

# Emission of biogenic hydrogen sulfide

By R. A. RASMUSSEN, *Air Pollution Research Section, College of Engineering  
Research Division, Washington State University, Pullman, Washington 99163*

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## ABSTRACT

Until recently sulfur has been assumed to occur in the atmosphere principally in three forms:  $\text{H}_2\text{S}$ ,  $\text{SO}_2$  and sulfates. The principal processes by which  $\text{H}_2\text{S}$  is believed to be generated on a global scale are (1) nonspecific reduction of organic sulfur and (2) from sulfate reduction by anaerobic bacteria. Unfortunately, these assumptions have not been verified by analyses of the volatile sulfur compounds emitted by the micro-organisms that are supposed to be responsible for sulfur gas exchange in natural systems. To the contrary, gas chromatographic analyses using a flame photometric detector specific for sulfur at 394 nm indicates that dimethyl sulfide (DMS) and dimethyldisulfide (DMDS) are the major compounds in the gaseous emissions from bacteria and fresh water green and blue-green algae isolated from different soil types and eutrophic as well as naturally clean waters. Analysis of the sulfur gases in a wide variety of sea water samples and those released by red, green and brown seaweeds indicate that organic sulfur emissions dominate over  $\text{H}_2\text{S}$ . The data suggest the hypothesis that the micro-biota in the various natural systems like soil, leaf litter, lakes, ponds, rivers, and the oceans produce predominantly organic sulfides and that the reported observation of  $\text{H}_2\text{S}$  emission from water-logged soils and anaerobic muds is related to their low pH. Presumably the biogenic production of gaseous sulfur compounds constitutes a major input in the sulfur cycle. Unfortunately, the source of strength and spatial distribution of these sources are unknown.

## Introduction

Sulfur is assumed to occur in the atmosphere principally in three forms:  $\text{H}_2\text{S}$ ,  $\text{SO}_2$  and sulfates. Of these three,  $\text{SO}_2$  is the principal gaseous sulfur compound in the atmosphere. In remote, unpopulated environments, its concentration ranges from less than 1 to about 2 ppb ( $10^{-9}$ ). The atmospheric precursor of biogenic  $\text{SO}_2$  is believed by many to be hydrogen sulfide, but there are no reports of successful attempts to detect it in these regions at the very low concentration—about 0.1 ppb or less—at which it may be present.

The principal processes by which  $\text{H}_2\text{S}$  is believed to be generated on a global scale are (1) non specific reduction of organic sulfur and (2) from sulfate reduction by anaerobic bacteria. The identification of the production of  $\text{H}_2\text{S}$  by anaerobic bacteria is established. The breadth of these pertinent aquatic environments includes wet soils, swamps, the muddy bottoms of lakes, coastal wet lands and estuaries, organic rich muds and some fiords and ocean deeps where

physical barriers prevent flushing with oxygenated waters (Hitchcock & Wechsler, 1972).

Evidence favoring a bacteriogenic source for large quantities of atmospheric sulfur has been reviewed and accepted by many authors (Conway, 1943; Eriksson, 1960, 1963; Junge, 1963; Robinson & Robbins, 1968, 1970; Koyama et al., 1965; Jensen & Nakai, 1961; Berner, 1971). However, none of these authors critiqued the fact that no measurements of  $\text{H}_2\text{S}$  had been made in remote atmospheres.

It is also significant to note that no source measurements of  $\text{H}_2\text{S}$  concentrations in swamps, estuaries or near tidal flats have been reported in the literature to confirm the preeminent biogenic position  $\text{H}_2\text{S}$  occupies as the major biogenic sulfur compound released to the atmosphere. Rather the presence of  $\text{H}_2\text{S}$  is reported to be identified by its odor in such environments. In addition there are grounds for doubting whether or not  $\text{H}_2\text{S}$  can be emitted into the atmosphere from anaerobic muds through the intervening aerobic surface waters in quantities

