DOE/ER/61010--T9

FORMATION AND EMISSION OF METHANE IN RICE SOILS: EXPERIMENTAL DETERMINATION AND MODELING ANALYSIS

Tulane University

Final Report

Submitted to

National Institute for Global Environmental Change
Southern Region
Tulane University

August 31, 1993

Victor J. Law, Principal Investigator
Sanjoy K. Bhattacharya, Co-Principal Investigator

Nancy L. Johnson, Research Assistant

Note: The Louisiana State University work on this contract is covered in a separate document.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

ABSTRACT

Rice paddy soils have been identified as a major source of methane emissions contributing to the observed atmospheric increase in methane. This points to the need for a method of quantifying and predicting methane emissions for the widely varying conditions used in rice agriculture throughout the world. In the present work, a mathematical model for estimating the emission of methane from rice paddy soils is developed and refined. Kinetic parameters for methanogenesis in a Louisiana rice soil are determined from laboratory data on methane production from acetic acid substrate. Use of a stirred reactor allows simultaneous measurement of acetate consumption and methane production while minimizing mass transfer limitations.

An existing model for rice plant growth is utilized to provide data on the availability of root exudates as a carbon source for the methanogens. The final methane model includes the kinetic parameters, plant data, and estimated transport parameters. With adjustments in these parameters, it provides an acceptable match to field data.

MATERIALS AND METHODS

CULTURE DEVELOPMENT

An enrichment culture was started with samples of rice field soil from the Louisiana State University Agricultural Research Station in Crowley, Louisiana. Soil cores were collected using a plexiglass sampling tube (inner diameter 12 cm) with floodwater added to maintain the anaerobic environment [Todd, 1992]. The soil was a silt loam containing 12% clay and 71% silt. Phosphorus, potassium, and urea fertilizer were applied to the field prior to flooding. Typical conditions for this rice field are presented in Table 1 [Lindau et al., 1991].

TABLE 1. Louisiana Rice Field Conditions

Soil Temperature	- 36°C
External Temperature	- 36°C
Floodwater Depth	- 10 cm
Soil pH	
Soil Nitrogen Content	g total N kg^{-1} soil
Soil Carbon Content	g total C kg^{-1} soil

TABLE 2. Composition of Nutrient Solution [Bhattacharya, 1986]

Constituent	Concentration (mg/L)
NH_4Cl	
$MgCl_2$	
KCl	
$CaCl_2 \cdot 2H_2O$	
$(NH_4)_2HPO_4$	
$FeCl_2 \cdot 4H_2O$	
$CoCl_2 \cdot 6H_2O$	
KI	
$(NaPO_3)_6$	
$MnCl_2 \cdot 4H_2O$	
NH_4VO_3	
$ZnCl_2$	
$Na_2MoO_4 \cdot 2H_2O$	
H_3BO_3	
$NiCl_2 \cdot 6H_2O$	
Cysteine	
$NaHCO_3$	

In the laboratory, two enrichment cultures were started with the soil and floodwater in 20-L carboys (Nalgene). The volume was gradually increased over the first few months with the addition of alkaline inorganic nutrient solution, the composition of which is outlined in Table 2. Minimal sodium sulfide was added to achieve strict anaerobiosis [Todd, 1993]. After 2-3 months, the two enrichment cultures were being fed approximately 1000 ppm of acetate per week.

EXPERIMENTAL KINETICS DETERMINATION

The enrichment culture was used to conduct batch studies with the goal of determining the kinetic behavior of the rice soil methanogens growing on acetate. A 2-liter reactor was built which provides continuous stirring, monitoring of Eh and measurement of gas production. The reactor was constructed with a 2-liter Erlenmeyer flask which had been modified for a wider neck. It was sealed with a #13 rubber stopper with ports for liquid and gas sampling, a gas outlet, thermometer, and a redox combination electrode. This setup is shown in Figure 1. In the gas sampling port, an autosampler vial was inserted, which had the bottom cut off and was topped with a septum and screwcap. The reactor was started with 1.8 L of the rice soil enrichment culture, with nitrogen bubbled through the solution after closing the vessel. The stopper provided a good fit, but silicone sealant was added around the edge and around the ports. The vessel was placed on a plexiglass shelf mounted a few inches above a magnetic stirrer. With a stirring bar in the culture solution, this allowed stirring with a minimum of heating effect.

