Effect of glutaraldehyde on sulphide production and sulphate reduction by sulphate-reducing bacteria

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Glutaraldehyde at low concentrations (0.5–1.0 mM) inhibited the production of sulphide from sulphate without affecting the growth of sulphate-reducing bacteria. With higher concentrations (25–50 mM), growth was also inhibited.

SULPHATE-REDUCING bacteria have been implicated in numerous instances of damage[1]. One of the most important of these situations is the corrosion of buried pipelines. Sulphate is a common component in industrial and municipal waste waters. Under anaerobic conditions, sulphate is reduced to sulphide by sulphate-reducing bacteria (SRB). Several approaches for alleviating the problems associated with sulphate reduction have been proposed. The known compounds which interfere with sulphate reduction to H_2S are all analogues of SO_4, including monofluorophosphate and group VI oxyanions[2, 3, 4]. Other than SO_4 analogues, transition metals[5], antibiotics[6], bacteriocides[7, 8], etc., have been shown to inhibit selectively SO_4 reduction. Our preliminary studies indicated that formaldehyde and glutaraldehyde affected sulphide production[9]. In this study we report the inhibitory effect of glutaraldehyde on growth and production of sulphide by SRB.

Sewage and biofilm samples from two corroded sites upstream and downstream were collected in bottles outgassed with N_2. For enumeration isolation and inhibition studies of SRB, the medium described by Sleat *et al.* was used[10], with Na lactate as the carbon source. All medium preparations were done under strict anaerobic conditions using the method of Hungate[11] as modified by Bryant[12]. Axenic cultures were isolated by agar shake method[13].

Inhibitors studies were carried out in 20 ml culture tubes under anaerobic conditions, with head space flushed with $N_2 : CO_2$ (70 : 30). Tubes were sealed with butyl rubber stoppers and crimped in place with aluminium rings. Sulphate was determined by turbidimetric method after precipitation with $BaCl_2$, measuring at 420 nm[14]. Sulphide was measured by the methylene blue method[15]. Glutaraldehyde at concentrations of 0.25, 0.50, 0.75 and 1.0 mM was used for fresh samples and axenic cultures. For enrichment cultures (isolated by picking up a black colony from agar roll tubes and transferring it to a similar medium), glutaraldehyde at concentrations of 12.5, 25.0, 37.5 and 50 mM was added. Growth was determined using biomass A_{600} developed by the method described by Koch[16] using which a correlation was established between a particular value of biomass (mg dry/l) and the corresponding absorbance value. All absorbance studies were made using a Perkin–Elmer spectrophotometer junior model 35.

