

SUCCESSFUL APPLICATION OF DEXTRANASE IN SUGAR BEET FACTORIES

Gillian Eggleston^{1*}, Andy Dilks², Michael Blowers² and Kevin Winters²

¹USDA-ARS-SRRC, 1100 Robert E. Lee Boulevard, New Orleans, LA 70124 and
British Sugar plc, Wissington Factory, Wissington, King's Lynn, Norfolk, UK

ABSTRACT

Dextranases are sometimes applied to hydrolyze dextran polysaccharide in sugar manufacture when bacterial (mainly *Leuconostoc*) deterioration of sugar beet has occurred. Unfortunately, dextranases only have a small market and low volume sales compared to many other industrial enzymes. Consequently, research and development efforts to engineer properties of dextranases to specific conditions of sugar beet processing have not occurred and are not expected soon. Less than optimum application previously existed because of confusion about where to add the dextranase in the factory and which commercial dextranase to use. The wide variation in activity of commercially available “non-concentrated” and “concentrated” dextranases in the US, Europe, and other parts of the world, and a standardized titration method to measure activities at the factory are discussed. The titration method to measure the activity of dextranases at the factory is currently an ICUMSA (International Commission for Uniform Methods in Sugar Analysis) Tentative method. Optimization by applying “concentrated” dextranase as a working solution to juice is described. The results and conclusions from a trial of dextranase addition to draft raw juice at Wissington factory in the UK are discussed with emphasis on the impact on factory throughput and other key operational parameters. The trial demonstrated a significant benefit on second carbonation filtration which resulted in increased throughput, reduction in process chemicals usage, improved operational stability, a reduction in limesalts and the amount of water discharged to the site effluent treatment plant. A concentrated dextranase gave better cost in use, because an addition rate below that recommended by the suppliers was achieved making the product significantly cheaper.

Additional key words: *Beta vulgaris*, dextranases, optimized processing, precipitated calcium carbonate (PCC)

INTRODUCTION

Background Information on Dextranases

The major contributor to sugar beet and sugarcane deterioration is *Leuconostoc mesenteroides* infections (De Bruijn, 2000, Eggleston and Monge, 2005), particularly when humid and warm environmental conditions prevail. *L. mesenteroides* produce dextrans (α -(1→6)- α -D-glucans) and other deterioration products including mannitol and D-lactic acid, which in moderate and severe cases can disrupt normal processing operations. Dextrans are polydisperse by nature, i.e., they exist as a wide range of molecular weights. The high viscosity associated with the high molecular weight HMW portions (> 1000 KDa) of dextran mostly affects processing. Dextrans possess a largely linear structure (Khalikova et al. 2005) comprised of ~95% glucose units linked by (1→6) glycosidic bonds, but also containing ~5% branching through (1→4), (1→3) and some (1→2) linkages.

Freezes and subsequent thawing injure sugar beets and leave them susceptible to microbial infections, particularly from *L. mesenteroides* if warmer weather subsequently occurs.

The formation of the HMW dextran detrimentally affects the crystallization of calcium carbonate during the clarification processes. This results in the formation of smaller calcium carbonate particles that negatively impact second carbonation filtration by increasing filtration pressures. This in turn, causes a reduction in beet processing rates. The increased viscosity observed at high dextran concentrations can also lead to problems with sugar crystallization, however, this issue is more prevalent in the sugarcane industry.

Commercial dextranases (1→6- α -glucan hydrolases, EC 3.2.1.11) have been used in sugar beet and sugarcane factories to break down dextran by hydrolyzing α -(1→6) linkages at random endogenous sites (Khalikova et al. 2005). Most commercial dextranases in the United States are produced from *Chaetomium gracile* or *Chaetomium erraticum* fungi, generally recognized as safe (GRAS), and formulated as liquids. Some commercial dextranases are produced from some *Penicillium* species fungi, but these are not allowed in the US for safety reasons. The hydrolysis of dextran by dextranase is not an “all or nothing” mechanism. Instead, there is a gradual decrease in the average molecular weight of the various dextran fragments produced from the original HMW dextran, and these fragments in turn are continuously hydrolyzed (Fig. 1).

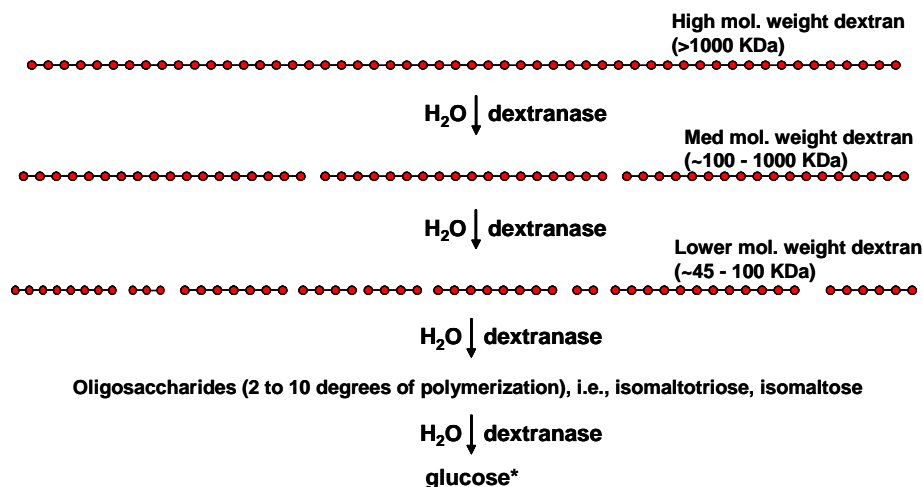


Fig. 1. Mode of endohydrolysis action of dextranase on α -(1→6) glycoside linkages in random sites of HMW dextran. Dots and connecting lines represent chains of glucose molecules linked by α -(1→6) bonds in the dextran molecule. Reaction time is not represented. * In dextranase application in the sugar industry, glucose as the end product rarely or never occurs. From Eggleston et al. (2007a).

