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Microbial processes of CH₄ production in a rice paddy soil: Model and experimental validation

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Abstract—The importance of different anaerobic processes leading to CH₄ production in rice paddies is quantified by a combination of experiments and model. A mechanistic model is presented that describes competition for acetate and H₂/CO₂, inhibition effects and chemolithotrophic redox reactions. The model is calibrated with anaerobic incubation experiments with slurried rice soil, monitoring electron donors and electron acceptors influencing CH₄ production. Only the values for maximum conversion rates (V_{\max}) for sulphate and iron reduction and CH₄ production are tuned. The model is validated with similar experiments in which extra electron donors or electron acceptors had been added. The differences between model estimates without kinetic parameter adjustments and experiment were not significant, showing that the model contains adequate process descriptions. The model is sensitive to the estimates of V_{\max} , that are site dependent and to the description of substrate release, that drives all competition processes. For well-shaken systems, the model is less sensitive to chemolithotrophic reactions and inhibitions. Inhibition of sulphate reduction and methanogenesis during iron reduction can however explain acetate accumulation at the start of the incubations. Iron reduction itself is most probably retarded due to manganese reduction. Copyright © 2001 Elsevier Science Ltd

1. INTRODUCTION

In rice fields, small organic compounds become available by root exudation, root decay, organic fertilizer decomposition and soil organic matter mineralization. Methane (CH₄) production is the terminal microbial process in anaerobic organic matter degradation in the absence of alternative electron acceptors like O₂, NO₃⁻, Fe(III), and SO₄²⁻. CH₄ production is suppressed directly or indirectly by the presence of alternative electron acceptors (e.g., Achnich et al., 1995a; Jakobsen et al., 1981). This suppression seems to be caused by competition for common substrates, particularly H₂ and acetate, with micro-organisms using alternative electron acceptors and by several direct inhibitions on CH₄ production caused by accumulated products of anaerobic respiring micro-organisms. Quantification and significance of these processes for CH₄ production are a major uncertainty in CH₄ production prediction.

Due to complex interactions, it is difficult to obtain complete quantitative insight in all interactions by experiments alone, despite the fact that many experimental data are available. A combination of experimental data and a quantitative mechanistic model may lead to a better quantitative understanding of the interactions. In this paper, we present a new mechanistic model, calibrated and validated by experimental data. This model differs from other models in several important aspects: (i) the model describes the conversions of frequently measured anaerobic alternative electron acceptors, in contrast to other models which summarize the alternative electron acceptors by one

alternative electron acceptor (Segers and Kengen, 1998), neglect all alternative electron acceptors (Grant, 1998; Vavilin et al., 1994) or treat only SO₄²⁻ (James, 1993; Lovley and Klug, 1986). (ii) We describe substrate competition by Michaelis–Menten rate expressions, which are more appropriate than first- or second-order rate expressions (used by Boudreau, 1996; van Cappellen and Wang, 1996) if different limitations occur in time or if saturation for a certain compound can occur. (iii) In addition to competition effects, direct inhibitions due to alternative electron acceptors are introduced. These effects are usually neglected in competition descriptions (e.g. Gupta et al., 1994; James, 1993). (iv) Contrary to e.g. Grant (1998) and James (1993), it is assumed that changes in microbial biomass are small. A constant biomass is taken, which simplifies model parameterization and model extrapolation, because scarce data on mortality and growth of anaerobic bacteria are not needed. (v) The model includes chemolithotrophic redox reactions. Omission of these reactions may cause overestimation of organic matter oxidation.

The objective of this paper is to obtain more quantitative insight in the importance of microbial interactions leading to CH₄ production in well-shaken incubation systems. A quantitative model is described, calibrated, and validated by data from several incubation experiments. The model summarizes mechanistic interactions between bacteria using alternative electron acceptors and CH₄ producing micro-organisms. Transport descriptions are not included in the model, because it is applied to well-shaken systems only and the model cannot be used to predict field gradients (Hunter et al., 1998). Various aspects of the microbial interactions are discussed in more detail and quantified by model sensitivity analyses. These analyses also reveal gaps in knowledge.

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2. MATERIALS AND METHODS

2.1. Model Calibration

Model potential conversion rates were calibrated using experimental data of van Bodegom and Stams (1999). In that study, soil slurries collected from rice paddies were incubated for 60 days at 14°C, 20°C, and 30°C. CH₄ and CO₂ production, accumulation and degradation of intermediate fatty acids and concentration changes in various electron acceptors were measured.

2.2. Incubation Experiments for Model Validation

2.2.1. General experimental setup

The same soil, described previously (van Bodegom and Stams, 1999), was used to prepare soil slurries in 1 liter serum bottles by mixing 250 ml sterilized distilled water with 100 g d.w. homogenized soil. The bottles were closed with butyl rubber stoppers, were repeatedly (6×) evacuated and flushed with N₂ gas to a final pressure of 150 kPa. The anoxic soil slurries were incubated in the dark in triplicate at 20°C and 30°C, while shaken gently at 100 rpm. Additionally, slurries with 3.75% v/v formaldehyde were prepared in duplicate for each experiment to determine abiotic dynamics. All incubations were monitored daily for lactate, fatty acids, Fe(II), NO₃⁻, NO₂⁻, SO₃²⁻, SO₄²⁻, CH₄, H₂, NO, N₂O, CO₂, sulphide, and pH-H₂O. Samples were taken as described previously (van Bodegom and Stams, 1999).

2.2.2. Short-term validation experiment with substrate additions

A validation incubation experiment was carried out for 20 days to test the effects of several substrate additions. The four treatments were the addition of 7.5 g/l rice straw (equivalent to 9 tons/ha) at day 3, addition of 15 mM acetate (below the inhibiting concentrations mentioned by Fukuzaki et al., 1990) at day 3, addition of 0.15 mmol H₂/l (below the thermodynamically inhibiting concentrations) to the head-space at days 3, 5, 7, 9, 11, and 14 and a control treatment without additions.

2.2.3. Long-term validation experiment with sulphate addition

A validation experiment was carried out to test the effects of sulphate addition. Air-dried samples were incubated at 10°C for 2 months, to decrease the amount of readily degradable carbon. Then, slurries were prepared and incubated for 60 days. In the first treatment 4 mM ammonium sulphate was added at day 0. The second treatment was a control without additions.

2.2.4. Short-term experiment on factors determining the lag phase for iron

Lag phases for iron reduction have been found to depend on temperature and preincubation conditions. It was hypothesized that an unmonitored electron acceptor, either humic acids or reducible manganese, caused this lag phase. Only the effects of reducible manganese were tested, because soil humic acid content was low. Soils incubated for 20 days were therefore monitored additionally for Mn(II). Both adsorbed and dissolved Mn(II) were extracted as described for Fe(II) by van Bodegom and Stams (1999).

2.2.5. Analytical techniques

Lactate, fatty acids, Fe(II), NO₃⁻, NO₂⁻, SO₃²⁻, SO₄²⁻, CH₄, H₂, NO, N₂O, CO₂, sulphide, and pH-H₂O were analyzed as described by van Bodegom and Stams (1999). No other low molecular weight organic acids could be detected in the soil slurries. In the short-term incubation on factors determining the lag phase for iron, reducible iron and reducible manganese were analyzed by inductively coupled plasma atomic emission spectrometer (ICP-AES).

