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ASSAY OF

endo-1,4-β-Xylanase

using

XYLAZYME TABLETS

T-XYZ 05/19

SUBSTRATE:

The substrate employed is azurine-crosslinked arabinoxylan (AZCL-Arabinoxylan), which is prepared by dyeing and cross-linking highly purified wheat-flour arabinoxylan to produce a material which hydrates in water but is water insoluble. Hydrolysis by *endo*-(1,4)-β-D-xylanase (xylanase) produces water soluble dyed fragments, and the rate of release of these (increase in absorbance at 590 nm) can be related directly to enzyme activity. The substrate is supplied commercially in a ready-to-use tablet form, Xylazyme tablets.

BUFFER CONCENTRATE:

[(Sodium acetate buffer, 200 mM, pH 4.7) containing sodium azide (0.02%)].
Add 12.1 g of glacial acetic acid (1.05 g/mL) to 900 mL of distilled water. Adjust the pH of this solution to 4.7 by the addition of 2 M (8 g/100 mL) sodium hydroxide solution (approx. 50 mL is required). Adjust the volume to 1 L. Add 0.2 g of sodium azide as a preservative.

EXTRACTION/DILUTION BUFFER:

[(Sodium acetate buffer, 25 mM, pH 4.7) containing sodium azide (0.02%) and BSA (0.5 mg/mL)].
Add 125 mL of extraction buffer to 850 mL of distilled water and adjust the pH 4.7 if necessary by dropwise addition of 2 M hydrochloric acid or 2 M of sodium hydroxide solution. Add 0.2 g of sodium azide and 0.5 g of BSA. Adjust the volume to 1 L with distilled water and store in a sealed Duran bottle at 4°C.

NOTE:
Do not add the sodium azide until the pH is adjusted. Acidification of sodium azide releases a poisonous gas.

ENZYME EXTRACTION AND DILUTION:

Liquid preparation:

Using a positive displacement dispenser, add 1 mL of liquid enzyme preparation to 99.0 mL of extraction/dilution buffer (25 mM, pH 4.7) containing sodium azide and BSA and mix thoroughly. Dilute an aliquot of this solution 10-fold by transferring 0.5 mL to 4.5 mL of extraction/dilution buffer. Mix thoroughly. Repeat this process of 10-fold dilution until a concentration of enzyme suitable for assay is obtained. A typical further dilution for industrial enzyme preparations is 200- to 500-fold.

Powder samples:
Add 1.0 g of the material to 100 mL of extraction/dilution buffer (25 mM, pH 4.7) containing sodium azide and BSA and mix on a magnetic stirrer for 10 min, or until the sample is completely dispersed or dissolved. Clarify the solution by centrifugation (1,000 *g*, 10 min) or filtration through Whatman No. 1 (9 cm) filter circles. Dilute an aliquot of this solution 10-fold by transferring 0.5 mL to 4.5 mL of extraction/dilution buffer. Mix thoroughly. Repeat this process of 10-fold dilution until a concentration of enzyme suitable for assay is obtained. For example, with the industrial enzyme preparation Bioxylanase 10P (from *Aspergillus niger*; Kerry Ingredients, Cork, Ireland) a further dilution of the original extract of 2000-fold is required.

ASSAY PROCEDURE:

1. Pre-equilibrate an aliquot (1.0 mL) of suitably diluted enzyme preparation in sodium acetate buffer (25 mM, pH 4.7) containing sodium azide and BSA at 40°C for 5 min.
2. Initiate the reaction by adding a Xylazyme tablet. The tablet hydrates rapidly. Do not stir the suspension. Incubate at 40°C for exactly 10 min.
3. Terminate the reaction by adding 10.0 mL of Tris buffer salt (2% w/v, pH ~ 10, Megazyme cat. no. **B-TRIS500**) with vigorous stirring on a vortex mixer.
4. Leave the tubes at room temperature for about 5 min, and then stir them again.
5. Filter the slurry through a Whatman No. 1 (9 cm) filter circle.
6. Measure the absorbance of the filtrate at 590 nm against a substrate blank.

If the absorbance is above 2.0, dilute an aliquot of the enzyme extract with an equal volume of extraction/dilution buffer and repeat the assay.

Prepare a **substrate/enzyme blank** by adding 10 mL of Tris buffer salt solution to the enzyme solution before adding the Xylazyme tablet. After adding the tablet, stir the tube vigorously and allow the tube to stand at room temperature for approx. 10 min. Then filter the slurry through Whatman No. 1 filter paper.

NOTE:
A single blank is required for each set of determinations and this is used to zero the spectrophotometer. The absorbance of the reaction solutions are measured against this blank.

STANDARDISATION:

A standard curve relating the activity of purified *Aspergillus niger* xylanase on wheat arabinoxylan and Xylazyme (Lot 150601) is shown in Figure 1. Xylanase activity was standardised using wheat arabinoxylan (10 mg/mL) in 100 mM sodium acetate buffer (pH 4.7) as substrate, performing incubations at 40°C and measuring increase in reducing sugar level using the Nelson/Somogyi reducing sugar procedure.

