

## DOSING OF STARCH HYDROLYSING ENZYMES INTO A DIFFUSER

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### Abstract

Sugarcane starch is a naturally occurring polysaccharide in sugarcane and consists of a mixture of linear and branched glucose molecules. Starch varies with cane variety and growing conditions and is particularly high in immature sugarcane. The presence of excessive starch in a cane sugar factory is known to cause, among other things, boiling and viscosity related problems and raw sugar filterability impairment, particularly in carbonatation refineries.

Since starch is introduced into the sugar factory with the cane, at particular times of the year the factory has little choice but to deal with high levels of starch in the milling process itself. The application of modified strains of *Bacillus licheniformis*  $\alpha$ -amylase (high temperature stable enzymes) to juice in the third or fourth evaporator effects has been practised in South Africa for more than 30 years, and is currently used as needed by five of the 14 South African factories.

The prevalence of diffusers in the South African sugar industry allows for the possibility of dosing  $\alpha$ -amylase before clarification. This has the advantage that the stable  $\alpha$ -amylase will be deactivated and possibly removed in the clarifier mud. It would also allow for the use of higher dosage rates of the enzyme at times when excessive starch enters the factory.

The paper discusses some work in this regard with results from two factory trials during consecutive seasons (2006-2007 and 2007-2008).

*Keywords:* sugar factory, factory process, starch,  $\alpha$ -amylase, diffuser, diffuser juice, hydrolysis, enzyme, activity

### Introduction

Starch is a naturally occurring polysaccharide in many flora and consists of a mixture of linear (amylose) and branched (amylopectin) glucose molecules, in a ratio of *ca* 20 to 80 in sugarcane, although this ratio varies according to the source and maturity of the plant (Vignes, 1974).

Starch levels of above 400 ppm on Brix in cane juice will generally result in starch levels of above 150 ppm on Brix in raw sugar (unpublished data<sup>1</sup>). Starch levels higher than this may cause severe processing difficulties related to boiling and viscosity problems in the raw house. Excessive levels of starch in raw sugar cause filterability problems in the refining process, particularly where carbonatation is involved, and are therefore highly undesirable (Boyes, 1960; Eggleston *et al.*, 2003; Godshall *et al.*, 2004; Madsen, 1974; Meade and Chen, 1977; Murray *et al.*, 1974; Simpson and Davis, 1998; Wood, 1962). High starch levels in raw sugar

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<sup>1</sup>Novozymes/Enzymes SA, 'Use of Termamyl® for break-down of starch in sugar cane juice', Information brochure, 1980s.

result in a product of lower quality and market value, and are thus subject to penalties in the SASMAL export quality scheme (currently above 130 ppm in raw sugar).

Since starch (as a factor of cane quality) is introduced into the sugar factory with the cane, the factory has to deal with starch in the manufacturing process itself. Most South African factories monitor starch levels and apply  $\alpha$ -amylase enzymes only when necessary, while some have to use starch reduction measures throughout the season (Schoonees, 2006). Starch levels in South Africa become prominent at the height of the rainy season, possibly due to poor cane burning conditions in the wet cane fields, resulting in more green leaves entering the factory. The seasonal accumulation of starch in the sugarcane plant is also affected by other climatic conditions such as the average or maximum daytime temperature, stress, diurnal temperature variations, cane variety, soil type and aspect (Alexander and Matic, 1974; Cuddihy *et al.*, 2001; Eggleston *et al.*, 2007; Meade and Chen, 1977; Wood, 1962).

Refineries initially dealt with high starch in raw sugar by installing larger filtration units. This was, however, a very expensive option. Over the years a number of processes and processing aids have therefore been used to reduce the starch content of raw cane juice.

