Indian Journal of Science and Technology, *Vol 9(21), DOI: 10.17485/ijst/2016/v9i21/95244, June 2016*

ISSN (Print) : 0974-6846
ISSN (Online) : 0974-5645

Purification of High Value Succinic Acid from Biomass Fermentation Broth Via Nanofiltration

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Abstract

Background/Objectives: Succinic acid purification from biomass fermentation experiences difficulty due to similar properties of acids presence. Thus purification work is conducted using nanofiltration (NF) membranes, and further characterizing its filtration performances. **Methods/Statistical Analysis**: The membranes used were NF1 and NF2 from AmforInc and NF270 from Filmtec. The investigated filtration performance parameters were different acids concentration and operating pressure of 5, 10, 20 and 50 g/L and 2, 5, 10 and 15 bar, respectively. **Findings:** From the results, NF270 showed the most efficient in purifying the succinic acid at 20 g/L and 50 g/L, which were 76% and 64.4% purification, respectively from 33% concentration in the feed solution. Meanwhile for different pressure, NF2 shows the highest purification percentages which were 82% and 81% at the respective 5 and 10 bar followed by NF270 membrane at 68% and 67% at the respective 5 and 10 bar. The characterization of the membranes using DSC, FTIR, contact angle and SEM method shows that there was no fouling on the membranes observed after the filtration when there were no differences shown on the analysis results. This indicates that the range of pressure and concentration used in this study are suitable to perform the purification. Contact angle has found to influence the percentage of purification of each membrane where the less hydrophilic membrane shows the highest purification percentage. **Application/Improvements:** Thus NF270 and NF2 membranes were considered as the best candidate for purifying the succinic acid from fermentation broth.

Keywords: Fermentation, Nanofiltration, Organic Acids, Polymer Membranes

1. Introduction

Over recent years, there has been an explosive growth of succinic acid for its utilisation in food (flavouring agent in drinks[1], chemical industry such as deodorant agent and anti-carcinogenic in a form of calcium succinate and as precursor for variety of chemicals such as Tetra Hydro Furan (THF)[2]. Meanwhile in pharmaceutical industry[3], succinic acid is also used as raw material in the production of vitamin A, anti-inflammatory drug and antidote for toxic[4].

Succinic acid can be produced from different routes either via chemical/biochemical oxidation of fat, fermentation process in the presence of succinic acid producing bacteria and catalyst oxidation. Traditionally, succinic acid is produced from chemical route via liquefied petroleum gas (LPG) process. In this process, n-butane acts as a raw material is converted into maleic anhydride by catalytic oxidation process based on the following chemical equation[5]:

$$C_4H_{10} + 3.5\ O_2C_4H_2O_3 + 4H_2O$$

Due to the high product value of succinic acid, the market demand of succinic acid has increased yearly, which resulted in the depletion of its raw material, n-butane. Additionally, the price of crude oil is rapidly increasing, which affecting the price of the succinic acid produced chemically. Moreover, as the chemically derived succinic acid is produced from non-renewable sources, the process is less environmental friendly due to its preparation conditions such as utilisation of expensive catalyst, and

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application of high operation temperature and pressure, therefore the process is found to be not sustainable for long term operation[6,7].

Therefore the demand for clean technology for biological production from fermentation process has gained significant interest as an alternative succinic acid production route using renewable raw material from abundant biomass such as xylose, molasses and glucose[8] which commonly found in oil palm biomass. The recovery from fermentation broth has gaining significant interest in the chemical industry worldwide. High production of succinic acid in between 40 – 100 g/L using different producing bacteria have been reported[8-10].

In the production of succinic acid via fermentation process, glucose is converted into succinic acid based on Tricarboxylic acid cycle (TCA) presented in Figure 1. High yield of succinic acid has been intensively reported via fermentation process using variety of microorganisms namely *Anaerobiospirillumsucciniciproducens, Mannheimiasucciniciproducens,* and *A. succinogenes*[11]. Among all microorganisms, *A. succinogenes*is one of the most studied microorganisms. It shows high level of tolerance to high concentration of raw material, high endurance to inhibitor product and this microorganism is able to produce succinic acid from a wide range of raw materials such as glucose, fructose, xylose, maltose, and cellobiotose[12]. The typical production of succinic acid from *A. succinogenes* is in between 30 - 60 g/L for 48 hours fermentation period. However, in the bio-based route, along with the production of succinic acid, by-products

