DEXTRO-ROTARY BODIES IN BAGASSE

EXPERIMENTS CARRIED OUT BY THE CHEMICAL STAFF AT EMPANGENI SUGAR FACTORY

By G. C. DYMOND.

Mr. G. C. Dymond read his paper on the above subject, as follows:—

As every stick of cane is a variable, its variations depending on factors of variety, soil conditions, climate, etc., so bagasse partakes physically and chemically of the characteristics of the cane before milling.

On first thought this would appear to be a truism, but actually it is not, for both the physical condition and chemical composition of bagasse is to a degree dependent on the quality of the milling; the use of cold or hot imbibition; or the practice of semi-diffusion in hot water baths by maceration.

Primarily, the cane stalk can be divided roughly into three parts; the hard rind composed of cuticle partly covered with wax impervious to water, a thick epidermis which gradually passes into the second or third parts—the parenchyma or soft pith cells and the fibro-vascular bundles which are instrumental in carrying out the transport of materials necessary in the growth of the plant.

It follows then that the poorer the crushing—such as dry crushing without shredders—the more the resulting bagasse will partake of the characteristics of the original cane. On the other hand, cane which is subjected to shredding, heavy crushing, hot water compound imbibition or semi-diffusion will have many of its chemical and colloidal impurities removed from it. This applies particularly to Uba, which is notorious for the refractory milling qualities, so-called gums, mealy bugs and generally refractory juices which are intensified under such conditions. It is to be expected therefore, that if dextro-rotary bodies other than sucrose do exist in bagasse, they will be found more extensively in bagasse not subjected to intense milling.

The method used for estimating the sucrose in bagasse is an arbitrary one, based on the extraction by means of boiling water of all substances capable of rotating the plane of polarised light.

Geerligs states that "the constituent which hot water dissolves from the fibre has a decided dextro-rotary power. For this reason the sucrose content of bagasse is found to be too high if the boiling is too prolonged."

Browne contends that hot water extracts optically active dextrins—gums and hemi-celluloses. Naudet believes that heat may induce a spontaneous maturing in the plant cells, while Miss Zafari theorises on the retention of sucrose by enzymes at low temperatures. Sidersky opposes this theory and attributes the increase of polarisation after long boiling to the presence of optically active substances derived from the hydrolysis of hemi-celluloses. Haddon recommends the use of baryta which he states nullifies the effect of other substances than sucrose.

EXPERIMENTS.

The first experiment carried out on bagasse shows how different results can be obtained by varying the procedure.

Bagasse with cold water brought to 100° C.	3.55%	Sucrose
Bagasse with cold water boiled for 40 mins.	3.95%	„
Bagasse with boiling water simmered for 30 minutes	3.65%	„

Second Series—different sample.

Bagasse with hot water 75° C., boiled gently for 15 minutes	3.60%	Sucrose
30 minutes	4.00%	„
45 minutes	4.10%	„
60 minutes	4.20%	„

Repeat experiment on another sample:

Bagasse with boiling water boiled gently for 45 minutes	3.45%	Sucrose
75 minutes	3.55%	„
105 minutes	3.65%	„
135 minutes	3.8 %	„

These results indicate that either the Sucrose has not been totally extracted in the first case or else other dextro-rotary bodies have gradually passed into solution.

The extract from the first sample (3.45) was now completely inverted with HCl and Invert calculated to a basis of Sucrose % Bagasse. Original Sucrose — 3.45; Calculated from Invert — 3.48.

Repeated on another sample:

	Sucrose.	Calc. from Inv.
Bagasse with boiling water 15 minutes	2.80	2.86
30 minutes	3.20	3.25

These experiments suggest an absence of dextro-rotatory impurities after boiling for 15, 30 and 45 minutes.

The next series of experiments was designed to establish if possible a point when a constant polarization is obtained.

a.	Boiled gently for	1	hour	3.55%	Sucrose
b.	„ „ „	2	„	3.80%	„
c.	„ „ „	3	„	3.70%	„
d.	„ „ „	4	„	3.80%	„
e.	„ „ „	5	„	3.75%	„

In this case a practical constant is obtained after two hours' boiling.

At the same time a series from the same bagasse was treated with water between 55 and 60° C.

