enzymic catalyses. When a pathway involves a novel sequence, such as the reduction of carbon dioxide to methane, then nature appears to have evolved a series of special coenzymes. The biochemical chapter on coenzymes was supposed to have been closed; we have been forced to reopen it. Methanogens appear unusual in that they apparently carry out electron transport phosphorylation in the absence of quinones, since they lack these compounds[17]. The work of Woese and colleagues has shown that the 16S rRNA of methanogens is only distantly related to typical procaryotes[18]. Kandler's laboratory has documented the wide diversity of cell-wall types among the methanogens[19]. No D-amino acids have been found, and muramic acid is absent; in one species N-acetyltalosaminuronic acid replaces muramic acid. Tornabene and Langworthy[20] have shown that the polar lipids of methanogens are non-saponifiable diphytanyl and dibiphytanyl glycerol ether-linked lipids. Squalene is found as a major component of the neutral lipids. Klotz's laboratory[21] has shown that the DNA complexity of a methanogen approaches ⅓ that of *Escherichia coli.* The mechanism of carbon dioxide activation for fixation into cell carbon is unknown. It would appear that we have only scratched the biochemical surface of these interesting organisms. For example, at the present time not a single mutant or phage has been isolated. The technology for handling these organisms is now at hand, and more of nature's biochemical secrets should be revealed in the near future. Perhaps we shall eventually understand nature's strategy for maintaining such a unique group of organisms. Why have the methanogens remained as an isolated biochemical island apparently not in genetic equilibrium with the microbial world?

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Engineering, operation and economics of methane gas production

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Processing of biomass for the production of a fuel gas containing methane requires a complex system. The degree of complexity is, in part, a function of the biomass utilized. In general, this system consists of 3 main subsystems;

- Raw material preparation
- Methane fermentation
- Residue processing, utilization and/or disposal

Gas scrubbing for carbon dioxide removal to produce a gas that is essentially 100% methane is not considered in this discussion.

Certain biomass materials such as animal manure from a confined and enclosed beef feeding operation can be added directly to the fermentation subsystem without any preparation. Conversely, urban solid waste requires extensive preparation including size reduction and various separation processes for removal of those materials that have the potential for creating operational difficulties with the physical processes employed in the fermentation and residue processing subsystems.

The essence of this processing system is the methane fermentation subsystem. The ability to convert a major portion of the organic material to methane is paramount to the success of this system. This conversion efficiency has an impact on 3 separate costs. First is the raw material cost. If the biomass cost is $20 per t, the methane cost at a 75% conversion efficiency will be about $6.5 per 100 m^3. At a 50% conversion efficiency, the raw material cost alone is $10 per 100 m^3.

A 2nd cost factor is associated with the reactor

volume. When processing a relatively dry material, the required reactor volume for a given retention time is fixed by the feed slurry solids concentration. The feed slurry solids in turn are a function of the conversion efficiency and the concentration of solids in the reactor slurry that will permit good mixing.

The 3rd cost factor relates to the cost associated with the residue processing. The fermentor slurry must be dewatered and either processed for material recovery or disposed in an acceptable manner. The processing costs are directly related to the mass and/or volume of slurry in the effluent from the fermentor.

Substrate characteristics

The chemical and physical characteristics of the biomass will have a significant effect on the process conversion efficiency and the economics of the system. Several substrate characteristics should be considered and they are listed as follows: - biodegradability, - chemical composition and structure, - moisture content.

Each of these characteristics affects one or more of the costs associated with the processing system. The biodegradability is the most important factor. In addition to the lower product yield per unit of substrate processed, this factor will have a significant impact on the reactor volume, size of dewatering system and residue disposal costs.

