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BLACK AND WHITE LAHAR AS INORGANIC SUPPORT FOR THE IMMOBILIZATION OF YEAST INVERTASE

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ABSTRACT

Volcanic ejecta or lahar can serve as an inorganic support for the immobilization of invertase. Pampanga and Bicol lahar samples were pretreated by ignition at 550°C for 5 hrs followed by concentrated hydrochloric acid treatment, activated by reaction with aminopropyltriethoxysilane (APTS) and then covalently bound to invertase using glutaraldehyde as linker. Chemical tests confirmed the attachment of APTS to lahar and glutaraldehyde to silanized lahar. The quantity of immobilized invertase on Pampanga white, Pampanga black and Bicol black lahar were 98.73%, 96.73% and 84.27%, respectively. Conditions for maximum activity of invertase immobilized on Pampanga white lahar were pH 3.5, 45°C and 0.3 M sucrose concentration. The K_m and V_{max} for free invertase and immobilized invertase on Pampanga white lahar were 2.37 M and 48.75 mmol/min, and 3.88 M and 38.87 mmol/min, respectively. Invertase bound to Pampanga white lahar was most stable towards repeated and continuous use and towards storage with intermittent use as indicated by its relatively greater activity.

1. INTRODUCTION

Table sugar, the most abundant food sugar and sweetening agent, is 99.9% sucrose. The high cost of sucrose has allowed the incursion of alternative sweeteners like invert sugar [1], an equimolar mixture of glucose and fructose that can be obtained by a one step hydrolysis of sucrose catalyzed by invertase (β-fructofuranosidase, EC 3.2.1.26). It is 1.4 times sweeter than sucrose thus its greater demand in the food industry such as the soft drinks and confectionery industries. In the latter, invert sugar is used to prevent crystallization and grittiness.

The conversion of sucrose to invert sugar, a high-value product, through hydrolysis by invertase is quite justifiable. Immobilization of invertase on porous solid supports is a practical approach to the production of invert sugar. Enzyme immobilization may be accomplished through the attachment of the protein portion of the enzyme either by physical or chemical means onto an insoluble solid support thereby restricting enzyme mobility. Immobilized enzymes have numerous advantages over free enzymes, thus, are more attractive for processes on the industrial scale. First, the enzyme can be easily separated from the product. Second, there is improved

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stability and retention because the enzyme is localized within a reactor. Third, continuous operation over extended periods is possible and superactivity may be attained [2].

Due to its greater thermostability and reusability, porous glass that is composed primarily of silicon dioxide, SiO_2, has been the most common inorganic support used in immobilization through covalent bonding [3 - 6]. However, a major drawback in the use of porous glass is its expense. Thus, it would be advantageous to use a porous glass substitute that contains the same major component. Lahar, the result of mudflows of monsoon rains on volcanic ejecta, can be a good inorganic support substitute. Lahar is in abundance and may be acquired without any cost. It has different chemical and physical properties from volcanic ash due to the amalgamation and sedimentation of different ashes and sediments including geological deposits [7]. Chemical analyses of lahar from Mt. Pinatubo [8, 9] and from Mt. Mayon in Bicol [10] showed that the major component of lahar is SiO_2 ranging at 58 - 72%. Lahar, therefore, possesses the necessary functional group for reactions that will activate its surface to allow it to react with a bifunctional reagent or linker like glutaraldehyde. The linker could then react with the enzyme and thus covalently link it to the solid support. Mendoza [11] covalently immobilized invertase on lahar. Studies on the immobilization of invertase on nata de coco, a locally available material, have also been done [12 - 14].

The study aimed to compare the performance of black and white lahar as inorganic solid support in the immobilization of invertase and to relate observed differences to physical structural variations of the raw materials. Kinetic parameters of invertase immobilized on white lahar were compared to those of the free enzyme.

2. MATERIALS AND METHODS

Lahar samples from Mt. Pinatubo and Mt. Mayon were obtained from Pampanga and Bicol, respectively. Crude invertase from baker's yeast was obtained from Sigma Chemical Co., Mo. USA and analytical grade sucrose was used as substrate.

2.1 Immobilization of Invertase

The procedure for immobilization of invertase was based on the method developed by Mendoza [11].

Pretreatment of lahar samples. Approximately 500 g of previously washed, dried, sieved (40-mesh) and ignited lahar samples were treated with 500 mL concentrated HCl with intermittent stirring for 5 hrs. The white lahar that settled as a top layer was manually separated from the black bottom layer. The separated samples were then washed several times by suction filtration.

Derivatization of lahar solid support. The pretreated lahar samples were reacted with APTS (10% v/v) with pH adjusted to 3.0 - 4.0 with concentrated HCl. The reaction mixture was heated at 75^{o}C for 2 hrs with stirring. The silanized lahar was cooled, washed with excess distilled water and dried at 115^{o}C for 2 hrs. Activation was done by suspending the silanized lahar in 2.5% glutaraldehyde solution in 0.05 M phosphate buffer (pH 7) for at least 1 hr. The activated lahar was washed with excess distilled water and dried.

Covalent linking of invertase to derivatized lahar. The activated lahar was mixed with 0.05 M phosphate buffer (pH 6.5) and pre-equilibrated at 5 - 10^{o}C. An invertase solution (1 mg/mL) was added and the mixture stirred for 3 - 4 hrs. The mixture was filtered and the filtrate collected. The residue was washed twice with 10 mL acetate buffer and the washings collected.

