Volatile Sulfur Compounds Produced by Methionine Degrading Bacteria and the Relationship to Concrete Corrosion

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Pseudomonas fluorescens, *Proteus vulgaris*, and *Serratia marcescens*, members of the microflora of soil and waste water, attacked methionine in the presence of glucose. The sulfur of methionine was released as methane thiol, dimethyl sulfide and dimethyl disulfide. The volatile sulfur compounds were qualitatively and quantitatively investigated by gas chromatography. Dimethyl disulfide was formed of methane thiol by various bacteria to a different extent. Growing in the presence of oxygen, *S. marcescens* oxidized most of the methane thiol to dimethyl disulfide. In the presence of glucose, *P. fluorescens* dissimilated methionine with production of methane thiol and dimethyl disulfide. The dissimilation was stimulated with decreasing glucose concentration.

1. Introduction

Methionine is decomposed by various microorganisms with production of methane thiol and dimethyl disulfide [1–9]. These volatile sulfur compounds were detected reproducibly in the atmosphere of Hamburg's sewage systems [10], where the surface of the sewer pipes above the waste water level showed severe concrete corrosion. The corrosion was caused by sulfuric acid produced by sulfur oxidizing thiobacilli [11–16]. It is not known, whether thiobiacilli utilize volatile sulfur compounds produced by decomposition of methionine. As reported by Sivelä and Sundman [15] unidentified strains of thiobacilli were able to grow with dimethyl sulfide and dimethyl disulfide as the only energy source. On the other hand in the presence of oxygen methane thiol is oxidized to dimethyl disulfide and higher polysulfides. These compounds might release elemental sulfur, which could be utilized by thiobacilli. In this case these compounds would be important in addition to hydrogen sulfide for microbial sulfuric acid production and concrete corrosion.

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This report is concerned with the production of volatile sulfur compounds at dissimilation of methionine by methionine decomposing bacteria.

2. Results

Influence of oxygen on the production of volatile sulfur compounds

P. fluorescens, *P. vulgaris*, *S. marcescens* and a population of microorganisms from waste water (Stellinger Moor, Hamburg) were incubated for 24 h in septum-sealed flasks with methionine medium under different gas atmosphere (oxygen, air, argon).

The headspace over the cultures was investigated gas chromatographically for hydrogen sulfide, methane thiol, dimethyl sulfide, and dimethyl disulfide. In relation to bacterial growth the contents of the volatile sulfur compounds were determined and expressed as µg/g cell protein. The detection limit was in the range of 30 ppb.

Pseudomonas fluorescens

Fig. 1 a shows the production of volatile sulfur compounds by *P. fluorescens* under different gas atmospheres. Growing under oxygen, air, and argon *P. fluorescens* degraded methionine with production of methane thiol, dimethyl sulfide, and dimethyl

