Bull. Environ. Contam. Toxicol. (2004) 72:850–857

Bulletin of Environmental Contamination and Toxicology

Emission of Biogenic Sulfur Gases from the Microbial Decomposition of Cystine in Chinese Rice Paddy Soils

J. Zhang,[1] L. Wang,[2] Z. Yang[1]*

[1] Department of Environmental Science and Engineering, Nanjing University of Science and Technology, Nanjing 210094, People's Republic of China
[2] Shanghai Institute for Design and Environmental Engineering, Shanghai 200232, People's Republic of China

Received: 27 May 2003/Accepted: 6 January 2004

The cycling of sulfur through the atmosphere has gained increasing attention for currently serious environmental problems: the increasing acid precipitation has implied that the natural sulfur cycle has been seriously disturbed by anthropogenic gaseous emissions. Sulfur gases are also known to be the precursors of sulfate particles and cloud condensation nuclei, which affect global climate by scattering solar radiation and changing the albedo of clouds (Andreae and Crutzen 1997). In order, therefore, to be able to estimate the significance of man-made activities over the sulfur cycle and its environmental consequences, it is necessary to have a detailed understanding of natural sulfur emission. Soils are also major sites of biogenic sulfur release (Cooper et al. 1987) and play an important role in the global sulfur cycle. The generalization of biogenic sulfur emission trend in the soils suffers from substantial variabilities both geographically and temporally (Cooper et al. 1987) for their involvement in biological activity. Therefore, to accurately define the contribution of biogenic sulfur gases from soils to the atmosphere's sulfur budget, the sulfur source and environmental factors that control soil biological activity must be taken into consideration.

The main goal of this study is to investigate the effects of variable factors such as cystine quantity in soil, air condition, temperature, soil moisture, soil pH, and light regimes on the biogenic sulfur emission from rice paddy soil, and to provide data for model-based calculations.

MATERIALS AND METHODS

Top-soil samples of rice paddies, which can represent the typical south area of the Yangtze River, were collected from Xiao Ling Wei, Nanjing, Jiangsu Province, China. Chemical fertilizer and organic manure had been applied to the collection plot for 9 years. The application amounts of fertilizer and manure were approx 80 kg N ha^{-1} yr^{-1}. Each sample was air-dried and ground and passed through 2 mm sieve. Total carbon and total nitrogen contents in soil were determined by the dry

Present address: KTL Testing Labs. Inc., Othello, WA 99344, USA
Correspondence to: J. Zhang

Table 1. Detected volatile sulfur compounds from waterlogged paddies under aerobic and anaerobic headspace for 7 days (ng g^{-1} soil)

incubation air condition	cystine treated (mg g^{-1}soil)	COS	H_2S	CS_2	CH_3SH	DMS
Aerobic	0	136(5)	1 (1)	0	0	44(2)
	2.5	361(5)	19 (1)	10(2)	0	51(2)
	5	490(5)	27(1)	16(2)	0	270(4)
	7.5	826(4)	23(1)	19(2)	50(1)	143(4)
	10	1445(5)	59(2)	20(2)	89(1)	485(4)
Anaerobic	0	146(6)	19 (1)	0	0	57(2)
	2.5	308(4)	22 (1)	16(2)	0	92(3)
	5	1028(5)	23(1)	17(2)	4(1)	313(4)
	7.5	1654(5)	88(2)	18(2)	21(1)	436(4)
	10	2624(5)	120(3)	41(2)	13(1)	465(5)

Note. Figures in parentheses indicate the day the maximum amount of sulfur compound was detected. Follows is the same.

combustion method using a CN analyzer (Yanagimoto Co. Ltd., MT-500, Japan). Total sulfur contents in the soil were determined turbidimetrically according to the method of Sansum and Robinson (1974) after dry ashing. Total carbon, organic carbon, total nitrogen and total sulfur contents of soil were determined respectively as 2.41 %, 1.85 %, 0.16 % and 0.15 %. Soil pH was 7.49.

