose, and their mode of action. Clin Chim 1975;21:119–24.
[20] Nelson N. A photometric adaptation of the Somogyi method for the determination of glucose. J Biol Chem 1944;153:375–80.
[21] Somogyi M. Notes on sugar determination. J Biol Chem 1952;195:19–23.
[22] Compendium of International methods of wine and must analysis. Alcoholic strength by volume (pycnometry, frequency oscillator, hydrostatic balance) (A2; 8/2000; 24/2003; revised by 377/2009)–OIV-MA-AS312-01A: R2009 1. Method OIV-MA-AS312-01A (Resolution Oeno 377/2009) Printed RUE D'AGUESSEAU 75008 PARIS. http://www.oiv.int/oiv/info/enmethodesinternationalesvin?lang=en; 2009.
[23] Revin VV, Atykyan NA. A method of ethanol production. RU patent 2407798. Application N 2009115345. Registered in the State Register of Inventions of the Russian Federation. http://www.freepatent.ru/images/patents/53/2407798/patent-2407798.pdf; December 27, 2010.
[24] Hall M, Bansal P, Lee JH, Realff MJ, Bommarius AS. Biological pretreatment of cellulose: Enhancing enzymatic hydrolysis rate using cellulose-binding domains from cellulases. Bioresour Technol 2011;102:2910–5. http://dx.doi.org/10.1016/j.biortech.2010.11.010.
[25] Kostylev M, Wilson D. Two-parameter kinetic model based on a time-dependent activity coefficient accurately describes enzymatic cellulose digestion. Biochemistry 2013;52:5656–64. http://dx.doi.org/10.1021/bi400358v.
[26] Chemat F, Zill-e-Huma, Khan MK. Applications of ultrasound in food technology: Processing, preservation and extraction. Ultrason Sonochem 2011;18:813–35. http://dx.doi.org/10.1016/j.ultsonch.2010.11.023.
[27] Siqueira G, Bras J, Dufresne A. Cellulosic bionanocomposites: A review of preparation, properties and applications. Polymers 2010;2:728–65. http://dx.doi.org/10.3390/polym2040728.

[28] Imai M, Ikari K, Suzuki I. High-performance hydrolysis of cellulose using mixed cellulase species and ultrasonication pretreatment. Biochem Eng J 2004;17:79–83. http://dx.doi.org/10.1016/S1369-703X(03)00141-4.
[29] Eriksson T, Börjesson J, Tjerneld F. Mechanism of surfactant effect in enzymatic hydrolysis of lignocellulose. Enzyme Microb Technol 2002;31:353–64. http://dx.doi.org/10.1016/S0141-0229(02)00134-5.
[30] Ho SH, Mai NL, An G, Koo YM. Microwave-assisted pretreatment of cellulose in ionic liquid for accelerated enzymatic hydrolysis. Bioresour Technol 2011;102:1214–9. http://dx.doi.org/10.1016/j.biortech.2010.07.108.
[31] Song X, Zhang M, Pei ZJ. Effects of ultrasonic vibration-assisted pelleting of cellulosic biomass on sugar yield for biofuel manufacturing. Biomass Convers Biorefin 2013;3:231–8. http://dx.doi.org/10.1007/S13399-013-0078-2.
[32] Karhumaa K, Wiedemann B, Hahn-Häagerdal B, Boles E, Gorwa-Grauslund MF. Coutilization of L-arabinose and D-xylose by laboratory and industrial *Saccharomyces cerevisiae* strains. Microb Cell Fact 2006;5:18. http://dx.doi.org/10.1186/1475-2859-5-18.