121. ON THE MECHANISM OF FERMENTATION OF TREHALOSE BY *FUSARIUM LINI* BOLLEY

By RITA C. O'CONNOR

*From the Department of Organic Chemistry, Fordham University,
New York, N. Y.*

(Received 27 May 1940)

It has been pointed out and experimentally proved by several authors [Krebs, 1935; Hofstetter *et al.* 1938; Macfarlane, 1939; Deuticke & Hollmann, 1939; Shaw & Barker, 1939] that the phase sequence of the enzymic conversions of different substrates is dependent on the actual state of the cells harbouring the enzymes concerned. These authors imply that there exists virtually no proof for the common assumption that evidence of the course of degradations or dissimilations of this kind, obtained with the aid of artificial preparations, represents the actual course of the phase sequence within the living cell.

Recent attempts to determine the actual phase sequence during the alcoholic fermentation of carbohydrates appear especially informative in this respect. In a recent review [Nord, 1940] of progress in regard to the mechanism of alcoholic fermentation by yeasts, fusaria, *Zygosarcina ventriculi* and *Thermobacterium mobile* Lindner, it is pointed out that not only do certain living fusaria possess the capacity to convert carbohydrates enzymically by three independent phase sequences, but also that ordinary living yeasts do not follow exclusively one path in the course of sugar dissimilation.

This does not necessarily mean that results obtained with an enzyme preparation have to be regarded as contradictory to the course of the reaction within the living cell. It suggests, however, the suitability of tracing the mechanism of degradation of certain compounds by using enzymically comparable living organisms where the existing data, obtained with the aid of artificial enzymic preparations, do not clarify the actual phase sequence within the original living system. A search of the literature [Armstrong & Armstrong, 1934; Myrbäck *et al.* 1937; Myrbäck, 1939; Nord & Engel, 1938; Dammann, 1938] revealed the following facts. First, maltose and lactose may be fermented by living *Fusarium lini* Bolley directly as well as indirectly, and are not, in the case of direct fermentation, necessarily phosphorylated. No observable phosphorylation accompanies the onset of fermentation of these disaccharides. Second, living *F. lini* Bolley contains an enzyme which enables it to ferment dextrin with the evolution of CO₂ and the formation of ethyl alcohol. Third, no decisive data whatsoever are available concerning the question of fermentability and the mechanism of fermentation of trehalose by yeasts.

At the suggestion of Mr Nord, I have undertaken a series of experiments to decide upon the fermentability of added, not native, trehalose by means of living *F. lini* Bolley.

EXPERIMENTAL

The following nutrient medium was used for all experiments:

100 ml. distilled water.

1.29 g. KH_2PO_4 (approx. 300 mg. P per 100 ml.).

0.08 g. $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$.

0.02 g. KNO_3 .

Carbohydrate in amounts designated in each experiment.

The initial pH of the medium was 4.4. The water content of crystalline trehalose was taken into consideration in calculation of amounts of trehalose used. All inoculations were made with mycelium from agar cultures of *F. lini* Bolley. CO_2 was determined according to the method of Truog [1915] with a modification whereby ascarite was employed for removal of CO_2 before aeration of flasks.

In preliminary experiments, measurement of CO_2 indicated that added trehalose ferments at a rate comparable with that of glucose (cf. Table 1).

In the series of experiments in Table 2, the carbohydrate concentration was 1%. The first measurements were made on the second day after inoculation. It is to be observed that the CO_2 evolution in the case of trehalose fermentation reaches a higher maximum than in glucose fermentation.

Table 1

Day	mg. CO_2 evolved daily from 0.75 % solutions of	
	Glucose	Trehalose
1	—	—
2	14.6	27.6
3	44.6	51.5
4	55.5	61.4
5	92.8	139.9
6	62.3	118.0
7	26.5	82.0

Table 2

Day	Daily CO_2 evolution in mg.			
	Glucose		Trehalose	
	I	II	III	IV
1	—	—	—	—
2	14.2	12.6	19.9	19.6
3	61.3	56.0	45.9	26.9
4	88.2	116.6	90.7	100.4
5	102.3	112.0	112.9	123.3
6	76.4	95.5	112.4	134.7
7	72.0	83.1	145.2	140.5
8	—	54.6	82.3	—
10	43.5	37.7	60.5	44.2
11	19.7	20.2	19.0	12.2
15	5.6	5.0	5.9	4.3

During the course of the fermentation process, the pH of the solutions changed to different degrees, the trehalose solutions more nearly maintaining the initial value of 4.4. After three weeks, the pH of the glucose solutions was 4.9 while the trehalose solutions showed a value of 4.6.

In the case of experiments where trehalose was the carbon source, growth was observed 6 to 8 hr. in advance of any perceptible development of mycelium in the glucose cultures. After three weeks, the mycelium from flasks used for CO₂ determinations was separated from nutrient medium, rinsed and dried in an oven for 1 hr. at 80° on filter paper which had been previously dried and weighed. The weights show very clearly the preferential growth of *F. lini* Bolley on trehalose. The dry weights of mycelium from two trehalose cultures were 120 and 138 mg., while those from two glucose cultures were 82 and 86 mg. The media contained 100 ml. of 1 % carbohydrate solution.

