Immobilization of endoglucanase Cel9A on chitosan nanoparticles leads to its stabilization against organic solvents: the use of polyols to improve the stability

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Abstract

The immobilization of enzymes improves their stability in non-conventional media such as organic solvents. In this work, the effects of solvents (DMSO, methanol, ethanol, and *n*-propanol) on the endoglucanase Cel9A activity and stability were studied. Then, the enzymes were stabilized by its immobilization on chitosan nanoparticles and also using polyols (sorbitol and glycerol) against organic solvents. The SEM results illustrated that the chitosan nanoparticles had about 40 nm diameter. The results indicated that the organic solvents, especially *n*-propanol, decreased the activity of the free and immobilized enzymes. The reduced activity of the immobilized enzyme was less than that of the free enzyme. Our studies about the enzymes' stability showed that the free and immobilized enzymes in hydrophobic solvents (with high log *P*) had the lowest stability compared to other solvents as we observed the half-life of the free enzyme in *n*-propanol solvent was 2.84 min, and the half-life of the immobilized enzyme was 4.98 min in *n*-propanol and ethanol solvents 4.50 min. Analysis of the combinatory effects of polyols (sorbitol and glycerol) and the solvents on the stability revealed that sorbitol and glycerol had the most stabilizing effect on the free enzyme in hydrophilic (DMSO) and hydrophobic (*n*-propanol) solvents, respectively. However, the stabilizing effects of polyols in the immobilized enzyme were independent of the solvents' hydrophobicity (or log *P*) due to the hydrophilic properties of chitosan nanoparticles. Therefore, one can conclude that the physiochemical properties of nanoparticles (such as hydrophilicity) influence the stabilizing effects of polyols on immobilized enzyme.

Keywords Immobilization · Chitosan nanoparticles · Endoglucanase Cel9A

Abbreviations

AaCel9A	Endoglucanase Cel9A from <i>Alicyclobacillus acidocaldarius</i>
ChNPs	Chitosan nanoparticles
DMSO	Dimethyl sulfoxide
DNS	Dinitrosalicylic acid
CMC	Carboxymethyl cellulose
TPP	Triphosphosphate
SEM	Scanning electron microscopy

Introduction

Enzymes are biological catalysts that are widely employed in various fields. However, enzymes are prone to be easily denatured by changes occurring in environmental conditions (Matsumoto et al. 1997). Therefore, the stability of enzymes is one of the essential criteria for commercial and industrial applications (Matulis et al. 1999; Ulbrich-Hofmann et al. 1999). Enzymes are widely used to catalyze a variety of biological and industrial reactions in organic solvents. Using enzymes in organic solvents has advantages over the aquatic environment; the benefits of using organic solvents include increased solubility of non-polar substrates, easy separation of products and recovery, enzyme reusability, reduced side reactions and elimination of microbial contamination in the reaction mixtures (Sellek and Chaudhuri 1999; Ogino et al. 2007; Klibanov 2001; Simon et al. 2001). In spite of the advantages, most organic solvents decrease the activity and stability of enzymes (Griebenow and Klibanov 1996; Miroliayi and Nemat-Gorgani 2002). Enzymes are surrounded by

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a hydrated layer of water molecules in aqueous solution. It has been suggested that organic solvents tend to strip water molecules from the hydrated layer of an enzyme, thereby destroying the interactions which are responsible for maintaining natural conformation of enzymes (Khmelnitsky et al. 1991). It has been indicated that the activity and stability of enzymes in an organic solvent are associated with the hydrophobic nature of the solvent, meaning that the destructive effects on the activity and stability of the enzyme can be raised by hydrophobicity (log *P*) of solvents (Pazhang et al. 2006). The maintenance of enzyme stability or activity in non-aqueous medium faces a significant challenge. Several approaches such as immobilization, the use of polyols, protein engineering and chemical modifications can be used for protein stabilization (Pazhang et al. 2006; Khajeh et al. 2001; Lee and Timasheff 1981; Pazhang et al. 2016).

