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Mass transfer in aqueous two-phases system packed column

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Abstract

The behavior of xylanase extraction in a packed column using polyethylene glycol (PEG) 4000 and dipotassium phosphate was studied. The possibility of using the packed column in continuous operations for enzyme extraction was studied since the previous work had only addressed the semi-continuous extraction of enzyme. The influence of several kinds of packings, Raschig rings, glass spheres and polystyrene rings were studied as well the superficial velocity ratio of the salt and the PEG phases. Packed column showed a good efficiency of overall mass transfer coefficient, around three times higher than sieve plate column, for xylanase extraction. The best selectivity was obtained with the polystyrene ring where 94% of xylanase was recovery to the polymeric whereas just 3% of contaminant was recovery to this phase. The residence time distribution was adjusted by the Model of Reactors in Series.

Keywords: Aqueous two-phase systems; Mass transfer; Xylanase

1. Introduction

Vertical extractors in liquid–liquid separations has been used for separating and purifying biomolecules and packed columns have been adapted for aqueous two-phase system (ATPS). Packed columns require small floor space and their operation is easy to control. The packing reduces the backmixing and breaks and distortes the droplets of the disperse phase increasing the mass transfer coefficients.

Packed columns are similar, in principle, to spray columns but are more efficient because the packing elements serve to reduce axial mixing, provide tortuous pathways for the two liquids, and can also cause distortions and breakup of the drops [1]. Due to these benefits Patil et al. [2] adapted, with success, a packed column for a semi-continuous protein extraction unit using ATPS.

The choice of the packing elements is markedly important. The packing should be wetted preferentially by the continuous phase, and its diameters limited to 1/8 of the column diameter [3] to random packings.

In the present work the xylanase was chosen as a model enzyme since extraction of this crude enzyme in a batch manner using ATPS had already been extensively studied and optimized by Bim and Franco [4], so the possibility of using a packed column with ATPS in a continuous process was investigated since the continuous operation decrease process time and costs, and increase process yield [5]. The influence of different kinds of packings (Raschig rings, glass spheres, and polystyrene rings) on the continuous enzyme extraction performance was investigated and the effect of inlet superficial velocity of the continuous salt phase was also studied.

2. Experimental

2.1. Materials

Pulpzyme HC, a commercial xylanase, was obtained from Novo Nordisk (Bagsvaerd, Denmark). The xylanase activity was 8035 U/ml, MW 25,000. In order to obtain 1 mg/ml of the contaminant protein, BSA (MW 67,000) from Sigma, ST. Louis, MO, USA was mixed with xylanase. Polyethylene glycol (PEG) 4000 and dibasic potassium phosphate (K_2HPO_4) were purchased from Synth, São Paulo, SP, Brazil. Glycine was purchased from Amersham Pharmacia Biotech (Uppsala, Sweden) and sodium hydroxide (NaOH) from Sigma. All other chemical reagents were of analytical grade.

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2.2. Tie line

The experiments were carried out with ATPS composed by 16% PEG 4000 and 10% K_2HPO_4 (global composition). The corresponding tie line was experimentally determined according to Snyder et al. [6]. The two equilibrium phases found were composed by 33.4% (w/w) PEG 4000 and 2.7% (w/w) K_2HPO_4 in the light phase and 3.3% (w/w) PEG 4000 and 14.6% (w/w) K_2HPO_4 in the heavy phase.

2.3. Xylanase assay

Xylanase activity was determined according to Bailey et al. [7] with Birchwood xylan (Sigma, St. Louis, MO, USA) 1% solution in 100 mM glycine–NaOH buffer at pH 10.0 and at 55 °C. The amount of reducing sugars was determined according to Miller [8]. One unit of xylanase activity was defined as 1 μmol of xylose produced per minute under the given conditions.

2.4. Equipment

The column was an acrylic tube with 36 mm internal diameter and 310 mm height. A schematic diagram of the packed column with the auxiliary equipments is shown in Fig. 1.

2.5. Packing

The packings characteristics are listed in Table 1. Glass beads smaller than 5 mm produced large head loss and, as a consequence, column malfunction.

Fig. 2 is a picture illustrating the shapes and relative size of the packings.

