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Peer-reviewed paper

Efficacy of FDA-approved biocides to inhibit microbial degradation of sucrose

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Abstract During the sugar-manufacturing process, undetermined sucrose loss from microbial activities especially occurs during milling train stage. Hence, good factory hygiene that limits microbial activities will improve sugar recovery. This project evaluated and compared the effectiveness of two types of FDA-approved biocides (P100 and P200) based on both laboratory- and factory-scale trials. Sucrose, glucose, fructose, mannitol, organics acids and polysaccharides contents of juices with and without biocide treatment were used as markers to determine sucrose degradation. Laboratory tests on primary mixed juice (PMJ) and secondary mixed juice (SMJ) from four mills clearly showed that P100 biocide is more effective in inhibiting sucrose degradation in PMJ than P200, while the reverse is true with SMJ. The factory trials at F2 Sugar Mill indicated that dosing P100 in the milling train station reduced the microbial sucrose degradation compared to the control, while at F4 Sugar Mill, the effectiveness of the two biocides could not be distinguished.

Key words Sucrose degradation, undetermined sucrose loss, microbial degradation, biocide

INTRODUCTION

Sucrose loss has long been a common problem for the sugar industry, estimated at 1-2% and indicating a huge financial loss to the industry (Rackemann and Broadfoot 2016). It occurs between harvesting and milling, and during the sugar-manufacturing processes. Three main reaction pathways result in sucrose loss: microbial, enzymatic, and chemical processes. An assessment on mixed juice (MJ) has indicated that 93% of sucrose deterioration is caused by microbial action, while 5.7% and 1.3% is by enzymatic and chemical processes, respectively, within the first 14 h of the deterioration process (Eggleston 2002). Microbial action in cane processing goes beyond sucrose loss, as the growth and accumulation of these microorganisms also lead to the formation of increasing proportions of non-sucrose impurities that detrimentally affect the evaporation and crystallisation rates, and molasses exhaustion (Daza *et al.* 2019).

Microbial infection of sugarcane juice results from the microorganisms that enter the mill with the cane supply, and those from the factory recovery stream and the filtrate etc. (Nel *et al.* 2019). Factors including the nature of cane varieties, harvesting protocols, cane maturity, seasonal variation (temperature), cut-to-crush lag, and the factory hygiene practices (including sanitizer application and biocide application) during processing impact on the colonization of microorganisms in juices (Solomon *et al.* 2006; Singh *et al.* 2008; Misra *et al.* 2020).

In Australian sugar mills, the hygiene practices vary from mill to mill. Periodic cleaning of filtration systems and the use of hot water to clean screens and drains are methods used to control, but not eliminate, the spread of microorganisms. Juice-deterioration studies carried out by Tilbury *et al.* (1997) indicated that even under Australian harvesting and processing conditions, where the lag time between harvesting and milling (i.e., cut-to-crush) is short, and there are good hygiene practices through sanitisation of the cane milling train, MJ screen, filters and drains, juice deterioration by microorganisms still occurs. Another approach to reduce/eliminate the microbial growth in sugarcane juice is to apply biocides which are applied in many oversea sugar factories but not routinely used in Australian sugar mills. Little recent work has been conducted by Australian sugar mills to accurately determine the extent of sucrose loss caused by microorganisms, and the benefits of the use of biocides approved by the US Food and Drug Administration (FDA) to mitigate sucrose loss.

Here, we assessed two FDA-approved biocides on typical juice streams, primary mixed juice (PMJ) and secondary mixed juice (SMJ), from four sugar factories under laboratory conditions to identify their efficacies to reduce microbial activities. Factory tests were then carried out at two Australian sugar factories to identify whether it is beneficial to use biocides in Australian factories.

MATERIALS AND METHODS

Biocides

Two FDA-approved biocides (P100 and P200) were purchased from TD Chemicals Pty Ltd. P100 is an aqueous solution of sodium dimethyldithiocarbamate ($C_4H_6N_2S_4.2Na$) with a concentration of 40–42% (w/w), and P200 is an aqueous solution of disodium ethylene bisdithiocarbamate ($C_5H_{10}NS_3Na$) with a concentration of $\geq 30\%$ (w/w). The suggested dosing rate of P100 and P200 are 7.5 ppm on cane and 10.0 ppm on cane, respectively.

Assessment of biocides under laboratory conditions

Composites of primary mixed juice (PMJ) were collected from F1 and F2 Sugar Mills and composite secondary mixed juice (SMJ, including floor washings and filtrate) were collected from F3 and F4 Sugar Mills. The juices were stored at $-20\text{ }^\circ\text{C}$ immediately after collection.

Juices were defrosted in the laboratory, and deterioration experiments for both PMJ and SMJ were conducted at laboratory scale at ambient temperature ($\sim 23\text{ }^\circ\text{C}$). Interval samples were withdrawn from the system at 0 h, 4 h, 6 h, 16 h, 24 h, 48 h, and were quenched with NaN_3 to stop the microbial activity, and instantly stored at $-20\text{ }^\circ\text{C}$ for subsequent analysis. Tests were conducted without biocide and with P100 and P200.

Concentrations of sugars (sucrose, glucose, fructose), 1-kestose, mannitol, organic acids (oxalic acid, cis-aconitic acid, citric acid, D-gluconic acid, L-malic acid, trans-aconitic acid, succinic acid, glycolic acid, lactic acid, formic acid, acetic acid) and total polysaccharides were monitored throughout the degradation process to determine the sucrose degradation rate and to identify the effectiveness of the biocides.