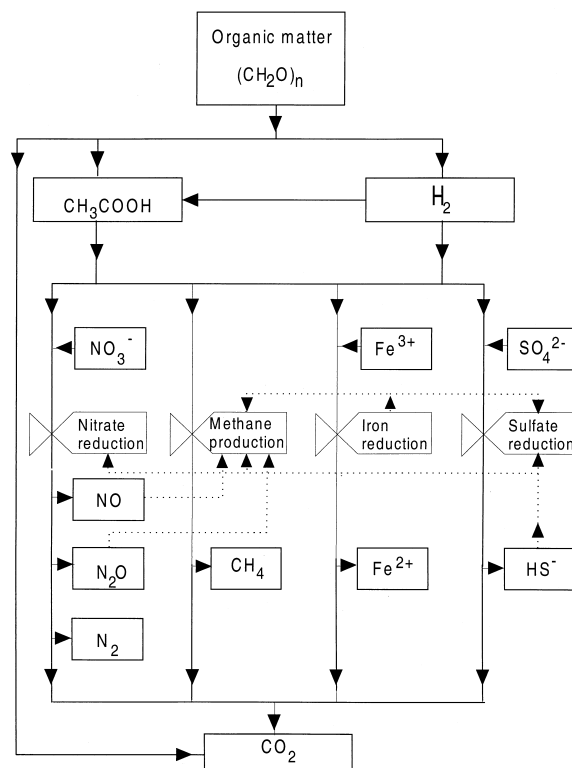


Fig. 1. Schematic representation of the interactions accounted for in the model. Lines indicate compounds flows and dashes indicate inhibitory effects. Chemolithotrophic reactions are not indicated separately in this scheme.

2.3. Model Description

The model, summarized in Fig. 1, describes the different microbial processes involved in anaerobic organic matter degradation in well-shaken soil slurries.

2.3.1. Substrate release

Basis of the model is the production of acetate and H₂/CO₂. These substrates are produced by soil organic matter mineralization and by the decomposition of complex organic substances. This can be summarized by



This reaction rate changes in time, because of decreases in easily accessible carbon. For a system that starts at the introduction of anaerobiosis (e.g., by the preparation of a rice paddy field after a dry fallow period), the reaction rate P_{\min} can be described by (Yang, 1996):

$$P_{\min} = C_{\min} \cdot (1 - S) \cdot K_d \cdot e^{-K_d \cdot \text{time}} \quad (2)$$

where C_{\min} is the constant soil carbon content (mol C m⁻³ water) and K_d is the relative decomposition rate, given by

$$K_d = R \cdot \text{time}^{-5}, \quad (3)$$

where R (time^{S-1}) and S (–) are empirical parameters. More details about the parameters and parameter value determinations are described in van Bodegom et al. (2000). Rice straw decomposition is described by a similar set of equations, changing C_{\min} into the amount of rice straw carbon. Substrate production from other sources, like root exudation, is not explicitly modelled, because such sources were absent in the calibration and validation experiments.

Table 1. Kinetic microbiological parameters used in this study at reference temperature -30°C.

Reaction	K_M electron donors (mol m ⁻³ H ₂ O)		K_M electron acceptor (mol m ⁻³ H ₂ O)	$V_{\max(\text{total})}$ (mol e ⁻ acc m ⁻³ H ₂ O s ⁻¹)
	Acetate	H ₂		
Nitrate reduction	0.09 ^a	0.1×10^{-3} ^f	0.42 ^l (NO ₃)	1.2×10^{-4} ^o
	0.09 ^b	0.1×10^{-3} ^f	5.93 ^b (NO)	8.6×10^{-5} ^b
	0.09 ^a	0.1×10^{-3} ^f	5.93 ^a (N ₂ O)	8.6×10^{-5} ^a
Iron reduction	0.23 ^c	0.22×10^{-3} ^g	61 ^m (Fe ³⁺)	5.0×10^{-4} [*]
Sulphate reduction	0.79 ^d	2.87×10^{-3} ^h	0.23 ⁿ (SO ₄ ²⁻)	0.8×10^{-5} [*]
Methanogenesis	2.56 ^e	13.3×10^{-3} ⁱ	n.a.	2.0×10^{-5} [*]
Denitrification/HS oxidation	1.68 ^j (HS ⁻)		1.75 ^j (NO ₃ ⁻)	6.8×10^{-5} ^p
Denitrification/iron oxidation	0.9 ^k (Fe ²⁺)		3.6 ^a (NO ₃ ⁻)	1.1×10^{-5} ^q
Product inhibition		Threshold concentration (mol m ⁻³ H ₂ O)		Maximum concentration (mol m ⁻³ H ₂ O)
HS ⁻ inhibition on NO ₃ ⁻ reduction		0.65 ^r		10 ^r
HS ⁻ inhibition on SO ₄ ²⁻ reduction		3.75 ^s		27.2 ^s
HS ⁻ inhibition on methanogenesis		3.46 ^t		13.7 ^t
N ₂ O inhibition on methanogenesis		6.3×10^{-2} ^u		0.21 ^u
NO inhibition on methanogenesis		1.8×10^{-3} ^u		0.01 ^u

^a Leffelaar and Wessel, 1988.

^b Assumed to be equal to (a).

^c No data, estimated between $K_M(\text{SO}_4)$ and $K_M(\text{NO}_3)$ from thermodynamics (Conrad, 1996).

^d Brandis-Heep et al., 1983; Middleton and Lawrence, 1977; Visser et al., 1996.

^e Jetten et al., 1990; Powell et al., 1983; Zehnder et al., 1980.

^f No data, estimated from thermodynamics using Conrad (1996).

^g Klüber and Conrad, 1993.

^h Kristjansson et al., 1982; Lupton and Zeikus, 1984; Robinson and Tiedje, 1984.

ⁱ Kristjansson et al., 1982; Lupton and Zeikus, 1984; Robinson and Tiedje, 1984; Zehnder and Wuhrmann, 1977.

^j Estimated from Brunet and Garcia-Gil 1996.

^k Estimated from Straub et al., 1996.

^l Klemendtsson et al., 1977; Leffelaar and Wessel, 1988; Murray et al., 1989.

^m Estimated from Achnich et al., 1995a; Lovley and Phillips, 1986; Roy et al., 1997.

ⁿ Brandis-Heep et al., 1983; Ingvorsen et al., 1984; Middleton and Lawrence, 1977.

^o Brunet and Garcia-Gil, 1996; Leffelaar and Wessel, 1988; Sweerts et al., 1990.

^p Estimated from Bak and Pfennig, 1991; Brunet and Garcia-Gil, 1996.

^q Estimated from Benz et al., 1998; Straub et al., 1996.

^r Brunet and Garcia-Gil, 1996; Sørensen et al., 1980.

^s McCartney and Oleskiewicz, 1993; Okabe et al., 1995; Visser et al., 1996.

^t McCartney and Oleskiewicz, 1993; Visser et al., 1996; Winfrey and Zeikus, 1977.

^u Balderston and Payne, 1976; Klüber and Conrad, 1998.

* Calibrated in this study.

2.3.2. Substrate competition

In the presence of alternative electron acceptors, methanogens have to compete for acetate and H₂ with anaerobically respiring micro-organisms using NO₃⁻, SO₄²⁻, and Fe(III) as electron acceptors. This competition is described by substrate conversion rates (V_i : mol m⁻³ water s⁻¹). For methanogens (m_i), substrate conversion is described by Michaelis–Menten kinetics:

$$V_{m_i} = Q_{\max, m_i} \cdot B_{m_i} \cdot \frac{[\text{substrate}]}{K_{M, m_i} + [\text{substrate}]}, \quad (4)$$

where B is the microbial biomass (mol biomass m⁻³ water), Q_{\max} is the specific microbial activity (mol mol⁻¹ biomass s⁻¹) and K_{M, m_i} is the affinity constant (mol m⁻³ water) while i is H₂ (hydrogenotrophic methanogens) and acetate (acetoclastic methanogens), respectively. The substrate conversion rates for the other anaerobic micro-organisms (e_i) is summarized by double Michaelis–Menten kinetics:

$$V_{e_i} = Q_{\max, e_i} \cdot B_{e_i} \cdot \frac{[e^- \text{ donor}]}{K_{M, d_i} + [e^- \text{ donor}]} \cdot \frac{[e^- \text{ acc}]}{K_{M, a_i} + [e^- \text{ acc}]}, \quad (5)$$

