*Technical paper*

# An Economical and Simple Bioaffinity Support for the Immobilization and Stabilization of Tomato (*Lycopersicon Esculentum*) Peroxidase

**Mahreen Matto, Sarah Naqash and Qayyum Husain***

*Department of Biochemistry, Faculty of Life Sciences, Aligarh Muslim University, Aligarh-202002. INDIA*

** Corresponding author: E-mail: qayyumhusain@yahoo.co.in; qayyum.husain@amu.ac.in, Phone No. (R) # 91-571-2720135; (O) # 91-571-2700741; Fax No. # 91-571-2721776*

*Received: 24-01-2008*

## Abstract

Ammonium sulphate fractionated tomato proteins were used for the direct immobilization of peroxidase on bioaffinity, Con A-cellulose support. Con A-cellulose bound peroxidase retained 77% of the original activity. The immobilized enzyme showed very high stability against denaturation mediated by heat, pH, organic solvents and inhibitors. Soluble and immobilized peroxidase exhibited maximum activity at 40 °C and pH 6.0. Con A-cellulose bound peroxidase retained 44% and 51% activity against the exposure to 50% DMF and *n*-propanol for 1 h, respectively; however soluble enzyme showed only 21% and 20% activity under similar exposure to organic solvents, DMF and *n*-propanol. Immobilized peroxidase retained significantly higher activity against sodium azide and sodium sulphite as compared to soluble enzyme.

**Keywords:** Ammonium sulphate, concanavalin A, cellulose, tomato, *Lycopersicon esculentum,* immobilization.

## 1. Introduction

Oxidoreductive enzymes such as peroxidases and polyphenol oxidases have shown their potential in the remediation of aromatic pollutants from wastewater.[1,2] Due to certain inherent limitations like susceptibility to inactivation by its own product, non-reusability and instability, soluble enzymes cannot be employed at large scale to treat huge volume of effluents.[3,4,5] In order to overcome such limitations, enzyme immobilization is one of the best alternatives to exploit the enzymes at industrial level.

Immobilized enzymes offer several advantages such as enhanced stability, easier product recovery, protection of enzymes against denaturants, proteolysis and reduced susceptibility to contamination.[6,7] This is due to their enhanced resistance to unfolding provided by multipoint covalent/non-covalent attachment with the matrix. However, most of the immobilized enzyme preparations use commercially available enzyme or expensive supports.[8,9] The high cost and low yield of immobilized enzymes are two major limitations in their applications at industrial level.[6] Immobilized enzyme preparations must be obtained by a cost effective and technologically convenient method.

Bioaffinity-based supports have several merits over the other known conventional methods of enzyme immobilization.[10] Ease of immobilization, easy regeneration of the support, lack of chemical modification and usually accompanying an enhancement in stability are some of the advantages offered by the bioaffinity based immobilization. Besides the mentioned advantages, there is an additional benefit that enzyme gets properly oriented on bioaffinity support.[11,12]

These supports have been used for the high yield and stable immobilization of glycoenzymes/enzymes. This procedure can be employed for the immobilization of enzymes directly from crude homogenate or partially purified enzyme preparations.[13] Affinity binding offers very mild, controlled adsorption of biocatalysts onto the supports and is likely to be of continuing value for immobilization of delicate biocatalysts.

In this study, an effort has been made to prepare bioaffinity support by incubating cellulose with jack bean extract; a source of concanavalin A (Con A). Con A bound cellulose support has been used to immobilize peroxidase directly from ammonium sulphate fractionated tomato proteins. A comparative stability study of soluble (S-TMP) and immobilized tomato peroxidase (I-TMP) has

been carried out against pH, heat, water-miscible organic solvents and some inhibitors.

# 2. Materials and Methods

## 2. 1. Materials

Bovine serum albumin was obtained from Sigma Chemicals Co., (St. Louis, MO) USA. Cellulose was the product of Serva Chemical Co., Heidelberg, Germany. *o*-dianisidine HCl was procured from IGIB, New Delhi, India. The chemicals and other reagents employed were of analytical grade and were used without any further purification. Tomato used in the study was purchased from a local vegetable market.

