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Immobilization of Acid Phosphatase in Agar-agar and Gelatin: Comparative Characterization

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Abstract: **Acid phosphatase from wheat germ was immobilized by entrapment in agar-agar and gelatin gel. Blocks of 5x5 mm size were employed for characterization. The maximum immobilization yield was achieved at 3.0% (w/v) of agar-agar and 20% (w/v) gelatin cross-linked with 10% (v/v) glutaraldehyde for 1h incubation. The optimum pH for both immobilized enzymes was observed at 5.5. Both the immobilized enzymes displayed a temperature optimum at 60 °C. The activation energies for agar-agar and gelatin immobilized acid phosphatase were 36.6 and 36.8 kJmol^{-1}. The K_m values for agar-agar and gelatin immobilized enzymes were 0.282 and 0.3 mM, respectively. The V_{max} values for agar-agar and gelatin immobilized acid phosphatase were 2.15 and 2.1 µmol/min/mg protein, respectively. The agar-agar and gelatin immobilized enzyme retained 73.2 and 75.7% activity on the 56th day of storage at 4 °C. Gelatin immobilized enzyme could be repeatedly used for more than 21 cycles (48%) whereas the agar-agar immobilized enzyme could be reused for 15 cycles (36.1%) only. The method of immobilization reported here is easy, profitable and will be suited for wider application.**

Index terms: **Acid phosphatase, Agar-agar, Gelatin, Immobilization, Stability.**

I. INTRODUCTION

Acid phosphatase (Orthophosphoric-monoester phosphohydrolase (acid optimum) EC 3.1.3.2) (APase) belongs to class hydrolase, and catalyzes the hydrolysis of orthophosphoric monoester to yield orthophosphate and alcohol (Dixon & Webb, 1979). APases are extensively spread in nature and are found in animals, plants and microbes. APases are involved in several processes of plant metabolic activities, such as P assimilation, transport, and distribution (Delhaize & Randall, 1995). Further, the enzyme has a crucial role in the assimilation of external Pi from the rhizosphere as well as the recycling of internal Pi (Duff et al., 1994; Tang et al., 2013). Other applications of APase include plant growth promotion (Schoebitz et al., 2013; Farhat et al., 2014), waste remediation and metal recovery (Macaskie et al., 2000), antagonistic activity against plant pathogens (Chakraborty et al., 2010), and hydroxyapatite biosynthesis (Sammon et al., 2014). In human beings, an increase in APase level is associated with certain diseases like prostate cancer, and Gaucher disease, and kidney, veins, and bones related disorders (Na et al., 2016).

Enzyme immobilization technique has brought a revolution for better application of enzymes. The soluble enzyme once used in the assay cannot be retrieved from the reaction mixture. On the other hand, the immobilized enzyme can be retrieved from the reaction mixture, and reused for several cycles. Immobilized enzymes usually have better storage stability and can withstand extreme conditions in comparison to the soluble enzyme. The methods and applications have been discussed (Mosbach, 1971; Spahn & Minteer, 2008).

Agar-agar is a naturally occurring polysaccharide complex of agarose and agaropectin. Agarose is electrically neutral, while the presence of sulfate on agaropectin imparts a negative charge. It is derived from the cell wall of red algae, commonly from *Gracilaria* and *Gelidium*. It has the capacity to form very strong gels even at a low concentration and has hydrophilic nature. The gelation process does not require metal ions. Agar-agar does not dissolve in cold water, but dissolves in hot conditions. It forms a gel around 43 °C. Once the gel is formed, it does not melt below 85 °C (Armisen & Galatas, 1987). Due to its high porosity, it has a better capacity for enzyme immobilization by entrapment (Prakash & Jaiswal, 2011; Bilal et al., 2016a). It is non-toxic and has an application in the food industry. The cost of this matrix is also low in comparison to other matrices commonly employed for immobilization. Further, agar-agar gel can act as a cover for enzyme to withstand against different drastic conditions common in industrial set-up (Nawaz et al., 2015).