The most important stabilization method to reduce direct contact of enzyme molecules with the organic solvent is to immobilize enzymes on different supports. Enzyme immobilization is a method used for increasing the efficiency of enzymes and the possibility of enzymes' recovery (Mardani et al. 2018). Also, the immobilization of enzymes on support can decrease the destabilizing effect of organic solvent on enzyme structures. Therefore, immobilization improves both the activity and stability of the enzyme in organic solvents (Trevan 1980). The other advantages of the enzyme immobilization are the possibility of reusing enzymes, continuous operations, enzyme separation from reaction mixtures, and increased stability in different temperatures and pH (Kumari et al. 2008). The type of immobilization and supports can be selected based on the application. Covalent binding is a conventional method for immobilization (Datta et al. 2013). The covalent binding is generally formed between the functional group in the support matrix and the enzyme surface that contains amino acid residues (Wong et al. 2008). In the covalent immobilization, bifunctional reagents such as glutaraldehyde are used for immobilization of enzymes onto different surfaces (Datta et al. 2013). Among different supports, chitosan is a natural polyamine–saccharide obtained by N-deacetylation of chitin. The availability of chitosan and its unique chemical and biological properties such as hydrophilicity, nontoxicity, biocompatibility, and biodegradability make it a very attractive biomaterial for enzyme immobilization (Chen et al. 2013; Krajewska 2004; Rampino et al. 2013). Immobilization of enzyme on chitosan support provides an appropriate microenvironment for the enzymatic reaction where enzyme reactivity is well preserved against different solvents (Dong et al. 2017). Nanoparticles have unique characteristics due to their small size and large surface area to volume ratio, which makes them ideal for the enzyme immobilization (Ansari and Husain 2012).

Using polyols is another enzyme stabilization approach. This method has drawn much more attention because of its

relatively low cost and easy usage half (Timasheff 1998; Pazhang et al. 2006). The polyols usually exert their stabilization effects via preferential hydration of proteins (Timasheff 1998, 2002; Kumar et al. 2011; Street et al. 2006). Among polyols, sorbitol and glycerol are frequently used for protein stabilization (Timasheff 1998; Kumar et al. 2011; Petersen et al. 2004). The stabilizing effects of osmolytes (polyols) on proteins correlate with the physiochemical properties of solvents. It has been shown that the stabilizing effect of polyols on pyrazinamidase correlates with the hydrophobicity of solvents (Pazhang et al. 2018a).

Cellulases are multicomponent enzymes that consist of three different enzymes including endoglucanase (β -1,4-glucanase), cellobiohydrolase and β -D-glucosidase which work synergistically to degrade cellulose for producing monomeric sugars to provide renewable fuels (Lynd et al. 2002; Hong et al. 2014; Hemsworth et al. 2014). The cellulases possess several desirable qualities for a wide range of applications, ranging from pulp and paper, textile, laundry, food and feed industry, agriculture and production of biofuels from renewable sources (Kuhad et al. 2011; Sadhu and Maiti 2013; Sarkar et al. 2012). Endoglucanase Cel9A from *Alicyclobacillus acidocaldarius* (AaCel9A) is a monomeric enzyme with 537 residues, which belongs to the family nine glycoside hydrolases (Eckert et al. 2002). This enzyme has an Ig-like domain composed of the first 85 amino acid residues in the N-terminal domain followed by its catalytic domain (Beguin 1990; Pereira et al. 2009; Bayer et al. 2006). In the structure of the catalytic domain, there are metal ions such as calcium and zinc (Eckert et al. 2009). It has been shown that the Ig-like domain contributes to the dependence of the enzyme stability on calcium (Pazhang et al. 2018b).

The present study was done to investigate the effects of chitosan as a carbohydrate polymer support on endoglucanase enzyme behavior under different stability conditions. For this, endoglucanases were immobilized onto chitosan nanoparticles (ChNPs) by glutaraldehyde as a linker. Then, the effects of organic solvents (DMSO, methanol, ethanol, and *n*-propanol) on the activity and the stability of the free and the immobilized enzymes were investigated. Eventually, the stabilizing effects of the polyols (sorbitol and glycerol) on the free and the immobilized enzymes in the presence of organic solvents were studied.

Materials and methods

Chemicals

Chitosan with medium molecular weight (190000–310000 Da) and the degree of deacetylation ranging from 75 to 85% was purchased from Sigma-Aldrich, USA. Glutaraldehyde (25%), carboxymethyl cellulose

(CMC), dinitrosalicylic acid (DNS), dimethyl sulfoxide (DMSO), methanol, ethanol, and *n*-propanol were purchased from Merck (Darmstadt Germany). Tripolyphosphate (TPP) was purchased from Sigma-Aldrich. All other chemicals were of analytical grade.

