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The formation of methane from biomass - ecology, biochemistry and applications

R.E. Hungate explains why methane as a product and cellulose as a substrate command so much attention today; he then examines the ecology of one natural methane producing system, the rumen. Other natural ecosystems are discussed by K. Wuhrmann.
The known biochemical pathways of methanogenesis are reviewed by R.S. Wolfe. It is astonishing how some of the structural and chemical properties of the cells are uniquely and specifically restricted to the group of methanogens. The practical engineering, operational and economic aspects of the methane production are reviewed by J.T. Pfeffer who discusses substrate properties, process characteristics, residue disposal and costs.
The special case of biogas production from agricultural wastes, especially those from animals, is presented by P.N. Hobson and practicable high- and low-technology systems are compared, particularly in regard to their application in underdeveloped countries.
The article by M. Gandolla and co-workers describes a small experimental landfill which, in producing methane, is a solid state fermentation system. This paper stresses many of the practical problems and considerations associated with such a system (composition of gas, leakage at the landfill, purification, storage, utilization and safety).

Methane formation and cellulose digestion - biochemical ecology and microbiology of the rumen ecosystem

by R.E. Hungate

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In postulates[1] on the Earth's origin, gaseous chemical elements combined with each other during cooling; compounds with the highest boiling points condensed first, followed by those containing lighter elements. Living material, formed gradually by chemical reactions of the lighter elements and traces of the heavy ones, was peculiar in its tendency to revert to non-living material unless chemical work maintained its living state. Abundant non-living compounds of C, H, and O also formed in various proportions. The relatively large energy changes involved in the oxido-reduction of these elements equipped them to be agents for the chemical work.
Because the Earth was initially anaerobic, with insufficient oxygen to combine completely with the available carbon and hydrogen, much of the carbon must have been in an intermediate state of oxidation. In the absence of O_2, energy was not available through the oxidation of carbon to CO_2 and H_2O, but it could be derived by converting C atoms at an intermediate state of oxidation to CO_2 and CH_4. These molecules are in a low energy state anaerobically, incapable of further redox reactions except that CO_2 can be reduced with H_2 to CH_4. Thus methane was, presumably, an important waste product of early metabolism, and methanogenesis was a primitive phenomenon, accomplished by possibly many diverse forms.

The primitive carbon compounds at intermediate states of oxidation do not have an equal potential for chemical work. This is evident from the following comparisons of single-C compounds containing both H and O[2].

$$4\,HCOOH\ (\text{formic acid}) \rightarrow 3\,CO_2 + CH_4 + 2\,H_2O \qquad (I)$$
$$\Delta G'_0 = -120\ \text{kJ}$$
184 daltons $\qquad -0.65$ kJ/dalton

$4\ CH_3OH$ (methanol) $\rightarrow 3\ CH_4 + CO_2 + 2\ H_2O$ (II)

$\Delta G'_0 = -311$ kJ

128 daltons -2.43 kJ/d

$2\ CH_2O$ (formaldehyde) $\rightarrow CO_2 + CH_4$ (III)

$\Delta G'_0 = -176$ kJ

60 daltons -2.93 kJ/d

Simultaneous oxidation and reduction of the intermediate state, CH_2O, gives 20% more work potential per unit weight than does that of methanol. This may have been sufficient to select carbohydrate as a preferred substrate for metabolic reactions supporting primitive anaerobic life, with CO_2 and CH_4 as low energy waste products[3]. This low energy state anaerobically can be inferred also from the fact that they are the end products of the anaerobic fermentation of most kinds of organic matter, not just carbohydrates.

$C_6H_{12}O_6 \rightarrow 3\ CO_2 + 3\ CH_4$ (IV)

$\Delta G'_0 = -393$ kJ

This is the maximum energy derivable anaerobically from glucose, as compared to the 2650 kJ released aerobically by oxidation to CO_2 and H_2O, or the 2255 kJ available from the oxidation of 3 CH_4.

Phosphorus and nucleic acids were drawn early on into metabolism, and participated in oxido-reductions in such a way that adenosine triphosphate (ATP) became a convenient high energy (44 kJ) molecule[2] for accomplishing biochemical work.

