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# The Viability of Forward Osmosis in the Concentration of Biologically Produced Fumaric Acid Using L-Alanine as a Draw Solution

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Separation and concentration processes of fumaric acid from industrial products and down streams have been a subject of wide range in research. With the emergence of Forward Osmosis (FO) technology attention has shifted to utilising it in downstream processing. This study has investigated the viability of FO technology in concentration a fumaric acid solution produced by continuous microbial fermentation process using L-Alanine as a novel draw solution (DS). Thin Film Composite (TFC) aquaporin protein flat sheet membrane was used in the FO set up. L-Alanine was used as a DS at a concentration of 0.085 g/mL. The DS concentration was chosen based on preliminary studies which were conducted to determine the optimal DS concentration which achieved the highest water flux with the lowest reverse solute diffusion.
The fumaric acid produced by continuous microbial fermentation process was investigated as a Feed Solution (FS). The biologically produced Fumaric acid was obtained from a continuous microbial fermentation process developed by the Bioreaction Engineering group at University of Pretoria. Rhizopus oryzae fungus was utilised in the fermentation process with glucose as the substrate. The influence of temperature on the efficiency of the FO process was also investigated. The results show that fumaric acid solution concentrated by 26.00 % and 19.80 % in 32 ℃ and 17 ℃, respectively. The results demonstrate FO technology to be an effective way to concentrate the fumaric acid solution produced by continuous microbial fermentation process. However, achieving high concentration has been limited because of high residual concentration of glucose and other minerals in the biological process. This lead to reduce the net driving osmotic force through the membrane. The initial concentration of glucose contributed with 50.50 % of overall osmotic pressure of FS, while the initial concentration of fumaric acid contributed only with 12.20 %. Minimising the residual glucose concentration could lead to a doubling of the fumaric acid concentration in the FO process.

## 1. Introduction

Fumaric acid is widely being produced by petrochemical route. The first step of the chemical reactions is producing maleic anhydride from the oxidation of Butane in the presence of the catalyst vanadyl pyrophosphate. Thereafter, the maleic anhydride is hydrolysed to maleic acid which is finally isomerized to produce Fumaric acid, as demonstrated as Equation 1 (Brar et al., 2016).

$$\underset{\text{Butane}}{C_4H_{10}} + 3.5\,O_2 \xrightarrow{\text{vanadyl pyrophosphate},(VO)_2P_2O_7} \underset{\text{Maleic anhydride}}{C_4H_2O_3} + 4\,H_2O \xrightarrow{\text{Hydrolysis}} \underset{\text{Maleic acid}}{C_4H_4O_4} \xrightarrow{\text{Isomerization}} \underset{\text{Fumaric acid}}{C_4H_4O_4} \quad (1)$$

However, due to the global environmental impacts of the petrochemical industry, the tendency of producing platform chemicals from renewable biomass through fermentation process is strongly promoted (Zhang et al., 2014). Fumaric acid is considered one of the best platform chemicals and can be produced by glucose fermentation conversion. The fermentative production of fumaric acid has also obtained greater attention by increasing the awareness in food safety. The microbial fermentative route does not carry the toxicity risk of the