#### *Natural enzymes*

Cane juice contains natural amylase enzymes (diastases) that will hydrolyse starch once it is gelatinised (above 68°C) and under the right conditions. The attraction of this process is the relatively lower cost. However, the quality and type of enzyme that is naturally present in cane juice is not constant, so that there is little control over this process. The natural enzyme is also not heat tolerant, and is believed to be inactive above 80°C (Madsen, 1974). Special retention tanks for juice were needed at *ca* 70°C for this natural enzyme process. Unfortunately, the natural enzyme invertase, which causes the inversion of sucrose to fructose and glucose, is also active under these acidic conditions. After hydrolysis, high temperatures in clarification deactivate and possibly remove the natural enzymes to prevent these protein-rich impurities from causing problems in subsequent processes (Alexander and Matic, 1974; Carter, 1967; Madsen, 1974).

#### *Amylase*

Development of a relatively heat-stable  $\alpha$ -amylase enzyme that could be added to the process allowed for a different approach. For the enzyme to be active, specific conditions are required in terms of temperature, pH and Brix, as well as sufficient residence time. Amylase also requires the presence of calcium ions – not a problem after liming, although the amounts needed sometimes exceed the liming dosage.

Higher temperatures favour the enzyme activity only up to a temperature where the enzyme is thermally deactivated. Enzyme activity is also favoured by low Brix conditions that are typically found in the first evaporator effect. However, the high temperatures of the first and second effects would deactivate the enzyme instantaneously. Conditions in the third, fourth or fifth evaporator effects are usually suitable where temperatures are between 65 and 85°C. The higher sugar concentrations in these effects also tend to protect and stabilise the enzyme against thermal deactivation, although the activity is reduced. Enzyme holding tanks were sometimes installed between the third and fourth effects to increase residence times.

#### *Heat-stable amylase*

The development of thermally stable amylase enzymes resulted from specific needs in the starch industry. Since these were developed for industries other than the sugar industry (considered to be a small enzyme market) they are not tailored to sugar industry conditions.

The enzymes can usually tolerate temperatures up to 105°C before they are thermally deactivated (Eggleston *et al.*, 2008) and can therefore be added to the second evaporator effect. These enzymes are also no longer dependent on the presence of large amounts of calcium. Since mixed juice does contain some calcium, further addition is not necessary (Bruijn, 1973; Madsen, 1974). One drawback of the thermally stable products is that they may remain active, which will in turn cause problems in subsequent processes and downstream industries (Eggleston *et al.*, 2008).

#### *Enzymes in a diffuser*

Before the introduction of thermally stable enzymes, the temperatures in diffusers (>85°C) were simply too high for enzyme addition. Diffusers are currently operated at high temperatures (85-95°C) to avoid sucrose losses through bacterial degradation. However, thermally stable enzymes will be active at these diffuser temperatures and might be able to hydrolyse most of the starch before being thermally deactivated in the juice heaters.

Although the temperatures across a milling tandem (40-60°C) are low enough for natural enzymes to work, the starch granules have not been gelatinised by exposure to higher temperature, and hence the use of enzymes would not be effective unless special procedures (including provision of adequate retention time) are implemented.

Graham *et al.* (1968) compared the extraction of starch in a cane and a bagasse diffuser at low temperatures (53-67°C) and high temperatures (75-96°C) when the first two diffusers were introduced in the South African sugar industry (1966/67). Diffusers were initially operated at 70 to 75°C. The authors concluded that diffuser starch levels were lower than mill starch levels when the diffuser was operated at low temperatures, but that mill and diffuser starch levels were similar when the diffuser was operated at higher temperatures. A study by Sahadeo *et al.* (2002) comparing the starch content of five mixed juice sets assumed to be extracted from the same cane by diffusion and by milling, clearly showed much lower levels of starch in the diffuser juices. The reason for this was not clear.

