Immobilization and characterization of inulinase from *Ulocladium atrum* on nonwoven fabrics

TAREK M MOHAMED[1,*], SOAD M ABU EL-SOUOD[2], EHAB M ALI[1], MOHAMMED O EL-BADRY[3], MAI M EL-KEIY[1] and ALY SAYED ALY[4,†]

[1]*Biochemistry Department and* [2]*Botany Department, Faculty of Science, Tanta University, Tata, Egypt*

[3]*Molecular Biology Department and* [4]*Textitle Research Division, National Research Centre, Cairo, Egypt*

**Corresponding author (Fax, +2- 040-33-50-804; Email, tarek967@hotmail.com)*

[†]*Deanship preparatory year, Shaqra, Shaqra University, KSA.*

Ulocladium atrum inulinase was immobilized on different composite membranes composed of chitosan/nonwoven fabrics. Km values of free and immobilized *U. atrum* inulinase on different composite membranes were calculated. The enzyme had optimum pH at 5.6 for free and immobilized *U. atrum* inulinase on polyester nonwoven fabric coated with 3% chitosan solution (PPNWF3), but optimum pH was 5 for immobilized *U. atrum* inulinase on polyester and polypropylene nonwoven fabrics coated with 1% chitosan solution. The enzyme had optimum temperature at 40°C for immobilized enzyme on each of polyester and polypropylene composite membranes coated with 1% chitosan, while it was 50°C for free and immobilized enzyme on polypropylene nonwoven fabric coated with 3% chitosan solution. Free *U. atrum* inulinase was stable at 40°C but thermal stability of the immobilized enzyme was detected up to 60°C. Reusability of immobilized enzyme was from 38 to 42 cycles of reuse; after this, the immobilized enzyme lost its activity completely. In conclusion, immobilized *U. atrum* inulinase was considerably more stable than the free enzyme, and could be stored for extended periods.

[Mohamed TM, Abu El-Souod SM, Ali EM, El-Badry MO, El-Keiy MM and Aly AS 2014 Immobilization and characterization of inulinase from *Ulocladium atrum* on nonwoven fabrics. *J. Biosci.* **39** 785–793] **DOI** 10.1007/s12038-014-9477-1

1. Introduction

Fructose and fructo-oligosaccharides are fast emerging as important ingredients in the food, beverage and pharmaceutical industry because of their beneficial effects in diabetic patients, low carcinogenicity, increased iron absorption in children and higher sweetening capacity compared with sucrose. Additionally, they enhance the flavor, colour and product stability of food and beverages (Hanover 1993; Roy *et al.* 2000; Treichel *et al.* 2009; Yewale *et al.* 2013). Inulin, a fructan, is used either as a macronutrient substitute or as a supplement mainly for its nutritional properties. Inulin consists of fructose molecules linked by β-(2→1) glycosidic bonds, which are responsible for its nutritional characteristics (Diaz *et al*. 2006).

Inulinase (2,1-β-D-fructanfructanohydrolase; EC 3.2.1.7) hydrolyses inulin in a single enzymatic step and yields up to 95% fructose. This process is an excellent alternative for production of fructose syrup (Catana *et al.* 2005; Singh and Gill 2006; Singh and Lotey 2010; Chi *et al.* 2011; Yewale *et al.* 2013). Inulinases are classified as endoinulinase and exoinulinase, depending on their mode of action. Endoinulinases (2, 1-β-D-fructanfructanohydrolase; EC 3.2.1.7) are specific to inulin and hydrolyse it by breaking the bonds between fructose units that are located away from the ends of the polymer network, to produce oligosaccharides. Exoinulinases (β-D-fructohydrolase; EC 3.2.1.8) split

Keywords. Chitosan; composite membrane; immobilization; inulinase; nonwoven fabric; *U. atrum*

Published online: 20 October 2014

terminal fructose units from the non-reducing end of the inulin molecule to liberate fructose (Chi *et al.* 2009).

Enzyme immobilization is a method to keep enzyme molecules confined or localized to a certain region of space with retention of their catalytic activities. In comparison with their native form, immobilized enzymes offer several advantages, such as enhanced stability, easier product recovery and purification, the potential for repeated usage and continuous process technology (Shahidi *et al.* 1999; Sobral *et al.* 2003; Coghetto *et al.* 2012).

It seems that, today, the main effort in developing new hyper-filtration membranes is concentrated on thin film composite membranes. The work is mainly directed at new polymers with better chemical stability, which allows them to be used as basic material for membrane preparation by the phase inversion process (Strathmann *et al.* 1975). The first thin film composite membrane was made by coating a cellulose acetate solution of 0.2% to 2% polymer on a micro-porous membrane (Loeb and Sourirajan 1962).

Chitosan is a high-molecular-weight polysaccharide composed mainly of (1,4)-linked 2-deoxy-2-aminod-glucopyranose units and partially of (1,4)-linked 2-deoxy-2-acetamido-D-glucopyranose. It has the properties of biocompatibility and biodegradability, among others, and hence, chitosan has been used in many areas (Zeng and Ruckenstein 1996; Klein *et al.* 2002; Mohamed *et al.* 2008).

