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Turnover of propionate in methanogenic paddy soil

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Abstract

Samples from planted Italian paddy soil exhibited most probable numbers (MPN) of about 10^7 anaerobic propionate utilizers. In anoxic soil slurries that were either unamended or amended with rice straw production of CH_4 was measured together with concentrations of H_2, acetate and propionate. After a lag phase, during which ferric iron was depleted, CH_4 was produced at a constant rate which was slightly higher in the straw-amended than in the unamended soil. Propionate concentrations were relatively low at about 5–15 μM. However, in the straw-amended soil propionate transiently accumulated to about 35 μM just after onset of methanogenesis. During the period of propionate accumulation H_2 partial pressures were elevated and the Gibbs free energy (ΔG) of propionate consumption to acetate, bicarbonate and H_2 was endergonic or higher than -3 kJ mol^{-1} propionate. Propionate concentrations decreased again when the ΔG decreased to more negative values. In unamended paddy soil, propionate did not accumulate transiently and ΔG was always < -6 kJ mol^{-1} propionate. Propionate radiolabelled in the C-1 or C-2 position was utilized with turnover times of 30–60 min. Propionate turnover rates approximately accounted for the rates of H_2/CO_2-dependent methanogenesis that were measured in experiments with [^{14}C]bicarbonate. The only radioactive product of [1-^{14}C]propionate was $^{14}CO_2$. However, [2-^{14}C]propionate was converted to radioactive acetate, CO_2 and CH_4. This observation indicates that propionate was consumed via a randomizing pathway to CO_2 and acetate, the latter being then further degraded by acetotrophic methanogens to CO_2 and CH_4. Turnover of [1-^{14}C]propionate was almost completely inhibited by high H_2 concentrations, chloroform or molybdate. The MPN of bacteria that utilized propionate either in syntrophy with methanogens or by reduction of sulfate was identical. All these observations suggest that propionate was consumed by a syntrophic randomizing pathway, probably by bacteria that have also the capacity to reduce sulfate.

Keywords: Methanogenesis; Propionate degradation pathway; Gibbs free energy; Syntrophy; Hydrogen; Acetate; Iron

1. Introduction

Projected global human population levels indicate that the demand for rice as food will increase from 460 to 760 Tg $year^{-1}$ by the year 2020 [1]. This growing demand can only be met by intensified rice production and will most likely increase the production and emission of CH_4 from the wetland rice fields if current agricultural management technolo-

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gies continue [1]. Methane is an important greenhouse gas and its atmospheric abundance is presently increasing [2]. For the development of mitigation options for CH_4 emissions from rice fields it is necessary to gain a detailed knowledge of the processes that are involved in the production of CH_4 in the paddy soil.

Methane is a product of the anaerobic degradation of organic matter. It is produced by a complex microbial community that consists of many different hydrolytic, fermenting, acetogenic, syntrophic and methanogenic bacteria [3–6]. In paddy soil, acetate and H_2 are the two most important immediate precursors for CH_4 formation [7–9]. Carbon flow through acetate contributes about 50–90% to the total carbon flow to CH_4 [8,10,11]. A significant part of the acetate seems to be produced by homoacetogenic bacteria [10,11] which ferment many different substrates (e.g. sugars, alcohols, aromatic compounds) to acetate as the only product [12,13].

Acetate may, however, also be produced by syntrophic bacteria that convert the products (e.g. fatty acids and alcohols) of fermenting bacteria to acetate, CO_2 and H_2 [4,12]. Some syntrophs produce formate in addition to or instead of H_2 [14,15]. In paddy soil, syntrophic bacteria play an important role in the turnover of H_2 which takes place by interspecies H_2 transfer within microbial associations of syntrophic and methanogenic bacteria [16,17]. Syntrophic bacteria apparently contribute significantly to the production of both acetate and H_2 (or formate), the immediate precursors of methanogenesis. However, the turnover of syntrophic substrates in methanogenic paddy soil has not yet been investigated.

Next to acetate, propionate is the most abundant fatty acid in rice field soils [18] and is also found in Italian rice fields [9]. Propionate was shown to be an intermediate in the degradation of glucose to CH_4 [19] and to accumulate when CH_4 production is inhibited [11]. It also accumulated transiently when syntrophic metabolism was inhibited by thermodynamically non-permissive concentrations of H_2, suggesting that syntrophic bacteria were involved in the degradation of propionate [11].