The use of  $\alpha$ -amylase enzymes in some of the South African sugarcane factories to control high levels of seasonal starch in evaporator syrup has become routine over the past 30 years. All of these factories currently use the same enzyme from genetically modified strains of *B. licheniformis*, which were developed specifically for their heat stable properties. A recent laboratory study indicated that the use of these enzymes in the South African industry is reasonably optimised (Schoonees, 2004). The enzymes are added before or after the fourth effect evaporator and are for the most part effective in reducing raw sugar starch levels to below 130 ppm. However, this study strongly reiterated previous suggestions that conditions in a diffuser might be better suited to the current generation of enzymes. This topic seems to surface at regular intervals, especially since the 14 factories in South Africa currently operate 16 cane diffusers and only three tandem mills. KwaZulu-Natal province, where most of the diffusers are operated, is therefore the ideal area to investigate the use of thermally stable  $\alpha$ -amylase enzymes in a diffuser.

## **Experimental**

#### *Enzyme activity*

The activity of the enzymes was measured using the method described in the Laboratory Manual of the South African Sugar Technologists' Association (Anon, 2005a).

#### *Natural enzymes in diffuser juice*

A sample of diffuser mixed juice was taken from a mixed juice tank and sub-sampled into six portions. Mercuric chloride preservative was added to three of the samples. Two of these and

one of the unpreserved samples were immediately put on ice. A sample of clear juice was then taken exactly one hour later (to account for the residence time) and sub-sampled into two portions. Mercuric chloride preservative was added to one of the portions. The samples were left overnight either on the bench or in the freezer and analysed for starch (Anon, 2005b) and some for sucrose (Anon, 2005c). Duplicates were included in the analysis set.

#### *First factory trial*

A factory trial to investigate the possibility of dosing heat-stable enzymes (*B. licheniformis*) into one of two diffusers was conducted over two weeks (2006-2007). During the two weeks, samples of draft juice were taken from both diffusers (A and B) and a sample of mixed juice was taken after the mixed juice tank. Samples were taken every eight hours. No measures were taken to stop any potential enzyme activity, and the samples were immediately placed in a freezer. The samples were thereafter composited into daily samples and analysed for starch (Anon, 2005b) and Brix (Anon, 2005d). An attempt was made to keep the diffusers running as consistently as possible. Diffuser conditions over the two weeks of the trial are shown in the Appendix.

During the second week of sampling, the heat-stable amylase enzyme was dosed into the middle of the A diffuser to allow a reasonable enzyme residence time. The enzyme was not diluted and a dosing rate of 23 ppm enzyme on cane (in the A extraction line only) was used to keep enzyme usage exactly the same as when dosing into the evaporators at this specific factory. After two days of dosing the factory laboratory mixed juice, starch results showed an upward trend contrary to expectations; the dosing point was therefore moved to the beginning of the diffuser to establish whether the mixed juice tank would act as a suitable enzyme mixing vessel in case the enzyme was absorbed onto the bagasse fibres. The Brix and starch levels in the beginning of the diffuser were also much higher, which would generally encourage enzymatic action.

#### *Second factory trial*

A second factory trial was conducted during the 2007-2008 season to assess the dosing of larger amounts of the same enzyme (*B. licheniformis*) into the front of a diffuser. The enzyme was diluted (1:10) prior to use according to supplier recommendation. The enzyme was dosed into the first tray of one of the diffusers, after which about half of the diffuser juice was removed from the diffuser as draft juice and pumped to the mixed juice tank. Samples were taken after the mixed juice tank and after clarification with an estimated one hour holding time (70-90°C) between the two sampling points to correlate the data with the same consignment of cane.

Two different dosage rates were used in two sets: (i) twice the dosage normally used by the factory, and (ii) five times the usual dosage. Samples were taken at 10 minute intervals for 30 minutes before the first dosing set, for 30 minutes between the sets and for an hour after the second dosing set. A residence time of one hour was allowed for the batch of juice to move between the mixed juice tank and the exit to the clarifier, in order to compare the samples using relative time. It was thus possible to assume that the same consignment of juice was being sampled.

## **Results and Discussion**

#### *Enzyme activity*

The activities of the enzymes used were such that at least 98% starch hydrolysis was achieved at the specified analytical conditions (Anon, 2005a).

