Korean J Biol Sci 8: 281-288, 2004

Relationships between Methane Production and Sulfate Reduction in Reclaimed Rice Field Soils

Juhwan Lee and Kang-Hyun Cho*

Department of Biological Sciences, Inha University, Incheon 402-751, Korea

Key Words:
Anaerobic carbon mineralization
Methane production
Reclamation
Rice field
Sulfate reduction

The change in relationships between methane production and sulfate reduction was investigated in reclaimed rice field soils at different time points after reclamation of tidal flat in Korea. Sulfate concentrations of soils in the ca. 60-year-old and 26-year-old reclaimed rice fields were much lower than that in a natural tidal flat. During 60 d of anaerobic incubation, total methane production and sulfate consumption of the soil slurries were 7.0 μmol CH_4/g and 8.2 μmol SO_4^{2-}/g in the 60-year-old rice field, 5.6 μmol CH_4/g and 12.7 μmol SO_4^{2-}/g in the 26-year-old rice field, and ca. 0 μmol CH_4/g and 22.4 μmol SO_4^{2-}/g in a natural tidal flat. Relative percent electron flow through sulfate reduction in the 60-year-old rice field was much lower (50.8%) compared with the 26-year-old rice field (69.3%) and the tidal flat (99.9%). The addition of an inhibitor of methanogenesis (2-bromoethanesulfonate) had no effect on sulfate reduction in the soil slurries of the reclaimed rice fields. However, instant stimulation of methane production was achieved with addition of an inhibitor of sulfate reduction (molybdate) in the soil slurries from the 26-year-old reclaimed rice field. The specific inhibitor experiments suggest that the relationship of methanogenesis and sulfate reduction might become mutually exclusive or syntrophic depending on sulfate content in the soil after reclamation. Sulfate, thus sulfate reduction activity of sulfate-reducing bacteria, would be an important environmental factor that inhibits methane production and determines the major pathway of electron and carbon flow in anaerobic carbon mineralization of reclaimed rice field soils.

Recently, there have been concerns around the world on methane because of its strong influence on the climatic change. With the exception of carbon dioxide, methane is regarded as the major greenhouse gas affecting global heat balance. The atmospheric methane originates mainly from biogenic sources, among which rice fields are the major anthropogenic source (Khalil and Rasmussen, 1994). Methane emissions from rice fields are estimated between 20 and 100 Tg/yr, averaged 60 Tg/yr, which accounts for approximately 11% of the worldwide methane release (IPCC, 1995). In Korean rice fields, the total emission of methane was estimated to be 0.49 Tg/yr (Shin et al., 1996).

Rice field soils undergo periodic waterlogging resulting in anoxic conditions (Yao et al., 1999). The anoxic conditions lead to anaerobic degradation of organic materials in flooded soils. The anaerobic degradation of organic materials to methane requires the cooperation of several bacteria types (Conrad, 1996). The hydrolytic bacteria and fermenting bacteria convert polymers such as cellulose and hemicellulose into monomers, which are in turn fermented to smaller molecules such as alcohols, volatile fatty acids, H_2 and CO_2. Methanogens can convert H_2/CO_2, formate, acetate, and a few other simple compounds into CH_4 and CO_2. Sulfate-reducing bacteria can also consume H_2 produced in fermentative processes and produce hydrogen sulphide in environments containing sufficient levels of sulfate (Raskin et al., 1996). Methanogenesis and sulfate reduction have been regarded as mutually exclusive in previous studies on profiles of methane, sulfate, and sulfide in soils and sediments (Ingvorsen and Brock, 1982; Iversen and Jϕrgensen, 1985). In sulfate-rich marine or brackish environments, sulfate-reducing bacteria effectively outcompete methanogens and rates of methane production and flux are uniformly low in such environments (Oremland et al., 1982; Capone and Kiene, 1988). Sulfate reduction may therefore have a primary role in the overall oxidation of organic matter in sulfate-rich soils (Jϕrgensen, 1982). This is mainly because they have more efficient uptake systems (lower K_m) for common substrates, thermodynamic advantages over

---

*To whom correspondence should be addressed.
Tel: 82-32-860-7698, Fax: 82-32-874-6737
E-mail: khcho@inha.ac.kr

methanogens, and the greater trophic flexibility (Capone and Kiene, 1988; Ingvorsen et al., 1984). In contrast to sulfate-reducing bacteria, methanogens are considered as a dominant anaerobic carbon oxidation process in sulfate-poor or organic matter-rich freshwater environments (Capone and Kiene, 1988).

In Korea, the area of newly formed land by reclamation was 38,526 ha during 1970-1994, and above 50% of the land was used for agriculture such as rice cropping (Rural Development Corporation, 1996). One of the chemical characteristics of reclaimed soils is that its salt content tends to be lowered during the early stage of reclamation. As salinity and, hence, sulfate concentration decreases, methanogenesis may displace sulfate reduction as the dominant pathway of terminal carbon metabolism. However, studies have focused on methanogenesis and sulfate reduction in many non-saline rice fields. There have been relatively fewer studies on the relative contribution of methanogenesis and sulfate reduction in reclaimed rice field soils. In this study, we determined the relative importance of methanogenesis and sulfate reduction in anaerobic carbon mineralization during the course of desalinization after reclamation of tidal flat.

