II.—NOTE ON A SIMPLE METHOD FOR CONCENTRATING ENZYME SOLUTIONS.

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Solutions of colloids and crystalloids when subjected to temperatures below their freezing points, throw down crystals of the solvent, with the result that a more concentrated solution remains. This property of solutions, more or less universal, can be utilised in concentrating dilute solutions of enzymes.

The method consists essentially in freezing the dilute enzyme solution under mechanical stirring. This stirring facilitates the precipitation of ice in fine crystals, thereby preventing the enclosure of the mother liquor and consequent loss of enzyme. The vessel containing the enzyme solution is placed in a freezing mixture of ice and common salt and kept in an ice-chest. The freezing vessel is provided with an inverted Buchner funnel resting on the bottom and containing a pad of glass wool, the stem of the funnel being connected through a filter flask to a filter pump by means of thin pressure-tubing. The solution is kept constantly stirred by a simple mechanical device and, when the temperature has fallen below 0°, a small quantity of ice is added in order that the solvent may crystallise out without any supercooling. When part of the solvent has separated the filter flask is evacuated, whereby the mother liquid containing the enzyme and free from the separated ice collects in the flask. It is evident that enzyme losses, if any, are mainly due to an imperfect removal of the mother liquor from the freezing vessel due to inefficient suction, there being little or no adsorption of enzyme by the separated crystals. This is readily proved by the fact that, when the separated ice is transferred to a Buchner funnel and about half allowed to melt and drain away, the remaining ice contains practically no enzyme which would not be the case if any enzyme were adsorbed. The amount of enzyme left in the residue in the freezing vessel is in fact very small, less than 5 per cent. and even such losses can be effectively minimised by powerful centrifugal separation.

A repetition of the process with the once concentrated liquor effects a further concentration and the operations can be repeated until the desired concentration is attained.

The method has been successfully applied in concentrating yeast autolysates and barley malt-extracts, a nearly five-fold concentration being achieved in three successive operations.

The following table illustrates the progress of the concentrations, when yeast autolysates are treated by this method, in a very carefully conducted experiment:—

Progress of Concentration.

Enzymic material	Activity	Total solids per cent.	Ash per cent.	Liquor density	Freezing point depression	Relative viscosity η'/η
Original yeast-liquor ...	− 1·49°	7·6	1·20	1·014	− 2·10°	1·121
I Concentration ...	− 2·85°	10·6	1·37	1·034	− 2·93°	1·499
II do. ...	− 4·94°	13·2	1·64	1·042	− 3·80°	1·920
III do. ...	− 6·64°	15·8	1·90	1·052	− 4·01°	2·081

The figures in the activity column, refer to the fall in rotation of a 10 per cent. cane-sugar solution, when 20 c.c. of it are treated with 1 c.c. of the yeast-liquor at 28°, the action being stopped at the end of 30 minutes by the addition of 5 c.c. of 2-*N* sodium carbonate. Starting with 1000 c.c. of yeast-liquor of activity − 1·64°, 120 c.c. of liquid having activity − 6·64° was obtained.

The special value of the method lies in the fact that the enzyme concentration is effected under conditions least harmful to the stability of enzymes. The method is very simple to conduct and is of general applicability.

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