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Ion-exchange properties of mildiomycin on HZ110™ resin

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Abstract−Mildiomycin (MIL) is a novel nucleoside agro-antibiotic which shows a specific and strong inhibitory activity against fungi, especially, powdery mildews. Because of its low action dosage, excellent environmental compatibility and remarkably low toxicity to human and animals, MIL is regarded as a kind of green biological pesticides. In this paper, the recovery and purification of MIL from fermentation broth were investigated by ion-exchange separation technique. The ion-exchange properties of MIL on HZ110™ resin, including ion-exchange equilibrium and dynamics in column process, were discussed. Results showed that the equilibrium exchange capacity of MIL was 26.5 mg per gram of wet resin at optimal pH value of pH 7.0. Ion-exchange isotherms of MIL can be well correlated with Freundlich equation. The ion-exchange capacity increased with raising the temperature. In the dynamic column process, breakthrough capacity was 29.3 mg per gram of wet resin. According to the ratio of desorption and eluent concentration of MIL, 2% ammonia aqueous solution was chosen as the eluent. And the total recovery ratio of MIL in ion-exchange process was up to 94.5%, and the enriched factor was about 8.

Key words: Mildiomycin, Ion-exchange, Freundlich Equation, Dynamic Column Process

INTRODUCTION

Mildiomycin (MIL) is a product from fermentation with *Streptoverticillium rimofaciens*, and a valuable nucleoside agro-antibiotic [Kishimoto et al., 1997; Suzuki et al., 1982] with strong capability to prevent and cure the infection of powdery mildew [Mujeebur et al., 1998; Reuveni et al., 2000] of various plants. The powdery mildew is caused by the mildew fungus, and is widely spread all over the world. Because it is seriously pernicious to many kinds of plants, it is considered to be one of the highly controlled diseases in agriculture. With the growth of environmental consciousness, a bio-pesticide showed its high-speed developing tendency and bright future in the agricultural application. Mildiomycin, a green environmental conservative bio-pesticide, is the very bio-pesticide, which is especially effective to prevent and cure powdery mildew and has the advantage of low effective dosage (effectively inhibiting wheat powdery mildew at 3.12-62.5 ppm) [Mujeebur et al., 1998], no toxic and side effects on fish and mammals, good consistency of environment; it was a new pesticide worth extensively promoting [Takeda Pharmaceutical Company Limited, 1994].

MIL is a water-soluble alkali antibiotic. According to the molecular structure as shown in Fig. 1, in each MIL molecule, there are four groups which can be dissociated, and the pK values are 2.8 ($-COO^-$), 4.2($3-NH^+=$), 7.2($2'-NH_3^+$), 13.5(guanidyl), respectively. So the organic solvent extraction is not suitable in theory, while the ion exchange technology is a good choice for recovering MIL from fermentation broth. Suzuki [Suzuki et al., 1982] and Harada [Harada et al., 1978] studied the recovery of MIL from fermentation broth by Amberlite IRC-50, which was a kind of weak-acid ion exchange resin. However, in their research, the ion exchange properties were not described in detail. In this paper, the exchange properties of MIL on HZ110™ resin, including ion-exchange equilibrium and dynamic column process, were discussed.

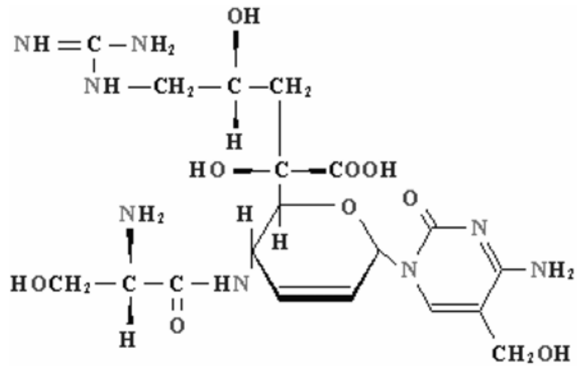

Fig. 1. Structure of MIL.

