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Immobilization of *Streptomyces olivaceoviridis* E-86 xylanase on Eudragit S-100 for xylo-oligosaccharide production

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Abstract

A 47 kDa xylanase (FXYN) from *Streptomyces olivaceoviridis* E-86 was immobilized on Eudragit S-100 and an immobilization efficiency of 90% was obtained. The optimum pH of the immobilized xylanase shifted from 5.8 to 6.3 while its optimum temperature moved from 60 to 65 °C. The immobilized enzyme also showed a higher thermal stability than the free xylanase. Only a marginal increase in the K_m values of free enzyme was observed upon immobilization. However, V_{max} values of the immobilized enzyme were 82–161% higher than those of the free xylanase. The immobilized xylanase was also evaluated for its application in hydrolyzing the corncob powder pre-treated with 2% NaOH solution for xylo-oligosaccharide production. The final extent of xylan hydrolysis using pre-treated corncob powder was 84% for the immobilized enzyme after 24 h of incubation at 55 °C. The immobilized xylanase retained 81% of its initial hydrolysis activity even after being recycled four times. Therefore, the immobilized xylanase is suitable to produce xylo-oligosaccahrides from corncob powder pre-treated with dilute alkaline solution.

Keywords: Eudragit S-100; Immobilization; *Streptomyces olivaceoviridis*; Xylanase; Xylo-oligosaccharide

1. Introduction

Second only to cellulose in natural abundance, xylan is a major component of the hemicellulose fraction in the plant cell walls. Xylanase (EC. 3.2.1.8; 1,4-β-D-xylanase, xylanohydrolase) can hydrolyze β-1,4-glycosidic linkages of the xylan backbone to produce short chain xylo-oligosaccharides of various lengths-hence, the crucial enzyme component of microbial xylanolytic systems [1]. Recently, xylanases have attracted considerable research interest because of their potential industrial applications. One of the exciting applications of xylanases is the production of xylo-oligosaccharides [2]. Xylo-oligosaccharides have been found to have a stimulatory effect on the selective growth of human intestinal *Bifidobacteria*, and are frequently defined as pre-biotics [3]. They also show a remarkable potential for practical utilization in many fields, including food additives, pharmaceuticals, feed formulations and agricultural applications [4]. Xylan exists abundantly in the lignocellulosic materials (LCM) such as corncobs which contain approx 35% xylan. Unfortunately, such the native xylan in LCM is known to be resistant to the xylanase action. Xylan can be extracted from LCM with a highly concentrated alkaline solution such as 10–15% NaOH, but this method is impractical in industrial application as it involves great difficulties and high cost [4–6]. However, there are also many reports described that xylo-oligosaccharides are produced by enzyme hydrolysis of the isolated xylan [6,7].

Several reports on the immobilization of fungal xylanases such as *Aspergillus* xylanases and *Trichoderma* xylanases have been published [8–12]. The efficiency of xylanase immobilized on solid supports decreased because the bound enzyme is not accessible to the bulky insoluble substrate. Recently, reversibly soluble–insoluble polymers (i.e. Eudragit S-100 and L-100, etc.) have been utilized as carriers for

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immobilization of various xylanases [13,14]. Immobilization of xylanases to reversibly soluble–insoluble polymer facilitates better hydrolyses of insoluble substrate. Moreover, the xylanases immobilized on such polymers can be used as a soluble form for mediating the desired reaction.

Streptomyces olivaceoviridis E-86 was previously reported to produce two kinds of xylanase (47 kDa FXYN and 23 kDa GXYN, the family F/10 β-xylanase and the family G/11 β-xylanase, respectively) [15–17], more than 94% of the xylanase activity present as FXYN [16]. It was also shown that FXYN could be used to being produce xylo-oligosaccharides effectively from xylan [18]. Moreover, *S. olivaceoviridis* E-86 is also a good xylanase producer and is able to produce a high level of xylanase (1653 U ml^{-1}) in the corncob xylan medium [19]. Xylanase has been used industrially for xylo-oligosaccharide production from corncobs. In this process, the use of immobilized enzyme will enable recycling of the xylanases and simplification of the process. Thus, the main purpose of the present study is to investigate the immobilization of *S. olivaceoviridis* E-86 FXYN on Eudragit S-100 and to produce further xylo-oligosaccharides by directly hydrolyzing corncob powder pre-treated with dilute alkaline solution. This is the first report of the immobilization of xylanase on Eudragit S-100 for xylo-oligosaccharide production.