## 2. 2. Ammonium Sulphate Fractionation of Tomato Proteins

Tomato (50 g) was homogenized in a blender with 100 ml of 0.1 M sodium phosphate buffer, pH 6.0. The filtrate was centrifuged at 10,000 *g* on a Remi R-24 Cooling Centrifuge. The solution thus obtained was subjected to salt fractionation by adding 10–90% (w/v) $(NH_4)_2SO_4$. The mixture was stirred overnight at 4 °C to obtain the maximum precipitate. The precipitate was collected by centrifugation at 10,000 *g* on a Remi R-24 Cooling Centrifuge. The obtained precipitate was re-dissolved in appropriate volume of 0.1 M sodium phosphate buffer, pH 6.0 and dialyzed against the same buffer.[9]

## 2. 3. Adsorption of Con A on Cellulose

Jack bean meal (10 g) was suspended in 100 ml of 0.1 M sodium phosphate buffer, pH 6.2. The mixture was kept on magnetic stirrer for about 12 h at room temperature (28–30 °C) and then centrifuged at 3000 *g* for 30 min and supernatant was collected. Cellulose (5.0 g) was added to 50 ml of distilled $H_2O$ and kept overnight for swelling. It was decanted and then mixed with jack bean extract; the mixture was kept overnight on magnetic stirrer. Con A adsorbed cellulose was collected by centrifugation.[12]

## 2. 4. Immobilization of Tomato Peroxidases (TMP) on Con A-cellulose

Con A-cellulose (5.0 g) was incubated with tomato peroxidase (1500 U) overnight on a magnetic stirrer at room temperature (28–30 °C). The immobilized enzyme was collected after centrifugation at 3000 *g* for 20 min at room temperature and washed thrice with 0.1 M sodium phosphate buffer, pH 6.0. Finally the immobilized enzyme was suspended in the assay buffer and analyzed for enzyme activity.

## 2. 5. Effect of Temperature

Activity of soluble and immobilized TMP (1.27 U) was determined at various temperatures (20–80 °C) in 0.1 M sodium phosphate buffer, pH 6.0. The percent remaining enzyme activity was calculated by taking activity at temperature-optimum as control (100%).

In another set of experiment, tomato peroxidase preparations were incubated at 60 °C for varying times in 0.1 M sodium phosphate buffer, pH 6.0. After each incubation period the enzyme was quickly chilled in crushed ice for 5 min. The enzyme was brought to room temperature and then the percent remaining activity was determined as described in text. The activity of enzyme without exposure at 60 °C was considered as control (100%) for the calculation of remaining percent activity.

## 2. 6. Effect of pH

Soluble and immobilized TMP (1.27 U) were taken for determining the activity of enzyme in the buffers of different pH. The buffers used were glycine-HCl (3.0), sodium acetate (4.0–6.0), sodium phosphate (7.0 and 8.0) and Tris HCl (9.0 and 10.0). The remaining percent activity was calculated by taking activity at optimum-pH as control (100%).

## 2. 7. Effect of Organic Solvents

Soluble and immobilized TMP (1.27 U) were incubated with 10–60% (v/v) of water-miscible organic solvents; *n*-propanol and DMF in 0.1 M sodium phosphate buffer, pH 6.0 at 37 °C for 1 h. Peroxidase activity was determined at all the organic solvent concentrations indicated. The activity obtained without exposure to organic solvent was taken as control (100%) for the calculation of remaining percent activity.

## 2. 8. Effect of Inhibitors

Soluble and immobilized TMP (1.27 U) were incubated with inhibitors; sodium azide (0.01–0.1 mM) and sodium sulphite (0.1–1.0 mM), in sodium phosphate buffer, pH 6.0 at 37 °C. Peroxidase activity was determined at all the inhibitor concentrations indicated. The activity obtained without exposure to inhibitor was taken as control (100%) for the calculation of remaining percent activity.