where K_{M, d_i} and K_{M, a_i} are the affinity constants (mol m⁻³ water) for the electron donor (e^- donor) and electron acceptor (e^- acc), respectively. Acetate and H₂ are electron donors and NO₃⁻, NO, N₂O, reducible iron

and SO₄²⁻ are included as electron acceptors. All K_M values are summarized in Table 1. For iron the total reducible iron concentration is used and not the Fe(III) in the solution, thus assuming that ferric iron desorption and iron dissolution from amorphous iron oxides is not the rate-limiting step. Concentrations of gaseous compounds (NO, N₂O, CO₂, CH₄) are corrected for the equilibrium with the gas phase by Henry's law. Microbial biomass changes are not included in the model. Arguments for a constant biomass for methanogens and bacteria using alternative electron acceptors are given elsewhere (van Bodegom and Stams, 1999; Asakawa et al., 1998; Segers and Kengen, 1998). The constant microbial biomass allows a simplification of Eqn. (4) and (5), which is used throughout the remainder of the paper:

$$Q_{\max} \cdot B = V_{\max}, \quad (6)$$

where V_{\max} is a potential conversion rate (mol m⁻³ water s⁻¹).

It is assumed that K_M values are independent of temperature, although both thermodynamic considerations (van Bodegom and Stams, 1999) and experimental data (Westermann et al., 1989) falsify this assumption. There are however not enough quantitative data to describe such changes with temperature. Temperature influence on V_{\max} values is described using a Q_{10} value, indicating the increase in reaction rates at a temperature increase of 10°C:

$$V_{\max}(T) = V_{\max}(T_{\text{ref}}) \cdot Q_{10}(T - T_{\text{ref}})/10, \quad (7)$$

where T_{ref} is the reference temperature, which is 30°C in this study.

In Table 1 the V_{max} values at the reference temperature are indicated for the microbial processes. This table presents the total V_{max} , which is the sum of conversions via acetate and via H_2 . To avoid too many degrees of freedom in the model calibration, it is assumed that $V_{\text{max}}(\text{via } \text{H}_2) = V_{\text{max}}(\text{via acetate})$, because there are not enough published data that allow a further specification. Only for CH_4 production it is assumed that $V_{\text{max}}(\text{via acetate})$ is twice the value of $V_{\text{max}}(\text{via } \text{H}_2)$. This assumption allows 70–80% of the CH_4 to be produced via acetate (compared to a theoretical 67%) (Chin and Conrad, 1995; Rothfuss and Conrad, 1993). Due to homoacetogenesis, a H_2/CO_2 to acetate conversion that mainly occurs after sulphate depletion (van Bodegom and Stams, 1999), aceticlastic methanogenesis is more important than hydrogenotrophic methanogenesis. By correcting the methanogenic V_{max} in this way, it is not necessary to model homoacetogenesis explicitly. The competition outcome is completely determined by differences in V_{max} values, affinity constants and reactant concentrations.

2.3.3. Chemolithotrophic redox conversions

Electron acceptor kinetics is complicated by the occurrence of chemolithotrophic redox conversions, occurring when inorganic compounds act as an electron donor. Such conversions are included, but only two conversions occurring in freshwater systems could be quantified: Coupled nitrate reduction to N_2 /sulphide oxidation to sulphate (e.g., Dannenberg et al., 1992) is included and described by the following substrate conversion rate description:

$$V = V_{\text{max}} \frac{[\text{HS}^-]}{K_{M,\text{HS}} + [\text{HS}^-]} \cdot \frac{[\text{NO}_3^-]}{K_{M,\text{NO}_3} + [\text{NO}_3^-]} \quad (8)$$

Additionally, coupled nitrate reduction to N_2 /iron oxidation to ferric iron (e.g., Straub et al., 1996) is described by the following description:

$$V = V_{\text{max}} \frac{[\text{Fe}^{2+}]}{K_{M,\text{Fe}} + [\text{Fe}^{2+}]} \cdot \frac{[\text{NO}_3^-]}{K_{M,\text{NO}_3} + [\text{NO}_3^-]} \quad (9)$$

The experiments were initiated under anaerobic conditions. Therefore, aerobic respiration and electron acceptor reoxidation by oxygen are not included in the model. The absence of aerobic respiration and the absence of transport limit the possibilities of the above-mentioned chemolithotrophic conversions due to the lack of oxidized substrates.

2.3.4. Lag time and inhibition during iron reduction

In a previous study we observed a lag phase of a few days before iron reduction started (van Bodegom and Stams, 1999). Other incubation experiments (Acht nich et al., 1995a; Ratering and Conrad, 1998; Roy et al., 1997) also showed a slow start of iron reduction. This lag time cannot be explained from competition with nitrate reducers (results not shown). We therefore apply a fully empirical lag time for iron reduction as a function of temperature, based on experimental data (van Bodegom and Stams, 1999).

Sulphate reduction and CH_4 production were severely inhibited during iron reduction (Ratering and Conrad, 1998; Roy et al., 1997). Neither substrate competition (Ratering and Conrad, 1998) nor redox effects (van Bodegom et al., 2000; Ratering and Conrad, 1998) could explain this inhibition. Therefore, we postulate an empirical direct inhibition by iron on sulphate reduction and CH_4 production. This inhibition is described by a constant threshold reducible iron concentration above which no sulphate reduction or CH_4 production occurs.

2.3.5. Direct inhibitions

Direct inhibitions are inhibitory effects on CH_4 production caused by intermediates produced by anaerobic respiring micro-organisms. This inhibition is described by two parameters, a threshold concentration of toxic intermediates below which no inhibition occurs and a maximum concentration above which complete inhibition occurs. Inhibition increases linearly in between these concentrations. Inhibition of CH_4 production by sulphide, NO and N_2O , inhibition of sulphate reduction by sulphide, and inhibition of nitrate reduction by sulphide are included (Table 1). Inhibition of N_2O on iron reduction may occur (Klüber and Conrad, 1998), but cannot be quantified. Inhibitions by SO_3^{2-} and NO_2^- are not included either, because these compounds were never detected experimentally. Data for half-saturation constants for inhibition were not always available, but results are not affected if such description is used when available (results not shown).

3. RESULTS

3.1. Model Calibration

Model V_{max} values were calibrated with an anaerobic incubation of slurried paddy soil (van Bodegom and Stams, 1999; reorganized in Fig. 2). Both experimental data and model calculations (Fig. 2) showed a sequential, but partly overlapping, reduction of electron acceptors; first nitrate reduction (data not shown), followed by iron reduction. After these processes, sulphate reduction and methane production started simultaneously. Low rates of sulphate reduction and methane production were already measured, but not modelled, during nitrate reduction and iron reduction. At the end of the incubation, only methanogens consumed acetate and H_2/CO_2 and methanogenesis was solely controlled by substrate production rates. The sequence proceeded faster at higher temperatures (Fig. 2). Model calculations were not significantly different from experimental results at any temperature for all electron acceptors, $P > 0.50$ (all statistical analyses are based on paired student's t -test), with $r^2 > 0.90$.

The combined reduction processes caused a CO_2 release (Fig. 2), which was corrected for abiotic CO_2 release and CO_2 release coupled to propionate and butyrate accumulation, because these processes were not modelled explicitly. The model calculates a high mineralization rate of readily accessible carbon at the start of the incubation. These high mineralization rates are found in the measurements in a quick and high release of H_2 , acetate and CO_2 . Not all released H_2 and acetate was consumed immediately and accumulated. H_2 was already below the detection limit, 10 Pa, after 4 days (data not shown). Acetate disappeared when methane production became the dominating process according to both model and data. Modelled acetate disappearance was qualitatively correct, but concentrations were significantly different from measured acetate concentrations at 14°C and 30°C ($P < 0.01$). Acetate accumulation is difficult to predict, because it equals the difference between soil mineralization and soil reduction rates and is thus very sensitive to small deviations in these descriptions. Moreover, soil mineralization is difficult to predict. Modelled CO_2 release, which is the result of both mineralization and reduction, is however not significantly different ($P = 0.81$) from measured CO_2 at any temperature with $r^2 = 0.96$.

