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Methane from Anaerobic Fermentation

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Summary. Conventional anaerobic digestion is an established technology for wastewater stabilization, but methane production rates and net energy yields are generally too low to make the process competitive as a source of methane. Numerous improvements are being developed to make conversion of plant biomass to methane and simultaneous waste stabilization-methane production practical. Among these improvements are innovative digester designs and process configurations. Efforts to commercialize modern anaerobic digestion technology are progressing.

Methane production by anaerobic fermentation (also called anaerobic digestion) involves the conversion of organic material at modest temperatures, ambient pressures, and nearly neutral *p*H to methane and carbon dioxide in the absence of exogenous electron acceptors such as oxygen, nitrate, and sulfate through a complex series of microbial interactions. During this process, which is promoted almost exclusively by mixed bacterial populations, the yield of new microbial cells is relatively small. Most of the substrate's chemical energy ultimately resides in the methane despite the fact that up to about an equal number of carbon dioxide molecules can be formed when the fermentation is proceeding in a balanced, steady-state mode. For example, ignoring the small amount of substrate that is used to replace cellular biomass and to provide cellular maintenance energy, the gross stoichiometry of the methane fermentation of glucose can be approximated by

$$C_6H_{12}O_6(aq) = 3CH_4(g) + 3CO_2(g)$$

The standard Gibbs free energy and enthalpy changes for this conversion under physiological conditions (*p*H 7, 25°C, unit activities) per mole of glucose are about −418 and −131 kJ, and the mass and energy contents of the methane expressed as fractions of the glucose converted are about 27 and 95 percent. Thus, the thermodynamic driving force is large; the exothermic energy loss is small; the energy is transferred at a higher energy density to a simple gaseous hydrocarbon that is the main component of natural gas fuel; methane is easily separated from the aqueous system and, if desired, from the coproduct carbon dioxide; methane and carbon dioxide selectivities are high; and the mass of substrate is significantly reduced if the conversion proceeds reasonably well. These are the primary reasons why anaerobic digestion has been used for about the past 100 years for waste stabilization and disposal and as a source of fuel gas, particularly in the developing countries. Prior to about 1970, anaerobic digestion was considered to be useful in the United States mainly as a waste stabilization process. Considerable effort has been made since then to apply it to the biological gasification of plant biomass for the production of intermediate- and high-Btu gas for fuel applications and to combined waste stabilization–fuel gas production (*1*).

Methanogenic Bacteria

The origin of methane in fermenting materials was suggested by Volta in 1777 (*2*). In the late 1800's, evidence was presented showing that methane is formed in the biological decomposition of cellulose (*3*) and that microorganisms in river muds cause the formation of methane from cellulose and fatty acid salts (*4*). Since then, methane fermentation has been recognized in many ecosystems including lake sediments, sewage, marshes, and peat bogs. In nature, methane fermentation is most conspicuous where plants die and decompose under water. The water layer acts as a blanket to exclude oxygen and promote the growth of many species of anaerobic organisms. Methane also forms in large amounts in the digestive tracts of ruminants. The rumen is supplied with ample quantities of food, is well buffered, has a nearly neutral *p*H, and is almost free of oxygen. Methane-producing bacteria (methanogens) develop rapidly and commonly form 100 to 500 liters of methane daily per cow.

Until 1936, all attempts to isolate pure cultures of methanogens or even to obtain colonies grown on solid media were unsuccessful (*5*). Consequently, much of the early work was carried out with enrichment cultures, in which substrates and environmental conditions were chosen to selectively promote the growth of certain microbial species. By enrichment culture techniques, it is possible to obtain valuable information about the morphology of methane-producing bacteria, the environmental conditions that favor their development, and the types of substrates utilized. A roll-tube method (the Hungate technique) proved to be the most successful for cultivation of methanogens (*6*). Several taxonomically identified species of methanogens have now been isolated and studied in pure culture, and numerous strains have been isolated but remain to be described in more detail before their taxonomic assignment can be established (*6*). Some of the notable species that have been classified are

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Table 1. Estimated free energy changes of selected biological reactions in anaerobic digesters under physiological conditions. Calculated from standard Gibbs free energies of formation (*48*). Conditions are 25°C, *p*H 7, and aqueous solutions at unit activity where possible. Methane, hydrogen, and carbon dioxide are in the gaseous state. Cellulose is assumed to have same standard free energy of formation per unit of glucose as glycogen, and hydrolysate is assumed to be α-D-glucose.

Reaction	$\Delta G^{o\prime}$ (kJ)
Fermentative bacteria	
$(C_6H_{10}O_5) + H_2O = C_6H_{12}O_6$	−17.7
$C_6H_{12}O_6 = 3CH_3CO_2^- + 3H^+$	−311
$C_6H_{12}O_6 + 2H_2O = CH_3CH_2CO_2^- + H^+ + 3CO_2 + 5H_2$	−192
$C_6H_{12}O_6 = CH_3CH_2CH_2CO_2^- + H^+ + 2CO_2 + 2H_2$	−264
$C_6H_{12}O_6 + 6H_2O = 6CO_2 + 12H_2$	−25.9
Acetogenic bacteria	
$C_6H_{12}O_6 + 2H_2O = 2CH_3CO_2^- + 2H^+ + 2CO_2 + 4H_2$	−216
$CH_3CH_2CO_2^- + H^+ + 2H_2O = CH_3CO_2^- + H^+ + CO_2 + 3H_2$	+71.7
$CH_3CH_2CH_2CO_2^- + H^+ + 2H_2O = 2CH_3CO_2^- + 2H^+ + 2H_2$	+48.3
$CH_3CH_2OH + H_2O = CH_3CO_2^- + H^+ + 2H_2$	+9.7
$2CO_2 + 4H_2 = CH_3CO_2^- + H^+ + 2H_2O$	−94.9
Methanogenic bacteria	
$CH_3CO_2^- + H^+ = CH_4 + CO_2$	−35.8
$CO_2 + 4H_2 = CH_4 + 2H_2O$	−131
$HCO_3^- + H^+ + 4H_2 = CH_4 + 3H_2O$	−136

