CELLULOSE CHEMISTRY AND TECHNOLOGY

VERSATILE THERMOBARIC SETUP AND PRODUCTION OF HYDROTROPIC CELLULOSE THEREIN

IGOR N. PAVLOV, MARINA N. DENISOVA, EKATERINA I. MAKAROVA,
VERA V. BUDAEVA and GENNADY V. SAKOVICH

Bioconversion Laboratory, Institute for Problems of Chemical and Energetic Technologies, Siberian Branch of the Russian Academy of Sciences (IPCET SB RAS), Biysk 659322, Altai Krai, Russia
✉ *Corresponding author: Igor Pavlov, pawlow-in@mail.ru*

Received May 7, 2014

The application of cellulosic biomass makes it possible to avoid problems of utilizing food resources in the biofuel technology and to enhance the competitiveness of biofuels by using cheap and available raw materials. The development of technologies for lignocellulose-to-biofuel conversion by fermentolysis methods is connected with employing a complex processing operation to release cellulose and improve its surface bioaccessibility. A versatile thermobaric setup has been developed to implement pretreatments conforming to the principles of environmental safety, effective delignification, and related costs' reduction. *Miscanthus sinensis* Andersson was subjected to hydrotropic pulping with an aqueous solution of sodium benzoate under autoclaving conditions. This involves treatment at 180 °C under excess pressure of 1.0 MPa and isolation of cellulose from the reaction mass. The resultant fibrous residue was hydrolyzed using commercial enzymes. The fibrous fractions were eventually produced in a high yield of 43-46% on a feedstock basis, the content of α-cellulose and acid-insoluble lignin being 85-86% and 7.0-7.6%, respectively. The substrate was found to have a satisfactory reactivity to fermentolysis, the yield of reducing agents being 65.5% of the weight in 72 h hydrolysis.

Keywords: *Miscanthus sinensis* Andersson, hydrotropic delignification, fermentolysis, pretreatment, lignocellulosic biomass

INTRODUCTION

Studies conducted over the past decades to produce various cellulose-based products from non-woody raw materials make such materials an important raw source of cellulose.[1-2] For this type of biomass, technologies for chemical cellulose are tested first. The manufacture of chemical cellulose is known to require a large amount of chemicals, which may adversely affect the environment.[3] To isolate cellulose, modern researchers strive therefore to apply techniques devoid of sulfur- and chlorine-containing reagents. However, the individuality of each cellulosic non-woody source necessitates the development of alternative methods to achieve more effective destruction of the lignocellulosic matrix wherein all the constituents (cellulose, hemicellulose, and lignin) are chemically bound. The most rational solution in this respect is to create universal technologies that permit fractionation of a feedstock into a single polymer or several native polymers (cellulose, lignin) in high yields, followed by conversion into valued products.

The finding of delignification methods for plant raw materials takes on particular importance for the second-generation biofuel technology.[4] The feedstock recalcitrance to enzyme activity requires that the feedstock be pretreated to isolate cellulose and break down lignin. The application of chemical pretreatments of cheap cellulosic non-woody biomass (food industry residues, renewable fast-growing plants, etc.) does not afford biofuels competitive with conventional processes for fossil fuels.[5-6] So, the technologies can be improved through the search for new chemical pretreatments (use of mineral acids, Lewis acids, composite microemulsions) and application of physical pretreatments (mechanical comminution, steam explosion, extrusion) as well as combined physical chemical pretreatments (autohydrolysis, carbon dioxide explosion) and biodelignification.[7-12] The methods listed are needed first of all to increase the delignification rate and accessibility (porosity) of the fiber's inner surface, which in turn enhances the fermentolysis efficiency.