The impact on reactor volume can be illustrated as follows. There is a limit to the level of solids in the reactor slurry that can be efficiently mixed. This level will vary with the type of substrate, but in general, will be between 5 and 10%. Preliminary studies on mixing of reactors receiving urban refuse for methane production suggest that a slurry containing 8% solids is the near optimum for mixing. This solids level and the biodegradability control the solids concentration in the feed slurry. This in turn sets the feed volume for a given quantity of substrate and the required reactor volume for a given reactor retention time. Table 1 shows the reactor volume required per t of dry substrate containing 15% ash. The reactor has a 10-day retention time resulting in 80% conversion of the biodegradable solids to methane and carbon dioxide. The limit on the reactor slurry solids concentration was set at 8%.

In addition to a significant reduction in the reactor cost, other savings result from the smaller reactor volume and feed slurry volume.

Heat losses are lower with a smaller reactor volume. The thermal energy required to elevate the temperature of the feed slurry is reduced since less water is needed to maintain the solids level in the reactor slurry. Lower feed slurry volumes result in lower pumping costs because less water is circulating in the process. Also, less power is required to mix the smaller reactor volume associated with a substrate having a high biodegradability. These cost reductions are not of the magnitude of those associated with the reduction in reactor volume, but they will result in lower costs.

The substrate biodegradability is a major factor in determining the costs of fermented residue processing and disposal. As shown in table 2 the mass of dry solids as well as the volume of slurry remaining after the fermentation is complete is much less when the organic solids in the raw material are more biodegradable. Slurry dewatering processes are sized either on a volumetric flow rate or a solids mass flow rate. In either case, a much larger dewatering system is required for the less biodegradable material. Direct application of the fermented slurry to land will also be more expensive because of the larger volume of slurry to be handled from the less biodegradable substrate.

The chemical nature of the raw material to be used as a substrate for the production of fuel gas will affect the costs in 2 areas. The chemical structure of the material can significantly alter the rate of conversion to gas. Carbohydrates in the form of simple sugar have a much higher rate of conversion than cellulose. Even the crystalline nature of cellulose will affect the kinetics of cellulose fermentation. The fermentation process is a biological process that requires a balanced substrate. If the raw material does not contain adequate nitrogen, phosphorus and micronutrients, it will be necessary to add these nutrients for fermentation.

Control of the fermentation pH may also require chemical addition. The gas produced by the fermentation contains 30–50% carbon dioxide. Maintaining a neutral pH will require a significant level of alkalinity. With some substrates, sufficient natural alkalinity is formed to maintain the pH. In other cases, lime, soda ash or caustic must be added to obtain the desired pH.

The moisture content of the substrate can alter the economics in much the same way as biodegradability. When the moisture content is very high, as with sewage sludges, the reactor volume per t of dry solids

Table 1. Effect of substrate biodegradability on reactor volume

Biodegradability (% organic solids)	Feed slurry Solids (%)	Volume (m^3/t)	Reactor volume (m^3/t)
40	10.7	9.37	93.7
60	12.8	7.81	78.1
80	16.0	6.24	62.4
100	21.4	4.68	46.8

Table 2. Effect of substrate biodegradability on quantity of residue

Biodegradability (% organic solids)	Residue Dry solids (t)	Volume (m^3/t substrate)
40	0.73	9.1
60	0.59	7.4
80	0.46	5.7
100	0.32	4.0

processed will be between 200 and 300 m^3. This imposes a severe cost penalty on gas production. In general, a substrate with a moisture content of 90% or greater will not yield cost competitive gas. Credits such as those applied to waste disposal systems are necessary to cover the costs of gas production from these sludges. Because of the cost in energy for drying these wet substrates, this is not a viable option. The substrate will have to be used as received. Conversely, the moisture content of the dry substrate can be increased by the addition of water. Frequently, this water originates from an internal recycle and, as such, does not impose an added cost.