Methanobacterium formicicum, M. bryantii, M. thermoautotrophicum; *Methanobrevibacter ruminantium, M. arboriphilus, M. smithii*; *Methanococcus vannielii, M. voltae*; *Methanomicrobium mobile*; *Methanogenium cariaci, M. marisnigri*; *Methanospirillum hungatei*; and *Methanosarcina barkeri* (*7*).

All of these methanogens can use hydrogen as the sole electron donor for methanogenesis and growth (*7*). Carbon dioxide is reduced to methane when hydrogen is the substrate. Some species can use formate as a carbon and energy source, while *M. barkeri* can use methanol, methyl amines, and acetate for growth and methane production. The nutritional requirements of methanogens can be very simple. Pure cultures generally grow well in media containing the usual mineral nutrients needed for growth of living organisms, a reducing agent, and ammonium ion as the nitrogen source. The addition of extracts containing amino acids, growth factors, and other nutritional supplements to synthetic media may not have a beneficial effect, although some species require complex media for growth (*M. mobile, M. voltae, M. ruminantium*, and *M. smithii*). Several species of methanogens need large amounts of carbon dioxide because it is used as a major carbon source. Generally, growth is best in the *p*H range 6.4 to 7.4; inhibition may occur at higher *p*H. There are exceptions to the generalization that an alkaline medium is unfavorable. The formate-fermenting species *M. vannielii* grows best between *p*H 7 and 9, and some methanogens grow well at a *p*H of 7.6 or above.

Despite their diverse morphology, which consists of many different cell shapes and structures, all pure methanogenic isolates are unique in that all use simple substrates for energy and growth and are specialized in their ability to produce methane. Even though only a few species of methanogens are believed to be capable of utilizing acetate as a substrate (*8*), about 70 percent of the methane formed in anaerobic sewage digesters and from lake sediments is derived from the methyl group of acetate (*9–12*). The carboxyl group yields carbon dioxide. Because of the multiplicity of anaerobes in these systems as well as in other methane fermentations, it is probable that there are many yet-to-be-identified methanogens that utilize acetate.

Fermentative and Acetogenic Bacteria

It is obvious that to degrade complex organic substrates such as wastes and biomass to methane by anaerobic fermentation, other organisms are necessary because of the limited number of substrates catabolized by the methanogens. These organisms are the fermentative species that convert the carbohydrates, proteins, and lipids in the complex substrates to lower molecular weight fragments. These fragments are then utilized by obligate, hydrogen-producing (proton-reducing) acetogenic bacteria to form acetate and hydrogen for consumption by the methanogens. A second group of acetogenic bacteria converts hydrogen and carbon dioxide to acetate and sometimes other acids (*8*). Because of the broad variety of organic structures in complex substrates, many different bacterial species are necessary to facilitate degradation. The fermentative bacteria found in operating methane fermentations supplied with complex substrates are usually obligate anaerobes in genera such as *Bacteroides, Clostridium, Butyrivibrio, Eubacterium, Bifidobacterium*, and *Lactobacillus* (*8*).

The first step in the fermentation of complex substrates is the extracellular enzyme-catalyzed hydrolysis of polysaccharides to oligosaccharides and monosaccharides, of proteins to peptides and amino acids, of triglycerides to fatty acids and glycerol, and of nucleic acids to nitrogen heterocycles, ribose, and inorganic phosphate. The sugars are degraded through pyruvate, a key intermediate in the bacterial metabolism of carbohydrates, to acetate, higher fatty acids, carbon dioxide, and hydrogen. At low partial pressures of hydrogen, acetate is favored. At higher partial pressures, propionate, butyrate, ethanol, and lactate are favored, generally in that order (*8*). The amino acids and glycerol are degraded by the glycolysis pathway to the same products and by other routes. After hydrolysis and glycolysis, some of the fermentation products are suitable substrates for the methanogens; others are not.

Further degradation of the unsuitable substrates is caused by another group of anaerobes, collectively called acetogenic bacteria. This group is known to exist on the basis of experimental data collected with several cocultures containing one hydrogen-using species such as a methanogen. The acetogens convert the alcohols and higher acids produced on glycolysis to acetate, hydrogen, and carbon dioxide. The isolation of "S" organism from *Methanobacterium omelianskii* is the first documented evidence of species in the acetogenic group. Originally, *M. omelianskii* (*13*) was believed to be a methanogen that catabolized ethanol by

$$2CH_3CH_2OH + CO_2 = 2CH_3CO_2H + CH_4$$

Later, this was shown to result from the syntrophic association of S organism and a methanogen (*14*). The S organism converts ethanol to acetate and hydrogen, and the methanogen uses the hydrogen to reduce carbon dioxide by

$$2CH_3CH_2OH + 2H_2O = 2CH_3CO_2H + 4H_2$$

$$CO_2 + 4H_2 = CH_4 + 2H_2O$$

It has now been established that propionate and longer fatty acids are catabolized by similar syntrophic associations (*15*).