3.2. Model Validation

Two model validation experiments were carried out to test the model applicability. Both validation experiments were per-

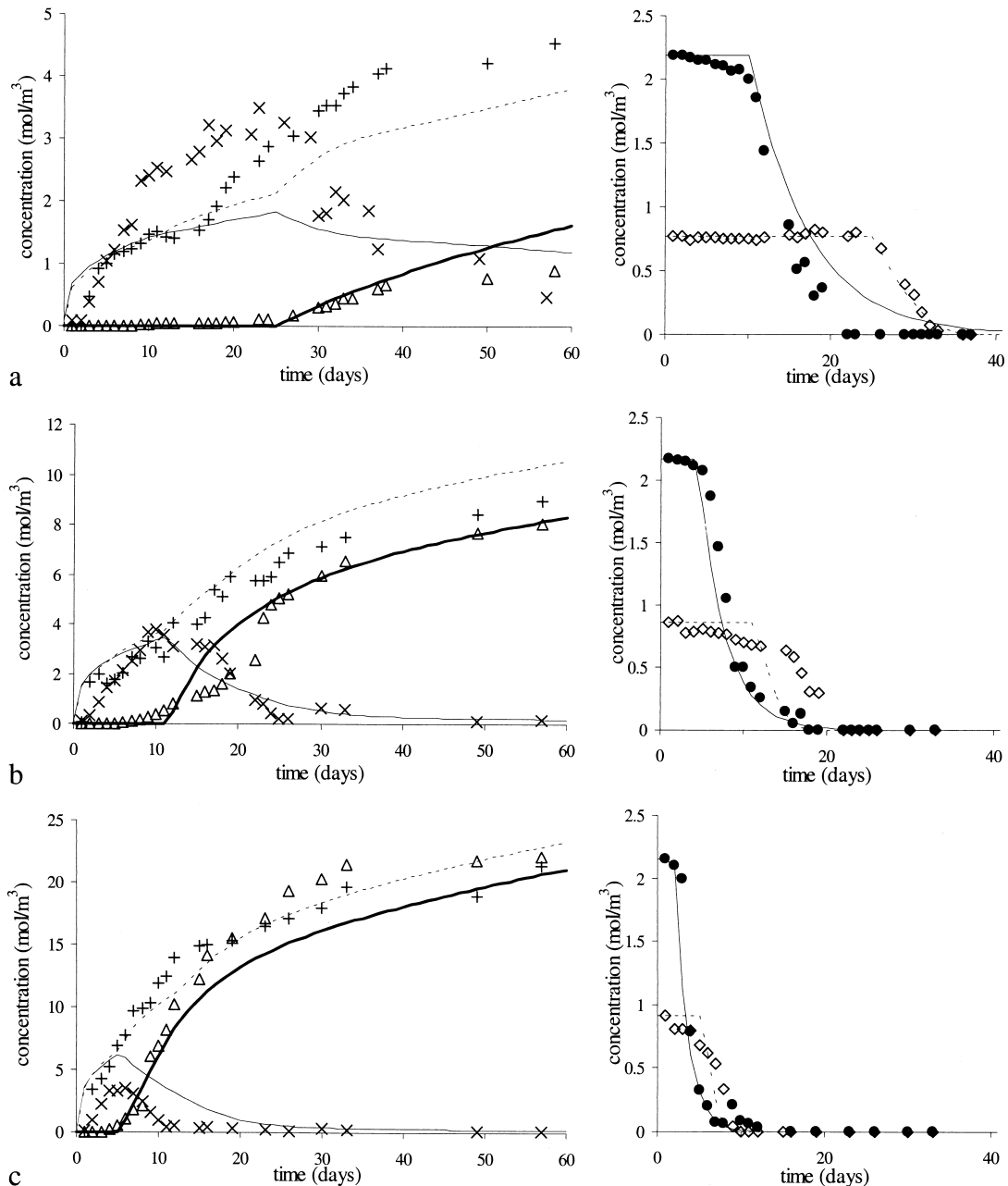


Fig. 2. Comparison of experiment (symbols) and model (lines) for the calibration of a long-term incubation experiment with rice paddy soil at (a) 14°C, (b) 20°C, and (c) 30°C. Indicated are CH₄ (open triangles and thick lines), CO₂ (pluses and dashed lines), and acetate (crosses and thin lines) in the left figures and sulphate (open diamonds and dashed lines) and reducible iron (closed circles and thin lines) in the right figures. Note the different scales.

formed with the same soil. Calibrated V_{\max} values from Sect. 3.1 were used for the model, because soil storage does not affect microbial numbers (Mayer and Conrad, 1990). All kinetic parameters were kept at the same values (Table 1) as in the model calibration.

3.2.1. Short-term validation experiment with substrate additions

The reduction sequence was accelerated—lag phases of CH₄ production were reduced—by substrate additions (shown for

20°C in Figure 3. Trends are similar at 30°C). Modelled CO₂ release under influence of substrate additions was not significantly different ($P = 0.52$) from the measurements, while no microbial parameter value had been changed. Substrate release was least increased in the H₂ treatment and most in the acetate treatment as is shown by the changes in CO₂ and CH₄ in time (Fig. 3). The increase in available substrate also increased CH₄ release rates. Measured and modelled CH₄ production rates were not significantly different ($P = 0.76$) for the control, straw addition and H₂ addition. In case of acetate addition [Fig.

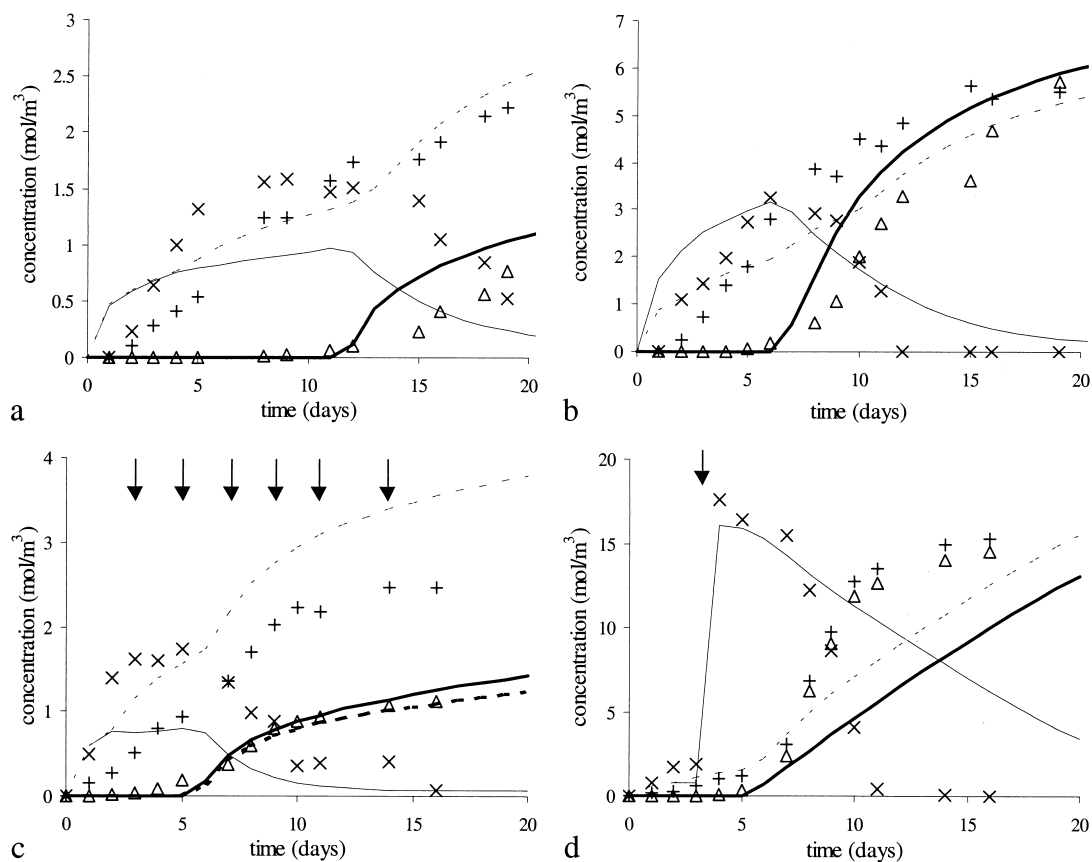


Fig. 3. Comparison of model and experiment for the short-term validation experiment at 20°C for (a) control, (b) addition of rice straw, (c) repeated addition of H_2 , and (d) addition of 15 mM acetate. Symbols are as indicated in Figure 2.

