Successful Application of Dextranase in Sugar Beet Factories

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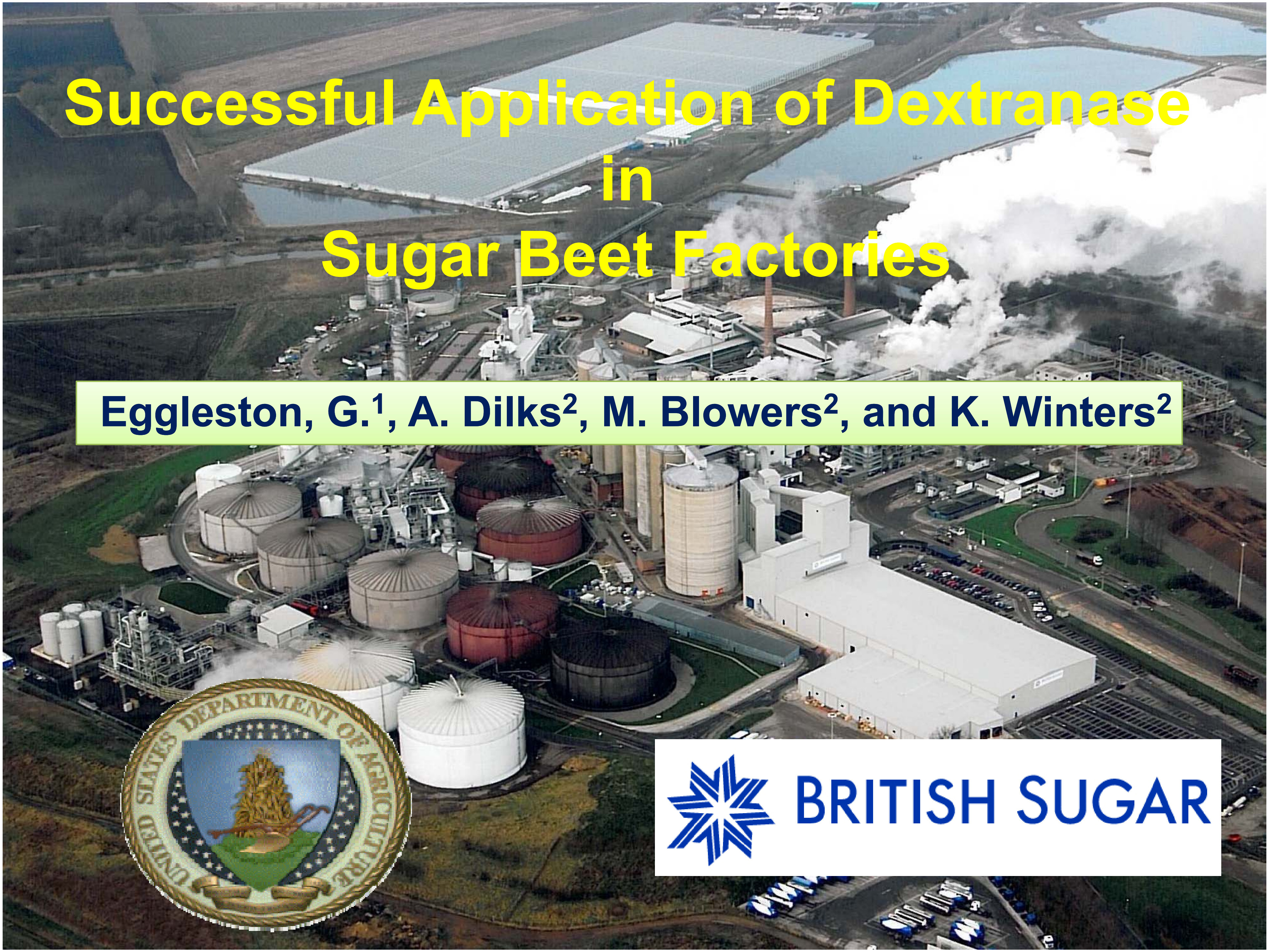
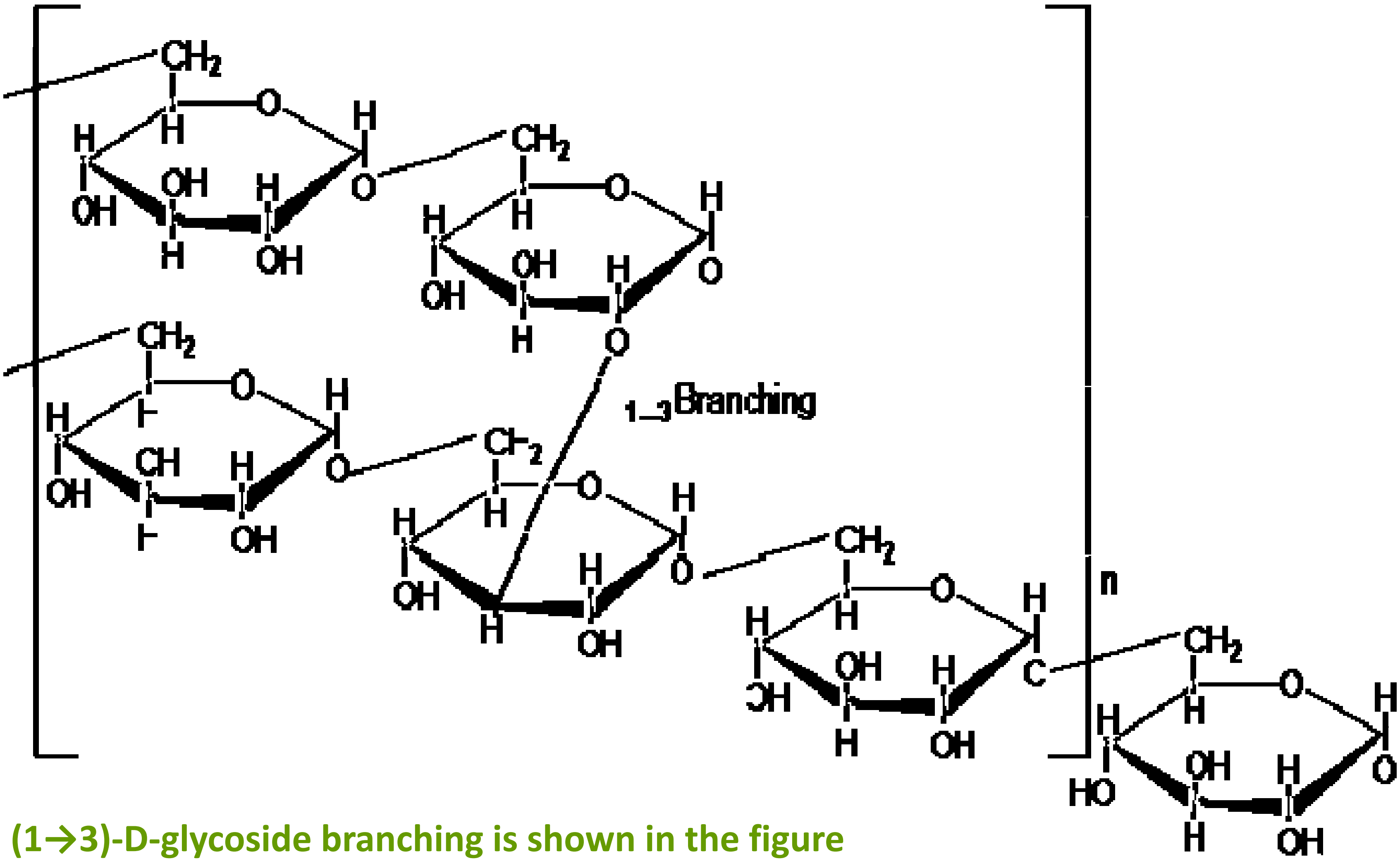


Presentation Outline

- **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

Basic chemical structure of dextran (α-(1→6)-α-D-glucan)

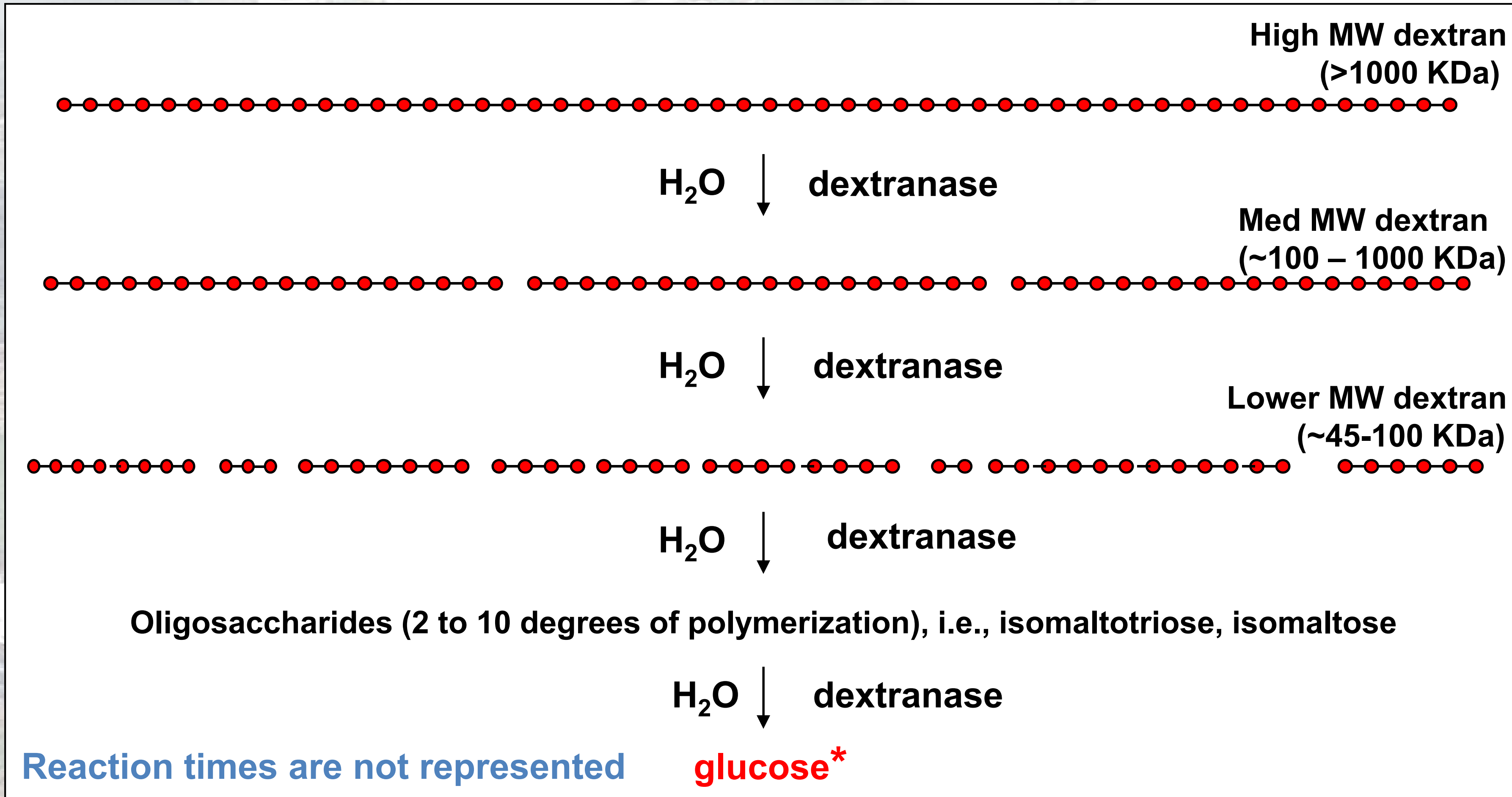


(1→3)-D-glycoside branching is shown in the figure

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.

