CXXXVI. THE REMOVAL OF SUGARS FROM DILUTE SOLUTIONS.

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MOST of the recorded investigations on the fermentation of sugar solutions by the yeast cell involve the use of a relatively small initial seeding yeast rate and a large amount of sugar. The converse of this, *viz.* a high seeding rate acting on very dilute sugar solutions, has received less attention, and but little is known as to the rate at which sugar disappears under such conditions. The subject presents many points of interest, and a study of it should throw some light on the mechanism of fermentation in its initial stages. Somogyi [1926-27] in the course of some work on the estimation of "true sugar" in blood, urine, *etc.*, considered that a protein and a protein precipitant were necessary factors in the removal of sugar by yeast from such fluids. Later, however [1928], he apparently modified his ideas and reverted to the older practice of fermenting the de-proteinised filtrates.

Preliminary experiments showed that dextrose and maltose were completely removed from 150 cc. of a 0.2 % aqueous solution of these sugars by exposing them for 30 minutes to the action of 30 g. of pressed brewer's yeast. This is equivalent to a yeast rate of 100 g. of yeast per g. of sugar. Under similar conditions of working sucrose and invert sugar were removed to a slightly less degree, whilst lactose, galactose and arabinose were unaffected. Subsequently, the experimental methods employed by us were as follows.

Approximately 0.2 % solutions of the sugars used were prepared, and 150 cc. of these were well stirred up with the quantity of washed yeast required to give the desired yeast rate. The suspension was kept at 15.5° with frequent stirring for the required time, the whole was then centrifuged, and the supernatant liquid filtered and titrated with Fehling's solution. From the difference between the sugar concentration so found and that in the original solution is calculated the amount of sugar removed by the yeast.

The following results were obtained with dextrose, sucrose and maltose, the time and yeast rate being arranged so as to permit of the removal of only a portion of these sugars.

Table I.

Sugar	Sugar per 100 cc. g.	g. of yeast per g. of sugar	Time of action in minutes	% of sugar removed	Calculated weight of sugar lost per 100 g. of yeast per hour
Glucose	0.1946	33.3	30	51	3.06
„	0.1946	66.6	10	32.9	2.96
Sucrose	0.1987	33.3	30	53.8	3.23
„	0.1987	66.6	10	32	2.88
Maltose	0.199	33.3	30	50	3.00
„	0.199	66.6	10	30	2.70

It will be seen from the figures in the right-hand column that under the experimental conditions employed (1) the capacity of yeast to remove up to 50 % of the three sugars is approximately equal, and (2) that such removal is proportional both to time and yeast rate or, in other words, that time and yeast rate are interchangeable factors in controlling the degree of sugar removal.

If the time and yeast rate used result in a removal of more than half the sugar this relationship no longer holds, as the following example shows:

Table II. *Sucrose 0.2 % solution.*

g. of yeast per g. of sugar	Time in minutes	Percentage of sugar removed	Calculated weight of sugar lost per 10 g. of yeast per hour
33.3	10	16.5 A	2.97
33.3	30	46.2 B	2.77
33.3	60	76.7 C	2.30
66.6	10	33.2 A	3.00
66.6	30	76.7 C	2.30
66.6	60	90.6	1.36
99.9	10	45.6 B	2.74
99.9	30	87.6	1.75
99.9	60	99.0	1.0

The interchangeability of yeast rate and time is well shown in the figures marked A, and B above, but the departure from the linear relationship becomes more marked as complete removal of the sugar is approached (C).

In the course of this work it soon became evident that samples of yeast collected from day to day for use in brewery fermentations varied considerably in their capacity for removing sugar under our conditions of experiment. Some of the possible factors which might give rise to these variations such as age, washing, exposure to air and inert gases *etc.*, were studied, but so far we have been unable to elucidate the causes.

A measure of this variability can be made by testing the yeast with a solution of maltose (0.2 %) under defined conditions of yeast rate (33 %) and time (30 minutes). This as a rule removes about 50 % of the maltose, but from the results of working with many samples of yeast, it was found that the figure may rise to 70 or fall to 20 %.

It will be noted that the loss of sugar arising from the action of yeast under the conditions described has been referred to as "removal"¹ as it seems unlikely that true fermentation (entailing the formation of CO₂ and alcohol) can have taken place in so short a time even with the high yeast rates employed when bearing in mind the period required for the "lag phase" which occurs with normal seeding rates. A reference to Table II shows for instance that nearly 50 % of the sucrose present disappeared from a 0.2 % solution in 10 minutes when using a yeast rate of 100. This means the loss of 0.15 g. of sugar equivalent to the formation of 0.07 g. of CO₂ (37 cc. at 15° and 760 mm.).

