SUCROSE LOSS IN DIFFUSION WITH REFERENCE TO THERMOPHILIC BACTERIA AND LACTIC ACID

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Abstract

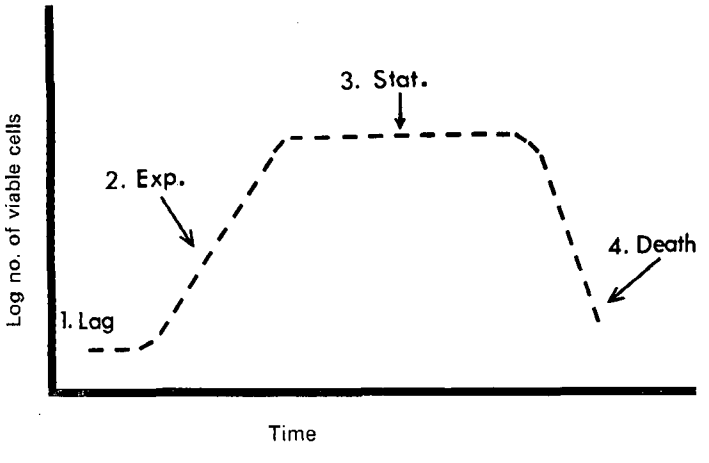
The potential degradation of sucrose by hyperthermophilic bacteria during the diffusion process was investigated. It was found that the organic acids formed on bacterial fermentation of sucrose comprised predominantly lactic acid. The observed weight ratio of approximately two parts sucrose destroyed to one part lactic acid produced is in good agreement with that found in beet diffusers. It was observed that the actual lactic acid and bacterial count levels in diffusers tended to be higher when diffusion temperatures were not maintained above approximately 75°C. The use of formalin was shown to be an effective method for controlling bacterial activity in a diffuser.

Introduction

In 1966 the first diffusers were introduced in the South African sugar industry as an alternative to conventional milling for the extraction of sucrose from cane or bagasse. Although diffusion is still relatively new in South Africa, it has long been used in the beet sugar industry and extensive research has been conducted into many facets of the process, including its microbiological aspects. The latter type of research has revealed that bacteria are undesirable inhabitants in a diffuser as they may be held responsible, under certain conditions, for substantial and costly sucrose losses.

The high temperatures associated with diffusion permit only the development of a special class of bacteria, the hyperthermophiles, and temperatures in the range 60° to 70°C could present ideal conditions for the active growth of an infinite number of these micro-organisms. Generally the bacterial population within a diffuser may be divided into two groups, namely those permanently growing and circulating in the diffuser and a heterogeneous group introduced continually with the incoming cane; not all of the latter group will be hyperthermophiles.

Graphically the growth of a bacterial species may be depicted as shown in the following sketch.



The life cycle falls into four distinct sections. Initially, during the lag phase there is little increase in cell number whilst the bacteria adapt to their environment. This is followed by the exponential growth phase, where the growth of cells is rapid and the population increases.

The third stage is the stationary phase in which the growth rate drops and the number of cells reaches a plateau. Finally this is followed by the death phase, where the viable cells die.

The exponential growth phase is usually terminated by a decrease in the availability of nutrients and by an accumulation of toxic metabolic by-products. Under low temperature conditions in a diffuser, viable bacteria may be considered to be in a permanent state of exponential growth, as a fresh source of nutrients is continually entering while toxic by-products are simultaneously removed in the extracted juice.

Research on beet diffusion has indicated that hyperthermophiles ferment sucrose as a substrate to produce organic acids, the major constituent being lactic acid[5,6]. The weight ratio of sucrose destroyed to lactic acid produced is normally taken as 2 to 1.[5,10,11] The use of formaldehyde as a bactericide is practised in the beet industry.[6,7,9] With these factors in mind, it was decided to conduct investigations along similar lines on cane/bagasse diffusers.

