ADSORPTION ISOTHERM OF GLUCOSE OXIDASE

Orozco Alvarez Carlos, García Salas Sergio and Cartas Romero Adaluz
Departamento de Bioingeniería. Unidad Profesional Interdisciplinaria de Biotecnología. Instituto Politécnico Nacional. Av. Acueducto S/N. Col. La laguna Ticomán. G. A. Madero. México, D.F.
e-mail: tepoztlan61@yahoo.com.mx

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Introduction. Currently the final purification of the glucose oxidase (GOX) is carried out by ion exchange chromatography resins are used which are only used at laboratory by its low mechanical resistance and are very costly (1). In this work, the only criterion is the degree of purification aside the economy and efficiency of operation (2). Therefore, in this paper we study two resins for industrial use, high strength and low cost for the purification of the enzyme in order to improve the economy of the chromatographic operation.

Methods. We used two anionic ion exchange resin, Amberlite IRA-400 and IRA-96. Experiments were carried out at 25 ° C and with an agitation of 30 rpm. To determine the adsorption isotherms multiple assays were performed. For each test, 1.0 g was placed in a glass resin and hydrated for 1 h with 9 mL of a 0.1 M solution of TRIS-HCl pH 7.0.
Is removed and TRIS-HCl is added 9 mL of enzyme solution at different concentrations (GOX) and stirring maintained for 1h. Resin is allowed to settle and the enzyme activity quantified and liquid phase protein.

Results. First was tested using IRA-400 resin obtained from the fermentation GOX with *A. niger*. In Fig.1 shows the results. The degree of adsorption is very low, 0.4 U/g, for GOX concentrations of 0.2 U/L. Through a balance is obtained adorbe the GOX is only 10%.

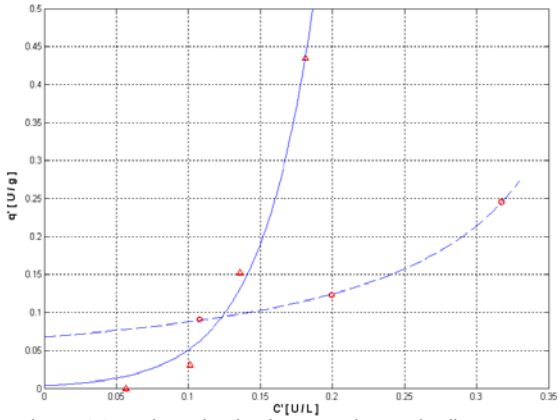

Fig. 1. GOX adsorption isotherms using Amberlite IRA-400. O clarified broth. Δ ruptured mycelium.

GOX concentrations higher than 0.5 U/mL adsorption causing a null in the ion exchange resin. Subsequently worked with IRA-96 resin using GOX concentrations of 0.1 to 3000 U/mL. The results are presented in Fig 2. The resin has a high degree of adsorption can adsorb up to 1000 U/g when in equilibrium with a GOX from 3.5 U / mL. The resin adsorbed 97% of the enzyme when used in concentrations up to 100 U/mL. When using an initial GOX 3.000 U/ mL, the resin can adsorb up to 12,000 U/g and remains in equilibrium with a concentration of 1600 U/mL. Through the resin adsorbs a balance 50% of the enzyme when the initial GOX is between 100 and 3000 U/mL.

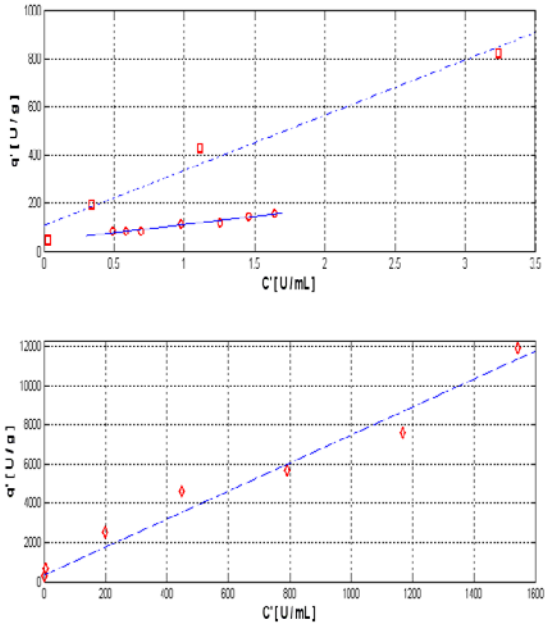

Fig. 2. GOX.adsorption isotherms using Amberlite IRA-96 Purity: ○ 60 000 U/g. □ 307 000 U/g. ◊ 193 000 U/g

Conclusions. The IRA-96 resin has a high degree of adsorption and can adsorb up to 12,000 U per gram of resin at 25 ° C.

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