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ABSTRACT

The aim of this study was to investigate the integration of nanofiltration in succinic acid production based on a fermentation. An experimental investigation was 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, a two-step process including NF in a diafiltration mode followed by reverse osmosis was proposed to perform the purification of succinate from a synthetic fermentation broth containing acetate. 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%.

1. Introduction

Succinic acid is considered as a high value organic acid which could be manufactured from feedstocks [1]. Succinic acid is a precursor of many specialty chemicals for food, pharmaceuticals, green solvent and biodegradable plastics [2,3]. As a result, the total market size for uses of succinic acid and its derivatives is around 20,000–30,000 tones per year [4]. A commercialized succinic acid is produced by chemical process from butane or oxidation of benzene through maleic anhydride. Due to the price of crude oil rapidly increasing and to environmental concerns, the succinate production moves to fermentation based processes, including the fermentation step itself followed by several downstream operations to recover succinic acid. Thus, to make fermentation-based succinate production competitive with petrochemical processes, the development of optimized producing strains and fermentation processes is required [5–8]. Currently, a metabolically engineered *E. coli* KJ122 was originally developed to ferment glucose into succinate with high yields [8]. The first fermentation step carried out with lignocellulosic materials can produce up to 0.7 M succinate. However, the fermentation generates a broth containing succinate and impurities including residual sugar (glucose), remaining ions (chloride, phosphate) and other organic acids (0.1–0.05 M of acetate).

The development of an efficient process to separate succinic acid from fermentation broth is very important because this step represents about 50% of the production costs and it is still difficult to achieve high purity and yield [2,9]. Different operations of separation and purification can be used to recover succinate from fermentation broth such as reactive extraction [10–12], ion exchange [13,14], crystallization [15–17] and membrane operations like electrodialysis [18,19] and nanofiltration (NF) [20–24]. Among them NF was successfully used for the recovery of organic acids from fermentation broth. It has been investigated in organic acids purification step at different stages depending on the composition of fermentation broth [20–23]. When carried out in a diafiltration mode, NF can be used to recover a target product while simultaneously decreasing the concentration of impurities [22,25–27]. To our knowledge, only Kang and Chang (2005) reported that NF membrane can efficiently remove impurities including acetate, formate and lactate from succinate under diafiltration mode [22]. However, because succinate retention is lower than 80%, this purification results in a loss of succinate.

In the present work, NF is investigated as a purification step to recover succinate produced by fermentation. The objective is to improve

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the process performances, i.e. the succinate yield and purity. 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 is 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. Finally, a two-step process is proposed to achieve the purification of succinate from fermentation broth.

2. Experimental procedures

2.1. Membranes and Chemicals

A Filmtec NF45 membrane supplied by Dow Chemicals as flat sheet, was used. It is negatively charged at pH higher than 5.1. Average molecular weight cut-off was about 150–300 g mol^{-1}, and 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 a hydraulic permeability about 5 L h^{-1} m^{-2} bar^{-1}.

Feed solutions were prepared from high purity succinic acid (H_2Suc), acetic acid (HAce), glucose (Glu), potassium phosphate (K_3PO_4) and potassium chloride (KCl) dissolved in ultra-pure water.

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 [8] given in Table 1. Solutes molecular weight, pK_A are also reported in Table 1.

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.

2.2. Analytical methods

Succinate, acetate and glucose concentrations were determined by high performance liquid chromatography using a Shodex SUGAR SH1011 column (Showa Denko). 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 (ionic chromatography) 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 $CH_4O_3SO_4$ at 1 mL min^{-1}) for anions and cations respectively.

2.3. Filtration unit and experimental procedure

2.3.1. Membrane pre-treatment

In this work, the new membrane was pre-compacted by ultra-pure water at a constant temperature of 25 ± 0.5 °C, flow rate of 400 L h^{-1} and pressure of 20 bar until the water permeation flux was constant (1.5 h). At the end of each run, the membrane was cleaned by RO water until the conductivity of water in the feed tank was below 20 µS cm^{-1} and then with ultra-pure water until the conductivity of water in the feed tank was below 5 µS cm^{-1}. The cleaning steps were operated at 25 ± 0.5 °C, 10 bar and a flow rate of 150 L h^{-1}.

Table 1
Composition of the fermentation broth and size properties of the solutes.

Compounds	Concentration (M)	Molecular weight (g mol^{-1})	pK_A
Succinate	0.35	116.09	4.2/5.6
Acetate	0.065	59.05	4.79
Glucose	0.027	180.16	12.28
K^+	0.8	39.1	–
PO_4^{3-}	0.017	95.0	–
Cl^-	0.0045	35.45	–

2.3.2. Filtration set-up

The experiments were carried out using a cross-flow filtration system described in previous papers [21,23]. The experimental set-up is described in Fig. 1. The total membrane area in the filtration cell was 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 were 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 was not recycled back to the retentate tank and the retentate volume was maintained constant by adding ultra-pure water. The initial retentate volume was fixed at 2 L. The diafiltration has been carried out at 20 bar during 26 h. The permeation flux as well as the solutes concentrations were measured every 30 min.

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. In that case, only retentate is recycled back to the feed tank whereas the permeate is collected in the permeate tank. This concentration step has been also carried out at 20 bar using the XLE reverse osmosis membrane. Starting with 2 L of the diafiltrated synthetic fermentation broth, the operation was carried out during 2.5 h. The permeation flux as well as the solutes concentrations were measured every 30 min.