The course of the dissimilation of trehalose and of glucose was followed polarimetrically. Solutions containing 1 g. of carbohydrate per 30 ml. were used in these experiments in order to give readings sufficiently large to permit detection of slight differences. Samples for this determination were removed by means of a sterile pipette, filtered rapidly and transferred without dilution to polarimeter tubes. Results of such a series of experiments are shown in Table 3. The specific rotations for crystalline trehalose and for glucose are +178.3° and +52.6° respectively.

Table 3

Day	Polarimeter readings				Sugar content calculated in g. per 30 ml.			
	Trehalose		Glucose		Trehalose		Glucose	
	I	II	III	IV	I	II	III	IV
0	+6.394°	+6.388°	+1.765°	+1.698°	1.08	1.08	1.00	0.97
1	6.426	6.462	1.684	1.698	1.08	1.08	0.96	0.97
2	6.498	6.308	1.636	1.666	1.09	1.06	0.93	0.95
3	6.128	6.075	1.515	1.505	1.03	1.02	0.86	0.86
4	5.394	5.456	1.466	1.472	0.91	0.92	0.83	0.84
5	4.512	4.546	1.418	1.456	0.76	0.76	0.81	0.83
6	3.43	3.45	1.398	1.45	0.58	0.58	0.80	0.73
7	2.04	2.05	—	—	0.34	0.34	—	—
8	0.79	—	1.21	—	0.15	—	0.69	—
9	0.12	0.125	1.08	1.11	0.002	0.002	0.62	0.63
15	0.00	0.00	1.00	1.01	0.00	0.00	0.57	0.57

It is significant that the rate of disappearance of trehalose exceeds the rate of glucose dissimilation. Likewise the rate of disappearance of trehalose conforms closely to the rate of CO₂ evolution in trehalose fermentation (cf. Fig. 1).

In several series of experiments undertaken with the idea of determining whether trehalose can be phosphorylated by means of added inorganic P, or to compare the speed of esterification of trehalose with that of the phosphorylation of glucose at an initial pH of 4.4, it was found that trehalose is esterified practically at the same rate and to the same extent as glucose (3-4 % on the 6th day). Phosphate was determined according to Fiske & Subbarow [1925]. It appears, however, to be questionable whether this observation, in connexion with the optical measurements of the rate of disappearance of trehalose in the course of its dissimilation, can be reconciled, at least at present, with the considerations of Lipmann [1935] in regard to the glycolysis of polysaccharides with the aid of rabbit muscle extracts.

DISCUSSION AND CONCLUSIONS

Unlike living yeasts, living *F. lini* Bolley ferments trehalose more rapidly than glucose. Growth of the organism is more rapid and more abundant on trehalose than on glucose. Since the rate of trehalose dissimilation is more rapid

than that of glucose the occurrence of the latter as an intermediary is excluded, for, in such a case, the dissimilation rate of glucose would be the controlling step. The indications thus far observed point toward a *direct* fermentation of this disaccharide. Glucose has never been observed in a fermenting trehalose mixture and, consequently, we cannot say that under our experimental conditions there was a trehalase active or present in *F. lini* Bolley.

The culture of *F. lini* Bolley No. 5140 was received from the Biologische Reichsanstalt in Berlin-Dahlem through the courtesy of Dr Wollenweber. The trehalose for our experiments was obtained from two sources. Pure crystalline

Fig. 1. Comparison of optical activity and CO₂ evolution in the course of trehalose fermentation.

× — × Measured according to optical activity.
 ● — ● Measured according to CO₂ evolution.

trehalose was supplied through the assistance of Dr Gudernatsch, Nutley, N.J., by Messrs Hofmann-La Roche and Co. Ltd. in Berlin. A pure product was also isolated from dried top yeast, furnished through the kindness of Dr C. N. Frey of Fleischmann Laboratories, New York.

REFERENCES

- Armstrong & Armstrong (1934). *The Carbohydrates*. London: Longmans, Green & Co.
 Dammann (1938). *Ber. dtsh. chem. Ges.* **71**, 1865.
 Deuticke & Hollmann (1939). *Hoppe-Seyl. Z.* **258**, 175.
 Fiske & Subbarow (1925). *J. biol. Chem.* **66**, 375.
 Hofstetter, Leichter & Nord (1938). *Biochem. Z.* **295**, 414.
 Krebs (1935). *Biochem. J.* **29**, 1639.
 Lipmann (1935). *Biochem. Z.* **276**, 236.
 Macfarlane (1939). *Biochem. J.* **33**, 574.
 Myrbäck, Örténblad & Ahlborg (1937). *Enzymologia*, **3**, 210.
 — (1939). *Svensk. kem. Tidskr.* **51**, 36.
 Nord & Engel (1938). *Biochem. Z.* **296**, 153.
 — (1940). *Handbuch der Enzymologie*, Leipzig, p. 968.
 Shaw & Barker (1939). *Biochem. J.* **33**, 1809.
 Truog (1915). *J. Industr. Engng. Chem.* **7**, 1045.