Study of the Little Trunk Microbial Mat Community

Microbial Diversity 1999

Tracy B. Norris
University of Oregon
Institute of Molecular Biology
Eugene, OR 97403

Abstract

In this project the microorganisms of a microbial mat community were studied. A mat sample from Trunk River was cultivated in an aquarium setting that attempted to mimmick the environmental conditions, particularly those of sulfide concentration and water flow. The mat was dominated by colorless sulfur oxidizing bacteria and several species of cyanobacteria. Attempts were made to cultivate and identify a marine species of the filamentous sulfur oxidizing bacteria Thiothrix. Several species of cyanobacteria were also cultivated and their ability to carry out anoxygenic photosynthesis was tested. Molecular techniques were used to identify these and other species in the mat.

Introduction

A combination of factors make the Trunk River outlet of Oyster Pond (Woods Hole, MA) an interesting site for the study of microorganisms. The Trunk River connects Oyster Pond with the ocean (Figure 1). Seasonal high tides provide an influx of salt water, while at low tides fresh water flows to the ocean. The water column near the river outlet is stratified, having the salinity of freshwater at the surface and becoming closer to that of marine water near the sediment. The concentration of sulfide in the water at this site is very high. I recorded concentrations of up to 9 μM near the sediment. In fact, we observed a unusual phenomena when walking in this area that we attribute to the prevalence of high sulfide; Any skin exposed to the lower depths would turn bright red for a period of at least 15 minutes after leaving the water. We have named this affliction "Trunk Foot" and hypothesize that the sulfide may be crossing the skin and binding hemoglobin.

The original motivation for this work was to search for a marine form of the sulfide oxidizing bacteria Thiothrix. The combination of high concentrations of

sulfide and flowing water make this site a likely habitat for this filamentous bacteria. Freshwater forms of Thiothrix have been cultured (Larkin, 1980), but to date marine forms have only been described as symbionts of marine organisms and are uncultivated (Brigmon, 1998).

In addition to finding candidates for Thiothrix at this site we observed an unusual microbial mat that warranted further study (Figure 2). This mat was composed almost entirely of what looked like elemental sulfur and cyanobacteria. A sample of this mat was brought back to the lab and cultivated in an aquarium setting in such a manner to attempt to replicate the conditions of water flow and sulfide concentrations observed in the field. The goal was to try and cultivate Thiothrix in this aquarium setting. As the project progressed I expanded my studies to other microorganisms in this community as well, particularly a number of species of cyanobacteria that thrived on the high sulfide concentrations. It has been known for a number of years that some species of cyanobacteria are capable of anoxygenic photosynthesis (Castenholz, 1977; Jorgensen, 1986). In these organisms H_2S rather than H_2O is used to reduce CO_2. Microelectrode experiments on the mat and chemical inhibition of photosystem II in cultures were used to determine whether the cyanobacteria from the Trunk mat were capable of using sulfide for photosynthesis. Molecular techniques were used to try and identify the members of this mat community, particularly Thiothrix and cyanobacterial species.

Materials and Methods

Laboratory Mat Aquarium: Little Trunk

A mat sample was collected from Trunk River and placed in a Tupperware dish. A sulfide generator was constructed by filling a 10 gallon bucket with sediment and organic material from the Trunk River mat site. A hole had been

drilled near the bottom of the bucket and fitted with an outlet tube. The sulfide generator was fed from the top with seawater. At the bottom outlet the water flowed over a small stream made of a Plexiglas half-pipe filled with rocks before flowing over the mat (Figure 3). The generator was "fed" at the start and then on a weekly basis with potato starch and cellulose such that a high concentration (roughly 3 mM) of sulfide was maintained in the outflow.

Enrichments

Enrichments for cyanobacteria were set up using a standard cyanobacterial media (Kurt Hanselmann) with 2 mM sulfide in 50 % seawater. To test for anoxygenic photosynthesis enrichments were also set up with the addition of 5 or 10 μM 2,4-D or DCMU to inhibit photosystem II. The source of innoculum for the cyanobacterial enrichments was the Little Trunk Mat. Enrichments of for Thiothrix were made using agar slants or agar plugs with a low agar content overlay. The slants and plugs contained 5 mM Na_2S. The innoculum for Thiothrix came from either Trunk River or Salt Pond Inlet.

Microelectrodes

Microelectrode experiments were conducted with the assistance and expertise of Pieter Visscher. Microelectrodes were used to measure sulfide, oxygen and pH in the Little Trunk Mat.

Phylogenetic Analysis

DNA was extracted from environmental samples using the Mo Bio soil extraction kit (a bead beating method). Cyanobacteria were collected from the

"ramp" flowing into the Little Trunk Mat. Microscopy revealed that these cyanobacteria were mostly of two types, one with a "wavy" morphology and another that was a straight filament. Both appeared to be Plectonema like organisms. Also, both contained vesicles at regular intervals in the filaments. A fresh sample of Thiothrix was collected from Salt Pond Inlet attached to a red algae. Both the cyanobacteria and the Thiothrix samples were washed several times in sterile seawater prior to extraction. Thiothrix used for extraction remained attached to the algae. Universal eubacterial primers were used to amplify Thiothrix DNA samples and cyanobacterial primers were used to amplify the cyanobacterial samples. The Invitrogen TOPO TA cloning kit was used to create a clone library of PCR products. After RFLP analysis, unique sequences were sequenced on an ABI DNA sequencer.