*Natural enzymes in diffuser juice*

Results are shown in Table 1.

**Table 1. Mixed juice and clear juice starch results.**

Treatment	Brix (°Bx)	Starch (ppm on Brix)	Sucrose (%)
<b>Mixed juice</b>			
Preservative + Freezing (rep 1)	12.7	1 096	11.1
Preservative + Freezing (rep 2)	12.8	1 093	11.1
Preservative + No freezing	13.0	1 060	11.2
No preservative + Freezing	12.7	415	11.1
No preservative + No freezing (rep 1)	13.0	146	5.0
No preservative + No freezing (rep 2)	13.1	144	4.1
<b>Clear juice</b>			
Preservative + No freezing	12.8	734	–
No preservative + No freezing	12.8	670	–

From the mixed juice starch results in Table 1, it is clear that a bioreactive entity which is deactivated by the preservative and by freezing (albeit delayed), such as natural starch enzymes that are known to be present in the cane, was acting on the juice and hydrolysed as much as 90% of the starch. It appeared therefore that not all of the natural enzymes are thermally deactivated in a diffuser as was suggested by Madsen (1974), and that these could be responsible for starch hydrolysis in the mixed juice tank. Unfortunately, since sucrose is inverted by natural invertase at the same conditions, harnessing these enzymes to hydrolyse starch on a large scale would be problematic.

The same hydrolysis was not exerted on the starch in the unpreserved clear juice sample, indicating that this entity is either deactivated or removed by clarification. The lower clear juice starch levels (as compared to mixed juice) corroborate reports by Eggleston *et al.* (2003) that some of the starch is removed by precipitation during clarification.

*First factory trial*

Measurements taken at the Sugar Milling Research Institute (SMRI) are shown in Figure 1.

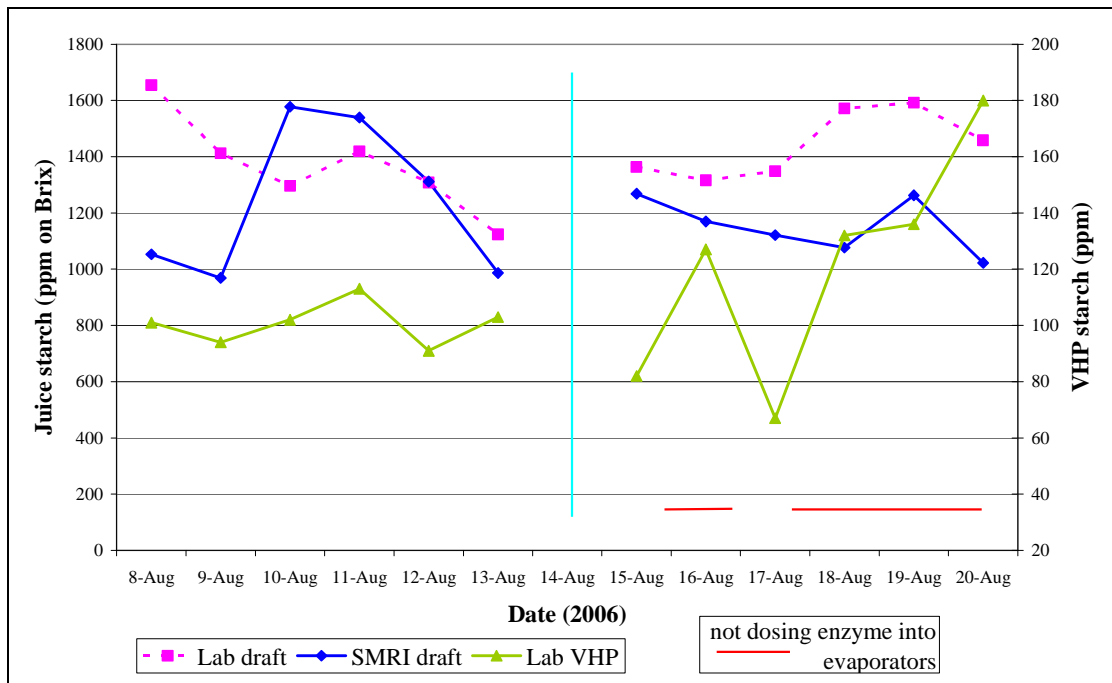
Although starch levels in the A draft juice were variable, statistical t-tests indicated that there was no significant difference between starch levels in the A and B diffusers during the first week. There was, however, a significant difference between starch levels in the A and B diffusers during the second week, as well as between starch in the mixed juice during the first and second weeks, with the starch being lower during the second week when the enzyme was dosed into the A diffuser. Moving the dosing point from the middle to the front of the diffuser did not appear to have any effect on the draft juice starch levels. It may have had an effect on the mixed juice starch levels, which were lower during the second part of the week, indicating that the enzyme may have been more effective in the mixed juice tank, since it reached the tank quicker and would have had less time to degrade.