The emission of volatile sulfur gases effected by cystine source in soil was studied using the method described by Yang et al.(1997): 20g of each soil sample added different amount cystine was placed into pre-silanized glass bottle (~170ml), 30 ml of water was added to each bottle to simulate waterlogged conditions, the bottle was sealed with a silicone rubber septum, then incubated either aerobically (bottle air was ambient atmosphere, 21% O_2 and 78% N_2) or anaerobically (bottle air was purged with N_2) at 35°C under cool-white fluorescent light for a week. Air samples were taken twice a day at 9:00 a.m. and 3:00 p.m. using Pressure-Lock gas syringes (PS CO., Ltd., USA) analyzed as described below. The bottles were not shaken before sampling.

The effects of soil incubation temperature (°C), moisture level, pH and light regime on sulfur emission were investigated using the similar proceeding procedure as above.

The collected air from the headspace of the incubation bottles was analyzed using a gas chromatograph (GC-4000A, Beijing Institute of East-West Electronic Technology, China) equipped with a double-flame photometric detector (FPD) operating in the sulfur mode. Sulfur speciation was separated on a glass column

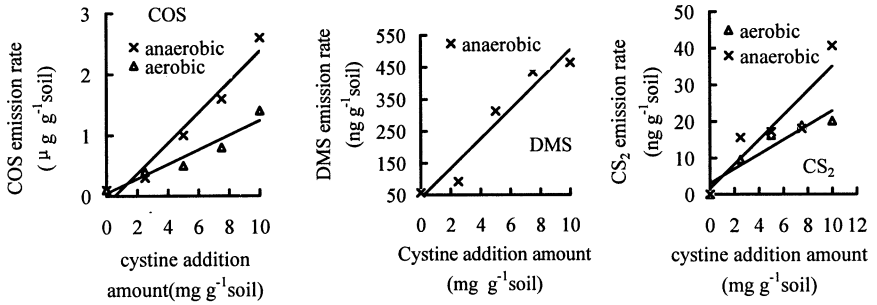

Figure 1. The relationship between the cystine addition quantity and sulfur emission rate under aerobic and anaerobic atmosphere conditions

(2.6 mm I.D., 3 m length) packed with 25% β,β'-oxydipropionitrile(ODPN) on 60～80 mesh Chromosorb W (AW DMCS treatment). Column temperature was programmed from 50°C (isothermal, 2 min) to 80°C at a rate of 30°C min^{-1} and maintained for 2 minutes. Each analysis required 12 minutes to separate sulfur speciation.

Certified standard H_2S and COS gases (Nanjing Analytical Instrument Factory, China) were diluted using the standard gas generator (S-tec Co., SGGU-62L, Japan). The permeation method was used for the calibration of other standard gases (Andrew and Ortman, 1966). The certified permeation tubes for CH_3SH and DMS, and a diffusion tube for CS_2 were obtained from Gastec. Co., Ltd.. The permeation apparatus (Gastec Co., P-1, Japan) was operated at 35°C.

The detection limit of the FPD is 3 ng H_2S, 6 ng COS, 4 ng CH_3SH, 4 ng CS_2 and 6 ng DMS, respectively. The number of replicates of sample is 3.

RESULTS AND DISCUSSION

The following five volatile compounds were identified as emission products from the waterlogged paddy soils under either aerobic or anaerobic condition (Table 1): carbonyl sulfide (COS), hydrogen sulfide (H_2S) and dimethyl sulfide (CH_3SCH_3 or DMS), methyl mercaptan (CH_3SH), carbon disulfide (CS_2). COS was the major product obtained, the second most abundant product was DMS. These two gases were considered to be the more abundant reduced organic sulfur compounds emitted to the atmosphere (Kuhn et al. 1999; Yang 2000). Their higher concentrations were not unexpected. H_2S accounted for less than 5 % of the total emission of sulfur gases. A low amount of detected H_2S does not mean that this gas was not produced during incubation. Soil has substantial capacity for sorption of H_2S. The solubility of H_2S in water is larger than COS or CS_2, and this gas is

