Daphnia magna demonstrated sufficient sensitivity in techno-economic optimization of lignocellulose bioethanol production

Marek Vochozka¹ · Vojtěch Stehel¹ · Anna Maroušková¹

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Abstract Notable progress has been achieved in the past two decades regarding production of different enzymatic mixtures for hydrolysis of the lignocellulose matrix. Nevertheless, the hydrolysing mixtures remain slow and require tempering, which results in high-energy demands and bad financial results. Use of acids or alkali at a very high temperature and pressure accelerates the process more than ten times wherein the energy requirements are approximately equal. However, these elevated reaction conditions might cause the breakdown of complex lignin formula into substances that have the potential to inhibit subsequent fermentation processes. Formation of these breakdown products may be prevented by selecting the optimum process parameters, but their acquisition requires either a large number of expensive analytical techniques or equally large amounts of slow fermentation tests. An inexpensive and time saving alternative that is based on the sensitivity of chosen organisms to these inhibitors was designed and financially assessed. It was confirmed that the method is technically feasible and economically viable with significant potential to reduce the bioethanol production cost.

Keywords Bioethanol · Process management · Valuation · Financial analysis · Techno-economical assessment

Introduction

The new reserves of fossil fuel reserves, found using the state-of-the-art technologies, are not plentiful enough to meet the increasing demand for them (Shahir et al. 2015). Research efforts have been directed towards finding an inexpensive alternative that is also environmentally friendly (Maroušek et al. 2015). Bioethanol or bioethanol–gasoline blends represent an emerging direction (Manzetti and Andersen 2015). It appears that the cost reduction is best achieved via complex biorefineries that will integrate biomass conversion processes to produce bioethanol, power, heat, and other value-added chemicals (Moreno et al. 2015). It was repeatedly proven that bioethanol production from corn starch provides higher yields (Zabed et al. 2016); however, utilization of farming residues is considered more socially responsible, since lignocellulose feedstock does not compete with food or feed production (Maroušek 2013a). Nevertheless, utilization of the lignocellulose is complicated by mandatory pre-treatment techniques to disintegrate and subsequently to hydrolyze (depolymerization of cellulose into fermentable sugars) the cellulose matrix (Mood et al. 2013). Techno-economic assessment of the most common mechanical and thermo-chemical methods was presented (Maroušek 2013b). There is wide consensus steam-explosion and grinding which are the most reasonable techniques, whereas microwave, shockwave, or freeze pre-treatment are hardly economically viable. The subsequent depolymerization can be carried out by the following methods: (1) enzymatic hydrolysis; (2) ionic liquids; (3) organic solvents; (4) ozonolysis; and (5) acid or alkali hydrolysis. The enzymatic hydrolysis (1) is carried out most often by cocktails of xylanases, cellulases, endoglucanases, or glucosidases (Mardoyan and Braun 2015). There is wide consensus that

✉ Marek Vochozka
vochozka.marek@gmail.com

¹ The Institute of Technology and Businesses in České Budějovice, Okružní 517/10, 370 01 České Budějovice, Czech Republic