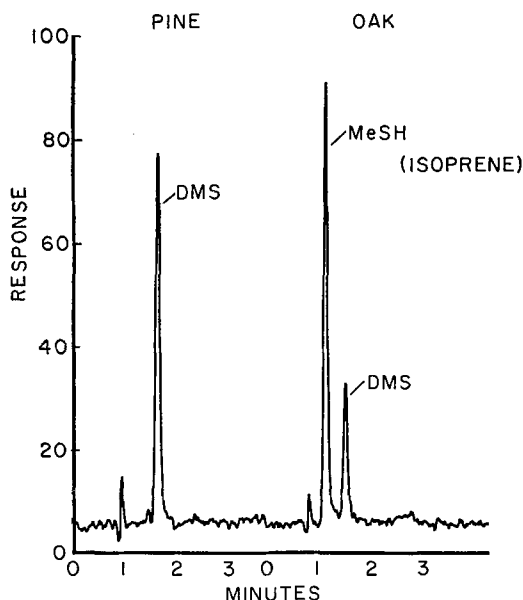


Fig. 1. Foliage emitted sulfur gases, both foliages held in 500 ml flasks for 40 hours, Pine, 50 ml air sample, DMS peak = 1.18 ng: Tissue weight 2 g oven-dried. Oak, 20 ml air sample, DMS peak 0.7 ng. Isoprene peak 850 ppm (47 600 ng) tissue weight 1.8 g oven-dried.

sufficient to account for the quantities of excess sulfate estimated to be in circulation (Ostlund & Alexander, 1963). Brinkman & Santos (1973) have also observed that under normal meteorological conditions the main part of  $H_2S$  formed in bottom muds is imprisoned in the bottom layers of eutrophic lakes and the  $H_2S$  that does occasionally escape upwards is rapidly oxidized and partly recycled in the aquatic sulfur standing crop.

The chemical analyses made in our laboratory of the volatile sulfur compounds emitted by microorganisms and the natural systems supposedly responsible for  $H_2S$  emission lead us to reject the  $H_2S$  hypothesis originated by Conway (1943).

## Experimental

### Gas chromatographic analysis

Gas chromatographic analyses of the organic sulfur compounds in the air samples were made using packed columns in a Varian-Aerograph 1490-5 sulfur gas analyzer with a Flame Photo-

metric Detector (FPD). The operating conditions were optimized as follows: column 10-ft  $\times$  1/8-in. FEP Teflon® tubing packed with 5% Triton X-305 coated on 100- to 120-mesh Chromosorb G; column temperature: 50°C isothermal, with a programmed temperature rate of 12°C/min to 80°C and hold. Column adsorption stabilization was obtained by conditioning the system to 63 ppb  $H_2S$ , 40 ppb  $CH_3SH$ , and 75 ppb  $(CH_3)_2S$  at 50°C for 72 hours. No ghosting or memory peaks were obtained after 2 hours of secondary conditioning with SCOT®<sup>1</sup> "zero" air. Conditions were as follows: carrier gas flow: air 78 ml/min, hydrogen 64 ml/min, make-up air 38 ml/min; attenuation:  $2 \times 10^{-9}$  AFS; samples size: variable from 25 to 1 000 ml. All samples were injected via the freeze-out loop.

For read-out, a 1-mv potentiometric recorder was used at a chart speed of 30 in./hr. The component peaks in the chromatograms were quantitated using an AutoLab model 6300-02 electronic integrator. The concentrations of the sulfur species were calculated using external standards.

### Sampling procedure

The air samples described in this paper were handled using a 50-ml Pressure-Lok®<sup>2</sup> magnum syringe construction of Teflon® and micro-finished drawn borosilicate glass. The freeze-out systems consisted of an external loop (1/8-in.  $\times$  18-in. FEP Teflon®) packed with 10 in. of 40- to 60-mesh glass beads immersed in liquid oxygen ( $-183^\circ C$ ). Subsequently, the trapped materials in the loop were released by 60°C water and flushed onto the head of the packed column. Light sulfur gases, such as  $H_2S$ , carbon disulfide ( $CS_2$ ) and methyl mercaptan ( $CH_3SH$ ), were allowed to elute from the column prior to initiating the programmed temperature analysis necessary to resolve dimethyl sulfide (DMS), dimethyl disulfide (DMDS) and other higher-molecular-weight organic sulfur species.

A six-port rotary gas sampling valve constructed of a Roulon-filled Teflon® core with a stainless-steel outer body provided the switching unit needed to inject the sample into the GC system.

<sup>1</sup> Scott Research Laboratories, Plumsteadville, PA, 18949.

<sup>2</sup> Precision Sampling Corporation, Baton Rouge, LA, 70815.