AaCel9A enzyme production and purification

To produce the AaCel9A, *E. coli* BL21 (DE3) cells were transformed by pET-21a (+) containing AaCel9A gene, which was previously constructed in our laboratory (Rahimi-zadeh et al. 2015). Then, the expression and purification of the recombinant enzymes were performed as previously described. The purified enzyme concentrations were determined by the Bradford method using BSA as standard (Bradford 1976; Sambrook et al. 1989).

ChNPs preparation and activation with glutaraldehyde

ChNPs were prepared by ionic gelation method where inter- and intracross-linking (gelation) of protonated amine groups with the negatively charged tripolyphosphate (TPP) anions form spontaneously on the chitosan molecules, which lead to the formation of the chitosan particles (Dong et al. 2013). First, 0.023 g chitosan powder was dissolved in the acetic acid solution (2%) and Tween 80 as a surfactant by

continually stirring and then aqueous TPP (10%) solution was added during sonication. In the next step, ChNPs were placed on a stirrer for 2 h and, to separate from the rest of the substances in the reaction and samples, were centrifuged at $15,000\times g$ for 5 min. Following this, the precipitant was washed three times with phosphate buffer (50 mM and pH 7). To functionalize the nanoparticles, glutaraldehyde (1.25%) was added to nanoparticles. The immobilization was done by different concentrations of glutaraldehyde, among which 1.25% of glutaraldehyde had the most efficiency for the immobilization (data not shown). Finally, ChNPs were washed with distilled water for the complete removal of unreacted glutaraldehyde until the absorbance was lower than 0.01 at 280 nm and then they have washed three times with phosphate buffer again and stored at 4 °C in phosphate buffer. Eventually, the morphology, size, and structural properties of obtained nanoparticles were characterized by scanning electron microscopy (SEM).

The AaCel9A enzyme immobilization

The AaCel9A was immobilized onto ChNPs by using glutaraldehyde as a linker. The enzyme immobilization on the ChNPs required at least 16 h at 4 °C to complete the immobilization reaction. Then, the nanoparticles were washed three times with phosphate buffer (50 mM and pH 7) to remove free (unbound) enzyme molecules (Fig. 1).

Fig. 1 Schematic view of the process of preparation of ChNPs containing the enzyme

The determination of the free and the immobilized AaCel9A activity

The activity of the free and immobilized AaCel9A was determined by incubating the free and immobilized enzymes for 10 min with 1% (CMC) in phosphate buffer (pH 7) at 65 °C. Enzymatic reactions were stopped by adding of DNS reagent followed boiling for 5 min (Miller 1959). The produced reducing ends of sugars were measured at 540 nm by spectrophotometer, using glucose as a standard. One unit (U) of the enzyme activity was defined as the amount of enzyme that produces 1 μ mol of glucose equivalent per minute under the conditions mentioned.

The effect of organic solvents on the activity of free and immobilized AaCel9A

For determining the activity of the free and the immobilized AaCel9A in the organic solvents, different concentrations (from 0 to 25% v/v of each solvent) of the organic solvents including DMSO, methanol, ethanol and *n*-propanol in the phosphate buffer (50 mM and pH 7) were prepared and then enzymes activity was determined as described above. The solvents were selected according to their hydrophobicity (log *P*) (Asghari et al. 2011; Badoei-Dalfard et al. 2010). The activity of the free and immobilized enzymes in the reaction without solvents was considered as 100%.

The effects of organic solvents on the free and the immobilized AaCel9A stability

To study the effects of the organic solvents on thermal stability (or irreversible thermal inactivation) of the free and immobilized AaCel9A, the enzyme solutions containing the organic solvents [10% (v/v)] were prepared. It should be noted that the stability of the enzymes at the different concentrations of the solvents was evaluated, which proved that the stability of the enzymes at 10% (v/v) of each solvent was prominent (data not shown). Therefore, organic solvents with a concentration of 10% were selected to evaluate the enzyme stability. The enzyme solutions were incubated at 65 °C at different times (0, 5, 10, 15, 20 and 25 min) and the residual activity of each sample was determined after being on the ice for 30 min (for protein refolding and termination of the enzyme thermal inactivation). The residual activity of the enzymes in the reactions without solvents was considered as a control.

