Utilization of Sugars by *Chlorella* under Various Conditions

By M. RODRÍGUEZ-LÓPEZ

Institute 'G. Marañón' Velázquez 138, Madrid-6, Spain

SUMMARY

The consumption and utilization of a series of carbohydrates by a strain of *Chlorella pyrenoidosa* under different conditions of culture was examined. Only glucose, fructose and mannose were avidly consumed; galactose was consumed in small quantities. Light was highly stimulatory for the consumption of sugars. The carbohydrates assimilated were polymerized to starch; this induced gigantism in the organisms, a considerable increase in the dry weight, and inhibition of division.

INTRODUCTION

The various conclusions that have been arrived at about the nutrition and assimilation powers not only of related species of the genus *Chlorella*, but also within a given species, made us ponder on the great capacity of this organism to alter or change itself biochemically, thus making its taxonomic characteristics more complex. These considerations prompted us to make a study of the behaviour of our strain of *C. pyrenoidosa* when assimilating a series of sugars under different cultural conditions. Previously (Rodríguez-López, 1963) we showed that sugars induced gigantism in chlorella; but we felt that it would be interesting to know the behaviour of the alga towards the sugars studied; by growing cultures of it under various conditions, we investigated what sugars it could use, what quantities of these it consumed and how this consumption affected the yield, division and growth of the organism.

METHODS

An inoculum equivalent to 17 mg. dry wt. *Chlorella pyrenoidosa* was placed aseptically in 250 ml. Erlenmeyer flasks containing 100 ml. of basal culture medium A (Rodríguez-López, 1964). Each flask contained a 16 mM concentration of the sugar to be studied: glucose, fructose, mannose, galactose, xylose, arabinose, rhamnose, sorbose, lactose, sucrose, maltose (Sigma Chem. Co.).

The cultures were grown in daylight and aerated with an 8 l./hr mixture of air containing 5 % (v/v) CO₂; other cultures were grown in daylight without aeration and still others in darkness without aeration. The temperature was maintained at 24° and all flasks were periodically shaken. The experiments lasted for 4 days and controls without sugar were run. Each experiment was repeated 10 times.

Consumption of carbohydrates was determined, by difference, by the Somogyi-Nelson colorimetric method (1944, 1952) (the sucrose being previously hydrolysed).

Growth of the cultures was measured by counting the number of organism with a Burkner-ruled haemocytometer slide and the growth index (*GI*) determined:

$$GI = \frac{\text{No. organisms/mm.}^3 \text{ at end of experiment}}{\text{No. organisms/mm.}^3 \text{ at beginning of experiment}}$$

The size of organisms was also measured by using a previously calibrated micro-meter eye-piece.

The dry weights of organism were obtained by desiccation in an oven at 105° to constant weight. The results of consumption of carbohydrates and increase in dry wt. are recorded with reference to 100 mg. dry wt. alga.

RESULTS

Table 1 shows the consumption of the various carbohydrates under the various culture conditions. Of the sugars studied, only glucose, fructose and mannose were consumed in significant quantities when cultures were grown in daylight without aeration. The lowest consumption was by cultures kept in darkness. Galactose and xylose were also consumed, but in smaller quantities, especially the latter. None of the other sugars was consumed. The increase in the dry wt. of alga was proportional to the consumption of carbohydrates (Table 2). Nevertheless, the growth index presents very peculiar features, in accordance with the conditions under

Table 1. *Carbohydrate (mg) consumed in 96 hr by equiv. 100 mg. dry wt. of chlorella cultivated under various conditions, in a mineral medium with the addition of 16 mM concentration of a sugar*

Sugar added	In daylight, aerated, and 24°	In daylight, no aeration, and 24°	In darkness, no aeration, and 24°
Glucose	528 ± 26	1228 ± 86	426 ± 22
Fructose	441 ± 11	1162 ± 88	448 ± 35
Mannose	480 ± 33	1114 ± 40	346 ± 24
Galactose	102 ± 6	162 ± 31	152 ± 22
Xylose	47 ± 26	40 ± 22	0
Ribose	0	0	0
Arabinose	0	0	0
Rhamnose	0	0	0
Sorbose	0	0	0
Lactose	0	0	0
Sucrose	0	0	0
Maltose	0	0	0

Table 2. *Increase in weight (mg. dry wt.) of equiv. 100 mg. dry wt. of chlorella cultivated under various conditions, in a mineral medium with the addition of a 16 mM concentration of a sugar*

Sugar added	In daylight, aerated, and 24°	In daylight, no aeration, and 24°	In darkness, no aeration, and 24°
Glucose	454 ± 26	707 ± 57	235 ± 50
Fructose	444 ± 23	617 ± 43	231 ± 48
Mannose	450 ± 45	617 ± 58	246 ± 30
Galactose	307 ± 19	86 ± 10	51 ± 3
Xylose	215 ± 4	0	0
Ribose	222 ± 6	0	0
Arabinose	211 ± 5	0	0
Rhamnose	212 ± 3	0	0
Sorbose	216 ± 3	0	0
Lactose	255 ± 23	0	0
Sucrose	212 ± 4	0	0
Maltose	238 ± 17	0	0
Control	260 ± 27	47 ± 14	0

which the culture was kept (Table 3). Those cultures which contained assimilable sugar and were grown in daylight without aeration showed inhibition of growth to a marked degree when they contained either glucose, fructose or mannose. These experiments showed that only assimilated sugars produced giant forms (Table 3). Controls without sugar did not grow if placed in the dark.