Determination of amount of immobilized invertase. The amount of immobilized invertase was determined by taking the difference between the initial protein concentration used for

immobilization and the protein content of the combined filtrate and washings. Protein content was determined by the Bradford method [15].

Enzyme assay. The immobilized invertase was packed into a column and a 0.30 M sucrose solution was passed through for inversion. The free invertase was mixed with 50 mL 0.30 M sucrose solution in 0.10 M acetate buffer (pH 4.5) at 45^{o}C and incubated at the same temperature. Invertase activity was assayed by determining the amount of reducing sugars liberated by the enzyme from the sucrose solution using the Nelson-Somogyi method [16].

One unit (U) of invertase was defined as the amount of enzyme that hydrolyzed 1 μmole of sucrose in 1 min in 0.10 M acetate buffer (pH 4.5) at 45^{o}C. Specific activity of the immobilized invertase was expressed as one unit invertase per mg lahar bound protein (U mg^{-1} protein).

2.2 Verification of covalent attachments of activating reagent and linker

The Hinsberg and nitrous acid tests were used to determine the covalent attachment of APTS to lahar while the attachment of glutaraldehyde was verified by the Benedict's and Tollen's tests. FT-IR analyses were also conducted to further verify the attachments of the reagents.

2.3 Scanning electron microscopy of acid treated lahar

Black and white lahar were subjected to scanning electron microscopy (SEM) to examine the physical attributes of the particles. To obtain electron micrographs, dried lahar was coated directly with gold by mounting on an aluminum stub. The coating process was done using an Ion sputter (JFC 1100) for several minutes at 1.5 kV. Using a Hitachi S-510 scanning electron microscope, the coated samples were immediately subjected to SEM imaging and photomicrography because the samples were prone to charging-up, that is, the gold coating was easily removed because of the silicates in the sample.

2.4 Optimization of conditions for maximum activity of immobilized invertase

Pampanga white lahar (PWL) was used as the inorganic solid support to optimize conditions for maximum activity of immobilized invertase. Activity of the immobilized enzyme was compared with that of the free enzyme.

Optimum pH. Enzyme activity was determined using different sucrose concentrations at 45^{o}C at varying pH. The pH at which the highest enzyme activity was measured was considered the optimum pH.

Optimum temperature. Enzyme activity was measured using different sucrose concentrations at optimum pH but at varying temperature. The optimum temperature was that at which the highest enzyme activity was obtained.

Optimum substrate concentration. The activity of the enzyme was determined at optimum temperature and pH at different sucrose concentrations. The sucrose concentration at which the highest enzyme activity was measured was considered the optimum concentration.

2.5 Determination of invertase stability on immobilization

The effect of continuous and repeated use as well as storage with intermittent use on the activity of the immobilized enzyme was determined using the optimum conditions obtained for PWL-immobilized invertase. One-gram samples of lahar with the immobilized enzyme were packed into columns.

Effect of repeated and continuous use on the stability of immobilized invertase. The stability of immobilized invertase was determined on repeated and continuous use by measuring enzyme activity at 2-hr intervals over a period of 10 hrs. The determination was repeated for 5 days. The evaluation was conducted at room temperature (RT) by continuously feeding the column with 0.30 M sucrose solution in 0.10 M acetate buffer (pH 4.5) with a flow rate of 0.5 mL per min. After use, the column was washed and stored in 0.10 M acetate buffer. Prior to use, the column was again washed with buffer. The amount of immobilized enzyme on the carrier after each use was also determined by taking the difference between the initial amount of invertase immobilized and the protein content of the combined eluate and washings. The Bradford method [15] of protein determination was used in the analysis.

Effect of storage and intermittent use on the stability of immobilized invertase. A 0.30 M sucrose solution in acetate buffer (pH 4.5) was passed through the column continuously for 10 hrs, with aliquots collected every 2 hrs and assayed for activity. The immobilized enzyme was stored in 0.10 M acetate buffer (pH 4.5) at 5 - 10^{o}C for 4 weeks and its activity determined weekly.

