Estimates of global methane production from rice paddies based on substrate requirement

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Estimates of methane emission from rice paddies are highly variable and hence debatable. Most of the estimates were made without consideration of the substrate requirements. A mole of glucose should normally produce 2 moles of methane yielding 178 mg methane from 1 g of glucose. In our incubation experiment only 10–11% of this potential efficiency was realized. Estimates of biomass of globally produced rice were made from the yield of rice and thus of the potential leaching of the organic substances which gives an upper limit of 13 Tg global methane production from rice paddies.

RICE paddies have been identified as an important source of methane[1]. This was based on some experiments which did not simulate rice-growing conditions of Asia where 92% of the world's rice is grown. The experiments in Europe and US had high organic matter and nitrogen in the soil (200 kg N ha^{-1}) and continuous standing water with an yield of 7000 kg ha^{-1} as against Asian soils with low organic matter and low fertilizer input, which varies from none to 150 kg N ha^{-1} with an yield average of hardly 2000 kg ha^{-1}. In most of the experiments the emphasis was on developing suitable techniques of sampling and gas chromatography. However, the important observations on diurnal variation in methane emission, and the maximum flux of methane coinciding with the maximum biomass of the crop were not utilized for understanding the limits of methane production. The microbial and biochemical basis of methane production has been known[2], according to which a source of carbon is essential for methane production. Here we report the estimates of methane emission from rice paddies on the basis of requirement of carbon source.

Rice-paddy soils are the source of methane, for which there ought to be a source of carbon. This could be either organic carbon or constant supply of carbon source (carbohydrate or organic acids) from the crop itself[3]. Since soil organic carbon is apparently not a major source of methane because about no methane is produced from soils without a rice crop, hence, it was assumed that a carbohydrate such as glucose would simulate rice-paddy soil for methane production.

Soil samples were taken at random from a depth of 0–15 cm from a plot of 500 m^2 after the rice harvest. The samples were air-dried and sieved through 2 mm mesh screen. The soil samples were incubated at 30± 1°C and 35± 1°C temperature in conical flasks of 1 l capacity each. This range of temperature was chosen because at this temperature rice cultivation in India takes place and methanogenic activity is maximum. Provisions were made in the flask for measurements of pH and Eh, collection of gas samples for methane estimation and collection of liquid samples for estimation of acetate content.

Two hundred and fifty grams of air-dried soil was taken in each flask and distilled water was added to cover the soil with a 5 cm deep layer of water. Nitrogen gas (99.998%) was flushed every 3 days for 5 min to ensure complete anaerobic conditions. Two treatments, viz. control (no glucose) and 1 g glucose, were given to the soil (250 g) in different incubation flasks in three replicates.

Gas samples were collected using 1 ml gas-tight syringe through a three-way valve at a regular interval of 24 h for 30 days. Supernatant water samples were also drawn from the side port of the flask to estimate acetate content. Volume of samples was replenished by equal amount of water.

Gas samples were analysed for methane on a Packard Gas Chromatograph (Model 438) using flame ionization detector (FID)[4]. Stainless steel column packed with molecular sieve 5 Å 60–80 mesh at a temperature of 80°C was used for methane analysis. The injector and detector were kept at 100°C. Nitrogen at a rate of 15 ml min^{-1} and H_2 at a rate of 25 ml min^{-1} were passed through as carrier and fuel gases respectively. The equipment was calibrated and frequently checked against the standard gas samples.

Five ml sample of supernatant aqueous phase above the soil was obtained using a syringe through a side port and was passed through Whatman No. 1 filter paper. The acetate concentration was measured using an Ion Chromatograph (Dionex, Dionex Corporation, USA) fitted with a suppressed conductivity detector and integrator. The column used was 4S 4A-5C. The eluent was a mixture of 1.7 mM sodium bicarbonate and 1.8 mM sodium carbonate in the ratio of 1 : 1 with a flow rate 2 ml min^{-1}. The pump pressure was 1160 psi. Samples were injected through a loop of 25 µl automatically by a pneumatic pressure-operated injector and the excess volume was flushed out. The anions were separated in the column and detected by the conductivity detector and the chromatograms were recorded by the integrator. The acetate concentration in the samples was determined with reference to a standard solution of 30 ppm acetate prepared from ammonium acetate (AR grade).