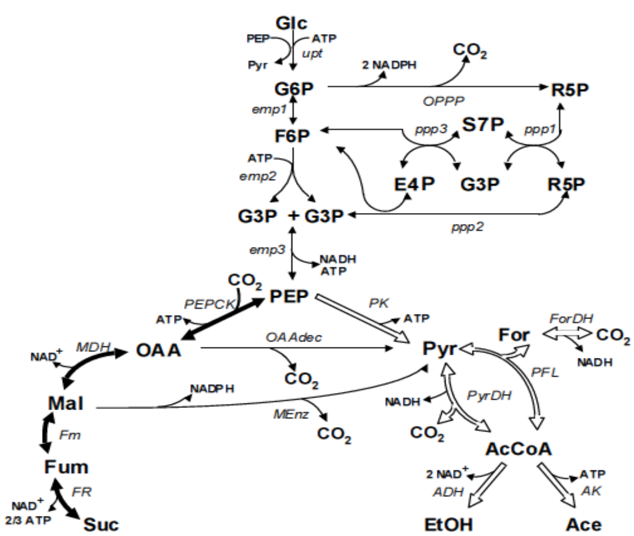


Figure 1. Summary production of succinic acid by typical microorganism via fermentation process (27)

of similar physicochemical properties such as acetic acid and formic acid were also generated[13]. This consequently imposes difficulty in recovery of the desired product.

To date, many processes have been developed to separate and purify succinic acid from the fermentation broth including reactive extraction[14], ion exchange resin[15], electro-dialysis, precipitation and pressure filtration[16,17]. Among all, filtration technology[18] especially Nano Filtration (NF) has drawn much attention in the field of separation and purification compared to other technologies as it offers flexibility in manipulating the product yield and purity by either adjusting the process parameters or/and by changing the membrane properties. NF was also reported to have high selectivity towards divalent compared to monovalent anions, which gives advantage in succinate separation of divalent anion from multivalent anions solution[8]. Presently, many have reported the separation and purification of succinate from fermentation broth using variety of commercial membranes namely NF45, ESNA1[8] and NF270[19]. Study by Kang and Chang (2005) shows that NF45 shows better performance compared to ESNA1 in succinate recovery. The former membrane shows large rejection difference between monovalent anion, Cl^- and the divalent anion, SO_4^{2-} of 58.9% compared to the later membrane of 23.5 %. For the succinate rejection, 85.6 % rejection was successfully achieved with filtration using NF45 membrane from quaternary organic acids salts using diafiltration at 150 h of operation at 200 psig and 25^{o}C. In the study .[18] high rejection of succinic acid was obtained by manipulating more on the process parameters rather than membrane.[19] investigated the effect of pH, pressure and concentration of salt solutions. They found out that the rejection of succinic acid was over 90% irrespective of the operating pressure and salt solution concentration due to the close molecular weight (MW) of succinic acid to the molecular weight cut off (MWCO) of NF270 membrane used. However, it was found that pH has high influenced on the succinic acid rejection. The rejection increases with increasing pH, depending on the degree of organic acid dissociation[19].

In a pressure driven membrane processes, fouling is considered as one of the biggest obstacles in the application of separation and purification as it limits and affect the performance of membrane. Biofouling is critical in the separation and purification of fermentation broth due to its complex mixture that contains significant amount of dissociated and non-dissociated forms of metabolites, dissolved organics, bacteria and residual mineral salts[20].

The deposited layer of foulant formed on the membrane surface could increase or decrease the solute rejection depending on the solute, membrane and type of foulant[21]. It has been reported that fouling increases the concentration polarization consequently facilitated the rejection of small inert organic compound. Therefore, NF membrane is seen as one of the emerging environmentally friendly technologies that have the ability of selectively recovering succinic acid by manipulating both the type of membrane used and the process parameters for maximum separation and purification. Many have reported the lower rejection of monovalent anions such as format, acetate and lactate, while higher rejection of divalent anion such as succinate for NF, giving an advantage of selective recovery of divalent anions such as succinate from a mixed salts solutions containing multivalent ions.

The motivation of this work is to investigate the performance of three different NF membranes, NF1, NF2 and NF270. The recovery of succinate from synthetic mixed salt solutions containing three different organic acids in salts form (acetic, formic and succinic) were investigated in relation to the NF operation parameters (mixed salt solution concentration and pressure) and the properties of the membrane used. Membrane fouling was also investigated using SEM, FTIR, DSC and contact angle.

2. Experimental

2.1 Materials

Three different commercial membranes were used in this study namely NF1, NF2 and NF270, as shown in Table 1. The manufacturers and properties of membranes are presented in Table 1. The synthetic solution used in this filtration study is based on the composition of organic acids obtained from fermentation using *A. sucdniciproducens*[8]. The synthetic solution contains succinic acid with 99 % purity and acetic acid with 99.89 % purity purchased from MERCK and formic acid with 85 % purity purchased from PC LAB. The synthetic solution is prepared at different concentration of 5, 10, 20 and 50 g/L and mixed at equal volume. All mixture solutions were neutralize with sodium hydroxide (NaOH, R&M Chemicals) until pH 7 resembling the fermentation broth pH ranging from 6.8 – 7.