FISH

Fluorescent In Situ Hybridization (FISH) to identify Thiothrix was done according to standard lab protocols (Scott Dawson). Thiothrix samples were the same as used for the DNA prep. Probes used for FISH were gamma-proteobacteria, delta-proteobacteria, universal and a negative control with no probe.

Results

Enrichments

Enrichments for Thiothrix were unsuccessful. Enrichments for cyanobacteria were quite successful, and several healthy cultures were grown. In the photosystem II inhibition experiments I found that 2,4-D did not inhibit growth under any conditions. The addition of DCMU inhibited growth, but not completely. Cultures with both sulfide and DCMU grew more vigorously than those with DCMU alone.

Microelectrode Experiments

Oxygen and sulfide depth profiles were constructed by measuring concentrations at 0.25 mm intervals as the probe was lowered into the mat (Figure 4). Oxygen concentration was very low in the water column (~45 μM) above the mat and decreased to zero by 1.0 cm below the mat. Sulfide concentrations decreased from 60 to 10 μM as the probe approached the mat, and then increased again rapidly after passing through the mat.

In an experiment using the oxygen microelectrode we were able to correlate the production/consumption of oxygen with oxygenic photosynthesis (Figure 5). In this experiment an intense light source was focused on the mat at time zero and oxygen concentration in the cyanobacterial layer of the mat was measured over a time course of several minutes. Initially, an increase in oxygen production was observed as the rate of photosynthesis increased. At around 300 seconds the production and consumption of oxygen reached an equilibrium as the rate of photosynthesis reached a new steady state. The light source was then turned off and the production of oxygen was observed to decrease as the rate of photosynthesis decreased.

In another experiment an attempt was made to detect anoxygenic photosynthesis in the Little Trunk Mat. If the cyanobacteria in this mat were capable of using sulfide as a reductant then in theory it should be possible to measure changes in the rate of sulfide consumption when photosynthesis is stimulated. For these experiments the flow on the sulfide generator was turned off at time zero to prevent the mat from being continuously replenished with sulfide. Sulfide concentrations were measured under various light conditions over a time course with the probe again centered in the cyanobacterial layer of the mat (Figure 6 A,B). Under high light conditions we expected to see an increased consumption of sulfide if the cyanobacteria were capable of sulfidogenic photosynthesis. The results of this experiment were less clear. There were differences in the rate of consumption

of sulfide under light and dark conditions, but without repeating the experiments it is difficult to draw any sound conclusions.

Molecular Techniques

I was successful in identifying filaments that bound to a gamma-proteobacterial probe (Figure 7 A,B). 16s rRNA sequences from other species of Thiothrix fall into this group. Sequencing of environmental samples for Thiothrix identified a red algae chloroplast. Sequencing of environmental samples for the cyanobacteria also identified a eukaryotic chloroplast sequence.

Discussion

The importance of these results is not so much in what was learned through quantitative measurements, but rather the insight gained by observing a variety microoganisms that exist together in a community. In the Trunk Mat, both in the lab and in the field, concentrations of sulfide, water flow and salinity have important consequences for the types of microorganisms that thrive. In this mat the dominant species of microorganisms seem to be colorless sulfur oxidizing bacteria and cyanobacteria that utilize (or at least tolerate) high concentrations of sulfide.

I originally focused on the cultivation of the sulfur oxidizing bacteria Thiothrix. Although enrichments for Thiothrix were not successful, the use of FISH indicates that we have likely identified a marine form of this organism. At the time of this report I was still awaiting results of further molecular sequence analysis. Another prevalent sulfur oxidizer that was observed in the Little Trunk mat was a species of Aquabacter. This bacterium was a dominant species in the Little Trunk Mat, forming large gelatinous matrices of elemental sulfur. This species has previously been identified by Carl Wirsen (1997) who found similar species in Eel pond (Woods Hole) and in hydrothermal vents.

The cyanobacteria from the Little Trunk Mat were also an interesting subject for study. We were unable to determine conclusively whether these cyanobacteria were capable of anoxygenic photosynthesis, but some of our observations indicate that they may be capable of this type of metabolism. Although it is known that cyanobacteria are capable of anoxygenic photosynthesis, the biochemistry of this process is not well studied. Even less seems to be know about what they do in the dark. Cultivation of these cyanobacteria could provide an avenue for further investigation of sulfidogenic photosynthesis with pure cultures.

References

Brigmon, R.L., and Ridder, C. (1998), *Appl. and Env. Micro.,* Vol. 64 (9), 3491-3495.

Castenholz, R.W. (1977), *Microbial Ecology*, Vol. 3, 79-105.

Jorgensen, B.B., Cohen, Y., and Revsbech, N.P. (1986), *Appl. and Env. Micro.*, Vol. 51 (2), 408-417.

