REVISTA ESPAÑOLA DE FISIOLOGIA, 39, 339-340, 1983

CARTA AL EDITOR

Evaluation of *Clostridium thermocellum* Strains for Ethanol Production

Society is confronted today with dwindling sources of fossil fuels and chemical feedstocks and the proliferation of wastes generated by municipalities, agriculture and industries. The conversion of renewable resources or wastes to chemicals and fuels by microbial fermentations or enzymes represents a tremendous challenge for microbiologists today and in the near future (9). The attractive features of low cost, abundance and renewable nature of a variety of cellulosic residues such as corn stover, wheat straw and rice straw, results in their frequent consideration as alternate energy sources. A growing need for alternate transportation fuels has fostered renewed interest in processes for the hydrolysis and fermentation of these materials to liquid fuels such as ethanol.

The concept of producing a liquid fuel, ethanol, from biomass is not new. If a yeast such as *Saccharomyces cerevisiae* is utilized for ethanol production then only the hexose portion of the biomass can be fermented. In addition these sugars must be hydrolyzed prior to fermentation. This has previously been accomplished by high temperature acid treatment or more recently, by the addition of cellulase from *Trichoderma reseii* (1). However, recurring drawbacks to these approaches have been high costs as well as low yields of fermentable sugars. As an alternative to these multi-stage methods, an original approach (1), may be to achieve conversion of cellulose to fermentable sugars and the fermentation of these sugars to ethanol in one single step, greatly reducing capital costs. In this context ethanol producing thermophiles with cellulolytic activity and high metabolic rates (1, 9), are specially interesting.

This investigation focuses on *Clostridium thermocellum*, a thermophilic anaerobic bacterium, with cellulolytic capabilities (7). In this microorganism (8), the final catabolic products from cellulose are ethanol and organic acids. It was the objective of this work to quantitatively asses the potential of different *C. termocellum* strains to produce ethanol. During growth of this microorganism on cellulosic substrates, cellobiose seems to be the only sugar consumed under non-pH controlled conditions (3). Therefore, cello-

Table 1. *Growth characteristics of various strains of* C. thermocellum *on cellobiose.*

Strain	O. D. at 660 nm Initial	O. D. at 660 nm Final	Doubling Time (h)
OI	0.03	0.55	5.0
TET	0.03	0.60	3.0
157	0.03	0.66	3.0
ATCC-27405	0.03	0.71	2.7

Table II. *Ethanol production by different strains of* C. thermocellum *on cellobiose.*

Strain	Ethanol, Maximum Concentration (g/l)	Specific Ethanol Productivity (g ETOH/g cell-h)
QI	0.24	0.04
TET	0.24	0.06
157	0.34	0.10
ATCC-27405	0.48	0.12

biose was assumed to be a good model substrate to follow growth and ethanol production.

The strains of *C. thermocellum* available at the beginning of this investigation were the QI, TET, 157 and ATCC-27405 isolates. The strain QI, came from Dr. J. G. Zeikus, University of Wisconsin. Strains TET and 157, came from Dr. J. K. Alexander, Hahneman Medical College, Philadelphia, Pa. The strain ATCC-27405, was from the American Type Culture Collection, Rockville, Ma. All of the following results were obtained with 10 ml anaerobic test tube cultures with CM-4 complex medium (2, 4), containing 6 g/l of cellobiose. Growth was followed by changes in the optical density of the liquid medium at 660 nm. Cell mass, expressed as cell dry weight, was obtained from optical density measurements and a calibration curve. Ethanol was quantitatively analyzed (2, 5) by absorption gas chromatography with a 6 ft Teflon column, packed with Chromosorb 101 in a Hewlet Packard 5839-A gas chromatograph.

Table I shows the growth characteristics of various strains of *C. thermocellum* on cellobiose. The strain ATCC-27405, showed the most prolific and rapid growth, both in terms of higher final cell concentrations and lower doubling times. Table II shows the ethanol production of the same strains, when growing on cellobiose. Again, the strain ATCC-27405 was the best ethanol producer, both in terms of maximum attainable ethanol concentration and maximum specific formation rate. Accordingly, from all the *C. thermocellum* strains available at the beginning of this investigation, *C. thermocellum* ATCC-27405, exhibited the most extensive and rapid growth and the best ethanol production.

References

1. AVGERINOS, G. C., FANG, H. Y., BIOCIC, I. and WANG, D. I. C.: In «Advances in Biotecnology». Vol. 2. Pergamon Press Canada Ltd., Toronto, 1981, pp. 119-124.
2. GÓMEZ, R. F. and HERNÁNDEZ, P. E.: In «Advances in Biotechnology». Vol. 2. Pergamon Press Canada Ltd., Toronto, 1981, pp. 131-136.
3. GORDON, J., JIMÉNEZ, M., COONEY, C. L. and WANG, D. I. C.: In «Biochemical Engineering: Renewable Sources of Energy and Chemical Feedstocks». Vol. 74. AIChE Symp. Ser. 181. AIChE, New York, 1977 pp. 91-97.
4. HERNÁNDEZ, P. E.: *J. Gen. Appl. Microbiol.*, 28, 469-477, 1982.
5. HERNÁNDEZ, P. E.: *Rev. esp. Fisiol.*, **38**, 473-474, 1982.
6. MCBEE, R. H.: *J. Bacteriol.*, **50**, 653-663, 1948.
7. NG, T. K., WEIMER, P. J. and ZEIKUS, J. G.: *Arch. Microbiol.*, **114**, 1-7, 1977.
8. WEIMER, P. G. and ZEIKUS, J. G.: *Appl. Environ. Microbiol.*, 33, 289-297, 1977.
9. ZEIKUS, J. G.: *Ann. Rev. Microbiol.*, **34**, 423-464, 1980.

P. E. HERNÁNDEZ*

Massachusetts Institute of Technology
Cambridge, Massachusetts (U.S.A.)

(Received on April 28, 1983)

*Present address:
Instituto Tecnológico para Postgraduados
P.º Juan XXIII, 3
Madrid-3 (España)