Paths to Methane

Interestingly, the catabolism of ethanol by acetogenic S organism to form acetate is inhibited by hydrogen and proceeds at good growth rates only when a hydrogen utilizer is present (*8*). This can be explained by use of the standard Gibbs free energy changes for the dominant reactions of the major groups of bacteria in methane fermentation (Table 1). Ethanol conversion to acetate by acetogenic bacteria has a slightly positive free energy change, so coupling of this reaction with a methanogenic reaction that reduces carbon dioxide to methane and that has a strongly negative free energy change is thermodynamically favorable. Other trends can also be perceived from the free energy changes. The thermodynamic driving force for several of the major acid-forming reactions promoted by acetogenic bacteria is positive, while that for direct conversion of glucose to acetate is strongly negative. For fermentative bacteria, the free energy changes listed in Table 1 are negative, but cellulose hydrolysis is the least favorable reaction. Complete conversion of glucose to carbon dioxide and hydrogen in dark fermentations, while offering a slightly negative free energy change, has not been considered an efficient process for the production of hydrogen energy since every fermentation reaction must be coupled with the synthesis of adenosine triphosphate (ATP) from adenosine diphosphate and inorganic phosphate (*16*). ATP synthesis requires about 42 to 50 kJ per mole of ATP formed; the glucose-to-hydrogen reaction supplies only about 26 kJ per mole of glucose. It is interesting that each of the methanogenic reactions in Table 1 exhibits a negative free energy change. Methanation of carbon dioxide and carbonate is more favored than direct conversion of acetate, which produces about 70 percent of the methane in anaerobic fermentation. Thermodynamic data are quite useful for making predictions and explaining methane fermentation, but judgment should be exercised in interpreting them.

Information accumulated from the examination of pure compounds and natural products as substrates for methane fermentation and work done by many investigators on the characterization of anaerobic organisms indicate that at least three groups of bacteria are involved, as illustrated in Fig. 1. The fermentative bacteria accomplish hydrolysis and conversion of the substrates to intermediates and their transformation to acetate, higher acids, hydrogen, carbon dioxide, and other lower molecular weight compounds. Additional acetate, hydrogen, and carbon dioxide are produced by the acetogenic bacteria, and the methanogenic bacteria yield methane and carbon dioxide from acetate, and methane and water from hydrogen and carbon dioxide. Observations of operating methane fermentations are in accord with this scheme. For example, when a steady-state fermentation is upset by an undesirable change in environmental conditions or an operating parameter that reduces gas and methane production, the *p*H decreases while the volatile acids in the fermentation broth and carbon dioxide production increase. This, as well as several other features of methane fermentation, can be predicted and explained with this model.

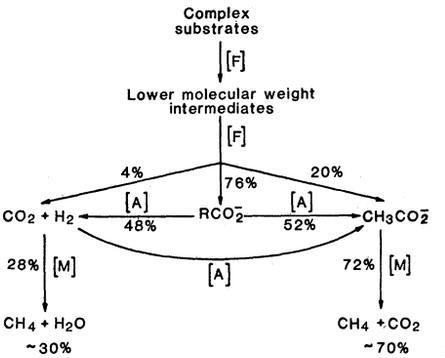

Fig. 1 (left). Important routes to methane by anaerobic fermentation of polysaccharides, proteins, lipids, and other components of complex substrates. Bracketed letters denote mixed cultures of bacteria; *F*, fermentative; *A*, acetogenic; *M*, methanogenic. The percent conversion in each step is from Zehnder *et al.* (*49*), except that fatty acid conversion to hydrogen and carbon dioxide was revised from 24 to 48 percent. Fig. 2 (right). Variation of gas composition with time for 36-day batch digestion of *Macrocystis pyrifera*. Data from Klass and Ghosh (*18*). Conditions: 2.0 liters liquid volume in 2.0-liter fermentor, continuous mixing, *p*H 6.8 to 7.2 with NaOH additions, mixed inoculum, chopped kelp passed through a 3/8-inch screen, 1.41 percent (by weight) volatile solids in charge. Results: maximum gas production rate as volume(n) per liquid volume per day—CH_4, 0.294; CO_2, 0.188; H_2, 0.079; time to maximum gas production rate in hours—CH_4, 294; CO_2, 103; H_2, 12.6; energy in gas as percentage of substrate energy—20.4 as CH_4, 0.45 as H_2; volatile solids reduction, 20.3 percent.

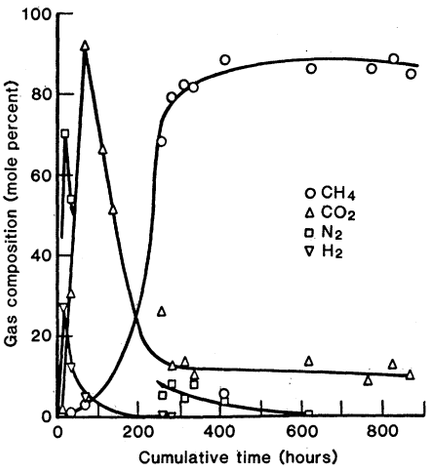

Fig. 1 (left). Important routes to methane by anaerobic fermentation of polysaccharides, proteins, lipids, and other components of complex substrates. Bracketed letters denote mixed cultures of bacteria; *F*, fermentative; *A*, acetogenic; *M*, methanogenic. The percent conversion in each step is from Zehnder *et al.* (*49*), except that fatty acid conversion to hydrogen and carbon dioxide was revised from 24 to 48 percent. Fig. 2 (right). Variation of gas composition with time for 36-day batch digestion of *Macrocystis pyrifera*. Data from Klass and Ghosh (*18*). Conditions: 2.0 liters liquid volume in 2.0-liter fermentor, continuous mixing, *p*H 6.8 to 7.2 with NaOH additions, mixed inoculum, chopped kelp passed through a 3/8-inch screen, 1.41 percent (by weight) volatile solids in charge. Results: maximum gas production rate as volume(n) per liquid volume per day—CH_4, 0.294; CO_2, 0.188; H_2, 0.079; time to maximum gas production rate in hours—CH_4, 294; CO_2, 103; H_2, 12.6; energy in gas as percentage of substrate energy—20.4 as CH_4, 0.45 as H_2; volatile solids reduction, 20.3 percent.