3(d)], the model significantly underestimated acetate consumption rates ($P = 0.03$) and the concomitant release of CH_4 ($P = 0.007$).

3.2.2. Long-term validation experiment with sulphate addition

Sulphate was added in the second model validation experiment. Methanogenesis was only partly inhibited by sulphate addition (Fig. 4). Modelled initiation and release of CH_4 under influence of sulphate additions was not significantly different from the measurements ($P = 0.84$), although modelled methane production rates in Figure 4d were lower than measured. The model significantly underestimated sulphate reduction rates after $t = 20$ days if sulphate was added ($P = 0.001$). Sulphate reduction rates were not significantly different in the control ($P = 0.33$).

In all incubations of Figure 4, organic substrate release—shown by the release of CO_2 and CH_4 —was much lower than in the calibration experiment (Fig. 2). The model corrected for the loss of easily accessible carbon during storage and CO_2 release was not significantly different ($P = 0.28$) from measured values, although modelled CO_2 release rates were in all cases slightly higher than measured values.

3.2.3. Short-term experiment on factors determining the lag phase for iron

The model includes an empirical temperature dependent description for the lag time for iron reduction. This lag phase could not have been caused by substrate limitation, because substrate concentrations were high during the first few days nor can it be explained by direct inhibition, because N_2O and NO had disappeared one day after all nitrate had been consumed. The lag phase dependence on temperature (van Bodegom and Stams, 1999), on preincubation redox conditions (Ratering and Conrad, 1998) and an unexplained CO_2 release in the period before iron reduction (van Bodegom and Stams, 1999) suggest the influence of a more competitive electron acceptor, like reducible manganese. The results of a short-term incubation experiment on the initiation of iron reduction (Fig. 5) show that iron reduction only started after almost all manganese had been reduced. This suggests that the lag phase is indeed caused by manganese reduction.

4. DISCUSSION

4.1. Calibration of the Model

Model and experiment performance were not significantly different for any process, except for acetate accumulation, while most kinetic microbial parameters (Table 1) resembled

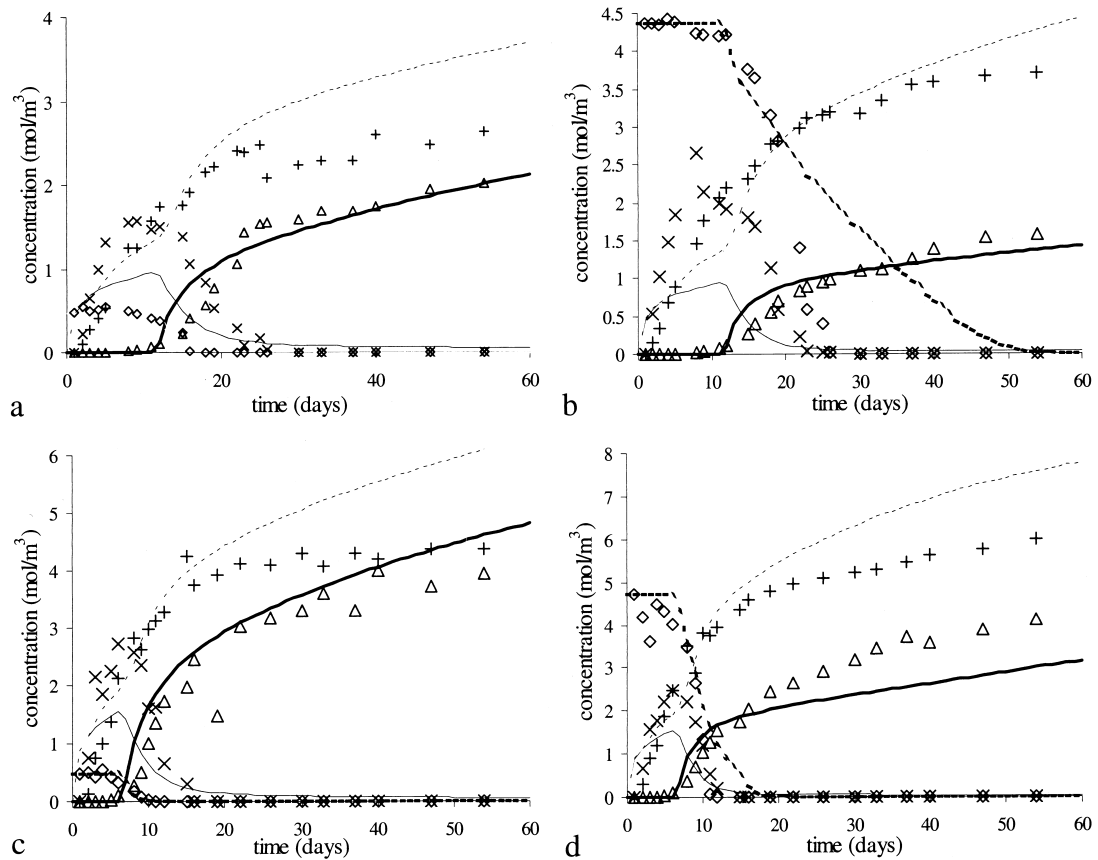


Fig. 4. Comparison of model and measured data for the long-term validation experiment for (a) control at 20°C, (b) addition of 4 mM sulphate at 20°C, (c) control at 30°C, and (d) addition of 4 mM sulphate at 30°C. Symbols are as indicated in Figure 2.

average values from published data that were kept constant in all simulations. Temperature effects were described by Q_{10} values, based on values obtained previously (van Bodegom and Stams, 1999) for nitrate, sulphate, and iron reduction and CH₄

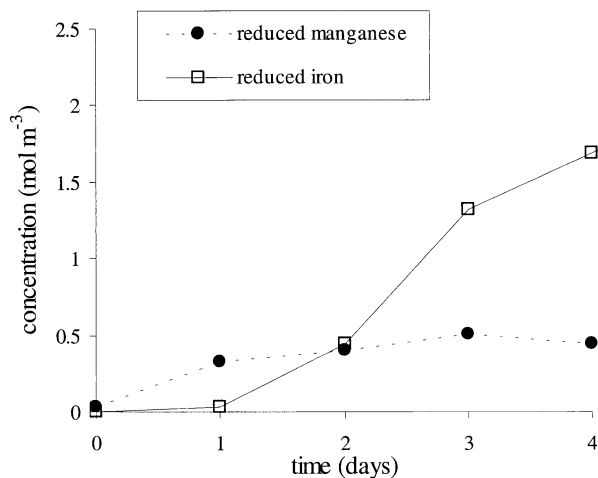


Fig. 5. Sequence of iron reduction and manganese reduction in the short-term validation experiment at 20°C.

production and a Q_{10} of 2 for other processes. Only V_{\max} for sulphate and iron reduction and for CH₄ production were calibrated, because V_{\max} is system dependent as it includes a measure of microbial biomass and activity, which again depend on site history and soil adsorption characteristics. V_{\max} values for other processes were not calibrated, because there were not enough experimental data to justify this. Calibrated V_{\max} values are all in the range of values derived from published data. The V_{\max} for iron reducers is similar to the V_{\max} calculated from Achnich et al. (1995a), Lovley and Phillips (1986; 1987; 1988), and Roy et al. (1997) assuming that acetate was not rate limiting. The same applies for the modelled V_{\max} for sulphate reducers (Crill and Martens, 1986; Lovley and Phillips, 1987; Roy et al., 1997) and for methanogens (Crill and Martens, 1986; Sass et al., 1990; Sigren et al., 1997).