EXPERIMENTAL

1. Materials

The cation exchange resins used in this work were provided by ZeGuang Resin Factory (S-9, A-D0-1, 001×4 and 110 strong-acid ion-exchange resins), by NuKang Resin Factory (D151 and D152 weak-acid ion exchange resins) by HuaZhen Resin Factory (D113, D115, HZ110™ and DK110 weak-acid ion exchange resin). The basic physicochemical properties of HZ110™ resin are listed in Table 1. All reagents in the experiment were in AR grade or better. The fermentation broth of MIL for this research was prepared from *Streptoverticillium rimofaciens* grown in our laboratory.

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Table 1. Basic physicochemical properties of HZ110™ resin

Average particle diameter (mm)	Skeleton	Functional group	Exchange capacity (m mol/g)	Regeneration
0.65	Acrylic acid	Carboxyl	12.0	1 mol/L NaOH 1 mol/L HCl

2. Methods

2-1. Measurement of Static Ion-exchange Capacity

The static ion-exchange capacity of various resins was measured in a triangular flask on a thermostatic oscillator (Model DHZ-D, TaiCang Experimental Equipment Factory of JiangSu Province, China). In the triangular flask, 25 ml of MIL solution (pH 7.0, initial concentration 1.07 g/*l*) was contained, and then 1.0 gram of wet resin was added. After ten hours and attaining equilibration, a sample of solution was taken and the MIL concentration was measured by HPLC: then the static ion-exchange capacity was calculated by the following equation:

$$q=\frac{(c_0-c)\times v}{w} \quad (1)$$

2-2. Experiments of Ion-exchange Isotherms

The ion-exchange isotherms experiments were performed on the thermostatic oscillator at different temperatures. In the triangular flask, MIL solution with equal volume and different initial concentration was contained, and then the HZ110™ resin with equal weight was added. After attaining equilibrium, the sample of solution was taken and the equilibrium concentration of MIL was measured by HPLC.

2-3. Measurement of Ion-exchange Velocity

The ion-exchange velocity of MIL on HZ110 resin was measured in a beaker on a thermostatic magnetic stirrer (Model 78HW-1, HangZhou Instrument and Meter Plant, China). In the beaker, 50 ml of MIL solution (pH 6.0, initial concentration 1.07 g/*l*) was contained, and then 2.0 gram of wet resin was added to start the ion exchange process. The samples were taken and analyzed at a certain time interval. The ion-exchange capacity (q_t) and the fractional attainment of equilibrium (F) were calculated with following equation:

$$F=\frac{(c_0-c_t)\times v}{wq_\infty}=\frac{q_t}{q_\infty} \quad (2)$$

2-4. Experimental Procedure of Dynamic Ion-Exchange Column

The dynamic ion-exchange properties were performed with Gradi-Frac System chromatograph system (Amersham Pharmacia Biotech), and the chromatography column was type XK16 (16×200 mm). The MIL solution of concentration $\mathbf{c_0}$ was pumped into a chromatographic column with a jacket via peristaltic pump at a flow rate of 0.5 mL/min. The MIL concentration $\mathbf{c_t}$ at the exit of the column was analyzed at time interval until complete breakthrough of MIL.

The saturated ion-exchange column was washed thoroughly with deionized water, and then was eluted by different eluents at the flow rate of 0.5 mL/min. The desorption ratio (η) was calculated with the following equation:

$$\eta=\frac{c_1\times v_1}{q_t w} \quad (3)$$

Table 2. Results of resins selection

Resin brand[1]		Static exchange capacity (mg/g wet resin)	Desorption ratio[2] (%)	Total yield (%)
Strong-acid	S-9	22.0	59.2	49.2
resins	A-D_0-1	20.6	79.9	62.1
	001×4	21.0	48.1	38.0
	110	22.0	57.8	48.0
Weak-acid	D151	19.5	90.6	80.3
resins	D152	20.4	93.1	86.2
	D113	19.3	99.4	87.2
	D115	19.9	100	91.7
	HZ110	26.5	100	96.5
	DK110	19.0	98.2	86.4

[1]Before application, all resins were pretreated to H^+ type.
[2]2 N sodium chloride solution was used to elute MIL after absorption.