Larkin, J.M. (1980), Current Microbiology, Vol. 4 , 155-158.

Taylor, C.D. and Wirsen, C.O. (1997), Science, Vol. 277(5331), 1483-1485.

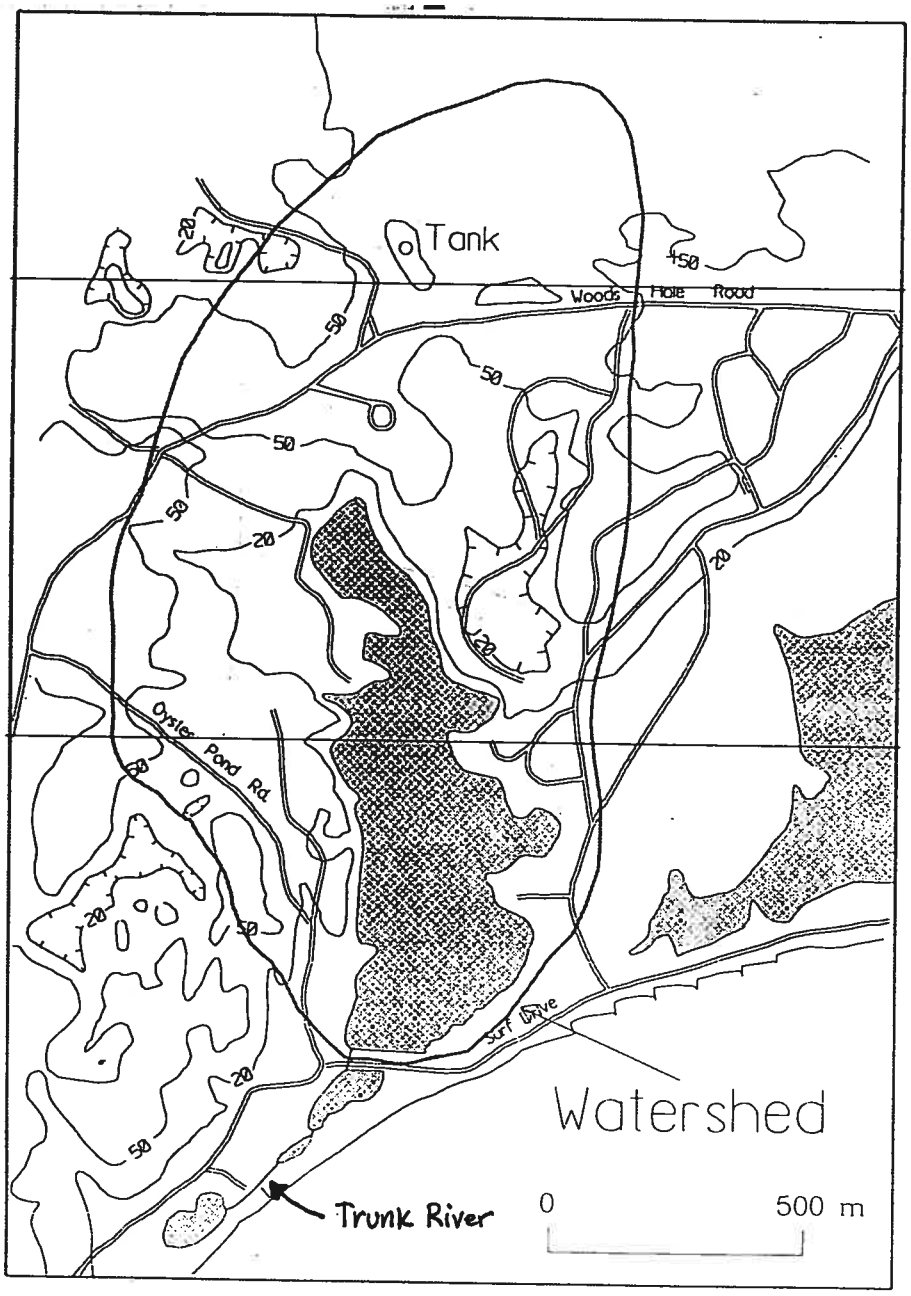