2.2 Salt Rejection

Rejection of mixed salt solutions were carried out using dead-end filtration cell (HP4750 Stirred Cell) manufacture by STERLITECH with a filtration area of 0.00146 m^2. The schematic diagram is presented in Figure 2. Rejections were carried out at pressure ranging from 2 – 15 bar using nitrogen gas with gas line attached at the top of the cell. The permeate is collected at the permeate site. Prior to filtration, all membranes were soaked in ultra pure water (UP) overnight and compacted for 30 minutes at 20 bar. After every run, membranes used were rinsed with UP water until the initial water flux was recovered. The flux and rejection were measured at various mixed-salt solution concentrations between 5–50 g/L at 15 bar. All mixed-salt solution concentrations were carried out at 1:1:1 ratio of succinic: formic: acetic for simplification (33.33 : 33.33 : 33.33 ratio in %). The rejection and flux were calculated based on the following equations:

$$R = \left(1 - \frac{Cp}{Cf}\right) \times 100\% \quad (1)$$

where R is rejection (%), C_p is the concentration at permeate (g/L) and C_f is concentration at the feed (g/L).

$$J = \frac{\Delta V}{A.t} \quad (2)$$

where J is the flux (L/m^2.h), V is the volume of permeate (L), A is the effective area of membrane (m^2) and t is time (h).

The organic acid salts were measured using a high performance liquid chromatography (G1313A series, Agilent) with a separating column (REZEX ROA ORGANIC ACID H+ (8 %), 300 x 7.8 mm, PHENOMENEX). The analysis was run with a refractive index (RI) detector at column temperature of 60 °C.

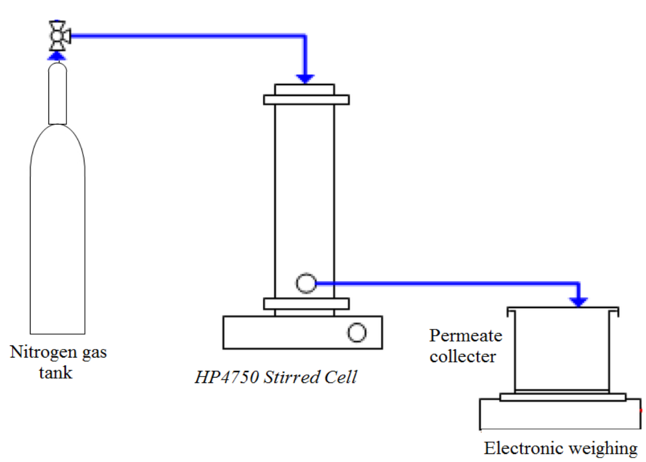


Figure 2. Schematic diagram of the dead-end filtration system

0.005 N H_2SO_4 was used as the mobile phase at 0.6 ml/min of flow rate[8].

2.3 Membrane Characterization

All membranes were characterized before and after filtration to determine fouling effect on the membrane using SEM, FTIR, DSC and contact angle. For the surface morphology of the membranes, membranes were analysed using Scanning Electron Microscope (Carl Zess EVO MA10, UK). All samples were sputter coated with gold using putter coater (QUORUM Q150RS, UK).

The chemical composition of membrane before and after filtration were determined using Fourier transform infrared (Nicolet 6700 Thermo Scientific FTIR). This test is conduct in the transmittance mode in a wave number range from 4000 – 450 cm^{-1} with resolution of 4 cm^{-1}.

The effect of fouling on the thermal properties of membranes was also investigated using Differential Scanning Calorimetry (DSC) analysis (DSC 822e Mettler Toledo). Samples were cut into small pieces of between 5 – 10 mg, placed in aluminium pans and sealed using lid with sealing press (Mettler Toledo). The samples were heated over a temperature ranging between 30 – 350 °C at a heating rate of 10°C/min.

Contact angle was used to investigate the effect of filtration process on the membrane hydrophilicity. The contact angle of membranes was measured using Kruss GmbH FM12MKE Easy drop Contact Angle aided by video image Digitization technique. A drop of pure water was injected slowly by a needle to the membrane surface under a constant pressure and monitored by contact angle system.