2-5. Analytical Method

The HPLC analytical conditions of MIL are referred to the literature [Chen et al., 2004].

RESULTS AND DISCUSSION

1. Screening of Ion-exchange Resin

Certain factors, such as the properties of functional groups of the antibiotics, the intensity of the charge and the difficulty of eluting, must be taken into consideration in the screening of proper ion-exchange resin. MIL is a strong alkaline antibiotic with positive charge, so acidic ion-exchange resin should be suitable for MIL separation from fermentation broth. Table 2 lists the static equilibrium exchange capacity and total yield for various resins. The results indicate that the differences in static equilibrium exchange capacity of MIL are relatively insignificant, whereas the desorption yield for the weak-acid resins was much higher than those for strong acid resins, showing that MIL is easier to be eluted from weak-acid resins. Among the weak-acid ion-exchange resins, HZ110™ was the best one for MIL separation.

2. Effect of Commutative Ions and pH on the Exchange Capacity of HZ110™ Resin

2-1. Effect of Commutative Ions

The effect of commutative ion of HZ110™ resin on the exchange capacity of MIL was evaluated--hydrogenous (H^+) type, sodium (Na^+) type and ammoniac (NH_4^+) type--and the results are shown in Fig. 2. It is clear that the hydrogenous (H^+) type shows the highest ion-exchange capacity, while the sodium (Na^+) type and ammonia (NH_4^+) type only have little ion-exchange ability. The results indicate that the affinity of commutative ions for HZ110™ resin was $H^+<MIL^+<Na^+<NH_4^+$. Because the affinity of NH_4^+ for HZ110™

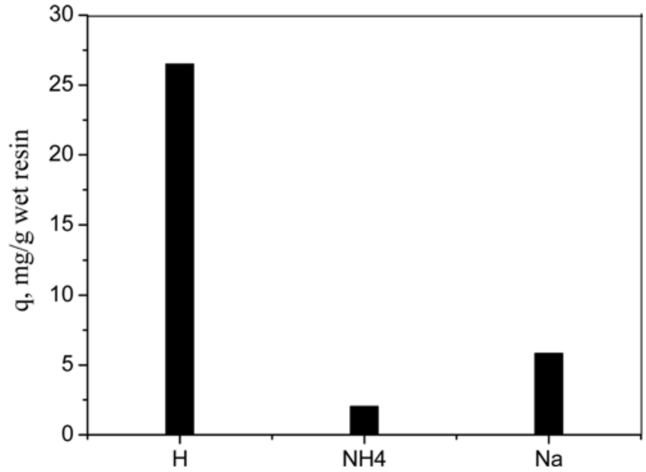

Fig. 2. Effect of commutative ion on adsorption capacity.

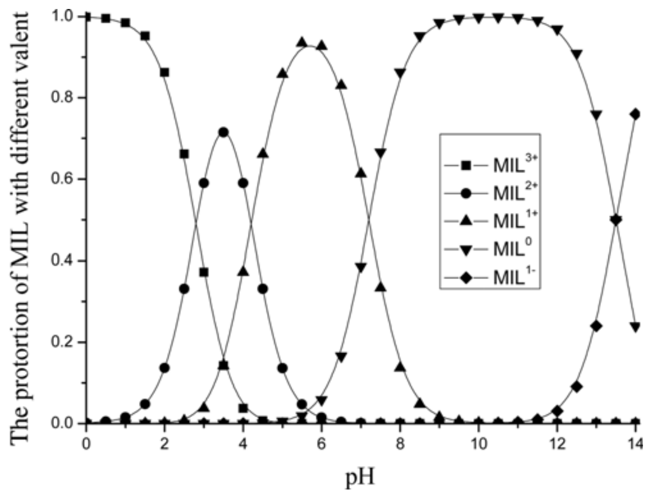

Fig. 3. Dissociation curves of MIL in aqueous solution at different pH value.

resin was even more intensive, we could choose NH_4^+ as the desorption agent.

