

RESEARCHES ON UTILISATION OF CANE MOLASSES.

PART II. Preparation of mixed calcium salts of organic acids from cane molasses and its technical applications.

By T. R. Bhaskaran, S. C. Pillai and V. Subrahmanyam.

Attention has already been drawn to the fact that under certain controlled conditions, the major part of the sugar of cane molasses can be fermented to yield a mixture of organic acids. Evidence has also been adduced to show that the mixed acids and their salts are highly potent in fixing atmospheric nitrogen and are nearly thrice as efficient as when molasses is applied directly to land (*Vide* Part I).

The object of the present enquiry was to extend the foregoing observations and to prepare solid products containing mixed salts of organic acids.

EXPERIMENTAL.

Methods.—Reducing sugars and sucrose were estimated by the Bertrand method after caltrification with alumina cream. Organic carbon was determined according to Bhaskaran *et al.*, (*Jour. Ind. Inst. Sci.*, 1936 *19A*, 45). The other estimations were carried out according to A.O.A.C. (1935).

Materials.—The molasses used in the present study was obtained through the courtesy of Messrs. The Mysore Sugar Company and had the same composition as given in Table II in Part I of this series.

Influence of H-ion concentration on the production of acids.—An active culture of the mixed flora of the soil was first prepared by inoculating 25 g. of garden soil into 500 cc. of one per cent. glucose solution. At the end of 36 hours, when the fermentation was at its height, aliquots (10 c.c. each) of the suspension were inoculated into 200 c.c. lots of 5 per cent. molasses. The reactions of the latter were

variously adjusted by suitable additions of alkali at 24-hour intervals. The quantities of alkali thus required at each stage were recorded as also the amounts required for final titration (after 20 days) to pH 8.0. The total amounts of acids found at 4-day intervals were then calculated (Table I).

TABLE I.

Time in days	Acid produced in c.c.N/4 in 200 c.c. medium				
	8	7	6	5	Control (pH not adjusted)
4	10.6	16.6	8.8	6.8	2.5
8	74.6	82.6	54.4	41.8	20.0
12	114.6	120.6	76.4	63.8	29.8
16	168.9	180.6	122.4	93.8	36.0
20	189.0	228.0	181.0	167.0	35.0

Maximum acid production was observed at pH 7.0 and that was nearly seven times as much as formed in the untreated control.

Relative merits of caustic soda, slaked lime and calcium carbonate for neutralisation.—It was found that regulated addition of caustic soda or lime to adjust the reaction led to the highest yields of organic acids. A precaution to be observed was that excessive alkali had to be carefully avoided, as, otherwise, acid production was adversely affected. There was less production of acid in presence of the calcium carbonate but that treatment was found to be very much more convenient in practice. All the carbonate could be added in one lot and no further precaution was needed.

Effect of initial concentration of molasses on acid production.—Molasses solution of different concentrations were prepared. To 200 c. c. of each, excess calcium carbonate was added and the suspension inoculated in the same manner as in the previous series. They were then incubated at 30° and the quantities of free as well as combined acids present at different stages determined (Table II). The latter were calculated from the quantities of calcium present in solution.

TABLE II.

Time in days	Total organic acids in c. c. N in 200 cc. medium				
	Concentration of molasses				
	2	5	10	15	20
4	15.0	33.5	29.6	45.1
10	20.6	51.1	50.0	96.8	15.8
14	28.8	45.5	54.4	100.4	88.6
20	24.2	46.5	58.8	110.5	108.4
25	21.6	36.7	56.4	100.9	126.0

The efficiency of acid production decreases with increasing concentrations of molasses. At the lower concentrations the maximum acid production is observed in about a fortnight, but after that period, the quantities tend to diminish on standing.

Influence of calcium acetate on acid production.—It appeared probable that increasing quantities of calcium salts were inhibiting further production of acids, especially at the higher concentrations of molasses. With a view to verifying this, varying proportions of calcium acetate (one of the products of fermentation) were added to 200 c. c. lots of 2 per cent. molasses and acid production followed as before (Table III).

TABLE III.

