Archaebacterial Fuel Production: Methane From Biomass

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> . . . with the future prospect of scarcity and high price of oil and related fuels, [in the context of] political events of the last few months, attention has been increasingly focused on the prospect of using gas from microbial digestion of organic matter as a fuel.

This observation from Hobson, Boufield, and Summers (1974) seems even more relevant in light of the current world situation. At the 1980 NABT convention in Boston, Barry Commoner observed that our unchecked consumption of nonrenewable energy resources ensnares us in a trap of upwardly spiraling cost and increasing dependency on other nations. Commoner's prescription to this problem is to redirect our nation's energy consumption toward "soft" energy paths, principally toward solar technologies which include both direct (photovoltaic cells and passive heating) and indirect (wind, hydroelectric, and biomass) sources.

Of the enormous energy emitted by the sun, only about 20 parts per billion strike the Earth, and absorption by water and ozone reduce this still further so that only about 48% of the light energy penetrating the atmosphere reaches the Earth's surface (Merrill 1978). Much of this energy falls upon barren soil, ice, or mid-ocean where plant life is sparse. Still more is in the form of heat or wavelengths of light not readily absorbed by photosynthetic organisms. Plants and photosynthetic bacteria are rather inefficient in converting sunlight into chemical energy. Nevertheless, about 1/10 of 1% of the Earth's incident light energy is captured by green plants and a few other photosynthetic organisms and is converted into chemical energy. The dry weight of plant material thus produced, that of all animal species at higher trophic levels, and of all degradative organisms which draw energy directly or indirectly from these primary producers is referred to as "biomass."

Hammond (1977) suggests that "biomass is such an obvious and ubiquitous resource that its energy potential has been largely overlooked." Recently, however, national dependency on petroleum products has stimulated interest in renewable biomass and in the waste products of biomass utilization (wood chips, organic garbage, manure) as alternative energy sources. The energy of biomass may be utilized directly by consumption as food or by burning to produce heat. Alternatively, the energy may be converted to more versatile liquid and gaseous fuels. When biomass is subjected to microbiological digestion, end products possess more energy per mole than the starting materials (Lipinsky 1978).

Staggering quantities of biomass are produced annually. In 1978 it is estimated that one billion tons of primary biomass (crops and wood) were produced in the United States and an equal amount of derived residues (crop residues, forest residues, feedlot manures, municipal garbage, and sewage wastes)

TABLE 1. Types of Methane-Producing Bacteria Known from Pure Culture

Organism	*Source*	*Morphology*	*Gram Reaction*	*Autotrophy*	*Comments*
Methanobacterium arbophilicum	wet wood of living trees, fresh water sediments and soil	short straight rods non-filamentous	positive	yes	
Methanobacterium formicicum	mud and sewage sludge	irregularly curved rods	variable	yes	
Methanobacterium ruminantium	rumin and sewage sludge	coccus to short rods "streptococcus-like"	positive	no	
Methanobacterium mobilis	rumin	short rods	negative	no	motile
Methanobacterium strain MOH	from coculture with an ethanol-oxidizing bacteria. (Formerly *Methanobacillus omelianskii*)	long slightly curved rods	variable	yes	isolated as the autotrophic member of a symbiotic pair of bacteria
Methanobacterium thermoautotrophicum	sewage sludge, manure, thermal springs		positive	yes	thermophilic optimum 65-70°C
Methanosarcina barkeri	mud and sewage sludge	Sarcina-type irregular size and shape	positive	yes	gas vesicles present
Methanococcus vanniellii	mud	cocci, spherical to pear shaped	?	N.D.*	quite sensitive to osmotic shock, a motile coccus
Methanospirillum hungatii	sewage sludge, pear waste digester	cells are rods with blunt ends. Growth results in long helical filaments	negative	N.D.*	polar flagellae in tufts, striated cell surface

*Not determined.

(Zeikus 1980). Unfortunately, most of this is so widely distributed that its collection would be economically impossible. Nevertheless, a study reported by Maugh (1972) estimates that perhaps 136 million tons of dry organic wastes could reasonably be collected. Converted by anaerobic fermentation, this amount would yield 1.36×10^{12} standard cubic feet of methane which, at 1021 BTU/SCF, would satisfy nearly 7% of our nation's annual natural gas requirement of 20.4 quadrillion BTU. Currently, the disposal of biomass residue such as manure requires a net energy expenditure and represents a significant source of environmental pollution. The development of appropriate fermentation technology and its application in converting these wastes into an energy resource represents not only a novel and renewable source of energy but a partial solution to the problem of environmental deterioration as well.

Skeptics question the advisability of diverting land from food production to energy production in a hungry world. In fact, no such diversion is contemplated since plant residue now generally left unused (e.g., corn stalks and husks, wood chips, etc.) would suffice. Additional biomass crops might be supplied by gathering the rapidly growing water hyacinth which threatens to clog our inland waterways or by harvesting kelp or blue green algae cultured on municipal sewage waste (Hammond 1977).

The lack of interest in the production of methane from biomass might lead one to assume that the process is impractical or requires some advanced and sophisticated technology, yet millions of simple generators producing methane from animal manure and human sewage are currently in operation in India and China (Commoner 1979). Closer to home, many U.S. farmers and feedlot operators have capitalized on formerly untapped resources such as agricultural wastes and animal manure by building their own methane generators to provide heat and electricity. Several enterprising cattle feedlot owners in Guymon, Oklahoma, are even selling their excess methane to Peoples Gas Company of Chicago. The by-products of this methane fermentation are sold as feed and fertilizer (Commoner 1979).

The ability to turn potential pollutants, requiring for their disposal enormous expenditures of municipal funds, into nearly unlimited and renewable resources capable of meeting at least a portion of our nation's

energy requirement is a sow's ear to silk purse concept which captures the imagination. A demonstration of methane production using a readily assembled apparatus serves as an interesting point of departure for the study of the unique organisms responsible, the ecological niches they inhabit, and a comparison of the economic and environmental advantages of employing renewable as opposed to nonrenewable sources of energy. [*Editor's Note*: A transparency master for this organism was printed in *ABT* last year (Moore and McCormack 1982).]