From Eggleston et al (2005). Process. Biochem.

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

Current Different Units of Strength or Activity

u/g
Du/g
U/mL
Du/mL
kDu-A/g

- 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

Commercial Dextranase	Dextranase Activity DU/mL				Classification
	2003	2004	2008	2009	
A	52000	51920	52000	52000	“Concentrated”
B	5499	6500	2500	nd	“Non-Concentrated”
C	4786	2750			“Non-Concentrated”
D 5X				8000	“Non-Concentrated”
D				3000	“Non-Concentrated”

Eggleston Classification:

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

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

Eggleston et al (2007) ACS Book Chapter.

Activity per Unit Dollar Varies Enormously Among Commercial Dextranases

Commercial Dextranase	Dextranase Activity DU/mL/$				Classification
	2003	2004	2008	2009	
A	2832.2	2827.9	2813.9	2813.9	"Concentrated"
B	916.5	583.3	416.6		"Non-Concentrated"
D 5X				490.5	"Non-Concentrated"
D				735.3	"Non-Concentrated"

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

Eggleston and Monge (2004). SPRI Proc.

Principle

	Reaction End Color
(a) $K_3Fe(CN)_6$ + Reducing Sugar → Na_2CO_3 → $K_4Fe(CN)_6$	yellow
(b) $2K_3Fe(CN)_6$ + 2KI → Acetic acid → $2K_4Fe(CN)_6$ + I_2	orange
(c) $2K_4Fe(CN)_6$ + $3ZnSO_4$ → $K_2Zn[Fe(CN)_6]_2$ + $3K_2SO_4$	orange
(d) I_2 + starch indicator → starch-I_2 complex	dark blue
(e) Starch-I_2 complex + $2NaS_2O_3$ → $Na_2S_4O_6$ + 2NaI	white

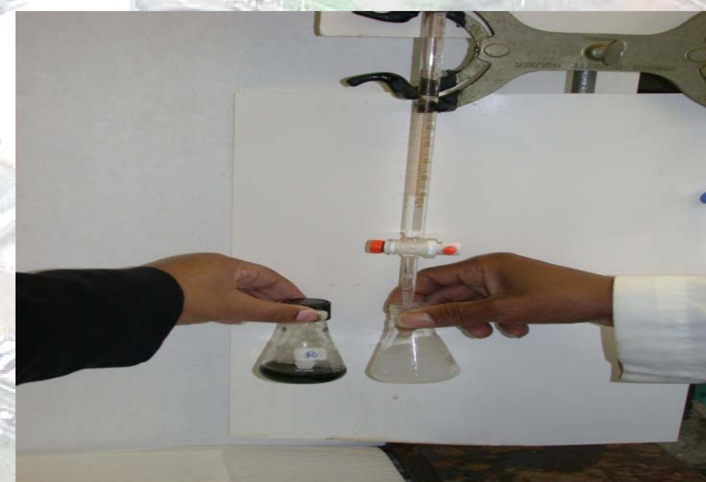
Measuring Dextranase Activity Using Simple Equipment at the Factory

Incubation at 37°C

Add reagents

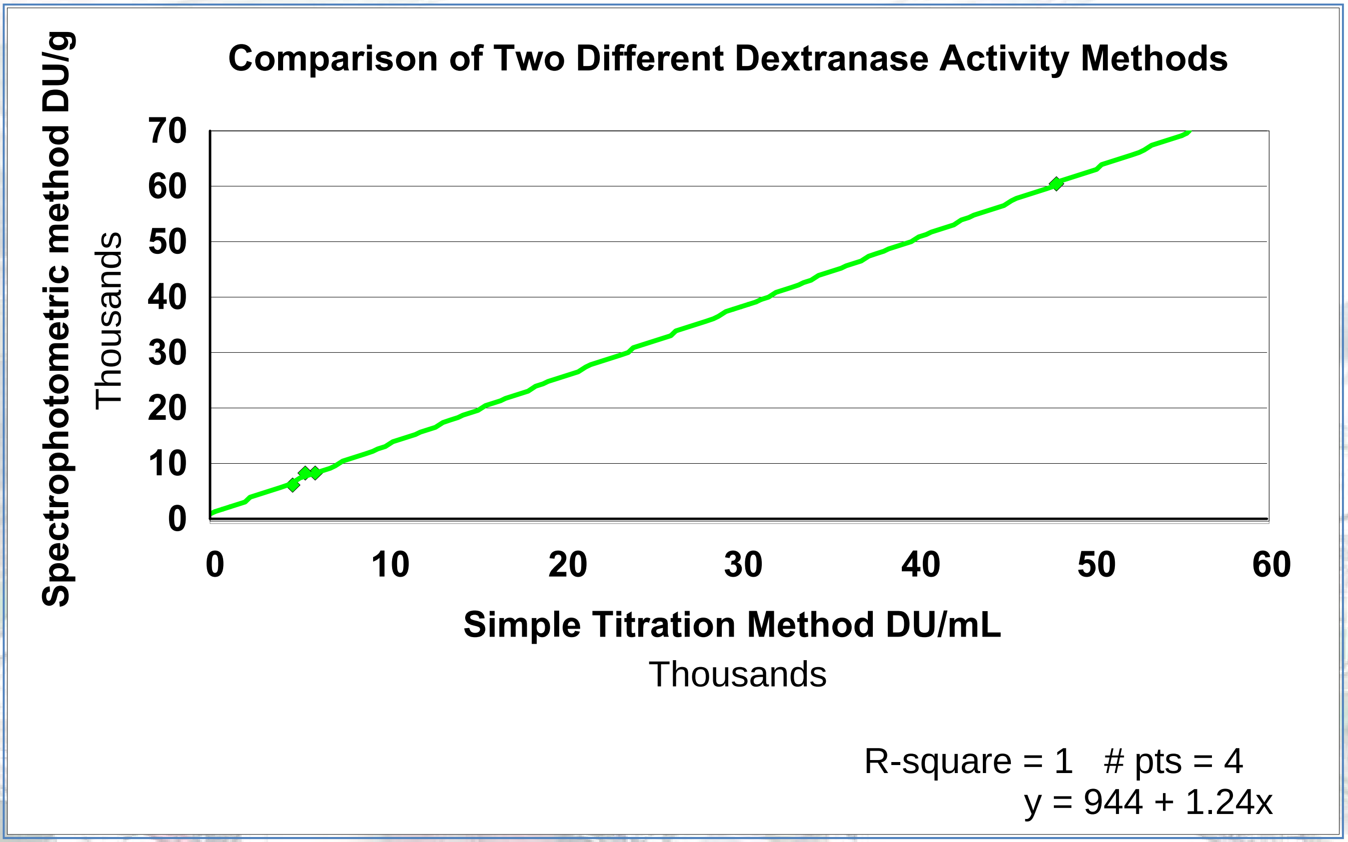
Boiling

Adding reagents



Simple Titration

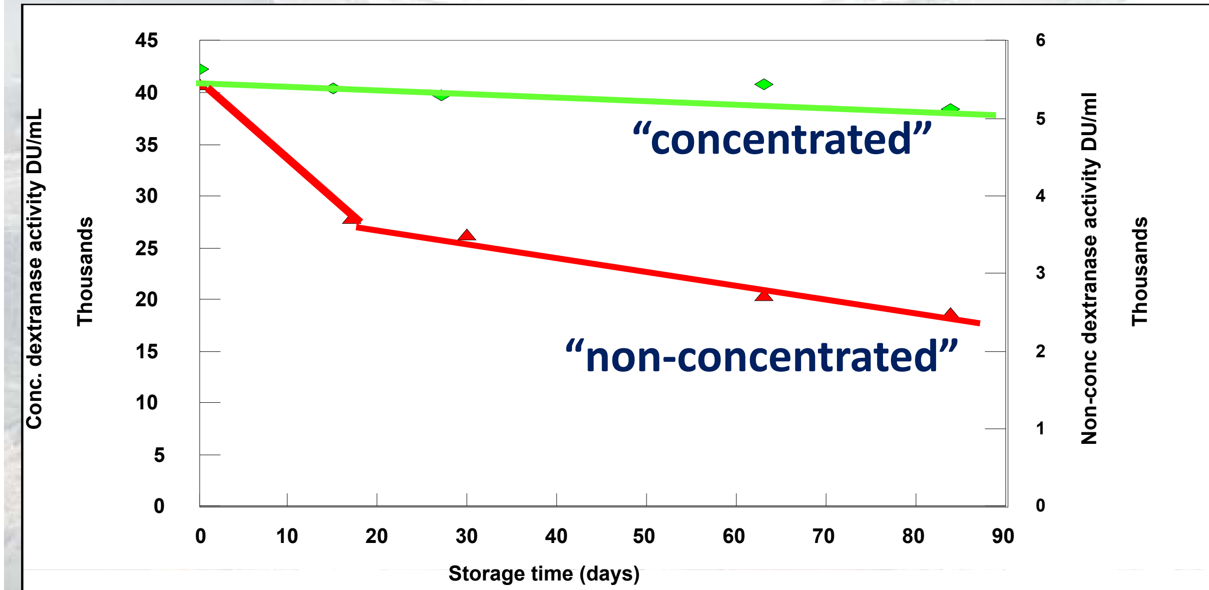
Accuracy



- **The excellent correlation between the two methods, confirms the accuracy of the simple titration method.**