Nonwoven fabric is a fabric-like material made from long fibres, bonded together by chemical, mechanical, heat or solvent treatment. In recent years, nonwovens have become an alternative to polyurethane foam. Surgical garment production from nonwoven fabrics is a new application, where the advantage of using nonwoven materials includes shorter production cycles, lighter weight, good absorbency, liquid repellency, stretchability, softness, flame retardancy and filtering, and it used as a bacterial barrier and sterility (Shalaby 1996; Mohamed *et al.* 2008). Nonwoven fabrics have attracted attention in many industrial fields because of their durability, light weight, easy handling and low cost. Nonwoven fabrics are widely used to support the formation of flat-sheet membranes by electrospinning (Li *et al.* 2011). Enzymes such as β-galactosidase, α-amylase, peroxidase and lipase have been immobilized on nonwoven fibers or membranes (Albayrak and Yang 2002; Meryem *et al.* 2007; Mohamed *et al.* 2008; Li *et al.* 2011). Extensive studies on inulinase immobilization was chosen on different organic supports as: amino-cellulofine, CNBr- activated Sepharose 4B, cellulose carbonate, bromoacetyl-cellulose, Carboxy-Cellulofine, and on inorganic support as: natural montmorillonitesodiumalginate,glutraldhde and activated coal and on chitosan (Vandamme and Derycke 1983; Nakamura *et al.* 1995; Ettalibi and Baratti 2001; Ricca *et al.* 2007; de Paula *et al.* 2008; Coghetto *et al.* 2012; Yewale *et al.* 2013). However, there are no reports on the immobilization of inulinase on nonwoven fabric.

The aim of this study was to develop a method of immobilizing inulinase on different nonwoven fabrics to enhance stability of inulinase activity. The biochemical properties of immobilized enzymes were evaluated and compared with the free enzyme.

2. Materials and methods

Ulocladium atrum inulinase was partially purified in Biochemistry Lab, Biochemistry Division, Tanta University, according to the methods of Abu El–souod *et al.* (2014).

2.1 *Inulinase assay*

Inulinase activity was measured according to Miller (1959). 3,5-Dinitrosalicylic acid (DNS) reacts with reducing sugars that were produced from inulin degradation (fructose) by inulinase enzyme in alkaline solution to form 3-amino-5-nitrosalicylic acid (which absorbs light strongly at 540 nm). The reaction mixture contained 0.2 mL enzyme solution and 0.8 mL of 2% (w/v) inulin dissolved in 0.05 M sodium acetate buffer (pH 5.5) at 37°C for 60 min, and then 2 mL DNS was added to the mixture; the mixture was boiled for 10

Table 1. Type and coating conditions of nonwoven fabrics

Code sample	Types of substrate	Coating condition
PPNWF1	Polypropylene nonwoven fabric	1% chitosan, 2.5 % polyethylene glycol, 0.1 mL gluteraldehyde
PPNWF3	Polypropylene nonwoven fabric	3% chitosan, 7.5% polyethylene glycol, 0.3 mL gluteraldehyde
V/PENWF3	Viscose/polyester nonwoven fabric	3% chitosan, 7.5% polyethylene glycol, 0.3 mL gluteraldehyde
PENWF1	Polyester nonwoven fabric	1% chitosan, 7.5% polyethylene glycol, 0.3 mL gluteraldehyde

Table 2. Immobilization of *U. atrum* inulinase on different activated materials

Support material	Protein immobilized (mg/g support)	Activity immobilized (Units/g support)	Specific activity (units/mg protein)
PPNWF1	0.0232	30.1	1297.4
PPNWF3	0.0453	60.8	1342.1
PENWF1	0.0324	50	1543.2
V/PENWF	0.0323	10.7	331.2

min, after which cooling absorbance was measured at 540 nm. One enzyme unit was defined as the amount of enzyme that liberate 1 µmol of fructose from inulin per minute under standard assay conditions (Yun *et al.* 2000).

2.2 *Protein determination*

The protein was determined by the method of Bradford (1976) using bovine serum albumin as a standard.

2.3 *Preparation of immobilized substrate*

Chitosan/nonwoven composite membranes were prepared by the phase inversion method. The nonwoven fabrics were subsequently stored in 1% chitosan solution overnight. Then, 8 mL of chitosan solution containing appropriate amount of polyethylene glycol-600 (PEG) as porogen (used as pore formers to be removed from the blend), and 1 mL (0.01%) glutraldehyde solution was poured over a pretreated nonwoven fabrics placed in a Petri dish (100 mm diameter). The resulting mixture was agitated for 1 day, and then precipitated with 1 mol/L NaOH to extract the porogen and to generate a micro-porous membrane. The so-formed composites membranes were washed with distilled water until the solution became neutral. Finally, the membranes were treated with 10% glycerol solution to protect the composite membrane from shrinkage and were dried at 50°C. The same procedure was followed for the other nonwoven fabrics. The type and the coating conditions used are listed in table 1.

2.4 *Immobilization procedure*

A solution of native enzyme (about 2 mL) was added on the 0.5 g of the composite membrane, and the mixture was left at 4°C overnight with end-over-end stirring. The next day, the membrane immobilized enzyme was thoroughly washed with 0.05 M acetate buffer and with gradient of NaCl (0.1–0.5 M).