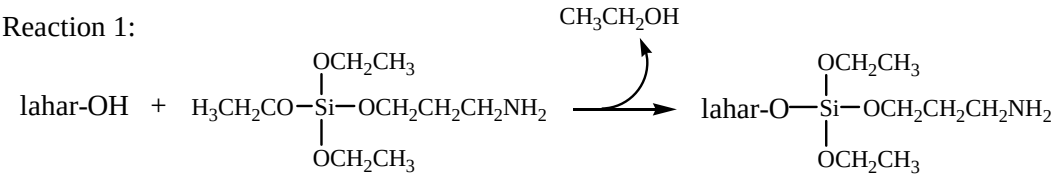
3. RESULTS AND DISCUSSION

3.1 Immobilization of Invertase

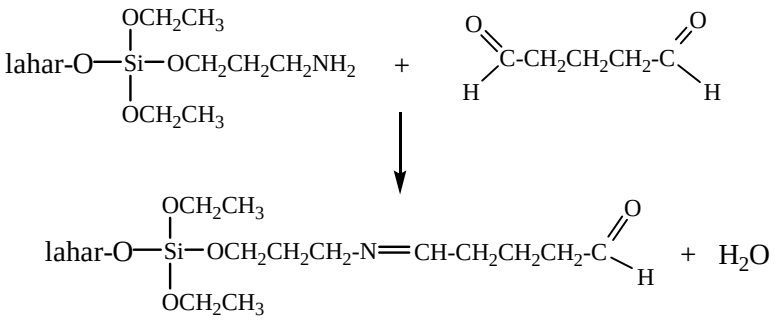
Lahar samples, washed and sieved, were ignited in a furnace to eliminate any organic material present. Further removal of other substances that may interfere with the immobilization process was done by treatment with HCl. Acid treatment separated the Pampanga lahar into a black and a white component. The white component formed the top layer and the black component the bottom layer. This observation may imply that white lahar was less dense than black lahar. The Bicol sample consisted only of black lahar. In this paper, the Pampanga lahar components are referred to as Pampanga white lahar (PWL) and Pampanga black lahar (PBL) while the Bicol sample as Bicol black lahar (BBL).

Activation of lahar. The most crucial phase in the immobilization process is activation. It involves two steps: silanization and the addition of glutaraldehyde. The bifunctionality of APTS is advantageous because its inorganic group readily attaches to lahar leaving its amino group unreacted. The reactions involved in activation are as follows:

Reaction 1:



Reaction 2:



Reaction 1 is the silanization reaction wherein the hydroxyl groups on the surface of lahar particles react with APTS. This provides amino groups to which the linker, glutaraldehyde, may covalently bind, as shown in Reaction 2. The free aldehyde group of glutaraldehyde may then react with amino groups on the side chains of invertase. Monsan et al. [17] reported that lysine residues of ribonuclease reacted with a polyglutaraldehyde carrier.

The adjustment of pH to 3 - 4 using concentrated HCl upon treatment of the ignited lahar with APTS was required for the catalytic action of the hydronium ion in Reaction 1 [11]. Upon reaction of glutaraldehyde with the silanized lahar the pH of the mixture had to be adjusted to 7 to prevent protonation of the free amine. It also prevented the precipitation of the linking reagent through aldol condensation at higher pH [17].

Linkage of activating agent and linker to lahar. Chemical tests to determine the covalent attachment of APTS and glutaraldehyde were conducted as described by Mendoza [11].

Silanized samples were subjected to tests involving its free amino group. The Hinsberg and nitrous acid tests indicated the attachment of silane to lahar. The recovery of crystals of benzenesulfonamide in the Hinsberg's test was a positive test for the attachment of APTS to the lahar. Benzenesulfonyl chloride reacts with primary amines in basic solution and the product upon acidification forms insoluble needle-like crystals of benzenesulfonamide [18]. This reagent can react with the primary amine group of APTS. Further confirmation of this attachment was the evolution of nitrogen gas in the nitrous acid test because the reaction of amines with nitrous acid produces nitrogen gas.

The covalent attachment of glutaraldehyde to silanized lahar was verified using Benedict's and Tollen's tests. Benedict's solution, which contains copper bound in a complex anion, reacts with aldehydes and functions as a selective oxidizing agent. The appearance of a red precipitate is a positive result [18]. The Tollen's test further confirmed the attachment of glutaraldehyde on silanized lahar. In this test, the aldehyde is oxidized to an acid and silver is reduced from Ag^{+1} to Ag^{o}, the latter deposits as a silver mirror or colloidal silver in the reaction vessel.

The three lahar samples were subjected to the above tests. Results are summarized in Table 1.

Table 1: *Results of confirmatory tests for the attachment of silane and glutaraldehyde to lahar*

Tests	Derivatized Lahar			Acid-washed Lahar		
	PWL	PWL	BBL	PWL	PBL	BBL
For silane attachment						
Hinsberg's test	+	+	+	-	-	-
Nitrous acid test	+	+	+	-	-	-
For glutaraldehyde attachment						
Benedict's test	+	+	+	-	-	-
Tollen's test	+	+	+	-	-	-

Legend: + Positive result PWL – Pampanga white lahar BBL– Bicol black lahar
- Negative result PBL – Pampanga black lahar

IR analysis further confirmed the attachment of APTS and glutaraldehyde to lahar. The decreasing intensity of the hydroxyl band in the 3000 - 3500 nm region in the IR spectra was an important parameter to consider. This implied that hydroxyl groups in lahar were used up suggesting that the activating agents were successfully attached. Bands appearing in the 1000 - 1100 nm region may be attributed to oxygen containing molecules in the lahar samples. Bands appearing in the 750 - 800 nm region indicated a Si-C stretch arising from the attached APTS.

Attachment of invertase to activated lahar samples. Immobilization of invertase to lahar was done after successfully attaching the activating agents to the lahar samples. Coupling of the invertase solution was done for a minimum of three hours. This length of time was assumed to be sufficient for the invertase to bind to lahar [11].

The quantity of enzyme immobilized on lahar (Table 2) was determined using the Bradford method [15]. PWL had the largest amount of invertase immobilized implying that there are more sites for attachment of the enzyme on the carrier. A porous structure would provide a greater surface area and therefore more sites for attachment. Of the black samples, PBL had more invertase immobilized than BBL. Again, this implies the presence of a greater number of binding sites in the former than in the latter.

Table 2: *Amount of invertase immobilized on lahar*

Lahar Samples	Immobilized Invertase (%)*
Pampanga White	98.73
Pampanga Black	94.73
Bicol Black	84.27

*Mean of four replicates.