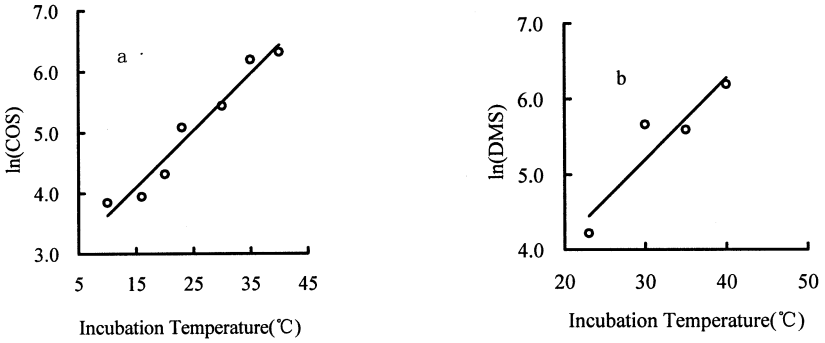

Figure 2. The relationship between the natural logarithm value of the sulfur emission rate and the incubation temperature

rapidly converted to ferrous sulfides (FeS). Its lifetime is very short in an aerobic soil atmosphere. Therefore H_2S emission rate was very low, and even could not be detected. Other sulfur compounds account approximately for 5% of the total natural emission of sulfur gases.

The next days, after incubation had started, we detected the emission of volatile sulfur gases from the incubation bottle and found that the concentration of each sulfur speciation increased with time. The highest values of volatile sulfur gases appeared at 1-5 days after incubation (Table 1). After being emitted from soil, the reduced gaseous sulfur compounds undergo oxidation in the atmosphere to sulfur dioxide, primary through reaction with the hydroxyl and other radicals. The atmospheric lifetime of most gaseous sulfur speciations is in the order: $COS>DMS>CS_2>CH_3SH>H_2S$. After that time, detected sulfur gases decrease rapidly. After 6-7 days generally there was no sulfur gas detected except COS. COS is the most stable reduced sulfur gas in the atmosphere (Kuhn, 1999). Attention has been focused on COS as a precursor of stratospheric sulfate particles, due to (a) the influence of the stratospheric sulfate aerosol layer on the earth's radiation budget (Turco et al., 1980) and (b) its role in the heterogeneous reaction chemistry leading to ozone destruction (Roche et al. 1994; Fahey et al. 1993).

Our results (Table 1) showed that the emissions from the cystine-added paddy soils were significantly higher than from the paddy soils without added cystine under either aerobic or anaerobic condition, therefore the cystine concentration in soil influences the amount of emission. The volatile sulfur gases emitted from soil are produced by the microorganism decomposition of cystine, addition of cystine increases the population and activity of soil microorganisms and causes the increasing of biogenic sulfur emissions in comparison with the unamended soils.

The emission of sulfur gases also positively correlated to the cystine content in the soil. This observation reminded us that a relationship might exist between the emission amount of sulfur and cystine concentration in soil. Linear regression of the sulfur emission rate with the cystine concentrations shows a good correlation with the correlation coefficients ranging from 0.91 to 0.98 (Fig.1 COS, DMS, and CS_2). The results indicate that the microbial decomposition of cystine in waterlogged soil can act as one source of biogenic sulfur gases emission.

Under anaerobic headspace (nitrogen atmosphere), the sulfur gases were detected after one day of incubation. Most detected speciations of volatile sulfur gases were higher than under the aerobic condition (ambient atmosphere) — H_2S was most obvious (Table 1). 1-2 days after incubation, the H_2S emission rate reached a higher level. The production of other gases, such as COS, CS_2, CH_3SH, and DMS also increased. It is believed that the main reason for reduced sulfur depletion in the atmosphere is a daytime reaction with the OH radical and the nighttime reaction with the NO_3 radical (Jensen et al. 1992). Because of no oxygen content in the incubation bottle under anaerobic condition, the rate of oxidation and exhaustion of sulfur gases were slowed enormously. However, even under anaerobic condition, reduced sulfur gases were still depleted slowly. This maybe due to the sorption of paddy water and paddy soils for sulfur gases. Along with the decrease in the potential decomposable sulfur-containing organic matter and reducible sulfate in soil, the production decreased gradually too. The wall effect also influences the sulfur gas emissions. DMS and CS_2 were depleted 4-7 days after incubation, varied with the cystine amount.

