

RESEARCHES ON UTILISATION OF CANE MOLASSES.*

PART I. DISSOLUTION OF BONE BY FERMENTATION OF CANE MOLASSES.

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During the past few decades, several attempts have been made to dissolve or disintegrate bone by simple and inexpensive methods and thus converting it into more readily available forms (Lipman and McLean, *Soil Sci.*, 1916, 1, 533; Waksman and Joffe, *J. Biol. Chem.*, 1922, 50, 35; Joffe, *Soil. Sci.*, 1922, 14, 479; *Ibid.*, 1923, 15, 41, 93; Hutchinson, *Agric. Jour. India*, 1924, 19, 4; Ramaswami Iyer and Perumal, *Jour. Ind. Inst. Sci.*, 1928, 11A, 85; Sen and Hossain, *Agric. and Live-Stock in India*, 1931, 1, 151; Hossain, *Agric. and Live-Stock in India*, 1933, 3, 152; Vyas, *Agricultural Research Institute, Pusa, Bulletin* 1930, 204; Sahasrabuddhe, *Agric. and Live-Stock in India*, 1933, 3, 264). Some of these enquiries, like those of Sen and Sahasrabuddhe, were undertaken with the definite object of disintegrating bone and in this they were successful. The others which were designed to dissolve the bone either failed altogether or have been only partially successful, so that there has been practically no improvement on the original sulphuric acid method of manufacturing superphosphate. The rapid growth of the cane sugar industry in different parts of the world, and more recently in India, has led to enormous accumulation of molasses—which is the chief by-product—in most of the factories. The present production of molasses in India alone may be rated at between 500,000 and 600,000 tons per annum. Only a part of this is utilized for the manufacture of alcohol and other technical purposes, while the rest has either to be dumped on land or otherwise disposed of.

It is well known that molasses undergoes rapid fermentation in the soil yielding acid products. The nature of these acids has been

* Previous communications from these laboratories relating to fermentation of molasses in the soil have been published in the *Proc. Ind. Acad. Sci.* (Bhaskaran, Narasimhamurti, Subrahmanyam and Sundara Iyengar, 1934, 1B, 155; Narasimhamurti and Subrahmanyam, 1935, 1B, 823; Sundara Iyengar and Subrahmanyam, 1935, 1B, 868) and *Agric. and Live-Stock in India* (1936, 6, 488). Papers relating to production of organic acids and to nitrogen fixation have appeared in the *Proc. Ind. Acad. Sci.* (Bhaskaran and Subrahmanyam, 1936, 3B, 143; Bhaskaran, 1936, 3B, 320) and in the *Proc. Nat. Inst. of Sci., India* (1937, 3, 163). Contributions bearing on utilisation of molasses for preparation of yeast, as also in composting, have appeared in the *Proc. Soc. Biol. Chem., India* (1937, 2, 39, 41) and in the *Proc. Assor. of Sugar Technologists, India* (Oct. 1937). In addition to these, a number of technical as well as semi-technical contributions bearing on various aspects of this subject have been published by members of this Department.

determined (Bhaskaran *et al.*, *Proc. Ind. Acad. Sci.*, 1934, 1B, 155) and, more recently, Bhaskaran (*Proc. Ind. Acad. Sci.*, 1936, 3B, 320) has shown that, under certain favourable conditions, the sugar of the molasses can be mostly converted into organic acids. Since these acids are known to dissolve calcium phosphate, it was considered possible that the acid fermentation of molasses can be utilised for the dissolution of bone and rock phosphate and for the preparation of more readily available phosphatic fertilisers.

EXPERIMENTAL.

Materials: Bone.—This was obtained in the form of small broken bits from Messrs. The Presidency Manure Works of Ranipet. It was crushed in a disintegrator and the portion passing 10-mesh sieve, but not 20-mesh sieve, used for the experiments.

Rock Phosphate.—This was a specimen of phosphatic nodule occurring in Trichinopoly District in the Madras Presidency. It was ground to a fine powder passing the 80-mesh sieve.

Molasses.—This was obtained through the courtesy of Messrs. The Mysore Sugar Company from Mandya.

Methods.—Methods for the estimation of sugars and most of the other constituents were the same as prescribed by the A.O.A.C. (1935). Phosphate in solution was estimated by the Neumann method after destroying organic matter by digestion with sulphuric and nitric acids.

TABLE I.
Composition of bone and rock phosphate.