enzymatic hydrolysis enables excellent yields of fermentable sugars (referred as glucose equivalents); however, the process is relatively slow (Meng and Ragauskas 2014) and requires tempering (40–60 °C), which results in high-energy demands and worse financial results (Maroušek 2015). Among ionic liquids (2) investigated, 1-allyl-3-methylimidazolium chloride, 1-ethyl-3-methylimidazolium-acetate, 1-butyl-3-methylimidazolium chloride, and 1-ethyl-3-methylimidazolium diethyl phosphate have recently received much attention due to their remarkable cellulose dissolution capability (Zavrel et al. 2009). However, the melting point of these organic salts is above 100 °C, which hampers transfer of this energy-demanding technology into commercial operation (Maroušková and Braun, 2014). Organic solvents (3), i.e., methanol, ethanol, acetone, ethylene glycol, and tetrahydrofurfuryl alcohol (with or without the addition of organic or inorganic acids or sodium hydroxide, ammonia and lime as a catalyst) were repeatedly investigated (Mesa et al. 2011). The main drawbacks of this method are the low-boiling point of organic solvents, high risk of high-pressure operation, and the flammability and volatility of such solvents (Sun and Chen 2008). Ozone gas (4) is a powerful oxidant soluble in water and capable of breaking down lignin and hemicelluloses and increasing the overall cellulose biodegradability (García-Cubero et al. 2009). On the other hand, the main disadvantage is the cost of ozone used, as a large amount of ozone is employed to treat lignocellulosic materials (Sun and Cheng 2002). Acid and alkali hydrolysis (5) proved outstanding yields at elevated temperature and pressure (Mood et al. 2013). However, the intensification of process parameters results in formations of breakdown products of lignocellulose degradation that can significantly inhibit the subsequent fermentation processes (Maroušek et al. 2013). Detailed analysis and subsequent optimization of the key process parameters with regard to the essential biochemical parameters of the treated lignocellulose allows minimizing formation of these inhibitors (for a comprehensive review, see Palmqvist and Hahn-Hägerdal 2000a, b). Nonetheless, such robust analysis requires either a large number of expensive analytical techniques or equally large amounts of slow fermentation tests (Maroušek 2012).

The hypothesis is whether the routine tests of biological inhibition could replace these expensive or time consuming techniques.

Materials

Barley straw of 89.8% volatile solids (hereinafter VS) was analyzed on acidic-detergent lignin (hereinafter ADL, %) and acidic-detergent fiber (hereinafter ADF, %) by the

Fibertec 1020 (M6) fiber analyzer (FOSS Ltd., Denmark). Labile (hereinafter LP1, %) and partly resistant (hereinafter LP2, %) fractions of organic matter were analyzed using the acid hydrolysis method (H₂SO₄) as modified by Maroušek (2014a) using the NC-90A automatic high-sensitive N/C analyzer (Shimadzu, Japan).

Methods

The conveyor belt transported bales of barley straw into the M2 continuous macerator (BiomassTechnology a.s., Czech Republic) that turned them (200 kg h⁻¹; 85 °C) into soggy pulp (details of the apparatus traceable in Maroušek et al. 2012). Subsequently, the pulp was pressurized by a high-pressure screw pump into the TTP-07 high-pressure reactor (AIVOTEC, s.r.o., Czech Republic) that ends with an expansion turnstile. Nitric acid was used for computer-controlled acidification of the internal environment of the high-pressure reactor to pH = 1.65. The hydraulic retention time in the high-pressure reactor was set to 4 min; the speed of the expansion turnstile was set to 0.5 L per revolution (1 revolution per 5 s); temperature and pressure ranged, as depicted in Fig. 1. The efficiency of hydrolysis was determined by analysis of glucose equivalents (hereinafter GE, %) as traceable for example in (Vanoye et al. 2009). The presence of fermentation inhibitors (weak acids; furan derivatives, and phenols) was analyzed via the conventional gas chromatography and the flame ionization detector by Maroušek (2014b), and for comparison also via biological inhibition. *Daphnia* (*Daphnia magna*) were analyzed on immobilization (%) according to Rosa et al. (2006). The overall process efficiency was classified using the bioethanol yields (hereinafter BE). Unless otherwise stated, all laboratory, financial (Net Present Value) and static methods were carried out according to Maroušek et al. (2014) in agreement with routine standards ($n = 20$; $\alpha = 0.05$).

Results and discussion

Analysis on biodegradability (Table 1) revealed that the barley straw is well comparable with other feedstock traceable in literature (Mood et al. 2013), which is a good prerequisite for the formulation of general conclusions. The analysis also indicates that under usual conditions, only 3% of ADF resisted the hydrolysis, which is an economically important finding for further optimization efforts. It is worth mentioning that the operating conditions are capable of decomposing up to 2% of ADL, which is a source of potential inhibitors, such as weak acids, furan derivatives, and phenols. It has been statistically proven that nitric acid