2.4. Retention, separation factor and purification performances

For each component, retention R (%) is defined as:

$$R_{obs} = 1 - \frac{C_P}{C_r} \quad (1)$$

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

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

$$SF = \frac{(C_{Ace}/C_{Suc})_p}{(C_{Ace}/C_{Suc})_r} = \frac{1-R_{obs,Ace}}{1-R_{obs,Suc}} \quad (2)$$

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

For a given solute, the mass balance for the diafiltration mode is given by the following equation, assuming that the solute retention remains constant:

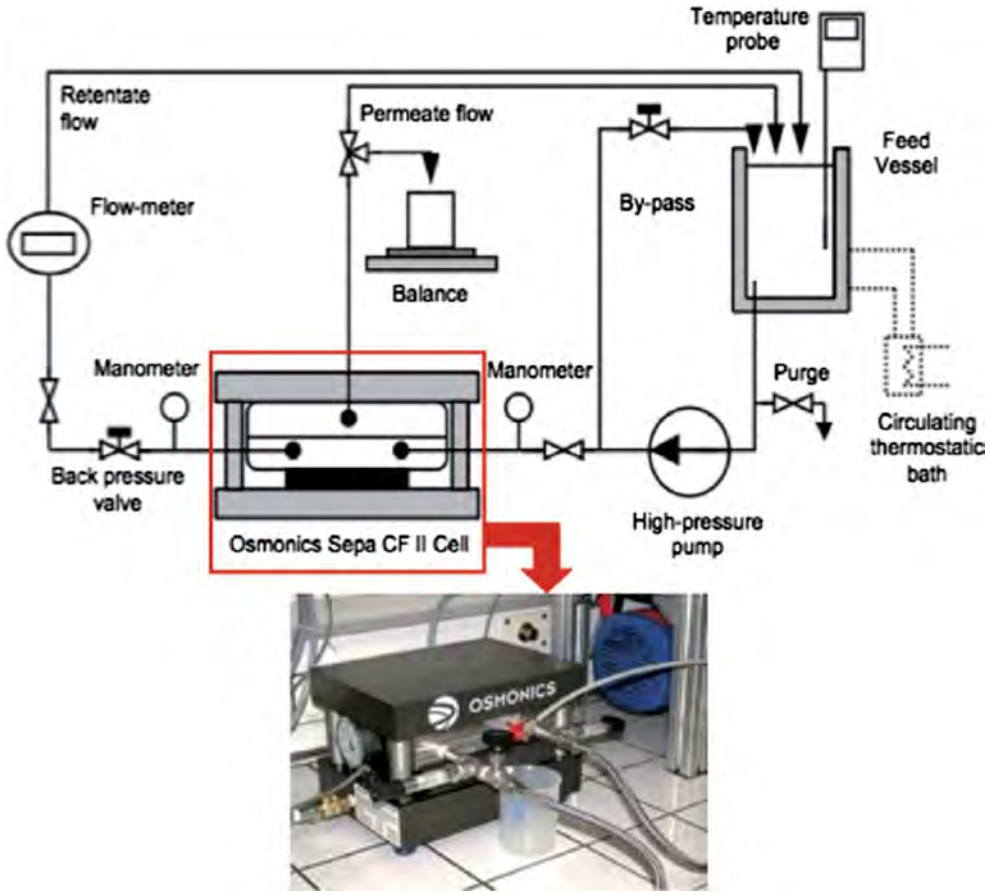

Fig. 1. Schematic diagram of the nanofiltration system set-up [21].

$$C_r = C_0 \exp[-V^*(1-R_{obs})] \tag{3}$$

$$\text{with} \quad V^* = \frac{V_p}{V_0} \tag{4}$$

where C_0 is the initial concentration of solute, R_{obs} is the solute retention, and V^* is the number of diavolumes. The number of diavolumes is defined as the total volume of ultra-pure water added during the diafiltration, V_p, divided by the initial volume of solution, V_0.

For the concentration mode, the mass balance equation is expressed as:

$$\left(\frac{V_0}{V_r}\right)^{R_{obs}} = \frac{C_r}{C_0} \tag{5}$$

where V_r is the retentate volume and C_r is the concentration of solute in the retentate.

The process performances have been also evaluated according to the succinate yield in the retentate, defined as the succinate concentration in the retentate compared to that in the feed solution:

$$\%\ Yield = \frac{C_{r,Suc} V_r}{C_{f,Suc} V_f} \times 100 \tag{6}$$

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

Finally, the succinate purity, defined as the ratio of the succinate concentration to the sum of succinate and acetate concentration in the retentate, has been also determined:

$$\%\ purity = \frac{C_{r,Suc}}{C_{r,Suc} + C_{r,Ace}} \times 100 \tag{7}$$

3. 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 succinate has been evaluated.

3.1. Mass transfer investigation

3.1.1. 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 condition, 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. 2. 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 [20].

The variation of the acetate retention in acetate/succinate solutions versus the permeate flux for various concentrations of succinate are

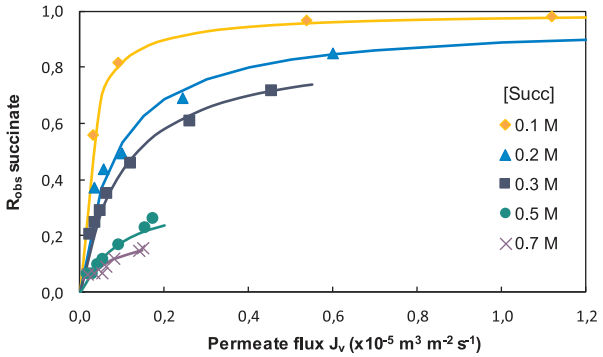

Fig. 2. Retention of succinate vs. permeate flux in single solutions: Influence of succinate concentration – pH 7.

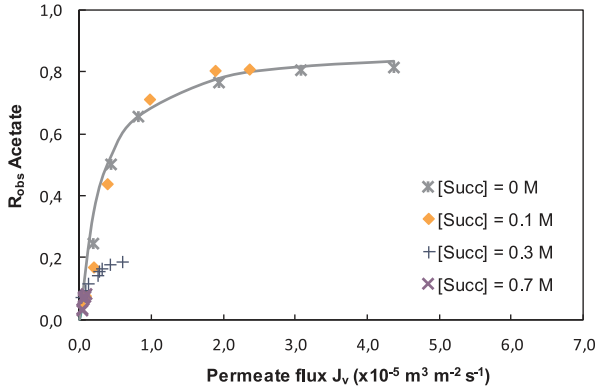

Fig. 3. Retention of acetate vs. permeate flux in binary solutions of acetate and succinate: influence of succinate concentration [Ac-] = 0.1 M – pH 7.