Time in days	Total acids in c. c. N. in 200 c. c. medium			
	Concentration of calcium acetate %			
	1	2	5	10
0
4	4.5	4.0	1.3	0.2
8	7.6	5.4	1.9	0.2
12	12.0	9.0	2.0	0.4

Increasing concentrations of calcium acetate had a definite inhibiting effect on acid production.

Concentration of the mixed calcium salts.—This presented a very difficult problem especially as the salts were all water soluble. Prolonged evaporation yielded a dark, sticky product. Addition of extra quantity of lime followed by further heating yielded a dry product, but the process was, on the whole, found to be expensive.

With a view to overcoming the above defect, the fermentation was conducted in presence of soil. It was hoped that this would provide larger surfaces and richer flora, that the fermentation would be quicker and that higher concentrations of molasses can be used. The procedure consisted in first mixing soil with liquid molasses in the desired proportion followed by addition of calcium carbonate (one-third weight of the molasses) and sufficient water to make a homogenous paste. A number of mixtures were thus prepared and allowed to ferment in open, shallow, containers (length $2\frac{1}{2}'$, breadth $2\frac{1}{2}'$, and depth 9") improvised by embanking Cuddappah slab floor with brick and clay.

After the disappearance of sugar from the first batch of molasses, a second batch (together with the necessary amount of water and calcium carbonate) were added and the fermentation allowed to proceed as before. In this manner, a number of successive fermentations were conducted. The distribution of organic matter at different intervals has been presented in Tables IV to VI. (The figure for 'carbon as products of fermentation' includes the non-sugar portion of the organic matter in molasses).

TABLE IV.

Series A—Initial concentration of molasses to soil :: 1 : 3

Molasses addition- instalment No.	Expressed as percentage on oven dry basis				
	Time in days	Total organic carbon	Carbon present as sugar	Carbon as products of fermenta- tion	Loss on ignition
1	0	8.25	6.40	1.85	28.2
	4	7.80	5.86	1.94	24.6
	8	6.70	3.83	2.87	26.4
	17	5.38	1.83	3.55	18.2
2	22	9.25	5.68	3.57	33.7
	40	8.70	4.60	4.10	33.1
3	49	12.60	5.00	7.60
	65	10.30	Nil	10.30	37.7
4	80	13.00	3.60	9.40	39.8
	95	12.70	3.00	9.70	39.0

TABLE V.

Series B—Initial concentration of molasses to soil :: 1 : 4

Molasses addition- instalment No.	Expressed as percentage on oven dry basis				
	Time in days	Total organic carbon	Carbon present as Sugar	Carbon as products of fermenta- tion	Loss on ignition
1	0	6.95	5.65	1.30	22.5
	4	6.25	4.70	1.55	25.1
	8	4.90	2.50	2.40	17.0

	22	7.80	5.10	2.70	26.5
2	31	7.05	3.70	3.35	26.2
	40	8.50	3.10	5.40	25.8
3	49	7.80	1.10	6.70	24.2
	65	10.02	3.00	7.02	31.0
4	90	8.80	1.30	7.50	27.8

TABLE VI.

Series C—Initial concentration of molasses to soil :: 1 : 5

Molasses addition- instalment No.	Time in days	Expressed as percentage on oven dry basis			
		Total organic carbon	Carbon present as Sugar	Carbon as products of fermenta- tion	Loss on ignition
	0	5.60	4.70	0.90	18.2
1	4	5.20	4.30	0.90	16.2
	8	4.10	2.23	1.83	15.9
	17	3.60	0.88	2.72	14.5
	22	6.24	3.40	2.84	22.4
2	31	21.5
	40	5.20	2.30	2.90	21.1
	49	7.20	3.00	4.20	26.2
3	65	6.20	Nil	6.20	27.1

When the proportion of molasses to soil was as 1:3 the fermentation was rather tardy but a large proportion of sugar was converted into acids. By successive addition of molasses in the same proportion, a high ratio of 1:1 was eventually attained. Further addition of molasses was not very useful as it was not easily decomposed. It was found convenient to stop the fermentation when total organic carbon in the product was of the order of 10 per cent.

