Nutrient recovery from the dry grind process using sequential micro and ultrafiltration of thin stillage

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ABSTRACT
The effectiveness of microfiltration (MF) and ultrafiltration (UF) for nutrient recovery from a thin stillage stream was determined. When a stainless steel MF membrane (0.1 μm pore size) was used, the content of solids increased from 7.0% to 22.8% with a mean permeate flux rate of 45 L/m²/h (LMH). Fat increased and ash content decreased. UF experiments were conducted in batch mode under constant temperature and flow rate conditions. Permeate flux profiles were evaluated for regenerated cellulose membranes (YM1, YM10 and YM100) with molecular weight cut offs of 1, 10 and 100 kDa. UF increased total solids, protein and fat and decreased ash in retentate stream. When permeate streams from MF were subjected to UF, retentate total solids concentrations similar to those of commercial syrup (23–28.8%) were obtained. YM100 had the highest percent permeate flux decline (70% of initial flux) followed by YM10 and YM1 membrane. Sequential filtration improved permeate flux rates of the YM100 membrane (32.6–73.4 LMH) but the percent decline was also highest in a sequential MF + YM100 system. Protein recovery was the highest in YM1 retentate. Removal of solids, protein and fat from thin stillage may generate a permeate stream that may improve water removal efficiency and increase water recycling.

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1. Introduction
In the corn dry grind ethanol industry, thin stillage is recycled as backset which is added in the slurry tank. The rest of the thin stillage is sent to evaporators for concentration which involves significant energy inputs and results in evaporator fouling problems. To decrease the load upon evaporators and reduce demand for fresh water, recycle of water in the thin stillage stream should be increased. However, there are concerns with recycling thin stillage above a certain percentage, generally 30–50% depending on plant operating conditions. As percent recycle is increased, the concentrations of various compounds, especially lactic and acetic acids, inhibit yeast growth and reduce ethanol yields (Chin and Ingledew, 1993; Ingledew, 2003).

Ultrafiltration (UF) is an efficient process for selective removal of compounds by convective solvent flow through a membrane. Membrane filtration involves no evaporation of water; hence energy consumption is lower than with thermal methods. Application of UF membranes to process thin stillage obtained from conventional and an enzymatic corn dry grind (E-Mill) processes have been described (Arora et al., 2009, 2010). Total solids recovered through batch UF membrane separation were similar to solids levels obtained from commercial evaporators. The membrane fouling issue has also been addressed during thin stillage concentration and found to be primarily reversible using filtration parameters and cleaning methods (Arora et al., 2009). In small scale experiments, the flux rates of thin stillage through MF and UF membranes was high enough to appear economically feasible (Arora et al., 2009). However, application of microfiltration (MF) and sequential MF + UF processes have not been used to filter and recover nutrients from commercial thin stillage; distribution of yeast inhibitors in membrane retentate and permeate streams have not been investigated. Water recycling rates could be increased if MF and UF methods were effective in removing compounds that inhibit yeast growth and metabolism. We evaluated nutrient recovery using MF and UF membranes and evaluated permeate streams on the basis of organic compound removal. Specific objectives were to: (1) compare filtration characteristics of thin stillage for MF, UF and sequential MF + UF processes, (2) evaluate solids recovery and nutrient compositions of permeate and retentate streams and (3) evaluate the permeate streams for potential water recycling based on organic acid contents.
2. Methods

2.1. Experimental material

Thin stillage was collected from a commercial dry grind ethanol facility and stored at 4 °C. To characterize thin stillage and retentate streams, one 500 mL sample was analyzed for total solids (TS) using a two stage oven method (Approved Method 44-15A, AACC International, 2000). Composition of thin stillage and retentate streams obtained from MF and UF processes were analyzed for protein (total nitrogen × 6.25), fat and ash content using standard methods (AOAC, 2003) at the University of Missouri, Columbia. Compositional analyses were performed in duplicate.

