Biotechnol. Bioprocess Eng. **2001**, 6: 200-204

Concentration of 6-Aminopenicillanic Acid from Penicillin Bioconversion Solution and Its Mother Liquor by Nanofiltration Membrane

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Abstract In this study, nanofiltration was applied to the concentration of the 6-aminopenicillinic acid (6-APA) from bioconverted penicillin solution and also to its mother liquor. The 6-APA in the solution was concentrated from 0.211 mol/L to 0.746 mol/L by nanofiltration. The final maximum concentration was 3.6 times higher than the initial concentration and the recovery yield was 97% to 99% of the original 6-APA. The concentrated solution was crystallized with the yields of 88.9-90.2% and the purity of the crystallized product was about 98%. The concentration of 6-APA in the mother liquor after crystallization was 0.014 mol/L and thus was concentrated 20-30 fold by nanofiltration and crystallization. The recovery of 6-APA was over 98%. The salts contained in the mother liquor, such as NH_4Cl and KCl, could be removed by allowing them to permeate through the membrane.

Keywords: nanofiltration, 6-APA, crystallization, mother liquor

INTRODUCTION

Nanofiltration (NF) membranes are similar to reverse osmosis (RO) membranes, which pass monovalent salts while retain polyvalent salts and uncharged solutes larger than 200 daltons. The primary bases for separation involves the rejection of solutes depending on their sizes and the charge of membranes [1,2]. Unlike ultrafiltration (UF) membranes, NF membranes retain most salts as well as uncharged solutes. NF membrane systems are generally operated in the tangential flow mode and allow small solutes to be concentrates by removing water. NF membrane can also be used in diafiltration mode to remove monovalent salts from larger solutes in aqueous solution [3,4].

Penicillin bioconversion solution can be crystallized after being concentrated so that the working volume may be reduced and product yield improved. Vacuum film concentration is usually performed at 45-60°C. The method has some drawbacks such as high energy consumption, deepened color of the product due to the high temperature, and non-removable inorganic salts in solution. On the other hand, nanofiltration process can be carried out at 5-15°C, univalent inorganic salts can be removed by permeation, the product has good quality, and energy consumption is accordingly reduced.

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The mother liquor contains about 0.014 mol/L 6-APA after penicillin bioconversion solution is crystallized. Therefore, an additional 237.44 kg of 6-APA can be obtained from 100 tons of mother liquor every day if the residual 6-APA could be recovered with an yield of 80%.

Danzig *et al.* [5] concentrated penicillin bioconversion solution using DDS 95 HR membrane (DOW, Nakkshov, Denmark). The NF process shows excellent promise. The retention is better than 98.5% up to a concentration of 0.3 mol/L. In this study, we have investigated concentrating penicillin bioconversion solution and its mother liquor using a nanofiltration membrane (Nanomax 50, Millipore Corporation). Results on rejection, permeation flux, concentration factor, product quality and yield were obtained.

MATERIALS AND METHODS

Materials

6-APA standard product was obtained from North China Pharmaceutical Corporation (China); 6-APA bioconversion solution and its mother liquor were from LIVCON (group) Syntpharm factory, Zhuhai, China. Prolab Nanofiltration System and Nanomax 50, Nanomax95 membrane (spiral mode) with a filtration area of 0.4 m^2 were provided by Millipore Corporation (USA). Digital auto-polarimeter was purchased from Shanghai Physical Optical Instrument Corporation

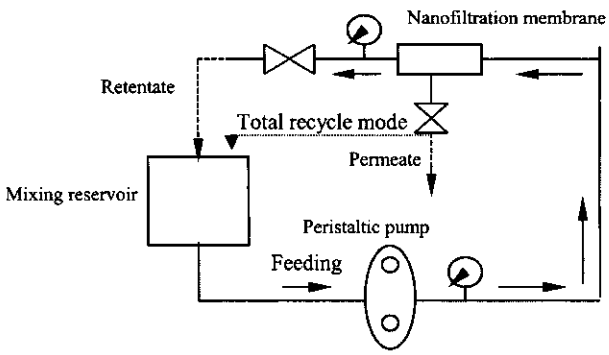

Fig. 1. Schematic diagram of 6-APA nanofiltration process.

(Shanghai, China), and conductivity meter was purchased from Shanghai No.2 Analytical Instrument Corporation. (Shanghai, China).

Assay Method

6-APA concentrations were determined by iodine [6] and polarimeter methods $[a]_{25}^{D}$= +273°(0.1 M HCl). Concentrations of KCl and NH_4Cl were measured by electric conductivity.