Materials and Methods

Study site and soil sampling

This study was conducted in a coastal region of the central Korean Peninsula toward Yellow Sea. Two reclaimed rice fields (site R60 and R26) and an adjacent tidal flat (site T) were selected for our study. The site R60 was estimated to be formed about 60 years ago and the site R26 in 1973 by tideland reclamation (Rural Development Corporation, 1996). Soil qualities of the reclaimed rice fields have been continuously improved for rice cropping by the introduction of puddling.

Soil samples of R60 and R26 were taken from the plow layer (0 to 15 cm in depth) in May 1999 and that of T in February 2000. The soils were air-dried, mechanically crushed, sieved (mesh size = 1 mm), and stored in plastic containers. Organic matter content was determined after combustion at 550°C for 5 h using a furnace (Thermolyne 67100). Electrical conductivity and pH in the 1:2 (w/v) soil extract were measured with a conductivity meter (Orion 162) and a pH meter (Orion 920A), respectively. Soil texture was determined using the hydrometer method (Sheldrick and Wang, 1993). Total nitrogen was determined following the Kjeldahl procedure (McGill and Figueiredo, 1993). For determination of cation-exchange capacity, the soils were saturated with ammonium using 1 M CH_3COONH_4 (pH = 7) and then extracted with 5% KCl. The extracts were distilled by the Kjeldahl distillation and titrated with 1/140 NH_2SO_4 (Hendershot et al., 1993). Exchangeable cations (Na, Mg, Al, K, Ca, Mn, and Fe) were extracted from the soils using the Mehlich III method (Tran and Simard, 1993) and determined with ICP-mass spectrophotometer (VG Elemental/PQ-2TURBO).

Soil slurry experiments

Soil slurries were prepared by adding 10 ml of distilled and sterilized water to 10 g dry soil in a sterile 120 ml serum bottle. The bottles were then closed with sterile butyl rubber stoppers and crimped with aluminum caps. The gaseous headspace was exchanged to N_2. To investigate the relative contribution of methane production and sulfate reduction on anaerobic carbon mineralization according to different time lapse after reclamation of tidal flat, the soil slurries of R60, R26, and T were incubated at 25°C for 60 d without any treatment. For inhibition experiments of methanogenesis and sulfate reduction, the soil slurries were pre-incubated to obtain constant anoxic conditions at 25°C for 10 d. After the incubation, the gaseous headspace was re-exchanged with N_2. An appropriate volume from a stock solution of inhibitors was injected into the bottles using a 1 ml syringe and mixed into the soil slurries by gentle shaking. A 2-bromoethanesulfonate (BES) (sodium salt, 98% purity, Sigma) was added to a final concentration of 20 mM for inhibition of methanogenesis. Molybdate (dihydrate, 99.95% purity, Sigma) was added to a final concentration of 5 mM for inhibition of sulphate reduction.

Gas analysis

One set of slurry bottles was used to measure the partial pressures of CH_4 (n=5) and H_2S (n=3) which were allowed to accumulate over the incubation. After vigorous shaking of soil slurries for 30 seconds to allow equilibration between the liquid and gas phases, gas samples were taken from the headspace of the bottles using a gas-tight pressure-lock syringe (1 ml, Hamilton, USA). Methane concentrations were analyzed using a gas chromatography (MODEL 600D, YongLin, Korea) equipped with a flame ionization detector (FID). The column (Porapak Q) was heated at 35°C and the temperatures of injector and detector were 110°C and 140°C, respectively. H_2S was analyzed by gas chromatography (HP 5890 series plus II, Hewlett Packard, USA) equipped with a flame photometric detector (FPD). The column (HP-1, internal diameter 0.25 mm, length 30 m, film thickness 0.25 μm) was heated at 35°C and the temperatures of injector and detector were 100°C and 200°C, respectively.

Chemical analysis

Another set of slurry bottles (n=3) was used for chemical analyses of soil slurries. The pH and redox potential (E_h) of the soil slurries were measured before centrifugation and filtration with a pH meter (Orion 920A, USA) and an ORP meter (TOA RM-12P, Japan). For sulfate and chloride analysis, liquid portions of slurries were centrifuged at 13000 rpm for 20 min and then the supernatant was filtered through 0.2 μm membrane filter. Sulfate and chloride in the filtrate were determined by ion chromatography (Waters 510, USA) with an IC Pac™ anion column (diameter 46 mm, length 50 mm, Waters, USA) and a conductivity detector (Waters 432, USA). The eluent was sodium borate/gluconate solution with a flow rate of 1.2 ml/min.

Results

Initial soil characteristics

Electrical conductivity and concentrations of sulfate, chloride, and sodium of air-dried soils showed the most remarkable decreases during reclamation (Table 1). In terms of soil salinity assessed by electrical conductivity, soil types of the 60-year-old (R60) and 26-year-old (R26) reclaimed rice fields and tidal flat (T) were classified as 'non saline', 'slightly saline' and 'very saline', respectively (USDA 1954). Initial pH value of soil slurries of R60 and R26 ranged 5.9-6.0, and that of T was 8.0. Total nitrogen contents of R60, R26 and T were 0.77, 0.69 and 0.33 mg/g, respectively. Organic matter content, soil texture (percent of sand, silt and clay) and cation exchange capacity (CEC) did not show a significant difference among the sites.

