Reports

Anaerobic Digestion: The Rate-Limiting Process and the Nature of Inhibition

Abstract. *Although attractive as a method for creating preferred fuel from cellulose wastes, the anaerobic digestion process is very slow. A new hypothesis is that phase transfer of products is rate-limiting and product gases inhibit the methanogenic bacteria. In a phase transfer–assisted digester, bacterial reproduction is very rapid.*

Bioconversion in general and anaerobic digestion in particular would hold much greater promise if faster processes could be developed (*1*). A major advantage of anaerobic digestion is that methane, a preferred fuel with a high energy content, having a combustion energy of 50 kjoule/g, is created from cellulose, which has a combustion energy of only 15 kjoule/g. The methane production rate, however, is only about 0.8 to 2.1 g per liter of digester per day (*2*), and the corresponding process capital and operating expenses are significant, with the result that methane is an expensive fuel (*3*).

There are four potentially rate-limiting steps in the anaerobic conversion of cellulose to methane (*4–6*): (i) insoluble cellulose is converted by extracellular cellulases into soluble carbohydrates such as glucose; (ii) nonmethanogenic organisms moderate the reaction of carbohydrates to form predominantly acetic and propionic acids; (iii) these acids are converted by methanogenic bacteria into dissolved methane and carbon dioxide; and (iv) the dissolved products undergo transfer from the liquid to the gas phase. The third step is usually considered to be rate-limiting. However, it is our hypothesis that the transfer to the gas phase is actually rate-limiting and, furthermore, that the normal metabolic activity of the methanogenic bacteria is inhibited by product gases.

The volatile acid concentration within digesters increases as the biological solids retention time (SRT), the average residence period of a bacterium, is reduced from 20 to about 2 days (*5, 7, 8*). This observation is used as primary evidence that the biological conversion of the volatile acids to methane and carbon dioxide is rate-limiting. Such buildups are thought to occur because of the slow reproduction rate of the methanogenic bacteria; at SRT's near 2 days, these microbes are thought to become totally displaced from the digester.

Such evidence, however, is insufficient to distinguish between biological conversion of acids and phase transfer of products as candidates for the rate-limiting step. As the displacement period is lowered, individual bacteria must reproduce more rapidly, which means that their gas production and transport requirements also increase. Accordingly, if transport of gaseous products away from individual bacteria were rate-limiting, a decreasing SRT would also result in the observed acid concentration increases.

The precursor of about 70 percent of the methane formed in the digestion of sewage sludge is acetic acid (*6*) and its conversion was therefore selected for further study. Following Monod (*9*), the mechanism is usually given as

$$\mathrm{HAc(aq)} + \mathrm{B_m} \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} \mathrm{HAc\text{-}B_m} \xrightarrow{k_2} \mathrm{CH_4(aq)} + \mathrm{CO_2(aq)} + \mathrm{B_m} \qquad (1)$$

where HAc(aq) is aqueous acetic acid, B_m is a methanogenic bacterium, and the k's are specific rate constants. This mechanism gives a rate expression similar to that obtained by using the steady state assumption for enzyme kinetics

$$-\frac{d(\mathrm{HAc})}{dt} = \frac{k_2(\mathrm{B_m})(\mathrm{HAc})}{K_s + (\mathrm{HAc})} \qquad (2)$$

with K_s equal to $(k_2 + k_{-1})/k_1$, the substrate concentration at which the rate attains its half-maximum value. Monod noted that Eq. 2 was similar in form to both the Michaelis-Menten expression and the Langmuir adsorption isotherm. However, the literature on anaerobic digestion does not distinguish between rate limitation by internal enzymatic processes or by cell wall permeation.

Conversion of substrate into cells and bacterial decay (*10*) may be described by

$$\frac{d(\mathrm{B_m})}{dt} = Y\frac{d(\mathrm{HAc})}{dt} - k_d(\mathrm{B_m}) \qquad (3)$$

where Y is the bacterial yield or fraction of substrate converted to cells and k_d is the first-order bacterial decay constant. For a steady state digester, the SRT is the reciprocal of the specific bacterial growth rate, $[d(B_m)/dt]/(B_m)$. Accordingly, the dynamic constants, Y, k_2, k_s, and k_d, may be evaluated when effluent bacterial masses and acetic acid concentrations are measured as a function of the SRT. A most interesting feature of such measurements is the strong dependence exhibited by k_2 and K_s on substrate feed rates. For example, when Lawrence and McCarty (*11*) increased their influent feed concentration from 1568 to 3135 mg/liter, they found that k_2 and K_s each decreased by about 25 percent. There is some experimental evidence that the degree of inhibition may be related to cation concentrations (*12*). Andrews (*13*) has proposed that inhibition is caused by an increase in the proportion of nonionized acids present at low *p*H.