Concentrations of sucrose, glucose, fructose, 1-kestose and mannitol in the samples were determined using high-performance liquid chromatography (HPLC). The samples were diluted by 50 times to achieve a sucrose concentration within the appropriate range and filtered with a $0.45\text{ }\mu\text{m}$ nylon filter prior to analysis. An Aminex HPX-87P Column with a guard column was used to separate each of the compounds equilibrated at $60\text{ }^\circ\text{C}$ and detected by a Waters 2414 refractor index detector. The eluent was MilliQ water at a flow rate of 0.5 mL/min .

Contents of organic acids and polysaccharides in juice samples were analysed using the methods reported in our previous research work (Shi *et al.* 2020).

Factory trials at F2 Sugar Mill

To further demonstrate the efficacy of using biocide, another factory trial was conducted at F2 Sugar Mill. P100 biocide weeks ON and OFF mode were applied during the crushing season.

F2 Sugar Mill has a milling train (A side) and a diffuser (B side) in the milling stage (Figure 1). The processing capacities of A and B side are 300 t/h and 270 t/h , respectively. No. 1 mill, No. 5 mill of A side, and No.1 mill and the front end of diffuser of B side were the biocide dosing points. Based on the suggested dosing rate, the pump rate at A side dosing points was 1.125 L/h , and that at the B side dosing points was 1.0125 L/h . The biocide dosing points, dosing rate and sampling point are shown in Figure 1.

Each test period was set as 2 weeks except for unexpected stoppages, which was to accommodate the regular factory periodic cleaning practices (the factory stops for one day every 2 weeks for the regular cleaning of the evaporators). The detailed test time frame was: (1) 1–2 weeks, P100 biocide test; (2) 3 week, P100 biocide test; (3) 4–5 weeks, no biocide test; (4) 6–7 weeks, P100 biocide test; (5) 7–8 weeks, no biocide test; (6) 8–9 weeks, P100 biocide test.

SMJ and ESJ samples were collected for at least 5 days at the end of the testing period, except for unexpected stoppages, and each juice sample were collected 4 times a day (with 0.05% NaN_3 added to stop the microbial

activity) and immediately placed on ice before being stored at 4°C, and were combined into a composite sample before storage at -17 °C.

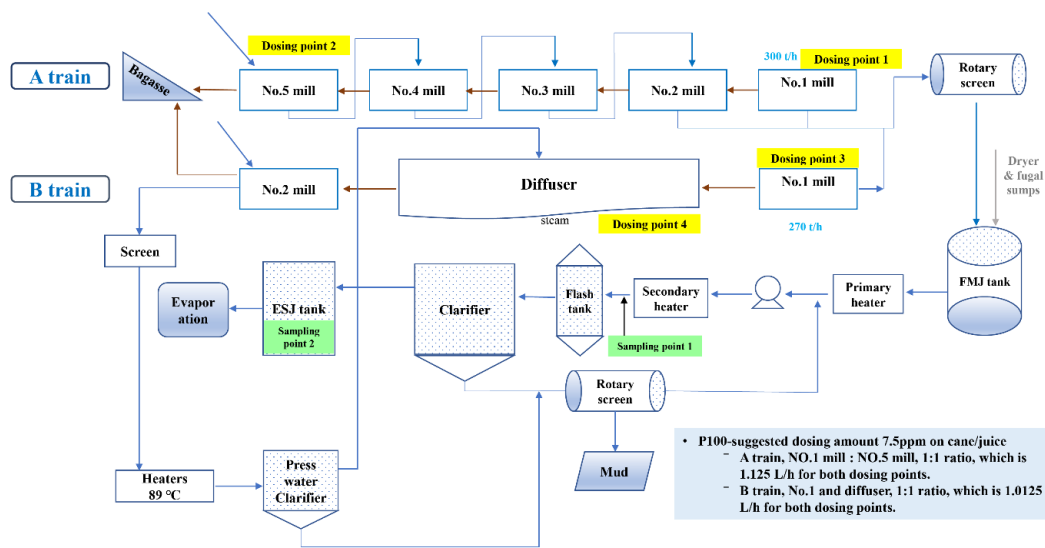


Figure 1. Schematic processing diagram and the detailed biocide dosing test parameters at F2 Sugar Mill.

Factory trials at F4 Sugar Mill

The effectiveness of P100 and P200 biocides was compared at a factory scale in F4 Sugar Mill. The trials were carried out by a 5-week test dosing with P100, followed by a 5-week test dosing with P200.

No. 1 and No. 5 mills were set as the biocide dosing points, and the dosing rate was based on the suggestion from the supplier (P100 7.5 ppm on cane, and P200 10 ppm on cane). The crushing rate at F4 Sugar Mill was 320 t/h, so the dosing rate at each dosing point was 2.4 L/h for P100 biocide and 3.2 L/h for P200 biocide. The schematic processing diagram and the detailed biocide dosing points, dosing rate and sampling point are shown in Figure 2.

