Microbial processes of CH_4 production in a rice paddy soil: Model and experimental validation

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Abstract—The importance of different anaerobic processes leading to CH_4 production in rice paddies is quantified by a combination of experiments and model. A mechanistic model is presented that describes competition for acetate and H_2/CO_2, 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 CH_4 production. Only the values for maximum conversion rates (V_{max}) for sulphate and iron reduction and CH_4 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 (CH_4) production is the terminal microbial process in anaerobic organic matter degradation in the absence of alternative electron acceptors like O_2, NO_3^-, Fe(III), and SO_4^{2-}. CH_4 production is suppressed directly or indirectly by the presence of alternative electron acceptors (e.g., Achtnich et al., 1995a; Jakobsen et al., 1981). This suppression seems to be caused by competition for common substrates, particularly H_2 and acetate, with micro-organisms using alternative electron acceptors and by several direct inhibitions on CH_4 production caused by accumulated products of anaerobic respiring micro-organisms. Quantification and significance of these processes for CH_4 production are a major uncertainty in CH_4 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 SO_4^{2-} (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 CH_4 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 CH_4 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. CH_4 and CO_2 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 N_2 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), NO_3^-, NO_2^-, SO_3^{2-}, SO_4^{2-}, CH_4, H_2, NO, N_2O, CO_2, sulphide, and pH-H_2O. 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 H_2/l (below the thermodynamically inhibiting concentrations) to the headspace 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), NO_3^-, NO_2^-, SO_3^{2-}, SO_4^{2-}, CH_4, H_2, NO, N_2O, CO_2, sulphide, and pH-H_2O 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).
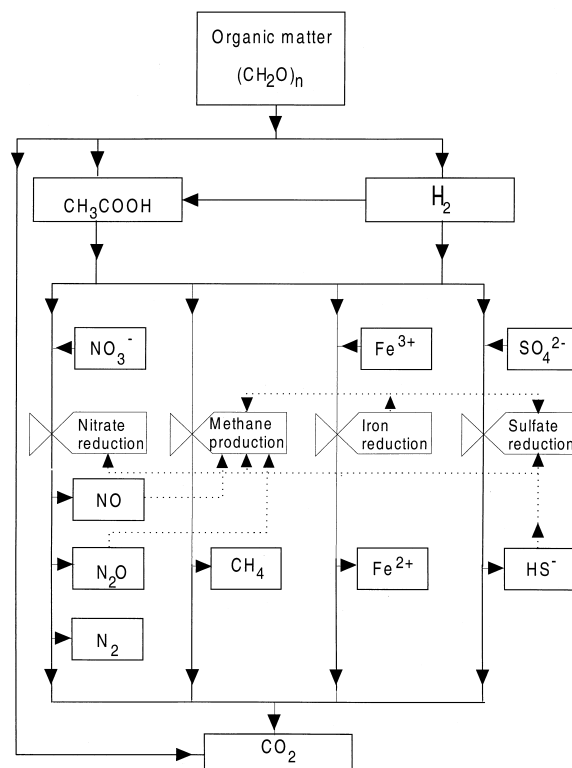

Fig. 1. Schematic representation of the interactions accounted for in the model. Lines indicate compounds flows and dashes indicate inhibitory effects. Chemolithotrophic reactions are not indicated separately in this scheme.

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 H_2/CO_2. These substrates are produced by soil organic matter mineralization and by the decomposition of complex organic substances. This can be summarized by

$$(CH_2O)_n + \tfrac{1}{3}\, nH_2O \rightarrow \tfrac{1}{3}\, nCH_3COOH + \tfrac{1}{3}\, nCO_2 + \tfrac{2}{3}\, nH_2. \quad (1)$$

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):

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

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

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

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)	$V_{max(total)}$ (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(SO$_4$) and K_M(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 Achtnich 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 H$_2$ with anaerobically respiring microorganisms using NO$_3^-$, SO$_4^{2-}$, and Fe(III) as electron acceptors. This competition is described by substrate conversion rates (V: mol m^{-3} water s^{-1}). For methanogens (m_i), substrate conversion is described by Michaelis–Menten kinetics:

