Communications to the Editor

Kinetics of Methanogenesis from Whey Permeate in Packed Bed Immobilized Cells Bioreactor

Shang-Tian Yang and Meining Guo
Department of Chemical Engineering, Ohio State University, 140 West 19th Avenue, Columbus, Ohio 43210

Accepted for publication February 5, 1990

Whey, a by-product from the manufacture of cheese and casein, contains about 5% lactose, 1% protein, 1% salts, and 0.1–0.8% lactic acid. The annual production of liquid whey in the U.S. has continuously increased to about 57 billion pounds in 1988.[1] Recovery of whey proteins by ultrafiltration has become a relatively well-established process. However, the lactose stream (whey permeate), having a biological oxygen demand (BOD) of greater than 3.2×10^4 mg/L, remains a major disposal problem. Recently, growing concern about environmental pollution has generated interest in producing methane from whey and whey permeate through anaerobic digestion.[2-10] The major advantages of this process are low costs, high energy efficiency, and process simplicity as compared to other waste treatment methods. In addition, the produced biogas (methane) may be used as a process fuel in cheese manufacturing, or used in a power plant to generate electricity.[5] However, despite the waste reduction and energy potential, anaerobic digestion is not a highly regarded process in the dairy industry, largely due to the problems of slow reaction rate and poor process stability. In order to solve these problems and to develop a better methanogenic process, it is necessary to understand the fermentation kinetics of the methanogenic process first.

The anaerobic methanogenic process degrades organic matter to the gaseous products, CH_4 and CO_2[11] which occurs as a result of three distinct but simultaneous metabolic phases.[12-15] First, in the hydrolytic phase, large, complex organic matter is hydrolyzed and fermented to C_1 to C_5 organic acids, alcohols, H_2, and CO_2. In the second (acetogenic) phase, C_3 to C_5 organic acids and alcohols are further reduced to acetate, formate, methanol, CO_2, and H_2. These products are then used for methane formation in the last (methanogenic) phase. The interspecies hydrogen transfer between H_2 producers and H_2-utilizing methanogens is a key to allow some organic compounds, such as propionate, to be completely degraded to CH_4 and CO_2.[14,16] In general, acetic acid is the major immediate precursor for methane formation,[17] accounting for more than 70% of total methane formed in anaerobic digesters.[18] The other 30% is mainly from CO_2 reduction with H_2. In addition to acetate, propionate and butyrate are two major intermediary products most frequently found in anaerobic digesters.[19,20] Lactate and ethanol were also found as important intermediates in some anaerobic digesters.[21-23]

The kinetics of anaerobic digestion of whey and whey permeate has been extensively studied, recently.[9,21,24-32] However, the intermediary products found in these sudies are not consistent,[9,21,24] and varied with reactor startup procedures and operating conditions.[24,25] This variation could be attributed to the variation in bacterial populations in the digesters. The predominant populations in whey digestors reported from two different research groups are quite different.[26,27] Apparently, the fermentation kinetics largely depends on the environmental conditions and the seeding cultures used. Although the complex nature of methanogenic fermentation is now well appreciated, to date, the detailed fermentation kinetics has not been completely established. Most works were done on the free cells system; very little is known for the immobilized cells system. Furthermore, all the previous kinetic studies provided, more or less, only qualitative information, lacking in quantitative data required for engineering modeling and design.

The purpose of this study was to elucidate the fermentation kinetics of methane production from whey permeate in packed bed immobilized cell bioreactors. The major intermediary products formed and key biological reactions taking place during whey permeate fermentation were identified by studying batch fermentations of various key compounds, including lactose, lactate, butyrate, propionate, and acetate. A three-step reaction mechanism was proposed for modeling methanogenesis of whey permeate and was tested in this work.

MATERIALS AND METHODS

Reactor Construction, Startup, and Operation

Three packed bed immobilized cell bioreactors were used in this work. Each bioreactor had a total volume of 1.5 L before packing, and was made from a water-jacketed glass column. These bioreactors were packed with 0.25-in. ce-

Biotechnology and Bioengineering, Vol. 36, Pp. 427–436 (1990)

CCC 0006-3592/90/040427-010$04.00

ramic intalox saddles (U.S. Stoneware, Inc.) for cell immobilization. After packing, the void volume of each bioreactor was ~1000 mL. These reactors were operated with external liquid recirculation to provide complete mixing, and at a constant temperature between 30 and 37°C (±0.5°C) controlled by a water circulating bath. Unless otherwise noted, the reactor pH was maintained at 7.0 ±0.5 with a pH controller. The produced gas was collected and measured in an inverted plastic 4-liter graduate cylinder floating in acidified water.

These reactors were seeded with sewage sludge originally obtained from an anerobic digester in a municipal waste treatment plant at West Lafayette, IN. About 600 mL sludge and 400 mL diluted whey permeate (~1.2% lactose, reconstituted from dry whey permeate powder obtained from Food Ingradient, Inc.) were added to each reactor initially. After four days, fresh diluted whey permeate (0.6–2.0% lactose) was added intermittently to gradually bring the reactors to a steady operation, as monitored from the reactor pH and gas production rate. Thereafter, they were operated at continuous mode (retention times: 4 h to 4 days) for about a year. Before these reactors were converted to batch reactors for the kinetic study, they were operated on and off for several years with a longest shut-off time being six months.

