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Characterization of Glucoamylase Immobilized on Celite

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ABSTRACT

Immobilization of glucoamylase (EC 3.2.1.3) on Celite R649 biocatalyst carrier for hydrolysis of maltose and maltodextrin has been investigated in both packed bed and recirculated batch reactors. The kinetics parameters on the hydrolysis of maltose were estimated from the packed bed reactor. It is found that this immobilized enzyme is as efficient as the soluble enzyme in catalyzing hydrolysis of maltose. However, it is less efficient than the soluble enzyme in hydrolyzing 30% (w/v) maltodextrin, giving a maximum dextrose equivalent (DE) value of 96.0% instead of 98.2%.

Index Entries: Immobilized enzyme; glucoamylase; celite; hydrolysis of maltose; hydrolysis of maltodextrin; kinetics.

INTRODUCTION

Glucoamylase is one of the enzymes employed in the production of high fructose corn syrups (HFCS) from corn starch. Currently, soluble glucoamylase is being used in the HFCS process to convert oligosaccharides into glucose.

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One alternative that may potentially compete with the current process for hydrolysis of oligosaccharides is the use of immobilized glucoamylase (*1*). The immobilized enzyme can not only prolong the half-life of enzyme but also increase the process efficiency when the packed bed immobilized enzyme reactor is employed for continuous operation. This may result in high enzyme activity/U reactor volume, short reaction time and low capital and energy costs (*2*). Hence, a great number of papers dealing with the use of immobilized glucoamylase for the hydrolysis of oligosaccharides have been published (*3,4*) and some of them were studied in pilot-plant scale reactors (*5,6*).

However, immobilization of enzyme onto porous particles imposes two additional diffusional processes, i.e., external and internal mass transfer, upon the reaction system. This results in the difficulty of diffusion of large oligosaccharide molecules into the carrier and the acceleration of reversed reactions on account of the high glucose concentration inside the pore (*3*). Hence, the final DE value of the sugar solution from the immobilized glucoamylase would be slightly lower than that from the soluble enzyme. To minimize these mass transfer effects, the selection of an inexpensive carrier with a large mean pore-size would help increase the final glucose concentration for this immobilized enzyme system.

The aim of this study is to investigate and evaluate specifically the utilization of Celite R649 (silicon diatomite) particles for immobilization of glucoamylase. Celite is much cheaper than controlled-pore glass beads and it possesses good mechanical properties and high chemical durability. In addition, it possesses a highly porous structure with a mean pore size of 0.25 μm. In this paper, the following tasks are undertaken

1. Immobilization of glucoamylase onto Celite particles at different particle sizes;
2. Kinetics study with maltose as substrate; and
3. Comparison of maximum DE values obtained on the hydrolysis of maltose and maltodextrin by soluble and immobilized glucoamylases.

MATERIALS AND METHODS

Materials

Glucoamylase from *Aspergillus niger* was provided by Finnsugar Biochemicals, Inc. (Spezyme GA200; Elk Grove Village, IL). The protein content of the enzyme solution was determined to be 158 mg/mL by the UV method (at 275 nm) with bovine serum albumin as reference. The specific activity of this enzyme was determined to be 19.30 U/mg of protein by using 30% (w/v) maltose as substrate in acetate buffer. Here, one unit of enzyme activity (U) is defined as the ability to produce 1 μmol of glucose/min at 50°C from the maltose solution in 0.06 *M* acetate buffer (pH 4.5).

Celite R649, obtained from Manville Sales Corporation (Denver, CO), was used for enzyme immobilization. This carrier has mean pore size of 2500 Å and a surface area of 39 m^2/g. Original mixture of Celite R649 was screened to obtain the following three particle sizes (mean diameter): 165 μm, 275 μm, and 365 μm.

Either Maltose (Grade I, Sigma Co.) or maltodextrin (Maltrin-M100, Grain Processing Corp.) was used as substrate in all experiments.

Method of Immobilization

The carrier (6.5 g) was treated with 5% HNO_3 solution (25 mL/g Celite) at 75°C for three h and then washed three times with 150 mL of distilled water. A 3% (v/v) γ-aminopropyltriethoxysilane solution (25 mL/g Celite) was treated batchwise with Celite for 3.5 h at 75°C. After washing three times with 150 mL of distilled water, a solution of 2.5% (w/v) glutaraldehyde in 0.1 *M* phosphate buffer at pH 7.0 was added and in shaker for one hour at 25°C. The sample was then washed with distilled water twice at room temperature and left in 150 mL of distilled water in the refrigerator overnight (*7*).