Scanning electron microscopy of the lahar samples supports these observations. The PWL particles (Fig. 1) have a rough surface with deep crevices and pores indicating a more porous structure. This structure would provide more possible sites for activation, thus, more points of attachment of invertase. Although the SEM micrographs for PBL (Fig. 2) show a comparatively porous surface as the PWL, this is not the characteristic of all particles. In fact, not all PBL particles had rough surfaces. BBL particles (Fig. 3) were observed to have the least number of pores of the three samples. Most particles showed smooth surfaces.

3.2 Optimum conditions for maximum activity of immobilized invertase

Catalytic activity of enzymes depends on the maintenance of their native structure. Any slight alteration in structure may cause a significant change in its catalytic activity [19]. Changes in enzymatic properties after immobilization may be brought about by changes in the enzyme itself such as modification of the amino acid residues in the active site, conformational changes and changes in charge, thereby altering the activity of the enzyme. The physical and chemical properties of the solid support used for immobilization can also change the properties of the enzyme. Formation of diffusion layers around the immobilized enzyme, steric hindrance effects and electrostatic attraction between carrier and substrate may also cause changes in activity. According to Chibata [20], observed changes in enzymatic properties result from complicated interactions of these factors, and it is difficult to determine a definite effect of a given factor in terms of changes in enzymatic properties.

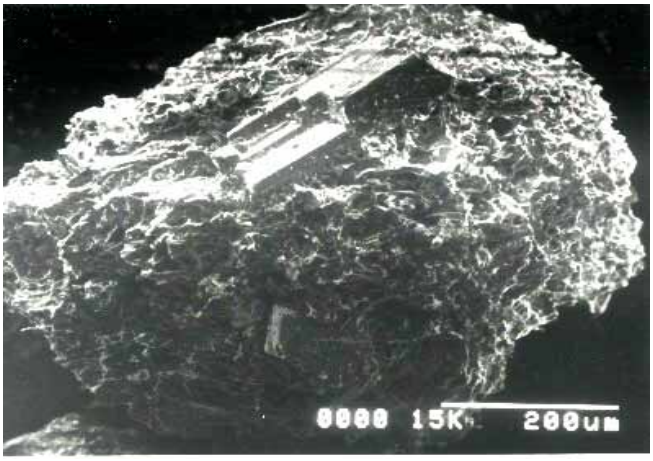


Fig. 1: *Scanning electron micrograph of Pampanga white lahar (150×)*

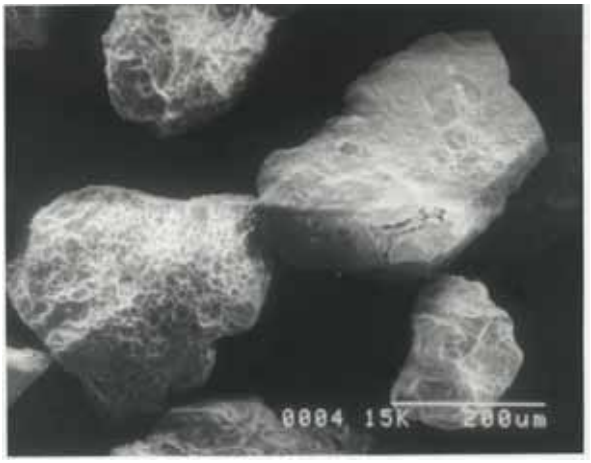


Fig. 2: *Scanning electron micrograph of Pampanga black lahar (150×)*

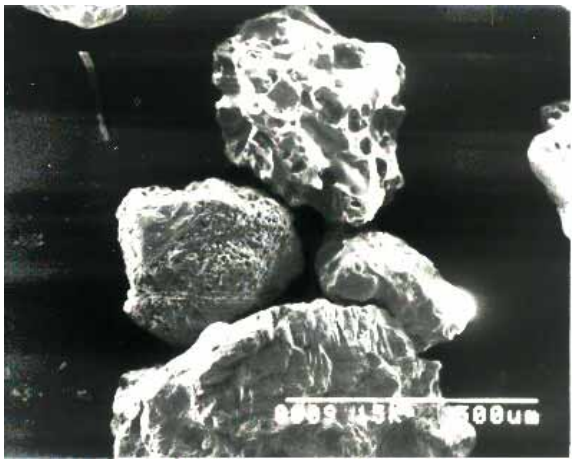


Fig. 3: *Scanning electron micrograph of Bicol black lahar (100×)*

Effect of pH. The efficiency and stability of enzymes are considerably influenced by pH [21]. The specific activity and relative activity of the free and immobilized invertase were statistically shown to be significantly different using Duncan's Multiple Range Test at a 5% level of significance. Immobilized invertase exhibited lower activity at pH 4 – 6 than the free enzyme (Fig. 4). The bound enzyme only showed a higher activity than the free enzyme at the more acidic pH of 3.5. This may be attributed to the microenvironment about the enzyme on immobilization. Electrostatic interactions between lahar and invertase may affect the activity of the enzyme. Also, access to the active site may be restricted because of steric hindrance or structural perturbations in the protein thereby reducing the catalytic efficiency of the enzyme. The free enzyme had an optimum pH of 4.5.

