THE OCCURRENCE OF LACTIC ACID AND ASSOCIATED MICRO-ORGANISMS IN CANE SUGAR PROCESSING

Lynn McMaster and A.B. Ravno
Huletts Sugar Ltd., Mount Edgecombe, South Africa

ABSTRACT

Results obtained from a three year survey of the lactic acid and associated bacterial levels at various sugar mills in Natal are presented. Preliminary research involved establishing a suitable method for the qualitative and quantitative determination of lactic acid in various factory streams. The relationship between sucrose lost and lactic acid produced was investigated and an average weight ratio of 2:1 is postulated. It was also confirmed that lactic acid is the principal organic acid formed on the fermentation of sucrose by thermophilic bacteria.

The results from the surveys at the various mills indicate that, while lactic acid is absent in freshly harvested cane, it is usually to be found in the incoming cane by the time it reaches the mill. Further lactic acid is formed by bacterial fermentation at the expense of sucrose during juice extraction especially in the case of diffusion processes. Various methods for inhibiting the production of lactic acid are discussed. Quantitative lactic acid balances indicate that most of the lactic acid present in mixed juice ends up in the final molasses stream, presumably as calcium lactate.

INTRODUCTION

Cane juice provides a natural and highly nutritive chemical environment, rich in both organic and inorganic materials which are ideal for the growth of a variety of micro-organisms. It is therefore not surprising to find that micro-organisms develop during the extraction and processing of cane juice. The relatively specialised environment prevailing at various stages of the process selects for the development of particular micro-organisms. For example, the fairly low pH of juice in a milling tandem restricts viable micro-organisms to the acidophiles such as Leuconostoc and Lactobacillus. On the other hand the higher temperature and pH values associated with diffusion limits the growth of microbial flora to the thermophiles, such as Bacillus.

The most disturbing factor related to the activity of these micro-organisms is that sucrose is one of the principal nutrients utilised for their growth. The fermentation of sucrose by these bacterial genera results in the production of lactic acid as the principal organic acid formed.

Other micro-organisms which utilise sucrose, but do not produce lactic acid as a metabolic byproduct, are also present during juice extraction. These have been reported to be various yeasts and Aerobacter.
philic actinomycetes. While their role in sucrose destruction cannot be ignored, it is generally acknowledged that the primary organisms responsible for sugar loss in the cane sugar industry are the lactic acid bacteria.

A considerable amount of research has been done on the occurrence of lactic acid in beet sugar factories. It was found that the low temperatures and lengthy residence times often associated with beet diffusion resulted in serious bacterial fermentation with a resultant sucrose loss and pH reduction in the juice. Several American and British workers have reported that the principal organic acid formed by thermophilic bacteria is lactic acid. Attempts have been made to correlate the amount of sucrose lost with the weight of lactic acid produced. Reported values vary between 0.81:1 and 4.44:1, the average being approximately 2:1 on a weight basis.

It is now generally accepted that the formation of lactic acid during sugar manufacture is highly undesirable due to the costly sucrose losses which it incurs. However, very few details are available as to the extent of bacterial sucrose fermentation and lactic acid production in the cane, as opposed to the beet sugar industry. As a result, it was decided in 1973 to embark upon a detailed investigation on the occurrence of lactic acid and its associated micro-organisms at several Hulett's mills in Natal.

At the outset, it was considered necessary to conduct a number of preliminary investigations. These centred around the development of a reliable qualitative and quantitative method for the determination of lactic acid in both juice and molasses streams. Secondly, whilst it is known that the principal organic acid formed by Leuconostoc and Lactobacillus during conventional milling is lactic acid, it was necessary to ascertain whether this was the case for the thermophilic bacteria associated with sugar cane diffusion. Thirdly, the relationship between the weight of sucrose lost and the amount of lactic acid produced was investigated.

Once these preliminary studies were completed, detailed surveys on the presence of lactic acid were undertaken at several mills during the 1974/75, 1975/76, and 1976/77 crushing seasons. Lactic acid levels in the incoming cane, across the extraction plant and in the boiling house were monitored.