To advance alternative pretreatments, we have been devising a versatile thermobaric setup that can ensure the creation of new technologies to produce biofuels. This setup allows testing conditions of chemical pretreatment (neutral hydrotropic cooking) and physicochemical pretreatment (autohydrolysis, carbon dioxide explosion). In this work, we discuss the application of the versatile thermobaric setup for the hydrotropic pulping of *Miscanthus* biomass, followed by the examination of the reactivity of the resultant fibrous fraction to fermentolysis. The pulping with concentrated solutions of hydrotropic salts, such as sodium benzoate, xylenesulfonate or toluenesulfonate, which ensures a satisfactory quality of the pulp in 8-12 h cooking, was described.[13] The hydrotropic destruction is known to allow the isolation of two native polymers (cellulose and lignin) from the feedstock, which are suitable for further conversion into end products. The use of the hydrotropic cooking with 36% aqueous solution of sodium xylenesulfonate will help mitigate the environmental impact of the chemical pretreatment and improve the technical economic indicators of the production, as claimed by Gabov *et al.*, who, in addition, succeeded in isolating 42% of the lignin present in birch wood by the modified hydrotropic process.[14] However, the study of the hydrotropic method as a pretreatment for successful fermentolysis in the biofuel technology has not received enough consideration so far.

EXPERIMENTAL

Equipment

Figure 1 illustrates a diagram of the versatile thermobaric setup. The main unit is a 2.3-L reactor (Fig. 1a) designed for a maximum rated pressure of 10.0 MPa. The reactor can be arranged in two operating positions depending on the pretreatment process in question. For the study of physicochemical pretreatments, the reactor (1) is mounted onto a collector (Fig. 1c) by means of a flange (2), whereas for the study of chemical pretreatments, the reactor is positioned on a D-shaped platform (Fig. 1b) within a hinged element (3). The reactor contents are heated by an electric strip heater (4) located on the outer wall along the reactor height. To reduce heat losses, the reactor is lined with a heat insulator (5). The D-shaped platform is meant for studies under steady-state conditions and dynamic impact on the feedstock. The reactor is either rigidly fixed on the platform or makes a wide-amplitude swinging motion during the operation. The reactor swings within the hinged element when the rod of a pneumatic cylinder (7) moves out. Excess working pressures are attained upon pressurization of the reactor by means of a cover (8) and upon autoclaving at a preset temperature. The temperature of the reactor contents is measured by a temperature gauge (9) centered inside the reactor vessel and fastened on the reactor's cover. The reactor pressure level is controlled by a pressure gauge (10) hooked up through the Perkins tube. A general view of the setup is demonstrated in Figure 2.

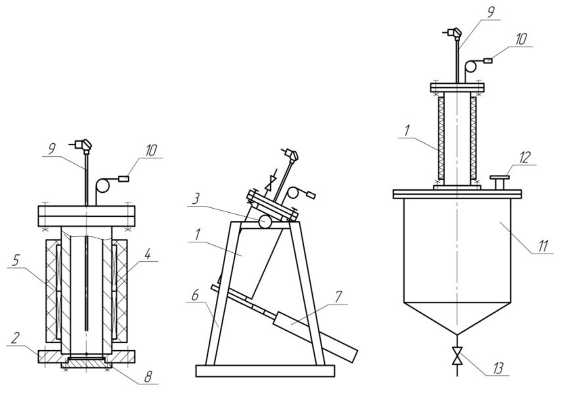

Figure 1: Versatile thermobaric setup diagram; (a) reactor; (b) reactor on D-shaped platform; (c) reactor on collector; ([1]Reactor, [2]reactor-mounting flange on the collector, [3]hinged element, [4]strip heater, [5]reactor heat-insulation, [6]D-shaped platform, [7]pneumatic cylinder, [8]cover, [9]pressure gauge, [10]temperature gauge, [11]storage

Figure 2: General view of the versatile thermobaric setup

tank, [12]pipe sleeve, [13]product discharge valve)

The swing intensity is defined by the pneumatic drive system settings, which provide the reciprocating movement of the rod at a specified rate. The rate of vibrations is regulated by using a control console. Such an operation causes the reactor to swing throughout the entire process.

Depending on the extent the reactor's volume is filled to, the feedstock in the reaction solution is under various hydrodynamic conditions due to vibrations. Upon completion of the treatment process, the product is discharged through the bottom flange of the reactor into a reservoir.

Raw material and reagent

Miscanthus sinensis (*Miscanthus sinensis* Andersson) harvested in 2008 from a plantation of the Institute of Cytology and Genetics SB RAS (Novosibirsk, Russia) was used here.[15] The whole above-ground part of the plant, including stems with internodes, leaves, and panicle, was utilized in the experiment. *Miscanthus* was preliminarily chopped to a size of 10-15 mm.

The chemical composition of *Miscanthus* was as follows: 57.4% Kürschner cellulose (on an oven-dry basis), 23.3% pentosans (on OD basis), 19.1% acid-insoluble lignin (on OD basis), and 3.9% ash (on OD basis).