## 2. 9. Assay of Peroxidase Activity

Peroxidase activity was estimated from the change in the optical density ($\lambda_{460}$ nm) at 37 °C by measuring the initial rate of oxidation of *o*-dianisidine HCl by $H_2O_2$ using the two substrates in saturating concentrations.[14]

Appropriate aliquots of tomato peroxidase were taken in a set of test tubes. The volume was made up to 2.8 ml with 0.1 M sodium phosphate buffer, pH 6.0, $H_2O_2$ (18 mM) and *o*-dianisidine HCl (6.0 m*M*), 100 µl each, was added to the tubes. The total reaction volume was 3 ml in all the tubes. The reaction mixture was properly mixed and incubated at 37 °C for 15 min. The reaction was stopped by adding 1.0 ml 6 N HCl in each tube. The reaction volume was again mixed and the absorbance was taken at 460 nm against the reagent blank.

The reaction mixture with immobilized TMP was continuously stirred for entire duration of assay. The assay was highly reproducible with immobilized enzyme.

One unit (1.0 U) of peroxidase activity is defined as the amount of enzyme protein that catalyzes the oxidation of 1.0 µmol of *o*-dianisidine HCl per min at 37 °C into colored product ($\varepsilon_m$ at 460 nm = 30,000 $M^{-1}cm^{-1}$).

## 2. 10. Protein Estimation

Proteins were estimated by the procedure described by Lowry *et al.*[15] Bovine serum albumin was used as standard.

## 2. 11. Data Analysis

Each value represents the mean for three independent experiments performed in duplicates, with average standard deviation, < 5%.

# 3. Results

## 3. 1. Bioaffinity Based Immobilization of TMP on Con A-cellulose

Con A-cellulose matrix was selected as a bioaffinity media for the direct immobilization of glycoenzymes from ammonium sulphate fractionated tomato proteins. In view of the glycoproteinic nature of tomato peroxidases, these enzymes could be directly immobilized on Con A-cellulose support from ammonium sulphate fractionated proteins or from the crude homogenate of tomato. Con A-cellulose adsorbed 687 U peroxidase/g of the matrix (Table 1).

Immobilized peroxidase preparation retained significantly very high activity as it is evident from high effectiveness factor, η 0.77.

## 3. 2. Effect of Temperature

Fig. 1 demonstrates the temperature-activity profiles of soluble and immobilized enzymes. S-TMP and I-TMP showed maximum activity at 40 °C. However, the immobilized enzyme retained greater fraction of catalytic activity at higher temperatures as compared to the soluble enzyme.

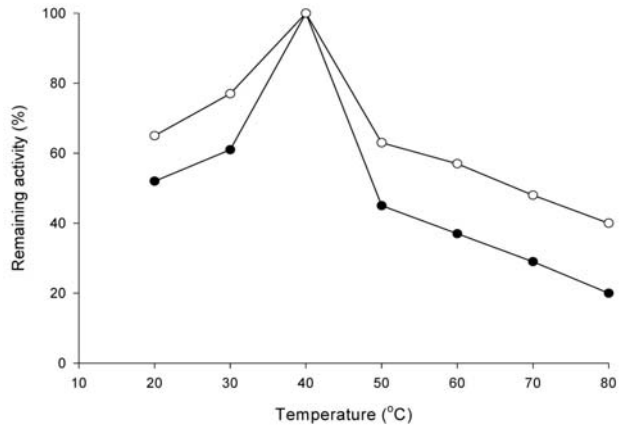


**Figure 1:** Temperature-activity profiles of soluble and immobilized peroxidase. Soluble and immobilized peroxidase (1.27 U) activity was determined in 0.1 M sodium phosphate buffer, pH 6.0 at various temperatures (20–80 °C). Activity expressed at 40 °C was taken as control (100%) for the calculation of remaining percent activity. Symbols indicate, the S-TMP (●) and I-TMP (○).

Soluble and immobilized tomato peroxidase preparations were incubated at 60 °C for various time intervals. Soluble enzyme lost over 70% of its initial activity after 2 h exposure, whereas immobilized enzyme retained about 58% of the original activity under identical exposure to heat (Fig. 2).

