ELSEVIER

Desalination 154 (2003) 17–26

DESALINATION

www.elsevier.com/locate/desal

Recovery of glutamic acid from ultrafiltration concentrate using diafiltration with isoelectric supernatants

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Received 10 October 2001; accepted 12 June 2002

Abstract

A new diafiltration method for recovery of glutamic acid (GA) from ultrafiltration concentrate by continuously feeding isoelectric supernatants was developed. A developed model of GA recovery ratio was used to simulate the process of diafiltration with isoelectric supernatants, which demonstrated higher recovery ratio and water saving than the traditional dilfiltration with water. Coupled with the technique of distillation and isoelectric crystallization, the GA purity and yield were increased by 9.3% and 3.35% respectively. Then, a novel flow sheet of GA isolation from fermentation broth was formed.

Keywords: Glutamic acid; Ultrafiltration concentrate; Diafiltration; Isoelectric supernatants; Distillation; Isoelectric crystallization

1. Introduction

L-glutamic acid (GA), in the form of monosodium salt, is widely used as a seasoning throughout the world. Nowadays, the production of monosodium salt in China is higher than any other country in the world. The total Chinese production of monosodium glutamate in 1998 exceeded

590,300 tons [1]. Traditionally, GA is isolated from fermentation broth by isoelectric crystallization with or without prior removal of biomass. However, the presence of biomass would reduce the yield of crystallization, favoring formation of β -form crystals, especially at higher concentrations [2]. Ultrafiltration (UF) process has been widely used for concentration, separation and purification. An attractive feature of UF is its simplicity in

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operation and does not require a phase change. Ultrafiltration and diafiltration (DF) have been used to concentrate and purify many types of products in different fields. One of the drawbacks associated with UF is that the recovery of some useful substances contained in UF concentrate is limited. Kuo and Chiang [3] used ultrafiltration to remove bacterial from GA fermentation broth. The retentivity of the UF membrane with glutamic acid was practically zero, thus the cell-free glutamic acid could be recovered in the UF permeate. The higher the volume concentration ratio (VCR), the higher recovery for glutamic acid could be accomplished by UF. Although the recovery ratio increased with increasing VCR, the low permeate flux at high VCR may entail processing difficulties. Therefore, DF process mode should be used at a certain VCR of UF process to “wash out” the remaining GA in the UF concentrate, thus enhancing GA recovery.

DF is simply an alternative method of operating an ultrafilter where a solvent, typically water, is combined with the feed to the ultrafilter. This has the effect of conveniently “washing out” any substances not retained by the membrane [4]. DF is a very versatile operation and is not only limited to water. Any compatible solvent will work just as well. In fact, DF can be used to transfer GA from UF concentrate to UF permeate. The constant volume batch diafiltration mode was used, in which the diafiltrating water was added to the feed tank at a rate equal to UF permeate flux to maintain a constant volume [3]. The additive in DF process conventionally was water, and no GA was introduced into the UF unit. The recovery of GA was always less than 100% due to the residual GA in ultrafiltration concentrate. In addition, much water was needed in order to obtain a certain recovery ratio and thus the total permeate volumes were increased in UF and DF processes. About 1.0–2.0% residual GA, usually contained in isoelectric supernatants, needed to be put into ion exchange resins to recover the residual GA and then discharged, which caused the loss of GA. In this work, UF was initially used to clarify the

broth. After the distillation and crystallization of UF permeate, some parts of the isoelectric supernatants were continuously recycled to wash out the residual GA in the UF concentrate during diafiltration process, which reduced water consumption in DF process and the burden of resins subsequently. The purpose of this work was to enhance the recovery of glutamic acid while reducing the total permeate volumes in UF and DF processes. This novel technology process is favorable for GA production.

2. DF mathematical model of recovery fraction

The system employed in the experimental tests was modeled as continuous stirred tank reactor (CSTR) whose flow sheet was shown in Fig. 1. Some experiments proved perfect agreement between the experimental response to a step variation and the one calculated by a CSTR model.

