

## **Separation of reducing sugars from lignocellulosic hydrolysate: Membrane experiments & system dynamic modelling**

A.R.P.P. Weerasuriya Arachchige\*, L. Mezule and T. Juhna

Riga Technical University, Faculty of Civil Engineering, Department of Water Science and Technology, Paula Valdena street 1-204/205, LV-1048 Riga, Latvia

\*Correspondence: antonrayan@hotmail.com

**Abstract.** Separation of fermentable sugars after hydrolysis of lignocellulosic biomass plays a vital role in second-generation biofuel production. Byproducts and solid fractions generated during pretreatment and hydrolysis can have adverse effects on fermentation efficiency. Previous studies have shown that a maximum of 40% (w/w) of sugar yield can be obtained by sequential UF and NF permeate recovery. This study aimed to introduce a multi-step membrane filtration process to recover fermentable sugars while removing inhibitory bi-products. Fermentable sugar recovery was investigated using a recirculation flow between various stages of separation. The experimental results demonstrated that by introducing NF permeate recirculation to the UF unit a sequential UF/NF system can achieve 60% (w/w%) recovery of reducing sugars. Based on the experimental results, a ‘Simultaneous ultrafiltration and nanofiltration model’ was developed using system dynamics. The model was used to predict the final sugar concentration and sugar yield using sugar permeability in each membrane as the dynamic variability. The model predicts that high sugar permeability (or selective permeability) through the ultrafiltration mostly affects the efficiency of the system, which still is a challenge.

**Key words:** lignocellulosic biomass, fermentable sugars, membrane separation, system dynamic modelling.

### **INTRODUCTION**

The expanding human population and the rapid development of industries are significant causes for high energy demand which leads to many problems such as environmental pollution and depletion of fossil fuel resources (Mahapatra & Kumar, 2019). According to ‘Global Energy Statistical Yearbook 2019’ by the end of the year 2018, total global energy consumption has reached 14,391 million tons of oil equivalent which is a 2.3% increment concerning the year 2017. The repercussions from excessive use of fossil fuel have raised the importance of increasing the share of renewable energy. Driven by these factors the EU Renewable Energy Directive II mandates at least 32% of renewable energy share by the year 2030 (European Union, 2018). Moreover, use of food crops is no more favoured. According to Annex IX of the RED, alternative resources should be used for fuel production.

Lignocellulosic biomass is becoming a valuable resource for bioenergy production due to its high abundance and constant regeneration. In agriculture, after harvesting and processing of crops, the residues are still rich in cellulose and hemicellulose (35%–55%

and 25%–40% by weight, depending on the source) (Adhikari et al., 2018). Currently, most of it is left on the fields, used for soil enrichment or landfilled. However, research has shown that agricultural residues prove to have a great potential towards liquid biofuel production (Blaschek et al., 2010; Nguyenhuynh et al., 2017a). Despite the extensive research, an economically feasible system to produce second-generation bioethanol (Roomi et al., 2017) or any other modern biofuel, which can compete with fossil fuel derivatives is still a challenge.

A typical process for liquid fuel production from lignocellulose consists of pretreatment, hydrolysis, fermentation and subsequent product recovery. Pretreatment is necessary to depolymerise lignin which covers cellulose and hemicellulose. Currently, a wide range of chemical, biological, oxidative and physical methods or their combinations have been used (Kumar & Sharma, 2017). In hydrolysis, the use of enzymes (biological hydrolysis) has been preferred to chemical one, however, commercial enzymes generally contribute to approximately 20% of the total costs in second-generation biofuel production (Tu et al., 2007). Thus, recirculation of enzymes, separation of hydrolysis products and their subsequent concentration to produce carbohydrate concentrations useful for fermentation is of high importance. This can be achieved by the integration of ultrafiltration (UF) and nanofiltration (NF) within the conversion process (Dalecka et al., 2015). Even though there is no effect on the separation of sugars by UF due to high MWCO, it is essential to separate enzymes and use for subsequent hydrolysis reactions (Nguyenhuynh et al., 2017a).