**Table 1:** TMP immobilized on Con A cellulose support

| Amount of enzyme loaded (X) (U) | Amount of enzyme activity in washes (Y) (U) | Activity bound/g Con A-cellulose support (U) | | | Activity yield (B/A x 100) (%) |
|---|---|---|---|---|---|
| | | Theoretical (X-Y=A) (A) | Actual (B) | Effectiveness factor (η) (B/A) | |
| 1500 | 608 | 892 | 687 | 0.77 | 77% |

The effectiveness factor ($\eta$) value of the immobilized preparation represents the ratio of actual and theoretical activity of the immobilized enzyme. Each value represents the mean for three-independent experiments performed in duplicates, with average standard deviation, < 5%.

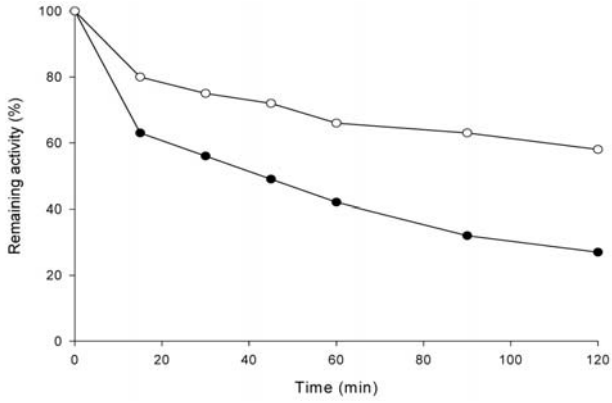


**Figure 2:** Thermal denaturation of soluble and immobilized peroxidase. Soluble and immobilized peroxidase preparations (1.27 U) were incubated at 60 °C for various times in 0.1 M sodium phosphate buffer, pH 6.0. Aliquots were removed at indicated time intervals and chilled quickly in crushed ice for 5 min. The enzyme activity was determined after bringing enzyme preparations at 37 °C. Un-incubated samples were taken as control (100%) for the calculation of remaining percent activity. For symbols please refer to legend of figure 1.

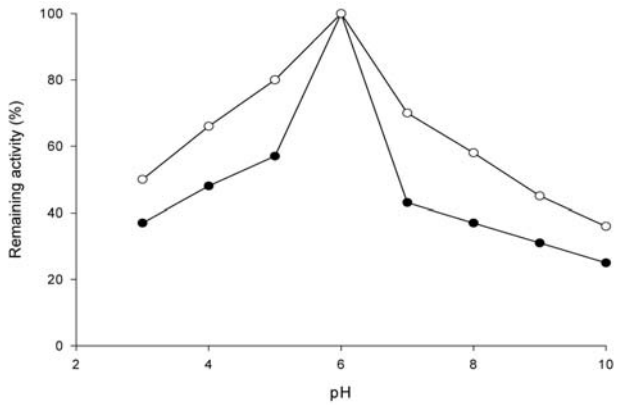


**Figure 3:** pH-activity profiles of soluble and immobilized peroxidase. Soluble and immobilized peroxidase (1.27 U) activity was measured in the buffers of varying pH (3.0–10.0). The molarity of each buffer was 0.1 M. The activity at pH 6.0 for both preparations was taken as control (100%) for the calculation of remaining percent activity. For symbols please refer to legend of figure 1.

## 3. 3. Effect of pH

Immobilized tomato peroxidase showed broadening in pH-activity profiles indicating a marked increase in stability. The pH-optimum was the same for both soluble and immobilized TMP preparations, pH 6.0 (Fig. 3).

## 3. 4. Effect of Water Miscible Organic Solvents

Incubation of soluble and immobilized peroxidase with increasing concentrations of DMF (10–60%, v/v) resulted in decreased activity. Soluble enzyme lost nearly 79% of its activity; however, immobilized enzyme retained over 44% enzyme activity even after exposure to 50% (v/v) DMF for 1 h.

Table 2 demonstrates the effect of increasing concentration of *n*-propanol (10–60%, v/v) on the activity of soluble and immobilized TMP. The activity of soluble enzyme was decreased to 80% upon exposure to 50% *n*-propanol, while immobilized enzyme retained more than 50% activity under similar exposure.