The pathway of propionate degradation has mostly been studied in defined bacterial cultures, methanogenic enrichment cultures and anaerobic digesters [20–27]. Propionate degradation has to our knowledge not been studied in natural methanogenic environments, with one exception (two lake sediments [24]). Propionate degradation in these methanogenic systems was usually found to follow a pathway in which C-2 and C-3 of propionate are randomized before being converted to the methyl (C-2) and carboxyl (C-1) groups of acetate [20–27]. Such a randomization is typical for the succinate pathway [4,6]. In contrast, C-2 of propionate would be only converted to C-1 of acetate if no randomization takes place, as is the case for propionate degradation via acrylyl-CoA [28]. In both pathways, the carboxyl group (C-1) of propionate is always liberated as CO_2, by decarboxylation of either oxaloacetate or pyruvate. However, Tholozan et al. [29,30] observed, in a methanogenic digester, a non-randomizing degradation of propionate that involved a reductive carboxylation so that C-1 of propionate was converted to C-2 of butyrate. Degradation by this pathway coupled to methanogenesis would result in the production of CH_4 from C-2 of propionate.

In methanogenic paddy soil the pathway of propionate degradation has so far not been investigated. Therefore, we studied the turnover of ^{14}C-labelled propionate in Italian paddy soil.

2. Materials and methods

The soil samples were collected during the unflooded (winter) season from rice fields of the Italian Rice Research Institute in Vercelli, Italy. The characteristics of the soil have been reported previously [31]. The soil was filled into plastic containers, flooded, planted with germinated rice seedlings (*Oryza sativa*, var. Roma, type japonica) and incubated under flooded conditions in the greenhouse of our institute. Soil samples from microcosms were used to determine the population size of bacteria. The samples were taken 10 days after planting the rice seedlings.

Soil slurries were prepared at the end of the growth season (about 120 days after planting the rice seedlings). The rice plants were cut, the submerged soil was drained and allowed to dry under air. The dry soil lumps were then broken and passed through a stainless steel sieve (mesh size = 2 mm). One portion of the soil was amended with rice straw

(1 mg straw g^{-1} dry weight (dw) soil). Soil samples (150 g dw) were suspended at a weight ratio of 1:1 in distilled degassed sterile water (giving 0.75 g dw soil ml^{-1} slurry) and incubated in sterile Erlenmeyer flasks (1120 ml volume) which were closed with sterile latex stoppers and incubated under an atmosphere of N_2 at 30°C without shaking. The temporal change of gases (CH_4, H_2, CO_2), of dissolved compounds (acetate, propionate, sulfate), of iron species, and of pH was followed with time by taking samples. The gases were analyzed by gas chromatography [17,32]. After brief vigorous shaking by hand, gas samples (1 ml) were taken from the headspace using gas-tight pressure-lock syringes and analyzed immediately. Liquid samples (about 5 ml) were taken from an outlet at the bottom of the flasks. An aliquot was centrifuged at $13\,000 \times g$ for 7 min, filtered through 0.2 μm membrane filters (regenerated cellulose, Sartorius, Göttingen, Germany) and stored frozen at −20°C until analysis of fatty acids by high pressure liquid chromatography [19]. Another aliquot was used to analyze ferrous iron by photometric measurement of the red-violet complex formed with ferrozine, and to analyze total extractable iron after its reduction to ferrous iron by treatment with hydroxylamine [33]. The concentration of ferric iron was calculated from the difference between total iron and ferrous iron.

After CH_4 production had reached a constant rate and propionate and acetate concentrations had reached steady state, subsamples were taken from the soil slurries for the measurement of propionate turnover and of CH_4 production from H_2/CO_2. For measuring propionate turnover, aliquots (15–20 ml) of the slurry were transferred from the Erlenmeyer flasks into serum bottles (50 ml volume) which were closed with black rubber stoppers and gassed with N_2. CO_2 was injected to give approximately the same partial pressure (11–13 kPa) which existed in the headspace of the Erlenmeyer flasks. In some experiments, 200 μM $CHCl_3$ (inhibitor of methanogens) or 5 mM sodium molybdate (inhibitor of sulfate reducers) was added, or H_2 (inhibitor of syntrophs) was added to the gaseous headspace to give an overpressure of 0.5 bar. Experiments were started by injection of 15–20 μl of carrier-free sodium [^{14}C]propionate (about 14–18.5 kBq) into each bottle and incubation at 30°C. The [1-^{14}C]propionate