A kinetics study with this reactor was started by feeding 750-1000 ppm of acetic acid and stirring at about 200 rpm for the duration of the fermentation, usually about 20 hours. The solution pH ran from 6.5 upon feeding to approximately 7.0 at the end of the fermentation, with the bicarbonate alkalinity in the range of 1500 to 2000 ppm. The reactor temperature was maintained at approximately 35 °C by location of the apparatus in a constant

temperature room. Liquid samples, about 3 ml, were taken at regular intervals from a tube extending below the liquid level. The samples were filtered immediately through a 0.45 micron filter into small vials. This filtration was performed to remove solids and cells from the sample. Samples were subsequently stored in a refrigerator until they could be analyzed by gas chromatography (GC) to determine the acetate concentration. In preparation for GC analysis, the samples were acidified to a pH of about 3 (determined with pH paper) by adding 85% phosphoric acid in 1-drop increments from a syringe with a 20-gauge needle. Gas samples of approximately 1 ml were taken through the septum on top of the reactor with a syringe equipped with a Luer-lock, and immediately injected on the GC to determine methane composition. The volume of gas produced was measured by connecting the gas outlet tube to a water column constructed from a graduated cylinder.

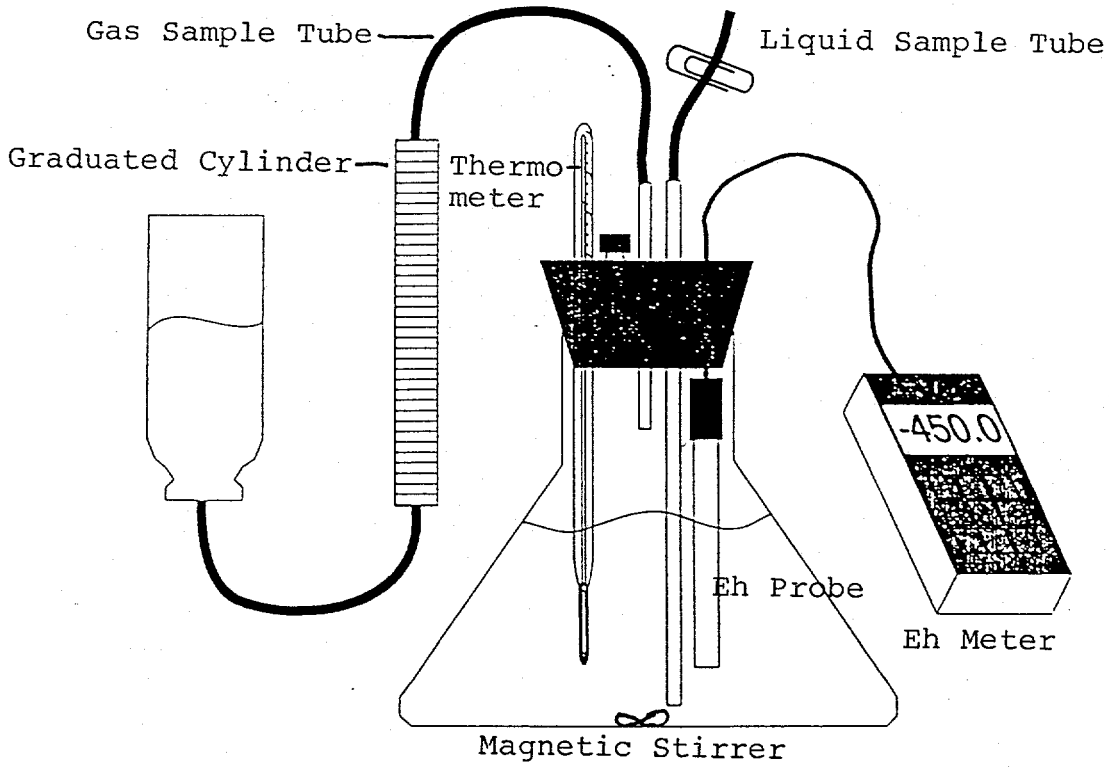

Figure 1. Diagram of Reactor

ANALYTICAL METHODS

Gas chromatographic analysis of liquid samples was done with a Shimadzu GC-14A on a glass column packed with 60/80 Carbopack C (Supelco). The column temperature was 120 °C while the injector and FID detector were run at 200 °C. Standards were prepared with 99% glacial acetic acid by serial dilution to cover the range of concentrations present in the samples. Calibration curves were used to determine the acetate concentration in samples from the peak areas determined for standard solutions. Gas chromatographic analysis of gas samples was done with a Shimadzu GC-8A on a 1/8 inch-diameter stainless steel column packed with 100/120 Haye Sep D. The column was operated at 60 °C and the injector temperature was 170 °C. A prepared gas standard with 39.86% methane and CO_2 making up the balance (Quality Gas Products, Inc.) was used to calibrate for methane concentration each time the instrument was used.