Process characteristics

In an attempt to improve the kinetics of conversion of organic material to gas, much effort has been invested in trying to determine the optimum reactor type and geometry. The following reactors have received the most attention:
CSTR - no cell recycle
CSTR - cell recycle
Fixed film
Plug flow - multi stage
(CSTR refers to a completely-stirred-tank-reactor).
Since it has been recognized that methane fermentation is a multiphase process, researchers have attempted to separate the acetogenic from the methanogenic phase. With this approach, it should be possible to operate each stage under conditions that optimize the growth of the specific cultures. However, cellulose hydrolysis rather than acetogenesis or methanogenesis has been found to be the rate limiting step when fermenting complex natural substrates[1]. McBee[2] found thermophilic cellulolytic bacterial growth occurs in a pH range of 6.4 to 7.4, which is generally the optimum range for acetogens and methanogens. Stranks[3] reported extremely high rates of cellulose hydrolysis when using a mixed culture of thermophilic microorganisms. Pure culture cellulose hydrolysis rates were much lower[2].
Cellulose hydrolysis as well as acetogenesis is an enzymatic reaction. The simplest enzyme reaction (equation 1) can be described by the Michaelis-Menten relationship.

$$S+E \underset{k_2}{\overset{k_1}{\rightleftarrows}} SE \overset{k_3}{\rightarrow} P+E \qquad (1)$$

The substrate (S) and enzyme (E) are in equilibrium with substrate-enzyme complex (E-S). However, an irreversible reaction resulting in the product (P) and enzyme is assumed. This reaction is the rate limiting step having a constant, k_3. The Michaelis-Menten expression (equation 2) is developed from this equilibrium.

$$-\frac{dS}{dt} = \frac{k_3(S)(E_0)}{K_a+(S)} \qquad (2)$$

Many enzyme reactions do not satisfy the restriction that the enzyme-substrate complex breaks down irreversibly. The complex may also form from the product side as shown in equation 3.

$$S+E \underset{k_2}{\overset{k_1}{\rightleftarrows}} SE \underset{k_4}{\overset{k_3}{\rightleftarrows}} P+E \qquad (3)$$

As the concentration of the product increases, most enzymatic reactions slow down. This is due to the phenomenon of product inhibition. The rate equation shown in equation 4 is developed from equation 3. This equation shows that the substrate utilization rate is a function of not only the substrate and enzyme concentration, but also the product concentration.

$$-\frac{dS}{dt} = \frac{dP}{dt} = \frac{[k_1k_3(S)-k_2k_4(P)]E_0}{[k_4+k_3]+k_1(S)+k_4(P)} \qquad (4)$$

In any basic biochemistry text such as Mahler and Cordes[4], one will find information that an enzymatic reaction slows down as equilibrium is approached, not only by virtue of the thermodynamic back reaction, but also because, as the product concentration increases, an increasing proportion of the enzyme is immobilized as an EP complex. This kinetic effect of product inhibition is thus an intrinsic property of any realistic, i.e. reversible, mechanism of enzyme catalysis.
Consequently, process configurations that approach a plug flow reactor will be much less efficient than completely mixed reactors. With a multistaged biochemical process such as methane fermentation, the best reactor design is one that allows these reactions to occur concurrently with the final product being methane and carbon dioxide. These gases have limited solubility and are lost from the reacting medium thereby reducing the effect of product inhibition on kinetics.
A completely stirred reaction tank is generally the most efficient reactor design. One limitation of this reactor type is the inability to operate with a mean cell residence time greater than the hydraulic retention time of the tank. This deficiency has been overcome by employing sludge (cell) recycle, either internal or external, or by adding a packing material to the reactor vessel to form a fixed-film reactor. These reactor types have been successfully applied to substrates that are either soluble or essentially 100% biodegradable.
However, if the substrate contains a quantity of suspended biologically inert material, the fixed film or CSTR with cell recycle may not be able to efficiently process the material. The packing material in the fixed film reactor provides a multitude of small quiescent settling chambers where the suspended material can accumulate. If these solids are nonbiodegradable, the reactor will fill with these solids.
A similar problem exists with the CSTR-sludge recycle system. A recirculation factor (R), defined as the

ratio of mean cell residence time (θ_c) to the hydraulic residence time (θ), is used to calculate the accumulation of inert material in a reactor employing sludge recycle. In a system operating with θ of 1 day and θ_c of 10 days, the value of R is 10. If the feed stream to the reactor contained 10 g/l of inert solids, the equilibrium concentration of these solids in the reactor would be 100 g/l or 10%. The volume of the reactor is simply occupied by these inert solids. Most of the substrates available for the production of a fuel gas will contain a substantial quantity of inert material and the only reactor type that can be expected to function efficiently would be the CSTR.