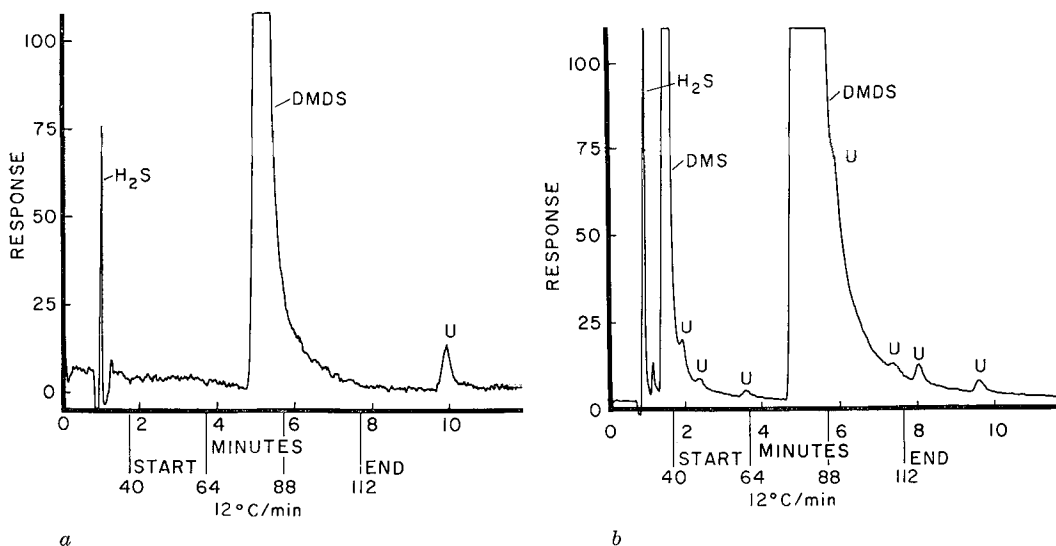


Fig. 2a. Volatile sulfur compounds in atmosphere above *Pseudomonas aeruginosa*, no organic sulfur in media. Freezeout of 10 ml of headspace. Attn:  $4 \times 10^{-9}$  AFS. Organism grown on Drake's enrichment medium for *Pseudomonas* ( $\text{MgSO}_4$  0.05 %).

Fig. 2b. Volatile sulfur compounds in atmosphere above *Pseudomonas aeruginosa*, organic sulfur in media. Freezeout of 10 ml of headspace. Attn:  $16 \times 10^{-9}$  AFS. Organism grown on Brain Heart Infusion (Difco).

## Results

The purpose of the data presented is to attempt to unravel the natural ecological role organic sulfur compounds play in the interactions of the biosphere with the atmosphere. Therefore the majority of the studies made were concerned with the biogenic emission of  $\text{H}_2\text{S}$ ,  $\text{CH}_3\text{SH}$ , DMS, and DMDS from aerobic habitats simulating natural systems.

Previously it was reported (Lovelock et al., 1972) that the emission of DMS from living intact leaves of oak, cotton, spruce and pine trees ranged from 2 to  $43 \times 10^{-12} \text{ g g}^{-1}$  of oven dried tissue per hour; the rate of emission for decaying leaves from the same species was 10 to 100 times greater. In Fig. 1 the sulfur gasses emitted from the foliages of Scot pine (*Pinus sylvestris*, L.) and red oak (*Quercus ruber*, L.) are shown. Originally the dominant peak in the gas chromatogram of the oak foliage was tentatively identified as methylmercaptan. Later this identification was proven wrong by GC-MS studies and the component correctly identified as isoprene. The reason isoprene was measured by the FPD, which is normally insensitive to hydrocarbons compared to sulfur species at similar concentrations (10 000:1 specificity for

sulfur) is that the concentration range of isoprene accumulated in the study chambers was 30 000 to 60 000 fold greater than the sulfur species. It is also interesting that these same foliages were producing carbon monoxide. The oak foliage which was an actively metabolizing tissue from the greenhouse produced a maximum concentration of only 1.1 ppm, whereas the pine needles, which were obtained from dormant over-wintering trees out-of-doors, released CO to a concentration of 32.4 ppm. It is especially significant to note that the measured organic sulfur compounds as well as CO, isoprene and other hydrocarbons represent only a few of the possible chemical species emitted by the tissues studied.