Carbohydrates such as glucose, less reactive than formaldehyde, have the advantages that they are less toxic and that the energy of redox reactions can be channeled through ATP to transform individual carbon atoms into the carbon skeleton precursors of amino acids and other cell components. The sugars can also be polymerized into large insoluble molecules such as starch and glycogen, well suited for storage, and common in anaerobes.

Photosynthesis with O_2 evolution made carbohydrate extremely cheap, manufacturable in great quantity from light, water and CO_2. In the form of cellulose it was used to strengthen the walls of aquatic plant cells, a non-energy-requiring mechanism preventing osmotic plasmoptysis. At the time land plants appeared, cellulose was used to support aerial organs, and has become an abundant and almost universal component of higher plants. Although digestible to sugar by many organisms, it is hydrolyzed less readily than starch or glycogen.

Lignin. Do we then have huge supplies of cellulose? The answer is yes. Annual world power consumption has been estimated at 10^{17} kJ. The maximum annual agricultural production (possibly 50% cellulose) has been estimated to be equivalent to 2×10^{17} kJ, if oxidized. This is a large amount, but unfortunately much of it is not readily exploitable through microbial fermentation. The lignins, less digestible than cellulose, are linked with it in a way that diminishes cellulose digestibility.

It is commonly believed that lignin cannot be digested and fermented anaerobically, but the aromatic ring structure may actually be susceptible, as is benzoate[4], to microbial oxido-reduction to CO_2 and CH_4. Perhaps the insolubility of lignin is the impasse blocking lignin fermentation. The achievement of successful fermentation of lignin would greatly increase the amount of fermentable cellulose. Alkali treatment of lignified plant materials increases their digestibility, but the process is still incomplete.

Cellulose conversion to methane. Methanogenesis is commonly encountered in fermenting systems containing cellulose. Early investigators assumed that methane was a waste product of the cellulolytic bacteria, but lacked knowledge of anaerobiosis and of the nutrients needed to grow axenic cultures of the cellulolytic and the methanogenic bacteria. In their agnotobiotic enrichment cultures containing pure cellulose, aerobic and euryoxic bacteria mopped up the traces of O_2 initially present. Various members of the microbial consortium produced small amounts of needed nutrilites, and the metabolic products of the total biota helped to create a highly reduced environment.

Because of the slow digestibility of the cellulose and the limited synthesis of nutrilites, the acid fermentation products of the cellulolytic bacteria did not exceed the buffering capacity of the medium. Methanogens could develop fast enough to keep pace with acid production, and the cellulose was gradually converted to CO_2 and CH_4.

Improved culture methods have yielded pure cultures of both methanogenic[5,6] and cellulolytic[7,8] bacteria, and revealed the existence of the microbial consortium concerned. Modern methanogens are stenotrophic. All except one[12] isolated pure cultures can grow at the expense of the reaction[2],

$4\ H_2 + HCO_3^- + H^+ + \frac{1}{2}\ P_i + \frac{1}{2}\ ADP \rightarrow CH_4 + 3\frac{1}{2}\ H_2O + \frac{1}{2}\ ATP$ (V)

$\Delta G'_0 = -96$ kJ

Some strains can use also formate, methanol and acetate[5,6]. Also, most of the actively cellulolytic anaerobic bacteria are stenotrophic, fermenting cellulose and its derivatives and only a few other substrates. Various organic nutrilites are either required by most strains of both these groups or are stimulatory.

The rumen ecosystem

The rumen is an essentially anaerobic ecosystem[7] containing hundreds of kinds of microbes in total concentrations as high as 2×10^{10} bacteria and 10^6 protozoa/ml. 40–60% of the cellulose and hemicellulose and most of the starch and pectin in the plant

material are digested, and the sugars formed, together with those in the feed, are fermented to volatile fatty acids (vfa), CO_2, CH_4 and microbial cells.

The ruminant is an open system fermentor, not requiring sterilization or expensive procedures for growing pure cultures. It supports continuous fermentation by taking in water, gathering plant bodies, comminuting and retaining them for microbial action in a large fermentation chamber; it maintains a constant favorable temperature and anaerobiosis; regulates the pH with bicarbonate and by absorption and utilization of fermentation acids; transports and voids undigested residues; and digests the microbial cells and converts them, with the acids, into meat, milk, wool and hides[8].

Each fermentation unit is small and mobile enough for adequate substrate collection, yet the total vast capacity on earth, realized through a multiplicity of units, produces enough methane to meet the average requirements of one-fourth of the world's human population. Unneeded units have a high sale value, and pairs of units can periodically produce new fermentors, all this with no human intervention except for exploitation.

