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Production of fermentation feedstock from lignocellulosic biomass: applications of membrane separation

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Abstract. The development of cost-efficient, highly productive technologies for fermentation feed production from lignocellulose biomass is still a challenge. In this paper, the production of fermentable sugars from lignocellulosic biomass using hydrolysis techniques with membrane separation systems is studied. The research was conducted on both a laboratory and pilot level to evaluate and optimize the efficiency of the proposed technology. The results demonstrated that UF and NF permeate recovery increased efficiency, and the highest sugar recovery rates were obtained when secondary waste recirculation was introduced after NF and UF, reaching an almost 40% yield from all produced sugars.

Key words: fermentable sugars, lignocellulosic biomass, pre-treatment, membrane separation.

INTRODUCTION

Due to increasing energy demands and pollution problems caused by the use of non-renewable fossil fuels, it has become necessary to introduce alternative energy sources into the global energy turnover (Karmakar et al., 2010). Lignocellulosic biomass, such as wood, grass, agricultural and forest residues, has been regarded as a potential resource for the production of biofuels for many years (Sanchez & Cardona, 2007; Xu & Huang, 2014), since it is both renewable and available in large quantities all around the world. Large-scale processing technologies allowing to ferment biofuels from lignocellulose have been used for decades (Hamelinck et al., 2005). However, the extensive application of this resource is still linked to technological challenges and high production costs (Laser et al., 2001). Generally, the conversion of lignocellulosic biomass to biofuel consists of four major operations: pre-treatment, hydrolysis, fermentation, and product separation/purification (Moiser et al., 2005), where the effective conversion of biomass to fermentable sugars requires a combination of chemical, mechanical and/or enzymatic processes (Dhabhai et al., 2012).

The most commonly used pre-treatment methods are acid pre-treatment with high-pressure steam explosions, enzymatic hydrolysis, milling, etc. (Hamelinck et al., 2005; Hendriks & Zeeman, 2009; Alvira et al., 2010; Tutt et al., 2014). Nevertheless, cost-efficient, highly productive technologies for fermentation feed production from lignocellulose biomass still need to be developed. Membrane separation processes such as ultrafiltration (UF) and nanofiltration (NF) have gained much attention in the biotechnology industry due to their simplicity, high selectivity, low energy costs and

reduced chemical usage (Cho et al., 2012; Gryta et al., 2013). UF membranes can selectively remove not only large molecules such as proteins, viruses, and microorganisms from the biological environment through size sieving mechanisms but can also substantially reduce emulsion to improve the successive solvent extraction efficiency (Li et al., 2006; Yasan et al., 2009). However, after passing the UF membrane system the permeates are, in general, very diluted and great in volume. Therefore, NF membranes are suitable not only for the separation of small molecules like organic acids and salts but also for concentration (Bruggen et al., 1999; Yasan et al., 2009).

The aim of this study was to optimize and combine the available lignocellulosic biomass pre-treatment and hydrolysis techniques with UF–NF membrane separation systems to increase the product yields of enzymatic hydrolysis and decrease the production costs related to enzyme recovery as reported previously (Mezule et al., 2012). The research was conducted on both a laboratory and pilot level to evaluate the efficiency of the proposed technology.

MATERIALS AND METHODS

Lignocellulosic biomass

Hay mown in late June from lowland hay meadows located in Latvia was used as reference material. After drying, the lignocellulosic biomass was stored at room temperature until further processing. The dried biomass was ground (Retsch, GM200) to obtain the desired biomass particle size of < 0.5 cm (Hamelincik et al., 2005).

Batch scale substrate pre-treatment and hydrolysis

Batch scale tests were prepared to estimate the production yields of enzymatic hydrolysis with fungal enzymes. In brief, the biomass was diluted in a 0.05 M sodium citrate buffer (3% w v^{-1}) and boiled for 5 min to neutralize unnecessary microorganisms. After cooling, an enzyme (0.2 FPU ml^{-1}, 20 FPU g^{-1}, Mezule et al., 2012) was added to the diluted substrates and incubated on an orbital shaker for 24 hours at 30 °C. All tests were prepared in triplicate and sugar yields were estimated after hydrolysis.

Pilot tests

The tests were carried out in the pilot system developed by the Riga Technical University (Latvia). The main technological processes involved in this study are presented in Fig. 1. Hay biomass (3% w v^{-1}; with constant mixing) was boiled until the hydrolysis reactor temperature reached 120 °C (~ 1 h) and then cooled down to 40 °C. Subsequently, the substrate from the hydrolysis reactor was pumped through a rough water filter system (Geyser, Russia) to the UF ceramic tank. Then the substrate was filtered through the UF membrane system to the NF tank. Eventually the substrate was filtered through the NF membrane system to get the concentrated sugar solution into a collector tank. Sugar concentration was measured at all process stages. During all pilot tests enzymatic hydrolysis was omitted and sugars were either generated during grinding and heating or artificially added glucose (Bacteriological grade, Oxoid Ltd) was used.