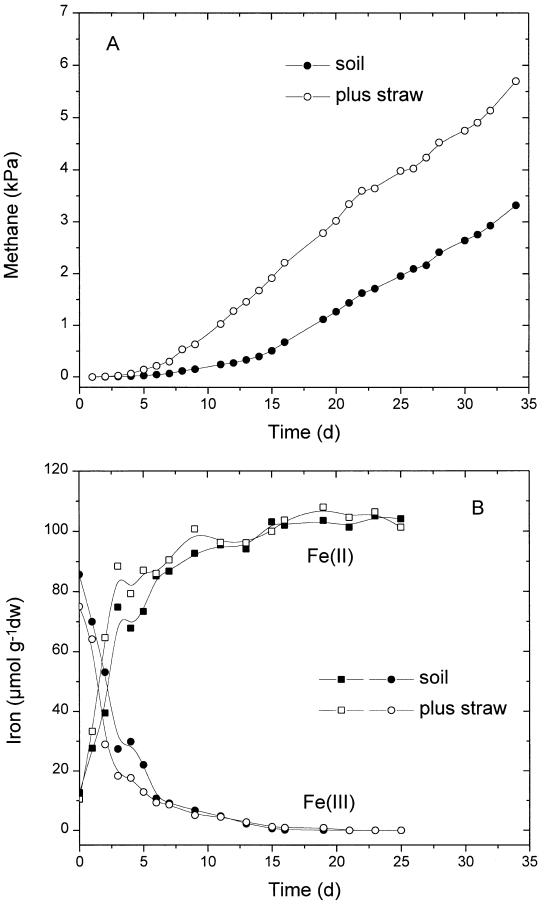

Fig. 1. (A) Production of methane and (B) changes in ferrous and ferric iron contents in anoxic slurries of unamended and straw-amended Italian paddy soil.

(1.850 GBq $mmol^{-1}$) was obtained from ICN (Costa Mesa, CA, USA), the [2-^{14}C]propionate (1.998 GBq $mmol^{-1}$) was obtained from Hartmann Analytic (Braunschweig, Germany). After addition of the [^{14}C]propionate, gas samples (0.5 ml) were taken repeatedly and analyzed for radioactive and non-radioactive CH_4 and CO_2 using a gas chromatograph with a radioactivity detector [17]. Liquid samples (1 ml) were also taken repeatedly with a syringe, the soil slurry was centrifuged and the supernatant stored frozen until analysis of radioactive and non-radioactive dissolved compounds using high pressure liquid chromatography with refractive index and radioactivity detectors [19]. The recovery of radioactivity from the gas phase and the pore water was in a range of 80–120%.