These attributes are not easy to simulate. In times when energy and materials (also representing much energy) are to be conserved, industrial conversions of plant materials can profitably be compared with the fiber economy of the ruminant. In turn, industry might conceive of ingenious means to collect ruminant methane. If ruminant husbandry moves toward the massive battery stage already characteristic of pig and fowl production, methane collection may not be entirely fanciful.

Most plant material is fibrous and fiber cannot be fermented until it has been digested to soluble sugars. Digestion is slow because enzymes act only at the surface, and even there they do not act until the surface of ingested fibers is covered by fibrolytic enzymes and bacteria. Electron microscopy has disclosed that some plant tissues digest completely without bacterial attachment but bacteria attach to other tissues, eat out 'Frassbetten' in the cell walls, multiply and ultimately dissolve out the digestible fibrous components. Attachment may increase the chance of absorbing the soluble products. Enzyme action is hampered also by the chemico-physical bonding of lignin in the fiber.

In spite of these limitations microbes can ultimately digest and metabolize most plant material. The holes digested through cell walls of lignified wood by higher fungi suggest the presence of a formidable array of enzymes. These fungi grow slowly and use fixed nitrogen with great economy. Could this be associated with high energy costs for enzyme elaboration?

The present analytical categories for plant fiber (cellulose, hemicellulose and lignin) do not correspond to biological digestibility. An improvement in the knowledge of plant fiber composition might be obtained by applying various permutations and combinations of pure enzymes to the fiber itself as substrate. The false concept of a C_1 enzyme (not cellulolytic but necessary to prepare 'native' cellulose for digestion) has arisen largely from use of artificial soluble derivatives of fiber instead of the more slowly digested natural material.

Microbes can use the sugars from fiber digestion faster than they can be formed. The usual low sugar concentrations in the rumen limit the speed of microbial growth, with biochemical mechanisms increasing the growth rate through maximal microbial synthesis per unit of substrate fermented.

Apparently a single species cannot perform at maximal rate all possible biochemical work reactions. A community of specialized microbes, linked by various interactions, is more effective. Possible interactions include: a) excretion by some of wastes used by others, b) leakage of useful metabolites into the milieu, c) commensalism, as in the escape of fiber digestion products before they are captured by the cell elaborating the responsible enzymes, d) predation, e) parasitism, and f) synergistic colony formation.

Changes in the ruminant's feed can induce changes in its microbiota, but as long as the substrate remains limiting, the biochemistry remains stable, in that ATP production is maximal for a wide variety of feeds. Acetic, propionic and butyric acid are the chief volatile fatty acid products of the rumen fermentation, in the proportions 65, 20 and 15%, respectively.

The biochemical work possible when acetate is formed can be summarized as follows[2]:

$$C_6H_{12}O_6 + 4\,P_i + 4\,ADP \rightarrow 2\,CH_3COO^- + 2\,HCO_3^- + 4\,H^+ + 4\,H_2 + 4\,ATP \qquad (VI)$$

$$\Delta G_0' = -51\ kJ$$

Pyruvate (2 molecules) is an intermediate. 2 ATP and 4 H (2 NADH × 2 H^+) arise during its formation, and 2 ATP and 2 H_2 are formed in the conversion of the pyruvate to acetate. In theory the 8 H can reduce 1 CO_2 to 1 CH_4, a 3rd of that in equation IV, but the actual rumen methane production is always less because some H is used for cell synthesis (cells are more reduced than carbohydrate) and some for other reductions, the chief one being propionate formation.

When a molecule of hexose is fermented to propionate, the 2 H atoms needed can be generated endogenously along with acetate, giving

$$C_6H_{12}O_6 + 4\,P_i + 4\,ADP \rightarrow \tfrac{4}{3}\,CH_3CH_2COO^- + \tfrac{2}{3}\,CH_3COO^- + 2\,H^+ + \tfrac{2}{3}\,CO_2 + 4\tfrac{2}{3}\,H_2O + 4\,ATP \qquad (VII)$$

$$\Delta G_0' = -131\ kJ$$

(or exogenously) from the H_2 in rumen liquid,

$$C_6H_{12}O_6 + 2\,H_2 + 4\,P_i + 4\,ADP \rightarrow 2\,CH_3CH_2COO^- + 2\,H^+ + 6\,H_2O + 4\,ATP \qquad (VIII)$$