Glucoamylase solution (2.5 mg protein/mL) was added to the activated Celite particles in the amount of 60 mg protein/g Celite and was treated with particles for 3 h at 35°C. Enzyme concentration in the bulk solution was monitored by measuring the UV absorbance at 275 nm. After immobilization, Celite particles were washed with 5% (w/v) maltose solution (150 mL), 6 *M* urea solution (150 mL), and 1 *M* acetate buffer (150 mL), NaCl solution (150 mL) and again with 5% (w/v) maltose solution (150 mL) several times. The total amount of enzyme washed out by these posttreatment steps was also determined spectrophotometrically.

Determination of Enzyme Loading and Activity

The amount of enzyme attached, physically and covalently, to the carrier was determined by material balance according to the amount of enzyme in the bulk solution before and after the immobilization. The amount of enzyme covalently attached to the carrier was also determined by taking into account the enzyme loss during the posttreatment after the immobilization, and the results are as follows: 165 μm, 29.4 mg/g Celite; 275 μm, 23.4 mg/g Celite; 365 μm, 22.6 mg/g Celite.

The activities of immobilized enzyme particles were determined by passing the maltose solution (2–30%, w/v) continuously through a packed bed reactor. The reactor was made of stainless steel (0.515 cm × 10.0 cm) and loaded with 0.75–0.85 grams of immobilized Celite particles. The glucose concentration in the effluent was analyzed by using a YSI-27 glucose analyzer (YSI Inc.; Yellow Springs, OH). When maltodextrin is used, a recirculated batch process was employed in order to determine the final DE value of the sugar solution. This process was slightly modified from continuous packed-bed operation by adding a reservoir between the

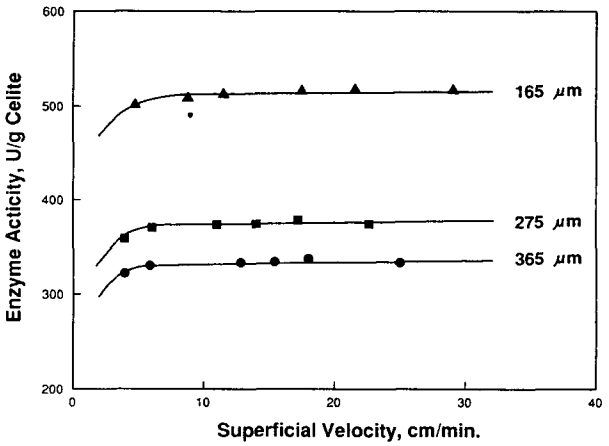

Fig. 1. Dependence of activity of immobilized glucoamylase on the liquid flow rate with T=50°C, pH=4.5, and 30% (w/v) maltose.

packed bed reactor and the recirculation pump. Maltose and other oligosaccharides were analyzed by a high performance liquid chromatograph (Waters, Inc.) equipped with a refractive index detector and an Aminex Carbohydrate HPX-42A column (Bio-Rad Laboratories; Richmond, CA) operated at 85°C.

RESULTS AND DISCUSSION

Hydrolysis of Maltose

Dependence of Enzyme Activity on Flow Rate

The effect of flow rate on the conversion of maltose to glucose was investigated in order to determine conditions under which external mass transfer resistance can be neglected. The result is shown in Fig. 1 for three different particle sizes. It can be seen that the conversion increases with flow rate and levels off at higher flow rates, suggesting that the liquid film resistance can be neglected if the superficial liquid velocity is above 5–10 cm/min. Therefore, a superficial velocity of 20 cm/min was used for the rest of experiments to eliminate the liquid film resistance.

Dependence of Enzyme Activity on Temperature

The effect of reaction temperature on the activity of immobilized glucoamylase was studied in the range of 35 to 75°C with maltose (30%, w/v) as substrate. The Arrhenius plots for free and immobilized glucoamylases are shown in Fig. 2. The activation energies determined by linear regression for three Celite particles are listed in Table 1, that shows that the activation energies are almost immaterial to the particle sizes and they are slightly lower than that of soluble enzyme. However, the difference of

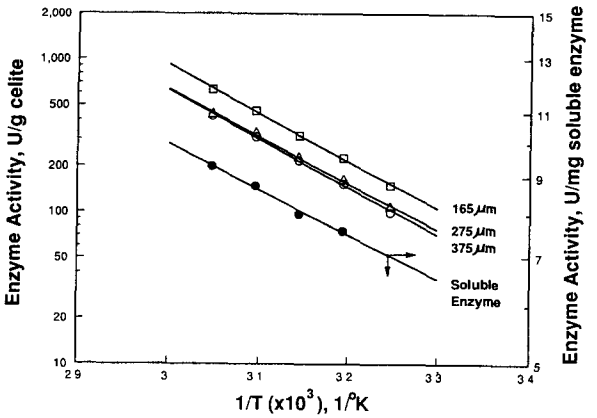

Fig. 2. Arrhenius plots for soluble and immobilized glucoamylases with pH=4.5 and 30% (w/v) maltose.