Batch Kinetic Study

To study the fermentation kinetics, the bioreactors were fed with concentrated sweet whey permeate (from Kraft, Inc.), which contained about 10% lactose, 0.1% lactic acid, and very little (<0.1%) protein, at a fed-batch mode until the reactors reached pseudosteady state. About 50 mL whey permeate was added to each reactor at the beginning and it took 3–5 days to complete each batch cycle, which was determined as gas production ceased. After whey addition, 0.5 mL liquid sample was collected at every 0.5–1.0 h for the first 5 h and every 2–6 h thereafter. These liquid samples were stored in a freezer for future high-performance-liquid-chromatography (HPLC) analysis. The pH value in the reactor was measured as liquid samples were collected. Also, gas production was monitored by the accumulated gas volume in the gas collector, and a 0.5-mL gas sample was withdrawn from the head space of the bioreactor and analyzed for methane content by a gas chromatographic method.

Similar batch experiments were also conducted with lactose, acetate, propionate, lactate, and butyrate as growth substrates. To each batch experiment, 2–5 g of one substrate were added in the solution form. Between two batch experiments, at least one 20-mL whey permeate was added and the reactors were given 2–3 days to restore pseudo-steady-state conditions. Experiments with each growth substrate were at least duplicated.

Sample Analyses

Concentrations of lactose, organic acids, and alcohols in liquid samples were analyzed by the HPLC method described elsewhere.[33] Gas composition was analyzed with a gas chromatograph (Varian 3300) equipped with a thermal conductivity detector (TCD) and two 0.125-in. stainless-steel columns, 2-m Porapack N (80/100 mesh), and 4-m molecular sieve 5A (40/60 mesh), in series operated at 100°C. The carrier gas was He at a flow rate of 30 mL/min. The detector polarity was switched after CO_2 was eluted from the first column (about 3 min after injection).

The cell concentration in the bioreactor was determined from cell dry weight as well as optical density at 660 nm (OD_{660}). Liquid samples (200 mL each) were centrifuged at 1.3×10^4 rpm in a centrifuge (Beckmen J2-21) at 4°C for 40 min. The cell precipitates were then collected and dried overnight at 103°C. The value of OD_{660} was measured with a spectrophotometer (Turner model 350) in a cuvette of 10-mm light path length. Samples were diluted to the OD_{660} range of lower than 0.45, where one unit of OD_{660} was approximately equivalent to 0.65 g/L cell. The amount of cell attached to the ceramic packing was also determined after completing all the batch experiments. Part of the packing material was removed from the reactor, washed with tap water until the washing water was nearly clear, and then cells present in the washing water were collected and determined by measuring either the dry weight or OD_{660}.

RESULTS AND DISCUSSION

Reactor Startup

Figure 1 shows the dynamic concentration changes of various intermediary products in a bioreactor during reactor startup. Initially, butyrate, propionate, and acetate were three major intermediary products accumulated as lactose was fermented. Large amounts of iso-valerate and some formate and other unidentified C_3–C_5 organic acids were also detected in the first 24 h, but they disappeared soon after they were formed. After continuously feeding the reactor at a dilution rate of 0.02 h^{-1} for 10 days, butyrate was no longer detected in the reactor, indicating the occurrence of dynamic microbial population changes. It is known that bacteria with a long doubling time would be washed out if the dilution rate was high. The butyrate-producing bacteria could have been washed out during startup. It has been reported that the formation of mixed-culture aggregates during startup of an anaerobic gas-lift reactor was coupled with a general metabolism change from acetate-butyrate production to acetate-propionate production, and this change was attributed to a selection by washout at a high dilution rate, which favored propionate-producing bacteria with superior adhesive properties.[34]

It was also found that the bacteria immobilized in these reactors could recover their methanogenic activity within a week even after several months without feeding them with any organic substrates. However, whether the predominant populations have changed after the starvation period is not known.

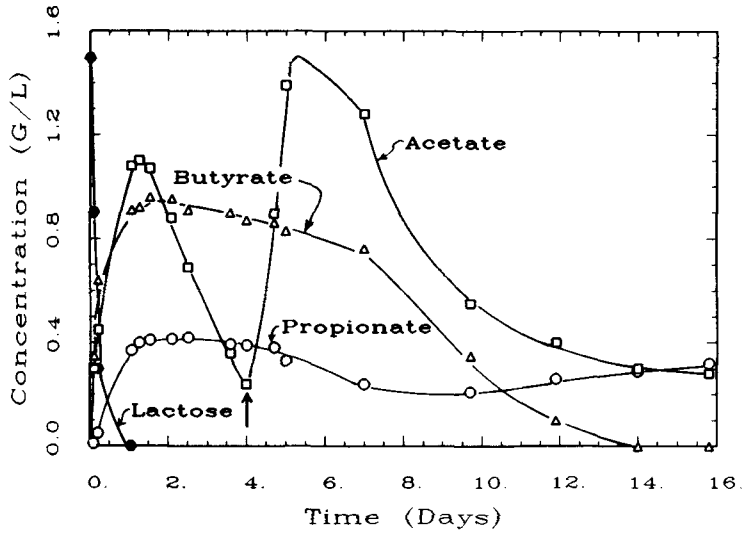


Figure 1. Concentration changes of intermediary products in methanogenic fermentation of whey permeate during reactor startup. The arrow indicates when the reactor was fed continuously with whey permeate.

Batch Fermentation Kinetics

To avoid population changes during the experiments, batch fermentations using reactors that had been operated at a low dilution rate (~0.02 h^{-1}) were conducted to elucidate the methanogenic kinetics. The population changes during batch experiments were negligible as the amounts of substrate added were so low that the increased cell mass was negligible as compared with the total cell mass present in the reactors. The total cell mass in each reactor was approximately 5–10 g, of which less than 5% was free cells suspended in the liquid phase.