Table 1. Comparisons of physicochemical characteristics of the air-dried soils in this study

Soil property	Study Site		
	R60[a]	R26[a]	T[a]
pH[b]	5.9±0.04	6.0±0.02	8.0±0.02
Electrical conductivity (mS/cm)[b]	0.9±0.04	2.8±0.02	12.5±0.01
Sand (%)	5±0.6	2±0.5	10±0.4
Clay (%)	24±0.0	25±0.7	18±0.1
Silt (%)	71±0.6	73±0.7	72±0.4
Organic matter content (%)	1.25±0.03	1.48±0.05	1.18±0.03
Total nitrogen (mg/g)	0.77±0.06	0.69±0.07	0.33±0.06
Sulfate (μmol/g)	7.1±1.2	8.2±2.4	32.9±3.8
Chloride (μmol/g)	17±0.2	67±1.3	265±3.3
CEC[c] ($\mu mol_c/g$)	124±1.6	126±1.5	107±1.6
Sodium (μmol/g)	31±0.0	74±0.0	307±0.0
Magnesium (μmol/g)	33±0.0	41±0.0	54±0.0
Aluminum (μmol/g)	37±0.0	32±0.0	28±0.0
Potassium (μmol/g)	10±0.0	12±0.0	29±0.0
Calcium (μmol/g)	43±0.0	22±0.0	24±0.0
Total free Manganese (μmol/g)	3±0.0	4±0.0	5±0.0
Total free iron (μmol/g)	8±0.0	8±0.0	6±0.0

[a] R60 indicates ca. 60-year-old reclaimed rice field, R26 26-year-old reclaimed rice field, and T a natural tidal flat; [b] Soil slurry (soil: water=1:2 W/V); [c] Cation exchange capacity. Mean±SE, n=5.

Temporal changes in methanogenesis and sulfate reduction during anaerobic incubation

As the anaerobic incubation progressed, pH of the soil

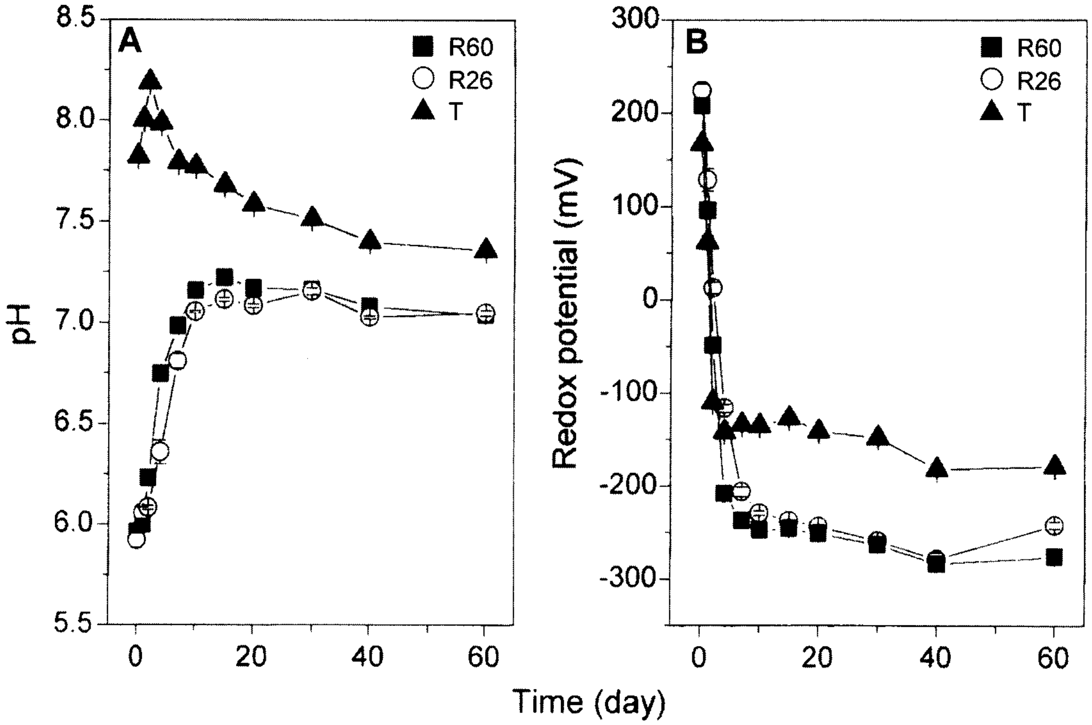

Fig. 1. Temporal changes in pH (A) and redox potential (B) during the anaerobic incubation of the soil slurries in ca. 60-year-old (R60) and 26-year-old (R26) reclaimed rice fields and a natural tidal flat (T). Mean±SE, n=3.

slurries increased from acidic in R60 and R26 while it decreased from calcareous in T, stabilizing between 7.0 and 7.4 (Fig. 1A). Redox potential of R60 and R26 decreased rapidly from +205 mV to ca. -280 mV (Fig. 1B). However, redox potential of T was reduced to only -180 mV at the end of incubation. Both pH and redox potential became relatively stable after 10 d of incubation.