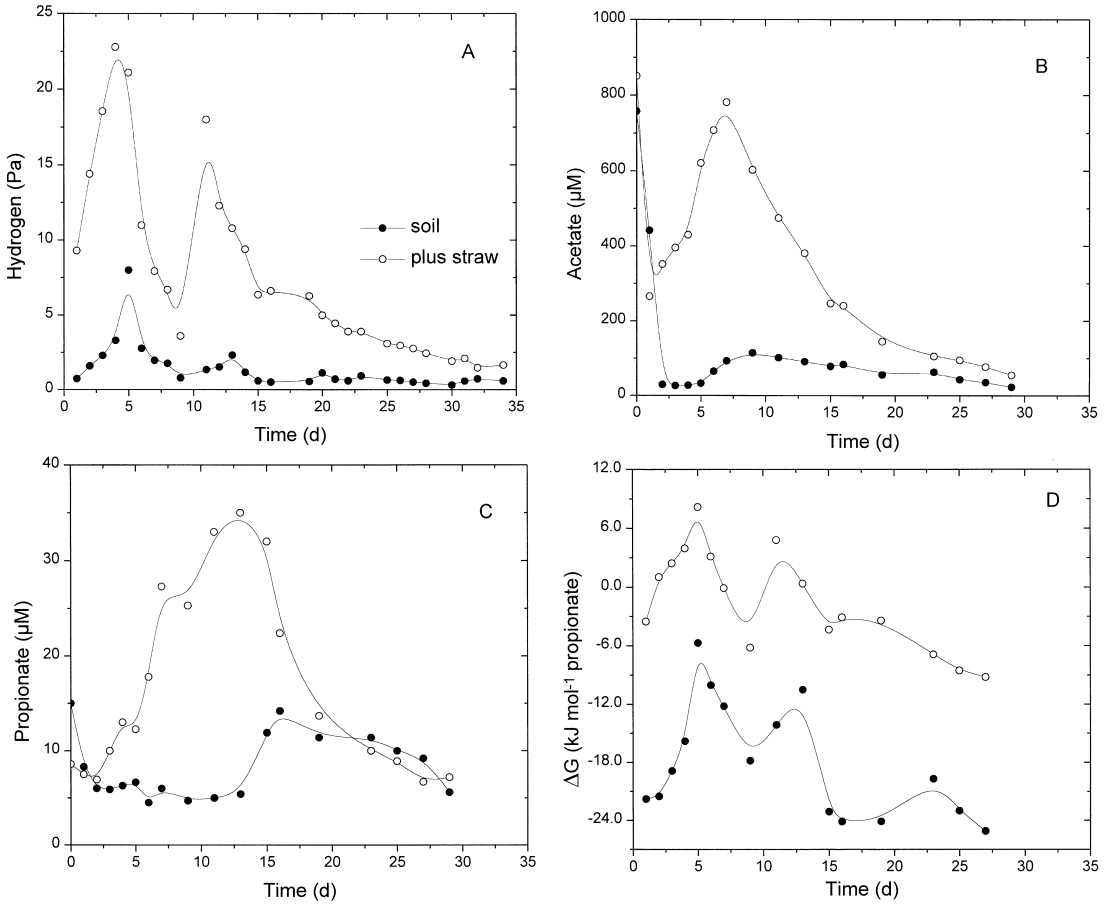

Fig. 2. (A) Partial pressures of hydrogen, concentrations of (B) acetate and (C) propionate, and (D) Gibbs free energy changes of syntrophic propionate consumption in anoxic slurries of unamended and straw-amended Italian paddy soil.

For determination of the H_2/CO_2-dependent methanogenesis, aliquots (15 ml) of the slurry were transferred into pressure tubes (27.5 ml) which were closed with black rubber stoppers, gassed with N_2 and incubated at 30°C. The experiment was started by injection of approximately 200 μl of carrier-free $NaH^{14}CO_3$ (about 72–85 kBq; 1.92 GBq $mmol^{-1}$; Amersham-Buchler, Braunschweig, Germany). Gas samples (0.5 ml) were taken repeatedly and analyzed for radioactive and non-radioactive CH_4 and CO_2 using a gas chromatograph with a radioactivity detector [17]. The fraction of H_2/CO_2-dependent methanogenesis was calculated from the specific radioactivities of CH_4 and CO_2 obtained after addition of $NaH^{14}CO_3$ [17].

All experiments give mean values of duplicate experiments. The variation between the duplicate determinations was typically 2% for CH_4, 1% for CO_2, 20% for H_2, 1% for iron, 6% for acetate, 8% for propionate.

The Gibbs free energies of propionate consumption at 30°C were determined from the actual H_2 and CH_4 partial pressures, the actual propionate, acetate and bicarbonate concentrations and the pH, as described by Chin and Conrad [11].

Bacteria were counted by the most probable number (MPN) technique using 10-fold serial dilutions in growth medium [34] and testing the tubes for production of CH_4, H_2S and/or acetate after 11 weeks incubation at 25°C. The growth media consisted of the dilute medium (DM) of Janssen et al. [35] amended with (1) 10 mM sodium propionate plus

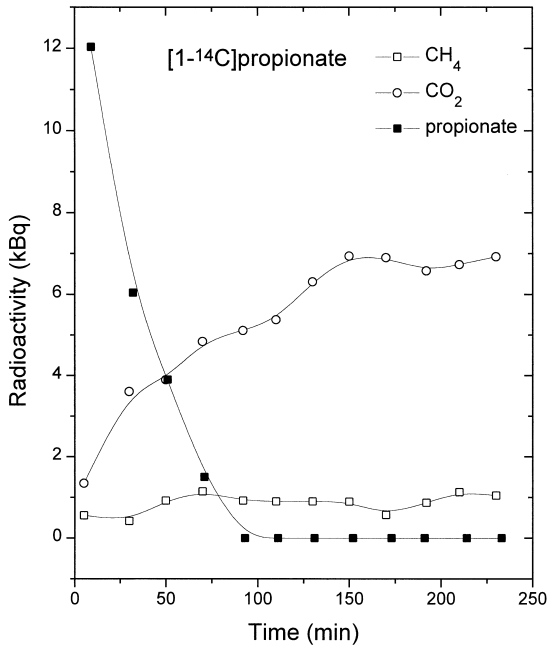

Fig. 3. Conversion of [1-^{14}C]propionate to ^{14}C-labelled products in straw-amended methanogenic paddy soil. The values give total radioactivity per bottle, but do not include the dissolved CO_2 and bicarbonate.

1 ml of a culture of *Methanospirillum hungatei* JF1 (DSM 864); (2) 10 mM sodium propionate plus 10 mM sodium sulfate; (3) a gas phase of 80% H_2 plus 20% CO_2 (including 1 mM acetate as additional carbon source); or (4) 4 mM glucose, for counting syntrophic propionate utilizers, sulfate-reducing propionate utilizers, methanogens and anaerobic heterotrophic bacteria, respectively. Tubes were scored as positive when >500 ppmv CH_4, >1 mM acetate or H_2S (color test by [36]) were detected, and the MPN calculated from published tables [37].