The phasic or stepwise nature of methane fermentation shown in Fig. 1 is also supported by observations of the behavior of individual substrates. For example, when pure glucose was digested in the batch mode with an inoculum from an active sewage sludge digester, almost all the glucose was assimilated in the first 30 hours of fermentation; the product gas during this period was 70 to 100 percent carbon dioxide (*17*). No methane was detected for the first 6 hours, and most of it was collected after about 95 percent of the glucose had been consumed (*17*). The gas production data indicate that the methanogenic organisms function at a much lower rate than the nonmethanogenic fermentative and acetogenic bacteria that rapidly catabolize glucose. Other observations (Fig. 2) that support stepwise methane fermentation have been made with *Macrocystis pyrifera* (giant brown kelp), a complex substrate (*18*). Some denitrification occurred, as shown by the nitrogen peak. Maxima in production rates were observed for hydrogen at 13 hours and carbon dioxide at 103 hours; methane was essentially absent during this period.

Low- and High-Rate Digestion

Conventional methane fermentation takes place in the batch, semicontinuous, or continuous modes of operation. In the latter two modes, the digester is intermittently or continuously supplied with an aqueous slurry of the substrate and an equal amount of fermentor broth is withdrawn. Individual and multistage digesters, in which all phases of methane fermentation occur, are used. In batch systems, steady-state conditions cannot be achieved because the components within the digester are constantly changing. In the semicontinuous and continuous modes, methane fermentation can take place in the steady state as the organisms grow at the maximum rate permitted by the inflow of substrate and nutrients. For methane fuel recovery, the batch mode is not ordinarily used, except for small systems, because the gas composition varies with time and the equipment costs are usually higher than

Table 2. Digester gas utilization of West-Southwest Sewage Treatment Plant of the Metropolitan Sanitary District of Greater Chicago (*50*). High-rate digestion conditions are used at a mesophilic temperature of 35°C.

Plant description	
Digester size	9462 m^3
Number of digesters	12
Design capacity	22.7 tons per day per digester
Activated sludge feed	3.3 percent total solids
Average gas production	72.5×10^3 m^3(n)/day*
Average methane production	47.3×10^3 m^3(n)/day
Gas utilization	
For digester heating	45.6×10^3 m^3(n)/day
For low-pressure steam	26.9×10^3 m^3(n)/day
Gas utilization economics	
Capital cost for steam plant	$830,000
Annual operating cost	$150,000
Annual natural gas cost for steam	$569,400
Approximate payout from digester gas use	2 years

The "n" denotes International Gas Union normal reference condition of 273.15 K (0°C) and a pressure of 101.325 kPa, dry.

those of continuous systems for the same throughput rates. The important independent operating parameters are the composition, physical form, and energy content of the substrate; inoculum source and activity; amounts and types of nutrients; feeding frequency and rate of nutrient and substrate addition to the digester; hydraulic and solids retention times (HRT and SRT) within the digester; *p*H, temperature, and mixing rate within the digester; gas removal rate; and recycling. Many studies have been conducted on how these parameters affect methane production rate and yield, substrate reduction, volatile acid formation, gas composition, energy recovery, and inhibition of fermentation. Reactor configuration and design also play an important role in the performance of methane fermentations.

The details of these interactions are beyond the scope of this article, and only a few general remarks are presented here. So-called standard or low-rate digestion is utilized for wastewater stabilization; it takes place in semicontinuously fed units at mesophilic fermentation temperatures of 25° to 40°C or thermophilic fermentation temperatures of 50° to 60°C, total retention times of 30 to 60 days, and loading rates of about 0.5 to 1.6 kg of volatile solids (VS, organics) per cubic meter of digester capacity per day. Stratification usually occurs, resulting in layers of digesting sludge, stabilized sludge, and a supernatant, which often has a scum layer. High-rate digestion is conducted in a similar manner, except that mixing is used to provide homogeneity, and the retention times are about 12 to 20 days. Under these conditions, loading rates can be increased to about 1.6 to 6.4 kg/m^3-day. Modifications of these processes are used in some wastewater treatment plants; an example is the anaerobic contact process, in which digester sludge is separated for recycling from the digester effluent. Generally, the volatile solids fed to a sewage digester will yield 0.8 to 1.1 m^3 of digester gas per kilogram of VS destroyed in a well-operated, balanced digester. The gas contains 50 to 65 mole percent methane. Many wastewater treatment plants in the United States are now making maximum use of digester gas for process heat, steam production, or electric power generation (*1*). Table 2 summarizes the gas utilization program under way at the West-Southwest Treatment Plant of the Metropolitan Sanitary District of Greater Chicago. The economics of low-pressure steam production are quite favorable. About 63 percent of the product gas is used for digester heating.