The average difference between starch levels in the A and B diffusers during the second week, when the enzyme was dosed into the A diffuser, was 20%. The average difference between starch in the mixed juice during the first and second weeks was 25%. The difference between the average starch coming out of the diffusers (combined draft juice) in the second week and mixed juice was 30%. More starch was therefore removed in the mixed juice tank than in the diffuser. It is possible that some of the enzyme had been absorbed onto the bagasse fibre in the diffuser and had inhibited the enzymatic hydrolysis of the starch.

For comparison, the factory laboratory results (daily analysis of a composite draft juice and very high pol (VHP) sample) are shown in Figure 2.



Figure 1. Starch results for the two-week trial.



VHP = very high pol

Figure 2. Comparison of Sugar Milling Research Institute (SMRI) and factory laboratory results.

The overall average difference between the SMRI and factory laboratory results for draft juice was 15%; a 9% difference during the first week and a 20% difference during the second week. The factory results were consistently higher during the second week. t-Tests showed that the results were significantly different for the second week, but not for the first week ( $p=0.05$ ). Overall, the results were not significantly different.

Compared to the first week samples, the SMRI results showed a reduction of 7% in the draft juice starch during the second week; using the same comparison the factory laboratory mixed juice starch actually showed an increase of 5%. It must be noted that the samples collected by the SMRI and by the factory laboratory were taken at different times; the SMRI took catch samples every eight hours from the two diffusers and composited these into two daily samples. The average of these two samples was used in the above comparisons. The laboratory took hourly samples from their continuous sampling system (combined draft juice from the two diffusers) and composited these into a daily sample. The factory laboratory samples are therefore more representative.

### Second factory trial

Results are shown in Figure 3.

