

Uniform and Simple Measurement of the Activity of Dextranases at the Sugarbeet Factory

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ABSTRACT

Currently, the activities or strengths of commercial dextranases in the U.S. cannot be directly compared because there is no uniform method used by vendors and/or distributors to measure the activity. A very wide variation exists in the activities of commercial dextranases, and this is compounded by the fact that activities and prices change regularly. Moreover, the factory storage characteristics of commercial dextranases differ enormously, which further highlights the need for not only a uniform method to (a) measure and economically compare the activities of different commercial dextranases, but (b) one which can be used simply at the sugarbeet factory to measure the activity of factory delivered batches of enzymes, and their changing activities on factory storage. A dextranase activity method, based on simple titration, was identified and modified for easy use at the sugarbeet factory. This method does not need any sophisticated equipment and there is no need for standards and a standard curve.

Objective

The objective of this research was to deliver a standard method to measure dextranase activity to sugarbeet factory staff, which could be undertaken easily with existing factory laboratory equipment.

Materials and Methods

Equipment required

Waterbath or constant temperature oven at 37°C

Stoppered conical flasks (50ml)

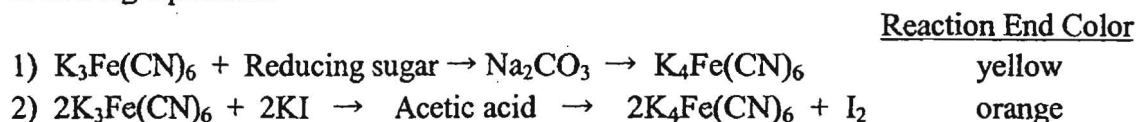
Boiling pan and heater

Burette (25ml)

Test-tubes and volumetric flasks

Measurement of dextranase activity using a modified titration method

A very simple method (Anon, 2002a) to measure dextranase activity at the method was identified and modified for use at the factory (Eggleston and Monge, 2004). One dextranase unit (DU/ml) is the amount of enzyme which degrades dextran T2000™ to produce reducing sugar corresponding to the reducing power of one μM of sodium thiosulfate in 1min at 37°C and pH 5.8. The reducing sugar is determined by the following equations:



- 3) $2K_4Fe(CN)_6 + 3ZnSO_4 \rightarrow K_2Zn[Fe(CN)_6]_2 + 3K_2SO_4$ orange
 4) $I_2 + \text{starch indicator} \rightarrow \text{starch-}I_2 \text{ complex}$ dark blue
 5) $\text{Starch-}I_2 \text{ complex} + 2NaS_2O_3 \rightarrow Na_2S_4O_6 + 2NaI$ white

The dextranase first has to be prediluted. The more “concentrated” or active the dextranase, the more predilution is required and vice versa. It is recommended that factories start at 0.5g/l distilled or de-ionized water if they are unsure of the activity. For “concentrated” dextranases, a dilution of 0.065g/l is recommended. Prediluted dextranase (1ml) is added to a solution of dextran (10ml; 1% T2000™) and 0.1M acetate buffer (4ml; pH 5.8) in a test-tube (2 replicates) and incubated in a waterbath at 37°C for 30min. The control is 1ml water instead of dextranase. After 30min, a 2ml aliquot of the reaction mix is added to 5ml of a potassium ferricyanide/sodium carbonate solution and water (3ml) in a stoppered conical flask. The yellow colored mix in the flask is then boiled for 15min and cooled. A mix of potassium iodide/zinc sulfate (5ml) and dilute acetic acid (3ml) are added successively to give an orange color. Five drops of soluble 1% potato starch are then added to react with the excessive liberated iodine to produce a dark blue color (starch-iodine complex), which is then titrated against 0.01N sodium thiosulfate until the blue color turns completely white. Calculation of dextranase activity is based on the following equation:

$DU/ml = (C - T) \times 2.5 \times N$, where C = titration ml of control, T = Average titration ml of the two dextranase replicates, and N = dilution multiple of the dextranase enzyme.

Results and Discussion

Most commercial dextranases currently available in the U.S. are produced from a fungal source: *Chaetomium gracile* or *erraticum*, and have GRAS (Generally Recognized as Safe status). A simple titration method (Anon, 2002a) to measure dextranase easily and uniformly at the factory was identified and modified for factory use. The method was compared with a more complicated spectrophotometric method (Anon, 2002b), and the correlation between the two was excellent ($R^2=0.999$) which confirm the method’s accuracy. Considerable variation existed amongst the dextranases currently being used in the U.S. Dextranases could be categorized into “concentrated” and “non-concentrated” forms, and an approximate 8-10 fold difference in activity existed between the two forms, but this did not always reflect the corresponding price per lb of enzyme (Eggleston and Monge, 2005). “Non-concentrated” activities ranged from 4,780 – 5,999 DU/ml and “concentrated” dextranases ranged from 48,072 – 57,687 DU/ml.

Conclusions

A dextranase activity method, based on simple titration, was identified, modified, and verified for easy use at the sugarbeet factory. The advantages of this method are that it is (a) easy to use, (b) has no need for sophisticated equipment, and (c) has no need for standards and a standard curve. A very wide variation exist in the activities and storage characteristics of commercial dextranases, with “non-concentrated” dextranases losing partial activity in a matter of a few days under factory storage conditions (Eggleston and

Monge, 2005). This method allows factory staff to measure and economically compare the activities of different commercial dextranases, as well as monitor the different batches of dextranase delivered to the sugarbeet factory, and storage characteristics.

Literature Cited

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