Table 1
Kinetic Parameters for Soluble and Immobilized Glucoamylases at 50°C and pH 4.50 with Maltose

	Soluble Enzyme	Immobilized Enzyme (Celite 165 μm)	Immobilized Enzyme (Celite 275 μm)	Immobilized Enzyme (Celite 365 μm)
K_M, % w/v Maltose	0.14	0.28	0.27	0.27
V_{max} μmole glucose/min/mg enzyme	19.30	16.35	14.37	13.28
E_A, kcal/mole	15.25	14.22	14.11	14.54

activation energies between soluble and immobilized enzymes is within 7.5%. Such a small discrepancy suggests that the intraparticle diffusion resistance is not the rate-limiting step for this particular reaction system. Hence, the use of Celite R649 on the hydrolysis of maltose removes limitation of intraparticle diffusion on the overall rate of reaction in the cases we investigated.

Kinetics of Immobilized Enzyme and Parameter Estimation

The above studies show that the rate controlling step can be the reaction itself if the experimental conditions are well controlled. In such a case, the equation proposed by Ber-Eli and Katchalski (*8*) and Lilly et al. (*9*), for plug flow reactor can be directly applied.

$$FXS_o = K_M Fln(1-X) + V_{max}V \quad (1)$$

in which S_o, is the inlet substrate concentration, X is the conversion of the reaction at the column exit, F is the volumetric flow rate, K_M is Michaelis constant, V_{max} is the maximum activity and V is the effective reactor volume.

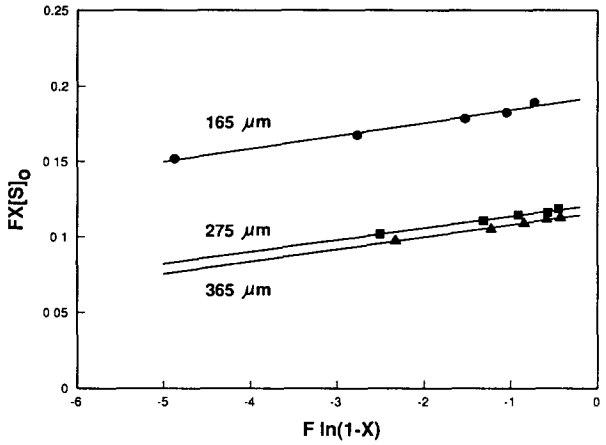

Fig. 3. FXS_0 vs. Fln(1-X) plot for three sizes of Celite particles with pH=4.5 and T=50°C.

Eq. (1) shows that a plot of FXS_0 against Fln(1-X) should have a straight line of which the slope is equal to K_M and the intercept is equal to $V_{max}V$. Figure 3 gives such a plot for three Celite particles and the parameters obtained are listed in Table 1. The result shows that K_M is independent of particle size and its value is twice as large as the soluble enzyme, indicating that the association ability for the formation of enzyme-substrate complex is reduced after immobilization. The specific maximum rate of reaction is also smaller than the soluble enzyme by a factor of 0.15 to 0.30 for particle size 165 μm, 275 μm, and 365 μm, respectively.

The validity of neglection of mass transfer resistances in this immobilized enzyme system was further verified using a mathematical model that considers both liquid-solid mass transfer and intraparticle diffusion (*11, 12*). The simulation was carried out on a DEC VAX 11/780 machine using the kinetic parameters listed in Table 1 and estimated mass transfer parameters. We found that the effectiveness factor, which is based on the substrate concentration on the outer surface of the particle, is always higher than 0.97 in each case we simulated. This concludes that the mass transfer resistances are indeed negligible in this case.

Batchwise Operation

Maltose (DE=48%) was hydrolyzed by free and immobilized glucoamylases using batch and recirculated batch processes, respectively. The experimental conditions were adjusted so that the initial specific enzyme activities in both cases were the same. The DE profiles for both soluble and immobilized enzymes are shown in Fig. 4. It can be seen that there is no difference on the DE profiles for soluble and immobilized glucoamylases, further confirming that the immobilized enzyme system we studied is not rate-limited by external mass transfer and intraparticle diffusion.

