Reducing microzones and sulfide production in marine snow

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ABSTRACT: Using tetrazolium salts we tested the hypothesis that reducing microzones can form within detrital aggregates (i.e. marine snow). Even when the ambient waters were well oxygenated, patches of reduced tetrazolium salts were found in marine snow indicating that strongly reducing microzones were present in these aggregates. Further, in both laboratory-made and field-collected marine snow we found measurable amounts of sulfide. Sulfide was present in marine snow on 9 out of 10 sample dates and the sulfide concentration within marine snow ranged from 1.3 to 25 μmol S l^{-1}. On only 1 out of 10 dates was sulfide detected in the oxygenated waters surrounding the aggregates. The sulfide in the marine snow was probably produced by anaerobic microbes in the reducing microzones in the marine snow. Our data suggest that the paradoxical production of sulfide in aerobic water columns is probably due to production within anoxic microzones within marine snow.

INTRODUCTION

Microbial production of methane and sulfide only occurs in anoxic environments. Paradoxically, in the oxygenated waters of the ocean microbes produce both chemicals (Brooks et al. 1981, Burke et al. 1983, Cutter & Krahforst 1988). How can these microbial reactions that are dependent on a reducing environment occur in oxygenated waters? This paradox might be resolved if anoxic microzones form in detrital aggregates suspended in the aerobic water column.

Macroscopic detrital aggregates (marine snow) are ubiquitous in the world's oceans (Alldredge & Silver 1988). Alldredge & Cohen (1987), using oxygen microelectrodes, found that the oxygen concentration in marine snow in the dark could drop as low as 46 % below the surrounding ambient oxygen concentration. Alldredge & Cohen (1987) did not, however, find anoxic zones in the aggregates they investigated.

They did not observe anoxic conditions, perhaps, because their oxygen measurements were made at 100 μm intervals and in seawater with a high ambient oxygen concentration. Researchers working in benthic environments have frequently found anoxic microzones scattered throughout aerobic sediments (Jørgensen

1977). Similar microzones might form in marine snow. Because of the potentially small size of the anoxic zones, the sampling regime of Alldredge & Cohen (1987) may have inadvertently missed anoxic microzones.

Near the ocean's surface, marine snow is suspended in water with a high ambient oxygen concentration, but as the aggregates sink they can enter deeper waters with quite low oxygen concentrations (e.g. < 1 mg l^{-1}), the oxygen minimum layer (Sverdrup et al. 1942). In the oxygen minimum layer, because of the lower ambient oxygen concentration, there will be a reduced flux of oxygen into aggregates. Under these conditions the microbial community in the marine snow might be able to reduce the oxygen concentration in an aggregate to zero. We used laboratory-made artificial marine snow and tetrazolium salts to investigate the hypothesis that reducing microzones can form in aggregates.

METHODS

Tetrazolium salts are water-soluble under oxidized conditions, but form insoluble colored formazan crystals when reduced (Paerl 1984). We used the tetra-

zolium salts INT [2-(4-iodophenyl)-3-(4-nitrophenyl)
-5-phenyltetrazolium chloride] and TV (3-naphthyl-
2,5-diphenyl-2H-tetrazolium chloride), which are re-
duced at an Eh of +50 and –290 mV respectively.

Laboratory-made artificial marine snow was gener-
ated following the methods of Shanks & Edmondson
(1989). Cylindrical tanks (14 cm diameter × 6.5 cm
deep) of unfiltered seawater from Charleston Harbor
Estuary, South Carolina, USA (79°55' W, 32°45' N),
were rotated on a roller table until the particles in the
seawater formed aggregates (about 0.5 to 1.5 h). A pre-
vious study demonstrated that these artificial aggre-
gates are a good biological model of natural marine
snow (Shanks & Edmondson 1989). The marine snow
was formed and maintained in the laboratory at room
temperature (20 °C) and under room lights. Before
sampling marine snow a photograph was taken of each
tank while it was rolling on the roller table. These pho-
tographs were used to determine the size and abun-
dance of aggregates in the tanks. Prior to the formation
of marine snow, nitrogen gas was bubbled through
some water samples to reduce the ambient oxygen
concentration.

Experiments with the tetrazolium salts began in
October 1991. One or two ml of a 1 % solution
(weight/volume) of INT and/or TV was added to each
tank. The dyes were allowed to react with the marine
snow for anywhere from 3 to 24 h. At intervals (one to
several hours) after the addition of the dye, samples of
marine snow (aggregates of 1 to 5 mm diameter) were
removed from the tanks with a pipette, placed on a
slide, and inspected under a compound microscope.