The above type of fermentation requires very little attention. A felt of microorganisms forms on top and thus effectively controls the air supply in the medium during fermentation.

Since the addition of molasses in the liquid form has been carried in instalments along with varying amounts of water, it is not possible to obtain any direct simple estimate of the efficiency of the conversion of the molasses carbon into these products. The percentage efficiency e of conversion of molasses carbon into the products of fermentation is calculated from the following two equations.—

$$l = \frac{m (21.9 - 0.73 c) - s c}{100 - 3.3 c} \quad \dots \quad (1)$$

$$e = \frac{0.219 m - l}{0.219 m} \times 100 \quad \dots \quad (2)$$

where m = the quantity of molasses added,

s = amount of soil taken plus calcium carbonate added as (CaO),

c = the organic carbon content of the final product as % after making correction for the soil carbon,

and l = loss of carbon from the system.

The organic carbon content of the molasses on oven dry basis was 30.0 per cent and the water in the liquid product used for the fermentation was 27.0 per cent. By substituting values for m , s and c for the different series the efficiencies in each case are calculated. The results are presented in Table VII.

TABLE VII.

Efficiency of conversion of molasses at the end of 65 days

Series No.	Initial concentration Soil : Molasses	Quantity of molasses added (<i>m</i>)	Organic carbon content of the final product (<i>c</i>)	Sugar C in the final product %	% Efficiency of fermentation (<i>e</i>)
A	3 : 1	108.0	10.0	Nil	82.8
B	4 : 1	81.0	6.8	Traces	60.9
C	5 : 1	64.5	5.9	Nil	70.2

It may be noted from the above results that the fermentation was the quickest and the loss of carbon the least when the ratio of molasses to soil was 1 : 3. The fermentation was successfully repeated three times, the total quantity of molasses thus added corresponding to the same weight as the soil originally started with. The final dry product (obtained after the 65th day) had a carbon content of 10.3%. Further addition of molasses was not however very useful, a considerable part of the sugar remaining unattacked even after 95 days.

When the initial ratio of molasses to soil was 1 : 4, three successive batches were first fermented. In this case also, the fermentation of the fourth batch (after 65 days) was tardy. The carbon content (as products of fermentation) after 49 days was 6.7% and that after 90 days after the fourth addition) 7.0%.

When the ratio of molasses to soil was 1 : 5, three successive fermentations were completed in 65 days. The final dry product had a carbon content of 6.2%

A comparative study of the recovery of carbon at the three proportions showed that it was 82.8% when the starting ratio was 1 : 3, 60.9% when it was 1 : 4 and 70.2% when it was 1 : 5.

Effect of stirring on the economy of carbon.—Two sets of experiments were conducted in one of which A the soil-molasses paste

was left undisturbed (except at the time of addition of fresh quantities of molasses) while in the other B it was stirred once in 24 hours. The conditions were otherwise the same in both the sets. Total organic carbon and loss of ignition of the sample obtained from these sets are presented in Table VIII.

TABLE VIII

Molasses addition- instalment No.	Time in days	Expressed as percentage on oven dry basis			
		A		B	
		Total Organic C	Loss on ignition	Total Organic C	Loss on ignition
1	0	5.30	21.2	5.20	20.6
	4	4.90	17.0	4.20	16.0
	11	3.50	14.0	2.00	8.8
2	27	4.60	16.3	2.60	10.1
	32	3.90	15.8	1.40

It was found that although the fermentation was rendered slightly quicker by frequent stirring, this was accompanied by much greater loss of carbon than the undisturbed set. Thus, whereas the carbon content of the former came down to 1.4% at the end of 32 days, it was nearly three times (3.9%) in the latter.

DISCUSSION.

The present enquiry has revealed an elegant method⁴ of converting the major part of the organic carbon of molasses into mixed calcium salts of different organic acids. The use of soil as the medium facilitates not only fermentation at very high concentration but also quicker drying. Fermentation proceeds spontaneously and requires very little attention.

Even under the above conditions fermentation is comparatively slow. It takes more than two months to ferment the same weight of molasses as the soil. Moreover, the fermentation is accompanied by considerable emission of smell which is rather objectionable.

