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THERMOPHILIC METHANOGENESIS FROM SUGAR BEET PULP BY A DEFINED MIXED BACTERIAL CULTURE

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SUMMARY

Thermophilic degradation of sugar beet pulp was studied in batch cultures at 55°C by different associations of bacteria, including Clostridium thermocellum, Methanobacterium sp. and Methanosarcina MP. C.thermocellum produced acetate, succinate, methanol, ethanol, H_2 and CO_2. The coculture of C.thermocellum and Methanobacterium sp. produced trace amounts of ethanol and succinate; acetate concentration was about three times higher than in the C.thermocellum monoculture. The association of this coculture with Methanosarcina MP produced 5.5 mmol CH_4/g dry weight sugar beet pulp.

INTRODUCTION

Biogas production from agricultural wastes has gained increasing interest over the last decade. The use of thermophilic anaerobic digestion has been extensively studied (Cooney and Wise, 1975; Shelef et al., 1980) since this process offers several advantages over mesophilic digestion including increased fermentation rates. Few reports mentioned the degradation of cellulosic wastes as compared to pure cellulose by defined methanogenic mixed cultures of bacteria (Weimer and Zeikus, 1977; Khan, 1980; Laube and Martin, 1981; Mountfort et al., 1982). Mountfort et al. (1982) reported the fermentation of plant fibrous materials by the coculture of an anaerobic rumen fungus with Methanobrevibacter sp. in the absence and presence of Methanosarcina barkeri.

Beet pulp is available in large amounts as a by product of sugar manufacture. It consists mainly of carbohydrates (cellulose),

pectin and proteins (Arntz et al., 1985). Methane production from sugar beet pulp is generally studied by natural undefined mixed bacterial cultures (Labat et al., 1984; Lescure and Bourlet, 1984; Stoppok and Buchholz, 1985). The purpose of our work was to study the fermentation of this by product by a defined mixed culture including the cellulolytic bacterium Clostridium thermocellum and two methanogenic bacteria: Methanobacterium sp. and Methanosarcina MP. The part played by each methanogenic partner in the mixed culture is discussed.

MATERIALS AND METHODS

Organisms. Clostridium thermocellum NCIB 10682 and Methanosarcina MP (Ollivier et al., 1984) were used during this work. The hydrogenophilic methanogenic bacterium Methanobacterium sp. was isolated in our laboratory.

Medium. The culture medium contained 10 g dry weight of grounded sugar beet pulp; its composition was: $(NH_4)_2SO_4$, 1.3 g; K_2HPO_4, 0.3 g; $MgCl_2.6H_2O$, 1.0 g; $CaCl_2$, 0.15 g; L-cysteine-HCl, 0.5 g; yeast extract (Difco Lab.,Baltimore), 2.0 g; resazurin, 0.001 g; 5 % $FeSO_4$, 0.03 ml; distilled water, 1,000 ml. Anaerobic media were prepared as previously described (Ollivier et al., 1985). 20 ml media were dispensed into 60 ml serum bottles. After sterilization, 0.2 ml of 2 % $Na_2S.9H_2O$ and 0.9 ml of 10 % $NaHCO_3$ were added to each vial. N_2-CO_2 (80-20 %) was the gas phase.

Experimental cultures were prepared by inoculating 0.5 ml of an 1-day culture of Methanobacterium sp. grown on H_2-CO_2 (80-20 %) at 60°C, 0.5 ml of a 4-days culture of C.thermocellum grown on cellulose at 60°C and 2 ml of a 5-days culture of Methanosarcina MP at 55°C. The mixed cultures were incubated at 55°C.

Product analysis. Average value of triplicate vessel was reported. Gases, alcohols, volatile fatty acids and organic acids were measured as previously described (Garcia et al., 1982). Total carbohydrates were measured by anthrone reaction (Hanson and Philipps, 1981).

RESULTS AND DISCUSSION

Mixed cultures of C.thermocellum, Methanobacterium sp. and Methanosarcina MP converted 75 % of total carbohydrates contained in beet pulp (Table 1) mainly to methane (Fig.1). The results suggest that this triculture may be useful in a thermophilic bioconversion. All methane was produced in the triculture after 10 days of incubation (Fig.1).

Table 1 . Effect of different associations of C.thermocellum with methanogenic bacteria on the degradation of carbohydrates contained in sugar beet pulp and on the final culture pH

	C.thermocellum	C.thermocellum + Methanobacterium sp.	C.thermocellum + Methanobacterium sp. + Methanosarcina MP
% carbohydrates	64	81	75
final culture pH	5.9	5.6	6.5

. Initial culture pH was 7.2; initial carbohydrates concentration was 5.3 g/l.

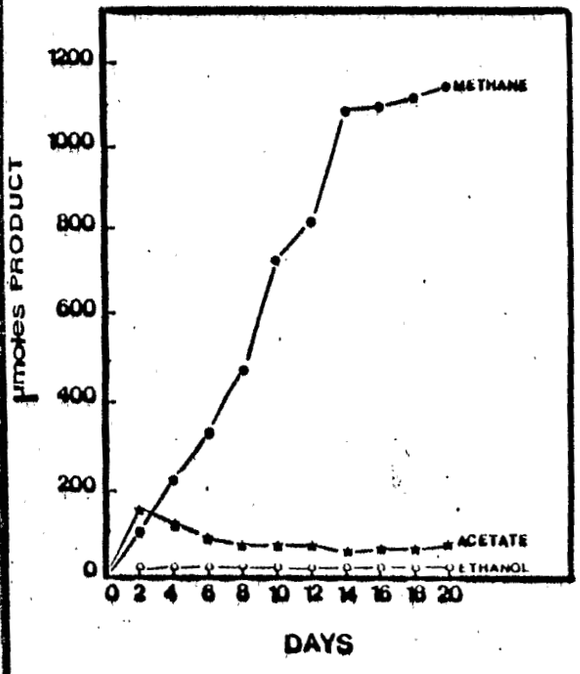

Fig.1 . Products of sugar beet pulp fermentation by the triculture C.thermocellum-Methanobacterium sp.-, Methanosarcina MP. Results are expressed in µmol per 20 ml medium.