Sodium benzoate (CAS No. 532-32-1), a sodium salt of benzoic acid, a dietary supplement, pertains to a group of preservatives; an odor-free white powder.

The mass fractions of acid-insoluble lignin, Kürschner cellulose, α-cellulose, pentosans, and ash were quantified by standard analytical protocols for feedstock and its conversion products.[16]

The following enzyme preparations were employed as biocatalysts:

- BrewZyme BGX (fungal origin), cellulase-standardized (manufactured by Tarchomin Pharmaceutical Works Polfa S.A., Poland); enzyme activities: 1500 U/cm^3 cellulase activity, 6500 U/cm^3 xylanase activity, and 1700 U/cm^3 β-glucanase activity.
- CelloLux A (*Trichoderma longibrachiatum* producer), hemicellulose-standardized (manufactured by Sibbiopharm Ltd., Berdsk, Russia); enzyme activities: 2000 U/g cellulose activity, 8000 U/g xylanase activity, and 1500 U/g β-glucanase activity.

Experimental cooking

The hydrotropic cooking was run as follows: a weighed portion of *Miscanthus* (100 g) was charged into the reactor and a 35% sodium benzoate solution in a ratio of 1 to 10 was poured thereto; the reactor was pressurized by means of a gasket and the bottom flange (6). The reactor was then connected to the pneumatic cylinder and its swinging intensity was set by feeding compressed air into the system; afterwards, the reactor heating was switched on. After the temperature was raised to 180 °C, the cooking was run for 3-5 h under a reactor pressure of 1.0 MPa. When the cooking time was over, the heating was switched off, and the reactor was naturally cooled to room temperature. The reaction mass (fibrous fraction in the hydrotropic liquor) was discharged into a reservoir through the bottom flange after the apparatus was vertically positioned.

The cooking liquor was separated from the fibrous fraction by squeezing, and the residue was washed in several steps. The squeezing was run on a basket press in the form of a cylindrical vessel whose bottom has a perforated diaphragm and a vertical piston fastened on the vertical screw rod. Briefly, the suspension of the fibrous fraction was placed into the press, onto the perforated diaphragm with a filter fabric preliminary laid atop it. The hydrotropic solution was removed by means of creating a tightening force of the piston rod. By adjusting the pressure generation, the release of the spent cooking liquor was attained with a desired intensity. The spent cooking liquor was used to liberate hydrotropic lignin.

Special attention was paid to washing cellulose in the hydrotropic process in order to prevent lignin from precipitating and sticking to the cellulose fiber. The squeezed fibrous fraction was washed with a fresh 35% sodium benzoate solution in the vertical vessel while stirring at a low speed. The washing was performed at 90-95 °C for 1 h in a ratio of 1:20. The stirring was run using a two-bladed stirrer installed on a vertical shaft connected to the drive unit at the top of the vessel. By adjusting the stirrer's speed, the required stirring intensity of the solution was specified.

The squeezing was again performed in the same manner, after which the washing was repeated with a 20% sodium benzoate solution under the same conditions. Prior to final squeezing, the fiber fraction was preliminarily washed with distilled water at room temperature to colorless wash waters. The resultant fibrous residue was air-dried to a moisture content of 7-10%. All wash waters were employed to prepare the sodium benzoate solution.

After being used three times, the spent cooking liquor was utilized to isolate lignin (water dilution, filtration or centrifugation, drying). The hydrotropic liquor can be recovered by concentrating the filtrate via an available method (evaporation or membrane technology).

The fermentolysis was run in triplicate with the substrate (fibrous fraction) derived at a mean cooking time of 4 h. The fermentolysis of the substrate at a concentration of 33 g/L in a 4.7-pH acetate buffer using CelloLux A and BrewZyme BGX (each being injected in a quantity of 0.04 g/g substrate) was performed by the reported procedure.[17,18] The concentration of reducing agents in the hydrolyzates on a glucose basis was quantified spectrophotometrically on a UNICO UV-2804 (USA) instrument using a reagent based on 3,5-dinitrosalicylic acid (Panreac, Spain), the relative error of the measurement being 3.5%. The pentose concentration in the hydrolyzates on a xylose basis (after 72 h) was determined on the same instrument using orcinol monohydrate (Acros Organics, USA), the relative error being 5.0%.