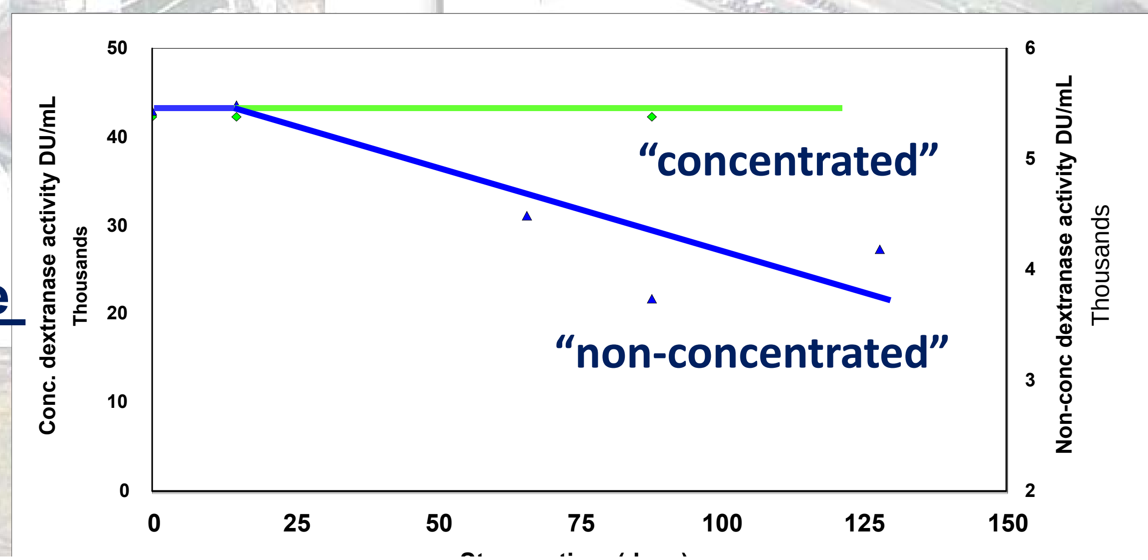
- **ICUMSA Method GS7-8 (2010): "Standard Measurement of the Activity of Dextranases at Sugarcane or Sugar Beet Factories using a Simple Titration Method" – Tentative Status**

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



Simulated Factory Storage

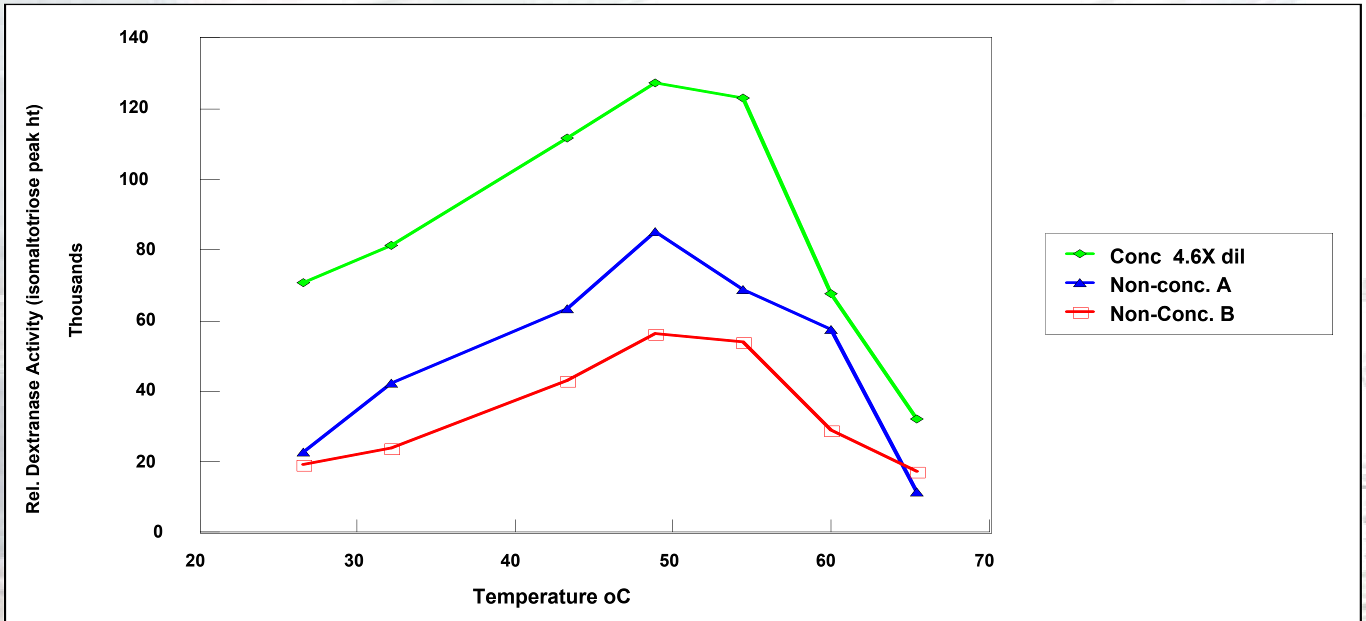
Stored at room temperature in shaded place (~25 °C) over 90 day processing season



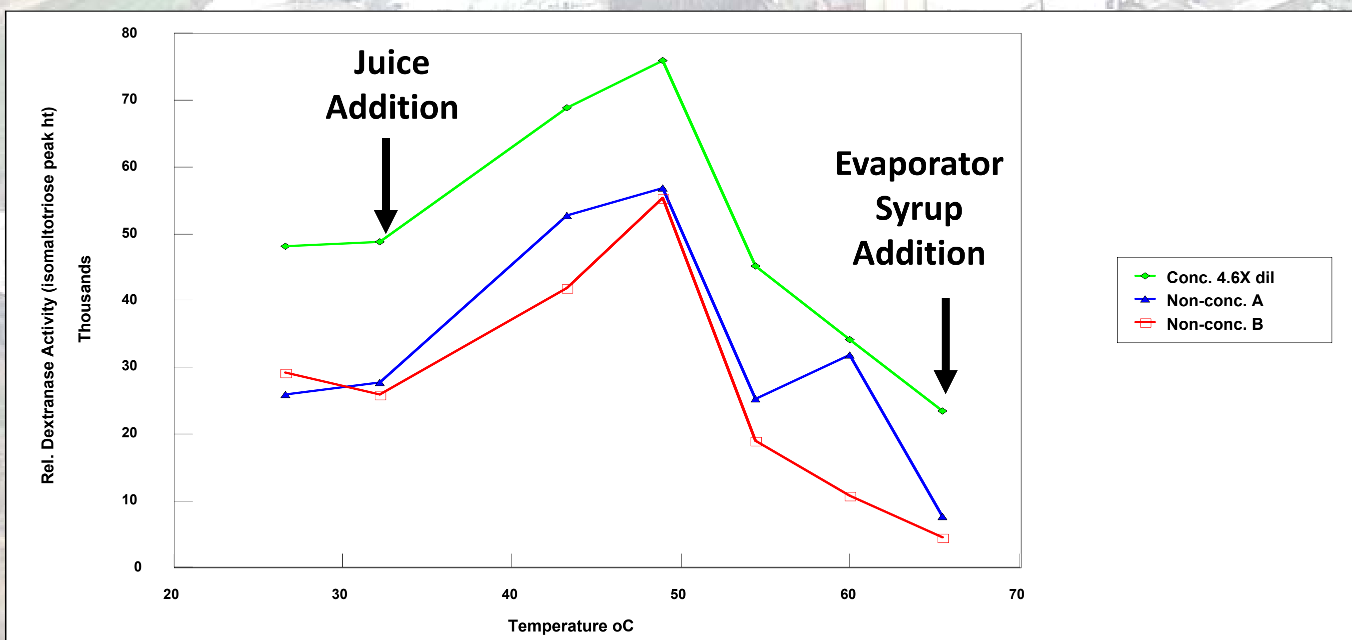
Refrigerated Temperature (4 °C)