2.2. Microfiltration equipment

Microfiltration (MF) was carried out using a bench top membrane unit. A tubular stainless steel module with six tubes having 0.61 m length, nominal diameter of 0.64 cm, 0.1 μm pore size, 0.31 cm wall thickness and 0.07 m² membrane area (Scepter model 2.5-250A-2P6, Graver Technologies, Glasgow, DE) was used in a crossflow filtration arrangement. The unit was equipped with a batch tank of 15 L capacity, a heat exchanger and positive displacement pump (model M-03, Hydra-Cell, Minneapolis, MN). The unit was operated in total recycle mode (permeate returned to tank) or batch concentration mode (permeate collected separately and retentate recycled) with 4.75 m/s crossflow velocity. The permeate that passed through the membrane was termed MFP and the material that was retained and returned to tank was termed retentate. Permeate was collected in a graduated cylinder during batch concentration. Thin stillage (15 L) was used for each batch experiment; permeate flux rates measurements were taken for protein (total nitrogen × 6.25), fat and ash content using standard methods (AOAC, 2003) at the University of Missouri, Columbia. Compositional analyses were performed in duplicate.

2.3. Stirred cell ultrafiltration unit

A stirred ultrafiltration cell (400 mL Amicon, model 8400, Millipore Corporation, Bedford, MA) was used for concentrating thin stillage at room temperature. An argon gas cylinder was used to apply pressure to the stirred cell. A magnetic stir bar was used to simulate crossflow filtration. Two regenerated cellulose membranes, YM10 and YM100 (Millipore Corporation, Bedford, MA) with pore sizes of 10 and 100 kDa, respectively, and effective membrane area of 41.8 cm² were used. Five replicates of thin stillage (300 mL) were used in filtration.

2.4. Membrane selection

Since thin stillage contains a range of molecular weight compounds such as amino acids, peptides, proteins, fat and minerals (Jones and Ingledew, 1994; Kim et al., 2008; Arora et al., 2009), it is important to choose a membrane or membranes that recover solids and produce cleaner permeate stream with higher flux rates. Therefore, four membranes with different pore sizes were chosen to evaluate permeate flux rates and solids recovery. In phase I filtration experiments (Fig. 1), thin stillage batches were filtered through stainless steel MF (0.1 μm pore size) and regenerated cellulose UF membranes YM1, YM10 and YM100 with 1, 10 and 100 kDa molecular weight cutoff (MWCO), respectively. Permeate flux rates were measured for all membranes. Thin stillage, retentates and permeates were analyzed for compositions. Five replications were used for each treatment.

In phase II, permeates obtained from MF runs were filtered further using YM100, YM10 and YM1 membranes (Fig. 2) and analyzed for lactic and acetic acid concentrations. UF experiments were conducted at 25 °C (room temperature) and used pressures recommended by the manufacturer for each membrane (380, 207 and 70 kPa for YM1, YM10 and YM100 membranes, respectively). Both MF (15 L batch) and UF (350 mL/batch for phase I, 300 mL/batch for phase II) experiments were operated in batch concentration mode and experiments were continued until the material was exhausted. Permeate flux rates were measured using a graduated cylinder during MF experiments. During UF, permeate flux rates were determined gravimetrically by measuring the cumulative weight permeated, collected from the bottom of the cell as a function of time using an electronic balance. In sequential filtration, MF permeate was further filtered using a UF membrane. Permeate flux rates for UF and MF + UF membranes were calculated from start of the UF experiments.

2.5. Measurement of membrane separation performance

The average permeate flux rate \( J_{av} \) was calculated by

\[
J_{av} = \frac{V}{At}
\]

where \( J_{av} \) was the average flux rate (LMH), \( V \) was the total volume (L) of permeate, \( A \) was the effective area of the membrane, and \( t \) (h) was the permeate collection time.

![Fig. 1. Thin stillage filtration through various membranes (phase I).](image-url)
Average flux rates of permeate streams and compositions of retentate streams for thin stillage and permeate streams were determined. Thin stillage and permeate streams obtained from various pore sized membranes were analyzed using HPLC. Five replicates for each process (MF, UF, and MF + UF) were used. A completely randomized design was used in each case. Results were expressed as means and standard deviations. Statistical analyses were performed by using statistical software (SAS, 1989) using a significance level of \( p < 0.05 \). One-way ANOVA was used to detect differences among means. The LSD method was used to compare mean compositions of streams and HPLC results.

### 3. Results and discussion

#### 3.1. Flux profiles

MF had the highest average flux rates compared to UF membranes (Table 1) at 25 °C. \( J_{av} \) for MF + YM100 was higher than for the YM100 membranes alone because removal of suspended solids using MF reduced fouling resistance and increased \( J_{av} \) for the YM100 membrane by more than 200% (32 and 73 LMH for YM100 and MF + YM100 membranes, respectively). In the case of YM10 and YM1 membranes, sequential filtration (MF + YM10 and MF + YM1) did not increase permeate flux rates. The YM1 membrane had higher solids recovery (87%) than MF (81%) as calculated using Eq. (2). YM100 had the highest percent flux decline in UF (Figs. 3 and 4).