In the rice soil reactor, a combination redox electrode from Orion was used to measure Eh with a Fisher Scientific Accumet 950 pH/ion meter. Reactor tubing was Tygon brand. External pH measurements were made with a Fisher Accumet 910 pH meter, and titrations for volatile acids were done with 0.2 N H_2SO_4 using the method of Jenkins et al. (1983) from which the total and bicarbonate alkalinity and volatile acids content can be determined. For the microfiltration of samples, 0.45 micron filters (25 mm diameter) from Gelman Sciences were used.

DETERMINATION OF KINETIC CONSTANTS

The reactor described in the METHODS section provides continuous stirring, monitoring of Eh and measurement of gas production. Stirring the culture solution is important since the goal is to measure kinetic rates of reaction, and thus mass transfer limitations must be eliminated as much as possible. The production of methane is dependent on a low redox

potential, as previously stated, making the monitoring of Eh useful. Another goal was to measure the rate of methane production, which, combined with acetate consumption data, should yield more accurate determination of kinetic constants. Use of this reactor has eliminated the erratic behavior noted in previous data obtained from serum bottle kinetics studies.

The Monod kinetic expressions were used for the change in substrate and cell concentrations, as given by the following.

$$\frac{dS}{dt} = -\frac{kSX}{K_s + S}$$

$$\frac{dX}{dt} = Y\left(\frac{dS}{dt}\right) - bX = Y\frac{kSX}{K_s + S} - bX$$

where S = substrate concentration

K_s = Monod half velocity constant

k = maximum specific uptake rate of substrate

X = cell concentration

Y = yield coefficient (yield of cells from substrate)

b = decay coefficient

An equation for the change in methane concentration (M) is derived as detailed by Todd (1992), and the final equation is as follows.

$$\frac{dM}{dt} = \frac{dS}{dt} \cdot \left[1 - \left(Y - \frac{0.8b(K_s + S)}{kS}\right)\right] \cdot \frac{16}{59}$$

The differential equations presented above were solved using a fourth order Runge-Kutta algorithm. With the experimental data, a FORTRAN program called ConReg [Law, 1991], which performs constrained nonlinear regression analysis, was used to determine the

kinetic constants. The use of two sets of data, for changes in both substrate and methane concentrations with time, made it possible to solve for six unknown parameters - the kinetic constants K_s, k, b, and Y, along with the initial cell and substrate concentrations (X_o and S_o). The "best fit" values for these parameters are shown in Table 3. The excellent fit of the data provided by the regression analysis can be seen in Figures 2-5; however, the parameters K_s and b are not being determined to an acceptable degree of certainty, as evidenced by their standard deviations. In addition, the yield coefficient calculated for reactor study #3 has a large standard deviation. The values obtained for Y from this analysis are significantly greater than those previously reported. Zehnder et al. (1982) cite a value of 0.043 g cells/g substrate for the yield coefficient of a mixed culture grown on acetate.

TABLE 3. Summary of Kinetic Parameters

	Reactor Study #2		Reactor Study #3	
Parameter	Value	Standard Deviation	Value	Standard Deviation
K_s	mg/L		mg/L	
k	hr^{-1}		hr^{-1}	
b	x 10^{-5} hr^{-1}	---	x 10^{-5} hr^{-1}	
Y	mg/mg		mg/mg	
X_o	mg/L		mg/L	
S_o	mg/L		mg/L	