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Gelatin is an edible, non-toxic, hydrophilic and biodegradable natural material. It has an extensive use in industries related to pharmacy and food, and is used as a gelling agent. It has been used as a preferred material for generating microcapsules in bio-applications pertaining to drug delivery (Patel et al., 2013). It has wide applications in agriculture, paper industry, photography, cosmetics, etc. Other advantages include its abundance, availability and cheapness. The hydrolysis of collagen results in the formation of gelatin, which is a mixture of proteins and peptides. Glycine is the major constituent in addition to proline, hydroxyproline and alanine (Devi et al., 2018). Since the gelation process is reversible with temperature, there is a lack of effective immobilization. To accomplish an irreversible gelation process, the use of cross-linking agents, has been suggested (Bilal et al. 2019). Glutaraldehyde has been used in dual capacities for cross-linking and enzyme coupling (Kennedy et al., 1984). The reaction between gelatin and glutaraldehyde goes along with a change in color with the formation of the aldimine linkage with the lysine residues of the protein (Tanriseven & Dogan, 2002). Formaldehyde is another molecule involved in cross-linking for immobilization (De Alteriis et al., 1988; Kharkrang & Ambasht, 2013). Water retained in normal gelatin is uniformly distributed, while in formaldehyde hardened gelatin, water is within the empty spaces of spongy structure (Dhulster et al., 1983).

The available literature on immobilization of APase on different matrices is relatively limiting in comparison to other enzymes (Vaidya et al., 1987; D'Urso & Fortier, 1996; Kurita et al., 1997; Marzadori et al., 1998; Bautista et al., 1999; Yamato et al., 2000; Chang & Juang, 2004; Zhu et al., 2010; Belho et al., 2014; Homma et al., 2014; Srivastava & Anand, 2014; Kalita & Ambasht, 2019). In most of the studies on APase, immobilization were on different matrices (Vaidya et al., 1987; Bautista et al., 1999; Yamato et al., 2000; Chang & Juang, 2004; Belho et al., 2014). Earlier, we described the immobilization of APase inside agarose gel (Kalita & Ambasht, 2019). In the present work, we report the immobilization of APase inside two other matrices, namely agar-agar and gelatin. We report a comparative study on kinetic characterization, storage, and reusability aspects and comparison to the soluble enzyme.

II. MATERIALS AND METHODS

Important chemicals procured were described in earlier publication (Kalita & Ambasht, 2019). Other chemicals were gelatin, and agar-agar (Hi-Media, India), and glutaraldehyde (Sigma Aldrich, USA). Milli-Q system from Millipore, USA was used for getting de-ionized water. The latter was used in the preparation of solutions. Quantification of protein was done using Bradford's reagent (Bradford, 1976). Assay buffers I and II (0.05 M sodium acetate, pH 5.0 and 5.5) were prepared. Assay buffer II is also referred to as the storage buffer for further experiments.

A. Entrapment of APase in agar-agar

The immobilization process of APase in agar-agar was optimized. Different solutions of agar-agar (1.0-4.0%) were prepared by suspending agar-agar in assay buffer I and heating them at 60 °C for 20 min. The above solutions were brought to 37 °C. The enzyme (1.82 U/mg protein), (1.0 mL) was transferred to 9.0 mL of different agar-agar solution, was mixed and immediately casted on a pre-assembled glass plate. After solidification, the gel was cut into small blocks of 5x5 mm. These blocks were washed several times with assay buffer I. Finally, the blocks were stored in assay buffer I at 4 °C. The activity and protein contents were determined in washings and specific activity.

B. Entrapment of APase in Gelatin

The process of immobilization of APase inside gelatin was optimized. The parameters were variation of gelatin and glutaraldehyde concentration, and time of incubation. Different solutions of gelatin (10.0-30.0%) were prepared by suspending gelatin in de-ionized water and heating them at 70 °C for 40 min. Solutions were then brought to 37 °C. The enzyme (1.0 mL) (1.82 U/mg) was transferred to different solutions of gelatin (9.0 mL) with gentle mixing. The solution was casted on pre-assembled glass plates. When the gelatin-enzyme solution got jellified, 10.0 mL of glutaraldehyde solution (5-15%) was added to different sets of glass plates. These were left for complete hardening. The gelatin gel was then cut into small blocks of 5x5 mm and washed thoroughly with assay buffer I. Finally, the blocks were stored in assay buffer I at 4 °C. The activity and protein contents were determined in washings and specific activity. The % immobilization was determined as described previously (Belho et al., 2014).

