Nutrient Cycling in Agroecosystems **64:** 59–69, 2002.

Control of microbial methane production in wetland rice fields

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Key words: methanogenic archaea, microbial community, mitigation, rice root, rice straw, syntrophy, temperature, thermodynamics

Abstract

Methane emission rates are a function of production, transport and oxidation of CH_4 in the rice field. Production of CH_4 is the prerequisite for any flux. The most important variables that control CH_4 production include soil type, rice variety, temperature, soil redox potential, water management and fertilization with organic carbon or nitrogen. The effects of these variables have empirically been assessed on a macroscopic scale. However, the actual mechanisms by which these variables affect the microbial CH_4 production on a microscopic scale are little understood. The purpose of the present contribution is to review existing knowledge of microbiological data and microscopic processes that are relevant for the control of CH_4 production. These include the flow of carbon and electrons during the anaerobic degradation process, thermodynamic constraints of reactions in-situ and changes in the composition of the microbial community.

Introduction

Wetland rice fields are an important source in the global budget of atmospheric CH_4 which has been increasing in abundance and contributes to global warming (Schütz et al., 1991; Shearer and Khalil, 1993; Crutzen, 1995). Since rice fields are managed environments, it is feasible to contemplate possible strategies for mitigation of CH_4 emission (Buendia et al., 1997; Yagi et al., 1997; Mosier et al., 1998). For this purpose it is important to know by which factors CH_4 emission is controlled. The number of studies measuring CH_4 fluxes in rice fields under different conditions, in different regions and at different times of the year have increased dramatically since the first field study in 1981 (Cicerone and Shetter, 1981). These field observations and a number of laboratory studies have been used to describe the general patterns of CH_4 emission and to deduce the major variables that affect CH_4 emission. This kind of data has resulted in IPCC guidelines for determination of national CH_4 inventories (Houghton et al., 1997) and has been used for the development of empirical and semi-empirical models (Cao et al., 1995; Potter, 1997; Huang et al., 1998; Khalil et al., 1998a).

The most important variables that control CH_4 emission from rice fields have been described and reviewed (Conrad, 1993; Minami, 1994; Sass and Fisher, 1997; Segers, 1998; Neue and Roger, 2000; Aulakh et al., 2001). These variables include soil type, rice variety, temperature, soil redox potential, water management, fertilization with organic carbon or nitrogen. The effects of these variables have empirically been determined. They affect either one of the principal processes that control CH_4 emission, i.e. the production, the transport and the oxidation of CH_4 in the rice soil-plant system (Conrad, 1989, 1993; Schütz et al., 1991). However, although production, transport and oxidation of CH_4 are obvious processes on a macroscopic scale (e.g. rice fields), they are again the result of a multitude of processes that operate on a microscopic scale (e.g. soil microsites, root surface). It is these microscopic processes that are ultimately controlling the flux of CH_4 form rice fields into the atmosphere. Although most of these microscopic processes are too detailed and sophisticated to be included in mechanistic flux models, the understanding of these processes will increase the confidence in the accuracy of the flux models.