The emission of sulfur gases increased exponentially with the temperature from 10 to 40 °C (Fig.2). Fig.2 shows that the natural logarithm values of the COS and DMS emission rate from waterlogged soils were proportional to the incubation temperature, with the linear regression correlation coefficients 0.96 and 0.93

Table 2. Detected biogenic sulfur gases under various moisture level condition (ng g^{-1} soil)

Incubation air condition	Moisture level (%)	COS	CS_2	CH_3SH	DMS	H_2S
Aerobic	25%	110(4)	11(2)	0	84(2)	0
	50%	2466(5)	29(1)	56(3)	366(4)	62(2)
	75%	2150(5)	13(2)	13(4)	208(4)	32(1)
	150%	490(5)	0	0	270(4)	27(1)
Anaerobic	25%	165(4)	0	2(2)	92(2)	0
	50%	4854(5)	34(1)	67(2)	475(2)	214(1)
	75%	4332(5)	12(2)	51(2)	392(2)	72(1)
	150%	1028(5)	17(2)	4(1)	313(4)	23(1)

respectively (Fig.2a and b). Kanda et al. (1992) also found that the logarithmic values of the DMS fluxes from Japanese paddy field were proportional to the air temperature within the chamber. This is presumably due to the enhancement of the activities of soil microorganisms associated with an increase in soil temperature.

Emissions of sulfur gases were also markedly affected by soil moisture level (Table 2). The emission amount of each sulfur species is the highest from the moisture level-50% soil sample. No matter the soil moisture level is higher or lower than 50%, the sulfur emission is lower than 50%. This means the activities of microorganisms involving the sulfur emission required a moderate soil moisture level. When soil moisture level is over low, the humid condition required by microorganism's growth can't be met, the metabolism of microorganisms obviously slowed down, many microorganisms converted into inactive state or dead, the production of sulfur gases decreased. On the other hand, water has the capacity of absorption solubility for reduced sulfur gas, therefore over high moisture level hinders the detection of sulfur gases emission from soil.

Table 3. Detected sulfur gases under aerobic conditions at various pH (ng g^{-1} soil)

pH	COS	H_2S	CS_2	DMS
3.5	86(7)	25(5)	51(5)	38(7)
5.5	117(5)	27(5)	26(5)	29(5)
7.5	136(5)	22(1)	0	44(2)
8.5	106(4)	13(1)	0	76(2)

Table 4. The most suitable and available pH ranges for sulfur emission

	COS	H_2S	CS_2	DMS
The most suitable pH	7.5	5.5	3.5	8.5
available pH ranges	3.5-8.5	3.5-7.5	3.5-5.5	3.5-8.5

The results (Table 3) show that emission of sulfur varies with the changing soil pH, this means that soil pH has effects on the emission of sulfur gases. The most suitable and available soil pH ranges for sulfur emission are listed in Table 4.

Table 5.Detected biogenic sulfur gases under aerobic conditions with various light conditions (ng g^{-1} soil)

Soil moisture level (%)	Light condition	COS	H_2S	CS_2	CH_3SH	DMS
150	No light	321(4)	0	0	5(3)	33(2)
150	Light	756(5)	28(1)	17(2)	0	148(4)
50	No light	654(3)	0	48(3)	33(3)	87(3)
50	Light	1694(6)	25(1)	97(1)	59(2)	166(3)

Doubtless, only under appropriate pH conditions, the microorganism can decompose cystine and leads to the production of sulfur gases. Over high or low soil pH influences the activity of microorganism. This result is not unexpected.

Previous field experiments on sulfur emission (Staubes et al. 1989; Kanda et al. 1992) showed that, the emission of sulfur gases in the daytime was higher than that during the night. They noticed the effect of higher temperature in daytime. Our results (Table 5) indicate that the light regime is also a reason caused that fact. The production of COS under light condition was far higher than under no light condition at both soil moisture levels 150% and 50%, this is because other sulfur gases, such as DMS and CS_2 can be converted to COS by photochemical oxidation (Yang et al. 1997; Brugger et al. 1998). Photochemical oxidation is also the main sink of DMS . Therefore no detected DMS doesn't mean that DMS was not produced from soils. On the other hand, this may suggest that, the microorganisms of producing COS may be photochemical trophic facultative. Under with and without light conditions, the productions of CS_2 do not change significantly. It suggests that the microorganism producing CS_2 might be a typical facultative trophic bacterium. The emission of sulfur gases is commonly higher under light condition than that under no light condition.