2. Materials and methods

Eudragit S-100 is a product of Röhm Pharma GmbH (Weiterstadt, Germany) and is an anionic copolymer composed of methacrylate and methacrylic acid, with a nominal molecular weight of 135 kDa. The ratio of free carboxyl groups to the ester groups of the polymer is approx 1:2. Birchwood xylan, oat-spelt xylan and beechwood xylan were purchased from Sigma (St. Louis, USA). Silica gel plates (Merck Silica Gel 60F 254) were purchased from E. Merck, Darmstadt, Germany. All other chemicals were of analytical grade.

2.1. Microorganisms and xylanase preparation

S. olivaceoviridis E-86 was used in this investigation. An actively growing 5–6-day-old colony (0.5 cm^2 agar block) was inoculated into 100 ml of the following medium (g l^{-1}): corncob xylan, 20; peptone, 14; yeast extract, 1; KH_2PO_4, 10; $MgSO_4{\cdot}7H_2O$, 0.5. The culture was then incubated at 30 °C with shaking (150 rpm) for 6 days. The crude extracellular xylanases were then obtained by centrifuging the culture broth at 10 000 × *g* for 15 min at 4 °C. Ammonium sulphate was added slowly with agitation at 30–60% saturation. The precipitate obtained by centrifugation (10 000 × *g*) was dissolved in 50 mmol l^{-1} sodium citrate buffer (pH 5.8). The re-suspended protein was dialyzed (12–14 kDa cut-off membranes), concentrated with polyglycol (20 000) and stored at 4 °C for further use.

2.2. Determination of enzyme activity

Xylanase activity was determined by the dinitrosalicylic acid (DNS) method of Bailey et al. [20]. The reaction mixture (1 ml) containing 0.1 ml of appropriately diluted enzyme solution and 0.9 ml of 1.0% birch wood xylan solution in 50 mmol l^{-1} McIlvaine buffer (a mixture of 0.1 mol l^{-1} citric acid and 0.2 mol l^{-1} Na_2HPO_4), pH 5.8, was incubated at 55 °C for 10 min. The reaction was terminated by adding 1 ml DNS. The amount of reducing sugar liberated was determined by a DNS method using xylose as the standard [21]. One unit of xylanase activity (U) was defined as the amount of enzyme that produces 1 μmol xylose equivalent per minute under the assay conditions.

2.3. Xylanase immobilization

Eudragit S-100 solution was prepared according to the method of Sardar et al. [22]. Eudragit S-100 (5 g) was dissolved by constant stirring in 90 ml distilled water and dropwise addition of 3 mol l^{-1} NaOH until pH 11.0. After the polymer was fully solubilized, the pH of the solution was decreased to 7.0 by adding 3 mol l^{-1} HCl. Volume of the solution was made up to 100 ml with distilled water. The solution was stored at 4 °C until further use.

One millilitre of xylanase solution described above was mixed with 4 ml of Eudragit S-100 solution at different concentrations (concentration of the polymer varied between 0.1 and 2%). The polymer was precipitated after 2 h by adjusting the pH of the solution to 4.5 with addition of 0.5 mol l^{-1} acetic acid at 25 °C. After 30 min, the suspension was then centrifuged for 30 min at 12 000 × *g*. The precipitate obtained was washed three times with 50 mmol l^{-1} acetate buffer (pH 4.5). The final precipitate was dissolved in 50 mmol l^{-1} acetate buffer, pH 5.8, to a final volume of 5 ml; this solution was used to calculate the expressed activity.

2.4. SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE of the protein samples was performed in a 10% (w/v) polyacrylamide gel by the method of Laemmli [23]. The molecular weight standards used were the low molecular weight calibration kit for SDS electrophoresis (Amersham). The immobilized enzyme was dissolved and eluted according to the method of Gawande & Kamat [24]. After a complete elution, the protein sample was subjected to SDS-PAGE and characterization.