Item	Expressed as percentage on air-dry sample	
	Bone	Rock phosphate
Phosphorus (as P_2O_5) ..	24.95	26.50
Calcium (as CaO) ..	30.15	37.20
Iron (as F_2O_3) ..	0.90*	4.50
Organic carbon ..	30.01	0.15
Nitrogen ..	2.80	0.01

* Includes Al_2O_3 and SiO_2 .

TABLE II.
Chemical composition of molasses.

Item	Percentages on moisture-free basis
Sugars :	
Sucrose ..	35.8
Reducing sugars (as invert sugar) ..	32.0
Total Phosphoric acid (as P_2O_5) ..	0.1
Total Potash (K_2O) ..	3.6
Total Lime (CaO) ..	1.4
Total Carbon ..	30.1
Total Nitrogen ..	0.3

Dissolution of tricalcium phosphate in different organic acids.—
This experiment was carried out with a view to obtaining a preliminary idea of the extent of dissolution of pure tricalcium phosphate in different acids known to be formed during fermentation of cane molasses. The results represent the quantities brought into solution by shaking 10 g. lots of the phosphate with 200 c.c. portions of N/4 acids for 30 minutes in each case.

TABLE III.

Organic acid used	Phosphorus in 200 c.c. solution (as mg. of P_2O_5)	Percentage dissolution
1. Lactic acid ..	1604	34.8
2. Acetic acid ..	763	16.5
3. Propionic acid ..	625	13.5
4. Butyric acid ..	543	11.7
5. Mixture of 1, 2, 3 and 4 in equal pro- portions ..	908	19.2

The following results were obtained on using varying strengths of the same (acetic) acid.

TABLE IV.

Conc. of acid	N	N/2	N/4	N/8	N/12
Phosphorus brought into solution (P_2O_5) as mg. in 200 c.c. ..	1224	1150	763	458	308

It may be seen from the above that (1) among the acids that were tried, lactic acid was the most efficient; (2) in the case of volatile fatty acids, the efficiency decreased on going higher up the series, and (3) the efficiency of dissolution decreased as the concentration of acid was raised above N/8.

Dissolution of bone and rock phosphates during the fermentation of cane molasses.—The samples (10 g. each) were weighed into 250 c.c. conical flasks and treated with 200 c.c. each of 5 per cent. solution of molasses. The fermentation was initiated by inoculating each flask with 10 c.c. of an active suspension obtained as follows:—Glucose solution (1 per cent.; 500 c.c.) was treated with 25 g. of fresh garden soil. Fermentation started in less than 24 hours (at 30° C.) and was fairly vigorous at the end of 36 hours. It was at this stage that 10 c.c. portions of the vigorously fermenting suspension was used for inoculating the molassed flask. The results are given in Table V.

TABLE V.

Phosphate used	Phosphorus in 200 c.c. solution (as mg. of P_2O_5)						
	Time in days						
	0	2	3	5	8	12	16
Rock phosphate ..	9.1	17.0	16.2	44.2	..	34.2	29.0
Bone ..	10.2	70.3	113.4	197.3	483.2	499.1	553.5
Tricalcium phosphate $Ca_3(PO_4)_2$..	6.8	275.6	450.0	571.6	546.7	589.8	630.6

The maximum dissolution was observed in the case of pure tricalcium phosphate. The quantities dissolved from bone would probably have been more if it had been ground to finer division. There was very little dissolution from rock phosphate, so the subsequent experiments were carried out with bone only.

Dissolution of bone in relation to decomposition of sugars.—The conditions were similar to those in the previous experiment.

TABLE VI.

Time in days	Weight of residual bone in g.	In 200 c.c. solution		Percentage dissolved	
		Total sugar mg.	Phosphorus as mg. P_2O_5	Whole bone	Phosphate of bone
0	10.0	5920	25.7	Nil	..
4	9.6	4340	267	4.0	10.6
10	9.1	1440	432	9.0	17.2
14	8.8	Traces	609	12.0	24.4
20	8.7	Nil	633	13.0	25.3
26	8.5	„	623	15.0	24.9

Dissolution of phosphate proceeded proportionately faster than that of the other solid constituents of the bone. The extent of dissolution of phosphate cannot therefore be accurately estimated by merely determining the residual weight at each stage. In fact, the organic matter of the bone is left largely unattacked in the beginning and it is not until the fermentation is repeated a number of times that those forms are brought into solution. A further observation of interest is that the dissolution of phosphate increases with the decomposition of sugar, but is not directly proportional to it.