In previous studies, it has been shown that a maximum of 40% yield of sugar recovery from the sugars generated during the pre-treatment and hydrolysis can be recovered (Dalecka et al., 2015). This study aims to determine the maximum sugar yield extractable from the enzymatic hydrolysis of lignocellulose biomass using a laboratory-scale reactor consisting of a subsequent UF-NF filtration system. Laboratory pilot measurements and adjustments in operational system will be combined with the design of system dynamic (SD) model to enable process control. Moreover, mechanically pretreated hydrolysates obtained with laboratory made enzymes from white rot fungi were used as liquids for separation tests. Using the results from pilot experiments, a system dynamic (SD) was developed to model the change of sugar yield over time.

## MATERIALS AND METHODS

### Biomass and enzymes

Dried hay (dry weight (DW): 92.8% ± 1.3%; 6.02% ash) from semi-natural grasslands was collected and stored at room temperature, then milled by a mechanical cutting mill (Retsch SM100, Haan, Germany) with 1.5 kW drive and a parallel section rotor with a peripheral speed of 9.4–11.4 m s<sup>-1</sup> to obtain particle size < 0.5 cm. Lignocellulose degrading enzyme mix was prepared from cultures of *Irpex lacteus* IBB 104 according to a protocol described by Mezule et al. (Mezule et al., 2015).

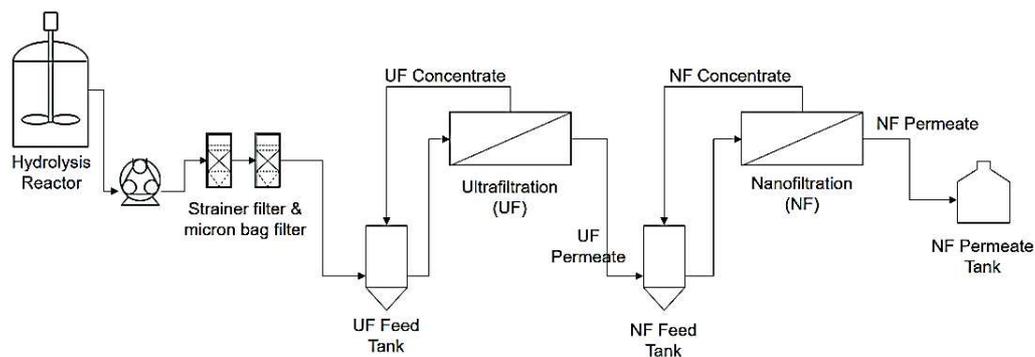
### Pilot Tests

All experiments and production of hay biomass hydrolysates were performed in a laboratory-scale pilot reactor (Fig. 1). The pilot reactor consists of a hydrolysis reactor with a capacity of 30 L per batch, followed by subsequent filtration units to concentrate sugars extracted in the hydrolysate. Each UF and NF filtration system have one

membrane element particularly selected for protein and sugar rejection respectively. The ground hay was mixed (3% w/v) with 20 L of nano filtered permeate water and boiled in a closed hydrolysis reactor unit until the temperature reached 120 °C, then it was cooled until 37 °C. After cooling, the stock enzyme mixture (25 FPU per mL) was added into the reactor to obtain a final enzyme concentration in the ranges of 0.1–0.4 FPU per mL of reaction liquid. The enzymatic hydrolysis was carried out for 24 hrs at 37 °C. After completion of the hydrolysis, the liquid hydrolysate was pumped into the UF feed tank through a rough filter system (1 micron) to remove biomass particles. Further, the pre-filtered hydrolysate was filtered through an UF membrane where UF permeate was collected in the NF feed tank until a minimum volume (1.5–2 litres) of UF concentrate retains in the UF feed tank. UF feed rate was continuously maintained at 1.5 m<sup>3</sup>hr<sup>-1</sup> at feed pressure of 0.3 MPa. Subsequently, NF filtration of UF permeate was carried out to obtain concentrated carbohydrates. Feed flow rate of NF was maintained 210 L hr<sup>-1</sup> at initial feed pressure of 30 bar. Depending on the type of the experiment, the process flow was modified with either batch or continuous recirculation of NF permeate into the UF feed tank. All the nanofilter circulations were maintained at a constant feed and concentrate flowrates to maintain a constant flux. since flux declination over recirculations or model the fouling effects on the membrane were not addressed in this study. From each unit process step, samples were collected and analysed for the concentration of reducing sugars with the Dinitrosalicylic Acid (DNS) method (Ghose, 1987) according to a previously described protocol (Mezule et al., 2019).