2-2. Effect of pH on the Ion-exchange Capacity

The multilevel dissociation equilibrium of MIL occurred in the aqueous solution as follows:

$$MIL^{3+} \xleftrightarrow{pK_1'} MIL^{2+} \xleftrightarrow{pK_2'} MIL^{1+} \xleftrightarrow{pK_3'} MIL^{0} \xleftrightarrow{pK_4'} MIL^{1-}$$

The calculated distribution curves of four kinds of ionic forms as well as the molecular MIL, according to an equation derived by Handerson-Hasselbalch, are shown in Fig. 3. The dissociation of MIL is very complex and depends greatly on the pH value. The ionic form of MIL at different pH value is a major factor affecting the ability of ion exchange. Thus, the pH value is an important factor affecting the dissociation of ion-exchange resin as well as the ion-exchange capacity. The effects of pH value on the ion-exchange capacity of MIL on HZ110™ resin are shown in Fig. 4.

It was found that when the pH value was raised from pH 3.0 to pH 6.0, the ion-exchange amount at equilibrium increased from 4.05 mg/g wet resin to 25.58 mg/g wet resin. Table 3 lists the calculated fractions of various MIL dissociation patterns at different pH value.

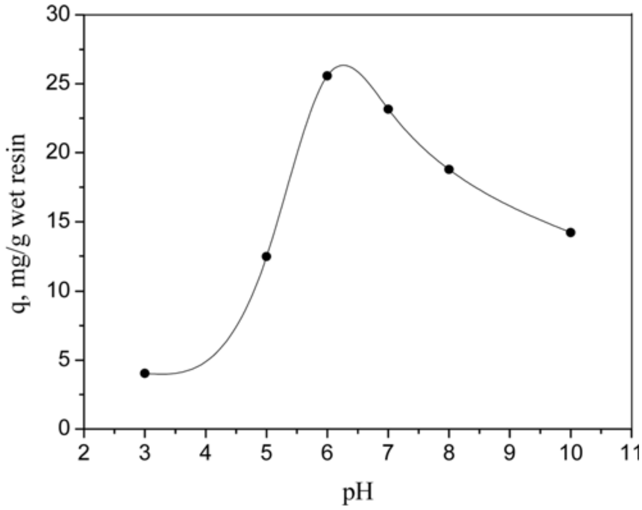

Fig. 4. Effect of pH on the exchange capacity of MIL on HZ110™ resin (c_0=1.07 g/L, T=298 K).

Table 3. Equations of ion-exchange isotherms at different temperature

Temperature	Freundlich ion-exchange isotherms
25 °C	$q=0.2075c^{0.5343}$, $R^2=0.9965$
35 °C	$q=0.2313c^{0.6177}$, $R^2=0.9997$
45 °C	$q=0.2780c^{0.6156}$, $R^2=0.9968$

At pH 3.0, the fractions of MIL^{3+}, MIL^{2+} and MIL^{1+} are 37.3%, 59.0% and 3.7%, respectively, whereas <0.5%, 1.5% and 92.7% at pH 6.0. The ionic radii of MIL^{3+} and MIL^{2+} were larger than that of MIL^+, and ion exchange of each MIL^{3+} or MIL^{2+} would occupy three or two active sites in HZ110™ resin, thus resulting in low ion-exchange capacity. Also the low pH value was not preferred for the dissociation of weak acidic ion-exchange resin HZ110™. Therefore, it was unfavorable for ion exchange of MIL at low pH value. Further raising the pH value to 10.0 would cause the decrease in the ion-exchange capability as shown in Fig. 4. From Fig. 3, it is clear that the proportion of MIL^+ rapidly dropped, while the proportion of MIL^0 fast increased. The MIL^0, neutral form of MIL, did not have the ability of ion exchange. Therefore, when the pH value further rose from pH 7.0 to pH 10.0, the ion-exchange amount at equilibrium decreased from 23.15 mg/g wet resin to 14.20 mg/g wet resin. Moreover, the MIL was both not stable in the acidic condition (pH<2) and in the alkali condition (pH>9) [Harada et al., 1978]. So the pH values between 6 and 7 are suitable for the ion-exchange process of MIL.

