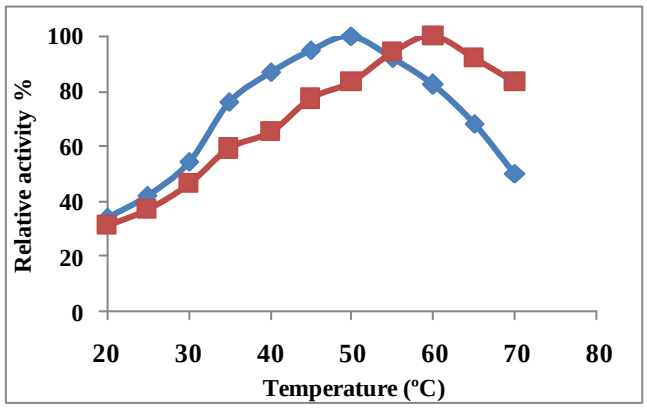

Figure 3: Effect of temperature on activity of (♦) free and (■) immobilized α-amylase.

4.3 Effect of substrate concentration on immobilized α-amylase

K_m and V_{max} for the free and immobilized α-amylase were calculated using Lineweaver-Burk plot with starch as substrate. The calculated K_m value of the immobilized enzyme were higher than that of the free enzyme. Alpha amylase entrapped in alginate beads showed an apparent K_m of 2.8×10^{-3}g/ml (Fig. 4). The K_m of amylase entrapped in alginate beads is approximately 1.7 times higher than that of the free enzyme. V_{max} on immobilization was decreased from 1388 (units/min/mg) for free enzyme to 1295 units/min/mg for immobilized enzyme. The increase in K_m values when compared with that of the free enzyme may be due to the lower accessibility of the substrate to the active site of the immobilized enzyme.

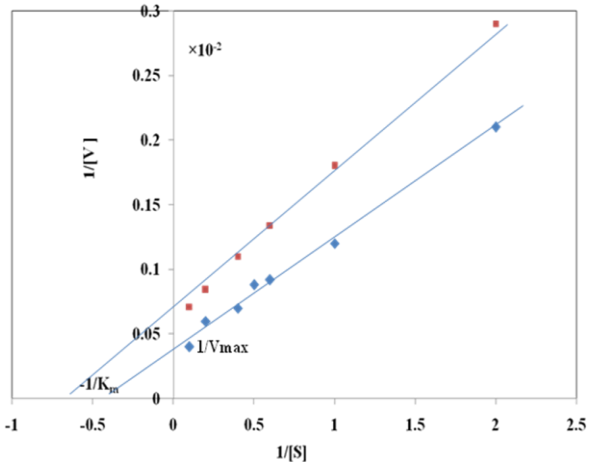

Figure 4: Determination of K_m for ■free and ■immobilized α-amylase by Lineweaver-Burk plot method.

4.4 Operational stability of immobilized α-amylase

The operational stability of immobilized enzymes is one of the most important factors affecting the utilization of an immobilized enzyme system. The α-amylase activity in calcium alginate beads remained unchanged during the first three to four cycles. Immobilized α-amylase retained 72.5% activity upto seven cycles (Fig. 5). After the seventh cycle, a decrease in the enzyme activity may be due to enzyme denaturation or may also be due to physical loss of enzyme from the carrier.

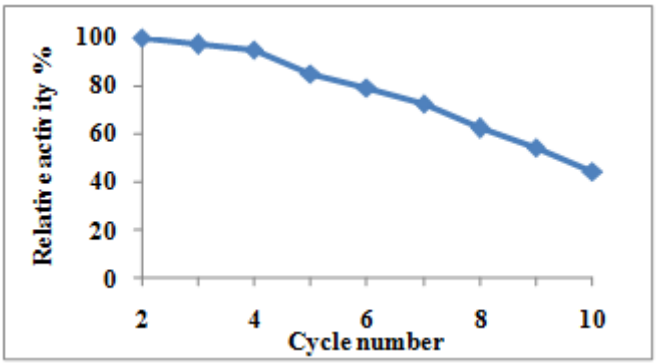

Figure 5: Reusability immobilized α-amylase.