The anaerobic microbiology of flooded rice fields

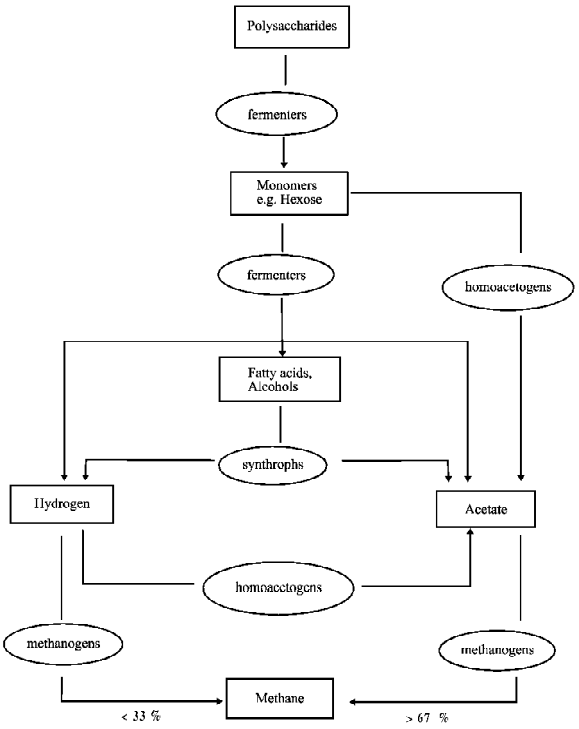

Figure 1. Scheme of the pathway of anaerobic microbial degradation of polysaccharides to methane.

has recently been reviewed in detail by Kimura (2000) and Liesack et al. (2000). The purpose of the present article is to review existing knowledge that explains the effects on CH_4 emission by macroscopic variables (e.g. soil type, water management etc.) on a smaller, microscopic scale. I will focus on processes that are involved in the production of CH_4.

Methanogenic degradation of organic matter

Biological CH_4 production is exclusively caused by anaerobic methanogenic microorganisms that are phylogenetically affiliated with the domain *Archaea*, one of the three domains of life (*Bacteria*, *Archaea*, *Eucarya*). Methanogenic archaea can only use simple substrates. Thus, methanogens living in freshwater ecosystems produce CH_4 from either acetate or H_2/CO_2 (Conrad, 1989, 1999). Acetate, H_2, and CO_2 are products of the microbial degradation of organic matter (in rice fields mainly polysaccharides) under anoxic conditions. The entire methanogenic degradation process involves the following reactions of which each is catalyzed by a specific physiological group of microorganisms (Figure 1): (1) Hydrolysis of polysaccharides followed by fermentation of sugars to fatty acids (mainly propionate), acetate, H_2, and CO_2; (2) alternatively fermentation to only acetate (homoacetogenesis); (3) syntrophic conversion of fatty acids (e.g. propionate) to acetate, H_2, and CO_2; (4) homoacetogenic conversion of H_2/CO_2 to acetate; (5) methanogenic conversion of H_2/CO_2 to CH_4; and (6) methanogenic disproportionation of acetate to CH_4 and CO_2.

Under balanced conditions, the polysaccharides are anaerobically degraded to 50% CO_2 and 50% CH_4, the latter originating by >66% from acetate and <33% from H_2/CO_2 (Conrad, 1999). However, balanced conditions, when all the individual degradation operate in quasi steady state, are only established when the soil stays anoxic for several weeks. Quasi steady state conditions are also characterized by constant and low concentrations of the methanogenic substrates H_2 (<2 Pa) and acetate (<100 μM). Concentrations of H_2 and acetate are much higher in the beginning of anoxia (Table 1). At this time, processes are not yet balanced, and fermentation reactions produce acetate faster than it can be consumed by the methanogens (Chidthaisong et al., 1999). In the end, however, quasi steady state is reached during which CH_4 and CO_2 are produced at equal rates. Deviation from this degradation pattern indicates an imbalance in the electron budget which, for example, can occur when the oxidation status of soil organic matter changes during decomposition (Yao and Conrad, 2000a).

Initiation of CH_4 production upon flooding of rice fields

Textbooks describe methanogenic archaea to be killed by exposure to O_2 and to be highly sensitive to redox potentials of $E_h > -200$ mV. This characteristic seems to be consistent with the observation that CH_4 production in rice fields is inversely correlated to the E_h of soil (Wang et al., 1993; Yagi et al., 1996). However, this concept is not completely correct.