The intermediary products formed during batch fermentation of whey permeate were studied first. Two typical batch fermentations of whey permeate are shown in Figure 2. As shown in Figure 2, in addition to the initial substrates lactose, propionate and acetate were the only two major compounds found throughout the anaerobic digestion of whey permeate. Low concentration of lactate was also detected initially, as lactate was also present in whey permeate. There were some unidentified C_3–C_5 organic acids (which were similar to those belonging to the TCA cycle, and were possibly produced by aerobic bacteria) formed from lactose initially, but they were soon degraded (but slower than lactose) to smaller compounds like acetate and propionate. Only very low concentrations of butyrate and ethanol were occasionally detected. Significant amounts of butyrate were found only when propionate concentration was high (>2 g/L) and the pH value was low (<6.0). Neither formate nor methanol was found throughout these batch fermentations.

As also shown in Figure 2, propionate and acetate were produced simultaneously from lactose; they were then converted to biogas (~50% CH_4 and 50% CO_2). At pH values around 7.0, the concentration of propionic acid was always higher than that of acetic acid throughout the fermentation. The conversion of propionate was also slower than acetate. These results suggest that the conversion of propionic acid to acetic acid was the rate-limiting step in this methanogenic fermentation. The lactose consumption rate ranged from 2.1–4.8 g/h · L. The large variation in the reaction rate was mainly due to the difference in cell density in different reactors.

The apparent yields of propionic acid and acetic acid were found to be respectively ~0.28 and 0.18 g/g lactose fermented. However, since both propionic and acetic acids were also consumed to form other compounds during the fermentation, the actual yields of propionic and acetic acids from lactose fermentation should be higher than these values. The total biogas volume collected in the gas collecting device was lower than expected. This was largely due to gas leak and gas retention in the bioreactor system and gas (CO_2) dissolution in liquid (both in the reactor and in the gas collector). It was noted that less biogas was collected when the reactor pH was higher, due to large solubility of CO_2 in water at pH > 7. The produced biogas contained about 50% of methane and the methane yield from lactose was about 300 mL/g lactose or 80% of the theoretical yield.

Based on the intermediary products identified in these batch fermentations, it is conceivable that whey lactose was mainly converted to propionic acid, acetic acid, CO_2, and (presumably) H_2 by the hydrolytic fermentative bacteria. Lactate, butyrate, ethanol, methanol, and formate were not produced in significant amounts, and thus may be assumed to be unimportant in the methanogenic system studied. Based on these results, a three-step reaction mechanism for methanogenic fermentation of whey permeate (lactose and lactate) is proposed and shown in Figure 3. Lactate is also present in whey permeate, although in much lower amount, and was found to follow the same reaction mechanism as the lactose.

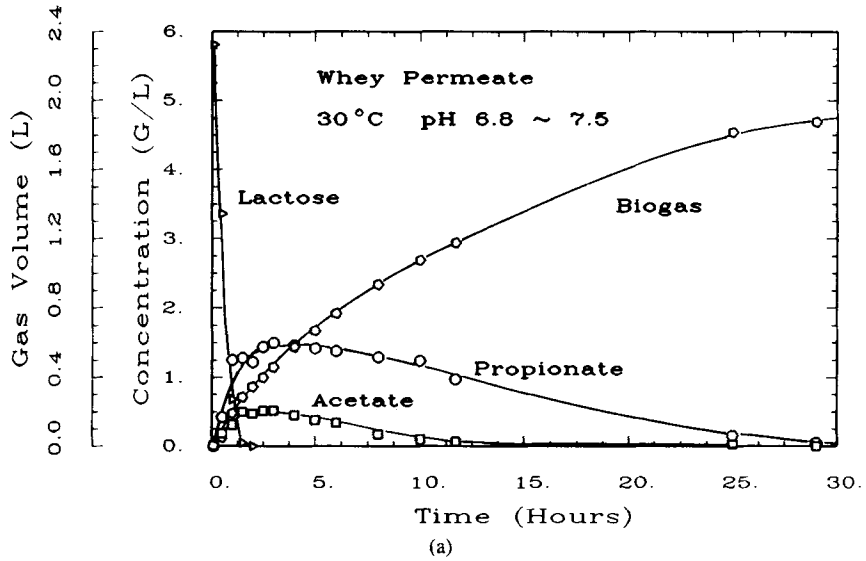


Figure 2. Batch methanogenic fermentation of whey permeate in (a) reactor I and (b) reactor II.

Further batch fermentation experiments were conducted with lactose, acetate, and propionate as the initial substrate, respectively, to evaluate the proposed methanogenic mechanism and to show that propionic and acetic acids were the only two major intermediates formed during anaerobic lactose fermentation. Lactate and butyrate were also used as the growth substrate, respectively, to show that these two organic acids were not produced in significant amounts during anaerobic lactose fermentation. The results from these experiments are shown in Figure 4 and are discussed in the following sections.

Lactose Fermentation

As shown in Figure 4(a), lactose, added as the initial growth substrate, was fermented to propionic acid, acetic acid, CO_2 and CH_4, with yields similar to those found in whey permeate fermentation. The conversion of propionic acid was also slower than that of acetic acid.