Tank
Woods Hole Road
Oyster Pond Rd.
Surf Drive
Watershed
Trunk River
0
500 m

FIGURE 1

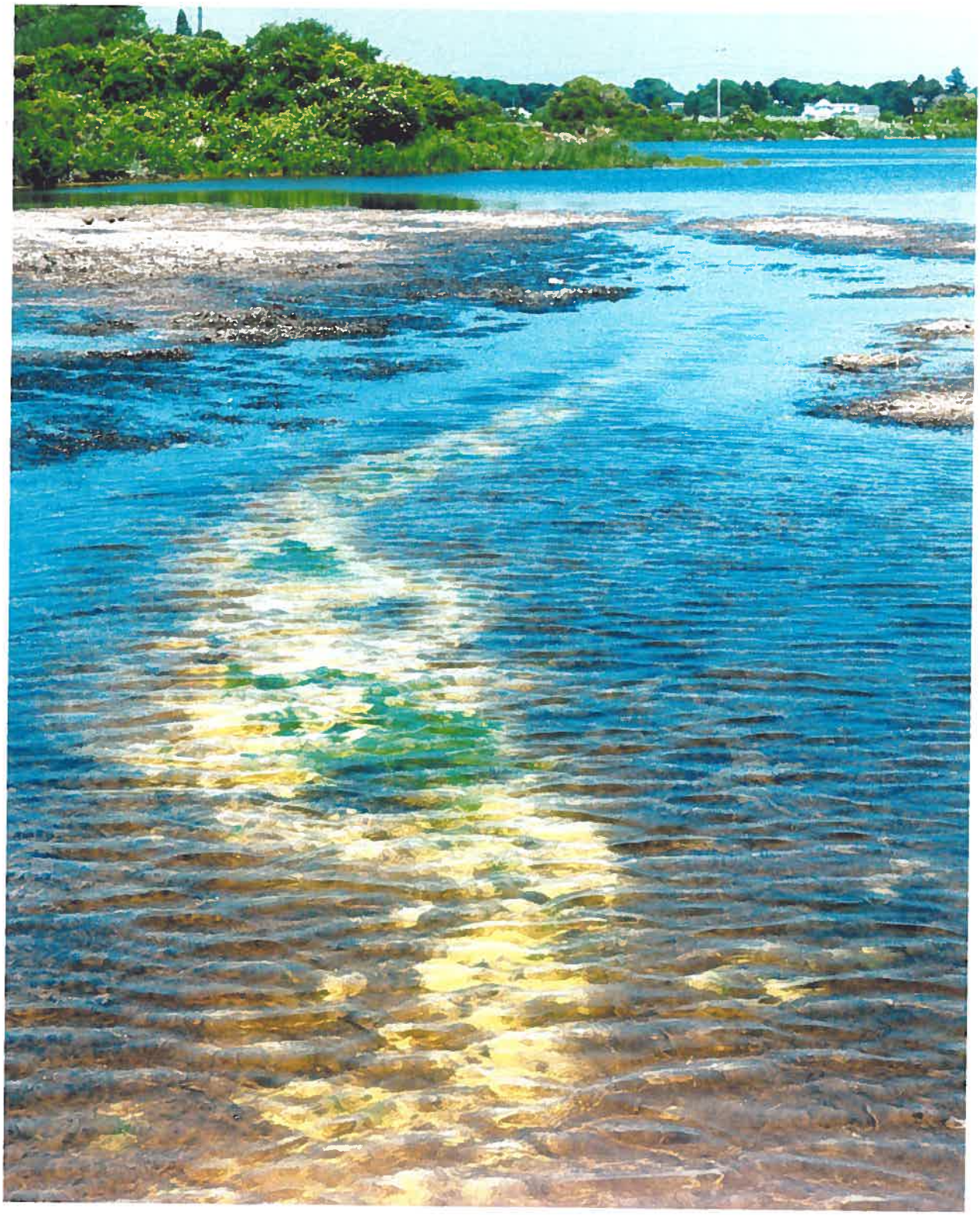
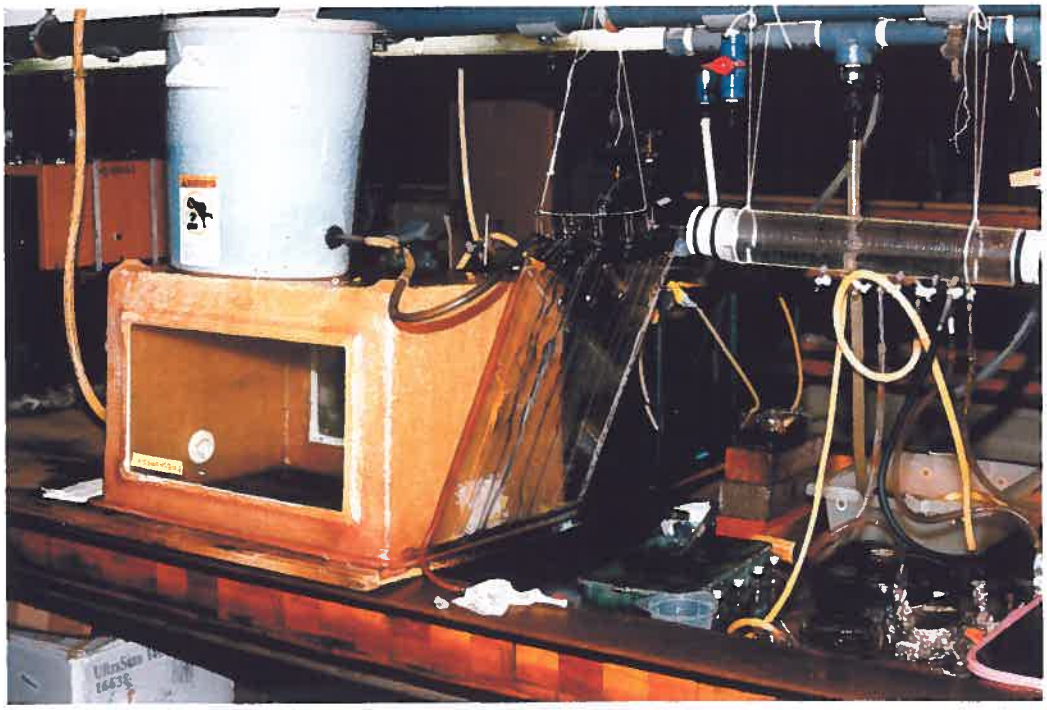