The pH was measured using the combined electrode in the soil solution through the side port of the flask using digital pH meter, Century-model CP 901. Eh (redox potential) was recorded using the same pH cum mV meter. A platinum micro electrode prepared in the

laboratory was installed permanently in the flask and a calomel electrode through side port served as a reference electrode.

Methane production from control (absence of glucose) at 30°C showed a steep rise from day 7 onwards and reached a maximum value of 0.07 $\mu g\ g^{-1}\ day^{-1}$ by day 18. The production then declined to 0.01 $\mu g\ g^{-1}\ day^{-1}$. The pH during the experimental period varied between 7.1 and 7.9. However, Eh reached – 157 mV by day 13 and then remained between – 183 and – 234 mV from day 17 onward (Figure 1). The correlation coefficient between methane emission and Eh and pH independently was – 0.90 and 0.16 respectively (Table 1).

The incubation of the soil with 1 g glucose gave an emission of 8.88 $\mu g\ g^{-1}\ day^{-1}$ on day 15, being 130 times higher than the control (Figure 1). The Eh also declined sharply to – 480 mV by day 10 and reached – 498 mV by day 25. Initially, the pH was reduced to 6.2 but with the decline in methane production it increased and reached a value of 7.9. The correlation coefficient between emission and Eh was – 0.82, and between methane emission and pH it was – 0.14. Table 1 gives the correlation coefficient between methane emission with Eh and pH. At 35°C, the maximum production of methane (9.03 $\mu g\ g^{-1}\ day^{-1}$) was observed on day 10. The Eh of the system declined below –200 mV by day 12 and reached –410 mV on day 14. The production of methane declined to 0.154 $\mu g\ g^{-1}\ day^{-1}$ by day 30.

Acetate accumulation started by day 5 and day 6 when the Eh was still above –100 mV. In the treatments of 1 g glucose at 30 and 35°C acetate accumulation preceded methane emission. Figure 2 shows this relationship at 35°C.

Methane production from rice paddies has been estimated considering microbiological processes and also the potential requirements of substrate for methane emission[3]. Since plants do release organic substances, such as, sugars and organic acids, these substances would serve as substrates for methane production and other microbial activities such as growth, multiplication and production of metabolites. Assuming that eventually glucose is the substrate, then 32 g methane would be produced from 180 g glucose if there was no reduction of CO_2 to methane or 178 mg methane would be produced for 1 g of glucose. In our experiment the maximum amount of methane production was 17.49 mg and 19.16 mg from 1 g glucose at 30 and 35°C respectively. This means that the substrate utilization efficiency was only 10% and 11% at 30 and 30°C respectively.

The cause of such low efficiency of the conversion of glucose to methane may be due to the following reasons: a) A part of the glucose may be converted to carbon dioxide or got bound in clay organic matter complexes, thus reducing efficiency; b) Inhibitory nature of some of the end product in the process of methane formation may reduce efficiency; c) Methane oxidation in the upper layers of the soil by methanotrophic bacteria may reduce efficiency.