**Experimental Procedure**

**Preliminary Studies**

The quantitative determination of lactic acid was based upon a slightly modified form of the high precision ion-exchange/colorimetric method developed by Oldfield and Shore. The evaluation of the proportion of lactic acid in the total organic fraction formed from the bacterial fermentation of sucrose in cane juice was based on the procedure suggested by Carruthers and his co-workers. A slight variation of the method used by these workers was adopted for the determination of the ratio of sucrose lost to lactic acid produced.
**Bacterial Counts**

On primary and mixed juice 250 ml catch samples were collected aseptically from which duplicate 1 ml aliquots were diluted separately in series to 1 in 10,000 concentration in 1% sterile saline. A 1 ml subsample was removed from each of the final dilutions and pipetted into petri-dishes. Molten cane juice agar (pH 5.5) was poured into the dishes and the contents well mixed. After solidifying, the plates were incubated for 48 hr or 72 hr at 30°C. Colonies were counted using a Gerber Colonicont.

For diffuser juices, 250 ml catch samples were also collected and 1 ml sub-samples were immediately diluted 1 to 10 in 1% sterile saline. The diluted samples were then filtered aseptically through Sartorius membrane filters (pore diameter 0.45 microns) and after washing twice with sterile distilled water, the membranes were placed onto Oxoid dextrose tryptone agar plates. These were subsequently incubated for 24 hr at 65°C, after which bacterial colonies were counted.

**Lactic Acid Analysis**

Full details of the method of sample preparation and the ion exchange and resin regeneration procedures are given in Appendix A.

**RESULTS AND DISCUSSION**

**Preliminary Studies**

After trials on various methods for determining lactic acid, the ion-exchange/colorimetric procedure of Oldfield and Shore\(^\text{13}\) was selected as being the most suitable. Extensive testing indicated that the reproducibility on duplicate analyses was better than 5% and that there was virtually no interference from various other acids (viz. aconitic, succinic and malic) which may be present in cane juice. The precision of the method was checked by adding a known amount of lactic acid to a juice sample and determining its recovery. A typical set of results is shown in Table I.

**TABLE I. Precision of the lactic acid method.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Eluate volume (ml)</th>
<th>Lactic acid measured (mg)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 ml juice (+) 50 ml distilled water</td>
<td>562</td>
<td>1.41</td>
<td></td>
</tr>
<tr>
<td>50 ml juice (+) 5 mg added lactic acid standard</td>
<td>555</td>
<td>6.40</td>
<td>99.8</td>
</tr>
<tr>
<td>5 mg lactic acid standard only</td>
<td>580</td>
<td>4.93</td>
<td>98.6</td>
</tr>
</tbody>
</table>
In a similar series of tests a number of final molasses samples were spiked with known amounts of lactic acid and recoveries of 96 — 99% were obtained in the ion-exchange columns.

From a separate set of experiments it was found that an average of 91% of the total organic acid formed by thermophilic bacteria, isolated from a diffuser, was lactic acid. Actual values ranged between 88 and 99% and this variation can probably be attributed to the bacterial types within the culture which determine whether the fermentation is of the homo- or heterolactic type. These results are similar to those obtained by Car ruthers and Oldfield in beet diffusers.

The third stage of the preliminary work involved the attempt to determine the correlation between the amount of sucrose lost and lactic acid produced. It has always been extremely difficult to obtain an accurate measure of the quantity of sucrose lost due to micro-organism growth at a sugar mill. Hence it would be very useful if this could be deduced from measurements of the lactic acid content of juice. The results obtained during the present study showed ratios which varied between 0.77 : 1 and 4.3 : 1, the average being, 2.15 : 1. These values are very similar to those reported for the beet industry.