Industrial Conditions That Affect The Efficiency of Dextranases

Effect of Temperature

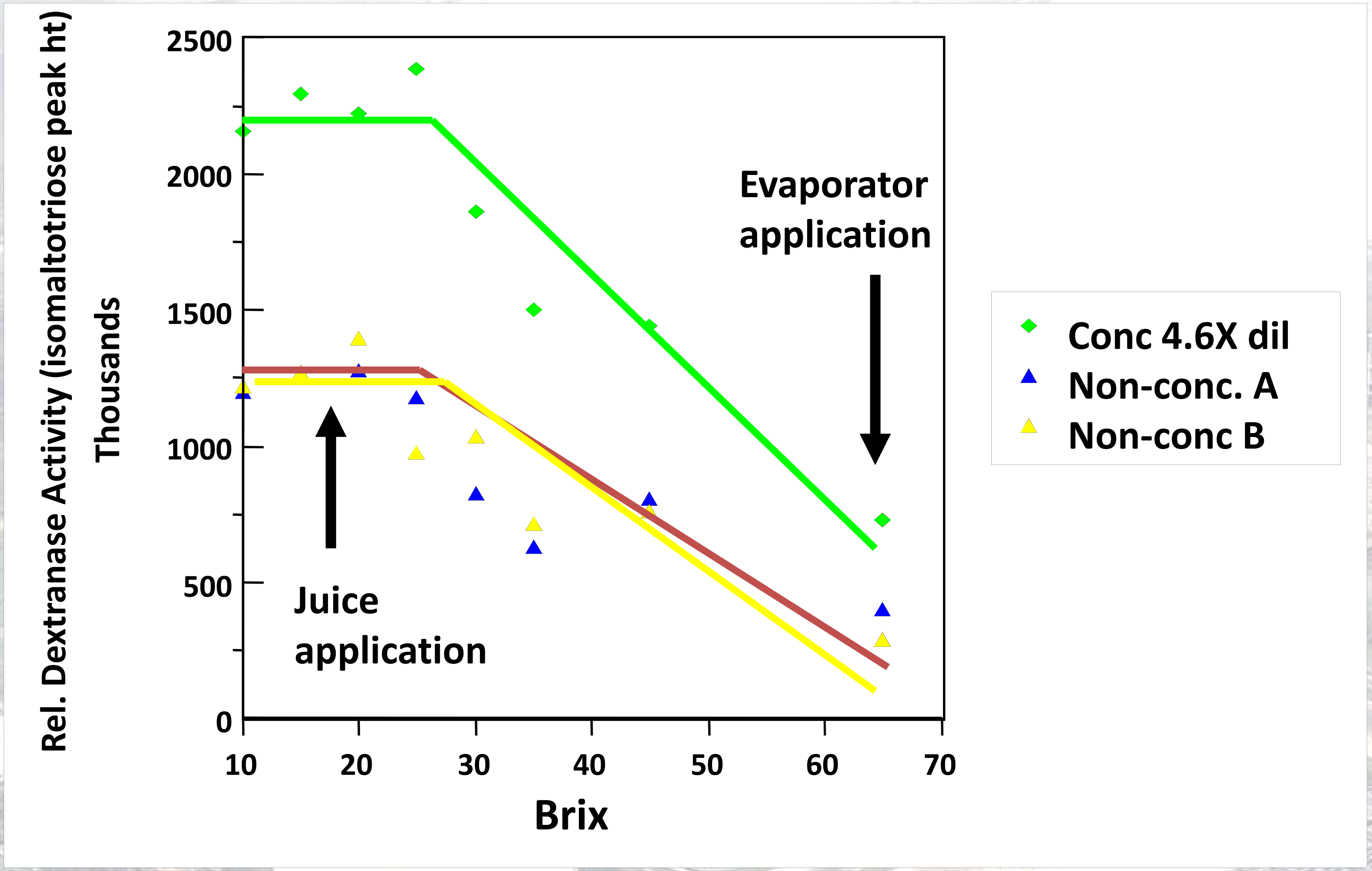


Pure Dextran T2000 - 2000ppm
50ppm dextranase
pH 5.4
25 min



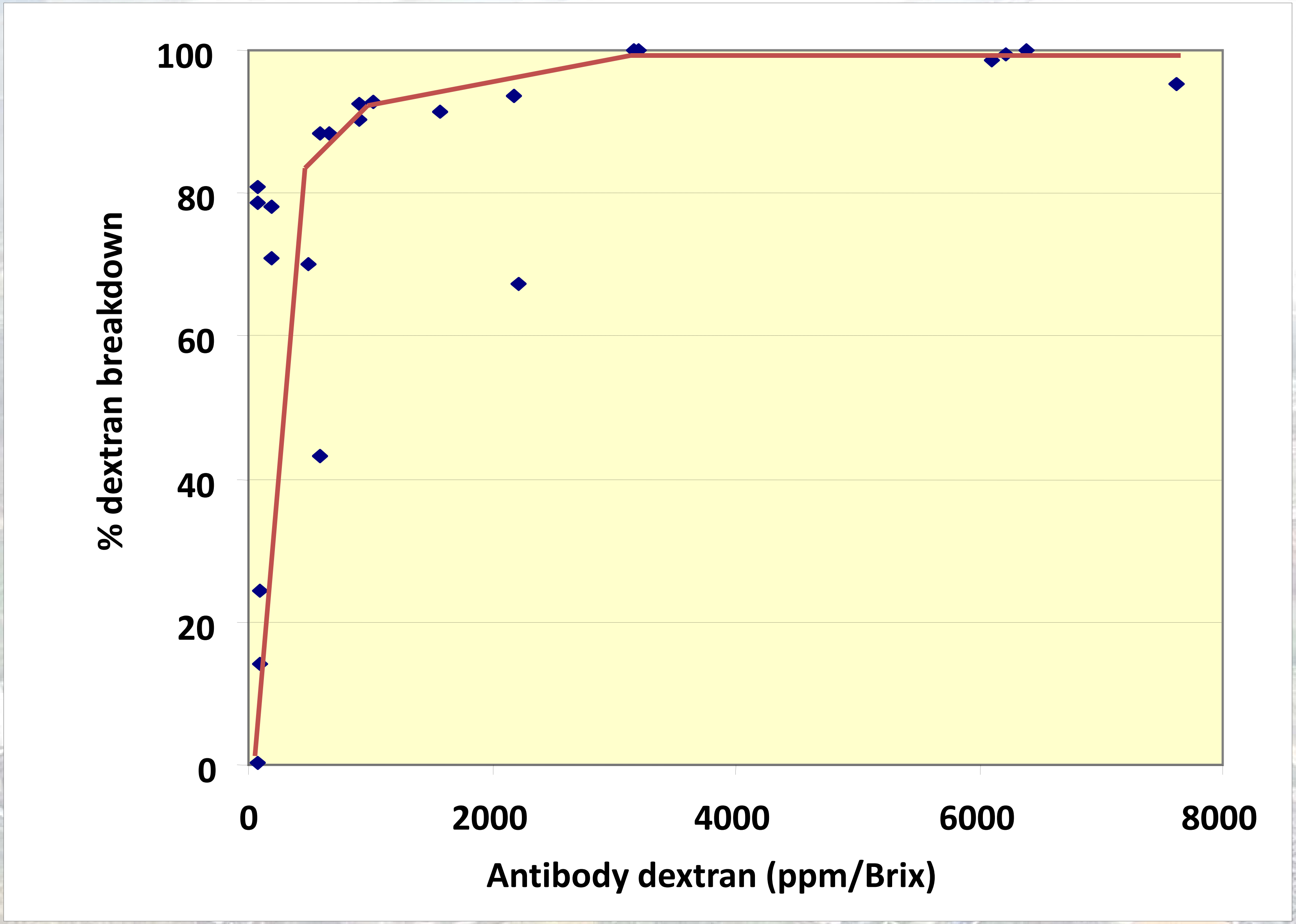
Dextran in Juice
3177ppm/Brix
100ppm dextranase
pH 5.4
25 min

Effect of Brix (% Dissolved Solids)



- pH optimum is between 5 and 6

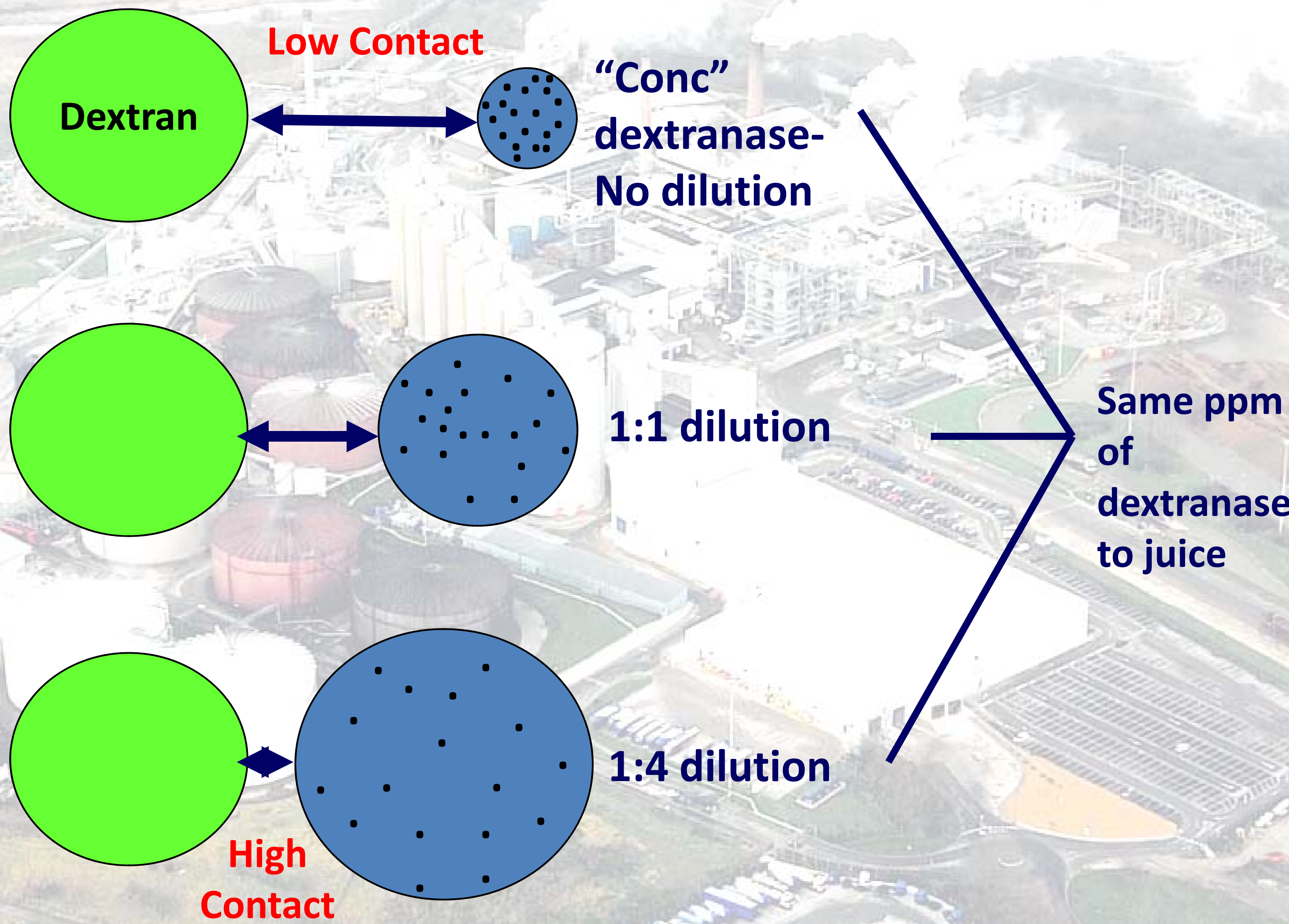
Effect of Dextran Concentrations on Dextranase Action



- 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



Studies into the Use of Dextranase at Wissington Factory During the 2009/10 Campaign

BRITISH SUGAR

Problem

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

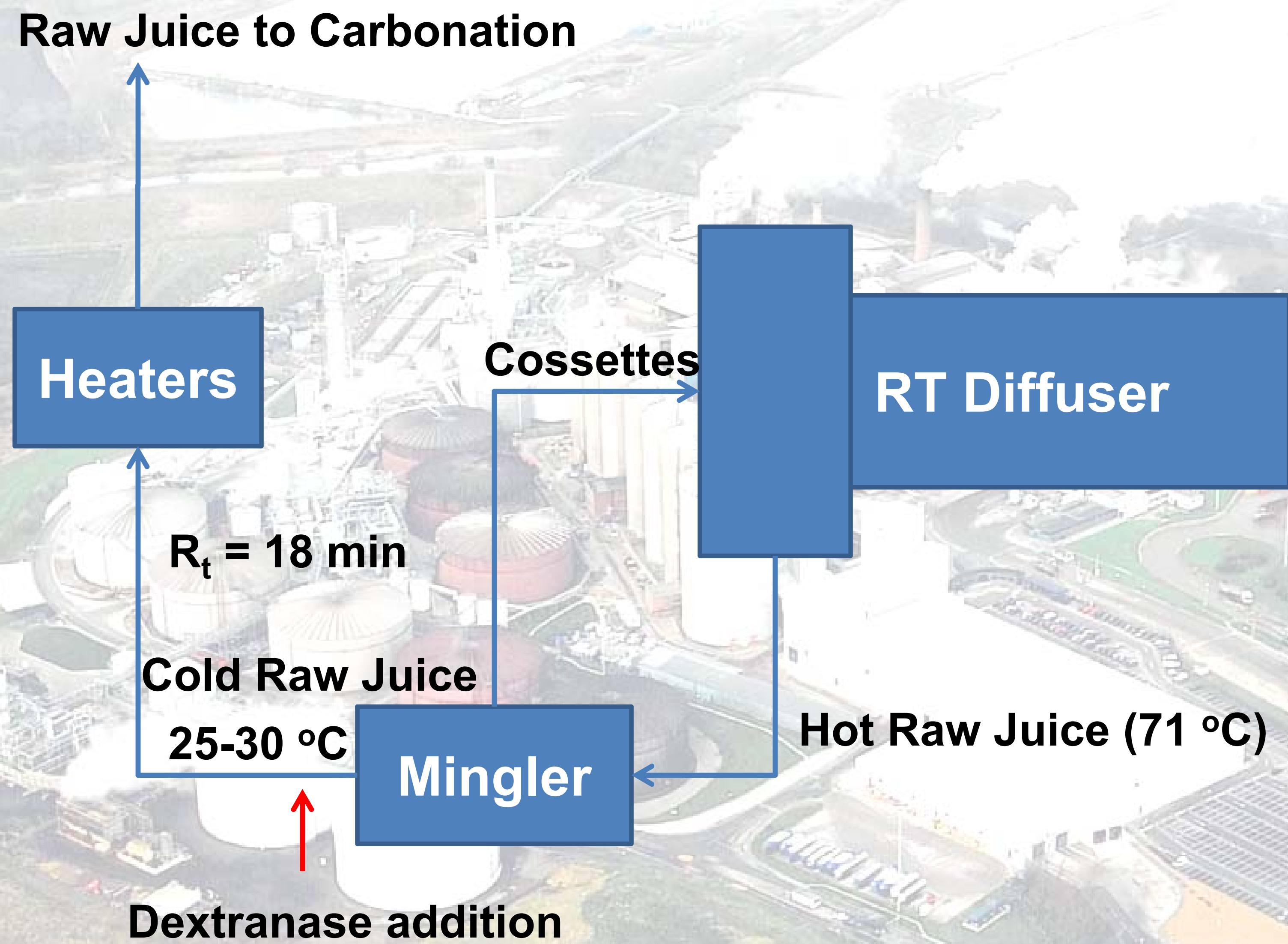
- The large crop and long campaign lengths (up to 180 days) also led to difficult beet processing conditions later in the campaign