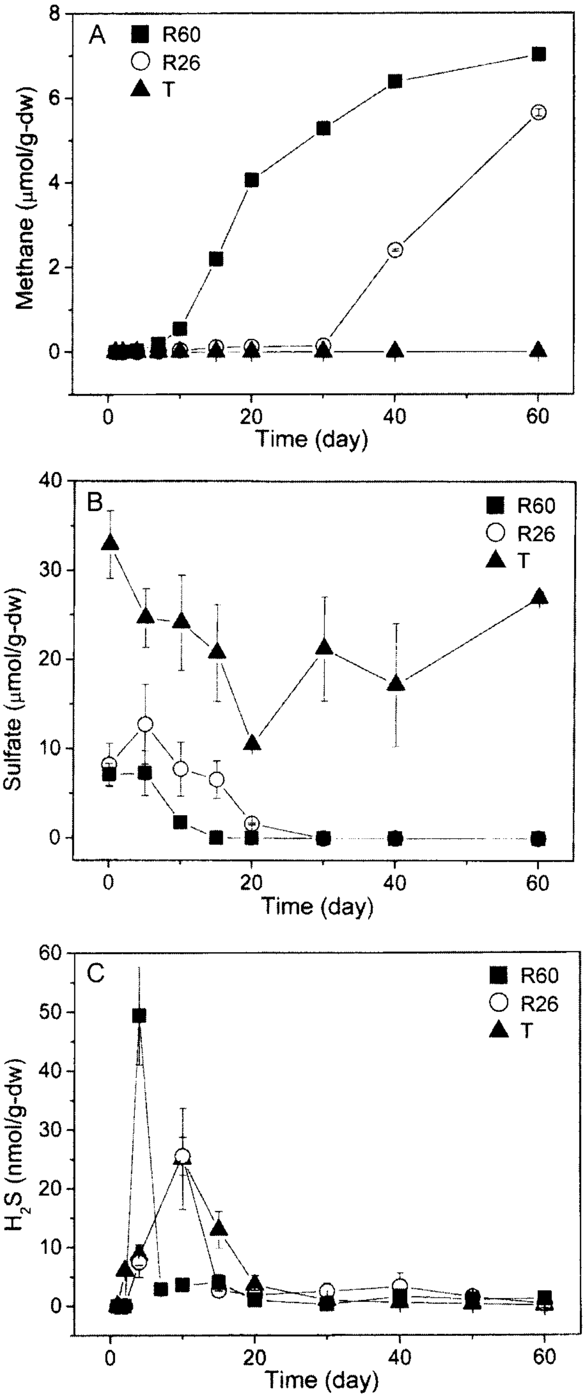

Fig. 2. Temporal changes in methane production (A), sulfate concentrations (B), and hydrogen sulfide (H_2S) production (C) during the anaerobic incubation of the soil slurries in ca. 60-year-old (R60) and 26-year-old (R26) reclaimed rice fields and a natural tidal flat (T). Mean±SE, n=5.

The total amount of methane production of R60 (7.0 μmol/g) was higher than that of R26 (5.6 μmol/g) at the end of the anaerobic incubation (Fig. 2A). The methane production in the slurries of R60 and R26 generally showed similar patterns: a lag-phase and then a period of exponential increase of methane. However, the major difference was the starting time for exponential increase of methane production. The methane production of R60 began on the 7th day of incubation, while methane production of R26 increased slowly until the 30th day of incubation. Tidal flat soil (T) had virtually no methane production throughout the incubation (less then 1 nmol/g).

The sulfate (SO_4^{2-}) in the slurries was consumed as the anaerobic incubation progressed (Fig. 2B). The sulfate in R60 was consumed at a rate of 0.72 μmol g^{-1} day^{-1} and depleted completely by 15-d of incubation. The sulfate in R26 was consumed at a rate of 0.69 μmol g^{-1} day^{-1} and depleted completely by 30-d of incubation. The sulfate concentration of T was decreased from 32 μmol/g to 10 μmol/g during first 20-d of incubation. After endogenous SO_4^{2-} was almost depleted in R60 and R26, the stable methane production immediately took place. The temporal change in hydrogen sulfide (H_2S) production as a result of SO_4^{2-} reduction generally had a similar pattern in R60, R26 and T (Fig. 2C). The amounts of hydrogen sulfide produced in R60, R26 and T were measured to the maximum values of 26, 49 and 25 nmol g^{-1}, respectively. The stable hydrogen sulfide production in R26 and T lasted for a longer time than that in R60.