Because of the system dependence of V_{\max} values, it is important to know the sensitivity of the processes for these estimates. CH₄ production rates are enhanced by 40% during CH₄ production initiation if V_{\max} of CH₄ production is doubled (Fig. 6a), causing a concomitant decrease in acetate and increase in CO₂ concentrations. At the same time, sulphate reduction rates decrease slightly at low sulphate concentrations, when the competitiveness of sulphate reducers is negatively affected (results not shown). A correct estimation of V_{\max} is thus important, while V_{\max} values are not always available.

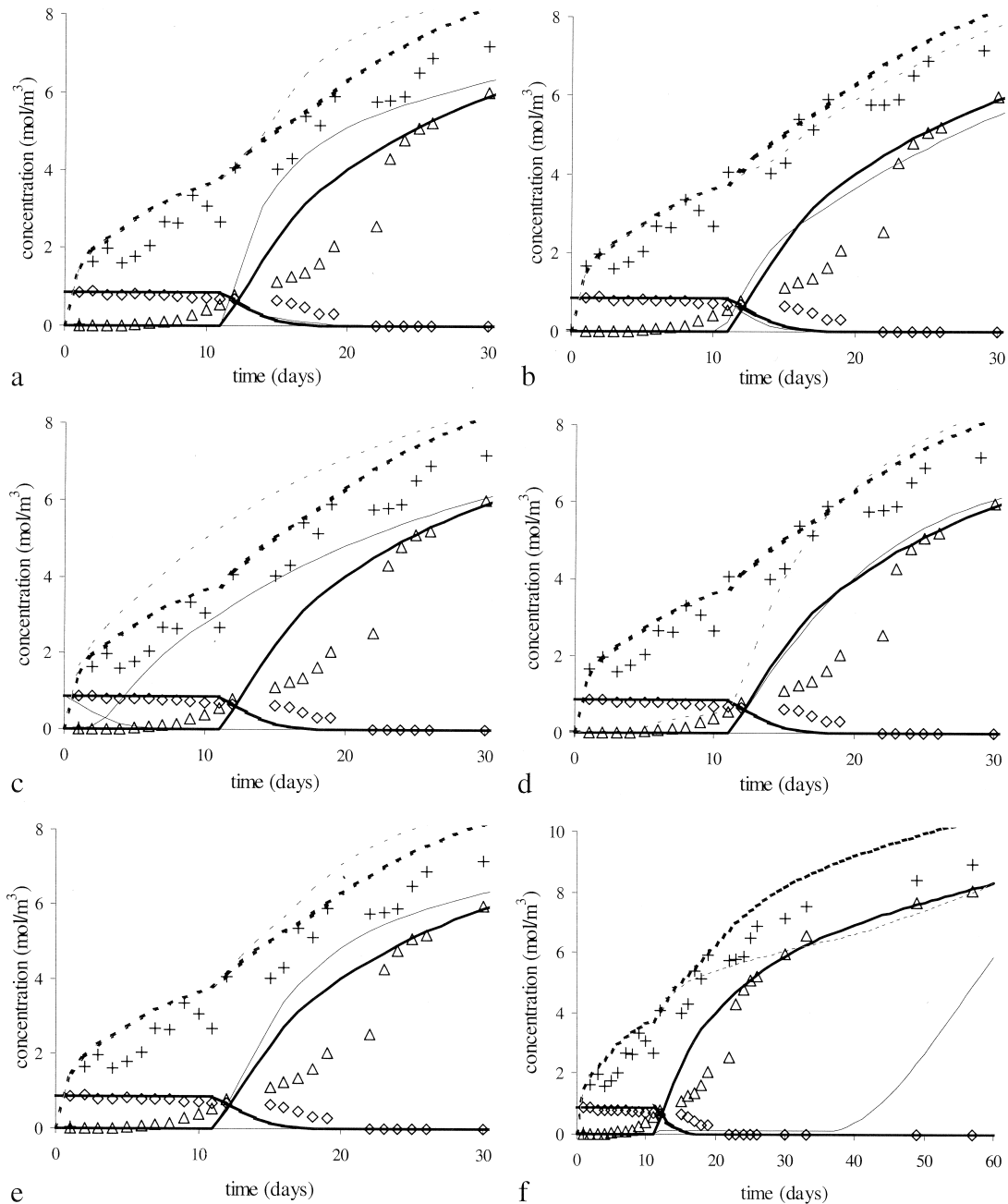


Fig. 6. Model sensitivity analysis at 20°C for the experimental results of the calibration experiment and modelled results of the default model (thick) and of the sensitivity analysis (thin) for CH₄ (triangles), CO₂ (pluses) and sulphate (diamonds) for (a) double V_{\max} values for CH₄ production, (b) equal V_{\max} for hydrogenotrophic and acetoclastic methanogenesis, (c) no inhibition of sulphate reduction and methane production by reducible iron, (d) without introducing H₂, (e) introducing K_M values for CH₄ production as a function of temperature, (f) use of sulphide inhibition parameters on methane production based on Cappenberg (1974)—note the different scales—and (g) no introduction of a lag phase for iron reduction.

This limits the general applicability of the model. Estimation of V_{\max} from direct estimates of microbial numbers [B, Eqn. (4)] is difficult, because such estimates are affected by similar errors.

Another issue is the distribution of V_{\max} for methanogenesis. Acetoclastic methanogenesis V_{\max} was assumed to be twice as high as the hydrogenotrophic methanogenesis V_{\max} to account implicitly for homoacetogenesis in the CH₄ production phase.

On the other hand, hydrogenotrophic methanogens are more active than acetoclastic methanogens at the start of the rice-growing season (Roy et al., 1997). If V_{\max} of hydrogenotrophic and acetoclastic methanogenesis are taken equal, then CH₄ production rates decrease 8% (Fig. 6b) while sulphate and iron reduction rates increase less than 2% (results not shown). Methanogens are less competitive for H₂ than for acetate (Acht nich et al., 1995b; Jakobsen et al., 1981; Lovley and

and suggest that a proper description of substrate availability is more important for CH₄ production explanation than kinetic microbiological parameters.

The only situation for which the model was significantly different ($P < 0.05$) from the experimental results, was for acetate addition. A possible explanation is that the calibrated V_{\max} for CH₄ production (Sect. 3.1) was not precise enough, while the system was more sensitive to V_{\max} than in other cases, because other limitations were absent in this case. Another explanation is that growth of methanogenic biomass occurred in this special case, thus causing an increase in V_{\max} . Especially the biomass of *methanosarcina*, a fast growing acetate consuming methanogen, might have increased in this period. This complication is not important for field situations, because acetate never accumulates to such high concentrations and the model could describe the effects of acetate accumulation under influence of high rice straw additions.

4.2.2. Long-term validation experiment with sulphate addition

Model validation with sulphate additions is an important test, because sulphate reducers are kinetically the most comparable competitors to methanogens. CH₄ production was only partly inhibited during sulphate reduction, showing that sulphate reducers are not able to outcompete the methanogens. Inhibiting sulphide concentrations were never reached. Only if methane production inhibition is described by the sulphide values of Capenberg (1975), then methane production is inhibited for 38 days (Fig. 6f), which is much larger than in reality.