Many methanogens, those isolated from soil in particular, are neither very sensitive to high redox potentials nor to exposure to O_2 (Fetzer et al., 1993; Fetzer and Conrad, 1993). Some methanogenic species were even found to live specifically in oxic habitats (Leadbetter and Breznak, 1996) and apparently are even able to consume O_2 if supplied at low concentrations (Brune, pers. comm.). Indeed, methanogens in rice field soil easily survive desiccation after drain-

Table 1. Microbial processes determining the different phases of CH_4 production after flooding of soil

Phase	Processes	Duration (d)
0	General lag phase	<1
1	Fermentation produces H_2 and acetate	<10
	Iron reducers and sulfate reducers are still inactive	
	Production of CH_4 is limited by the methanogens themselves	
2	Iron reducers and sulfate reducers become active	<30
	They deplete H_2	
	Production of CH_4 is suppressed by the deficiency of H_2	
3	Iron(III) and sulfate are depleted	<30
	Iron reducers and sulfate reducers become inactive	
	Concentration of H_2 increases again (acetate still high)	
	Production of CH_4 is thus possible at a relatively a high rate	
4	Fermentation is getting limited by hydrolysis of biopolymers	<100
	Acetate and H_2 reach relatively low but constant values	
	Rates of CH_4 production decrease to relative constant value (steady state)	

age and maintain their population size at a constant level throughout the year (Mayer and Conrad, 1990; Asakawa and Hayano, 1995). Molecular analysis of environmental DNA indicates that the relative composition of the major methanogenic and archaeal taxa in rice field soil changes only slightly between dry-oxic and wet-anoxic soil states (Lueders and Friedrich, 2000; Ramakrishnan et al., 2000).

Methanogens in rice field soil are inhibited, though not killed, by exposure to O_2. However, they are apparently not inhibited by positive E_h provided that the substrate H_2 is available at a concentration that is sufficient for exergonic CH_4 production (Roy et al., 1997; Yao and Conrad, 1999). Studies of numerous rice field soils from China, the Philippines and Italy demonstrated that CH_4 production is initiated immediately after inundation at still positive E_h values. This early initiation was found in virtually all soils tested and was permitted due to active H_2 production by fermenting bacteria (Table 1). Production of CH_4 continued as long as the Gibbs free energy of the CH_4-producing reaction was more exergonic than about -25 kJ mol^{-1} CH_4 (Yao and Conrad, 1999). This amount of energy corresponds to the minimum energy quantum that bacteria theoretically can use (Schink, 1997). The availability of H_2 and thus the magnitude of the Gibbs free energy depends on whether other H_2-utilizing microorganisms become active. Bacteria that utilize H_2 by reducing ferric iron or sulfate are able to deplete H_2 to concentration levels that are no longer permissible for exergonic CH_4 production (Table 1).

The extent and duration for which CH_4 production is suppressed mainly depends on the ratio of available organic matter to reducible ferric iron (Yao et al., 1999; Watanabe and Kimura, 1999). In many rice field soils, this ratio is so low, that after an initial production phase, methanogenesis stops until most of the ferric iron is reduced. Then, methane production starts again, and it is this second and persistent phase of CH_4 production which coincides with a low E_h. At constant temperature, this phase sooner or later turns into steady state with relatively constant CH_4 production rates (Yao et al., 1999). However, since many soils exist which have a high ratio of organic matter to ferric iron (or sulfate), CH_4 production often starts right from the beginning of inundation (Yao and Conrad, 1999).

Stimulation of CH_4 production by rice straw

Addition of organic matter such as rice straw to the soil stimulates the production of CH_4 and enhances CH_4 emission from rice fields (Schütz et al., 1989; Yagi and Minami, 1990; Sass et al., 1991; Denier van der Gon and Neue, 1995; Chidthaisong et al., 1996; Rath et al., 1999). Rice straw consists of cellulose (32–37%), hemicellulose (29–37%), lignin (5–15%) and contains inorganic components such as silica (Tsutsuki and Ponnamperuma, 1987; Watanabe et al., 1993). Rice straw is rapidly colonized by bacteria (Kimura and Tun, 1999) which mainly consist of clostridia belonging to the taxonomic clostridial