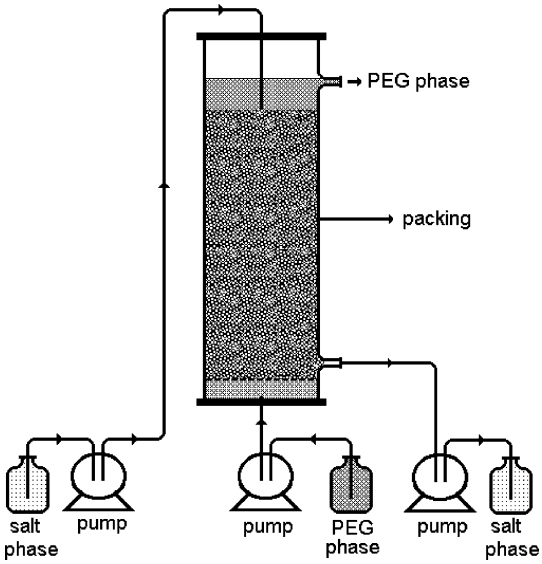

Fig. 1. Experimental set up of the packed column with the auxiliary equipments.

Table 1
Characteristics of the packing used in the extraction column

Packing	Material	Size	Void fraction
Sphere	Glass	5.0 mm diameter	0.44
Raschig rings	Glass	11.63 mm height 6.05 mm diameter	0.67
Rings	Polystyrene	3.2 mm diameter 0.7 mm thickness	0.85

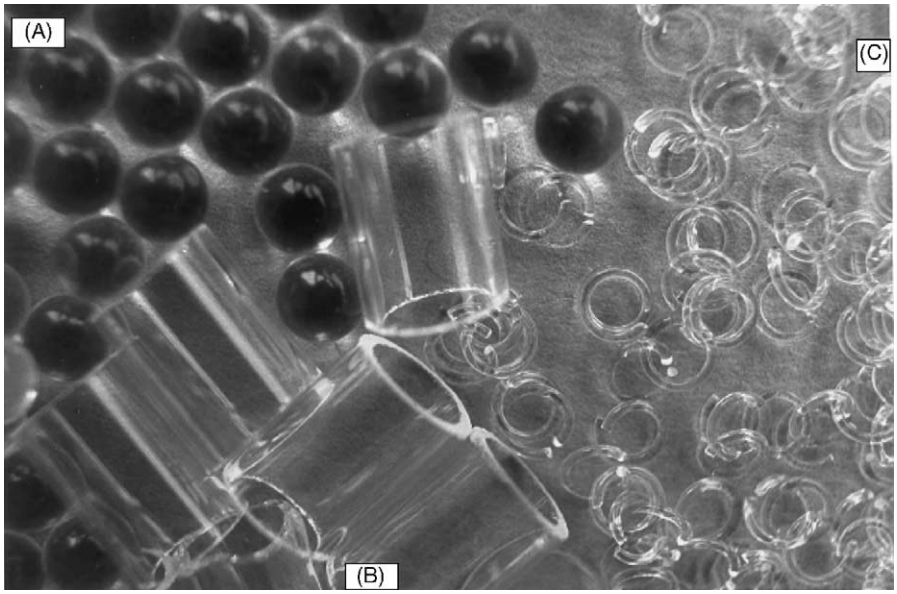

Fig. 2. Packings used in packed column. (A) Glass spheres, (B) Raschig rings, (C) polystyrene rings.

2.6. Experimental procedure

The phases of the aqueous system made with PEG 4000 and K_2HPO_4 were prepared separately according to the equilibrium phase compositions given by the tie line. The packed column was operated in a continuous fashion with the lighter phase (PEG-rich) being continuously dispersed at the bottom of the column, delivered by a peristaltic pump Pharmacia LKB (Uppsala, Sweden) and the heavier phase (salt-rich) being continuously fed at the top of the column. The xylanase was previously dissolved in the salt phase and the xylanase activity in the PEG and salt phase was determined in samples collected at the inlet and outlet of the system. The concentration of xylanase was assumed to be proportional to the xylanase activity. The temperature operation was fixed in 20 °C.

A superficial velocity range of 0.05–0.19 mm/s of the salt phase was studied, keeping the superficial velocity of PEG phase constant (0.12 mm/s). The superficial velocity of PEG phase was kept in 0.12 mm/s because the overall mass transfer coefficient was kept constant above this superficial velocity [9]. The influence of different contact times between the two phases was studied for the three kinds of packings.

2.7. Tracer

Methylene blue, Methyl red both from Aldrich (Milwaukee, WI, USA) and Drimaren A-4G from Clariant (Resende, RJ, Brazil) were tested in order to choose the best tracer to the experiments.