2.5 *Enzyme characterization*

Enzyme characterization was performed before and after immobilization. The Km values of free and immobilized inulinase activity were determined by using different substrates concentrations of inulin ranging from 2 to

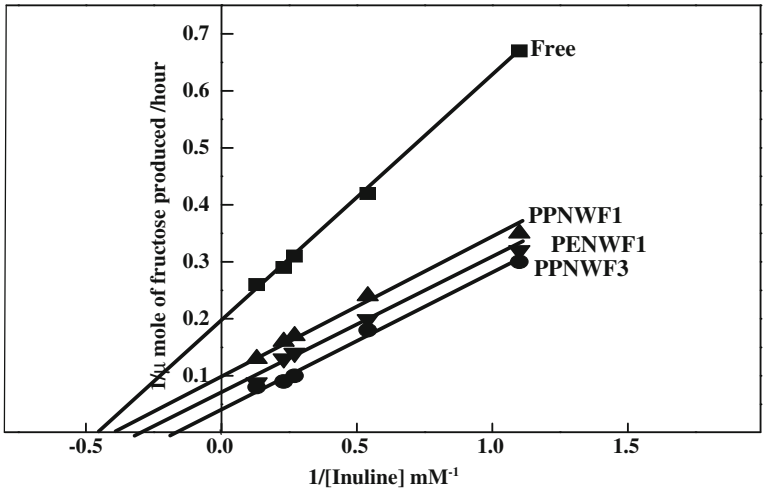

Figure 1. Linweaver-Burk plot of free, and immobilized PPNWF1, PPNWF3 and PENWF1 *U. atrum* inulinase reaction velocity to inulin concentration. The reaction mixture in 1 mL contained (0.9 – 7.5 mM) inulin dissolved in 0.05 M acetate buffer pH 5.5 and 0.2 mL of free inulinase or 0.3 g of immobilized enzyme. The reaction was incubated for 1 h at different substrate concentrations. The experiment was carried out three times.

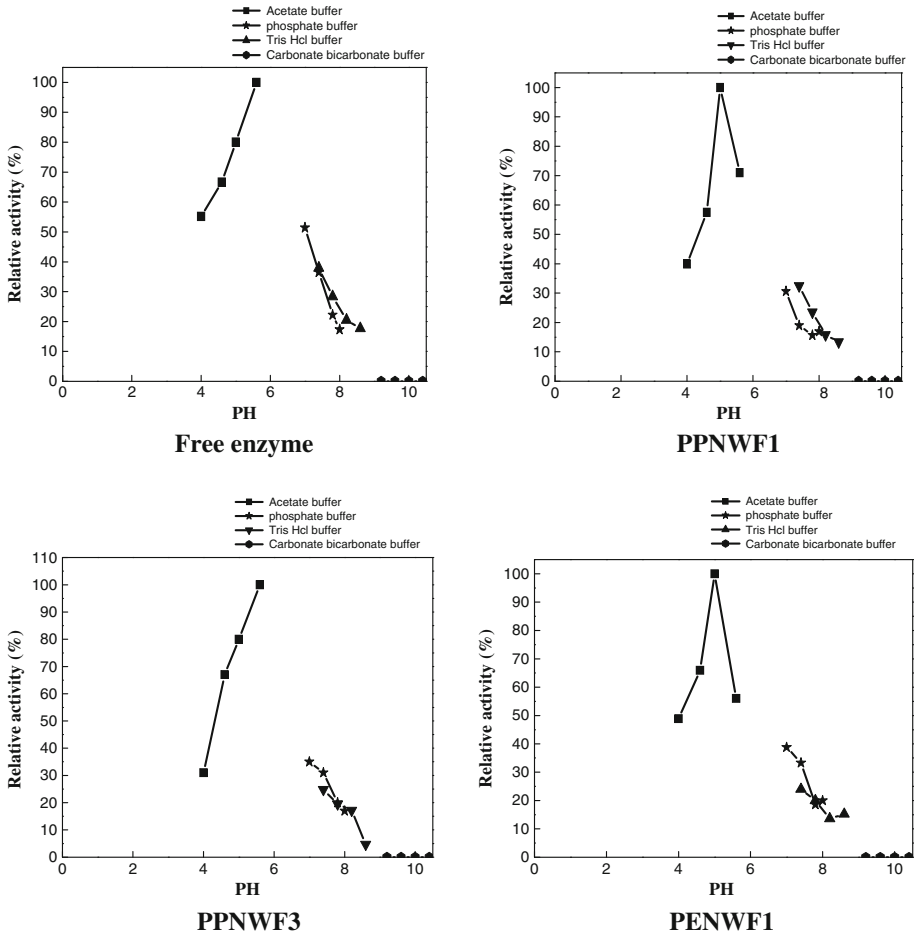

Figure 2. pH optima of free, and immobilized PPNWF1, PPNWF3 and PENWF *U. atrum* inulinase. The reaction mixture in 1 mL contained 15 mg inulin, and 0.05 M acetate buffer (pH 4.0–5.6), 0.1 M sodium phosphate buffer (pH 7.0–8.0), Tris HCl buffer (pH 7.4–8.6), and 0.1 M carbonate bicarbonate buffer (pH 9.2–10.4) with suitable amount of free or immobilized inulinase.

16 mg inuline. The free and immobilized inulinase activity profile was analyzed using 16 mg of inulin as substrate and 0.05 M acetate, phosphate and Tris HCl buffer in pH range 5.0–9.0 at 40°C. The free and immobilized enzyme profiles were also analysed at temperature ranges from 10°C to 80°C. The thermal stability was also investigated by measuring the residual activity of the free and immobilized enzyme after 15 min of incubation at different temperatures ranged from 40°C to 90°C prior to substrate addition. The effect of metal cations was performed by incubating the enzyme for 15 min at 37°C with 2 mM cations prior to substrate addition. The percentage of enzyme activity was calculated considering 100% as the highest activity detected in the assay.