3. Results

Most probable numbers of propionate-utilizing anaerobic bacteria were determined in planted rice soil. The 95% confidence limit was about one order of magnitude. Syntrophic propionate utilizers and sulfate-reducing propionate utilizers had the same titer (10^7 cells g^{-1} dw). That of H_2-utilizing methanogenic bacteria (5×10^7 cells g^{-1} dw) was similar. However, heterotrophic anaerobic bacteria (utilizing glucose) exhibited a much higher titer of 2×10^{10} cells g^{-1} dw.

Incubation of straw-amended and unamended paddy soil slurries under anoxic conditions resulted in linear production of CH_4 after a lag phase of about 7 and 15 days, respectively (Fig. 1A). The CH_4 production rate was somewhat higher (19.9 nmol h^{-1} g^{-1} dw) in the straw-amended than in the unamended soil (13.1 nmol h^{-1} g^{-1} dw). Reduction of Fe(III) started right from the beginning of the incubation; about 95% of the available Fe(III) was reduced after the first 6 days in both the straw-amended and the unamended soil (Fig. 1B). Sulfate had been completely reduced during this period (not shown). The start of linear CH_4 production in the straw-amended soil coincided with the time when 95% of the Fe(III) had been reduced. In the unamended soil, however, the lag phase of CH_4 production lasted until all of the Fe(III) had been reduced, i.e. after 15 days.

The shorter length of the lag phase of CH_4 production in the straw-amended compared to the un-

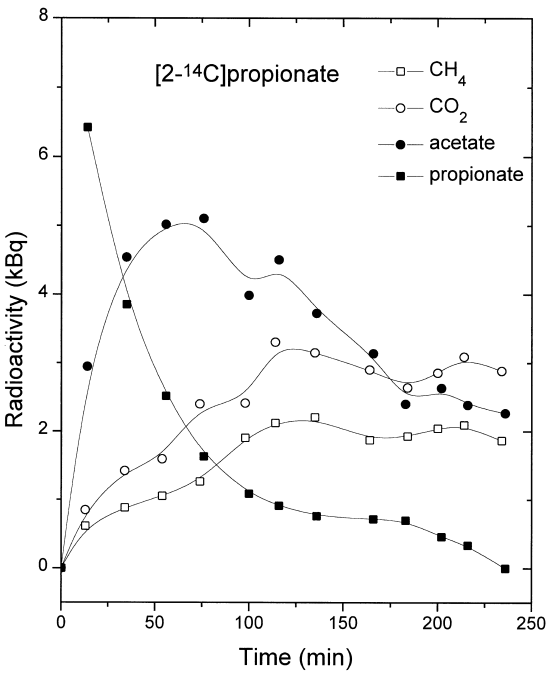

Fig. 4. Conversion of [2-^{14}C]propionate to ^{14}C-labelled products in straw-amended methanogenic paddy soil. The values give total radioactivity per bottle, but do not include the dissolved CO_2 and bicarbonate.

Table 1
Turnover rate constants (k), transformation rate constants (k_g), and turnover rates (P, P_g) of propionate determined with [1-^{14}C]propionate and [2-^{14}C]propionate in paddy soil slurries at 30°C, compared to rates of CH_4 production from H_2

Parameter	Unamended soil		Straw-amended soil	
	[1-^{14}C]	[2-^{14}C]	[1-^{14}C]	[2-^{14}C]
k [h^{-1}]	1.59	1.10	2.79	1.52
k_g [h^{-1}]	0.92	1.40	1.11	1.35
P [nmol h^{-1} g^{-1} dw]	7.95	5.50	13.9	7.60
P_g [nmol $h^{-1}g^{-1}$ dw]	4.60	7.00	5.55	6.75
CH_4 production [nmol $h^{-1}g^{-1}$dw]	13.1		19.9	
CH_4 from H_2[a]	5.4		6.6	
CH_4 from propionate via acetate[b]	5.5–7.0		6.7–7.6	
CH_4 from propionate via H_2[c]	4.1–5.2		5.1–5.7	

[a]Calculated from the rate of total CH_4 production times the fraction of CH_4 produced from $H_2/H^{14}CO_3^-$.
[b]Identical to the rate of propionate turnover.
[c]Calculated from the stoichiometric conversion of 4 propionate to 12 H_2 to 3 CH_4.

amended soil was probably caused by the generally higher partial pressures of H_2 (Fig. 2A) and higher concentrations of acetate (Fig. 2B). However, at the end of incubation, the H_2 partial pressures and acetate concentrations approached a similar steady state value in the two soil treatments. Propionate transiently accumulated in the straw-amended soil between day 3 and 18 (Fig. 2C). At this time Fe(III) reduction had already slowed down (Fig. 1B) and methanogenesis slowly started (Fig. 1A) so that propionate was probably being consumed by syntrophic bacteria. At the end of incubation (day 30), propionate concentrations reached a similarly low concentration of 6–8 μM in both soil treatments (Fig. 2C) with a tendency to approach 5 μM, i.e. the detection limit of our analytical system.