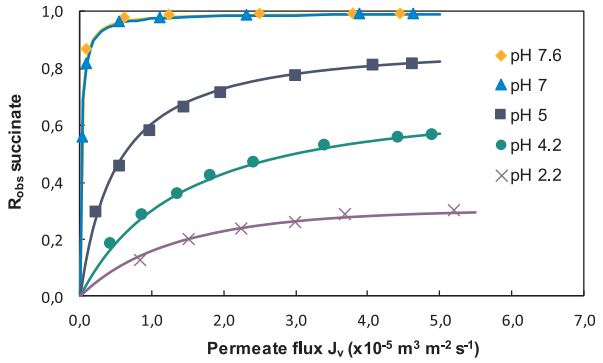

Fig. 4. Retention of succinate vs. permeate flux in single solutions: Influence of the pH - [Succ] = 0.1 M.

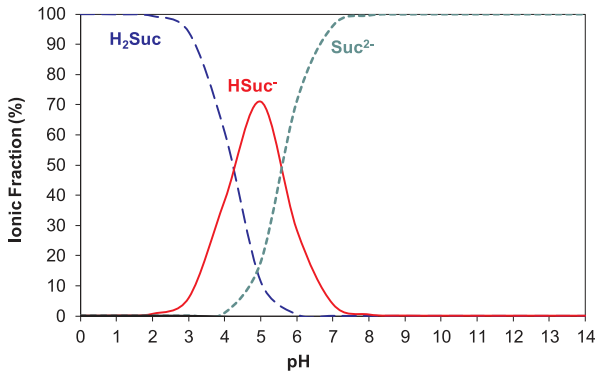

Fig. 5. Distribution of the different forms of succinic acid versus pH.

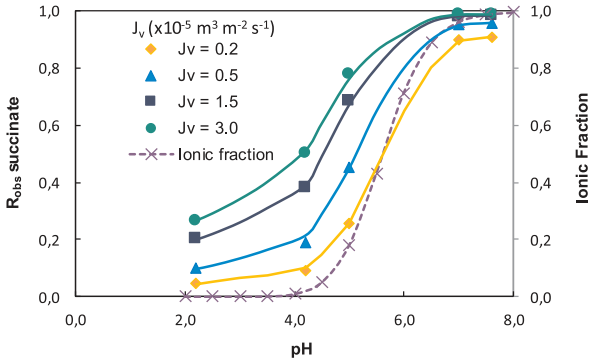

Fig. 6. Variations of succinate retention & ionic fraction of divalent succinate versus pH [Succ] = 0.1 M.

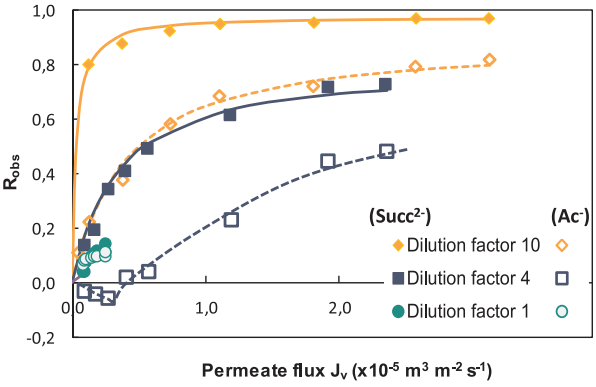

Fig. 7. Retention of succinate and acetate vs. permeate flux: Influence of the dilution factor [Succ2-] = 0.7 M – [Ac-] = 0.1 M – pH 7.

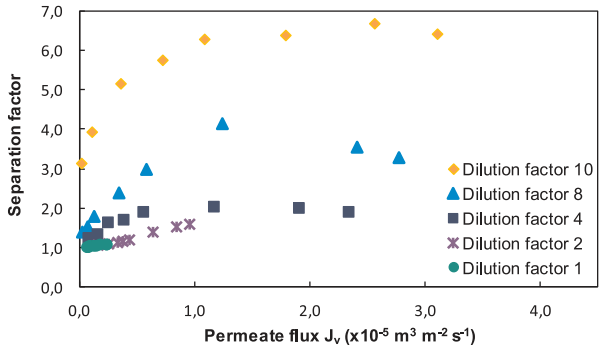

Fig. 8. Separation factor vs. permeate flux: Influence of the dilution factor [Succ] = 0.7 M – [Ac] = 0.1 M – pH 7.

depicted in Fig. 3. One can also observe that the retention of acetate decreases for increasing succinate concentrations. Again, this is due to the screening effect.

These results are in agreement with the ones obtained previously reported in the literature [20,21,23]. 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.

3.1.2. Influence of the pH on the succinate retention

It was previously shown that the transfer of succinate at low salt concentration depends on the electrostatic interactions. 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. Thus, the influence of the pH is investigated at low concentration (0.1 M) where the charge effects are dominant. Fig. 4 shows that the