Experimental procedure

Raw juice from a cane diffuser (Amatikulu) and two bagasse diffusers (Empangeni and Dalton) was examined. In each case 250 ml juice samples were drawn at various points along the diffusers for bacterial count and lactic acid estimations. Full details of the experimental method for these two determinations are given in Appendix I and II respectively.

Freshly-crushed sterile raw juice was used for the laboratory studies on the organic acids formed during fermentation. The method used for the analysis of the proportion of lactic acid to total organic acids was that published by Carruthers *et al.*[6] For the determination of the ratio of sucrose destroyed to lactic acid produced, the procedures suggested by Carruthers and his co-workers[5] were followed except that juice pH's were maintained at 6,9 or 6,1 by the use of 2N NaOH.

Discussion of results

(1) *Analysis of diffuser samples*

A considerable amount of work was done to check the lactic acid determination which showed it to be both reliable and reproducible. As a result it is suggested that this provides a more accurate assessment of bacterial activity within a diffuser than laboratory colony counts at 65°C on artificial media. Many of the bacteria present in cane juice are of the spore-forming genus *Bacillus*. These spores remain dormant under unfavourable conditions (e.g. high temperature, lack of suitable nutrients, etc.) and only germinate when the environment is suitable, as on commercial media. Hence the bacteria which grow on the laboratory membranes need not necessarily have been active in the diffuser.

The results of the bacterial count and lactic acid determinations on the various diffuser samples are given in Table 1.

TABLE 1

Bacterial counts and lactic acid contents of diffuser samples

Date	Sample	Temp. °C	Themophiles/ml juice at 65°C	Lactic acid mg/litre juice
15.11.74	AK Cell 2	65	TNTC	1 390
15.11.74	AK Cell 5	70	TNTC	1 620
15.11.74	AK Cell 8	70	TNTC	1 950
27.11.74	AK Cell 2	63	30	95
27.11.74	AK Cell 5	76	130	95
27.11.74	AK Cell 11	74	400	41
4.12.74	UC Cell 1	77	410	160
4.12.74	UC Cell 6	82	890	92
4.12.74	UC Cell 11	76	750	170
12.12.74	AK Cell 2	69	10	760
12.12.74	AK Cell 5	72	650	770
12.12.74	AK Cell 11	65	TNTC	790
12.12.74	EM Cell 2	71	30	370
12.12.74	EM Cell 5	72	2 230	400
12.12.74	EM Cell 11	61	TNTC	410
18.12.74	UC Cell 1	78	180	97
18.12.74	UC Cell 6	83	60	183
18.12.74	UC Cell 11	76	300	241
5. 2.75	EM Cell 2	75	380	341
5. 2.75	EM Cell 5	73	80	405
5. 2.75	EM Cell 11	63	TNTC	397
13. 2.75	AK Cell 1	70	800	203
13. 2.75	AK Cell 5	75	900	321
13. 2.75	AK Cell 11	72	TNTC	393

Key: AK — Amatikulu diffuser (cane)
EM — Empangeni diffuser (bagasse)
UC — Union Co-op. diffuser Dalton (bagassse)
TNTC — too numerous to count

It is readily apparent that the bacterial population in these diffusers varies very widely, both along the length of the diffuser at any one time and from day to day. To a lesser extent the lactic acid concentration shows a similar pattern. With the continual circulation of juice within the diffusers, bacterial numbers and acid content would not be expected to show a constant gradation through the process. Bacterial metabolism would vary from cell to cell depending on the environmental conditions.

From the limited results obtained the lactic acid concentrations at both Empangeni and Dalton lie in the range 100–400 mg/litre, whilst those at Amatikulu vary widely on either side of this band. This can undoubtedly be allied with the high frequency of unscheduled mill stops experienced at Amatikulu during the past season as a result of the inevitable teething problems associated with the commissioning of a new and very large diffuser. However, the results do tend to indicate that higher operating temperatures result in lower lactic acid production which is consistent with the fact that hyperthermophilic activity is reduced by higher temperatures, becoming negligible above 80°C.[13] This serves to emphasise the important role which adequate temperature control can play in retarding microbial fermentation and hence sucrose losses.