Recoveries of the reducing agents were calculated as the substrate weight subtracted non-hydrolyzable components, with allowance for a factor of 0.90 attributable to the water molecule addition to anhydroglucose residues of the corresponding monomer units as a result of fermentolysis. The pentose recovery was calculated as the weight of pentosans in the substrate with allowance for a factor of 0.88 attributable to the water molecule addition to anhydroxylose residues of the corresponding monomer units.

RESULTS AND DISCUSSION

Hydrotropic cooking

This process is of interest in that it uses water-soluble and eco-benign reagents. Owing to its advantages, the hydrotropic method has been considered since the middle of the last century as a new technique that could become an alternative to the existing pulping technologies. It is especially attractive as a way of fractionating biomass into lignin and cellulose considering the high demand for biomaterials to replace the petroleum ones. This leads to an enhanced cellulose yield. Another native polymer, lignin, can be isolated from the hydrotropic liquor and transformed into different chemicals (polyurethanes, acrylates, epoxy resins, composites, engine oil additive).[19] With increased utilization of biomass, lignin is regarded as a potential starting material for the manufacture of adhesives, epoxy and phenolic resins, and polyolefins because of its polyphenolic chemical structure.[20]

Previous studies of the hydrotropic *Miscanthus* cooking at a reduced temperature of 140-160 °C revealed that the resultant fibrous fractions had inclusions of uncooked feedstock.[21] The undercooked pulp was made up of solid parts of the plant, such as stem and internodes. In order to decrease the portion of the undercooked pulp, the cooking temperature in the versatile thermobaric setup was fixed at 180 °C, with the same cooking time of 3-5 h, using additional washing with the hydrotropic solution. The yield and characteristics of the fibrous fractions are listed in Table 1.

The high yield of the fibrous fractions ranging from 43 to 46% on a feedstock basis (Kürschner cellulose, 57.4%) indicates a maximum preservation of the cellulose. The hydrotropic cooking proceeds according to the scheme illustrated in Figure 3, where the lignin formula was borrowed from the literature.[20]

The plant matrix (I) composed of the bound constituents, such as cellulose, hemicellulose, and lignin, was subjected to the hydrotropic solution, a concentrated aqueous solution of C_6H_5COONa, at a specified temperature and for a specified time. The reaction mass resulting from the cooking process comprised a solid phase—cellulose (II) with residual hemicellulose and lignin, as well as a liquid phase—lignin solution (IV), and a hemicellulose hydrolysis product—xylose (III). The lignin dissolution is due to the high hydrophobic affinity of the sodium benzoate molecule to lignin leading to a solvate. The cooking liquor was squeezed from the solid phase to afford two target products: cellulose (II) and lignin solution whose further dissolution with a 3-fold water amount destroys the solvate causing the lignin to precipitate (IV). It should be noted that well water-soluble xylose (III) remains in the solution, not contaminating the resultant solid-state lignin. The initial results of the study on the hydrotropic *Miscanthus* lignin obtained by the above-mentioned procedure were reported previously.[19]

Table 1
Yield and characteristics of *Miscanthus* fibrous fractions

Cooking time (h)	Yield[a] (%)	Components [b] (%)			
		α-Cellulose	Pentosans	Lignin	Ash
3	46.1±1.0	85.8±0.5	7.2±0.1	7.6±0.1	3.6±0.05
4	44.6±1.0	86.1±0.5	7.0±0.1	7.2±0.1	3.6±0.05
5	43.4±1.0	86.4±0.5	6.7±0.1	7.0±0.1	3.4±0.05