2.5. Characterization of immobilized and free enzymes

The effects of pH on the free and immobilized xylanases were studied by assaying both preparations at different pH values (50 mmol l^{-1} McIlvaine buffer for pH 2.0–8.0; 50 mmol l^{-1} boric–NaOH buffer for pH 8.0–9.5; 50 mmol l^{-1} phosphate–NaOH buffer pH 9.5–11.0 and

50 mmol l^{-1} Piperdine for pH 11.0–12.0). To determine pH stability of the free and immobilized xylanases, the enzymes were diluted in appropriate buffers (final concentration 50 mmol l^{-1}) of different pH values (as mentioned above) and incubated at 35 °C for 30 min. The residual activities of these treated enzymes were measured by standard assay procedure.

Effect of temperature on xylanase activity was determined by assaying its activity at different temperatures in 50 mmol l^{-1} McIlvaine buffer (pH 5.8 for the free enzyme and pH 6.3 for the immobilized enzyme). The thermal stability of the enzyme was studied at 50, 55, 60 and 65 °C by incubating both the free and immobilized enzymes for 120 min in 50 mmol l^{-1} McIlvaine buffer, pH 6.3 and 5.8, respectively. Aliquots of free and immobilized xylanases were withdrawn at different time intervals, and the remaining activities were measured under the standard conditions.

2.6. Determination of kinetic parameters

For the kinetic experiments, substrate was prepared in six different concentrations using 50 mmol l^{-1} McIlvaine buffer (pH 6.3 and 5.8 for the immobilized and free enzymes, respectively) and incubated with the free or immobilized xylanase at 50 °C for 5 min. The amount of reducing sugars produced was estimated by the DNS method [21]. The K_m and V_{max} values were calculated from the kinetics data using the ''GraFit'' software [25].

2.7. Hydrolysis of pre-treated corncob powder for xylo-oligosaccharide production

Corncobs were collected from a local market and milled to powder form (60–80 mesh size). Corncob powder with 32.5% xylan, 8.9% moisture content and 3.1% ash was treated with 2% NaOH solution at a liquid to solid ratio of 6:1 (kg/kg) overnight at room temperature [5]. After washing thoroughly with distilled water, the sample was air-dried at room temperature. The pre-treated corncob powder (PCP) with 40.7% xylan, 7.8% moisture content and 1.1% ash was further used for xylo-oligosaccharide production. The xylan composition in corncob powder or PCP was analyzed by the method of Kusakabe et al. [5]. Aliquots of corncob samples were subjected to both moisture (drying at 105 °C to constant weight) and ash contents determination (calcinations at 575 °C for 6 h).

Four grams of native corncob powder or PCP in 100 ml of 50 mmol l^{-1} McIlvaine buffer (pH 6.3 and 5.8 for the immobilized and free enzyme, respectively) were hydrolyzed by the immobilized or free xylanase (600 U) for 24 h at 55 °C under shaking (210 rpm) on a water-bath shaker. At different time intervals, samples were withdrawn for sugar analysis. The samples were boiled for 5 min and the undegraded substrates were removed by centrifugation at 3000 × g for 10 min.

2.8. Operational stability of the immobilized enzyme

The reusability of the immobilized enzyme was assessed by hydrolyzing 4% PCP in 100 ml 50 mmol l^{-1} McIlvaine buffer (pH 6.3) containing 600 U immobilized xylanase at 55 °C under shaking (210 rpm). After each cycle of hydrolysis, the un-degraded PCP was removed by centrifugation at 3000 × g for 10 min. The pH of the supernatant was adjusted to 4.5 by adding 0.5 mol l^{-1} acetic acid and precipitated the immobilized enzyme collected by centrifugation at 12 000 × g for 20 min. For running the next cycle, the immobilized enzyme was re-dissolved in 5.0 ml fresh buffer, added to fresh substrate and processed the same way as above. The experiment was repeated for four cycles. Each cycle consisted of 6 h of continuous hydrolysis.

2.9. Analysis methods

Total sugar was determined according to an orcinol–HCl method [26]. The extent of xylan hydrolysis was calculated as follows: extent of xylan hydrolysis (%) = xylose produced (g) × 0.88/initial xylan (g) × 100. Thin-layer chromatography (TLC) and HPLC were used to analyze the hydrolysis products according to the method of Jiang et al. [27].