Treated for	1	hour 55 to 60° C.		3.1%	Sucrose
„ „	2	„ „		3.3%	„
„ „	3	„ „		3.4%	„
„ „	4	„ „		3.4%	„
„ „	5	„ „		3.4%	„
„ „	6	„ „		3.4%	„
„ „	21	„ „		3.5%	„

A sample of the same bagasse was also treated with cold water as follows:

Treated cold water	2	hours		2.95%	Sucrose
„ „ „	4	„		3.10%	„
„ „ „	5	„		3.25%	„
„ „ „	6	„		3.2 %	„
„ „ „	19	„		3.4 %	„

The official method gave 3.55% sucrose, which after two hours' boiling rose to 3.80%. With water at 55° C. a constant of 3.40% was obtained after three hours. With cold water the same figure was obtained after 19 hours.

Experiment to find out if the extract undergoes muta-rotation. Sucrose determined by official method (3.55) and large quantity of extract withdrawn :

Allowed to stand for	½	hour		3.5%	Sucrose
„ „	1	„		3.5%	„
„ „	2½	„		3.5%	„
„ „	3½	„		3.6%	„
„ „	5	„		3.55%	„
„ „	19	„		3.5%	„

No signs of muta-rotation were observed.

The next attempt was to ascertain whether the constant obtained with water at 50—60° C. was the same for all types of bagasse.

Time.	Sucrose %	Time.	Sucrose %	Time.	Sucrose %
1 hour	3.15	1½ hours	3.95	1 hour	3.3
1¼ „	3.20	2 „	3.90	1½ „	3.4
1½ „	3.30	2¼ „	3.95	2 „	3.4
1¾ „	3.40	2½ „	3.95	2¼ „	3.45
2 „	3.40	2¾ „	4.00	2½ „	3.45
2¼ „	3.40				

With ordinary bagasse these experiments indicate that a certain constancy is obtained after treating the bagasse for two hours with water at 50° C., but that the figure obtained is appreciably lower than that obtained on boiling.

Experiments as above (digestion at 50° C. and boiling), using totally different types of bagasse.

Sticks of cane were divided into rind and pith and treated as follows:—

Rind (from burnt cane).—Treated with water at 50° C.:

2½	hours	5.4%	Sucrose.
3	„	5.6%	„
3½	„	5.7%	„
4	„	5.8%	„
4½	„	5.8%	„

At 100° C.:

2½	hours	8.7%	Sucrose.
3	„	9.5%	„

Pith (passed through a hand mill once).—Treated with water at 50° C.:

2½	hours	10.1%	Sucrose.
3	„	10.1%	„

The same at 100° C.:

½	hour	11.6%	Sucrose.
1	„	12.5%	„
1½	„	12.7%	„
2	„	12.8%	„
2½	„	10.0%	(Inversion).

Repeat Experiment.

Rind.—Treated at 50° C.:

1	hour	3.7%	Sucrose.
1½	„	4.3%	„
2	„	4.3%	„
2½	„	4.4%	„
3	„	4.5%	„
3½	„	5.0%	„

Pith.

1	hour	6.5%	Sucrose.
1½	„	6.9%	„
2	„	7.1%	„
2½	„	7.3%	„
3	„	7.5%	„
3½	„	7.7%	„

Cane without division into pith and rind but crushed in a hand mill first, gave the following results:—

Official Method.	Separate sample kept at 50° C. for 2 hrs.	Then boiled for:— ½ hr.	1½ hrs.	2½ hrs.	3½ hrs.
9.5% Suc.	8.9	10.2	—	—	—
10.6% Suc.	9.8	10.8	11.4	11.7	11.7
Tops 5.8% Suc.	4.3	5.1	5.3	5.3	5.3

These experiments demonstrate the irregularities which are obtained when widely different types of material are used.

The next experiments were carried out to check the official method against extraction at 50° C. and subsequent boiling.

Official Method.	Extraction at 50° C.	Same boiled for ½ hour.	Boiled for 1½ hours.
3.8% Suc.	3.7% Suc.	4.1% Suc.	—
3.4% „	2.7% „	3.3% „	—
3.2% „	3.0% „	3.3% „	—
3.7% „	2.9% „	3.2% „	—
2.8% „	2.6% „	2.9% „	—