When fresh samples were used, there was no

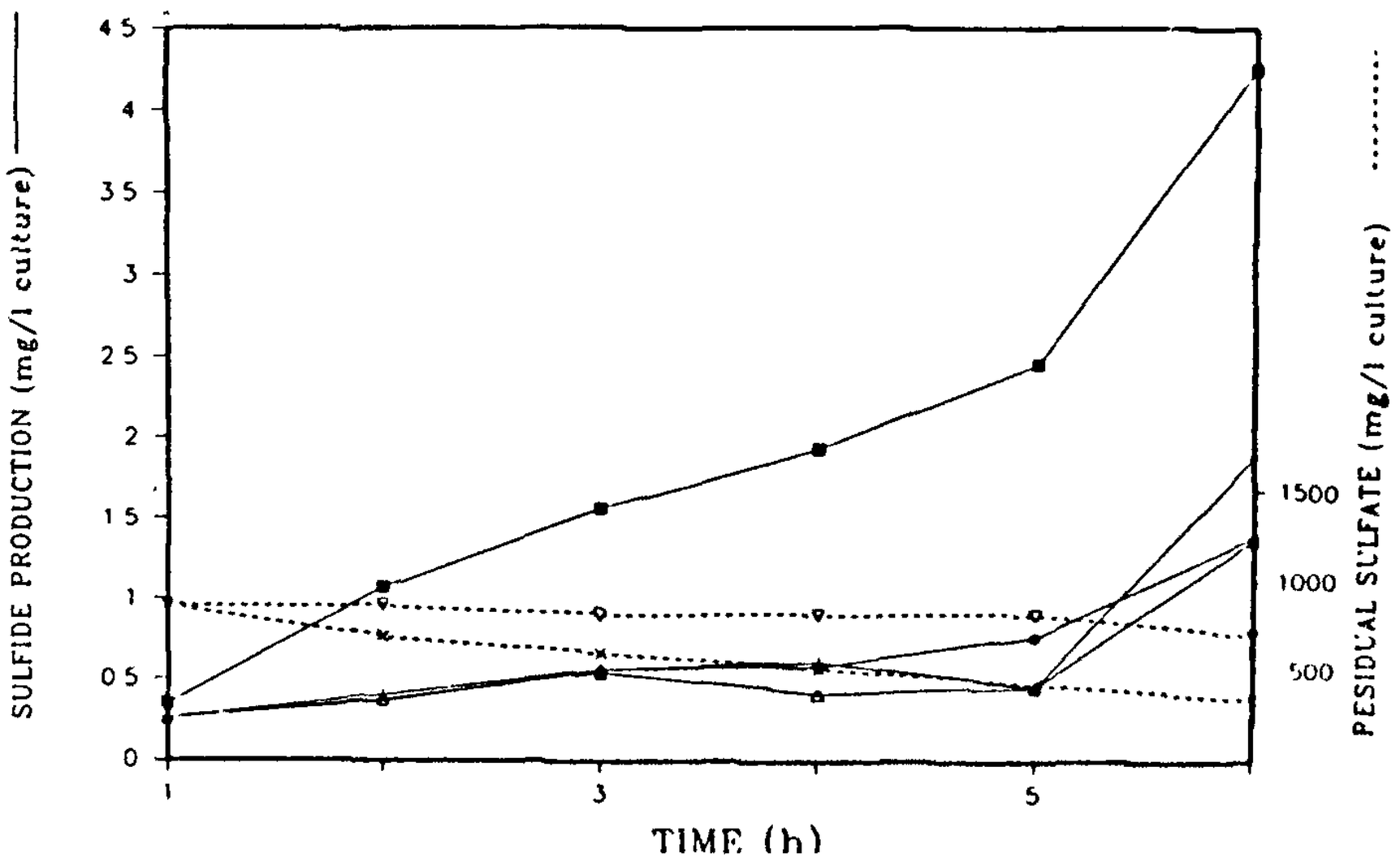

Figure 1. Reduction in sulphide levels by glutaraldehyde added to fresh downstream sewage samples with the substrate. Sulphide levels with (■) 0 (+) 0 5 (◊) 0 75 (Δ) 1 0 mM of glutaraldehyde added. Sulphate at 0 time (×) and 6 *h* (V) after the addition of 1 mM glutaraldehyde