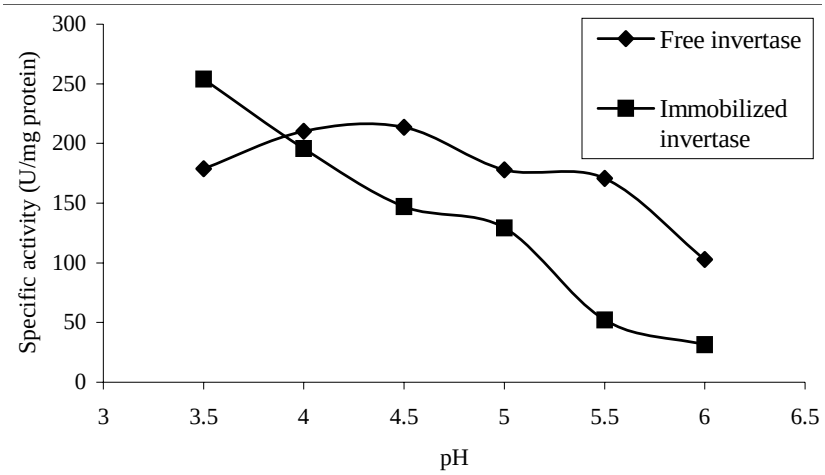


Fig. 4: *Effect of pH on the specific activity of free invertase and invertase immobilized on Pampanga white lahar*

Pitcher [22] reported a similar pH optimum for free invertase while Fundador et al. [12] and Sabularse and Parayno [14] reported the same pH optimum for both free and immobilized invertase on derivatized cellulose from nata de coco as carrier. The PWL-immobilized invertase had maximum activity at pH 3.5. Activity measurements at lower pH values were not conducted thus no pH optimum for the bound enzyme may be stated. However, this increase in activity at the acidic range may be due to changes in the ionic state of the amino acid residues of the enzyme and of the substrate molecules causing variations in the efficiency of binding of the substrate [19]. A shift in the position of the pH optimum towards acidic pH has been observed for enzymes bound to a polycationic carrier [20, 21]. The insertion of a spacer between the carrier and the enzyme allowed the protein to be positioned in a microenvironment completely different from if it were directly attached to the carrier resulting in the alteration of the pH dependence of the enzyme [21].

Effect of temperature. Fig. 5 shows the effect of temperature on the activity of free and PWL-immobilized invertase. Increases in temperature increase the rate of an enzyme catalyzed reaction within certain limits. However, this is also accompanied by an increase in the rate of enzyme denaturation resulting in the loss of secondary and tertiary structures. The point of balance between the acceleration of the enzyme catalyzed reaction and the effect of denaturation by increasing temperatures is the temperature optimum [23]. The temperature optimum for both free and PWL-immobilized invertase was 55^{o}C.

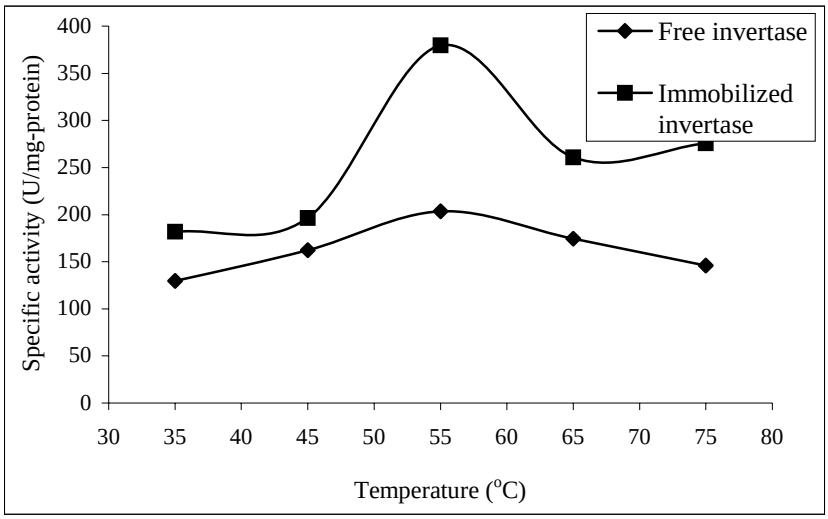


***Fig. 5**: Effect of temperature on the activity of free invertase and invertase immobilized on Pampanga white lahar*

Although both the free and immobilized enzymes have the same temperature optimum, the bound enzyme showed higher activity at all temperatures. It may be deduced that immobilization rendered the enzyme more stable to variations in temperature. This finding contrasts that observed by other researchers. Fundador et al. [12] noted that invertase immobilized in agar-carboxymethyl cellulose from nata de coco beads exhibited lower activity than the free enzyme at varying temperatures. Tomato invertase embedded in acrylamide was significantly sensitive to temperature changes from 12 - 30°C [24]. Likewise, yeast invertase immobilized in polyvinyl alcohol showed increased sensitivity to temperature changes [25].

The activation energy (E_a) of the free and immobilized enzyme catalyzed reactions was determined from Arrhenius plots. The E_a was calculated using the equation:

$$E_a = \tan \beta * R$$

where tanβ is the slope of the plot of the reciprocal of absolute temperature vs. ln relative activity and R is the gas constant equivalent to 8.3 J/mol - K.

The E_a of the reaction involving the immobilized enzyme was greater than that of the free enzyme (Table 3). The greater the E_a, the greater is the extent of acceleration of the reaction with increase in temperature [21]. Hence, the immobilized enzyme exhibited better catalytic action because it catalyzed the reaction more efficiently even at lower temperatures, implying that less energy is required for catalysis. This is of considerable importance because the immobilized enzyme may be used efficiently for inversion even at room temperature thereby reducing the cost for energy requirements since temperature need not be brought to optimum.