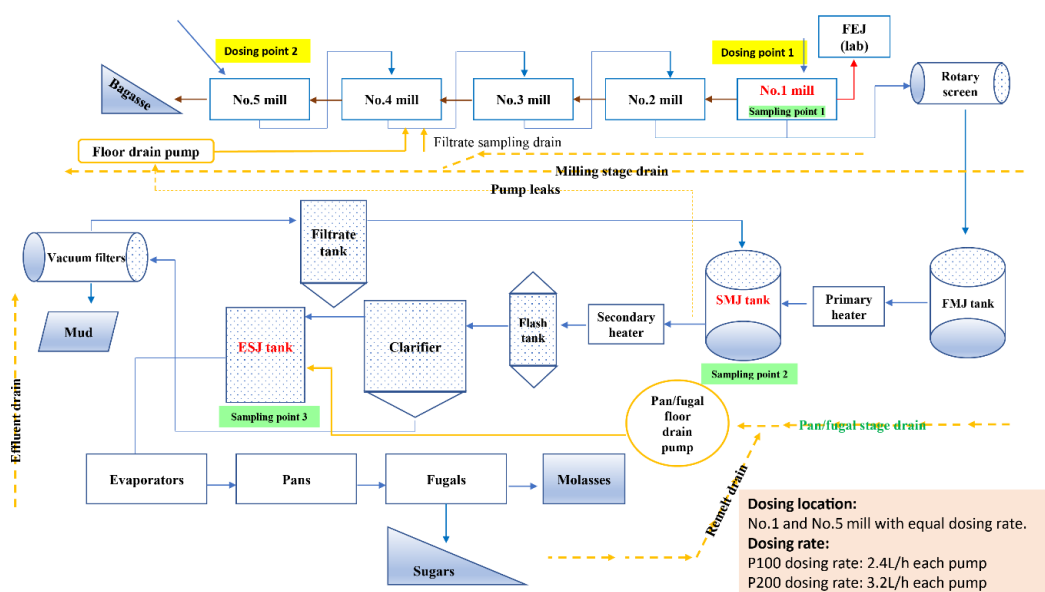


Figure 2. Schematic processing diagram and the detailed biocide dosing test parameters at F4 Sugar Mill.

No.1 Mill juice (#1 Mill juice), SMJ and evaporator supply juice (ESJ) were collected for at least 7 days at the end of each testing duration. Sampling and storage procedures were the same as in F2 Sugar Mill.

For SMJ and ESJ collected from F2 Sugar Mill, and # 1 Mill juice, SMJ and ESJ collected from F4 Sugar Mill, sucrose, glucose, fructose, mannitol and 1-kestose were analysed. In addition, organic acids and total polysaccharides content were analysed for the SMJ collected from the two factory trials.

RESULTS

Assessment of biocide under laboratory conditions

Primary mixed juice samples (PMJ) were collected from F1 and F2 Sugar Mills and SMJ samples were collected from F3 and F4 Sugar Mills. We carried out a 48-h degradation test on these samples to determine the effectiveness of the biocides P100 and P200. A control without the addition of biocide was also assessed.

The presence of metabolic products such as mannitol, lactic acid and ethanol etc. are indicators that can be used to predict sucrose loss (Jones *et al.* 1997; Lionnet and Pillay 1987; Eggleston 2002) and are used as tools to compare the rate and extent of juice deterioration. Therefore, in addition to sucrose degradation rate, the formation of the metabolic products was monitored throughout the degradation process.

The composition of PMJ and SMJ are listed in Table 1. Mannitol was not detected in both PMJ and SMJ which indicated that there is no noticeable degradation in the two types of juices collected from the four mills. The PMJ collected from F1 Sugar Mill contains the drains from evaporation, fugals, and scrubbers, while PMJ collected from F2 Sugar Mill contains the drains from the dryer and fugals. The most significant difference between the PMJ and SMJ is that the latter is obtained by heating the juice via the primary heaters (76 °C). As the SMJ has been heated, some of the mesophilic microorganisms entering with PMJ may have been killed, and so most of the survived microorganisms may only be thermophilic microorganisms.

Table 1. Compositions of the PMJ samples collected from F1 and F2 Sugar Mills, and SMJ samples collected from F3 and F4 Sugar Mills.

Sample	Sucrose (mg/mL)	Glucose (mg/mL)	Fructose (mg/mL)	Mannitol (mg/mL)	1-kestose (mg/mL)	Total organic acids (mg/kg·Bx)	Total polysaccharides (mg/kg·Bx)
PMJ	146-192	1.3-3.9	1.3-4.0	0	0.2-2.3	12-15	25-65
SMJ	143-152	0.5-0.8	0.5-0.9	0	0.2-0.35	10-20	44-46

Figure 3 shows the representative results for the degradation of PMJ (from F2 Sugar Mill). The degradation of sucrose occurred quickly with PMJ, within 2 h for all the three groups (control, P100 and P200). There was no significant variation among the three testing groups in the first 2 h.

In general, across the three testing groups, sucrose concentration decreased, while glucose, fructose, 1-kestose and mannitol, total organic acids and polysaccharides concentrations increased. The fructose content was slightly higher than that of glucose throughout the degradation process. The degradation rate of PMJ ranged from 80.1% to 96.8% in 48 h (Figure 3a) and may be due to the activities from both mesophilic and thermophilic microorganisms.