## 3. 5. Effect of Inhibitors

Incubation of soluble and immobilized peroxidase with increasing concentrations of sodium azide (0.01–0.1 mM) resulted in decreasing activity of enzyme. Soluble enzyme lost 91% of the initial activity, whereas immobilized preparation retained slightly higher activity (21%) after exposure to 0.1 mM sodium azide for 1 h at 37 °C (Table 3).

Effect of increasing concentrations of sodium sulphite (0.1–1.0 mM) on the activity of soluble and immobi-

**Table 2:** Effect of water miscible organic solvents on soluble and immobilized peroxidase

| Organic solvent (%, v/v) | Remaining activity (%) | | | |
|---|---|---|---|---|
| | DMF | | *n*-propanol | |
| | S-TMP | I-TMP | S-TMP | I-TMP |
| 10 | 45 | 65 | 54 | 79 |
| 20 | 38 | 61 | 43 | 74 |
| 30 | 32 | 56 | 37 | 68 |
| 40 | 26 | 49 | 29 | 60 |
| 50 | 21 | 44 | 20 | 51 |
| 60 | 15 | 37 | 11 | 42 |

Soluble and immobilized TMP preparations (1.27 U) were incubated with increasing concentrations of DMF/*n*-propanol (10–60%, v/v) in 0.1 M sodium phosphate buffer; pH 6.0 at 37 °C for 1 h and enzyme activity was determined as described in text. The activity of soluble and immobilized peroxidase without exposure to organic solvent was considered as control (100%) for the calculation of remaining activity. Each value represents the mean for three independent experiments performed in duplicates with average standard deviation, <5%.

**Table 3:** Effect of inhibitors on soluble and immobilized peroxidase

| Sodium azide (mM) | Remaining activity (%) | | Sodium sulphite (mM) | Remaining activity (%) | |
|---|---|---|---|---|---|
| | S-TMP | I-TMP | | S-TMP | I-TMP |
| 0.01 | 63 | 80 | 0.1 | 69 | 86 |
| 0.02 | 58 | 72 | 0.2 | 63 | 83 |
| 0.03 | 51 | 71 | 0.3 | 55 | 79 |
| 0.04 | 43 | 65 | 0.4 | 47 | 74 |
| 0.05 | 37 | 58 | 0.5 | 39 | 69 |
| 0.06 | 30 | 50 | 0.6 | 31 | 63 |
| 0.07 | 23 | 41 | 0.7 | 22 | 57 |
| 0.08 | 18 | 33 | 0.8 | 18 | 50 |
| 0.09 | 13 | 27 | 0.9 | 11 | 45 |
| 0.10 | 9 | 21 | 1.0 | 8 | 39 |

Soluble and immobilized TMP preparations (1.27 U) were incubated with increasing concentrations of sodium azide (0.01–0.10 mM)/sodium sulphite (0.1–1.0 mM) in 0.1 M sodium phosphate buffer, pH 6.0 at 37 °C for 1 h and enzyme activity was determined as described in the text. Each value represents the mean for three independent experiments performed in duplicates with average standard deviation, <5%.

lized peroxidase was observed. The soluble enzyme lost nearly 92% of activity while the immobilized enzyme retained 39% of its initial activity in the presence of 1.0 mM sodium sulphite for 1 h at 37 °C (Table 3).

## 4. Discussion

The present study aimed to work out an inexpensive, simple and high yield procedure for the immobilization of tomato peroxidase that would be of extreme interest in the remediation of several types of aromatic compounds present in polluted water or industrial effluents.[16] Partially purified peroxidase was adsorbed on Con A-cellulose support, retaining 77% of the original activity (Table 1). It indicated that the major peroxidases from tomato are glycosylated. This bioaffinity-based procedure provides higher mechanical and operational stability to the enzyme[10,17] while retaining the basic biochemical activity of the free enzyme, thus making it more stable to withstand the handling procedures. In this technique enzyme is immobilized directly from crude homogenate. Thus, the cost of enzyme purification is avoided.[12,18,19]