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

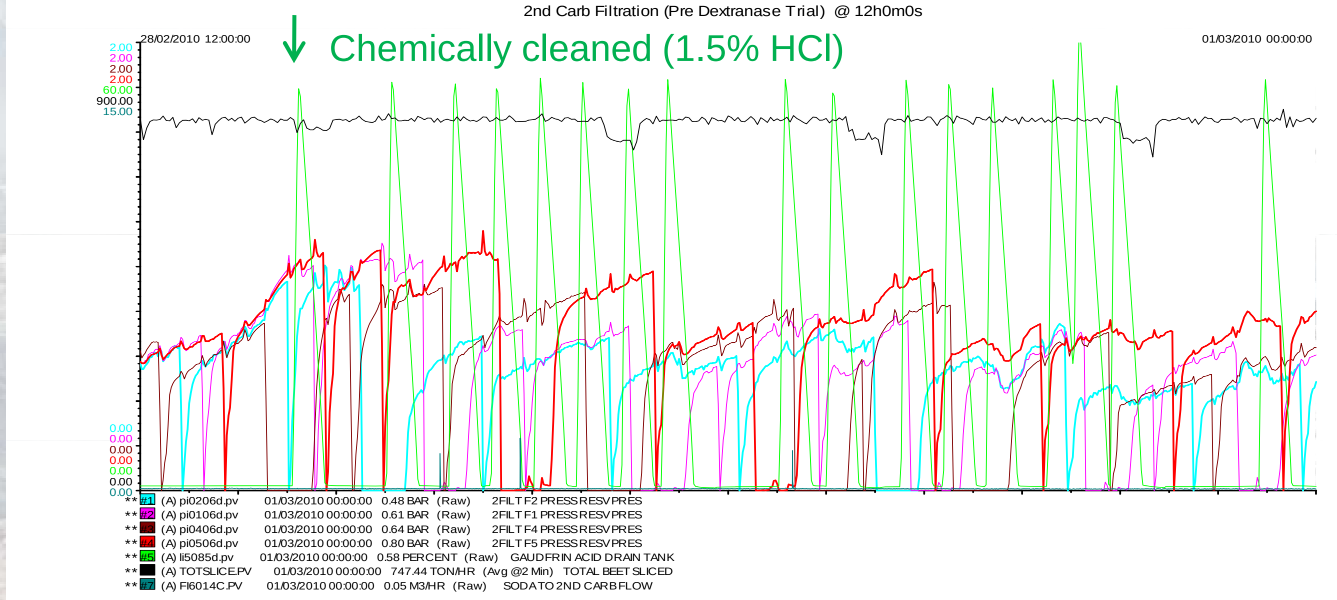
Simple Scheme Showing Dextranase Addition Point



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

First Trial

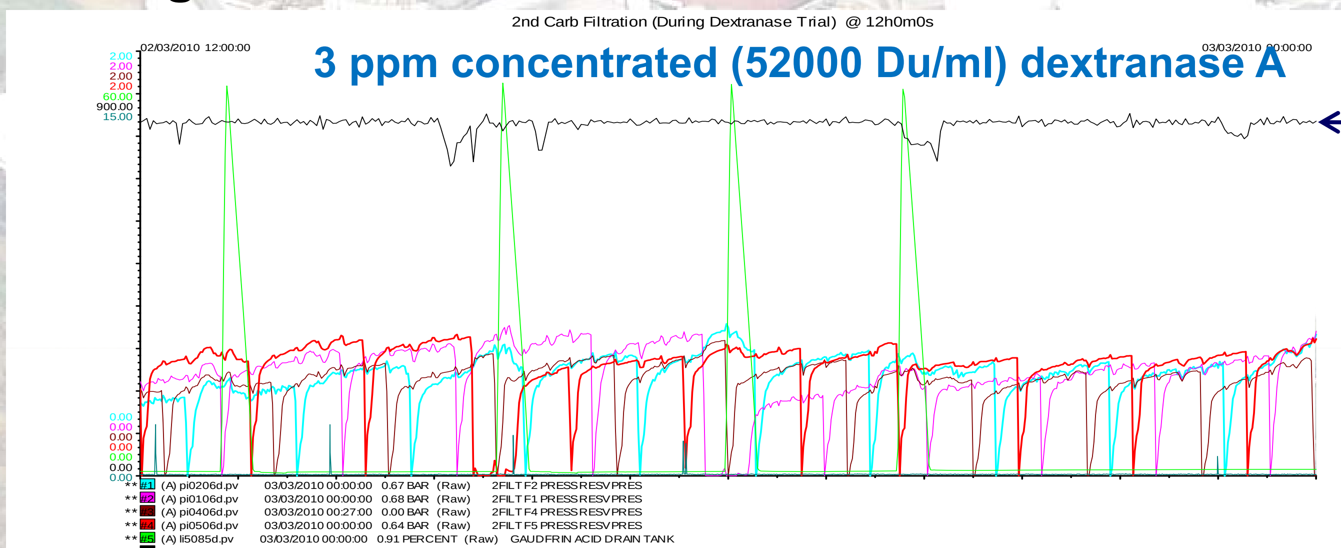
Prior to First Dextranase Trial



← factory throughput

~30 cleanings per day of carbonation filters

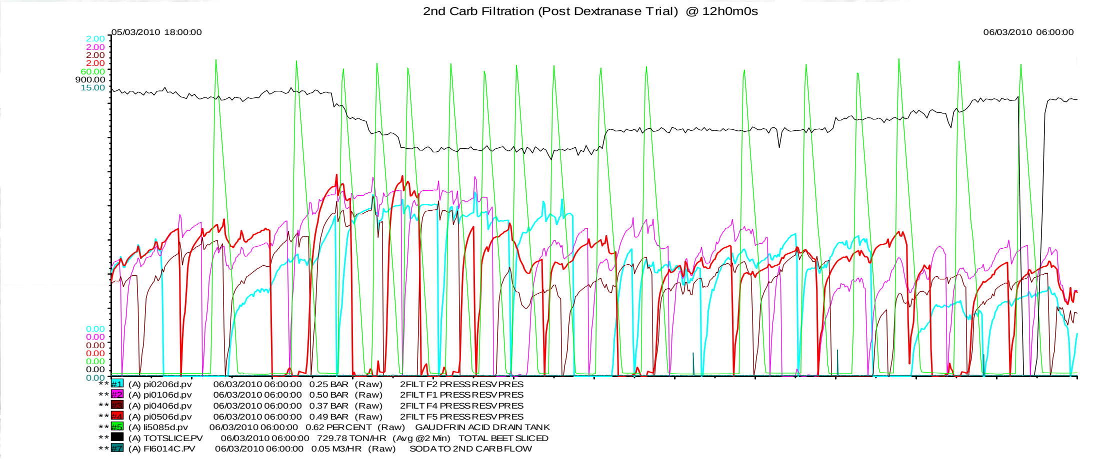
During the First Dextranase Trial



No change in factory throughput because of existing limits on the diffuser

~ 8 cleanings per day

- 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

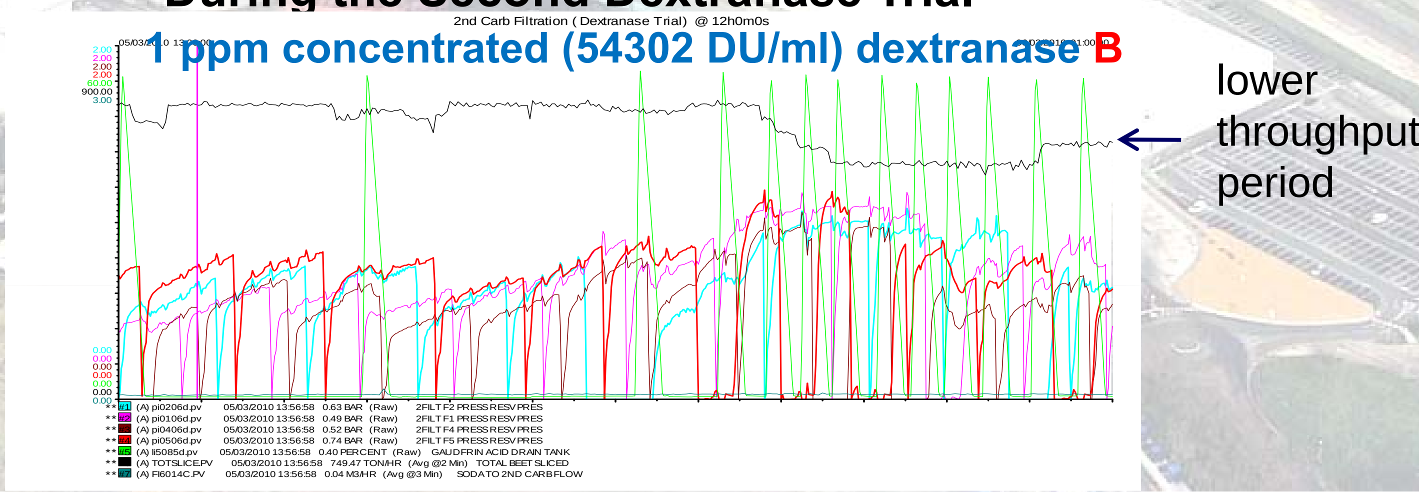


Second Trial

- 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:

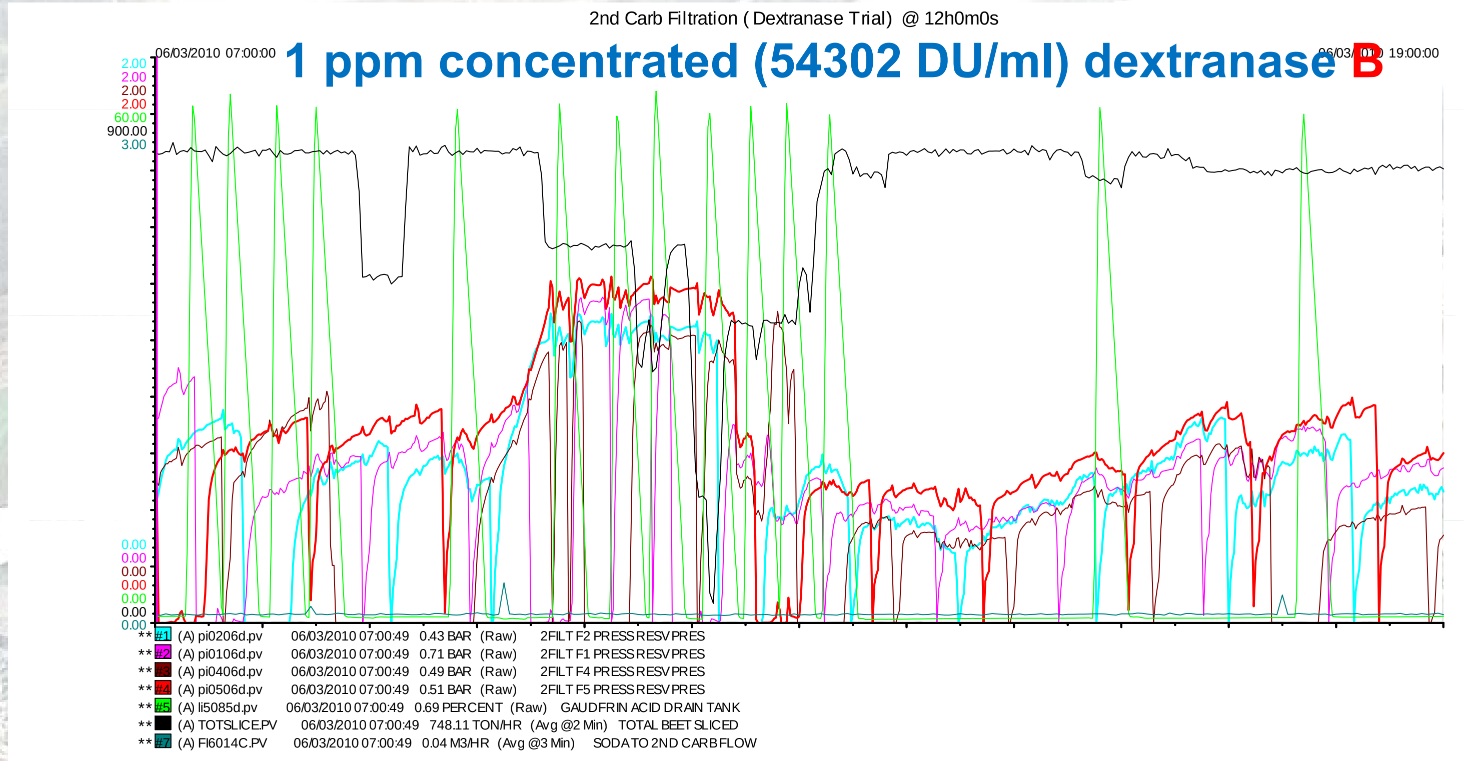
During the Second Dextranase Trial

1 ppm concentrated (54302 DU/ml) dextranase B



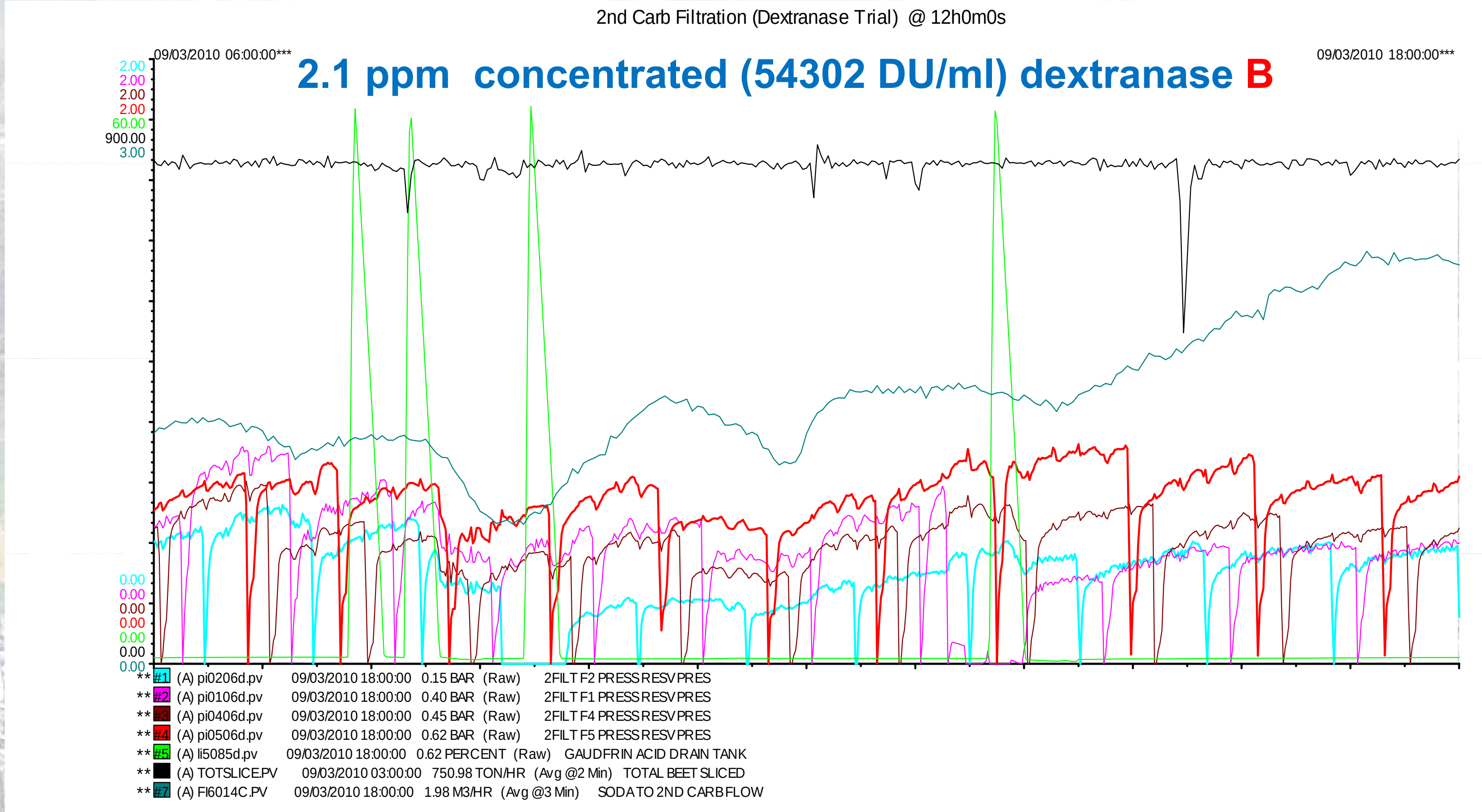
Second Trial

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



Second Trial

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

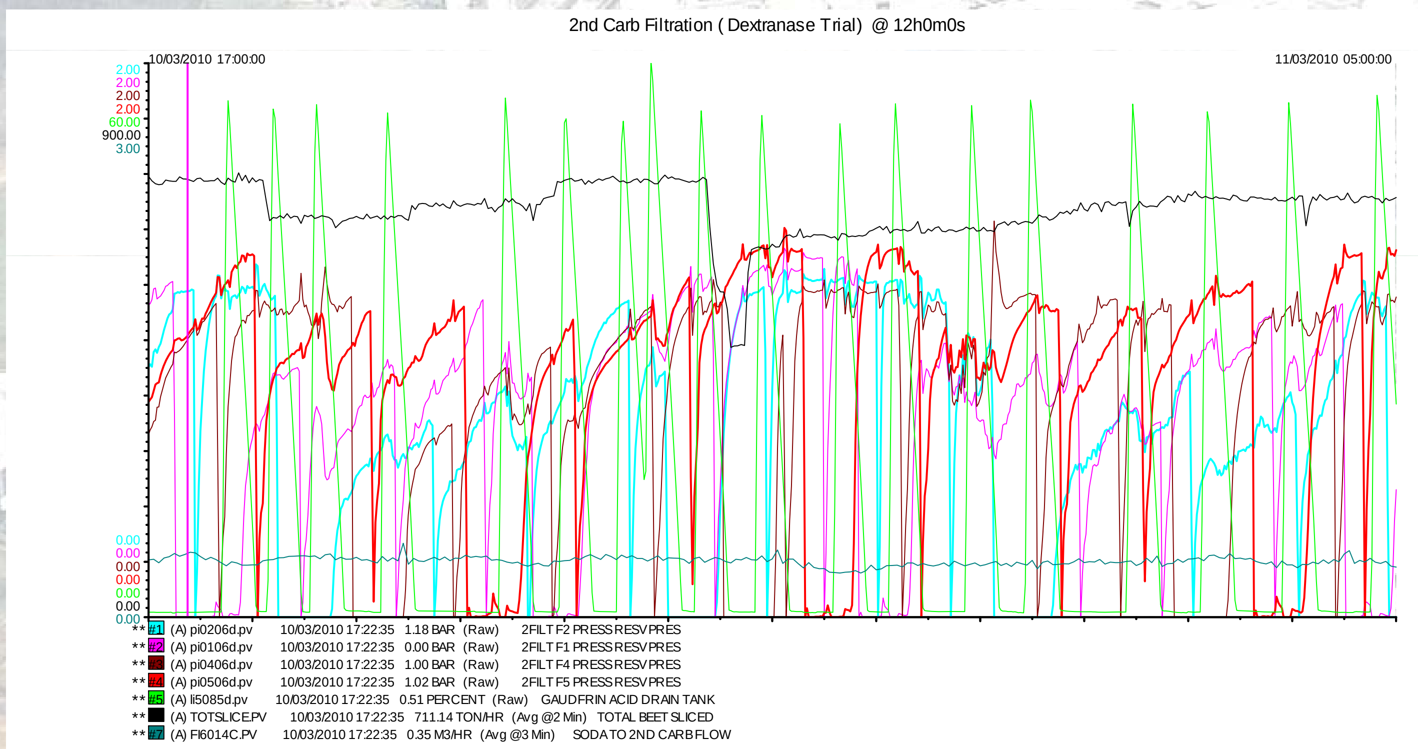


- Improved conditions allowed the increase in alkali addition to the 2nd carbonation vessel to aid limesalts control
- Sodium carbonate addition increased 4-fold without any detrimental impact on 2nd carbonation filtration and allowed a reduction in filtered 2nd carbonation limesalts from ~0.01g to 0.086gCaO/100Brix

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.