Selection of Nanofiltration Membrane

Four liter of penicillin bioconversion solution, 0.014 mol/L 6-APA, 1.0 mol/L NH_4Cl and 0.24 mol/L KCl were operated under total recycle mode (Fig. 1). The rejection of 6-APA, NH_4Cl and KCl by Nanomax 50 and Nanomax 95 was tested by measuring their concentrations in retentate and permeated solution. Rejection was calculated by:

$$R = 1 - \frac{C_P}{C_0} \quad (1)$$

R, C_0 and C_p are rejection, feed concentration and permeation concentration, respectively.

Effect of Pressure on Permeation Flux

Ten liter of mother liquor was concentrated by 20-fold at 2.5 MPa and 6-10°C. The concentration was performed under total recycle mode (Fig. 1), permeation flux was tested at different pressure (0.5-2.5 MPa).

Effect of Concentration on Permeation Flux

Ten liter of 6-APA mother liquor and four little penicillin bioconversion solution were concentrated at 2.5 MPa and 6-10°C (Fig. 1). Permeate flux was tested at different times of concentration.

The Concentration and Crystallization of Mother Liquor and Penicillin Bioconversion Solution

Four liter of penicillin bioconversion solution and ten liter of mother liquor were concentrated at 3.5-4.0 MPa. The bioconversion solution was concentrated three-fold and the mother liquor 20-30 fold. A half of volume of dichloromethane was added to the concentrated solution. The pH of the system was adjusted to 2.5 using 2.0 M HCl. Then organic phase was separated from aqueous phase. pH of aqueous phase was adjusted to 4.1-4.2 with 2.0 M NaOH and stood overnight and then the suspension was filtered. 6-APA precipitate was washed with acetone. It was dried at 60°C. The organic phase was mixed the same volume of water and pH was adjusted to 6.0-7.0, and aqueous phase was separated from the organic phase. pH of aqueous phase was adjusted to 1.5-2.0 and the phenylacetic acid was recovered.

RESULTS AND DISCUSSION

Selection of Nanofiltration Membrane

The mother liquor contained 0.014 mol/L 6-APA, 0.014 mol/L phenylacetic acid, 0.005 mol/L penicillin G(K), 0.05 mol/L boric acid, 1.0 mol/L NH_4Cl, 0.24 mol/L KCl, and 0.24 mol/L CH_2Cl_2. 6-APA is the most interesting product, while others need to be removed by permeation.Particularly, 1 mol/L NH_4Cl and 0.24 mol/L KCl were required to be removed because they increase osmosis pressure obviously. A satisfactory membrane should reject 6-APA and permit NH_4Cl and KCl to permeate through for a successful concentration. The selection of a nanofiltration membrane which satisfies the above demands is crucial. Rejection and permeation fluxs for 6-APA, NH_4Cl and KCl with Nanomax-50 and Nanomax-95 membranes were tested. The results are shown in Table 1. Two 6-APA concentrations were tested. The one is low concentration (0.014 mol/L) and another is high concentration (0.274 or 0.264 mol/L). Change of rejection could be observed at different 6-APA concentrations.

In the case of Nanomax-50 membrane, 6-APA rejection was more than 99%. The salt components, NH_4Cl and KCl in the mother liquor easily permeated through the membrane. Permeation flux was more than 60 L $h^{-1} \cdot m^{-2}$ at 1.0 MPa. For Nanomax-95 membrane, although 6-APA rejection was more than 99.5%, NH_4Cl and KCl could not easily permeate through the membrane and permeation flux was very low at high pressure. The flux of 1 mol/L of NH_4Cl solution was only 9.0 L $h^{-1} \cdot m^{-2}$ at 4.0 MPa pressure. It was difficult to use Nanomax-95 membrane in practice. On the other hand, the Nanomax-50 membrane met with technical demand and was used in later study.

The penicillin bioconversion solution was concentrated using Nanomax-50 membrane. 6-APA rejection at its original concentration and about three-fold concentration solution was tested. The results are shown in Table 2.