Given that reducing microzones may be present in
aggregates, we investigated the possibility that the
byproducts of anaerobic metabolism were present in
aggregates. We tested this hypothesis by measuring
the concentration of sulfide in marine snow made in
the laboratory and that collected in the field. We also
measured the concentration of sulfide in the water
surrounding the marine snow. These experiments
were done in April and May 1992.

Artificial marine snow was made in the laboratory
and sampled when it was about 10 h old. Using a 3 ml
syringe inserted through a serum cap, 10 to 20 aggre-
gates were collected from each rotating tank. About 30
ml of water surrounding the marine snow was sampled
with a 50 ml syringe. On 13 and 21 April, surrounding
water samples were collected as the tanks rotated on
the roller table. It was impossible to avoid inadver-
tently collecting some aggregates during this sampling
procedure. Subsequently, the tanks were removed
from the roller table and allowed to stand until the ma-
rine snow had sunk to the bottom of the tank (this took
about 5 min) at which time the surrounding water was
sampled. Marine snow and surrounding water samples

were immediately injected with the reagents used in
the sulfide analysis.

Field sampling of marine snow took place in the
waters around the San Juan Islands, Washington, USA
(48°40' N, 123°0' W), in August 1992. In the field, pho-
tographs were taken to determine the average size and
abundance of marine snow (Shanks & Edmondson
1990). Five replicate samples of about 30 ml of water
surrounding marine snow and 30 ml of marine snow
(100 aggregates per sample) were collected in syringes
(Shanks & Edmondson 1990) at about 8 m depth.
Approximately 1 h elapsed between sample collection
and the addition of reagents.

The sulfide analysis used was a modification of
Cline's (1969). This technique for measuring sulfide in
seawater uses a 10 cm path-length cell and is consid-
ered to be accurate down to a concentration of 1 μmol
$S\ l^{-1}$. We found, however, that with care we could use
a 1 cm path-length cell and that the useful range of this
technique is down to a concentration of 0.2 μmol $S\ l^{-1}$.
Between 0.2 and 3.0 μmol $S\ l^{-1}$, we found a linear rela-
tionship between the concentration of sulfide and ab-
sorbance (n = 7, r = 0.9978, p < 0.01). Marine snow and
surrounding water samples were analyzed using 1 and
10 cm path-length cells respectively. All samples were
filtered through 0.45 μm Millipore filters prior to read-
ing on the spectrophotometer.

RESULTS

Over a wide range of ambient oxygen concentrations
(0.6 to 10.6 mg $O_2\ l^{-1}$) the tetrazolium salts INT and TV
were reduced in the laboratory-made marine snow
(Table 1). Several hours after the addition of tetra-
zolium salts formazan crystals formed within many, but
not all, of the aggregates in a tank. Reduced tetra-
zolium salts were usually found in tiny patches (i.e. 100
to 300 μm diameter) scattered throughout the aggre-
gates. Often the patches of reduced dye were inside
pieces of detritus, especially diatom frustules.

INT is reduced at a lower Eh than TV (Paerl 1984).
INT tended to be reduced more quickly and produced
larger patches of formazan crystals than TV. Patches of
reduced TV tended to be more frequently found inside
detrital particles. As anticipated, reduced patches of
INT and TV tended to be more abundant in marine
snow in tanks with low ambient oxygen concentra-
tions. These observations indicate that highly reducing
and probably anoxic microzones were present in
laboratory-made marine snow.

Throughout the observations of sulfide in laboratory-
made marine snow, oxygen did not drop below 6.8 mg
$O_2\ l^{-1}$ S (Table 2). Sulfide was detectable in most of the
marine snow samples (Table 2), but decreased steadily

Table 1. Results of experiments with the tetrazolium salts INT and TV. Salts were added to tanks of seawater in which artificial marine snow was suspended. INT and TV: time from the addition of the dye until a reaction was observed. NR: no reaction

Date	Age of aggregate (h)	Oxygen (mg l^{-1})	INT (h)	TV (h)
2 Oct 1991	24	0.9	2	2
4 Oct 1991	72	6.8	NR	NR
		2.4	1	1
		0.6	1	1
23 Oct 1991	24	7	3	3
		1.5	24	24
		0.7	24	24
31 Oct 1991	3	7.6	NR	NR
		1.1	NR	NR
		0.7	2	2
4 Feb 1992	24	10.6	5	5
		2.4	3	3
		1.4	3	3
2 Mar 1992	24	10.8	5	–
		2.3	5	–
		0.6	5	–
22 July 1992	24	8.1	–	3
21 Aug 1992	24	7.5	3	3

over the period of the observations. On the first 4 sampling dates (Table 2) the sulfide concentration in the marine snow samples were above the detection limit of the technique. On the last laboratory sampling date (27 May) sulfide was not detectable. On 12 and 15 May sulfide was detectable (readings were well above the reagent and turbidity blanks), but concentrations were slightly below the usable range of the modified sulfide technique (0.2 μm S l^{-1}). Sulfide concentrations in these samples were estimated by assuming that the linear relationship between absorbance and concentration held at concentration below the usable range of the modified technique.