2.8. Residence time distribution (RTD)

The RTD of the dispersed phase (PEG) was determined by a stimulus/response method, using the pulse injection of a tracer. Samples were taken at intervals of 30 s and the concentration, C, was determined by absorbance using the spectrophotometer GBC Equipment UV-Vis 911A (Victoria, Australia). The residence time distribution and the mean residence time were determined according to equations given by Levenspiel [10]. The tanks in Series Model was used to fit the experimental data [10] with N, the number of reactors as fitting parameter.

2.9. Enzyme recovery (%)

The enzyme recovery by the PEG phase was determined according to Eq. (1). The xylanase activity in the PEG phase and salt phase were determined after the steady state was reached.

$$\text{recovery (\%)} = \frac{(\text{xylanase activity} \times \text{flow rate})_{(\text{PEG phase})\text{out}}}{(\text{xylanase activity} \times \text{flow rate})_{(\text{salt phase})\text{in}}} \quad (1)$$

2.10. Mass transfer coefficients

The overall volumetric mass transfer coefficient (K_Da) was calculated with Eq. (2) obtained from a mass balance [11].

$$K_Da = \frac{L_{\text{salt}}}{V_d}\left[\left(\frac{1}{1 - L_{\text{salt}}/KL_{\text{PEG}}}\right) \times \ln\left(\frac{C_{\text{salt in}} - (C_{\text{PEG in}}/K)}{C_{\text{salt in}} - (C_{\text{PEG out}}/K)}\right)\right] \quad (2)$$

3. Results and discussions

3.1. Hydrodynamic characterization

3.1.1. Tracer

Three kinds of tracers were tested; methylene blue, methyl red and Drimaren A-4G, in order carry out the RTD experiments. Table 2 shows the characteristics of tracer in ATPS.

It was described in Table 2 that the best tracer to the RTD in polymeric phase was Drimaren A-4G, since it presents a low interaction with salt phase ($K > 200$).

Fig. 3 showed that the maximum absorbance was obtained using wavelength 530 nm, so it will be used in the RTD experiments.

Table 2
Tracer characteristics in ATPS composed by 16% PEG 4000 and 10% K_2HPO_4

Tracer	Color	Water soluble	Affinity	K^*
Methylene blue	Blue	Yes	Polymeric phase	50
Methyl red	Red	No	–	–
Drimaren A-4G	Pink	Yes	Polymeric phase	>200

K^*: tracer concentration in polymeric phase/tracer concentration in salt phase (experimental data). Separation temperature was 20 °C.

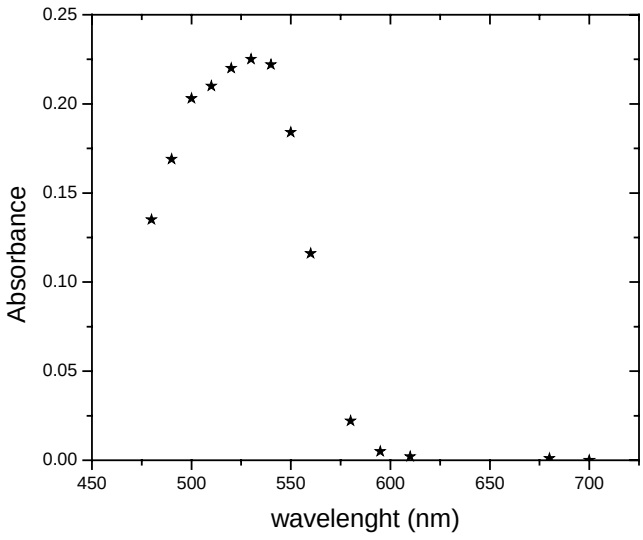

Fig. 3. Spectrum of Drimaren A-4G.

3.1.2. Residence time distribution (RTD)

The hydrodynamic characterization of the packed column was established through the RTD under operational conditions. The experimental results obtained for the RTD to the different kinds of packing were well adjusted by the tanks in Series Model. This model has just one parameter, N the number of tanks, and represents the real flow where the fluid flows through a series of tanks with same size and agitated. The packed column flow conditions can be approximated by a plug flow reactor since N obtained was larger than 10 [10]. The Peclet number for all superficial velocity and packings were higher than 20. In this case the axial mixture is negligible and an empistonate flow can be accepted [12].