[a] On feedstock basis, [b] on OD basis

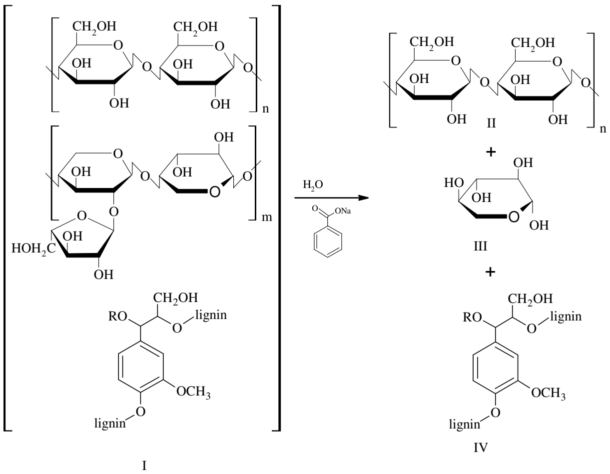

Figure 3: Hydrotropic cooking scheme

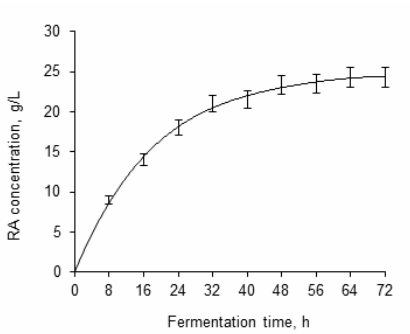

Figure 4: Reducing agents concentration versus fermentolysis time of *Miscanthus* fibrous fraction

The presence of weak acids in the cooking liquor, high temperature, and prolonged cooking time exert a hydrolytic effect on the lignin-carbohydrate complex and further on the easily hydrolyzable carbohydrates (hemicelluloses). The hydrolysis of pentosans during hydrotropic delignification eventually diminishes their content from 23% in the feedstock to 7% in the fibrous fraction, the biomass weight loss being 55% (see Table 1). The decrease in acid-insoluble lignin from 19.1% in the feedstock to 7.0-7.6% in the fibrous fraction is due to the high solubility of the *Miscanthus* lignin in the hydrotrope.

Enzymatic hydrolysis

Sodium benzoate is utilized in the hydrotropic cooking because it is available and relatively cheap. The sodium salt of benzoic acid is known to be widely used in food industry as a preservative. Its action is particularly connected with a strong inhibition of any enzymes. A reasonable concern is that the washed specimens of the fibrous fractions may carry the salt residues, which adversely affect the activity of the enzyme preparations. The surface accessibility of the resultant fibrous fractions to enzymes is a matter of discussion as well.

To evaluate the suitability of the fibrous fractions obtained after the hydrotropic cooking in the versatile thermobaric setup, the product fermentolysis after 4-h cooking was investigated. The reactivity of the studied substrate to fermentolysis was assessed against the concentration of the resultant reducing agents present in the hydrolyzate and their accumulation rate. Figure 4 illustrates the concentration of reducing agents (RA concentration) versus fermentolysis time as a result of the enzymatic hydrolysis of the hydrotropic cellulose.

The hydrolysis is characterized by an average process rate at the onset of the reaction with growing RA concentration from 0 to 10 g/L for the initial 8 h. The RA concentration rises to 14 g/L for the next 8 h. As the process proceeds, the amount of reducing agents moderately increases to 20 g/L (yield greater than 50% of the substrate weight) at 32 h. The process further proceeds with a slow gain and attains a maximum possible RA concentration of 24 g/L at 64 h, which corresponds to the yield of 65.5% of the substrate weight. The overall character of the accumulation curve of the RA concentration for 72 h is indicative of favorable conditions for the enzyme activity, which is in agreement with the general ideas of the successful enzymatic hydrolysis of the cellulose-rich product, and suggests a classical progress of the process.[22,23] The recovery of reducing agents subtracted non-hydrolyzable components (ash and lignin) was 73.5%. It should be noted that the xylose content in the hydrolyzate after 72 h was 1.3 g/L, which is consistent with the yield of 49.0% of the weight of pentosans in the substrate. The findings on the digestibility of the cellulose produced by the hydrotropic cooking in a sodium benzoate solution indicate that the cellulose has a positive accessibility to enzymes.

As the earliest researchers of the hydrotropic wood cooking reported that the spent cooking liquor could be not only recovered, but also repeatedly reused until the hydrotrope is saturated with lignin,[13] the feasibility of recycling the sodium benzoate solution and thereby reducing the reagent consumption to isolate the two polymers from *Miscanthus* is beyond doubt.

