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Thermophilic degradation of cellulose by a triculture of *Clostridium thermocellum, Methanobacterium* sp. and *Methanosarcina* MP

(Methanogenesis; defined mixed culture)

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1. SUMMARY

The fermentation of cellulose at 55°C by different associations of the 3 bacteria *Clostridium thermocellum, Methanobacterium* sp. and *Methanosarcina* MP was studied. *C. thermocellum* alone

abundant natural polymer and comprises the majority of solid waste material. The anaerobic degradation of cellulose to methane and carbon dioxide is of current interest in terms of renewable energy for the future. To improve the overall performance of anaerobic digestion from cellulose

3. MATERIALS AND METHODS

3.1. Chemicals

Gases were purchased from Airgaz (Marseille). All chemicals were of reagent quality unless otherwise stated. cellulose MN 300 (Machery and Nagel 300, thin layer chromatography grade) was used during this work.

3.2. Organisms

The organisms used were *C. thermocellum* NCIB10682 and *Methanosarcina* MP [9]. *Methanobacterium* sp. was isolated in our laboratory.

3.3. Culture media

The anaerobic techniques described by Hungate [10] and Balch et al. [11] were used throughout this study. A sodium bicarbonate-buffered medium was used instead of the phosphate-buffered medium of Weimer and Zeikus [4] since the total cellulose degradation (6 g/l) to CH_4 by the mixed defined culture *C. thermocellum-Methanobacterium* sp. *-Methanosarcina* MP was only successful in the bicarbonate medium.

The culture media contained the following compounds (g/l): cellulose, 6; $(NH_4)_2SO_4$, 1.3; K_2HPO_4, 0.3; $MgCl_2 \cdot 6H_2O$, 1.0; $CaCl_2$, 0.15; L-cysteine-HCl, 0.5; yeast extract (Difco, Baltimore, MD, U.S.A.), 2.0; resazurin, 0.001 and 0.03 ml of 5% $FeSO_4$ solution. The medium was

of *C. thermocellum* (60°C) and 2 ml of a 5-day-old culture of *Methanosarcina* MP (55°C). Average values of triplicate vessels are reported. All experiments were repeated at least twice. Results are expressed per vial (20 ml medium).

3.4. Analytical techniques

H_2 and CH_4 were measured by gas chromatography [13]. Volatile fatty acids were measured using a gas chromatograph equipped with a flame ionisation detector [13]. Cellulose concentrations were determined by measuring total carbohydrates by anthrone reaction [14].

4. RESULTS

4.1. Cellulose fermentation

Fig. 1 shows the time-course of cellulose utilisation in monoculture, co-culture and triculture. Cellulose degradation in the *C. thermocellum* culture or in the *C. thermocellum-Methanobacterium* sp. co-culture was essentially linear up to 4 days.

Addit on of *Methanobacterium* sp. to *C. thermocellum* h ad no effect on cellulose utilisation. In the tricul ure including *Methanosarcina* MP, cellulose was d egraded slower than in the co-culture. In all associ ations, more than 90% of the cellulose was degra ded.

With all 4 culture systems, cellulose fermentation was accompanied by a decrease in pH (Fig. 1). The pH drop was more pronounced when *C. thermocellum* was associated with *Methanobacterium* sp. In the triculture, the pH was maintained at 6.3 and decreased after 10 days of culture. When methanol was added to the triculture, the pH began to rise after 8 days and levelled off at pH 6.6.