Figure 1. Schematic diagram of the experimental set-up. 1: hydrolysis reactor (HR, working capacity 15 l), 2: rough filters (RF), 3: ultrafiltration (UF), 4: nanofiltration (NF), 5: collector (CC) for concentrated liquid.

Analysis of total reducing sugars

A reducing sugar analysis was performed for all the collected samples using the Dinitrosalicylic Acid (DNS) Method (Ghose, 1987). In brief, all samples were centrifuged (6,600 g, 10 min). Then 0.1 ml of the supernatant was mixed with 0.1 ml of the 0.05 M sodium citrate buffer and 0.6 ml of DNS. For blank control, distilled water was used instead of the sample. Then all samples were boiled for 5 min and transferred to cold water. Next, 4 ml of distilled water was added. Absorption was measured with the spectrophotometer M501 (Camspec, United Kingdom) at 540 nm. To obtain absolute concentrations, a standard curve against glucose was constructed.

RESULTS AND DISCUSSION

The effect pre-treatment has on lignocellulosic materials has been recognized for a long time (Ye & Cheng, 2002). Depending on the biomass source, different harvesting times and treatment methods used, sugar yields vary from 12% to 98% (Dhabhai et al., 2012; Tutt et al., 2012; Tutt et al., 2013). Enzymatic hydrolysis generally gives lower product yields than other hydrolysis methods, however, the technology is regarded as environmentally friendly and is less inhibitory to fermenting microorganisms (Ye & Cheng, 2002; Behera et al., 2014). Batch scale studies with hay and cellulolytic enzymes produced at laboratories generated 15% to 19% of sugar yields (45%–57% of the theoretical cellulose/hemicelluloses content), showing that the direct application of the technology in large-scale fermentation systems might not be productive enough. Thus, a combined UF–NF system for filtrating fermentation substrates (Yasan et al., 2009) was introduced as a technique to produce fermentation feed.

Initially the treatment involved the direct transfer of hydrolysis products through the filtration system. Heating to 120 °C prior to filtration did not produce more than a 15% increase in sugar; thus, the additional pre-treatment was accepted more as a step for substrate sterilization to remove indigenous microorganisms than a process for releasing sugar. Besides, previous studies have shown that pre-treatment at less than 150 °C does very little damage to plant cell walls, therefore, cellulose cannot be accessed for degrading it to glucose (Raud et al., 2014; Tutt et al., 2014). Sugars produced in the reactor after mixing and heating were regarded as a 100% sugar yield. The results of a direct hydrolysate transfer through the membrane showed that 46% of the generated sugar yield was lost in permeate-waste (Fig. 2, single) after UF. At the same time, rough filters and NF attributed to an 11% and 19% decrease respectively, thus producing only a 24% yield (6.55 g l^{-1}; initial yield 2.49 g l^{-1}) after sugar concentration. Generally, UF membranes can selectively remove large molecules such as proteins, viruses, and microorganisms through size sieving mechanisms and can substantially reduce emulsion to improve the successive solvent extraction efficiency (Li et al., 2006; Yasan et al.,

2009). However, the permeates after using the UF membrane system are, in general, very diluted and great in volume. To increase the product yields and subsequently decrease the sugars lost in the waste, a recirculation system was introduced into the pilot system where the UF permeate was transported back to the hydrolysis reactor. The collected UF permeate was mixed with 3 l of nanofiltred water to increase the product volume critical for the system. No improvements were observed when comparing these two setups, and the final sugar percentage yield in both attempts was less than 25% when compared to the initial yield (Fig. 2, double). At the same time, double recirculation generated more waste within the NF and required higher resource inputs (water, electricity). Thus, it was not considered to be potentially applicable.

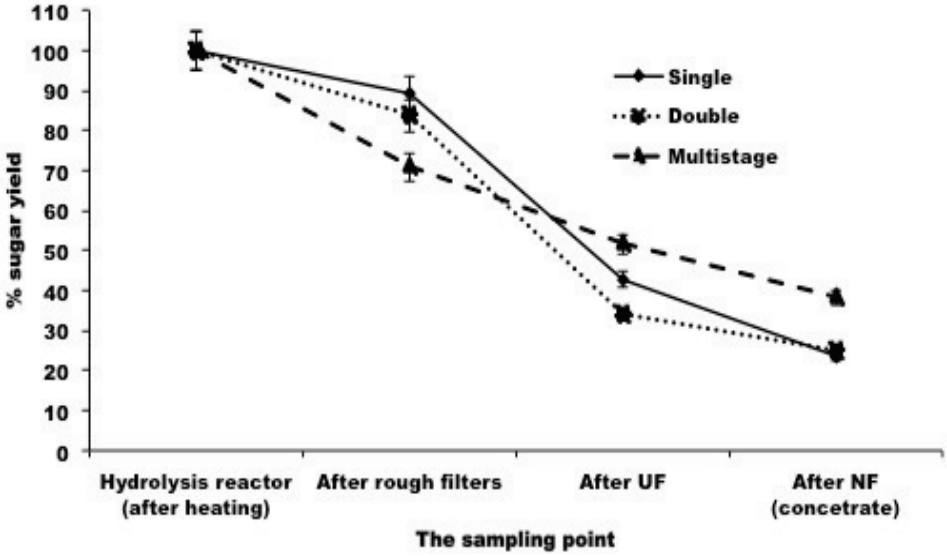

Figure 2. Sugar percentage yield changes in the pilot system with direct filtration (single), recirculation from the UF to a hydrolysis reactor (double) and multistage recirculation (multistage). Standard deviation represents the average from at least two repetitive measurements.