clusters I, III and XIVa (Weber et al., 2001). These clostridial clusters are known to consist of hydrolytic and fermenting bacteria, and thus explain the rapid accumulation of a large variety of fatty acids upon addition of straw to rice soil (Glissmann and Conrad, 2000). Aromatic compounds also accumulate (Tsutsuki and Ponnamperuma, 1987). However, the accumulation of these fermentation products is only transient, and after a few weeks, when the easily degradable straw polysacharides are exhausted, the fermentation pattern in straw-amended soil is virtually the same as in unamended soil, with acetate and propionate being the only important fermentation products (Glissmann and Conrad, 2000). One may speculate that much of the degradable soil organic matter is nothing else than tiny straw particles that have been left over from previous years, thus explaining why the degradation of more recent rice straw is qualitatively not different from that of soil organic matter. This conclusion is supported by the observation that the bacterial community colonizing rice straw is only moderately different from that present in unamended soil (Weber et al., 2001; Chin et al., 1999a; Hengstmann et al., 1999). In the end, rice straw is becoming an integral part of soil organic matter and, with a mean half life about 2 years, is becoming increasingly recalcitrant to further degradation (Neue and Scharpenseel, 1987).

Nevertheless, CH_4 production keeps being enhanced after a single addition of straw over periods of weeks and months, only gradually decreasing with time. Methane production seems to be limited by the hydrolysis of polysaccharides which itself is limited by the accessibility of the polymers rather than be the activity of the hydrolytic enzymes (Glissmann and Conrad, 2002). Hence, the entire degradation process to CH_4 seems to be in steady state, but at a quantitatively higher level than in unamended soil. This concept is in agreement with the observation that although the density of microbial colonization of straw increases with time (Kimura and Tun, 1999), the diversity and activity of the bacterial colonizers does not (Weber et al., 2001).

Syntrophic propionate degradation

Next to acetate propionate is usually the most important fermentation product in rice field soil, the production being stimulated by addition of rice straw (Krylova et al., 1997). Many of the fermenting bacteria that are abundant in rice soil seem to produce propionate as a fermentation product (Chin et al., 1999a). Propionate can only be degraded in syntrophy with H_2-consuming methanogens because of thermodynamic constraints (Krylova and Conrad, 1998). Even in the presence of H_2-consuming methanogens, the Gibbs free energy available to propionate-degrading syntrophs (catalyzing: Propionate$\rightarrow$Acetate+CO_2+3 H_2) is usually not more exergonic than about -10 kJ mol^{-1} propionate in microbial culture (Scholten and Conrad, 2000) and is on the average only about -7 kJ mol^{-1} propionate in various rice field soils (Yao and Conrad, 2001). Hence, the syntrophic propionate degraders are tightly constrained by thermodynamics. Nevertheless, they can provide nearly all the H_2 that is required to drive CH_4 production from CO_2 reduction which is up to a third of total CH_4 production (Krylova et al., 1997). Besides rice straw, propionate metabolism is also enhanced on rice roots. There, accumulation of H_2 plus acetate from fermenting bacteria can result in propionate synthesis by the reversal of the syntrophic degradation reaction (Conrad and Klose, 1999, 2000).

Control of CH_4 production by water management

Drainage of rice fields results in decrease of CH_4 emission rates. This immediate reaction is plausible, since O_2 can better penetrate into non-flooded soil and thus suppress CH_4 production. However, suppression of CH_4 production usually persists for quite some time even when fields are flooded again (Yagi et al., 1996). Even short-term drainage is sufficient for rather long-term suppression of CH_4 emission. The reason for this behavior was found in the regeneration of oxidants during the short drainage and aeration period (Sigren et al., 1997; Ratering and Conrad, 1998). The penetration of O_2 into the soil allows the oxidation of reduced sulfur to sulfate and of ferrous iron to ferric iron. Sulfate and Fe(III) allow the operation of sulfate-reducing and iron-reducing bacteria, respectively. These bacteria utilize acetate and H_2, the two most important methanogenic substrates, more efficiently than the methanogens. As a consequence, concentrations of H_2 and acetate decrease to values that are no longer thermodynamically permissive for CH_4 production, and CH_4 production stops (Sigren et al., 1997; Ratering and Conrad, 1998). The same effect is observed when sulfate or ferric iron are added to methanogenic soil (Achtnich et al., 1995; Chidthaisong and

