IMMOBILIZATION OF XYLANASE AND XYLANASE–β-CYCLODEXTRIN COMPLEX IN POLYVINYL ALCOHOL VIA *ELECTROSPINNING* IMPROVES ENZYME ACTIVITY AT A WIDE PH AND TEMPERATURE RANGE

JAQUELINE POZZADA DOS SANTOS[1]; NATHAN LEVIEN VANIER[2]

[1]*Federal University of Pelotas – pozzadaj@gmail.com*
[1]*Federal University of Pelotas – nathanvanier@hotmail.com*

1. INTRODUCTION

The utilization of xylanolytic enzymes for xylan degradation into shorter sugar residues is of relevance for several industrial purposes (SHAHRESTANI et al., 2017). A limitation of xylanase application in food and beverage industries is the low enzyme activity and stability at a wide pH and temperature range.

Enzyme immobilization possess a key role for increasing enzyme activity, stability and shelf–life, broadening their application as natural catalysts. The immobilization by electrospinning technique is a common and versatile method for spinning fibers from pure solutions of polymers or blends of polymers (NEO et al., 2013).

An alternative support for enzymatic immobilization is the use of cyclodextrins, in special beta–cyclodextrin (β–CD), which offers functional solutions by creating complex structures that exhibit unique properties, such as improved solubility and non–toxic nature (CAMBOLAT et al., 2017).

In the present study, different concentrations of xylanase were immobilized in PVA fibers, using or not β-CD, by electrospinning technique. Morphological characteristics of obtained fibers were investigated by scanning electron microscopy (SEM).The activity of free, PVA– immobilized, and PVA–β-CD–immobilized enzyme was studied with respect to xylan hydrolysis at varying pH and temperature values.

2. METHODOLOGY

Xylanase (E.C. 3.2.1.8 from *Aspergillus oryzae*, ≥ 2500 U g^{-1}), beta-cyclodextrin (β-CD) and PVA of high molecular weight were used in this study. The inclusion complex (IC) of xylanase and β–cyclodextrin was prepared as described by PETROVIC et al. (2010) in a ratio of 1:1 (w:w). Pure xylanase or xylanase previously complexed with β-CD was added to the PVA solution (8 w v^{-1}) until get the final electrospinning solution (0.5, 1.0, 1.5, 2.0 and 2.5% w v^{-1}) of enzyme.

The electrospinning process was conducted adding the polymer solutions in a 1 mL syringe, which had a 0.7 mm diameter needle. The horizontally distance between the tip to the collector plate during the fiber production was 20 cm, with feeding rate of 0.5 mL h^{-1}, being controlled by an infusion pump. The amount of enzyme loaded on the PVA membrane was measured using the Bradford assay, as described by IVANOVA et al. (2010). Activity of xylanase was determined as xylose formation activity, according to the method reported by BAILEY et al. (1992), using beechwood xylan as substrate. The morphology of the fibers, as well as the average diameter, was investigated using a SEM (Jeol JSM 6010LV, Japan). Thermal and pH stability of the free and immobilized xylanase were estimated as described by MEHNATI-NAJAFABADI et al. (2018).

3. RESULTS AND DISCUSSION

The activity and the loading capacity of pure and immobilized xylanase are shown in Table 1. The amount of immobilized enzyme per gram of electrospun fiber increased as the initial enzyme concentration increased. However, the xylanase activity of the PVA-immobilized and PVA–β-CD-immobilized fiber did not accompany the increases in loading capacity to a similar extent. This explains the reduction in specific activity (activity per mg of immobilized enzyme) of immobilized xylanase as the initial enzyme concentration increased.

Table 1. Activity and loading capacity of pure and immobilized xylanase

Treatment	Xylanase concentration (%)	Loading capacity (mg immobilized xylanase/g fiber)	Xylanase activity (µM/min)	Specific activity (µM/min/mg of immobilized xylanase)
Pure xylanase	1.5	-	184.44	-
XY in PVA fiber	0.5	5.55	331.50	59.73
	1.0	10.66	336.85	31.60
	1.5	12.45	341.53	27.43
	2.0	15.99	393.68	24.63
	2.5	18.06	406.71	22.52
XY–β-CD in PVA fiber	0.5	6.04	181.76	30.09
	1.0	9.52	196.13	20.601
	1.5	11.72	294.40	25.11
	2.0	13.59	307.77	22.65
	2.5	16.03	337.86	21.08

Legend: Xy: Xylanase; PVA: Polyvinyl alcohol; Xy–β-CD: Xylanase–β-cyclodextrin

The SEM images of fibers from PVA, xylanase–PVA (XY–PVA), and xylanase–β-CD–PVA (XY–β-CD–PVA) treatments at enzyme levels of 1.5% are shown in Figure 1. In general, homogeneous distribution and compact surface of fibers were found in pure PVA fibers (control), as well as in fibers from XY–PVA and XY–β-CD–PVA treatments. The average diameter was higher in fibers prepared with XY–β-CD inclusion complex. This fact is a result of the larger molecular dimensions of XY–β-CD complex than the dimensions of pure XY.