Whether the metabolism of the leaf is directly responsible for this emission of DMS, or whether it is a secondary result from the microbial populations living on the surface of the leaves, is unknown. However, it appears that dormant or senescent foliages are better candidate tissues for studying the release of organic sulfides than healthy fully photosynthetic tissues.

The previously reported (Lovelock et al., 1972) measurements of organic sulfides in sea water and of its emission by land plants, soils

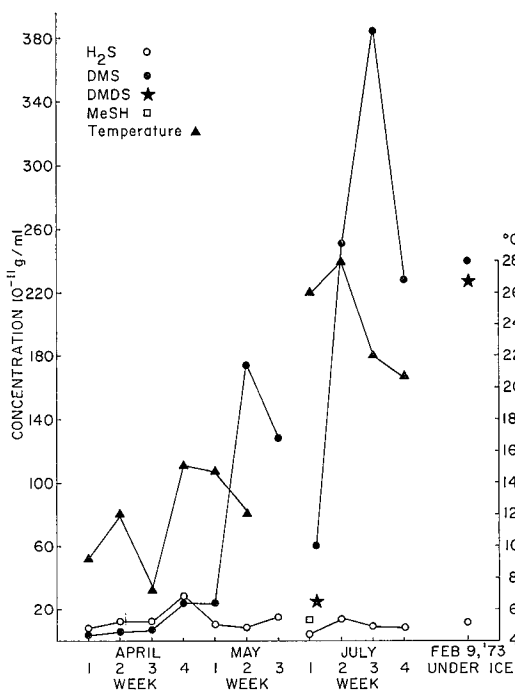


Fig. 3. Weekly changes in the level of H<sub>2</sub>S and DMS in College Pond.

and marine algae were concerned primarily with dimethylsulfide. In the analyses of these soil samples representative of (garden, pasture, and woodlands) ecosystems collected in diverse areas of the United States from November through April, 1971–1972, all of the soils produced DMS if contained in flasks sealed with glass wool, allowing restricted exchange of gases with the surrounding air. If the flasks were completely sealed, DMS production fell and hydrogen sulfide was produced. These observations have been repeated with soil samples collected from tidal flats and the organic muds obtained from shallow ponds with the same results. In addition, depending upon the amount of organic matter in the mud, other organic sulfides such as CH<sub>3</sub>SH and DMDS, were measured.

Thus it would appear that DMS is not the only organic sulfide that is released naturally to the atmosphere. Further corroboration of this was obtained in the analysis of the sulfur gases emitted by aerobic cultures of *Pseudomonas*, a ubiquitous genus of bacteria. Gas analyses of the head space above cultures of this genus showed that: *Ps. fluorescens* (soil and aquatic

habitat) and *Ps. aeruginosa* (aquatic and sewage habitat) (Fig. 2a and b) produced almost exclusively DMDS when grown on a non-organic sulfur media (S source = MgSO<sub>4</sub>) and both DMS and DMDS with minor traces of H<sub>2</sub>S and eight other unidentified sulfur compounds when grown on a media containing organic sulfur (S source = peptone). Similar observations were obtained for two common Pacific tidal zone seaweeds *Entromorpha compressa* and *Fucus furcatus*.

The production of organic sulfides from both the *Pseudomonas* cultures and the seaweeds ceased when the cultures and/or the tissues died. It is interesting to note that analyses of the volatile sulfur compounds emitted by an anaerobic dairy manure lagoon into an aerobic atmosphere produced 100 fold more organic sulfur as CH<sub>3</sub>SH, DMS, DMDS, and diethylsulfide than hydrogen sulfide.

The analysis of sulfur gases displaced from water samples using the method of McAuliffe (1971) has provided further information on the types of sulfur compounds produced by fresh water green and blue green algae isolated from eutrophic as well as naturally clean waters. The rationale for making these studies is that since fresh water algae are a major constituent of the biomass in aquatic ecosystems they should contribute significantly to the types and amounts of trace gases dissolved in aquatic ecosystems and subsequently released to the atmosphere. Large populations of fresh water algae in water impoundments are often correlated with eutrophic conditions. The unpleasant odors associated with these eutrophic waters suggests the presence of hydrogen sulfide and organic compounds. Unfortunately, although many species of algae are associated with eutrophic conditions in water impoundments, the gaseous emissions from fresh water algae have not been critically analyzed. Rather it has been assumed that the principal form of sulfur emitted by microorganisms and algal blooms was hydrogen sulfide.