Conrad, 2000). Sulfate, for example, may be added by application of ammonium sulfate fertilizers. Normally, however, iron is the quantitatively more important oxidant in rice field soils, usually accounting for >50% of the organic carbon that is oxidized to CO_2 during the season (Yao et al., 1999). Therefore, any regeneration of ferric iron during drainage may result in a dramatic reduction of total CH_4 production.

Recent studies in Italian rice fields showed that relatively high concentrations of ferric iron (and to a minor extent also of sulfate) in soil resulted in acetate conversion to CO_2 rather than to CH_4. In parallel, acetate concentrations decreased and so did CH_4 production rates (Krüger et al., 2001). The residual CH_4 production was then largely due to CO_2 reduction, which in turn influenced the ^{13}C-isotopic composition of the produced CH_4 (Krüger et al., 2002). Hence, drainage not only affects the quantity but also the quality of CH_4 production.

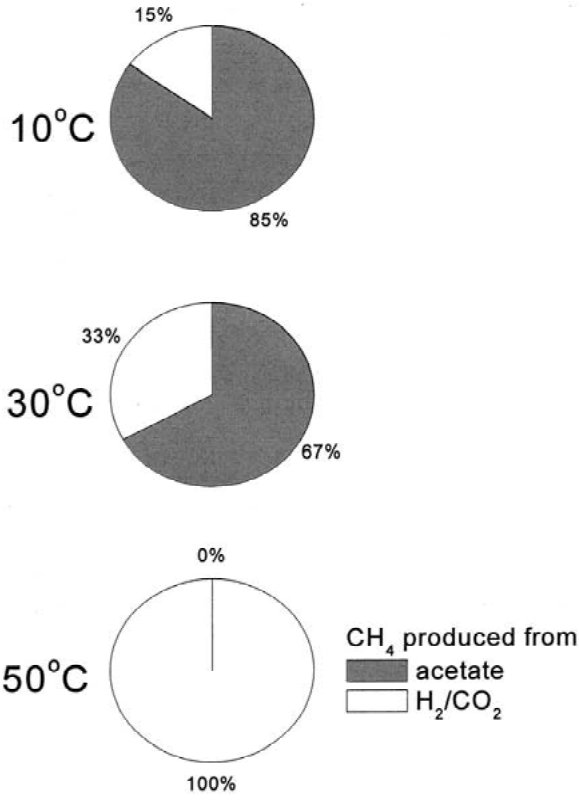

Figure 2. Effect of temperature on the percentage contribution of acetate and H_2/CO_2 to methanogenesis in anoxic rice soil.

Control of CH_4 production by temperature

Temperature affects the rates of all microbiological reactions similarly as chemical reactions. Temperature dependence can in principle be described by the Arrhenius equation, ideally by assuming a constant apparent activation energy or a constant Q_{10}. This is trivial. However, temperature effects are more complex. Immediately after flooding, the reduction of ferric iron and sulfate is enhanced by increasing temperature so that steady state CH_4 production is reached earlier (VanHulzen et al., 1999; Yao and Conrad, 2000b). Thus, the beginning of CH_4 production critically depends on temperature.