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

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 $K_{M,mi}$ is the affinity constant (mol m^{-3} water) while i is H$_2$ (hydrogenotrophic methanogens) and acetate (aceticlastic methanogens), respectively. The substrate conversion rates for the other anaerobic micro-organisms (e_i) is summarized by double Michaelis–Menten kinetics:

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

where $K_{M,d}$ and $K_{M,a}$ are the affinity constants (mol m^{-3} water) for the electron donor (e^- donor) and electron acceptor (e^- acc), respectively. Acetate and H$_2$ are electron donors and NO$_3^-$, NO, N$_2$O, reducible iron

and SO$_4^{2-}$ 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, N$_2$O, CO$_2$, CH$_4$) 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:

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

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:

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

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_{max}(via H_2) = V_{max}(via acetate), because there are not enough published data that allow a further specification. Only for CH_4 production is it assumed that V_{max}(via acetate) is twice the value of V_{max}(via 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:

$$V = V_{max} \frac{[HS^-]}{K_{M,HS} + [HS^-]} \cdot \frac{[NO_3^-]}{K_{M,NO_3} + [NO_3^-]}. \quad (8)$$

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

$$V = V_{max} \frac{[Fe^{2+}]}{K_{M,Fe} + [Fe^{2+}]} \cdot \frac{[NO_3^-]}{K_{M,NO_3} + [NO_3^-]}. \quad (9)$$

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 (Achtnich 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-
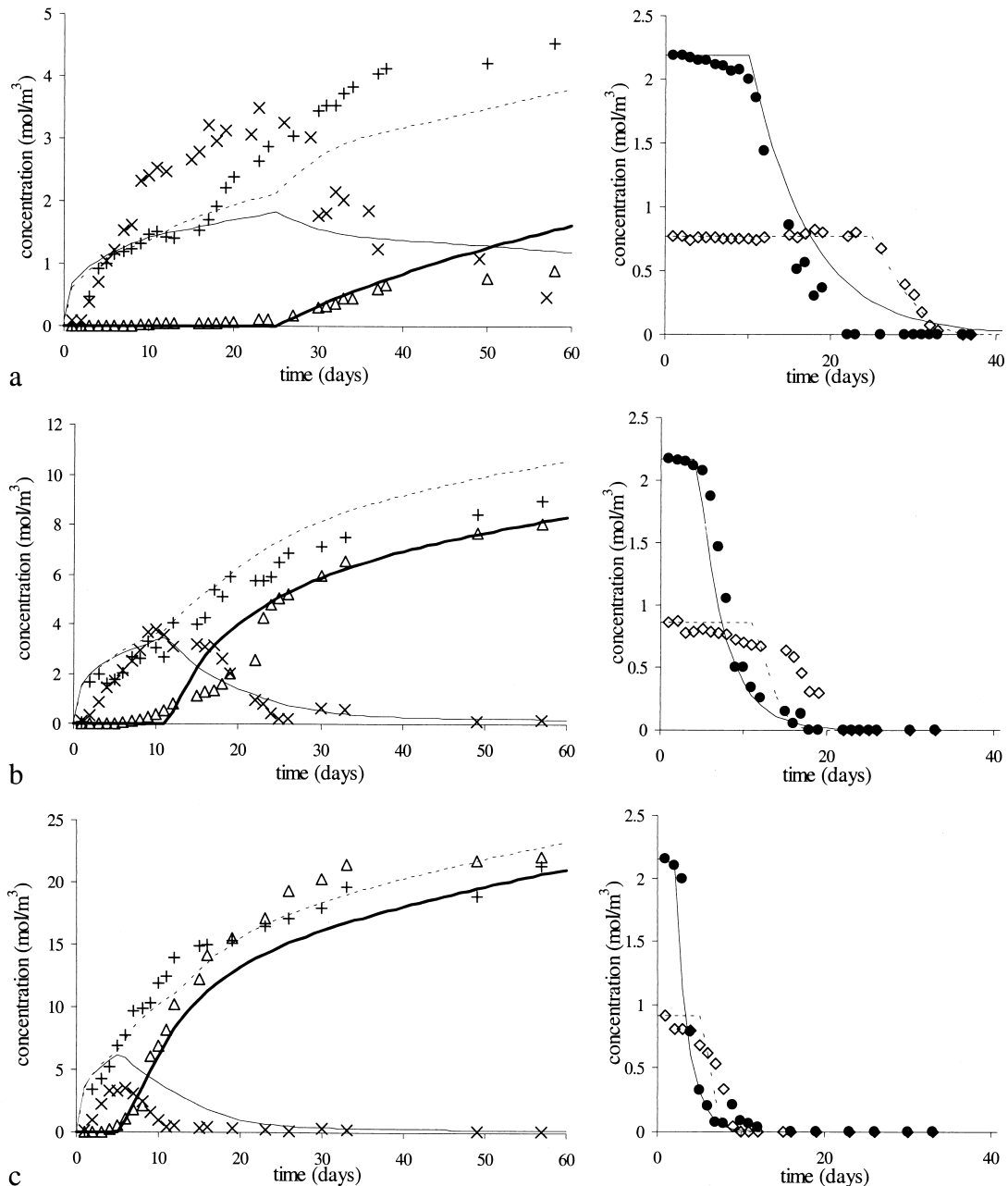
Fig. 2. Comparison of experiment (symbols) and model (lines) for the calibration of a long-term incubation experiment with rice paddy soil at (a) 14°C, (b) 20°C, and (c) 30°C. Indicated are CH$_4$ (open triangles and thick lines), CO$_2$ (pluses and dashed lines), and acetate (crosses and thin lines) 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 CH$_4$ production were reduced—by substrate additions (shown for