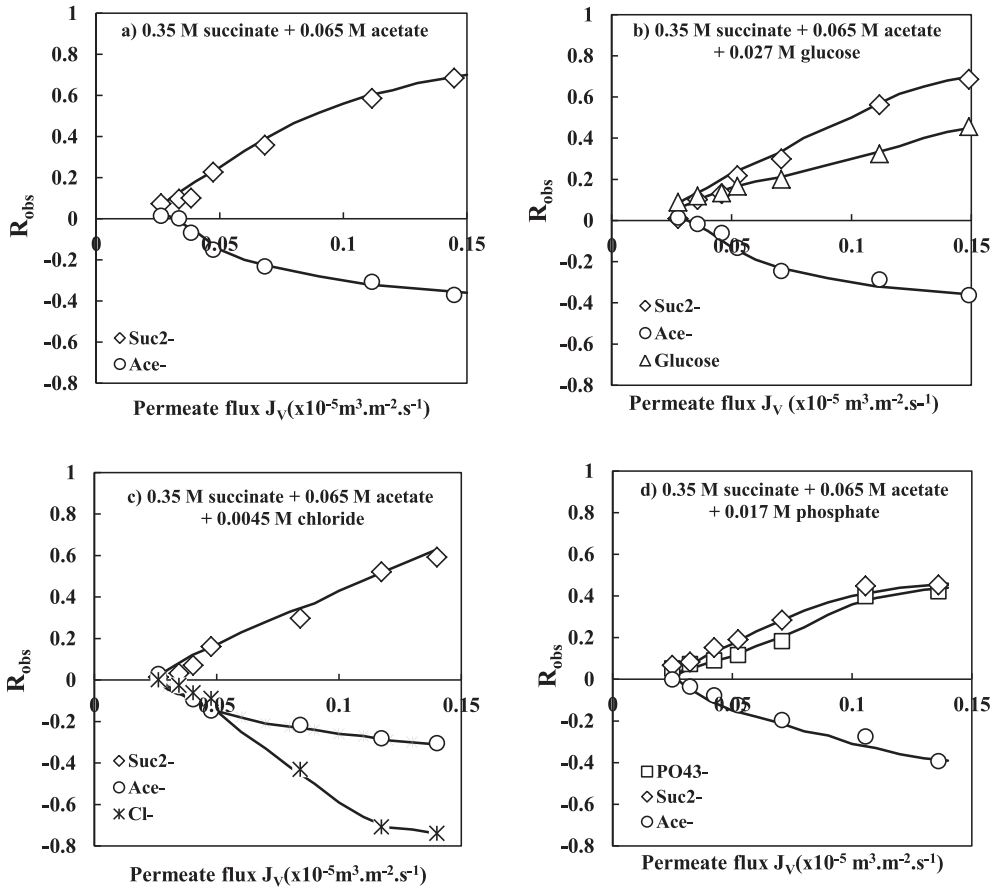

Fig. 9. Retention of succinate and impurities as function of the permeate flux with increasing complexity of feed solutions.

succinate retention is 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 increases from 0.25 to 1 when the pH increases from 2.2 to 7.6.

These results are in agreement with previous work published in the literature [28–30]. It was found that the retention of acetic acid, lactic acid, glutamic acid and fumaric acid increases with increasing pH from 3 to 7 due to more dissociated form of organic acids as well as more negatively charged membrane surface. Additionally, high retention of amino acid such as L-glutamate (Mw = 146 g mol^{-1}, pK_{A1} = 2.17 and pK_{A2} = 9.13) was observed at pH 9 since the amino acid is mainly in a divalent form at pH 9 [31]. Then, at low concentration, increasing pH results in a higher retention because of increasing electrostatic repulsions.

Succinic acid is a dicarboxylic acid, then it can exist in three forms i.e. neutral, monovalent and divalent (pK_{A1} = 4.2 and pK_{A2} = 5.6). The distribution of the different forms versus the pH is plotted on Fig. 5. At pH 2.2, succinic acid is totally neutral. At pH 4.2, succinic acid is shared equally between the neutral and monovalent forms. At pH 5, it is mixed in the three forms, neutral, monovalent and divalent. At pH 5.6, it is a mixing between mono and divalent forms. At pH higher than 7, it is mainly in divalent form. Then, the low retention observed at pH 2.2 corresponds to the retention of the neutral form (size effect). For increasing pH from 2.2 to 5, the retention increases since succinic acid becomes more monovalent form and the retention is fixed by a combination between size and charge effects. At pH above 7, the high retention is obtained according to the high fraction of divalent form.

The variation of the succinate retention versus the pH for various permeate fluxes are reported in Fig. 6. The corresponding ionic fraction of the divalent form is also plotted for comparison. One can observe 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. From this result, one can consider that at low succinate concentrations, where charge effects are involved, the retention increases due to the increasing of divalent succinate ions.

3.1.3. *Succinate/Acetate separation: Influence of the dilution factor*

From the previous results, one can conclude that the separation of succinate and acetate from 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%) (Figs. 2 and 3). However, the separation could be achieved at low concentration and pH 7 since succinate is then completely retained contrarely to acetate (Fig. 4). Then, in order to evaluate the influence of the broth concentration on the transfer of both solutes and the separation efficiency, nanofiltration of a binary-solute solution (0.7 M succinate/0.1 M acetate) has been performed at pH 7 with different dilution factors (1 – 2 – 4 – 8 – 10), to decrease the total concentration.

The variations of succinate and acetate retentions versus permeate flux were in agreement with those previously observed at various concentrations (Fig. 7). 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. However, 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 achieved for diluted solutions. Similar results have been previously reported [31]. It was also found that the retention of L-glutamine increased with increasing dilution factor due to increasing electrostatic repulsion.

Moreover, at a dilution factor of 4, negative values are obtained for the retention of acetate. This means that in these conditions, the acetate