If, however, it is assumed that phase transfer of products is rate-limiting, a mechanism emerges which also accounts for inhibition. Phase transfer is achieved by bubble transport from solution. The product bubble density should initially increase even more rapidly than the acid substrate concentration. It is plausible, therefore, that at very high substrate concentrations gas bubbles actually surround a bacterium, interfering with substrate diffusion into intracellular spaces.

In general, if product transport were rate-limiting, observed reaction rates would be expected to be inversely proportional to the solubility of the more soluble product gas, carbon dioxide. For gases of moderate solubility, such as carbon dioxide, the slow step is often diffusion across the liquid side of the bubble membrane (*14*). The corresponding diffusion rate, R_t, may be expressed from Fick's first law as

$$R_t = \frac{D_1}{t}(C_s - C_1) \qquad (4)$$

where t is the membrane thickness, D_1 is the diffusion coefficient, and C_s and C_1 are the average solution and membrane carbon dioxide concentrations, respectively.

Since the concentrations of carbon dioxide between the bubble interior and the liquid membrane are in equilibrium, Henry's law [$C_1 = k_H(T)P$, where P is the carbon dioxide partial pressure and $k_H(T)$ is Henry's constant] is applicable, giving

$$R_t = \frac{D_1}{t}[C_s - k_H(T)P] \qquad (5)$$

Equation 5 formally expresses the fact that gas transfer is facilitated by agitation, which reduces the film thickness, t; by elevated temperatures, which decrease $k_H(T)$; and by low carbon dioxide partial pressures.

In the past most laboratory studies of the effect of temperature on digestion rates have been conducted at a total pressure of about 1 atm, the carbon dioxide partial pressure being about 1/3 atm. However,

since Henry's constants and carbon dioxide concentrations for digesters are unknown, we compared the relative inverse carbon dioxide solubility in pure water to the relative maximum digestion rates observed by Golueke (*15*) for digestion of volatile solids and by Lawrence (*16*) for acetic acid conversion (Fig. 1). We regard the observed correlation in the mesophilic temperature range as evidence supporting a rate limitation by gas transfer (*17*).

Our first experimental effort was directed toward determining the kinetic parameters of the methanogenic bacteria in a highly mixed, low-pressure environment. The digester was similar to that employed by Andrews and Pearson (*8*) except that a splash plate, located in the direct path of flow within the 3.10-liter stainless steel digester, assisted in continuous mixing of the phases. Vigorous agitation and surface renewal were achieved by having liquid flow rates of 11.4 liter/min, controlled by an external centrifugal pump. In addition, the digester and its 25.5-liter gas storage elements were vacuum tight to less than 1 torr.

The initial sewage sludge loading, acetic acid culture medium equilibration, and data accumulation procedures were similar to those described elsewhere (*18*). Effluent analyses were conducted for acetic acid (*19*), organic nitrogen, and methane gas (*20*). The factor for converting organic nitrogen to volatile bacterial mass, 11.4, was the same as that employed by Lawrence and McCarty (*11*); our correction for bacterial mass adhering to the walls was done differently from theirs, but we obtained 23 mg/liter, similar to the value they reported. Independent mass spectrometric analysis gave 77 percent methane and 23 percent carbon dioxide in the effluent gas; there was 20 percent more methane than expected for a strict mass balance from Eq. 1.

Conditions for the experiment with a feed rate of 4.29 g liter^{-1} day^{-1} are given in the legend of Table 1. Least-squares fitting of these data to Eqs. 2 and 3 gave Y = 0.044, K_s = 250 mg/liter, k_2 = 56 day^{-1}, and k_d = 0.44 day^{-1}. In contrast, for a digester operating at a constant influent feed concentration of 3135 mg/liter, Lawrence and McCarty (*11*) determined Y = 0.040, K_s = 166 mg/liter, k_2 = 9.6 day^{-1}, and k_d = 0.019 day^{-1}.

