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REVIEW

Dynamics of the microbial community during continuous methane fermentation in continuously stirred tank reactors

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Methane fermentation is an attractive technology for the treatment of organic wastes and wastewaters. However, the process is difficult to control, and treatment rates and digestion efficiency require further optimization. Understanding the microbiology mechanisms of methane fermentation is of fundamental importance to improving this process. In this review, we summarize the dynamics of microbial communities in methane fermentation chemostats that are operated using completely stirred tank reactors (CSTRs). Each chemostat was supplied with one substrate as the sole carbon source. The substrates include acetate, propionate, butyrate, long-chain fatty acids, glycerol, protein, glucose, and starch. These carbon sources are general substrates and intermediates of methane fermentation. The factors that affect the structure of the microbial community are discussed. The carbon source, the final product, and the operation conditions appear to be the main factors that affect methane fermentation and determine the structure of the microbial community. Understanding the structure of the microbial community during methane fermentation will guide the design and operation of practical wastewater treatments.

[**Key words:** Microbial community; Continuous stirred tank reactor; Continuous methane fermentation; Anaerobic digestion; Phylogenetic analysis; Chemostat]

Methane fermentation is an anaerobic process during which organic carbon is converted into its most oxidized (carbon dioxide) and most reduced (methane) states through a series of oxidations and reductions. Methane fermentation has been utilized to treat organic waste for many years. The push to find clean and renewable energy sources that will reduce our dependency on fossil fuels and minimize environmental pollution has led to a renewed interest in methane fermentation. Novel reactors with novel treatment processes and increased reaction rates have been developed recently. However, methane fermentation is still considered to be an unstable and unmanageable technology. Advances in molecular biology have enhanced our understanding of the microbial ecology relevant to methane fermentation. Understanding the microbial mechanism of methane fermentation will contribute to the development of improved processes for methane fermentation.

Many reports cover microbial communities during methane fermentation. Microbial communities under various conditions have been examined. These conditions include the treatment of wastewater with complex components (1–6) or with single carbon sources (7–13), the treatment under mesophilic (7–13), thermophilic (1), or hyperthermophilic (14) conditions using continuously stirred tank reactors (CSTRs) (1,7–13), fixed-bed reactors (4,5), or fluidized-bed reactors (6). In theory, the large volume of data available on the subject should inform the analysis of how various factors, such as carbon source, temperature, retention time, loading rate and reactor type, affect the microbial community. However, it is difficult to reach clear conclusions. One important reason for this difficulty is the complexity and flexibility of the microbial community during methane fermentation. Correlating various factors with the microbial community during methane fermentation will become much easier, if variables are studied systematically, fixing some of the key factors while varying others.

In this paper, we review reports on the dynamics of microbial communities in chemostats treating synthetic wastewaters each containing only one carbon source. The tested carbon sources include propionate, butyrate, long-chain fatty acids, glycerol, protein, glucose, and starch, which are general substrates and intermediates of methane fermentation. These reports used the same CSTR, the same basic medium, identical TOC concentrations in the synthetic wastewater and identical operating temperatures. Only the carbon substrate was varied. The performance and microbial community of each chemostat are reviewed. Factors that affect the microbial community are discussed. The carbon source, the final product of fermentation, and the operating conditions are the main factors affecting the structure and stability of the microbial community during methane fermentation in chemostats. The review also outlines the significance of studying microbial community structures with respect to process design and operation in wastewater treatment.

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MECHANISM OF METHANE FERMENTATION

The anaerobic conversion of organic waste to methane is facilitated by a consortium of microorganisms comprised of chemoheterotrophic, non-methanogenic bacteria and methanogenic archaea. Because methanogens utilize only a limited range of substrates, the anaerobic breakdown of organic matter is carried out by bacterial communities comprised of anaerobic bacteria with different physiological types. Fig. 1 illustrates the three different phases of the anaerobic digestion process: hydrolysis-acidogenesis, acetogenesis, and methanogenesis. Biopolymers, such as polysaccharides, proteins, nucleic acids, and fats, are first hydrolyzed by extracellular enzymes. The resulting monomers and oligomers (sugars, amino acids, purines, pyrimidines, and glycerol) are then fermented by a wide variety of bacteria. The resulting products include hydrogen, formate, and acetate, which in turn are converted into methane by methanogens. Other products include propionate, butyrate, and higher fatty acids. The higher fatty acids need to be anaerobically oxidized to methanogenic substrates prior to being converted to methane and carbon dioxide; however, the $\Delta G^{0'}$ values of these conversions are highly positive (Table 1, reaction nos. 2, 5, and 7).

Short chain fatty acids, such as acetate, propionate, and butyrate, are the major intermediates produced during methane fermentation. It is estimated that approximately 70–80% of the methane is derived from acetate and that 6–35% is derived from propionate (8,15–17). Acetate and propionate degradation is rate-limiting during methane fermentation. Therefore, acetate and propionate are the main fatty acids that accumulate when either reactors are operated under a high loading rate or problems occur during treatment.