3. Results and Discussion

3.1 Membrane Flux

Figure 3 shows the fluxes of pure water and mixed salt solution at 50 g/L as a function of pressure. Overall it was observed that the fluxes of all membranes increase as the pressure increased with NF1 being affected most. Significant increase in flux was observed when the pressure was increased from 10 – 15 bar, with NF1 shows the highest increase in flux followed by NF270 and NF2. For pure water flux, NF1 and NF270 display close values at increasing pressure. Similar trend of plots as pure water flux was also observed for the permeate flux, as expected based on the Molecular Weight Cut-Off (MWCO) values presented in Table 1. Figure 3 also shows that there are

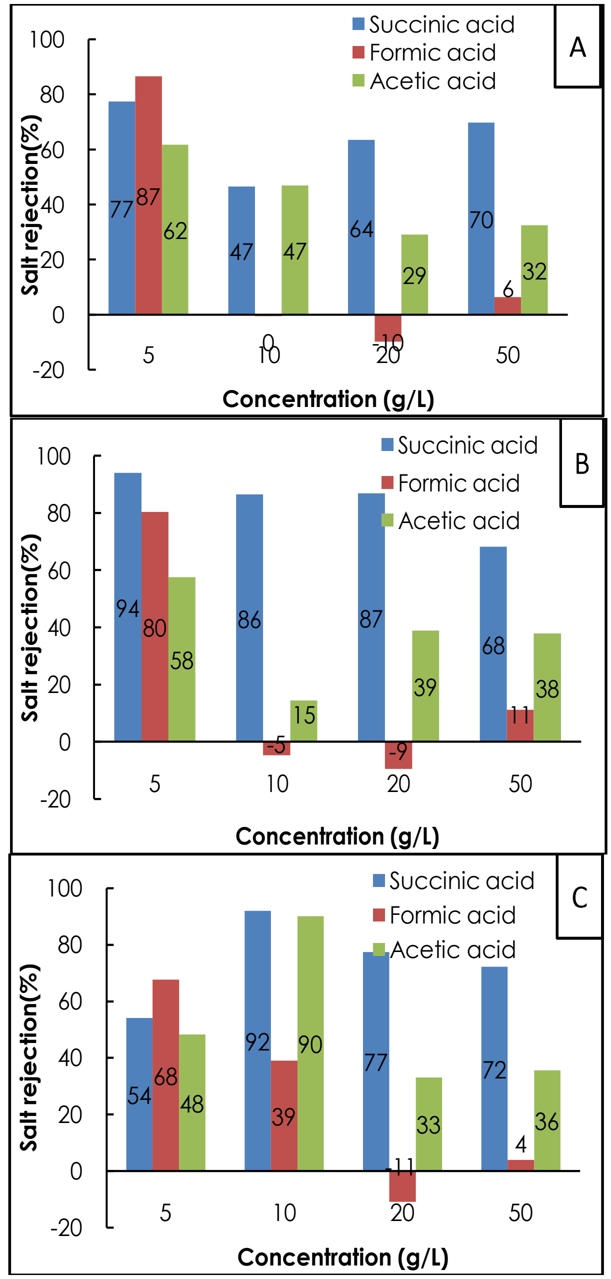


Figure 3. Rejection (%) of mixed salt solution containing succinic acid, formic acid and acetic acid at different concentration using three different membranes A) NF1, B) NF2 and C) NF270. (P = 15 bar, pH = 7, T = 25 oC).

Table 1. Properties and manufactures of the tested membranes

Type of membrane	NF1	NF2	NF270
Manufacture	AmforInc	AmforInc	Filmtec
Material	Polyether sulfone	Polyamide	Polyamide
MWCO (g/mol)	200-400	200	200-300

concentration polarization and osmotic pressure occurred during the rejection process based on the much smaller permeate flux compared to the pure water flux[22].

3.2 Salt Rejection

3.2.1 Effect of Mixed Salt Solution Concentration on Succinic Acid Retention and Flux

The rejection of organic salt solution at different concentration were carried out using three different membranes, NF1, NF2 and NF270. Figure 4 shows the rejection of mixed salt solution containing succinic, formic and acetic acid at different concentration ranging from 5 – 50 g/L. Overall, Figure 4 shows that selective rejection of succinate and removal of formic and aceticacid increases with increasing mixed salt solution concentration and was observed to be significant at higher concentration. The rejection difference between succinic acid (divalent anion) and formic and acetic acid (monovalent anion) was found to be the highest at 10 g/L of 89 % for NF2 and at 20 g/L of 76 % and 77 % for NF1 and NF270 respectively. The purity of succinic acid at different mixed salt solution concentration is presented in Table 2. Meanwhile for formic acid and acetic acid, the rejection was observed to decrease significantly from 87, 80, and 68 % to 6, 11 and 4 % and from 62, 58 and 48 % to 32, 38 and 36 % for NF1, NF2 and NF270 respectively when the concentration of mixed salt solution was increased from 5 to 50 g/L. In a mixed salt solution the increasing rejection of divalent anion and decreasing rejection of monovalent anion at increasing mixed salt solution concentration can be explained by the pumping effect of monovalent ions by the divalent ion in the solution. The pumping effect can be explained by Donnan exclusion and electron neutrality with the counter ion, Na^+. In a solution containing mono- and di-valent ions, the divalent ions will tend to go further away from the membrane surface due to strong repulsion, simultaneously pushing the monovalent ions to the surface to meet electro neutrality in the membrane phase. Thus more monovalent ions exist in the membrane phase and permeate preferentially at higher fraction compared to the divalent ions resulting in the observed lower rejection of acetic and formic acid compared to succinic acid[8]. Meanwhile for more significant decrease of formic acid compared to acetic acid at increasing mixture solution concentration observed, this is due to the smaller degree of dissociation of formic acid (pK_a 3.8) compared to acetic acid (pK_a 4.7) regardless of the similar molec-