Lactate Fermentation

Whey permeate usually contains some lactic acid, which also can be used as a methanogenic substrate. Lactic acid was also reported as a major intermediary metabolite of lactose fermentation during anaerobic degradation of whey.[21] As shown in Figure 4(b), lactate, added as the initial growth substrate, was converted to propionate, acetate, and biogas. However, concentrations of propionate and acetate were quite low as compared to the initial lactate concentration. This implies that significant amounts of propionate and acetate formed from lactate might have

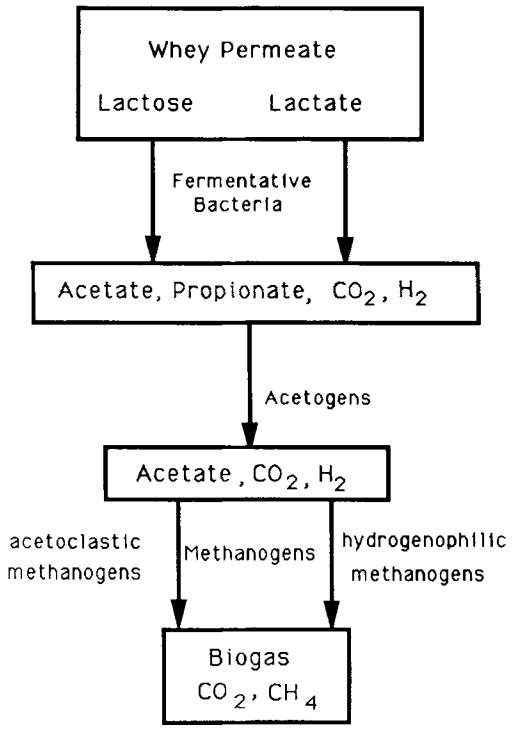


Figure 3. Proposed three-step reaction mechanism for methanogenesis of whey permeate (lactose and lactate).

been further degraded to methane soon after they were produced.

The methanogenic mechanism for lactate fermentation is thus similar to that for lactose fermentation and involves at least the following reactions:

$$\text{Lactic acid} \longrightarrow \underset{\text{acetic acid}}{\text{propionic acid}} \longrightarrow \text{acetic acid} \longrightarrow CH_4 + CO_2$$

The lactate consumption rate was slow, only ~1.0 g/h · L initially and 0.18 g/h · L afterwards. The dramatic drop in lactate consumption rate might be caused by propionate inhibition. The lactate consumption rate was much slower than those for lactose and acetate. If significant amounts of lactate were also produced during methanogenic fermentation of whey permeate (lactose), some lactic acid would have been accumulated before acetic acid accumulation occurred since the consumption rate of lactate was slower than that for acetate. Therefore, it can be concluded that lactate was not an important intermediary product formed in these reactors. However, it should be noted that lactate may become a major product from lactose acidogenesis when the reactor pH was low (pH 4.5) and the dilution rate was high.[25,31,32] The methane yield from lactate was similar to that from lactose, ~300 mL/g lactic acid fermented or 80% of the theoretical yield.

Butyrate Fermentation

Butyric acid is another intermediate acid usually found during anaerobic digestion of organic wastes including whey.[9] Butyrate methanogenesis was reported to be faster than that of acetate and much faster than that of propionate.[19] Significant amounts of butyrate might have been produced during whey fermentation but were immediately converted to acetate and biogas. As a result, only very low butyrate concentrations, often below detectable limits, were found in stable, continuous digesters. However, results from batch experiments with butyrate as the initial growth substrate indicated that butyrate was not readily consumed for methane production [Fig. 4(c)]. This suggests that butyrate was not produced in significant amounts during methanogenesis of whey permeate in the bioreactor system studied. This finding is also consistent with our previous observation that butyrate was no longer detected after 10 days from the reactor startup. Apparently, both butyrate-producing and butyrate-degrading bacteria had been washed out during reactor startup.

Propionate Fermentation

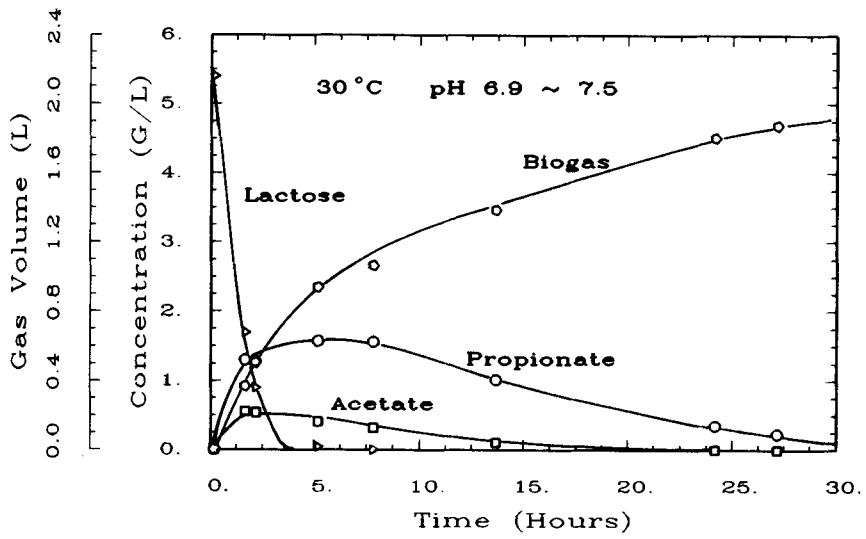
It is known that the methanogenic mechanism for propionate fermentation generally involves the following reactions:[35,36]

$$\text{Propionic acid} \longrightarrow \text{acetic acid} + H_2 + CO_2 \longrightarrow CH_4 + CO_2$$