Unfortunately, dextranases only have a small market and low volume sales compared to many other industrial enzymes. Consequently, research and development efforts to engineer properties of dextranases to specific conditions of sugar beet processing have not occurred and are not expected soon. Less than optimum application has previously existed because of confusion about where to add the dextranase in the factory and which commercial dextranase to use. This confusion is further compounded by the activity of commercial dextranases being quoted in many different units by suppliers/vendors, which does not allow the direct comparison of activities by the factory user. Furthermore, the commercial dextranase market is very dynamic and activities and prices change regularly. To solve this, Eggleston (2004) identified and modified a simple titration method to measure the activity (DU/ml units) of dextranases at

the factory. This method is currently being used by several sugarcane and sugar beet factories in the US and around the world. The method is easy to use and there are no requirements for sophisticated equipment or a standard curve (Eggleston, 2004; Eggleston and Monge, 2004). Although the titration method measures the activity of dextranases under more optimal conditions than factory juice applications, it is highly correlated to a spectrophotometric method, and higher industrial temperatures made no relative difference (Eggleston and Monge, 2005). In 2010, the method became ICUMSA Method GS7-8 (2010): “Standard Measurement of the Activity of Dextranases at Sugarcane or Sugar Beet Factories Using a Simple Titration Method” with Tentative status (Huet, 2011).

The urgent need for a standard method to measure the activity of dextranases at the factory is highlighted by the measurement of wide variations (up to 20-fold) in the activity of commercial dextranases that do not always reflect the unit costs of the enzymes (Eggleston and Monge, 2004). Commercial dextranases occur in a wide range of activity, that Eggleston (2004) classified into “non-concentrated” (<25,000 DU/ml but usually <6,000 DU/ml) or “concentrated” (25,000-58,000 DU/ml but usually between 48,000-58,000 DU/ml) forms (Table 1).

Table 1. Variation in Activities and Activities per Unit Cost of Commerical Dextranases Available Worldwide to the Sugar Beet Industry

Commerical Dextranase	Dextranase Activity DU/ml (Dextranase Activity per \$ cost)				Classification
	2003	2004	2008	2009	
A	52000 (2832)	51920 (2828)	52000 (2814)	52000 (2814)	Concentrated
B	5499 (917)	6500 (583)	2500 (417)		Non-Concentrated
C	4786	2750			Non-Concentrated
D 5X				8000 (491)	Non-Concentrated
D				3000 (735)	Non-Concentrated

The monitoring of activity on storage is also extremely important, as the storage characteristics of commercial dextranases vary widely (Eggleston and Monge, 2005). On storage at room temperature (~25 °C) and even refrigerated temperature (4 °C), non-concentrated dextranases lose activity in days and weeks, whereas a concentrated dextranase does not lose activity at 4 °C up to several years and only little at room temperature. The dramatic loss in activity on storage for non-concentrated dextranases is because more water is available to deactivate and denature the enzyme protein structure and increase its conformational mobility. Moreover, because non-concentrated dextranases rapidly lose their activity, transport conditions (particularly temperature) can affect their activity, e.g., they have been known to arrive at the factory with no activity (Eggleston et al. 2006). Therefore, the activity of delivered batches to the factory should be monitored too.

Another problem for the sugar industry is the availability of commercial dextranases. Because dextran problems are often sporadic, i.e., do not occur every beet campaign, the market for dextranases is small and limited. Thus, there is a supply and demand problem. However, as discussed above, concentrated dextranase (which can be purchased as in pails or drums) stores very well for several years, therefore, if it is not used one campaign it can be used in subsequent campaigns. A central, refrigerated storage unit for multiple factories is another possibility to mitigate this problem.

Industrial Conditions That Affect the Efficiency of Dextranases

The efficiency of dextranases in the factory depends on the pH, Brix, temperature, retention time (R_t), agitation, dextran concentration, and source, activity, and dosage of the dextranase applied (Eggleston et al. 2007b). The optimum pH range for dextranase activity is pH 5.0-6.0, with the lower end of the range more preferable. It is easier to break down larger rather than smaller amounts of dextran due to increased contact between the dextran and number of dextranase molecules (Eggleston et al. 2006). This is of particular concern to the sugar beet industry as much lower dextran concentrations affect processing, i.e., second carbonation, than for the cane industry. Fig. 2 illustrates the effect of temperature on commercial dextranases. Both non-concentrated (5,999 DU/ml) and concentrated (52,000 DU/ml) dextranases at juice pH 5.4, showed similar maximum activity at $\sim 50^\circ\text{C}$. The lowest activity occurred at 65.5°C because of the partial denaturation of dextranase. Dextranase activity was also low at $26-32^\circ\text{C}$, typical ambient temperatures of juice, but still better than at 65.5°C (Eggleston and Monge, 2005).

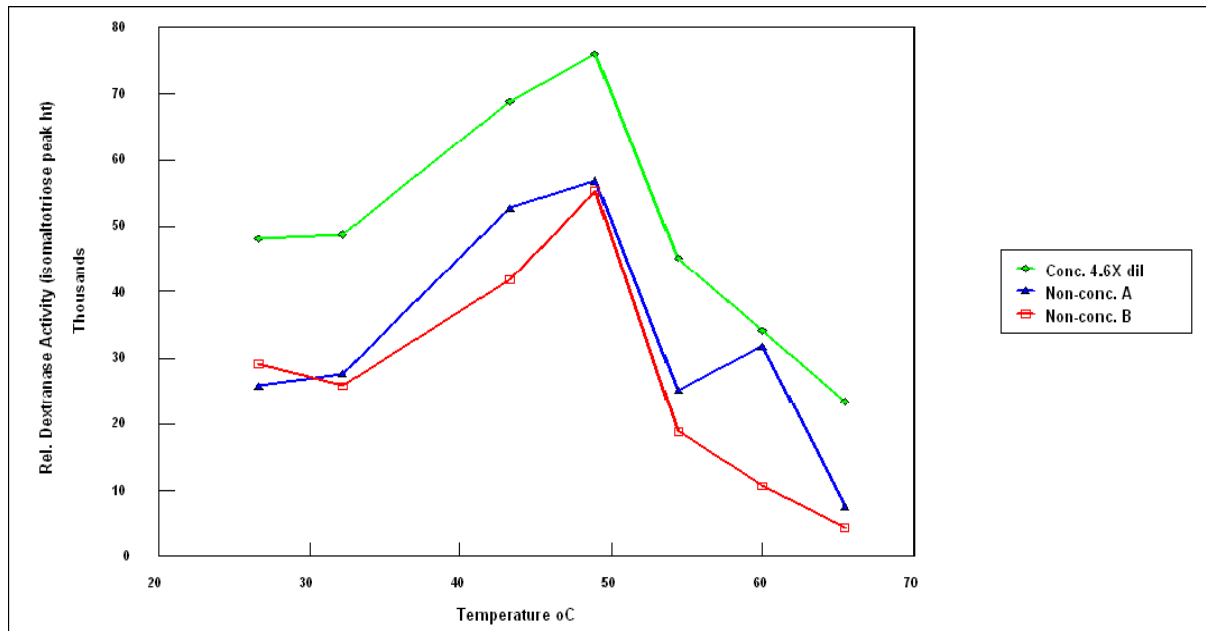


Fig. 2. Effect of temperature on dextranase activity to break down dextran in cane juice. Dextran 3177 ppm/Brix; 100 ppm dextranase; pH 5.4; 25 min. Commercial dextranases were sourced from *Chaetomium* fungi. From Eggleston and Monge (2005).

