Measurement of Monosaccharides and Conversion of Glucose to Acetate in Anoxic Rice Field Soil

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Degradation of glucose has been implicated in acetate production in rice field soil, but the abundance of glucose, the temporal change of glucose turnover, and the relationship between glucose and acetate catabolism are not well understood. We therefore measured the pool sizes of glucose and acetate in rice field soil and investigated the turnover of [U-¹⁴C]glucose and [2-¹⁴C]acetate. Acetate accumulated up to about 2 mM during days 5 to 10 after flooding of the soil. Subsequently, methanogenesis started and the acetate concentration decreased to about 100 to 200 μ M. Glucose always made up >50% of the total monosaccharides detected. Glucose concentrations decreased during the first 10 days from 90 μ M initially to about 3 μ M after 40 days of incubation. With the exception at day 0 when glucose consumption was slow, the glucose turnover time was in the range of minutes, while the acetate turnover time was in the range of hours. Anaerobic degradation of [U-¹⁴C]glucose released [¹⁴C]acetate and ¹⁴CO₂ as the main products, with [¹⁴C]acetate being released faster than ¹⁴CO₂. The products of [2-¹⁴C]acetate metabolism, on the other hand, were ¹⁴CO₂ during the reduction phase of soil incubation (days 0 to 15) and ¹⁴CH₄ during the methanogenic phase (after day 15). Except during the accumulation period of acetate (days 5 to 10), approximately 50 to 80% of the acetate consumed was produced from glucose catabolism. However, during the accumulation period of acetate, the rate of acetate production from glucose greatly exceeded that of acetate consumption. Under steady-state conditions, up to 67% of the CH₄ was produced from acetate, of which up to 56% was produced from glucose degradation.

Acetate is the main intermediate in anaerobic mineralization of organic carbon in many aquatic ecosystems (22, 27, 29, 43). Even in upland environments, such as prairie and forest soils, where anaerobic conditions are restricted to microsites in soil aggregates, acetate was found to play an important role in the turnover of carbon (20, 37). In rice field soil, acetate is well known as the most dominant fatty acid, and it has frequently been observed to accumulate up to millimolar concentrations within 2 weeks after soil flooding (14, 17, 19, 33, 39). It is also known that during methanogenesis in rice field soil, >60% of the produced CH₄ is derived from acetate (28, 31, 34).

Although considerable research has emphasized the consumption of acetate, especially in relation to CH₄ formation (17, 31, 33), only a few studies have documented the production and consumption of acetate in the same set of experiments (18, 35). Furthermore, the relationship between accumulation of acetate and catabolism of complex acetate precursors in rice field soil has not seriously been examined. Thebrath et al. (35) investigated the process of reductive acetogenesis from CO₂ in Italian rice soil and concluded that this process produces only a small amount of acetate compared to the amount of acetate turned over during methanogenesis. Thus, it is assumed that acetate is produced from other processes to balance its consumption by the ongoing acetoclastic methanogenesis. A study using position-labeled glucose demonstrated that glucose is a potential substrate for acetate production in Italian rice soil (18). To our knowledge, measurement of the glucose concentration in rice field soil has not yet been reported. As a result, the quantitative relationship between glucose catabolism and acetate accumulation is not known.

Sugar analysis with a pulsed amperometric detector (PAD) allows the measurement of the in situ concentrations of various sugars (15, 40, 41). Using this technique, we report here the results of measurement of dissolved monosaccharides in Italian rice field soil. The importance of glucose as a potential acetate precursor and the temporal change of glucose and acetate turnover in anoxic Italian rice field soil were investigated by using [U-¹⁴C]glucose and [2-¹⁴C]acetate.

MATERIALS AND METHODS

Preparation of anoxic soil. Rice field soil was obtained in 1993 from the plow layer (0 to 15 cm in depth) of the experimental fields of the Italian Rice Research Institute in Vercelli. The main soil characteristics were 60% sand, 25% silt, 12% clay, 1.49% organic carbon, and 0.15% total nitrogen (12). The soil was air dried, mechanically crushed, passed through a sieve with a mesh size of 0.5 mm, and stored at room temperature until use. Storage of the soil under oxic conditions does not affect the initiation of CH₄ production and the population size of methanogenic bacteria (23). Soil slurry was prepared by adding 28 ml of sterilized water to 28 g of air-dried soil in a sterile 120-ml serum bottle. All treatments were duplicates. The bottles were then closed with a sterile black rubber stopper, and the headspace was exchanged with pure nitrogen gas. The incubation of the soil slurry was performed at 30°C without shaking to avoid damage of the methanogenic community (8). At given time intervals, gas samples were taken from the headspace after vigorous shaking of the bottles by hand, and then the samples were analyzed for CO₂ and CH₄. Two additional bottles were prepared for slurry sampling and analysis of organic acids. For analysis of monosaccharides, 20 additional bottles were prepared, and 2 were sacrificed at each time point. The pH of the soil slurries increased from an initial pH of 6.0 and eventually stabilized at pH 7.1.