***Table 3:** Energy of activation of free and Pampanga white lahar immobilized invertase*

	Activation energy, Jmol-K^{-1}
Free invertase	52.43
Immobilized invertase	49.13

Effect of sucrose concentration. The activity of both free and immobilized enzyme increased with increasing sucrose concentration (Fig. 6). The rate of an enzyme catalyzed reaction is influenced by the concentration of its substrate. However, there is a limit to the increase in enzyme activity with increasing substrate concentration. A certain maximum concentration is reached beyond which no increase in the rate of reaction is observed. At this point the enzyme is saturated with substrate thus a maximum concentration of the enzyme-substrate complex is reached. Statistical analysis showed that the specific activity and relative activity of both free and PWL- immobilized invertase were not significantly different using 0.3 M and 0.5 M sucrose solutions. Thus, maximum enzyme activity is reached using 0.3 M sucrose solution.

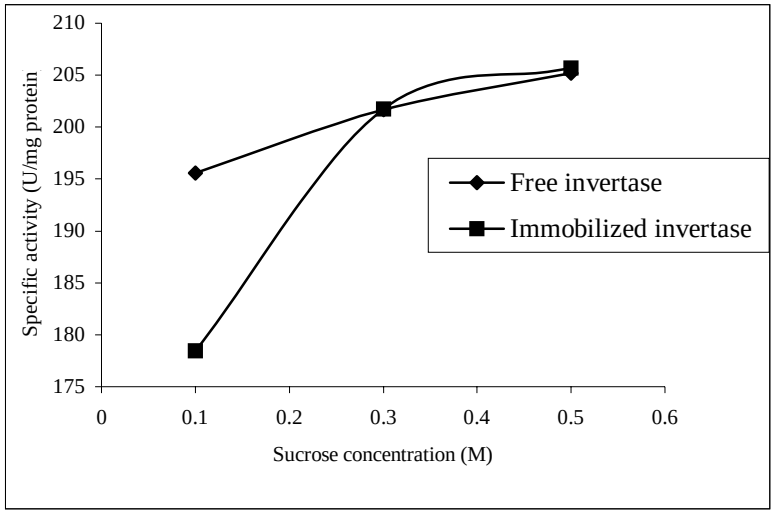


Fig. 6: *Effect of sucrose concentration on the activity of free invertase and invertase immobilized on Pampanga white lahar*

3.3 Kinetic parameters of free and Pampanga white lahar immobilized invertase

The K_m and V_{max} of the free and immobilized enzyme are given in Table 4. The K_m of the immobilized enzyme was higher than that of the free enzyme. This indicated that the affinity of the substrate for the invertase-bound enzyme was less than that for the free enzyme. The V_{max} of the free invertase was higher that that of the immobilized enzyme. According to Hornby et al. [26] changes in reactivity, as indicated by apparent K_m and V_{max}, of enzymes when immobilized may be due to electrostatic interactions of charged substrates and charged solid supports. Interactions of these charges will determine the substrate concentration in the microenvironment about the immobilized enzyme. A decrease in substrate concentration, and thus an increase in apparent K_m may result when like charges exist in both substrate and solid support.

Table 4: *Kinetic parameters of the free and Pampanga white lahar immobilized invertase*

	Free invertase	Immobilized invertase
K_m, M	2.37	3.90
V_{max} (μmol/min)	48.75	38.87

Another factor that influences reactivity of an immobilized enzyme is diffusional effects. According to Fundador et al. [12] not all of the enzyme molecules entrapped in the carrier matrix are accessible to the substrate particularly those in the innermost part of the carrier. Hornby et al. [26] stated that although conformation changes that may occur upon coupling of enzyme and substrate may account for the increase in K_m of an immobilized enzyme, the increase may be more likely due to a diffusion-limiting layer existing in the microenvironment of the bound enzyme. Changes in kinetic parameters may also be attributed to changes in enzyme conformation during immobilization.

3.4 Stability of immobilized invertase towards repeated and continuous use

Table 5 shows the percentage of immobilized invertase left in the column after repeated and continuous use. For PWL the amount of immobilized enzyme remained constant from the 2nd day to the 5th day of use while that of PBL was unchanged from the 3rd to the 5th day. These indicated covalent bonding of invertase to the solid support. The existence of noncovalent interactions aside from covalent bonding was indicated by the decrease in the amount of immobilized invertase from the 1st to the 2nd day and from the 1st to the 3rd day for PWL and PBL, respectively, and by the gradual decrease shown by BBL. Weaker interactions may have been disrupted with repeated and continuous use as well as washing of the column. Noncovalent interactions may have arisen from electrostatic attraction of the charged side chains of the enzyme and the charges present on the surface of the lahar particles. The presence of hydrogen bonding cannot be discounted. The relatively higher concentration of protein present in the column on day 1 and day 2 may be due to some protein trapped in the lahar matrix that was not washed off during the preparation process. The smaller quantity of immobilized invertase in BBL supports the assumption that there are less binding sites on its surface.