3. Results and discussion

3.1. Immobilization of S. olivaceoviridis E-86 xylanase

To optimize the immobilization conditions, different concentrations of the polymer (Eudragit S-100) were added to the same amount of *S. olivaceoviridis* E-86 xylanase. Table 1 shows the yield of immobilized xylanase calculated in terms of theoretical and expressed activities. The recovery activity gradually increased up to 92% as the polymer concentration was increased to 1%. The recovery activity was almost stable at approximately 90% when the polymer concentration was more than 0.8%. The best immobilized preparation which yielded 92% recovery activity corresponded to 1% Eudragit S-100 concentration. SDS-PAGE of ammonium sulphate precipitate and complete elution of immobilized protein performed in a 10% (w/v) polyacrylamide gel showed significant purification of the enzyme upon immobilization (Fig. 1). The immobilized enzyme preparation showed a major single band on SDS-PAGE. The molecular weight of the immobilized xylanase was found to be 47 kDa, which agrees well with that reported FXYN earlier [17].

Immobilization of *Aspergillus* sp. xylanases and *Thermomyces lanuginosus* SSBP xylanase on Eudragit S-100 was reported by Gawande et al. [13] and Edward et al. [28] but the yield of enzyme immobilization was >10% lower than that of this study. It has also been reported that Eudragit S-100 was applied for purification of *Aspergillus* sp.

Table 1
Immobilization of *S. olivaceoviridis* E-86 xylanase on Eudragit S-100

Polymer concentration (%)	Unbound activity in supernatant (U)	Bound enzyme (theoretical) (U) (A)	Expressed activity (U) (B)	Extent of enzyme immobilization [$(BA^{-1}) \times 100$] (%)
0.1	45.8	362.8	138.4	38
0.3	4.7	403.9	259.5	64
0.5	4.1	404.5	296.7	73
0.8	2.4	406.2	367.9	91
1.0	2.0	406.6	373.4	92
1.5	2.4	406.2	361.7	89
2.0	2.6	406.0	365.2	90

Enzyme units used for immobilization: 408.6 U ml^{-1}.

xylanases by affinity precipitation [24]. SDS-PAGE of the immobilized xylanase indicates that Eudragit S-100 seemed to bind selectively and efficiently FXYN. Therefore, the immobilized xylanase prepared at the polymer concentration of 1% was arbitrarily regarded as the immobilized FXYN, and was further characterized and applied in the following study.

3.2. Effect of pH on activity and stability of immobilized and free xylanases

Fig. 2 shows the activity of the immobilized and free *S. olivaceoviridis* E-86 xylanases at different pH values. The optimum pH values of the immobilized and free xylanases were pH 6.3 and 5.8, respectively. The pH stabilities of the immobilized and free xylanases were compared in the pH range between pH 2.3 and 11.8 at 35 °C during 30 min incubation periods (Fig. 3). Immobilization of the enzyme had marginal effect on its pH stability as compared to free xylanase. Both the immobilized and free enzymes exhibited good stability at pH 4–10.

There were no changes in the optimum pH values for α-amylase, and β-glucosidase immobilized on Eudragit S-100 [22]. Immobilization of enzymes on the reversibly soluble–insoluble polymers often has marginal effect on the enzymes optimum pH and pH stability [13,14,28,29]. *S. olivaceoviridis* E-86 xylanase immobilized on Eudragit S-100 showed the same pH stability as the free xylanase and had only a minimal shift in optimum pH.

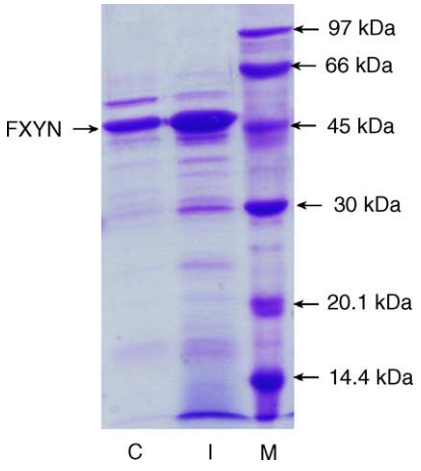

Fig. 1. SDS-PAGE of the crude and immobilized *S. olivaceoviridis* E-86 xylanases. Lane C: ammonium sulphate precipitate; lane I: elution of immobilized xylanase; lane M: low molecular weight standards.