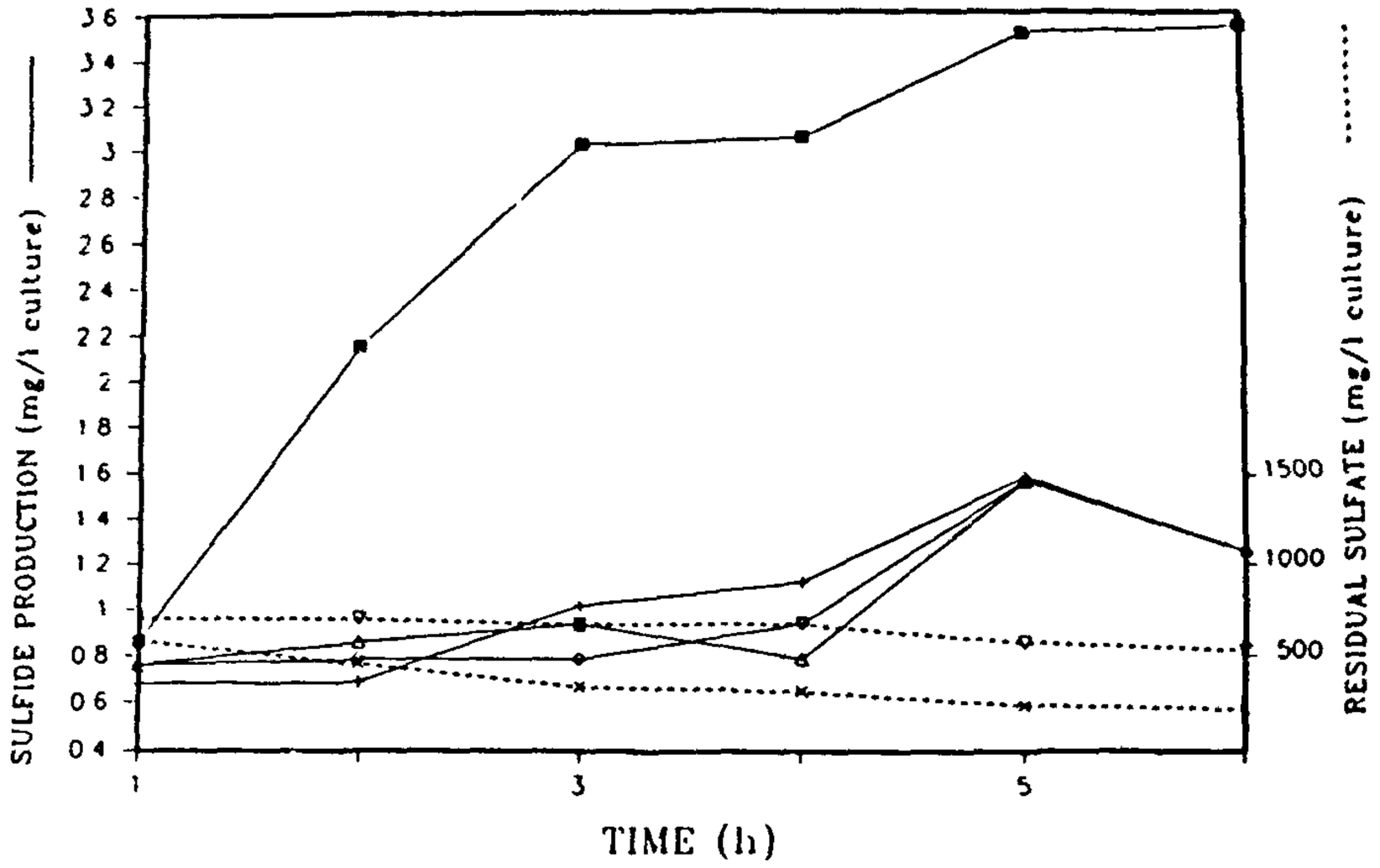

Figure 2. Reduction in sulphide levels by glutaraldehyde added to fresh upstream sewage sample with the substrate. Sulphide levels with (■) 0 : (+) 0.5 (◊) 0 75 : (Δ) 1 0 mM of glutaraldehyde added Sulphate at 0 time (×) and 6 h (∇) after the addition of 1 mM glutaraldehyde.

measurable amount of sulphide produced in 6 h even in controls, and hence further studies were carried out with a concentrated medium which did not alter the composition and proportion of the organisms present.

The results are given in Figures 1 and 2. Concentrations of 0.5–1.0 mM of glutaraldehyde reduced sulphide production by 70% compared to controls in 4 h. Inhibitor studies with enrichment and axenic cultures were carried out 24 h after the cultures were inoculated and incubated. Glutaraldehyde inhibited sulphide production after 3 h by 66% at all concentrations tested (12.5–50 mM). Table 1 gives the effect of glutaraldehyde on enrichment culture from downstream sewage. At the end of 24 h, the quantity of detectable sulphide was reduced by 90% compared to control and after 48 h none was detected. At the same time, growth was inhibited by about 70% while only 14% of the sulphate was reduced. Similar results were obtained with enrichment studies with the other samples.

Axenic cultures isolated from the sewage and biofilm samples were inoculated into the medium containing lactate as the carbon and energy source and incubated for 24 h prior to the addition of glutaraldehyde. Measurements were made for growth, sulphide and sulphate just after the addition of aldehyde and after 3 and 6 h of incubation following the introduction of aldehyde. After the addition of aldehyde, growth continued as sulphide decreased, while there was no change in sulphate levels. The results shown in (Table 2) on axenic cultures isolated from upstream sewage and biofilm samples indicate that any sulphide already present in the system was bound to glutaraldehyde added. No significant quantity of sulphide appeared to be formed at the expense of sulphate reduction since essentially no sulphate disappeared from the medium. At the same time, the growth continued. The growth was higher than or similar to that of the controls. Similar results were obtained for axenic cultures obtained from downstream samples.