For the multistage setup (Fig. 2, multistage), sugar production was performed according to the method description with added UF and NF permeate recirculation. Retained large particles and molecules were initially separated from the substrate with RF and UF and then further filtered with NF, while permeates from UF and NF were retained and filtered once more. The results showed that UF and NF permeate recovery increased efficiency and the final sugar percentage yield reached almost 40% (7.01 g l^{-1}; initial yield 1.89 g l^{-1}) which was higher than with single and double filtration setups (Fig. 2). A certain decrease (up to 30%) in recovery was observed after RF. However, this was attributed to the potential shift in biomass size and mixing properties during hydrolysis. Thus there is a need for the further investigation of the process at this stage to minimize recovery fluctuations. At the same time, no significant difference ($p > 0.05$) in recovery was observed after NF for all three setups.

Since batch scale enzymatic hydrolysis produced 4.8–5.6 g l^{-1} of fermentable sugars on average, the multistage filtration treatment was tested on these concentrations on the pilot level. This was achieved by adding glucose to the hydrolysis reactor. The results showed that sugar recovery yields at multistage UF–NF separations do not differ significantly ($p > 0.05$) when the initial sugar concentration is changed (Fig. 3). Again, RF showed the highest decrease in recovery (around 30%) when compared to other process steps, and NF did not cause a more than 20% decrease.

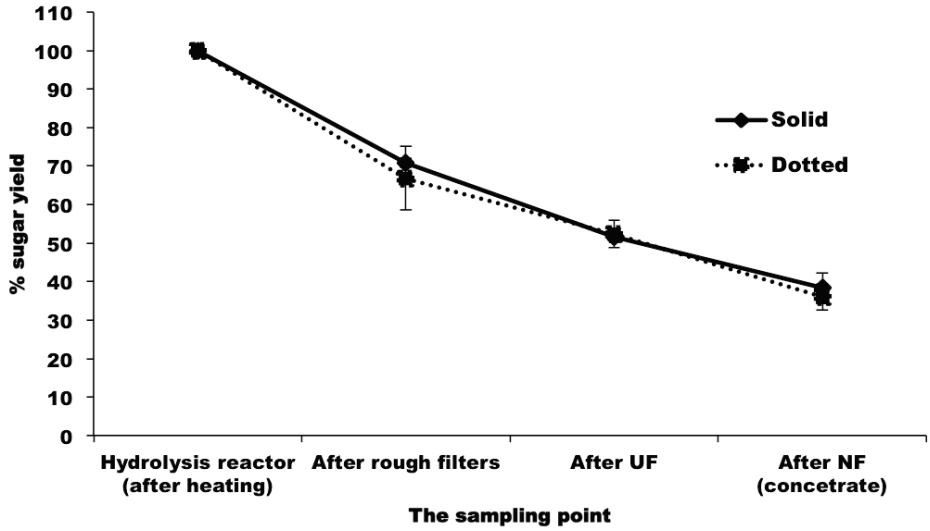

Figure 3. Sugar percentage yield changes during membrane multistage filtration processes in a system with added glucose (dotted) and without added glucose (solid). Standard deviation represents the average from two pilot runs.

The enzymatic hydrolysis of lignocellulosic biomass for biofuel production is a technology that has been thoroughly investigated over the years. Despite of its high potential, it is still not competitive enough due to low product yields when compared to production costs (Alvira et al., 2010). Effective fermentation feed production is closely linked to the separation of sugars from the generated waste. As suggested (Olofsson et al., 2008), the issue can be overcome by introducing simultaneous saccharification and fermentation techniques. However, the selection of the most favourable process conditions as well as enzyme and fermenting microorganism recovery are still challenging. In order to aid the separation of fermentation feed from waste, recover enzymes and decrease separation costs, multistage membrane separation can be introduced after enzymatic hydrolysis. Furthermore, when compared to the classical separation processes, membrane processes have several advantages as they work at low temperatures without phase change, and without the addition of chemicals (Karakulski & Morawski, 2002). The results of this research project showed that it is possible to apply membrane separation techniques to purify sugars produced during hydrolysis. Nevertheless, further research is still needed to increase the recovery rate and produce higher amounts of fermentation feed.

CONCLUSIONS

The results of this research project show that it is possible to introduce multistage separation techniques to generate fermentation feed for biofuel production from lignocellulosic biomass. The highest sugar recovery rates were obtained when secondary waste recirculation was introduced after NF and UF, and this yielded almost 40% of all produced sugars. Moreover, changing the initial sugar concentration did not significantly affect the efficiency of the process, which is more connected to various substrates and changing hydrolysis conditions.

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