INTEGRATION OF NANOFLTRATION IN THE PRODUCTION OF SUCCINIC ACID FROM FERMENTATION OF LIGNOCELLULOSIC MATERIAL

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Key Words: Biorefinery, Nanofiltration, Retention mechanisms, Succinate purification

The aim of this study is to investigate the integration of nanofiltration in succinic acid production based on a fermentation from lignocellulosic material. An experimental investigation is reported carried out with NF 45 membrane and synthetic fermentation broths of increasing complexity containing succinate salt and different impurities like inorganic salts, glucose or other organic acid salts like acetate. The influence of the operating conditions (pH, pressure...) as well as of the broth composition on the NF performances was studied. The mechanisms governing the transfer of the solutes through the membrane were investigated in order to explain the different solute retentions observed according to the fermentation broth composition. Finally, according the knowledge of the mechanisms, a methodology was proposed to perform the purification of a succinate fermentation broth. The succinate/acetate separation has been carried considering the operations proposed in this study, i.e. dilution, NF in a diafiltration mode and RO concentration steps. It was shown that it is possible to increase the succinate purity from 85 % to 99.5 % while maintaining the total yield higher than 92 %.

I- Introduction

Current trend is to move from primary carbohydrate resources to more complex ones, like lignocellulosic materials as a bio renewable feedstock, to produce biofuels or chemical building blocks, like organic acids. This evolution requires significant modifications at different stages in the process, including fermentation and downstream processes, among which membrane operation can play an important role [1]. Previous work has also demonstrated that nanofiltration can be used for the purification of lactic acid from fermentation broth [2,3]. Succinic acid is an important building block for the synthesis of high added-value products such as biopolymers, pharmaceuticals or food additives. Since the lignocellulosic material is hardly fermentiscible, research is carried out to optimize the fermentation step, using for instance specific modified strains, like KJ122 a mutant obtained from *Escherichia coli* [4], or implementing a pretreatment before the fermentation. The first fermentation step carried out with lignocellulosic materials produces a broth containing, besides the succinic acid salt, some impurities like remaining carbon sources, salts and other organic acid salts. For instance, KJ122 can produce about 0.7-0.3 M succinate but with about 0.1-0.05 M acetate [4].

In this work, nanofiltration is investigated as a purification step in the production of succinic acid from fermentation. An experimental investigation is carried out with synthetic fermentation broths of increasing complexity containing beside succinate salt different impurities like salts, glucose or other organic acid salts like acetate. The influence of the operating conditions (pH, pressure...) as well as of the broth composition on the NF performances are studied. The mechanisms governing the transfer of the solutes through the membrane are investigated in order to determine, for different broth compositions, the best conditions to be used to achieve the purification of succinate.

II- Experimental materials and methods

II-1 Membrane and Chemicals

A Filmtec NF45 membrane (Dow Chemicals) was used to investigate the mass transfer mechanisms as well as the succinate/acetate separation. It is a thin film composite membrane negatively charged at pH higher than 5.1. The average molecular weight cut-off was about 150-200 g mol⁻¹, and the hydraulic permeability about 5.5-7.1 L h⁻¹ m⁻² bar⁻¹.

A Filmtec XLE membrane (Dow Chemicals) was used to concentrate the succinate solution after the separation step. This reverse osmosis membrane has an hydraulic permeability about 5 L h⁻¹ m⁻² bar⁻¹.

Solutions were prepared using succinic acid (H₂Suc), acetic acid (HAc), glucose (Glu), potassium phosphate (K₃PO₄) dissolved in ultra-pure water. The relevant characteristics of the different solutes are listed in Table 1.
Table 1: Physical characteristics of the investigated compounds

<table>
<thead>
<tr>
<th>Compounds</th>
<th>(g mol(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Succinic acid</td>
<td>118.09</td>
</tr>
<tr>
<td>Acetic Acid</td>
<td>60.05</td>
</tr>
<tr>
<td>Glucose</td>
<td>180.16</td>
</tr>
<tr>
<td>K(^+)</td>
<td>39.1</td>
</tr>
<tr>
<td>PO(_4^3-)</td>
<td>95.0</td>
</tr>
<tr>
<td></td>
<td>4.2 / 5.6</td>
</tr>
</tbody>
</table>

Table 1: Physical characteristics of the investigated compounds

The initial feed concentration of succinate and acetate and the pH were selected in accordance to the final compositions of the real succinate fermentation broth (0.7 - 0.3 M succinate and 0.1 - 0.05 M acetate; pH 7).

