Immobilized α-Galactosidase in the Biochemistry Laboratory W

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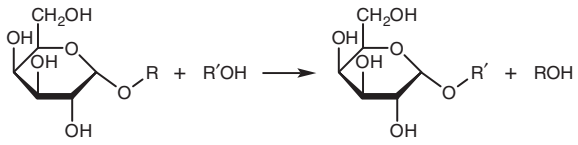
Enzymes are natural catalysts and their ability to catalyze biochemical reactions under mild conditions in a highly specific and efficient manner has led to interest in their exploitation as industrial catalysts. Enzyme processing traditionally has been accomplished using soluble enzymes. But soluble enzymes are not economical because they must be used for single operation since conventional recovery methods are either expensive or cause denaturation and loss of catalytic activity. To improve the stability of enzymes, an inexpensive non-destructive recovery method is needed. One of the major activities in the field of biotechnology over the past two decades has been the immobilization of enzymes. Enzyme immobilization is defined as "the enzyme physically confined or localized in a certain defined region of space with retention of its catalytic activity, which can be used repeatedly and continuously" *(1).* Many methods exist for the immobilization of the enzyme, but usually one of four methods is used: entrapment, physical binding, adsorption, and cross-linking. For industrial applications, covalent binding, adsorption, and cross-linking methods are extensively used because of stability of the immobilization *(2).*

Many enzymes are used in industry. Among them, α-galactosidase (α-D-galactoside galactohydrolase; EC 3.2.1.22) has drawn great attention in the food industry because of its potential applications. α-Galactosidase is widely distributed among microorganisms, plants, and animals and is able to catalyze the hydrolysis of a variety of simple galactosides as well as more complex polysaccharides such as galactomannan and glycoconjugates (e.g., glycoproteins and glycolipids). The raffinose family oligosaccharides consist of linear chains of galactopyranosyl residues attached to the glucose moiety of sucrose via an α(1→6)-galactopyranosidic linkage *(3).* α-Galactosidase is potentially important in the hydrolysis of raffinose family oligosaccharides (raffinose, stachyose, verbascose, and ajugose) of pulses. Pulses are defined by the Food Agricultural Organization of the United Nations (FAO) as annual leguminous crop yielding from one to twelve grains of seeds of variable size, shape, and color within a pod. α-Galactosidase catalyzes the following reaction:



The hydroxyl acceptor molecule R′OH is commonly water, although R and R′ can be aliphatic or aromatic groups.

The structural relationship between raffinose family sugars is represented in Figure 1. Legumes are good sources of protein required for the daily diet, but very poor sources compared with meat, fowl, and fish *(4).* Among the leguminous plant seed, chickpea (*Ciceratietinum*), red gram (*Cajanus cajan*), soybean (*Glycine max*), black gram (*Phaseolus mungo*), cowpea (*Vigna unguiculata*), horse gram (*Dilichos biffarus*), and dry beans (*Phaseolus valgaris*) are known to contain antinutritional factors including oligosaccharides of the raffinose family sugars. Since human intestinal juice lacks α-galactosidase these raffinose family sugars are not digested and as a result they are passed in to the large intestine where intestinal micro flora act on them leading to intestinal discomfort and flatulence *(5).* Soymilk whose raffinose sugars have been hydrolyzed can be an alternative for lactose intolerant individuals and nutrient supplement for daily diet.

Immobilized enzyme experiments are not found in most biochemistry laboratory textbooks, although Boyer does include an experiment using immobilized peroxidase *(6).* Some articles have appeared in the literature that describe the use of immobilized enzymes in undergraduate laboratories *(7, 8).* A previous report in the literature describes polyacrylamide as a matrix to teach enzyme immobilization in biochemistry laboratory *(9),* but this involves the use of neurotoxins. We have selected chitosan as a matrix because of its promising characteristics including hydrophilicity, lack of toxicity, low cost, and antimicrobial activity. Chitosan is a polysaccharide mainly made up of 2-amino-2-deoxy-glucose units that are joined by β(1→4) linkages (Figure 2). Glutaraldehyde was used as cross-linking agent. One end of the glutaraldehyde combines with the NH_2 groups of chitosan and the other aldehyde group binds the enzyme resulting in formation of covalent bonds. More than 70% of the enzyme was immobilized on chitosan.