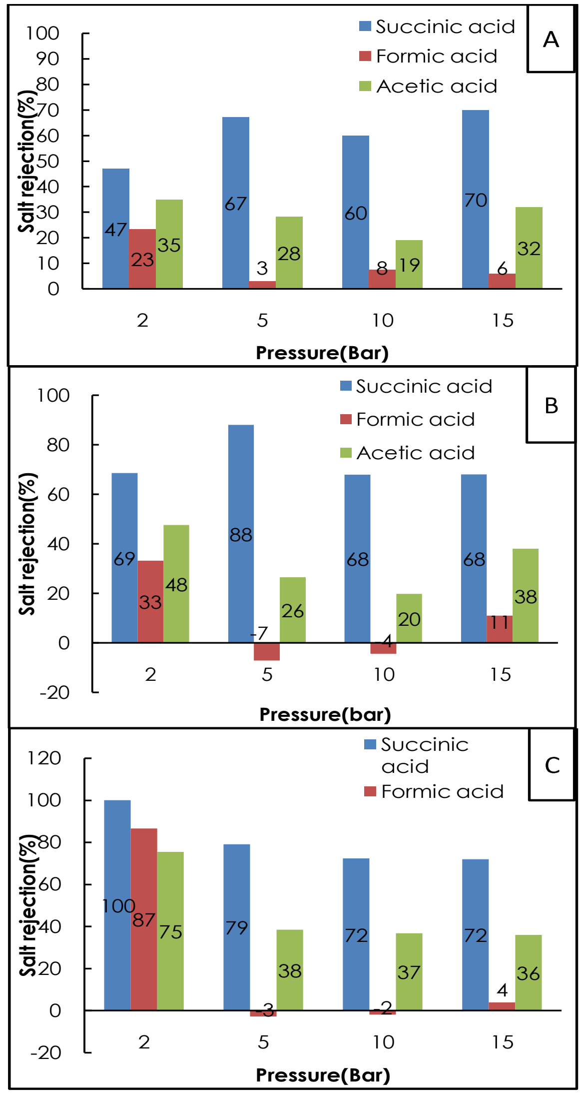


Figure 4. Rejection (%) of mixed salt solution containing succinic acid, formic acid and acetic acid at different pressure using three different membranes A) NF1, B) NF2 and C) NF270. (Concentration = 50 g/L, T = 25 oC).

Table 2. Purity percentage of succinic acid for NF1, NF2 and NF270 at different concentration of mixed salt solutions (P = 15 bar, pH = 7, T = 25 oC)

Type of membrane	NF1				NF2				NF270			
Concentration (g/L)	5	10	20	50	5	10	20	50	5	10	20	50
Purity (%)	34	50	77	64	41	89	75	58	32	42	78	64

ular weight[19,23], thus formic acid was more completely dissociated than acetic acid at tested pH.

Figure 4 shows the rejection (%) of mixed salt solution containing succinic acid, formic acid and acetic acid at different concentration using three different membranes A) NF1, B) NF2 and C) NF270. (P = 15 bar, pH = 7, T = 25 °C)

3.2.2 Effect of Pressure on Succinic Acid Rejection

NF is a pressure-driven process; the operating pressure is one of the main factors contributing to the retention performance besides the feed concentrations. Figure 5 shows the rejection of succinic, formic and acetic acid at different applied pressure between 2 to 15 bar for feed concentration of 50 g/L using three different membranes; NF1, NF2 and NF270. Overall the retention of organic salts decrease at increasing pressure with acetic and formic acid being affected most. A significant decrease in retention was observed for acetic and formic acid when pressure was increased from 2 to 5 bar but above 5 bar, the applied pressure seems to have little influence on the rejection performance. However for succinic acid, applied pressure shows small impact on the retention performance due to the close succinic acid MW to the MWCO of the membranes[19]. Below pK_a, the neutral form of organic acids seems to limit the amount of electrostatic repulsion between membrane and organic solute, thus sieving effect due to the molecular size of the compound become the important mechanism in the selective separation[23]. It was also observed that the maximum purity of succinic acid was obtained at pressure of 10 bar for all membranes as presented in Table 3.