These data can now be used for assessing the carbo-

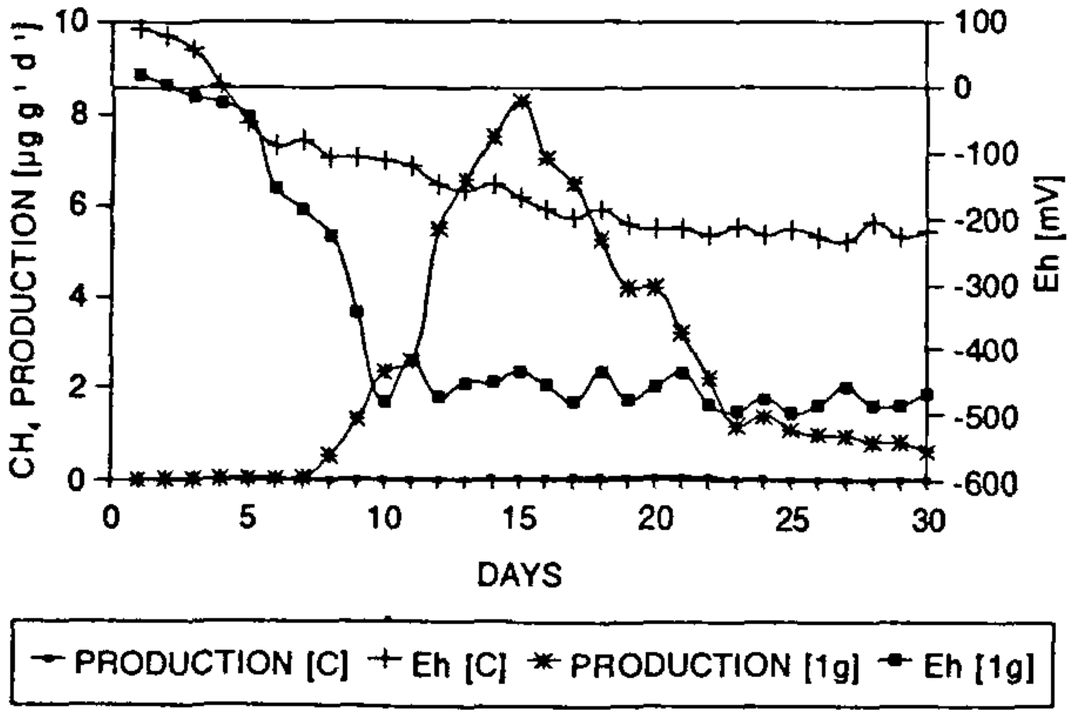

Figure 1. Time-course of redox and methane production with or without glucose amendment.

Table 1. Correlation coefficients for the relationship of methane emission with Eh and pH in soils with and without glucose amendment

Incubation temperature	Treatments	Eh	pH
At 30°C	Control	–0.90*	0.16
	1 g glucose	–0.82*	–0.14
At 35°C	Control	–0.91*	0.04
	1 g glucose	–0.90*	0.03

*Significant at 1%.

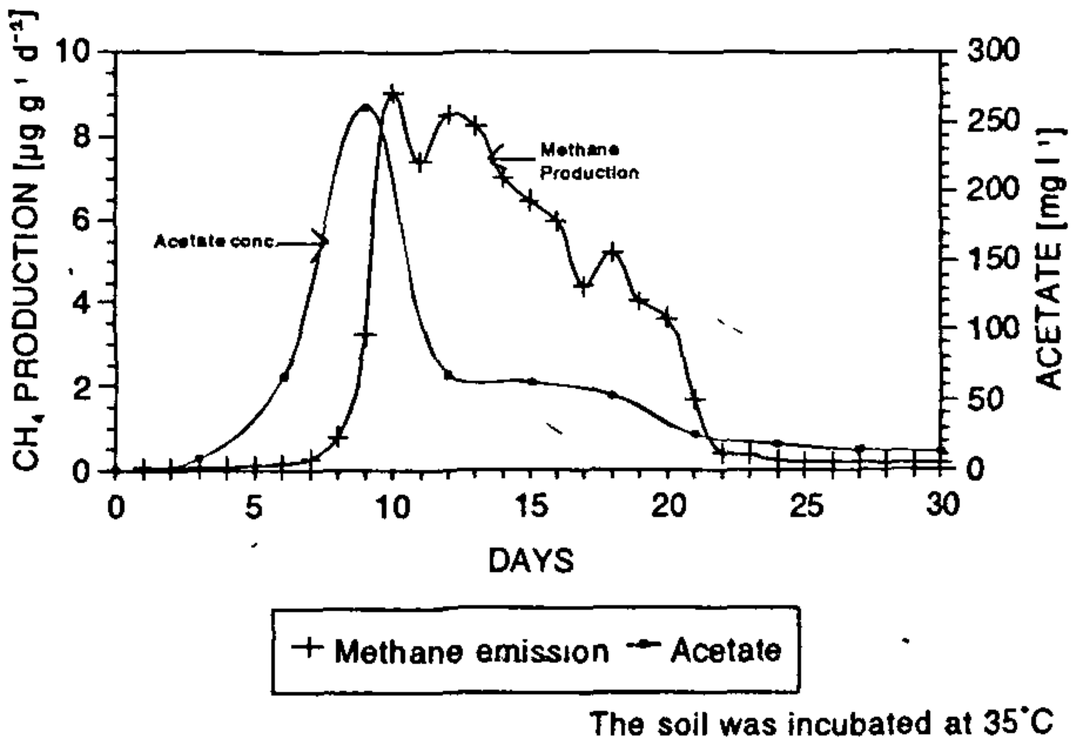

Figure 2. Effect of 1 g glucose as a substrate on methane emission and acetate accumulation.