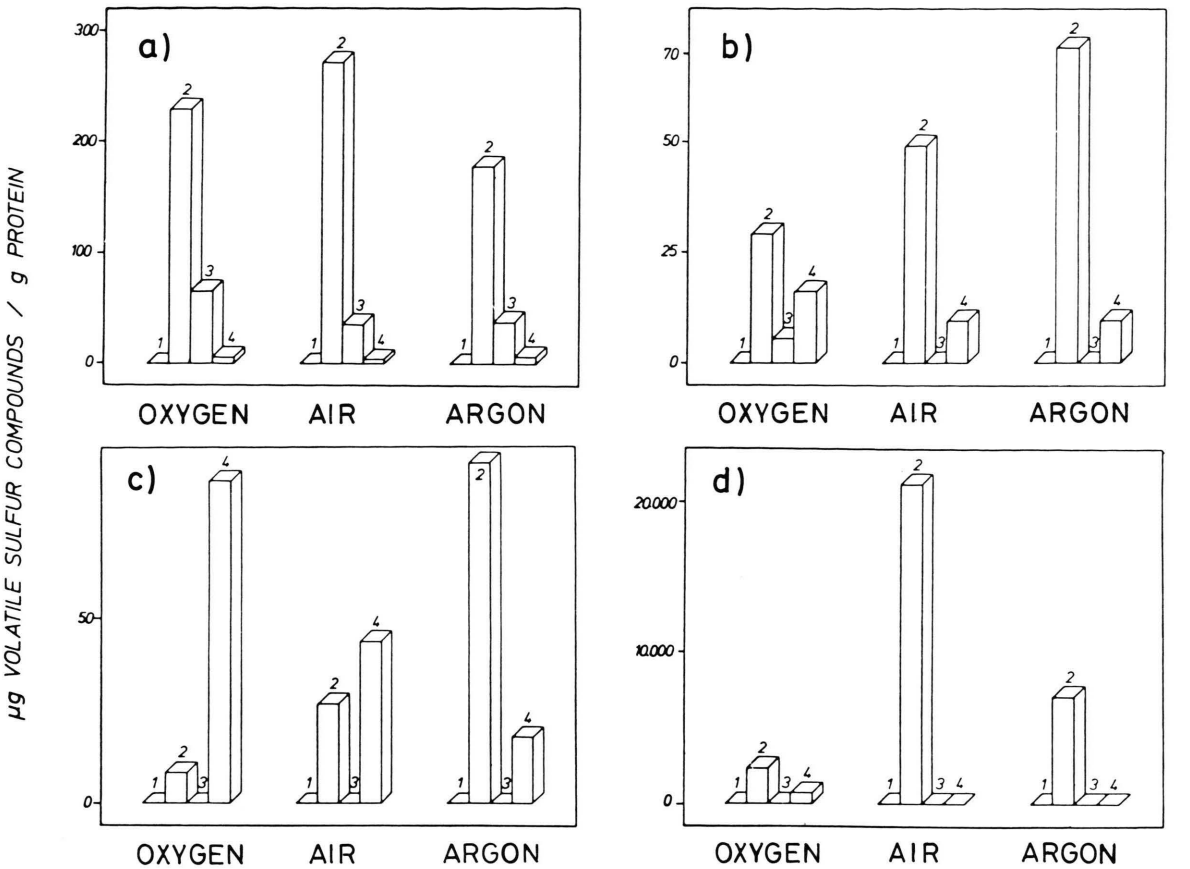

Fig. 1. Release of volatile sulfur compounds from methionine by a) *Pseudomonas fluorescens*, b) *Proteus vulgaris*, c) *Serratia marcescens*, and d) microorganisms from waste water under oxygen (left side), air (center), and argon (right side). 1 = hydrogen sulfide, 2 = methane thiol, 3 = dimethyl sulfide, 4 = dimethyl disulfide.

disulfide of nearly the same concentration. Hydrogen sulfide was not formed.

Proteus vulgaris

With *P. vulgaris* methane thiol and dimethyl disulfide were the only volatile sulfur compounds produced under an atmosphere of argon and air (Fig. 1 b). Cells grown under oxygen also formed dimethyl sulfide. Fig. 1 b shows the increase of methane thiol production from aerobic to anaerobic conditions, whereas the production of dimethyl disulfide was highest under aerobic conditions.

Serratia marcescens

Growing on methionine medium *S. marcescens* produced methane thiol and dimethyl disulfide as the only volatile sulfur compounds (Fig. 1 c). In contrast to *P. fluorescens* and *P. vulgaris* the extent of dimethyl disulfide production was high under oxygen as well as under air. Under oxygen almost all methane thiol was oxidized to dimethyl disulfide, showing that this oxidation is not only a pure chemical process.

Microorganisms from waste water

The results from the experiments with waste water organisms are in contrast to those with *P. fluorescens*, *P. vulgaris*, and *S. marcescens*. Growing on methionine medium the waste water microorganisms produced high amounts of methane thiol but no dimethyl disulfide in the presence of air (Fig. 1 d).