Official Method.	Extraction at 50° C.	Same boiled for ½ hour.	Boiled for 1½ hours.
2.45% Suc.	2.4% Suc.	2.5% Suc.	—
2.85% „	2.85% „	2.85% „	—
3.0% „	3.1% „	3.55% „	—
2.6% „	2.55% „	3.0% „	—
2.75% „	2.5% „	2.8% „	3.0% Suc.
4.4% „	4.9% „	5.2% „	5.3% „
3.1% „	3.1% „	3.55% „	—
3.6% „	3.6% „	4.3% „	4.3% „
3.6% „	3.4% „	4.0% „	4.2% „
3.6% „	3.6% „	3.8% „	4.0% „
3.4% „	3.4% „	3.5% „	3.6% „
3.3% „	3.2% „	3.4% „	3.5% „
Average— 3.27% Suc.	3.14% Suc.	3.48% Suc.	—

It becomes increasingly evident from these experiments that varying types of bagasse, or varying types of fibre in bagasse, give variable results when subjected to any treatment.

In two cases of the above experiments an aliquot quantity of the extracts after digestion at 50° C. were concentrated on a water bath and an estimation of the sucrose content by Clerget inversion method carried out.

Pol.	Clerget Sucrose.
13.65	11.74
7.50	7.20

This was not pursued further.

Finally bagasse was lixiviated at 50° C. until no rotation was observed. The requisite amount of water was now added and the whole boiled gently for half-an-hour. Result: 0.15% Sucrose. Repeat experiments showed 0.15 and 0.1% Sucrose.

Attempts to identify the sugar present by formation of its osazone by the action of phenylhydrazine were unsuccessful owing probably to the extreme dilution.

Now the main points emanating from these experiments are:—

1. Lengthening the time of heating gives an increase in optical activity.
2. There appears to be no time constant for boiling. In some cases an apparent constant is reached while in other cases an increase is continuously observed.
3. Extraction with water at 50° C. appears to reach a fairly constant figure after 2—3 hours, but the figure obtained is appreciably below that obtained by boiling.
4. Irregular results are due to variations in the types of bagasse.

In addition to the above experiments a large number of ordinary bagasse routine tests were filtered with the addition of 7 cc. of alcohol and compared with ordinary results. An average of over 70 such tests showed no practical difference, though individual examples showed differences in a few cases of up to 0.3 in either direction.

CONCLUSIONS.

The general opinion of several authorities is that the increased rotation obtained on prolonged boiling is not sucrose. The principal point emanating from the above experiments is the indication of a constant obtained on treatment with water at 50° C. which is appreciably lower than any results obtained on boiling. The only supporting evidence that the difference is not sucrose is the fact that when bagasse is extracted first with ether and then boiled as usual the polarization drops from 0.1 to 0.6. This was described in a paper on Mealy Bugs in 1929.

In further support of the opinion that extraction with water at 50° C. represents a truer sucrose % than that obtained by boiling, it should be noted that crystalline substances (crystalloids) diffuse into water with comparative rapidity, whilst non-crystalline substances (colloids), such as gum, starch, gelatin, etc., diffuse with extreme slowness. The rates of diffusion, however, of all these substances are greatly increased with a rise of temperature.

It may be contended that diffusion of sucrose and non-crystalline bodies takes place in bagasse under varying conditions of temperature at varying rate.

At 50° C. it is contended that all the sugar has diffused after 2½ hours, and that increases obtained by boiling are not due to sucrose but to other dextro-rotatory bodies.

CHAIRMAN: Mr. Dymond has given us a paper which shows a considerable amount of careful experimental work. He has evidently gone into this question very thoroughly and has been very painstaking in arriving at conclusions. I am interested to see his results on continuous boiling, and that he usually gets a constant after two hours' boiling and sometimes a continuous rise. Last year we did a certain amount of experiments ourselves on bagasse and we found in a very great number of cases that we got a curiously shaped curve. There would be a sharp rise, we would then get a constant after two or three hours, then it would be a flat, but after four or five hours another sharp rise would start. The first rising part of the curve is where the sucrose was being diffused, but the diffusion of these other dextro-rotatory substances was very much slower and did not start until after long continued treatment was made; later on that diffusion became apparent and you got the further rise in the curve. We noticed that especially at Gledhow in our experiments where quite a large number of samples were done in this way. There was no doubt from the experiments we did that these dextro rotatory bodies are things which have a very definite effect on our sucrose determination in bagasse. I would

like to ask Mr. Dymond whether he has done anything in the way of isolating any of these bodies or identifying them?

Mr. DYMOND: I think when you read the conclusions of some of the world's authorities you will appreciate the great difficulty in getting this thing so that you can see it. We have tackled the problem from various points and it certainly was our hope that we would extract the substance and be able to definitely show it. But in that we have been entirely unsuccessful.