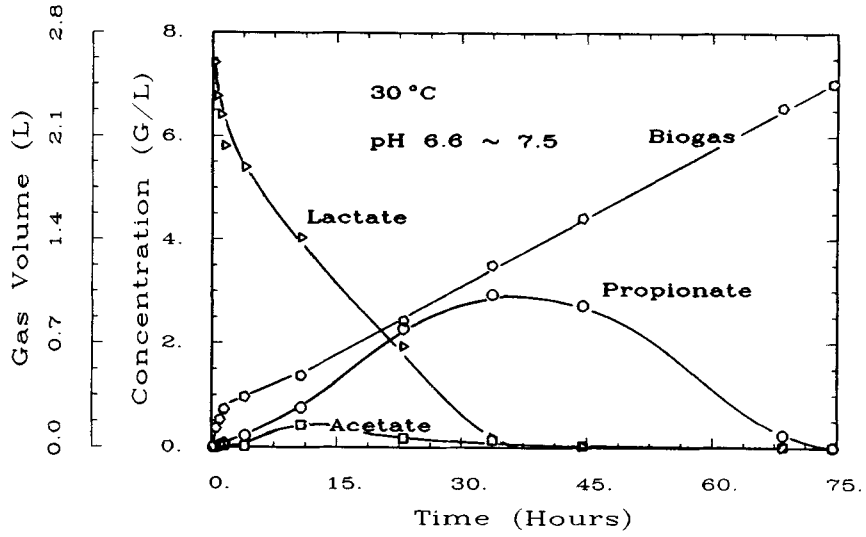
Experimental results with propionic acid as the initial substrate are shown in Figure 4(d). Only very low concentrations of acetic acid were detected throughout the fermentation. The consumption rate of propionate was <0.22 g propionic acid/h · L, which was smaller than the acetate consumption rate found in the experiment with acetate as the initial growth substrate [Figure 4(e)]. Apparently, most of acetic acid produced from propionic acid was immediately converted to methane and CO_2. This suggests that the conversion of propionate was the rate-limiting step in the methanogenic system studied. These results are consistent with previously reported work on propionate degradation using methanogenic consortia.[35] The total biogas yield from propionic acid was ~510 mL/g propionic acid fermented, of which 75% was methane. This corresponds to 72% of the theoretical yield.

Acetate Fermentation

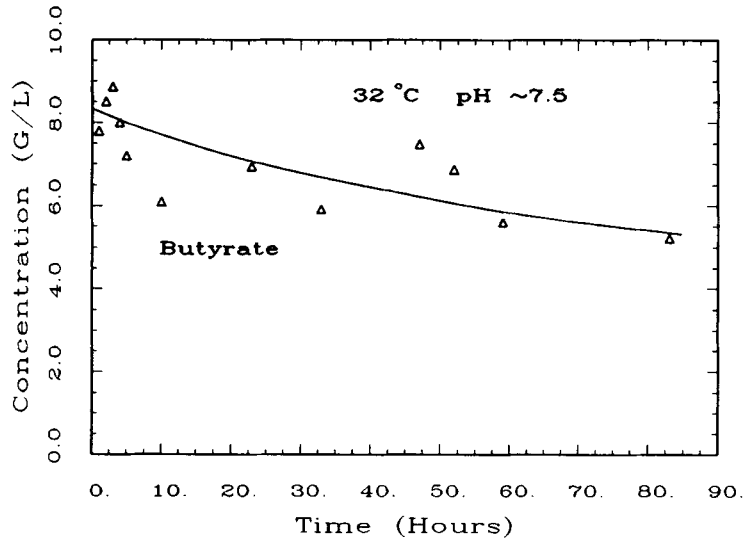
In a methanogenic environment, acetate is converted to equal molar methane and carbon dioxide by acetoclastic methanogens.[17] Results from experiments with acetic acid as the initial substrate are shown in Figure 4(e). About 720 mL biogas were produced from each gram of acetic acid consumed. The methane content was about 50%. The methane yield was close to the theoretical yield, 373 mL methane/g acetic acid. The consumption rate of acetate was ~0.3 g acetic acid/h · L, which was much faster than the consumption rate for propionate. It is clear that the conversion of acetate to methane was not the rate-limiting step in the methanogenic system studied.



(a)

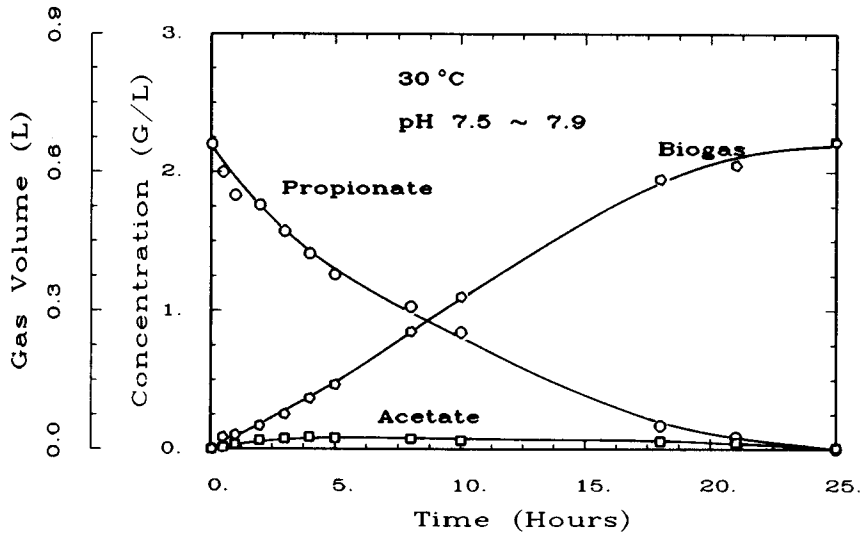


(b)