3.3. Effect of temperature on activity and stability of immobilized and free xylanases

Temperature dependence of the activities of the immobilized and free *S. olivaceoviridis* E-86 xylanases was studied at different temperatures in 50 mmol l^{-1} McIlvaine buffer (pH 5.8 for the free enzyme and pH 6.3 for the immobilized enzyme). The free enzyme had an optimum temperature of 60 °C, whereas that of the immobilized enzyme was shifted to 65 °C (Fig. 4).

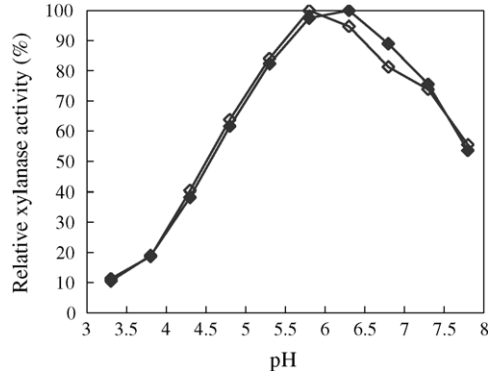

Fig. 2. Effect of pH on the activities of immobilized and free *S. olivaceoviridis* E-86 xylanases. (◆), immobilized enzyme; (◇), free enzyme.

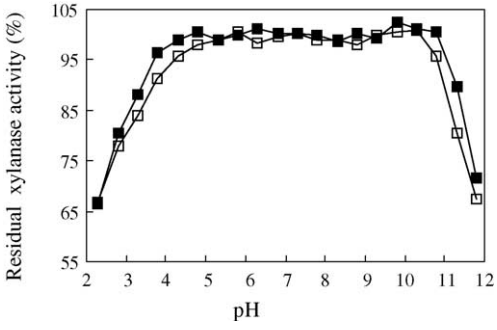

Fig. 3. pH stability of immobilized and free *S. olivaceoviridis* E-86 xylanases. (■), immobilized enzyme; (□), free enzyme.

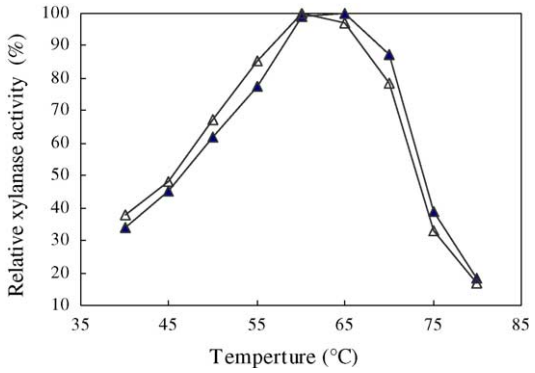

Fig. 4. Effect of temperature on the activities of immobilized and free *S. olivaceoviridis* E-86 xylanases. (▲), immobilized enzyme; (△), free enzyme.

The rates of heat inactivation of free and immobilized enzymes were investigated in the temperature range between 50 and 65 °C. The plot of log % remaining activity versus time is linear at the temperature range tested, indicating the first-order inactivation kinetics for both the immobilized and free FXYN (Fig. 5). Half-time and stabilizing factor values (protective effect) showed that the immobilization of the enzyme had significant effect on the thermal stability of the enzyme (data not shown). Both the immobilized and free xylanases were reasonably stable at 50 °C, but inactivation at 60 °C was rapid. The half-life of the enzyme at 60 °C was prolonged from 39 to 64 min due to immobilization. Similarly, several researchers also reported the increase in half-life of *Aspergillus* xylanases at 60 °C upon immobilization [10,13]. Thermal stability data at 65 °C even showed that immobilization resulted in significant stabilization compared to the free enzyme. The first order rate constant for thermal inactivation at 65 °C for the immobilized and free enzymes was calculated as 0.038 min^{-1} and 0.062 min^{-1}, which gave the half-time values of 15 min and 10 min, respectively. Therefore, immobilization significantly enhanced the thermal stability of the xylanase.