Calculation of half-life and k_{in} of the free and immobilized AaCel9A in the presence of organic solvents

For calculation of the half-life and rate constant of enzyme inactivation (k_{in}), the time course of a reduction in the free

and immobilized enzymes, residual activity in the presence of organic solvents are necessary to be calculated. In the present study, a decrease in the residual activity was a linear function of the enzymes incubation time, which indicated the first-order reaction for enzymes inactivation. Accordingly, for estimation of the rate constant of enzymes inactivation (k_{in}) and the half-life, the following equations were used, respectively:

$$\text{Half-life} = 0.693/k_{in} \quad \text{and} \quad \ln(\text{Activity}) = \ln(\text{Activity})_0 - k_{in}t.$$

The effects of polyols on the free and the immobilized AaCel9A stability

The effects of polyols (sorbitol and glycerol) on the stability of the free and the immobilized AaCel9A in the presence of organic solvents were evaluated. The enzyme solutions containing sorbitol [5% (w/v)] and glycerol [20% (v/v)] were prepared in the presence of the organic solvents (the results for determining the optimum concentration of glycerol and sorbitol for enzyme stabilization are not shown). The enzyme solutions were incubated at 65 °C for different times (0, 5, 10, 15, 20, and 25 min) and the residual activity of each sample was measured as described previously. Similarly, for these experiments, enzyme/organic solvent solutions kept on ice were considered as control.

Results

Purification of the recombinant AaCel9A

After overexpression, the recombinant AaCel9A was purified using an Ni-NTA affinity chromatography column. As shown in Fig. 2, SDS-PAGE analysis revealed a band with a molecular weight of 59 kDa whose size was consistent with the predicted mass of the recombinant enzyme, which had 537 residues.

Chitosan nanoparticle size analysis

The synthesized ChNPs were studied using SEM to determine their average particle size and morphology. It was observed that the particle size of the product depends on the designed parameters during the synthesis procedure. The results indicated that the particles had a 40 nm approximate diameter (Fig. 3). According to the shape and size, the particles were suitable for the immobilization of enzymes.

The effects of organic solvents on the free and immobilized AaCel9A activity

To evaluate the activity of the free and the immobilized AaCel9A in the presence of organic solvents, the activities

Fig. 2 SDS-PAGE analysis of the enzyme expression and purification. Lane 1: marker (kDa), lane 2: crude enzyme extract (crude enzyme extract from induced recombinant *E. coli* BL21 by sonication), lane 3: partially purified enzyme using heat treatment, lane 4: purified AaCel9A on the Ni-NTA column

of the enzymes were measured in different concentrations (from 0 to 25% v/v of each solvent) of the solvents (DMSO, methanol, ethanol, and *n*-propanol). As shown in Fig. 4a, DMSO increased the free enzyme activity at 5% and 10% concentrations, while the higher concentrations of DMSO decreased the enzyme activity. The other organic solvents reduced the free enzyme activity from low concentrations. However, the organic solvents decreased the immobilized enzymes' activity less than that of the free enzymes (Fig. 4b).

The effects of the organic solvents on the free and immobilized AaCel9A stability

To determine the irreversible thermal inactivation of AaCel9A in the presence of organic solvents, the free and the immobilized enzyme solutions containing 10% (v/v) of each solvent were incubated at 65 °C for different times (0–25 min). As can be seen in Fig. 5a, the organic solvents reduced the free enzyme stability, while this reduction was lower in the immobilized enzymes, especially in the presence of DMSO and methanol (Fig. 5b). DMSO decreased the free and immobilized enzyme stability less than other solvents (Fig. 5a, b). Also, the half-life of the immobilized enzymes was approximately five times more than that of the free enzymes in the presence of DMSO (Table 1). On the other hand, the stability of the enzymes decreased more than that of the others in the presence of *n*-propanol (Fig. 5a, b). However, the results showed that the immobilized enzymes in the presence of organic solvents were more stable than the free forms (Fig. 5a, b). As shown in Table 1, the half-life of the immobilized enzymes was higher than that of the free enzymes in the presence of organic solvents.

The effects of polyols on the free AaCel9A stability in the presence of organic solvents

Polyols can stabilize proteins against various stresses. To study the stabilizing effect of sorbitol and glycerol on the free and immobilized enzymes, the residual activity of enzymes was investigated in the presence of solvents. As shown in Fig. 6, the effects of sorbitol on the stability of free

Fig. 3 The high-resolution SEM imaging of ChNPs. The ChNPs were obtained by ionic gelation method (a). These particles exhibited nearly spherical shape, and their diameters were approximately 40 nm (b)

Fig. 4 The activity of the free (a) and the immobilized (b) enzymes in the presence of organic solvents with different concentrations. The free and the immobilized enzyme activity in reactions without solvents was considered as 100%

AaCel9A were more in the presence of DMSO and methanol than glycerol. Among the used solvents, glycerol showed the most stabilizing effects in the presence of ethanol. Also, the results showed that polyols had no significant effects on the stability of the free enzymes in the presence of the *n*-propanol.