Production of organic acids during dissolution of bone.—These determinations were similar to those in the previous trials except that in one series molasses was fermented in presence of bone, while, in the other, it was fermented by itself. In addition to colorimetric determination of hydrogen-ion-concentration, the total quantities of organic acids were also determined. In addition to free acids, the quantities present in solution as the corresponding calcium salts were also estimated.

TABLE VII.
Production of organic acids and changes in reaction.

Time in days	Hydrogen-ion-concentration		Total organic acids (as c.c. N)			
	In presence of bone	In the absence of bone	In presence of bone		In the absence of bone	
			Free	In combination	Free	In combination
0	5.5	5.5	2.1	Nil	2.2	Nil
4	4.3	4.3	21.6	4.6	12.8	„
10	4.4	4.3	27.6	16.4	20.0	„
14	4.3	4.3	26.1	18.1	25.8	„
20	4.3	4.3	27.2	19.3	20.0	„
26	4.2	4.1	28.2	20.9	26.8	„

TABLE VIII.
Relationship between production of organic acids and dissolution of bone.

Time in days	0	4	10	14	20	26	33
Organic acid (in c.c. N)	2.1	26.2	44.0	46.2	49.1	49.1	49.5
Phosphorus dissolved (as mg. P ₂ O ₅)	25.7	267	432	609	633	623	595

It may be seen from the above that (1) the changes in reaction were not affected by the presence of bone; (2) the production of organic acids was almost doubled in presence of bone; (3) the dissolution of bone generally increased—though not on proportionate basis—with the production of acid; and (4) there is evidence of some reprecipitation of dissolved phosphate in the later stages.

Influence of size of bone on the extent of dissolution.—Same weights (10 g. each) of bone crushed or powdered to different sizes were treated with 5 per cent. molasses and the fermentation conducted in the usual way (Table IX).

TABLE IX.

Size of bone	Time in days					
	0	2	5	8	14	18
	Phosphorus brought into solution in 200 c.c. (as mg. of P_2O_5)					
Size 1"-2"	16.9	37.4	95.8	199.8	226.9	136.7
Between 10- and 20-mesh	26.1	54.4	78.4	182.0	255.9	188.8
Passing 60-mesh	22.6	62.9	165.6	355.2	..	231.3
Passing 100-mesh	18.0	114.0	314.8	623.8	510.4	243.9

With increasing fineness of division, there was correspondingly greater dissolution of bone. There was also distinct evidence of re-precipitation of dissolved bone after the 8th day.

Effect of varying the proportion of bone to molasses.—To the same quantities of molasses as used in the previous experiments, varying amounts of bone (passing 10–20 mesh sieve) were added and the quantities passing into solution estimated at intervals (Table X).

TABLE X.

Solubilisation of phosphorus at varying concentrations of bone in the medium.

Time in days	Phosphorus dissolved from					
	5 g. of bone		7½ g. of bone		10 g. of bone	
	P_2O_5 (in mg.) in 200 c.c. of medium	Per cent. dissolved	P_2O_5 (in mg.) in 200 c.c. of medium	Per cent. dissolved	P_2O_5 (in mg.) in 200 c.c. of medium	Per cent. dissolved
2	60	4.8	55	2.9	60	1.6
4	118	9.4	120	6.4	114	3.0
8	195	15.4	200	10.6	150	4.0
12	165	13.2	160	8.5	158	4.2
19	160	12.3	176	9.4	163	4.6

More or less the same quantities were dissolved in all the cases, so that there is very little advantage in increasing the proportion of bone to molasses above a certain minimum.

Effect of increasing the concentration of molasses on dissolution of bone.—To 10 g. lots of crushed bone, the same volumes (200 c.c. each) of molasses solution of varying concentrations were added and the fermentation conducted as usual (Table XI).

TABLE XI.
Dissolution of bone at varying concentrations of molasses.

Time in days	Phosphorus (as mg. P_2O_5) in 200 c.c. of medium when the concentration of sugar was			
	5 per cent.	10 per cent.	15 per cent.	20 per cent.
4	25.7	25.7	25.9	26.8
10	267	312.0	400	32.6
14	432	388.0	428	302.6
20	609	510.0	463	310.0

There was greater dissolution at the lower concentrations of molasses than at the higher ones. In the latter, the fermentation came, more or less, to a stand-still after some time.