The sugar yield was calculated as the ratio of the amount of sugars collected as NF concentrate in respect to the amount of sugars produced after the hydrolysis (Formula 1):

$$\text{Sugar yield} = \frac{\text{Amount of sugar obtained in NF concentrate (g)}}{\text{Amount of sugar obtained in hydrolysis reactor (g)}} \times 100\% \quad (1)$$



**Figure 1.** Process flow diagram of the pilot (laboratory-scale) reactor.

### System Dynamic (SD) Model

System dynamic modelling has been widely used as a tool for demonstrating the behaviour of a particular system. Though the application of SD is more prevalent among socio-economic aspects, it has also been used to model chemical/biological systems (Park et al., 2014). ‘Stella Architect’ software was used to model a ‘Simultaneous UF & NF filtration process’, which can be used as an empirical model to predict the sugar yield over time in the filtration system.

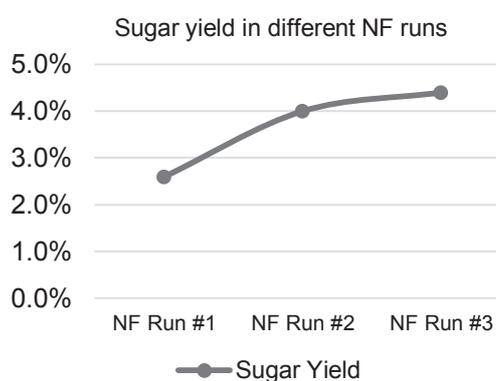
## RESULTS AND DISCUSSION

Dalecka et al., 2015 has demonstrated that a single pass (one-stage filtration) of the hydrolysate has a high amount of sugar loss and could only recover 24% of the yield produced during hydrolysis. Multistage filtration (secondary waste recirculation after NF and UF) produced the highest sugar yield, which is, 40% from all the produced sugar. From these studies, it was observed that a significant amount of sugar loss occurs as a waste of UF concentrate and waste of wet biomass.

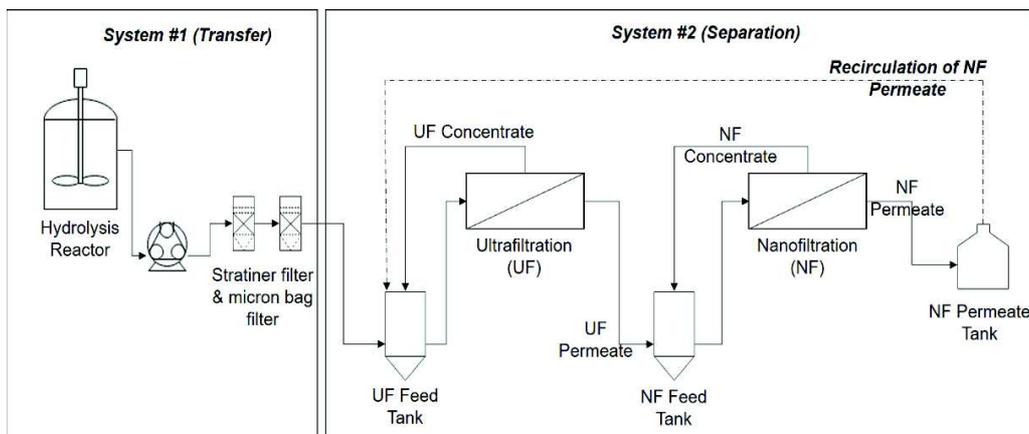
Based on the previous observations, it was assumed that recirculation of NF permeate into UF feed tank will produce higher sugar yield. To test this, an experiment was performed to recirculate equal volume (6 litres) of NF permeate into UF feed tank two times as batch. Sugar yield, which is collected in NF concentrate, was analysed after each recirculation (Fig. 2). Without any NF permeate recirculation, the recovered sugar yield was only 24% of the sugars generated in the hydrolysis. The consecutive recirculations increased the sugar yield. After two recirculations the maximum sugar yield observed was 44%.