To examine the effect of a strictly chemical non-biological reaction of aldehyde on binding sulphide in our experiments, we tested the removal of sulphide from sterilized, pregrown culture fluid. The axenic cultures grown on medium were sterilized by autoclaving and used as a basal medium for addition of glutaraldehyde at the same concentrations as those used for inhibitor studies. The samples were incubated for 6 h at 37°C and sulphide was estimated at 6 h. The results (not shown) support the conclusion that glutaraldehyde does indeed bind sulphides.

The mechanism of the binding between glutaraldehyde and sulphide is not known. Aldehydes are known to bind to sulphides stoichiometrically[17–19] and to sulphites[20]. Our results with axenic cultures show that there is a binding between the two, and two moles of sulphide combine with one mole of glutaraldehyde stoichiometrically. Growth was not affected and when incubation was prolonged for 24 h with glutaraldehyde (0.25–1.0 mM), sulphate reduction was resumed, indicating that probably enzyme activity was not affected by glutaraldehyde. Thus, our report indicates that glutaral-

Table 1. Biomass, sulphate and sulphide levels in enrichment culture from downstream sewage sample. Glutaraldehyde was added after 24 h of growth in regular medium

Time ⟶	0 h			3 h			24 h		
	CM*	SO_4 (mM)	$S^=$ (mM)	CM	SO_4	$S^=$	CM	SO_4	$S^=$
Control	35	8 5	2.5	45	7 0	3 0	144	1 5	7.5
Glutaraldehyde (mM)									
12.5	35	8 5	2.5	49	8 5	1 6	56	8.5	0 4
25 0	35	8 5	2 5	49	8 5	1.5	56	8 5	0 2
37 5	35	8 5	2 5	49	8 5	1 4	49	8.5	0 16
50 0	35	8 5	2 5	49	8 5	1.16	49	8 5	0 03

*Cell mass mg dry/l.

Table 2. Biomas, sulphate and sulphide levels in axenic cultures isolated from upstream sewage and biofilm samples Glutaraldehyde was added after 24 h of growth in medium

Time ⟶	0 h			3 h			6 h		
	CM*	SO_4 (mM)	$S^=$ (mM)	CM	SO_4	$S^=$	CM	SO_4	$S^=$
Sewage									
Control	70	5 8	4.2	77	4 4	5 6	98	4.2	5 8
Glutaraldehyde (mM)									
0 25	70	5.8	4.2	87	5 8	3.4	120	5 8	2.9
0 50	70	5.8	4 2	87	5.8	3.7	120	5 8	2.6
0 75	70	5 8	4 2	87	5 8	3 7	132	5 8	2 6
1 00	70	5 8	4.2	87	5 8	3 7	132	5 8	2 6
Biofilm									
Control	70	5.8	4 2	80	4 8	5.2	100	3 2	6.8
Glutaraldehyde (mM)									
0 25	70	5 8	4 2	90	5.8	3 6	123	5.8	2.6
0 50	70	5 8	4 2	90	5 8	3 6	123	5 8	2 6
0.75	70	5.8	4.2	90	5 8	3 6	130	5 8	2 6
1 00	70	5 8	4 2	90	5 8	3.6	130	5 8	2 2

*Cell mass mg dry/l

dehyde may be used as an effective inhibitor of sulphide production without affecting the growth. Glutaraldehyde at 2% concentration (0.2 M) is used as a bacteriocide; however, at lower concentrations it does not affect the growth of SRB.

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Radiocarbon dates of sediment cores from inner continental shelf off Tainga-patnam, southwest coast of India

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Radiocarbon dating of carbonized wood samples from three sediment cores from the inner continental shelf off Taingapatnam, in the southwestern coast of India, indicates ages in the bracket 8400–9400 YBP. These radiometric ages correlate well with the ages of carbonized wood from inner continental shelf off Ponnani (Kerala) and Karwar (Karnataka). The occurrence of carbonized wood in widely spread offshore areas probably represents a regional transgressive event in the west coast which resulted in submergence and destruction of coastal mangroves.

The rate of sedimentation in the study area varies between 0.12 and 0.37 mm/yr, much lower than those reported from shelf areas north of Mangalore. The slow accumulation of sediments in the southern parts of the western continental shelf of India, as exemplified from the present study, may be due to very poor discharge and low bed load sediments of the west-flowing small rivers of this part of the peninsula and low concentration of suspended particulate matter in them.

THOUGH a large number of radiocarbon dates are available for the outer continental shelf sediments, particularly