The standard Gibbs free energy at 30°C of propionate consumption (propionate$^-$ +3 $H_2O \rightarrow$ acetate$^-$ +H^+ +bicarbonate$^-$ +3 H_2) is endergonic (ΔG° = +115.1 kJ mol^{-1} propionate; see [11]). The actual Gibbs free energies (ΔG) in the soil slurries were calculated from the actual concentrations and partial pressures of the reactants and products. Values of ΔG (Fig. 2D) generally increased when H_2 partial pressures increased (Fig. 2A). The ΔG values were more positive in the straw-amended than in the unamended soil. Propionate consumption in straw-amended soil was either endergonic or only slightly exergonic, whereas in the unamended soil it was always exergonic. In the straw-amended soil, ΔG values were greater than approximately −3 kJ mol^{-1} propionate until day 15 (Fig. 2D), i.e. in the time during which propionate transiently accumulated (Fig. 2C).

Radiolabelling experiments were started from day 35 of the incubation onward. Incubation of the soil slurries with $NaH^{14}CO_3$ resulted in the production of $^{14}CH_4$. The percentage contribution of H_2/CO_2 to total CH_4 production that was measured between day 2 and day 8 of the incubation was 33 ± 2% (mean ± S.E.M.) and 41 ± 2% in the straw-amended and unamended soil, respectively.

Turnover of ^{14}C-labelled propionate was measured with [1-^{14}C]propionate (Fig. 3) and [2-^{14}C]propionate

Table 2
Residual activity of the turnover of [1-^{14}C]propionate after addition of different inhibitors

Inhibitor	Residual activity [%]	
	Unamended soil	Straw-amended soil
H_2 (5 kPa)	12	4
$CHCl_3$ (200 μM)	3	8
Molybdate (5 mM)	5	4

The turnover rate constants of the uninhibited controls (= 100% residual activity) are given in Table 1.