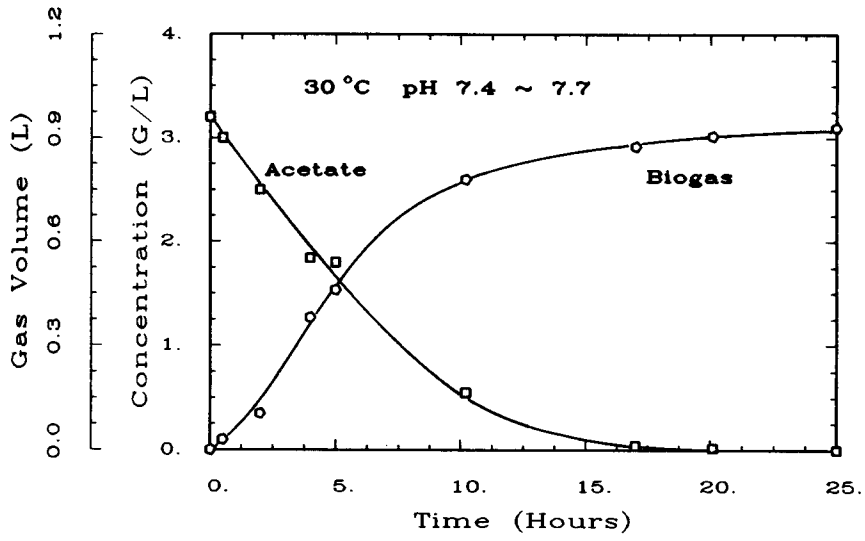


(c)

Figure 4. Batch methanogenic fermentation of (a) lactose, (b) lactate, (c) butyrate, (d) propionate, and (e) acetate.



(d)



(e)

Factors Affecting Fermentation Kinetics

The nature of fermentation kinetics is determined by the populations as well as the growth conditions. The environmental factors, including pH value,[24] temperature, retention time,[31] oxidation–reduction potential, E_h,[20] and partial pressure of H_2,[16] have profound effects on methanogenic fermentation. It is known that all anaerobic digesters have a marked pH optimum somewhere around 7, and a pH below 6 adversely affects waste degradation and methane formation. Depending on the growth conditions, the distribution of fermentation products could be quite different, even the same seeding culture was used. Cohen et al.[20] suggested that two fermentation types occurred complementary to each other in anaerobic acidogenesis of glucose. The first one (butyric acid type) was characterized by the production of butyrate, acetate, hydrogen, and carbon dioxide as the main fermentation products. The second one (propionic acid type) showed the formation of mainly propionate and acetate, with little gas production. They also found that high butyrate production were related to low E_h values of about -300 mV, while high propionate formation coincided with high E_h values of around -120 mV.

In a study of acidogenic fermentation of lactose, Kisaalita et al.[24] reported that no gaseous products (CO_2 and H_2) were formed from lactose if the reactor pH was kept above 4.5 during startup, but there was gas production even at higher operating pH values if the reactor pH fell below 4.5 during startup. They also found that butyrate and acetate were two main acid products, with butyrate predominated at pH < 5.0 and acetate predominated at pH > 5.5. More recently, Kissalita et al.[31,32] reported that acetate, propionate, *n*-butyrate, and lactate were the major products from lactose acidogenic fermentation in a continuous stirred tank digestor, with their concentrations strongly dependent on the dilution rate. Lactate appeared and then be-

