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Purification of L-Lysine in Simulated Moving Bed and Fixed-Bed Chromatography

ROBATJAZI Seyed Mortaza[1], SHOJAOSADATI Seyed Abbas[1], KARBASY Seyed Mojtaba[2]

(*1. Biotechnology Group, Department of Chemical Engineering, Tarbiat Modarres University, Tehran, Iran; 2. Institute of Jahad Engineering, Tehran, Iran*)

Abstract: L-Lysine was produced by a microbial process utilizing a *Corynebacterium glutamicum* (ATCC 21799) strain. L-Lysine was purified from the cultivated medium by fixed-bed and simulated moving bed (SMB) chromatography. The separation conditions including pH, eluent concentration and Lys^+ and Lys^{2+} adsorption isotherms were studied in batch adsorption. The column capacity, eluent flow rate and eluent concentration have been studied in fixed-bed chromatography. Maximum purification rate of lysine was obtained as 0.066 g/(g · h) (per gram resin and per hour) at an eluent flow rate of 10 mL/min in fixed-bed chromatography. The results obtained from SMB were 0.11 g/(g · h) for L-lysine purification rate and 96% for L-lysine recovery.

Key words: ion exchange chromatography; simulated moving bed; purification; L-lysine

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L-Lysine is an essential amino acid used as a feed additive in any balanced poultry diets, and it is produced by a microbial process that utilizes a *Corynebacterium glutamicum* strain. Commonly L-lysine is recovered from culture broth using cationic exchange[1-3]. The batch and fixed-bed adsorption often cause high consumption of pure mobile phase. The discontinuity of elution chromatographic operation and the dilution of collected analytes have adversely discounted the attractiveness for pilot and full process scale separation. Thus engineers and chemists who need to scale up their separation for production purposes often turn to continuous chromatographic techniques. Among the continuous chromatographic techniques that have received considerable attentions is simulated moving bed (SMB) chromatography. It has been applied for almost forty years in the hydrocarbon and sugar industry for large-scale separations. It has been widely recognized as a solvent-saving and efficient technology. The applications of SMB chromatography have been expanded greatly. With the advent of several commercial systems, most of which are customized for user requirements, it is now an established preparative technique for production scale applications[4]. Several experimental investigations indicate that, when compared to the classical batch preparative chromatography, SMB units most often profit from the countercurrently contact between the stationary phase and the mobile phase, and exhibit better performance in terms of solvent consumption and productivity per unit mass of stationary phase[5-7].

The aim of this study is to evaluate such a SMB for the purification of L-lysine from the cultivated medium by ion exchange chromatography. The results obtained with SMB system are compared to a fixed-bed system.

1 Experimental

1.1 Production of L-lysine

L-Lysine was produced by auxotrophic strain of *Corynebacterium glutamicum* ATCC 21799. Batch culture was carried out in a 13 L fermentor (INFORS AG, Switzerland). Fermentation medium had the following composition: glucose, 100 g/L; yeast extract, 3 g/L; pepton, 5 g/L; NH_4SO_4, 50 g/L; $MgSO_4 \cdot 7H_2O$, 0.1 g/L; KH_2PO_4, 1 g/L; Mn^{2+}, Fe^{2+}, 2 μg/g; biotin, 500 μg/L; thiamin HCl, 500 μg/L; calcium pantotenat, 5 mg/L. Temperature was maintained at 31 ℃ and pH was adjusted to 7.2 by addition of concentrated NH_4OH.

1.2 Purification of L-lysine

A strong acid cation resin (Amberlite IR-120)

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First author: ROBATJAZI Seyed Mortaza, male, master, Tel/Fax: +9821/8005040.

Corresponding author: SHOJAOSADATI Seyed Abbas, professor, Tel: +9821/8011001, Fax: +9821/8006544, E-mail: shoja_sa@modares.ac.ir.

was used to adsorb L-lysine as a cationic species at low pH. L-Lysine is a basic amino acid with an isoelectric point at pH of 9.74. During the adsorption stage, free biomass cultivated medium was contacted with resin at a pH of 1.5 – 6 where L-lysine had positive charge. This allowed the adsorption of L-lysine onto the strong cationic resin. The separation conditions, including pH, eluent concentration, Lys^+ and Lys^{2+} adsorption isotherms were evaluated in the batch adsorption system. The L-lysine solutions were contacted with resin in glass tubes. The tubes were shaken, then the amount of free L-lysine was measured. The column capacity, eluent flow rate and eluent concentration have been studied in fixed-bed chromatography by a glass column, with dimensions of 15 cm × 3 cm i. d.. The column was packed with 60 g of strong cation-exchange resin Amberlite IR-120 in ammonium form. The column capacity was obtained in breakthrough point where L-lysine concentration in effluent was 5% of influent concentration. The column capacities for Lys^+ and Lys^{2+} were obtained at pH 4 and 1.5, respectively.