FIGURE 2

Little Trunk Mat



Sulfide Generator

FIGURE 3

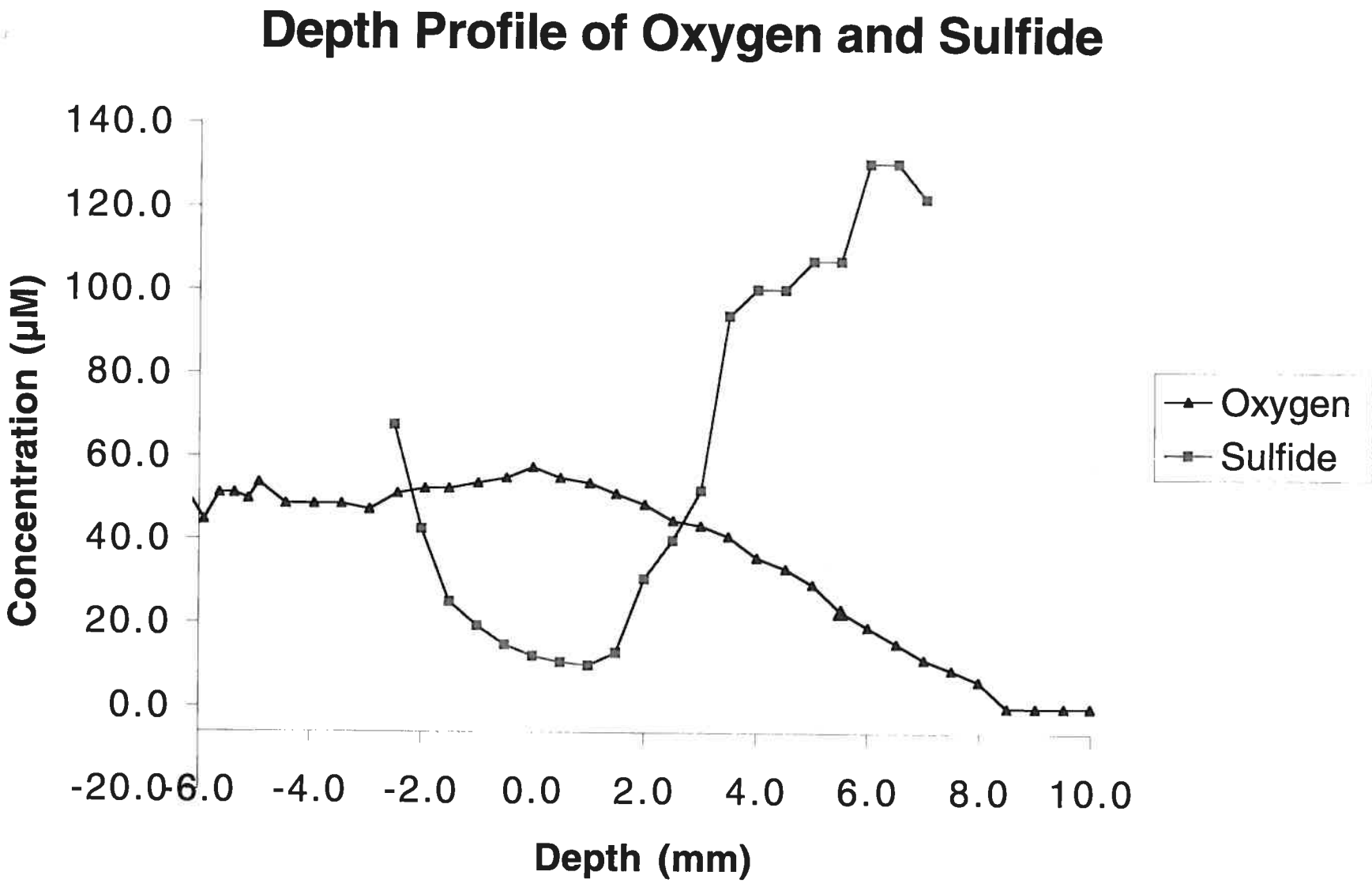

Depth Profile of Oxygen and Sulfide
Concentration (µM)
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
-20.0
-6.0
-4.0
-2.0
0.0
2.0
4.0
6.0
8.0
10.0
Depth (mm)
Oxygen
Sulfide

