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Dynamics of changes in methanogenesis and associated microflora in a flooded alluvial soil following repeated application of dicyandiamide, a nitrification inhibitor

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Summary

Influence of repeated application of the nitrification inhibitor dicyandiamide (DCD), on CH_4 production and associated microflora in a flooded alluvial soil, was investigated in a laboratory incubation study. Application of DCD at the time of soil incubation resulted in a substantial reduction in CH_4 production (31% over that of untreated control). Second repeat application of DCD, on the contrary, annulled the inhibitory effect on CH_4 production, restoring it to the level of unamended soil. Application of the third dose of DCD maintained CH_4 production almost to the same extent as that of second application. The alleviation of the initial inhibitory effect of DCD on CH_4 production was linked to the enhanced degradation of DCD following its repeated application to the flooded soil. Admittedly, abatement of the initial inhibitory effect of DCD on CH_4 production in soil repeatedly amended with DCD was also related to the inhibition of CH_4-oxidizing bacterial population and noticeable stimulation of heterotrophic bacterial population. Results suggest that repeat application of DCD with fertilizer-N to flooded rice soils might not be effective in controlling CH_4 production under field condition.

Introduction

Concentrations of CH_4, a potent greenhouse gas, have been increasing in the atmosphere at the rate of ~0.5% per year (Dlugokencky, 2001). Increased

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anthropogenic activity is generally given as the reason for this increase in the atmospheric concentration of CH_4. Flooded rice fields, mostly in the tropics, is considered to be one of the largest sources of atmospheric CH_4 with an estimated contribution of approximately 15% of the global budget (IPCC, 2001). Projected increase in rice production during the coming decades (IRRI, 1999), is anticipated to result into further increase in CH_4 fluxes to the atmosphere if the prevalent cultivation practices are continued (Houghton et al., 1995). Methanogenesis is an anaerobic microbial decomposition process of organic carbon and any parameter affecting the chemical, physical or biological characteristics of the flooded rice environment will influence the production of CH_4. It is suggested that through appropriate interference – physical, chemical or biological, CH_4 emission from rice paddies could be abated to the extent of 10–30% (Watson et al., 1995).

Urea is the dominant form of N fertilizer applied to rice in Asia (Vlek and Byrnes, 1986), but is subjected to various forms of loss including nitrification-denitrification (DeDatta, 1995). Nitrification inhibitors are being recommended for intensive agriculture to regulate fertilizer N losses (Prasad and Power, 1995) from flooded paddy. In addition to their acknowledged role in controlling various processes of N losses (McCarty, 1999), nitrification inhibitors like acetylene (wax coated calcium carbide) and nitrapyrin have been shown to inhibit CH_4 emission from flooded soil planted to rice (Bronson and Mosier, 1991; Keerthisinghe et al., 1993). Dicyandiamide (DCD), a well-known inhibitor of nitrification, was reported to inhibit CH_4 production in a flooded alluvial soil that appeared to be a combined result of higher redox status, lower pH, lower Fe^{2+} and readily mineralizable carbon contents as well as lower population of methanogenic bacteria and their activity (Bharati et al., 2000).

DCD is a commercially available non-volatile nitrification inhibitor suitable for use with solid fertilizer and is a good nitrification inhibitor for rice (Gorelik et al., 1992). It is less volatile than nitrapyrin and is easier to blend with fertilizers (McCarty and Bremner, 1989). However, the bioactivity and effectiveness of DCD mainly depends on the concentration applied (Guiraud et al., 1989). In rice cultivation, fertilizer-N is usually applied in several splits to enhance its efficiency and since nitrification inhibitors are used along with the fertilizer, the rice soil is subjected to repeated application of the nitrification inhibitors. Repeated application of DCD to field soil at 80% water-holding capacity has been shown to enhance its degradation that followed zero order kinetics (Rajbanshi et al., 1992). In a laboratory incubation study, we investigated the effect of repeated additions of DCD on the production of CH_4 and the associated microflora in a flooded alluvial soil.

Materials and methods

Soil and chemicals

The soil, used in the study, was an alluvial soil (an aeric endoaquept), collected from the experimental farm of the Central Rice Research Institute, Cuttack, India. The soil was a sandy clay loam in texture (clay 25.6%, silt 21.6%, sand 52.8%) with pH (H_2O) 6.2, cation exchange capacity 15 mEq g^{-100}, electrical conductivity 0.6 dS m^{-1}, organic C 0.68% and total N 0.09%, SO_4–S 34.3 mg kg^{-1}, Olsen's P 8 mg kg^{-1}. The soil, collected from the plough layer (0–15 cm), was air-dried in shade, ground, sieved (<2 mm) and stored at 4 °C until used in the study. Both methanogens and CH_4-oxidizing bacteria survive drying for prolonged periods (Frenzel, 2000; Le Mer and Roger, 2001). DCD was obtained from M/s Loba-Chemie, Mumbai and used as aqueous solution prepared in sterile distilled water.

CH_4 production studies

Potential CH_4 production was quantified following two approaches. In the first approach (Wassmann et al., 1998), 20 g portions of air-dried soil samples were placed in 100 ml spouteless beakers and amended with urea (40 mg N kg^{-1}). The effect of DCD on CH_4 production, in the presence of urea, was measured by incubating 20 g soil amended with required quantity of DCD in a 100 ml beaker to provide a final concentration of 2 mg kg^{-1} soil (equivalent to 5% of applied N). Soils without any amendment served as control. To each beaker, sterile distilled water to make a final volume of 40 ml was added to flood the soil. Following flooding, the beakers were closed with a rubber stopper with provisions for gas ports for headspace gas sampling and placement of platinum electrode and pH electrode assembly. Soil samples in beakers were incubated under N_2 atmosphere at 28+2 °C for 80 d. Headspace gas samples (5 ml) were analyzed at regular intervals by gas chromatography for quantification of CH_4 produced.

The effect of repeated application of DCD on CH_4 production was measured by an alternate incubation method (Adhya et al., 1998). In brief, portions (5 g) of the soil samples placed in B–D vacutainer