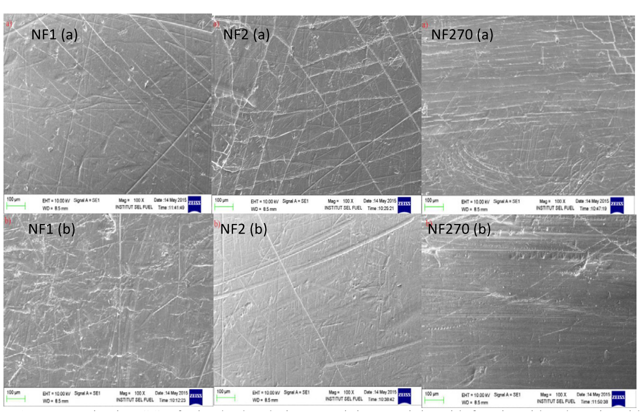


Figure 5. SEM surface images of NF1, NF2 and NF270 (**a**) before and (**b**) after filtration process

3.3 Membrane Characterizations

3.3.1 SEM Analysis

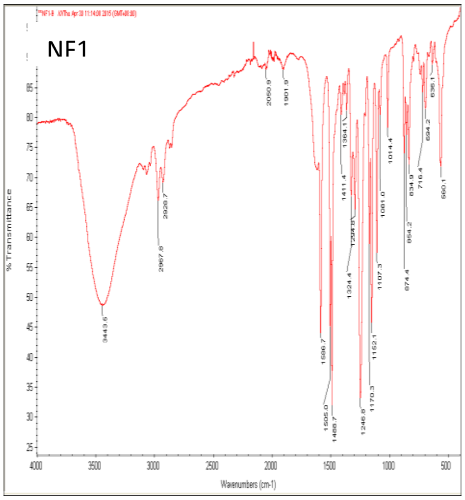
SEM analysis was conduct to study the effect of filtration on the morphology of the membrane. Figure 6 shows the SEM images of membranes before and after filtration process. From the images, it was observed that no fouling has occurred on the membranes surface during the filtration process. This maybe due to the synthetic solution used only contained known organic acids and/or due to the short filtration time that avoid the accumulation of foulant on the membrane surface. FTIR and contact angle were also carried out to further confirm the results.

3.3.2 FTIR Analysis

Figure 7–9 show the FTIR spectrum of all membranes, NF1, NF2 and NF270 before and after the filtration process. Based on the FTIR spectrum of the membranes, it was observed that the intensity of peaks representing water is increased at around 3500 cm^{-1}indicating the water component has increased after filtration. Meanwhile the main peak that represent carboxylic acid (C=O) commonly observed at 1730 cm^{-1} [24] was not observed even after the filtration process. The absence of this peak may indicate that there was no or very minimum fouling occurred thus preserving the chemical composition of the membranes. The fouling effect is too small to effect the filtration process as confirmed by the flux measurement (Figure 3) and therefore the effect can be possibly neglected. Similar observation was also reported.[24]. In their study, new peaks were observed in the FTIR spectrum for membrane after filtration compared to before filtration process indicating the deposition of foulant on the membrane surface[25].

Table 3. Purity percentage of succinic acid for NF1, NF2 and NF270 at different pressure (Concentration =50 g/L, T = 25 oC)

Type of membrane	NF1				NF2				NF270			
Pressure (bar)	2	5	10	15	2	5	10	15	2	5	10	15
Purity (%)	44	68	50	58	45	82	81	62	38	69	67	53



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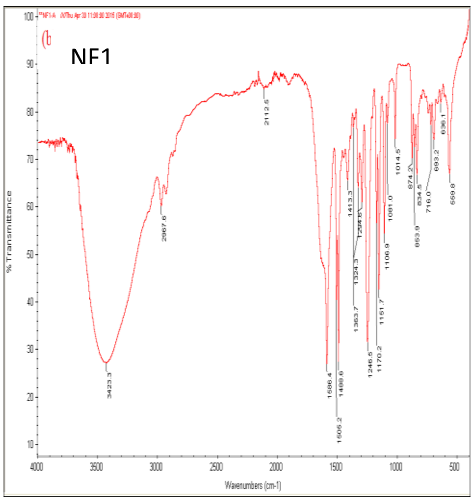
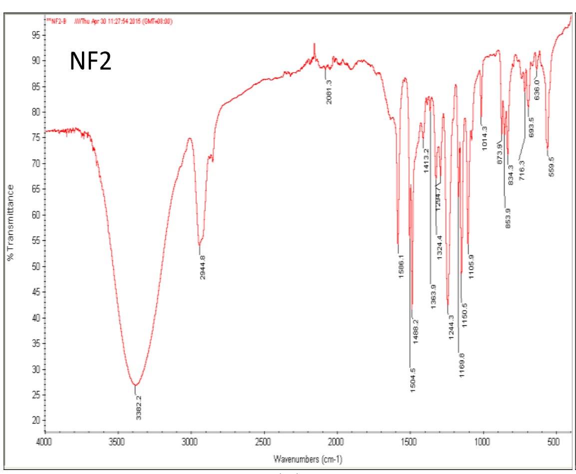