Mr. CHRISTIANSON: I would like to point out that the time taken to reach that constant polarization was 40 minutes not two hours. There was a very temporary constant which lasted about 20 minutes and thereafter the curve began to rise again.

Mr. DYMOND: I take it you refer to boiling?

Mr. CHRISTIANSON: Yes.

Mr. DYMOND: You will notice from this paper that the extraction referred to was at 50° C. only.

Mr. CHRISTIANSON: In connection with extraction at 50° C. I would like to ask Mr. Dymond if any preservative was used? 50° C. is about the optimum temperature at which enzymes begin to work.

Mr. DYMOND: No; just the usual carbonate. We did not take steps to add any mercuric chloride.

Mr. CHRISTIANSON: It is rather an important point. We all know bagasse ferments very rapidly and this figure obtained at 50° C. might therefore be too low.

Mr. RAULT: Putting aside the idea of these tests being done in order to investigate whether our methods of analysis are right or wrong, we may consider the experiments in another light. We may say that if we do not go over 50° C. in lixiviating or treating bagasse with water we are not able to extract a certain amount of rotatory substance. We do not know exactly whether this rotatory substance is sugar, but we find out that in other lands the subject of maceration has been studied with the idea of finding whether the effects of temperature are what the theory demands. Not many years ago all text books maintained that whether you treated your bagasse with hot or cold water there was no difference at all in your extraction because it was supposed that all the cells had been ruptured. Nowadays we do not think that this theory is right, and we have even the authority of Herzfeld, who maintains that after testing thoroughly the bagasse by microscopic analysis it has been revealed that many cells are not broken by the ordinary milling, and these cells can only be touched if we subject the bagasse to very high temperatures, over 50° C. That possibly is what Mr. Dymond has found. When you are treating your bagasse with higher temperatures you are really extracting more sugar; it opens our eyes, and it opens possibilities of changing our ideas on milling. At Natal Estates two years ago we were using boiling hot water and we had very good extraction, but I do not remember that the sugar that was extracted was not found in bagasse, so that the rotary substance taken away by the hot water may really have been final sugar.

Mr. BECHARD: I would like to express the same opinion as Mr. Rault. Mr. Dymond mentioned Naudet's theory; it is more than a theory. Miss Zafari put it down to enzyme action. I did some work in that connection in collaboration with Mr. de Froberville, and we found that using inhibitory reagents this enzyme action was arrested; in other words, by treating bagasse with either chloroform or pure formaldehyde we arrested that increase of polarization. I would not like to say for certain that there is not a possibility of these reagents causing the tissues of the cells to harden and therefore preventing hydrolysis of hemi-cellulose. I have an open mind on this subject, but the indications are that enzyme action did take place. I have attempted since to investigate the possibility of thermo-synthesis by finding the effect of time and temperature, but so far my observations are not yet complete. But I certainly think that under certain conditions you will find sugar which would otherwise be an invisible loss.

Mr. CHRISTIANSON: Arising out of what has been said by Mr. Rault and Mr. Bechard, it seems that authorities generally are not in agreement as to whether this substance is sucrose or not. When you look at Mead's Handbook, 1929 Edition, he states that De Haan did extract a dextro-rotatory gum, but he further found this gum was completely precipitated by lead acetate, and he also mentioned that Norris of Hawaii found no such dextro-rotatory substances. So far as our methods are concerned, 2½ hours at 50° C. seems to be rather a cumbersome method. Hardly any of our results in the Sugar Industry are strictly speaking absolutely accurate. The best we can hope for is to get somewhere near accuracy. We also want quick results if we can get them. I found last year at Gledhow using a bagasse probably different from Mr. Dymond's, but which is probably nearer the average, that on boiling for about 40 minutes we got a temporary constant. That means if you vary the time within about five to ten minutes you will get that figure which is therefore a comparative figure, and I think that in any bagasse determinations we do we should arrive at some such constant.

Mr. DYMOND: One point I think you forget and that is the determination of sucrose in bagasse means a lot of money to-day, and if we were to alter our comparative methods to two hours it would mean the Milling Companies would have to pay out quite a considerable amount of money as the sucrose in question would rise. I have a perfectly open mind on the subject which I am the first to concede is a very difficult one. There is another

point we must not lose sight of in quoting authorities from other countries; in this country we have a cane which is different from any other type in the world.