C. Activity assay of soluble and immobilized APase

The soluble APase activity was assayed in assay buffer I, while the immobilized APase activity was assayed in assay buffer II. The details were described earlier (Kalita & Ambasht, 2019).

D. Fourier transform infrared (FTIR) spectroscopy studies

The FTIR spectrum of agar-agar alone and enzyme entrapped in agar-agar was recorded. The other FTIR spectrum recorded was of gelatin alone and enzyme entrapped inside gelatin in the presence of glutaraldehyde. The method has been described earlier (Kalita & Ambasht, 2019).

E. Kinetic characterization

The effect of variation of pH and temperature on soluble and immobilized enzymes was studied. The kinetic parameters of immobilized enzymes were determined. The method of kinetic characterization has been described earlier (Kalita & Ambasht, 2019).

F. Storage stability and reusability study of immobilized APase

APase immobilized in agar-agar and gelatin gel blocks were routinely stored in storage buffer at 4 °C. The methods were according to our earlier publication (Kalita & Ambasht, 2019).

III. RESULTS AND DISCUSSION

A. Optimization of APase immobilization inside agar-agar blocks

The results of variation of agar-agar (w/v) on % immobilization are shown in Table I.

Table I: Effect of agar-agar concentration on the % immobilization of APase

[Agar-agar] %	% Immobilization
1.0	58.29±2.44
2.0	62.24±1.72
3.0	71.82±1.17
4.0	69.05±1.16
5.0	Gel got solidified before the addition of enzyme

It is evident that at 3.0% agar-agar, maximum % immobilization (71.8%) was achieved. The blocks obtained were stable. In some other enzymes, the maximum % immobilization was also achieved at 3.0% agar-agar (Rehman et al., 2014; Asgher et al., 2017; Sattar et al., 2018). At lower agar-agar concentrations (1.0 and 2.0 %), the % immobilization was low. The reason suggested for the lower % immobilization is greater pore size leading to enzyme leaching. Further, blocks obtained were fragile. Above the optimal agar-agar concentration (4.0%) resulted in a decrease in immobilization yield, which could be due to the reduced porosity of the agar-agar gel and also causing limited movement of *p*-NPP. Further, the gel got solidified before the addition of the enzyme. In some other reports, highest % immobilization was obtained at 4.0% agar-agar concentration (Prakash & Jaiswal, 2011; Nawaz et al., 2015; Bilal et al., 2016a; Pervez et al., 2019). The immobilization of urease has been reported at 5% agar-agar though immobilization yield was low (51.7 %) (Mulagalapalli et al., 2007). In some other reports, agar-agar: enzyme ratio was 1:1 (Rehman et al., 2014; Bilal et al., 2016a). The agar-agar: enzyme ratio was kept 9:1 in the present work, like in α-amylase (Prakash & Jaiswal, 2011).

B. Optimization of APase immobilization inside gelatin blocks

The results of optimization of APase immobilization have been summarized in Table II. It includes the effect of variation of gelatin concentration (10-30% w/v), glutaraldehyde concentration (5-15% v/v) and time of incubation (0.5-2.0 h) on % immobilization. The maximum % immobilization (83.4%) was achieved when gelatin concentration was 20% and glutaraldehyde 10% with a 1 h incubation period. The blocks were stable with uniform distribution of enzyme. In some other enzymes also, maximum % immobilization was achieved at 20% gelatin (Jaiswal et al., 2012; Bilal et al., 2016a).

Table II: Optimization of immobilization of APase in gelatin

[Gelatin] (%)	[Glutaraldehyde] (%)	Time (h)	% Immobilization
10	10	1	73.57±2.57
20	10	1	83.45±0.875
30	10	1	78.16±2.08
20	5	1	81.8±2.37
20	15	1	80.93±2.82
20	10	0.5	81.34±2.82
20	10	2	78.37±2.19

At lower gelatin concentration (10%), % immobilization was also low. It may be due to the larger pore size that leads to enzyme leaching. Further, there was the formation of unstable and fragile blocks. Lower % immobilization at low gelatin (10%) was also observed with α-amylase (Jaiswal et al., 2012). In some other cases, maximum % immobilization was achieved at low gelatin concentration (6%) (Kennedy et al., 1984; Asgher et al., 2017) and (0.05%) (Tanriseven & Olcer, 2008).