Influence of pre-treatment of bone on dissolution.—Samples of crushed bone were first autoclaved with water, slaked lime or caustic soda under different conditions. They were then washed with water and fermented with 5 per cent. molasses in the usual way (Table XII).

Pre-treatment of bone—at any rate, under the above-mentioned conditions—did not appreciably improve the dissolution. The very low values obtained after autoclaving with lime were traced to the presence of varying quantities of the hydroxide and carbonate of calcium which could not be entirely washed out. These had reacted preferentially with the acids, thus reducing the extent of dissolution of bone phosphate.

Effect of successive fermentation of molasses on the dissolution of bone.—This experiment was conducted with a view to determining whether the entire quantity of phosphorus in bone can be eventually brought into solution. After the initial fermentation with 5 per cent.

TABLE XII.

Time in days	Phosphorus brought into solution (as mg. P_2O_5 in 200 c.c.) after treatment				
	A	B	C	D	E
4	267	202	33	198	258
8	372	385	126	355	356
12	508	526	170	532	516
16	642	588	192	623	636

A.—autoclaved with 50 c.c. distilled water at 15 lbs. for 30 minutes.
 B.—" " " 3 per cent. NaOH
 C.—" " " " Lime
 D.—" " " " NaOH at 5 lbs. for 30 minutes.
 E.—Untreated (Control).

molasses had progressed for 7 days, the supernatant containing the dissolved bone was decanted out and fresh quantities of molasses of the same strength added. This again was estimated after a further period of 7 days. The quantities of phosphate solubilised by four successive fermentations were thus determined (Table XIII).

TABLE XIII.

Effect of successive extraction on phosphorus dissolution in bone.

Treatment No.	Total phosphorus dissolved as mg. of P_2O_5			
	1st Set		2nd Set	
		As percentage of total phosphorus		As percentage of total phosphorus
1	293.6	11.7	285.6	11.4
2	557.0	22.3	583.8	23.4
3	803.2	32.1	817.2	32.7
4	1004.0	40.2	1046.8	41.9

The above observations were then extended to show that by repeated fermentations almost the entire quantity of phosphorus can be brought into solution. This finding does not support the suggestion made by some previous workers that only a portion of the bone phosphate is acid-soluble.

Precipitation of the dissolved phosphate.—The dissolved bone will not have much commercial value unless it can be converted into some solid form which will keep reasonably well. Concentration of the solution by evaporation is hardly satisfactory. Apart from being expensive, it does not yield a dry, solid product. A dark, sticky, resinous mass is obtained and this readily absorbs moisture, thus rendering the product quite inconvenient to handle. The dissolved phosphate was therefore precipitated out with slaked lime and the product filtered, washed and dried at 55° C. The resulting product which was greyish in colour was analysed for some of its constituents and the composition compared with that of the original bone (Table XIV).

TABLE XIV.

Item	Expressed as percentage on oven-dry basis	
	Original bone	Precipitate
Loss on ignition ..	30.0	32.9
Calcium (as CaO) ..	30.2	20.8
Phosphorus (as P ₂ O ₅)	25.0	28.6

The precipitated product bore some resemblance to the original bone in composition. It was, however, very much more available than the latter, being fully soluble in one per cent. citric acid.

Further experiments showed that the precipitated product was a mixture of substances. In addition to calcium phosphate, it contained a part of the organic matter of the original bone. There was also a small quantity of calcium carbonate together with still small quantities of calcium salts of organic acids.

The form in which phosphorus is present in the fermenting solution.—With a view to determining whether the phosphorus in the fermented liquor is present in true solution, aliquots were passed

through the ultra-filter. The phosphorus content of the filtrate was compared with those of (a) the original liquor, and (b) the liquid obtained on filtering (a) after neutralisation.

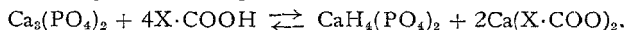
TABLE XV.

Treatment	Phosphorus in 50 c.c. solution (as mg. of P ₂ O ₅)	
	1 Set	2nd Set
Untreated ..	56.5	56.5
Ultra-filtered and phosphorus in filtrate estimated ..	54.8	52.6
Neutralised with alkali and ultra-filtered ..	Nil	Nil

The results (Table XV) would show that the phosphorus in the fermenting liquor was present in true solution as acid phosphate. On addition of lime, neutral phosphate was formed and separated out as a precipitate.