The fact that this ratio is not constant can be attributed to an interplay between the following factors:

a) There are numerous active bacteria developing at any given time during juice extraction. No two living micro-organisms can be assumed to assimilate simultaneously the same amount of carbohydrate in an identical manner. It has been demonstrated by Klaushofer and Pollach that the ratio varies even when pure cultures are inoculated into chemically identical media.

b) The environmental conditions prevailing in the juice (e.g. temperature, pH, oxygen content etc.) will influence the rate and type of bacterial sucrose metabolism at any given time.

c) The breakdown of sucrose to lactic acid is an energy-releasing process associated with micro-organism growth. However various other growth processes (e.g. the formation of bacterial slimes and enzymes) also require energy and may consume sucrose without producing lactic acid. Hence the utilisation of sucrose for various biosynthetic processes will interfere with the ratio between sucrose lost and lactic acid produced.

d) Sucrose is not the only fermentable carbohydrate in cane juice which can be fermented to lactic acid. It has been found that glucose and fructose can also be utilised by the micro-organisms in cane juice to produce lactic acid.

Nevertheless it is suggested that a 2 : 1 ratio can be used to provide an estimate of sucrose losses by thermophilic bacteria.
Lactic Acid in Cane Deliveries

No lactic acid was found in the juice extracted in a laboratory press from freshly harvested, mature cane stalks. This was not unexpected since lactic acid has not been reported as a natural constituent of sugar cane. However, once cane has been harvested, it is prone to attack by invading micro-organisms which can ferment sucrose to lactic acid.

A short series of tests was undertaken at Mount Edgecombe mill in an attempt to obtain some typical figures for the level of lactic acid in delivered whole-stick cane. All cane consignments in South African mills are sampled just prior to the first mill for purposes of poll distribution to the growers. Over a period of a week a number of 4 hr composite samples was preserved from the juice extracts from each of these prepared cane samples. Lactic acid content of each composite was determined and the relevant figures are presented in Table II.

### Table II. Lactic acid content of delivered cane.

<table>
<thead>
<tr>
<th>Refracto brix</th>
<th>Apparent purity</th>
<th>% Burnt cane</th>
<th>Lactic Acid % brix</th>
</tr>
</thead>
<tbody>
<tr>
<td>14.43</td>
<td>85.17</td>
<td>83</td>
<td>0.171</td>
</tr>
<tr>
<td>15.06</td>
<td>84.53</td>
<td>46</td>
<td>0.018</td>
</tr>
<tr>
<td>15.78</td>
<td>85.23</td>
<td>60</td>
<td>0.049</td>
</tr>
<tr>
<td>15.57</td>
<td>83.69</td>
<td>71</td>
<td>0.042</td>
</tr>
<tr>
<td>15.71</td>
<td>85.17</td>
<td>54</td>
<td>0.082</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td></td>
<td></td>
<td><strong>0.073</strong></td>
</tr>
</tbody>
</table>

It is immediately apparent that delivered cane does contain lactic acid and that the amount varies considerably. There are a number of factors which will influence the lactic acid content of delivered cane. Firstly, the greater the delay between harvesting and crushing the greater the level of bacterial contamination and hence lactic acid. This effect would normally be worse for chopper harvested cane than for whole-stick cane. Secondly, when cane has been burnt, bacterial infection of the stalk is rapid and hence burnt cane would be expected to contain more lactic acid than trashed cane. The results in Table II appear to confirm this trend. Finally, the prevailing weather conditions during the time interval between harvesting and crushing will also affect lactic acid levels, since it has been reported that Leuconostoc activity appears to increase under wet conditions.

Lactic Acid in Milling

The Mount Edgecombe milling tandem was monitored regularly over a period of six months during the 1976/77 crushing season in order to assess the level of micro-organism activity. Altogether 54 composite 24 hr samples...
of first expressed juice and mixed juice were collected for lactic acid analyses, whilst 103 catch samples of juice were taken for viable plate counts. The average values recorded over this period are listed in Table III.