The model underestimation of sulphate reduction at high sulphate levels might imply that growth of sulphate reducers could be important if sulphate concentrations are increased above concentrations naturally found in the field. Such situations might occur in rice paddies if inorganic fertilizers containing sulphate are applied.

The experiment also showed that organic substrate release was reduced by a factor 4–5 by storage. Model predictions for this decreased release were not significantly different from measured values, but to fully account for losses during storage the model should be expanded with a more detailed description of soil mineralization.

4.2.3. Short-term experiment on factors determining the lag phase for iron

The results of Figure 5 suggest that manganese reduction might explain the empirical lag time for iron reduction introduced in the model to describe the late start of iron reducers. Iron reduction, sulphate reduction, and CH₄ production start about 4 days earlier than experimentally found if no lag phase is introduced (Fig. 6g). This again leads to an overestimation of CO₂ in the early phase. A lag phase is thus necessary to describe the redox sequence. A mechanistic description of this lag phase is not yet possible, because kinetic parameters for manganese reduction are highly uncertain. This limits the applicability of the model because, if manganese reduction causes the lag phase, the lag phase will be dependent on reducible manganese concentrations and substrate release rates and thus upon soil type.

5. CONCLUSIONS

Combination of model and experiments showed the quantitative importance of different microbial interactions leading to CH₄ production in well-shaken systems. The mechanistic model was calibrated with incubation experiments at different temperatures by tuning only the V_{\max} values for sulphate and iron reduction and for CH₄ production. The model thus balances mechanistic detail and available information. Model results agreed with the validation experiments without the adjustment of any kinetic parameter.

The competition for acetate and H₂/CO₂ is the most important factor determining methane production. The model is therefore very sensitive to the description of substrate release. Competition outcome is partly determined by differences in V_{\max} values, which are system dependent. The V_{\max} should be estimated separately for H₂ and acetate, as can be determined in experiments with labelled acetate and bicarbonate. This limits model applicability.

On the other hand, the model showed that chemolithotrophic reactions and direct inhibitions of NO, N₂O, and S²⁻ are not important in these well-shaken systems. The inhibition of sulphate reduction and methanogenesis during iron reduction explains acetate accumulation, but the mechanisms are unknown. The retardation of iron reduction is probably caused by the, uncertain, manganese reduction. A final gap in knowledge is the temperature dependence of affinity constants, while this has a clear influence on the outcome.

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REFERENCES

- Acht nich C., Bak F., and Conrad R. (1995a) Competition for electron donors among nitrate reducers, ferric iron reducers, sulfate reducers and methanogens in anoxic paddy soil. *Biol. Fertil. Soils* **19**, 65–72.
- Acht nich C., Schuhmann A., Wind T., and Conrad R. (1995b) Role of interspecies H₂ transfer to sulfate and ferric iron-reducing bacteria in acetate consumption in anoxic paddy soil. *FEMS Microbiol. Ecol.* **16**, 61–70.
- Asakawa S., Akagawa-Matsushita M., Koga Y., and Hayano K. (1998) Communities of methanogenic bacteria in paddy field soils with long-term application of organic matter. *Soil Biol. Biochem.* **30**, 299–303.
- Bak F. and Pfennig N. (1991) Microbial sulfate reduction in littoral sediment in Lake Constance. *FEMS Microbiol. Ecol.* **85**, 31–42.
- Balderston W. L. and Payne W. J. (1976) Inhibition of methanogenesis in salt marsh sediments and whole-cell suspensions of methanogenic bacteria by nitrogen oxides. *Appl. Env. Microbiol.* **32**, 264–269.
- Benz M., Brune A., and Schink B. (1998) Anaerobic and aerobic oxidation of ferrous iron at neutral pH by chemoheterotrophic nitrate-reducing bacteria. *Arch. Microbiol.* **169**, 159–165.
- Boudreau B. P. (1996) A method-of-lines code for carbon and nutrient diagenesis in aquatic sediments. *Comput. Geosci.* **22**, 479–496.
- Brandis-Heep A., Gebhardt N. A., Thauer R. K., Widdel F., and Pfennig N. (1983) Anaerobic acetate oxidation to CO₂ by *Desulfobacter postgatei*. 1. Demonstration of all enzymes required for the operation of the citric acid cycle. *Arch. Microbiol.* **136**, 222–229.
- Brunet R. C. and Garcia-Gil L. J. (1996) Sulfide-induced dissimilatory nitrate reduction to ammonia in anaerobic freshwater sediments. *FEMS Microbiol. Ecol.* **21**, 131–138.
- Capenberg Th.E. (1975) A study of mixed continuous cultures of sulfate-reducing and methane-producing bacteria. *Microbiol. Ecol.* **2**, 60–72.