20°C in Figure 3. Trends are similar at 30°C). Modelled CO$_2$ 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 H$_2$ treatment and most in the acetate treatment as is shown by the changes in CO$_2$ and CH$_4$ in time (Fig. 3). The increase in available substrate also increased CH$_4$ release rates. Measured and modelled CH$_4$ production rates were not significantly different ($P = 0.76$) for the control, straw addition and H$_2$ addition. In case of acetate addition [Fig.
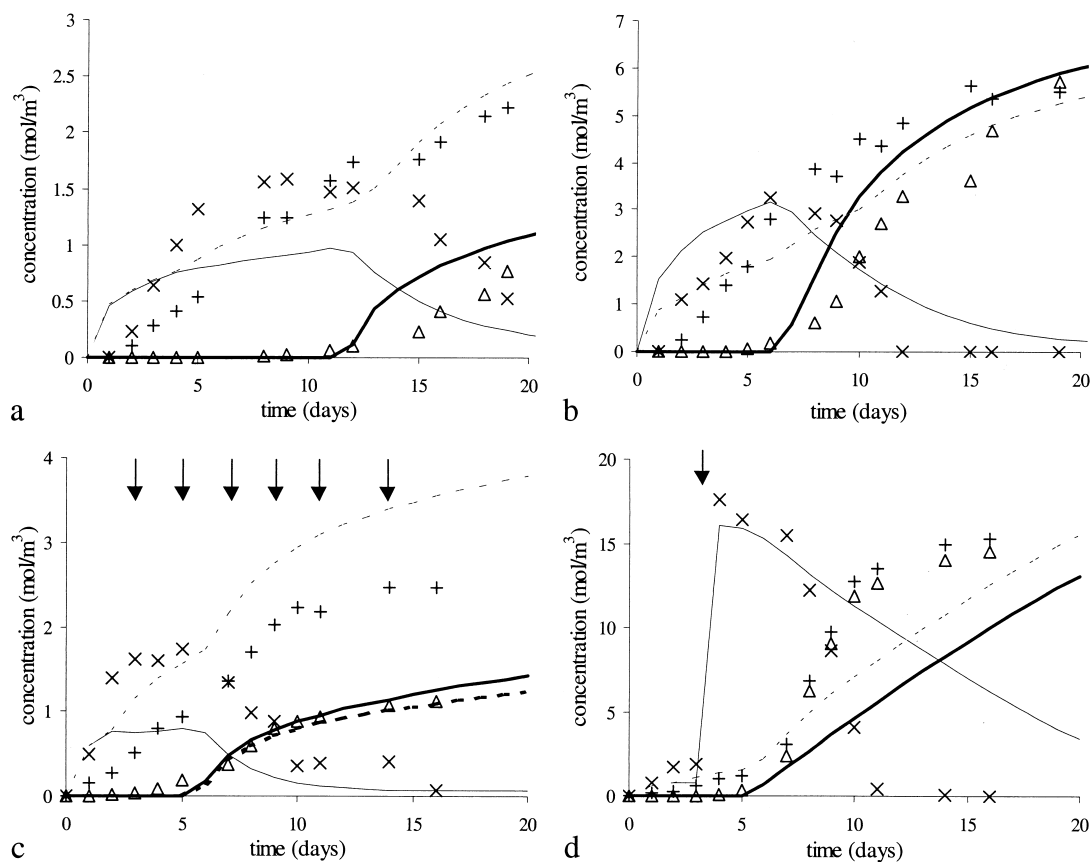
Fig. 3. Comparison of model and experiment for the short-term validation experiment at 20°C for (a) control, (b) addition of rice straw, (c) repeated addition of H$_2$, and (d) addition of 15 mM acetate. Symbols are as indicated in Figure 2.

