P-024 **Immobilization of invertase produced by a new isolate *Saccharomyces* sp.**

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INTRODUCTION AND OBJECTIVES

It is mainly used in confectionary, beverage industries and bakeries (Basha and Palanivelu,2000). Invert sugar can be produced by a chemical process (using acid as a catalyst) or biochemical process (using invertase as a catalyst). Nowadays, the biochemical process is preferred as the resulting product contains less coloured by-product and salt (Bergamasco *et al.*, 2000). Moreover, this process requires less energy than the chemical process. Immobilization of enzymes on solid supports by adsorption (D'Souza 1986) and their industrial applications are receiving great attention in the recent years.

A variety of microorganisms, including candida and yeast have been reported to produce invertase (Belcarz *et al*., 2002). The immobilized enzymes are more stable at higher temperatures and are active over a wide pH range (Kennedy and White, 1985). In the present study an extra-cellular invertase produced by a new isolate *Saccharomyces* sp. was selected for immobilization and characterization by adsorption method on glass beads.

MATERIALS AND METHODS

Microorganism and inoculum preparation
The chemicals used in immobilization (3-amino propyltriethoxysilane and polyethylenimine) were from Supelco Analyticals, USA. The glass beads were from Biomatrix Technologies, India. All other chemicals were of analytical grade. *Saccharomyces* sp. used in the present study was isolated from local sweet shop fermented starter inoculum sample and maintained on nutrient agar containing sucrose (1.0% w/v) at 40°C for 24 h.

Enzyme production and assay
One percent of 24h old seed culture (OD_{660}= 1.0) inoculated in production medium containing beef extract 0.3%, yeast extract 0.5% and sucrose 1.0% (pH 9.0) and incubated at 40°C for 48 h at 150 rpm. The fermented broth was centrifuged at 5000 rpm for 15 min at 4°C and the supernatant used as a crude enzyme. Invertase activity was determined by the DNS method (Miller, 1959) using D-glucose as a standard. Enzyme assayed at 40°C using 0.5% (w/v) sucrose (50 mM Acetate buffer, pH 9.0) as a substrate. One unit of invertase activity is expressed as 1µg of reducing sugar (glucose equivalent) released/ml/min under assay conditions.

Characterization of immobilized invertase
The activation of glass beads (4-5 mm) was carried out using Bisswanger (2004) method. Immobilization of invertase on glass beads was carried out by D'Souza *et al*. (1986) method. For Immobilization five ml of enzyme (128 UmL^{-1}) was added to activated support and activity was determined by using 0.1g mL^{-1} of immobilized support under assay condition. The activity yield and characteristics of immobilized enzyme were compared with that of free enzyme and yield determined using the formula:

$$\% \text{ Enzyme yield} = \frac{\text{Activity in immobilized support}}{\text{Activity in free enzyme}} \times 100$$

The activity of free and immobilized invertase was determined at different temperature (30-70°C) and pH (6.0-10.0) under experimental conditions. Thermostability profile of free and immobilized was studied up to 4 h after incubation at different temperatures (30°C-60°C) and assaying samples at regular intervals.

The immobilized invertase was tested for its reusability and percent relative activity was determined.

RESULTS AND DISCUSSION

The invertase produced by *Saccharomyces* sp. showed optimal activity at pH 9.0 and 40°C respectively. Glass beads have functional groups like hydroxyl, carboxyl, amino, etc., for binding the enzymes. This enzyme was immobilized successfully on to glass beads. All the experiments were carried out in triplicate and analyzed. The immobilization was preceded by silanization to introduce reactive groups onto inert glass surface to increase the surface area for immobilization (Bisswanger, 2004). Immobilization of enzyme with polyethylenimine is one of the very quick and cheap procedures and reported to improve the catalytic and stability characteristics of the biocatalyst (D'Souza *et al*., 1986).

After immobilization the enzyme activity yield was found to be 80%. The loss of enzyme activity after immobilization is normal phenomenon (Rosevear, 1988). At least 49% of the activity was retained in controlled pore glass immobilized with *Thermus Rt* 41A enzyme (Wilson *et al*., 1994). The extra cellular invertase produced by *Sacchromyces* sp. had optimum activity at 40°C and invertase immobilized on glass beads also showed optimal activity at 40°C (100%) and at 50°C it was 80% (Fig. 1a). The enzyme immobilized support showed optimum catalytic activity at pH 9.0 and retained 66% at pH 8.0 (Fig. 1b.) also the free enzyme showed optimal activity at pH

9.0. Taylor *et al*. (1977) recorded high activity with papain immobilized on silanated controlled pore glass, alumina and titania.