For sequential processes, MF + YM100 had the highest percent flux decline relative to the amount of permeate collected; only 30% of initial flux was recorded after 285 ± 5 mL of permeate was recovered from 300 mL of input stream volume (Fig. 5). The YM1 membrane had the lowest percent flux decline for UF and MF + UF filtrations. About 60% of initial flux was observed after collection of 275 ± 5 mL permeates (Fig. 5).

Permeate flux rate values declined more slowly over time for YM10 compared to YM100 membrane (Fig. 3). Decline in permeate flux rate was expected due to gel layer formation on the membrane surface as thin stillage concentration increased and deposition occurred (Zeman and Zydney, 1996; Cheryan, 1998; Ghosh, 2003; Yorgun et al., 2008). For pressure driven processes, a greater flux decrease signified build up of solute at the interface. The YM100 membrane had lower flux rates than MF + UF membranese for permeate and retentate total solids concentrations, respectively.

#### 2.6. Permeate composition and effect on water recycling

Organic acids such as lactic and acetic acid above a certain concentration (lactic acid > 0.8% w/v and acetic acid > 0.05% w/v) affect yeast performance and therefore reduce ethanol production (Narendranath et al., 2001). Original thin stillage and permeate streams obtained from different pore sized membranes were analyzed for lactic and acetic acid concentrations using HPLC. Based on these concentrations from previous work, the effectiveness of membrane filtration methods used in reducing lactic and acetic acids was evaluated. If lactic and acetic acid concentrations were above the thresholds described in Narendranath et al. (2001), it is likely that MF, UF and UF + MF permeate streams would need additional separation through membranes such as nanofiltration or reverse osmosis in order to affect the amount of recycling within the plant.

#### 2.7. HPLC analyses

Thin stillage and permeate samples were collected to determine concentrations of ethanol, lactic acid, acetic acid, glucose, fructose, maltose, maltotriose, DP4+ and glycerol using HPLC. Each sample was centrifuged at 17896 g for 5 min (Centra CL3, Thermo IEC, Needham Heights, MA); supernatant was filtered through a 0.2 μm syringe filter into 1 mL vials. Filtrate was injected into an ion exclusion column (Aminex HPX-87H, Bio-Rad, Hercules, CA) maintained at 50 °C. At a rate of 0.6 mL/min, sugars, alcohols and organic acids were eluted from the column with HPLC grade water containing 5 mM sulfuric acid. Components were detected with a refractive index detector (model 2414, Waters Corporation, Milford, MA). Data processing was performed using HPLC software (Waters Corporation). For calibration, we used standards containing the above components at known concentrations. Two replicates of each sample were injected for analysis.

#### 2.8. Statistical analysis

Average flux rates of permeate streams and compositions of retentate streams for thin stillage and permeate streams were determined. Thin stillage and permeate streams obtained from various pore sized membranes were analyzed using HPLC. Five replicates for each process (MF, UF, and MF + UF) were used. A completely randomized design was used in each case. Results were expressed as means and standard deviations. Statistical analyses were performed by using statistical software (SAS, 1989) using a significance level of \( p < 0.05 \). One-way ANOVA was used to detect differences among means. The LSD method was used to compare mean compositions of streams and HPLC results.

#### Table 1

<table>
<thead>
<tr>
<th>Membrane Type</th>
<th>Pore size</th>
<th>( J_{av} ) (LMH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microfiltration (MF)</td>
<td>Stainless steel</td>
<td>0.1 μm</td>
</tr>
<tr>
<td>YM100</td>
<td>Regenerated cellulose</td>
<td>0.01 μm</td>
</tr>
<tr>
<td>MF + YM100</td>
<td>Regenerated cellulose</td>
<td>0.01 μm</td>
</tr>
<tr>
<td>YM10</td>
<td>Regenerated cellulose</td>
<td>0.1 μm</td>
</tr>
<tr>
<td>MF + YM10</td>
<td>Regenerated cellulose</td>
<td>0.1 μm</td>
</tr>
<tr>
<td>YM1</td>
<td>Regenerated cellulose</td>
<td>0.1 μm</td>
</tr>
<tr>
<td>MF + YM1</td>
<td>Regenerated cellulose</td>
<td>0.1 μm</td>
</tr>
</tbody>
</table>