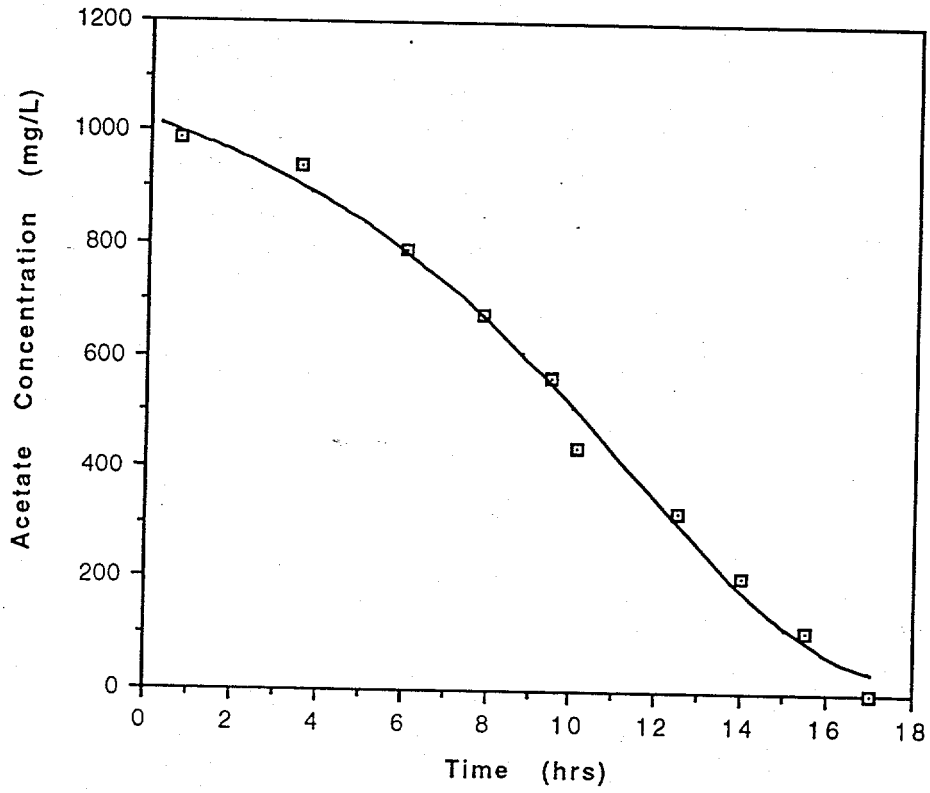

Figure 2. Comparison of Measured with Predicted Values for Acetate Concentration in Reactor Study #2

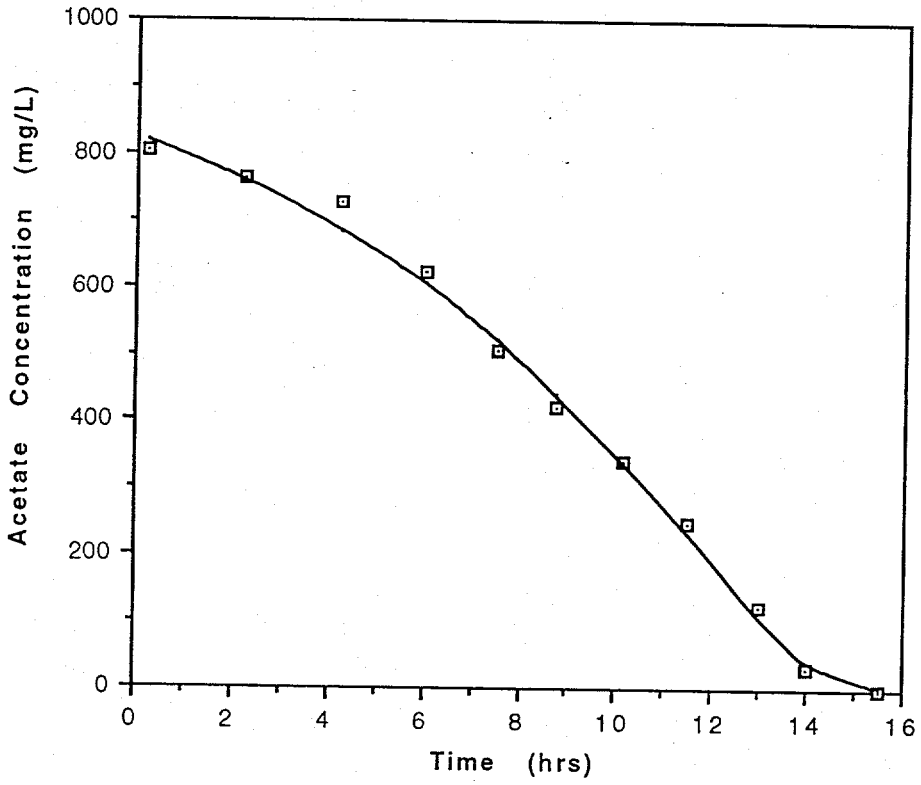

Figure 3. Comparison of Measured with Predicted Values for Acetate Concentration in Reactor Study #3