Two processes that convert acetate to methane have been described. In the first process, acetate-utilizing methanogens of the genera *Methanosaeta* and *Methanosarcina* use the aceticlastic cleavage pathway to convert acetate to methane and carbon dioxide (Fig. 2, reaction no. 1; Table 1, reaction no. 1) (18). The second process involves the syntrophic oxidation of acetate to carbon dioxide and hydrogen by one organism and the subsequent reduction of carbon dioxide to methane by a hydrogenotrophic methanogen (Fig. 2, reaction nos. 2 and 3; Table 1, reaction no. 4) (19,20). Under methanogenic conditions, either propionate or butyrate is degraded by the syntrophic association of propionate- or butyrate-oxidizing bacteria and hydrogenotrophic methanogens (Table 1, reaction no. 7) (21).

TABLE 1. Degradation reactions of acetate, propionate and butyrate under methanogenic conditions.

Reaction	$\Delta G^{0'}$ (kJ/reaction)
(1) Aceticlastic methanogen	
$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^-$	−31.0
(2) Acetate-oxidizing bacteria	
$CH_3COO^- + 4H_2O \rightarrow 2HCO_3^- + 4H_2 + H^+$	+104.6
(3) Hydrogenotrophic methanogen	
$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$	−135.6
(4) Reaction 2 + reaction 3	
$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^-$	−31.0
(5) Propionate-oxidizing bacteria	
$CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + HCO_3^- + 3H_2 + H^+$	+76.0
(6) Reaction 5 × 4 + reaction 1 × 4 + reaction 3 × 3	
$4CH_3CH_2COO^- + 7H_2O \rightarrow 7CH_4 + 5HCO_3^- + H^+$	−224.8
(7) Butyrate-oxidizing bacteria	
$CH_3CH_2\ CH_2COO^- + 2H_2O \rightarrow 2CH_3COO^- + 2H_2 + 2H^+$	48.3
(8) Reaction 7 × 2 + reaction 1 × 4 + reaction 3	
$2CH_3CH_2\ CH_2COO^- + 5H_2O \rightarrow 5CH_4 + 3HCO_3^- + H^+$	−158.1

ANAEROBIC CHEMOSTATS AS A TOOL FOR THE SYSTEMATIC ANALYSIS OF MICROBIAL COMMUNITY DYNAMICS

A chemostat operated under anaerobic conditions using CSTR is ideal for studying microbial communities. Systematic studies were conducted with chemostats to understand the overall structures of the microbial communities relevant to methane fermentation (7–14,22). Each chemostat contained synthetic wastewater with only one type of substrate as the carbon source. The total organic carbon concentration in all the synthetic wastewaters was identical (8000 mg/L). $NiCl_2 \cdot H_2O$ (21.3 mg/L) and $CoCl_2 \cdot H_2O$ (24.7 mg/L) were added to the chemostats when required. Synthetic wastewater was continuously pumped into the reactor; the loading rate was adjusted based on the feeding rate. For each chemostat, the concentrations of volatile suspended solids (VSS), total organic carbon (TOC), volatile fatty acids (VFAs), methane and carbon dioxide were measured. Gas production was also measured. Microbial communities in each chemostat were operated under different dilution rates (different loading rates) and supplemented with different carbon sources. Additionally, these communities were analyzed using various molecular biological techniques, including fluorescent *in situ* hybridization (FISH), clone library analysis, quantitative real-time polymerase chain reaction (PCR), and denaturing gradient gel electrophoresis (DGGE).

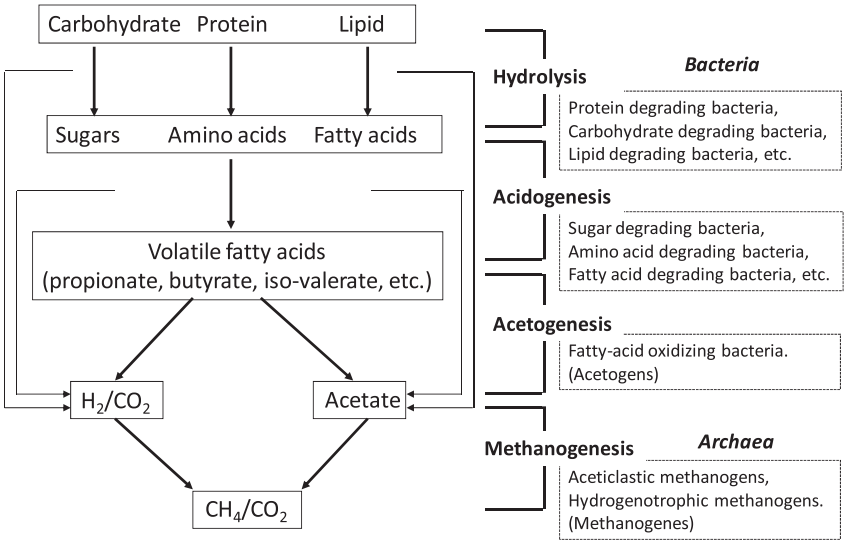

FIG. 1. Flow chart of methane fermentation and the microorganisms involved in each step.