As the activity of many commercial dextranases reaches its maximum at $\sim 50^\circ\text{C}$, heating juice to this temperature at the factory may improve dextranase efficiency (Fig. 2) and, to some extent, overcome insufficient retention time. Heating juice to $\sim 50^\circ\text{C}$ is beyond the optimum temperature for *Leuconostoc* growth and dextran formation. However, if processors are concerned about microbial growth at this temperature, Eggleston and Monge (2005) showed that dextranase still works in the presence of up to 20 ppm sodium carbamate biocide. A sugarcane factory trial (Eggleston et al. 2007b) showed that, just heating juice from 27 to 37°C

dramatically improved average dextran hydrolysis from 50.8 to 83.8%. Furthermore, this improvement, generally, occurred irrespective of the initial dextran concentration. Although heating juice would be expected to increase factory energy inputs and costs, these will be negligible as existing heated juices could be re-circulated into the juice tank or juice pipes. Moreover, because heating the juice will reduce the dextranase dosage, any costs from increased energy or biocide requirements will be significantly lower than costs for the relatively expensive dextranase.

There is also a dramatic effect of Brix on the activity of dextranase (Eggleston and Monge, 2005) which is illustrated in Fig. 3. Dextranase activity is stable up to 25-30 Brix (Fig. 3), but afterwards decreased rapidly because of the low concentration of water reactant. Overall, the pH, temperature, and Brix conditions in sugar beet factory evaporators are sub-optimal for dextranase reactions.

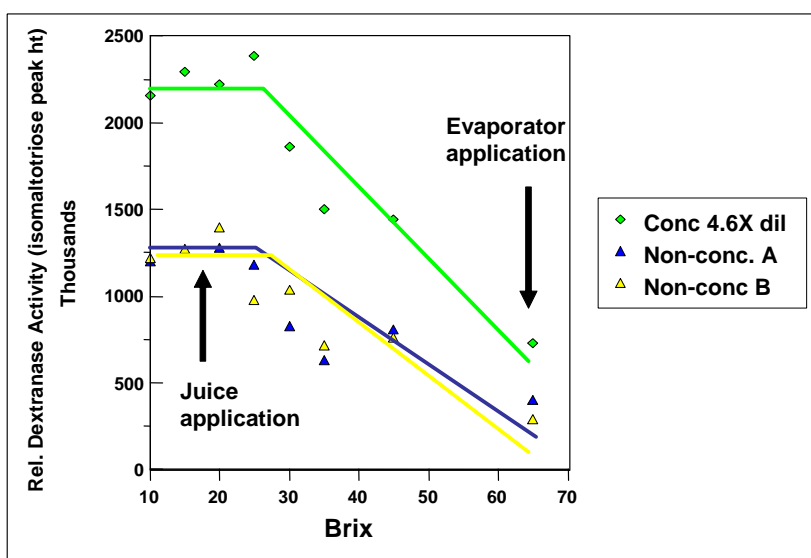


Fig.3. Effect of Brix on dextranase activity. The concentrated dextranase was diluted 4.6X to make it economically equivalent to the nearest priced non-concentrated dextranase. Commercial dextranases were sourced from *Chaetomium* fungi. From Eggleston and Monge (2005).

Improving Contact Between the Dextranase and Dextran at the Factory

Eggleston et al. (2006, 2007a) showed that concentrated dextranases are more cost effective than non-concentrated dextranases, but smaller volumes have to be added which will take longer to disperse in the juice tank. To ensure there is sufficient contact between the concentrated dextranase and dextran, a working solution of the concentrated dextranase has to be applied (Eggleston et al. 2006; Eggleston et al. 2007b). Working solutions are prepared at the factory and represent the same final concentration of dextranase but at larger volumes to improve contact. (Note: applying working solutions of concentrated dextranase are much more cost effective than adding non-concentrated dextranase undiluted). If working solutions of concentrated dextranases are to be useful, they need to be stable. Sucrose is a known stabilizer (Davidson, 2001) of many industrial enzymes and, fortunately, is readily available at the factory as sugar. A concentrated dextranase (52,000 DU/mL) diluted 5-fold with a 24 Brix sugar solution effectively stabilized the dextranase activity over 5 days, i.e., the activity decreased by

only ~2% after ~140 h (Eggleston et al. 2006). Furthermore, concentrated dextranase diluted 2-fold with distilled or tap (mains) water, is still stable up to 48 h, and even 5-fold dilutions are stable for 24 h. Because the least expensive and most readily available source of water at the factory is tap water, it is conservatively recommended that a working solution be prepared with tap water and stored for 12 to 24 h maximum at room temperature (Eggleston et al. 2006). If factory staff prefer, they can store the working solution for up to 60 h if it is prepared with a 24 Brix sugar solution.

This paper reports dextranase trials at British Sugar's Wissington Factory, UK, during the 2009/10 beet campaign. Concentrated dextranases were applied as working solutions (Eggleston et al. 2006) and results are reported. The trials were planned to understand the benefits of using dextranase when processing both frost damaged and naturally deteriorated beets due to long campaign lengths. Dextranase had not been used within BSUK previously due to a combination of the high cost and the somewhat milder winters normally experienced compared to more Northern European countries.

MATERIALS AND METHODS

Dextranase activity

The modified titration method of Eggleston (2004) was used to measure the activity of dextranases. One dextranase unit (DU/ml) is the amount of enzyme which degrades dextran T2000TM to produce reducing sugar corresponding to the reducing power of one micromole of sodium thiosulfate in one min at 37 °C and pH 5.8. Averages of duplicates are reported.

Dextran determination in factory trials

The level of dextran in filtered second carbonation juice was measured using British Sugar method METHOD-CF-098 based on the Nordic Sugar method which, in turn, is a modification of the ICUMSA Method GS1/2/9-15 (2009): "The Determination of Dextran in Sugars by a Modified Alcohol Haze Method." The sampled juice is acidified to pH 2.0 by the addition of conc. HCL; after filtration dextran is precipitated by the addition of absolute ethanol. The concentration was determined by measuring the resultant turbidity at absorbance 720 nm.