The Methanogens

The bacteria responsible for the biological production of methane are found in nearly any aqueous environment, provided only that there is an abundant source of organic carbon and that their requirement for strict anaerobic conditions is met. These organisms have been isolated from the rumens and intestinal tracts of mammals, the sediments of fresh and salt water environments, saturated soils, waterlogged trees, and anaerobic sewage digesters.

The methanogens are a morphologically diverse group. Some species form *Sarcina*-type cellular arrangements with spherical cells in octets; others are rods, spheres, or spirals (table 1). Within a morphological type, cell size and shape may vary considerably. Most species have a cell wall which, under electron microscopy, appears to be of a typical gram positive type. Despite this, some strains give gram negative or gram variable reactions and chemical analysis reveals that the typical peptidoglycan of eubacteria is absent.

Even given optimal conditions of temperature and reduced oxygen concentration, the methanogens are slow growers. *Methanobacterium arbophilicum* and *Methanospirillum hungataii*, both mesophilic bacteria, are typical with doubling times of 10 hours and 17 hours respectively (Zeikus 1977).

Ammonia can serve as the sole nitrogen source for these organisms although some other nitrogen-containing substance such as rumen fluid, yeast extract, and certain amino acids are stimulatory.

The major natural carbon source for growth and methane production appears to be acetate and perhaps certain other low molecular weight, volatile fatty acids. Some species are known to incorporate carbon dioxide, but the determination of true autotrophy is difficult due to the slow growth of these organisms in the absence of organic compounds. The methanogens with exception can use hydrogen as their sole source of reducing power for methane synthesis. In natural environments of extremely low oxidation, reduction potential such as those occupied by methanobacteria hydrogen is readily available (Zeikus 1977).

Despite years of research activity, the mechanisms leading to the reduction of carbon dioxide or the methyl group of acetate to methane are only poorly understood. The unusual nature of this pathway is emphasized, however, by the finding that its enzymes require co-factors found, to date, nowhere else in Nature. One of these is a fluorescent compound called diazoflavine or F 420 because it is at 420 nm that it absorbs light most strongly. This compound is probably protein bound in its active state and is believed to serve as an electron carrier in CO_2 reduction (Zeikus 1980). Coenzyme F 420 is fluorescent and, when illuminated with 420 nm radiation, gives off a blue green light. This property has been found to be useful in identifying methanogens *in situ* (Vogels, Hoppe, and Stumm 1980). Upon exposure to oxygen, F 420 loses both its absorbence and fluorescence and presumably its biological activity as well. It is possible that the extreme sensitivity of methanogens to aerobic conditions is due to the inactivation of F 420 by oxygen.

A second enzyme co-factor unique to methanogens is coenzyme M (Co M) or 2 mercaptoethane sulfonic acid. This compound is believed to be the terminal methyl transfer coenzyme which is reduced by the enzyme methyl reductase to methane gas (Mah *et al.* 1977).

The pathway to carbon dioxide fixation present in methanogens appears to be unique in that the enzymes found in one carbon transfer in the photosynthetic dark reactions of the Calvin-Benson cycle (ribulose 1, 5-biphosphate carboxylase, for example) are absent. Thus the methane-synthesizing bacteria may represent an alternate autotrophic pathway, distinct from that of green plants (Zeikus 1977).

Just how unique a position the methanogens occupy in the biological scheme of things is the subject of a series of papers by George Fox, Carl Woese, and their colleagues. As these investigators point out, the methanogens, due to their morphological variety, have customarily been treated as a heterogeneous group. Fox *et al.* (1977) and Woese and Fox (1977) arrived at quite another conclusion following their analysis of the 16S ribosomal fragment of methanogens, other bacteria, and eukaryotes. Ribosomes, according to their logic, are ubiquitous in distribution, ancient in evolutionary terms, and highly constrained in nucleotide sequence by their crucial function in protein syntheses. The 16S fragment of ribosomes can be obtained from essentially any organism. If one assumes that the number of mutational changes as measured by nucleotide differences from the 16S fragment of one organism to that of another is a measure of the time since the two diverged evolutionarily, then one can estimate the degree of relatedness of pairs of organisms regardless of their taxonomic position. These investigators concluded that the methanogens not only represent a "coherent phylogenetic grouping" but that they are as distantly related to conventional bacteria

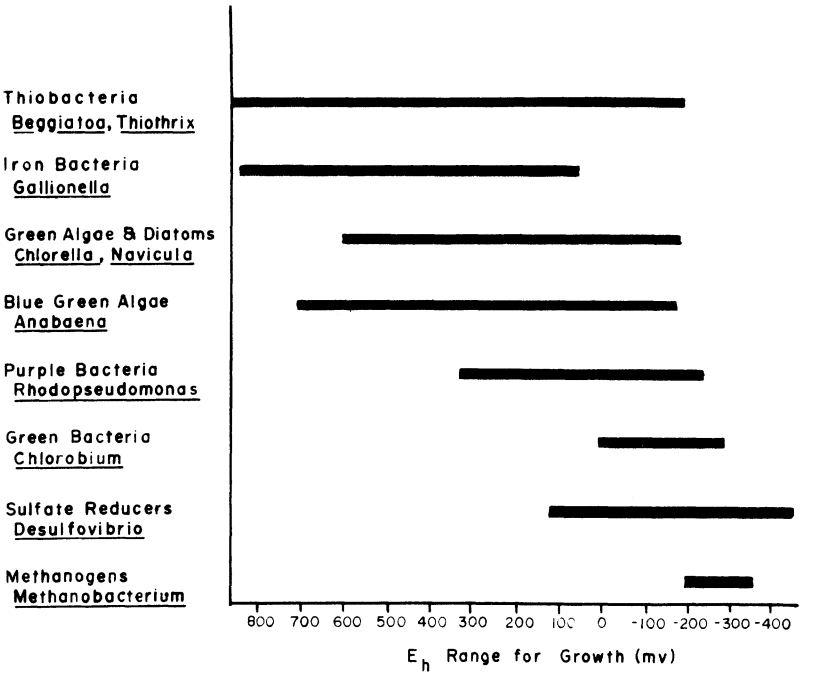


FIGURE 1. When compared to a variety of other microorganisms, methanogens are found to occupy environmental niches of very low redox potential (E_h). An environment with a positive E_h is oxidizing while one with a negative E_h is reducing. Data for Methanogens from Mah (1982); all other from Bass-Becking, Kaplan, and Moore (1960).

as conventional bacteria are to the cytoplasmic components (ribosomes) of eukaryotes (Fox *et al.* 1977).