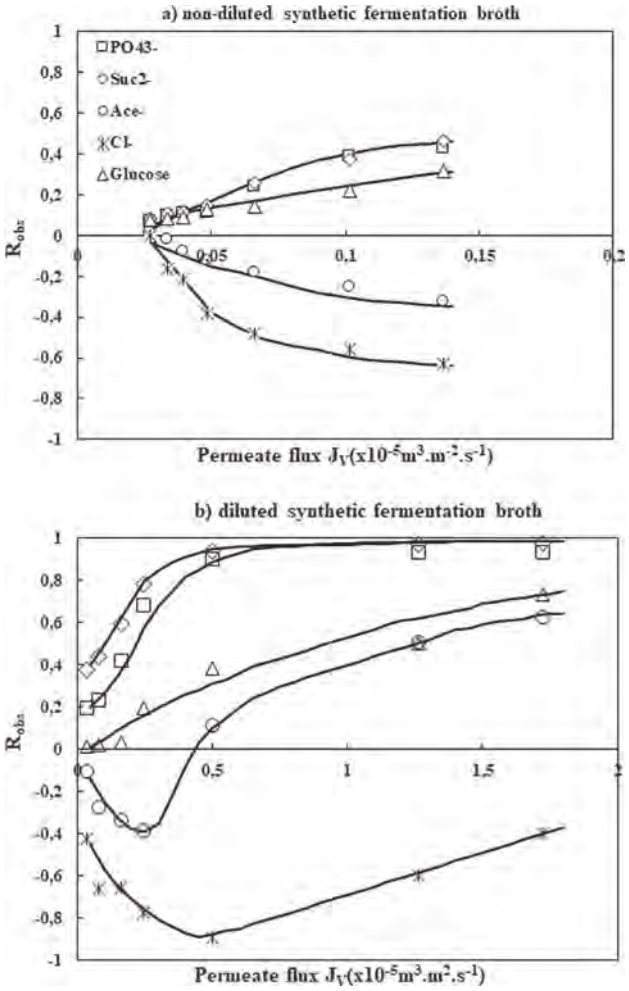

Fig. 10. Observed retention of succinate and impurities as function of the permeate flux: (a) non-diluted synthetic fermentation broth; (b) diluted synthetic fermentation broth (Dilution factor 2).

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 for instance mono- and divalent ions [32,33]. It is due to the competition for permeation between ions of the same sign of charge. Negative retention is reported for the less retained ion, like acetate in the present case, for some experimental conditions. For increasing dilution, because succinate retention increases due to lower concentration, the retention of acetate is expected to decrease in order to maintain electroneutrality in the permeate. 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. 7 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. 8. One can first observe that as expected the separation factor is close 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 $J_v = 1$ 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 total concentration.

3.1.4. *Succinate and acetate transfer in synthetic fermentation broth*

The composition of the fermentation broth, given in Table 1, shows that acetate is the major impurity. The transfer of succinate and acetate were investigated with feed solutions of increasing complexity containing succinate, acetate and other impurities such as glucose, chloride and phosphate.

In presence of glucose, one can observe that both succinate and acetate retentions are similar to that observed without glucose (Fig. 9a and b). Similar results have been reported in previous investigations. It was found that the addition of neutral solutes like glucose has no impact on the transfer of charged solutes [23,34].

Fig. 9c shows the variation of the retention of succinate, acetate and chloride with the permeate flux. The retention of succinate is slightly lower than that observed in binary-solute solution containing succinate and acetate, i.e. without chloride. The acetate retention is less negative in the presence of chloride since acetate is more retained than chloride.

In presence of phosphate, the retention of succinate is lower than that observed in succinate/acetate binary-solute solution. The retention of succinate and phosphate are similar and less than 60% at $J_V = 0.1 \times 10^{-5}\,m^3\,m^{-2}\,s^{-1}$ (Fig. 9d). Also, a negative retention of acetate is observed in the ranges of flux tested.

It was previously pointed out that the separation of succinate and acetate was not possible for a succinate concentration higher than 0.35 M (see Fig. 8). On the contrary, it was shown that the succinate was strongly retained by the membrane at succinate concentrations lower than 0.2 M at pH higher than 7, whereas the acetate retention is low. Then, in order to evaluate the impact of the dilution factor on the separation of succinate and acetate, experiments were carried out with non-diluted and diluted synthetic fermentation broth. Fig. 10 shows that the retention of succinate was less than 60% and the acetate retention was negative for non-diluted synthetic fermentation broth (Fig. 10a). However, for decreasing feed concentrations by a dilution

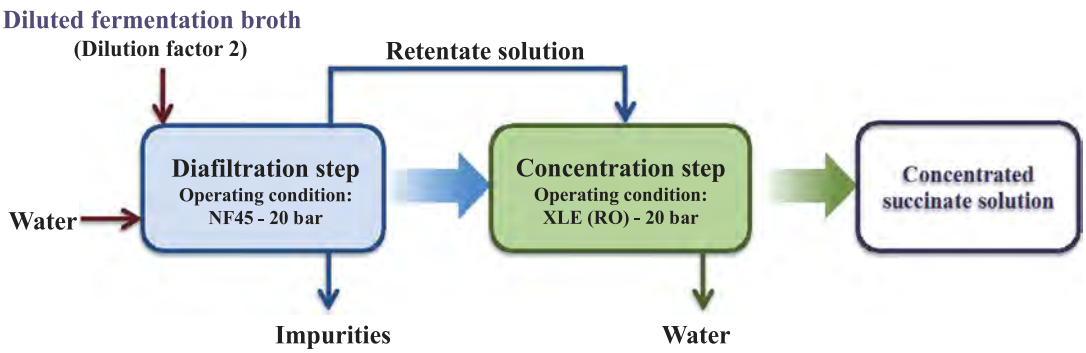

Fig. 11. Two stages filtration recovery process for succinate purification.