In the laboratory experiments, sulfide was only detected in the surrounding water samples from 21 April, a date on which the surrounding water samples were contaminated by marine snow (see 'Methods'). Except for these surrounding water samples, all of the sulfide detected in the experimental tanks was in the aggregates.

Sulfide was not present in any of the field-collected surrounding water samples, but was present in the samples of marine snow (Table 2). On both dates sulfide was detectable (readings

Table 2. Results of observations on sulfide sulfur in marine snow. Laboratory observations were made on artificial marine snow maintained in tanks rolling on a roller table. Marine snow in the tanks were globular in shape; their diameter is reported, and volume was calculated assuming they were spheres. Field observations were made on marine snow collected in the San Juan Islands, Washington, USA. Marine snow sampled in the field tended to be long and stringy; both their diameter and length are reported, and volume was calculated assuming they were cylinders. Values are means (SD); 5 replicate marine snow and surrounding water samples were collected on each date. Agg.: aggregate; Surr. water: surrounding water

Date	Oxygen (mg l^{-1})	Agg. size (mm) Diameter	Length	Agg. volume (ml)	Density (no. l^{-1})	Sulfide sulfur conc. Surr. water (μmol S l^{-1})	In agg.[a] (μmol S l^{-1})	Total[b] (nmol S l^{-1})
Laboratory observations								
13 Apr	6.9 (0.7)	>1		>0.0005	–	0	25 (0.1)	–
21 Apr	8.6 (0.2)	1.6 (0.1)		0.003 (0.0006)	240 (80)	0[c]	21 (9.8)	16 (4.6)
28 Apr	8.5 (0.3)	1.6 (0.2)		0.003 (0.001)	78.4 (21.1)	0	21 (13)	5.1 (2.9)
30 Apr	7.9 (0.1)	1.5 (0.1)		0.002 (0.0003)	75.1 (21.3)	0	9.6 (8.2)	1.5 (1.1)
1 May	7.4 (0.2)	1.4 (0.0)		0.002 (0.0002)	151 (44.8)	0	4.2 (3.5)	1.4 (1.5)
12 May	7.6 (0.2)	1.6 (0.2)		0.007 (0.0057)	93.2 (36.4)	0	1.5[d] (2.0)	0.1 (0.1)
15 May	6.8 (0.2)	1.9 (0.2)		0.005 (0.002)	358 (102)	0	1.3[d] (0.9)	1.9 (1.4)
27 May	7.4 (0.4)	1.8 (0.2)		0.006 (0.0028)	72.8 (13.8)	0	0	0
Field observations								
6 Aug	8.9 (0.1)	0.7 (0.4)	4.1 (2.9)	0.009 (0.02)	2.6 (1.7)	0	3.7[d]	0.1
10 Aug	8.8 (0.2)	0.9 (0.3)	4.3 (1.7)	0.071 (0.13)	29.6 (14.9)	0	18[d]	0.5

[a] Calculated as: [(average μmol S l^{-1} in agg. sample)/(no. agg. in sample)]/[volume of average agg. (l)]
[b] Calculated as: (surrounding water μmol S l^{-1}) + (μmol S agg.$^{-1}$ × no. agg. l^{-1})
[c] Samples contaminated with marine snow
[d] Sulfide was present, but the concentration was slightly below the range of the technique used to measure sulfide (0.2 μmol l^{-1}). Sulfide was estimated in these samples by assuming that the linear relationship between sulfide concentration and absorbance held at sulfide concentrations below 0.2 μmol S l^{-1}

were well above the reagent and turbidity blanks), but concentrations were slightly below the usable range of the modified sulfide technique (0.2 μm S l^{-1}). Sulfide concentrations in these samples were estimated by assuming that the linear relationship between absorbance and concentration held at concentrations below the usable range of the technique. The concentration of sulfide within the aggregates was around 3.7 and 18 μmol S l^{-1} on 6 and 10 August respectively (Table 2). Despite the high ambient oxygen concentration (almost 9 mg O_2 l^{-1}), sulfide was present in the natural marine snow.

DISCUSSION

Our results suggest that highly reducing microzones can exist in marine snow. These microzones formed even in aggregates suspended in well oxygenated waters. Sulfide was present in marine snow samples from 8 of 9 laboratory experiments and on both dates on which field samples were collected. Sulfide was detected only in those surrounding water samples which had been contaminated with marine snow. Sulfide present in the aggregates was probably the result of the metabolism of anaerobic microbes in reducing microzones in the marine snow.