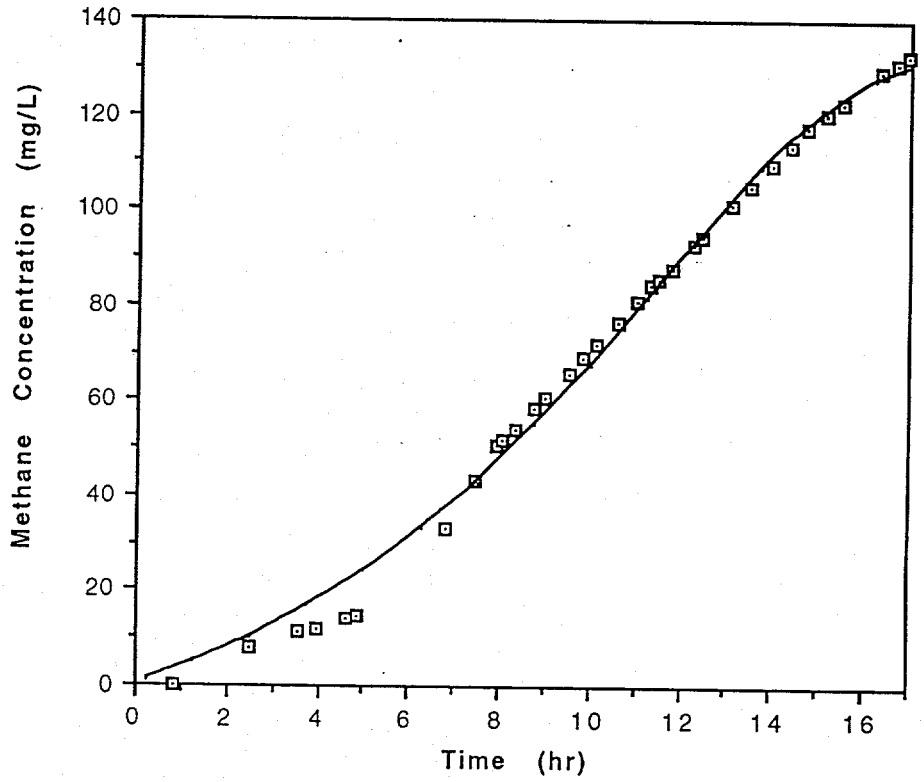

Figure 4. Comparison of Measured with Predicted Values for Methane Concentration in Reactor Study #2

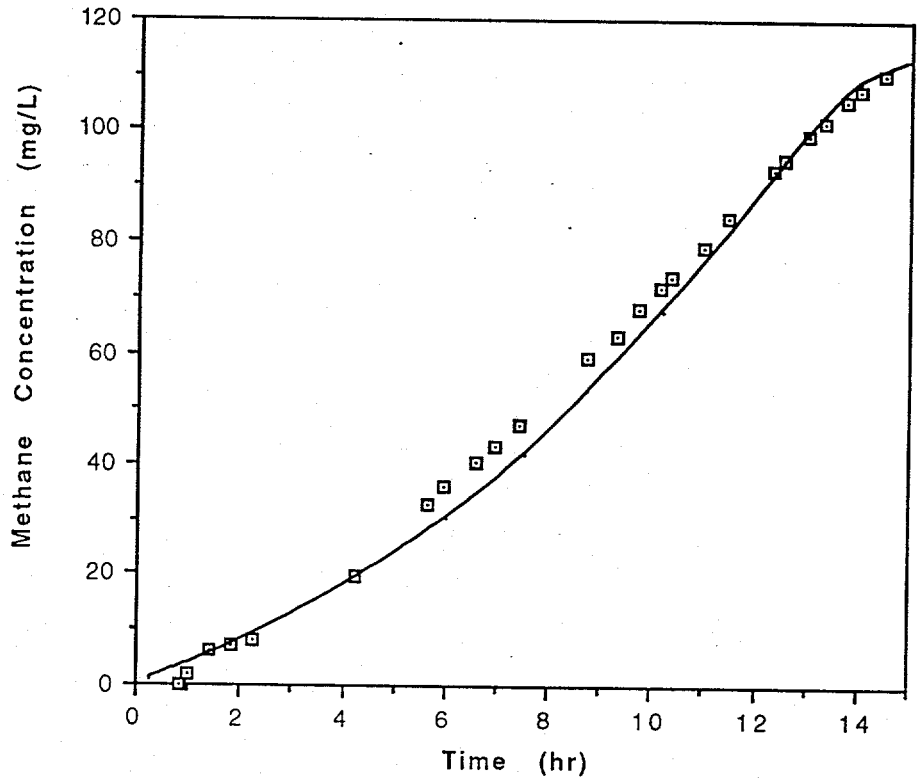

Figure 5. Comparison of Measured with Predicted Values for Methane Concentration in Reactor Study #3

MODEL DEVELOPMENT

MODEL EQUATIONS

In modeling the rice soil system, there are four major processes that must be included - (1) Kinetics of methanogenesis, (2) Carbon substrate availability, (3) Mass transfer of methane through the plant, and (4) Oxidation of methane. The Monod equations described previously are taken as the starting point of the model. These differential equations are expanded to include terms accounting for the addition of substrate in the form of root exudates, and the disappearance of product from the reaction zone by diffusion to the rice plant. The mass balance equations are presented here.

