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Methanogenic Transformation of Methylfurfural Compounds to Furfural

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The metabolic conversion of 5-methylfurfural and 2-methylfurfural to furfural by a methanogenic bacterium, *Methanococcus* sp. strain B, was studied. This bacterium was found to use methylfurfural compounds as a growth substrate and to convert them stoichiometrically to furfural. For every mole of methylfurfurals metabolized, almost 1 mol of furfural and 0.7 mol of methane were produced. Several methanogenic bacteria did not carry out this conversion. The metabolic conversion of methylfurfurals is likely to be of value in the anaerobic treatment of methylfurfural-containing wastewaters such as those produced by the paper and pulp industries and oatmeal processing industries. This study adds to the list of the limited number of compounds that are known to serve as electron donors for methanogenesis.

In nature, furan compounds are commonly present (23). Furan compounds are produced commercially from lignocellulosic plant materials, such as oat husks or corn cobs, by treatment with hot sulfuric acid (27). Furan compounds are also formed during the concentration of certain aqueous wastes containing pentosans; in paper mills, which use the bisulfite pulping process, sulfite evaporate condensate is a by-product of concentrating sulfite-spent liquor (4, 11). Sulfite evaporate condensate may contain 30 mM or more furfural compounds (10). Furan derivatives are also formed during the heat treatment of municipal wastes (19).

Only a few microbial transformations of furan compounds are known. *Saccharomyces* spp. are known to reduce furfural to furfuryl alcohol by $2e^-$ reduction (17). Under aerobic conditions, *Acetobacter ascendens* dismutes the aldehyde to alcohol and acid (17). Several enteric bacteria are known to reduce furfural to furfuryl alcohol and 5-hydroxy methylfurfural to 5-hydroxy methylfurfuryl alcohol (7). Abdulrashid and Clark (1) have demonstrated that a mutant strain of *Escherichia coli* can grow with 2-furoic acid as the sole carbon and energy source. Hong et al. (16) described *Pseudomonas* sp. strain Fs-1, which degraded 2-furoic acid. Koenig and Andreesen (18) reported aerobic degradation of 2-furoic acid by *Pseudomonas putida* Fu1. Degradation of furfural under anaerobic sulfate reducing conditions has been reported earlier by several workers (8, 11, 15). There was no report in the literature on the transformation of methylfurfurals such as 5-methylfurfural and 2-methylfurfural (5-MF and 2-MF, respectively) by methanogenic bacteria. In this paper, I describe a methanogenic bacterium, *Methanococcus* sp. strain B, which uses the methyl groups of methylfurfurals as the sole carbon source for growth and methanogenesis.

Organisms. The methanogenic bacterium *Methanococcus* sp. strain B, isolated from a lake sediment (9), was used for the study. *Methanococcus deltae*, *Methanococcus thermolithotrophicus*, *Methanobacterium thermautotrophicum*, *Methanosarcina barkeri*, and *Methanobrevibacter ruminantium* were obtained from L. Daniels (University of Iowa, Iowa City).

Media and growth conditions. *Methanococcus* sp. strain B and the other methanogenic bacteria were grown in serum tubes (no. 2048-00150; Bellco Glass, Vineland, N.J.) or serum bottles (no. 223950; Wheaton Scientific) according to the anaerobic techniques described previously by Balch and Wolfe (2) and Daniels et al. (12). The basal medium used for *Methanococcus* sp. strain B was described previously (6, 9), except 10 mM either 5-MF or 2-MF was substituted for the electron donor, depending on the specific experiment. The gas phase was N_2 (100%), and there was no carbonate in the medium to avoid any carbon other than 2-MF or 5-MF. The other methanogenic bacteria were grown as described previously (6), except that the electron donor was replaced with methylfuran compounds (5-MF or 2-MF). In all of the experiments, a 5% pregrown inoculum was used. The pregrown cells were centrifuged, and the pellets were resuspended with fresh media to avoid any carryover carbon. A duplicate culture of killed control (autoclaved) cells was used in all the experiments to monitor any abiotic degradation of methylfurfurals. All of the culture bottles and tubes were covered with aluminum foil to prevent any photodegradation and were incubated at room temperature (20 to 22°C) on a shaker table kept at 150 rpm. All data represent average values from duplicate cultures.

Analytical methods. Bacterial growth was monitored by measuring culture turbidity at 600 nm in a spectronic 20 spectrophotometer (Milton Roy, Rochester, N.Y.). Methane production in the culture bottles was measured by gas chromatography (3). The concentrations of furan compounds, such as furfural, 5-MF, and 2-MF, were analyzed by high-performance liquid chromatography (HPLC) with a Waters Associates (Milford, Mass.) liquid chromatograph equipped with two model 6000A solvent pumps, a model 490E programmable multiwavelength detector set at 276 nm, a data module, and a model 600E system controller. The column was a reverse-phase C_{18} μBondapak. Injection volumes via an autosampler were 10 μl. Elution was with a gradient of solvent A (0.9 ml of H_3PO_4 per liter of water) versus solvent B (acetonitrile-water, 80:20 [vol/vol], containing 0.9 ml of H_3PO_4 per liter). The flow rate was 1 ml/min at room temperature.