At the initial concentration of 0.218 mol/L, 6-APA rejection was 99.4%, but at three times high concentration (0.546 mol/L) the rejection was 98.9%. This shows

Table 1. Rejection ratio and flux of Nanomax-50 and Nanomax-95 versus main components of mother liquor

Components	6-APA (low conc.)	6-APA (high conc.)	NH_4Cl	KCl
Nanomax-50 membrane				
Retentate conc.	0.014 (mol/L)	0.274 (mol/L)	1.0 (mol/L)	0.24 (mol/L)
Permeate conc.	Not detectable	0.0024 (mol/L)	0.98 (mol/L)	0.22 (mol/L)
Rejection ratio	1.00	0.991	0.0233	0.08
Permeate flux ($L\ h^{-1} \cdot m^{-2}$)	69.75	37.50	63	66
Nanomax-95 membrane				
Retentate conc.	0.014 (mol/L)	0.264 (mol/L)	1.0 (mol/L)	0.24 (mol/L)
Permeate conc.	Not detectable	0.0014 (mol/L)	0.26 (mol/L)	0.20 (mol/L)
Rejection ratio	1.00	0.995	0.74	0.17
Permeate rate ($L\ h^{-1} \cdot m^{-2}$)	24.0	–	9.0	22.5

Nanomax 50 membrane: Pressure = 1.0 MPa, temperature = 20°C
Nanomax 95 membrane: Pressure = 4.0 MPa, temperature = 20°C

Table 2. 6-APA rejection ratio of Nanomax 50 membrane

Constitutes	6-APA (beginning)	6-APA (end)
Retentate conc. (mol/L)	0.218	0.546
Permeation conc. (mol/L)	0.0014	0.0061
Rejection	0.9935	0.9888

that Nanomax 50 membrane is feasible for concentration of bioconversion solution of penicillin. Danzig *et al.* [5] carried out RO concentration experiment of penicillin bioconversion solution. The rejection was 98.5% at 0.3 mol/L 6-APA, but rejection sharply decreased when 6-APA concentration exceeded 0.4 mol/L. In this study, 6-APA rejection was better at concentration exceeding 0.5 mol/L.

Data in Table 1 and Table 2 show that 6-APA rejection slightly decreases with the increase of 6-APA concentration. The mathematical model derived for nanofiltration by Spiegler and Kedem [7] was little changed. The mathematical model is based on irreversible thermodynamics, and the membrane is assumed to be uncharged. The salt rejection fraction R, predicted by this model, is given by:

$$R = \frac{\sigma(1-e^{-Jv(1-\sigma)/Ps})}{1-\sigma e^{-Jv(1-\sigma)/Ps}} = 1 - \frac{C_p}{C_m} \quad (2)$$

Jv is volume flux. C_m is the feed solution concentration on the membrane surface and C_p is the permeate concentration. For neutral membrane, salt permeability P_s and the reflection coefficient σ have constant values characterizing a given membrane system. Salt concentration has no effect on P_s and σ so that R is not affected by solution concentration.

For charged membrane, ion exclusion arises from Donnan-equilibrium effect and salt rejection is concentration dependent. Salt transport in pressure-driven charged membrane has been theoretically analyzed by Hoffer and Kedem [8,9]. Their "fixed charge model" showed that both Ps and σ are influenced by solution concentration, charge density and valency of the co-ion and counter-ion. In this study, the rejection fraction was tested from industrial point of view. The R value changes with salt concentration, water flux, and membrane properties, but the change is very small from the view point of industrial application.

Molecular weight of phenylacetic acid is not so different from 6-APA. Therefore, unfortunately, it is impossible to exploit the difference of molecular weight as a way to selectively remove phenylacetic acid.

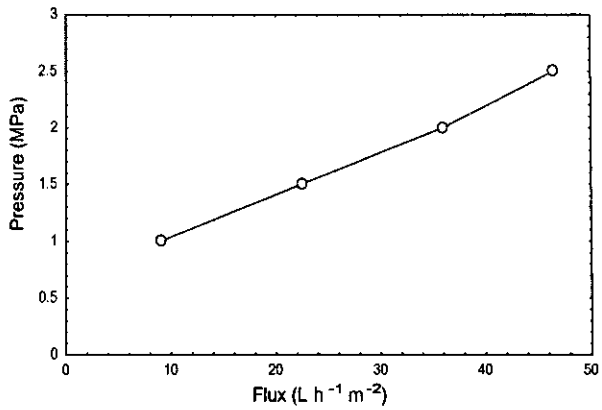

Fig. 2. Effect of working pressure on permeate flux. Ten little of mother liquor containing 0.014 mol/L 6-APA was concentrated 20-fold at 2.0 MPa, 6-10°C, and then the 0.5 L of concentrated solution was treated under total recycle mode. Permeation flux was tested at different pressures (0.5-2.5 MPa)