**TABLE III.** Bacterial assay of Mount Edgecombe tandem.

<table>
<thead>
<tr>
<th></th>
<th>First expressed juice</th>
<th>Mixed juice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>Std. dev.</td>
<td>Average</td>
</tr>
<tr>
<td>Lactic acid % brix</td>
<td>0.016</td>
<td>0.008</td>
</tr>
<tr>
<td>Bacteria/ml juice</td>
<td>4.50x10⁶</td>
<td>1.78x10⁶</td>
</tr>
</tbody>
</table>

At first glance it appears that the average level of micro-organisms was higher in first expressed juice at the front end of the tandem. However this should not be taken to indicate that no development of micro-organisms takes place along the tandem, since the lower average value for mixed juice is largely due to the dilution effect of the added imbibition water. One serious drawback in attempting to gauge the level of micro-organism activity from viable plate counts results from the use of catch, rather than composite, samples (since no preservative may be added). This introduced the possibility that the sample is not fully representative of the actual conditions prevailing.

The lactic acid figures in Table III show that there is apparently a large increase in lactic acid content across the milling tandem. However the average lactic acid value of 0.016% on brix for first expressed juice is considerably lower than the mean value of 0.073% reported in Table II for the incoming cane. The average for mixed juice, collected over the same period as the data in Table II, was 0.083% on brix which points to only a slight increase across the length of the tandem. One possible explanation for the low value observed in first expressed juice is that sucrose is preferentially extracted in the first mill giving rise to a low level of lactic acid extraction at that stage.

The foregoing results tend to indicate that the average lactic acid content of mixed juice at Mount Edgecombe is around 0.07% on brix and that the bulk of this probably enters the mill with the incoming cane. This only serves to emphasise the need to reduce delays between harvesting and crushing to a minimum. However further work in this area is necessary in order to verify the trends reported above.

**Lactic Acid in Diffusion**

The 400 tch cane diffuser at Amatikulu and the 220 tch bagasse diffuser at Empangeni were monitored for both lactic acid and micro-organism levels during the 1974/75, 1975/76 and 1976/77 crushing seasons. Both catch and composite samples were taken at various points along the diffusers and from the mixed juice tanks as described in the experimental procedure.
Some typical values for the thermophilic bacterial counts recorded along the length of the diffusers are given in Table IV.

**TABLE IV.** Bacterial counts on diffuser samples.

<table>
<thead>
<tr>
<th>Date</th>
<th>Sample location</th>
<th>Temperature (°C)</th>
<th>Thermophiles/ml juice at 65°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>15th November</td>
<td>AK - cell 2</td>
<td>65</td>
<td>TNTC</td>
</tr>
<tr>
<td>1974</td>
<td>AK - cell 5</td>
<td>70</td>
<td>TNTC</td>
</tr>
<tr>
<td>1974</td>
<td>AK - cell 8</td>
<td>70</td>
<td>TNTC</td>
</tr>
<tr>
<td>27th November</td>
<td>AK - cell 2</td>
<td>63</td>
<td>30</td>
</tr>
<tr>
<td>1974</td>
<td>AK - cell 5</td>
<td>76</td>
<td>130</td>
</tr>
<tr>
<td>1974</td>
<td>AK - cell 11</td>
<td>74</td>
<td>400</td>
</tr>
<tr>
<td>12th December</td>
<td>AK - cell 2</td>
<td>69</td>
<td>10</td>
</tr>
<tr>
<td>1974</td>
<td>AK - cell 5</td>
<td>72</td>
<td>650</td>
</tr>
<tr>
<td>1974</td>
<td>AK - cell 11</td>
<td>65</td>
<td>TNTC</td>
</tr>
<tr>
<td>12th December</td>
<td>EM - cell 2</td>
<td>71</td>
<td>30</td>
</tr>
<tr>
<td>1974</td>
<td>EM - cell 5</td>
<td>72</td>
<td>2230</td>
</tr>
<tr>
<td>1974</td>
<td>EM - cell 11</td>
<td>61</td>
<td>TNTC</td>
</tr>
<tr>
<td>5th February</td>
<td>EM - cell 2</td>
<td>75</td>
<td>180</td>
</tr>
<tr>
<td>1975</td>
<td>EM - cell 5</td>
<td>73</td>
<td>60</td>
</tr>
<tr>
<td>1975</td>
<td>EM - cell 11</td>
<td>63</td>
<td>300</td>
</tr>
<tr>
<td>13th February</td>
<td>AK - cell 1</td>
<td>70</td>
<td>900</td>
</tr>
<tr>
<td>1975</td>
<td>AK - cell 5</td>
<td>75</td>
<td>800</td>
</tr>
<tr>
<td>1975</td>
<td>AK - cell 11</td>
<td>72</td>
<td>TNTC</td>
</tr>
</tbody>
</table>