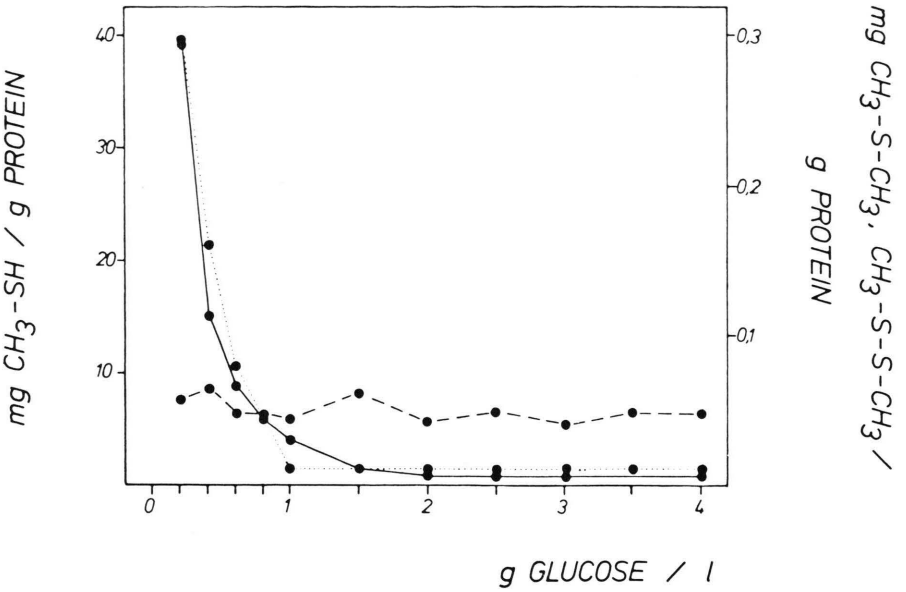

Fig. 2. Dependence of the production of volatile sulfur compounds from the glucose concentration in the culture medium (—— ≡ methane thiol, ···· ≡ dimethyl disulfide, ---- ≡ dimethyl sulfide).

Dissimilation of methionine at different glucose concentrations

P. fluorescens was grown on glucose in the presence of methionine. The glucose concentration before inoculation was varied in the range from 0.2–4.0 g/l. Fig. 2 shows the increase of methane thiol and dimethyl disulfide concentration with decreasing glucose concentrations.

Discussion

When glucose was provided methionine was degraded by various microorganisms by production of methane thiol [1, 6]. The capability to oxidize methane thiol to dimethyl disulfide differed with various organisms. In the presence of oxygen *S. marcescens* oxidized nearly all methane thiol while *P. fluorescens* oxidized only parts of it. We suggest that this oxidation is a detoxification of methane thiol and no energy generating process.

Dimethyl sulfide was mainly produced by *P. fluorescens*. We suppose that *P. fluorescens* is able to methylate methionine by production of methyl methionine [18]. The demethiolation of methyl methionine would lead to dimethyl sulfide [18]. Kadota and Ishida [1] reported that the release of methane thiol from methionine increased under anaerobic conditions. These results are in accordance with the results obtained with *P. vulgaris*. When methionine is degraded oxidation products (2-oxomethionine, 2-oxobutyric acid) are formed [6]. It might be possible that these products and their tautomers could be used under anaerobic conditions as electron acceptors in transport-mediated phosphorylation [19].

The degradation of methionine was noted as an example of codissimilation [3] when glucose was provided, methionine was deaminated and demethiolated. *P. fluorescens* increased demethiolation with decreasing glucose concentration. So evidence is provided that *P. fluorescens* has developed a regulatory mechanism to dissimilate methionine in the presence of glucose.

When methionine is degraded methane thiol, dimethyl sulfide, and dimethyl disulfide are produced, while the dissimilation of cysteine only gave hydrogen sulfide [5]. The oxidation of hydrogen sulfide to elemental sulfur and its consecutive microbial oxidation to sulfuric acid was reported to be the reason for concrete corrosion in sewer systems [11, 13]. It is conceivable that the volatile sulfur compounds produced by decomposition of methionine contribute to the release of elemental sulfur. This would mean that an increase in the protein content of the waste water would aggrevate concrete corrosion.

Experimental

Media and cultural conditions

P. fluorescens, *P. vulgaris*, and *S. marcescens* were cultivated on methionine medium containing 1.0 g L-methionine, 4.0 g glucose, 0.25 g $MgSO_4 \cdot 7\,H_2O$, 0.02 g $CaCl_2 \cdot 2\,H_2O$, 7.0 g $Na_2HPO_4 \cdot 2\,H_2O$, 3.0 g KH_2PO_4, 0.5 g NaCl, 1.0 g NH_4Cl. The volume was brought to 1 liter with water and the final pH was 6.9. 100 ml septum-sealed flasks were filled with 40 ml liquid medium and shaken during incubation at 28 °C. After 24 h, when the cells were in the stationary phase, the gas volume of the flasks was analyzed gas chromatographically with the headspace-technique.

Analytical procedures

The protein content of whole cells was determined by the method of Bradford [20] using bovine serum albumin as a standard.

Volatile sulfur compounds were analyzed gas chromatographically with the headspace-technique. Gas samples taken from glass flasks through a silicon rubber septum with a gas tight syringe were injected into a Carlo Erba series 2300 gas chromatograph. It was equipped with a 2.5 m glass column (4 mm i.d.) packed with graphitized carbon (Carbopack B-HT-100, 40–60 mesh, Supelco Inc., Bellefonte, USA) [21]. A flame photometric detector [22] Carlo Erba SSD Control Mod. 250 was used for detection of sulfur compounds and nitrogen served as carrier gas at a flow rate of 125 ml/min. The gas chromatograph was run isothermally at 60 °C and 130 °C. A Shimadzu C-R1A integrator was used for peak integration.

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