Effect of Work Pressure and Concentration on Permeation Flux

Flux is another problem in nanofiltration process. High flux results in a short processing time and high productivity. Effect of work pressure and concentration on flux was investigated. The relationship between work pressure and flux is shown in Fig. 2. The mother liquor was concentrated under 2.0 MPa pressure at 6-10°C. After concentrating 25-fold, the concentrated solution was operated under total recycle mode (Fig. 1) at different work pressure. The flux was proportionately increased with the increase of pressure. The transport phenomena of ultrafiltration, nanofiltration and reverse osmosis membranes in pressure-driven processes can be described by the irreversible thermodynamics. The relationship between the volume flux (J_v) and the solute flux (J_s) through a membrane is given by the following equations:

$$J_v = A \cdot (\Delta P - \Delta \pi) \quad (3)$$

$$J_s = B \cdot \Delta C \quad (4)$$

A is the permeability parameter of the solvent, which

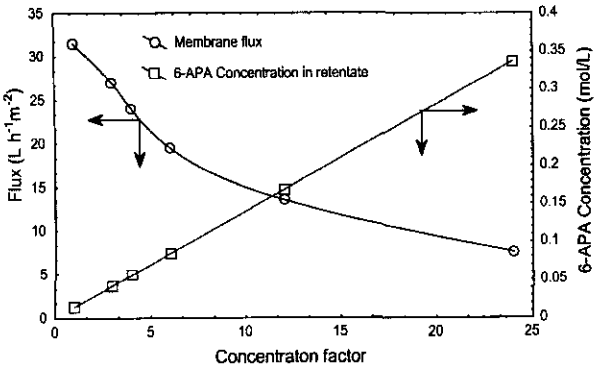

Fig. 3. Effect of concentration factor of 6-APA mother liquor on permeate flux. Ten little of mother liquor containing 0.014 mol/L of 6-APA was concentrated at 2.5 Mpa, 6-10°C. Permeate flux was measured at different concentration factors and 6-APA concentrations.

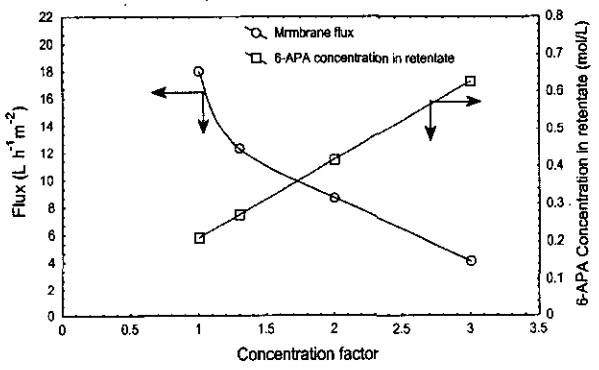

Fig. 4. Effect of concentration factor of penicillin bioconversion solution on flux. Four little of penicillin bioconversion solution containing 0.211 mol/L of 6-APA was concentrated to 1.3 L at 2.5 MPa, 6-10°C. Permeate flux was measured at different concentration factors and concentrations.

can be estimated from pure water permeability measurements and B is considered to be a single parameter, namely the solute transport parameter. ΔP, $\Delta\pi$ and ΔC are pressure, osmosis and concentration differences across membrane, respectively. In this study, J_v increased with the increase of pressure proportionally at given concentrations of solute. Equation (3) described the flux changes with pressure at given solute concentrations. When ΔP is fixed, J_v decreased with the increase of con-centration. Relationship between concentration factor of mother liquor and flux is shown in Fig. 3. The flux decreased with the increase of concentration factor. Initially, the flux was 31.5 L h^{-1} m^{-2} and concentration was 0.014 mol/L, while at the end of the process with 24-fold concentration, flux was 7.5 L h^{-1} m^{-2} and concentration was 0.336 mol/L. Flux was decreased by four times more when concentration increased 24-fold.

In the case of penicillin bioconversion solution, osmotic pressure is high, and flux determines concentration rate. Effect of concentration factor on flux of the bioconversion solution is shown Fig. 4. The flux was decreased rapidly with the increase of concentration factor. In the beginning of concentration process, flux and concentration were 18.0 L h^{-1} m^{-2} and 0.211 mol/L, respectively. When three-fold concentration was performed, the flux and concentration were 4.05 L h^{-1} m^{-2} and 0.63 mol/L, respectively. Flux was decreased by four times more, and concentration process could hardly be continued because of high osmotic pressure. However, three-fold concentration is enough in industrial scale, and the process was completed within 30 min. Operation time was short and productivity was high enough.