To investigate the influence of the operating conditions (pH, pressure...) as well as of the broth composition on the NF performances, experiments were carried out with synthetic solutions of increasing complexity (single, binary, ternary... -solute solutions). The pH values of synthetic solutions were adjusted by adding KOH.

A synthetic fermentation broth was used to investigate the purification of a succinate based on the composition of a real fermentation broth given in Table 2.

Table 2: Composition of the synthetic fermentation broth

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Succinate</th>
<th>Acetate</th>
<th>PO(_4^3-)</th>
<th>K(^+)</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration (M)</td>
<td>0.35</td>
<td>0.065</td>
<td>0.017</td>
<td>0.8</td>
<td>0.027</td>
</tr>
</tbody>
</table>

II-2 Analytical methods

Succinate, acetate and glucose concentrations were determined by high performance liquid chromatography using a Shodex SUGAR SH1011 column (Showa Denko) and a refractive index detector. The column temperature was set at 50 °C and the mobile phase was 0.01 M sulfuric acid at a flow rate of 1 mL min\(^{-1}\).

The inorganic ions were analyzed by HPLC with a Dionex system. The ion concentrations were determined using a CD20 conductimetric detector with an Ionpac AS11 column (mobile phase: 5 mM NaOH at 1 mL min\(^{-1}\)) and an Ionpac CS12 column (mobile phase: 20 mM CH\(_3\)O\(_3\)SO\(_4\) at 1 mL min\(^{-1}\)) for anions and cations respectively.

II-3 Filtration unit and experimental procedure

II-3-1 NF set-up

The experiments were carried out using a cross-flow filtration system described in previous papers [2.3]. The total membrane area in the filtration cell is 137 cm\(^2\). Feed solution was contained in a 5 L feed vessel maintained at a constant temperature of 25 ± 0.5 °C. A high-pressure pump was used to pull the feed solution into the membrane cell. The transmembrane pressure was controlled by a pressure valve (stainless steel control valve), mounted on the retentate outlet. Experiments were performed at a constant cross-flow rate of 400 L h\(^{-1}\) with increasing transmembrane pressures from 2 to 20 bar. A volume of 5 mL of permeate was collected for each pressure and timed to estimate the permeation flux. The flux values reported later are those obtained at steady state. The feed and permeate concentrations were determined by the analytical methods previously presented.

The investigation of the mass transfer has been carried out in constant concentration mode, with both retentate and permeate streams recycled back into the feed tank.

The purification of the synthetic fermentation broth was operated using a two-step process. The first one is a nanofiltration step carried out in a diafiltration mode. This mode of operation is well-known to improve the removal of non-retained impurities and the recovery of retained target species, like succinate is this work. In that case, the permeate is not recycled back to the retentate tank and the retentate volume is maintained constant by adding ultra-pure water. The initial retentate volume is fixed at 2L. The diafiltration has been carried out at 20 bar during 26 h.

The diafiltration mode using the NF membrane has been followed by a concentration step using the RO membrane in order to increase the succinate concentration in the purified synthetic fermentation broth. This concentration step has been also carried out at 20 bar using the same experimental set-up.

II-3-2 Retention, separation factor and purification performances

In NF experiments, the observed retention, \(R_{obs} \), of each component is usually defined as:

\[
R_{obs} = 1 - \frac{C_p}{C_r}
\]

where \(C_p\) and \(C_r\) are the permeate and retentate (or feed) concentrations respectively.

In order to estimate the succinate/acetate separation efficiency, one can also use another parameter, the
separation factor, \( SF \), which is expressed by the solute concentration ratio in the permeate divided by the concentration ratio in the retentate. The separation factor can be also calculated from the succinate and acetate retentions as:

\[
SF = \frac{(C_{Ac}/C_{Suc})_p}{(C_{Ac}/C_{Suc})_r} = \frac{1-R_{obs,Ac}}{1-R_{obs,Suc}}
\]  
(2)

\( SF \) values higher than 1, like those obtained in this work, mean that the NF retentate is a solution enriched in succinate, which is the target specie, compared to the feed.