The electron flow through methanogenesis and sulfate reduction during anaerobic decomposition of organic matter can be calculated using the equations of Ingvorsen and Brock (1982) and Isa et al. (1986). The electron flows through both methane production and sulfate reduction were calculated from the total methane production and total sulfate consumption during the incubation (60 d). The electron flow through methanogenesis averaged 56.1 μmol e$^-$/g in R60, 45.2 μmol e$^-$/g in R26, and 0.009 μmol e$^-$/g in T (Table 2). The electron flow through sulfate reduction of R60, R26, and T averaged 58.0, 101.8, and 179.4 μmol e$^-$/g, respectively. The relative percent of electron flow through sulfate reduction in R60 was much lower compared with R26 or T. Also, the contribution of methanogenesis coincided with sulfate reduction during the incubation.

Inhibition of methanogenesis and sulfate reduction

Addition of 2-bromoethanesulfonate (BES) to the slurries of R60 resulted in 98.4% reduction of methane (Fig. 3A) down to 0.001 μmol g^{-1} day^{-1} (Table 3). The inhibition of methane production by BES did not stimulate the sulfate

Table 2. Electron flows through methane production and sulfate reduction during anaerobic decomposition of organic matter in the soil slurries of ca. 60-year-old (R60) and 26-year-old (R26) reclaimed rice field, and a natural tidal flat (T) for 60 days of incubation at 25°C

Process	R60	R26	T
Methane production (μmol/g-dw)[a]	7.0	5.6	0.001
Sulfate consumption (μmol/g-dw)[b]	7.2	12.7	22.4
Electron equivalents (μmol e^-/g-dw)[c]			
through methane production	56.1 (49.2%)	45.2 (30.7%)	0.009 (<0.01%)
through sulfate reduction	58.0 (50.8%)	101.8 (69.3%)	179.4 (>99.9%)

[a] Methane production was determined as the total content of methane at the end of incubation; [b] Sulfate consumption was determined from the total content of sulfate decreased between 0 and 30 days of incubation; [c] Calculation of electron equivalents was based on that methane produced by methanogenesis and H_2S by sulfate reduction both contain 8 electron equivalents. n=5, coefficient of variation<35%

consumption while the hydrogen sulfide production increased (Fig. 3A). Addition of molybdate resulted in complete inhibition of the sulfate consumption and H_2S production. Molybdate did not stimulate the methane production (0.294 μmol g^{-1} day^{-1}) compared with the control (0.307 μmol g^{-1} day^{-1}) (Table 3).

The addition of BES to the slurries of R26 resulted in 99.2% inhibition of total methane production (Fig. 3B). The sulfate consumption rate (0.45 μmol g^{-1} day^{-1}) by BES was similar to the control (0.47 μmol g^{-1} day^{-1}) (Table 3), therefore BES did not stimulate sulfate consumption. The H_2S production was also not stimulated by BES. The addition of molybdate resulted in an increase of the methane production rate (0.228 μmol g^{-1} day^{-1}). Molybdate inhibited the sulfate consumption by 98.7% compared with the control (Table 3). The stimulation of methane production in R26 coincided with an inhibition of sulfate consumption and hydrogen sulfide production. The exponential increase of methane production by molybdate in R26 was similar to that of the control in R60 (Fig. 3, Table 3).

Discussion

Relative distribution and concentration of salts may play an important role in determining soil characteristics in reclaimed rice fields. The desalinization of main salts such as SO_4^{2-}, Cl^-, and Na^+ after reclamation resulted in decreasing electrical conductivity, and pH decreased due to the relative decrease of basic cations such as Na^+, K^+, and Mg^{2+} after reclamation (Table 1). The desalinization process in reclaimed rice field soil is dependent on irrigation management and agricultural practices, which are commonly used to accelerate reclamation of highly saline area for rice cropping (Häfele et al., 1999).

The patterns of the temporal change in methane production in our study (Fig. 2) were generally similar to the results of rice field soils from China, the Philippines, and Italy (Yao et al., 1999). The major difference was that the onset of stable methanogenesis in non-saline rice field soil (R60) proceeded much faster than in slightly saline rice field soil (R26) in the present study. This shows that the entire reduction process might be dependent on soil salinity changes with the different time lapse after reclamation. We did not observe rapid methanogenesis in the reclaimed rice field soils until the endogenous sulfate was depleted (Fig. 2). This phenomenon has also been reported by several other researchers (Winfrey and Ward, 1983; Iversen and Jϕrgensen, 1985). Generally, the inhibition of methane production by sulfate is accompanied by a linear decrease of the sulfate concentration indicating sulfate reduction activity (Achtnich et al., 1995). The temporal changes of H_2S as a result of sulfate reduction roughly coincided with the sulfate consumption (Fig. 2). Nearly all produced hydrogen sulfide is immediately recycled to sulfate and again available for reduction *in situ* sediments (Bak and Pfennig, 1991). However, we believe that most of hydrogen sulfide was probably transformed to pyrite in this study, due to the sedimentation of sulfide by interaction between sulfide and Fe.