At steady state, temperature not only affects the rate of CH_4 production, but also significantly affects the pathway of carbon flow (Chin and Conrad, 1995; Fey and Conrad, 2000). The pathway changes since some microbial processes are more sensitive to temperature than others. At low temperature, for example, the formation of acetate is favored relative to the formation of propionate and thus, CH_4 is increasingly produced from acetate rather than from H_2/CO_2 (Figure 2). At intermediate temperature, on the other hand, H_2/CO_2 contributes about 33% to total CH_4 production, as theoretically expected for methanogenic degradation of polysaccharides (Conrad, 1999). Rice soil apparently contains thermophilic microorganisms which allow, albeit after adaptation, CH_4 production at temperatures as high as 50 °C. At this high temperature, CH_4 is exclusively produced from H_2/CO_2, while acetate is no longer consumed and accumulates (Fey et al., 2001). The temperature-dependent shift in the methanogenic pathway greatly affects the stable isotopic signature of the produced CH_4, as the fractionation of carbon and hydrogen isotopes depends on the biochemical mechanism of CH_4 formation (Krzycki et al., 1987; Burke, 1993; Gelwicks et al., 1994; Botz et al., 1996; Summons et al., 1998; Whiticar, 1999). Indeed, the $\delta^{13}C$ of CO_2, CH_4 and acetate in rice field soil was found to depend on temperature (Fey and Conrad, in prep.).

Temperature also affects the growth of microbial populations and consequently may result in a nonlinear response of CH_4 production to temperature (Schütz et al., 1990). Indeed, the community of methanogens in Italian rice field soil was found to be different at low (15 °C) than at high (30 °C) temperature (Chin et al., 1999b). *Methanosaeta* species in rice soil seem to be able to adapt to a larger temperature range than *Methanosarcina* species which seem to prefer higher temperatures (Chin et al., 1999b; Wu

et al., 2001). The two groups of methanogens have also different kinetic characteristics (K_m, threshold) with respect to acetate utilization, and *Methanosaeta* species are able to exploit lower acetate concentrations than *Methanosarcina* species (Jetten et al., 1990). The ratio of *Methanosaeta/Methanosarcina* indeed was found to dynamically change with temperature and acetate concentration in rice soil slurries (Chin et al., 1999b; Lueders and Friedrich, 1999; Fey and Conrad, 2000). Such changes in the methanogenic community were found to affect the pathway and rate of CH_4 production, and in addition also the resistance and resilience towards stress situations, e.g. caused by aeration, water management or temperature change (Wu and Conrad, 2001; Wu et al., 2002).

Role of rice plants for the production of CH_4

Rice plants have different effects on CH_4 production. The extent of these effects strongly depends on the type of rice cultivar. For example, Fe(III) is permanently regenerated from Fe(II) in the rhizosphere of the plants. Rice plants not only allow the diffusion of CH_4 from the soil into the atmosphere (Holzapfel et al., 1985; Schütz et al., 1991; Nouchi and Mariko, 1993), but also allow the diffusion of O_2 from the atmosphere through their aerenchyma system into the roots. From there O_2 partially leaks into the soil (Armstrong, 1979; Frenzel et al., 1992; Revsbech et al., 1999) and thus allows various oxidation processes in the rhizosphere, e.g. of CH_4 to CO_2 (Bosse and Frenzel, 1997; Gilbert and Frenzel, 1998), ammonium to nitrate (Reddy et al., 1989; Arth et al., 1998), sulfide to sulfate (Wind and Conrad, 1997), and Fe(II) to Fe(III) (Begg et al., 1994). The regeneration of Fe(III) in the rhizosphere results in partial suppression of CH_4 production within the upper soil layers where most of the rice roots are found (Frenzel et al., 1999; Ratering and Schnell, 2000).

Rice cultivars with increased ventilation capacity were claimed to emit more CH_4 over the season (Butterbach-Bahl et al., 1997; Watanabe and Kimura, 1998). This effect was discussed to be caused by a more efficient CH_4 oxidation in the rhizosphere when CH_4 is less rapidly ventilated (Watanabe and Kimura, 1998). It is unclear, whether decreased ventilation would also impede the regeneration of oxidants, Fe(III) in particular, and thus release the suppression of CH_4 production.