Fig. 1 Dynamics of GE, BE, and inhibitors in relation to the operating parameters of acid hydrolysis technology. All abbreviations, other process parameters, and statistical details are traceable in “Materials” and “Methods” sections

Table 1 Column A represents raw feedstock, whereas column B represents average data after acid hydrolysis

	A	B
ADF	36.2 ± 3.1	1.1 ± 5.9
ADL	26.3 ± 4.5	25.8 ± 4.8
LP1	4.2 ± 2.5	0.0 ± 0.0
LP2	17.3 ± 5.8	0.9 ± 0.1
GE	2.4	3.8
BE	3.8	4.4

All abbreviations, other process parameters, and statistical details are traceable in “Materials” and “Methods” sections

completely decomposes LP1 wherein LP2 is decomposed from 95%. All these observations are in good agreement with Palmqvist and Hahn-Hägerdal (2000a, b). Analysis on the depolymerization of cellulose into fermentable sugars (GE) is stated in the upper part of Fig. 1. The plot can be read as that by elevating both, pressure and temperature

accelerates the hydrolyzing effect of the nitric acid. However, subsequent analysis on the BE (see middle part of Fig. 1) reveals that there are some critical borders (pressure of 1.6–1.7 MPa and temperature of 220–230 °C) that significantly limit the yield. It is probably not a coincidence that certain analogies were also observed by other authors (Maroušek et al. 2016). A possible explanation is provided by the chart in the lower part of Fig. 1, which depicts the sum of inhibitors as traced by the gas chromatography and flame ionization detector. It is possible to observe an analogous barrier in the pressure range of 1.6–1.7 MPa, behind which the concentration of the inhibitors sharply rises. With reference to Meng and Ragauskas (2014), it can be derived that pressure is probably more important for the formation of inhibitors than the operating temperature. Such findings, however, do not have a commercial application yet, because the gas chromatography and flame ionization detector can hardly manage hundreds of samples at low operating costs. Efforts to replace the costly and

Fig. 2 Sensitivity of *Daphnia magna* to the products of lignin breakdown during hydrolysis that was carried out by nitric acids reveals that this cheap and fast biological immobilization technique is sufficiently precise to be used for the optimization of process parameters on a commercial scale

timely conventional methods by cheap and fast analysis on *D. magna* inhibition are depicted in Fig. 2. The upper part of the figure shows that daphnia are sensitive to very small quantities of inhibitors (as low as 50 ppm). The chart also reveals that concentrations over 1100 ppm result in 100% immobilization. If these elevated concentrations are neglected (see the lower part of the Fig. 2), the data can be approximated by $0.0943 I - 17.264$ ($R^2 = 0.9214$; where I represents the sum of inhibitors). Two hundred optimization cycles by *Daphnia magna* led to the finding of optimal process parameters in the immediate surroundings of 1.7305 MPa and 219.6857 °C which represented 1.8% increase on BE yield in comparison to previous findings. Regarding the mass that was highly loaded with inhibitors, the easiest solution (from the techno-economical point of view) is to dilute this mass with another mass that is contaminated by inhibitors much less. Another alternative is to use this material for other processes, such as composting, since a consortium of aerobic organisms may solve this problem (Maroušek et al. 2016).

Conclusions

It was confirmed that HNO_3 is a technically suitable hydrolyzing agent for turning raw lignocellulose into glucose equivalents. It was repeatedly observed that the amounts of glucose equivalents are much higher provided that the hydrolysis is carried out under critical conditions (elevated pressure and temperature). At the same time, it was

confirmed that the conditions of the high-pressure reactor increase formations of lignin breakdown products (represented by the sum of furan derivatives and phenols). Regarding subsequent fermentation to bioethanol, it was revealed that these inhibitory properties significantly increase if their sum exceeds 0.03 g L^{-1} . Another new finding is that the organisms of *D. magna* are sensitive enough (0.008 g L^{-1}) to serve as fast and cheap analytical methods for multifactor process optimization. Use of this optimization technique increased the Net Present Value of the pilot-scale bioethanol plant by 0.93%.

Compliance with ethical standards

Conflict of interest Authors declare no conflict of interest.

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