Further increase in gelatin concentration (30%), resulted in a decline in % immobilization. It may be due to smaller pore size and difficulty of the substrate to diffuse inside the enzyme blocks.

Since gelatin itself is very soft, glutaraldehyde was used to strengthen the gel. In most cases, 10% glutaraldehyde was used (Jaiswal et al., 2012; Asgher et al., 2017). In some other studies, very low glutaraldehyde concentration (2.5%) was used (De Paula et al., 2008) and 0.25% (Bilal et al., 2016b).

C. FTIR

The result of the FTIR of matrices alone and in the presence of enzyme has been summarized in Table III. Agar-agar is primarily made up of agarose and agaropectin. The main ingredient is galactose and 3,6-anhydrogalactose. The functional groups are due to –OH str., C-O-C str., sp^3 C-C str. and sp^3 C-H str. In the case of agar-agar, bands are near identical except the emergence of a new band at 2356 cm^{-1}. The band between 3550-3200 cm^{-1} represents an alcoholic group which is evident from the band at 3447 cm^{-1}. In the corresponding agar-agar, in the presence of the enzyme, the band at 3447 cm^{-1}, represents the –OH group from galactose, Ser and Thr. The other bands are due to C-O-C str. (1050-1085 cm^{-1}; 1074 cm^{-1}) and C-H str. (3000-2840 cm^{-1}). The other specific bands for agar-agar are at 1070 and 930 cm^{-1}, anhydrogalactose bridge vibration, and 890 cm^{-1}, a typical agar peak (Armisen & Galatas, 1987). In the present FTIR result, there are little deviations. The overall result suggests that there is no change in the features of agar-agar upon enzyme immobilization.

Table III: FTIR of Agar-agar alone and agar-agar in the presence of APase; FTIR of gelatin alone and gelatin the presence of enzyme and glutaraldehyde.

Agar-agar Band Wave number cm^{-1}	Agar-agar and enzyme Band Wave number cm^{-1}	Gelatin Band Wave number cm^{-1}	Gelatin, enzyme and glutaraldehyde Band Wave number cm^{-1}
3447.45	3447	3448.26	3447.78
2928.75	2926.64	2960.15	2961.49
	2356.58	2883.14	2883.54
1642.56	1643.02	2123.12	2101.88
1559.80	1557.96	1649.95	1653.47
1414.62	1414.19	1549.18	1547.81
1154.05	1154.22	1454.24	1454.94
1074.26	1074.43	1403.95	1404.52
967.04	966.92	1337.14	1337.08
933.02	933.30	1243.21	1244.21
890.26	890.44	1203.53	1203.67
		1166.16	1166.59
		1080.93	1081.35
		1033.50	1033.67
		977.94	
		874.04	
		700.04	702.39

In the case of gelatin, a similar FTIR result is indicative of the protein nature of gelatin and enzyme. The presence of three amide bands at 1700-1600; 1600-1500 and 1350-1200 cm^{-1} indicate Amide I, II and III, respectively (Singh, 2000). These bands reflect the presence of peptide bond and amide functional groups of Asn and Gln residues. The presence of the –OH group is indicated by the band at 3550-3200 cm^{-1}, while the presence of another band at 1615-1650 cm^{-1} indicated the formation of the Schiff base. However, the band is little deviated (1653 cm^{-1}) in the present study. The latter is indicative of cross-linking of gelatin in the presence of glutaraldehyde.

D. Effect of pH

Fig. 1 exhibits the pH profile results of soluble and immobilized enzymes. It is evident that immobilized enzymes exhibited pH optimum at 5.5, with a 0.5 pH unit shift towards the right side in comparison to the soluble enzyme (5.0). In other reports on wheat germ APase, immobilized on chitosan beads and in agarose gel blocks, a similar shift in pH optimum was also reported (Belho et al., 2014; Kalita & Ambasht, 2019).