FIGURE 4

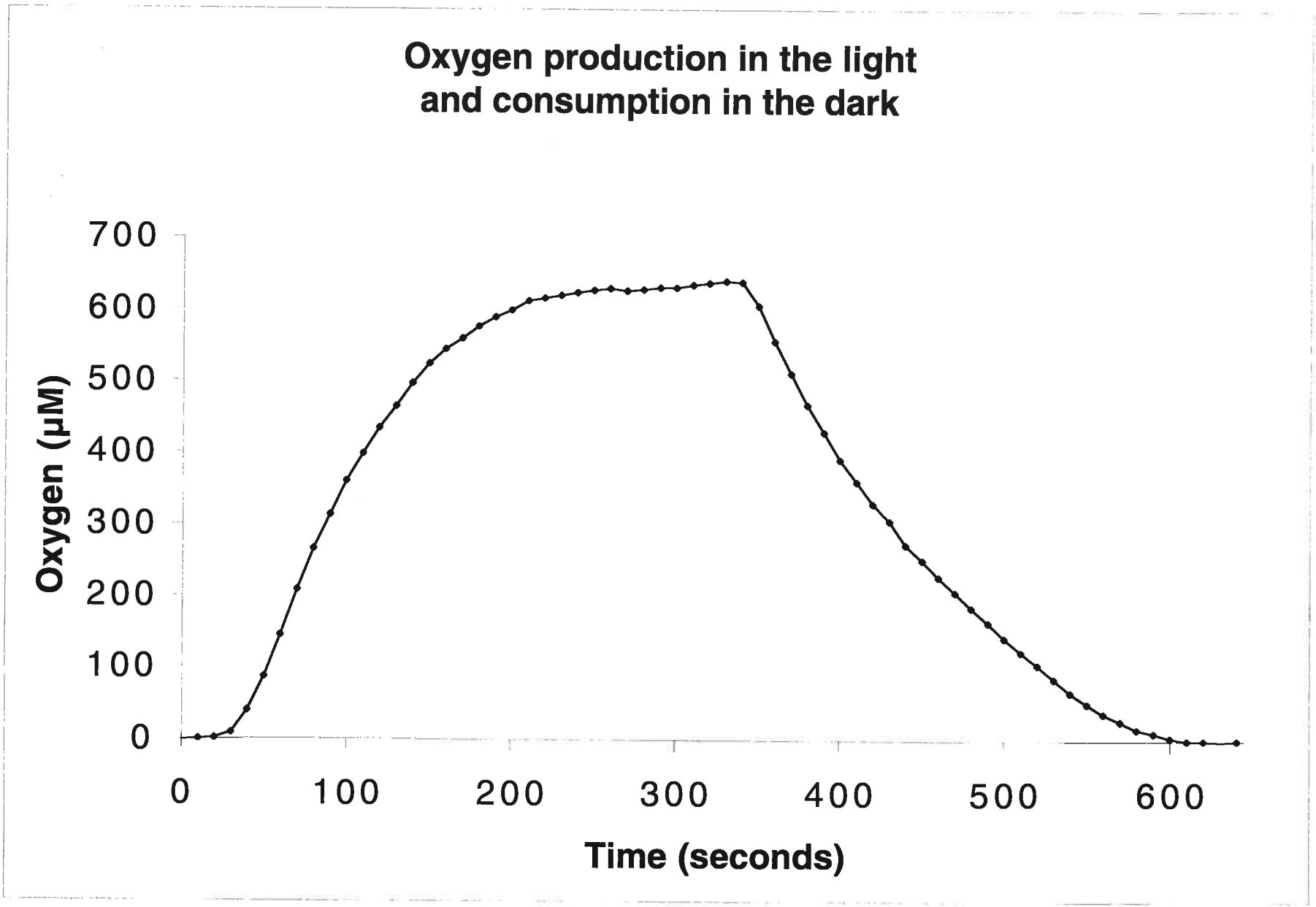

Oxygen production in the light
and consumption in the dark
Oxygen (µM)
700
600
500
400
300
200
100
0
0
100
200
300
400
500
600
Time (seconds)