Many organic wastes and plant biomass species as well as blends have been evaluated as substrates for methane fermentation. Although there are major differences in energy, moisture, volatile solids, and ash contents between the various raw materials, the gas production parameters, volatile solids reductions, and energy recovery efficiencies as methane span a relatively narrow range under high-rate, balanced digestion conditions. This is illustrated by the data in Table 3, which show composition and digestion performance for each of several complex substrates under similar high-rate conditions. This kind of performance is somewhat unexpected,

Table 3. Comparison of compositions and methane fermentation performance under high-rate mesophilic conditions. Data of Klass *et al.*, adapted from (*1*). Conditions were daily feeding, continuous mixing, 35°C, *p*H 6.7 to 7.2, 12-day retention time, 1.6 kg VS/m^3-day except for kelp, which was 2.1 kg VS/m^3-day. All biomass substrates 1.2 mm or less in size; substrate blends are described in detail in original references. RDF is refuse-derived fuel.

Component or measure of performance	Primary sewage sludge	Primary-activated sludge	RDF-sludge blend	Biomass-waste blend	Coastal Bermuda grass	Kentucky bluegrass	Giant brown kelp	Water hyacinth
Carbon, percent by weight (dry)	43.7	41.8	42.1	43.1	47.1	46.2	26.0	41.0
Nitrogen, percent by weight (dry)	4.02	4.32	1.91	1.64	1.96	4.3	2.55	1.96
Phosphorus, percent by weight (dry)	0.59	1.30	0.81	0.43	0.24		0.48	0.46
Ash, percent by weight of total solids	26.5	23.5	8.4	17.2	5.05	10.5	45.8	22.7
Volatile matter, percent by weight of total solids	73.5	76.5	91.6	82.8	95.0	89.8	54.2	77.3
Heating value, MJ/kg (dry)	19.86	18.31	17.20	20.92	19.04	19.19	10.26	16.02
C/N ratio	10.9	9.7	22.0	26.3	24.0	10.7	10.2	20.9
C/P ratio	74.1	32.2	52.0	100	196		54.2	89.1
Gas production rate, volume(n)/liquid volume-day	0.74	0.84	0.59	0.52	0.56	0.52	0.62	0.47
Methane in gas, mole percent	68.5	65.5	60.0	62.0	55.9	60.4	58.4	62.8
Methane yield, m^3(n)/kg VS added	0.313	0.327	0.210	0.201	0.208	0.150	0.229	0.185
Volatile solids reduction, percent	41.5	49.0	36.7	33.3	37.5	25.1	43.7	29.8
Substrate energy in gas, percent	46.2	54.4	39.7	38.3	41.2	27.6	49.1	35.7

because not all the organic components are present in each substrate, and those that are may not be present in the same concentration. Compositional differences would be expected to control methane fermentation and lead to dissimilar gas production rates and methane yields per unit of volatile solids added to the digester.

The major organic fractions in most wastes and terrestrial biomass species are proteins, celluloses, hemicelluloses, and lignins. Marine biomass (kelp) contains reduced monosaccharides (mannitol), cellulose, and other carbohydrate polymers (algin). Conversion of individual components is shown in Table 4 for a few of the substrates from Table 3. Lignin conversion is small, as expected because of its polyaromatic structure, but most substrates have a much smaller percentage of lignin than of other components. On the basis of these and other data, the general order of decreasing anaerobic degradability is: monosaccharides (glucose, mannitol, and so on), hemicelluloses, algins, cellulose, proteins (crude), and lignins. Several factors should be kept in mind when considering data of this type. The first is that reduction in concentration of a particular fraction does not necessarily mean that the material was gasified. The organic structures in the substrate may have been modified during fermentation and therefore not detected in the analysis of the residual solids or detected in another fraction. The second factor is that a particular fraction in one substrate may not have precisely the same molecular structure as the corresponding fraction in another substrate, even though the analytical results are the same. As a result, the fractions identified as the same component in two substrates may have different degradabilities. This is supported by the cellulose data, which indicate that the fractions identified as cellulose in different substrates had different degradabilities. Cellulose is thought to exist in complexed form in biomass that contains components such as lignin. In this form it is less accessible and has lower degradability than free cellulose. Thus, cellulose conversion might be expected to vary greatly depending on plant species and maturity. Another factor concerns protein conversion, which was assayed in Table 4 by crude protein analysis (Kjeldahl nitrogen value times 6.25). Amino acid assays are necessary to determine true protein degradability, which is often high.

For high-rate digestion where all the basic steps of digestion—hydrolysis, fermentation, acetogenesis, and methanogenesis—take place simultaneously in the same vessel in the presence of each bacterial group, one of these steps might intuitively be thought of as rate-limiting. Considerable experimental work has been done over several years by many research groups to examine the kinetics of methane fermentation, and many reports of empirical observations, particularly with pure cultures, have led to proposals regarding methane fermentation kinetics. For example, as the SRT is reduced from 20 days to about 2 days, the volatile acids in the digester increase and the methanogens tend to be washed out in the digester effluent (*19–21*). This type of evidence led many investigators to conclude that the conversion of volatile acids to methane limits the rate of the overall process. In fermentations with a complex substrate containing large amounts of cellulosics, such as municipal solid waste (MSW) or refuse-derived fuel (RDF), cellulose hydrolysis might be rate-limiting in the overall process.

Table 4. Comparison of component conversion under high-rate methane fermentation conditions (*1*). Same fermentation conditions as in Table 3. All values are percentages by weight.