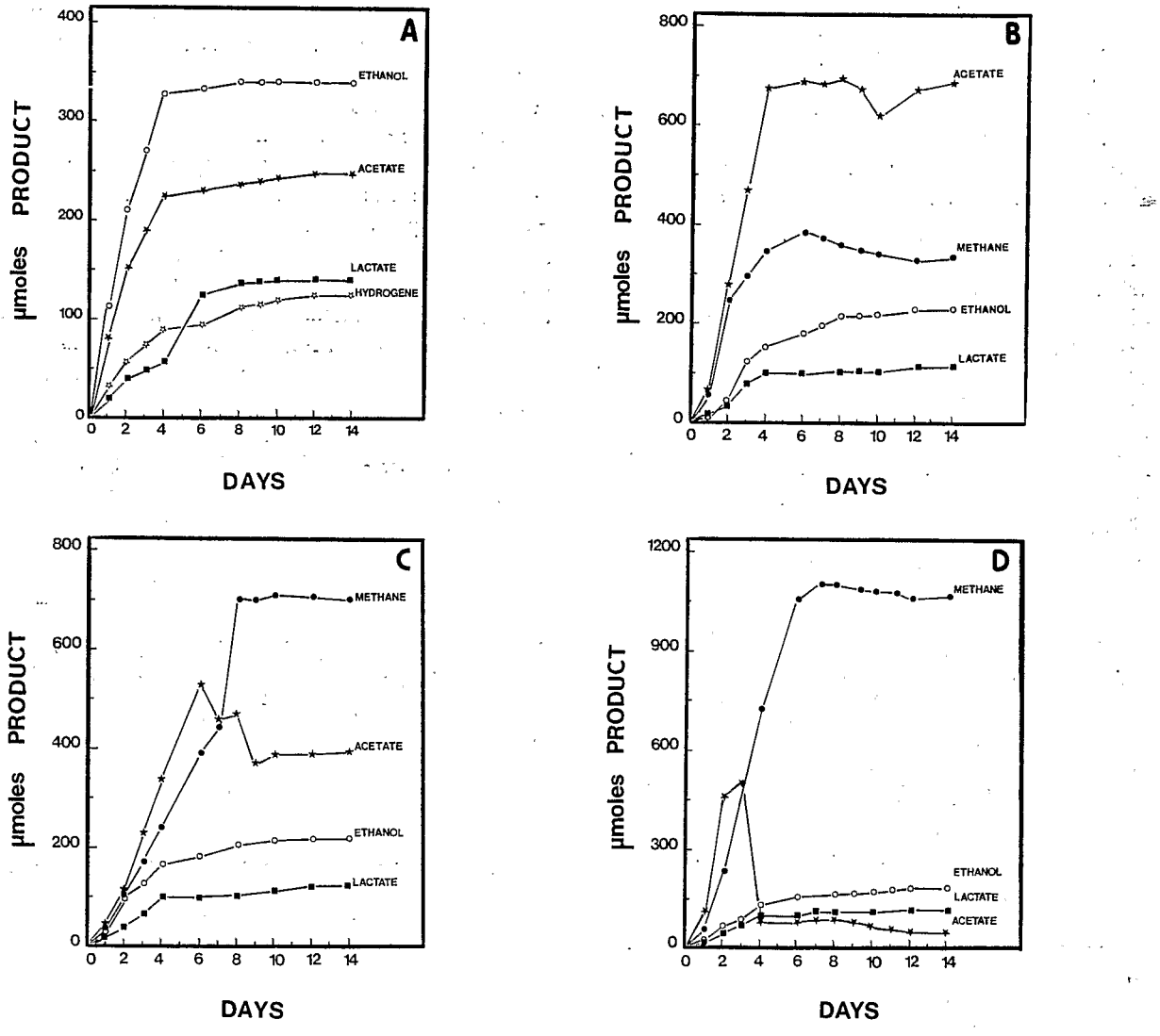

Fig. 2. Products of cellulose fermentation by *C. thermocellum* (A); *C. thermocellum* and *Methanobacterium* sp. (B); *C. thermocellum*, *Methanobacterium* sp. and *Methanosarcina* MP (C); *C. thermocellum*, *Methanobacterium* sp. and *Methanosarcina* MP+0.05 ml 2 M methanol (D). Culture vessels were incubated at 55°C. Results are expressed in µmol per 20 ml medium.

4.2. Cellulose fermentation p oducts

When grown on cellulose *C. thermocellum* produced large amounts of ethanol and acetate. Lactate and H_2 were also d etected (Fig. 2A). CO_2 was produced but not meas ured. Co-culture of the cellulolytic bacterium with *Methanobacterium* sp. resulted in an increase (from 240 to 650 μmol) in acetate concentration, whereas ethanol concentration decreased (Fig. 2B). H_2 did not accumulate at any stage of incubation. The fermentation products of the *C. thermocellum-Methanosarcina* MP co-culture were identical qualitatively and quantitatively to those of the *C. thermocellum* monocultures have been well documented with a variety of mixed cultures [15–18].

The focus of this work was to elucidate the role of *Methanosarcina* MP in the triculture system. Unlike mesophilic *Methanosarcina* species [19,20], *Methanosarcina* MP was unable to use H_2-CO_2 [9]. Thus, under thermophilic conditions, each methanogen had a well-defined role during cellulose degradation. In the triculture, the yield of methane was higher than for either co-culture. Furthermore, complete acetate degradation could be achieved in presence of methanol in the triculture. When both methanol and acetate were added

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