If a production of CO₂ of this order actually occurs it should readily be observable by carrying out the reaction in a closed vessel fitted with a narrow-bored U-tube manometer containing water, and noting the rise in the open limb after shaking from time to time. This was in fact done and the rise in mm. read off and plotted. The curve so obtained is linear and regular between the periods of 10 and 35 minutes—the time at which the experiment was stopped. It is of course necessary to carry out a control digestion using water in place of maltose

¹ Somogyi terms it "adsorption."

solution to allow of a correction being made for the CO_2 produced as a result of the intracellular respiration of the yeast. The gas so liberated amounted to 25 % of the total obtained in the yeast-maltose digest. When lactose was similarly tested, a rise of a few mm. only was obtained (after deducting the respiration control) and the solution showed no loss of sugar even after 3 hours when tested with Fehling's solution. There would thus seem to be little doubt that fermentation is the real cause for the removal of sugar, the more so as our experiments show that only "fermentable" sugars are in fact removed.

The employment of toluene in the digest should throw light on this question, as fermentation should be impossible in its presence, but as the figures in Table III show, the removal of sugar is much reduced but not entirely stopped

Table III. *Action of antiseptics.*

Sugar 0.2 % solution	Antiseptic used	Concentration of antiseptic	Yeast rate	Time	% of sugar removed
Maltose	Nil	—	33	30 min.	50
"	"	—	33	24 hr.	100
"	Toluene	Sat. aq.	33	30 min.	13
"	"	"	33	24 hr.	Dextrose formed
"	Salicylic acid	0.1 % aq.	33	20 min.	4
"	"	"	33	24 hr.	3
"	Alcohol	12 % by vol.	33	30 min.	26
"	"	"	33	24 hr.	100

in the first 30 minutes. If the digestion period is prolonged, dextrose is formed as the result of the action of yeast maltase on the maltose solution as shown by the titration; the preparation of glucosazone confirms this. Salicylic acid (0.1 % solution) however arrests fermentation completely, while 12 % (by vol.) alcohol weakens the yeast in the earlier stages, but does not prevent the entire removal of sugar in 24 hours.

It would appear probable therefore that toluene in saturated aqueous solution is not capable of entirely stopping fermentation, and the belief of many workers that it does so needs revision.

Behaviour of S. marxianus towards sugars.

This yeast, though capable of fermenting dextrose, is unable to ferment maltose, presumably owing to the absence of the maltose-hydrolysing enzyme, maltase, from its cells, and its action towards maltose under the conditions described should therefore be of interest. If it succeeded in "removing" maltose from solution, some action other than fermentation must be the cause, as for example adsorption on the surface of the yeast cell, or entry into the cell followed by the formation of a non-diffusible complex therein, while if it behaves as *S. cerevisiae* does to lactose (*i.e.* is without action), it becomes increasingly probable that fermentation is the cause of maltose removal by *S. cerevisiae*. Some *S. marxianus* was grown in bulk to test its action and it was found to be incapable of removing any maltose from solution under conditions which allowed of a 50 % removal of the sugar by *S. cerevisiae*.

The work described up to this point has been done with fresh brewery yeast, washed with tap-water and filter-pumped to a moisture content of 76–78 %. It seemed of interest to see if baker's yeast behaved similarly. It was found that while dextrose and sucrose are readily removed by this yeast only 5–15 % of

maltose disappeared under conditions which caused the loss of 50 % or more when brewer's yeast was employed. This result would suggest that baker's yeast is deficient in maltase, without which maltose cannot be hydrolysed to dextrose and so rendered fermentable (*cf. S. marxianus* and *S. exiguus*). It is difficult to reconcile this idea with the fact that baker's yeast undoubtedly ferments maltose formed from the action of the amylase of flour on wheat starch in the dough during bread making. The total gas yielded at this stage is much in excess of that due to the small amounts of sucrose and invert sugar already present in the flour. When brewer's yeast was kept together with toluene and maltose (see Table III) for 24 hours, dextrose in abundance was found in the digest owing to the action of the yeast-maltase. Baker's yeast when similarly tested, failed to produce dextrose and this appears to confirm the suggestion that its failure to remove maltose from solution under the standardised conditions is related to the deficiency of maltase. It is, however, possible that variation such as those met with in brewer's yeast may also be found with baker's yeast.

SUMMARY.

1. Conditions for the removal of dextrose, sucrose, invert sugar and maltose from dilute solutions are described. When conditions are arranged so as to limit the loss of sugar, time and yeast rate were found to be interchangeable factors in controlling the degree of such loss.

2. Experimental evidence is adduced showing that the removal of sugar in such short periods of time is due, not to adsorption, but to fermentation.

3. The behaviour of *S. marxianus* and baker's yeast towards sugars was investigated; the latter yeast, which resembles brewer's yeast in its behaviour to dextrose, has but little action on maltose under the conditions described.

REFERENCES.

- Somogyi (1926-27). *Proc. Soc. Exp. Med. Biol.* **24**, 320.
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