3. Ion-exchange Isotherms of MIL on HZ110™ Resin

The experiment investigated the ion-exchange isotherms of MIL on HZ110™ resin at 25 °C, 35 °C and 45 °C (The results are shown in Fig. 5). The exchange amount of MIL on HZ110™ resin increased with the increase of temperature, which indicated that the ion exchange process between MIL with the H^+ of HZ110™ resin was an endothermic reaction. The ion exchange isotherms could be simulated by Freundlich's equation as shown in Table 3. According to Freundlich's equation $q=k_f c^n$ (n<1), the absorption isotherm of MIL is the favorable type, namely in low concentration the exchange quality of MIL could reach a relatively high level. This property

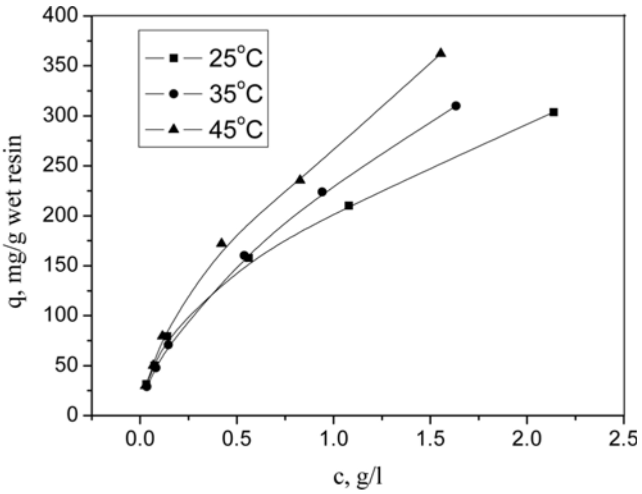

Fig. 5. Ion exchange isotherm of MIL on HZ110™ (pH 6.0).

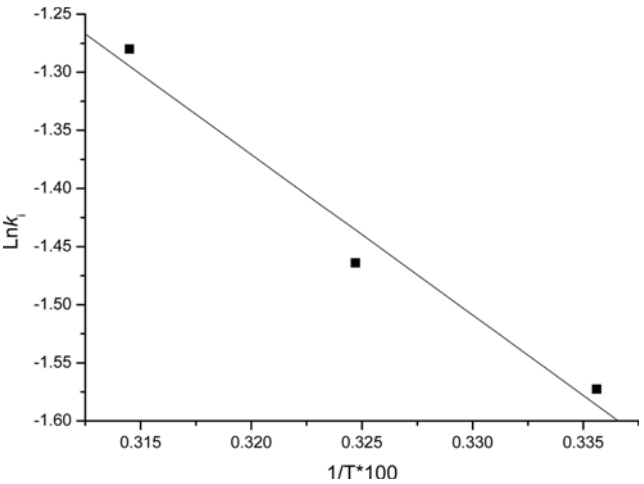

Fig. 6. Temperature dependence of k_i.

has the advantage of the recovery and purification of mildiomycin from fermentation broth.

In addition, ion-exchange equilibrium constant k_i and temperature T comply with Arrhenius' formula [Chen et al., 2005; Seung et al., 2002]:

$$k_i = k_0 \exp(-\Delta H_i / RT) \tag{4}$$

Fig. 6 shows the relationship between $\log k_i$ and 1/T. And the heat of the ion-exchange process calculated from Arrhenius' formula was quite low (11.49 KJ/mol).

4. Ion-exchange Velocity of MIL on HZ110™ Resin

Fig. 7 is the adsorption curve of MIL on HZ110™ resin at pH 6.0 when the initial MIL concentration was 1.07 g/L. It indicates that about 70% MIL in the solution had been adsorbed by HZ110™ resin after 60 min. The result made clear that the MIL showed well exchange property on HZ110™ resin.