Radioactive studies. An additional 13 duplicate bottles with soil slurry were prepared for each radioactive experiment, with one set for [U-¹⁴C]glucose utilization and another one for [2-¹⁴C]acetate utilization. Four radioactive experiments with both [U-¹⁴C]glucose and [2-¹⁴C]acetate were conducted (i.e., at 0, 5, 10, and 36 days after the start of the anoxic slurry incubation). Approximately 2.5 μ Ci (1 μ Ci = 2.22×10^6 dpm = 37 kBq) of carrier-free [U-¹⁴C]glucose or [2-¹⁴C]acetate (purity of >99%; specific radioactivity of 300 mCi mmol⁻¹ for glucose and 57 mCi mmol⁻¹ for acetate; American Radiolabel Chemical, Inc.) was added with a syringe to each bottle. Before the labeled compounds were added at day 0, the bottles were preincubated for 5 h at 30°C for acclimatization. With the exception of days 10 and 36, when the amounts of [U-¹⁴C]glucose added were 6 and 8% of the glucose pool size, respectively, the [U-¹⁴C]glucose

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FIG. 1. Dissolved monosaccharides detected in Italian rice field soil. The first data point represents the measurement in extracts of air-dried soil before slurring; 1 nmol g of dry soil⁻¹ is equivalent to a pore water concentration of 1 μ M.

and [2-¹⁴C]acetate added at the other dates were <1% of the substrate pools. Therefore, addition of labeled compounds did not significantly increase the endogenous pool sizes of both substrates.

The reaction in the bottles was terminated by addition of 3 ml of 7 N H₂SO₄. Slurry acidification also resulted in the release of ¹⁴CO₂ from dissolved radioactive bicarbonate and carbonate to the gas phase (9). When used below, the term "¹⁴CO₂" is assumed to represent total ¹⁴CO₂ (CO₂ plus bicarbonate plus carbonate). Recovery of the [U-¹⁴C]glucose added to the autoclaved soil was constant throughout the experiment at 97.66% $\pm$ 2.32% (mean $\pm$ standard error [SE]; n = 4). When [2-¹⁴C]acetate was added to autoclaved soil, its recovery was constant at 46.10% $\pm$ 1.57% (mean $\pm$ SE; n = 4). The relatively low recovery of acetate from the sterilized soil slurry indicates that this was due to physical factors, such as absorption to soil particles and/or exchange with tightly bound acetate pools, which were not accessible by the extraction procedure (5, 25, 31). When used below, the term "radioactivity" of glucose or acetate refers to the values that have been previously corrected by the radioactive recovery from autoclaved soil slurry.

Chemical analyses. To measure the concentrations of monosaccharides, the soil slurry was transferred to a sterile 30-ml centrifuge tube and centrifuged at 5,000 $\times$ g at 4°C for 10 min. The supernatant was filtered through 0.2- μ m-pore-size polycarbonate membrane filters (Nucleopore Corp., Pleasanton, Calif.) and immediately frozen at -20°C until analysis. For the analyses of acetate and propionate, 1 ml of soil slurry was taken at each given time and centrifuged at 14,000 $\times$ g at room temperature for 5 min. The supernatant was passed through polytetrafluoroethylene membrane filters with a pore size of 0.2 μ m (Sartorius AG, Göttingen, Germany).