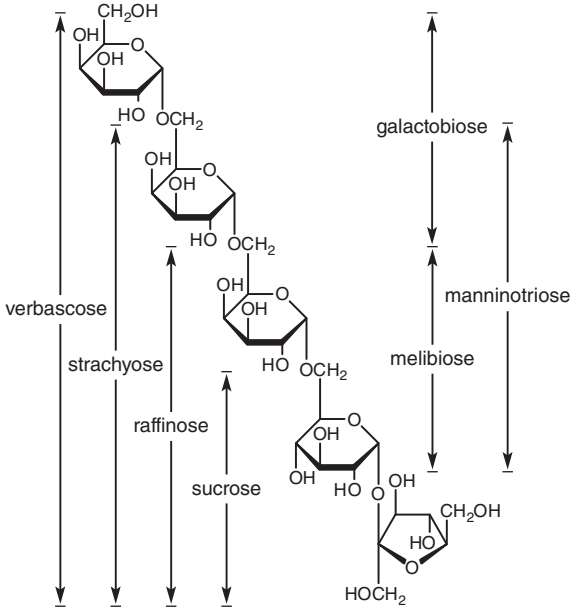


Figure 1. Structural relationship between raffinose family sugars.

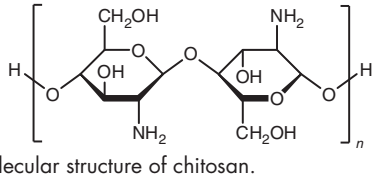


Figure 2. Molecular structure of chitosan.

Experimental Overview

The experiment was conducted during last few weeks of a postgraduate biochemistry laboratory course. Students, working in pairs, conducted the lab experiment at various time slots during a one-week period. They isolated the α-galactosidase from *Aspergillus oryzae* and then immobilized the α-galactosidase on the cross-linked chitosan. Students prepared the soymilk by the method outlined by Mulimani and Ramalingam *(10).* The performance of the free and immobilized α-galactosidase was tested in a stirred batch reactor. The ratio of oligosaccharides and monosaccharides was determined by TLC. Details of the experiment are in the Supplemental Material.[W]

Hazards

Hydrochloric acid and glutaraldehyde are harmful if swallowed, cause moderate eye irritation, and may cause respiratory, digestive tract, and skin irritation. The spraying reagent (α-naphthol) is a respiratory irritant. The solvent system (propanol:ethyl acetate:water) fumes are irritating; the apparatus has to be installed in a fume hood with efficient ventilation and a protective shield.

Results and Discussion

Student data from the hydrolysis of raffinose family sugars by free and immobilized α-galactosidase at different time intervals are shown in Figure 3. After 3 h incubation raffinose family sugars were hydrolyzed to 79% and 53% by free and immobilized α-galactosidase, respectively. After 6 h incubation soluble α-galactosidase led to 88% hydrolysis, whereas immobilized enzyme resulted in 73% reduction of raffinose family sugars. After 12 h incubation, hydrolysis of raffinose family sugar was 91% and 75% by free and immobilized enzyme, respectively. The data indicate that prolonged incubation does not significantly increase the percent hydrolysis. Students thus suggested that one could save time just by incubating for 6 h. For more advanced students, these methods could be used for the detailed investigations of enzyme stability, substrate specificity, pH, temperature, and repeated use of immobilized enzyme. Students gain an appreciation for the real-world applications of enzymes in food industries.

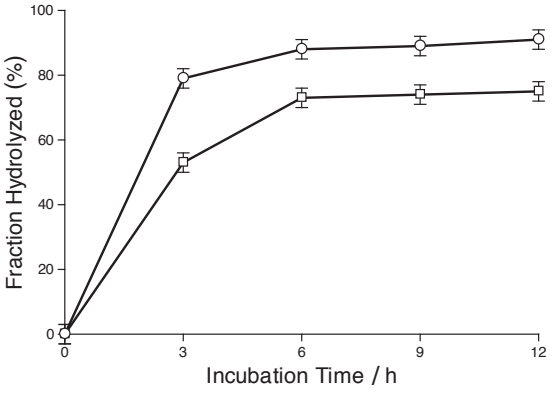


Figure 3. Hydrolysis of raffinose family sugars after treatment with free (○) and immobilized (□) α-galactosidase at different incubation periods.

There are points during the experiments at which students are not very busy, such as during the incubation for hydrolysis of oligosaccharides by the immobilized enzyme. This time can be used to discuss the immobilization techniques that could be used in the food industry and the reaction between chitosan and glutaraldehyde. The students found this experiment to be one of their favorite during the semester. The experimental results submitted by the students are assessed, based upon their understanding the basis of the experiment, as judged by the discussion of results and literature reports.

Conclusions

The experiment describes an inexpensive and versatile method for demonstrating enzyme immobilization. The reagents are classified as safe for use in the food industry.[1] This experiment is easy to set up and provides a good introduction to immobilization for undergraduate and graduate students of biochemistry, biotechnology, chemical engineering, and food technology students. The main pedagogic benefit of the experiment was to teach the basic concept of enzyme immobilization used for an industrial purpose.

Acknowledgment

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[W]Supplemental Material

Directions for the students and notes for the instructors are available in this issue of *JCE Online.*

Note

1. *Aspergillus oryzae* has GRAS (generally regarded as safe) status is considered as an excellent host for the safe production of harmless products *(11)* and chitosan can be used safely in food industry *(12).*

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