In the case of washing with water, mass balances yield the following expression for the component concentration in the UF permeate [5].

$$C_{V_D} = C_0 (1 - \sigma) e^{-(1-\sigma)V_D} \quad (1)$$

where V_D is volumes permeate/volume of solution to be treated, V_D can be defined as:

$$V_D = \frac{V_{PD}}{V_0} \quad (2)$$

V_{PD} is volume of permeate in the diafiltration process; V_0 – initial volume of solution to be treated;

Fig. 1. Simple DF mathematical model, CSTR.

C_{ID} – component concentration in the permeate at V_D time; C_0 – initial component concentration in the solution to be treated; σ – retentate coefficient of the component.

In the following, the equations for the recovery ratio model are presented. In this work, n is given by:

$$n = \frac{V_0}{V_0 - V_P} \quad (3)$$

where n is the volume concentration ratio (VCR) in UF process; V_0 – initial volumes of solution to be treated; V_P – volume of liquid permeated (l) in UF process without DF.

An alternative way of investigating ultra-filtration is to formulate the flux in terms of the transmembrane pressure driving force [6]. This is the osmotic pressure approach in which the flux is given by:

$$J = \frac{\Delta P - \pi}{\mu_s R_m} \quad (4)$$

where ΔP is transmembrane pressure; π – osmosis pressure in the UF concentrate; μ_s – solution viscosity (N.s/m²); R_m – membrane resistance (m⁻¹), π is the function of salt concentration. In the case of washing with water, UF flux will increase due to osmosis pressure changes with salt concentration changing. But in this work, in the case of washing with isoelectric supernatants, osmosis pressure would be approximately constant due to salt balance between the additive and the permeate. Consequently it is assumed that UF flux is not changed in the DF process.

In the case of washing with a solution containing a component, a mathematical model has to be developed for the component concentration in the retentate. Barba and Beolchini [7] have developed the following expression for the component concentration in the permeate:

$$C_{V_D} = C_{IN} - [C_{IN} - C_0(1 - \sigma)]e^{-(1-\sigma)V_D} \quad (5)$$

where C_0 is initial component concentration in the solution to be treated; C_{IN} – component concentration in the washing solution; σ – retentate coefficient of the component; V_D – volumes permeate/volume of solution to be treated.

For small changes in the liquid permeate, V_D , the change in the total recovery ratio of component in the system, expressed as η_t , is:

$$\eta_t = 1 - \frac{1}{n} + \frac{\int_0^{V_D} dV_{PD}}{V_0 C_0} \quad (6)$$

where $1 - 1/n$ is the recovery ratio of the component in UF without DF; $\int_0^{V_D} C_{ID} dV_{PD} / V_0 C_0$ the recovery ratio of the component when the washing solution, V_D was introduced to UF concentrate.

Substituting Eqs. (2) and (5) to Eq. (6) and integrating from 0 – V_D at limiting flux, recovery ratio can be calculated by the following equation:

$$\eta_t = 1 - \frac{1}{n} + \frac{C_{IN} V_D}{C_0} + \frac{C_{IN} - C_0(1 - \sigma)}{C_0(1 - \sigma)} (e^{-(1-\sigma)V_D} - 1) \quad (7)$$

It is essential to point out that Eq. (7) is only suitable to UF-DF unit but not to the whole process. Because the residual GA from isoelectric supernatants was introduced into UF unit, 100% recovery could be reached only for UF-DF unit. But for the whole process including the subsequent process of crystallization, GA recovery ratio was still less than 100% based on mass balances since there was always a little residual GA discharged.

3. Experiments

3.1. Flow sheet

The conventional and novel experimental technological processes were shown in Figs. 2a, b and c.

Fig. 2. Comparison of technological processes; a, conventional technological process without UF and DF; b, technological process including UF and DF with water; c, novel technological process including UF and DF with isoelectric supernatants.