A further effect of rice plants is the supply of additional organic matter to the soil microbial community. The organic matter is derived from rhizodeposition, i.e. root exudation, sloughed-off cells and decay of roots. The CO_2 that is photosynthetically assimilated by the rice plants is partially excreted from the roots, fermented to acetate and H_2, and finally converted into CH_4 (Dannenberg and Conrad, 1999). About a third of total CH_4 emission is derived from photosynthetically assimilated CO_2 (Watanabe et al., 1999), thus emphasizing the importance of rice plants for CH_4 production. Rice cultivars with more root exudation also tend to emit more CH_4 (Wang et al., 1997; Watanabe and Kimura, 1998).

Most of the CH_4 produced from root exudation is probably generated by the soil microflora in some distance to the roots. However, the root surface itself is also colonized by methanogenic archaea. The composition of the root methanogenic flora is different from the soil methanogenic flora (Großkopf et al., 1998a, b; Lehmann-Richter et al., 1999). The root surface is also colonized by fermenting bacteria which are able to produce H_2, acetate, propionate and butyrate at high rates (Conrad and Klose, 1999, 2000; Rosencrantz et al., 1999). More than 50% of CH_4 produced on the roots is derived from H_2/CO_2, the remainder from acetate (Lehmann-Richter et al., 1999; Conrad et al., 2000). However, much of the acetate is also produced homoactogenically, so that the conversion of H_2 plus CO_2 to acetate is followed by acetoclastic methanogenesis (Figure 3). Obviously, the methanogenic pathway on roots is different from that in soil and thus, may affect the $\delta^{13}CH_4$ of the produced CH_4 (Conrad et al., 2000). However, it is unclear how much the root-produced CH_4 is in comparison to the soil-produced CH_4.

Effect of fertilization and options for mitigation of CH_4 production

The acetate-utilizing methanogens that colonize the root surface are sensitive to phosphate (Conrad et al., 2000). This may explain why addition of phosphate fertilizer results in decreased CH_4 emission (Lu et al., 1999). However, phosphate fertilization also decreases the total amount of root exudation by decreasing the root/shoot ratio (Lu et al., 1999). Nevertheless, phosphate deficiency possibly stimulates CH_4 production and consequently, application of sufficient phosphate

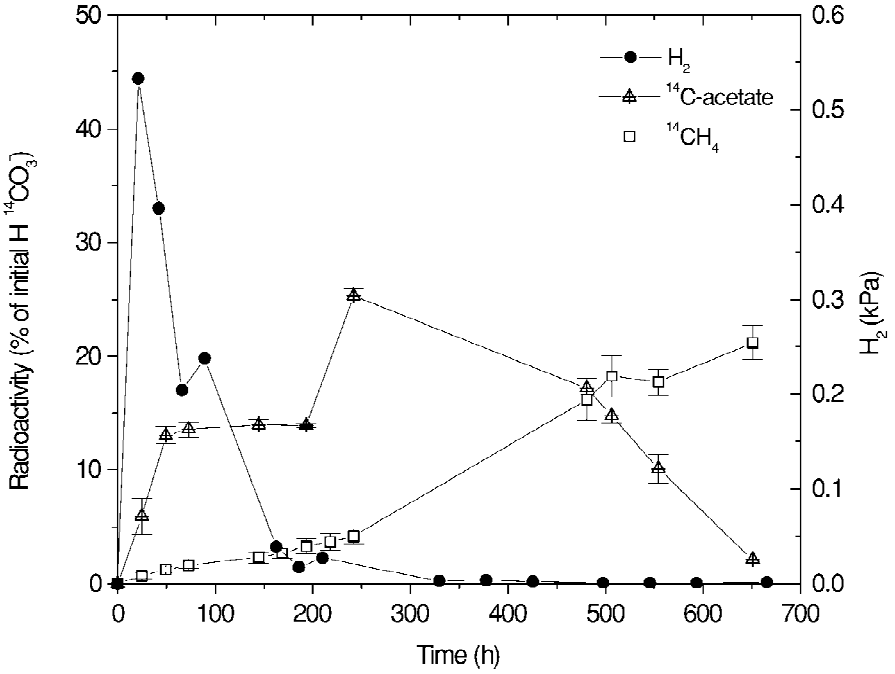

Figure 3. Homoacetogenesis from H_2/CO_2, followed by acetoclastic methanogenesis on rice roots shown by the conversion of radioactive bicarbonate to acetate and CH_4.

would mitigate CH_4 emission from rice fields. In addition, crop yields would be maximized.