5. Separation of MIL on Dynamic Ion-Exchange Column

5-1. Breakthrough Curve

Fig. 8 shows the breakthrough curve of MIL on HZ110™ resin. Generally speaking, the point, that the concentration of MIL in out-

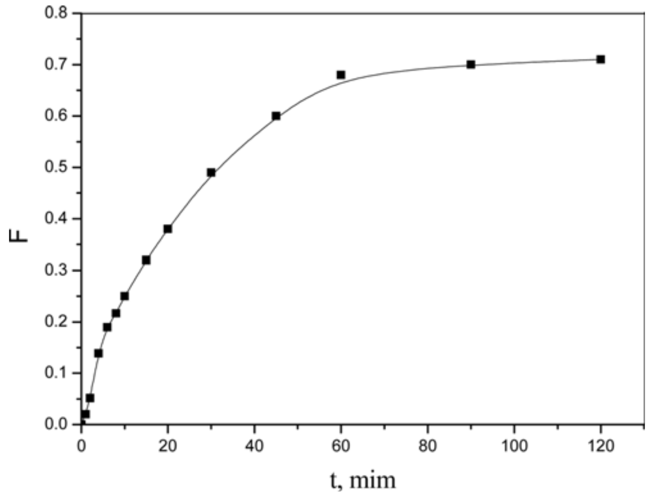

Fig. 7. Ion-exchange velocity curve of MIL on HZ110™ resin (c_0= 1.07 g/L, T=298 K, pH 7.0).

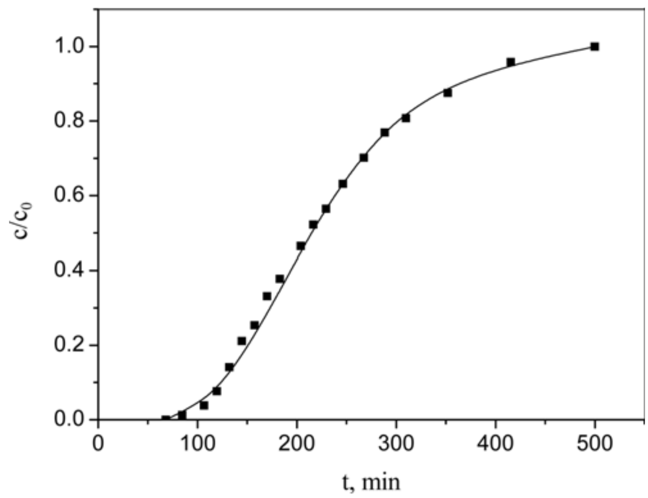

Fig. 8. Breakthrough curves of MIL on HZ110™ resin (c_0=1.07 g/L, T=298 K, pH 7.0).

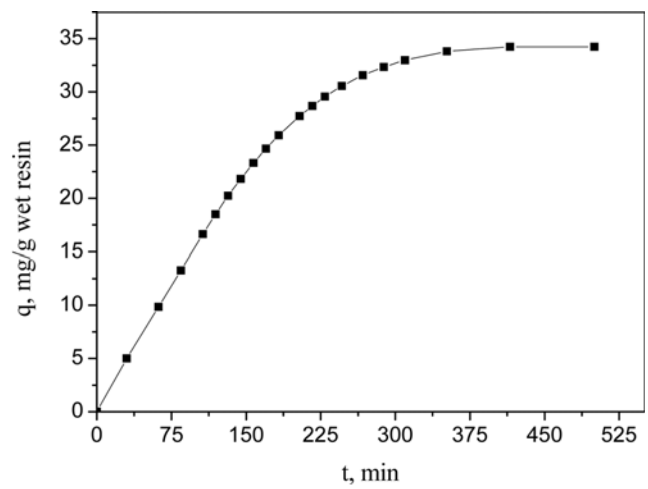

Fig. 9. Ion-exchange rate of MIL on HZ110™ resin (c_0=1.07 g/L, T=298 K, pH 7.0).

flow solution exceeds 10% of the inflow concentration, was considered to be the breakthrough point [He et al., 1995]. From Fig. 7, we could work out that the breakthrough capacity was about 29.3 mg/g wet resin; the saturation ratio of resin was 46.3%. Fig. 9 shows the relationship between average amount of adsorbed MIL and time. The dynamic exchange capacity was nearly a linear increase at first and the exchange velocity increased comparatively fast; afterwards the exchange capacity increased slowly and finally reached a constant.