FIGURE 5

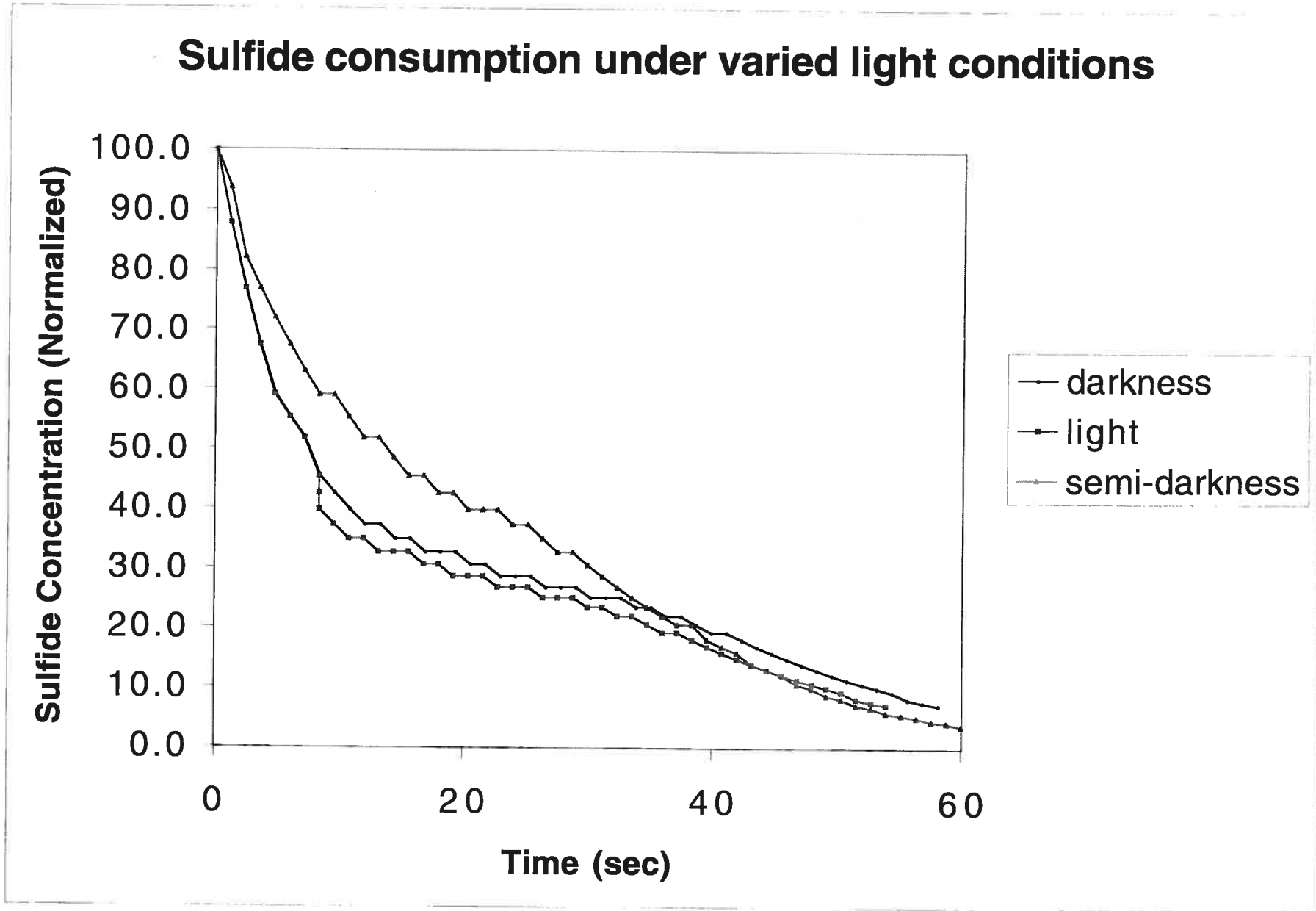

Sulfide consumption under varied light conditions
Sulfide Concentration (Normalized)
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
0
20
40
60
Time (sec)
darkness
light
semi-darkness