The fermentation temperature has also been found to significantly effect the conversion efficiency. When urban solid wastes were used as a substrate, thermophilic fermentation (60 °C) yielded much higher gas production[5]. These data were evaluated using a first-order kinetic expression for substrate utilization. A mass balance on a CSTR yields equation 5.

$$\frac{S_0}{S} = 1 + K\theta \tag{5}$$

In this equation, K is the first-order rate constant, S_0 is the initial substrate level, S is the final substrate level and θ is the hydraulic retention time.

Measurement of S_0 and S when the substrate is a complex material such as plant fiber is difficult. S_0 must be the initial biodegradable substrate. Volatile solids are frequently used as a measurement of S and S_0. In order to determine the portion of the volatile solids that are biodegradable, the following technique was employed. At an infinite retention time, θ, the value of S will be zero and $S_0\text{-}S = S_0$. A semi-log plot of volatile solids destroyed, S_0-S, versus θ^{-1} will provide the value for S_0 when θ^{-1} equals zero.

Once the value of S_0 is obtained for each fermentation temperature, the value of K can be determined by plotting S_0/S versus θ. The slope of the line is K. Table 3 lists the values for S_0 and K obtained from this analysis. The apparent increase in the biodegradability of the volatile solids is substantial, increasing from 44% at 40 °C to 55% at 60 °C. This is a 25% increase in the portion of the volatile solids that are biodegradable. When this is combined with an increased rate constant, the thermophilic fermentation temperature yields a more efficient and economical processing system.

Residue disposal

The discharge from the fermentor must be processed in order to eliminate adverse environmental effects. The unfermented solids must be removed and the liquid stream can be recycled back through the system, disposed by land application or treated for discharge to receiving water bodies. Recycling of this water offers significant cost savings as well as conservation of heat and of chemicals that may be necessary for pH control and for microorganism nutrition. The solids may have some value. For example, the organic solids still have energy that can be recovered by incineration. The calorific value of the residue from the fermentation of city refuse was found to be 18.5 MJ/kg total solids (24 MJ/kg volatile solids). Recovery of these solids with a centrifuge will produce a cake having a solids content between 30 and 40%. This cake will provide self-sustaining combustion in a properly designed incinerator. Recovery of a significant quantity of energy in the form of steam is possible[6].

Other material recovery may be possible. Based on studies reported by Turk and Coe[7], a number of researchers are investigating the recovery of protein from the residue of fermentors processing animal manures. The results have not been encouraging because of the poor efficiency of protein recovery with centrifuge systems. Hashimoto et al.[8] have only been able to recover about 20% of the protein (as measured by organic nitrogen) in the fermentor residue when using a commercial centrifuge operating at a centrifugal force of $2300 \times g$. Other uses may be found for the fermentor residue. This will in part depend upon the type of raw material fed into the process.

Economics of methane fermentation

The economics associated with methane recovery from biomass is highly dependent upon the raw material. There are 2 cost factors. First, the cost of the raw material is significant. When this material must be purchased, a significant cost is added to the methane production costs. However, if this is a waste material, the producer may pay a fee for disposal of this material. The 2nd factor is the degree of preparation required before the material can be added to the fermentation reactor. Urban solid waste must undergo size reduction, separation of undesirable constituents such as plastics, metals, glass, etc. and preparing a slurry with water. Conversely, animal manure from certain confined animal feeding units can be added directly to the fermentor.