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$_2$O 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
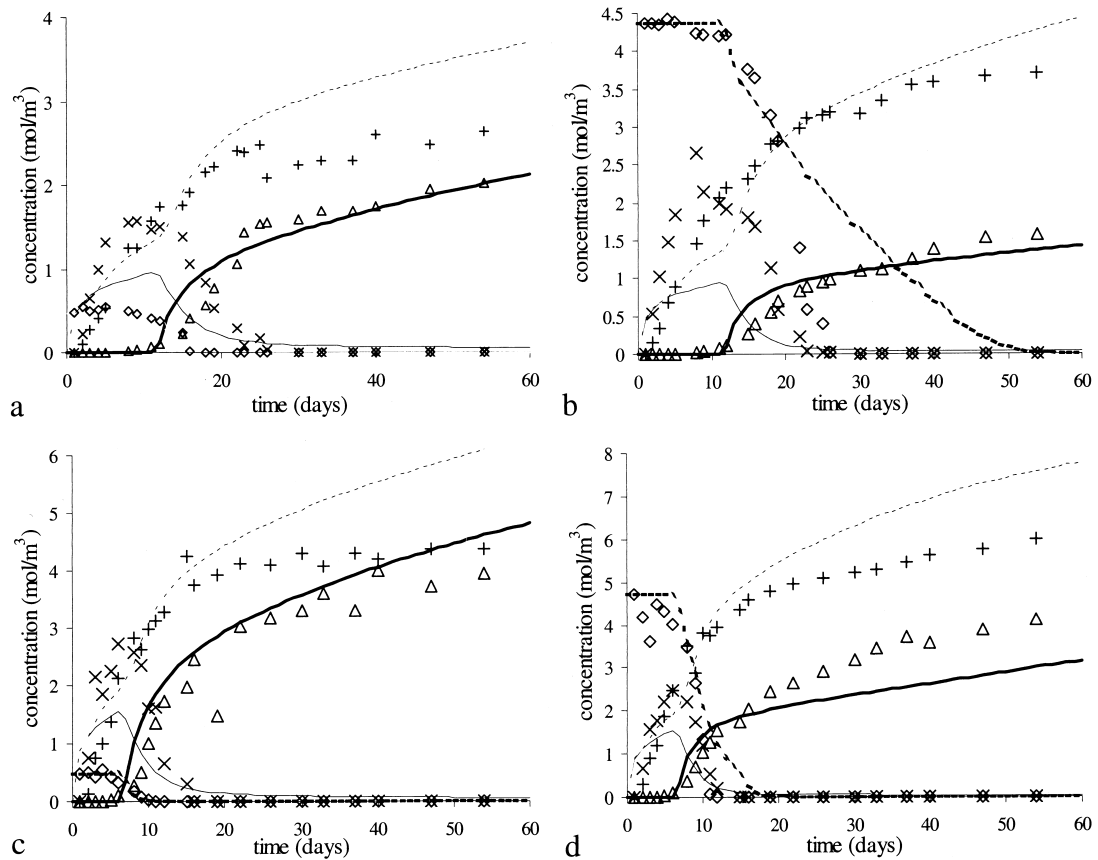
Fig. 4. Comparison of model and measured data for the long-term validation experiment for (a) control at 20°C, (b) addition of 4 mM sulphate at 20°C, (c) control at 30°C, and (d) addition of 4 mM sulphate at 30°C. Symbols are as indicated in Figure 2.