Results from this study demonstrated that: (1) A quite good correlation exists between the emission rate of sulfur and the cystine concentration in soil, therefore, when the content of material which contains cystine such as the remains of plant, animal, and manure in soil is higher, more biogenic sulfur gases will be emitted. (2) Under anaerobic headspace conditions the emissions of sulfur gases are commonly higher than under aerobic incubation conditions. (3) Linear-correlation exists between the natural logarithm values of the COS and DMS emission rates and the soil incubation temperature from 10 to 40 °C. (4) The soil moistures level has effects on the production of sulfur gases from the decomposition of cystine. The suitable water/soil ratio for sulfur gases emission is 50%. (5) Soil pH has a profound influence on the emission of sulfur gases. The soil pH ranges for the most suitable and available sulfur emission are detected. (6) Under light condition, the emission rates of most sulfur speciation are higher than under without light.

Acknowledgments. This research was supported by the International Foundation for Science (IFS), Sweden (No. C/2409-2) and The National Natural Science Foundation of China (No.29677007).

REFERENCES

Andreae MO, Crutzen PJ (1997) Atmospheric aerosols: biogeochemical sources and role in atmospheric chemistry. Science 276:1052-1058

Andre EO, Ortman GC (1966) Primary standards for trace gas analysis. Anal Chem 38:1083-1089

Brugger A, Slezak D, Obernosterer I, Herndl GJ (1998) Photolysis of dimethylsulfide in the northern Adriatic Sea: Dependence on substrate concentration, irradiance and DOC concentration. Mar Chem 59:321-331

Cooper WJ, Cooper DJ, Saltzman ES, De Mello WZ, Savoie DL, Zika RG, Prospero JM (1987) Emission of biogenic sulfur compounds from several wetland soils in Florida. Atmos Environ 21:1491-1495

Fahey DW, Kawa SR, Woodbridge EL (1993) In situ measurements constraining the role of sulphate aerosols in mid-latitude ozone depletion. Nature 363:509-514

Jensen NR, Hjorth J, Lohse S, Skov H, Restelli G (1992) Products and mechanisms of the gas phase reactions of NO_3 with CH_3SCH_3, CD_3SCD_3 and CH_3SSCH_3. J Atmos Chem 14:95-108

Kanda K, Minami K (1992) Measurement of dimethyl sulfide emission from lysimeter paddy fields. Ecol Bull 42:195-198

Roche AE (1994) Observations of lower-stratospheric $CINO_2$, HNO_3 ans aerosol by the UARS CLAES experiment between January, 1992, and April, 1993. J Atmos Sci 51: 2877-2902

Sansum LL, Robinson JBD (1974) Wet digestion, manual and automated analysis of total sulfur in plant material. Commun Soil Plant Anal 5:365-385

Staubes R, Gerogi HW, Ockelmann G (1989) Flux of OCS, DMS and CS_2 from various soil in Germany. Tellus 41B:305-313

Turco RP, Whitten RC, Toon OB, Pollack JB, Hamill P (1980) OCS, stratospheric aerosols and climate. Nature 283:283-286

Kuhn U, Ammann C, Wolf A, Meixner FX, Andreae MO, Kesselmeier J (1999) Carbonyl sulfide exchange on an ecosystem scale: soil represents a dominant sink for atmospheric COS. Atmos Environ 33:995-1008

Yang GP, Zhang ZB, Liu XT, Liu LS (1997) Kinetic study of photochemical oxidation reaction of dimethylsulfide in aqueous solution. Ocean Univ Qingdao 27:225-232

Yang GP (2000) Spatial distributions of dimethylsulfide in the South China Sea. Deep-Sea Res I 47:177-192

Yang Z, Kong L, Wang SM (1997) The emission of biogenic sulfur gases from incubated Chinese paddy soil and its relationship with carbon dioxide. Toxicol Environ Chem 64:33-43