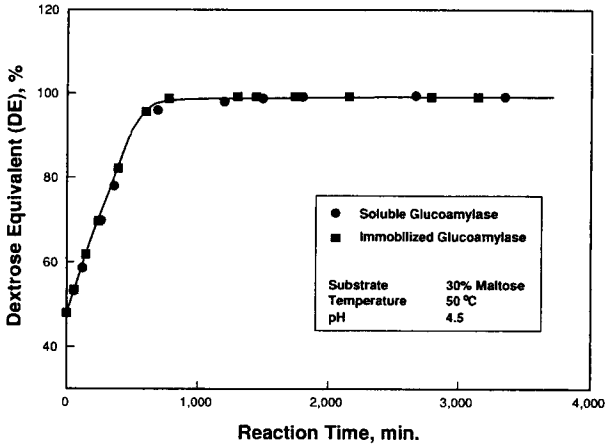

Fig. 4. Dextrose equivalent (DE) profiles on the hydrolysis of maltose with an initial enzyme activity of 12 U/g maltose for both soluble and immobilized glucoamylases.

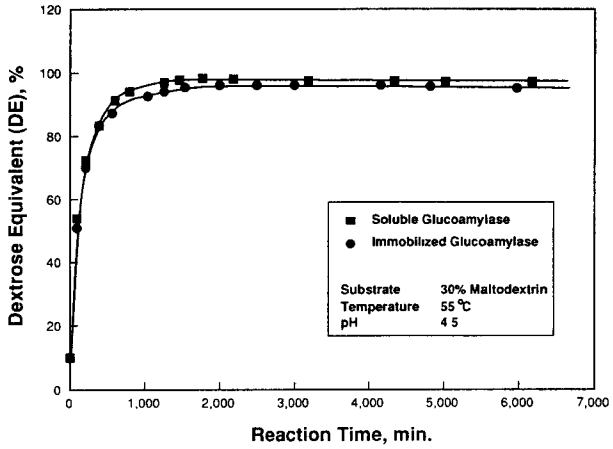

Fig. 5. Dextrose equivalent (DE) profiles on the hydrolysis of maltodextrin with an initial enzyme activity of 12 U/g maltose both soluble and immobilized glucoamylases.

Hydrolysis of Maltodextrin

The experiments were also conducted in batch and recirculated differential batch reactors for soluble and immobilized glucoamylases, respectively. Similarly, the experimental conditions were adjusted to have the same initial specific enzyme activities in both cases. The results are given in Fig. 5, which show that the DE values for soluble and immobilized enzymes are the same in the first 10 h; however, the DE values of soluble enzyme is higher than the immobilized enzyme as the reaction goes on. It also shows that in both cases the DE values reach maxima within 1600 to

2200 min and then drop slightly thereafter because of reversed reactions to convert glucose to maltose, isomaltose and others. The maximum DE values for soluble and immobilized glucoamylases are 98.2 and 96.0%, respectively.

The HPLC chromatograms of the hydrolysis samples from soluble and immobilized enzymes were compared. The HPLC column is capable of distinguishing molecules with less than 12 glucopyranose units and other larger oligosaccharides are lumped to a peak called G_{max}. The results show that there is no G_{max} peak for soluble enzyme after 55 h; however, this peak is still evident after 93 h when the immobilized enzyme is employed. This clearly shows that the catalytic ability of this immobilized enzyme to small oligosaccharide molecules is as efficient as that of soluble enzyme; however, its ability to hydrolyze large oligosaccharide molecules is much less efficient than the soluble enzyme. Such a discrepancy may result from the following reasons:

1. The intraparticle diffusion resistance increases when the size of oligosaccharide molecule increases;
2. The accessibility of large oligosaccharide molecules to the active site of the immobilized enzyme is retarded by the limited space inside the pore; and
3. The capability to form immobilized enzyme-substrate complex is much less than that of the soluble enzyme when large oligosaccharide molecules are used as substrate.

CONCLUSIONS

In this study, a cheap carrier, Celite R649, was chosen for immobilization of glucoamylase. The result is comparable to those best results using controlled pore glass beads and DEAE-cellulose as biocarriers (*6,10*). On the hydrolysis of maltose, the same maximum DE values (98.8%) were obtained for free and immobilized glucoamylases. On the hydrolysis of maltodextrin, the final DE value for immobilized enzyme (96.0%) is lower than that of soluble enzyme (98.2%).

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