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 CH$_4$
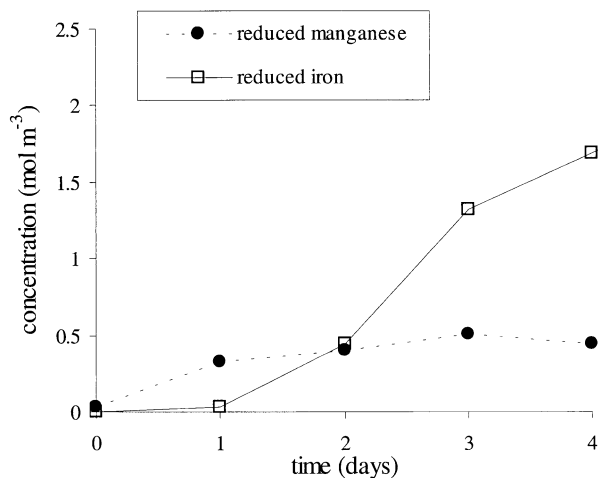

Fig. 5. Sequence of iron reduction and manganese reduction in the short-term validation experiment at 20°C.

production and a Q_{10} of 2 for other processes. Only V_{max} for sulphate and iron reduction and for CH$_4$ 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 Achtnich 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. CH$_4$ production rates are enhanced by 40% during CH$_4$ production initiation if V_{max} of CH$_4$ production is doubled (Fig. 6a), causing a concomitant decrease in acetate and increase in CO$_2$ 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.
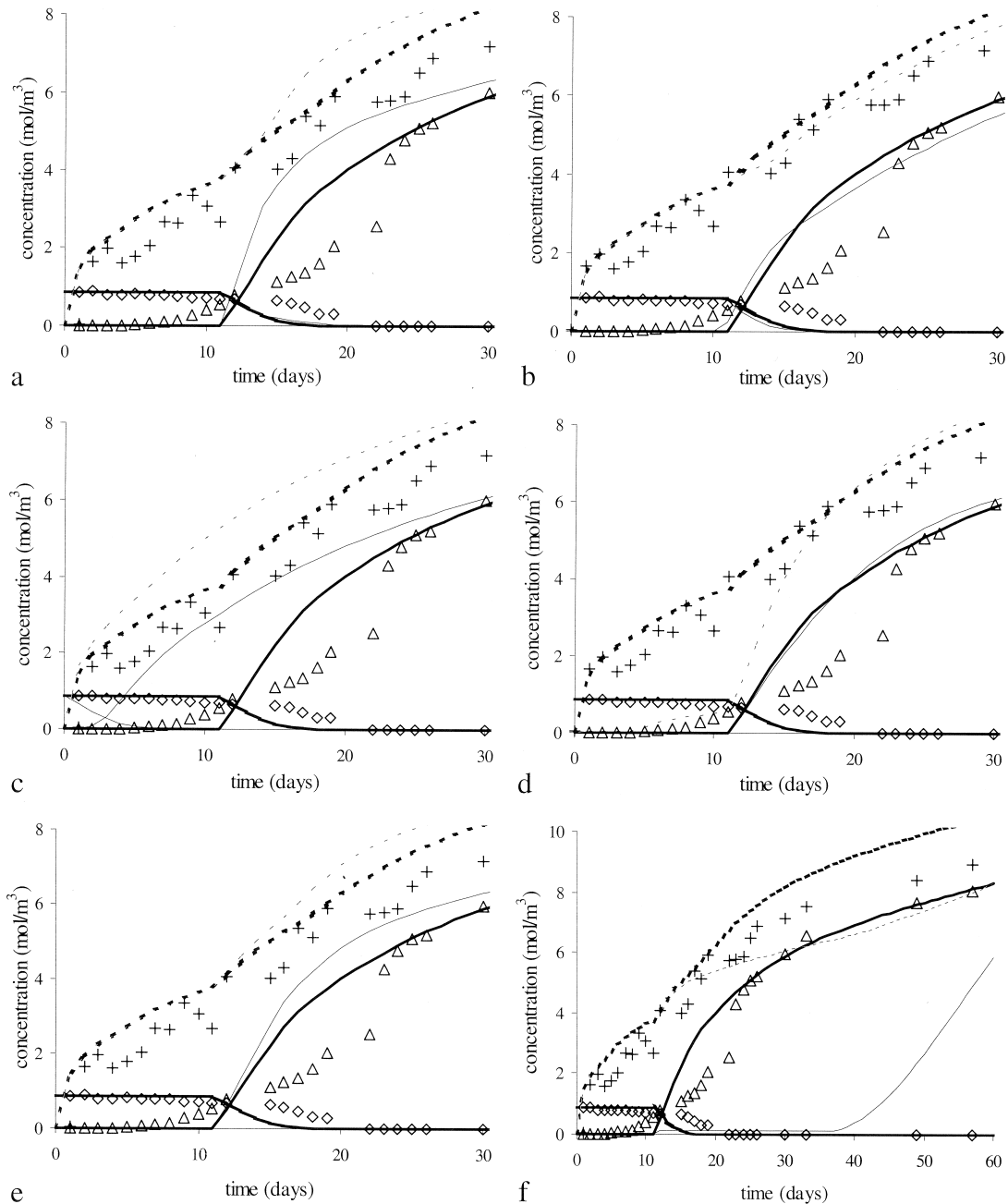
Fig. 6. Model sensitivity analysis at 20°C for the experimental results of the calibration experiment and modelled results of the default model (thick) and of the sensitivity analysis (thin) for CH_4 (triangles), CO_2 (pluses) and sulphate (diamonds) for (a) double V_{max} values for CH_4 production, (b) equal V_{max} for hydrogenotrophic and aceticlastic methanogenesis, (c) no inhibition of sulphate reduction and methane production by reducible iron, (d) without introducing H_2, (e) introducing K_M values for CH_4 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. Aceticlastic methanogenesis V_{max} was assumed to be twice as high as the hydrogenotrophic methanogenesis V_{max} to account implicitly for homoacetogenesis in the CH_4 production phase.

