Successful Application of Dextranase in Sugar Beet Factories

Eggleston, G.¹, A. Dilks², M. Blowers², and K. Winters²
Presentation Outline

• Background information on commercial dextranases:
  - Principle of dextranase action
  - Problem associated with different activity units of commercial dextranases
  - New ICUMSA method to measure dextranase activity at the factory
  - Industrial conditions that affect the efficiency of dextranases

• Trials on the use of dextranase at Wissington British Sugar factory in the 2009/10 campaign
  - Include effects on second carbonation filtration
  - Cost evaluations of the dextranase trial

• Major conclusions
Background Information on Commercial Dextranases
Glucose molecules are linked by (1→6)-D-glycoside bonds. Approximately 5% branching occurs in cane dextrans, mostly through (1→4), and (1→3), and to a lesser extent (1→2)-D-glycoside linkages.
Principle of Dextranase Action

Dextranase hydrolyzes (1→6)-D-Glycoside Linkages in Random “Endo” Sites of HMW Dextran

Dots and connecting lines represent *chains* of glucose molecules linked by (1→6) glycosidic bonds in the dextran molecule.

Current Problem

• At the present time, the activities or strengths of commercial dextranases as vendors use a multitude of methods with different units of activity to measure and quote the activity:

For Example

<table>
<thead>
<tr>
<th>Current Different Units of Strength or Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>u/g</td>
</tr>
<tr>
<td>Du/g</td>
</tr>
<tr>
<td>U/mL</td>
</tr>
<tr>
<td>Du/mL</td>
</tr>
<tr>
<td>kDu-A/g</td>
</tr>
</tbody>
</table>

• The dextranase market is very dynamic – activities and prices can change regularly
A Large Range in Variation Exists in Activities of Commercial Dextranases Available in the U.S. to the Sugar Industry

<table>
<thead>
<tr>
<th>Commercial Dextranase</th>
<th>Dextranase Activity DU/mL</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2003</td>
<td>2004</td>
</tr>
<tr>
<td>A</td>
<td>52000</td>
<td>51920</td>
</tr>
<tr>
<td>B</td>
<td>5499</td>
<td>6500</td>
</tr>
<tr>
<td>C</td>
<td>4786</td>
<td>2750</td>
</tr>
<tr>
<td>D 5X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Eggleston Classification:**

“Concentrated” Dextranases  
25,000 - 58,000 DU/mL

“Non-Concentrated” Dextranases  
<25,000 DU/mL

Activity per Unit Dollar Varies Enormously Among Commercial Dextranases

<table>
<thead>
<tr>
<th>Commercial Dextranase</th>
<th>Dextranase Activity DU/mL/$</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2003</td>
<td>2004</td>
</tr>
<tr>
<td>A</td>
<td>2832.2</td>
<td>2827.9</td>
</tr>
<tr>
<td>B</td>
<td>916.5</td>
<td>583.3</td>
</tr>
<tr>
<td>D 5X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Simple Titration Method to Measure Dextranase Activity at Sugar Beet and Sugarcane Factories

Advantages of this method:

1. Simple
2. No need for sophisticated equipment
3. No need for standards and a standard curve

<table>
<thead>
<tr>
<th>Principle</th>
<th>Reaction</th>
<th>End Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) $K_3Fe(CN)_6 + \text{Reducing Sugar} \rightarrow Na_2CO_3 \rightarrow K_4Fe(CN)_6$</td>
<td></td>
<td>yellow</td>
</tr>
<tr>
<td>(b) $2K_3Fe(CN)_6 + 2KI \rightarrow \text{Acetic acid} \rightarrow 2K_4Fe(CN)_6 + I_2$</td>
<td></td>
<td>orange</td>
</tr>
<tr>
<td>(c) $2K_4Fe(CN)_6 + 3ZnSO_4 \rightarrow K_2Zn[Fe(CN)_6]_2 + 3K_2SO_4$</td>
<td></td>
<td>orange</td>
</tr>
<tr>
<td>(d) $I_2 + \text{starch indicator} \rightarrow \text{starch-}I_2 \text{ complex}$</td>
<td></td>
<td>dark blue</td>
</tr>
<tr>
<td>(e) Starch-$I_2$ complex + $2NaS_2O_3 \rightarrow Na_2S_4O_6 + 2NaI$</td>
<td></td>
<td>white</td>
</tr>
</tbody>
</table>
Measuring Dextranase Activity Using Simple Equipment at the Factory

- Incubation at 37°C
- Adding reagents
- Boiling
- Adding reagents
- Simple Titration
The excellent correlation between the two methods, confirms the accuracy of the simple titration method.