3. Results and discussion

The free *U. atrum* inulinase partially purified from DEAE-cellulose INII (Abu El–souod *et al.* 2014) [containing 100 units with specific activity about 1678.9 units mg^{-1} (0.0595 mg protein/g support)] was immobilized on the different nonwoven fabrics matrices, and the specific activity of the enzyme are noted in table 2. The results showed that the specific activity of the *U. atrum* inulinase on the PPNWF1, PPNWF3, PENWF1 and V/PENWF was 1297, 1342, 1543.2 and 331.2 units mg^{-1} protein, respectively. The immobilization efficiency of the matrices were 30, 60, 50 and 10% respectively, indicating that PPNWF3 and PENWF1 were the most efficient matrices for *U. atrum* inulinase immobilization. Rhee and Kim (1989) immobilized

inulinase from *A. ficuum* on chitin. Also immobilized inulinase from *Aspergillus niger* on amino-cellulofine was chosen as support material among different materials (CNBr-activated Sepharose 4B, cellulose carbonate, bromoacetyl-cellulose and carboxy-cellulofine), for immobilization with different immobilization efficiencies depending on the supporting material (Nakamura *et al.* 1995). Inulinase from Kluyveromycesmarxianus NRRL Y-7571 was immobilized on the inorganic support, natural montmorillonite, with efficiency 63% (Coghetto *et al.* 2012), and sodium alginate, glutraldhde and activated coal with efficiency 60% (Richetti *et al.* 2012). Yewale *et al*. (2013) immobilized inulinase from *A. niger* on chitosan with immobilization efficiency 83%.

3.1 *Characterization of free and immobilized inulinase*

3.1.1 *Km value:* The Km value of the immobilized *U. atrum* inulinase was found to be higher than that of free enzyme. The Km values of free and immobilized enzymes on PPNWF1, PPNWF3 and PENWF were 2.2, 5.8, 2.6 and 3.6 mM, with V_{max} values 5.2, 22.2, 11.3 and 14.7 µmol/h, respectively (figure 1). The increase observed in Km after immobilization of *U. atrum* inulinase on PPNWF1, PPNWF3 and PENWF probably resulted from conformational modifications introduced by the covalent attachment of the enzyme to the support and to mass transfer resistances in the morphology of the support used, leading to reduced affinity of the substrate at the active site of the enzyme (Kusano *et al.* 1989; Abdel-Naby *et al.* 1999). The Km value of immobilized inulinase on barium alginate treated with glutaraldehyde *Kluyeromyces marxianus* was 0.522 mM and V_{max}=113.7 µmol/min (Florido *et al.* 2001), Novozyme immobilized on calcium aliginate beads was 184 mM (Catana *et al.* 2005). Coghetto *et al*. (2012) evaluated the kinetic parameters of the immobilized inulinase from *K. marxianus* on natural montmorillonite and obtained a Km value of 0.38 mM and Vmax value of 0.2396 mol/L min. The Km values of free *Aspergillus niger* and immobilized inulinase on chitosan were 0.25 and 0.21 mM, respectively. The V_{max} values of free and immobilized enzyme were 0.23 mM/min/mL and 0.21 mM/min/mL, respectively (Yewale *et al.* 2013).

3.1.2 *pH profile:* The optimum pH of the enzyme was not changed after immobilization on PPNWF3 (pH 5.6), whereas the optimum pH of the immobilized enzyme on PPNWF1 and PENWF1 shifted into a more acidic range (pH 5) (figure 2). This shift in pH probably could be attributed to the existence of a micro-environmental difference, such as the surface charge of the carrier material (Yun *et al.* 2000; Yewale *et al.* 2013). In another study the results indicated that the optimum pH of the immobilized enzyme was shifted to 5.2 in comparison with that of the free enzyme (pH 4.8). This reveals the fact that the ionization of the amino acid residues at the active site was affected by immobilization. Similarly, optimum pH of 5.2 was reported by Nakamura *et al.* (1995) for inulinase immobilized on amino-cellulofine. Theoptimum pH of inulinase from *K maxianus* var. bulgaricus immobilized in gelatin was 3.5 (Paula *et al.* 2008), from *A. ficuum* immobilized in porous glass it was 5 (Ettalibi and Baratti 2001), from novozymed immobilized on calcium aliginate it was 4 (Catana *et al.* 2005), and from *Aspergillus niger* and immobilized inulinase on chitosan it was 4 (Yewale *et al.* 2013).

3.2 *Effect of temperature on enzyme activity*

The effect of temperature on the activity of free and immobilized *U. atrum* inulinase showed a bell-shaped curve

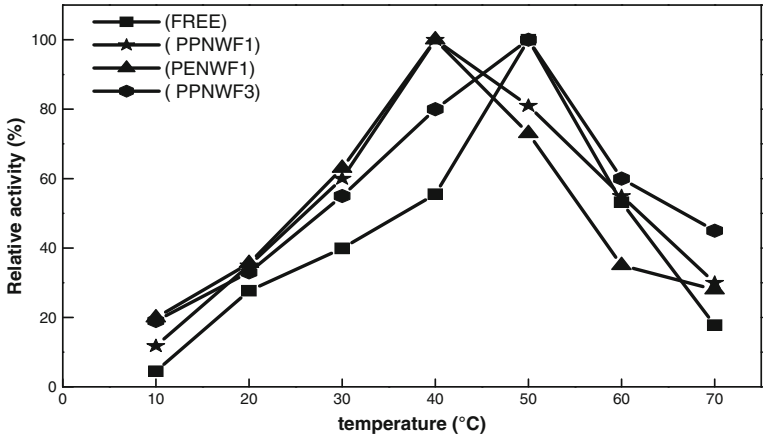

Figure 3. Effect of temperature on activity of free and immobilized *U. atrum* inulinase. The reaction mixture in 1 mL contained 15 mg inulin dissolve in 0.05 M acetate buffer (pH 5.5) and 0.2 mL of free inulinase and 0.3 g of immobilized inulinase. The reaction was incubated for 1 h at different temperatures ranging from 10°C to 70°C.