hydrate requirements for methane production. According to the IPCC (1992), the annual average production of methane from rice paddies is 110 Tg. If the efficiency, as experimentally observed, is taken into consideration, it would require 1100 million tonnes (10% efficiency) or 1000 million tonnes (11% efficiency) of glucose or glucose equivalent for producing 110 million tonnes of methane. Assuming the harvest index of 40%, with an annual rice paddy production of 527 million tonnes, the maximum biomass would be 1300 million tonnes. All estimates made so far suggest that plants can release organic substance up to 10% of their weight through roots[5]. Thus, a maximum amount of 130 million tonnes may be available as substrate for methane production. From this amout only 11 to 13 million tonnes of methane can be produced. Thus an upper limit of 13 Tg methane production from rice paddies can be expected. Any assessment ignoring biological aspects of the mechanism of methane production is an artifact of methodology.

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Bacillus thuringiensis, a biocontrol agent for major tea pests

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A preliminary screening was carried out to determine the efficacy of crystal proteins of nine strains of *Bacillus thuringiensis* to control two lepidopteran pests of tea plants. Among these strains *B. thuringiensis* var. *kurstaki* HD1 and HD73 were highly toxic, *B. thuringiensis* var. *thuringiensis* HD2 was moderately toxic, *B. thuringiensis* var. *aizawai* HD133 showed low toxicity, while the other strains showed no toxicity towards these pests. The LC_{50} values of the three toxic strains and that of the purified crystal protein of the highly toxic strain HD1 were determined by laboratory bioassays.

TEA is an important plantation crop of India and is affected by many pests. The two most economically important lepidopteran insect pests of tea are the leaf-feeding caterpillars, *Caloptilia theivora* (leaf roller) and *Cydia leucostoma* (flushworm), belonging to the family Gracillariidae and Olethreutidae respectively. They cause considerable damage to young leaves of the tea plant by destroying the apical bud and tender leaves, thereby arresting the shoot growth.

The choice of conventional insecticides to be used on this crop is limited because of concerns related to consumer protection and environmental safety. Hence the search for effective biopesticides for the management of tea pests becomes appropriate. We undertook a study on the feasibility of the use of *Bacillus thuringiensis*, a well-known entomopathogen, as a biopesticide in tea plantations.

We carried out a preliminary screening for the efficacy of the crystal proteins of various *B. thuringiensis* strains belonging to class I which are known to be toxic to lepidopterous insects (Table 1). These strains were cultured on nutrient agar plates (peptone, 5 g; NaCl, 5 g; yeast extract, 1.5 g; beef extract, 1.5 g; distilled water, 1000 ml; pH 7.4) for four days at 30°C. One plate was used for each strain. The resultant spore–crystal mixtures were scraped off the plates, washed once in 0.5 M NaCl, followed by three washings in sterile water and resuspended in 1 ml of sterile water containing a protease inhibitor phenylmethylsulphonyl fluoride, at a concentration of 1 mM. These crude preparations were stored at –20°C until further use.

The pure crystal protein preparation was made from the spore–crystal mixture of HD1 (ref. 1). The purity of the crystals was 95% as determined by light microscopy. The level of purity of crystals was also confirmed by SDS–PAGE analysis[2]. Protein concentration of the spore–crystal mixtures of all nine strains and the purified crystal fraction of the strain HD1 were estimated by the dye-binding method[3] after solubilizing the crystal inclusions at 37°C in 10 mM NaOH (pH 10.5) containing 25 mM dithiothretol.

Prior to setting up bioassays, the surface area of the leaves was measured. Preliminary bioassays were conducted using crude spore–crystal samples obtained from the nine strains of *B. thuringiensis* towards larvae of flushworm and leaf rollers. Assays were done at 28°C

Table 1. Various strains of *B. thuringiensis* used in the study

Strain	Serotype
B thuringiensis var *kurstaki* strain HD1	3a 3b
B thuringiensis var. *kurstaki* strain HD73	3a 3b
B. thuringiensis var *thuringiensis* strain HD2	1
B thuringiensis var. *aizawai* strain HD133	7
B. thuringiensis var *indiana* strain HD521	15
B. thuringiensis var *dakota* strain HD551	16
B thuringiensis var *pakistani* strain HD395	13
B thuringiensis var *morrisoni* strain HD12	8a 8b
B thuringiensis var. *galleriae* strain HD207	13