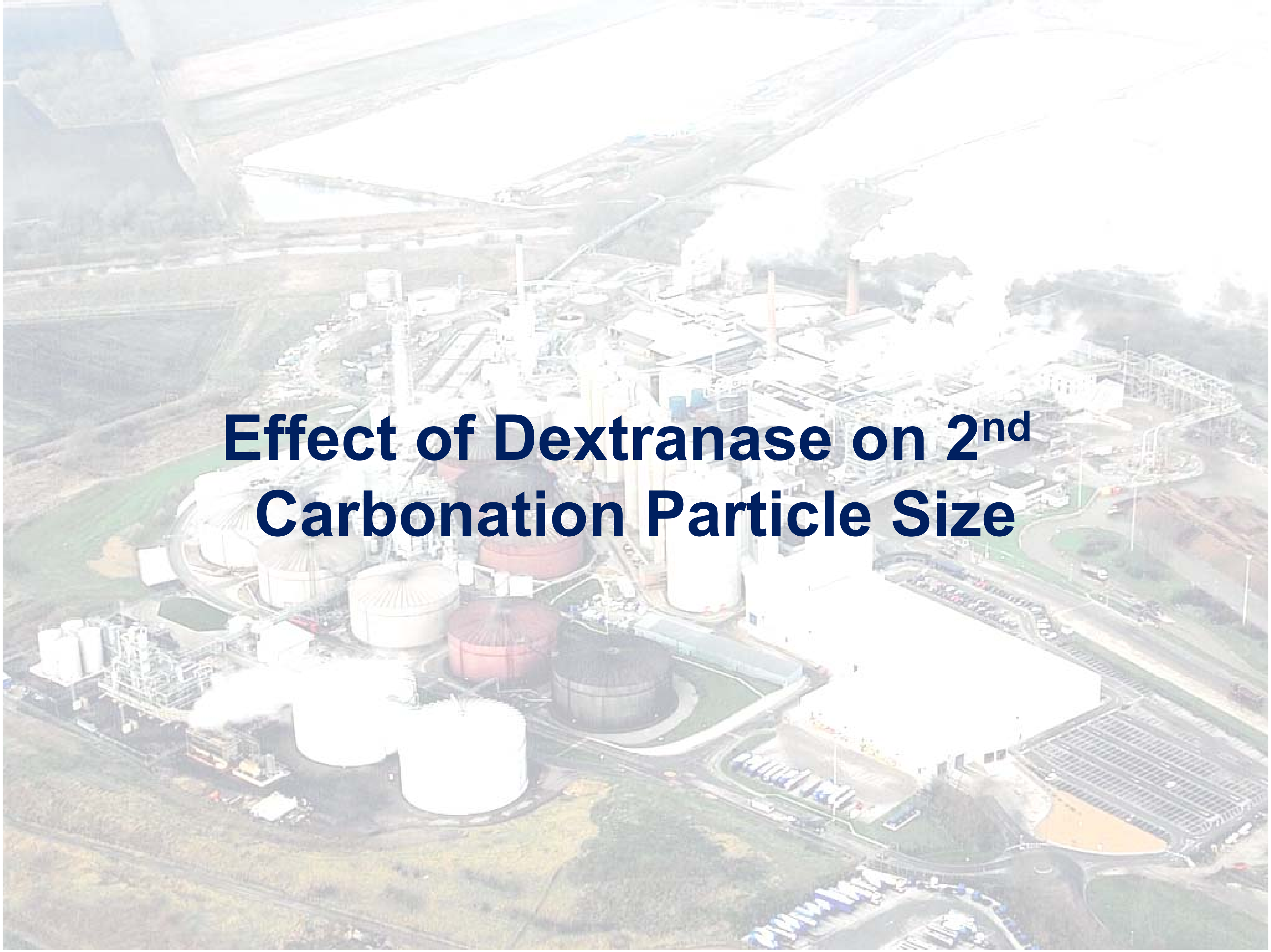
Trend of second carbonation filtration and throughput conditions after the end of the second dextranase trial



High rate of cleaning

Higher limesalts

Effect of Dextranase on 2nd Carbonation Particle Size



Continuous Precipitated Calcium Carbonate (PCC) Reactors

- Used during the 2009/10 campaign to aid 2nd carbonation filtration

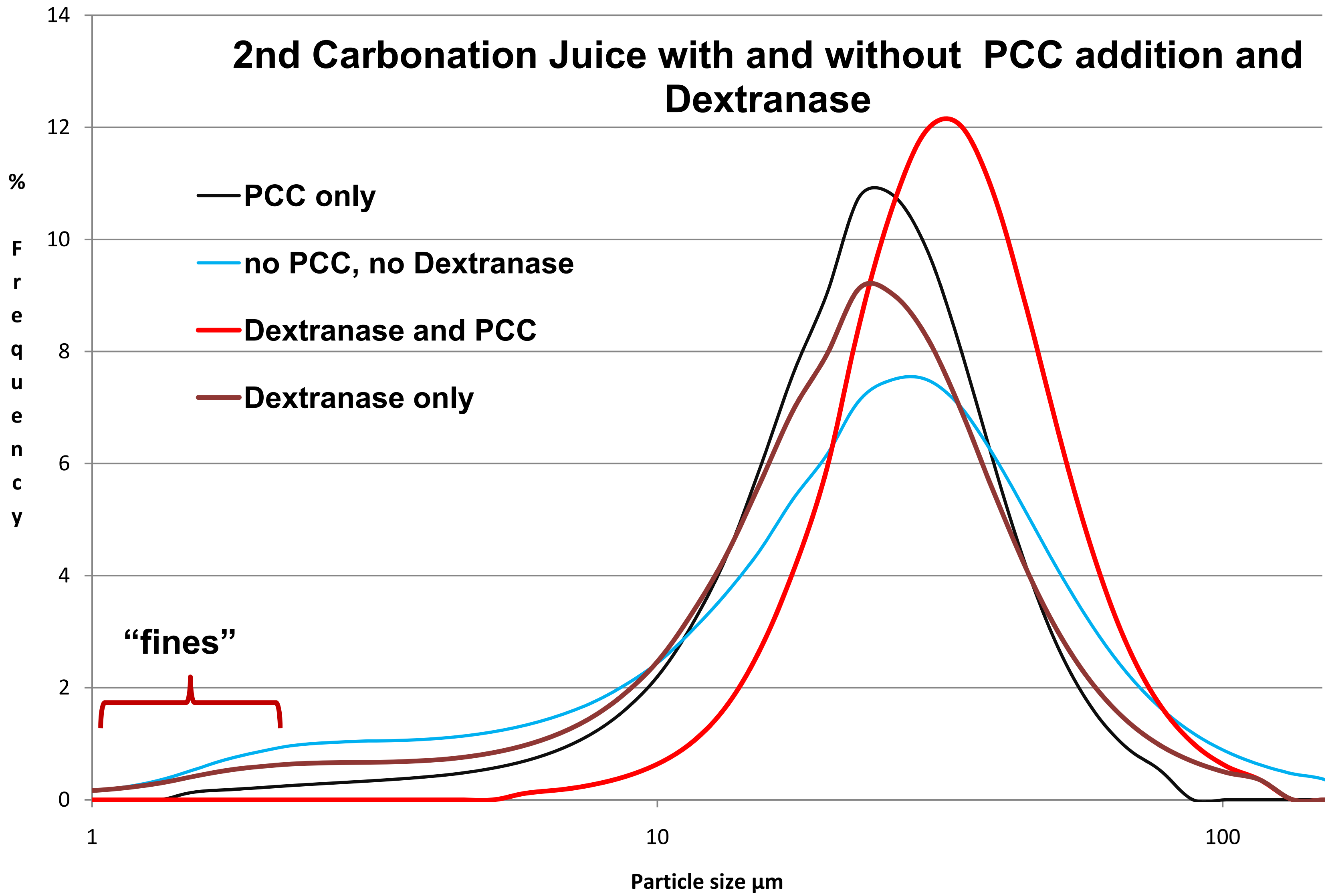
How Does It Work?

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 2nd carbonation juice leading to improved filterability