One Unit of activity is defined as the amount of enzyme required to release one micromole of D-xylose reducing-sugar-equivalents from wheat arabinoxylan (10 mg/mL) in 100 mM sodium acetate buffer (pH 4.7) per minute at 40°C.

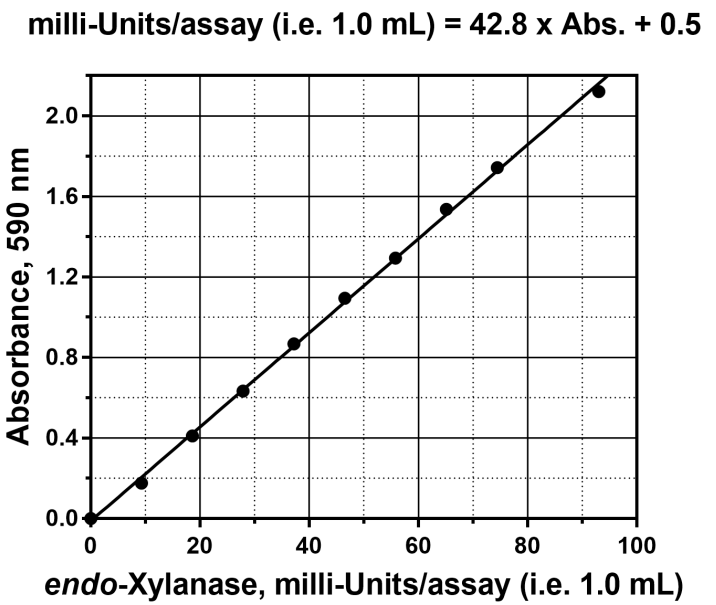

Figure 1. Standard curve for *Aspergillus niger* xylanase on Xylazyme 150601.

CALCULATION OF ACTIVITY:

Xylanase activity is determined by reference to the standard curve or regression equation to convert absorbance to milli-Units of activity per assay (i.e. per 1.0 mL), and then calculated as follows:

Units/mL or per gram of Original Preparation:

$$= \text{milli-Units per assay (i.e. per mL)} \times \frac{1}{1000} \times \text{Dilution}$$

where:

1/1000 = conversion from milli-Units to Units.
Dilution = the dilution of the original enzyme preparation.

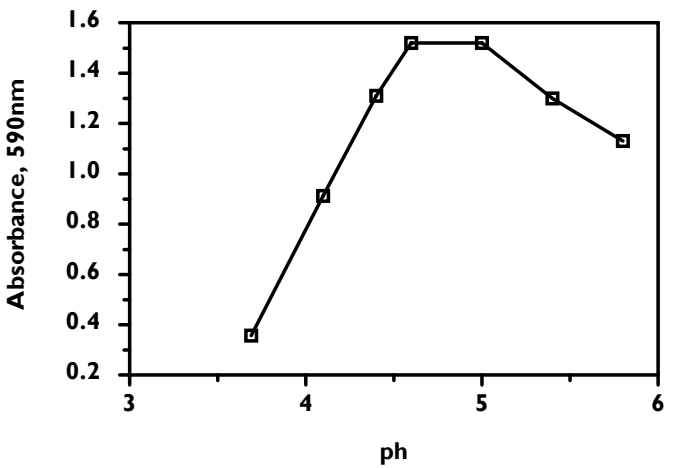

Figure 2. Effect of pH on the activity of *A. niger* xylanase on Xylazyme substrate tablets.

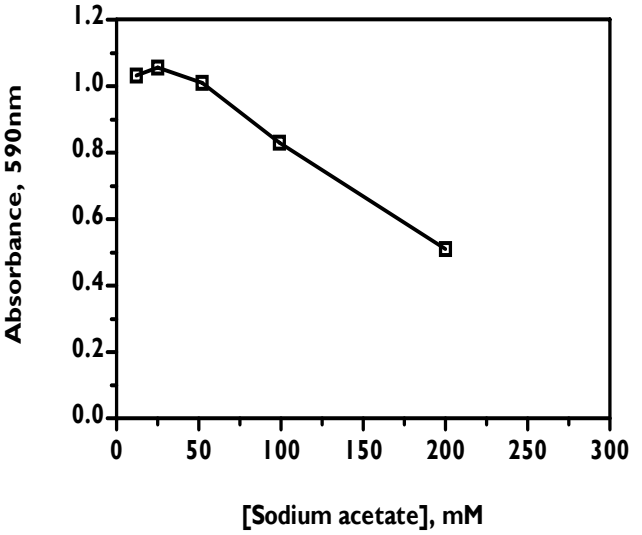

Figure 3. Effect of buffer salt concentration on the activity of *A. niger* xylanase on Xylazyme substrate tablets.

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