* Mean ± standard deviation. Values in same column with different letters are different \( (p < 0.05) \).
observed effect on the YM1 membrane permeate flux profile (Fig. 4). YM1 pores, 100 times smaller than those of the YM100 membrane, were too small for particles to cause plugging. Improved flux Profiles of YM10 following MF (MF + YM10) was noticed compared to permeate flux obtained using only YM10 membrane and demonstrates how solute size and pore size interacted during filtration. In the sequential MFP + YM10 process, the YM10 membrane was plugged by macromolecules that were able to penetrate the pores. YM10 pore size, intermediate between YM100 and YM1, rejected macromolecules larger than pore size and therefore had higher flux rate compared to YM10 alone.

The flux values observed during MF and UF experiments ($J_{\text{av}} = 9–73$ LMH, Table 1) were of similar magnitude observed in other work. Fukumoto et al. (1998) used ceramic MF and UF membranes to clarify apple juice in stirred cell systems. Most fluxes were between 30 and 75 LMH for membranes having 0.02–0.2 µm pore size and operating at 23°C and 276 kPa. They observed increased flux rates when using membranes with smaller pore sizes (0.2 µm). Padilla-Zakour and McLellan (1993) filtered apple juice in a crossflow filtration system using a 0.2 µm ceramic membrane at 50°C, 250 kPa, 14.6 m/s (relatively high velocity) and observed 285 LMH. Ben Amar et al. (1990) reported 100 LMH with 0.2 µm ceramic membrane operated at 50–55°C, 300 kPa and 2.8 m/s. Ghosh and Balakrishnan (2003) conducted a pilot scale study using sugarcane juice and using spiral wound UF modules (809 m² membrane area) operating at 91–97°C and observed 7 LMH. Yorgun et al. (2008) compared UF, NF and RO membrane filtration methods to treat whey from a cheese processing plant. Steady fluxes of 15, 10–15 and 2–3 LMH for UF, NF and RO, respectively, were observed.

Mean total solids in retentates were similar among MF and UF membranes (Table 2). YM1 membrane had higher solids recovery than the MF membrane. While these data indicate potential to increase solids contents of retentate streams, it should be kept in mind that MF and UF apparatus were not designed to determine maximum solids levels possible for each membrane type. MF and UF experiments were conducted in batch mode; thus, when material was depleted in the recirculation loop, each experiment was terminated. Therefore, higher concentrations of retentate solids may be possible. There were no differences detected among protein contents of MF, YM100 and YM10 membrane retentate streams. Retentate streams from the smaller pore size membranes (YM1 and YM10) had lower fat content values than retentate obtained from the larger pore size YM100 membrane but similar to the MF membrane. Ash contents were reduced more than 50% in retentates of all membranes. Reduction of ash contents in retentates, which would eventually be combined with streams to produce DDGS, may be an advantage from an animal nutrition standpoint. In thin stillage and DDGS, phosphorus contents are quite high relative to animal nutrient needs (Belyea et al., 2004, 2006; Rausch and Belyea, 2006). By reducing ash content, phosphorus content may also be reduced and improve DDGS value.

3.2. Permeate composition and effect on water recycling

There were no differences in lactic acid concentrations of thin stillage and permeate streams (Table 3). Thin stillage samples obtained from a dry grind facility at different periods of time had mean lactic acid concentrations (0.1% w/v) eight times lower than inhibitory level to the yeast (0.8% w/v; Narendranath et al., 2001). Acetic acid contents of original thin stillage samples were below the HPLC detection limit. Glycerol concentrations were not different between thin stillage and MF permeate. Since stillage is concentrated using evaporators and eventually added to DDGS, valuable coproducts such as organic acids and glycerol end up as a part of animal diets.
Table 2
Retentate compositions from various membranes.*