A lot of variation in the behavior of enzymes has been observed after immobilization inside agar-agar with respect to pH optimum. Some enzymes exhibited no change in pH optimum after immobilization (Kuroiwa et al., 2005; Li et al., 2008; Karim et al., 2014; Bibi et al., 2015; Sattar et al., 2018), while some exhibited 0.5 unit change towards right, similar to the present report (Nawaz et al., 2015; Pervez et al., 2019). The shift may be as less as 0.3 units (Mulagalapalli et al., 2007) or one unit (Bilal et al., 2016a) or as much as two units (Asgher et al., 2017). Similarly, immobilization of enzyme inside gelatin brought either no change in pH optimum (Tanriseven & Dogan, 2002; Tanriseven & Olcer, 2008; De Paula et al., 2008; Zhu et al., 2010; Vujcic et al., 2011) or a change of one unit towards right (Bilal et al., 2016b; Asgher et al., 2017) or a change of two units (Srivastava & Anand, 2014).

The change in pH profile is mostly dependent on the pH of the microenvironment around the immobilized enzyme. To some extent, the chemical and the physical nature of the supporting matrix, is also responsible (Mosbach, 1971; Srivastava & Anand, 2014).

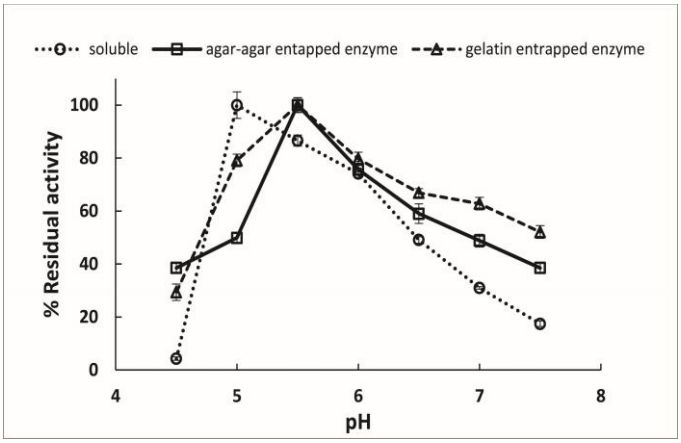

Fig. 1: Effect of variation of pH on soluble and entrapped agar-agar and gelatin APase

E. Effect of temperature

The results of the influence of temperature on the enzyme activities have been shown in Fig. 2. Soluble APase exhibited an optimum temperature at 50 °C. Both the agar-agar and gelatin immobilized APase showed the optimum temperature at 60 °C. Some other reports also exhibit a 10 °C rise in the optimum temperature of the immobilized enzyme with respect to the soluble enzyme (Li et al., 2008; Karim et al., 2014; Bibi et al., 2015; Asgher et al., 2017). The increase in optimum temperature indicates that APase entrapped inside agar-agar, and gelatin provides a shield to withstand higher temperatures. Further hydrophobic interactions may emerge, reduce the flexibility of enzyme and therefore higher temperature may be required for optimal activity (Munjal & Sawhney, 2002; Jaiswal et al., 2016).

Some enzymes exhibited no change in optimum temperature (Tanriseven & Dogan, 2002; Tanriseven & Olcer, 2008; Srivastava & Anand, 2014; Mesbah & Wiegel, 2018), while, others exhibited little increase in optimum temperature (5 °C) (De Paula et al., 2008; Rehman et al., 2014; Wahab et al., 2018; Sattar et al., 2018; Pervez et al., 2019). In some cases, there was increase of 20 °C or more (Ichikawa et al., 2002; Mulagalapalli et al., 2007; Nawaz et al., 2015; Bilal et al., 2016b). Some of the immobilized enzymes were functional at 70 °C and above

(Ichikawa et al., 2002; Karim et al., 2014). At 70 °C, the agar and gelatin immobilized enzyme retained 42 and 57% of initial activity, respectively, whereas, only 32% activity was left for the soluble enzyme. Consequently, it can be advocated that the native structure of the entrapped enzyme was shielded from denaturation due to elevated temperature, and might be useful for several industrial applications (Karim et al., 2014).