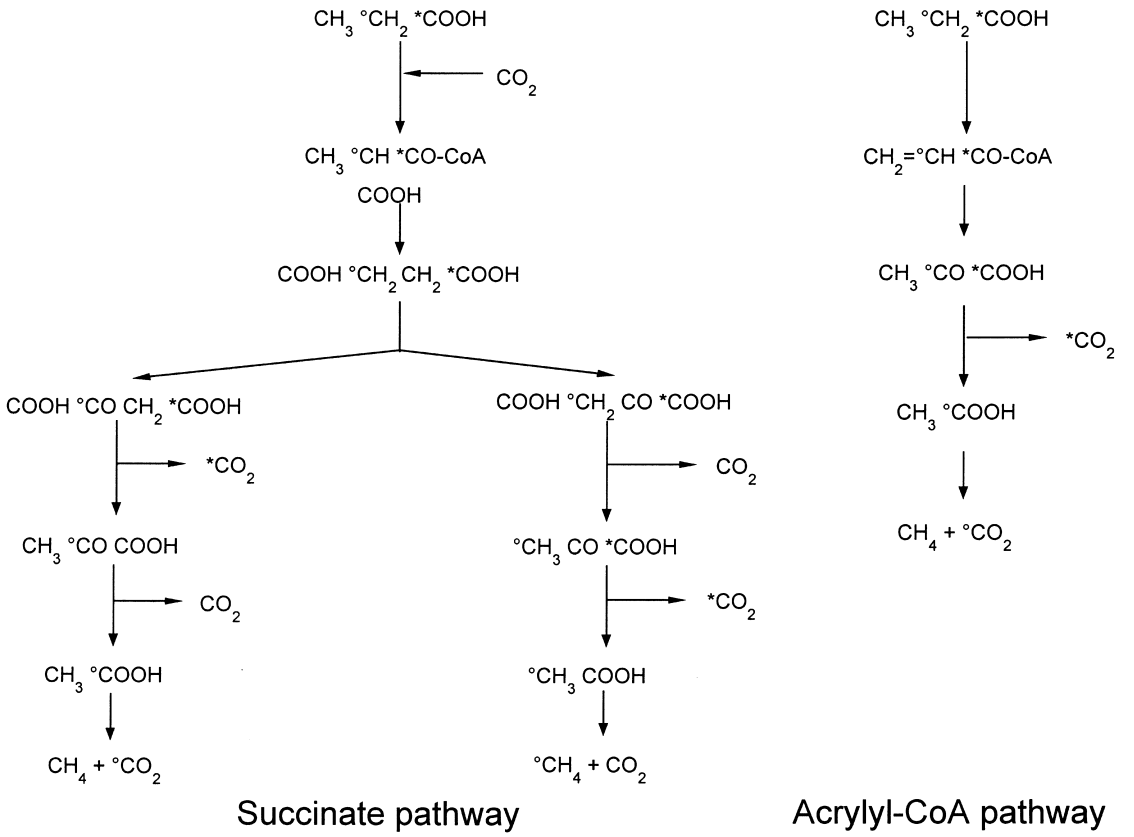

Fig. 5. Labelling scheme of intermediates and products of the methanogenic degradation of [1-^{14}C]propionate and [2-^{14}C]propionate via the succinate pathway and the acrylyl-CoA pathway.

(Fig. 4). The ^{14}C-labelled propionate was consumed within 100–200 min. With [1-^{14}C]propionate, the ^{14}C was recovered exclusively as $^{14}CO_2$ but not as $^{14}CH_4$ (Fig. 3). No radioactivity was detected in formate, acetate or butyrate. With [2-^{14}C]propionate, on the other hand, ^{14}C was incorporated into acetate, CO_2 and CH_4 (Fig. 4). No radioactivity was detected in formate or butyrate. The final radioactivity in CH_4 was about half that in CO_2. The propionate turnover rate constants were determined from the logarithmic decrease of the radioactivity in propionate. The propionate transformation rate constants were determined from the logarithmic increase of radioactivity in CH_4 plus CO_2. The results are summarized in Table 1. The turnover rate constants were slightly higher than the transformation rate constants and were both higher in the straw-amended than in the unamended soil. Propionate turnover rates were calculated by multiplying the rate constants with the steady state propionate concentrations which was at the time of measurement ≤ 5 μM for both unamended and straw-amended soil (Table 1).

The turnover of [1-^{14}C]propionate was inhibited by high H_2 partial pressures (Table 2) which also created a positive ΔG of propionate consumption to acetate, bicarbonate and H_2 (results not shown). Propionate turnover was also inhibited by chloroform, an inhibitor of methanogenic bacteria. However, inhibition was only achieved if the soil was preincubated with chloroform to allow the accumulation of H_2 (Table 2). Finally, propionate turnover was also inhibited by molybdate, an inhibitor of sulfate-reducing bacteria (Table 2). The residual activities were usually $< 10\%$ of the uninhibited control.

4. Discussion

Propionate was rapidly turned over in methanogenic paddy soil. The turnover time was in the order of 30–60 min and thus much faster than the turnover of acetate which is in the order of hours [8,10,19]. Because of this rapid consumption, propionate concentrations were relatively low in the methanogenic soil slurries, except in straw-amended paddy soil during a phase of transient propionate accumulation. During this phase, syntrophic propionate consumption was endergonic or only slightly exergonic. As soon as the Gibbs free energy became < -3 kJ mol^{-1} propionate, propionate concentrations decreased again. Interestingly, propionate consumption operated at a relatively small negative value of ΔG. Similar small values (i.e. ΔG of -3 to -15 kJ mol^{-1} propionate) during phases in which propionate was degraded were reported earlier in anoxic paddy soil [11], natural wetlands [38,39], and methanogenic digesters [40,41].

In our methanogenic paddy soil, $^{14}CO_2$ was the only detectable product of [1-^{14}C]propionate degradation. Therefore, the operation of reductive degradation of propionate via butyrate such as observed in a methanogenic digester [29,30] can be excluded. Radiolabelled butyrate was not observed in our experiments. Instead, experiments with [2-^{14}C]-propionate showed that propionate was degraded via acetate to CH_4 and CO_2. The fact that C-2 of propionate was recovered in both CO_2 and CH_4 shows that propionate degradation was largely due to a randomizing pathway such as the succinate pathway (Fig. 5). The operation of this pathway has already been reported for digester sludge and lake sediments [24]. In the paddy soil slurries, the rate of CO_2 production from C-2 of propionate was always somewhat greater than that of CH_4. However, it is unclear whether this observation really indicates the additional operation of a non-randomizing pathway such as the acrylyl-CoA pathway (Fig. 5). A slightly higher production of CO_2 relative to CH_4 was also observed during [3-^{14}C]propionate degradation in Lake Mendota sediment [24]. Similarly, Tholozan et al. [30] reported a slightly higher labelling of C-1 versus C-2 of acetate from [2-^{13}C]propionate in cultures of *Desulfobulbus elongatus* and cocultures of *Syntrophobacter wolinii* plus *Desulfovibrio*. Both degrade propionate by the succinate pathway (reviewed by Stams [6]). Therefore, more detailed tracer experiments, including the study of labelling patterns of C-1 and C-2 of acetate, will be necessary to clarify whether an additional non-randomizing pathway of propionate degradation is also operative in methanogenic paddy soil.