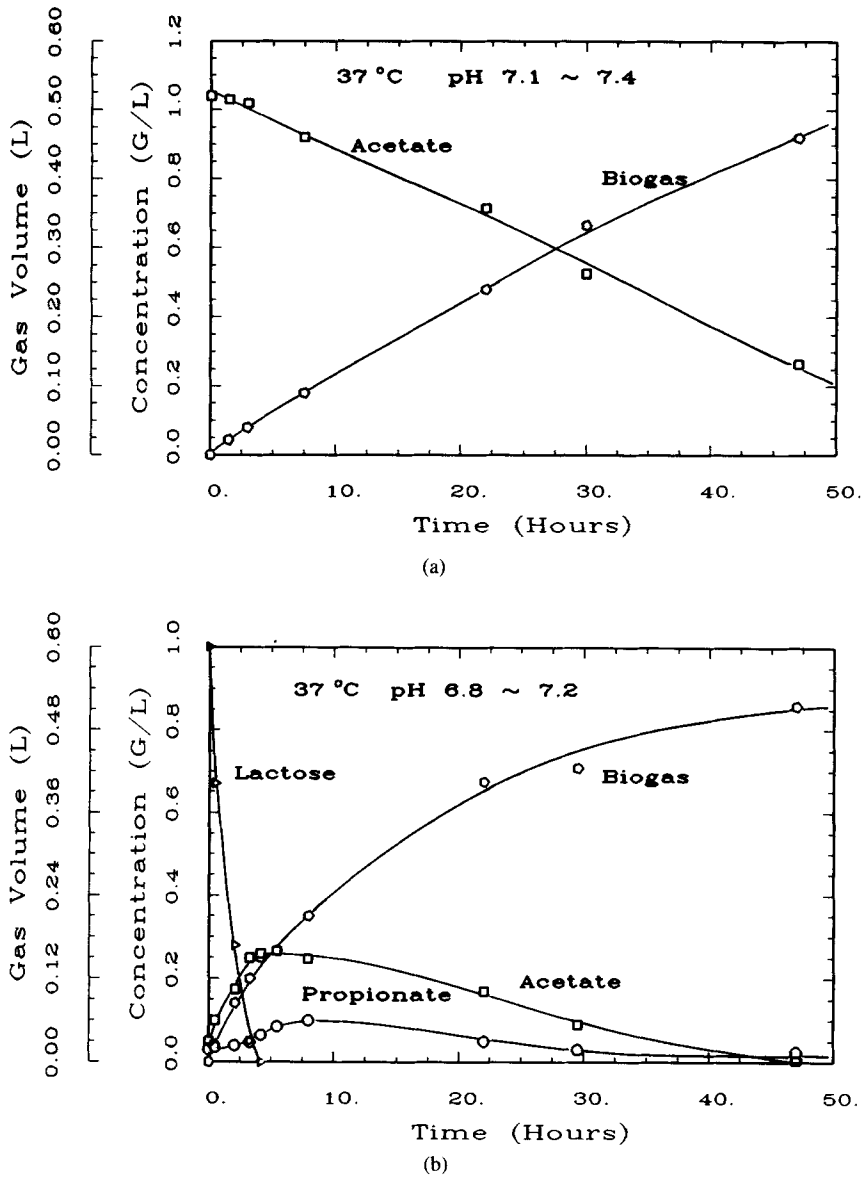


Figure 5. Batch methanogenic fermentation of (a) acetate and (b) lactose in a newly started bioreactor.

came predominant with increasing the dilution rate. A similar change in product distribution effected by the pH was also reported for glucose acidogenesis.[22] However, these studies were conducted at low pH values (<6.5) and under the conditions of no methane formation.

Under methanogenic conditions and at pH values around 7, the product distribution from lactose degradation could be very different due to the occurrence of interspecies hydrogen transfer.[16] In a laboratory chemostat study of whey biomethanation, Chartrain and Zeikus[21] found that lactose was metabolized primarily into lactate, ethanol, acetate, formate, and CO_2 when the retention time was 100 h and the reactor pH was maintained at 7.1, but as the retention time decreased to lower than 25 h, acetate and propionate were the first intermediary metabolites to accumulate, followed by formate and butyrate and then lactate and ethanol. In another whey biomethanation study using fixed-bed reactor, Winter and his co-workers[9] found that acetate and propionate started to accumulate at a hydraulic retention time (HRT) lower than 6.25 days (pH 6.5), while butyrate accumulation occurred only when HRT decreased to 3.75 days and pH value was 5.25 or lower. However, they identified butyrate as an important intermediary product produced from whey protein (not whey lactose).[26] They also pointed out the butyrate produced by *Eubacterium limosum* can be prevented by a well-functioning interspecies hydrogen transfer and low acetate levels.[9]

In this work, the reactor pH was always maintained at 6.0 or higher, and whey permeate was used as the substrate, which contained very little protein (<0.1%). Therefore, the conclusion from this work that butyrate and lactate are not among the major intermediates in the methanogenic

fermentation of whey permeate (lactose) is in agreement with aforementioned studies.

The change in product distribution could be attributed to changes in the metabolism and the composition of the bacterial population. In a newly started bioreactor, acetate consumption rate was slow [Fig. 5(a)] and large amounts of acetate was accumulated before propionate [Fig. 5(b)]. However, after running the same reactor for several months, methanogenic population was greatly enriched and acetate accumulation became less significant than propionate.

The relative amounts of propionate and acetate accumulated during whey permeate fermentation were largely affected by the pH. In the same reactor, the propionate levels were found to be much lower at pH $\approx$ 8 than at pH $\approx$ 7. This is because the pH influences on different groups of bacteria are not the same. In studying propionate degradation by a methanogenic enrichment culture, Boone and Xun[37] reported that propionate oxiders had a wide optimal pH range for growth (pH 6.8–8.5) while acetate-degrading populations had a narrow pH optima (pH 6.8–7.2). They also found that during propionate fermentation, large amounts of acetate were accumulated at pH 8.4, but not at pH 7.2. Apparently, the relative conversion rate of propionate to acetate increases with increasing the pH. This may partially explain the observed low propionate concentration at pH values higher than 8. The amounts of propionate produced from lactose (and lactate) degradation also decrease with increasing the pH value since most propionate producers have an optimal pH between 6.5 and 7.0.[38]

In anaerobic digesters, propionate may be converted to acetate by syntropic association of obligate proton reducers such as *Syntrophobacter wolinii*[39] and hydrogenophilic methanogens, or by sulfate reducers belonging to the genus *Desulfobulbus*.[40,41] However, acetogenesis from propionate is only feasible, thermodynamically, at a very low partial pressure of hydrogen.[16,42] Inhibition of propionate degradation may result in propionate accumulation to a level toxic to anaerobic digestion. Complete inhibition of anaerobic methanogenesis was observed in a reactor containing 10 g/L propionate at pH <6.0. The acetogenic population in a whey digester was also found to be very low, only consisted of ~5% of the total population, in comparison to 55% for fermentative bacteria and 41% for methanogens.[26] These may explain why the conversion of propionate to acetate was the slowest reaction. About 10% of propionate also may be converted to butyrate by reductive carboxylation occurred in methanogenic ecosystem.[42] Some butyrate formation observed at high levels of propionate accumulation in several experiments can be partially explained by this reaction.

This study was supported in part by a seed grant from the Office of Research and Graduate Studies, The Ohio State University.