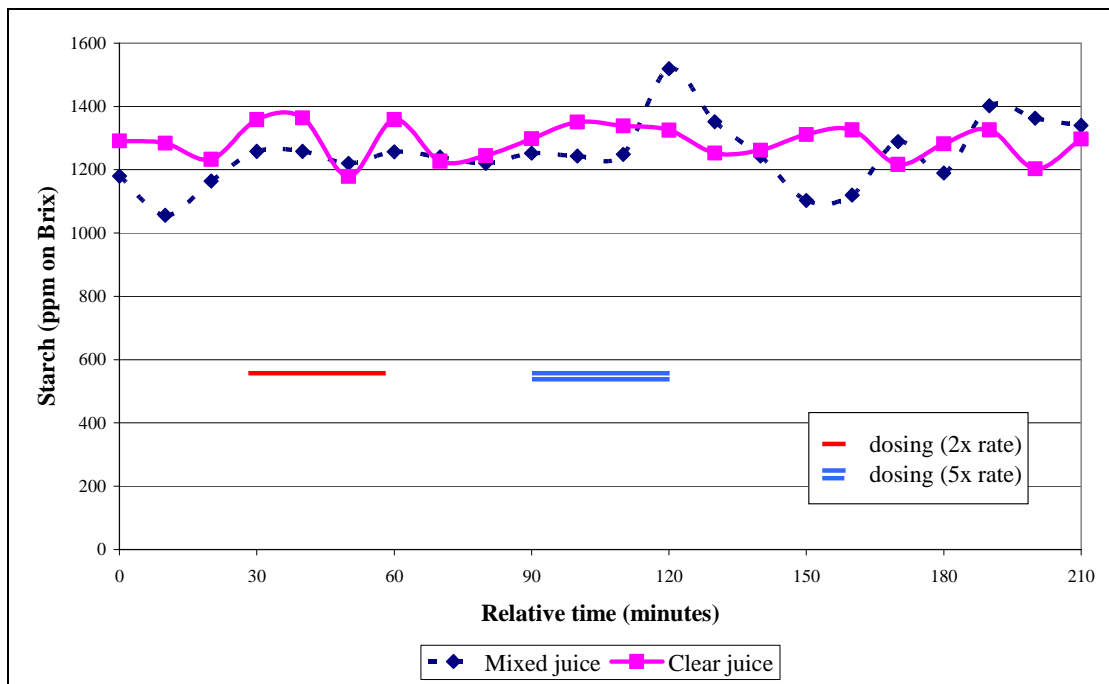


Figure 3. Starch levels in diffuser mixed and clear juices at relative times.

The mixed juice starch level before dosing was about 1 300 ppm on Brix. For the  $\alpha$ -amylase dosing trial to be successful, i.e. to confirm that the enzyme is efficient enough in the diffuser and mixed juice tank to substitute for the enzyme currently being dosed into the evaporator, a clear juice level of no more than 450 ppm on Brix should be obtained.

Statistical evaluation indicated that the effect of  $\alpha$ -amylase on starch levels was negligible. Therefore, although natural enzymes are active in diffuser mixed juice, it can be concluded that the commercially available, thermally stable *B. licheniformis*  $\alpha$ -amylase used in the South African sugar industry is not active towards sugarcane starch in diffuser mixed juice.

## Conclusions

From the results presented in this paper it can be concluded that the dosing of  $\alpha$ -amylase enzyme from genetically modified strains of *B. licheniformis* before clarification is not viable under the conditions investigated. It would appear that the enzymes are rendered inactive by a component of the raw juice, or are absorbed onto the bagasse fibres in the diffuser. The reduction in starch levels observed during the first factory trial could have been due to the natural enzymes that are active at the temperature found in the mixed juice tank.

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**APPENDIX**  
**Diffuser conditions during the first factory trial.**

Day	Temperature (°C)		Chain speed (m/min)		Bed level (m)		Crush rate (t/h)		Imbibition (t/h)	
	A	B	A	B	A	B	A	B	A	B
Tues	91	90	0.7	0.7	1.3	1.2	200	200	120	120
Wed	89	87	0.8	0.7	1.1	1.3	150	150	120	120
Thurs	89	89	0.8	0.9	1.3	1.3	220	220	130	130
Fri	92	92	0.8	0.7	1.2	1.2	220	220	130	150
Sat	92	92	0.7	0.7	1.3	1.2	210	200	130	140
Sun	87	90	1.3	1.2	0.9	0.7	210	210	130	140
Mon	<i>stop day</i>									
Tues	89	91	0.8	0.4	1.5	1.1	250	150	155	160
Wed	92	92	0.7	0.7	1.1	1.2	160	160	130	130
Thurs	87	90	0.7	0.7	1.2	1.2	180	180	130	150
Fri	89	87	0.8	0.9	1.2	1.3	250	220	130	130
Sat	90	89	0.8	0.8	1.3	1.2	220	220	140	140
Sun	90	90	0.9	0.9	1.2	1.3	230	230	130	140
<b>Average</b>	<b>90</b>	<b>90</b>	<b>0.8</b>	<b>0.8</b>	<b>1.2</b>	<b>1.2</b>	<b>208</b>	<b>197</b>	<b>131</b>	<b>138</b>