Carbon and sulfur balances for R60, R26, and T were calculated to determine the relative contribution of methane production and sulfate reduction on anaerobic carbon mineralization (Table 2). The percent of electron flow through sulfate reduction during the 60 days of incubation was 50.8%, 69.3% and 99.9% in R60, R26 and T, respectively. It appears that the increased sulfate enhanced the relative electron flow by sulfate reduction. Generally, it has been shown that sulfate-reducing bacteria may catalyze more than 50% of total carbon oxidation in salt marshes and coastal sediments (Jϕrgensen, 1982; Howarth and Giblin, 1983). Even in R60, a non-saline soil, sulfate reduction played an important role in the partial anaerobic carbon mineralization (Table 2). This supports the notion that sulfate reduction significantly limits methane production even in relatively low-sulfate freshwater systems (60-105 μM) (Lovely and Klug, 1983; Roden and Wetzel, 1996). Bak & Pfennig (1991) also showed that sulfate reduction rates in freshwater sediments could be high even in low sulfate content. In rice field soils, sulfate-reducing bacteria are present in relatively high numbers (Dalsgaard and Bak, 1994) and well adapted to low sulfate concentrations. Therefore, sulfate-reducing bacteria are potentially important in even non-saline rice field soils.

Specific inhibitors have been used to quantify the interaction of sulfate reducers and methanogens in a

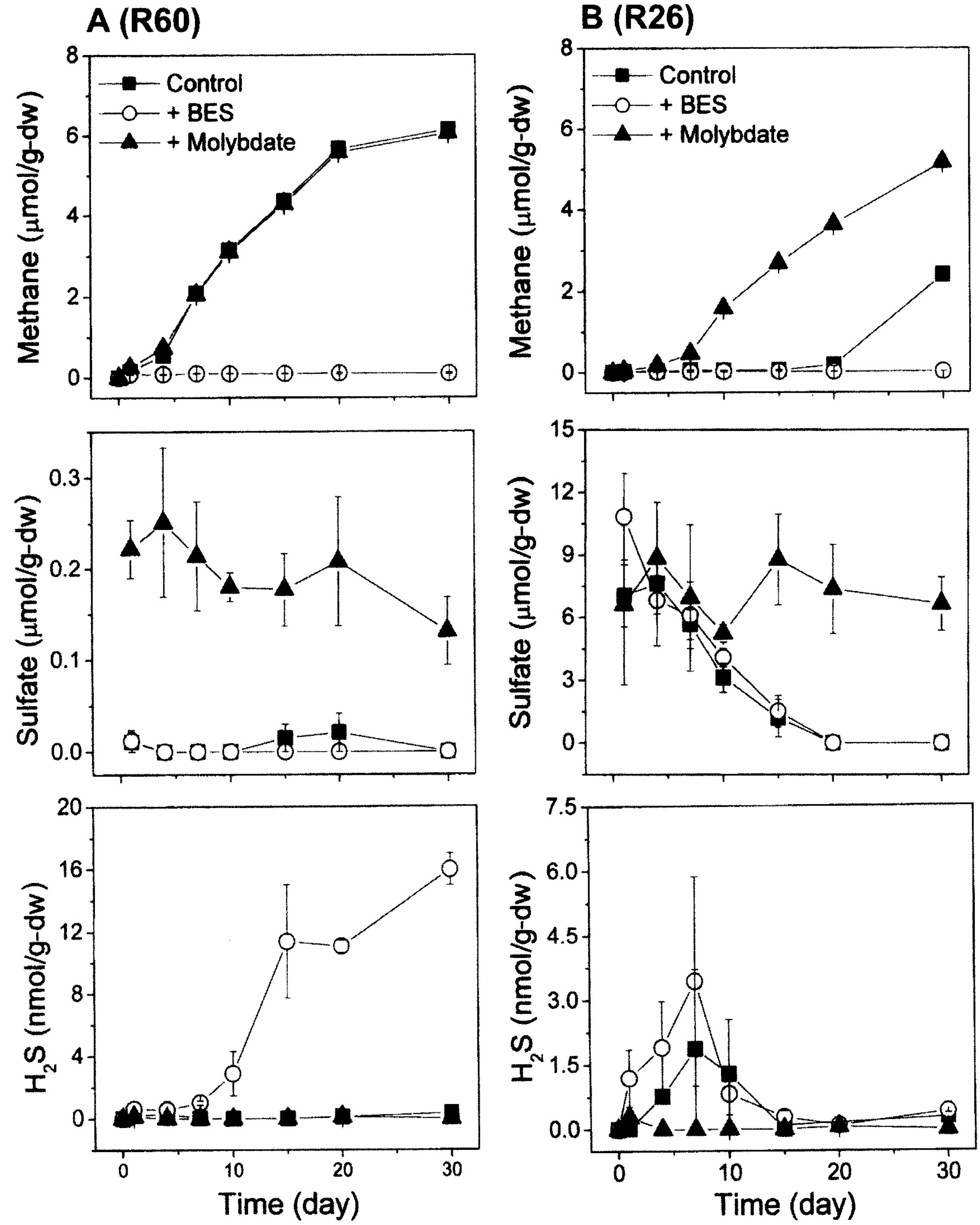

Fig. 3. Temporal changes in methane production, sulfate concentrations, and hydrogen sulfide (H_2S) production during the anaerobic incubation of the soil slurries after preincubation for 10 days in ca. 60-year-old (R60) (A) and 26-year-old (R26) (B) reclaimed rice fields with and without inhibitors, 2-bromoethanesulfonate (BES) and molybdate. Mean±SE, n=3.