Peaks of interest on the liquid chromatograph were collected and concentrated and analyzed by liquid chromatography-mass spectrometry. Liquid chromatography-mass spectrometry analysis was performed on the same μBondapak column as that described above. The injection volume of the sample was 50 μl. The liquid chromatograph was linked to a

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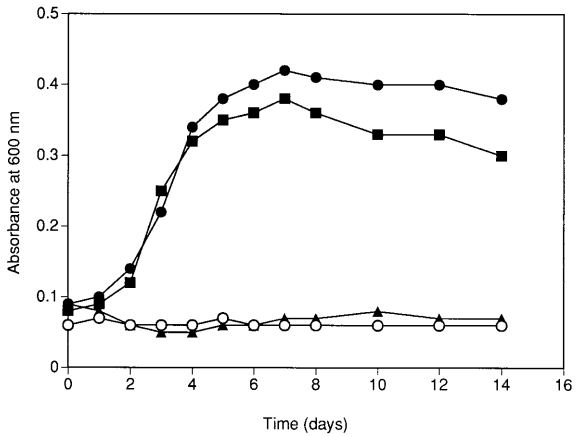

FIG. 1. Growth of *Methanococcus* sp. strain B on 5-MF, 2-MF, or furfural as the sole growth substrate. Data represent the average of two values. ○, killed control cells; ■, growth on 5-MF; ●, growth on 2-MF; ▲, growth on furfural.

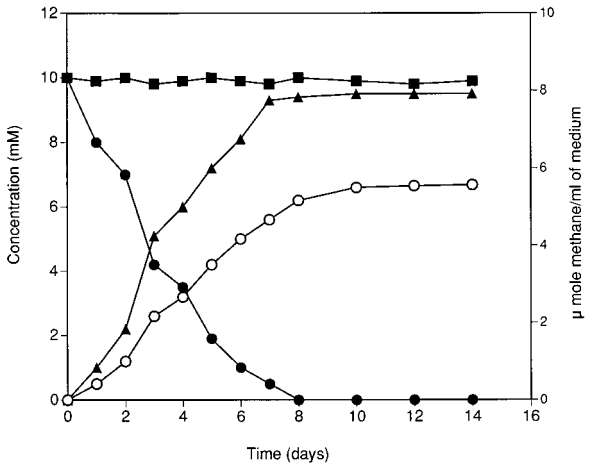

FIG. 2. Concentrations of 5-MF and furfural and cumulative methane production in the cultures of *Methanococcus* sp. strain B grown with 10 mM 5-MF as the sole carbon source. Data represent the average of two values. ■, 5-MF concentration in killed control cells; ●, 5-MF concentration in the active culture; ▲, furfural concentration in the active culture; ○, cumulative methane production in the culture bottle. There was no production of methane and furfural in the control bottle.

mass spectrum detector (Hewlett-Packard, Palo Alto, Calif.) equipped with a particle beam interface. Mass spectra were obtained at an ionizing voltage of 60 eV and an accelerating voltage of 4 kV.

Chemicals. All furan compounds were purchased from Aldrich Chemical Company (Milwaukee, Wis.). All other chemicals were of reagent grade.

Growth on methylfuran compounds. As described earlier, a methanogenic bacterium, *Methanococcus* sp. strain B, was isolated from a lake sediment (9). This isolate used formate or H_2-CO_2 as the sole source of carbon and energy. The culture purity was routinely evaluated by microscopic and nutritional characteristics to make sure that the culture was pure. The ability of the new isolate to use methylfurfurals as the sole carbon source was studied. Figure 1 shows the growth of *Methanococcus* sp. strain B in the presence of 10 mM 5-MF or 2-MF. Both of these compounds supported the growth of this bacterium. Maximum growth was observed on day 7 (A_{600}s of 0.38 for 5-MF and 0.42 for 2-MF). However, the isolate did not grow on furfural when it was used as the sole source of carbon in the medium, and the growth was similar to the growth with no added substrate. In killed controls, no growth was observed. All of the other methanogens (viz., *M. deltae*, *M. thermolithotrophicus*, *M. thermautotrophicum*, *M. barkeri*, and *M. ruminantium*) did not grow on either 5-MF or 2-MF as the sole carbon source (data not shown).