As shown in Table 1, the final pH was significatively higher when Methanosarcina was present, probably because of the utilization of acetate by this methanogenic bacterium. The C.thermocellum monoculture produced acetate as the major volatile fatty acid from sugar beet pulp (Fig.2). Succinate was the only non volatile organic acid detected. In contrast, when grown on pure cellulose, C.thermocellum produced lactate as the major non volatile organic acid and succinate was produced in trace quantities (Weimer and Zeikus, 1977).

H_2 and ethanol were other products of the fermentation of sugar beet pulp by C.thermocellum. The production of methanol, a major end product of pectin metabolism (Schink and Zeikus, 1980), indicated that C.thermocellum was probably pectinolytic, although this had never been reported for this Clostridium species. Figure 3 illustrates the rate of product formation during sugar beet pulp fermentation by the coculture of C.thermocellum with Methanobacterium sp.: no free H_2 was detected in the coculture, since H_2 was used by Methanobacterium to reduce CO_2 into CH_4. This interspecies H_2 transfer resulted in the production of higher amounts of acetate. Only trace amounts of ethanol and succinate were detected. The use of H_2 by methanogenic bacteria caused a qualitative and quantitative shift in the products formed by C.thermocellum. Similar results were reported from the C.thermocellum-Methanobacterium coculture grown on pure cellulose (Weimer and Zeikus, 1977).

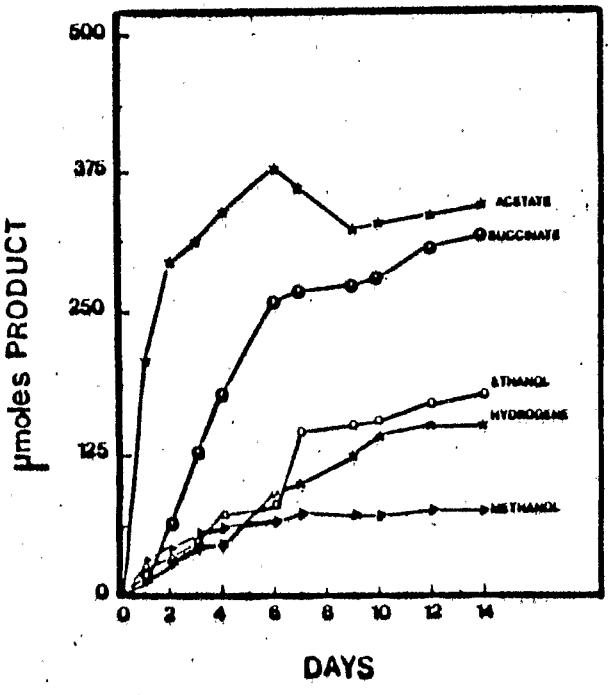

Fig.2 . Products of sugar beet pulp fermentation by C.thermocellum

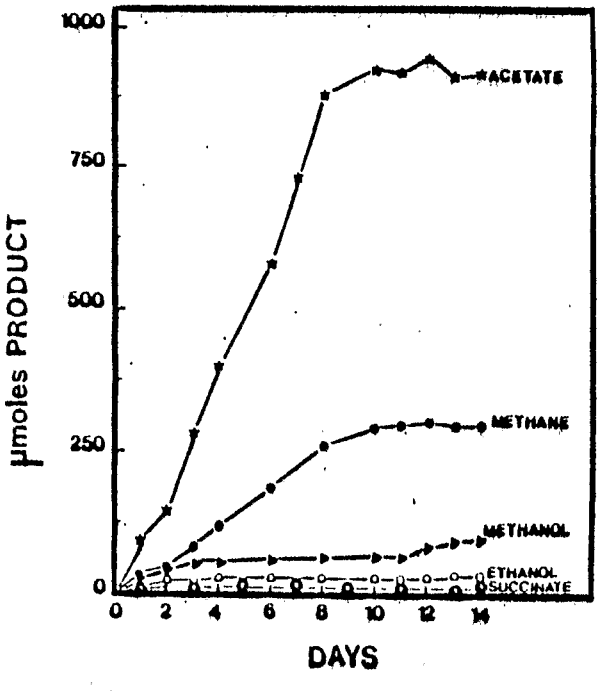

Fig.3 . Products of sugar beet pulp fermentation by the coculture C.thermocellum-Methanobacterium sp.

Results are expressed in µmol per 20 ml medium

Conversion of sugar beet pulp by the triculture of C.thermocellum, Methanobacterium sp. and Methanosarcina MP led to highest yields in methane production since H_2-CO_2 and acetate produced were converted into methane by Methanobacterium

sp. and Methanosarcina MP respectively (Fig.1). Furthermore, methanol was also degraded via methanogenesis by the methylotrophic Methanosarcina, so that the triculture finally produced 5.5 mmol CH_4 per g dry weight sugar beet pulp. However small amounts of acetate and ethanol were detected. The use of acetate by Methanosarcina MP did not change the products of sugar beet pulp fermentation by C.thermocellum in the triculture.

The studied triculture was a defined mixed culture capable to convert carbohydrates and also pectin contained in sugar beet pulp into methane. Experiments could be undertaken to know if this triculture can degrade other cellulosic waste materials at rates comparable to those for sugar beet pulp.

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