Concentrations of CH₄ and CO₂ were determined by gas chromatography (7). Radioactivity of gaseous products was measured by a gas proportional counter as described previously (7). Acetate and propionate concentrations were determined by high-performance liquid chromatography (HPLC; Sykam, Gauting, Germany) with a chromatograph equipped with a refractive index detector (18). The detection limit was approximately 5 μ M. The radioactivity of glucose and acetate was measured by connecting a scintillation monitor (a lithium-glass scintillator cell with a volume of 400 μ l; Raytest, Straubenhardt, Germany) to the outlet of the HPLC column. The limit of detection of radioactivity was approximately 1 kdpm ml of eluent⁻¹ (35). The concentration of glucose could not be determined by the refractive index detector due to the interference of an unknown peak. Thus, monosaccharides were determined in a parallel experiment by using PAD-HPLC. The PAD-HPLC was equipped with a Dionex ED40 PAD and a gold electrode, and the sugars were separated by CarboPac PA10 columns (250 by 4 and 50 by 4 mm) eluted with NaOH at different concentrations (15). Mannose and xylose could not be completely separated by these columns and, therefore, are shown as a combined peak (mannose plus xylose).

Calculations. The uptake rate constant (k) of glucose and acetate was estimated from a semilogarithmic plot of the radioactive glucose or acetate (disin-

tegrations per minute) versus the incubation time, and then the slope of the line was determined. The data points included in the calculation of glucose uptake rate constant were usually obtained within 20 min after the addition of [U-¹⁴C]glucose, except at day 0, when the data points between 10 and 120 min were used. Acetate uptake rate constants were calculated from the time period when accumulation of metabolic products was observed. These were between 120 and 720 min (n = 4) at day 0, between 60 and 720 min (n = 6) at day 5, between 120 and 1,440 min (n = 4) at day 10, and between 0 and 240 min (n = 7) at day 36. Actual turnover rates of glucose and acetate were obtained by multiplying the uptake rate constant by the pool size (pore water concentration) of the respective substrate. The fraction of CH₄ produced from the labeled acetate added was calculated from the specific radioactivity (SR; disintegrations per minute per nanomole) of the CH₄ produced and the SR of acetate (disintegrations per minute per nanomole). In addition, the respiratory index (RI) was used to compare the carbon flow toward CH₄ and CO₂: $RI = [(^{14}\text{CO}_2)/(^{14}\text{CO}_2 + ^{14}\text{CH}_4)]$.

RESULTS

Abundance of monosaccharides in rice field soil. When the soil condition was changed from air dried to prolonged flooding, the content of total dissolved monosaccharides decreased significantly from 136 μ M to 5 μ M. Glucose was the dominant monosaccharide (Fig. 1). The contribution of glucose to total dissolved monosaccharides was always >50%. Fructose was the second-most-abundant monosaccharide (21%). Relatively large amounts (>10% of total monosaccharides) of arabinose and deoxyribose were occasionally found. Mannose plus xylose made up <5% of total monosaccharides during the early period of incubation, but increased to >10% a month later. Other monosaccharides detected were galactose, rhamnose, fucose, and glucosamine. However, together these sugars made up <10% of total monosaccharides. On a carbon basis, the total dissolved monosaccharides in the present study were <1% of the total soil organic carbon content.

Sequential reduction processes and acetate and CH₄ accumulation. The reduction process in the Italian rice field soil has been well investigated (1, 17, 19). Generally, nitrate is consumed within less than a day of anaerobic incubation. Reduc-

FIG. 2. Temporal changes of production of acetate and CH_4 in anoxic rice soil slurry. The arrows indicate the dates when labeled glucose and acetate were added.

tion of ferric iron also starts immediately after onset of anaerobic incubation, but usually lasts until about days 10 to 15. Sulfate reduction, on the other hand, begins at about day 5 and continues until about days 10 to 15. Thus, the time from day 0 to day 15 when various oxidants are sequentially reduced is designated as the "reduction phase" (44). Accumulation of acetate during this reduction phase has often been observed and is regarded as a typical characteristic of anoxic rice field soil. In the present study, acetate started to accumulate at day 5 and reached its accumulation peak (ca. 2 mM) around day 10 (Fig. 2). CH_4 production started around day 10 and reached a constant rate of $34.5 \text{ nmol g}^{-1} \text{ h}^{-1}$ around day 15. The period after day 15 is therefore designated as the "methanogenic phase." The acetate concentration during the methanogenic phase was 100 to 200 μM . Propionate was also found to accumulate, but generally made up <5% of the acetate concentration and became undetectable after day 25 (data not shown).