On the other hand, hydrogenotrophic methanogens are more active than aceticlastic methanogens at the start of the rice-growing season (Roy et al., 1997). If V_{max} of hydrogenotrophic and aceticlastic methanogenesis are taken equal, then CH_4 production rates decrease 8% (Fig. 6b) while sulphate and iron reduction rates increase less than 2% (results not shown). Methanogens are less competitive for H_2 than for acetate (Achtnich et al., 1995b; Jakobsen et al., 1981; Lovley and
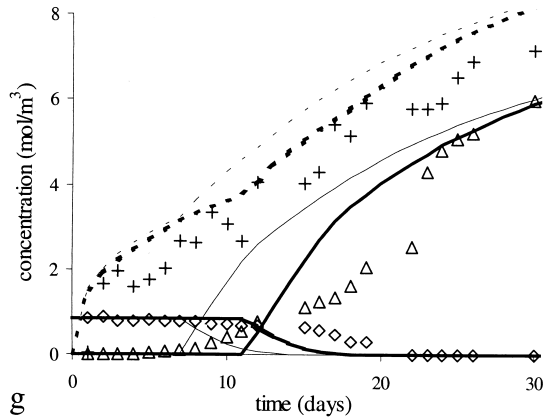
Fig. 6. (*Continued.*)

Phillips, 1987), while H$_2$ conversion is given more importance in this analysis. It is thus important to account for homoaceto-genesis and for community dynamics within a functional group. This further complicates the estimation of $V_{\max}$ values.

The redox sequence in the incubations was well predicted ($r^2 > 0.90$, Sect. 3.1) at various temperatures with only the calibration of $V_{\max}$ values. The redox sequence had overlap in both model and experiment, while a complete separation would be predicted based upon thermodynamically determined affin-ity constants. The additional influence of potential conversion rates, direct inhibitions, and lag times, however, induced se-quence overlap.

The combination of a high $V_{\max}$ and a very high affinity for both acetate and H$_2$ gave the nitrate reducers a large compet-itive advantage over bacteria using other electron acceptors (Table 1). In addition, NO but not N$_2$O concentrations were high enough, maximally 7×10^{-3} mol m^{-3}, to inhibit methane production during the first 2 days. This might be important in N-fertilized rice paddy fields.