RESULTS

Dextranase Trial at Wissington Factory, UK, During the 2009/10 Beet Campaign

During the 2009/10 campaign the UK experienced a very cold winter with temperatures dropping to below freezing for several consecutive days during January 2010; this was followed by gradual warming. This in conjunction with the large crop and long campaign lengths (up to 180 days) led to very difficult beet processing conditions with second carbonation filtration becoming difficult and limiting throughput rate at all British Sugar UK (BSUK) factories. At Wissington factory, second carbonation filtration started to become limiting from mid-January, 2010 with significant impacts on beet processing rate being observed.

Practical Application of Dextranase during the Trial

During the trial two different sources of dextranase were used. The first was a concentrated dextranase A with a manufacturer's recommended addition rate of 4 ppm on total weight of the raw juice (on average 15-16 Brix). The second was dextranase B, with a manufacturer's recommended addition rate of 2-3 ppm on raw juice. The stated activity of the

dextranase B was 100kDU-A/g and that of the dextranase A was 30,000 DU/ml. Thus, if activity units were assumed to be equal the same performance would be expected between a 3 ppm addition of dextranase A to a 1 ppm addition of dextranase B, however, this was not realized. Independent activity analysis of the dextranases A and B using the Eggleston (2004) Tentative ICUMSA method indicated that dextranase B had an activity of 54,302 DU/ml whereas dextranase A had a measured activity of 52,000 DU/ml, which indicates that the actual activity of dextranase B is similar to dextranase A and hence its cost in use somewhat higher than originally expected.

The normal practice when using dextranase in beet processing is to add the enzyme into the cossette/ juice mixture going to the diffuser, however, the temperature of this circuit has to be lowered to allow for the optimum activity of the enzyme to be realized. Alternatively addition to draft juice (the cold raw juice exiting the prescalding or mingler and going forward to carbonation clarification through a series of heaters and buffer tanks) can be used. The main draw back with draft addition is that in most UK beet factories there is limited retention time prior to either the temperature rising above 65 °C or the pH rising above 6.0. The reconfiguration of Wissington factory and the addition of further low grade heating has resulted in a significant increase in the retention time of juice below 65 °C, therefore, addition of dextranase into the draft juice was used as shown in Fig. 4.

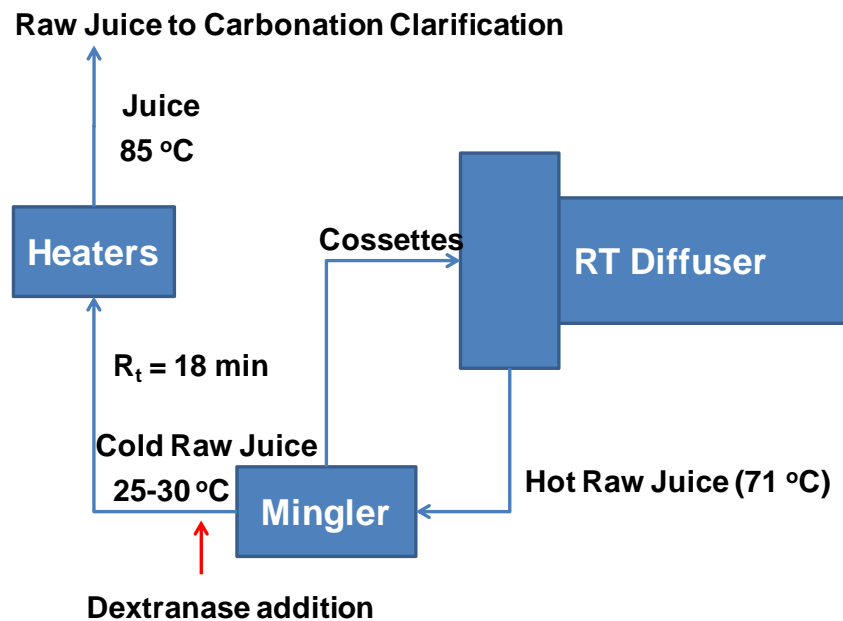


Fig. 4. Simple scheme showing the dextranase addition point.

Prior to the start of the trial, second carbonation filtration was not limiting the factory throughput. However, a significant amount of additional chemical cleaning of the second carbonation filters was required to maintain desired factory throughput (Fig. 5). The spikes on Fig. 5 represent significant increases in the level of the spent acid chemical cleaning tank and indicate that a filter has been chemically cleaned. Thus, the number of spikes is indicative of second carbonation filtration performance. On average each filter required chemical cleaning every 4 hrs. In addition, the factory had not been able to add the necessary volumes of sodium

carbonate (soda ash) to the second carbonation tank for thin juice buffering and the control of limesalts. Sodium hydroxide was being added to the second carbonation to allow for pH control because of its slightly less detrimental impact on second carbonation crystal particle size. The hard juice limesalts (post second carbonation filtration) were $>0.110\text{gCaO}/100\text{Brix}$ resulting in over exhaustion of the de-calcification plant.

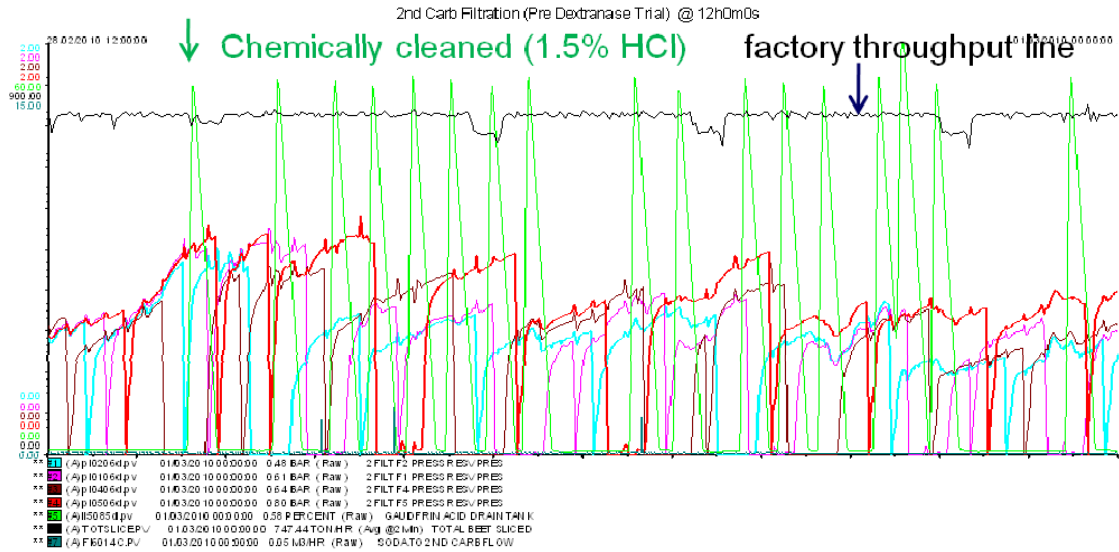


Fig. 5. Trend of second carbonation filtration and throughput conditions before the dextranase trial.