The conventional process of GA isolation from fermentation broth, as shown in Fig. 2a, is isoelectric crystallization without prior removal of biomass. GA recovery in Fig. 2a is less than that in Fig. 2b and 2c due to the effect of biomass on crystallization. Fig. 2b is the conventional process

of prior removal of biomass using UF and DF, which was investigated by KUO and others [2]. In this process, much water was needed to diafiltrate the GA of UF concentrates in order to obtain higher recovery ratio. Fig. 2c was the novel flow sheet in this work. Compared with Fig. 2b,

the supernatants were recycled as diafiltrating solution, which saved a great amount of water, and the crystallization was also different from conventional method, which enhanced the purity and yield of product. In addition, the burden of ion exchange resins would be lightened since some parts of the isoelectric supernatants were recycled as diafiltrating solution but not be put into resins.

3.2. Fermentation broth

The GA fermentation broth, supplied by Starlake Co. (ZhaoQing, GuangDong province), was produced by cultivating *corynebacterium crenatum* with hydrolytic sugar, corn steep liquor, urea, minerals, etc., as nutrients and polyoxalkylene glycerol ether as the antifoaming agent. The typical constituents of the broth are listed in Table 1.

Table 1

Some primary physical and chemical properties of the glutamic acid fermentation broth

GA, %, w/v	9.0–10.5
pH	6.4–6.9
Wet basis, %, w/v	3.5–4.5
Residual sugar, %, w/v	0.5–0.75

3.3. Ultrafiltration and diafiltration

The experimental set-up is shown in Fig. 3. The polyvinylidene fluoride (PVDF) and polyacrylic nitrile (PAN) membranes, made in our workshop were employed in this experiment. The molecular weight cut-off (MWCO) of the membranes was 60,000 and the effective membrane area was 1 m². The hollow fiber UF modules were cross-flow system. Rotameters were used to measure flow rates. A continuous agitation and temperature control was provided for the feed vessel. The feed solution at a given flow rate was supplied to the membrane module by means of centrifugal feed pump, the concentrate was recirculated to the feed vessel while permeate was collected in the permeate tank. Initial feed volumes was recorded and target permeate volume was calculated by substituting target VCR value to Eq. (3). When the UF permeate volumes reached a target value i.e., a target VCR value, water or isoelectric supernatants in DF process were added simultaneously to the beaker under agitation through a peristaltic pump.

3.4. Crystallization

3.4.1. Distillation and isoelectric crystallization

To simulate the industrial conditions, the UF and DF permeate was introduced into a 10 l

Fig. 3. Experimental apparatus employed for ultrafiltration and diafiltration.

beaker. Sulphuric acid was slowly added to the permeate at 30°C under constant agitation until the pH reached 4.5, then crystal seeds were added to the UF permeate which was concentrated by distillation to approximately 2.5-fold. Again sulphuric acid was slowly added to the solutions until the pH reached 3.0–3.2, the isoelectric point of glutamic acid. Thereafter, the solutions were standing for 1.5 h at 5°C to precipitate the crystals. Supernatants were taken from the beaker to diafiltrate UF concentrate, enhancing GA recovery.

3.4.2. Conventional crystallization

The conventional method of crystallization was also carried out as a contrast. Sulphuric acid was slowly added to the fermentation broth at 5°C under agitation at 200 rev min⁻¹ until the pH reached 3.0–3.2 for 5 h and the solution left standing for another 1–1.5 h.

3.5. Analytical methods

GA in the fermentation broth and isoelectric supernatants was quantified by measuring the carbon dioxide evolved through enzymic decarboxylation with a Warburg apparatus. For crystallized glutamic acid, purity was measured by polarimetric method and transmittance by spectrophotometric method at 590 nm in 2 mol.dm⁻³ hydrochloric acid solutions with 1 cm optical path. Residual sugar was analyzed by Fehling's method [8].

3.6. Sample preparation of isoelectric supernatants

The UF permeate was introduced into a 10 l beaker. After distillation and isoelectric crystallization, the GA crystals were filtered and the isoelectric supernatants were reserved. The composition of isoelectric supernatants was analyzed according to analytical methods above-mentioned. The GA concentration is about 2% and pH is about 3.1–3.2. Half of the isoelectric supernatants were diluted to 1% with water in order to investigate the effect of isoelectric supernatant concentration on GA recovery ratio in DF process.