The process performances can be also evaluated according to the succinate purity in the retentate, defined as the ratio of the succinate concentration over the sum of succinate and acetate ones in the retentate.

\[
\% Purity = \frac{C_{r,Suc}}{C_{r,Suc}+C_{r,Ac}} \times 100
\]  
(3)

Finally, the succinate yield, defined as the amount of succinate recovered in the retentate compared to the total succinate quantity in the initial feed solution, was also estimated:

\[
\% Yield = \frac{C_{r,Suc}V_r}{C_{f,Suc}V_f} \times 100
\]  
(4)

where \( V_f \) and \( V_r \) are the feed and retentate volumes, respectively.

III- Results and discussion

Experiments were first carried out with synthetic single-solute solutions as well as binary-solute solutions containing succinate and acetate. The influence of the operating conditions (pH, pressure...) as well as the broth composition on the nanofiltration performances were investigated. Then, according to the knowledge of the mechanisms governing the mass transfer of the solutes through the membranes, the best conditions to be used to purify the succinic acid have been evaluated regarding the purity of succinic acid to be obtained.

III-1 Mass transfer investigation

III-1-1 Influence of the succinate feed concentration

Firstly, the influence of the succinate concentration on the retention of both succinate and acetate salts has been investigated at pH 7 which is closed to the value of the real fermentation broth. In this conditions, both species are completely dissociated and negatively charged (see pK\(_A\) values in Table 1).

The variations of the succinate retention in single-solute solutions versus the permeate flux at different feed concentrations are plotted in Fig. 1. As expected, the retention of succinate continuously decreases with increasing concentrations. Indeed, the transfer of a charged solute depends on the combination of steric hindrance effects and electrostatic interactions between the charged solute and the fixed charge on membrane surface. At low concentrations, the electrostatic repulsions are dominant and thus high succinate retentions are obtained. Then, increasing succinate concentration results in a lower retention because of the screening effect that makes the electrostatic repulsion weaker [5].

The variation of the acetate retention versus the permeate flux for various concentrations of succinate are depicted in Fig. 2. One can also observe that the retention of acetate decreases for increasing succinate concentrations. Again, this is due to the screening effect.

![Fig. 1: Retention of succinate vs. permeate flux in single solutions: Influence of succinate concentration – pH 7](image1)

![Fig. 2: Retention of acetate vs. permeate flux in binary solutions: influence of succinate concentration \([Ac^-] = 0.1 M – pH 7\)](image2)

These results are in agreement with the ones obtained previously reported in the literature [2,3]. Finally, it was concluded that at low succinate concentrations, both acetate and succinate retentions are mainly fixed by their charge, while at high concentrations their retentions are mainly fixed by their size. In these conditions, one can observe that the retention of acetate, the smallest solute, is lower than that of succinate.
III-1-2 Influence of the pH on the succinate retention

The investigation of the influence of the succinate feed concentration points out the impact of the electrostatic interactions on the mass transfer. These interactions, which are fixed by the charge of the solute as well as that of the membrane, are expected to vary according to the pH of the solution. Then the influence of the pH has been investigated at low succinate concentration (0.1 M) in order to avoid the influence of the salt concentration. Fig. 3 shows that the succinate retention is indeed strongly affected by the pH. It is observed that the succinate retention continuously increases with the pH. For instance, at $J_v = 4 \times 10^{-5}$ m$^3$ m$^{-2}$ s$^{-1}$, the succinate retention is ranged between 25% to 100% when the pH increases from 2.2 to 7.6.

![Fig. 3: Retention of succinate vs. permeate flux in single solutions: Influence of feed solution pH - [Suc] = 0.1 M](image)

Fig. 3 shows that the succinate retention is indeed strongly affected by the pH. It is observed that the succinate retention continuously increases with the pH. For instance, at $J_v = 4 \times 10^{-5}$ m$^3$ m$^{-2}$ s$^{-1}$, the succinate retention is ranged between 25% to 100% when the pH increases from 2.2 to 7.6.