petroleum-based materials which used in the petrochemical route of fumaric acid production. The microbial fungus Rhizopus oryzae is preferably used to produce the fumaric acid in fermentation process due to its high productivity and low nutrient requirements (Brar et al., 2016). Rhizopus oryzae can generate large amount of fumaric acid as a main product compared to the other species (Rhizopus nigricans, Rhizopus formosa, and Rhizopus arrhizus) (Woźniak & Prochaska, 2014; Zhang & Yang, 2015). Liao et al. (2008) used Rhizopus oryzae to produce 31.00 g/L of fumaric acid as a fumarate salt with productivity of 0.322 g/L/h during the biological conversion of dairy manure as a carbon resource. Das et al. (2015) enhanced the production of fumaric acid by using Rhizopus oryzae 1526 species. He generated more than 47.22 g/L of fumaric acid with productivity of 1.675 g/L/h through the fermentation of brewery wastewater as a carbon source. The success of fermentation and bioprocesses is highly dependent on reducing the water content and consequently concentrating of downstream products (Woźniak & Prochaska, 2014). Relative low concentration of products at the downstream increases the fermentation processes cost. Therefore, concentration the effluent of fermentation processes via energy efficient technology improves the competence and the economy of the fermentative route of fumaric acid production (Kalafatakis et al., 2016). Forward Osmosis (FO) membrane technology has the separation potential of being utilised for extraction the water from various mixtures (Nicoll, 2013). FO is a natural process occurs due to the variance of osmotic pressure between the two solutions separated by semi-permeable membrane. This new osmotically driven membrane system allows movement of water molecules up-gradient to an extractable DS using non-degradable, natural, or artificial draw solutes. The advantage of FO lies in the low operation cost where a minimal energy consumption is required compared to pressure driven membrane processes (Zhao et al., 2012; Alsvik & Hägg, 2013). During the FO process, the osmotic pressure of the DS itself works as a driving force to create the water flux across the membrane. Furthermore, the absence of applied hydraulic pressure minimises the foulants accumulation on membranes which results in easy removed reversible fouling (Cai & Hu, 2016).

Many studies have been presented extensive investigations of using FO technology in wastewater reclamation, desalination, food industry, and other common applications (Zhang et al., 2017). However, further studies should be done to explore the efficacy of utilisation FO technology in promising applications. One of these promising applications is downstream bioprocessing of organic acids such as fumaric acid. This study has aimed at investigating the viability of FO technology in concentration a fumaric acid solution produced by continuous microbial fermentation process using L-Alanine as a DS. The study has focused on two major questions which form the structure of this manuscript: i) which is the best concentration of L-Alanine DS that achieves the highest water flux with the lowest reverse solute diffusion through the TFC aquaporin protein membrane and ii) whether the concertation of fumaric acid solution produced by microbial fermentation process can be achieved via FO.

## 2. Experimental section

### 2.1 Draw solutions

L-Alanine powder ($C_3H_7NO_2$) with purity $\geq$ 98 % and molecular weight: 89.09 g/mol (Sigma-Aldrich, South Africa) was used to prepare three draw solutions at concentrations 0.035, 0.085 and 0.120 g/mL. Distilled water with electrical conductivity of about 1.3 $\mu S/cm$ was produced by Water Still unit (WD-2008F, Daihan LabTech) and used to dissolve the L-alanine draw solute.

### 2.2 Feed solutions

Two Feed Solutions were investigated for the purpose of this work: distilled water and fumaric acid solution produced by microbial fermentation process. Distilled water was used in the first stage of experiments where the the highest water flux with the lowest reverse solute diffusion were determined. Later, the distilled water was replaced by the fumaric acid solution produced by microbial fermentation process. This fermentative FS was collected as a downstream of microbial fermentation process using the fungus, Rhizopus oryzae, to produce the fumaric acid during the bioconversion of glucose. The project is taken place by the Bioreaction Engineering group at University of Pretoria. The downstream was processed through filter paper with $2.5\ \mu m$ pore size (Whatman, 1442-110 Ashless, Grade 42) to produce filtrated FS.

### 2.3 FO membrane

TFC aquaporin flat sheet membrane (Sterlitech Corporation, USA) was used in the FO set up. TFC aquaporin flat sheet membrane is a thin film composite membrane with thickness equals 110 µm (±15 µm). It consists of thin dense polyamide active layer comprising aquaporin protein water channels deposited on porous polyethersulfone support layer. The integration of aquaporin water channels creates narrow passages with a diameter of only 2.4 nm in the thin active layer (Petrinić & Hélix-Nielsen, 2014). These narrow passages improve the water flux through the membrane structure and rejection of solutes (Mentzel et al., 2016).