Also Need an ICUMSA Dextranase Method Because the Activity of Commercial Dextranases Changes on Storage

Simulated Factory Storage
Stored at room temperature in shaded place (~25 °C) over 90 day processing season

Refrigerated Temperature
(4 °C)
Industrial Conditions That Affect The Efficiency of Dextranases
Effect of Temperature

Pure Dextran T2000 - 2000ppm
50ppm dextranase
pH 5.4
25 min

Dextran in Juice
3177ppm/Brix
100ppm dextranase
pH 5.4
25 min
Effect of Brix (% Dissolved Solids)

- pH optimum is between 5 and 6

![Graph showing the effect of Brix on dextranase activity](image-url)
Effect of Dextran Concentrations on Dextranase Action

- It is easier to breakdown large amounts of dextran than smaller amounts with dextranase.
Contact Between Dextran (Substrate) and “Concentrated” Dextranase (Enzyme)

Need Factory Working Solutions

- Low Contact
  - Dextran
  - “Conc” dextranase-No dilution

- 1:1 dilution
  - Same ppm of dextranase to juice

- 1:4 dilution
  - High Contact

- No dilution
Studies into the Use of Dextranase at Wissington Factory During the 2009/10 Campaign
Problem

• During the 2009/10 beet campaign the UK experienced a very cold winter with freezing temperatures for 15 days during January 2010, which was followed by gradual warming – conducive to *Leuconostoc mesenteroides* growth and dextran formation.

• The large crop and long campaign lengths (up to 180 days) also led to difficult beet processing conditions later in the campaign.

Problems Caused by Dextran in Factory

• Higher 2nd carbonation filtration pressures which significantly slow down beet processing rates.
  - High mol. wt. dextran detrimentally affects the crystallization of calcium carbonate - thus small calcium carbonate particles are formed which detrimentally impact 2nd carbonation filtration.

• Increased viscosity by high concentrations of dextran can cause sugar crystallization problems, but this issue is more prevalent in the cane industry.
Simple Scheme Showing Dextranase Addition Point

Raw Juice to Carbonation

Heaters

Cossettes

RT Diffuser

Cold Raw Juice
25-30 °C

Dextranase addition

Mingler

Hot Raw Juice (71 °C)

$R_t = 18$ min

- Concentrated dextranase added as a working solution (1:4 in tap water)
First Trial

Prior to First Dextranase Trial

Chemically cleaned (1.5% HCl)
~30 cleanings per day of carbonation filters

During the First Dextranase Trial

3 ppm concentrated (52000 Du/ml) dextranase A
~8 cleanings per day

No change in factory throughput because of existing limits on the diffuser
The First Trial finished on 5 March 2010 after which the 2nd carbonation filter pressures increased resulting in more acid washing and PCC reactor blockage.

The Second Trial

The Second Trial re-started with another dextranase B (54302 DU/ml) at 1 ppm, but the filtration conditions continued to be poor with some periods of markedly lower throughputs being experienced:

During the Second Dextranase Trial

1 ppm concentrated (54302 DU/ml) dextranase B
Second Trial

Trend of second carbonatation filtration and throughput conditions during the Second Trial showing effect of PCC reactor blockage.

1 ppm concentrated (54302 DU/ml) dextranase B
Second Trial

- Dextranase B addition rate was increased to 2.1 ppm and conditions improved:

  ![Graph showing 2.1 ppm concentrated (54302 DU/ml) dextranase B](image)

  - Improved conditions allowed the increase in alkali addition to the 2nd carbonation vessel to aid limesalts control
  - Sodium carbonate addition increased 4-fold without any detrimental impact on 2nd carbonation filtration and allowed a reduction in filtered 2nd carbonation limesalts from ~0.01g to 0.086gCaO/100Brix
End of Campaign

• During the remainder of the 2009/10 campaign (after dextranase trial) 2nd carbonation filtration continued to impact on factory throughput. This continued to lead to increased chemical cleaning of filters and higher 2nd carbonation limesalts due to the limited addition of alkali to the process.