Native peroxidase is quite stable to inactivation, however immobilization provides additional stability to denaturation mediated by different physical and chemical agents. Several earlier workers have also shown that the enzymes immobilized on bioaffinity support exhibited enhanced stability to heat inactivation.[13] Tomato peroxidase bound to Con A-cellulose exhibited high stability against denaturation induced by heat (Fig. 1,2). However, such enzyme preparations could be useful at relatively high temperatures, which is an important factor for industrial applications.[12]

Industrial effluents and municipal wastewater contain organic solvents together with other aromatic pollutants. The presence of organic solvents can influence the structure of enzymes, exploited for the treatment of wastewater.[17] Therefore; it is of utmost importance to investigate the role of some water-miscible organic solvents on the activity of immobilized enzymes. I-TMP showed remarkably very high stabilization as compared to S-TMP against exposure to DMF and *n*-propanol (Table 2). It goes in agreement with studies of other workers that immobilization of enzymes by multipoint attachment protects them from denaturation mediated by water-miscible organic solvents.[18,20,21] Magri *et al*.[22] have also reported that immobilized soybean seed coat peroxidase showed full activity over the organic solvent concentrations range (5–70% v/v) assayed whereas the free enzyme was almost inactive in 50% (v/v) of the solvents. More recently in our laboratory it has been shown that bitter gourd peroxidase immobilized on protein support was quite resistant to denaturation induced by various water-miscible organic solvents.[12] Thus bioaffnity based adsorption is the simplest method for enzyme immobilization and is especially suitable for preparing immobilized enzymes for use in organic solvents due to less desorption of the adsorbed enzymes in an organic environment.[23]

Sodium azide and sodium sulphite are potent inhibitors of peroxidases.[24] Peroxidase in the presence of sodium azide and $H_2O_2$ mediates one electron oxidation of azide ions forming azidyl free radicals, which bind covalently to the heme moiety thus inhibiting the enzyme activity.[25] Therefore we investigated the effect of these inhibitors on the activity of both soluble and immobilized tomato peroxidases. I-TMP exhibited high stability at higher concentration of inhibitors as compared to its soluble counterpart (Table 3).

Con A-cellulose bound TMP has pronounced stability against various denaturants. Earlier reports described that the immobilization of glycoenzymes on Con A support resulted in the stabilization of enzymes against several forms of denaturation.[11] However, Con A-cellulose ad-

sorbed enzyme has only non-covalent forces between support and enzyme molecules and sometimes it leads to desorption of enzyme or Con A or both from the support. Cross-linking of bioaffinity adsorbed enzyme could be done by using bifunctional or polyfunctional reagents to prevent the dissociation/desorption of enzyme or Con A-glycoenzyme complex from the cellulose support.[26]

## 5. Conclusions

Thus, adsorption procedures are significantly useful in the immobilization of enzymes directly from the crude homogenate and thus avoiding the high cost of enzyme purification. Moreover, this procedure emphasized the immobilization of TMP directly from the crude homogenate or ammonium sulphate precipitated proteins. It has further reduced the cost of immobilized enzyme preparation. Immobilized tomato peroxidase preparations with high stability against various physical/chemical denaturing agents could be exploited for the development of bioreactors for the treatment of phenolic and other aromatic pollutants present in wastewater derived from various agricultural and industrial activities.

## 6. Acknowledgements

The authors are thankful to the University Grants Commission and Department of Science & Technology, New Delhi, India for providing special grants to the Department in the form of DRS and FIST, respectively for developing infrastructure facilities.

**Abbreviations:** Con A, concanavalin A; DMF, dimethyl formamide; TMP, tomato peroxidase; S-TMP, soluble tomato peroxidase; I-TMP, immobilized tomato peroxidase.