### 2.4 FO system

FO experiments were conducted in a bench-scale experimental set up, as illustrated in Figure 1. FO membrane cell was designed with two cavities for both the draw and the feed solutions. Pre-cut TFC aquaporin flat sheet membrane was placed between the cavities. The effective membrane area for water transfer was fixed at 80.00 $\mathrm{cm}^2$ and orientated in FO mode, where the active layer faces the FS. Two pre-calibrated peristaltic pumps (Masterflex L/S, Model 77201-60) were linked the tanks to the FO membrane cell. The pumps drove the DS to flow in counter clockwise loop and the FS in clockwise loop across the two cavities of the FO cell with flow rate 1 L/min. The increase in DS weight was recorded by electronic scale (Radwag Model PS 4500/C/2), and used to calculate the water flux across the membrane. Meanwhile, The Total Dissolved Solid (TDS) concentration inside the FS tank was taken by handheld conductivity meter (FG3-FiveGo, Mettler Toledo) and used to calculate the reverse solute diffusion. The FS in all experiments were mixed continuously using magnetic stirrer.

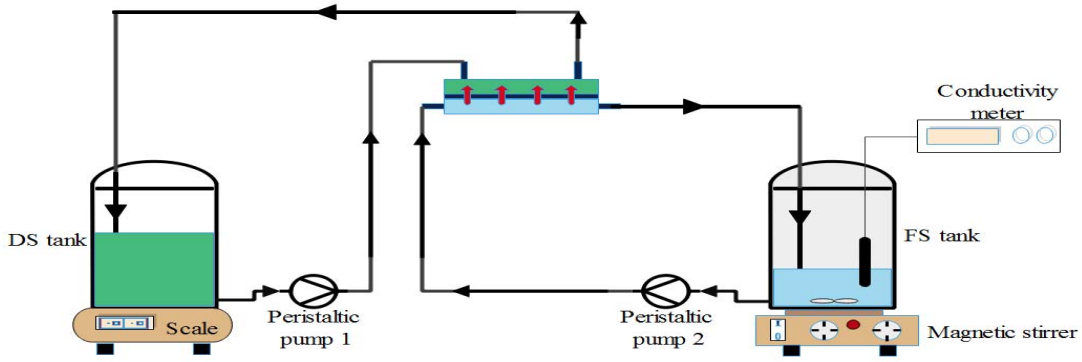


*Figure 1: Schematic diagram of the bench-scale FO system*

### 2.5 Measurements and analytical methods

#### Osmotic pressure and relative viscosity

The osmolality $(\mathrm{Osmol}\,/\,\mathrm{Kg})$ of the DS at various concentrations was tested at temperature of $(22℃ \pm 1℃)$ by freezing point depression Osmometer (Osmomat 030, cryoscopic osmometer). The osmotic pressure $(\mathrm{atm})$ of each solution was calculated by converting the osmolality to osmotic pressure using Equation 2.

$$\mathrm{OP} = \mathrm{RTc} \qquad (2$$

where OP $(\mathrm{atm})$ is the osmotic pressure, $\mathrm{RT}$ $(\mathrm{kg\,.atm\,/mol})$ = 24.22 at 22 ℃, and $\mathrm{c}$ $(\mathrm{Osmol/\,Kg})$ is the osmolality.

The relative viscosity $(\eta_r)$ of the DS compared to distilled water was calculated by Equation 3.

$$\eta_r = \frac{\eta_{DS}}{\eta_{water}} \qquad (3$$

where $\eta_{DS}$ (Pa.s) is the dynamic viscosity of DS measured at temperature of $(22℃ \pm 1℃)$ by MCR 301 rheometer (Anton Paar), and $\eta_{water}$ is the dynamic viscosity of distilled water at the same temperature, $(9.8 \times 10^{-4}\ \mathrm{Pa.s})$.