1.3 SMB apparatus

The operation principle and flowchart of the SMB system are shown in Fig. 1. The separator consists of four identical glass columns similar to

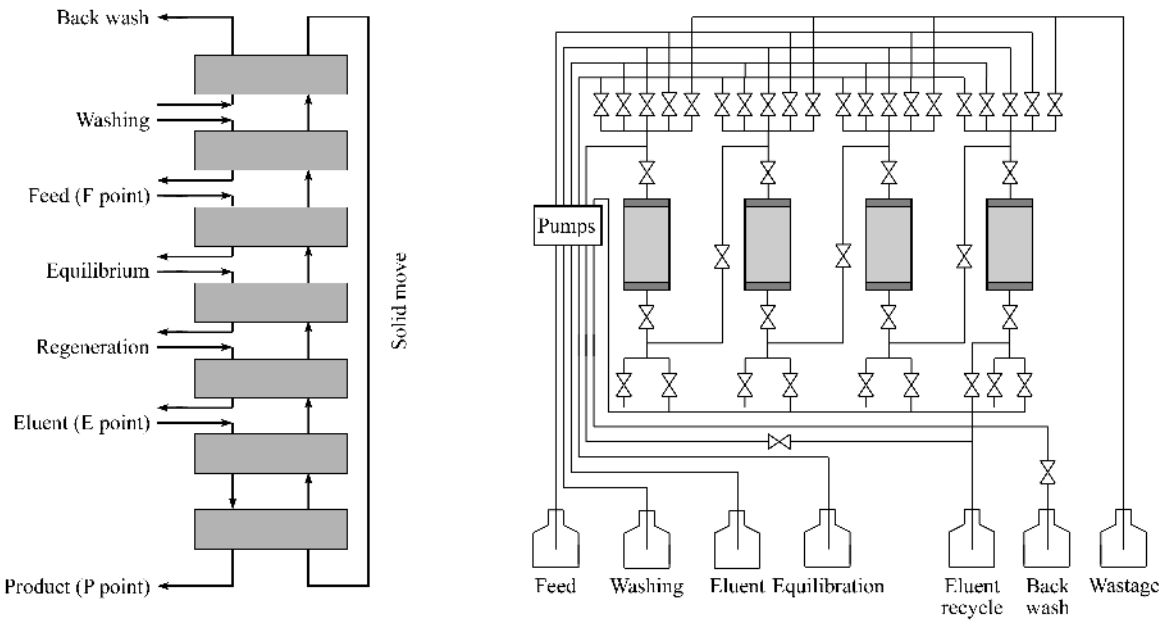

Fig. 1 Operation principle and flowchart of the simulated moving bed system

The process was started with equilibration stage and switch time was selected at 12 min.

fixed-bed system. The columns are connected in series through manual controlled valves for feed to be introduced, product to be withdrawn or liquid to be transferred to the next column. The solid movement was simulated by a stepwise shifting of all inlet and outlet streams by setting at " on " position which is equivalent to a movement of the column[8]. The flow rates were controlled by peristaltic pumps. The purification process included adsorption, washing, backwash, elution, regeneration and equilibration. Switch time was selected to be 12 min. To determine the amounts of L-lysine and glucose in the fluid phase, at each time of switch the effluent of each column was collected and analyzed. The operation conditions are summarized in Table 1. The free biomass cultivated medium was introduced at the adsorption stage (F point in Fig. 1) and L-lysine was adsorbed. The eluent was introduced at E point in Fig. 1 and L-lysine was leaving the SMB system device in ex-

Table 1 Operation conditions in SMB system

Stage	Mobile phase	Flow rate/ (mL/min)	Volume/ mL
Adsorption	feed (pH = 2)	5	60
Washing	H_2O	20	240
Back wash	H_2O	10	120
Elution	NH_4OH (4 mol/L)	11	132
Regeneration	H_2O	20	240
Equilibration	H_2SO_4 (0.1 mol/L)	10	120

tract stream (P point in Fig. 1). In this stage, eluent was injected continuously to the first column and effluent was passed through the second column. Effluent of the second column was then collected and analyzed. The similar SMB systems were also described previously by other researchers[9,10].