Conversion of 5-MF by *Methanococcus* sp. strain B. *Methanococcus* sp. strain B was found to be able to grow with 5-MF as a substrate. The HPLC analysis of culture media on day 8 showed one large metabolite peak with a retention time at 3.2 min. The liquid chromatography-mass spectrometry analysis of this peak perfectly matched that of furfural, and the standard furfural eluted at 3.2 min. This confirmed that the main metabolite of 5-MF metabolism was furfural. The concentrations of 5-MF and furfural and the cumulative methane production in the culture bottles are shown in Fig. 2. The 5-MF concentration slowly dropped from 10 mM to 0 mM within 8 days of incubation. On the other hand, the furfural concentration increased gradually in the culture medium and reached a maximum concentration of 9.4 mM on day 8. The cumulative methane production in the culture bottle reached a maximum of 6.7 μmol/ml of medium. In the killed control cells, the 5-MF remained at the initial concentration of 10 mM throughout this study, indicating that there was no abiotic transformation of 5-MF. This experiment showed that 5-MF was demethylated by *Methanococcus* sp. strain B, and an almost stoichiometric amount of furfural was produced. For every mole of 5-MF metabolized, almost 1 mol of furfural and 0.67 mol of methane were produced by this bacterium. The other methanogens studied did not metabolize 5-MF (data not shown).

Conversion of 2-MF by the methanogen. Similar to 5-MF, methanogenesis occurred when 2-MF served as the sole carbon source. The liquid chromatography-mass spectrometry analysis of the culture sample revealed one major metabolite. The mass spectrum of this metabolite perfectly matched that of the standard furfural. The concentrations of 2-MF, furfural, and methane in the culture bottle are given in Fig. 3. The concentration of 2-MF dropped from 10 mM to 0 mM within 7 days of

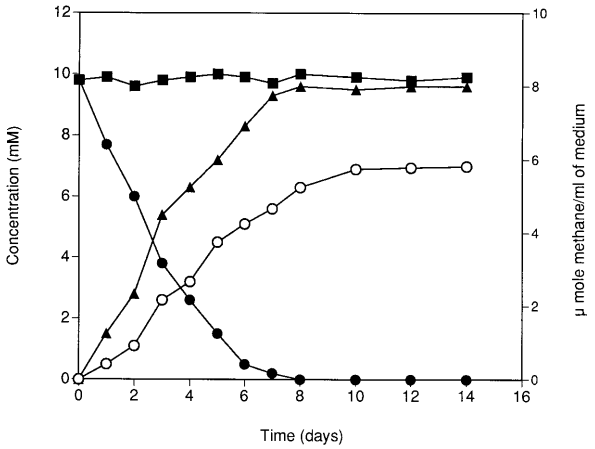

FIG. 3. Concentrations of 2-MF and furfural and cumulative methane production in the cultures of *Methanococcus* sp. strain B grown with 10 mM 2-MF as the sole carbon source. Data represent the average of two values. ■, 2-MF concentration in killed control cells; ●, 2-MF concentration in the active culture; ▲, furfural concentration in the active culture; ○, cumulative methane production in the culture bottle. There was no production of methane and furfural in the control bottle.

incubation, and in the same period, the concentration of furfural increased from 0 mM to 9.6 mM. The cumulative methane production in the bottle was 6.9 μmol/ml of medium. Similar to the 5-MF experiment, this study showed stoichiometric release of furfural from 2-MF and almost 0.7 mol of methane produced for every mol of 2-MF used by this bacterium. In the control, there was no change in the concentration of 2-MF, which remained at 10 mM throughout the study. The other methanogens studied did not metabolize 2-MF (data not shown).

This is the first study in which it has been conclusively proven that a pure culture of methanogenic bacterium can utilize methylfurans as substrate for growth. The metabolic transformation appeared to make use of the methyl groups as a carbon source and to demethylate them from the parent compounds and in the process formed an almost stoichiometric amount of furfural in the culture. This study also adds to the list of compounds that are known to be used for methanogenesis. The previously known compounds used by the methanogenic bacteria are H_2-CO_2, formate, CO, methanol, acetate, methylamines, dimethylsulfides, ethanol, butanols, pyruvate, and methylmercaptopropionate (5, 14, 20–22, 24, 26). The biochemical mechanism of demethylation of methylfuran is not clearly known. Some methanogenic bacteria are known to use methyl-*S*-coenzyme reductase to convert methyl-*S*-coenzyme M (25). Specific methyltransferases are known to be involved in the metabolism of methylamines (13). Recently, van der Maarel et al. (24) reported demethylation of 3-*S*-methylmercaptopropionate to 3-mercaptopropionate by a *Methanosarcina* sp. Various other well known methanogens should be studied for their ability to use methylated furan compounds as electron donors. Further work is needed to understand the mechanism of demethylation reaction of methylfurfurals by *Methanococcus* sp.

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