The trial commenced with an addition rate of 4 ppm of the concentrated dextranase A into the draft juice streams exiting the preclanders (heaters where the hot raw juice is used to heat the cosettes entering the diffuser; the raw juice is, therefore, cooled and then re-heated using lower grade heat; Fig. 4). As dextranase A was a concentrated dextranase it was added as a working solution (1:4 in tap water) as recommended by Eggleston et al (2006). Because the initial results were positive, i.e., lower 2nd carbonation filtration pressures resulting in longer run times between chemical cleaning, the addition rate was reduced to 3 ppm as shown on Fig. 6. To ensure sufficient throughput, the second carbonation filters require cleaning with a ~1.5% solution of HCl. After cleaning the contents of the filters are drained and pumped into the site effluent treatment ponds *via* a spent acid drains tank. During the trial the number of filters chemically cleaned reduced significantly, from approximately 30 to 8 per day as can be seen by comparing Figs. 5 and 6. No throughput benefits were realized during this part of the trial due to existing throughput limits on the diffusers.

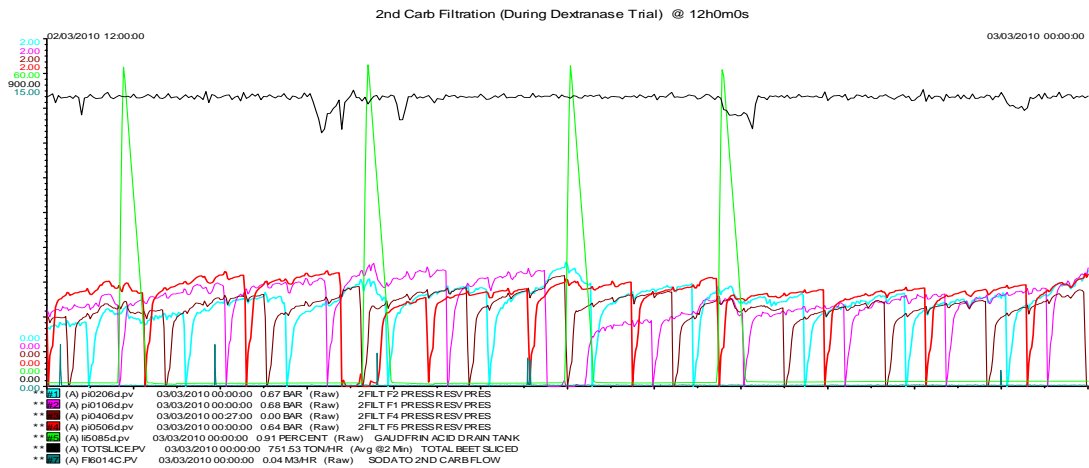


Fig. 6. Trend of second carbonation filtration and throughput conditions during the concentrated Dextranase A trial.

The initial trial with the concentrated dextranase A finished on 5 March 2010, after which the second carbonation filter pressures increased again, resulting in more acid washing being required. The trial started again with a commercial dextranase B supplied that was initially dosed at 1 ppm addition rate to evaluate whether the product could be used below the manufacturer's recommended addition rate of 2-3 ppm following the success seen with concentrated dextranase A. As dextranase B was also a concentrated dextranase it was similarly added as a working solution (1:4 in tap water). However, at 1 ppm the filtration conditions continued to be poor, with some significant periods of lower throughputs being experienced. Furthermore, during this initial period the factories precipitated calcium carbonate (PCC) reactor became blocked and was taken off line, therefore, PCC addition was stopped to the second carbonation vessel. As can be seen from Fig. 7, the interruption in PCC supply significantly impacted second carbonation filtration which resulted in a reduction in factory throughput. It can, therefore, be seen that dextranase addition alone did not overcome the second carbonation filtration issues and a combination of PCC and dextranase was required.

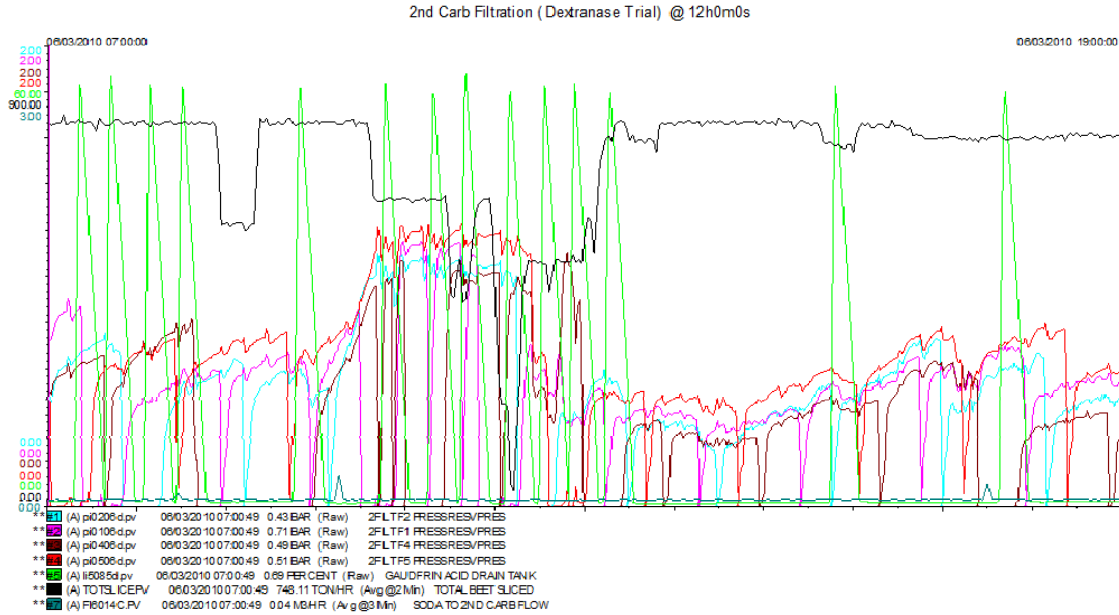


Fig. 7. Trend of second carbonation filtration and throughput conditions during the addition of dextranase B at 1 ppm addition rate showing effect of precipitated calcium carbonate (PCC) reactor blockage.