#### Water Flux

Water flux, $J_w$ $(\mathrm{L/m^2.h, cited\ to\ as\ LMH})$ across the membrane was calculated based on the observation of the DS weight change using Equation 4. The electronic scale reading was taken at zero time of experiments and then every two hours successively for 24 hours.

$$J_{w_{i+2}} = \frac{W_{i+2} - W_i}{\rho_{water} \times A \times (t_{i+2} - t_i)} \qquad (4)$$

where $J_{w_{i+2}}$ $(\mathrm{L/m^2.h})$ is the water flux on the top of every two hours, $W_{i+2} - W_i$ $(g)$ is the weight difference of the DS between every two hours, $\rho_{water}$ $(g/L)$ is the water density, $A$ $(m^2)$ is the effective membrane area, and $t_{i+2} - t_i$ $(h)$ is the time change.

**Reverse Solute Diffusion**

In FO process, the solutes diffuse reversely through the membrane from the side with high solute concentration (DS side) to the side with low solute concentration (FS side). This phenomenon is considered an inevitable challenge which causes a limitation in the efficiency of FO process (Nicoll, 2013; Akther et al., 2015). The reverse diffusion, $J_S$ $(g/m^2.h, \text{cited to as GMH})$ of L-Alanine solute was measured by monitoring the TDS concentration in the FS. The TDS was taken by the handheld conductivity meter at zero time of experiments and then every two hours successively for 24 hours. Then, the reverse solute diffusion was calculated by using Equation 5.

$$J_s = \frac{(C_t \times V_t - C_0 \times V_0)}{A \times t \times 1000} \quad (5)$$

where $C_0$ $(mg/L)$ & $V_0(L)$ are the initial TDS and the initial volume of FS, respectively, $C_t$ & $V_t$ are the TDS $(mg/L)$ and volume of FS at time t, respectively, $A$ $(m^2)$ is the effective membrane area, and t (h) is the time.

**High Performance Liquid Chromatography (HPLC) analysis**

Samples from the fermentation downstream were collected and analysed by the Agilent 1260 Infinity HPLC with refractive index RI detector (Agilent Technologies, USA). Fumaric acid, glucose, succinic acid, lactic acid, ethanol, etc. were separated and determined in a single injection by Aminex HPX-87H column (Bio-Rad Laboratories, USA). 0.3 mL/L $H_2SO_4$ aqueous solution was utilised as mobile phase with flow rate started at 0.2 mL/min increased in gradual increments to 0.6 mL/min at column temperature of 60 ℃.

## 3. Results and discussion

### 3.1 Characteristics of draw solutions

Osmotic pressure and relative viscosity of each DS concentration were investigated. Both characteristics have significant influence on the water flux and reverse solute diffusion. Table 1 demonstrated that the average osmotic pressure values of L-Alanine DS increased linearly with the concentration increase. The relative viscosity of the three DS concentrations are quite similar and close to the viscosity of distilled water. DS with high osmotic pressure generally achieves high water flux in the FO system, consequently increases the FS concentration. Meanwhile, DS with low viscosity improves the FO performance due to the low energy consumption needed to pump the solutions and the reduction of internal concentration polarisation impacts inside the membrane structure.

*Table 1: Relative viscosity and osmotic pressure values of L-Alanine draw solution at various concentration*

| | Relative viscosity | Osmotic pressure value 1 (atm) | Osmotic pressure value 2 (atm) | Osmotic pressure value 3 (atm) | Average osmotic pressure (atm) | Standard deviation of OP |
|---|---|---|---|---|---|---|
| 0.035 g/mL | 1.128 | 09.586 | 09.489 | 09.538 | 09.538 | 0.048 |
| 0.085 g/mL | 1.248 | 26.265 | 26.361 | 26.289 | 26.305 | 0.050 |
| 0.120 g/mL | 1.387 | 38.683 | 38.538 | 38.659 | 38.626 | 0.078 |