Products of the glucose and acetate metabolisms. When $[\text{U-}^{14}\text{C}]$ glucose was added to the soil at day 0, its consumption started only after a lag phase of about 20 min (Fig. 3). After glucose consumption started, ^{14}C acetate and, somewhat later, $^{14}\text{CO}_2$ started to accumulate. $^{14}\text{CH}_4$ was not produced. Glucose consumption and the formation of radioactive products followed the same pattern for days 5 (data not shown), 10 (data not shown), and 36 (Fig. 4), but some differences were found in the magnitude and time course of intermediate and end product formation. Besides acetate, propionate was occasionally detected from $[\text{U-}^{14}\text{C}]$ glucose catabolism, but the amount was too small to be accurately quantified.

In contrast to the results obtained at day 0 (Fig. 3), $[\text{U-}^{14}\text{C}]$ glucose was consumed without a lag after its addition

FIG. 3. Uptake and product formation from $[\text{U-}^{14}\text{C}]$ glucose and $[\text{2-}^{14}\text{C}]$ acetate in anoxic rice soil slurry at day 0. Values are means $\pm$ SD of duplicate determinations. Note the logarithmic time scale.

at days 5, 10, and 36 (Fig. 4). At day 5, consumption of $[\text{U-}^{14}\text{C}]$ glucose was completed after 10 min (data not shown). Again, only $^{14}\text{CO}_2$, but no $^{14}\text{CH}_4$, was produced. At day 10, however, a small amount of $^{14}\text{CH}_4$ was produced 2 days after glucose addition (data not shown), although the main products of glucose degradation were still labeled CO_2 and acetate. The appearance of $^{14}\text{CH}_4$ long after addition of $[\text{U-}^{14}\text{C}]$ glucose at this date indicates that the newly formed radioactive acetate was only slowly consumed. This result was in agreement with the uptake rate constant of acetate (see next section), which was slowest at day 10. At day 36, more $^{14}\text{CH}_4$ was produced from $[\text{U-}^{14}\text{C}]$ glucose, but ^{14}C acetate and $^{14}\text{CO}_2$ were still the main degradation products (Fig. 4).

The final recovery of $[\text{U-}^{14}\text{C}]$ glucose in the form of radioactive products (CO_2 plus acetate plus CH_4) ranged from 31% at day 0 to 84% at day 36 (Table 1), indicating that intracellular storage or assimilation into the biomass was approximately 70% at day 0 and was less than 20% at day 36. Despite the high variation in glucose uptake as described below, the maximum recovery of glucose as labeled acetate was fairly constant at 44 to 47% during the reduction phase and 62% during methanogenic phase, respectively (Table 2), assuming that two-thirds of glucose carbon was maximally incorporated into acetate as glucose was degraded by glycolysis (17).

Similar to glucose consumption at day 0, acetate was not consumed until 6 h after its addition (Fig. 3). When $[\text{2-}^{14}\text{C}]$ acetate was added to the soil at days 0 and 5, only $^{14}\text{CO}_2$, but no $^{14}\text{CH}_4$, was produced. Thus, similar to glucose metabolism, acetate was metabolized oxidatively to CO_2 . On the other hand, the end product of acetate metabolism at day 10 was mainly $^{14}\text{CH}_4$ (RI = 0.22; Table 1). This indicates that at day 10, acetoclastic methanogenesis was already established. During the active CH_4 formation at day 36, almost all of the $[\text{2-}^{14}\text{C}]$ acetate was converted to CH_4 (RI = 0.07; Fig. 4 and

FIG. 4. Uptake and product formation from $[U-^{14}C]$ glucose (A) and $[2-^{14}C]$ acetate (B) in anoxic rice soil slurry at day 36. Values are means $\pm$ SD of duplicate determinations. Note the logarithmic time scale.

Table 1). The rate of total CH_4 production measured during the radioactive experiment at day 36 was $39.7 \text{ nmol g}^{-1} \text{ h}^{-1}$. The recovery of radioactive acetate after 12 h generally ranged from 70 to 96%, indicating that only a small portion of acetate was assimilated into microbial biomass.

Turnover of glucose and acetate. We used $[2-^{14}C]$ acetate instead of $[U-^{14}C]$ acetate or $[1-^{14}C]$ acetate for two main reasons: (i) to account for acetate-dependent CH_4 production during the methanogenic phase of the soil (17, 35) and (ii) to avoid isotopic exchange between the carboxyl position of acetate and CO_2 . This exchange process is known to be rapid and may be a source of bias when acetate turnover is estimated from $[1-^{14}C]$ acetate or $[U-^{14}C]$ acetate (9).