The polysaccharides concentration at the end of the degradation process was ~250 mg/kg·Bx, which is 10 times higher than that at the beginning (~25 mg/kg·Bx) (Figure 3f). Microorganism metabolism also leads to the formation of organic acids. Lactic acid in raw juice has been a determinant for microbial activity and has been cited as a useful indicator for the process (Rein 2018). The total content of organic acids at the end of the degradation process was up to ~30 mg/kg·Bx, which is almost triple of that at the beginning (~10 mg/kg·Bx, Figure 3g). Ethanol and methanol were detected in the degraded juice as well. However, their concentrations fluctuated throughout the process, which could possibly be due to losses associated with the low boiling points of these alcohols (Shi *et al.*, 2020). Overall, the total alcohol content experienced an increasing trend for all the tests (Figure 3h).

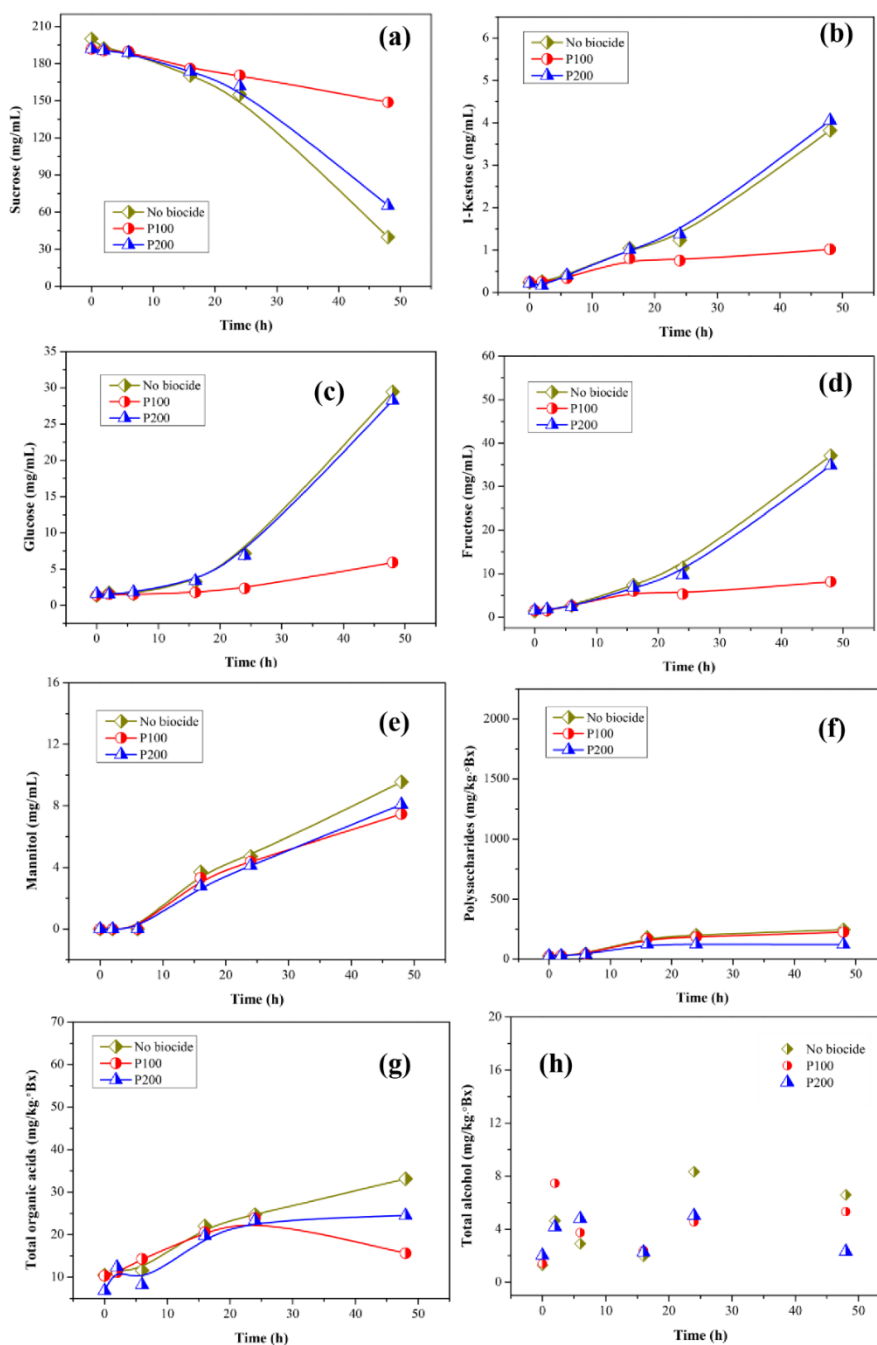


Figure 1. (a) Sucrose, (b) 1-kestose, (c) glucose, (d) fructose, (e) mannitol, (f) total polysaccharides, (g) total organic acids and (h) total alcohol content variation in PMJ from F2 Sugar Mill throughout the 48-h laboratory degradation test with and without the addition of biocides.

P100 was more effective than P200 to inhibit the growth of microorganisms and to reduce sucrose degradation in PMJ. Sucrose degradation rate was between 23% and 45% for P100 (7.5 ppm on cane), while that for P200 was between 66% and 93% (10 ppm on cane).

The use of P100 also resulted in lower concentrations of metabolic products, especially for 1-kestose, glucose and fructose as shown in Figure 3b,c,d. Contents of mannitol and total organic acids in PMJ treated with P100 were slightly lower than that in both the control and PMJ treated with P200. A slightly lower content of polysaccharides was observed for PMJ treated with P200, which could be due to its superior effectiveness in inhibiting the growth of microbes that secretes polysaccharides.