**Legend:** AK — Amatikulu cane diffuser; EM — Empangeni bagasse diffuser; TNTC — Too numerous to count. All cells numbered from front (feed) end.

It is readily apparent that the thermophilic bacterial population in the diffusers and from the mixed juice tanks as described in the experimental and from day to day. With the continuous circulation of juice within the diffuser, bacterial numbers would not be expected to show a constant graduation through the process. It did appear however that at certain times bacterial counts increased along the length of the diffuser from the front to the back-end.

This it not unexpected since it is probable that micro-organisms enter both at the head of the diffuser with the incoming cane/bagasse and at the tail-end with the presswater and/or imbibition. Conditions at the tail-end of the diffuser are probably more stable and temperatures are generally lower and hence it is considered likely that a reservoir of standing thermophilic bacteria would develop at the back-end. This would then be carried forward with the circulating juice to inoculate the whole diffuser.
The lactic acid surveys revealed some interesting trends. The distribution of lactic acid formation at various temperatures is shown in Table V.

**TABLE V.** Average lactic acid content of diffuser juice at various temperatures.

<table>
<thead>
<tr>
<th>Temperature range (°C)</th>
<th>Lactic acid % brix</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 - 55</td>
<td>0.188</td>
</tr>
<tr>
<td>56 - 59</td>
<td>0.170</td>
</tr>
<tr>
<td>60 - 65</td>
<td>0.399</td>
</tr>
<tr>
<td>66 - 69</td>
<td>0.556</td>
</tr>
<tr>
<td>70 - 75</td>
<td>0.430</td>
</tr>
<tr>
<td>76 - 79</td>
<td>0.111</td>
</tr>
<tr>
<td>80 - 85</td>
<td>0.114</td>
</tr>
</tbody>
</table>

It appears that lactic acid formation reaches a peak between 60° and 75°C. Below 60°C it is probable that the lower temperatures favour the development of acetic acid forming *Bacillus* strains while temperatures above 75°C are inhibitory to bacterial growth.

To ascertain whether there was any gradation in lactic acid formation along the length of the diffuser, representative composite samples were collected from cells 1, 7 and 11 at Amatikulu and analysed for lactic acid. The results are illustrated in Fig. 1.

**FIGURE 1.** Lactic acid distribution along a diffuser, and the effect of temperature on lactic acid production.
These results indicate two significant trends. Firstly lactic acid levels increase towards the back end of the diffuser, confirming that bacterial activity is probably higher in this area. Secondly there is a substantial increase in lactic acid content throughout the diffuser during operation at lower temperatures.

Results obtained at Amatikulu and Empangeni during the 1974/75 and 1975/76 crushing seasons indicated fairly high lactic acid levels in both diffusers.\textsuperscript{10,11} Hence it was decided to monitor the lactic acid content of weekly mixed juice composite samples at both mills throughout the 1976/77 season. The data obtained for the major part of this season are recorded in Fig. 2 together with the relevant weekly rainfall figures for each centre.

**FIGURE 2.** Lactic acid levels in mixed juice and rainfall figures at Amatikulu and Empangeni (1976).

It is interesting to note the parallel behaviour at both factories, which suggests that there is probably a seasonal trend in the lactic acid content of the incoming cane. There also appears to be a relationship between rainfall and lactic acid content. As in the case at Mount Edgecombe, this could be due to enhanced bacterial contamination of the harvested cane or to increased transport delays as a result of wet in-field conditions.