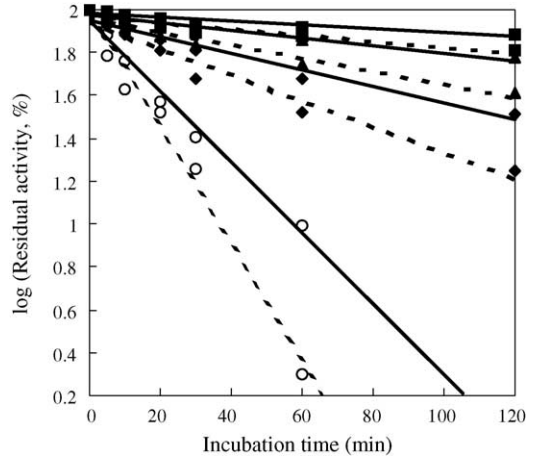

Fig. 5. Thermal stability of immobilized (solid lines) and free (dotted lines) *S. olivaceoviridis* E-86 xylanases at 50 °C (■), 55 °C (▲), 60 °C (◆) and 65 °C (○). Immobilized enzyme: 50 °C, $y = -0.0009x + 1.984$, $r^2 = 0.872$; 55 °C, $y = -0.0017x + 1.9712$, $r^2 = 0.9519$; 60 °C, $y = -0.0039x + 1.9499$, $r^2 = 0.9678$; 65 °C, $y = -0.0166x + 1.9482$, $r^2 = 0.9863$; free enzyme: 50 °C, $y = -0.0014x + 1.9667$, $r^2 = 0.8912$; 55 °C, $y = -0.0031x + 1.9552$, $r^2 = 0.9578$; 60 °C, $y = -0.0061x + 1.9392$, $r^2 = 0.9642$; 65 °C, $y = -0.0271x + 1.9801$, $r^2 = 0.9844$.

In general, the immobilization process of the enzyme protects the enzyme against heat inactivation [12,14,28]. Immobilization enhanced the optimum temperature and thermal stability of xylanase in this study. Activity and stability of the xylanase at higher temperature are expected to increase its suitability for industrial application [1,2]. Inactivation constants of the xylanase were higher than that of the free xylanase, indicating higher thermostability of enzyme due to immobilization (Fig. 5). The thermal inactivation process of the immobilized enzyme also corresponded well with the theoretical curves of the simple first-order reaction. The increase of the optimum temperature probably is a consequence of enhanced thermal stability.

3.4. Enzyme kinetics of immobilized and free xylanases

K_m and V_{max} values of immobilized and free enzymes were determined and results are shown in Table 2. The K_m values of the immobilized FXYN for birchwood xylan, beechwood xylan and soluble oat-spelt xylan were increased to 2.34, 2.07, and 1.36 mg ml^{-1}, compared to 1.54, 1.42, and 1.15 mg ml^{-1} of the free xylanase. The V_{max} values of the immobilized enzyme for birchwood xylan, beechwood xylan and soluble oat-spelt xylan showed significant increases to 171.8, 122.9, and 263.6 μmol ml^{-1} min^{-1} from 90.3, 67.6 and 101.2 μmol ml^{-1} min^{-1} of the free enzyme.

Changes in kinetic parameters upon immobilization are critical for evaluating the success of an immobilization protocol. Several researchers reported that the K_m did not change or only slightly increased after xylanases were immobilized on Eudragit S-100 or Eudragit L-100 [13,30].

Table 2
Kinetic parameters of immobilized and free *S. olivaceoviridis* E-86 xylanases

Substrate	Immobilized enzyme		Free enzyme	
	K_m (mg ml^{-1})	V_{max} (μmol ml^{-1} min^{-1})	K_m (mg ml^{-1})	V_{max} (μmol ml^{-1} min^{-1})
Birchwood xylan	2.34	171.8	1.54	90.3
Beechwood xylan	2.07	122. 9	1.42	67.6
Soluble oat-spelt xylan	1.36	263.6	1.15	101.2

Enzymatic reactions were carried out at 50 °C for 5 min in 50 mmol l^{-1} McIlvaine buffer, pH 6.3.

Only marginal increase in the K_m value upon immobilization with Eudragit S-100 was observed here. This confirms that the immobilized enzyme–substrate binding continued to be efficient. However, moderate increases in K_m value upon immobilization have been frequently reported when the matrix is insoluble or in solid form [9,10,12]. Increases in V_{max} upon immobilization are generally not observed. However, V_{max} values of the immobilized enzyme were 82–161% higher than those of free xylanase in this study. Roy et al. reported that there was a marginal increase for immobilized *Melanocarpus albomyces* IIS 68 xylanase on Eudragit L-100 due to the somewhat hydrophobic nature of the polymer [30].