The effects of polyols on the immobilized AaCel9A stability in the presence of organic solvents

As shown in Fig. 7a–d, glycerol and sorbitol increased the stability of the immobilized AaCel9A in the presence of all organic solvents. However, in the case of non-polar solvents (ethanol and *n*-propanol), the stabilizing effects of polyols (sorbitol and glycerol) on the immobilized enzymes were significant (Fig. 7c, d).

Discussion

The stability of enzymes in organic solvents is one of the significant challenges in industrial applications (Homaei et al. 2013). In this study, we immobilized AaCel9A on ChNPs to improve the enzyme activity and stability in the presence of organic solvents.

Fig. 5 The thermal stability of the free (a) and the immobilized (b) AaCel9A in the presence of 10% (v/v) of each solvent. The free and the immobilized AaCel9A activity in the reactions without solvent was considered as control

Table 1 The effect of the organic solvents on the half-life and $k_{\text{inactivation}}$ of the free and immobilized AaCel9A

Solvent (10% v/v)	Log <i>P</i>	Free AaCel9A		Immobilized AaCel9A ^a	
		k_{in} (min ⁻¹)	Half-life ($t_{1/2}$) (min)	k_{in} (min ⁻¹)	Half-life ($t_{1/2}$) (min)
–	–	0.0086	80.58	–	–
DMSO	– 1.34	0.0669	10.35	0.0136	50.95
Methanol	– 0.74	0.0845	8.20	0.0434	15.96
Ethanol	– 0.32	0.113	6.13	0.1538	4.50
<i>n</i> -Propanol	0.34	0.244	2.84	0.1389	4.98

^aIn the case of immobilized AaCel9A in the absence of solvents, the enzyme inactivation rate did not follow the first order of reaction, and then the half-life and $k_{\text{inactivation}}$ were not calculated

Organic solvents reduced the activity of both free and immobilized enzymes, but the activity of immobilized enzymes in the presence of solvents was more than that of its free form (Fig. 4). The reduction of the enzyme's activity in the presence of organic solvents can be due to the rigidity of the enzyme structure, denaturation, and inhibition (Klibanov 1997). The higher activity in the immobilized forms compared to the free enzymes can have many reasons. Pahujani

Fig. 6 The thermal stability of the free AaCel9A in the presence of organic solvents and polyols (sorbitol and glycerol). **a** The enzyme stability in the presence of polyols and DMSO; **b** the enzyme stability

in the presence of polyols and methanol; **c** the enzyme stability in the presence of polyols and ethanol; **d** the enzyme stability in the presence of polyols and *n*-propanol

Fig. 7 The thermal stability of the immobilized AaCel9A in the presence of organic solvents and polyols (sorbitol and glycerol). **a** The enzyme stability in the presence of polyols and DMSO; **b** the enzyme

stability in the presence of polyols and methanol; **c** the enzyme stability in the presence of polyols and ethanol; **d** the enzyme stability in the presence of polyols and *n*-propanol

et al. have shown that immobilization of *Bacillus coagulans* lipase on Nylon-6 support increases the enzyme activity in the presence of 2-propanol (Pahujani et al. 2008). Wang et al. studied the activity of free and immobilized laccase from *Rhus vernicifera* in the presence of organic solvents. Their results showed that the immobilized enzymes have a greater activity compared to free enzymes. It has been assumed that the microenvironment of the immobilized enzymes on chitosan helps the preservation of the active conformation of enzymes (Wan et al. 2010; Zhan et al. 1991). Presumably, the immobilization of enzymes on a chitosan support traps water molecule because of its hydrophilicity, which leads to preventing the entry of organic solvent molecules around the immobilized AaCel9A. Also, the immobilization prevents water molecules stripping by organic solvents from the hydration shell of the immobilized AaCel9A, which in turn results in the maintenance of enzyme structure and activity in the presence of high concentration of the solvents in comparison with the free enzymes (Tsuzuki et al. 2003), which is in line with our results.