These data and several other unique features have led Balch *et al.* (1979) to suggest a position for methanogens in the phylogenetic scheme equivalent to that assigned to typical bacteria (eubacteria) and eukaryotes. They propose a new taxon, the "archaebacteria," which would include all known methanogens, the extreme halophiles, and some thermoacidophiles which have similar properties.

Ecology of Methanogenesis

Methanogens have been isolated from many anaerobic, organotrophic natural environments: the rumen and intestinal tracts of warm-blooded animals (including man), marine and freshwater sediments, waterlogged soil, the heartwood of waterlogged but otherwise healthy trees, and from various sorts of anaerobic digesters of organic matter including sewage. Even the methane, which represents a serious hazard to safety in coal mines, is believed by some to have been produced in the mud of carboniferous swamps by methanogens and entrapped there during coal formation (Wolfe 1971).

Methanogens grow in only the most strictly anaerobic environments. A comparison with representatives of other bacterial groups (fig. 1) illustrates the methanogens' fastidiousness with respect to environments of low oxidation-reduction potential (E_h). The redox potential (E_h) is a measure of a system's tendency to yield electrons (positive E_h) or accept them (negative E_h). Natural systems in equilibrium with atmospheric oxygen have E_h values of +800 mv. Isolation from atmospheric oxygen causes a reduction in the E_h as does the activity of heterotrophic organisms. Microorganisms growing in freshwater and marine sediments, waterlogged soil, or the intestinal tracts of mammals scavenge oxygen and can result in E_h values as low as −450 mv. It is therefore not surprising to find methanogens in these anaerobic niches (Atlas and Bartha 1981).

The Ruminants

The domestic ruminants (cows, sheep, and goats) and the wild species (goats, deer, and antelope) are the world's dominant herbivores. Cellulose and starch constitute a major portion of their diet. Like nonruminants, these organisms lack the enzymes for hydrolyzing cellulose, but unlike them, ruminants possess a unique forestomach, actually an enlargement of the esophagus, called the rumen, which contains an extraordinarily rich collection of protozoa and bacteria. These microorganisms are enzymatically competent in digesting cellulose. The rumen is analogous to a continuous laboratory fermenter in which nutrient is constantly supplied, waste products are carried off through absorption, and elimination and conditions of constant temperature, low oxygen concentration, and agitation are maintained. An adult cow's rumen may contain 100 liters and may produce as much as 200 liters of methane gas daily (8.9 moles, assuming standard temperature and pressure). Normally this gas is removed by belching, but the failure of the process due to the consumption of certain crops may result in a condition called bloat (Wolfe 1971).

As a source of carbon, methane is unavailable to the ruminant and although oxidation of hydrogen to methane increases the crop of cells in the rumen, it

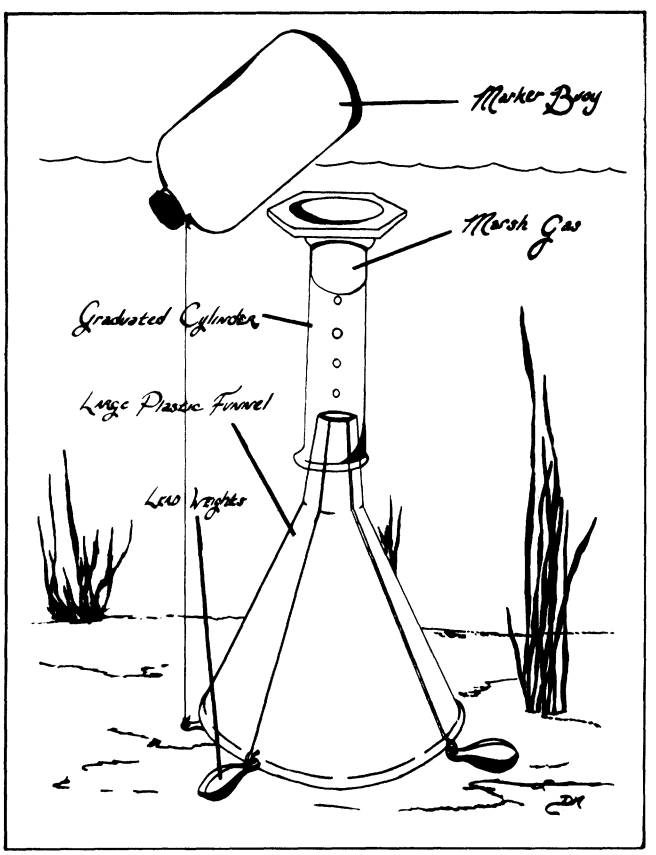
represents a loss of oxidizable substrate to the animal. These bacterial cells, on the other hand, represent a source of protein to the host and so the effect of methanogenesis on the host depends upon whether the concentration of volatile fatty acids (VFA) or microbial protein is the limiting factor.

Typical of the methane producers isolated from the rumen is *Methanobacterium ruminantium* (table 1). This organism is dependent upon the hydrolytic activity of other organotrophic rumen inhabitants for its supplies of VFA, carbon dioxide, and hydrogen. These requirements are met by organisms such as *Bacteroides succinogenes* and *Ruminococcus albus*, which can hydrolyze cellulose and cellobiose, and *Clostridium lochheadii* which hydrolyzes starch.