We found it surprising that following the addition of the tetrazolium salts it took at least several hours before we observed formazan crystals forming within the marine snow. The aggregates in the roller tanks, like those in the field, were falling through the water. Thus, there was advection of water around as well as through the aggregates (Logan & Alldredge 1989). We expected advection to fairly rapidly deliver the tetrazolium salts to reducing zones. The long delay between the addition of the tetrazolium salts and the formation of formazan crystals suggests that the tetrazolium salts were not delivered to the reducing microzones via advection. Most of the reduced tetrazolium salts were found in microzones bounded by detritus. For example, frequently formazan crystals were found within diatom frustules. The physical structure provided by the detritus could prevent the advection and greatly slow the diffusion of material in or out of a microzone. The formation of reducing microzones within marine snow is, in fact, probably dependent upon the reduced flux of oxygen into the microzone which is caused by the physical structure of the detritus.

Initially, sulfide concentrations within the laboratory-made marine snow were around 25 μmol S l^{-1}, but by mid-May sulfide had dropped below 1.5 μmol S l^{-1} and by 27 May sulfide was undetectable. Over this period there were no systematic changes in the size, abundance, or appearance of the laboratory-made marine snow. The reason for the steady decline of sulfide in the marine snow is unknown, but may be related to the seasonal warming of the coastal waters with concomitant changes in the water column microbial population.

Knowing the number of aggregates in the tanks or per liter in the field, along with the average concentration of sulfide in the marine snow, we calculated the overall or total sulfide concentration (i.e. the amount of sulfide in a liter of water contributed by the marine snow plus the surrounding water). In the laboratory, on those days when sulfide was present in the aggregates, the total concentration of sulfide in the experimental tanks ranged from 0.1 to 16 nmol S l^{-1}. In the field samples collected around the San Juan Islands, calculated sulfide concentrations were 0.08 and 0.53 nmol S l^{-1}. Our observations suggest that all of the sulfide in the water column could be due to that present in just the marine snow.

Cutter & Krahforst (1988), employing a far more sensitive technique, measured the concentration of sulfide in bulk water samples from the western Atlantic Ocean. These samples were collected with standard oceanographic sample bottles and would have contained disrupted marine snow (Trent et al. 1978) mixed with surrounding water. The highest sulfide concentration they observed (1.1 nmol l^{-1}) was found in summer near the mouth of the Chesapeake Bay. On a cross-shelf transect offshore from Charleston, South Carolina, sulfide was highest near shore. The total sulfide concentrations in two of our laboratory samples were significantly higher (5 to 10 × higher) than the highest values reported by Cutter & Krahforst (1988), but in the remainder of our laboratory samples the total sulfide concentrations were similar in magnitude to theirs. The total sulfide concentration we calculated for the field samples were also similar to those found by Cutter & Krahforst (1988).

Much of the downward flux of particles through the ocean is via marine snow (Shanks & Trent 1980, Asper 1987, Walsh & Gardner 1992, reviewed in Fowler & Knauer 1986). Sulfide present in marine snow may form insoluble complexes with a variety of metals (e.g. CuS; Dyrssen 1988). Complexed metals adsorbed onto the marine snow can be rapidly transported downward with the sinking aggregate (Cowen & Silver 1984). We predict that the removal of dissolved metals from the water column via this mechanism would be highest where marine snow is abundant (e.g. the surface waters or within an estuary) and/or where marine snow is producing an abundance of sulfide (e.g. perhaps within the oxygen minimum layer). The concentration of some metals dissolved in seawater may be controlled by the chemical reactions occurring in marine snow.

The presence of reducing microzones apparently capable of producing sulfide suggests that there were communities of anaerobic microbes within the marine snow we sampled. We predict that fermenters and sulfate-reducers will both be found in marine snow. When the ambient oxygen concentration in the water column is low (e.g. within the oxygen minimum layer) the reduced flux of oxygen into aggregates may lead to the formation of microzones within the aggregates which can support methanogens. Methane produced in the water column may originate in marine snow.

Inside an aggregate, reducing microzones were found immediately adjacent to oxic zones. The byproducts of anaerobic decomposition (CH_4, H_2S, H_2, etc.) produced in the microzone could diffuse into the surrounding oxic zones. The energy from the oxidation of these byproducts may fuel bacterial chemolithotrophy. Karl et al. (1984) observed bacterial chemolithotrophy associated with sinking particles. They suggested that the chemolithotrophy might have occurred within large particles. The data presented here suggest that these hypothesized large particles may have been marine snow.

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