submerged rice field soil (Chidthaisong and Conrad, 2000a) and the sediments of lakes (Jones et al., 1982; Scholten et al., 2000). Our results from the inhibitor studies (Table 3) suggest that the relative contribution of methanogenesis and sulfate reduction in anaerobic carbon mineralization may be determined according to sulfate content. Especially, the instant stimulation of methane production by the addition of molybdate in R26

Table 3. Comparison of methane production rate and sulfate consumption rates in the soil slurries of ca. 60-year-old (R60) and 26-year-old (R26) reclaimed rice fields with and without inhibitors, 2-bromoethanesulfonate (BES) and molybdate, during 30 days of incubation at 25°C

Site	Treatment	CH_4 production rate[a] (μmol g^{-1} day^{-1})	SO_4^{2-} consumption rate[b] (μmol g^{-1} day^{-1})
R60	Control	0.307 ±0.027	n.d.[c]
	BES	0.001 ±0.001	n.d.
	Molybdate	0.294 ±0.021	0.003±0.002
R26	Control	0.008 ±0.003	0.47 ±0.07
	BES	0.0002±0.0001	0.45 ±0.04
	Molybdate	0.228 ±0.017	0.006±0.13

[a]CH_4 production rate was calculated from the increase in CH_4 between 4 day and 20 day of incubation; [b]SO_4^{2-} reduction rate was calculated from the decrease in sulfate between 4 day and 20 day of incubation; [c]Not detectable. Mean±SE, n=3.

indicates that these microbial processes were mutually exclusive as a result of the competitive relationships (Fig. 3b). Molybdate is a specific inhibitor of sulfate reduction and if sulfate-reducing bacteria were competing with methanogens for substrates, molybdate should have relieved this competition and increased the production of methane (Boon and Mitchell, 1995; Ranade et al., 1999). Winfrey and Zeikus (1977) suggest that the carbon and electron flow during anaerobic decomposition of organic matter may be altered depending on sulfate content in soils. Raskin et al. (1996) suggest that some sulfate-reducing bacteria can grow syntrophically with hydrogen- or formate-consuming methanogens, eliminating their need to reduce sulfate.

The microbial pathway for terminal carbon mineralization in an anaerobic condition could be selected between methanogenesis and sulfate reduction by the change of sulfate content according to desalinization after reclamation of tidal flat (Table 2). Because sulfate reduction is thermodynamically favorable in sulfate rich conditions (Lovely and Klug, 1983), sulfate reduction may become a major pathway of electron and carbon flow when sulfate is rich in saline soils such as T (natural tidal flat). When sulfate is almost depleted with desalinization such as R60, methane production may become a dominant pathway of electron and carbon flow.

In conclusion, sulfate appears to be the most crucial factor that inhibits methane production in reclaimed rice field soils. The relative contributions of methanogenesis and sulfate reduction in anaerobic carbon mineralization appear to be dependent on the amount of endogenous sulfate content in reclaimed rice field soil. Therefore, major pathways of electron and carbon flow may be determined by sulfate content and thus sulfate reduction activity of sulfate-reducing bacteria. This study provides a basis for mechanistic understanding of methane production in reclaimed rice fields, and for developing effective mitigation strategies to reduce methane emission from rice fields after reclamation.

Acknowledgements

We thank Dr. Sangkyu Park (Korea Research Institute of Bioscience and Biotechnology) for reviewing the manuscript.

References

Achtnich C, Schuhmann A, Wind A, and Conrad R (1995) Role of interspecies H_2 transfer to sulfate and ferric iron-reducing bacteria in acetate consumption in anoxic paddy soil. *FEMS Microbiol Ecol* 16: 61-70.

Bak F and Pfennig N (1991) Microbial sulfate reduction in littoral sediment of Lake Constance. *FEMS Microbiol Ecol* 85: 31-42.

Boon PI and Mitchell A (1995) Methanogenesis in the sediments of an Australian freshwater wetland: comparison with aerobic decay, and factors controlling methanogenesis. *FEMS Microbiol Ecol* 18: 175-190.

Capone DG and Kiene RP (1988) Comparison of microbial dynamics in marine and freshwater sediments: contrasts in anaerobic carbon catabolism. *Limnol Oceanogr* 33: 725-749.

Chidthaisong A and Conrad R (2000) Specificity of chloroform, 2-bromoethanesulfonate and fluoroacetate to inhibit methanogenesis and other anaerobic processes in anoxic rice field soil. *Soil Biol Biochem* 32: 977-988.

Conrad R (1996) Soil microorganisms as controllers of atmospheric trace gases (H_2, CO, CH_4, OCS, N_2O, and NO). *Microbiol Rev* 60: 609-640.

Dalsgaard T and Bak F (1994) Nitrate reduction in a sulfate-reducing bacterium, *Desulfovibrio desulfuricans*, isolated from rice paddy soil: sulfide inhibition, kinetics, and regulation. *Appl Environ Microbiol* 60: 291-297.