- Chin K. and Conrad R. (1995) Intermediary metabolism in methanogenic paddy soil and the influence of temperature. *FEMS Microbiol. Ecol.* **18**, 85–102.
- Conrad R. (1996) Soil microorganisms as controllers of atmospheric trace gases (H₂, CO, CH₄, OCS, N₂O, and NO). *Microbiol. Rev.* **60**, 609–640.
- Crill P. M. and Martens C. S. (1986) Methane production from bicarbonate and acetate in an anoxic marine sediment. *Geochim. Cosmochim. Acta* **50**, 2089–2097.
- Dannenberg S., Kroder M., Dilling W., and Cyprianka H. (1992) Oxidation of H₂, organic compounds and inorganic sulfur compounds coupled to reduction of O₂ or nitrate by sulfate-reducing bacteria. *Arch. Microbiol.* **158**, 93–99.
- Fetzer S. and Conrad R. (1993) Effect of redox potential on methanogenesis by *Methanosarcina barkeri*. *Arch. Microbiol.* **160**, 108–113.
- Fukuzaki S., Nishio N., and Nagai S. (1990) Kinetics of the methanogenic fermentation of acetate. *Appl. Env. Microbiol.* **56**, 3158–3163.
- Grant R. F. (1998) Simulation of methanogenesis in the mathematical model ECOSYS. *Soil Biol. Biochem.* **30**, 883–896.
- Gupta A., Flora J. R. V., Sayles G. D., and Suidan M. T. (1994) Methanogenesis and sulfate reduction in chemostats—II. Model development and verification. *Water Res.* **28**, 795–803.
- Hunter K. S., Wang Y., and van Cappellen P. (1998) Kinetic modeling of microbially-driven redox chemistry of subsurface environments: Coupling transport, microbial metabolism and geochemistry. *J. Hydrology* **209**, 53–80.
- Ingvorsen K., Zehnder A. J. B., and Jørgensen B. B. (1984) Kinetics of sulfate and acetate uptake by *Desulfobacter postgatei*. *Appl. Env. Microbiol.* **47**, 403–408.
- Jakobsen P., Patrick W. H., Jr., and Williams B. G. (1981) Sulfide and methane formation in soils and sediments. *Soil Sci.* **132**, 279–287.
- James R. T. (1993) Sensitivity analysis of a simulation model of methane flux from the Florida Everglades. *Ecol. Modelling* **68**, 119–146.
- Jetten M. S. M., Stams A. J. M., and Zehnder A. J. B. (1990) Acetate threshold values and acetate activating enzymes in methanogenic bacteria. *FEMS Microbiol. Ecol.* **73**, 339–344.
- Klemendtsen L., Svenson B. H., Lindberg T., and Rosswall T. (1977) The use of acetylene inhibition of nitrous oxide reductase in quantifying denitrification in soils. *Swed. J. Agr. Res.* **7**, 179–185.
- Klüber H. D. and Conrad R. (1993) Ferric iron-reducing *Shewanella putrefaciens* and nitrogen fixing *Bradyrhizobium japonicum* with uptake hydrogenase are unable to oxidize atmospheric hydrogen. *FEMS Microbiol. Lett.* **111**, 337–342.
- Klüber H. D. and Conrad R. (1998) Effects of nitrate, nitrite, NO and N₂O on methanogenesis and other redox processes in anoxic rice field soil. *FEMS Microbiol. Ecol.* **25**, 301–318.
- Kristjansson J. K., Schönheit P., and Thauer R. K. (1982) Different K_s values for hydrogen of methanogenic bacteria and sulfate reducing bacteria: An explanation for the apparent inhibition of methanogenesis by sulfate. *Arch. Microbiol.* **131**, 278–282.
- Leffelaar P. A. and Wessel W. W. (1988) Denitrification in a homogeneous, closed system: Experiment and simulation. *Soil Sci.* **146**, 335–349.
- Lovley D. R. and Klug R. J. (1986) Model for the distribution of sulphate reduction and methanogenesis in freshwater sediments. *Geochim. Cosmochim. Acta* **50**, 11–18.
- Lovley D. R. and Phillips E. P. (1986) Organic matter mineralization with reduction of ferric iron in anaerobic sediments. *Appl. Env. Microbiol.* **51**, 683–689.
- Lovley D. R. and Phillips E. P. (1987) Competitive mechanisms for inhibition of sulfate reduction and methane production in the zone of ferric iron reduction in sediments. *Appl. Env. Microbiol.* **53**, 2636–2641.
- Lovley D. R. and Phillips E. P. (1988) Novel mode of microbial energy metabolism: Organic carbon oxidation coupled to dissimilatory reduction of iron or manganese. *Appl. Env. Microbiol.* **54**, 1472–1480.
- Lupton F. S. and Zeikus J. G. (1984) Physiological basis for sulfate-dependent hydrogen competition between sulfidogens and methanogens. *Curr. Microbiol.* **11**, 7–11.
- Mayer H. P. and Conrad R. (1990) Factors influencing the population of methanogenic bacteria and the initiation of methane production upon flooding of paddy soil. *FEMS Microbiol. Ecol.* **7**, 103–112.
- McCartney D. M. and Oleszkiewicz J. A. (1993) Competition between methanogens and sulfate reducers: Effect of COD:sulfate ratio and acclimation. *Water Env. Res.* **65**, 655–664.
- Middleton A. G. and Lawrence A. W. (1977) Kinetics of microbial sulfate reduction. *JWPCF* **229**, 1659–1670.
- Murray R. E., Parsons L. L., and Scott Smith M. (1989) Kinetics of nitrate utilization by mixed populations of denitrifying bacteria. *Appl. Env. Microbiol.* **55**, 717–721.
- Okabe S., Nielsen P. H., Jones W. L., and Characklis W. G. (1995) Sulfide product inhibition of *Desulfovibrio desulfuricans* in batch and continuous cultures. *Water Res.* **29**, 571–578.
- Powell G. E., Hilton M. G., Archer D. B., and Kirsop B. H. (1983) Kinetics of methanogenic fermentation of acetate. *J. Chem. Technol. Biotechnol.* **33B**, 209–220.
- Ratering S. and Conrad R. (1998) Effects of short-term drainage and aeration on the production of methane in submerged rice soil. *Global Change Biol.* **4**, 397–407.
- Robinson J. A. and Tiedje J. M. (1984) Competition between sulfate-reducing and methanogenic bacteria for H₂ under resting and growing conditions. *Arch. Microbiol.* **137**, 26–32.
- Rothfuss F. and Conrad R. (1993) Vertical profiles of CH₄ concentrations, dissolved substrates and processes involved in CH₄ production in a flooded Italian rice field. *Biogeochemistry* **18**, 137–152.
- Roy R., Klüber H. D., and Conrad R. (1997) Early initiation of methane production in anoxic rice soil despite the presence of oxidants. *FEMS Microbiol. Ecol.* **24**, 311–320.
- Sass R. L., Fisher F. M., Harcombe P. A., and Turner F. T. (1990) Methane production and emission in a Texas rice field. *Global Biogeochem. Cycl.* **4**, 47–68.
- Sass R. L., Fisher F. M., Turner F. T., and Jund M. F. (1991) Methane emission from rice fields as influenced by solar radiation, temperature and straw application. *Global Biogeochem. Cycl.* **5**, 335–350.
- Schütz H., Holzapfel-Pschorn A., Conrad R., Renneberg H., and Seiler W. (1989) A 3-year continuous record on the influence of daytime, season, and fertilizer treatment on methane emission rates from an Italian rice paddy. *J. Geophys. Res.* **94**, 16,405–16,416.
- Segers R. and Kengen S. W. M. (1998) Methane production as a function of anaerobic carbon mineralization: A process model. *Soil Biol. Biochem.* **30**, 1107–1117.
- Sigren L. K., Byrd G. T., Fisher F. M., and Sass R. L. (1997) Comparison of soil acetate concentrations and methane production, transport, and emission in two rice cultivars. *Global Biogeochem. Cycl.* **11**, 1–14.
- Srensen J., Tiedje J. M., and Firestone R. B. (1980) Inhibition by sulfide of nitric and nitrous oxide reduction by denitrifying *Pseudomonas fluorescens*. *Appl. Env. Microbiol.* **39**, 105–108.
- Straub K. L., Benz M., Schink B., and Widdel F. (1996) Anaerobic, nitrate-dependent microbial oxidation of ferrous iron. *Appl. Env. Microbiol.* **62**, 1458–1460.
- Sweerts J-P. R. A., de Beer D., Nielsen L. P., Verdouw H., van den Heuvel J. C., Cohen Y., and Cappenberg T. E. (1990) Denitrification by sulphur oxidizing *Beggiatoa* spp. mats on freshwater sediments. *Nature* **344**, 762–763.
- van Bodegom P. M. and Stams A. J. M. (1999) Effects of alternative electron acceptors and temperature on methanogenesis in rice paddy soils. *Chemosphere* **39**, 167–182.
- van Bodegom P. M., Wassmann R., and Metra-Corton T. M. (2000) A process-based model for methane emission predictions from flooded rice paddies. *Global Biogeochem. Cycl.* (in press).
- van Cappellen P. and Wang Y. (1996) Cycling of iron and manganese in surface sediments: A general theory for the coupled transport and reaction of carbon, oxygen, nitrogen, sulfur, iron, and manganese. *Am. J. Sci.* **296**, 197–213.
- Vavilin V. A., Vasiliev V. B., Pomomarev A. V., and Rytow S. V. (1994) Simulation model 'Methane as a tool for effective biogas production during anaerobic conversion of complex organic matter'. *Biosource Technol.* **48**, 1–8.
- Visser A., Pol L. W. H., and Lettinga G. (1996) Competition of methanogenic and sulfidogenic bacteria. *Water Sci. Technol.* **33**, 99–110.
- Westermann P., Ahring B. K., and Mah R. A. (1989) Temperature compensation in *Methanosarcina barkeri* by modulation of hydrogen and acetate affinity. *Appl. Env. Microbiol.* **55**, 1262–1266.

- Winfrey M. R. and Zeikus J. G. (1977) Effect of sulfate on carbon and electron flow during microbial methanogenesis in freshwater sediments. *Appl. Env. Microbiol.* **33**, 275–281.
- Yagi K., Tsuruta H., Kanda K., and Minami K. (1996) Effect of water management on methane emission from a Japanese rice paddy field: Automated methane monitoring. *Global Biogeochem. Cycles* **10**, 255–267.
- Yang H. S. (1996) Modelling organic matter mineralization and exploring options for organic matter management in arable farming in Northern China. Ph.D. dissertation, Wageningen Agricultural University.
- Zehnder A. J. B., Huser B. A., Brock T. D., and Wuhrmann K. (1980) Characterization of an acetate-decarboxylating, non-hydrogen-oxidizing methane bacterium. *Arch. Microbiol.* **124**, 1–11.
- Zehnder A. J. B. and Wuhrmann K. (1977) Physiology of *Methanobacterium*, strain AZ. *Arch. Microbiol.* **111**, 199–205.