Substrate or Carbon Balance

$$\frac{d(SV)}{dt} = -V\left[\frac{kSX}{K_s + S}\right] + K_r A_r f(t) \; ; \; S(0) = S_o$$

Methanogen Balance

$$\frac{d(XV)}{dt} = VY\left[\frac{kSX}{K_s + S}\right] - bXV \; ; \; X(0) = X_o$$

Methane Balance

$$\frac{d(MV)}{dt} = V\left[\frac{kSX}{K_s + S}\right]\left(1 - Y + \frac{0.8b[K_s + S]}{kS}\right)\frac{16}{59} - K_p M A_r \; ; \; M(0) = 0$$

where S = substrate concentration, g m^{-3}

S_o = initial value of S (initial concentration of carbon in the soil)

X = concentration of methanogenic bacteria, g m^{-3}

X_o = initial value of X

M = concentration of methane, g m^{-3}

t = time, days

V = reaction volume, m^3

k = maximum specific uptake rate of substrate, d^{-1}

K_s = Monod half velocity constant

Y = yield coefficient, (g cells/g substrate)

b = decay rate, d^{-1}

K_r = mass transfer coefficient of carbon from the roots, g m^{-2} d^{-1}

A_r = surface area of roots, m^2

= function representing root carbon availability, dimensionless

K_p = mass transfer coefficient of methane to the roots, m d^{-1}

The term $K_rA_rf(t)$ represents the carbon substrate provided by root exudates, and K_pMA_r represents the methane transported to the plant's roots. The use of these mass transfer coefficients is one simplifying assumption of the present model. Its physical significance can be enhanced by describing mass transfer as multicomponent diffusion through a tube, with the transport of CH_4 and O_2 occurring in opposite directions, or even better as multicomponent diffusion through porous media. The function f(t), representing the availability of root exudates, was previously input as a step function, but acquires physical significance with the use of a plant model as described in the section below.

In addition, methane oxidation should be accounted for with a kinetic equation. As previously noted, 60-80% of the methane produced in rice soils may be oxidized by the methanotrophic bacteria in the rhizosphere. The methane flux to the atmosphere must be the difference between K_pMA_r and $r_{ox}MV$, where r_{ox} (day^{-1}) represents a rate constant for the oxidation of methane, divided by the area of one plant, A_p (m^2). In the present work,

however, methane oxidation is not included in the model. This simplification is contained in the value of K_p.

To solve the above model equations for changes in substrate, biomass, and product, the Stella II program [High Performance Systems, Inc., 1990] was used on an Apple Macintosh computer. Stella uses a fourth order Runge-Kutta finite difference method to solve differential equations. The equation input is represented by a diagram, and results of a run can be displayed in tables or graphically. Changes are easy to make, allowing quick evaluation of the sensitivity of the model to various parameters.

USE OF RICE PLANT MODELS

A number of computer models are available which predict the yield of rice plants given soil, water, and weather conditions. No existing model includes the simulation of methane emissions. According to the U.S.E.P.A. review (1991), at least five rice models are interactive and could be adapted for methane studies: CERES-RICE [Alocilja and Ritchie, 1988; Godwin et al., 1990], MACROS [Penning de Vries et al., 1989], RICEMOD [McMennamy and O'Toole, 1983], RICESYS [Graf et al., 1990a,b], and the model of T. Horie [Horie, 1987]. The MACROS model was used for the present work since it was the first comprehensive model including coding and documentation made available. However, the CERES-RICE model, obtained more recently, appears to be of equal scope.

The importance of using a rice plant model lies in the requirement for a quantitative estimate, or at least a qualitative pattern for, the carbon substrate availability from root exudates. The documentation for MACROS states that root exudation is not included in the program, but could be incorporated as a fixed fraction of root growth or of gross photosynthesis. Estimates for this excretion run from 0-20% of the carbon assimilated by the plant [Penning de Vries, 1989]. MACROS is available as a FORTRAN program, with subroutines that can simulate potential crop growth, crop transpiration/soil evaporation, and

soil water balance. Subroutine L1QM, which gives growth rates for various parts of the plant with quarter day time steps, was chosen for the present work. Changes must be made in the weather data to correspond to the location and time period of interest. The six weather variables are daily global radiation, minimum and maximum air temperature, vapor pressure, wind speed, and rainfall.

To test the model's simulation capability, methane flux data from a Crowley, Louisiana rice field [Lindau et al., 1991] was chosen for comparison. A run of the program MACROS provided data for the growth rate of rice plant roots, which was used as the qualitative form of the function f(t) for the availability of root exudates. The growth rate function is shown in Figure 6.

MODEL RESULTS

The model equations presented in the previous section were solved using kinetic parameter values determined from experimental data, and estimated values for the mass transfer coefficients, initial values of the substrate and cell concentrations, and geometric parameters related to the plant. The root exudation function, f(t), was taken as a fraction of the root growth rate given by the rice plant model MACROS. The values used in the model solution are given in Table 4.