3.5. Hydrolysis of pre-treated corncob powder for xylo-oligosaccharide production

Kusakabe et al. reported that xylan-containing natural materials were treated with dilute alkaline solution to remove lignin, so that xylan in the pre-treated materials could be easily hydrolyzed by xylanase [5]. Therefore, attempts were made to produce xylo-oligosaccharides from corncob powder pre-treated with dilute alkaline solution. Fig. 6 shows the time-course of hydrolyses of non-treated (native) and pre-treated corncob powder by the immobilized and free *S. olivaceoviridis* E-86 xylanase. The xylan of PCP became easily hydrolysable compared to that of native corncob powder. The immobilized enzyme yielded 3.9 mg ml^{-1} and 15.5 mg ml^{-1} soluble sugar during the 24 h incubation of the native and pre-treated corncob powder at 55 °C, respectively. Specifically, 4.0 times (15.5 versus 3.9) more sugar was produced by hydrolyzing the pre-treated corncob powder compared to the native corncob powder. The hydrolysis of the xylan in PCP by immobilized enzyme proceeded at the same speed as the free enzyme

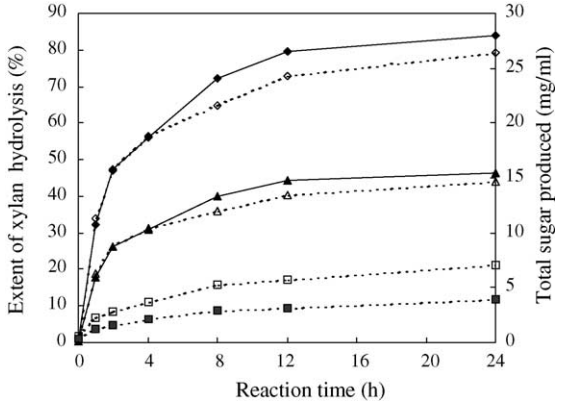

Fig. 6. Time-course of hydrolyses of non-treated (native) and pre-treated corncob powder (PCP) by the immobilized (solid lines) and free (dotted lines) *S. olivaceoviridis* E-86 xylanase. (▲), total sugar for PCP produced by immobilized enzyme; (△), total sugar for PCP produced by free enzyme; (◆), extent of xylan hydrolysis (%) for PCP by immobilized enzyme; (◇), extent of xylan hydrolysis (%) for PCP by free enzyme; (■), total sugar for native corncob powder produced by free enzyme; (□), extent of xylan hydrolysis (%) for native corncob powder by free enzyme.

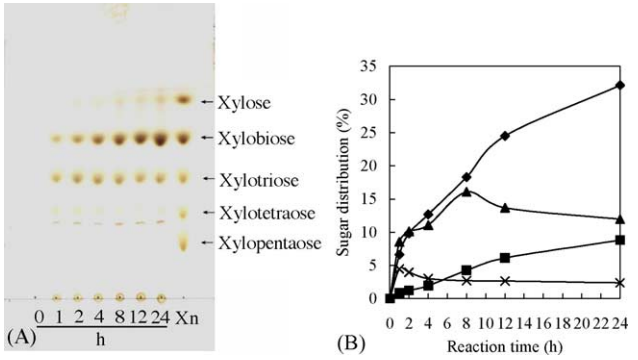

Fig. 7. Time-course of the xylo-oligosaccharide production from PCP (pre-treated corncob powder) by immobilized *S. olivaceoviridis* E-86 xylanase. PCP (4 g) was incubated with 600 U of the enzyme in 100 ml 50 mmol l^{-1} McIlvaine buffer (pH 6.3) and the reaction was carried out at 55 °C for 24 h and then the hydrolyzates were analyzed by TLC (A) and HPLC (B), respectively. Lane Xn, a mixture xylose to xylopentose; incubation time (h) is indicated. Symbols: (■), xylose; (◆), xylobiose. (▲), xylotriose; (×), xylotetraose.

during the early stage of reaction. The final extent of xylan hydrolysis of PCP by the immobilized enzyme was higher (84%) than that (79%) by the free enzyme. The difference in the free and immobilized enzyme performances possibly originated from their difference in thermal stability and this caused the immobilized FXYN to be more effective for xylo-oligosaccharide production.