Iron reducers had a higher $V_{\max}$ and higher affinity for both acetate and H$_2$ than sulphate reducers and methanogens, which gave them a competitive advantage. Effects of inhibition on CH$_4$ production and sulphate reduction during iron reduction, however, also played a significant role. Acetate (and H$_2$) con-centrations were too high to limit sulphate reduction initiation and if no empirical inhibition is included, then sulphate reduc-tion and CH$_4$ production are initiated immediately, which is much faster than experimentally found (Fig. 6c). This again leads to a faster release of CO$_2$. The inhibition could be predicted well by a single threshold concentration for reducible iron, independent of temperature and substrate (Figs. 3 and 4). This suggests indeed a direct inhibition, even though such effect has not been described and even though the mechanism for such inhibition is unknown. An effect of redox potential seems improbable, since this has been falsified for methano-gens (Fetzer and Conrad, 1993) and a redox potential effect on sulphate reducers is not known. The inhibition is an important cause for the ineffective acetate consumption and concomitant measured acetate accumulation.

One hypothesis to explain the apparent inhibition is the simultaneous occurrence of sulphate reduction and sulphide reoxidation coupled to iron reduction, in which case anaerobic

methane oxidation coupled to iron reduction should occur as well, although this process has never been proven. Other chemolithotrophic reactions seem not important in well-shaken systems. If $V_{\max}$ for the other chemolithotrophic reactions—coupled nitrate reduction/sulphide oxidation and coupled ni-trate reduction/iron oxidation—are put to zero, nothing changes in the model outcome (results not shown). In field situations, where various reduced and oxidized substances become avail-able by transport, such reactions might however be important. More research is needed to elucidate the role of chemolitho-trophic reactions.

CO$_2$ release was also well captured by the model ($r^2 > 0.90$). However, if substrate release is simplified to the release of only acetate—attributing all $V_{\max}$ to the $V_{\max}$ for acetate conversion and assuming that all H$_2$/CO$_2$ is transformed by homoacetogens—then CO$_2$ release is retarded and acetate ac-cumulation overestimated. Initiation and rate of methane pro-duction is however hardly influenced (Fig. 6d), because carbon substrate is not limiting methane production initiation.

Temperature effects were also described properly by the model. This means that the microbial community can react instantaneously to changes in temperature (without having to grow or induce enzymes). This information might be important to understand day and night rhythms in CH$_4$ emissions, found regularly in field studies (e.g., Sass et al., 1991; Schütz et al., 1989; Yagi et al., 1996). It was assumed that substrate affinity was constant with temperature. For methanogens it was how-ever shown that at 20°C the K_M for both acetate and H$_2$ is higher than at the reference temperature of 30°C (Westermann et al., 1989). Such higher affinity for methanogens (maintaining constant K_M values for other microbial processes, by a lack of quantitative data) increases CH$_4$ production rates by 21% dur-ing the initiation phase and methanogens compete more effec-tively with sulphate reducers (Fig. 6e). After sulphate is de-pleted, CH$_4$ production rates are limited by substrate production rates and become equal to default rates. The quan-titative influence of temperature on K_M values needs thus more consideration and might have to be determined separately for various functional groups and, e.g., for fast growing and slow growing acetate consuming methanogens.

4.2. Model Validation

4.2.1. Short-term validation experiment with substrate additions

The first validation experiment tested the model with differ-ent carbon substrate regimes—forced by the addition of straw, H$_2$ and acetate. This is an important validation, because organic substrate release drives the reduction sequence, providing the necessary electrons. The model could describe both the in-crease in CH$_4$ release rates—expected from Michaelis–Menten kinetics—and the decrease in the lag phase for CH$_4$ production and sulphate reduction without changing any microbial kinetic parameter value. Similar effects of substrate additions on the initiation of sulphate reduction and CH$_4$ production have been found by others (Achtnich et al., 1995b; Roy et al., 1997) and can be explained by the combination of a less severe compe-tition for substrates and a faster depletion of alternative electron acceptors. These effects of substrate availability are very large

and suggest that a proper description of substrate availability is more important for CH_4 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 CH_4 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. CH_4 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 Cappenberg (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 CH_4 production start about 4 days earlier than experimentally found if no lag phase is introduced (Fig. 6g). This again leads to an overestimation of CO_2 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 CH_4 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 CH_4 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 H_2/CO_2 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 H_2 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, N_2O, and S^{2-} 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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