Through the spring and summer of 1972 and the winter of 1973, water samples were taken periodically from three stations located around the periphery of a small (area approximately 1 hectare) pond that supports a large population of green and blue-green algae. The pond is located on the Washington State University campus adjacent to pasture land and receives

the run-off from a neighboring wheat field. The pond is spring fed and by mid-summer may be classified as eutrophic.

The objectives of these preliminary studies were to determine qualitatively and quantitatively the volatile sulfur gases contained in the pond. Also the same samples of pond water were examined for identifying the algae present in the pond. Accordingly the algal species present in samples containing organic sulfides were isolated and cultured.

Some of these data are shown in Fig. 3. The most obvious feature is the dominance of DMS over  $H_2S$ . This is especially true for the active algal growth periods from late spring through mid-summer. Conversely,  $H_2S$  was measured to be in slight excess over DMS from winter through early spring. However, in the summer the concentration of DMS compared to  $H_2S$  was higher by two orders of magnitude. In general the level of  $H_2S$  through the seasons did not vary significantly compared to the seasonal fluctuations in DMS. This generalization is true except in one instance, where in the third to fourth week of April a sudden drop in the temperature of the pond and associated winds resulted in a full circulation of the shallow body of water bringing to the surface a two fold higher concentration of  $H_2S$ . This type of turbulent transfer of bottom waters to the surface was reported for tropical lakes at this symposium by Brinkman and Santos.

The other organic sulfides associated with the pond water were a few isolated measurements of  $CH_3SH$  and DMDS. The concentrations of these sulfur species were much lower than that measured for DMS. Nevertheless their presence indicated that possibly changes in the dynamics and population mix of the algal and/or bacterial species in the pond at the time of sampling were responsible for the types of organic sulfides measured.

A very interesting measurement was made in the winter when the pond was frozen. Water samples taken beneath the ice were measured to have concentrations of DMS similar to those measured during the active growth periods of summer. Unexpectedly, the associated  $H_2S$  concentration was not greatly elevated above the mean value of the previous summer. It is also important to note that the concentration of DMDS in the samples obtained from beneath-the-ice was almost the same as that of DMS.

Table 1. *Sulfur gases emitted from axenic algal cultures*

*Cyanophyta*, Blue Greens

	Cell count (cells/ ml $\times$ $10^8$ )	$H_2S$	DMS	DMDS	MeSH
(concentration $10^{-11}$ g ml $^{-1}$ )					
<i>A. Unicellular form</i>					
Anacystis	3.5	15	31	2	0
Synecho- coccus	3.3	12	40	31	6
<i>B. Filamentous forms</i>					
	Dry wt. (g/l)				
Plectonema	0.45	12	450	30	2
Oscillatoria	2.5	17	20	196	—
Phormidium	1.9	15	112	64	—

Obviously the ice cap on the pond served as a lid which enabled the DMS and DMDS to accumulate under the isothermic conditions throughout the pond. However, since  $H_2S$  did not accumulate, it suggests that either  $H_2S$  was not being produced or that the production rate was equal to its destruction.

The types and classes of algae identified in the pond water samples associated with volatile sulfur gases were primarily green and blue-green algae. Preliminary results appear to show that DMS may indeed be a common metabolite for fresh water algae. It already is established to be the most common sulfur gas metabolite from marine algae. The gas chromatographic analyses indicated that water samples containing *Spirio-gyra*, a filamentous green algae, and *Pandorina*, *Eudorina* and *Gonium*, colonial green algae emitted moderate amounts of DMS and traces of methylmercaptan. Whereas the blue-green algae, like *Nostoc* and *Anabaena* produced much higher concentrations of DMS as well as DMDS.

Bacteria were always present with the algal types studied. This concurrence is associated both in the medium and as a symbiotic condition when the bacteria are adherent to the algae. Therefore axenic cultures (bacteria free) were established and the gases emitted from the axenic, clonal cultures analyzed.