An earlier paper[6] presented an economic analysis for a system designed to convert 900 t per day of urban refuse into methane and steam. The 1980 capital costs

Table 3. Biodegradability and rate constant as a function of temperature

Temperature (°C)	Biodegradability (% of volatile solids)	Rate constant - K (day^{-1})
35	36	0.53
40	44	0.58
45	40	0.47
50	49	0.63
55	51	0.78
60	55	0.95

for this plant are given in table 4. Costs should be reduced significantly by not processing the gas for removal of carbon dioxide. As produced, this gas is a good fuel gas that can be used in any system that is designed to burn gaseous fuels.

The allocation of the costs to the various unit processes is shown in table 5. Major capital expenditures are required for residue processing and disposal. The centrifuge and incineration account for 50% of the capital costs. The size of these units is directly related to the mass of the solids passing through the system. Consequently, the higher the efficiency of converting the raw material to methane, the lower the cost associated with these processes.

Gas processing also requires capital investment. The product gas from the fermentor has a calorific value of about 22.4 MJ/m^3 (600 Btu/ft^3). Removal of carbon dioxide increases the heating value of this gas to that commonly employed in the U.S. natural gas system. However, this is an unnecessary requirement as the gas as produced is an excellent fuel.

Energy recovery with this system is good if a use for the steam produced by the residue incineration can be found. A portion of this steam can be used for process heat. However, a considerable excess remains. The energy balance for the system is shown in table 6. Input energy includes all of the energy content of the organic material, the thermal energy and the electrical energy.

A cost analysis for fuel gas production is site- and raw material-specific. Such an analysis is shown in table 7 for the urban solid waste system. Capital costs are amortized at 10% interest over a 20-year period. Public financing is assumed so no tax or profit is included in this analysis. The fuel gas is priced at \$3.00/GJ and steam at \$1.00/GJ. The net processing cost is the dipping fee required for the process. This fee is substantially lower than most fees for refuse disposal, so there is a margin for profit in this analysis. Careful economic analysis must be conducted for any installation. In general, one can expect that methane production from any waste biomass that is relatively biodegradable will be economically attractive. However, if the biomass has an acquisition cost, it is probable that the economics will not be attractive unless the energy costs escalate to near \$10 per GJ.

The U.S. Department of Energy has funded 2 demonstration plants to determine the true economics associated with the methane recovery process. One plant located in Pompano Beach, Florida, processes city refuse for methane recovery. Operation of this plant was initiated in July 1978. A 2nd plant located in Bartow, Florida, is processing animal manure from a confined beef cattle feeding operation. This plant became operational in late 1978.

Table 4. 1980 capital cost for methane fermentation

Raw material processing system	\$5,248,000
Fermentation system	7,700,000
Incineration system	9,105,000
Gas purification system	3,655,000
Total	\$25,708,000
Additional capital required	4,890,000
Total capital	\$30,598,000

Table 5. Allocation of costs to unit processes used in methane recovery

Unit process	% Capital costs	% Operation and maintenance
Shredder	9.6	22.4
Separation	4.1	4.1
Storage	6.6	2.0
Fermentation	15.6	44.9
Centrifuge	14.3	10.0
Incineration	35.4	5.0
Gas processing	14.2	11.6

Table 6. Energy balance for refuse fermentation

Energy in	11.82 GJ/Mg
Methane produced	3.87 GJ/Mg
Steam produced	3.64 GJ/Mg

Table 7. Fuel gas production cost analysis - 1980 cost index

Annual capital cost (\$/yr)	3,121,000
Labor (\$/yr)	1,101,000
Operation and maintenance (\$/yr)	2,081,000
Total annual costs (\$/yr)	6,303,000
Tonnes/yr processed	331,000
Processing costs (\$/t)	19.04
Gas production (J/yr)	1.3×10^{15}
Gas value (\$/yr)	3,900,000
Steam production (J/yr)	1.2×10^{15}
Steam value (\$/yr)	1,200,000
Total revenue (\$/yr)	5,100,000
Net processing costs (\$/t)	3.65

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