Trend of second carbonation filtration and throughput conditions after the end of the second dextranase trial
Effect of Dextranase on 2\textsuperscript{nd} Carbonation Particle Size
Continuous Precipitated Calcium Carbonate (PCC) Reactors

- Used during the 2009/10 campaign to aid 2\textsuperscript{nd} carbonation filtration

**How Does It Work?**

By adding additional PCC to the second carbonation vessel, smaller calcium carbonate crystals formed within the gassing vessel agglomerate -this results in a lowering of the very small crystals “fines” in the 2\textsuperscript{nd} carbonation juice leading to improved filterability

**References**

Burrough and Wones (2003). The Effect of frost damaged beet and other factors on Dorr 2\textsuperscript{nd} Carbonation juice particle size, Proc. of European Society for Sugar Technology (ESST), pp 237-246

Typical 2nd carbonation particle size distribution, with no PCC and no dextranase, with PCC only, with dextranase only and with PCC and dextranase.
2nd Carbonation Juice with and without PCC addition and Dextranase

- PCC only
- No PCC, no Dextranase
- Dextranase and PCC
- Dextranase only

“fines”

<table>
<thead>
<tr>
<th>Condition</th>
<th>Modal Particle size/ µm</th>
<th>% &lt; 3µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>No PCC and no dextranase</td>
<td>27.8</td>
<td>7.3</td>
</tr>
<tr>
<td>With PCC addition only</td>
<td>24.3</td>
<td>1.9</td>
</tr>
<tr>
<td>With Dextranase only</td>
<td>21.4</td>
<td>5.4</td>
</tr>
<tr>
<td>With PCC and Dextranase</td>
<td><strong>31.8</strong></td>
<td><strong>0.0</strong></td>
</tr>
</tbody>
</table>

- Improved factory operations
- More stable beet-end flows
- Smoothing of vapor demands
- A reduction in the amount of recycle to raw juice from filter cleaning
Dextran Levels In Juice Streams

- British Sugar haze dextran method at absorbance 720 nm
- Dextran levels in 2\textsuperscript{nd} carbonation juice of $\geq 60$ ppm lead to filtration problems (information supplied by Nordic Sugar)
Cost Evaluation of the Dextranase Trial
Breakdown of Cost Evaluation of the Dextranase Trial

- based on 3 ppm of Concentrated (52000 DU/ml) Dextranase A addition as much more cost effective (activity per unit $ higher)

<table>
<thead>
<tr>
<th></th>
<th>% Reduction</th>
<th>Trial Saving $/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cost of conc. Dextranase</td>
<td>--</td>
<td>-2741</td>
</tr>
<tr>
<td>Cost of acid washing filters</td>
<td>73%</td>
<td></td>
</tr>
<tr>
<td>CaO to process*</td>
<td>11%</td>
<td></td>
</tr>
<tr>
<td>Anthracite</td>
<td>9%</td>
<td></td>
</tr>
<tr>
<td>Throughput costs (LOP)</td>
<td>84%</td>
<td></td>
</tr>
<tr>
<td><strong>Total Savings</strong></td>
<td></td>
<td><strong>3,180</strong></td>
</tr>
</tbody>
</table>

* Lime required to aid filtration

- Other Financial Benefit: Reduction in water to ponds and shorter campaign lengths
Major Conclusions

• Commercial dextranases occur in “concentrated” and “non-concentrated” forms; activities, and activities per unit $, vary widely

• A new ICUMSA tentative method is now available to uniformly and easily measure the activity of dextranases at the factory to (1) economically compare activities of different commercial dextranases, (2) monitor the changing activities of dextranases on storage, and (3) measure activity of delivered batches

• 2nd Carbonation filtration significantly improved by adding dextranase in a number of ways:
  - Frequency of 2nd carbonation filter chemical cleaning reduced by 73%
  - Reduced chemical usage
  - Reduction in the volume of water discharged to the effluent treatment plant with the used acid by 418 m³/day

• Adding dextranase significantly improved beet throughput

• $3180/day saved by using concentrated 52000 DU/ml dextranase (Commercial identification)
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