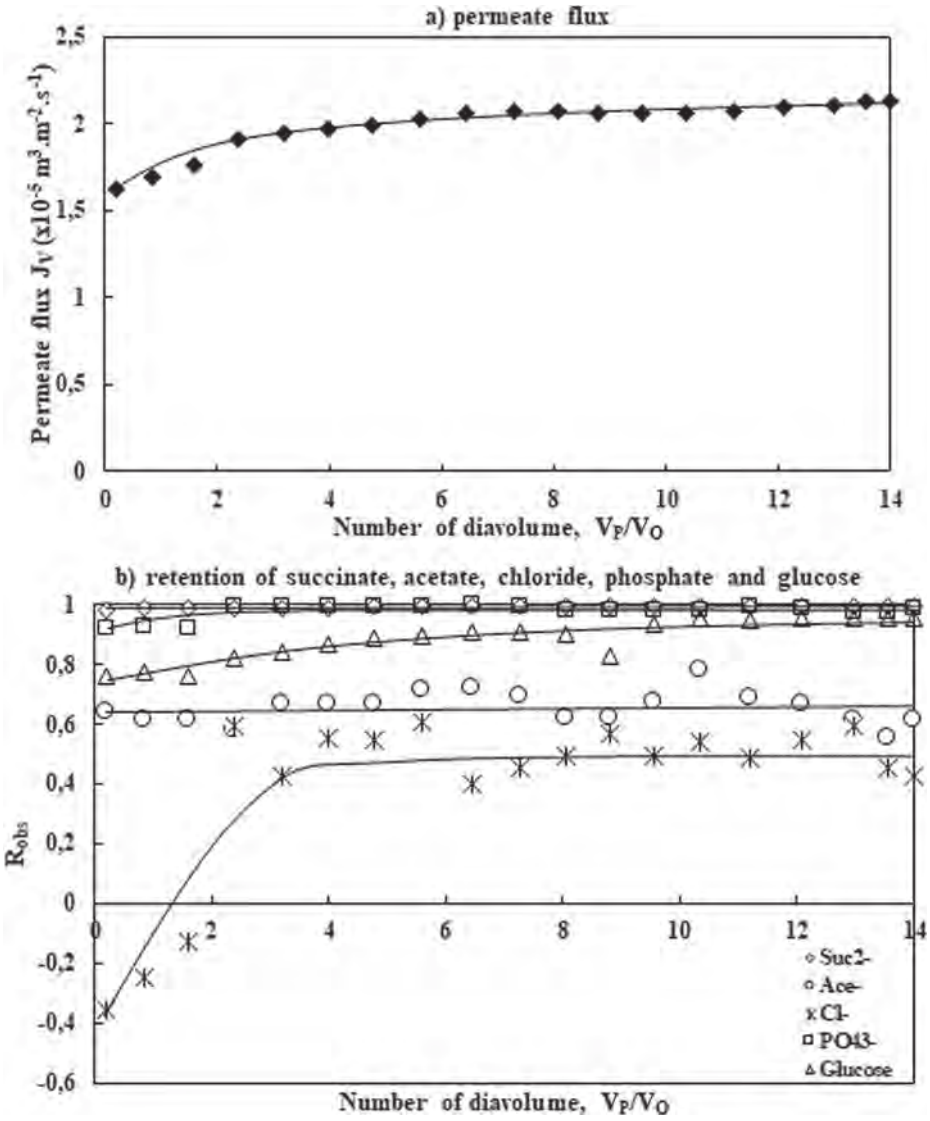

Fig. 12. Permate flux (a) and retention of solutes (b) as function of numbers of diavolumes in a diafiltration of diluted synthetic fermentation broth at pH 7 - ΔP = 20 bar - feed composition: 0.175 M succinate + 0.0325 M acetate + 0.0085 M phosphate + 0.0023 M chloride + 0.0135 M glucose (Dilution factor 2). (a) permeate flux; (b) retention of succinate, acetate, chloride, phosphate and glucose.

factor 2, it was observed that the increase of the succinate retention was higher than that of acetate (Fig. 10b). Therefore, the separation of succinate and acetate could be expected for diluted synthetic fermentation broth.

3.2. Purification of succinate from synthetic fermentation broth

Based on the previous mass transfer investigation, a methodology for the purification of succinate from the fermentation broth was proposed in order to determine the optimum condition to be used regarding the purity and the yield of succinate. The process to recover succinate from fermentation broth involves two steps of filtration, as represented in Fig. 11. A first step of nanofiltration was carried out in diafiltration mode to remove impurities contained in the fermentation broth. Before this NF step, the fermentation broth was diluted by water in order to obtain high succinate yield and purification during diafiltration. Indeed, it was previously demonstrated that the separation efficiency increases with dilution. Finally, the purified succinate solution (retentate of the first step) was concentrated using RO membrane to recover the initial succinate concentration.

For each step, the filtration performances are given in terms of flux, retention of succinate and impurities (glucose, chloride ions, phosphate ions and acetate) and separation efficiency such as separation factor, yield and purity of succinate.

3.2.1. Stage 1: Purification of succinate – NF in diafiltration mode

In this step, the diafiltration of the succinate fermentation broth diluted by a factor 2 has been carried out at 20 bar during 26 h. The results are firstly presented in terms of the variation of the permeate flux and retention of solutes versus the number of diavolumes (Fig. 12). A diavolume is defined as the total ultra-pure water volume (V_p) added during diafiltration divided by the initial retentate feed volume (V_0). The permeate flux first increased during the first eight diavolumes because of the decreasing concentration in the retentate and becomes almost constant for increasing diavolumes over eight (Fig. 12a). Furthermore, the variation of solute retention versus the number of diavolumes is reported in Fig. 12b. As expected, the succinate retention is about 99% and the retention of acetate is less than 70% all along the experiment. It is also observed that the retention of phosphate and glucose ranges between 92% to 99% and 75% to 95% respectively when the number of diavolumes increases from 0.2 to 14.