$$\Delta G_0' = -173\ kJ$$

The ATP in propionate production is formed by electron transport in fumarate reduction to succinate, a precursor of propionate. The $\Delta G'$ of -147 kJ in the combined equations V and VI,

$$C_6H_{12}O_6 + 4\tfrac{1}{2}\,P_i + 4\tfrac{1}{2}\,ADP \rightarrow 2\,CH_3COO^- + HCO_3^- + 3\,H^+ + CH_4 + 3\tfrac{1}{2}\,H_2O + 4\tfrac{1}{2}\,ATP \qquad (IX)$$

$$\Delta G'_0 = -147\ kJ$$

is about the same as in equations VII and VIII for propionate formation, but the theoretical yield of ATP is slightly more. This may explain the prevalance of acetate in the rumen, where the acetate/propionate ratio is 3, as compared to $\frac{1}{2}$ in equation VII.

This advantage in acetate production is a good example of increased growth through species interaction. The rumen methane bacteria have a high affinity for H_2[9], oxidizing it with CO_2 at 10^{-3} atm of H_2, at which concentration a conversion of $NADH + H^+$ to $NAD + H_2$ can occur.

It is difficult to understand biochemically how the work accompanying butyric acid production can compete with that when acetate and propionate are formed. The recent report[10] that CO_2 increases the growth yield of *Butyrivibrio*, the chief rumen producer of butyrate, may be pertinent.

In addition to the reactions summarized in equation IX, complete conversion of hexose to CO_2 and H_2O as in equation IV, must include the following:

$$2\,CH_3COO^- + 2\,H_2O \rightarrow 2\,HCO_3^- + 2\,CH_4 \qquad (X)$$

$$\Delta G'_0 = -62\ kJ.$$

The work of ATP synthesis which should be integrated into this reaction has not been measured, but it is even less than that in equation V. Perhaps for this reason methanogenic growth on acetate is extremely slow, slower than the average rumen dilution rate, k.

The rate of dilution of rumen contents is also important in fiber digestion. Slow dilution (passage) rates allow the fiber to remain in the rumen longer, and its digestion is more complete. But at the concomitant slower growth rate, with longer intervals between cell division, more work is expended for microbial maintenance, and the microbial cell yield per molecule of fermented substrate diminishes. This lower cell yield leaves more H for methanogenesis, and the proportion of methane formed increases.

As k increases, the concentration of soluble substrate increases slightly, and the microbes grow faster and more efficiently because of reduced maintenance costs. Young forages are rapidly digested, and transported (k is relatively large) and promote ruminant growth more efficiently than do the older, dried and more lignified plant materials composing most of earth's photosynthate.

Pure cultures of rumen bacteria often produce lactate, ethanol, formate, succinate and H_2, as well as the final rumen products, the vfa, CO_2, and CH_4. Formate appearing in the rumen is rapidly decarboxylated to CO_2 and H_2; succinate is decarboxylated to propionate, and H_2 reduces CO_2 to methane, all as part of efficient ATP-producing systems. But lactate and ethanol are not important intermediates in the rumen; their production is associated with formation of only 2 ATP per hexose.

When a forage-fed rumen is suddenly supplied an excess of sugar (or easily digested starch) the soluble carbohydrate concentration is no longer limiting, and lactacidigenic bacteria, developing almost explosively[11], metabolize hexose to produce ATP faster than can the predominant microbiota. The rate of lactic acid production soon exceeds the rate of its conversion by other microbes and by the ruminant, and the ecosystem is destroyed. But with gradual adaptation of a ruminant to starch and sugar a very dense microbial population slowly develops, capable of a fermentation sufficiently rapid to keep soluble sugar at limiting concentrations, and a highly productive though somewhat unstable rumen ecosystem with few lactic acid bacteria results.

The ability of the lactic acid bacteria to outgrow the other microbes indicates that anaerobic survival success is not due simply to the ability to obtain more ATP/hexose, but to obtain more ATP per unit time. At limiting rumen sugar concentrations, more ATP/hexose gives more ATP/unit time, but not when the sugar supply is ample. 'Too easy living' wrecks the biochemical stability of the rumen microbial ecosystem.

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