It would appear desirable therefore to modify the conditions in such a manner as to quicken the fermentation. It should be possible to work with much thicker layers of soil-molasses mixture than used in the present study.

Evidence has already been adduced to show that, with the addition of increasing quantities of molasses, the rate of fermentation drops considerably. This would be in keeping with the earlier observation that the products of fermentation retard further productions of acid. It would be useful therefore to remove these at least partially from time to time.

The use of the soil, however advantageous in other respects, still adds considerably to the bulk of the product. The colour also becomes darker because of the precipitation or oxidation of iron compounds which are brought into solution.

From the above point of view, fermentation in presence of sand appears to be more suitable. At convenient intervals the products of fermentation can be washed down with small additions of water and the concentrated extract thus obtained evaporated separately. In this manner, the cost of evaporation can be greatly reduced and a much purer product obtained than would be possible in presence of soil.

Although at the outset the sand medium is not so rich in active flora as the soil is, it soon builds them up so that after a few days, the reaction will proceed nearly as fast as in presence of soil. The initial stages of the process can be hastened by inoculating small quantities of active suspensions from a fermenting soil media.

Attention has already been drawn to the high nitrogen fixing capacity of the mixed calcium salts. It would be of interest to determine whether the nitrogen thus fixed is actually available for plant nutrition.

Evidence has already been adduced to show that the nitrogen fixed by direct application of molasses to land is not fully available to crop. This is largely due to the fact that a long period of rest is needed before the seed can be sown or the crop planted. Most of the nitrogen is fixed in the course of the first ten days. This ammonifies readily and a considerable part is soon lost—especially in a limed soil or one which is naturally rich in lime or alkali salts—from the soil system, so that by the time, the crop is ready for it, there is very little available nitrogen. Whether this observation also applies to the mixed calcium salts is still awaiting elucidation.

The possibility of separating organic acids or utilising them in other ways has not yet been investigated. Fermentation in sand medium will facilitate the separation of the mixed calcium salts in a concentrated form which can be used for subsequent distillation with mineral acid. The dry distillation of the mixed salts to yield the corresponding ketones may be of much practical value.

Further work along these and allied lines is in progress and will be reported in subsequent communications.

SUMMARY.

1. A comparative study of the production of organic acids from cane molasses at different hydrogen-ion-concentrations showed that the largest amount is produced at pH 7.0. pH 8.0 followed next and pH 6.0 and 5.0 later. The acid production was least when the reaction was left undisturbed and unadjusted.

2. Acid production was greatest when the sodium or calcium hydroxide was used for neutralisation. Even slight excess of alkali retarded acid production. Calcium carbonate was not so efficient as the other two but was very much convenient to handle.

3. The efficiency of the acid production decreased with increasing concentrations of molasses.

4. Increasing concentrations of calcium acetate (one of the products formed by neutralisation) had an adverse effect on further acid production.

5. Direct evaporation of the fermented liquor yielded a dark, sticky substance. This, on addition of extra quantity of lime followed by heating, yielded a dry solid substance.

6. Acid fermentation of molasses proceeds more quickly and at higher concentrations when suitably admixed with soil. The final product also dries quickly.

Advantage is taken of this observation to ferment thicker paste containing molasses and soil in the proportions of 1:3, 1:4 and 1:5. After a complete decomposition of sugars, fresh quantities of molasses were added and the fermentation allowed to proceed as before. The dry product thus formed after three successive fermentations at the initial ratio of 1:3 contained about 10.3% carbon, that at 1:4, 7.02% and that at 1:5, 6.2%.

7. Maximum acid production as well as retention of the acids was observed when the soil-molasses pastes were left undisturbed. Frequent stirring caused marked loss of organic matter.

8. The advantages of conducting the fermentation in presence of sand have been discussed. The possibility of separating acids or their salts by periodical extraction with water and thus obtaining concentrated products are discussed. The possible utilisation of the mixed calcium salts not only for the purpose of nitrogen fixation but also for the manufacture of the acids or their corresponding ketones have been indicated.

*Department of Biochemistry,
Indian Institute of Science,
Bangalore (India.)*

[Received, 21-3-1939]