Figure 4 details the representative results of SMJ (from F3 Sugar Mill) degradation process. Sucrose degradation in SMJ was observed after 6 h, which is slower than that in PMJ (Figure 3). There were no significant differences between the control and the biocide-treated juices in the first 6 h.

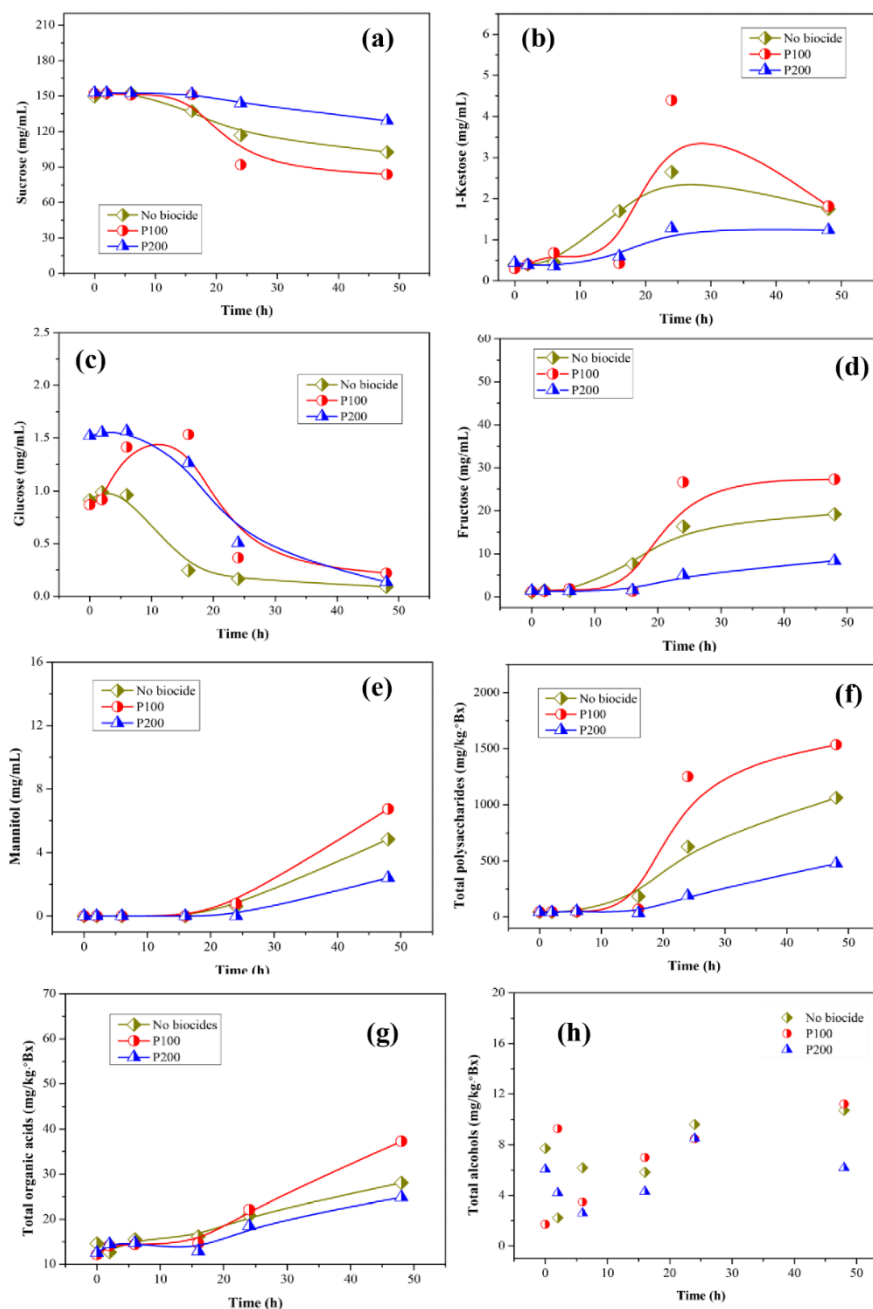


Figure 4. (a) Sucrose, (b) 1-kestose, (c) glucose, (d) fructose, (e) mannitol, (f) total polysaccharides, (g) total organic acids, and (h) total alcohol concentration variation in SMJ from F3 Mill throughout the 48-h laboratory degradation test with and without the addition of biocides.

Over the 48-h degradation process, with the three groups (P100, P200 and control), sucrose, fructose, mannitol, total organic acids, and polysaccharides concentration showed similar trend as those in PMJ. Whereas 1-kestose increased from the beginning to 24 h, followed by a decreasing trend towards the end. Unexpectedly, glucose content decreased in the process. The reduction of glucose is likely due to the polymerization of glucosyl moieties from sucrose by glucosyltransferase secreted by the *Leuconostoc*, resulting in the liberation of fructose (Eggleston 2002). This is consistent with the increased content of fructose that we observed. The sucrose degradation rate of SMJ was around 32% in 48 h (Figure 4a), which is much lower than that of PMJ. The lower degradation rate of

SMJ indicated a lower microorganism activity in SMJ, which further confirmed that the 76 °C heating process significantly reduced the number of microorganisms in SMJ, and those that survived could be thermophilic microbes.

