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SUSTAINABLE ENVIRONMENTAL SANITATION AND WATER SERVICES

Modelling of an AUF System from Anatomical Waste

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Introduction

Potentiality of using affinity ultrafiltration technique for separating large bio-molecules is now well understood because of its inherent advantages over the conventional techniques like salt pracipitation, solvent extractioin, gel chromatography etc. Now-a-days a considerable emphasis has been paid in downstream processing including isolation and purification of bio-molecules. Affinity ultrafiltration technique is expected to play a major role in the area in the forthcoming future[1]. While most of the developing countries pay a considerable emphasis on efficient utilization of clinical waste, there is enough scope in India to improve bio-medical waste management and handling techniques. Affinity ultrafiltration process uses an affinity ligand which in conjunction with a cross-linking agent covalently binds with one of the target bio-molecules making a larger conjugate molecule. Ultrafiltration of the above system using suitable membrane in a buffer medium in presence of inert pressure selectively excludes the target bio-nolecule conjugates as the retentate while it allows permeation of the other smaller size impurities through the membrane pores. In terms of operation, affinity ultrafiltration is characterized by two steps viz. washing phase and elution phase. In the washing phase the ligand unbound bio-molecules (target species) permeate through the ultrafiltration membranes. While, in the elution phase, the ultrafiltration operation is carried out to recover the target bio-molecule as permeate.

Literature survey shows that there are number of methods available for the recovery of valuable bio-chemicals from human anatomical waste[2-3]. The investigation carried out by our team shows that the continuous foam fractionation column can be used to isolate and purify proteolytic enzymes from placental extract. Human placentas were collected from Chittaranjan National Medical College and Hospital, Kolkata, kept on ice and were separated from membrane. It was then cut into small pieces and washed with ice-cold distilled water to remove blood. The tissues were homogenized with a suitable amount of Tris-HCI buffer (ph-8.0), centrifuged at 10,000 r.p.m. for 30 minutes in order to obtain 5% placental extract. With the help of equilibrium diagram evolved from the investigation, column design parameters have been established[4-5]. While foam fractionation technique may be one of the modern separation routes, it is felt that affinity ultrafiltration may be the other alternative. In the present investigation, an attempt has been made to study the feasibility of using affinity ultrafiltration system (washing phase) for utilisation of human anatomical waste. A simulated mixture of trypsin and $\bar{\alpha}$–chymotrypsin of the comparable concentration as that of placental extract has been used. Using soybean trypsin inhibitor as the affinity ligand and woodward's reagent K as the cross-linking agent, a semi-batch affinity ultrafiltration has been designed[6]. A 30k MWCO regenerated cellulose membrane has been used because of its high pH compatibility. It has been observed that the model equations so predicted for the washing phase can successfully represent the real time situation as determined from programmed experiments.

Experimental

Materials & Methods

Trypsin $\bar{\alpha}$–Chymotrypsin, S.T.I. and other chemicals were purchased from Sigma Chemical Co., U.S.A. Cross-linked S.T.I. was prepared using Woodward's reagent K and solutions of trypsin and $\bar{\alpha}$–chymotrypsin were made using 0.1 M Tris-HCI buffer of pH 8.3 following the method given by Bartling et al[7]. The ultrafiltration process was carried out at a pressure of 199.4 kPa. Spectral measurements were carried out at 25°C at 247nm. The set up is shown in fig.1.

Theoretical Analysis

The following assumptions have been made for both washing and elution phases:

1) Ideal mixing conditions exist within the vessel, thus the concentration polarization of ligand molecule on the membrane surface may be neglected.

2) Since the concentrations of enzymes are very low both membrane fouling and osmotic effect may be neglected.

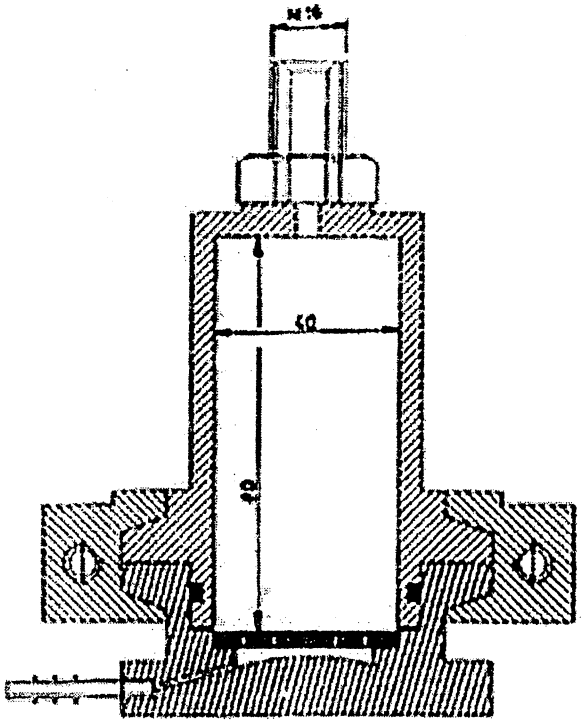

Fig 1. The Schematic representation of AUF with Magnetic Stirring.

Model of the Washing Phase

The washing phase model involves in determining the molar distribution of chymotrypsin, Trysin and STI-Trypsin complex at a particular instant within the module.