In newborn animals, the gut is virtually sterile and the acquisition of the endemic intestinal flora including strictly anaerobic forms is by ingestion. This implies that methanogens and other obligate anaerobes can at least pass through an aerobic environment. The oxygen intolerance of these bacteria suggests that they are protected from oxygen presumably in fecal particles or films. Intentional or accidental coprophagy or nursing on a feces-contaminated udder would be sufficient to insure inoculation of the young with methanogens and other anaerobes.

Nonruminant Intestinal Tract Environments

The production of methane has been described in the cecum of nonruminants as well as in the intestinal tracts of other animals including man. Although widely detected, the presence of methanogens in nonruminants is not universal and Mah *et al.* (1977) suggest that this may be due to the high rate of passage of intestinal contents through the digestive system. If the intestinal retention time is less than the average doubling time for the slow growing methanogens, the establishment of such a population would be unlikely.



Sediments of Freshwater Environments

The observation in 1776 by Alessandro Volta that the gas evolved from marshes is explosive is the first record of the activities of methanogens in anoxic freshwater sediments. Contemporary studies indicate that methane production in a sediment increases with depth to a few centimeters below the sediment water interface and then falls off with increasing depth. Apart from oxygen concentration, temperature appears to be the most significant variable, and thus the numbers and activities of methanogens undergo seasonal variation. In lakes in temperate climates (e.g., Lake Mendota, Wisconsin) there may be as much as a 400-fold difference in methane production between summer and winter and an equal difference in viable cell numbers (Zeikus and Winfrey 1976). The Mendota sediments contained a wide variety of methane-producing species. Zeikus and Winfrey successfully isolated species of *Methanobacterium, Methanospirillum, Methanosarcina,* and *Methanococcus* from the mud at lake bottom at all depths tested (table 1).

It is likely that methanogenesis in most lakes is limited by the small amounts of hydrogen and acetate present. In this regard, Lake Kivu, a lake in the African rift lying on the border of Zaire and Ruwanda, is interesting. The waters of this lake contain so much dissolved gas (principally methane and carbon dioxide) that samples brought up from depths greater than 100 m effervesce under the reduced pressure at the surface (Deuser, Dagens, and Harvey 1973). Here, the great amounts of methane formed are thought to be due to the lack of oxygen below depths of 50 m, the relatively high temperature (26°C), and the high concentration of carbon dioxide and hydrogen gas present as a consequence of volcanic activity along the African rift. These conditions are virtually ideal for the growth of methanogens.

The methane formed in lake sediments is liberated either by diffusion or ebullition into the overlying body of water. Gas collected by means of a funnel or plastic bag suspended at the sediment water interface may contain as much as 95% methane (fig. 2).

FIGURE 2. Apparatus demonstrating marsh gas production from fresh or salt water sediments. (Drawings by Dana Carpenter.)

Marine Sediments, Salt Marshes, and Estuarine Environments

Perhaps the most extensive habitats suitable for the growth of methanogens are the sediments of coastal marine, salt marsh, and estuarine environments. As was the case in freshwater locations, the evolution of methane is temperature-dependent and thus shows seasonal variation. Unlike the freshwater habitats, the production of methane in marine environments tends to be sparse at the sediment water interface, but increases with depth up to 1-5 m. Production among the *Spartina* grasses of salt marshes more closely resembles that found in fresh water with a peak of methane production occurring 5-7 cm below the sediment surface (Mah *et al.* 1977).

Flooded Soils and Living Trees

Soils frequently flooded naturally (e.g., flood plains) or by design (e.g., rice paddies) also support populations of methane-producing bacteria. Methane production is inhibited until alternative electron acceptors such as oxygen, ferric iron, nitrate, and sulfate are exhausted. If the soil is completely dried between floodings, the onset of methane production is also delayed.

A unique set of culture conditions for methanogenesis is described by Zeikus and Ward (1974). Introduction of a hollow increment borer into the heartwood of waterlogged trees such as cottonwood, elm, and willow resulted in the release of pressurized gas which, when ignited, burned with a clear blue flame. Analysis revealed hydrogen as well as methane, but as expected, oxygen was absent. The gas possessed a characteristic acrid odor described by the investigators as being "not dissimilar from rumen fluid." Incubation of aseptically removed heartwood samples from methane-containing trees in complex medium with mineral salts and a hydrogen carbon dioxide vapor phase resulted in the isolation of a methanogen of the genus *Methanobacterium*. *Methanobacterium arbophilicum* (table 1) was isolated from a similar source. The nutrient source for this fermentation is unclear. Methanogens are incapable of hydrolyzing the structural polysaccharides in wood, but the bacteria may be using a decomposition product of the wood produced by one of the numerous other microorganisms inhabiting this unusual microcommunity.

The Role of Methanogenesis in Nature

Despite their unusual methane-synthesizing ability, the methanogens are unable to exploit many naturally occurring energy sources. In the oxygen-free environments where decaying or digested plant materials are the major energy input, the methanogens are dependent upon other heterotrophic microorganisms for carbon dioxide and their energy source due to their total inability to attack cellulose, hemicellulose, or even their hydrolyzed products like simple sugars. The methane bacteria are, in fact, the terminal organisms in microbial food chains where they play an important and perhaps unique role in their anaerobic ecosystems (Zeikus 1977).