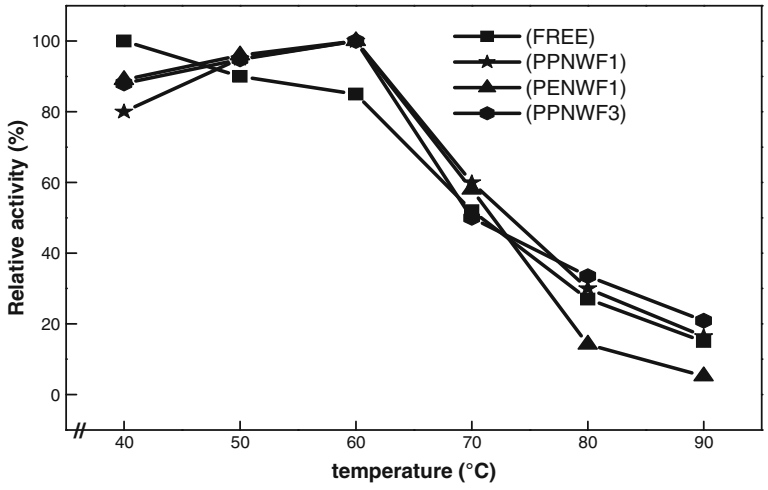

Figure 4. Effect of temperature on stability of free and immobilized *U. atrum* inulinase. The reaction mixture in 1 mL contained 15 mg inulin dissolved in 0.05 M acetate buffer (pH 5.5) and 0.2 mL of free inulinase and 0.3 g of immobilized inulinase. The reaction was pre-incubated at different temperatures ranging from 40°C to 90°C for 30 min prior to substrate addition. Activity of free enzyme at zero time was taken as 100%.

(figure 3). Normally, the enzyme activity increases with increase in temperature up to a certain point after which, due to denaturation of the enzyme protein, a decline in the activity is observed (Iyengar and Rao 1979). The maximum activity of the enzyme was reported at 50°C for free and immobilized enzyme on PPNWF3 and at 40°C for immobilized enzyme on PPNWF1 and PENWF1. The maximum novozyme inulinase activity immobilized on calcium alginate was at 60°C (Catana *et al.* 2005). The same result was obtained for *A. niger* and immobilized inulinase on chitosan (Yewale *et al.* 2013).

3.3 *Effect of temperature on enzyme stability*

Figure 4 shows the effect of temperature on the stability of free and immobilized *U. atrum* inulinase in the range between 40 °C and 90°C by incubation for 30 min prior to substrate addition. The results clarified that the free enzyme was stable until 40°C, while the stability of the immobilized enzyme was continued up to 60°C after which the immobilized inulinase showed a slight decreased in thermal stability; but at 90°C complete loss of the enzyme activity occurred. So, thermal stability of the immobilized inulinase was markedly enhanced compared to

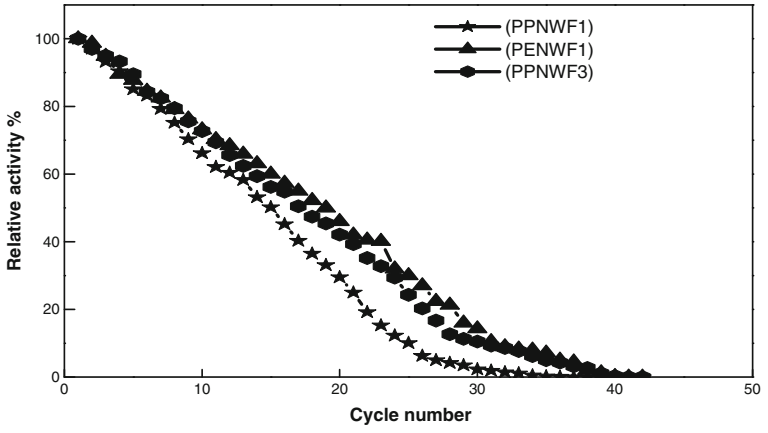

Figure 5. Reusability of immobilized on PPNWF1, PPNWF3 and PENWF1 *U. atrum* inulinase by wash several times after each assay. Enzyme activity was determined at 40°C in 15 mg inulin dissolved in 0.05 M acetate buffer (pH 5.5) and 0.3 g of immobilized inulinase.

that of free enzyme. The soluble inulinase was very unstable at temperatures over 40°C, while the immobilized enzyme was relatively stable up to 60°C (Kim *et al.* 1982). The increase in thermal stability may be a result of the fact that immobilization limits the thermal movement of the enzyme at higher temperature and, hence, decreased denaturation. Enhancement of thermal stability, due to immobilization, has also been reported earlier (Bajpai and Margaritis 1986; Wenling *et al.* 1999; Florido *et al.* 2001; Catana *et al.* 2005; Yewale *et al.* 2013). In general, immobilization of inulinase protected the enzyme against heat inactivation (Yewale *et al.* 2013).