The saturated ion-exchange capacity was 34.25 mg/g wet resin, which was 29.2% higher than that obtained in the static equilibrium experiment. The main reason was that in the static exchange experiment the H^+ ions exchanged by MIL still stayed in the solution to cause the reduction of pH value, which somewhat affected the further exchange of MIL on the resin. While in the dynamic exchange experiment the H^+ exchanged by MIL flowed directly out of the column, so the pH value remained unchanged; thus, the saturated exchange capacity was somewhat higher.

5-2. Desorption of MIL from the Ion-exchange Column

Fig. 10 and Table 4 present the elution curves with 1 mol/L NaCl, 2 mol/L NaCl, 1 mol/L NH_4Cl and 2% ammonia aqueous solution as the elutent when the flow rate was fixed at 0.5 BV/h, respectively. It was obvious that the ammonia aqueous solution was the best elutent with the sharpest peak in the effluent and the least requirement of elutant. The less the elutant was, the higher the enrichment that could be obtained for MIL. From Table 4, the total recovery of MIL reached 96.5% when ammonia aqueous solution was used as the elutent and the enriched coefficient was about 8. An additional advantage of using ammonia solution as elutant was that on salt was added into the system, which was favorable for further purification of MIL.

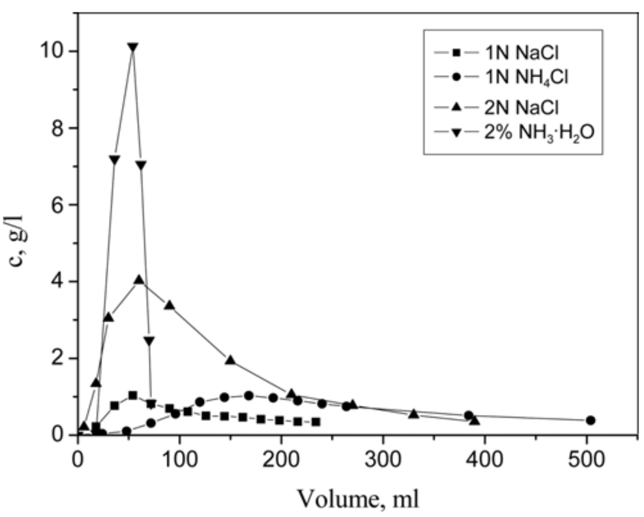

Fig. 10. Elution curves with different solutions.

Table 4. Desorption result of different eluents

Elution	Concentration of elution (g/L)	Volume of elution (mL)	Desorption ratio (%)
1 N NaCl	0.55	145	60.1
1 N NH_4Cl	0.36	384	62.9
2N NaCl	2.04	330	91.7
2% $NH_3\cdot H_2O$	4.12	90	96.5

CONCLUSIONS

Based on the strong alkalinity of MIL, the weak-acid ion exchange resin HZ110™ was selected for the separation of MIL from fermentation broth with high ion-exchange capacity and easy eluting behavior. The static ion-exchange isotherms of MIL on HZ110™ resin accorded with Freundlich's equation. The ion-exchange capacity increased with temperature and the optimal pH was 7.0. The static ion-exchange capacity of MIL on HZ110™ resin was 26.5 mg/g wet resin. The dynamic ion-exchange experiments indicated that the breakthrough capacity of MIL was 29.3 mg/g wet resin and the best elution was carried out with 2% ammonia aqueous solution. The total recovery of MIL was 94.5%, and the enriched coefficient was about 8.

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NOMENCLATURE

c_0 : initial concentration of MIL [g/l]
c : final concentration of MIL in the solution [g/l]
c_t : the concentration of MIL in the solution at t time [g/l]
c_1 : the concentration of MIL in the eluent [g/l]
k_i : ion-exchange equilibrium constant
q : the exchange capacity of MIL [mg/g (wet resin)]
q_t : ion-exchange capacity of MIL on HZ110™ resin at t time [mg/g (wet resin)]
q_∞ : equilibrium exchange capacity of MIL [mg/g (wet resin)]
v : volume of the solution [ml]
v_1 : volume of the eluent [ml]
w : weight of wet resin [g]
η : desorption ratio [%]

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