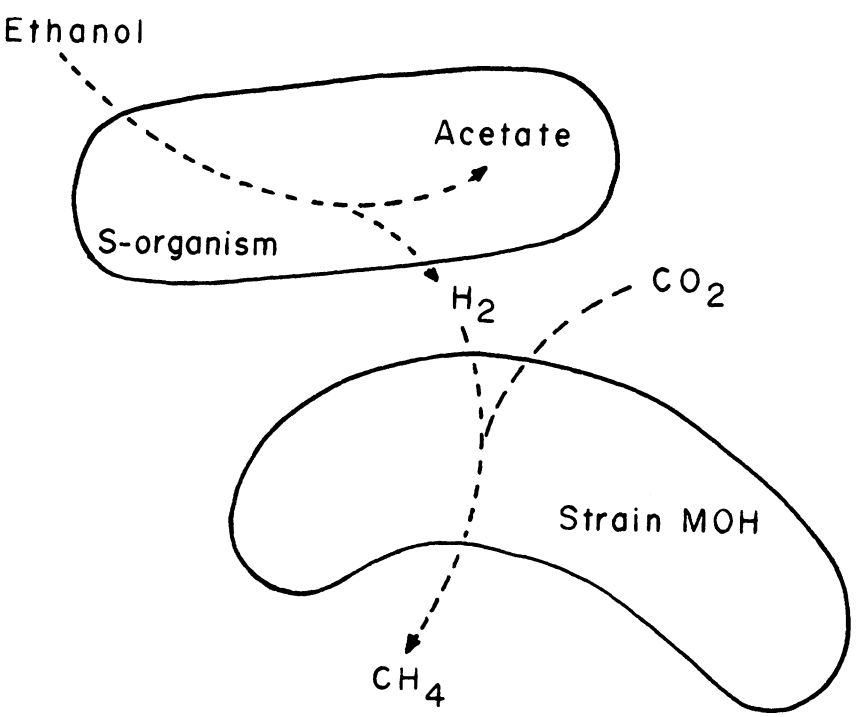


FIGURE 3. Mutualistic relationship between the hydrogen-producing "S" organism and the hydrogen-utilizing *Methanobacterium* strain MOH. This pair of organisms was previously referred to as *Methanobacterium omelianskii*.

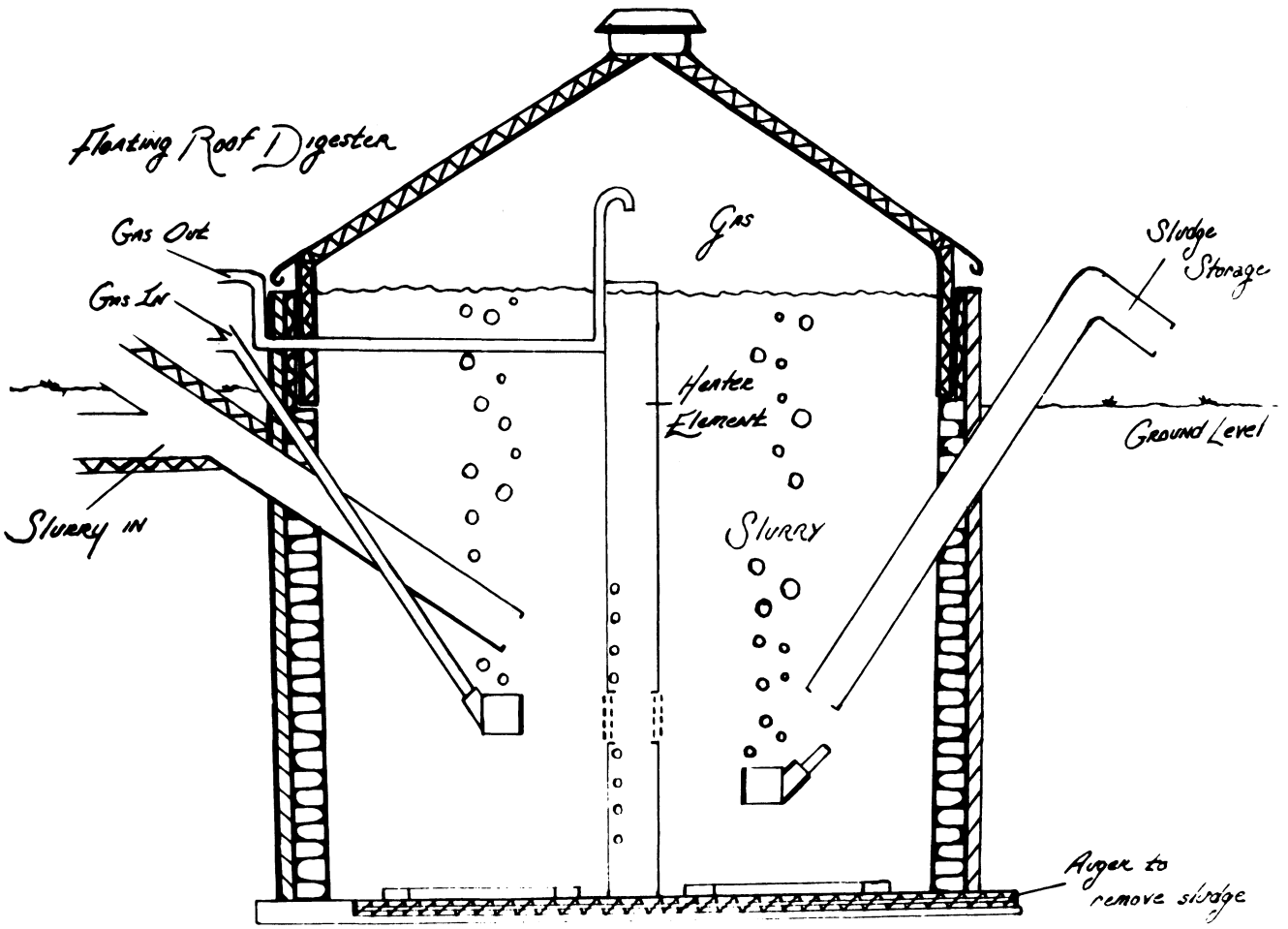


FIGURE 4. A floating roof methane digester. Adapted with permission from P.S.U. College of Agriculture Extension Service, Special Circular 260.