### 3.2 Variation of water flux and reverse solution diffusion over the operation time

The operation time was kept constant at 24 h for each experiment and distilled water was used as a FS in these experiments. On the one hand, Figure 2 presents the water flux produced by L-Alanine draw solutions at various concentrations through the TFC aquaporin protein flat sheet membrane. Similar water flux behaviour of the three concentrations was observed. Water flux decreased dramatically during the first 4 h of the experiments, afterward the water flux decreased relatively slowly over the remaining time of the experiment. The highest water flux was achieved by the DS at 0.085 g/mL concentration. Water flux of L-Alanine at concentration 0.085 g/mL started at 17.00 LMH as an initial value and reached to 4.50 LMH at the end of experiment. Whereas, the lowest water flux was achieved by the DS at 0.035 g/mL with 8.25 LMH as an initial value. The features of the membrane has also influenced the water flux. The porous support layer of TFC membrane combined with protein water channels enhances the water flux by allowing water molecules to diffuse easily through it. On the other hand, Figure 3 displays the value of reverse solute diffusion of L-Alanine with different concentrations through the TFC aquaporin protein flat sheet membrane over the operation time. The figure was plotted based on the calculations obtained by Equation 5. The reverse diffusion of L-Alanine at 0.035 and 0.085 g/mL was nearly constant over the operation time regardless of the variety in the

concentrations. The lowest reverse solute diffusion value through the membrane was observed when the concentration 0.085 g/mL was used. It fell from 0.05 GMH to 0.01 GMH through the membrane structure. The low reverse diffusion values of L-Alanine DS in FO process improves the water flux through the membrane and reduces the probability of the FS contamination.

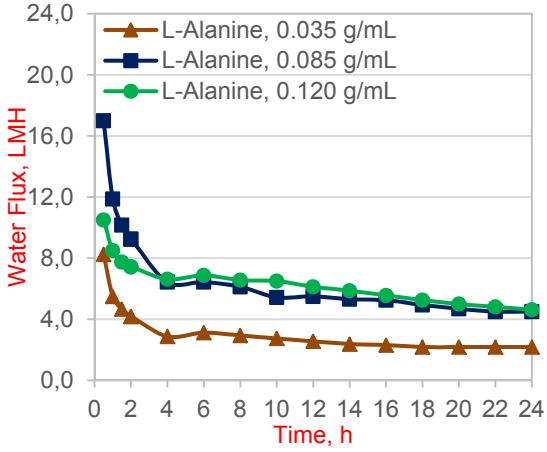


*Figure 2: Variation of water flux by L-Alanine DS using TFC aquaporin protein flat sheet membrane over the operation time.*

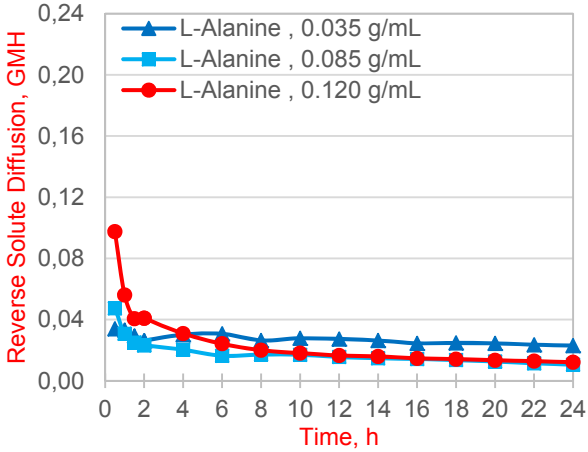


*Figure 3: Reverse solute diffusion of L-Alanine using TFC aquaporin protein flat sheet membrane over the operation time.*