(2) *Application of bactericides*

An alternate method of control which is widely used in the beet industry is the application of bactericides. Many compounds are available as bactericides, but in conforming to certain specifications, only a few may be considered. The bactericide must adhere rigidly to required food regulation standards, be inexpensive and easy to apply, preferably as a liquid. It should be non-volatile at diffuser temperatures, and able to withstand the presence of organic compounds long enough to be effective.

Busan 881 is widely used as a bactericide in the pulp and paper industry, but results reported on the application of this compound in the cane sugar industry have been mixed, some workers gaining success[2,3] and others failure.[8] When applied continually to diffusers in Cuba,[3] it was reputedly successful at concentrations of 10 ppm. It is completely degraded during defecation,[2] and therefore does not interfere with the final product.

Formalin is widely used, as a 40% formaldehyde solution in methanol in beet diffusers.[4,5,6,7,9,12] The formalin is usually applied by shock dosing at high concentrations at regular intervals in selected areas. It then moves throughout the diffuser and remains effective for several hours after application. It is completely degraded during the downstream processing to products similar to the alkaline decomposition products of fructose.[7]

Sodium metasilicate represses inversion in sugarcane juice[1] at a level of 30–60 μ moles silicate/ml raw juice for 48–60 hr. The method of repression is not certain, but one suggestion is that it combines with the fructose end of the sucrose molecule, and hence prevents bacterial fermentation (i.e. it could be acting as a bactericide).

Preliminary laboratory tests using these three materials indicated that formalin would probably provide the most favourable cost/benefit ratio for full scale trials on the Amatikulu diffuser. In these tests the formalin was applied in shock doses at various points along the diffuser; each cell received a slug dose of 8 kg of formalin every three hours. The cost of formalin is approximately 20 cents/kg. The results of the bacterial count and lactic acid determinations during these tests are shown in Table 2.

TABLE 2

Bacterial counts/lactic acid content at Amatikulu after formalin dosing

Date	Diffuser Cell No.	Temp. °C	Thermophiles/ml. juice	Lactic acid mg/litre juice
13.11.74	2	69	60	64
13.11.74	5	78	50	66
13.11.74	8	80	90	56
22.11.74	2	65	—	50
22.11.74	5	70	20	19
22.11.74	8	70	10	18
22.11.74	11	62	270	21
5. 2.75	1	73	140	4
5. 2.75	5	81	220	—
5. 2.75	11	69	TNTC	22

It is evident from the figures that formalin is very effective in inhibiting bacterial activity. However, it is unlikely that continuous shock dosing of the diffuser under normal running conditions with adequate temperature control is economically justifiable. Nevertheless the use of formalin can undoubtedly provide a very useful adjunct to temperature control at certain times and in selected areas in the optimisation of diffuser performance.

(3) *Lactic acid investigations*

A number of tests were undertaken in order to determine the proportion of lactic acid in the total organic acid fraction

from the bacterial fermentation of sucrose in cane juice. The results of these tests are shown in Table 3 which reveals that lactic acid comprises an average of 91% of the total organic acids.

TABLE 3
The proportion of lactic acid

Juice	Grams total acid produced/litre juice	Grams lactic acid produced/litre juice	Lactic Acid percentage
X	0,89	0,78	88
Y	0,87	0,75	86
Z	0,83	0,82	99

The variation in the proportion of lactic acid may be attributed to the bacterial types within the culture which determine whether the fermentation is of the homo or heterolactic type. The results obtained show a similar trend to those from beet diffusers[6] and confirm that the principal organic acid formed during the fermentation of sucrose by hyperthermophiles is lactic acid.