CONCLUSION

A versatile thermobaric setup has been developed to study alternative chemical and physicochemical delignification techniques for cellulosic biomass. The hydrotropic *Miscanthus* cooking was investigated in the versatile thermobaric setup under autoclaving conditions at preset temperature, pressure and reactor swinging intensity, with varying processing time from 3 to 5 h. The fibrous fractions were eventually produced in a high yield of 43-46% on a feedstock basis, the content of α-cellulose and acid-insoluble lignin being 85-86% and 7.0-7.6%, respectively. The study on the fermentolysis of the hydrotropic *Miscanthus* pulp using commercial enzyme preparations showed a satisfactory reactivity of the substrate to fermentolysis, and the yield of reducing agents was 65.5% of the weight in 72 h hydrolysis.

ACKNOWLEDGEMENTS: The work was accomplished with financial support from Basic Research Project #5, Program #3 of the Presidium of the RAS, Chemical Refinement of Renewable "Concentrated" Cellulosic Raw Materials in Different Media in Pressure Reactors.

REFERENCES

[1] M. M. Ibrahim, W. K. El-Zawawy, Y. Jüttke, A. Koschella, T. Heinze, *Cellulose*, **5**, 3518 (2013).

[2] R. M. K. Vala and L. Tichagwa, *Cellulose Chem. Technol.*, **47**, 565 (2013).

[3] P. B. Skalsi, A. Krabeki, P. H. Nielsen, H. Wenzel, *Int. J. LCA*, **13**, 124 (2008).

[4] Y. Sun and J. Cheng, *Bioresour. Technol.*, **83**, 1 (2002).

[5] M. O. Abdeen, *Cellulose Chem. Technol.*, **46**, 477 (2012).

[6] I. I. Moiseev, N. A. Platae, S. D. Varfolomeev, *Vestnik Rossiiskoi Akademii Nauk* (in Russian), **76**, 427 (2006).

[7] A. T. W. M. Hendriks and G. Zeeman, *Bioresour. Technol.*, **100**, 10 (2009).

[8] L. Li, B. Wang, D. T. Liu, F. Yang, R. Dang, *Cellulose Chem. Technol.*, **47**, 745 (2013).

[9] S. D. Razumovskii, V. V. Podmaster'ev, A. N. Zelenetskii, *Catalysis in Industry*, **3**, 23 (2011).

[10] R. Gupta, V. K. Saini, R. P. Bhatt, B. P. Thapliyal, S. Naithani, *Cellulose Chem. Technol.*, **47**, 59 (2013).

[11] V. P. Puri and H. Mamers, *Biotechnol. Bioeng.*, **25**, 3149 (1983).

[12] E. A. Emerhi, B. A. Ekeke, B. A. Oyebade, *Afr. J. Biotechnol.*, **7**, 1512 (2008).

[13] R. H. McKee, US Patent 2.308.564. Recovery of cellulose and lignin from wood, 19 Jan 1943.

[14] K. Gabov, P. Fardim, F. Gomes, *Bioresouces*, **8**, 3518 (2013).

[15] V. K. Shumny, S. G. Veprev, N. N. Nechiporenko, T. N. Goryachkovskaya, N. M. Slynko *et al.*, *Adv. Biosci. Biotechnol.*, **1**, 167 (2010).

[16] A. V. Obolenskaya, Z. P. Yel'nitskaya, A. A. Leonovich, in "Laboratory Works on Wood and Pulp Chemistry", Moscow Press, 1991, p. 320.

[17] E. I. Makarova, V. V. Budaeva, E. A. Skiba, G. V. Sakovich, *Catalysis in Industry*, **6**, 67 (2014).

[18] E. I. Makarova, *Chemistry for Sustainable Development*, **21**, 219 (2013).

[19] S. G. Il'yasov, V. A. Cherkashin, G. V. Sakovich, *Himija Rastitel'nogo Syr'ja* (in Russian), **4**, 21 (2013).

[20] K. Wang, S. Bauer, and R. C. Sun, *J. Agric. Food Chem.*, **60**, 144 (2012).

[21] R. Yu. Mitrofanov, V. V. Budaeva, M. N. Denisova, G. V. Sakovich, *Himija Rastitel'nogo Syr'ja* (in Russian), **1**, 25 (2011).

[22] A. P. Sinitsin, A. V. Gusakov, V. M. Chernoglazov, in "Bioconversion of Lignocellulosics", Moscow University Press, 1995, p. 224.

[23] S. D. Varfolomeev, in "Chemical Enzymology" (in Russian), Moscow Publishing Center "Academia", 2005, p. 480.