The uptake rate constant of glucose changed with the date of anaerobic soil incubation (Table 1). It was lowest at day 0 (0.3 h^{-1}), highest at day 5 (28.6 h^{-1}), and stayed relatively constant (6.7 to 7.9 h^{-1}) thereafter. Consequently, the turnover time of glucose ranged from 3 h at day 0 to about 2 min at day 5. The change in the uptake rate constant together with the change in the glucose pool size (Table 1) led to a change in the glucose turnover rate from $28 \text{ nmol g}^{-1} \text{ h}^{-1}$ at day 0 to $733 \text{ nmol g}^{-1} \text{ h}^{-1}$ at day 5 and then to $26 \text{ nmol g}^{-1} \text{ h}^{-1}$ at day 36. For acetate, the uptake rate constant was less variable than for glucose. The lowest acetate uptake rate constant ($k = 0.07 \text{ h}^{-1}$) was found at day 10, when the acetate pool size attained its maximum (Fig. 2). Relatively slow acetate uptake was also

TABLE 1. Turnover of glucose and acetate in anaerobic rice soil at different dates of soil incubation^a

Product	Rate constant (h^{-1}) ^b	r^2	n	P	Pool size (nmol g^{-1})	Turnover rate ($\text{nmol g}^{-1} \text{ h}^{-1}$)	Turnover time (h)	RI	Recovery of radioactivity in products (%)
Day 0									
Glucose	0.31	0.96	5	0.004	89	27.7	3.2	1.00	31.4
Acetate	0.07	0.97	4	0.015	309	22.9	13.5	1.00	68.5
Day 5									
Glucose	28.6	0.89	3	0.208	26	733.4	0.04	1.00	58.1
Acetate	0.03	0.94	6	0.001	233	6.5	35.7	1.00	78.5
Day 10									
Glucose	6.7	0.96	3	0.122	5	36.8	0.15	0.82	53.1
Acetate	0.02	0.98	4	0.009	1,336	21.4	62.5	0.22	95.7
Day 36									
Glucose	7.9	0.94	3	0.058	3	25.9	0.13	0.76	83.5
Acetate	0.22	0.98	7	<0.0001	130	28.7	4.5	0.07	68.9

^a Each value represents the mean of two replicates. The quality of the fit is given by r^2 and P .

^b Rate constants were calculated by linear regression of n data pairs.

TABLE 2. Acetate production from glucose catabolism at different times of rice field soil incubation^a

Day of incubation	Recovery of [¹⁴ C]glucose as [¹⁴ C]acetate (%) ^b	Rate of acetate production from glucose (nmol g ⁻¹ h ⁻¹) ^c	Total acetate turnover rate (nmol g ⁻¹ h ⁻¹)	% Acetate turned over via glucose	Total CH ₄ production (nmol g ⁻¹ h ⁻¹)	CH ₄ production from acetate (nmol g ⁻¹ h ⁻¹)	
						(1 - RI) × acetate turnover rate	(SR _{CH₄} /SR[2- ¹⁴ C] _{acetate}) × total CH ₄ production
0	44.5	12.3	22.9	54			
5	43.8	321.3	6.5	4,928			
10	47.2	17.4	21.4	81			
36	62.4	16.2	28.7	56	39.7	26.7	20.4

^a Each value represents the mean of two replicates.^b Recovery of [¹⁴C]glucose as [¹⁴C]acetate, assuming that two-thirds of the radioactivity of glucose is maximally converted to acetate.^c Glucose turnover rate × fraction (percent) of acetate production from glucose.

found at day 5 ($k = 0.03 \text{ h}^{-1}$), when glucose degradation was fastest. Acetate uptake was fastest at day 36 ($k = 0.22 \text{ h}^{-1}$). As a result, the acetate turnover times ranged from >60 h at day 10 to about 5 h at day 36. The pore water concentration of acetate varied over a range of 130 to 1,336 nmol g⁻¹. Thus, the turnover rates of acetate were between 6 nmol g⁻¹ h⁻¹ at day 5 and approximately 29 nmol g⁻¹ h⁻¹ at day 36.