Much controversy has centred around the determination of the ratio between sucrose destroyed and lactic acid produced. Norman and Rorabaugh[11] using beet juice as a culture medium at 60–65°C for 24 hours, obtained ratios varying between 0,81 and 4,44 to 1 with an average of 2,14 to 1. Carruthers and his co-workers[5] using raw beet juice inoculated with soil, reported values between 1,21 and 3,12 to 1, averaging 1,96 to 1. More recently Oldfield[13] found an average of 2,29 to 1 using figures fluctuating between 1,38 and 4,37 to 1. All these results suggest that approximately 2 parts by weight of sucrose is destroyed for every 1 part of lactic acid formed.

However, research by Klaushofer and Pollach[10] indicated that this ratio may be too high as they reported figures ranging between 1,00 and 1,39 to 1, which is far lower than either the British or American results. A general criticism of their work is that they used artificial culture media, and not beet juice, and hence their figures may not be as reliable as those determined when working with natural media.

The ratios calculated during the present study are shown in Table 4 and appear to be in good agreement with those reported by Carruthers, Oldfield and Norman.

TABLE 4
The sucrose destroyed to lactic acid produced weight ratio

Juice	pH	Brix	Percent decrease in Reducing Sugars	Sucrose Loss (gms)	Lactic Acid Formed (gms)	Sucrose/ Lactic Acid Ratio
A	6,9	13,94	0,58	5,00	1,67	3
B	6,9	17,78	0,13	6,00	1,4	4,3
C	6,9	15,58	0,08	3,47	1,46	2,4
D	6,9	15,53	n.d.	2,57	3,35	0,77
E	6,1	7,6	0,04	1,81	1,15	1,57
F	6,1	7,6	n.d.	1,22	1,4	0,87

Key: n.d. — not determined

Random inocula of soil and diffuser bacteria into raw cane juice at 65°C showed a scatter of results between 0,77 and 4,3 to 1, averaging 2,15 to 1. As with beet juice, the ratio of sucrose lost to lactic acid produced may be approximated at 2 to 1.

The criticism of Klaushofer and Pollach's work is valid and indicates that the spectrum of the ratios reported may be due to the nature of the culture medium employed, and the type of bacteria active within that culture. Intermediary and final metabolites of bacterial metabolic pathways do vary, depending upon the substrates available and the bacterial species. Furthermore, other end-products, apart from lactic acid, such as carbon dioxide are formed in the sucrose to lactic acid pathway.

It has been suggested that levan and dextran could be produced during this fermentation,[13] but neither has been detected. Carbon dioxide production is "insufficient to account for all carbon atoms represented by the sugar loss",[13] and it is therefore necessary to assume that unknown interconversions do occur, influencing the ratio.

Finally, examination of Table 4 also reveals that reducing sugars show a decrease over the incubation period. This might indicate that invert sugars also act as substrates for lactic acid production by hyperthermophiles. However, Oldfield *et al*[13] report that while an acid was formed from the fermentation of these sugars, it was not lactic, and work is in progress to identify this acid.

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Appendix I

Method for bacterial counts

A 1 ml sample of raw diffuser juice was immediately diluted 1 to 10 in water and filtered aseptically through a Sartorius membrane filter (pore diameter 0,45 μm). Membranes were removed after washing twice with distilled water and placed onto dextrose tryptone agar (D.T.A.) plates. These were then incubated for 24 hours at 65°C after which bacterial colonies were counted.

Appendix II

Method for lactic acid analysis

After removing the required amount of juice for bacterial counts, formalin at a concentration of 1% was added to the remaining sample to inhibit further bacterial activity during transport back to the laboratory. For subsequent analysis, 1 gm kieselguhr was added to 100 ml juice and this was filtered through Whatman No. 1 paper. Fifty millilitres of the filtrate was run through a cation exchange column (Amberlite IR 120), the eluate collected and passed through a second resin column (Amberlite IRA 402), which retained the acids present in the juice. The column was washed sugar free with distilled water and lactic acid eluted with 0,1 m NaCl. After extensive testing, the colorimetric method of Oldfield and Shore[14] was used to determine the lactic acid content of the eluate. The only deviation from their published method lay in the use of zinc lactate trihydrate instead of lithium lactate for the preparation of standard lactic acid solutions.

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