Production of CH_4 is inhibited by nitrate for the same reason as it is inhibited by sulfate or ferric iron, i.e. nitrate reducers utilize acetate and H_2 more efficiently than methanogens (Achtnich et al., 1995; Klüber and Conrad, 1998a; Chidthaisong and Conrad, 2000). In addition, CH_4 production is inhibited by nitrite, NO and N_2O that accumulate transiently during the reduction of nitrate and are toxic for methanogens (Klüber and Conrad, 1998b). Nitrate is not a customary nitrogen fertilizer in rice agriculture. Instead, ammonium or urea fertilzers are applied. However, nitrate is rapidly produced from these fertilizers in the rhizosphere (Reddy et al., 1998; Arth et al., 1998) and thus may contribute to suppression of CH_4 production in rooted soil. Hence, nitrogen fertilization contributes to the mitigation of CH_4 emission without compromising crop yields.

In addition, nitrogen fertilization stimulates the CH_4-oxidizing bacteria in the rhizosphere so that CH_4 emission can be further reduced (Bodelier et al., 2000a, b). Of course, nitrogen fertilizers must not be applied in too high quantities to avoid the eventual emission of the greenhouse gas N_2O which would be a trade-off to the mitigated CH_4. However, N_2O emission from flooded rice fields is usually low and only becomes significant during drainage periods (Bronson et al., 1997; Hua et al., 1997; Cai et al., 1997; Khalil et al., 1998b; Suratno et al., 1998). Nitrogen fertilization can also enhance the emission of NH_3 which, however, may largely be avoided by deep placement of the fertilizer (DeDatta, 1995; Gaudin and Dupuy, 1999).

A further mitigation option is fertilization with iron. Increased soil iron contents seem to guarantee that the rhizosphere always keeps sufficient ferric iron contents to suppress CH_4 formation (Watanabe and Kimura, 1999; Jäckel and Schnell, 2000). Field experiments with iron fertilization also resulted in decreased CH_4 emissions without compromising crop yields (Jäckel and Schnell, in prep.).

Conclusions

A large diversity of microorganisms is to some extent involved in the production of CH_4. This diversity is not only large with respect to the various functional groups of microorganisms (fermenting bacteria, iron reducers, methanogens etc.) but is also large with respect to the different populations being active within one particular functional group, e.g. the methanogens

which are dominated by different species at different temperatures, or when comparing soil and roots. The different population structures obviously affect the pathway and/or rate of CH_4 formation. However, CH_4 production is not only affected by the direct methane producers themselves, but also by other microbial populations that influence the availability of methanogenic substrates. It is the interaction between the various microorganisms which eventually determines the rate of CH_4 production over time. A good example for these interactions is the following. Upon flooding of soil, different phases of CH_4 production can be observed. Usually, CH_4 production starts after an extended lag phase, then CH_4 production rates accelerate and finally, rates decrease again and become more or less constant. Yao et al. (1999) called these three phases 'reduction phase', 'methanogenic phase' and 'steady state phase'. Table 1 illustrates these different phases in the light of the principal microbial processes that interact and ultimately limit CH_4 production. The phases 0–3 are analogous to the reduction phase, phases 3 and 4 to the methanogenic and steady state phases, respectively. The understanding of the ultimate causes of CH_4 production events on a microbial and microscopic process level will help to formulate process models with better confidence.

Acknowledgement

I thank Dr P. Frenzel for critically reading the manuscript.

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