TABLE 4. Values for Model Parameters

Kinetic Parameters		
	k	d^{-1}
	K_s	$g\ m^{-3}$
	b	d^{-1}
	Y	
Mass Transfer Coefficients		
	K_r	$g\ m^{-2}\ d^{-1}$
	K_p	$m\ d^{-1}$
Initial Values		
	X_o	$g\ m^{-3}$
	S_o	$g\ m^{-3}$
Geometric Parameters		
	A_r	m^2
	A_p	m^2
	V	m^3
Root Exudation Function		
		See Figure 6

The solution of the model equations with the parameters listed above is presented in Figure 7 with a comparison to field data from Crowley, Louisiana [Lindau et al., 1991]. The model contains a few adjustable parameters which must be changed to be representative of the location of interest, particularly the initial soil carbon concentration and the root exudation function. As previously stated, improvements are anticipated in the model's representation of methane mass transfer and methane sink mechanisms.

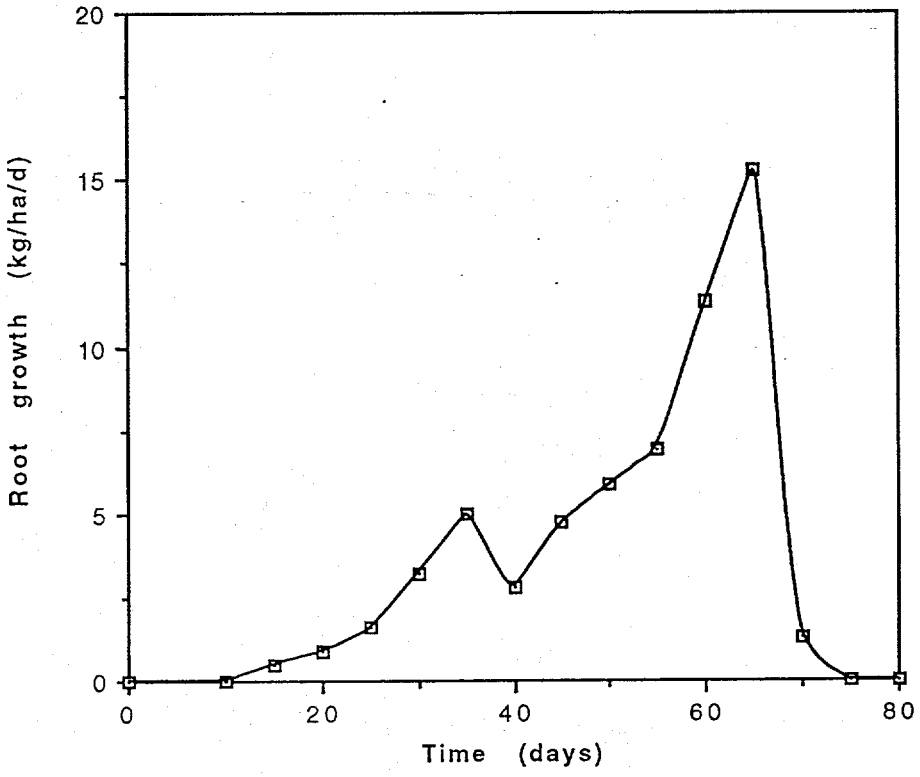

Figure 6. Growth Rate of Roots from MACROS

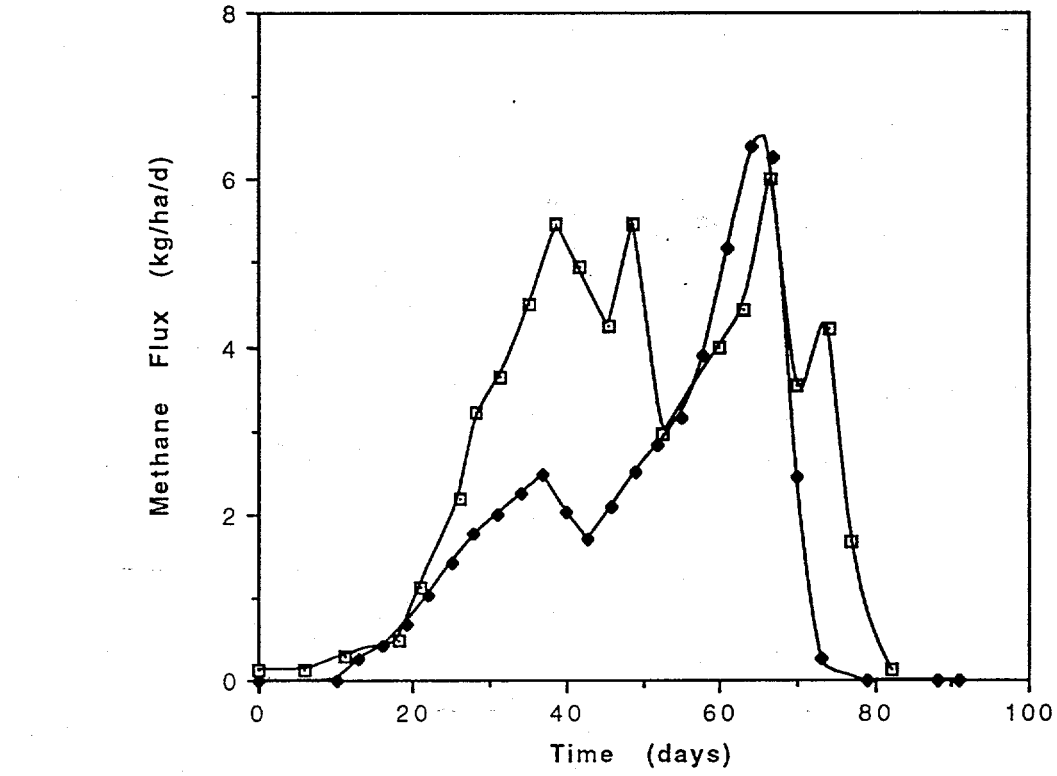

Figure 7. Comparison of Methane Flux Field Data to Model Predictions

CONCLUSIONS

Kinetic parameters for methanogenesis in a Louisiana rice paddy soil were determined from laboratory data on methane production from acetic acid substrate. The four kinetic parameters were found to be in the following ranges: $\mathbf{K_s}$, 57.2 - 287.6 mg/L; **k**, 6.5 - 8.6 d^{-1}; **b**, 4.8×10^{-4} - 9.1×10^{-4} d^{-1}; and **Y**, 0.49 - 0.51. The values obtained for Y are significantly greater than those previously reported. No satisfactory explanation has been found for the large Y values, but a potential reason would be that some of the produced methane was not being measured leading to a falsely high cell yield.

Use of a stirred reactor allowed simultaneous measurement of acetate consumption and methane production, while minimizing mass transfer limitations which would interfere with the determination of methanogenic reaction kinetics. In addition, with the use of a predictive model for rice plant growth, the representation of carbon substrate availability from root exudates, f(t), is now a function of the root growth rate rather than the previously estimated step function.

A mathematical model for predicting the methane flux from rice fields has been developed and refined. It includes the kinetic parameters, plant data, and estimated transport parameters, and, with adjustments in these parameters, it provides a reasonably good match to field data, at least for the second seasonal peak in the methane flux, though its prediction of the first peak is too low, about half that of the field data.