References

1. S. T. Yang, "Pollution prevention and waste minimization in the dairy industry through novel uses of whey permeat,"AIChE Pollution Prevention Conference, Washington, DC 1989.
2. P. H. Boening and V. F. Larsen, *Biotechnol. Bioeng.*, **24,** 2539 (1982).
3. J. P. Barford, R. G. Cail, I. J. Callander, and E. J. Floyd, *Biotechnol. Bioeng.*, **28,** 1601 (1986).
4. R. A. Messing, *Biotechnol. Bioeng.*, **24,** 1115 (1982).
5. M. Palmer, *Dairy Field*, Nov. 60 (1984).
6. M. D. Erdman and C. A. Reddy, *Biol. Wastes*, **22,** 23 (1987).
7. M. S. Switzenbaum and S. C. Danskin, *Agric. Wastes*, **4,** 411 (1982).
8. F. X. Wildenauer and J. Winter, *Appl. Microbiol. Biotechnol.*, **22,** 367 (1985).
9. G. Zellner, P. Vogel, H. Kneifel, and J. Winter, *Appl. Microbiol. Biotechnol.*, **27,** 306 (1987).
10. S. T. Yang, I. C. Tang, and M. R. Okos, *Biotechnol. Bioeng.*, **32,** 28 (1988).
11. P. L. McCarty, "The methane fermentation," in *Principles and Applications in Aquatic Microbiology,* H. Henkelekian and N. C. Dondero, Eds. (Wiley, New York, 1964), p. 314.
12. J. G. Zeikus, "*Microbial population in digesters,*" in *Anaerobic Digestion,* D. A. Stafford, B. I. Wheatley, and D. E. Hughes, Eds. (Applied Science Publishers, London, 1980), p. 61.
13. M. R. Winfrey, "Microbial production of methane," in *Petroleum Microbiology,* R. M. Atlas, Ed. (Macmillan, New York, 1984), pp. 153–219.
14. M. P. Bryant, *J. Animal Sci.*, **48,** 193 (1979).
15. R. A. Mah, "The methanogenic bacteria," in *Trends in the Biology of Fermentations for Fuels and Chemicals,* A. Hollaender, Ed., *Basic Life Science,* Volume 18 (Plenum, New York, 1981), pp. 357-373.
16. S. R. Harper and F. G. Pohland, Recent developments in hydrogen management during anaerobic biological wastewater treatment, *Biotechnol. Bioeng.*, **28,** 585 (1986).
17. M. R. Smith, S. H. Zinder, and R. A. Mah, *Process Biochem.*, **15**(5), 34 (1980).
18. A. W. Lawrence and P. L. McCarty, *J. Water Pollut. Control Fed.*, **41,** R1 (1969).
19. P. L. McCarty, J. S. Jeris, and W. Murdoch, *J. Water Pollut. Control Fed.*, **35,** 1501 (1963).
20. A. Cohen, J. M. van Gemert, R. J. Zoetemeyer, and A. M. Breure, *Process Biochem.*, **19,** 228 (1984).
21. M. Chartrain and J. G. Zeikus, *Appl. Environ. Microbiol.*, **51,** 180 (1986).
22. R. J. Zoetemeyer, A. J. C. M. Matthijsen, J. C. Vanden Heuvel, A. Cohen, and C. Boelhouwer, *Biomass*, **2,** 187 (1982).
23. S. T. Yang, I. C. Tang, and M. R. Okos, *Appl. Environ. Microbiol.*, **53,** 823 (1987).
24. W. S. Kisaalita, K. L. Pinder, and K. V. Lo, *Biotechnol. Bioeng.*, **30,** 88 (1987).
25. S. T. Yang, M. R. Okos, and J. C. Nye, "Kinetics of methane fermentation of whey," Meeting of the American Society of Agricultural Engineers, paper No. 82-3592, 1982.
26. G. Zellner and J. Winter, *System Appl. Microbiol.*, **9,** 284 (1987).
27. M. Chartrain and J. G. Zeikus, *Appl. Environ. Microbiol.*, **51,** 188 (1986).
28. M. Chartrain, L. Bhatnagar, and J. G. Zeikus, *Appl. Environ. Microbiol.*, **53,** 1147 (1987).
29. J. H. Thiele, M. Chartrain, and J. G. Zeikus, *Appl. Environ. Microbiol.*, **54,** 10 (1988).
30. J. H. Thiele and J. G. Zeikus, *Appl. Environ. Microbiol.*, **54,** 20 (1988).
31. W. S. Kissalita, K. V. Lo, and K. L. Pinder, *Biotechnol. Bioeng.*, **33,** 623 (1989).
32. W. S. Kissalita, K. V. Lo, and K. L. Pinder, *Biotechnol. Bioeng.*, **34,** 1235 (1989).
33. S. T. Yang, "Kinetic Study of Defined Methane Fermentation of Lactose," Ph.D. dissertation, Purdue University, West Lafayette, IN, 1984.
34. H. H. Beeftink and J. C. ven den Heuvel, *Biotechnol. Bioeng.*, **30,** 233 (1987).
35. H. Mucha, F. Lingens, and W. Trosch, *Appl. Microbiol. Biotechnol.*, **27,** 581 (1988).

36. M. Koch, J. Dolfing, K. Wuhrmann, and A. J. B. Zehnder, *Appl. Environ. Microbiol.*, **45,** 1411 (1983).
37. D. R. Boone and L. Xun, *Appl. Environ. Microbiol.*, **53,** 1589 (1987).
38. M. J. Playne, "Propionic and butyric acids," in *Comprehensive Biotechnology*, M. Moo-Young, Ed., Vol. 3 (Pergamon, New York, 1985), p. 731.
39. D. R. Boone and M. P. Bryant, *Appl. Environ. Microbiol.*, **40,** 626 (1980).
40. J. P. Guyot, I. Traore, and J. L. Garcia, *FEMS Microbiol. Lett.*, **26,** 329 (1985).
41. R. H. Heyes and R. J. Hall, *Appl. Environ. Microbiol.*, **46,** 710 (1983).
42. D. P. Smith and P. L. McCarty, *Biotechnol. Bioeng.*, **34,** 39 (1989).
43. J. L. Tholozan, E. Samain, J. P. Grivet, R. Moletta, H. C. Dubourguier, and G. Albagnac, *Appl. Environ. Microbiol.*, **54,** 441 (1988).