The mean residence time of the PEG phase increases with the increase in the superficial velocity of the salt phase as shown in Table 3. This result is expected, since larger salt superficial velocities produce a larger drag effect on the PEG phase drops, hindering their upward movement.

The mean residence time increases according to the sequence Raschig rings–glass spheres–polystyrene rings, a direct reflection of the increase in void fraction of the bed. The larger contact time in the column with polystyrene rings forecasts a better extraction performance for this kind of packing.

3.2. Mass transfer

In order to estimate the time necessary to steady operation, the xylanase activity of the light and heavy phase was monitored. In all situations, different superficial velocity ratio and packings a constant behavior was obtained after 40 min of operation, indicating that a steady state was achieved.

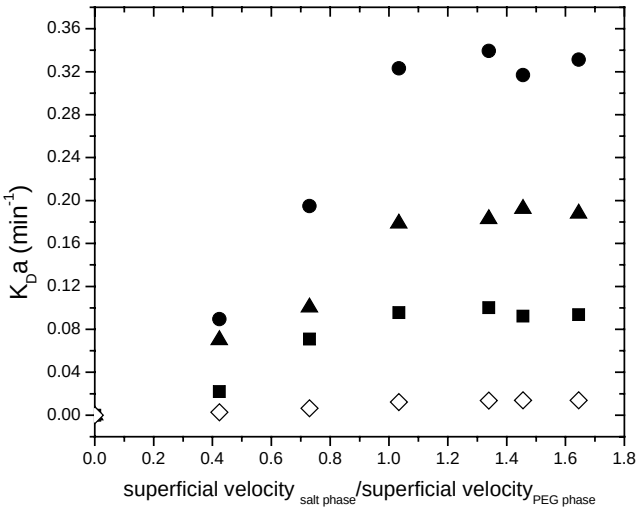

Fig. 4. Overall mass transfer coefficient of xylanase in packed column using Raschig rings (■), glass spheres (▲), polystyrene rings (●) and seven-sieve plate column (◇). ATPS composed by 16% PEG 4000 and 10% K_2HPO_4. Temperature operation was 20 °C.

Table 4 shows the recovery of xylanase into the PEG phase using Raschig rings and glass spheres, and Fig. 4 presents the corresponding overall mass transfer coefficient (K_Da) variations. The seven-sieve plate column data have also been plotted for comparison.

The extraction performance found for the Raschig rings is extremely poor, and a maximum recovery level of about 54% could only be achieved, at the higher superficial velocities. The values for the mass transfer coefficients are also correspondingly low, a result that was expected, due to the large rings dimensions (about 1/3 of the column diameter).

Table 3
Mean residence time of the PEG phase (t_m) and reactor numbers (N) with different packings using the tracer Drimaren A-4G

Superficial velocity of the salt phase (mm/s)	Superficial velocity ratio (salt phase/PEG phase)	Raschig rings		Glass spheres		Polystyrene rings	
		t_m (min)	N	t_m (min)	N	t_m (min)	N
0.05	0.42	9.3	23	11.1	12	15.0	18
0.08	0.72	13.2	13	16.3	20	19.2	13
0.12	1.03	16.5	19	19.5	15	21.2	15

ATPS composed by 16% PEG 4000 and 10% K_2HPO_4. Temperature operation was 20 °C.

Table 4
Recovery of the xylanase into the PEG phase with different superficial velocity of the salt phase, using Raschig rings, glass spheres and polystyrene rings in the extraction column

Superficial velocity of the salt phase (mm/s)	Superficial velocity ratio (salt phase/PEG phase)	Recovery (%)		
		Raschig rings	Glass spheres	Polystyrene rings
0.05	0.42	43.6	55.8	83.9
0.08	0.73	48.7	65.3	85.0
0.12	1.03	53.9	75.2	94.1
0.15	1.34	52.7	75.9	93.7
0.17	1.46	53.9	75.5	93.9
0.19	1.64	53.3	75.8	94.0

ATPS composed by 16% PEG 4000 and 10% K_2HPO_4. Temperature operation was 20 °C.

The dimensions and the shape of the packing showed a significant influence on the enzyme extraction. The extraction increased 40% using glass spheres in relation to Raschig rings, and a 75% increase with the small polystyrene rings.

These ratios are considerably higher than the ratios of the corresponding residence time, indicating that the shape and size of the packing inside the column gave a significant contribution increasing mass transfer rates, due to the break and distortion of the PEG droplets.