The methane model presented is still preliminary and requires further refinements to be a useful tool. Planned refinements include modeling methane mass transfer as multicomponent diffusion and modeling the oxidation of methane with kinetic equations for the reactions of methanotrophic bacteria. More information about rice plants, for example, on root exudates and changes in plant geometry, can be extracted from available plant models like MACROS, as long as data are available on weather and soil conditions. Future goals for the methane model include not only predictive capabilities but worldwide applications, with development of a global database.

TECHNOLOGY TRANSFER

Presentations:

Law, V. J., N. L. Johnson, and S. K. Bhattacharya, "Preliminary Efforts Involving Modeling Methane Emissions from Rice Soils," NIGEC Atmospheric Methane Conference, Irvine, CA, January 5-10, 1992.

Law,V. J., N. L. Johnson, A. Oyefodun, and S. K. Bhattacharya, "Modeling Methane Emissions from Rice Soils," ENVIROSOFT 92, Portsmouth, U.K., September 7-9, 1992.

Publications:

Law,V. J., N. L. Johnson, A. Oyefodun, and S. K. Bhattacharya, "Modeling Methane Emissions from Rice Soils," **Environmental Software, 12,** 123 (1993).

Lindau, C.W., P.K. Bollich, R.D. Delaune, W.H. Patrick, Jr. and V. J. Law, "Effect of Urea Fertilizer on Methane Emissions from a Louisiana, USA Rice Field." **Soil Science, 136,** 195 (1991).

Pending Publications:

Todd, J.C., S.K. Bhattacharya, and V. J. Law, "Biological Kinetics Modeling," submitted to **Environmental Software**, October, 1992.

Todd, J.C., S.K. Bhattacharya, and V. J. Law, "Biotreatment of Hazardous Chemicals: Stoichiometry Software," submitted to **Environmental Software**, October, 1992.

Law, V. J., N. L. Johnson, and S. K. Bhattacharya, "Modeling Methane Kinetics and Emissions from Rice Soils," submitted to **Environmental Progress**, August, 1993.

Academic Theses:

Todd, J. C., Modeling Biokinetics and Process Stoichiometry of Anaerobic Systems. M.S. Thesis, Tulane University, New Orleans, LA (1992).

Johnson, N. L., Modeling Methane Emissions from Rice Soils. M.S. Thesis, Tulane University, New Orleans, LA (1993).

Pending Academic Theses:

Oyefodun, A., Modeling Methane Transport in Aquatic Plants. Ph.D. Dissertation, Tulane University, New Orleans, LA (expected 1994).