The close agreement in yield constants indicates that an irregularity in the experimental procedure, chemical analysis, or data treatment does not account for the significantly different values determined for k_2 and k_d. The yield constant should be invariant because it is an internal cellular metabolic constant, the manifestation of which is the mass of cells produced per unit mass of acetic acid consumed.

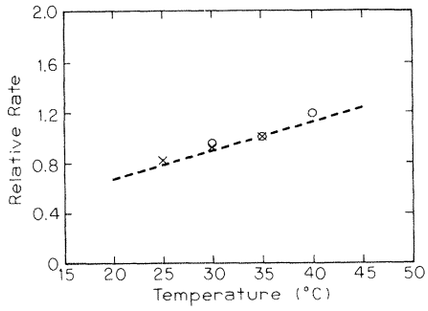

Fig. 1. Relative digestion rates and inverse carbon dioxide solubility as a function of temperature normalized at 35°C: (dashed line) 1/(CO_2); (×) data of Lawrence (*16*); and (○) data of Golueke (*15*).

It is also difficult to believe that the vigorous agitation and lower pressure employed could have caused an approximately 600 percent increase in k_2, if it applies to internal cellular enzymatic processes. Since the temperature data of Fig. 1 suggest rate limitation by the gas transfer step, our increased k_2 is probably the result of improved gas transfer characteristics in the region of the bacterial cell wall. This logic further dictates that the kinetic treatment in Eqs. 1 and 2 is normally applicable to cell wall activity and that the inhibiting factor could well be microbubbles, which decrease the bacterial surface area available for permeation processes (and thereby decrease k_2).

This explanation also accounts for the more modest increase we observed in K_s. Since K_s is a ratio of surface-related constants, it would be expected to vary much more slowly with available surface area than would k_2 alone. Our experiments, to date, are inconclusive regarding the difference between the measured bacterial decay constants. It appears plausible that our decay constant is significantly larger because of bacterial losses in the centrifugal pump, which revolved at 3600 rev/min.

From the more practical perspectives of preferred fuel production and pollution control, these results, and particularly our new value for k_2, indicate that faster, more efficient anaerobic digesters might be developed. The minimum doubling period, $T_d = 0.693/Yk_2$, of the methanogenic bacteria in our system was only 0.28 day, also lower than previous values. We tentatively suggest, as indicated by Eq. 5, that design specifications for faster systems might include vigorous agitation, low pressure, and elevated temperatures.

Table 1. Constant loading SRT study with acetic acid substrate. The conditions were: liquid temperature, 35° ± 1°C; gas temperature, 21° to 27°C; liquid volume, 3.10 liters; gas volume, 25.5 liters; initial pressure, 180.0 torrs; exposed liquid surface area, 4090 cm^2; and effluent *p*H, 6.4 to 7.4. (Data were accumulated at a specific SRT after an equilibration period equal to or exceeding three displacement periods.) Abbreviations: SRT, solids retention time; HAc, acetic acid; ΔP, pressure increment.

SRT (days)	Feed		Effluent (mg/liter)		
	Frequency (per day)	Concentration (mg/liter)	HAc	Organic N × 11.4	ΔP (torr/day)
2.00	2	8580	271	211	217
1.50	3	6430	146	153	207
1.00	4	4290	451	77.6	192
0.788	4	3380	584	58.5	181
0.394	8	1690	356	30.4	142

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References and Notes

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17. This conclusion may be intuitively appreciated by realizing that for Lawrence's (*16*) digester 2 at SRT = 12 days each bacterium (*16*, p. 175) produced a volume of gas equal to its own volume about every 160 seconds, and at SRT = 3 days this value dropped to 36 seconds. For the "faster" bacteria in our system (Table 1) at SRT = 1 day this time period fell to about 10 seconds. In this context it is interesting to watch (and time) the bubbling process from a glass of carbonated beverage.
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21. C.D.F. acknowledges discussions on bubbles with his teacher and friend, Dr. Frank C. Edwards. This report is dedicated to his memory.

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