Similar results have been previously reported [6-8]. It was found that the retention of acetic acid, lactic acid, glutaric acid and fumaric acid increases with increasing pH from 3 to 7 due to more dissociated form of organic acid as well as more negatively charged membrane surface. Then, at low concentration increasing pH results in a higher retention because of increasing electrostatic repulsions.

Succinic acid is a dicarboxylic acid, then according to the pH this component can be neutral, monovalent and divalent ($pK_{a1} = 4.2$ and $pK_{a2} = 5.6$). At pH lower than 2, the succinic acid is totally neutral, at pH 5.6 it is shared equally between the mono and divalent form. At pH higher than 7 it is mainly in the divalent form.

Then, the low retention observed at pH 2.2 corresponds to the retention of the neutral form (size effect). On the contrary, at pH higher than 7, the succinate is completely retained due to the strong electrostatic interactions between the divalent succinate ions and the negatively charged membrane (electrostatic effect). To demonstrate the role of the pH on retention of succinate according to the ratio between the neutral and dissociated form of the organic acid, we report the variation of the succinate retention versus the pH for various permeate fluxes in Fig. 4. The corresponding ionic fraction of the divalent form is also reported for comparison. Fig. 4 shows that the curve representing the variation of the succinate retention versus the pH is a S-shape curve which is completely similar to the variation of the ionic fraction of the divalent succinate form.

Consequently, one can conclude that at low succinate concentrations, the transfer of succinate is governed by the fraction of the divalent form.

III-1-3 Succinate/Acetate separation: influence of the dilution factor

From the previous experiments, one can conclude that the separation of succinate and acetate in a fermentation broth containing 0.7 M of succinate and 0.1 M of acetate is not achievable. Indeed, succinate and acetate retentions are too close and low (less than 20%) (see Figs. 1 and 2). However, one can expect that the separation is possible at lower succinate concentrations and pH about 7 since the succinate is completely retained contrary to the acetate (see Fig. 2). Then in order to evaluate the influence of the broth concentration on the separation efficiency, the NF of the synthetic fermentation broth (0.7 M/0.1M) was performed at different dilution factors ($1 - 2 - 4 - 6 - 8 - 10$) and pH 7.

The variations of succinate and acetate retentions versus permeate flux were in agreement with those previously observed at various concentrations (Fig. 5). For a dilution factor equal to 1, the retention of succinate and acetate are low and similar. Then, no separation is expected in this condition. But, for increasing dilution factors, i.e. decreasing feed concentrations, it is observed that the increase of the succinate retention is higher than that of acetate. Then the succinate/acetate separation can be achievable for diluted solutions.

Moreover, at a dilution factor of 4, negative values are obtained for the retention of succinate. This means that in
these conditions, the acetate concentration is higher in the permeate than in the feed.

Such negative values of the retention of ions were already reported during nanofiltration of synthetic solutions containing mono- and divalent ions [9,10]. It is due to the competition for permeation between the membrane co-ions (showing the same sign of charge as the membrane) which have different size and/or charge. Divalent ions are more retained than monovalent ones through a negatively charged membrane. Then, in a solution containing K₂Suc and KAc, the permeation of acetate increases in order to maintain electroneutrality on both sides of the membrane and negative retentions can be achieved. The permeation of acetate, which is the less retained co-ion, is facilitated by increasing the concentration of succinate ions, which is the more retained co-ion.

As previously mentioned, Fig.5 shows different succinate and acetate retentions for increasing dilution factor. Then, the variations of the corresponding separation factor versus permeate flux are reported in Fig. 6. One can first observe that as expected the separation factor is closed to 1 for the non-diluted solution (dilution factor 1) and that values higher than 1 are obtained for diluted feed solution. This means that nanofiltration gives a retentate solution enriched in succinate compared to the feed. Moreover, as expected, the separation factor increases for increasing dilution factor.

One can also observe that for any dilution factor, the separation factor passes through a maximum value. This maximum value increases from 2 to 6.5 for a dilution factor increasing from 4 to 10. The flux corresponding to the maximum value increases also from $J_v = 0.5$ to 2.5 $\times 10^{-5}$ m$^3$ m$^{-2}$ s$^{-1}$ when the dilution factor varies from 4 to 10.

These results point out that the separation performances (separation factor as well as permeate flux), are improved for increasing dilution factor, i.e. lower succinate concentration.