The methanogens serve in terminal electron removal (electron sinks) by accepting hydrogen produced by fermentative organisms via interspecies hydrogen transfer and by using this hydrogen as an energy source in the reduction of carbon dioxide. The relationship between methanogens and their associated microflora is thus a mutualistic one.

The first and most extensively studied example of this sort of interspecies transfer of hydrogen is that involving the "organism *Methanobacillus omelianskii*" isolated by H. A. Barker from mud in the canals of Delft and San Francisco Bay. This "organism" grew in ethyl alcohol-carbonate medium with the production of acetate and methane gas. Because of similarities of this "organism" to others studied by Omelianski in 1916, the designation *M. omelianskii* was proposed.

Phase contrast microscopy ultimately revealed that the "*M. omelianskii*" cultures included two morphologically distinguishable cell types. One type exhibited long, slightly curved, gram variable rods while the other was a short, gram negative, motile anaerobic rod. This culture was resolved into two microbial components each of which accounted for some of the properties of "*M. omelianskii.*"

The one component, designated the "S" organism, is the short gram negative rod that oxidizes ethanol to acetic acid and molecular hydrogen gas. Since the accumulation of hydrogen inhibits further oxidation, the "S" organism is dependent upon the constant removal of hydrogen from its environment for continued growth.

The other component, now called *Methanobacterium* strain M.O.H. (table 1), is the gram variable curved rod. Under anaerobic conditions and in the presence of hydrogen it can reduce carbon dioxide to methane (fig. 3).

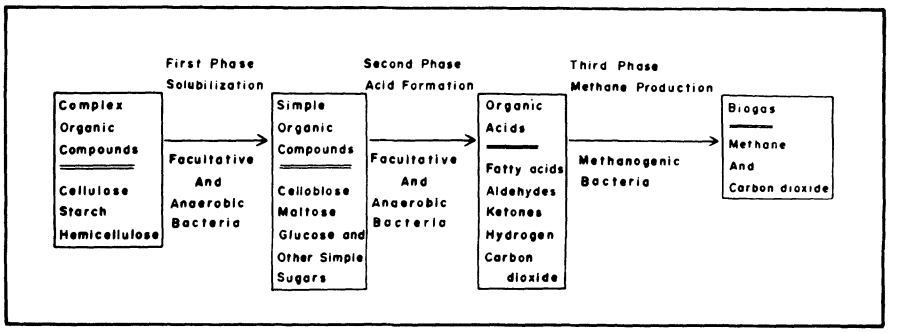


FIGURE 5. Phases in the microbial production of biogas. Modified from a figure in Persson, *et al.* (1979).

By using hydrogen in the formation of methane, methanogens keep its concentration low thus enabling certain chemical reactions to proceed which would otherwise have unfavorable equilibria (Wolfe 1971). Vogels, Hoppe, and Stumm (1980) demonstrated an intimate association between a variety of ruminant protozoa and bacteria believed to be methanogens on the basis of fluorescence at 420 nm which indicates the presence of the unique co-factor F 420. Such an association is mutualistic, permitting direct hydrogen transfer between the hydrogen-producing ciliates and the hydrogen-utilizing methanogens.

Methane Production: Digester Design and Operation

One of the few contemporary uses of methane production in the United States is that practiced in sewage treatment plants which employ anaerobic digesters in the treatment of municipal sewage sludge. The incubation of sewage wastes under anaerobic conditions results in the slow conversion of organic material to carbon dioxide, methane, and smaller amounts of other gaseous products. The function of such digesters is to lower the biological oxygen demand (BOD) of the sewage, and to an extent render it pathogen-free.

A digester is an airtight container in which the requirement of methanogens for a reduced environment, hydrogen gas, and substrate can be met. Some method of loading feedstock into the digester, of storing the methane produced, and of removing the exhausted sludge is also necessary. Most digesters also include mechanisms for mixing the sludge and maintaining a constant temperature.

Digesters for agricultural waste processing often are designed as a cylindrical tank with a fix roof. An external tank is required to store the gas. Sewage disposal plants and some agricultural digesters incorporate a floating roof which can rise and fall with the volume of gas evolved (fig. 4). In a third type of digester, wastes are introduced into an earthen lagoon covered with a flexible rubber or plastic sheet. Methane is trapped and stored beneath the cover.

Digesters may be loaded by several methods. In the batch process, the waste slurry is introduced all at once and the digester is maintained until gas production drops. At this time, the digester is emptied and recharged. In the plug flow type of digester, periodic increments of slurry are introduced into a tunnel-like digester without mixing. At each introduction, an equal amount of effluent is removed from the other end of the digester. The volume and timing of additions are calculated to equal the time required for maximal decomposition. A third method of charging a digester requires the frequent addition of slurry with some degree of mixing. Mixing usually increases the rate and efficiency of methane production (Smith 1978).

The rate of methane synthesis is also affected by the temperature of the digester. Digesters may be operated under either mesophilic (ambient to 43°C) or thermophilic conditions (43-70°C). Below 5°C almost no methane production occurs, but as temperature increases so does biological activity. Most of the organisms in the digester are of human or domestic animal origin, so the fact that most digesters run optimally at 37°C is easily understood. Some methanogenic bacteria like *Methanobacterium thermoautotrophicum* (table 1) grow best at temperatures between 56-70°C. Therefore, some digesters have been designed to operate at these high temperatures. Such high-temperature digesters produce greater amounts of methane but their requirement for fuel to warm the digester to 65°C reduces their efficiency (Smith 1978).

The material introduced to the digester, called a slurry, may consist of municipal or agricultural wastes (feces, urine, and bedding material). The major component is water (85% in dairy cow manure) and the remainder is solid matter consisting of organic (volatile solids) and inorganic materials (ash). The organic materials are a complex mixture of proteins, fats, starch, cellulose, and lignins.

The degradation products of carbohydrates supply the volatile fatty acids required in methane production while the proteins supply amino acids and ammonia, essential nutrients for the anaerobic bacteria. Proper functioning of the digester is also dependent on the carbon-to-nitrogen ratio (C/N). Estimates of the optimum carbon-to-nitrogen ratio vary from 16-30/1.