The average lactic acid content of mixed juice over the reported period was 0.249\% on brix at Empangeni and 0.173\% at Amatikulu, which can be compared with the figure of 0.062\% given for Mount Edgecombe in Table III. It is evident that although viable plate counts for thermophiles in...
the diffusers (Table IV) were much lower than for the mesophilic microorganisms in a milling tandem (Table III), the fermentation of sucrose to lactic acid is far more serious in the diffusers. This is probably due to the longer residence times and more favourable temperature and pH conditions which can prevail for thermophilic bacteria in a diffuser. It has also been noted that the lactic acid content of diffuser juice increases markedly during relatively short, non-scheduled stops (1 — 2 hours), particularly if temperatures are low (less than 75°C). It is likely that the considerably higher lactic acid levels at Empangeni are attributable to the lower average diffuser temperatures of around 70°C compared to those at Amatikulu, which are generally above 75°C.

The effect of temperature is amply demonstrated by the results shown in Fig. 2 for Amatikulu during weeks 24 and 25. Throughout this period diffuser temperatures, which are normally maintained above 75°C, were reduced for test purposes to a maximum of 70°C. As can be seen the lactic acid level in mixed juice increased almost three-fold.

Since lactic acid formation is accompanied by a loss of sucrose it is apparent that some form of control over the development of thermophilic bacteria is essential in diffusers. This can be achieved by maintaining temperatures high enough throughout the diffuser to inhibit bacterial activity (i.e. above 75°C)9,10 or by the judicious use of chemical bactericides. It was demonstrated at Amatikulu during 1974, that shock dosing each cell of the diffuser once every four hours with formalin (as a 40% formaldehyde solution) at a level of 300 p.p.m. on cane, was effective in controlling thermophilic activity.9,10 During 1976 a similar treatment was applied to the diffuser at Empangeni during weeks 21, 23 and 31. In the last two periods lactic acid levels were suppressed (see Fig. 2) but on the first occasion the formalin appeared to have no effect. This was due to the fact that in week 21 a high number of non-scheduled mill stops was recorded, during which juice circulation within the diffuser was interrupted and hence formalin distribution and mixing was poor.

**Lactic Acid Distribution/Recovery**

In view of the relatively high quantities of lactic acid present in mixed juice it was decided to monitor the distribution of lactic acid in the boiling house. The main purpose of this survey was to establish whether further lactic acid was produced, particularly across clarification, and also to trace the path of the lactic acid through the boiling house.

During the 1976/77 season, weekly composite samples of mixed juice and syrup (from Amatikulu and Empangeni) and of final molasses (from Amatikulu, Empangeni and Mount Edgecombe) were collected. In addition 54 composite 24 hr samples of clear juice were collected at Mount Edgecombe. All these samples were analysed for lactic acid. The average values over the six month period from May to November 1976 are shown in Table VI.
TABLE VI. Average lactic acid levels in boiling house.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Amatikulu Average</th>
<th>Std. dev.</th>
<th>Empangeni Average</th>
<th>Std. dev.</th>
<th>Mount Edgecombe Average</th>
<th>Std. dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixed juice</td>
<td>0.173</td>
<td>0.133</td>
<td>0.249</td>
<td>0.132</td>
<td>0.062</td>
<td>0.031</td>
</tr>
<tr>
<td>Clear juice</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.069</td>
<td>0.029</td>
</tr>
<tr>
<td>Syrup</td>
<td>0.147</td>
<td>0.138</td>
<td>0.210</td>
<td>0.139</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Final molasses</td>
<td>0.714</td>
<td>0.441</td>
<td>0.862</td>
<td>0.237</td>
<td>0.468</td>
<td>0.254</td>
</tr>
</tbody>
</table>

(All figures expressed as lactic acid % on brix).