3.7. Calculation methods of experimental recovery ratio

Experimental recovery ratio of GA, expressed as η_e , was calculated by comparing the pure GA quality before and after UF-DF process as follows:

$$\eta_e = \frac{V_P C_0 + V_{PD} C_{PD}}{V_0 C_0} \quad (8)$$

4. Results and discussion

4.1. UF performance

As UF proceeded, the biomass concentration, C increased and flux rate, J decreased, as shown in Figs. 4a and 4b. A plateau occurred on the curve, beyond this, the gel polarization model held and flux declined with $\ln C$ [2]. In other conditions, such as higher biomass concentration or lower circulation velocity, flux rate declined logarithmically from the beginning with no plateau region (data not shown).

In UF experiments, the average flux can be calculated by the following expression:

$$V_{av} = \frac{V_{total}}{t} \quad (9)$$

where V_{av} is average flux of different modules; V_{total} – total permeate volumes in one period; t – operation time in one period.

As shown in Fig. 5, the average fluxes of two kinds of membrane were compared. Some literature [9,10] have reported that hydrophilic membrane is more antifouling than hydrophobic membrane especially in the process of treating protein solution. In our experiments this conclusion was validated. Fig. 4 showed that the average flux (in situ UF experiments, about 5 h as an operation period) of PAN membrane was higher than that of PVDF membrane. Therefore, PAN UF membrane was employed in the DF experiments.

Fig. 4. Membrane flux rate changes with operation time in UF process; a, PVDF membrane flux rate changes with operation time; b, PAN membrane flux rate changes with operation time; $P_i = 2.6$ MPa; $P_o = 2.0$ MPa; P_i – retentate inlet pressure; P_o – retentate outlet pressure; fermentation broth 100–120 dm³; GA 9.0–10.5%, w/v; wet biomass concentration 3.5–4.5%, w/v; about 5 h as one operation period. After each period, cleaning with sodium hydroxide was carried out under 0.1 MPa pressure.

Fig. 5. Average flux of different membrane module; $P_i = 2.6$ MPa; $P_o = 2.0$ MPa; P_i – retentate inlet pressure; P_o – retentate outlet pressure; fermentation broth 100–120 dm³; GA 9.0–10.5%, w/v; wet biomass concentration 3.5–4.5%, w/v; about 5 h as one operation period.

4.2. DF performance

Continuous diafiltration involves adding washing solution at the appropriate pH and temperature to the feed tank at the same rate as the permeate flux, thus keeping the feed volume ($V_0 - V_p$) constant during the process. In this work, in order to meet with the company's request, which included the total permeate volumes in UF and DF processes must be no more than 1.2-fold initial

solution volumes and the recovery ratio of GA must be more than 98.5%, a 10-fold concentration of initial solution to be treated was chosen as the point to end UF and start DF. When the diafiltration volumes reached a target value, the operation was stopped. In Fig. 7, when diafiltrating solution is water, VCR is 10 and V_D is 0.3, the total permeate in UF-DF process is 1.2-fold initial solution volumes and the recovery ratio is less than 98.5% which cannot meet with the company's request. Therefore, a new diafiltration method using isoelectric supernatant to diafiltrate UF concentrate was proposed. Obviously, in the case of a washing solution containing GA at a certain concentration, as shown by Figs. 6 and 7, the higher GA concentration and recovery ratio of UF-DF process can be certainly reached with adding isoelectric supernatants rather than that with adding water in the same DF volumes. So, when obtaining the same recovery ratio, DF with isoelectric supernatants can reduce the total volumes of UF and DF processes.

Some continuous diafiltration tests have been performed in order to validate Eq. (7). The continuous lines have been calculated by Eq. (7) for washing with isoelectric supernatants (1% and 2%). The GA retentate coefficient was experimentally estimated according to the literature [7]

Fig. 6. Experimental, points, and predicated, lines, dimensionless GA concentration vs. diluted volumes profiles during diafiltration of a GA solution of 10% using distilled water and isoelectric supernatants, 1% and 2%, as washing solutions. Lines have been calculated by Eqs. (1) and (5).