In the case of stability, the enzymes were more stable in DMSO than in other solvents. Therefore, the free and the immobilized AaCel9A maintained more than 60% and 90% of their activity within 10 min, respectively. Besides, considerable stability was observed in the immobilized form of the enzymes in methanol (Fig. 5b). These results indicate that the AaCel9A exhibited more stability in the presence of polar solvents than in non-polar solvents. As the solvent log P increases, the enzyme stability decreases due to the unfolding of AaCel9A structure in the hydrophobic medium. It can be concluded that strong covalent bond formation between enzymes and support may induce the increased rigidity of enzymes, thereby raising the stability of the immobilized enzymes compared to the free enzymes in the presence of organic solvents. Tjernberg et al. showed that the enzymes tend to become more compact in low concentrations of polar solvents due to the reduction of the number of basic sites available for protonation, which gives rise to the greater stability (Tjernberg et al. 2006). Probably, the compactness that can be seen in the immobilized AaCel9a can be considered as the reason for more stability of the enzymes in the immobilized form. In the presence of *n*-propanol, the enzyme maintained 10% of itself stability during 10 min incubation at 65 °C. In the case of the immobilized AaCel9A, this amount was 14%. By increasing the log P of the solvent, the interaction of solvent molecules with the hydrophobic regions of enzyme increases. Therefore, the enzymes are likely to lose their natural structures, which triggers decreased stability as described by Pliura and Jones (1980).

In the case of polyols, sorbitol showed more stabilizing effects on the free AaCel9A in hydrophilic solvents, especially DMSO. With increasing hydrophobicity of solvents

(an increase of log P), the stabilizing effects of sorbitol decreased so that this effect was insignificant in non-polar solvent *n*-propanol. Pazhang et al. showed that with increase in log P of solvents, the stabilizing effects of sorbitol decreased in the trypsin enzymes (Pazhang et al. 2016), which is consistent with our findings. Also, Petersen et al. (2004) showed that sorbitol leads to preferential hydration of lysozymes whose structure has been unfolded due to preferential dislocation from hydrophilic surfaces of the lysozyme. It has been suggested that the preferential exclusion of sorbitol can decrease with increment in solvents' log P because of its polarity. This can indicate that the stabilizing effect of sorbitol on the free AaCel9A is likely to decrease with an increase in log P of the solvent.

In the free form of the AaCel9A, the stabilizing effects of glycerol in hydrophobic solvent (ethanol and *n*-propanol) were more than those of the hydrophilic solvent (DMSO and methanol). Glycerol is known to change the native protein group into more compact states. Vagenende and colleagues showed that glycerol preferentially interacts with the large fragment of contiguous hydrophobicity where glycerol acts as an amphiphilic interface between the hydrophobic surface of enzymes and the polar solvents (Vagenende et al. 2009). It has been shown that the stabilizing effects of glycerol were increased with increase in the solvent log P , which can correlate with a favorable amphiphilic orientation of glycerol in non-polar solvents. This leads to the prevention of unfavorable interactions between hydrophobic regions on the protein surface and non-polar solvents (Pazhang et al. 2016). Then, one can speculate that the stabilization effect of glycerol increases on the free AaCel9A with an increase in solvents' hydrophobicity.

The results indicated that the behavior of glycerol and sorbitol in the free form of the AaCel9A varied. However, this behavior in the immobilized form of the AaCel9A was somewhat similar. It has been shown that the chitosan support has no interaction with organic solvents (Lee et al. 2015) and also the hydrophilicity of chitosan should assist with retention of the essential water molecules in the enzyme molecular microenvironment due to the amino and hydroxyl groups (Wan et al. 2010; Milstein et al. 1989). Consequently, there are water molecules (no organic solvent molecules) in the vicinity of AaCel9A, which attached to the chitosan support. This can explain why the polyols' (sorbitol and glycerol) stabilizing effects are not dependent on the log P of the solvent.

Conclusions

The immobilized AaCel9A showed considerable stability in the organic solvents compared with the free AaCel9A. The stability of the free enzyme was reduced by increasing the

log P of the solvent. The stabilizing effects of the glycerol on the free enzyme increase with increase in the log P of the solvent, while the stabilizing effects of sorbitol increase with decrease in the log P of the solvent. In the immobilized AaCel9A, the stabilizing effects of sorbitol and glycerol are not dependent on log P of the solvent, which can be due to the hydrophilic properties of chitosan supports.

Finally, the immobilization method is an effective way to increase the stability of the enzymes in the presence of polar and non-polar organic solvents in comparison with polyols, since applying polyols for enzyme stability in organic solvents depends on log P and other properties of the solvent.

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

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