1.4 Analysis

L-Lysine was measured using the method described by Chinard[11]. Glucose concentration was determined colorimetrically by the glucose oxidase method.

2 Results and discussion

Lysine of 25.5 g/L was produced during 70 h of cultivation. Glucose concentration of 12 g/L was obtained at the end of fermentation.

In order to evaluate the effect of pH in batch adsorption, the L-lysine solutions of different pH value were added to the tubes, each of which contained 3 g of wet resin. The amount of free L-lysine was measured. The results showed that optimal pH for adsorption are in the range of 1 to 6. Desorption can be achieved by changing the pH to 13 using ammonia solution as eluent. A percentage of 99% of L-lysine was desorbed by using 2 mol/L NH_4OH solution. The adsorption isotherms for Lys^+ and Lys^{2+} are depicted in Fig. 2. The results show Langmuir isotherms with Eq. 1 and Eq. 2 for Lys^+ and Lys^{2+} respectively.

$$q = \frac{39.1y}{1.8 + y} \quad (1) \qquad q = \frac{119.5y}{2.1 + y} \quad (2)$$

where q is the final L-lysine content on the adsorbent (mg/g, milligram lysine per gram resin), and y is the mass concentration of L-lysine in solution (g/L).

The cultivated medium that contains L-lysine, glucose and other impurities was loaded into the column. The amounts of L-lysine and glucose were measured in the effluent of the column, as shown in Fig. 3 and Fig. 4. The column capacities for Lys^+ and Lys^{2+} were calculated at the breakthrough points to be 24.5 and 40.8 mg/g, respectively.

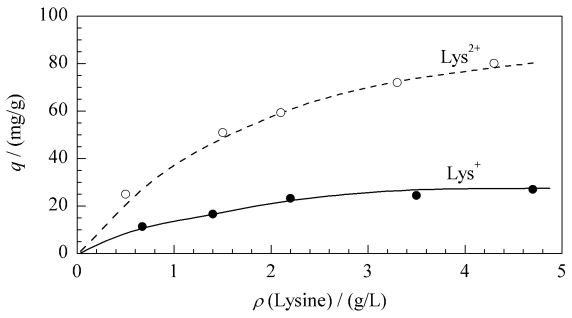

Fig. 2 L-Lysine adsorption isotherms

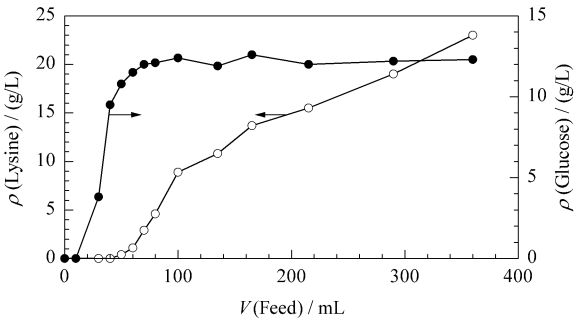

Fig. 3 Breakthrough curves of Lys^+ and glucose

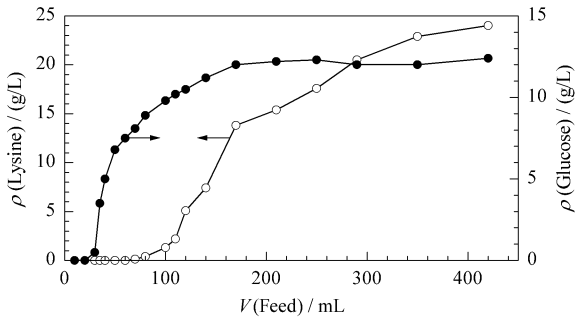

Fig. 4 Breakthrough curves of Lys^{2+} and glucose

Fig. 5 shows the effect of ammonia concentration in elution of the loaded resin at a flow rate of 10 mL/min in fixed-bed chromatography. The L-lysine recovery increased with the increase of ammonia concentration (see Table 2). The experiments showed an optimal ammonia concentration of 4 mol/L for elution of the loaded resin. Effect of eluent flow rate was studied with 4 mol/L ammonia solution as eluent. The results are shown in Table 3 and Fig. 6. The L-lysine