The addition rate of dextranase B was increased to 2.1 ppm (Fig. 8). Filtration conditions at this addition rate improved which allowed for more consistent factory throughputs. Given the improved filtration conditions it was possible to increase the alkali addition to the second carbonation vessel to aid the control of limesalts (Fig. 8). This had not been attempted during the first dextranase trial due to the limited availability of the concentrated dextranase A. Sodium carbonate addition was increased 4-fold without any detrimental impact on second carbonation filtration, which allowed a reduction in filtered second carbonation limesalts from ~0.13 to 0.086 gCaO/100Bx. At the conclusion of the second dextranase trial the soda ash addition was reduced markedly because it had a detrimental impact on second carbonation filtration. This was thought to be because of the rapid crystallization of very small calcium carbonate crystal due to the addition of sodium. The reduction in alkali addition resulted in the concentration of limesalts increasing to ~0.180 gCaO/100Bx. Fig. 8 shows the impact on second carbonation filtration and factory throughput at the end of the trial. There was a 4-fold increase in second carbonation filter chemical cleaning that led to a significant reduction in factory throughput (top black line on Fig. 8 trend).

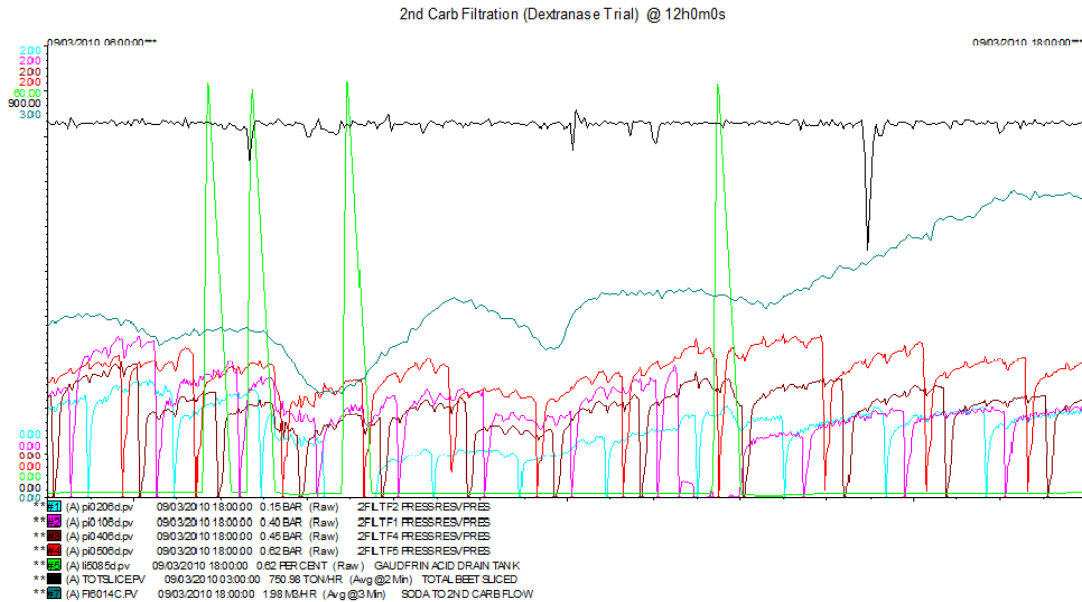


Fig. 8. Trend of second carbonation filtration and throughput conditions during the dextranase B trial at 2.1 ppm addition rate.

Effect of Dextranase on the Particle Size of Second Carbonation Filtration

During the 2009/10 campaign continuous precipitated calcium carbonate (PCC) reactors had been developed and used successfully at all British Sugar Factories to aid second carbonation filtration. PCC works by applying additional precipitated calcium carbonate to the second carbonation vessel to promote agglomeration of smaller calcium carbonate crystals formed within the gassing vessel. This results in a lowering of the amount of very small crystals in the second carbonation juice leading to an improved ‘filterability’ of the juice (Burroughs and Wones, 2003). When juice containing dextran is processed the agglomeration of calcium carbonate is negatively affected, which leads to a larger number of fines (the percentage of particles less than 3 μm) being present than normal (see Fig. 9). This phenomenon has been observed in BSUK during the 2009/10 campaign and elsewhere (De Bruijn, 2000, Nurmi, 2008, Struijs et al, 2009). Measurements during the dextranase trial showed that the addition of dextranase when PCC was present led to a *further reduction* in the concentration of fines in the second carbonation juice as illustrated by Fig. 9 and Table 2. The *average* modal particle size with dextranase addition increased by ~17% and the amount of fines was reduced 5.5-fold. The improved filterability observed during dextranase addition led to improved factory operations, allowing more stable beet-end flow throughputs, smoothing of vapor demands, and a reduction in the amount of recycle to raw juice from filter cleaning.