The products of hydrolysis of PCP were analyzed by TLC and HPLC. Xylobiose and xylotriose were produced within 1 h of the reaction time and was the major end product found (Fig. 7). Upon long-term incubation (more than 8 h), the xylose content increased steadily, indicating some of the xylotriose was gradually converted into xylose. In addition, the sugar located under xylotetraose was clearly distinguishable on TLC (Fig. 7A), and was possibly the xylo-oligosaccharide which included arabinose or glucuronic acid. After 24 h of incubation, about 32 and 12% of the total sugar from the reaction products were xylobiose and xylotriose, respectively. After the enzyme hydrolysis of PCP, the resultant hydrolyzate was filtered to separate the sugar solution from the un-degraded materials. Then the pH of the solution was adjusted to pH 4.5 for precipitating the enzyme and purifying the xylo-oligosaccharides. Thus, the immobilized xylanase described here can be advantageously applied to produce xylo-oligosaccharides from the pre-treated corncob powder.

3.6. Reusability of the immobilized S. olivaceoviridis E-86 xylanase

The reusability of the enzyme preparation was further assessed by carrying out hydrolysis of PCP using the immobilized xylanase at 55 °C (Fig. 8). It was seen that after the initial loss of 10.5% activity in the first cycle, the activity remained constant at 81% up to four precipitation cycles. This loss of enzyme activity in the first cycle may be

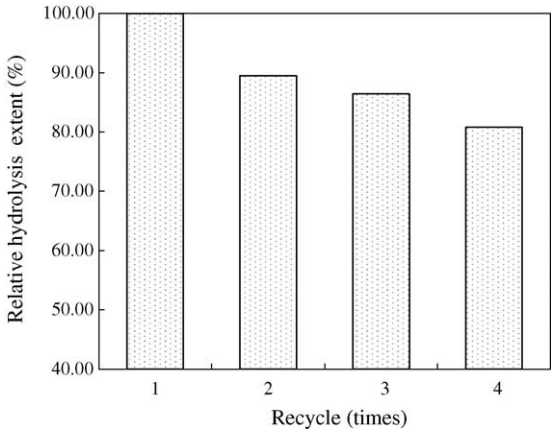

Fig. 8. Reusability of the immobilized *S. olivaceoviridis* E-86 xylanase. After one cycle of incubation of the immobilized enzyme and the substrate at 55 °C, un-degraded PCP was removed by centrifugation followed by removal of the polymer-bound enzyme, as described in Section 2. The immobilized enzyme was re-dissolved and incubated again with fresh substrate to initiate the second cycle of hydrolysis. Each hydrolysis cycle lasted 6 h.

attributed to the binding of some xylanase molecules to the low affinity sites on Eudragit S-100 [14]. Since the loss of activity during each precipitation is estimated to be about 1–1.5% [29], the gradual small loss of enzyme activity in the second, third and fourth cycles in this experiment probably occurred during each precipitation. Similar results were observed in other studies too. For instance, the initial hydrolysis activity of the immobilized trypsin on Eudragit S-100 decreased by 12% after being recycled three times [29]. Although *Aspergillus* sp. xylanases immobilized on Eudragit S-100 could be reused for three cycles without any loss in activity in hydrolyzing xylan and wheat bran, significant decrease in its activity was observed in the fourth cycle [13]. Immobilization of *Thermomyces lanuginosus* SSBP xylanase using Eudragit S-100 retained only 62% of its activity after six precipitations [28]. In the present study, xylanase immobilized on Eudragit S-100 can be recycled for four times with small loss of enzyme activity during applications. This shows that the cost of xylan conversion could be significantly reduced.

4. Conclusion

S. olivaceoviridis E86 xylanase (FXYN) can be non-covalently immobilized on an anionic copolymer Eudragit S-100 with a high immobilization efficiency of 90%. The overall performance of the immobilized xylanase with respect to catalytic activity and thermal stability is promising compared with that of the free enzyme. Therefore, the use of immobilized xylanase in xylo-oligosaccharide production enables its recovery and reuse. Accordingly, it is suggested that xylanase immobilized on Eudragit S-100 is suitable for practical xylo-oligosaccharide production.

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