Häfele S, Wopereis MCS, Boivin P, and N'Diaye AM (1999) Effect of puddling on soil desalinisation and rice seedling survival in the Senegal River Delta. *Soil Till Res* 51: 35-46.

Hendershot WH, Lalande H, and Duquette M (1993) Ion exchange and exchangeable cations. In: Carter MR (ed), Soil Sampling and Methods of Analysis, Lewis, Florida, pp 167-176.

Howarth RW and Giblin A (1983) Sulfate reduction in the salt marshes at Sapelo Island, Georgia. *Limnol Oceanogr* 28: 70-82.

Ingvorsen K and Brock TD (1982) Electron flow via sulfate reduction and methanogenesis in the anaerobic hypolimnion of Lake Mendota. *Limnol Oceanogr* 27: 559-564.

Ingvorsen K, Zehnder AJB, and Jφrgensen BB (1984) Kinetics of sulfate and acetate uptake by *Desulfobacter postgatei*. *Appl Environ Microbiol* 47: 403-408.

International Panel on Climate Change (IPCC) (1995) Climate Change 1994: Radioactive Forcing of Climate Change and an Evaluation of the IPCC IS92 Emission Scenarios. Cambridge University Press, Cambridge.

Isa Z, Grusenmeyer S, and Verstraete W (1986) Sulfate reduction relative to methane production in high-rate anaerobic digestion: microbiological aspects. *Appl Environ Microbiol* 51: 580-587.

Iversen N and Jφrgensen BB (1985) Anaerobic methane oxidation rates at the sulfate-methane transition in marine sediments from Kattegat and Skagerrak (Denmark). *Limnol Oceanogr* 30: 944-955.

Jones JG, Simon BM, and Gardener S (1982) Factors affecting methanogenesis and associated anaerobic processes in the sediments of a stratified eutrophic lake. *J General Microbiol*

128: 1-11.

Jφrgensen BB (1982) Mineralization of organic matter in the sea bed-the role of sulphate reduction. *Nature* 296: 643-645.

Khalil MAK and Rasmussen A (1994) Global emissions of methane during the last several centuries. *Chemosphere* 29: 833-842.

Lovely DR and Klug MJ (1983) Sulfate reducers can outcompete methanogens at freshwater sulfate concentrations. *Appl Environ Microbiol* 45: 187-192.

McGill WB and Figueiredo CT (1993) Total nitrogen. In: Carter MR (ed), Soil Sampling and Methods of Analysis, Lewis, Florida, pp 201-211.

Oremland RS and Polcin S (1982) Methanogenesis and sulfate reduction: competitive and noncompetitive substrates in estuarine sediments. *Appl Environ Microbiol* 44: 1270-1276.

Ranade DR, Dighe AS, Bhirangi SS, Panhalkar VS, and Yeole TY (1999) Evaluation of the use of sodium molybdate to inhibit sulphate reduction during anaerobic digestion of distillery waste. *Bioresource Technol* 68: 287-291.

Raskin L, Rittmann BE, and Stahl DA (1996) Competition and coexistence of sulfate-reducing and methanogenic populations in anaerobic biofilms. *Appl Environ Microbiol* 60: 3847-3857.

Roden EE and Wetzel RG (1996) Organic carbon oxidation and suppression of methane production by microbial Fe(III) oxide reduction in vegetated and unvegetated freshwater wetland sediments. *Limnol Oceanogr* 41: 1733-1748.

Rural Development Corporation (1996) Tideland Reclamation in Korea. Rural Development Corporation, Korea.

Scholten JCM, Conrad R, and Stams AJM (2000) Effect of 2-bromo-ethane sulfonate, molybdate and chloroform on acetate consumption by methanogenic and sulfate-reducing populations in freshwater sediment. *FEMS Microbiol Ecol* 32: 35-42.

Sheldrick BH and Wang C (1993) Particle size distribution. In: Carter MR (ed), Soil Sampling and Methods of Analysis, Lewis, Florida, pp 499-511.

Shin YK, Lee YS, Yun SH, and Park ME (1996) Methane emission measurement in rice paddy of Korea. In: Ping Q (ed), Environment and Biometeorology. The Proceedings of International Symposium on Environment and Biometeorology, China Agricultural Scientech Press, Beijing, pp 495-503.

Tran TS and Simard RR (1993) Mehlich III-extractable elements. In: Carter MR (ed), Soil Sampling and Methods of Analysis, Lewis, Florida, pp 43-49.

United States Department Agriculture (USDA) (1954) Diagnosis and Improvement of Saline-alkali Soils, USDA Handbook No. 60.

Winfrey MR and Ward DM (1983) Substrates for sulfate reduction and methane production in intertidal sediments. *Appl Environ Microbiol* 45: 193-199.

Winfrey MR and Zeikus JG (1977) Microbial methanogenesis in freshwater sediments. *Appl Environ Microbiol* 33: 275-281.

Yao H, Conrad R, Wassmann R, and Neue HU (1999) Effect of soil characteristics on sequential reduction and methane production in sixteen rice paddy soils from China, the Philippines, and Italy. *Biogeochemistry* 47: 269-295.

[Received August 2, 2004; accepted October 4, 2004]