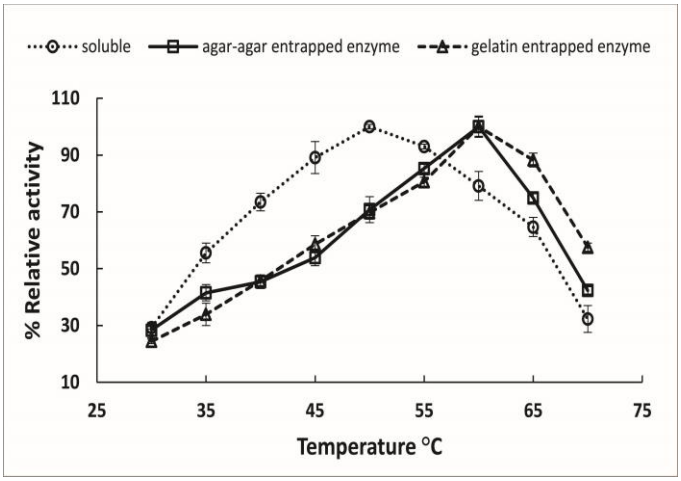

Fig. 2: Effect of variation of temperature (30-70 °C) on soluble and immobilized APase.

From the above data, Arrhenius plot was made (Plot not shown), and the energy of activation was determined. The activation energy values of agar-agar and gelatin entrapped APase were found to be 36.6 and 36.8 kJmol^{-1}, respectively. The result suggests that entrapment of enzyme inside agar-agar and gelatin is very similar. Further the activation energy of immobilized enzymes was a little higher than the soluble enzyme (33.1 kJmol^{-1}). In another report, the activation energy for entrapped invertase determined, was lower in comparison to the soluble enzyme (Vujcic et al., 2011).

F. Effect of substrate concentration on rate

The result of the effect of [*p*-NPP] on rate of agar-agar and gelatin immobilized APase has been presented as a Lineweaver-Burk plot in Fig. 3 A and B, respectively. The K_m and V_{max} values for soluble APase has been reported earlier (Kalita & Ambasht, 2019). The K_m values for agar-agar and gelatin entrapped APase were 0.282 and 0.3 mM, and the V_{max} values were 2.15 and 2.10 µmol/min/mg protein, respectively. It is interesting to note that K_m and V_{max} values are quite similar for immobilized APases. There are reports on a higher K_m value of immobilized enzymes (De Alteriis et al., 1988; Yamato et al., 2000; Srivastava & Anand, 2014; Nawaz et al., 2015; Mesbah & Wiegel, 2018; Sattar et al., 2018; Pervez et al., 2019). Several divergent reasons have been cited for the increase in K_m inside gelatin and agar-agar gel. APase uses *p*-NPP as substrate and has negative charges. The gelatin gel hardened in the presence of formaldehyde due to the formation of methylene bridges with basic residues of gelatin. The acidic amino acid residues are free; impart negative charges and repel *p*-NPP and therefore increase in K_m value was observed (De Alteriis et al., 1988). Another reason cited is the limited approachability of the substrate to the active site due to diffusional resistance and steric obstruction (Vujcic et al., 2011; Nawaz et al., 2015; Bilal et al., 2016a; Pervez et al., 2019). Formation of the Nernst layer around immobilized enzyme gel and a higher requirement of the substrate is essential to access the active site (Kumar et al., 2009; Sattar et al., 2018). Immobilization may bring about an alteration in the conformation of the enzyme as well (Goldstein, 1976).

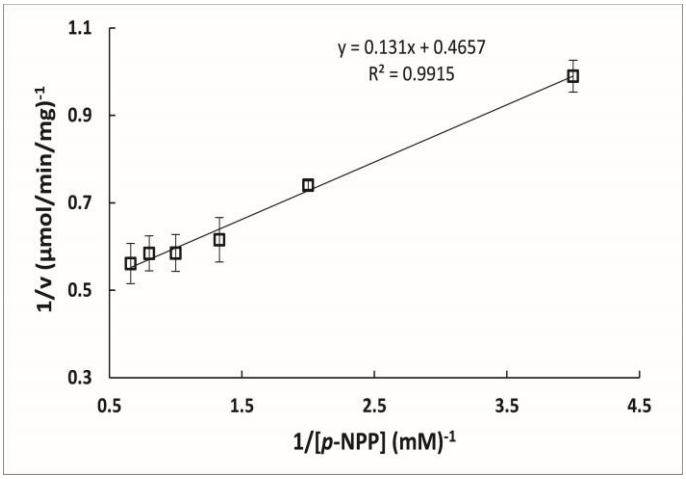

Fig. 3 (A) Lineweaver-Burk plot of Agar-agar immobilized APase (□)