### III-2 Purification of succinate from fermentation broth by nanofiltration

In this section, the objective is to propose a methodology for the purification of a succinate fermentation broth. This methodology is based on the knowledge of the previously investigated transfer mechanisms in order to determine the best conditions to be used regarding the purity of succinate.

The composition of the synthetic fermentation broth considered in this study is that given in Table 2 at pH 7. It was previously pointed out that the separation of succinate and acetate is not possible for succinate concentration higher than 0.4 M. On the contrary it was shown that the succinate is strongly retained by the membrane at succinate concentrations lower than 0.2 M and pH higher than 7 whereas the acetate retention is low (see Figs. 1 and 2). Consequently, the fermentation broth has to be diluted before the nanofiltration step since the separation is not achievable for the initial succinate concentration (see Figs. 5 and Fig. 6 dilution factor 1 to 4).

It was thus decided to carry out the nanofiltration of the diluted synthetic fermentation broth (dilution factor 2) in a diafiltration mode in order to improve the removal of acetate and thus the purity of the succinate. Finally, the purified solution (NF retentate) can be concentrated using a reverse osmosis membrane which is known to ensure a complete retention of organic salts.

The diafiltration of the fermentation broth diluted by a factor 2 has been carried out at 20 bar. The results are firstly presented in terms of the variation of the solute concentrations in the retentate versus the number of diaivolmes. A diavolume is defined as the total ultra-pure water volume added during diafiltration divided by the

**Fig. 5: Retention of succinate and acetate vs. permeate flux: Influence of the dilution factor**

\[
\text{[Succ]} = 0.7 \text{ M} - \text{[Ac]} = 0.1 \text{ M} - \text{pH 7}
\]

**Fig. 6: Separation factor vs. permeate flux: Influence of the dilution factor**

\[
\text{[Succ]} = 0.7 \text{ M} - \text{[Ac]} = 0.1 \text{ M} - \text{pH 7}
\]
initial retentate volume. As expected according to the retentions, Fig.7 shows that the concentration of succinate slightly decreases during the diafiltration operation ($R_{\text{Suc}} \approx 99\%$) while the acetate concentration decreases much more ($R_{\text{Ac}} \approx 65\%$). Consequently, as shown in Fig. 8, the purity of succinate increases, from 85\% (initial value in the feed) to 99.5\% for 14 diavolumes. Meanwhile the succinate yield remains higher than 93\% after 14 diavolumes.

![Graph showing concentration and purity variations](image)

Finally, the diafiltered fermentation broth (diavolume = 14, $[\text{Succ}] = 0.16\, \text{M}$) has been concentrated using the XLE reverse osmosis membrane at 20 bar. This operation has been carried out to recover the initial succinate concentration in the fermentation broth, i.e. 0.34 M (concentration factor $\approx 2$). The succinate purity and yield obtained with this operation are 99.5\% and 99.3\%, respectively.

Then, using the two step process proposed in this work, i.e. dilution/diafiltration/concentration operations, it was possible to achieve the purification of the fermentation broth, i.e. to increase the succinate purity in the fermentation broth from 85\% to 99.5\% while minimizing the succinate loss, keeping the total yield higher than 92\%.

IV- Conclusion

The aim of this work was to investigate nanofiltration as a purification step in the production of succinic acid from fermentation. Firstly, synthetic solutions of increasing complexity were used to investigate the influence of the operating conditions as well as of the broth composition on the transfer mechanisms. It was shown that both succinate and acetate transfer are strongly affected by the organic salt concentration due to charge effects. More precisely, a good correlation has been observed between succinate retention and its divalent ionic fraction. Considering the succinate /acetate separation it was shown that the nanofiltration performances are improved for decreasing salt concentration.

Then, based on these knowledge of the transfer mechanisms, a methodology has been proposed to achieve the purification of a succinate fermentation broth. The succinate/acetate separation has been carried out using the following operations. The broth was first diluted down to a given concentration to make the succinate/acetate separation feasible. NF was then used in a diafiltration mode in order to achieve the purification of succinate, i.e the removal of acetate. Finally, a concentration step by RO was used to recover the initial succinate concentration. In this manner, the succinate purity was increased from 85\% to 99.5\% while the total yield remained higher than 92\%.

![Graph showing concentration and purity variations](image)
References