ADSORPTION OF MICROBIAL LIPASES ON COMMERCIAL ADSORBENTS

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Introduction. Lipases are enzymes with a growing interest as biocatalyst in food and oleo-chemistry industries. Lipases tend to immobilize very rapidly on hydrophobic supports and has extensively used for the fractionation of this enzymes. However, very slight structural differences may recognize the interface in different way, altering the rate or strength of the adsorption.
In this work the adsorption of lipases from *Aspergillus niger*, *Candida rugosa* and *Rhyzophus oryzae* onto commercial adsorbents Octyl Sepharose and Dye-Matrex Blue A, were studied.

Methods. All solutions were buffered with ammonium sulphate (0-1.0 M) at pH 7.0, and the adsorption of lipase preparations was studied on well-stirred reactors and packed bed columns monitoring the protein concentration continuously with spectrophotometer (280 nm). Mathematical expressions for the protein solution uptake and breakthrough curve at initial concentration (c_o) were used to fit experimental data in order to obtain the correspondent parameters (3,4). All computers programs were run with MathCad 13.

$$y = y_A - \frac{(1-\varepsilon)}{\varepsilon}\left[\frac{(b+a)\left(1-\exp\left[\frac{-2a(1-\varepsilon)}{\varepsilon}k_1 t\right]\right)}{\left(\frac{b+a}{b-a}\right)-\exp\left(\frac{-2a(1-\varepsilon)}{\varepsilon}k_1 t\right)}\right]$$

$$a^2 = b^2 - \left(\frac{y_A \varepsilon}{(1-\varepsilon)}\right)q_0$$

$$b = \frac{1}{2}\left(\frac{y_A \varepsilon}{(1-\varepsilon)} + q_0 + \frac{K_d \varepsilon}{(1-\varepsilon)}\right)$$

$$\frac{y}{y_A} = \frac{J\left(\frac{n}{r}, nT\right)}{J\left(\frac{n}{r}, nT\right) + \left[1 - J\left(n, \frac{nT}{r}\right)\right]\exp[(1-r^{-1})(n-nT)]}$$

$$x = C/Co$$

$$n = q_0 \bullet k_1 \bullet z \bullet \frac{A}{F}$$

$$r = 1 + \frac{y_A}{k_d}$$

$$J(\alpha,\beta) = 1 - e^{-\beta}\int_0^{\alpha} e^{-\xi} I_0(2\sqrt{\beta\xi})\, d\xi$$

$$T = F \bullet t \bullet (k_d + y_A)(A \bullet q_0 \bullet z)$$

Results. The results shows that the rate of adsorption happens at short time interval (20 s) and when we increased the ammonium sulfate at high concentration this rate was significantly ($p<0.05$) increased that is related for a better adsorption capacity. The mathematical model (solid lines) fits the experimental data well for all kinetic study (R^2 > 0.97). A difference of the adsorption kinetic parameter was found between lipases and adsorbents. The rate and strength of the adsorption were altered by ammonium sulfate concentration (6).

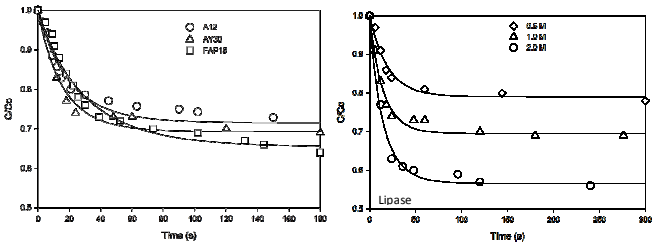

Fig.1 Kinetic of the adsorption of different lipases and salt concentration in batch experiments

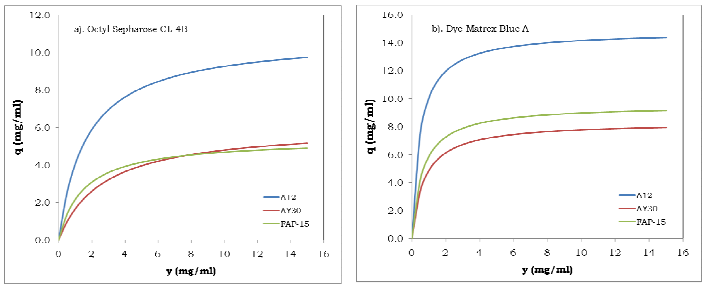

Fig.3. Isoterms of the adsorption of different lipases on commercial adsorbents

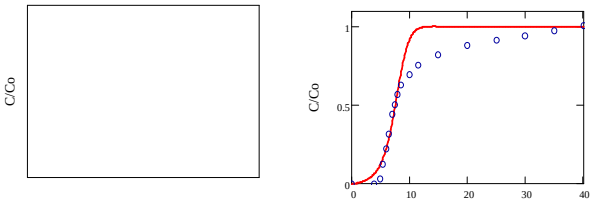

Fig.3 Frontal analysis of the breakthrough curves for the adsorption of different lipases on columns

Table I shows the kinetic parameters obtained in this work Greater adsorption capacity (q_m = 14.8 mg mL^{-1}), rapidity (6.24 mL mg^{-1} s^{-1}) and operational efficiency (75%) was observed for Dye Matrex adsorbent. This pseudo affinity ligand adsorbent is relative inexpensive, stable and versatile and can be used for the resolution of lipases from culture extracts.

Table 1. kinetic parameters for the adsorption of different lipases on commercial adsorbents.

	Octyl Sepharose CL-4B			Dye Matrex Blue A		
	A12	AY30	FAP-15	A12	AY30	FAP-15
k1 x 10^{-3} (mL/mg s)	2.2	2.5	2.88	2.6	5.8	3.2
k-1 x 10^{-2} (s-1)	3.8	6.7	1.8	1.3	4.7	1.9
Kd (mg/mL)	1.68	2.7	0.63	0.5	0.7	0.6
qm (mg/mL)	10.8	6.1	7.0	14.5	11.0	10.4
ϕ, efficiency (	61.4	50.0	39.9	75.0	65.0	63.3

Conclusions. Reactive Dyes are multifunctional ligands which contain both hydrophobic and charged groups that can be used for the separation of lipases from culture media.

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