The turnover of propionate in methanogenic paddy soil was inhibited at elevated H_2 partial pressures, indicating the involvement of syntrophic bacteria that require low H_2 concentrations for thermodynamic reasons. In straw-amended soil slurries, propionate concentrations increased until day 15, when syntrophic propionate degradation was endergonic or only slightly exergonic due to elevated H_2 partial pressures and acetate concentrations. However, a doubling of the H_2 partial pressure has a 4 times larger effect than a doubling of the acetate concentration. Propionate concentrations decreased again when the Gibbs free energy of propionate degradation became more negative than -3 kJ mol^{-1} propionate. Active methanogenesis was obviously required for maintenance of low H_2 partial pressures, since inhibition of CH_4 production by chloroform eventually resulted also in inhibition of degradation of [1-^{14}C]propionate. Apparently, chloroform inhibited hydrogenotrophic methanogenesis and the resulting increase in the H_2 partial pressure caused the inhibition of propionate degradation. Interestingly, degradation of [1-^{14}C]propionate was also inhibited by molybdate, an inhibitor of sulfate-reducing bacteria, although sulfate-reduction was not taking place. Molybdate has been shown to inhibit *Desulfovibrio desulfuricans* growing syntrophically with methanogens in the absence of sulfate [42]. These observations suggest that bacteria with the ability to reduce sulfate may act as the propionate-degrading syntrophic bacteria. This assumption is in agreement with the observed degradation pattern of position-labelled propionate in our paddy soil slurries, since this pattern was similar to that observed in cultures of either syntrophic bacteria or sulfate-reducing bacteria (reviewed by [6]). Furthermore, most probable number counts of propionate-utilizing bacteria indicated similar population sizes of syntrophic and sulfate-reducing propionate utilizers in

methanogenic paddy soil. Circumstantial evidence suggests that sulfate reducers may function as syntrophic partners in methanogenic aggregates in Lake Mendota sediment [43]. Sulfate reducers are obviously involved in syntrophic propionate degradation in methanogenic digesters [44,45]. *Syntrophobacter wolinii*, the classical syntrophic propionate utilizer, was found to be able to reduce sulfate [46]. A syntrophic propionate utilizer that was isolated from a methanogenic digester also turned out to be able to reduce sulfate [47]. Finally, syntrophic propionate utilizers in general seem to be phylogenetically related to sulfate-reducing bacteria [47–49].

The turnover rate constants obtained with [1-^{14}C]propionate were generally higher than those obtained with [2-^{14}C]propionate. The higher turnover may be due to an exchange reaction of the carboxyl group of propionate with CO_2 as shown in methanogenic digester sludge [23] and in cultures of propionate-utilizing bacteria [27]. Since $^{14}CO_2$ was the only product of [1-^{14}C]propionate degradation, the propionate transformation rate constant (calculated from the increase of $^{14}CO_2$) should be equal to the propionate turnover rate constant (calculated from the decrease of [^{14}C]propionate) [50]. The generally lower transformation rate constants may be due to a slow equilibration of the products (CO_2 and CH_4) with the gas phase, where the measurements were made. Conversely, the turnover rate constants may be overestimated due to exchange of labelled propionate with a bound (e.g. on mineral surfaces) fraction of unlabelled propionate. Since the discrepancies are smaller and there is no effect due to an exchange of the carboxyl group, we assume that the true turnover rate constant of propionate is between the transformation rate constant and the turnover rate constant of [2-^{14}C]propionate. Using these data we arrive at propionate turnover rates of 5.5–7.0 and 6.7–7.6 nmol h^{-1} g^{-1} dw for unamended soil and straw-amended soil, respectively (Table 1). Considering the uncertainties in the determination of propionate concentrations and turnover rate constants these rates compare reasonably well with the CH_4 production rates from H_2/CO_2 by assuming that all of the H_2 was produced by syntrophic propionate utilizers (Table 2).

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