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On the Utilization of Cellobiose and D-Glucose by *Clostridium thermocellum*

Clostridium thermocellum is an anaerobic, thermophilic and cellulolytic microorganism (5, 6) potentially useful for the direct conversion of cellulosic biomass to ethanol (2, 3, 7, 8). *C. thermocellum* can grow on cellulose, cellobiose and D-glucose as the sole sources of carbon and energy, but an extended lag time is observed before growth on D-glucose occurs (4). By successive transfers on glucose, cultures adapted to grow on glucose are obtained that no longer manifest that extended lag time, however the final cell concentrations are lower on D-glucose than on cellobiose (4). Thus, it was of interest to understand why the final cell concentrations were lower when this microorganism grew on D-glucose compared to cellobiose.

C. thermocellum, strain ATCC-27405, was obtained from the American Type Culture Collection (Rockville, Mar.). The growth of the microorganism and the sugar analyses were performed as previously reported (4). Ethanol and acetic acid were quantitatively analyzed by absorption gas chromatography with a 6 ft Teflon coated column, packed with Chromosorb 101 in a Hewlett Packard 5839-A gas chromatograph. For the assay of ethanol, the temperature was isothermal (110° C) with injector and detector temperatures at 200 and 250° C, respectively; the flow rate of carrier Helium was 60 ml/min. For the assay of acetic acid, the temperature was isothermal (170° C) with injector and detector temperatures and carrier gas flow as noted above. The lactic acid content was assayed with the Sigma, Diagnostic Kit No 826-UV. Cell mass, expressed as cell dry weight, was obtained from optical density measurements and a calibration curve.

The conversion efficiencies to final catabolic products were calculated after growth of the glucose-adapted cultures of *C. thermocellum* on cellobiose of D-glucose. The final products yields for ethanol, acetic acid and lactic acid were essentially the same when *C. thermocellum* grew on cellobiose or D-glucose, but the final cell concentrations and apparent, cell yields were lower on D-glucose than on cellobiose (table I).

It may be hypothesized that there are essentially two reasons for which the final cell concentrations and apparent cell yields may change depending on the carbon source utilized to sustain growth. One is dependent on the manner in which the sugars are transported inside the cell, and the other is dependent on the final catabolic products obtained. These data suggest that the above differences are a reflection of the way in which *C. thermocellum* transports these sugars. The lower cell yields obtained on D-glucose may be thought to be due to a large expenditure of metabolic energy to mediate the transport of this substrate. This means that D-glucose is less favorable as a substrate than cellobiose.

Recent results suggest that in this microorganism the transport of D-glucose is mediated by an ATP-dependent permease transport system (P.E.H., manuscript in preparation). In *C. thermocellum* ATCC-27405, the existence of an ATP-

dependent transport system for the uptake of D-glucose can hypothetically explain why D-glucose is energetically less favorable than cellobiose to sustain the growth of this microorganism. In effect, assuming that one mole of ATP is spent in transporting one of cellobiose, the conversion of cellobiose to two moles of glucose-6-phosphate would require two moles of ATP (1). On the other hand, if transport of glucose requires expenditure of ATP, then four moles of ATP are required for the production of two moles of glucose-6-phosphate from D-glucose. Therefore, a large expenditure of energy is required to mediate the transport and phosphorylation of D-glucose instead of this energy being used in the biosynthesis of cellular components.

Table I. *Fermentation, carbon and oxidation-reduction balances for glucose-adapted* C. thermocellum *when growing on cellobiose or D-glucose*[a].

	Cellobiose	Glucose
Fermentation time, h	14	12
Final pH	5.40	5.85
Sugar consumed, g/l	2.77	2.61
Cell dry weight, g/l	0.48	0.37
Ethanol, g/l	0.36	0.34
Acetic acid, g/l	0.22	0.22
Lactic acid, g/l	0.22	0.15
CO_2[b], g/l	0.50	0.48
H_2[c], g/l	0.02	0.02
Cell yield, g/g	0.17	0.14
Ethanol yield, g/g	0.13	0.13
Acetic acid yield, g/g	0.08	0.08
Lactic acid yield, g/g	0.08	0.06
Total carbon recovery, g/l	0.74	0.64
Total carbon recovery in % of theoretical value	63.70	61.53
Oxidation reduction index	0.84	0.86

[a] Cells were grown at 60° C in anaerobic Hungate tubes containing CM-4 medium and either cellobiose or D-glucose at 6.0 g/l. The pH was uncontrolled, the initial being 7.2. Each numerical value is the average result from three different tubes with the same inoculum.

[b] CO_2 evolution: assume 1 mole of CO_2 produced for each mole of ethanol or acetic acid produced.

[c] H_2 evolution: assume 1 mole of H_2 produced for each mole of CO_2 evolution.
Product concentrations as g/liter.
Product yield as g product formed/g sugar consumed.

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