Component	Coastal Bermuda grass		Giant brown kelp		Biomass-waste blend	
	Percent of VS	Percent converted	Percent of VS	Percent converted	Percent of VS	Percent converted
Crude protein	12.3		29.3	8	12.0	24
Cellulose	31.7	65	8.9	8	44.6	32
Hemicellulose	40.2	67			37.8	86
Lignin	4.1	9			5.5	0
Mannitol			34.5	71		
Algin			26.2	85		

Some investigators felt that these observations also supported transfer of the gaseous products to the gas phase as the rate-limiting step, and concluded that the design specifications for faster methane fermentations might include vigorous agitation, low pressure, and elevated temperature (*22*). However, with the exception of methane fermentation at thermophilic temperatures, which increase the methane production rate through increases in reaction rates, it has been known for many years that rapid, continuous agitation of anaerobic digesters is not necessary, and in some cases is even harmful (*23*). Reduced pressure also provides little or no benefit (*24*).

Two-Phase Digestion

Consideration of the requirements of mixed microbial groups in the anaerobic digestion process and the apparent rate-limitation of methanogenesis led to proposals that the acid- and methane-forming phases of methane fermentation be physically separated to take advantage of the stepwise nature of the process (*25–32*). The optimum environment for each group of organisms might then be maintained and the kinetics of the overall process improved. This could offer improvements over conventional high-rate methane fermentation, where the environmental parameters are chosen to satisfy the requirements of the limiting microbial population.

Techniques suggested for separating the acid- and methane-forming phases included selective inhibition of the methanogens in the acid-phase digester by manipulation of kinetic factors, addition of chemical inhibitors, and balancing of redox potentials (*27*); selective diffusion of the acids from the acid-phase digester through permeable membranes to the methane-phase digester (*26, 28, 29*); and kinetic control by adjusting dilution rates to preclude the growth of methanogens in the acid-phase digester (*30, 31*). Kinetic control is the simplest technique in concept and is likely to present the least operational difficulty. Kinetic control and acid-phase and methane-phase separation were demonstrated in 1971 with a soluble substrate, glucose (*31*), and then in 1975 with a particulate substrate, activated sewage sludge (*33*).

An example of the determination of the kinetic constants of the separate phases—acidogenesis (not acetogenesis) of pure cellulose (*33*), pure glucose (*17, 31*), and activated sludge (*19, 40*), and methanogenesis of acetate (*17, 33*)—is shown in Table 5. Comparison of the maximum specific growth rates (μ_{max}) showed that acid-phase fermentation of glucose was the fastest of the reaction steps studied. The other reactions in order of decreasing rate were acid-phase conversion of activated sludge and cellulose and methane-phase conversion of acetic acid. In an overall process supplied with hydrolyzable cellulosics, methanogenesis is rate-limiting, assuming that acetate is the main intermediate in the methane-phase reactor. The saturation constants (K_s) provided information on the effects of substrate concen-

Table 5. Comparison of kinetic constants for mesophilic acetogenic fermentation of glucose, cellulose, and activated sewage sludge and methanogenic fermentation of acetic acid at 35° to 37°C (*17, 33*). Kinetic constants are μ_{max}, maximum specific growth rate; g_{min}, minimum generation time; and K_s, saturation constant or substrate concentration at which the specific growth rate is 1/2 μ_{max}.

Kinetic constant	Acidogenesis of			Methanogenesis of acetate
	Cellulose	Glucose	Activated sludge	
μ_{max}, day^{-1}	1.7	7.2	3.84	0.49
g_{min}, hours	9.8	2.3	4.3	33.9
K_s, g/liter	36.8	0.4	26.0	4.2

tration on reaction rate (*17, 33*). The low K_s for glucose means that high acidification rates can be achieved at low concentrations, while the very high K_s for cellulose and activated sludge means that much higher concentrations of these substrates would be needed to reach conversion rates comparable to those of glucose. The K_s for acetate in the methane-phase reactor was much larger than that of glucose, but still much less than those of the insoluble substrates studied (cellulose and activated sludge). Theoretical substrate conversion rates per unit reactor volume were also estimated in this work (*17, 33*) from

$$R = \frac{S_0(\mu_{max}\,\theta - 1) - K_s}{\theta(\mu_{max} - 1)}$$

where R is the substrate converted per liquid volume at hydraulic retention time θ, and S_0 is the substrate concentration in the feed. For each substrate, plots of θ versus R yield a family of curves with maxima whose positions depend on S_0. The plots can be used to estimate the optimum θ to achieve maximum feed conversion in the shortest time at the lowest digester volume. At 30° to 37°C, application of this equation indicated that optimum conversion of glucose can be achieved at θ's of 4 hours and 4 days in the acid- and methane-phase reactors. For cellulosics and activated sludge, the corresponding θ's were much higher, about 1 to 2 days for acid-phase digestion and 5 to 8 days for methane-phase digestion.

Laboratory data for acid- and methane-phase digestion of activated sewage sludge in Table 6 (*17, 33*) illustrate the course of two-phase digestion by kinetic control. The acid-phase unit was operated at a short retention time and a high loading rate. Methane yield and production rate were very low in the acid phase. The low *p*H and short retention time in this unit precluded growth of methanogens. The methane-phase unit was operated on the liquid effluent from the acid phase at about half the retention time of a high-rate unit. Methane production rates were high, and the methane concentration in the gas from the methane-phase reactor was about 10 to 15 percent higher than that from a high-rate unit. When applied to a hypothetical commercial plant for two-phase digestion of sewage sludge at a rate of 231 tons of volatile solids (dry) per day, the operating parameters of the two-phase system indicated capital costs about 60 percent below those of a high-rate plant at the same throughput; this was because digester volume requirements were about 35 percent of those of the high-rate plant (*17*). In another example, conversion of glucose was studied in experimental reactors which were identical except that one was used for single-phase digestion and two were used for two-phase digestion; at the maximum specific loadings, the gas production rate (*34*) and chemical oxygen demand (COD) turnover rates (*35*) were about four times higher in the two-phase system than in the single-phase system.