From the results, it is conclusive that recirculation of NF permeate into UF concentrate gives a better yield than in a single pass filtration. Since molecular weight pore size of UF is higher than the molecular size of reducing sugars, UF does not have any effect on the separation of sugars (Dey et al., 2018). Hence, sugar separation largely depends on hydraulic recovery. Since UF concentrate consists of high molecular weight proteins such as enzymes, some sugars can adhere to these proteins as well (Qi et al., 2012). Recirculation of NF permeate into UF feed can increase recovery of sugars retained in the UF feed tank as UF concentrate. However, the yield increases at a decreasing rate with each filtration cycle demonstrating the impossibility to obtain 100% recovery. Introduction of diafiltration is an alternative option to increase efficiency with increase circulations (Wagner, 2001), where a buffer solution is used for the extraction of diluted solutes via several recirculations.

Further, the process flow was amended with a continuous recirculation of NF permeate into UF feed tank. The process can be denoted as 'simultaneous UF and NF' process. The process resembles an integrated diafiltration where NF permeate is used as the buffer solution for subsequent filtration in UF, eliminating the addition of a different buffer solution. The process flow (Fig. 3) was divided into two systems for the ease of analysis. In system 1, suspended solids were removed, and the hydrolysate was transferred into system 2, where the sugar separation happened. Similar steps were followed for hydrolysis. However, when transferring the hydrolysate (system 1), 5 litres of prefiltered NF permeate was added to recover sugars left in the wet biomass.



**Figure 2.** Final sugar yields in different NF runs.



**Figure 3.** Process flow diagram of ‘simultaneous UF and NF filtration’.

After a substantial filtration time, reducing sugar left in each tank was analysed (Table 1).

After the filtration of hydrolysate, the highest sugar yield achieved was 61.63%. The sugar loss wasted as UF concentrate is 3.13 g, which is 0.02% from all the sugars produced. However, most of the sugar waste (24.86%) occurred in system 1 as about 20% (v/v) of the hydrolysate is wasted with wet biomass. To recover this part, extensive washing of the material and physical separation could be introduced. Alternatively, the wet biomass waste produced after hydrolysis and still containing some unrecovered sugar can be used as a valuable feedstock for biogas production by anaerobic digestion; hence the lignocellulose has already partially depolymerised with enzymes (Karupiah & Ebenezer Azariah, 2019).

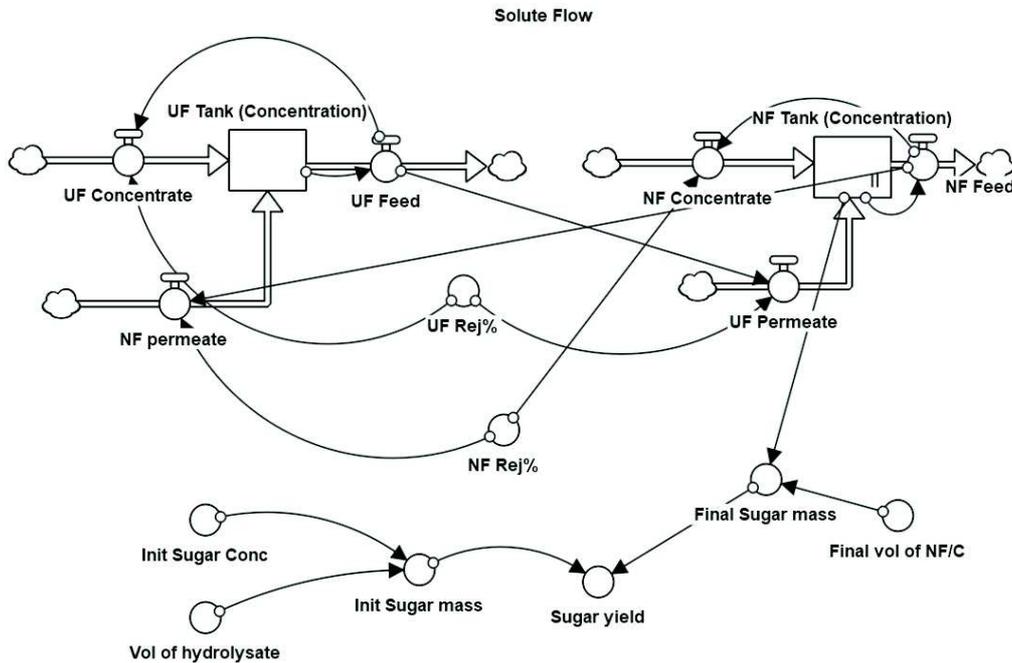
**Table 1.** Average reducing sugars in each tank after ‘simultaneous UF & NF filtration’