## 7. References

1. A. Bhunia, S. Durani, P. P. Wangikar, *Biotechnol. Bioeng.* **2001,** *72***,** 562–567.
2. A. A. Khan, Q. Husain, *J. Environ. Sci*. **2007**, *19*, 396–402.
3. F. Rojas-Melgarejo, J. N. Rodrßguez-López, F. Garcßa-Cánovas, P. A. Garcßa-Ruiz, *Process Biochem.* **2004**, *39*, 1455–1464.
4. B. Krajewska, *Enzyme Microb. Technol.* **2004,** *35*, 126–139.
5. A. E. David, N. S. Wang, V. C. Yang, A. J.Yang, *J. Biotechnol*. **2006**, *125*, 395–407.
6. *N. Duran, A. M. Rosa, A. D'Annibale, L. Gianfreda, Enzyme Microb. Technol.* ***2002****, 31, 907–931.*
7. Q. Husain, *Crit. Rev. Biotechnol.* **2006**, *60*, 201–221.
8. D, Norouzian, *Iran. J. Biotechnol*. **2003**, *1*, 191–205.
9. S. Akhtar, A. A. Khan, Q. Husain, *Chemosphere* **2005**, *60*, 291–301.
10. S. Dalal, M. N. Gupta, **2007**, *67*, 741–747.
11. D. P. Mislovicova, P. Gemeiner, J. Sandula, J. Masarova, A. Vikartovska, P. Doloomansky, *Biotechnol. Appl. Biochem*. **2000**, *31*,153–159.
12 Y. Kulshreshta, Q. Husain, *Biomol. Eng.* **2006,** 23, 291–297.
13 A. A. Khan, S. Akhtar, Q. Husain, *Process Biochem*. **2005**, *40*, 2379–2386.
14 S. Akhtar, A. A. Khan, Q. Husain, *Biores. Technol*. **2005**, *96***,** 621–626.
15 H. O. Lowry, J. N. Rosebrough, L. A. Farr, J. R. Randall, *J. Biol.Chem*. **1951**, *193*, 265–275.
16 A. A. Khan, Q. Husain, *Biores. Technol.* **2007**, *98*, 1012–1019.
17 S. Akhtar, A. A. Khan, Q. Husain, *J. Chem. Technol. Biotechnol.* **2005**, *80*, 198–205.
18 R. Batra, N. M. Gupta, *Biotechnol. Appl. Biochem*. **1994**, *19*, 209–215.
19 R. Batra, R. Tyagi, N. M. Gupta, *Biocatal. Biotransf*. **1999**, *15*, 101–119.
20 V. V. Mozhaev, V. M. Seregeeva, B. A. Belova, L. Y. Khmelnitsky, *Biotechnol. Bioeng.* **1990**, *35*, 653–659.
21 R. Fernandez-Lafuente, P. N. A. Wood, A. D. Cowan, *Biotechnol. Tech*. **1995**, *9*, 1–6.
22 L. M. Magri, V. M. Miranda, O. Cascone, *Biocatal. Biotrans*. **2005**, *23*, 339–346.
23 J. C. Wu, S. S. Lee, B. M. M. Mahmood, Y. Chow, R. M. M. Talukder, W. J. Choi, *J. Mol. Catal. B: Enzymatic*. **2007**, *45*, 108–112.
24 G. Stebbins, V. Gupta, *New Phytol*. **1969**, *108*, 421–432.
25 M. Tatarko, A. J. Bumpus, *Arch. Biochem. Biophys*. **1997**, *339*, 200–209.
26 U. Jan, A. A. Khan, Q. Husain, *World J. Microb. Biotechnol.* **2006**, *22*, 1033–1039.

## Povzetek

Z uporabo iz paradižnika pridobljenih proteinov smo raziskovali direktno imobilizacijo preoksidaze s (konkanavalin A) Con A-celulozo. Izkazalo se je, da peroksidaza, vezana na Con A-celulozo obdrži 77 % svoje prvotne aktivnosti. Imobiliziran encim kaže veliko stabilnost napram denaturaciji s toploto, spremembo pH medija, organskimi topili in inhibitorji. Topna in imobilizirana peroksidaza kaže največjo aktivnost pri 40 °C in pH = 6.0. Po enourni izpostavljenosti 50 % DMF in n-propanolu peroksidaza vezana na con A-celulozo kaže še 44 % oz. 51 % prvotne aktivnosti, v čistih topilih pa aktivnost pade na 21 % in 20 %. Imobilizirana peroksidaza ima kaže višjo aktivnost napram natrijevemu azidu in natrijevemu sulfitu kot topni encim.