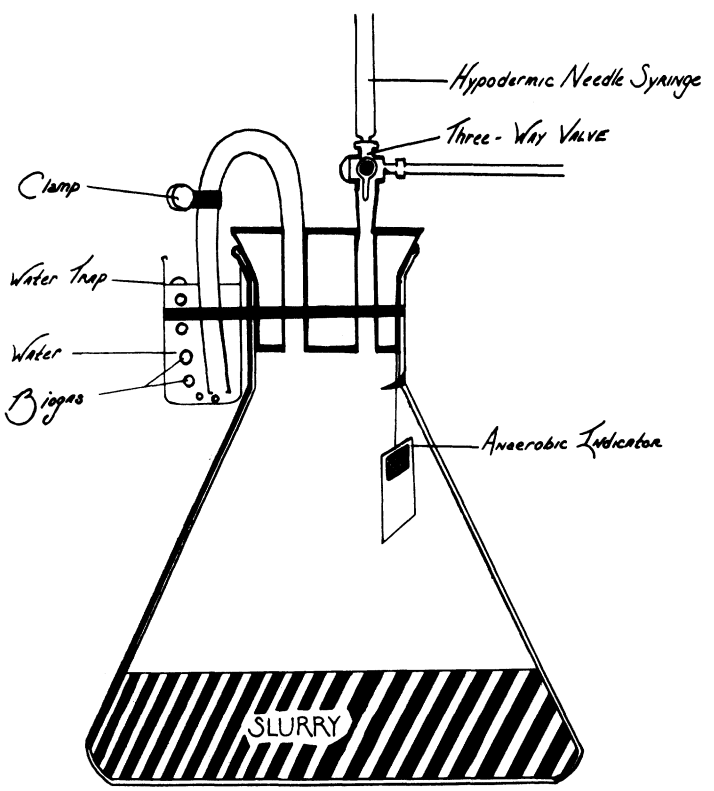
The amounts of carbon and nitrogen in the manure of an animal vary from one species to another. The carbon content of dairy cattle manure is slightly higher

than required for optimum functioning, but will work without added nitrogen. The manure of pigs and poultry has an excess of nitrogen and would ordinarily be mixed with some high carbon-containing substance such as bedding material or sawdust (Persson *et al.* 1979).

Of the total volatile solids in the manure slurry, only a portion can be degraded biologically and converted to gas. Much of the cellulose, for example, is complexed with lignin. Lignin itself is degraded only very slowly by microbial activity protecting much of the otherwise degradable cellulose from microbial action (Persson *et al.* 1979). The biogas produced is a mixture of about 60% methane and 40% carbon dioxide. This gas, when burned, releases about 60% of the energy of natural gas, that is, about 600,000 Btu/1,000 ft.^{3} Even so, for each pound of volatile solids introduced into a digester per day, about 3-4 ft.^{3} of biogas is produced. A farmer with 100 head of dairy cattle who digests all all of the manure these cows produce could reasonably expect to obtain 1.35×10^6 ft.^{3} of biogas annually. This is the energy equivalent of 5,365 gallons of fuel oil (Persson *et al.* 1979).

Incidentally, the processed sludge removed from the digester is nearly as good a fertilizer as the starting slurry. The volume of the slurry and its carbon content are reduced slightly. There is also some loss of nitrogen as ammonia gas, but that which remains is more available to plants. Since phosphorous and potassium compounds formed during digestion are not volatile, there is no loss of these minerals.

The production of biogas occurs in three stages. In the first (fig. 5), carried out by facultative and anaerobic bacteria, complex organic compounds such as cellulose, starch, and hemicellulose are solubilized; that is, they are hydrolyzed to simple organic compounds like cellobiose, maltose, glucose, and other simple sugars. The second phase, also carried out by nonmethanogenic bacteria, results in the fermentation of simple sugars to low molecular weight compounds including fatty acids (e.g., acetic, propionic, butyric), hydrogen, and carbon dioxide. These are the compounds from which the strictly anaerobic methanogens produce methane.



The Demonstration

Over the past three years, students in the introductory microbiology courses here have constructed and operated a number of methane digesters varying in size from 1 L to 9 L; published designs for digesters of larger sizes (55 gal. and 300 gal.) are also available (Moran 1975; Auerbach 1979).

The simplest digester (fig. 6) is made from a 1 L Erlenmeyer flask. A two-hole rubber stopper closing the mouth-held glass tube with a plastic tube is attached. The end of this flexible tube leads to a shell vial taped to the neck of the flask. Filling the vial with water forms a trap which permits the release of gas, but prevents the entry of oxygen. A clamp is attached to the plastic tube to permit closure. The function of this clamp is to prevent entry of air when the delivery tube is transferred from one container to another. It should be pointed out that this clamp must remain open at *all* other times to prevent expulsion of slurry through the influent tube. In the other hole of the stopper, a three-way plastic valve is inserted. The valve allows additions to the slurry by means of a Luer-lok syringe and removal of gas by the attached delivery tube. The valve and syringe can be replaced by a plastic delivery tube if no additions to the slurry are anticipated. When the digester flask is charged, a disposable methylene blue anaerobic indicator tied to a thread is inserted. This indicator is blue in the presence of oxygen, but turns colorless under anaerobic conditions. (Available as BBL Cat. No. 70504 from Beckton, Dickinson and Co., Cockeysville, Md.)

Aspirator bottles of various sizes make convenient and versatile digesters (fig. 7). Bottles of 1,350 and 2,150 ml capacity are closed with three-hole rubber stoppers. One hole holds the input tube, the bottom of which opens below the level of the slurry. To the top of this tube is attached a plastic tube, a funnel, and a clamp. The funnel is used for adding slurry and other constituents to the bottle. A second hole holds a glass tube to which is attached a flexible delivery tube. The third hole holds a thermometer which is used to record the digester's internal temperature. In this case, the anaerobic indicator may be taped to the input tube well above the level of the slurry. The ability to draw off samples of slurry through the aspirator tip for microscopic examination and measurement of pH, oxygen concentration, etc. is an advantage of this digester.