The results in Table VI show that there is perhaps a slight drop in lactic acid content across clarification and evaporation. This is not unexpected since the addition of lime during clarification will convert the lactic acid to calcium lactate which has a fairly high solubility product,\(^{19}\) and thus is unlikely to be precipitated. The considerably higher lactic acid levels in final molasses are consistent with the concentration effect resulting from the crystallisation and removal of sucrose, which forms a large proportion of the brix in mixed juice and syrup. The lactic acid content of Mount Edgecombe molasses appears somewhat higher than would be expected from the average mixed juice figure, but this can probably be attributed to the fact that the mixed juice/clear juice figures are not strictly comparable with those for molasses, since the sampling periods were not identical. The juice analysis refer to random 24 hr composite samples whilst those for molasses are weekly composites.

The fairly high standard deviations listed in Table VI indicate that there were considerable variations in lactic acid levels from week to week. However the relative trends from mixed juice through syrup to final molasses were very consistent, especially at the diffuser mills, as is illustrated in Fig. 3 by the results from Amatikulu.

The large increase in the lactic acid content of mixed juice and syrup during weeks 24 and 25 coincides with the period of low temperature operation on the diffuser. It is interesting to note that the higher levels in molasses show an offset of approximately one week, which corresponds with the normal process lag.

*Types of Micro-organisms Present*

Although the nutritive medium of cane juice is common to both milling and diffusion plants, the differing environmental conditions result in the development of different types of micro-organisms. Examination of the milling tandem at Mount Edgecombe showed that the predominant forms of
micro-organisms were bacteria of the *Leuconostoc* and *Lactobacillus* genera together with a variety of yeasts. This observation is in agreement with previous reports from the West Indies\(^1\) and Australia.\(^1\)

Whilst low pH is probably the limiting growth factor in a milling tandem, the high temperatures associated with diffusion restrict the development of microbial flora to the thermophiles. Altogether 44 different bacterial strains were isolated from the diffusers at Amatikulu and Empangeni. All these bacteria belonged to the spore-forming genus *Bacillus*. Fourteen of the isolates were identified as strains of *B. stearothermophilus* and the remainder as *B. coagulans*. These identification tests were carried out according to the methods of Gordon et al.\(^7\), Hollaus,\(^8\) Walker and Wolf\(^18\), and Wolfang and Barker.\(^20\)

**CONCLUSIONS**

Although the survey on the occurrence of lactic acid and its associated micro-organisms at various cane sugar factories in Natal is incomplete, several significant factors have clearly emerged.

It has been shown that although lactic acid is absent in freshly harvested cane stalks, it is usually present in cane upon receipt at the mill. The extent to which it is found probably depends inter alia upon the delay...
between harvesting and crushing, the prevailing weather conditions during this period and whether the cane was burnt prior to cutting.

Lactic acid is also present in mixed juice from a conventional milling tandem, but it appears that the bulk of this enters the mill with the incoming cane. However, there is considerable evidence that substantial amounts of lactic acid can be formed by the bacterial fermentation of sucrose during diffusion. It has been shown that this thermophilic activity and its resultant sucrose losses can be suppressed by maintaining temperatures above 75°C throughout the diffuser or by the addition of formalin. It is also evident that little or no additional lactic acid is formed during clarification or evaporation and that the lactic acid present in the juice is finally concentrated in the exhaust molasses stream.

ACKNOWLEDGEMENTS

The authors are indebted to Mr. J.B. Alexander of Hulett's Sugar Ltd. and to the staff of the Sugar Milling Research Institute and the University of Natal for their help and guidance. The kind assistance of Miss L. Bookstein and the factory personnel at Amatikulu, Empangeni and Mount Edgecombe for the execution of much of the analytical work is gratefully acknowledged. Finally, thanks are due to the Board of Directors of Hulett's Sugar Ltd. for permission to publish this paper.

REFERENCES


APPENDIX A

Determination of Lactic Acid

Sample Preparation

Juice samples of 250 ml volume were collected and filtered through Whatman No. 45 paper under vacuum using 2.5 g Kieselguhr as filter aid. Twenty ml of the filtrate was diluted 1 : 1 with distilled water. The sample was then ready for ion-exchange extraction. All juice samples were preserved by adding mercuric chloride and chilling.