Table 5: *Effect of repeated and continuous use on the amount of invertase immobilized on the different types of lahar*

Time (Days)	Immobilized Invertase (%)*		
	Pampanga White	Pampanga Black	Bicol Black
1	98.67	94.67	99.50
2	84.67	89.33	93.67
3	84.67	86.33	84.55
4	84.67	86.33	71.33
5	84.67	86.33	70.67

*Mean of 4 determinations.

The specific activity of invertase immobilized on lahar on repeated and continuous use is shown in Fig. 7. Statistical analysis showed that the specific activity of invertase immobilized on the different lahar differed significantly. PWL-immobilized invertase showed the highest activity while that immobilized on BBL gave the lowest activity. The activity curve for the PWL-immobilized invertase did not follow the typical gradually decreasing curve. This may be attributed to the fact that PWL is more porous than the other lahar types. The activity of invertase bound to the column was limited by diffusional factors [27]. Diffusion of substrates and products within the pores often become rate limiting because the pores may become easily clogged [28].

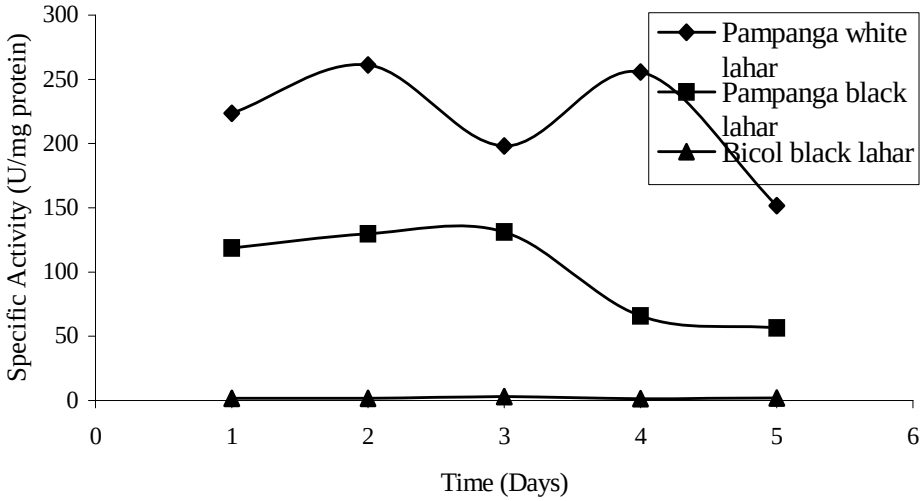


Fig. 7: *Effect of repeated and continuous use on the stability of lahar-immobilized invertase*

3.5 Stability of lahar immobilized invertase on storage with intermittent use.

Figure 8 shows the effect of storage with intermittent use on the specific activity of lahar-immobilized invertase. Statistical analyses indicated significant differences in specific activity of immobilized invertase with storage. PWL-immobilized invertase still gave the highest activity indicating that it is the best solid support of the three samples. However, its average specific activity on the fourth week was about 42.74% of its initial activity. Although both PBL- and BBL-immobilized invertase showed lower average specific activities than PWL-immobilized invertase, the average specific activity for both samples were higher than their initial values. These may again be attributed to greater diffusional barriers in the more porous PWL than in the other two less porous samples.

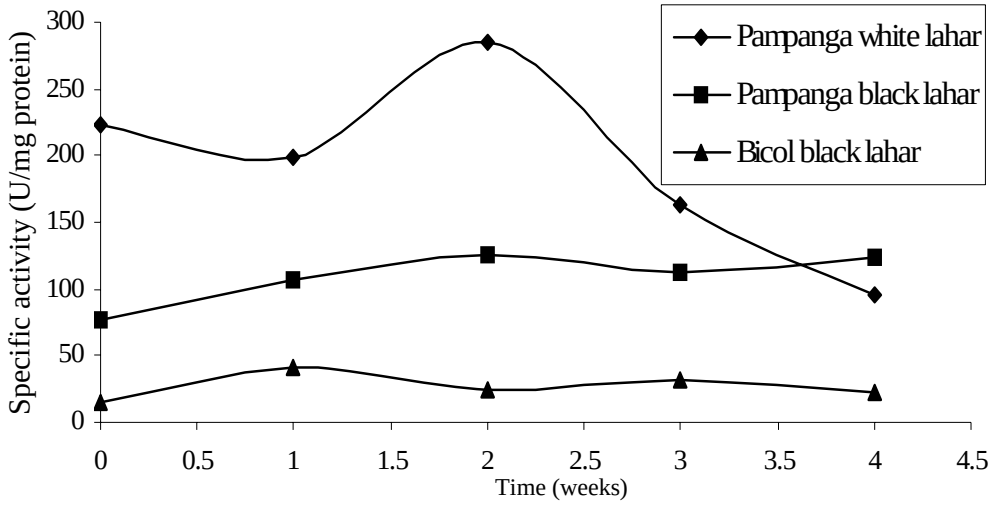


Fig. 8: *Effect of storage with intermittent use on the stability of lahar immobilized invertase*

4. CONCLUSIONS

Invertase was immobilized on Pampanga white and black lahar and on Bicol black lahar. The number of binding sites on the lahar samples were increased by activating their surfaces through treatment with APTS and glutaraldehyde. Hinsberg's and nitrous acid tests confirmed covalent bonding of APTS while Benedict's and Tollen's tests showed the attachment of glutaraldehyde. Infrared analysis further confirmed the covalent attachment of APTS and glutaraldehyde to lahar.