Mr. CHRISTIANSON: Still Geerligs stated that a hardy cane required a longer time for diffusion to take place. That probably applies to Uba. On the other hand, so far as the determination of sucrose per cent. bagasse affects the payment of cane, we have a certain amount of precedent. Meade gives methods in which the bagasse is boiled for one hour, and then we use Noel Deerr's apparatus. We don't use his methods altogether.

Mr. HAYES: Regarding the determination of bagasse involving quite a lot of money, the Fahey Agreement was based on the principle of the cost of manufacture and value of sucrose in cane on the methods which were then existing for determining these values. Should these methods change and we find different values for cane, I think the whole scale of payment will also have to be altered.

CHAIRMAN: Whatever should be the case in a thing like that we have to remember that the Fahey Agreement is a fixed thing now, and there will be no alteration in the values for another six years, but that does not debar us at any point in that six years bringing our methods up to a standard of greater accuracy. We must always bear in mind that it is accuracy we want, whichever way it goes. If it means more sugar reported in the cane or less sugar reported in the cane we are here considering it only from the technical point and the point of view of exactitude. So that the Fahey Agreement does not really affect the point at issue, which is that we want to get the most accurate method of determining sucrose in bagasse, and we are considering some of these things which are affecting it, in this case the dextro-rotatory bodies which do have a very sure effect on our determinations, but one which is not understood.

Mr. DYMOND: I might say the question of altering the method and its connection with the Fahey Agreement was never considered in making up these figures. It was rather on the point of view raised by Mr. Rault, and that is the importance of milling considerations—whether the use of maceration baths with boiling water was actually detrimental. It was rather on those lines we worked to find out whether it was better to use hot water or cold water for maceration.

CHAIRMAN: It might be interesting to get some experiments on this by extracting the bagasse to the limit and then determining the sucrose in the extract, to see of the total extractable substances in the bagasse what is sugar and what is gums. I don't know whether any experiments have been done in that direction at any time.

Mr. DYMOND: The nearest approach we got to actually handling the stuff is shown by these samples I have here. (Exhibits samples.) If you were to attempt to take some of this and boil it with ether and attempt to filter it, it would open your eyes to the retarding effect of these gums in filtration.

Dr. HEDLEY: I should like to ask whether Mr. Dymond at any time took a sample of bagasse and split it into two halves and carried out the same length of boiling on the two halves, and having done that, did you get the same figures? In other words, would duplicate results compare? Did you shred this bagasse or take it straight from the mill?

Mr. DYMOND: I did not shred it; it was taken straight from the mill.

Dr. HEDLEY: I have found when doing milling tests that it is an extremely difficult matter to take two samples of bagasse and get concordant results, on account of the size of the pieces of bagasse. You take a very small quantity of bagasse and call that representative of a very large bed passing through the mills, and it seems to me the very basis of this argument lies in the fact that one has got to get first of all duplicate analyses to agree, and the only way to get that will be by very intimate breaking up of your fibres. By that means it will show whether you are extracting more sucrose or whether you are inverting sucrose. Unless it is shredded I feel a little doubtful of any conclusions that are drawn from the result of such experiments. The only mill in the country which gets anything like a milling test which can be relied upon as accurate is at Mount Edgecombe on account of the fibre having been shredded. In the other mills the taking of a representative sample is very uncertain owing to the great variation in the size of the bagasse. What is a representative sample? How has Mr. Dymond solved his problem?

Mr. DYMOND: It is evident that Dr. Hedley has had the misfortune not to have visited the Empangeni Mill in the last year because I think the bagasse there, while not exactly shredded, is nevertheless broken up into very even particles, and it is quite simple to obtain a composite sample of bagasse, and in a few experiments we did of tests side by side you will notice the sucrose determinations under the same conditions.

Dr. HEDLEY: It does come down to the shredding of the bagasse to get the samples.

Mr. DYMOND: Yes, the condition of the bagasse.

Mr. ASKEW: I feel after listening to these gentlemen, in view of all the difficulties you have in arriving at a conclusion as to the value of sucrose in cane—and I think you will agree—the best thing is to go back to the old system and pay us so much a ton on the weight of our cane! (Laughter.)

Mr. CHRISTIANSON: It must be borne in mind that these differences do not affect the sucrose in cane very much, and if you boil bagasse 20 minutes to 45 minutes it comes out not even a halfpenny a ton.

Mr. ASKEW: Oh well, that is all right! (Laughter.)