A negative retention of chloride, that is the less retained anion in the

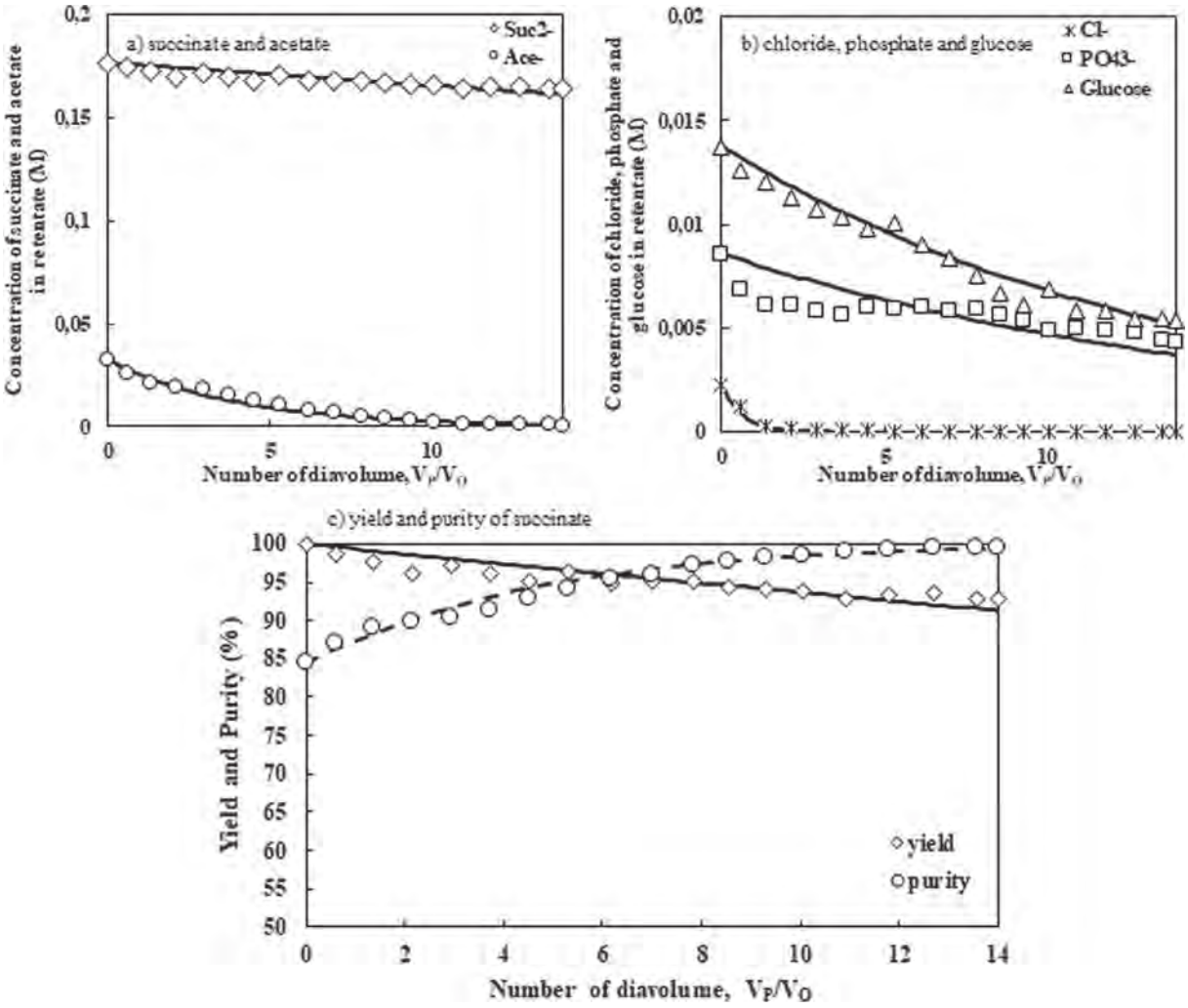

Fig. 13. Solutes concentration in the retentate, yield and purity of succinate as function of numbers of diavolumes in a diafiltration of diluted synthetic fermentation broth at pH 7 - ΔP = 20 bar - feed composition: 0.175 M succinate + 0.0325 M acetate + 0.0085 M phosphate + 0.0023 M chloride + 0.0135 M glucose (Dilution factor 2). (a) Succinate and acetate; (b) chloride, phosphate and glucose. The lines are calculated values.

feed, is obtained at the beginning of the diafiltration. Then, chloride retention increases to reach positive values. As previously explained this is due to the modification of the anion composition of the retentate, i.e. decreasing proportion of chloride.

The variation of the solute concentrations in the retentate versus the number of diavolumes is illustrated in Fig. 13. The calculated values from the mass balance equation (see Eq. (3)) are also plotted for comparison. It is observed that the concentration of succinate ($R_{Suc} \approx 99\%$) slightly decreases during the diafiltration operation while the acetate ($R_{Ace} \approx 65\%$) concentration decreases more (Fig. 13a).

Moreover, the concentration of phosphate and glucose continuously decrease with increasing the number of diavolumes. The concentration of chloride is close to zero after 2 diavolumes (Fig. 13b).

According to Fig. 13a, the decrease of acetate concentration is much higher than that of succinate. Consequently, the purity of succinate increases, from 85% (initial value in the feed) to 99.5%while the succinate yield remains higher than 93% after 14 diavolumes (Fig. 13c).

3.2.2. Stage 2: Concentration of the purified succinate - reverse osmosis

Finally, the diafiltrated fermentation broth containing the purified succinate (diavolume = 14, $[Succ^{2-}] = 0.16$ M) was concentrated using the XLE reverse osmosis membrane at 20 bar. This operation was carried out to recover the initial succinate concentration in the fermentation broth, i.e. 0.34 M (concentration factor ≈ 2). First, the results are presented in terms of the variation of solute retention and permeate flux versus the volume reduction factor (Fig. 14a). A volume reduction factor is defined as the initial feed volume (V_0) divided by the retentate volume (V_R). One can observe that the retentions of succinate and phosphate slightly decreases, from 99.7% to 99.2% with increasing the volume reduction factor. The retention of glucose is closed to 100% for the whole range of the volume reduction factor. Moreover, the retention of acetate decreases from 95% to 90% when the volume reduction factor increases from 1 to 2.1. As previously mentioned, the increasing salt concentration in the retentate results in a decrease of the charged solute retention. One can also observe that the permeate flux decreases during operation in concentration mode due to the increasing osmotic pressure.

As expected, the concentration of solute increases with increasing the volume reduction factor since retention of the solute is close to 100%. The variation of solute concentration in retentate versus the volume reduction factor is plotted in Fig. 14b and 14c. One can observe that the concentration of succinate ($R_{Suc} \approx 99\%$) increases from 0.16 M to 0.34 M when the volume reduction factor increases from 1 to 2.1, while the concentration of acetate ($R_{ace} \approx 93\%$) increases less (concentration factor ≈ 2). The concentration of phosphate ($R_{PO43-} \approx 99\%$) and glucose ($R_{glucose} \approx 99\%$) increase during the concentration step. Also, these results are in agreement with the calculated values (see Eq. (5)).