3.4 *Reusability and stability*

The ease of separation and increased stability are the advantages of using an immobilized biocatalyst. The immobilized biocatalyst was continuously recycled for 42 batches for PPNWF1 and 40 batches for PPNWF3 and PENWF1 (figure 5). The decrease in hydrolysis in the initial batches may be due to the loss of loosely bound inulinase during washings of the biocatalyst. The immobilized system showed a good stability in the batch system suggesting that it can be successfully used for the continuous preparation of high-fructose syrup (Mudd and Burris. 1959; Catana *et al.* 2005; Yewale *et al.* 2013). Study of the stability of free and immobilized *U. atrum* inulinase showed that the free inulinase lost nearly 92% of its activity at 4°C and 30°C within 121 and 48 days, respectively, while the immobilized inulinase lost nearly 92% of its activity at 4°C and 30°C within 150 and 65 days, respectively. However, on freezing, the free inulinase lost more than 50% of its activity within 140 days, while the immobilized enzyme lost about 60% of its activity within 150 days. This data indicated that immobilized inulinase was

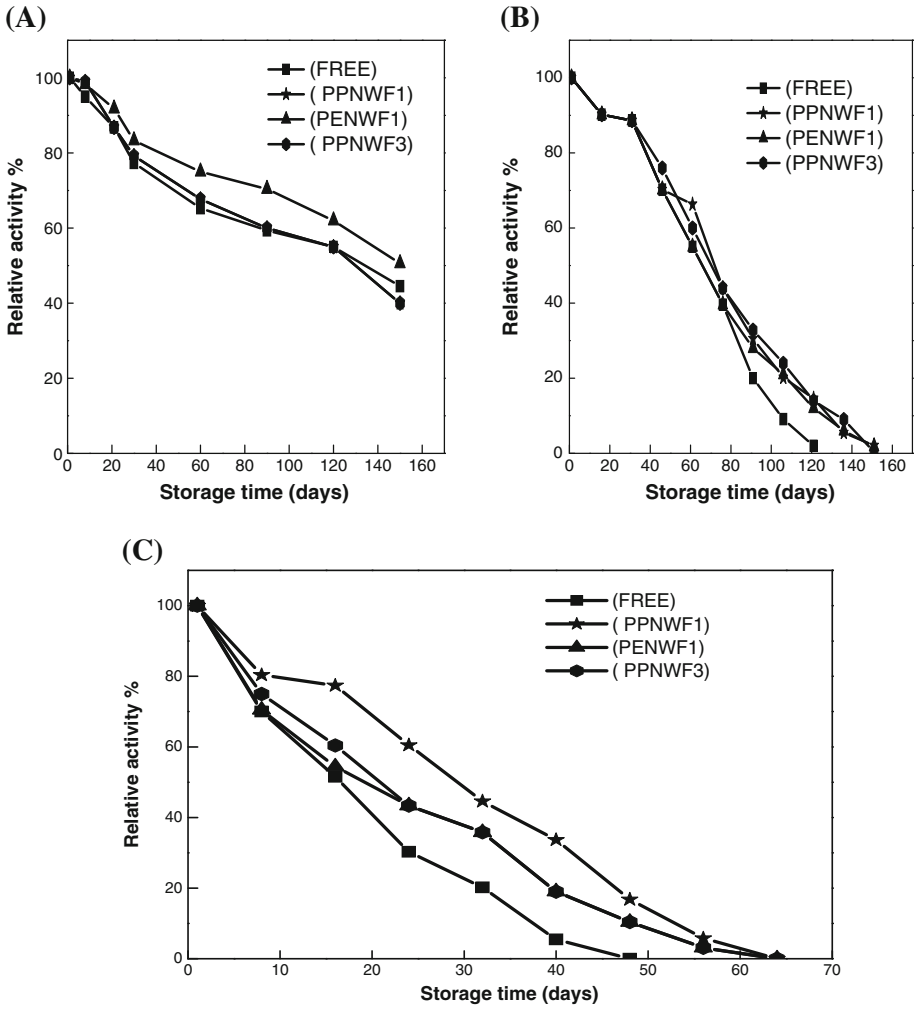

Figure 6. Storage stability of free and immobilized *U. atrum* inulinases at freezing (**A**), 4°C (**B**) and 30°C (**C**). Enzyme activity was determined at 40°C in 15 mg inulin dissolved in 0.05 M acetate buffer (pH 5.5) and suitable amount of free and immobilized inulinase.

Table 3. Effect of metal ions on activity of free and immobilized *U. atrum* inulinase

Metal ions	Salt used	Free enzyme Relative activity %	Immobilized enzyme on PPNWF1 Relative activity %	Immobilized enzyme on PPNWF3 Relative activity %	Immobilized enzyme on PENWF1 Relative activity %
None	None	100	100	100	100
Cu^{+2}	$CuCl_2$	83	75	85	80
Mg^{+2}	$MgCl_2$	87	102	102	103
Ca^{+2}	$CaCl_2$	78	70	67	65
Hg^{+2}	$HgCl_2$	55	50	55	45
Co^{+2}	$CoCl_2$	78	104	105	107
Ni^{+2}	$NiCl_2$	70	63	59	70
Ba^{+2}	$BaCl_2$	40	30	27	25
Mn^{+2}	$MnCl_2$	25	110	108	110
Fe^{+3}	$FeCl_3$	22	25	22	20

Immobilized enzyme was pre-incubated for 15 min with 2 mM of the listed metal as final concentration prior to substrates condition. Activity in the absence of metal ions was taken as 100%.

considerably more stable than the free enzyme, and could be stored for extended periods (figure 6).