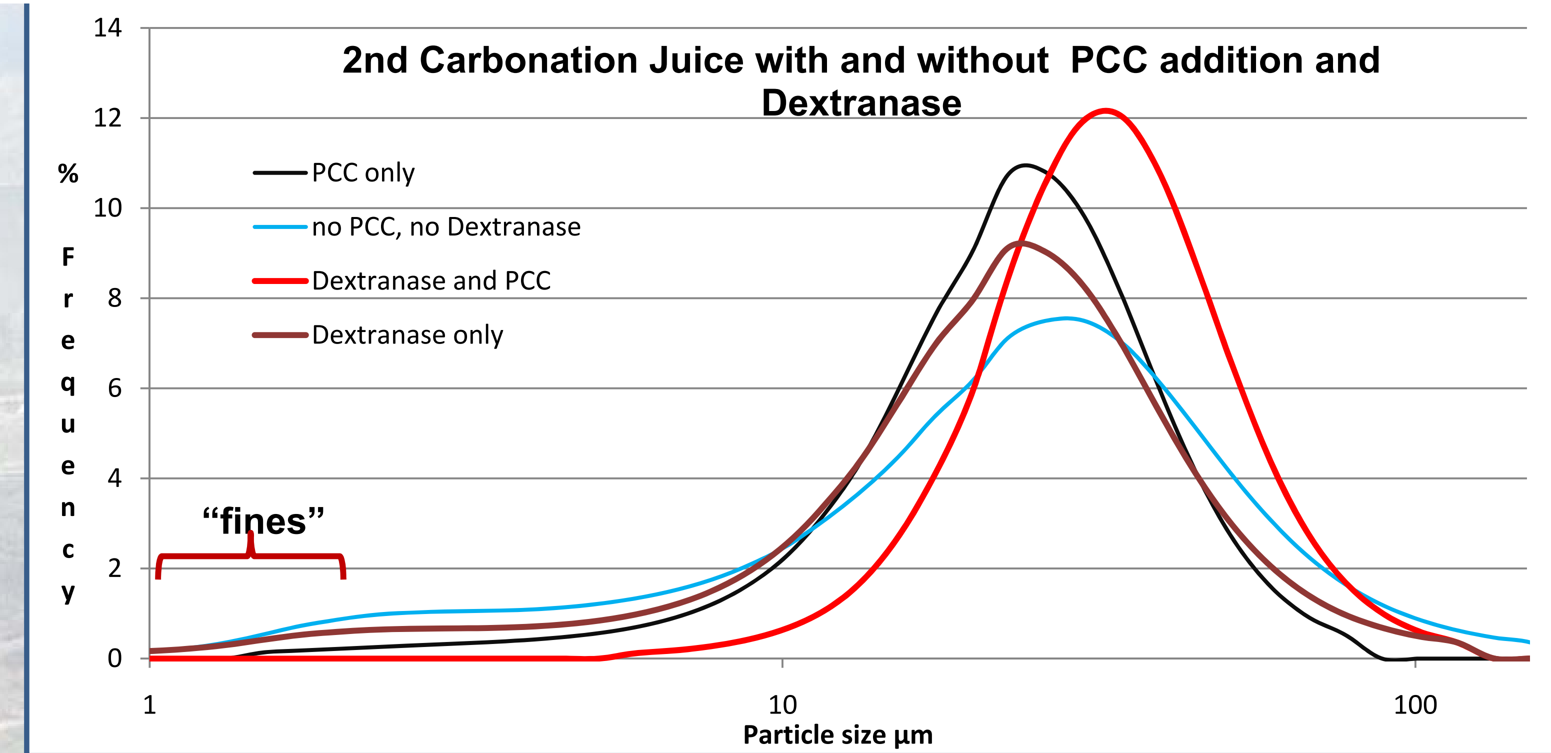
References

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

Struijs, Jaspers, van Dijk (2009). Methods used in the Netherlands to limit frost damage and to process frost-deteriorated beets,Proc. of European Society for Sugar Technology (ESST), pp 33- 38



Typical 2nd carbonation particle size distribution, with no PCC and no dextranase, with PCC only, with dextranase only and with PCC and dextranase.



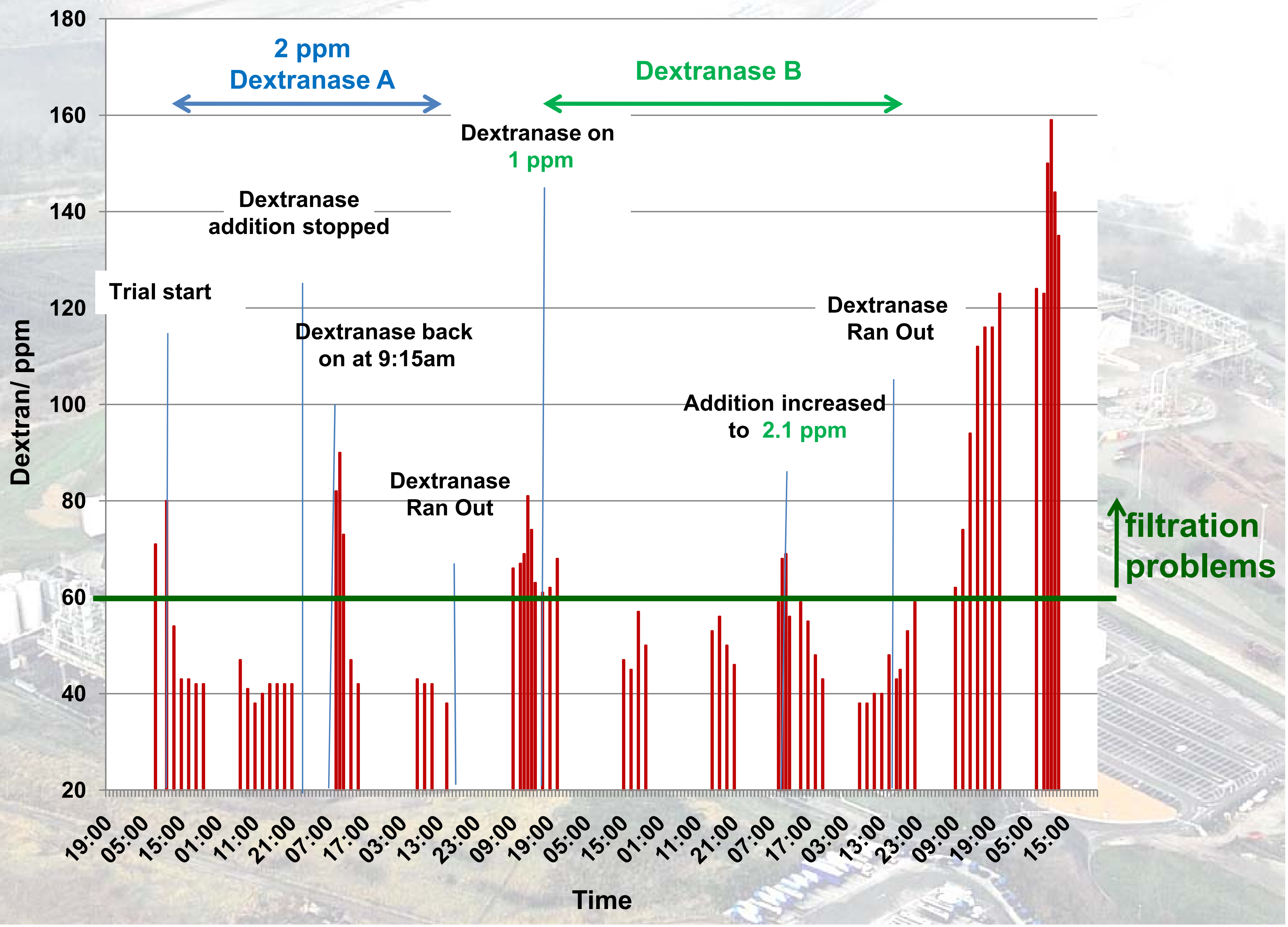
	Modal Particle size/ µm	% < 3µm
No PCC and no dextranase	27.8	7.3
With PCC addition only	24.3	1.9
With Dextranase only	21.4	5.4
With PCC and Dextranase	31.8	0.0

- **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 2nd carbonation juice of ≥ 60 ppm lead to filtration problems (information supplied by Nordic Sugar)**

Dextran Levels in Juice Streams



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)**

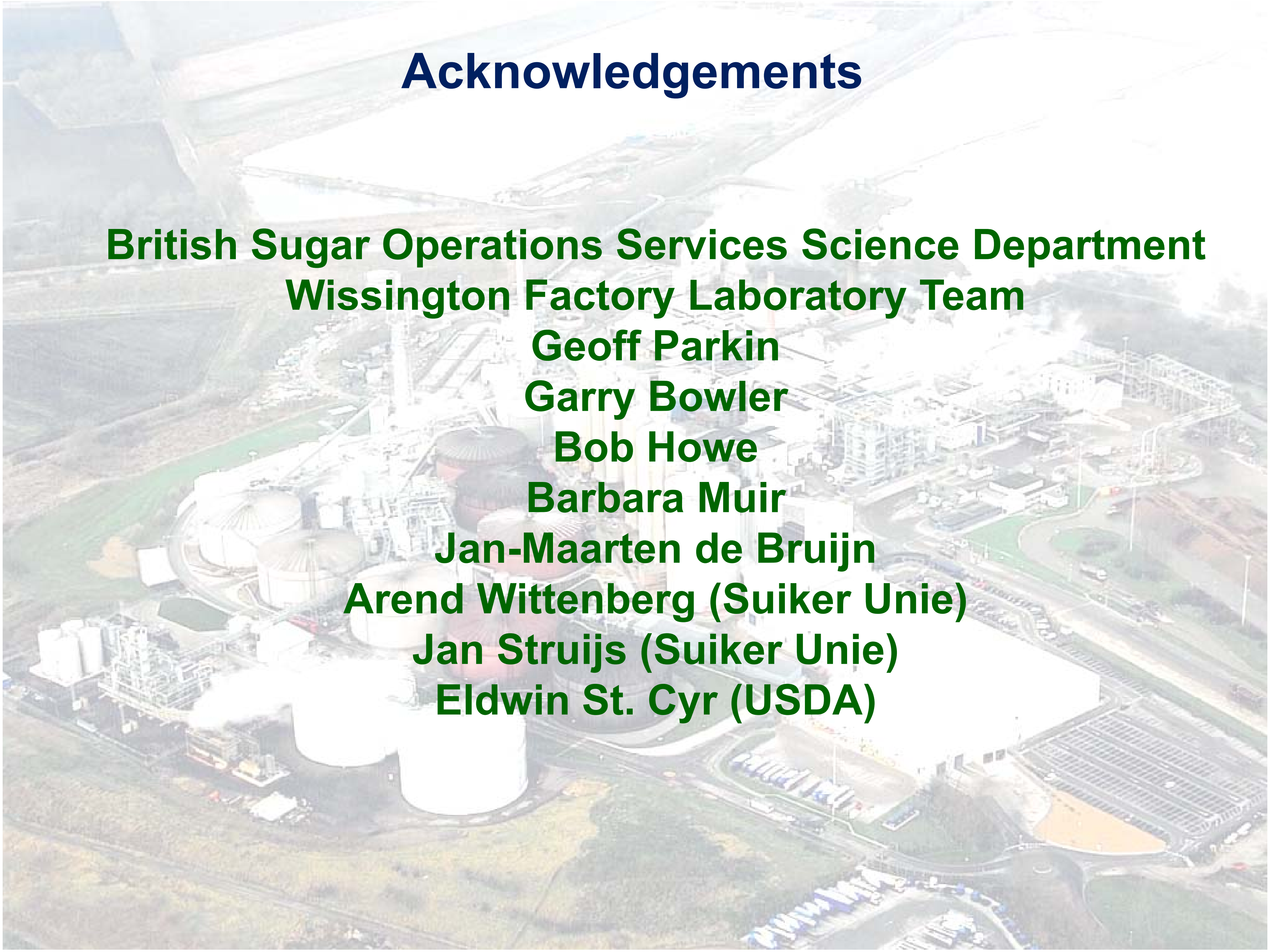
	% Reduction	Trial Saving $/day
Cost of conc. Dextranase	--	-2741
Cost of acid washing filters	73%	
CaO to process*	11%	
Anthracite	9%	
Throughput costs (LOP)	84%	
Total Savings		**3,180**

* Lime required to aid filtration

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

Major Conclusions

- Commmercial dextranases occur in “concentrated’ and “non-concentrated” forms; activities, and activites per unit $, vary widely
- A new ICUMSA tentative method is now available to uniformally and easily measure the activity of dextranases at the factory to (1) economically compare activites 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 m^3/day
- Adding dextranase significantly improved beet throughput
- $3180/day saved by using concentrated 52000 DU/ml dextranase



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