Results of a laboratory comparison of high-rate and two-phase digestion of an industrial waste are shown in Table 7 (*36*). Two-phase digestion facilitated conversion at much shorter retention times with a more concentrated feed. When these data were applied to a hypothetical commercial plant supplied with waste solids (dry) at 9 tons per day, the digester volume required for two-phase digestion was about one-third that of a high-rate system with the same throughput (*36*). Also, the net production of methane, after the digester gas needed for plant fuel is withdrawn, was 73 percent more than that of the high-rate plant. The increase in net methane yield is possible because less process fuel is needed for the two-phase plant due to the higher loading or concentration of volatile solids in the feed slurry. Less liquid is heated to maintain the process temperature.

The relation of the acid and methane phases to the scheme in Fig. 1 raises several interesting questions, such as where the acetogenic bacteria are located and whether methane is formed exclusively from acetate or from both carbon dioxide reduction and acetate in the methane-phase reactor. The experimental data useful in answering these questions include the observations that for a glucose-fed, two-phase digestion system, more than 96 percent of the products from the acid-phase reactor were hydrogen, carbon dioxide, acetate, and butyrate on complete assimilation of the glucose (*37*). The acid-phase gas represented about 12 percent of the influent COD and contained approximately equimolar amounts of hydrogen and carbon dioxide. No methane was detected. Butyrate was present in the acid-phase effluent at about three times the concentration of acetate. About 98 percent of the organic substances fed to the methane-phase reactor were converted to a small amount of cellular biomass and a product gas containing 84 mole percent methane and 16 mole percent carbon dioxide (*38*). These data support the views that the small amount of hydrogen from the acid-phase reactor (*39*) is derived mainly from fermentative bacteria because of the unfavorable thermodynamics of acetogenesis without coupling to a methanogenic reaction, which would have yielded methane; that acetogenic bacteria are

Table 6. Two-phase digestion of sewage sludge (*17, 33*). Charge was 90 percent activated sludge and 10 primary sludge from the Metropolitan Sanitary District of Greater Chicago. The vessel sequence was a 10-liter complete-mix, stirred tank reactor (CSTR) acid-phase digester, an effluent storage vessel, and a 10-liter CSTR methane-phase digester. The high heating value of the charge was 26.0 to 27.9 MJ/kg (dry).

Datum	Acid phase	Methane phase
Temperature, °C	37	37
*p*H controlled	No	No
*p*H	5.7–5.9	7.0–7.4
Retention time, days	0.5–1	6.5
Loading rate, kg VS/m^3-day	24–43.2	
Methane production rate, volume(n)/liquid volume-day	0.006–0.6	4.4–8.4
Methane concentration, mole percent	19–44	61–78
Methane yield, m^3(n) per kilogram of VS destroyed	0.006–0.07	0.50–0.76
Effluent volatile acids, mg/liter as acetic acid	3700–5100	100–150

present in the methane-phase reactor and convert butyrate to acetate and hydrogen, which is rapidly converted to methane since no hydrogen is detected in the product gas from the methane-phase reactor; and that a good portion of the methane is derived from carbon dioxide reduction because the methane concentration is much higher than 50 mole percent in the product gas and butyrate is the main carbon source in the acid-phase effluent. The observations of high butyrate concentrations in the acid-phase effluent and hydrogen in the acid-phase gas are also in accord with evidence that high hydrogen partial pressures promote the formation of higher fatty acids (*8*). The high rates and loadings of acid-phase digestion would be expected to lead to rapid generation of reduced nicotinamide adenine dinucleotide (NADH), which reduces carbon dioxide to methane when coupled to a methanogenic reaction. This makes the transfer of hydrogen (often called interspecies hydrogen transfer) thermodynamically favorable. But since there are few or no methanogens in the acid-phase reactor, the reducing power of NADH is transferred through fermentative routes to yield higher fatty acids and other products (lactate, ethanol). Thus, for glucose conversion by two-phase digestion, it seems reasonable to conclude that the acid-phase reactor contains fermentative bacteria as the dominant organisms, and the methane-phase reactor contains both acetogens and methanogens as dominant species. This probably also applies to other methane fermentations, because essentially the same gas compositions are obtained from other two-phase systems that are supplied with complex substrates such as sewage sludge and industrial wastes.

Table 7. Comparison of high-rate and two-phase digestion of soft-drink waste at 35°C (*36*). Charge was obtained from a modern soft-drink canning plant. The high-rate digestion was conducted in a 7-liter CSTR. The vessel sequence for two-phase digestion was a 2.5-liter CSTR and a 5.5-liter upflow anaerobic filter. The high heating value of the charge was 19.8 MJ/kg (dry).

Datum	High-rate	Two-phase
Loading, kg VS/m^3-day	0.64	4.8
Retention time, days	15	7.4
Methane yield, m^3(n) per kilogram of VS added	0.37	0.37
Gas production rate, volume (n)/liquid volume-day	0.39	2.74
Gas composition, mole percent		
Methane	61	63
Hydrogen	0	3
Volatile solids reduction, percent	72	64
COD reduction, percent	84	96
Feed energy in gas, percent	76	75

Commercial Development

Recent approaches to the development of advanced processes include digester operation at high loadings of solids; use of immobilized bacteria on solid supports; addition of materials such as activated carbon, fly ash, enzymes, lactobacillus cultures, and growth factors to digesters; pretreatment of the influent substrate or post-treatment of the residual digested solids to increase biodegradability before recycling or forwardcycling; integration of methane fermentation with thermal gasification of ungasified residuals from the digester in order to completely gasify the organic materials; and innovative digester designs such as plug-flow, fixed-film packed-bed, fixed-film fluidized-bed, upflow sludge blanket, and baffle-flow digesters that permit longer SRT's than HRT's (*1*, *40*–*42*). Many of these techniques merit further development and can be used with high-rate and two-phase digestion. Integration of advanced digester designs with techniques such as feed pretreatment for substrates that are resistant to enzyme-catalyzed hydrolysis and post-treatment of the residual digested solids should lead to optimum systems for methane production (*43*).