DISCUSSION

Incorporation of organic matter such as rice straw into rice field soil is a traditional farming practice for enhancing soil nutrient availability and productivity (13, 26). In Italian rice field soil, rice straw has been annually incorporated into the soil when the field is plowed after drainage and harvest. Microbial breakdown of rice straw (ca. 50% cellulose and 20 to 30% hemicellulose on a dry weight basis [36]) releases soluble carbohydrates which become part of the dissolved organic carbon pool available for soil microbes (21, 32, 38). Glucose was the most abundant monosaccharide in rice field soil, similar to the case in freshwater and marine ecosystems (3, 10, 11, 15, 16, 24, 41). The concentrations of glucose (3 to 90 μM) and total dissolved monosaccharides (5 to 136 μM) in the Italian rice field soil were in the same range as values reported in the literature.

The dominant glucose-utilizing microorganisms are not known definitely, but polysaccharolytic clostridia with the capacity to ferment glucose have been isolated (4). The formation of acetate and other fatty acids from glucose (18) indicates that fermentative microorganisms were important. Glycolysis has been shown to be the glucose degradation pathway in Italian rice field soil (18). Through this pathway, both CO₂ and acetate would be simultaneously produced. However, we observed that the labeled glucose was consumed relatively faster than the formation of the products and that acetate was released faster than CO₂. King and coworkers (16, 30) suggested that the observed difference between [U-¹⁴C]glucose uptake and ¹⁴C-end product formation is most likely due to differences in the specific radioactivity of extracellular and intracellular glucose pools. The slower formation of ¹⁴C-end product can be explained by the transport of [U-¹⁴C]glucose from extracellular pools with a relatively high specific radioactivity to intracellular pools with a lower specific radioactivity.

Uptake of glucose varied greatly throughout the incubation period. The most rapid uptake of glucose and acetate was found at days 5 and 36, respectively. At day 5, rapid glucose uptake was accompanied by a relative slow acetate uptake and therefore resulted in the accumulation of acetate during days 5 to 10 (Fig. 2). It is likely that a similar imbalance between glucose degradation and acetate consumption is the reason for the accumulation of acetate that has usually been observed in

other rice fields within 1 or 2 weeks of flooding (14, 33, 39). Nitrate reducers and ferric iron reducers were shown to be able to utilize acetate efficiently (1), while acetotrophic sulfate reducers were not so numerous and active in anoxic rice soil (2, 42). However, since soil nitrate is normally consumed within a few hours of anoxic incubation (1, 17), ferric iron reducers instead of nitrate reducers would play the most important role for acetate consumption during the reduction phase. We observed that acetate uptake was much faster during the methanogenic phase than during the reduction phase, probably due to activation and/or growth of acetate-utilizing methanogens. Hence, population shifts may be responsible for variations in glucose and acetate uptake during the incubation period.

The amount of [U-¹⁴C]glucose recovered as labeled acetate was used to estimate the rate of acetate production from glucose (Table 2). Glucose apparently served as an important precursor of acetate in rice field soil. Acetate production from glucose alone accounted for 54, 81, and 56% of the acetate turned over at days 0, 10, and 36, respectively. Thus, during these dates, it is assumed that acetate was additionally produced from other substrates (e.g., dissolved combined carbohydrates [11, 24]) to balance the amount of acetate that was simultaneously consumed. On the other hand, at day 5, the rate of acetate production from glucose was considerably greater than the rate of acetate consumption. It is noted that the rate of glucose consumption, and consequently the rate of acetate production from glucose at day 5, was so high that its persistence until day 10 would have resulted in the accumulation of 321 nmol of acetate per g of soil. However, the actual amount of acetate that accumulated between days 5 and 10 was much less (ca. 15 nmol g⁻¹), indicating that the high rate of glucose turnover at day 5 persisted only for a short time.

From the experiment using labeled acetate, we know that 93% of the acetate was converted to CH₄ (RI = 0.07; Table 1) at day 36. By assuming that CH₄ was only produced from acetate or H₂-CO₂, the rate of CH₄ production from acetate was estimated to be 20 to 27 nmol g⁻¹ h⁻¹, or 51 to 67% of the total rate of CH₄ production (Table 2). These fractions of CH₄ produced from acetate (51 to 67%) agree reasonably well with the value of 66% that is theoretically expected when carbohydrates are methanogenically degraded (6) and also agree with earlier estimates of fractions of CH₄ produced from H₂-CO₂ (28). We also estimated the flow of carbon from glucose to CH₄ (via acetate) by multiplying the rate of CH₄ production from acetate with the percentage of acetate produced from glucose resulting in 32 to 42% of total CH₄ formation. A similar range (30 to 40%) was also given by other studies (16, 22), indicating that glucose degradation via acetate to CH₄ is of general importance in methanogenic systems.

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