(**b**)

Figure 6. FTIR analysis of NF1 (a) before (b) after filtration



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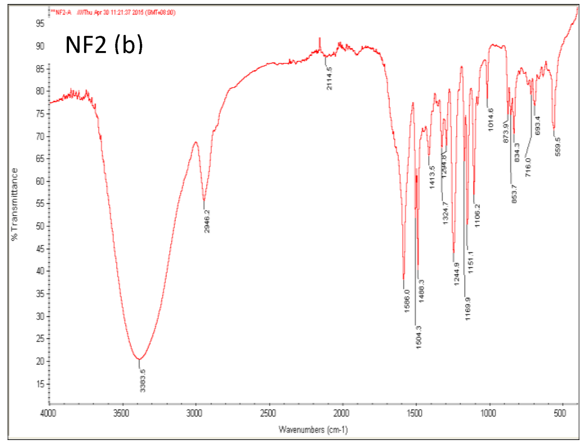

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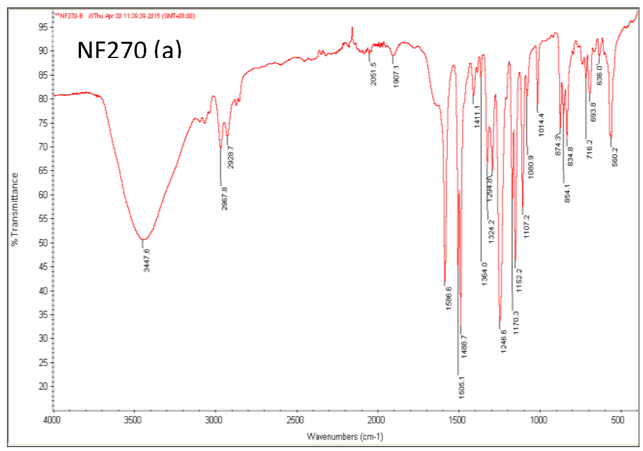
Figure 7. FTIR analysis of NF2 (a) before (b) after filtration



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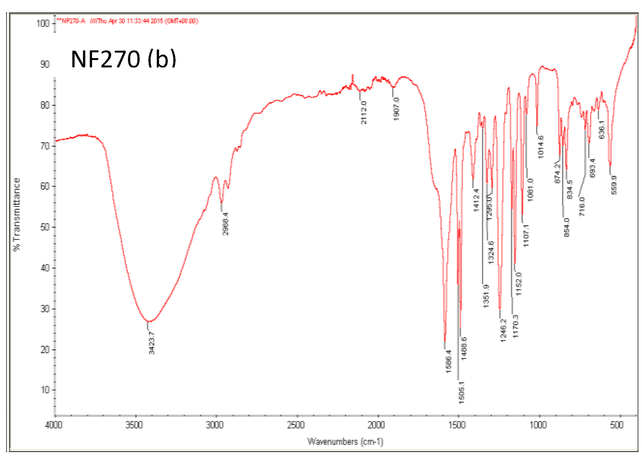


(**b**)

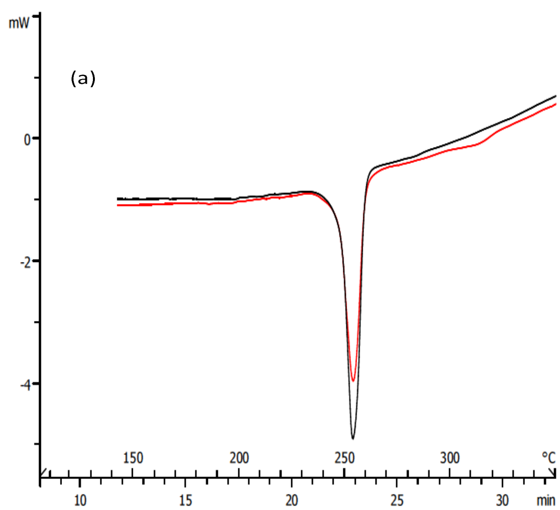
Figure 8. FTIR analysis of NF270 (**a**) before (**b**) after filtration

3.3.3 DSC Analysis

The effect of fouling on the thermal properties of membranes was also investigated using DSC. Figure 10 shows the result of DSC analysis of NF1, NF2 and NF270. Overall, all membranes show a melting point of approximately 250 °C before the filtration process. After the filtration process, only slight difference in the endothermic peak was observed compared to before the filtration process. There was no shift of peak indicating that the thermal properties is the same after the filtration. This indicates that the degree of fouling was too small to effect the thermal properties of the membranes[26].