Mechanism of dissolution of phosphorus: Equilibrium in the fermenting liquor.—It has already been shown that organic acids dissolve phosphorus of the bone only to a limited extent; that even large quantities of fermenting molasses cannot dissolve the entire quantity of phosphate in one instalment. These observations would show that there is some factor operating as a check to the complete dissolution of bone phosphate by the fermentation method.

It is well known that the reaction between calcium phosphate and organic acids is a reversible one. The stage of equilibrium will vary with the nature of the acid, but the reaction may be generally represented by the following equation:—



The nature of the equation would suggest that although the other conditions may be favourable, the dissolution of phosphate in the fermenting medium will stop at a certain stage. The only way in which the reaction can be allowed to proceed forward is to remove the products from time to time. That is what is being done by successive fermentation, so that the entire quantity of phosphate is eventually brought into solution.

The reversible character of the reaction will also explain the re-precipitation of the phosphate observed in some of the experiments. If the acid production is either arrested or reduced in quantity (as often happens through secondary fermentation in the later stages) the reaction will be reversed, thus resulting in reprecipitation of a part of the previously dissolved phosphate. If, therefore, maximum efficiency is to be secured, the fermentation should be repeated at short intervals and the dissolved product removed as quickly as possible.

DISCUSSION.

The results of the present enquiry would show that while it is theoretically possible to dissolve the entire quantity of phosphorus in bone by successive fermentation of molasses, the practical application of the process for purposes of manufacture involves a number of difficulties.

For the complete dissolution of bone phosphorus, (1) disproportionately large quantities of molasses have to be employed; (2) the molasses has to be used as a dilute solution; and (3) successive batches of fermentations have to be conducted. The related operations will require large containers. They will also involve much time. The cost of molasses and lime required for precipitation, though small, will not be quite insignificant.

Apart from this, the disposal of the residual liquor left after separation of phosphate will present a difficult problem. It is rich in organic matter and strongly smelly.

The fermentation can be utilised, however, in a different way. The production of acids as well as dissolution of bone proceed in presence of the soil, so an elegant method of utilising the process will be to apply a mixture of molasses and bone directly to the field. The molasses can be applied either as such or with irrigation water. It can also be used as a solid product containing lime and powdered bone. In that manner, the elaborate technical operations mentioned above can be entirely avoided. Work in this direction is already in progress and will be described in a later communication.

SUMMARY.

1. Pure tricalcium phosphate dissolves to a greater extent in lactic acid than in volatile fatty acids. In the case of the latter, the extent of dissolution decreases on going higher up the series. Maximum of efficiency in dissolution is obtained when the strength of acid corresponds to about N/8.

2. During fermentation of molasses by the mixed flora of the soil, bone dissolves to an appreciable extent while rock phosphate (phosphatic nodules from Trichinopoly District in South India) is only sparingly soluble.

3. The phosphate of the bone dissolves faster than the other constituents. The dissolution increases with the decomposition of sugar, but is not proportional to it.

4. The total production of organic acids from molasses is almost doubled in presence of bone. The dissolution of phosphate increases with the production of acid, but a part of it is reprecipitated after standing for some time.

5. The extent of dissolution of phosphate increases with the fineness of division of bone.

6. Increasing the proportion of bone to molasses above a certain limit does not lead to any appreciable increase in the dissolution of phosphate.

7. There is greater dissolution of bone at the lower concentration of molasses than at the higher ones.

8. Pre-treatment by autoclaving with water, caustic lime or soda does not lead to any appreciable improvement in the dissolution of bone phosphate.

9. By successive fermentation of dilute molasses, almost the entire quantity of phosphate in bone can be brought into solution.

10. The dissolved phosphate can be precipitated on treatment with lime. The product thus obtained has the following composition: loss on ignition, 32.9; Calcium (as CaO), 20.8; and Phosphorus (as P_2O_5), 26.8. It is completely soluble in 1 per cent. citric acid.

11. The phosphate in the fermented liquor is present in true solution.

12. The reaction between bone phosphate and the acids formed during fermentation is reversible. Accumulation of the soluble products arrests further dissolution, so that they have to be removed as rapidly as possible.

13. The fermentation method offers a number of practical difficulties and cannot be easily applied as a commercial process. Molasses can be utilised however for increasing the availability of bone and other phosphatic materials by direct application to land either as such or with irrigation water. It can also be applied to land as a solid product incorporating powdered bone or some other suitable form of phosphate.

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