Tank	Amount of sugar(g)	Sugar yield
Hydrolysis reactor	141.16	100%*
UF feed tank (before filtration)	106.08	75.14%
UF concentrate (after filtration)	3.13	N/A
NF feed tank (after filtration)	87.00	61.6%

\* 100% yield does not denote full conversion of all biomass oligosaccharides. This represents the value of all released carbohydrates in the current study.

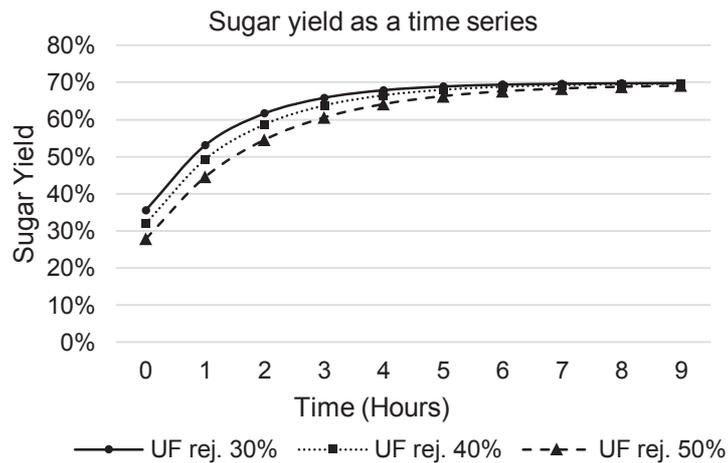
### SD MODEL

A significant problem of the proposed filtration method is the determination of the filtration time. Even though SD has numerous applications, this is the first time it has been used to model a sugar separation process, for the best our knowledge. SD model was built to study the change of sugar yield over time (Fig. 4) to aid the filtration time determination. The model was created and adjusted by using operational data collected from the laboratory pilot studies.



**Figure 4.** System dynamic model.

The validation of the model showed that it is able to predict that sugar yield increases with time at a decreasing rate (Fig. 5). Since the recirculation consumes energy, it is necessary to obtain the correct balance between the desired yield and the energy consumption when operating the system. Furthermore, the model indicates that the sugar permeation from the UF membrane plays a vital role in filtration efficiency.



**Figure 5.** Model result (sugar yield as a time series) with different sugar rejections from UF membrane.

As emphasised in this study UF is a vital operation in second-generation biofuel production. Though it has a lower effect in filtering sugars, it is essential to recover enzymes and recirculate to subsequent hydrolysis. The SD model developed for the specific purpose of sugar yield monitoring showed the importance of incorporating a selective UF membrane which has higher permeation for reducing sugars such as submerged UF filters and integrated membrane reactors, which still is a challenge (Rios et al., 2004; Nguyenhuynh et al., 2017b) in recovery processes.

## CONCLUSIONS

The study results demonstrated that a simultaneous UF and NF filtration system can recover 61% of the reducing sugars in lignocellulose hydrolysate, which is produced via enzymatic hydrolysis of biomass agricultural residue. SD model developed and applied for the first time within this study showed that the selection of UF membrane to permeate more sugars has a significant impact on filtration time. The simultaneous UF and NF filtration can be a valuable system when it comes to enzyme recovery and continuous process of sugar recovery. After the sugar separation, the higher molecular weight proteins such as enzymes are collected in the UF tank and will be diluted with the NF permeate. The diluted UF concentrate can be later transferred into the next batch of hydrolysis.

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