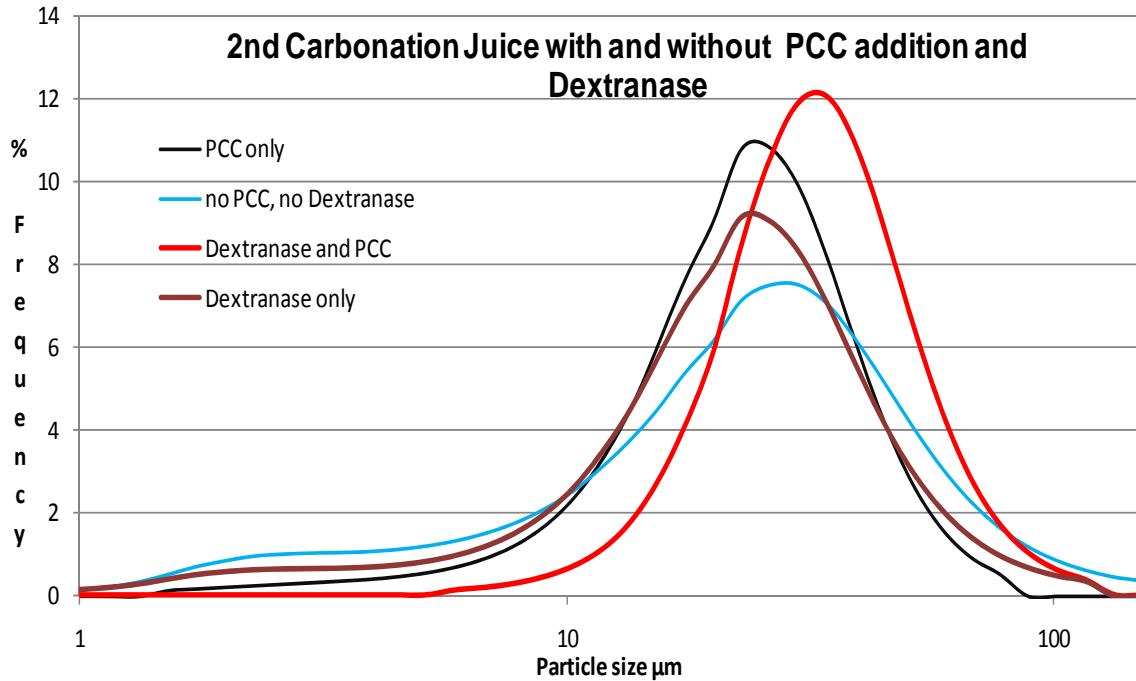


Fig. 9. Typical second carbonation particle size distribution, with no PCC and no dextranase, with PCC only, with dextranase only and with PCC and dextranase.

Table 2. Average modal particle size distribution and amount of fines (<3 μm) in second carbonation particle size distribution, with no PCC and no Dextranase, with PCC only, with Dextranase only and with PCC and Dextranase.

	Av. Modal Particle size/ μm	% < 3 μm
<i>No PCC and no dextranase</i>	27.8	7.3
<i>With PCC addition only</i>	24.3	1.9
<i>With Dextranase only</i>	21.4	5.4
<i>With PCC and Dextranase</i>	31.8	0.0

Dextran Levels in Factory Juice Streams

Haze dextran levels in second carbonation juice of ~60 ppm or above lead to second carbonation filtration issues (information from Nordic Sugar). As seen in Fig. 10 the haze dextran levels prior to the dextranase trial when filtration problems were occurring were above 70 ppm. On addition of the concentrated Dextranase A, dextran levels were reduced to consistently below 45 ppm; this level of dextran was achievable with a 2 ppm addition rate. In comparison, for Dextranase B an addition level of above 2 ppm was required to keep dextran levels below 45 ppm.

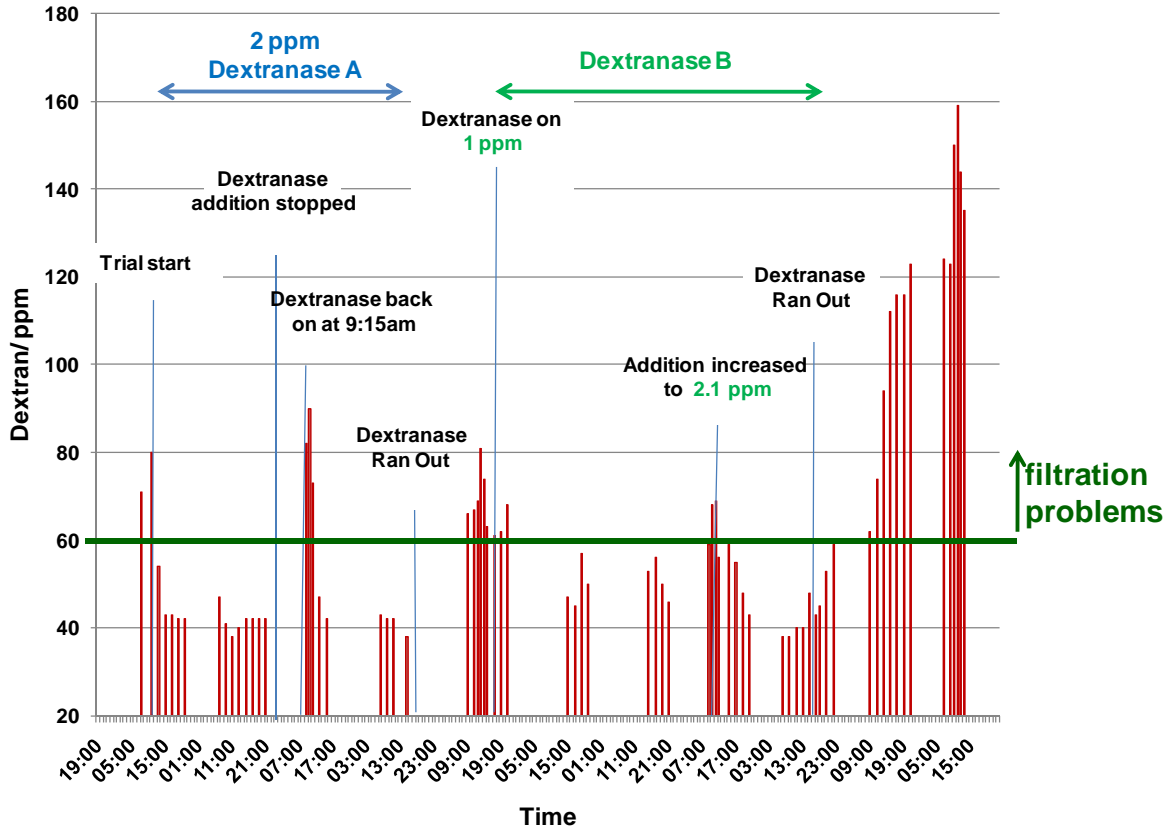


Fig. 9. Dextran concentrations in filtered second carbonation juice at Wissington factory before, during, and after dextranase trials.

Impact of the Application of Dextranase on the Level of Lime Salts

When processing deteriorated beet the concentration of limesalts typically increase in marked amounts. At Wissington factory lime salts increased to over 3-fold normal second carbonation levels which had a significant impact on the de-calcification plant because it was unable to soften the juice sufficiently to produce enough soft feed material for the resin separation plant. The addition of dextranase to the juice allowed the amount of sodium carbonate to be increased to aid the reduction in limesalts, whilst the measured limesalts were reduced by 34%, the levels of limesalts in the juice were still higher than normally observed. As filtration was not limiting during the addition of high levels of alkali it would be envisaged that the amount of alkali could be increased further allowing for a further reduction in the limesalts to the de-calcification plant which would assist in the production of soft juice. Due to the length of the trial it was not possible to increase sodium carbonate addition far enough to find the maximum addition rate.