By making a material balance for chymotrypsin one gets,

$$\frac{d(n_{EC})}{dt} = -QC_{EC} \qquad (1)$$

An attempt has been nade to predict the molar distribution of trypsin keeping the initial inhibitor concentration sufficiently high with respect to the initial enzyme concentration (160:1) the reaction will naturally to follow a pseudo first order irreversible kinetics represented as,

$$E + L \xrightarrow{k} EL \qquad (1)$$

The rate equation becomes,

$$-r_E = kC_E \qquad (2)$$

The integrated form of the above equation is :

$$\ln \frac{C_{EO}}{C_E} kt \qquad (3)$$

Evidently, a plot of $\ln \frac{C_{EO}}{C_E}$ against t yields a straight line passing through origin and having slope k, for the present investigation, the value of which has been found to be $0.0333s^{-1}$.

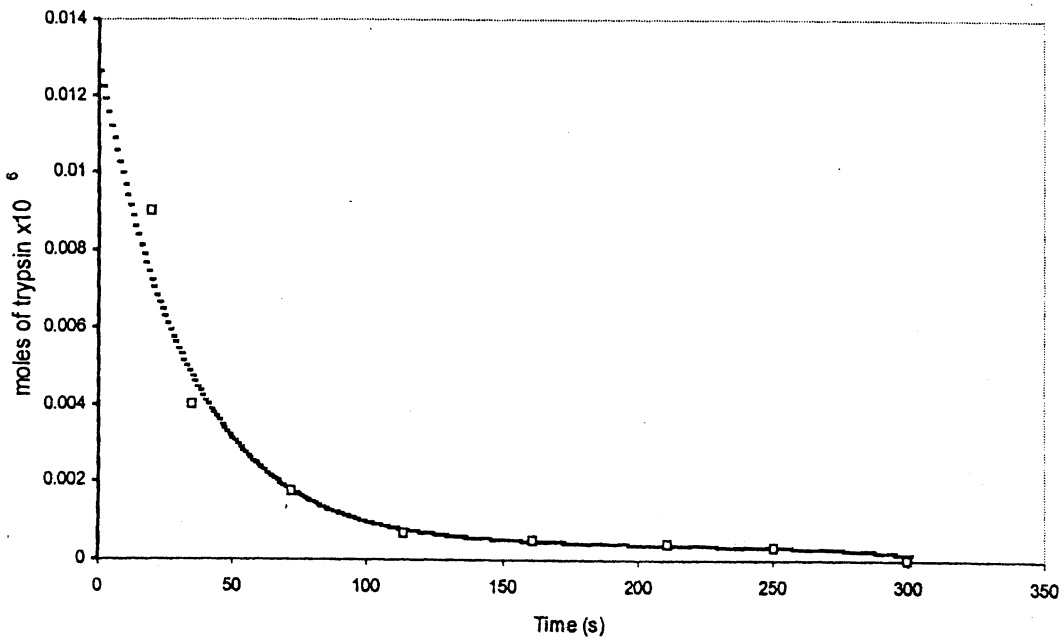

Fig 2. Progress curve of Trupsin (Legend : □ : Experimental Data Points)

The differential material balance of the components E and EL may be written as,

$$E: \frac{dn_E}{dt} - QC_E - V(kC_E) \qquad (4)$$

The initial boundary conditions are at t =0,

$$\begin{vmatrix} J & = & J_o \\ V & = & V_o \\ n_{EC} & = & n_{EC\,o} \\ C_{EC} & = & C_{ECO} \\ n_E & = & n_{EO} \\ C_E & = & C_{Eo} \\ n_{EL} & = & 0 \\ C_{EL} & = & 0 \end{vmatrix}$$

$$EL: \frac{dn_{EL}}{dt} - kVC_E \qquad (5)$$

The first order, first degree differential equations (1), (4) and (5) have been solved by 4th order Runge-Kutta method using a software developed by the present team to predict the dynamics of the system as well as molar distribution of participating bio molecules within the module at a particular instant.

Results and Discussions

The progress curve for trypsin during washing phase are shown in Fig. 2. Both simulated data and the experimental values were plotted. It is evident from the Fig. 2 that the experimental data fit well in the model equation, the correlation coefficient being 0.97. The fact that number of moles of trysin in the module is decreasing with time can be explained by noting that with progress of time gradual cross-linking of trypsin with the inhibitor STI takes place causing decrease of trypsin in the module. In the experimental run cross-linking of trypsin with STI was complete at 160 seconds.

Conclusions

On careful observation of the figure, it is evident that the proposed method of using affinity ultrafiltration for separation bio-molecules from its mixture is feasible. Although in the present modeling concentration polarization effect has been neglected, the model equations are expected to represent the real-time situation, since the concentrations of bio-molecules in clinical waste are so low, that the concentration polarization effect may safely be ignored.

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Nomenclature

C Concentration of biomolecule at any instant(M)
J Volumetric flux for ultrafiltration (m/s)
k Rate const. for pseudo-first order reaction (s^{-1})
n No. of moles of biomolecules at any instant
P Nitrogen pressure (kPa)
Q Volumetric flow rate (m^3/s)
r Rate of reaction of enzyme and STI (M/s)
t Time (s)
V Working volume of the module (ml)

Subscripts

E Enzyme trypsin
EC Enzyme chymotrypsin
EL Trypsin-STI complex-conjugate
O Intial condition (at t=O)

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