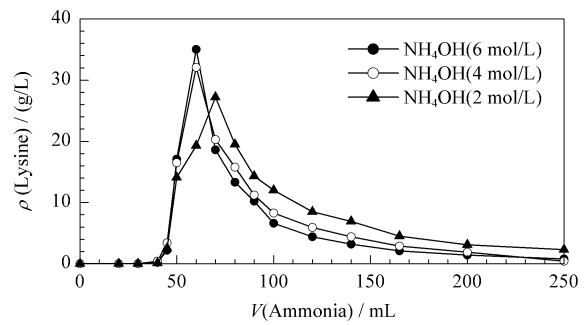

Fig. 5 Effect of ammonia concentration in elution of the loaded resin in fixed-bed system

recovery decreased with the increase of eluent flow rate. The maximum purification rate of L-lysine obtained was 0.066 g/(g · h) (per gram resin and per hour) at the eluent flow rate of 10 mL/min in fixed-bed chromatography.

Fig. 7 and Table 4 show results of L-lysine purification in SMB system. Fig. 7 shows the average concentrations of L-lysine for different fractions of purified L-lysine in elution stages in the SMB system. The L-lysine purification rate and L-lysine recovery were calculated to be 0.11 g/(g · h) and 96%, respectively.

Table 2 Comparison of L-lysine recovery for different eluent concentrations in the fixed-bed chromatography

c (NH_4OH)/(mol/L)	ρ(Purified L-lysine)/(g/L)	Recovery/%
2	5.4	92
4	6.6	95
6	6.9	96

The eluent volume was 250 mL.

Table 3 Comparison of L-lysine purification rate and L-lysine recovery for different eluent flow rates in the fixed-bed chromatography

Eluent		ρ(Purified L-lysine)/(g/L)	Purification rate/(g/(g · h))	Recovery/%
flow rate/(mL/min)	volume/mL			
2	165	10.4	0.021	98
10	250	6.6	0.066	94
15	380	4.1	0.062	93

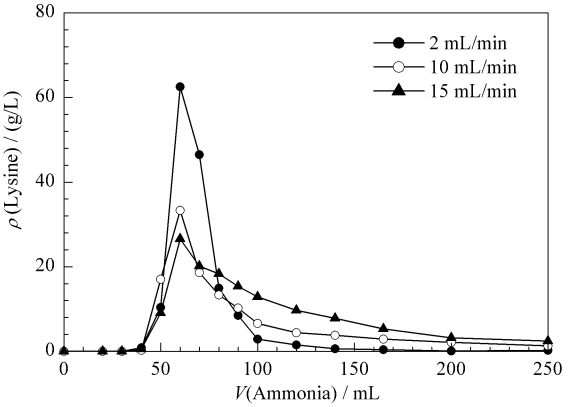

Fig. 6 Effect of eluent flow rate in elution of the loaded resin in the fixed-bed system

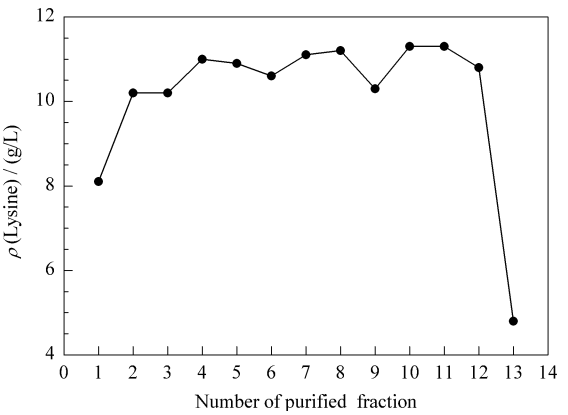

Fig. 7 Lysine mass concentration in purified fractions in the SMB system

Table 4 Results of purification in SMB system

Run	Medium		Purified		Purification rate of lysine/(g/(g · h))	Recovery of lysine/%
	ρ(lysine)/(g/L)	ρ(glucose)/(g/L)	ρ(L-lysine)/(g/L)	ρ(glucose)/(mg/L)		
1	24.7	11.6	9.5	6.7	0.1	92
2	25.2	12.2	10.1	8.1	0.11	96

The eluent was 4 mol/L NH_4OH and the consumption was 132 mL.

3 Conclusion

With the comparison of SMB chromatography and fixed-bed chromatography it can be concluded that SMB chromatography offers not only the advantage of a continuous process but also higher L-lysine purification rate. The L-lysine purification rate was increased to 167% in SMB system and the consumption of the eluent was decreased to 47%, and the process also provided a smaller dilution of the product, compared to fixed-bed system.

Acknowledgements

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