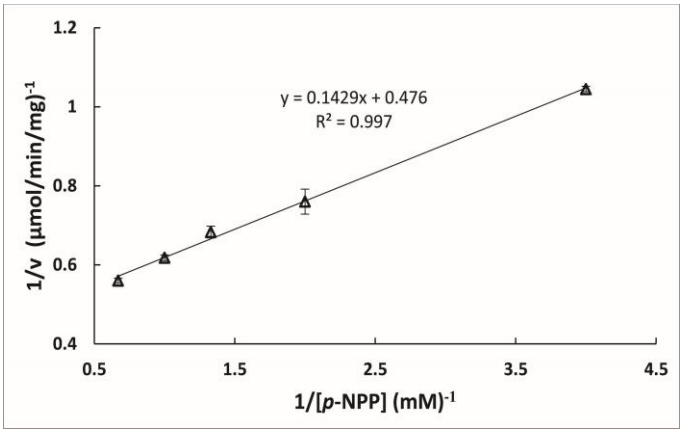

Fig. 3 (B) Lineweaver-Burk plot of Gelatin immobilized APase (Δ)

G. Storage stability

The storage stability of an enzyme provides the information on the duration that the enzyme can retain its catalytic activity when stored under particular conditions. The stability aspect of soluble and immobilized APase was investigated for 85 days at 4 °C. The results are exhibited in Fig. 4. The results indicated that immobilized enzymes possessed more than 65% activity after 84 days and loss in activity was gradual. The soluble enzyme, however, was labile and only 11% activity was retained under similar conditions. The latter enzyme exhibited a sudden loss

between 45 (56.9%) and 56 (23.5%) days. On the 56th day, agar-agar and gelatin immobilized APase retained 73.2 and 75.7% activity. There are some other reports where enzymes immobilized inside agar-agar and gelatin have been stored at 4 °C (Srivastava et al. 2001; Kharkrang & Ambasht, 2013; Asgher et al., 2017; Sattar et al., 2018; Pervez et al., 2019). α-Amylase immobilized inside gelatin bead, hardened in the presence of formaldehyde; exhibited 78.6% activity retention after 90 days under conditions of storage at 4 °C. The soluble enzyme retained only 50% activity after 90 days, when stored under similar conditions (Kharkrang & Ambasht, 2013). Laccase retained 60% activity on 42nd day when entrapped in agar-agar while only 38% entrapped in gelatin (Asgher et al., 2017). The storage of amyloglucosidase entrapped inside agar-agar was examined at 4 and 37 °C, and the activity retained after 60 days was 57 and 33%, respectively (Pervez et al., 2019). Entrapment of enzymes inside a gel improves the stability. α-Amylase entrapped inside calcium alginate beads retained 95% activity, after 90 days under conditions of storage at 4 °C (Kharkrang & Ambasht, 2019). Urease immobilized in gelatin exhibited $t_{1/2}$ value of 240 days (Srivastava et al. 2001).

There are reports on storage stability (4°C) study of APase immobilized on different matrices. For APase immobilized on layered double hydroxides (uncalcined and calcined Mg/Al-CO_3), the 50 % activity was retained after 55 and 79 days, respectively (Zhu et al., 2010). APase immobilized on chitosan retained 46.2% activity after 49 days (Belho et al., 2014) and 50 % activity after 60 days (Srivastava & Anand, 2014).