In the case of syrup samples, a representative volume was collected and after mixing thoroughly, 2-3 g were accurately weighed out and diluted to 25 ml with water. The sample was then ready for ion-exchange. Syrup samples were also preserved using mercuric chloride and chilled.

On final molasses, representative weekly composite samples were collected. After thorough mixing, approximately 1 g was accurately weighed out into a 25 ml volumetric flask. The molasses was first completely dissolved in 15 ml of distilled water and then made up to the 25 ml mark with water. The sample was then ready for ion-exchange.

Ion Exchange Procedure

The prepared samples were first run through a resin column containing a bed volume of 25 ml of Amberlite IR 120 resin for cation removal. The sample flow rate was 3 ml/min. The acidic effluent was collected and passed through a second column containing 25 ml of Amberlite IRA 402 at a rate of 3 ml/min. Any acids present in the sample were captured in this second column by the negatively charged resin. Before eluting the acids from this column, the resin was washed with distilled water at a rate of 6 ml/min until sugar-free. All washings were discarded. The acids were then stripped from the IRA 402 by eluting with 300 ml of 0.1 N NaCl at a rate of 3 ml/min. When elution with the sodium chloride was completed, distilled water was passed through the column until the final eluate volume was approximately 400 ml. The lactic acid content of the eluate was then determined according to the colorimetric method of Oldfield and Shore.13
Both resins were regenerated in 500 ml batches as follows:

**Amberlite IR 120** — The resin was slurried with distilled water and transferred into a large cylindrical separating funnel. Care was taken to eliminate all entrained air bubbles. Three bed volumes of 1 N HCl were run through the column at a rate of 3 ml/min. The column was then washed until acid free with distilled water. The regenerated resin was then ready for use.

**Amberlite IRA 402** — A slurry of the resin was prepared with distilled water and poured into a cylindrical separating funnel as before. One bed volume of 0.1 N HCl was run through the column at a rate of 3 ml/min to convert all the resin to the chloride form. The column was then washed with distilled water until acid-free before passing a 1 N NaOH solution through the column until the runnings were virtually free of chloride. It was found that this required approximately 15 bed volumes of caustic soda. The column was again washed with distilled water until free of hydroxyl ions before adding a 0.1 M Na$_2$CO$_3$ solution at 3 ml/min until the pH of the eluate was the same as for the soda ash solution. Finally the column was again washed with distilled water until the effluent was neutral in pH. The regenerated IRA 402 was then ready for use.

---

**EL ACIDO LACTICO Y MICRO-ORGANISMOS ASOCIADOS EN EL PROCESO DE ELABORACION DEL AZUCAR DE CAÑA**

L. McMaster y A.B. Ravnø

**RESUMEN**

Este trabajo presenta los resultados de un examen por tres años de duración sobre el ácido láctico y bacterias asociadas, en varios centrales azucareros de Natal. La investigación preliminar involucró establecer un método adecuado para la determinación cualitativa y cuantitativa del ácido láctico en distintos flujos de líquidos de las fábricas. Se investigó la relación entre la sacarosa perdida y el ácido láctico producido; quedando postulada una relación proporcional por peso de 2:1. Se confirmó que el ácido láctico es el principal ácido orgánico formado por la fermentación de la sacarosa por acción de bacterias termofílicas.

Los resultados del estudio en varios centrales indican que, entretanto el ácido láctico está ausente en la caña recien cosechada, generalmente aparece en la caña entrante cuando llega a la fábrica. Se forma ácido láctico adicional, a expensas de la sacarosa y por fermentación bacteriológica, durante la extracción del jugo, especialmente en el proceso por difusión. Este trabajo trata sobre varios métodos para inhibir la producción de ácido láctico. Los balances cuantitativos hechos del ácido láctico indican que la mayor parte del ácido láctico presente en el jugo mixto termina formando parte de las mieles finales, presumiblemente como lactato de calcio.