1.0 ABSTRACT

Use of immobilized AMG rather than soluble AMG would reduce the conversion time and equipment size on the saccharification of starch or dextrinized starch on an industrial scale. As most of the covalent methods of immobilization of enzymes use organic reagents not acceptable in food industry, physical immobilization of AMG to Amberlite IR-120 and Amberlite IRA-904 was tried.

The isoelectric pH of the AMG from *Aspergillus niger* was between 3.5 and 4.0. The feasibility of Amberlite IR-120 (cation exchanger) immobilized AMG for the hydrolysis of starch was studied at pH 2.5 and 3.3. With increasing concentration of protein from 5.0 to 25.0mg.ml^{-1} added to Amberlite IR-120, the maximum immobilization yields of protein and AMG units were 10 and 11% respectively at pH 3.3. At pH 2.5 immobilization yields of protein and AMG units were 14.6 and 15.1% respectively. Effective distribution coefficient (E.D.C is defined as the total amount of enzyme in aqueous phase divided by the total amount of enzyme in the resin phase at equilibrium at a particular temperature) for AMG units were 10.8 (+/- 3.0) and 6.40 (+/- 0.54) at pH 3.3 and 2.5 respectively. Retentivities of Amberlite IR-120 for AMG activity at 55^{o}C after 5 successive batch reactions (each 10 min. duration) were 1.6 (+/- 0.47) and 2.7 (+/- 0.47) respectively at pH 3.3 and 2.5. The poor retaining capacity of Amberlite

IR-120 for AMG from *A.niger* ruled out the feasibility of Amberlite IR-120 cation exchange resin in the immobilization of AMG.

It is advisable to immobilize an enzyme on an anion exchanger, provided that at suitable operational pH the net charge on the enzyme is negative. Further, macroporous resins like Amberlite IRA-904 could provide higher surface area for enzyme immobilization.

Hence studies were initiated with anion exchanger Amberlite IRA-904. As the isoelectric pH of the enzyme was between 3.5 and 4.0, pH 4.5 was selected for the immobilization. The capacity of Amberlite IRA-904 (1g wet wt) was 42mg protein (493 AMG units) at pH 4.5, giving an immobilization yield of 64% protein and 67.5% AMG units. Effective distribution coefficient at pH 4.5 was 1.18 (+/- 0.76) indicating high potentiality of Amberlite IRA-904 for the immobilization than that of Amberlite IR-120 (E.D.C = 6.40 at pH 2.5). The activity yield (a measure of functional fraction of expected immobilized enzyme activity) at pH 4.5 was 2%, which indicates the poor expression of immobilized AMG activity. Unlike Amberlite IR-120 (at pH 2.5) Amberlite IRA-904 (at pH 4.5) showed good retentivity for the immobilization of AMG 94.4% (+/- 2.3) (at possible operational pHs of the two immobilized enzyme preparations), when repeated batch hydrolysis of starch was performed similar to that of Amborlite IR-120

Relative activities of soluble AMG with starch (2%, W/V), dextrinized starch (2%, W/V; DE 36) and maltose (2% W/V) were 100, 52.2 and 37.5% respectively. The activity yield of Amberlite IRA-904 immobilized AMG were 22.3 and 28.0% with dextrinized starch (2%, W/V; DE 36) and maltose (2%, W/V) respectively. The effectiveness factor which is a measure of diffusional restriction of substrate to active site of an enzyme (E.F = Activity of bead preparation / Activity of ground preparation) molecule was 7.3, 1.6 and 1.1 for (2%, W/V) starch, dextrinized starch and maltose respectively at pH 4.5, indicating the high restriction for diffusion of starch molecules. The amount of Amberlite IRA-904 needed to obtain 97% immobilization yield from 6ml of enzyme solution having 762 AMG units (in 0.01M acetate buffer at pH 4.5 and 55^{o} C) was 2.5g. This proportion of carrier to enzyme ratio was used to prepare the immobilized AMG.

Continuous hydrolysis of (2%, W/V) starch, dextrinized starch (DE 36) and maltose was performed at pH 4.5 using a thermostated (30^{o}C) column reactor (1.7 x 15cm) having 7000 AMG units in 25g carrier. The amount of substrate hydrolysed expressed as glucose yields (glucose yield = glucose produced / glucose produced by acid hydrolysis x 100) were 37, 94 and 90% respectively at a flow rate of 1ml min^{-1}. The productivity, a measure of amount of product formed per hour per unit volume of the reactor under the specified conditions were 3.2, 8.25 and 7.9 g.l.$^{-1}$h^{-1}

respectively with starch, dextrinized starch and maltose. The productivity of the enzyme reactor for 2% (W/V) starch dextrinized starch (DE 36) and maltose increased from 3.2, 8.25 and 7.92 to 10.9, 49.5 and 47.5 respectively when the flow rate was increased from 1 to 6ml min^{-1}, While glucose yield for starch hydrolysis decreased from 37.0 to 20.8%. For the hydrolysis of dextrinized starch (20%, W/V; DE 36) glucose yield decreased from 82.5 (at 1ml min^{-1}) to 55% (at 6ml min^{-1}) at 30^{o}C. The productivities for dextrinized starch solutions 2, 4, 10 and 20% (W/V; DE 36) were 10.9, 98.2, 237.6 and 290.4 $g.l^{-1}$ respectively at a flow rate of 6ml min^{-1}at 30^{o}C.

At 55^{o}C and at a flow rate of 6ml min^{-1} the productivities for starch (2%; W/V) and dextrinized starch of concentrations 2, 4, 10 and 20% (W/V; DE 36) were 18.5, 52.3, 101.9, 250.8 and 353.6 respectively, while the glucose yields for dextrinized starch 2, 4 and 10% (W/V) were 98, 96.5 and 95% respectively. The glucose yield for starch (2%, W/V) and dextrinized starch (20%, W/V) at 55^{o}C decreased from 52 and 90% to 35 and 66.8% respectively when the flow rate was increased from 1 to 6 ml min^{-1}.

Temperature has a negligible effect on the productivity of the column reactor, when dextrinized starch solutions of 2, 4 and 10% (W/V; DE 36) were used. The glucose yields did not change with increase in flow rate up to 6ml min^{-1} (maximum attainable flow rate with the peristaltic pump

available in the laboratory), however productivity increased linearly. Productivities at 30 and 55^{o}C with 20% (W/V; DE 36) dextrinized starch were 290.4 and 353.6 $g.l^{-1}h^{-1}$ respectively. Thus temperature has an influence on the hydrolysis of higher concertrations of dextrinized starch under the experimental conditions.

The glucose yields for dextrinized starch (20%, W/V; DE 36) were 82.5% (at 1 $ml.min^{-1}$) and 55% (at 6 $ml.min^{-1}$) at 30^{o}C. The glucose yields for the same were 90% (at 1ml min^{-1}) and 66% (at 6ml min^{-1}) at 55^{o}C.

Amberlite IRA-904 immobilized AMG can be used for the cotinuous saccharification of dextrinized starch. The flow rate can be adjusted to obtain the desired degree of hydrolysis. At low flow rates high glucose yields are obtained.