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Recovery (%)</th>
<th>Total solids</th>
<th>Protein (% db)</th>
<th>Fat (% db)</th>
<th>Ash (% db)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thin stillage</td>
<td>65 ± 0.7a</td>
<td>23.5 ± 2.3a</td>
<td>16.7 ± 1.6a</td>
<td>10.5 ± 0.5a</td>
<td></td>
</tr>
<tr>
<td>MF</td>
<td>81 ± 2a</td>
<td>22.4 ± 1.2b</td>
<td>27.6 ± 1.3ab</td>
<td>31.1 ± 2.4b</td>
<td>3.8 ± 0.5b</td>
</tr>
<tr>
<td>YM100</td>
<td>82 ± 1a</td>
<td>24.6 ± 3.1b</td>
<td>28.4 ± 0.3b</td>
<td>39.0 ± 4.0c</td>
<td>3.1 ± 0.7b</td>
</tr>
<tr>
<td>YM10</td>
<td>84 ± 3ab</td>
<td>26.2 ± 2.0b</td>
<td>29.0 ± 7.6b</td>
<td>34.2 ± 1.7b</td>
<td>4.3 ± 1.8b</td>
</tr>
<tr>
<td>YM1</td>
<td>87 ± 1b</td>
<td>28.8 ± 1.4bc</td>
<td>32.2 ± 0.1c</td>
<td>31.5 ± 1.7b</td>
<td>5.1 ± 0.7c</td>
</tr>
</tbody>
</table>

* Mean ± standard deviation. Values in same column with different letters are different (p < 0.05).

Table 3
HPLC analysis of thin stillage and permeate streams using various pore sized membranes.a

<table>
<thead>
<tr>
<th>TS5</th>
<th>MF</th>
<th>YM100</th>
<th>YM10</th>
<th>YM1</th>
<th>MF + YM100</th>
<th>MF + YM10</th>
<th>MF + YM1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerol</td>
<td>1.66 ± 0.06c</td>
<td>1.68 ± 0.08c</td>
<td>1.94 ± 0.03a</td>
<td>1.94 ± 0.02a</td>
<td>1.96 ± 0.04a</td>
<td>1.86 ± 0.05ab</td>
<td>1.80 ± 0.03b</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>Bdl</td>
<td>Bdl</td>
<td>Bdl</td>
<td>Bdl</td>
<td>Bdl</td>
<td>Bdl</td>
<td>Bdl</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>0.1 ± 0.02a</td>
<td>0.09 ± 0.007a</td>
<td>0.14 ± 0.09a</td>
<td>0.11 ± 0.06a</td>
<td>0.11 ± 0.04a</td>
<td>0.10 ± 0.008a</td>
<td>0.10 ± 0.06a</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.04 ± 0.002</td>
<td>0.04 ± 0.002</td>
<td>0.08 ± 0.03</td>
<td>0.04 ± 0.008</td>
<td>0.03 ± 0.007</td>
<td>0.05 ± 0.004</td>
<td>0.04 ± 0.003</td>
</tr>
<tr>
<td>Maltose</td>
<td>0.21 ± 0.006</td>
<td>0.20 ± 0.001</td>
<td>0.23 ± 0.01</td>
<td>0.23 ± 0.001</td>
<td>0.16 ± 0.005</td>
<td>0.19 ± 0.002</td>
<td>0.20 ± 0.04</td>
</tr>
<tr>
<td>Maltotriose</td>
<td>0.05 ± 0.007</td>
<td>0.05 ± 0.007</td>
<td>0.05 ± 0.002</td>
<td>0.06 ± 0.001</td>
<td>0.04 ± 0.001</td>
<td>0.04 ± 0.001</td>
<td>0.05 ± 0.001</td>
</tr>
<tr>
<td>DP4+</td>
<td>0.95 ± 0.08</td>
<td>0.41 ± 0.02</td>
<td>0.64 ± 0.03</td>
<td>0.45 ± 0.01</td>
<td>0.36 ± 0.02</td>
<td>0.44 ± 0.04</td>
<td>0.40 ± 0.03</td>
</tr>
</tbody>
</table>

a Mean ± standard deviation. Values in same row with different letters are different (p < 0.05). Means were compared only for lactic acid and glycerol. N = 5.

4. Conclusions

During MF and UF membrane filtration experiments, MF had higher permeate flux rates. Solids concentrations obtained using membranes were similar to commercial condensed distillers solubles. Ash contents were reduced more than 50% in retentate membranes. Fat removal from retentate could increase retentate protein content, thus, a better value as an animal food. Sequential filtration increased permeate flux rates of YM100 membrane (32.6–73.4 LMH). Sequential membrane filtration (MF + UF) changed permeate flux rates significantly and may provide methods to increase water recycle within the dry grind process.

References