The impact of high limesalts in the feed to the resin separation plant will lead to a reduction in plant throughput due to the negative impact on filtration. In addition, high limesalts will lead to a reduction in the efficiency of the sucrose separation which will lead to an increase in the sucrose concentration in the raffinate (molasses) stream and, consequently, to the overall plant extraction. Whilst the dextranase trial was too short to realize any benefits for the resin separation plant, it may be envisaged that using dextranase would assist in controlling the

limesalts and minimizing the downstream impacts. It is likely that dextranase would need to be one of a number of operational strategies employed to reduce limesalts sufficiently

Cost Analysis

Evaluation of the cost in use of the dextranases used in the trial were undertaken to ascertain the true cost of using dextranase and to evaluate any financial savings and any other processing benefits observed. The concentrated dextranase B was purchased at a higher dextranase activity per unit dollar cost than the dextranase A. Based on the trial results discussed above it can be clearly seen that the concentrated dextranase A gave a significantly better cost benefit. Whether the lime reduction would be realized in practice would require a significantly longer trial. Factory throughput analysis indicated that the use of dextranase allowed the full beet slicing capacity to be realized, which was not the case after the trial had finished. Table 3 lists the breakdown of the cost evaluation produced from the trial data, the costs analysis is based on using the dextranase A product at an addition rate of 3 ppm.

Table 3. Breakdown of cost evaluation of the dextranase trial, based on 3 ppm of concentrated dextranase A (52000 DU/ml). Note: based on the US\$ currency exchange rate of 1US\$=0.63 pounds sterling on 28 Jan 2011.

	% Reduction	Trial Saving \$/day
Cost of conc dextranase A	--	-2,741
Cost of acid washing Filters	73	
CaO to process*	11	
Anthracite	9	
Throughput costs (LOP)	84	
Total Savings		3,180

*Lime required to aid filtration

In addition to the financial savings listed in Table 3 other benefits occurred during the trial including: a reduction in the amount of water discharged to the effluent treatment plant with the spent acid at 418 m³/day. The amount of acid washing in campaign has a significant impact on both the volume of water that needs to be treated and discharged and on the cost of alkali addition to the effluent circuits to maintain a neutral pH. The financial benefit of a reduction in water to ponds was measured at US\$398/day.

MAJOR CONCLUSIONS

- The activities of commercial dextranases and their activity per unit \$ vary widely. Commercial dextranases occur in “non-concentrated” and “concentrated” forms.
- 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 dextranase on storage, and (3) measure the activity of delivered batches

- Second carbonation filtration significantly improved by adding dextranase in a number of ways:
 - Frequency of second carbonation filter chemical cleaning was reduced by 73%
 - Reduced chemical useage
 - Reduction of the volume of water discharged to the effluent treatment plant with the used acid by 418 m³/day
- Adding dextranase markedly improved beet throughput
- Dextranase A (52,000 DU/ml) could be added at a dosage rate below that recommended by the suppliers which made the product significantly less expensive to use
- \$3,180 per day saved by adding concentrated dextranase A (52,000 DU/ml) at 3 ppm on weight of juice as a working solution

ACKNOWLEDGEMENTS

The authors thank Eldwin St. Cyr of USDA-ARS-SRRC for excellent technical assistance, British Sugar Operations Services Science Department, Wissington Factory Laboratory Team, Geoff Parkin, Garry Bowler, Bob Howe, Barbara Muir, and Jan-Maarten de Bruijn. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

In particular, and rather unusually, the first three authors wish to acknowledge the tremendous dedication and commitment to this project given by the fourth author, Kevin Winters. Kevin joined British Sugar's Wissington Sugar Factory in 1977 where he remains in post today. Over a number of years he has spent time teaching the beet sugar process to new process technicians, encouraging, and mentoring them with a passion which others aspire to.

LITERATURE CITED

- Burroughs, P and Wones, S. 2003. The effect of frost damaged beet and other factors on Dorr 2nd carbonation juice particle size distribution. Proc. CITS, 237-246.
- Davidson, P. S. 2001. Effect of sucrose/raffinose mass ratios on the stability of co-lyophilized protein during storage above the T_g. Pharm. Res. 18(4):474-479.
- De Bruijn, J.M. 2000. Processing of frost damaged beets at CSM and the use of dextranase. Zuckerindustrie, 125(11):892-902.
- Eggleston, G. 2004. Easy and uniform measurement of the activity of dextranase at the sugarcane factory or refinery. Sugar J. 67:32-33.
- Eggleston, G.; Monge, A. 2004. Optimization of factory applications of dextranases in the U.S. Proc. Sugar Proc. Res. Conf. p. 371-394.
- Eggleston, G., and A. Monge. 2005. Optimization of sugarcane factory application of commercial dextranases. Process Biochem., 40:1881-1894.

- Eggleston, G., Monge, A., Montes, B., Stewart, D. 2006. Factory trials to optimize the industrial application of dextranase in raw sugar manufacture: Part I. *Intern. Sugar J.* 108(1293): 528-537.
- Eggleston, G., Monge, A., Montes, B., Stewart, D. 2007a. Factory trials to optimize the industrial application of dextranase in raw sugar manufacture: Part II. *Intern. Sugar J.*, 109(1308): 757-764.
- Eggleston, G., Monge, A., Montes, B., Stewart, D. 2007b. Overcoming practical problems on enzyme applications in industrial processes. Dextranases in the sugar industry. In *Industrial Application of Enzymes on Carbohydrate Based Materials*. Eds.: Eggleston G and Vercellotti J R., *ACS Symposium Series 972*, Oxford Univ. Press, Chapter 6, 73-87.
- Huet, JM. 2011. General Subject 8. Beet Sugar Processing. Proc. 2010 Session of ICUMSA, USA, Bartens, Germany, 77-102.
- Khalikova, E., Susi, P., Korpela, T. 2005. Microbial dextran hydrolyzing enzymes: Fundamentals and applications. *Microbiol. Mol. Biol. Rev.* 69(2): 306-324.
- Nurmi, H. 2008. Experiences in using precipitated calcium carbonate at Danisco sugar. *Sugar Industry*, 133(8):508-511.
- Struijs, J., Jaspers, M., van Dijk, M. 2009. Methods used in The Netherlands to limit frost damage and to process frost deteriorated beets. *Proc. ESST*, 33-38.