Optimum conditions for PWL-immobilized invertase were pH 4.5, 55^{o}C and 0.3 M sucrose.

The effect of immobilization on the enzyme was evident from the kinetic constants of the enzyme. The K_m and V_{max} of free invertase were 2.33 M and 48.75 mmol min^{-1}, respectively. On the other hand, the PWL-immobilized invertase had a K_m of 3.90 M and V_{max} of 38.87 mmol min^{-1}. The E_A of the free invertase was higher (52.43 J mol-K^{-1}) than that of the PWL-immobilized invertase (49.13 Jmol-K^{-1}).

Immobilized invertase was stable towards repeated and continuous use and towards storage and intermittent use. Pampanga white lahar was noted to be the best inorganic carrier showing relatively higher activity than the other lahar samples.

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REFERENCES

1. Schallenberger, R.S. and Birch, G.G. **(**1975), Sugar Chemistry, Westport, Connecticutt: The Avi Publishing Co., 221.
2. Pavlou, O.P., Lye, G.J., and Stuckey, J.E. (1996), Biotechnol. Bioeng, vol. 51, pp. 69-75.
3. Dalvie, G.K. and Baltus, E.R. **(**1992), Biotech and Bioeng, vol. 40, pp. 1173-1180.
4. Lee, E.C., Senyk, G.F., and Shipe, W.F. (1987), J. Food Sci., vol. 52, pp.1124-1126.
5. Weetall, H.H. (1969), Science, vol. 166, pp. 615-617.
6. Yokote, Y., Fujita, M., Shimura, G., Noguchi, S., Kimura, K., and Samajima, H. (1985), Agric. Biol.Chem., vol. 49, pp.1545-1552.
7. Reyes, R.Y. and Neue, H.U. **(**1991), Phil. J. Crop Sci., vol. 16 (2), pp. 69-73.
8. Acda, R**.** (1996), Utilization of Mount Pinatubo Lahar/Pyroclastic Materials for Mineral Oxide Production, UPLB., pp. 4-22.
9. Bernardo, S.T., Marinez, E.R., Basilla, B.A., Celorico, J.R., Bedia, E.L., Salvador, J.B., Reynales, C.F., and Zambrano, C.B. (1993), Unpublished report by the Industrial Technology Development Institute, Department of Science and Technology, Taguig, Metro Manila, Philippines.
10. Galvez, N.L. (1938), The Phil. Agriculturist, vol. 27, pp. 765-774.
11. Mendoza, C.DG. **(**1996), Lahar as a Potential Inorganic Solid Support for Yeast Invertase Immobilization. Undergraduate thesis. UP Los Banos. College, Laguna.

12. Fundador, E.O.V., Sabularse, V.C., and Revilleza, M.J.R.R. (2003), ASEAN J. Sci. Tech. Dev., vol. 20 (1), pp. 37-47.

13. Sabularse, D.C. and Kinoshita, S. (1993), Annual Reports of International Center of BIOTECH, Vol. 15.

14. Sabularse, D.C. and Parayno, N.A. (1992), Microbial Utilization of Renewable Resources, P. Matangkasombut and Y. Oshima, (Eds.), International Center for Cooperative Research on Biotechnology, Japan. Osaka Univ., Osaka, Japan, vol. 8, 77.

15. Bradford, M. **(**1976), Anal. Biochem., vol. 72, pp. 248-253.

16. Nelson, N. (1944), J. Biol. Chem., vol. 153, pp. 375-380.

17. Monsan, P., Puzo, G., and Mazarguil, H. (1976), Analysis and Control of Immobilized Enzyme Systems, D. Thomas and J.P. Kernevez (Eds.), New York, North-Holland Pub. Co. pp. 275-282.

18. Shriner, R.L., Fuson, R.C., and Curtin, D.Y. (1998), The Systematic Identification of Organic Compounds. 7th ed. USA: John Wiley and Sons, 604.

19. Holme, D.J. and Peck, H. (1993), Analytical Biochemistry, 2nd (Ed.) USA, Longman Scientific and Technical, Essex, England, 507.

20. Chibata, I. (1978), Immobilized Enzymes, New York, John Wiley and Sons, 284.

21. Hartmeier, W. (1988), Immobilized Biocatalysts: An Introduction. New York, Springer-Verlag, 212.

22. Pitcher, Wayne H. (1980), Immobilized Enzymes for Food Processing, Boca Raton, Florida, CRC Press, 219.

23. Sumner, J.B. and Somers G.F. (1953), Chemistry and Methods of Enzymes, 3rd (Ed.), New York, Academic Press, 462.

24. Nakagawa, H., Arao, T., Matsuzawa, S., Ito, S., Oguran, N., and Takihama, H. (1975), Agri. Biol. Chem, vol 39, pp. 1-5.

25. Imai, K., Shiomi, T., Sato, K., and Atsuyoshi, F. (1983), Biotechnol. Bioeng., vol. 25, pp. 613-617.

26. Hornby, W.E., Lilly, M.D., and Crook, E.M. (1968), Biochem. J., vol. 107, pp. 669-674.

27. Mansfeld, J. and Schallenberger, A. (1987), Biotech and Bioeng, vol. 20 (1), pp. 72-78.

28. Wasserman, B.P. and Hultin, H.O. (1980), High-yield Method of Immobilization of Enzymes, Biochem. Biophys. Acta, vol. 22, pp. 271-287.