The concentration of polysaccharides in the control for SMJ at the end of the degradation process was ~1100 mg/kg·Bx, which is much higher than that at the beginning (~40 mg/kg·Bx) (Figure 4f) and is almost four times of that in PMJ, even though the degradation rate of SMJ was lower than that of PMJ. The higher concentration of polysaccharides in degraded SMJ indicated that the microorganisms survived in SMJ could be the main microbe species that secret polysaccharides.

The total content of organic acids at the end of the degradation process was ~30 mg/kg·Bx, which is almost double of that at the beginning (Figure 4g). Alcohol concentration in the degraded SMJ fluctuated throughout the process. Overall, the total alcohol content increased (Figure 4h).

When SMJ is tested, P200 biocide gave a better performance in reducing sucrose degradation. This means P200 biocide could be more effective in inhibiting the growth of thermophilic microorganisms in juice, particularly after 24 h. Sucrose degradation in SMJ (from F3 and F4 Sugar Mill) treated with P200 was in the range of 15.5% to 30.1%.

SMJ treated with P200 biocide showed a relatively lower sucrose degradation, and lower concentrations of metabolic products, i.e. 1-kestose, fructose, mannitol, polysaccharides and organic acids Figure 4b,d,e,f,g.

Based on the laboratory tests for the two types of biocides (P100 and P200), as there were no significant changes in the first few hours of the degradation process, in the real-world production process, when processing under normal condition where no stoppage occurs, and the quality of the cane is good, the use of biocides may not be necessary. However, if there is a wet-weather stop, or there is an unexpected stoppage, we recommended using P100 in milling train, and dose it as early as possible in the milling train (e.g., 1# Mill). P200 would be beneficial for use in SMJ.

Factory trials at F2 Sugar Mill

The trials at F2 Sugar Mill were to identify whether it is useful to use biocide in the sugar factory. In the 2020 crushing season, we performed 6 testing periods including 4 “ON” periods and 2 “OFF” periods.

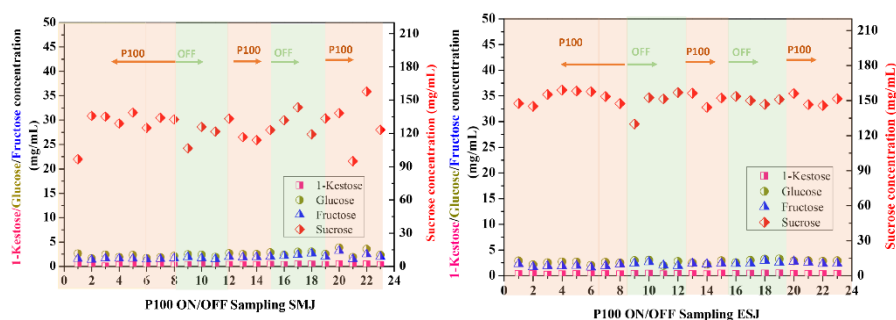


Figure 5. Sucrose, 1-kestose, glucose, fructose concentration in (a) SMJ and (b) ESJ samples collected from F2 Sugar Mill during P100 ON/OFF testing periods.

From the laboratory studies (see previous section), mannitol as an indicator of sugarcane juice degradation was detected only in juice samples that have been severely degraded. While it was not detected in the juice samples collected, which indicated that there was no significant juice degradation during the testing periods.

Figure 5a,b and Table 4 show the results of sugars (sucrose, glucose and fructose) and 1-kestose content in SMJ and ESJ collected during the testing periods. The first two testing periods were P100 “ON” mode, but the sample collecting process of the second ON period was disrupted by an unexpected stoppage, so the sample collection was only for the last two days. Similar disruption occurred with the third ON period. The average sucrose concentrations in SMJ samples collected from first ON, second ON, first OFF, third ON, second OFF, and fourth

ON periods were 126.3, 133.3, 121.9, 117.9, 132.0, and 128.6 mg/mL, respectively (Figure 5a). Glucose concentrations of the testing periods were 2.1, 2.0, 2.4, 2.6, 2.7, and 2.9 mg/mL, respectively, and fructose concentrations were 1.5, 1.6, 1.8, 1.9, 2.3, and 2.3 mg/mL, respectively. The average 1-kestose concentrations were similar between the ON and OFF period. No abnormal values were observed from the results.

The ratio between the glucose and fructose content (G/F ratio) has been used as an indicator to identify the sucrose microbial degradation (Eggleston 2002). Low G/F ratios indicate the occurrence of microbial degradation. Therefore, the G/F ratio of the juice samples (SMJ and ESJ) collected from the P100 “ON” and “OFF” testing periods were calculated and shown in Figure 6(a). No obvious differences were observed between the results of tests with and without use of the biocide, it is hard to identify the efficacy of biocide from the G/F ratio.

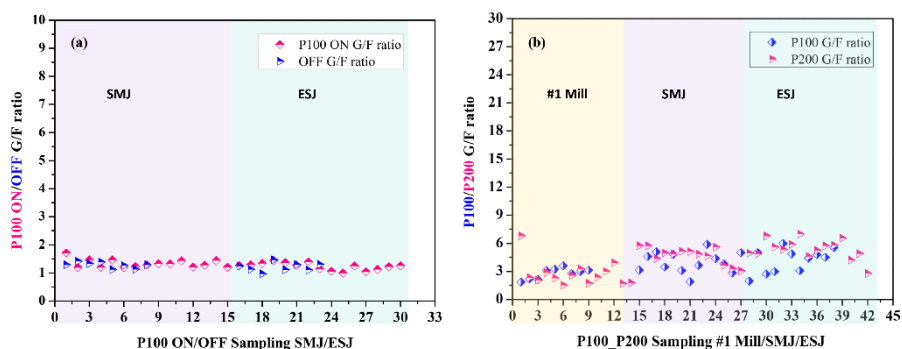


Figure 6. G/F ratio of (a) SMJ and ESJ samples collected from F2 Sugar Mill during P100 ON/OFF testing period and (b) #1 Mill juice, SMJ and ESJ samples collected from F4 Sugar Mill during P100_P200 testing period.