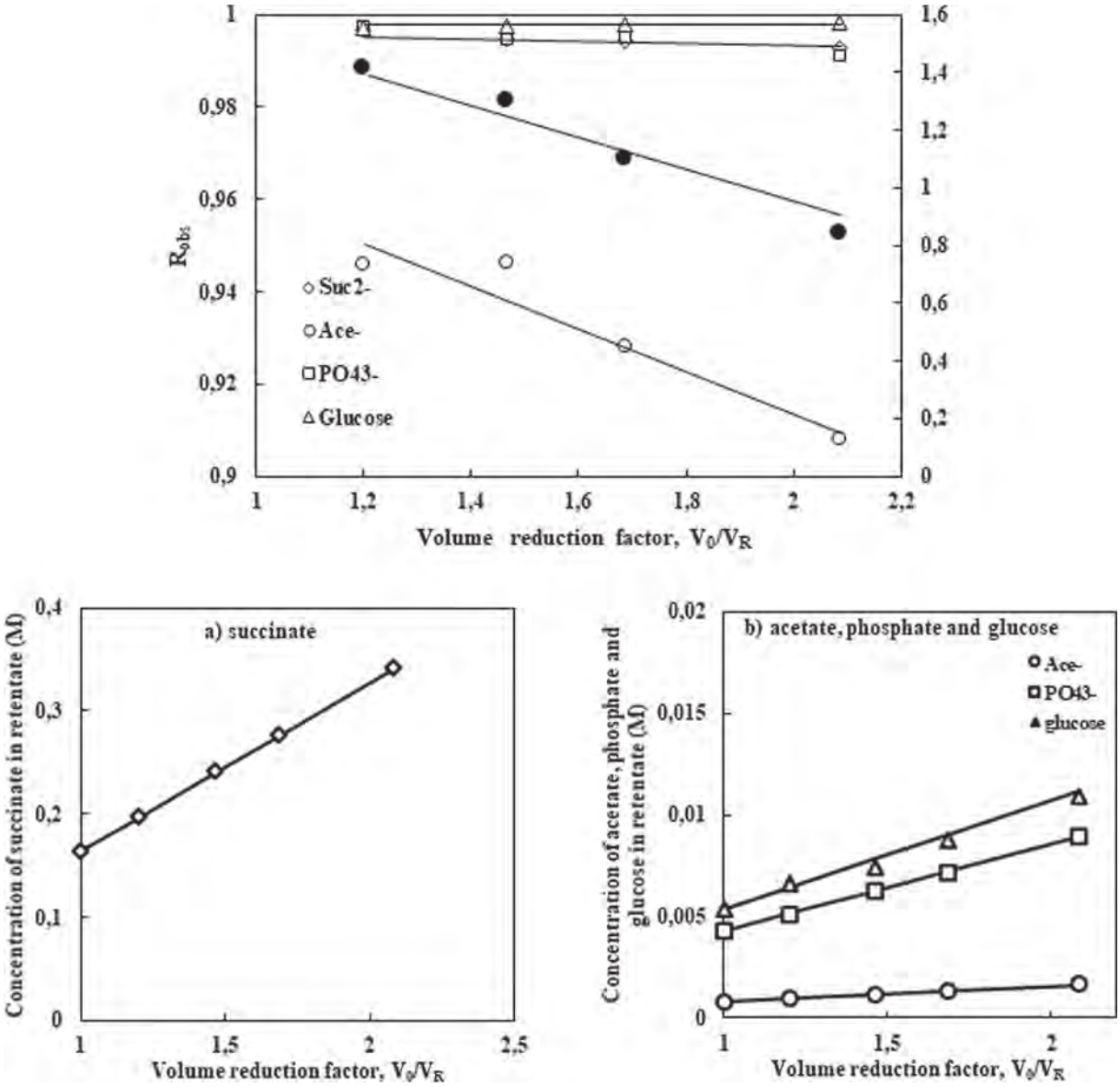

Fig. 14. Permeate flux, retention and concentration of solutes in retentate as function of the volume reduction factor in a concentration mode - at pH 7- $\Delta P = 20$ bar. (a) permeate flux and retention of solutes (b) concentration of succinate; (c) concentration of acetate, phosphate and glucose. The lines are the calculated values.

Table 2
The composition of initial fermentation broth, before/after diafiltration and after concentration step.

Feed composition	Initial fermentation broth	Before diafiltration (after dilution by 2)	After diafiltration (in the retentate)	After concentration (in the retentate)
Succinate	0.35 M	0.175 M	0.16 M	0.34 M
Acetate	0.065 M	0.0325 M	0.001 M	0.002 M
Chloride	0.0045 M	0.0023 M	–	–
Phosphate	0.017 M	0.0085 M	0.004 M	0.009 M
Glucose	0.027 M	0.0135 M	0.005 M	0.01 M

The composition of initial fermentation broth, purified fermentation broth before (retentate of diafiltration) and after (RO retentate) RO concentration are shown in Table 2. As expected, the concentration of solutes decreased during diafiltration. One can observe that chloride ions are completely removed from fermentation broth. After the concentration step, the solute concentrations increase by about 2 times their initial values for the volume reduction factor equal to 2.1 since the solute retentions ($\approx$ 99%). Then, it was possible to recover the initial succinate concentration by using concentration step.

The succinate purity and yield obtained with this operation are 99.5% and 99.3%, respectively.

Finally, using the two step process proposed in this work, i.e. dilution/diafiltration (NF)/concentration (RO) 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%.

4. 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 ion 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, ie the removal of acetate as well as other impurities. Finally, a concentration step by RO was used to recover the initial succinate concentration. With this two stage process, the succinate purity was increased from 85% to 99.5% with a total yield higher than 92%.

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