The immobilized invertase was quite stable and could be reused 2-3 times without any considerable loss in enzyme activity (Fig. 2). The loss in activity after three times was observed and this may be due to abrasion of supports during repeated use (Rosevear, 1988).The immobilized invertase was stable up to 3 h at all temperatures on glass beads and thereafter stability decreased with incubation time (Fig. 3). The enhancement in activity and stability of immobilized enzymes is important for their industrial applications (Rosevear, 1988). The immobilized invertase was evaluated for sucrose hydrolysis in syrup. It took 4h to prevent the crystal formation with immobilized glass beads at ambient room temperature under standard assay conditions.

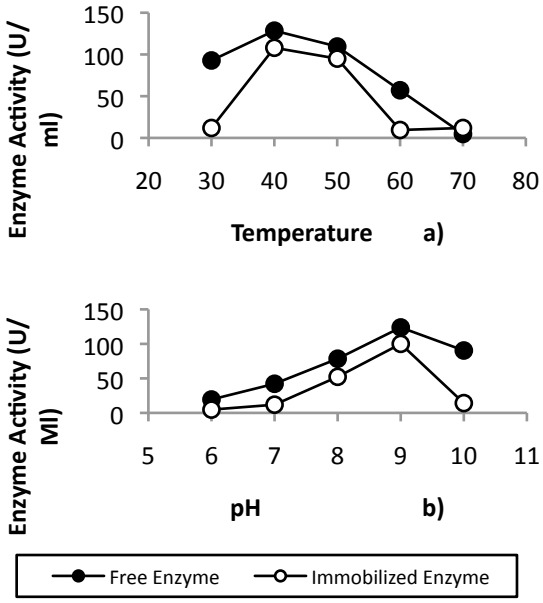

Figure 1: Effect of temperature and pH on activity of immobilized invertase. Optimum value corresponds to relative yield (100% = 123 Uml^{-1}).

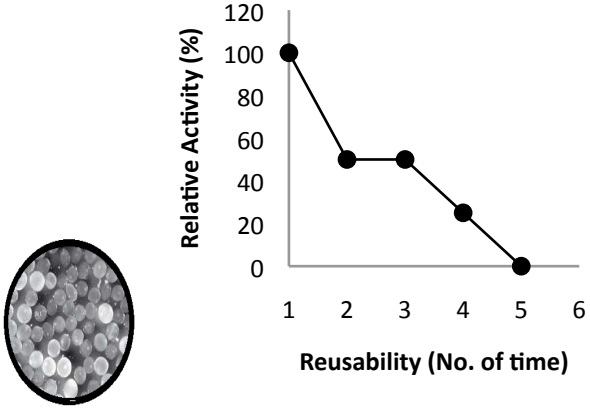

Figure 2: Immobilized glass beads and its reusability.

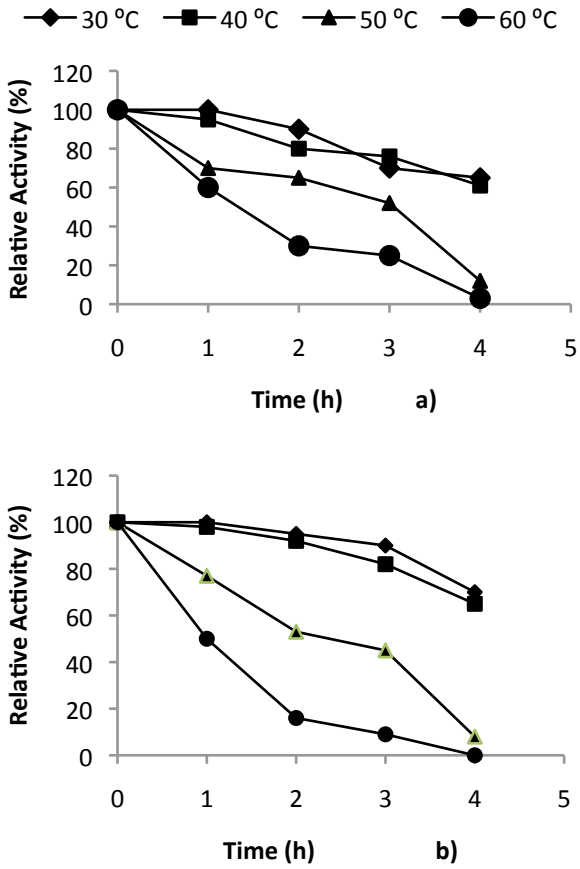

Figure 3: Thermostability of free (a) and immobilized (b) invertase under standard assay.

CONCLUSION

The present work describes that immobilized invertase of new strain of *Saccharomyces* sp. owing to its alkaline nature and activity at high temperature seem to be of considerable use in sucrose based syrup industry. Detailed characterization of the enzyme is in process.

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