5. Discussion

The immobilization yield is the key parameter, since it represents the general output of the efficiency of the immobilization process [22]. Immobilization of little millet α-amylase by entrapment with 2% sodium alginate gave an immobilization yield of 65%. Immobilization of *Bacillus acidocaldarius* α-amylase by entrapment with 2% sodium alginate gave an immobilization yield of 61.4% [23]. Dey *et al.* (2003) [24] reported that sodium alginate 4% gave the highest immobilization yield of 75% for *B. circulans* α-amylase. Immobilization of soybean urease by entrapment in sodium alginate gave an yield of 54% [25] and pigeon pea urease immobilized on 5% agar yielded an optimum immobilization of 51.7% [26].

The pH optima of little millet α-amylase did not alter on entrapment in calcium alginate beads. Arpana and Kayastha, 2011[16] reported a shift of pH towards the basic side for soybean α-amylase. Similar results were reported for mung bean α-amylase immobilized on chitosan and amberlite [17]. A shift towards acidic region has been observed when α-amylase was immobilized on zirconium dynamic membrane and poly (methylacrylate-acrylic acid) microspheres [27, 28]. Abdel-Naby *et al.* [29] reported the optimum pH of the immobilized enzymes was shifted to acidic range (optimum pH 5.25-5.75) in comparison to the free enzyme (optimum pH 6.0). The optimum pH of reaction was not affected by immobilization process (in case of covalent binding and ionic binding) [30]. El-Batal *et al.* (2005) [31] suggested that the α-amylase immobilized by ionic binding had the same pH optima as that of the free enzyme.

The temperature optima of little millet α-amylase showed a shift of 10°C on entrapment in calcium alginate beads. The higher temperature profile on entrapment in calcium alginate beads may be due to some conformational effects on enzyme entrapment, which protects the enzyme against heat denaturation. Free α-amylase from soybean reported an optimum temperature of 70°C [32], whereas α-amylase immobilized on amberlite showed an optimum temperature of 75°C [16]. Alpha amylase from *Bacillus circulans* immobilized in calcium alginate beads was reported to show an increase in operating temperature [24]. Temperature

optima of immobilized *Bacillus acidocaldarius* α-amylase activity shifted toward higher temperature from 50°C to 60°C [30].

Immobilized little millet α-amylase exhibited K_m values higher than the free enzyme. The maximum rate of the reaction catalyzed by the immobilized enzyme was lower than the free enzyme. An increase in K_m, clearly indicates an apparent low affinity of the enzyme towards its substrate compared to the soluble enzyme. Increased K_m values of amylases after the immobilization process were similarly reported [16, 17, 30, 33, 34, 35]. Decrease in V_{max} value of α-amylase after immobilization covalently on plastic supports was reported [36]. Kumar *et al*. (2006) [37] reported that K_m for both free and entrapped *Aspergillus oryzae* α-amylase enzymes remained the same. In this case alginate entrapment did not have any effect on the binding of substrate to the enzyme. However, V_{max} of the entrapped enzyme was decreased.

The operational stability of the immobilized little millet α-amylase was evaluated in repeated process. Calcium alginate immobilized α-amylase retained 72.5% activity upto seven cycles. With repeated use, the strength of binding between the matrix and enzyme is weakened, leading to leaching of enzyme from the matrix and loss in activity. *Bacillus circulans* α-amylase immobilized by entrapment in calcium alginate beads retained 83% of the initial activity after 7 cycles [24]. The immobilized *Bacillus acidocaldarius* α-amylase on a cation exchange resin and glass beads retained 70 and 73.4% from the initial activity after upto 6 cycles [30]. Soybean α-amylase immobilized onto chitosan and amberlite beads showed a residual activity of 38 and 58% respectively, after 10 reuses [16]. It was reported that the mung bean α-amylase immobilized onto amberlite beads and chitosan exhibited a residual activity of 43 and 27% respectively, after 10 cycles [17].

6. Conclusion

In this study *Panicum sumatrense* α-amylase was successfully immobilized by entrapment in calcium alginate beads. The immobilized enzyme showed a high operational stability by retaining utmost initial activity after several reuses. The immobilized enzymes showed enhanced thermal stability than the free enzyme. The easy accessibility of *Panicum sumatrense* α-amylase, the ease of its immobilization on low-cost matrices, increased stability upon immobilization make it a suitable product for future applications.

7. Acknowledgements

This work was supported by a grant from the University Grants Commission (UGC), New Delhi, India.

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