Efforts to commercialize modern anaerobic digestion processes for waste stabilization and methane production are progressing (*40*). Although the United States will probably never make such widespread use of anaerobic digestion for methane production as China, which now has over 7 million digesters in the rural areas and 560 power stations, or India, which has a 5-year program to install 1 million family units and 100 community units by 1985, digestion systems are being increasingly used in this country and package systems and processes are available for purchase and license (*41*). Many municipal wastewater treatment plants are now operating their anaerobic digestion units to maximize methane recovery for in-plant use. Commercial use of anaerobic digestion for methane production on farms has shown modest growth. Several manure-fueled high-rate systems are in operation. Recently, several small, manure-fueled, plug-flow systems have been built for operation on farms, where the methane is used for electric power production. The largest manure-to-methane plant was placed in operation in 1977 in Guymon, Oklahoma, for the production of animal feed and of pipeline-quality gas [16×10^6 m^3/year, 39.7 MJ/m^3(n), 1000 Btu per standard cubic foot] for transmission to Chicago. The digester gas is now used only for plant fuel. The only large-scale anaerobic digestion plant operating on refuse-derived fuel in the United States is a proof-of-concept plant in Pompano Beach, Florida, which is sized to process 90 tons per day; it has provided low to average methane production rates and yields and will probably have to be converted to an advanced design system to make the plant a net energy producer. Landfill-derived methane is the only methane currently produced from municipal solid waste by anaerobic digestion on a commercial scale in the United States. Landfills are batch analogs of anaerobic digesters and produce gas of about the same composition. Methane in the form of medium- and high-Btu gas is now recovered or produced at about two dozen landfills across the United States for use as industrial or utility fuel. Many landfill sites remain to be evaluated as sources of methane.

In 1982, there was a large increase in the number of commercial anaerobic digestion plants, particularly advanced technology systems for industrial wastes (*44*). Several of the new digesters for processing industrial wastes are of the fixed-film or upflow sludge blanket types. Full-scale upflow sludge blanket units ranging in size from 13,000 to 66,000 kg of COD per day have been installed for the treatment of beer, potato starch, and potato cooking wastes, and a downflow packed-bed system with a capacity of about 120,000 kg of COD per day was recently put in operation for the treatment of rum still bottoms. The COD reductions for these plants are about 80 to 85 percent.

Since 1977, four commercial and six pilot two-phase digestion plants have been built and used for several types of high-COD industrial liquid wastes in Belgium and West Germany (*45*). The pilot plants have capacities ranging from 45 to 180 kg of COD per day. They consist of a completely mixed acid-phase digester followed by an upflow sludge blanket methane-phase digester; are operated in

the mesophilic temperature range; and achieve COD reductions up to 90 percent. Three of these plants process beet sugar wastes, two process distillery wastes, and one processes citric acid wastes. The first full-scale, two-phase plant was built in 1980 in Belgium for the stabilization of liquid wastes generated during flax retting and has a capacity of 350 kg of COD per day; 87 percent COD reductions are obtained. The gas production rate is about 4.0 volumes(n) per liquid volume per day, and the yield is about 0.40 m^3(n) per kilogram of COD added. Three other full-scale, two-phase plants have been installed in West Germany to stabilize wastes from beet sugar, starch-to-glucose, and potato chip factories (*46*). Their capacities are 15,000, 20,000, and 32,000 kg of COD per day, respectively. A 9000-kg-COD-per-day plant for brewery wastes is under construction. Two-phase digestion process technology is available for license in the United States and Canada (*47*).

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Retinoblastoma: Clues to Human Oncogenesis

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Two major recent advances in human cancer research have been the detection of putative human oncogenes (*1*) and the delineation of tumor-specific chromosomal aberrations that might allow the expression of these oncogenes (*2*). In this article we discuss chromosomal evidence that supports the role of a diploid pair of "suppressor" alleles at the retinoblastoma locus in the development of this human tumor (*3–5*). This diploid retinoblastoma gene (wild-type alleles Rb+/Rb+) located in chromosomal region 13q14 (*6–8*) apparently functions in a fundamentally different way from the postulated mechanisms by which putative human cancer oncogenes are thought to produce tumors (*1*). In the case of the Rb gene it would appear that loss of function (Rb+/Rb+ → rb−/rb−) rather than gene activation or alteration as proposed for oncogenes is associated with the appearance of malignancy. Specific chromosomal changes in the retinoblast resulting in homozygosity or hemizygosity for the "mutant" or inactive allele appears to be a key mechanism leading to tumor formation (*3–5*). In addition, specific nonrandom chromosomal changes found in retinoblastoma suggest a potential role for an "expressor" gene (possibly an oncogene) in the etiology of this tumor. The evidence for both a suppressor and an expressor system in retinoblastoma will be presented in this article.

Genetics of Retinoblastoma

Retinoblastoma and certain other childhood tumors most likely arise from embryonal cells and could result from as

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