The previous experiments carried out with non-axenic algal cultures make the exact origin of the volatile sulfur gases uncertain. Since micro-

organisms are recognized (Ishida, 1968; Kadota & Ishida, 1972) to produce a variety of organic sulfides it was not too surprising that, when the gases emitted by axenic cultures of *Pandorina*, *Gonium*, and *Eudorina* were analyzed, no volatile sulfur gases were detected. However, the axenic cultures of the blue-green algae produced moderate to high concentrations of the  $\text{H}_2\text{S}$ , DMS and DMDS and occasionally  $\text{CH}_3\text{SH}$  (Table 1).

## Conclusions

The major gap in our knowledge of the sulfur cycle is the biogenic production of gaseous sulfur compounds, notably  $\text{H}_2\text{S}$ , DMS, and DMDS from terrestrial and marine surfaces. Presumably, these compounds constitute a major input; the source strength and spatial distribution of these sources, however, are poorly known. The techniques to measure the expected small ambient concentrations of  $10^{-12}$  are not available. Therefore, we are presently restricted to studying the biogenic emissions from candidate type ecosystems.

The evidence favoring a bacteriogenic source for large quantities of atmospheric sulfur is usually associated with *Desulfovibrio* and related sulfate-reducing bacteria under anaerobic conditions. In the fresh- and salt-water algal and bacterial systems studied, attempts to establish that  $\text{H}_2\text{S}$  is the major volatile sulfur compound failed. To the contrary, studies of these aerobic environments such as soil, intact foliages and in the fresh-water samples—collected from diverse ecosystems ranging from

clean mountain streams to eutrophic reservoir waters—show that the amount of  $\text{H}_2\text{S}$  measured was insignificant compared to the organic sulfides measured, as long as the system remained aerobic.

The previously recognized emission of  $\text{H}_2\text{S}$  from the waterlogged soils of rice paddies and estuarine muds is related to their anaerobic states. However, the mass transfer of  $\text{H}_2\text{S}$  to the atmosphere is normally through fresh and ocean surface waters that are much too oxidizing to permit the existence of  $\text{H}_2\text{S}$  at concentrations sufficient to sustain a significant transfer to the atmosphere. Also the existence of large anaerobic ecosystems that could release their  $\text{H}_2\text{S}$  to atmosphere through turbulent transfer conditions similar to that reported by Brinkman & Santos are inadequate to account for the standing concentrations of biogenic sulfur in the atmosphere.

It is therefore suggested that dimethyl sulfide and related compounds, derived from the turnover of both organic sulfur in plants and animals as well as inorganic sulfate, be considered as the candidate compounds responsible for the natural transfer of biogenic sulfur to the atmosphere.

## Acknowledgement

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## ЭМИССИЯ СЕРОВОДОРОДА БИОГЕННОГО ПРОИСХОЖДЕНИЯ

До последнего времени считалось, что сера существует в атмосфере главным образом в трех формах:  $H_2S$ ,  $SO_2$  и сульфаты. Предполагается, что главными процессами, с помощью которых  $H_2S$  генерируется в глобальном масштабе являются (1) реакция восстановления органической серы и (2) восстановление анаэробными бактериями. К сожалению, эти предположения не были подтверждены анализами летучих сернистых компонентов, испускаемых микроорганизмами, предположительно ответственными за обмен сернистого газа в природных системах. Наоборот, хроматографический анализ газа, использующий пламенный фотометрический детектор для серы при 394 нм, указывает, что диметилсульфид (ДМС) и диметилдисульфид (ДМДС) являются главными компонентами в газовой эмиссии бактерий, пресноводных зеленых и синезеленых морских водорослей, изолированных от различных

типов соли и автотрофных как естественная чистая вода. Анализ сернистых газов в широком диапазоне проб морской воды, содержащей красные, зеленые и коричневые водоросли, указывает, что органическая эмиссия серы превосходит эмиссию  $H_2S$ .

Из приведенных данных можно сделать вывод, что микробиологическая среда в различных естественных системах, таких как почва, опавшие листья, озера, пруды и реки, океаны, производит большую часть органических сульфидов и что опубликованные наблюдения эмиссии  $H_2S$  от болотистой почвы и анаэробного ила связаны с низкими содержанием рН. Повидимому биогенное производство газовых сернистых компонентов составляет большую часть поступлений в сернистый цикл. К сожалению, интенсивность и пространственное распределение этих источников не известны.