### 3.3 Concentration of Biologically Produced Fumaric Acid by FO system

Concentration a fumaric acid solution produced by fermentation process was investigated as a potential application of FO process. In these experiments, distilled water FS was replaced by the fumaric acid solution produced by fermentation process. Based on the abovementioned results, L-Alanine DS at 0.085 g/mL concentration was selected to extract the water from the potential FS and thus concentrate it. HPLC results demonstrated the existence of several components including fumaric acid, glucose, succinic acid, lactic acid, ethanol, etc. in the downstream. However, the highest concentrations among these components belong to the fumaric acid and glucose. Figures 4 and 5 show the changing of fumaric acid and glucose concentrations in the FS tanks after 32 h at two different temperatures; 32 ℃ and 17 ℃. At temperature 32 ℃, the concentration raised from 6.02 g/L as an initial concentration to 7.58 g/L at the end of the experiment with 26.00 % concentration percentage. Whereas, the Fumaric acid concentration percentage was 19.80 % at temperature 17 ℃. This observation indicated a positive effect of increasing the temperature in FO process. Better concentration percentage of fumaric acid was achieved by increasing the temperature. It was also observed a high residual concentration of glucose in the FS. This amount of glucose has a high osmotic pressure which reduces the net driving osmotic force through membranes and thus limits the concentration process. The overall osmotic pressure of the FS at the beginning of the experiments was 10.26 atm shared among its components. Glucose contributed with 5.18 atm (50.50 %), fumaric acid contributed with 1.25 atm (12.20 %), and the remaining value came from pH adjusted salts and other organic acids (37.30 %).

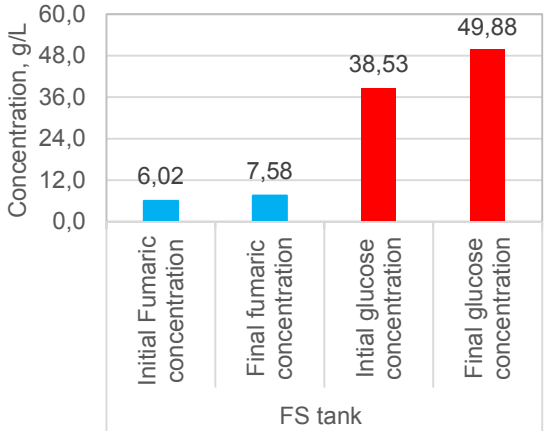


*Figure 4: Changing of fumaric acid and glucose concentrations in the feed solution tank at 32 ℃.*

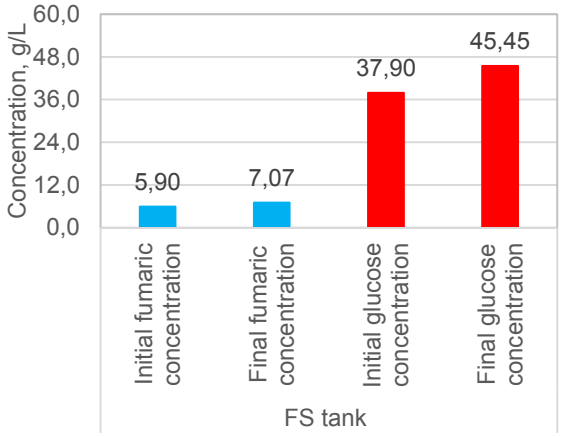


*Figure 5: Changing of fumaric acid and glucose concentrations in the feed solution tank at 17 ℃.*

## 4. Conclusions

Water flux and reverse solute diffusion in FO process were affected by the concentration of the DS. The highest initial water flux of 17.00 LMH and lowest reverse solute diffusion of 0.05 GMH were achieved with 0.085 g/mL L-Alanine as a DS. Therefore, this concentration of L-Alanine was chosen in this study to be utilised in the potential application. The concentration of fumaric acid solution produced by fermentation process with 0.085 g/mL L-Alanine DS via FO process is performed and illustrates a good results (26.00 % in 32 ℃ and 19.80 % in 17 ℃), suggesting the applicability of FO process for concentration the downstream of fermentation processes. However, the existence of glucose component with high osmotic pressure in the FS limited the concentration of fumaric acid. To enhance the performance of FO in concentrating the FS, glucose concentration should be minimised in the fermentation downstream and in the same time the biologically produced fumaric acid must be maintained without consuming.

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