3.3.4 Contact Angle

Contact angle was carried out to determine the effect of filtration process on the hydrophilicity of the membranes tested; the lower the contact angle the higher the hydrophilicity of the membrane[27]. Table 4 show the summary of the contact angle before and after filtration for NF1, NF2 and NF270. It was observed that NF1 shows the highest changes in contact angle after the filtration process followed by NF270 and NF2. This shows that fouling may have occurred on NF1 but the hydrophilicity of the membrane after filtration is still comparable to NF2 and NF270.



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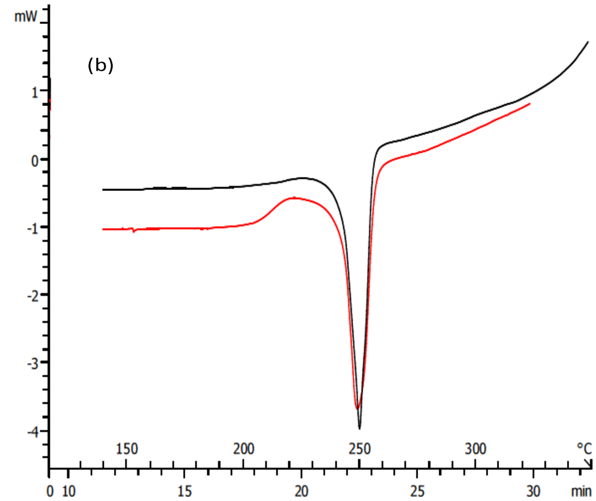

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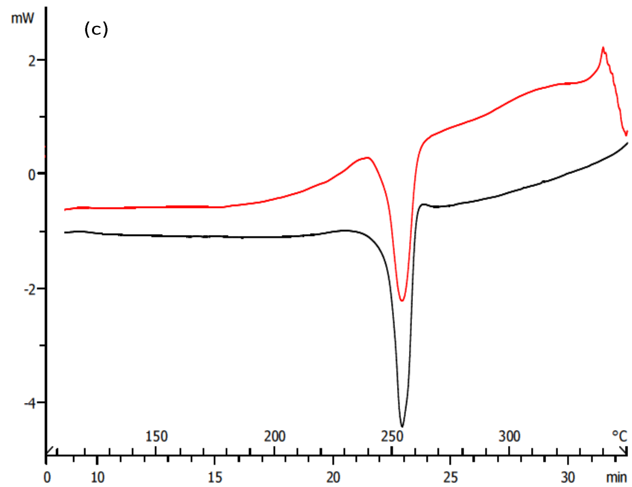

(**c**)

Figure 9. DSC analysis for (**a**) NF1, (**b**) NF2 and (**c**) NF270 before and after filtration process (Red line: before filtration; Black line: after filtration)

Table 4. Contact angle of membrane NF1, NF2 and NF270 before and after filtration process and the percentage difference

Membrane	Contact angle before filtration (o)	Contact angle after filtration (o)	Difference (%)
NF1	24.8	45.7	84.27
NF2	32.3	35.33	9.38
NF270	35.33	43.7	23.69

4. Conclusion

Overall, NF membranes show successful selective separation and purification of succinic acid from mixed salt solution. Different NF membranes were observed to give different capabilities of separation and purification of succinic acid and removal of formic and acetic acid at different mixed salt solutions concentration and pressure. At 15 bar pressure operation, NF1 and NF270 shows the best performance of 64 and 77 % rejection respectively and 77 and 78 % purity respectively at 20 g/L. Meanwhile NF2 shows the best performance of 86 % rejection and 89 % purity at 10 g/L mixed salt solution concentration. The different performance obtained for each membranes could be useful for the recovery of succinic acid from fermentation broth using different succinic acid producing bacteria, as each bacteria produces different amount of succinic acid. For the pressure variation, it was observed that NF1 and NF270 show no significant change in the rejection of succinic acid when the pressure is reduced. However for NF2 there is slight increase in succinic acid rejection from 68 to 87 % when the pressure is reduced to 5 bar. These results indicate that, the performance of separation and purification of succinic acid at high pressure, 15 bar could also be achieved at lower operation pressure, promising a reduction in operation cost. The characterization of all membranes before and after the filtration process by SEM, FTIR, DSC and contact angle also show that membranes were only slightly fouled, which could be due to the handling or the membranes rather than the foulant from the filtration process.

5. Acknowledgement

The authors gratefully acknowledgethe LRGS Project Future Biorefineries (LRGS/2013/UKM_UKM/PT/03) and Fundamental Research Grant Scheme (FRGS/2/2013/TK05/UKM/02/4) by Ministry of Science, Technology and Innovation (MOSTI),Geran GalakanPenyelidikan Muda (GGPM-074-2013) and GeranPenyelidikandanPengurusan (DPP-2015-FKAB) by UKM for providing financial support for this research project.

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