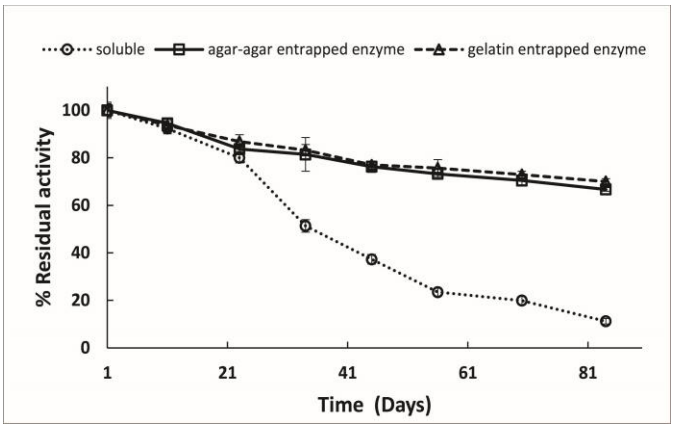

Fig. 4: Storage stability of soluble and immobilized APase

H. Reusability (Operational Stability)

The recycling efficiency or reusability is an important parameter from the economic viewpoint. Among the reports on the reuse of different enzymes immobilized inside agar-agar gel, invertase possessed in excess of 75% activity after 70 cycles of reuse (Vujcic et al., 2011). Amylase possessed more than 90% activity after 11 cycles and 58% after 16 cycles (Mesbha & Weigel, 2018). Most of the reports demonstrated less than 10 cycles of reuse. After 8 cycles of reuse, protease retained 50% (Sattar et al., 2018); pectinase retained 60% (Wahab et al., 2018); manganese peroxidase 27.6% (Bilal et al., 2016a) and β-glucanase 12% activity (Karim et al., 2014). Laccase immobilized in agar-agar and gelatin retained 24.8 and 22.8% residual activity, respectively after 8 cycles of reuse (Asgher et al., 2017). α-Amylase immobilized inside gelatin could be further used for 8 cycles with preservation of 60.7% activity (Kharkrang & Ambasht, 2013). The possible cause for activity decay may be leaching of enzyme as well as loss of matrix stability due to repeated use.

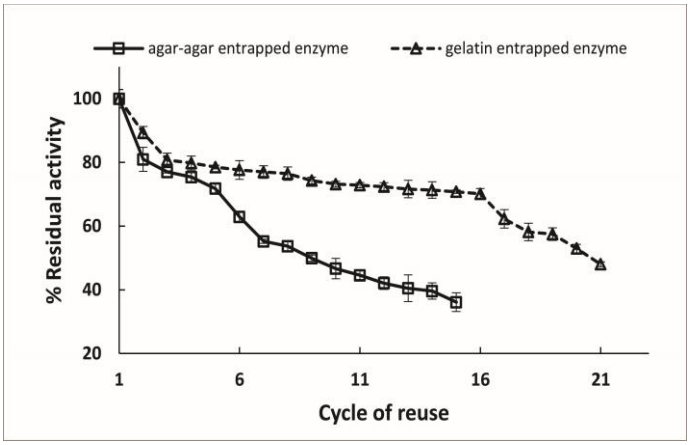

Fig. 5: Number of cycles of reuse of immobilized agar-agar and gelatin APase blocks

Here, the persistent usability of agar-agar and gelatin entrapped enzyme was carried out for different cycles and are displayed in Fig. 5. It was observed that the reuse capability of gelatin immobilized enzyme was much better as it could be reused for more than 21 cycles (48% retention) whereas, agar-agar immobilized enzyme could be reused for 15 cycles only (36.1% retention). In both immobilized enzymes, initial reuse brought a sudden drop in activity (2nd cycle 80.9% residual activity in agar-agar and 89% in gelatin). Thereafter, the rate of loss was slow. The activity retention after 15 cycles in agar-agar and gelatin were 36.1 and 70.8%, respectively.

CONCLUSION

In the current work, APase was entrapped inside agar-agar and gelatin gel. The % immobilization was more than 70% in the case of agar-agar and 80 % in the case of gelatin hardened in the presence of glutaraldehyde. The immobilization did not bring any major change in the properties of the enzyme. Only a little change in pH optimum (0.5 unit) in comparison to the soluble enzyme was observed. APase immobilized inside the above matrices functioned optimally at 60 °C and exhibited very similar activation energies. The K_m values were also very similar. Further, immobilized APase stored at 4 °C, retained more than 65% activity on 84th day. Both immobilized enzymes could be reused for more than 15 cycles efficiently.

ACKNOWLEDGMENTS, COMPLIANCE WITH ETHICAL STANDARDS AND CONFLICT OF INTEREST

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