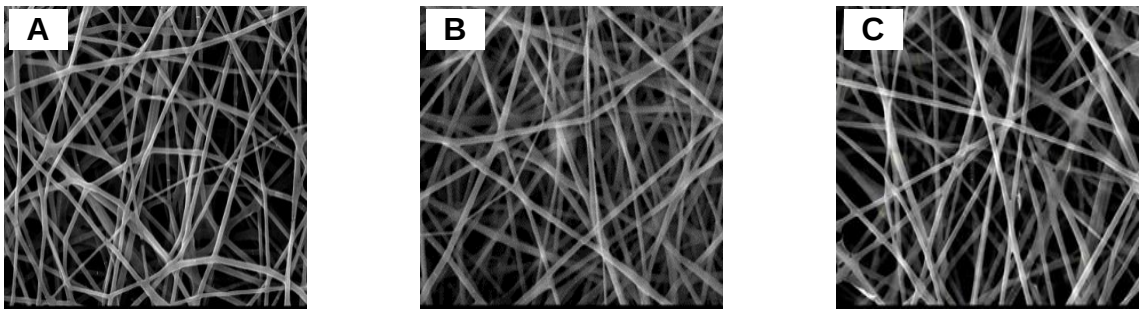

Figure 1. SEM images of electrospun fibers prepared with an 8% PVA solution (A), pure xylanase (B) and XY–β-CD complex (C) at enzyme levels of 1.5%.

The relative activity of the pure xylanase, XY–PVA and XY–β-CD–PVA fibers was assayed at temperatures in the range of 40 to 80 °C at pH 6.0 (Figure 2A) and also as a function of pH at 60 °C (Figure 2B). As shown in Figure 2A, there was a change in the maximum xylanase activity when enzyme was immobilized in PVA. Pure xylanase exhibited optimum temperature of 60 °C while 70 °C was determined as the optimum temperature for xylanase reaction when immobilized in the fiber XY–PVA and XY–β-CD–PVA.

It is of great relevance the significant increase ($P < 0.05$) in xylanase activity mainly at 70 and 80 °C, which supports the improvement of thermal stability of xylanase after immobilization in PVA electrospun fibers.

The maximum relative activity for free xylanase, XY–PVA, and XY–β-CD–PVA treatments was determined as a function of pH (Figure 2B). One hundred percent of relative activity was considered the best enzyme activity results for each one of the three treatments. It is important to notice that xylanase immobilized in PVA exhibited higher ($P < 0.05$) relative activity at a wide pH range, with greater values at pH 4, 5, 7 and 8, as compared to free xylanase.

Greater difference in the enzyme activity for xylanase immobilized in PVA was found at pH 8, as compared to free xylanase, where the average values were 313.79 and 144.33 μM/min, respectively.

According to MORENO-CORTEZ et al. (2015), improvements in the catalytic function of immobilized enzymes were attributed to a reduced mobility of the three-dimensional protein structure of the enzyme within electrospun fibers, reducing the pH and temperature effects on enzyme denaturation.

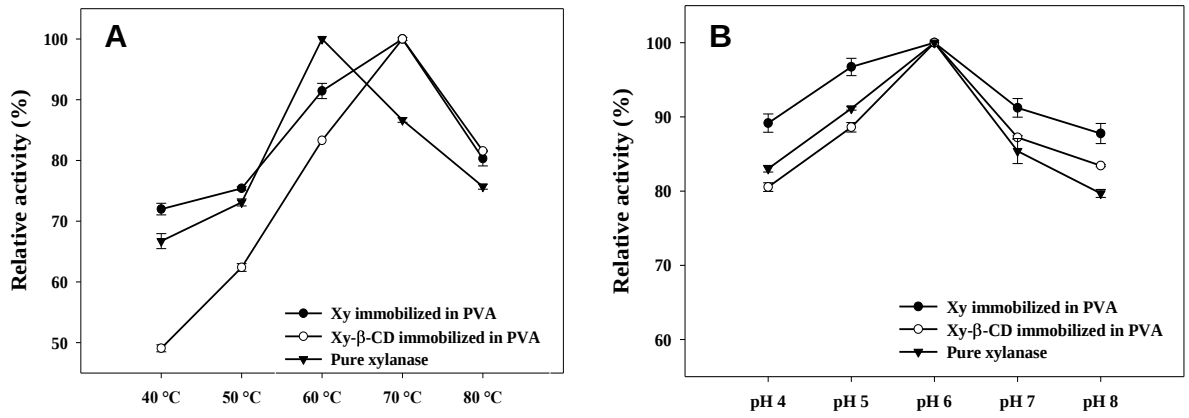

Figure 2. Relative activity of pure and immobilized xylanase as function of temperature (A) and Ph (B). Legend: Xy: Xylanase; PVA: Polyvinyl alcohol; Xy–β-CD: Xylanase–β-cyclodextrin.

4. CONCLUSIONS

The present study provided valuable information for food industries that demands xylanase, in order to reduce production costs and increase enzyme activity at extreme pH and temperature conditions. The use of inclusion complex of xylanase and β-cyclodextrin instead of pure xylanase in the inlet electrospinning solution favored the formation of thicker electrospun fibers. All fibers exhibited a smooth surface without beads. Future studies may be performed in order to evaluate the storage stability and release behavior of xylanase from obtained electrospun fibers.

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