Table 3. Concentration and crystallization of penicillin bioconversion solution

	1	2	3
Initial volume (L)	4.0	4.0	4.0
Initial conc. (mol/L)	0.211	0.32	0.218
Final volume (L)	1.1	1.28	1.415
Final conc. (mol/L)	0.746	0.995	0.606
Permeate volume (L)	2.900	2.720	2.725
Permeate conc. (mol/L)	0.008	0.0024	0.0052
Concentration yield (%)	97.2	99.3	98.4
Crystallization yield (%)	88.9	90.2	89.7
Products purity %	97.7	98.2	98.1

(pressure 2.0-3.0 MPa, temperature 6-10°C)

The Concentration and Crystallization of Mother Liquor and Bioconversion Solution

The results from three typical batches of penicillin bioconversion solution are shown in Table 3. Residual volume in the system was not included in finial volume. Concentration yield was calculated by initial total amount of 6-APA minus total amount of 6-APA in permeate and the difference was decided by initial total amount of 6-APA. The yield is expressed by following equation:

$$y = \frac{C_0V_0 - C_pV_p}{C_0V_0} \quad (5)$$

Y, C_0, V_0, C_p and V_p are 6-APA concentration yield, feed concentration, feed volume, permeate concentration and permeate volume, respectively.

Concentration recovery was 97-99% with concentration factor of 2.7-3.6-fold. Crystallization yield was 90% with purity of 97.7-98.1%. The same experiments were repeated over eight times, and similar results were obtained. It should be pointed out that pH should be adjusted to 5.0-6.0 using ammonia during concentration process because crystals could be produced with the increase of 6-APA concentration close to isoelectric point (PI 4.1). The results in Table 3 show that replacing vacuum film concentration with nanofiltration membrane has advantages as mentioned, namely, low energy consumption, good product quality, high yield and desalination. Therefore, this process could be recom-

Table 4. Concentration and crystallization of 6-APA mother liquor

	1	2	3
Initial volume (L)	9.84	15.00	20.00
Initial conc (mol/L)	0.014	0.013	0.0127
Terminal volume (L)	0.727	0.79	1.02
Terminal conc. (mol/L)	0.187	0.2450	0.248
Permeate volume (L)	9.113	14.21	18.98
Concentration yield (%)	98.5	99.5	99.7
Crystallization yield (%)	83.2	85.6	85.2
Products purity %	96.3	97.2	96.5

(pressure 2.0-3.0 MPa, temperature 6-10°C)

mended for industry.

Concentration results at three typical batches of mother liquor are shown in Table 4. 6-APA rejection was 99.6% with concentration yield of more than 98% at the end of the concentration process. Final volume includes residual volume in the system, which was calculated using volume of water in washing system and 6-APA concentration in it. Crystallization yields were 83.2-85.6 % with purity of 97%. The yield and purity were no better than those of bioconversion solution. The reason is perhaps that components of mother liquor are more complicated than those of bioconversion solution and crystallization conditions were not optimal. Moreover, as concentration factor is further increased crystallization yield may be improved. For example, the No. 2 and No. 3 batch had higher yield than No.1 batch due to concentration factors reaching 20-fold at 0.25 mol/L 6-APA. Color of products was pure white. The eight batches were repeated with similar results. These results demonstrate that nanofiltration process is a feasible and economical method for industry. Nanofiltration can processes a large volume of mother liquor in short work time with high product yield. A medium sized factory produces 15-20 tons of mother liquor every day. Using the technique 50 kilogram of 6-APA can be recovered from the mother liquor which has significant cost implications.

CONCLUSION

The nanofiltration technique was successfully applied to 6-APA concentration from penicillin bioconversion solution and its crystallization mother solution. Penicillin bioconversion solution was concentrated by nanofiltration so that solution volume was decreased and product yield improved. Nanofiltration concentration was carried out with no phase change. Salts permeated through nanofiltration membrane during the concentration process. The product had good quality and energy consumption was reduced. Moreover, consumption of acid and bases were reduced. Recovery of residual 6-APA in mother liquor could bring obvious economic benefit.

Acknowledgements We thank senior engineer Yiang Chun, LIVCON (group) for supporting this work. Ms. Mingjian Li, Mr. Saokun Huang, Mr. Jiande Tan and Ms. Chaoli Dong helped with part of experimental work carried out in Syntpharm Factory.

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[Received April 30, 2001; accepted June 13, 2001]