In addition to the sugars, non-sucrose impurities in SMJ that reflect the microbial activities were also analysed. As shown in Table 2, the overall average concentrations of polysaccharides and total organic acids in the SMJ samples during the P100 ON and OFF testing periods were 41.66 and 42.99 mg/kg·Bx, and 105.63 and 112.43 mg/kg·Bx, respectively. The P100 OFF period shows slightly higher content of impurities than that during the P100 ON testing period.

Furthermore, trend lines for the contents of polysaccharides and total organic acids in each of the testing periods were plotted and shown in Figure 7. The content of total organic acids decreased slightly in the four P100 “ON” testing periods (first, second, third and fifth), and there was also a similar decrease in content of total polysaccharides in the second, third and fifth P100 “ON” testing periods. However, when P100 was not used, both total polysaccharides and total organic acids showed an increasing trend. As in the laboratory degradation tests, the total organic acids content increased with time during the degradation process, so a higher concentration of total organic acids could be related to microbial activity. As a result, it is concluded that the addition of P100 reduced microbial degradation of the juice at F2 Sugar Mill.

Table 2. Average concentration of the impurities in SMJ and ESJ during P100 ON/OFF testing periods at F2 Sugar Mill.

Test	1-kestose (mg/mL)		Glucose (mg/mL)		Fructose (mg/mL)		Total polysaccharides (mg/kg·Bx)	Total organic acids (mg/kg·Bx)	Lactic acid (mg/kg·Bx)
	SMJ	ESJ	SMJ	ESJ	SMJ	ESJ	SMJ	SMJ	SMJ
P100 ON	0.29	0.30	2.42	2.63	1.82	2.16	41.66	105.63	2.94
P100 OFF	0.35	0.36	2.56	2.85	2.03	2.39	42.99	112.43	2.94

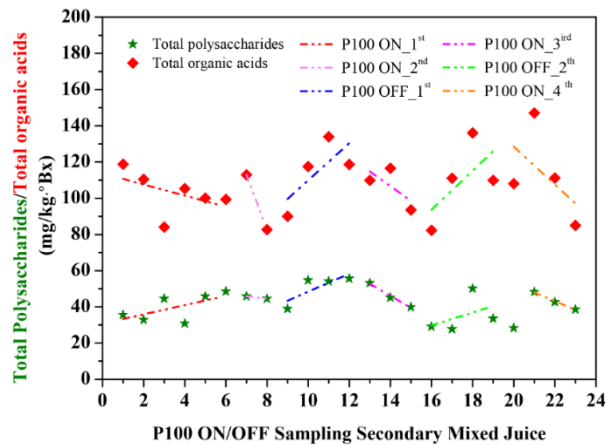


Figure 7. Concentration of total polysaccharides and total organic acids concentration in SMJ collected from F2 Sugar Mill P100 ON/OFF dosing periods.

Factory trials at F4 Sugar Mill

The effectiveness of P100 and P200 biocides was compared in factory scale test at F4 Sugar Mill. In this factory trials, mannitol was also not detected in all the juice samples, indicating no significant microbial degradation occurred in the during the testing periods.

Figure 8a,b,c and Table 3 present the sugars (sucrose, glucose, fructose and 1-kestose) results of #1 Mill juice, SMJ and ESJ. The average sucrose concentration in #1 Mill juice, SMJ and ESJ during the P100 and P200 testing periods were 196.71 and 192.40 mg/mL, 145.29 and 135.73 mg/mL, and 122.03 and 129.58 mg/mL, respectively. The variations could be due to changes of the incoming cane quality, which makes it difficult to judge the effectiveness of the biocides. The average concentrations for 1-kestose, glucose and fructose are generally higher with juice samples collected during P100 testing period.