3.5 *Effect of metal*

Some metal ions added to the reaction medium can promote inulinase activity, while others have inhibitory effect on the enzyme. The effect of some metal ions at 2 mmol/L on *U. atrum* inulinase activity is indicated in table 3. All examined ions had inhibitory effect on free *U. atrum* inulinase; the effectiveness of metal cations as inhibitors was sequentially $Fe^{+3} > Mn^{+2} > Ba^{+2} > Hg^{+2} > Ni^{+2} > Ca^{+2} > Co^{+2} > Cu^{+2} > Mg^{+2}$. Some of the metal ions tested had a low inhibitory effect (Ba^{+2}, Mn^{+2} and Fe^{+3}), which means that the inhibitory effect at the same concentration depends upon the chemical structure of the metal ions used (Mudd and Burris 1959). Also these metals inhibited inulinase production from *U. atrum*, which indicates that this effect may due to the formation of complex with ionized inulinase resulting in changing solubility and behaviour at the substrate interfaces (Dinarvand *et al.* 2012; Abu El–souod *et al.* 2014). In the immobilized enzyme on 1% and 3% CPNWF and PENWF, not all examined ions had inhibitory effect but there were some ions that had an activator effect. The effectiveness of metal ions as activators on an immobilized *U. atrum* inulinase was $Co^{+2} > Mg^{+2} > Mn^{+2}$.

References

Abdel-Naby MA, Ryad MR and Abdel Fattah AF 1999 Biosynthesis of cyclodextringlucosyltransferase by the free and immobilized cells of Bacillus amyloliquefaciens. *Biochem. Eng. J.* **3371** 1–9

Abu El–souod SM, Mohamed TM, Ali MM, El-badry MO, El-keiy MM 2014 Purification of Extracellular Exo-Inulinase from *Ulocladium Atrum. J. Gen. Biotechnol.* In Press

Albayrak N and Yang ST 2002 Immobilization of Aspergillusoryzae -galactosidase on tosylated cotton cloth. *Enzym. Microb. Technol.* **31** 371–383

Bajpai P and Margaritis A 1986 Immobilization of K. marxianus cells containing activity in open pore gelatin matrix: application for high fructose syrup production. *Enzym. Microb. Technol.* **7** 459–461

Bradford M 1976 A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of dye binding. *Analyt. Biochem.* **72** 248–254

Catana R, Ferreira BS, Cabral JMS and Fermandes P 2005 Immobilization of inulinase for sucrose hydrolysis. *Food Chem.* **91** 517–520

Chi Z, Chi Z, Zhang T, Liu G and Yue L 2009 Inulinase-expressing microorganisms and applications of inulinases. *Appl. Microbiol. Biotechnol.* **82** 211–220

Chi ZM, Zhang T, Cao TS, Liu XY, Cui W and Zhao CH 2011 Biotechnological potential of inulin for bioprocesses. *Bioresour. Technol.* **102** 4295–4303

Coghetto C, Scherer RP, Silva M, Goluniski S, Pergher SB, deOliveira D, Oliveira JV and Treichel H 2012 Natural montmorillonite assupport for theim mobilization of inulinase from *Kluyveromyces marxianus* NRRL Y-7571. *Biocatalysis Agric. Biotechnol.* **1** 284–289

dePaula FC, Cazetta ML, Monti R and Contiero J 2008 Sucrosehydrolysisby gelatin-immobilized inulinasefrom Kluyveromyces marxianus var. bulgaricus. *Food Chem.* **111** 691–695

Diaz EG, Catana R, Ferreira BS, Luque S, Fermands P and Cabral JMS 2006 Towards the development of a membrane reactor for enzymatic inulin hydrolysis. *J. Membr. Sci.* **273** 152–158

Dinarvand M, Ariff AB, Moeini H, Masomian M, Mousavi SS, Nahavandi R and Mustafa S 2012 Effect of extrinsic and intrinsic parameters on inulinase production by Aspergillus niger ATCC 20611. *Electron. J. Biotechnol.* **15** 1–11

Ettalibi M and Baratti JC 2001 Sucrose hydrolysis by termostable immobilized inulinases from Aspergillus ficuum. *Microbiol. Technol.* **28** 596–601

Florido EB, Garibay MG, Ruiz LG and Azaola A 2001 Immobilization system of *Kluy_eromyces marxianus* cells in barium alginate for inulin hydrolysis. *Process Biochem.* **37** 513–519

Hanover LWJ 1993 Manufacturing, composing and applications of fructose. *Am. J. Clin. Nutr.* **5** 7248–7328

Iyengar L and Rao AVSP 1979 Urease bound to chitin with glutaraldehyde. *Biotechnol. Bioeng.* **21** 1333–1343

Kim WY, Byun SM and Uhm TB 1982 Hydrolysis of inulin from Jerusalem artichoke by inulinase immobilized on aminoethylecllulose. *Enzym. Microb. Technol.* **4** 239–244