FIGURE 6. Simple methane digester assembled using a 1 liter Erlenmeyer flask.

In one series of experiments using freshly collected cow manure, dilutions were made in water to produce a slurry which could be easily handled. Published values for volatile solids in dairy cow manure average about 72%. Students made dilutions of 1:5, 1:7, and 1:11 to yield about 12%, 9%, and 6% volatile solids respectively. Samples of 500 ml of these dilutions were added to 1 L flasks and the flasks were incubated at 37°C. Two days later the flasks became anaerobic. Evolution of gas began within a few days, but at first no methane was detected. The first evidence of methane from the 1 L flasks ranged from 16-34 days with a mean of 24 days. Methane gas was produced in each of 18 digesters. The temperature of the digesters was maintained at 37°C by placing them in a 37°C incubator or waterbath.

Measurement of the Methane

If one assumes that the only gases present are carbon dioxide and methane, measurement of the methane is quite easy. A tube of gas collected by displacement of water is inverted in a graduated cylinder of 12% sodium hydroxide. The initial volume of gas is noted. The carbon dioxide reacts with sodium hydroxide to yield sodium bicarbonate which is water soluble. As the carbon dioxide is removed by chemical reaction, the liquid rises in the tube. With an excess of sodium hydroxide, virtually all the carbon dioxide is removed. The volume of gas remaining in the tube when the reaction is completed and the inside and outside liquid levels are equal represents the volume of methane gas. This technique is only approximate, but should yield the proportion of methane within a few percent. In our experimental digesters, the proportion of methane varied from flask to flask but was always between 50 and 66%.

The presence of methane can be demonstrated directly by bringing a flaming wooden split to the mouth of a tube from which the carbon dioxide has been removed. If this is done in a darkened room, methane will be observed to burn with a clear blue flame. CAUTION: mixtures of methane and air are explosive. One should be careful that air does not enter the tube prior to ignition and it is recommended that this step be performed by students only with supervision.

To determine the rate of biogas production, the digesters were connected to gas collection tubes and the gas evolved in a given time period measured. The volume of biogas evolved is not large and peak production in twelve 1 L digesters charged with diluted cow manure ranged from 4-7 cm^3/hr (200-300 u moles/hr).

The ability to add and remove substances to and from the flasks when in operation permits the possibility of a wide range of experiments involving the effects of additives on gas production. Methane production is reported to be inhibited by sulfate and nitrate ion as well as by low pH. The effects of antibiotics, disinfectants, oxygen, and specific feedstocks (sugars, starch, sawdust, etc.) and variations in temperature could also be investigated.

These simple digesters are quite stable and once in production will yield methane for weeks with little maintenance. We have experienced only two failures out of 22 digesters. One of these was operated at 28°C which is well below the optimum for methanogens. The other was a digester in which sulfate ion was added, a treatment known to be inhibitory to methanogenesis.

The value of the exercise lies in the demonstration that fuel commonly accepted as being nonrenewable is, in fact, easily manufactured from abundantly available wastes. The nature of anaerobic growth is demonstrated and the variety of natural anaerobic environments explored. Accurate measurements of a biological product can be made and the effects of environmental variables on the product determined.

Contrary to expectation, the digesters are not particularly smelly. The digester itself is sealed and the gas, which does have an odor, is collected. At one point, 18 such digesters were operating in one laboratory without complaints from students or other personnel. A hood is convenient if available but not essential. The students may at first be a bit squeamish about working with animal manure. The instructor's matter-of-fact example, and the use of volunteers, rubber gloves, and garden trowels can decrease their reluctance. Once into the project, most students are

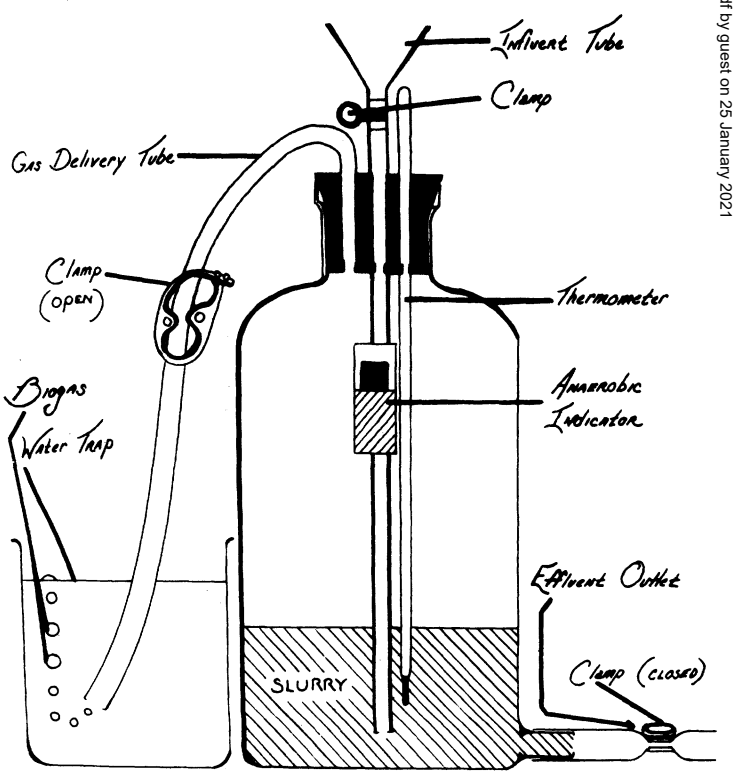


FIGURE 7. A methane digester assembled from an aspirator bottle facilitates sampling of the slurry.

fascinated by the experience of turning animal wastes into "the most natural gas."

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