Fig. 7. Experimental, points, and predicated, lines, dimensionless GA concentration vs. diluted volumes profiles during diafiltration of a GA solution of 10% using distilled water and isoelectric supernatants, 1% and 2%, as washing solutions. Lines have been calculated by Eq. (7); points have been calculated by Eq. (8).

at 0 for a pure GA. There is good agreement between the experimental (dots) and calculated (lines) data in both tests. In particular, the curves obtained with water and isoelectric supernatants as washing solution validate the modified model.

Fig. 8. pH changes with DF volume, V_D changing.

It should be pointed out that the relationships between C_{VD} , η_i and V_D are curvilinear in according to Eqs. (1), (5) and (7), but because V_D value is relatively low, the figures only show part of the straight line.

As shown in Fig. 8, because isoelectric supernatants, in the form of tiny trickles, were added to UF concentrate in the DF process, slight change of pH arose, but far from isoelectric point of GA. We cannot observe crystals precipitating in DF process and DF would also not influence the crystallization but reduce acid consumption in the subsequent crystallization process since the pH of isoelectric supernatants, which was added to UF concentrate, was about 3.1–3.2.

4.3. Advantage of proposed crystallization over conventional method

To simulate the industrial condition, the permeate in UF and DF processes was collected and then crystallized by the proposed method i.e. distillation and isoelectric crystallization. As a contrast, the conventional method was also carried out. Table 2 shows the results, indicating the proposed was superior in terms of product quality and yield. Thus, a novel flow sheet including UF-DF, distillation and isoelectric crystallization was formed.

Table 2

Comparison between present method and conventional crystallization

	Product quality		Yields, %
	Purity, %	Transmittance, %	
Present method	98.3	97.5	87.10
Conventional method	89.0	40.5	83.75

5. Conclusions

A new method for recovery of glutamic acid from ultrafiltration concentrate was presented in this work. Experimental results have validated the developed model of GA recovery ratio for a particular component concentration in the retentate during diafiltration where washing is performed with isoelectric supernatants. Mass balances and the modified mathematical model have allowed simulation of a diafiltration with isoelectric supernatants, which demonstrate higher recovery ratio and water saving with respect to a conventional diafiltration with water. This aspect is very interesting considering that water costs are quite important in the overall operating costs of diafiltration process. Furthermore, and considering that high recovery ratio, the diafiltration process with isoelectric supernatants might be realized in industry.

In comparison with the conventional technological process, a novel flow sheet including UF-DF, distillation and isoelectric crystallization can increase the product purity and yield of GA by 9.3% and 3.35%, respectively. The gains in GA would compensate for the additional cost of membrane system and the increased purity of GA would ease the subsequent process of conversion to the monosodium salt.

Symbols

C_0 — Initial component concentration in the solution to be treated, mg/l

C_{IN} — Component concentration in the washing solution, mg/l

C_{ID} — Component concentration in the permeate at V_D time

J — UF flux in DF process, l/m².h

n — Volume concentration ratio, VCR, in UF process

R_m — Membrane resistance, m⁻¹

t — Operation time in one period

V — Initial volume of solution to be treated

V_{av} — Average flux of different modules

V_D — Volumes permeate/volume of solution to be treated

V_{PD} — Volume of permeate in the diafiltration process

V_{total} — Total permeate volumes in one period

Greek

Δp — Transmembrane pressure

η_e — Experimental component recovery ratio, %

η_t — Theoretical component recovery ratio, %

μ_s — Solution viscosity, N s/m²

π — Osmosis pressure in the UF concentrate

σ — Retentate coefficient of the component

Acknowledgements

This work was supported by the Chinese Academy of Sciences, Project KZ951-A1-201-02, KZ95T-05. We would like to thank Dr. Yuansheng Pei for the preparation of this manuscript.

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