Klein E, Eichholz E, Theimer F and Yeager D 2002 Chitosan modified sulphonated poly(ethersuLfone) as a support for affinity separations. *J. Memb. Sci.* **205** 141–144

Kusano S, Shiraishi T, Takahashi SI, Fujimoto D and Sakano Y 1989 Immobilization of Bacillus acidopulluly ticusp inulanase and properties of the immobilized pullalanase. *J. Ferment. Technol. Bioeng.* **68** 233–237

Li W, Chen B and Tan T 2011 Comparative study of the properties of lipase immobilized on nonwoven fabric membranes by six methods. *Process Biochem.* **46** 1358–1365

Loeb S and Sourirajan S 1962 Hollow-fibers offer three primary advantages over flat-sheet or tubular. *Adv. Chem. Ser.* **38** 117

Meryem NB, Olga S, Saida SD and Hans B 2007 Co-Immobilization of different enzyme activities to nonwoven polyester surfaces. *Biotechnol. Bioeng.* **96** 623–630

Miller GL 1959 Use of dinitrosalicylic acid reagent in determination of reducing sugar. *Anal. Chem.* **31** 426–428

Mohamed SA, Said AA, Mohamed TM and Salah HA 2008 Immobilization of horseradish peroxidase on non-woven polyester fabric coated with chitosan. *Appl. Biochem. Biotechnol.* **144** 169–179

Mudd J and Burris R 1959 Participation of metals in peroxidase catalyzed oxidations. *J. Biol. Chem.* **234** 2774–2777

Nakamura T, Ogata Y, Shitara A, Nakamura A and Ohta K 1995 Continuous production of fructose syrups from inulin by immobilized inulinase from Aspergillusniger Mutant 817. *J. Ferment. Bioeng.* **80** 164–169

Paula FC, Cazetta ML, Monti R and Contiero J 2008 Sucrose hydrolysis by gelatin-immobilized inulinase from *Kluyveromyces marxianus* var. bulgaricus. *Food Chem.* **111** 691–695

Rhee SK and Kim CH 1989 Fructose production from Jerusalem artichoke by inulinase immobilized on chitin. *Biotechnol. Lett.* **11** 201–206

Ricca E, Calabro V, Curcio S and Iorio G 2007 The state of the art in the production of fructose from inulin enzymatic hydrolysis. *Crit. Rev. Biotechnol.* **27** 129–145

Richetti A, Munaretto CB, Lerin LA, Batistella L, Oliveira JV, Dallago RM, Astolfi V, DiLuccio M, *et al*. 2012 Immobilization ofinulinasefrom Kluyveromyces marxianus NRRL Y-7571 using modified sodium alginate beads. *Bioprocess Biosyst. Eng.* **35** 383–388

Roy I, Sardar M and Gupta MN 2000 Exploiting unusual affinity of usual polysaccharides on fluidized beds. *Enzym. Microb. Technol.* **27** 53–65

Shahidi F, Arachchi JK and Jeon YJ 1999 *Trends Food Sci. Technol.* **10** 37

Shalaby SW 1996 in *Biomaterials science: an introduction to materials in medicine* (eds) BD Ratner, AS Hoffman and F Schoen (SAN Diego: Academic Press) pp 118–124

Singh P and Gill PK 2006 Production of inulinases: recent advances. *Food Technol. Biotechnol.* **44** 151–162

Singh RS and Lotey S 2010 Enhanced exoinulinase production from Kluyveromycesmarxianus YS-1 using response surface methodology. *Braz. Arch. Biol. Technol.* **53** 1005–1013

Sobral KCA, Rodrigues RMO, de Oliveira RD, Olivo JE, De Moraes FF and Zanin GM 2003 Evaluation of supports and methods for the immobilization of the enzyme cyclodextrin glycosyl transferase. *Appl. Biochem. Biotechnol.* **102** 809–819

Strathmann H, Kock K, Amar P and Baker RW 1975 The formation mechanism of asymmetric membranes. *Desalination* **16** 179

Treichel H, Mazutti MA, Filho FM and Rodrigues MI 2009 Technical viability of the production, partial purification and characterisation of inulinase using pretreated agroindustrial residues. *Bioprocess Biosyst. Eng.* **32** 425–433

Vandamme EJ and Derycke DG 1983 Microbial inulinases: fermentation process, properties and applications. *Adv. Appl. Microbiol.* **29** 139–176

Wenling W, Huiying WWL and Shiyuan W 1999 Continuous preparation of fructose syrups from Jerusalem artichoke tuber using immobilized intracellular inulinase from Kluyveromyces sp. Y-85. *Process Biochem.* **34** 643–646

Yewale T, Singhal RS and Vaidya AA 2013 Immobilization of inulinase from Aspergillus niger NCIM 945 on chitosan and its application in continuous inulin hydrolysis. *Biocatalysis Agric. Biotechnol.* **2** 96–101

Yun JW, Park JP, Song CH, Lee JH, Kim S and Song K 2000 Continuous production of inulo-oligosaccharides from chicory juic by immobilized endoinulinase. *Bioprocess Eng.* **22** 189–194

Zeng X and Ruckenstein E 1996 Control of pore size in macroporous chitosan and chitin membrane. *Ind. Eng. Chem. Res.* **35** 4169–4175

MS received 07 March 2014; accepted 09 September 2014

Corresponding editor: B JAGADEESHWAR RAO