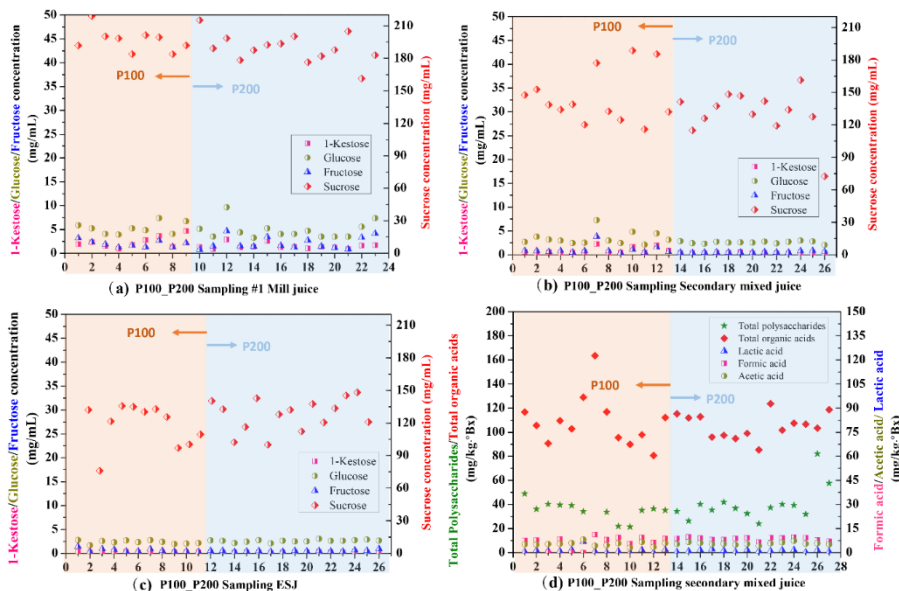


Figure 8. Sucrose, 1-kestose, glucose, fructose concentration in (a) # 1 Mill juice, (b) SMJ and (c) ESJ collected from F4 Sugar Mill during P100 and P200 testing period, and (d) the concentration of total polysaccharides and total organic acids concentration in SMJ collected from F4 Sugar Mill during P100 and P200 testing period.

Table 3. Average concentrations of sucrose, 1-kestose, glucose and fructose in #1 Mill juice, SMJ and ESJ collected from F4 Sugar Mill during P100 and P200 testing period.

Sample	Sucrose (mg/mL)		1-kestose (mg/mL)		Glucose (mg/mL)		Fructose (mg/mL)	
	P100	P200	P100	P200	P100	P200	P100	P200
#1 Mill juice	196.71	192.40	2.33	1.56	5.27	4.88	1.99	2.10
SMJ	145.29	135.73	0.86	0.39	3.45	2.60	1.04	0.57
ESJ	122.0	129.58	0.24	0.21	2.34	2.56	0.65	0.47

As shown in Figure 6(b), the overall average G/F ratios of SMJ and ESJ treated with P100 are slightly lower than that of the juices treated with P200, which indicated that P200 is more effective on the microorganisms that survive heat treatment. However, for the #1 Mill the average G/F ratios are very similar as the number of samples for comparison are small (Figure 6b).

The average polysaccharide content in SMJ samples during P100 and P200 testing periods was 35.14 and 39.91 mg/kg·Bx, respectively (Table 4 and Figure 8d). The average total organic acids content in SMJ samples from P100 and P200 testing period were 108.52 and 104.72mg/kg·Bx, respectively (Table 4 and Figure 8d). Like the results obtained from sugars analysis, there was no significant difference between the samples collected from the two testing periods. From these results, it is difficult to identify which one of these biocides is more effective under factory conditions.

Table 4. Total polysaccharides, total organic acids, and lactic acid content in SMJ samples collected from F4 Sugar Mill during the biocide testing periods.

Sample	Total polysaccharides (mg/kg·Bx)		Total organic acids (mg/kg·Bx)		Lactic acid (mg/kg·Bx)	
	P100	P200	P100	P200	P100	P200
SMJ	35.14	39.91	108.52	104.72	0.94	1.14

Economic analysis of biocide usage

To assess the economics of using biocide in the factory, we assumed a factory with the crushing capacity of 500 t/h, and the cost of the biocide was based on the price provide by the company from 2020-2021 crushing season. Without considering the drop in molasses yield, an increased sugar yield of 0.0375% covers the P100 biocide cost and an increase by 0.07% covers the P200 cost (Table 5).

Table 5. Economic assessment on the use of biocides.

Parameter	P100	P200
Biocide price (\$/kg)	2.5	3.5
Biocide dosage rate (ppm on cane)	7.5	10.0
Sugarcane crushing rate (t/h)	500	500
Biocide dosing rate (L/h)	3.75	5
Biocide cost (\$/day)	225	420
Assumed sugar yield (t sugar/t cane)	0.125	0.125
Sugar produced (t/day)	1500	1500
Sugar price (\$/t)	400	400
Sugar value (\$)	600,000	600,000
Biocide cost over sugar value	0.0375%	0.07%

CONCLUSIONS AND FUTURE RECOMMENDATIONS

P100 biocide is more effective in reducing the rate of sucrose degradation arising from mesophilic microorganisms. It could be more effective if applied to the milling-train stage. P200 is more effective in reducing the rate of sucrose degradation arising from thermophilic microorganisms, and so could be applied to secondary mixed juice.

When processing under normal condition where no stoppage occurs, and the quality of the cane is good, the use of biocides may not be necessary. However, if there is a wet weather stop, or there is an unexpected stoppage, it would be beneficial to use P100 in the milling train, and dose it as early as possible in the milling train (e.g. at 1# mill). P200 is preferred for use in SMJ.

There was no detectable sucrose loss, and no detectable amount of mannitol identified in the juice samples collected with the presence and absence of a biocide. P100 biocide could be used to reduce the non-sucrose impurities of polysaccharides and organic acids, as these are related to microbial activities.

Our preliminary data has indicated benefits in using biocides in Australian sugar mills. Recommendations for the future RD&A are:

- (1) Undertake extended factory trials with biocides in the “ON” and “OFF” modes in at least two different factories.
- (2) Conduct on-site small-scale degradation tests with the fresh PMJ and SMJ taken from the processing line at 1-h intervals with juices from similar rakes.
- (3) Test other types of FDA-approved biocides obtained from different suppliers and compare how they perform.
- (4) Estimate undetermined sucrose losses in a factory with and without biocides through extensive factory trials.

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