The values of K_Da were found to increase with a decrease in the packing size since it provides a reduction in drop size and the level of shear stress increased. For 1.65 velocity superficial ratio in the case of 3.2 polystyrene rings a K_Da of 0.33 min^{-1} was observed, while for 5 mm glass spheres, for the same velocity ratio the value of K_Da was found to be 0.19 and 0.09 min^{-1} for 6 mm glass Raschig rings. The seven-sieve plate columns generally have very poor K_Da values (0.014 min^{-1} at 1.65 velocity superficial ratio), since the existence of a plate and a coalesced layer inhibits the circulation of the continuous phase.

The variation of the values shown with different packing indicates a remarkably agreement: the extraction capacity leveled off at superficial velocities ratios of 1.0, in all situations studied. This condition represents compensation between contact time and area, turbulence and distortion and concentration differences.

It was observed that the continuous and counter-current process improved the mass transfer of xylanase. The K_Da obtained in the continuous process at 0.11 mm/s superficial dispersed phase velocity using 6 mm glass Raschig rings (0.1 min^{-1}) is 10 times higher than with the semi-batch operation (0.018 min^{-1}) using 6.7 ceramic Raschig rings [2]. The counter-current process causes a higher turbulence inside the equipment favoring the mass transfer phenomena.

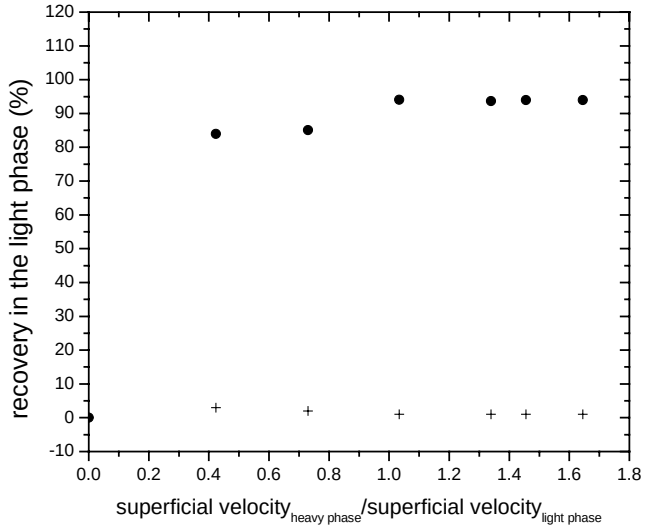

Fig. 5. Recovery of xylanase and BSA to the light phase in packed column using polystyrene rings and different superficial velocity ratios. ATPS composed by 16% PEG 4000 and 10% K_2HPO_4. (●) Xylanase, (+) BSA. Temperature operation was 20 °C.

3.3. Selectivity

Different kind of packing improves xylanase extraction. The extraction increased 75% with the small polystyrene rings instead of Raschig rings and 35% in relation to glass spheres, so the packed column using polystyrene rings was choose to model the behavior of a proteic contaminant in the enzyme extraction process. BSA was used as a contaminant model.

The increase in the inlet superficial velocity of the salt phase was negligible on the BSA extraction to the light phase, variance about 1–3%. The results given in Fig. 5 show that the amount of BSA extracted to the light phase is very small (about 3%) whereas about 94% of xylanase is presented in that phase. The inverse is presented in the heavy phase, around 97% of contaminant is present in this phase and just 6% of xylanase is extracted to heavy phase.

4. Conclusions

Packed columns are suitable for enzyme purification in aqueous two-phases systems. This investigation did not optimized packing sizes, but showed that the shape of the packing used is crucial for better extraction performance. An increase in salt phase superficial velocity improves significantly the xylanase extraction, for all packings, up to a ratio of the phase superficial velocities of 1.0. The continuous and counter-current process improved around 10 times the mass transfer of xylanase. The extraction of xylanase increased with a decrease in the packing size, 75% with the 3 mm polystyrene rings instead of 6 mm Raschig rings and 35% in relation to 5 mm glass spheres.

5. Nomenclature

ATPS	aqueous two-phase systems
BSA	albumin serum bovine
C	concentration of the solute
K	partition coefficient of the solute
K_Da	overall volumetric mass transfer coefficient
L	volumetric superficial velocity (m^3/s)
N	number of tanks
PEG	polyethylene glycol
RTD	residence time distribution
V_d	volume of dispersion (m^3)

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