Table 3. *Growth index over 90 hr of chlorella cultivated in a mineral medium with or without a sugar and under differing environmental conditions*

Sugar added	In daylight, aerated and 24°	In daylight, no aeration, and 24°	In darkness, no aeration, and 24°	Giant forms
Glucose	2.85	1.95	1.05	Yes†
Fructose	2.82	1.97	1.07	Yes
Mannose	2.89	1.92	1.06	Yes
Galactose	3.27	2.09	1.07	No
Xylose	4.78	2.14	0.98	No
Ribose	4.98	2.17	1.02	No
Arabinose	4.89	2.14	1.09	No
Rhamnose	5.04	2.20	1.00	No
Sorbose	5.08	2.20	0.97	No
Lactose	5.10	2.18	1.02	No
Sucrose	4.98	2.10	1.03	No
Maltose	4.90	2.13	1.00	No
Control*	5.44	2.21	0.98	No

* No sugar added.

† There are giant forms in all the cultures having glucose, fructose or mannose.

DISCUSSION

Of the various carbohydrates tested only glucose, fructose and mannose were consumed in relatively large quantities; these results differ from those of Samejima & Myers (1958). This supported our suspicion of the existence of biochemical modifications within the same species, that is to say that, as in other micro-organisms, including unicellular green algae, it reveals changes at the biochemical level induced by the environment and the cultural conditions.

Sucrose, which according to Milner (1948) is a component of *chlorella*, was not assimilable. This suggests the existence of barriers against penetration in the cell wall or cytoplasmic membrane.

The low consumption of xylose might have been due to impurities in the samples of this sugar. In the case of galactose, which was only used during the first 60 hr, perhaps a blocking mechanism is brought into play with the result that passage of this sugar is impeded.

Table 2 shows the high consumption of sugars in cultures exposed to light and not aerated as compared with that of those kept in darkness, so that light determined a difference in consumption. Griffiths (1961) referring to the light/cell-division relationship, suspected a 'participation of some photoreaction other than photosynthesis in the control of active cell division'. In our case also a factor dependent on light and distinct from photosynthesis, which acted as a stimulant of consumption of assimilable sugars, was shown.

The quantities of sugars consumed in the cultures kept in light and aerated

would in some way compensate for the autotrophic deficiencies of the cultures chiefly during the hours of darkness at night, when also CO_2 was denied to them, for it is well known that chlorella is capable of using both forms of nutrition, autotrophy and heterotrophy, simultaneously.

The increase in dry wt. in the cultures is due in our case to the reserve substances accumulated, and are in direct proportion to the quantity of sugar consumed (Tables 1 and 2), which they later polymerize, transforming it into starch, as we demonstrated previously (Rodríguez-López, 1965); for while the normal forms of chlorella contain 23 % of total carbohydrates, the giant forms contain 84 %. So that if the alga takes from the medium glucose, fructose or mannose in quantities determined by the conditions of the culture, these sugars are polymerized by it into starch, which accumulates in the cell, causing a great increase in volume and dry wt. and producing giant forms, of measurements given by us (1963). The small quantity of galactose consumed increased the cell volume to only a very insignificant extent (Table 2).

The inhibition of cell division in all cultures exposed to light, whether aerated or not, in the presence of assimilable carbohydrates is explained (Table 3) by the disequilibrium of the consumption of these in relation to nitrogen. The lower the N/C consumption ratio, the greater the decrease in the synthesis of nucleic acids and protein; and as a result, cell division will be negatively affected.

Giant forms produced in darkness or in light are discoloured in part in comparison with normal ones. We have shown (1965) that this phenomenon, mentioned by Ikuko Shihira-Ishikawa & Eiji Hase (1964), is due to the high concentration of starch in the organism, which completely disorganizes the chloroplast and distributes the pigment in a greater cellular volume.

Where inhibition of cell division by assimilable sugars is really manifest is in cultures exposed to light and aerated. The growth in cell population remains at less than half the normal level. Under these conditions of autotrophic growth little gigantism is to be seen, despite the fact that the alga has optionally at its disposal an assimilable sugar as a source of organic carbon.

Thus, in this work with our strain of *Chlorella pyrenoidosa* we arrive at results similar to those obtained by Griffiths (1963) in *C. vulgaris* under the influence of glucose, that is to say, that under the conditions pointed out in our work, chlorella avidly consumes glucose, fructose and mannose, light being a very stimulating factor in the consumption of sugar. Inhibition of cell division, a notable increase in the dry wt. and significant gigantism are the results of this consumption; while if the cultures are maintained in normal autotrophic conditions, the metabolizing sugars alone will be used to make good the deficiencies, producing an increase in the dry wt., very little gigantism and markedly inhibited cell division.

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