FIGURE 6 A

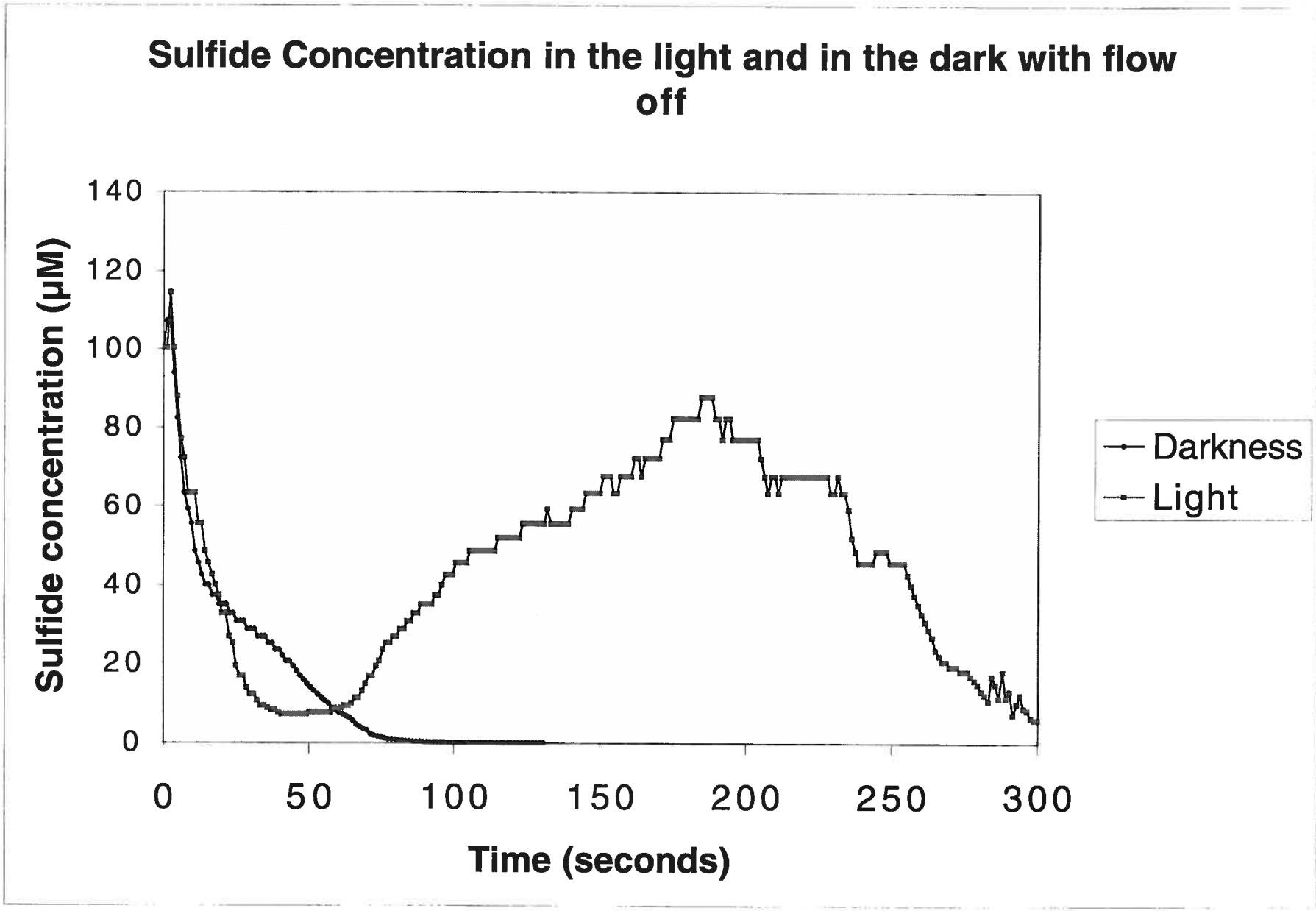
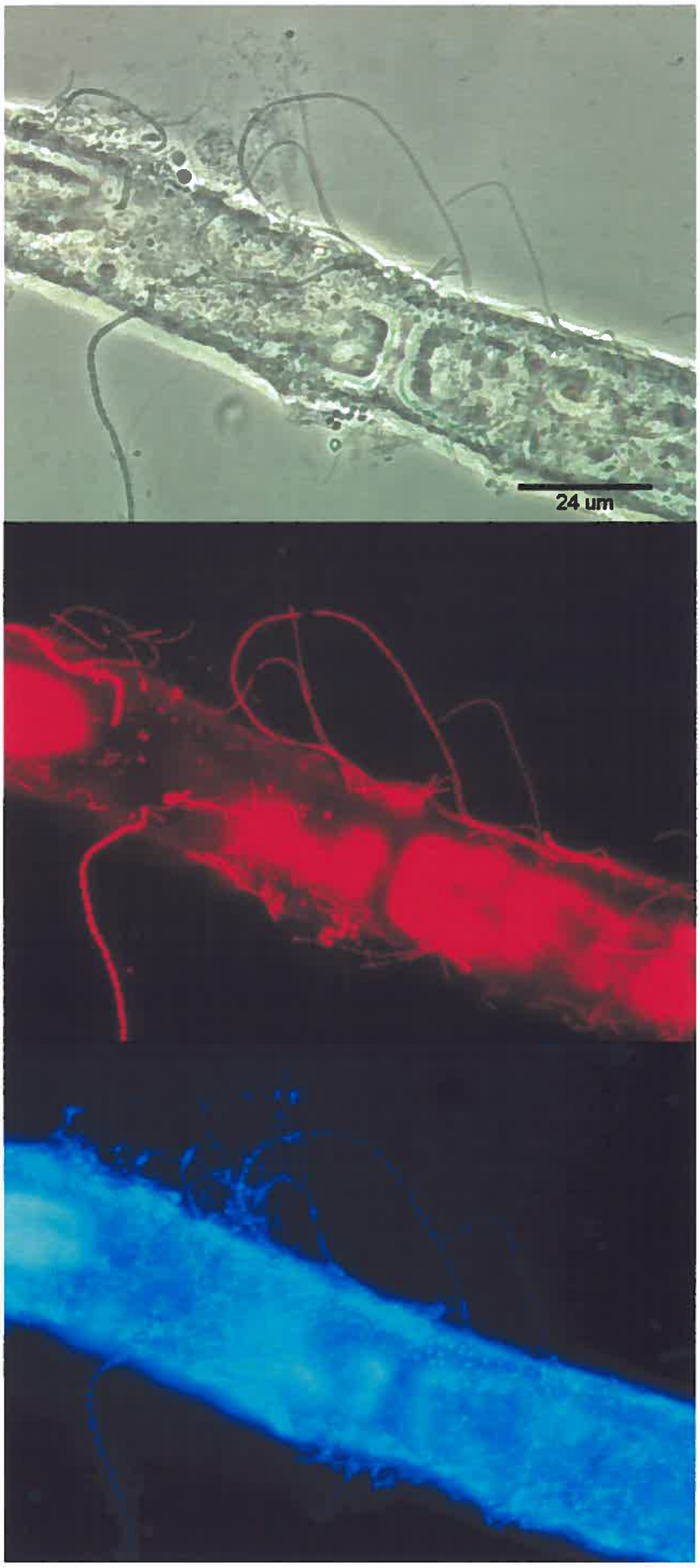

Sulfide Concentration in the light and in the dark with flow off
Sulfide concentration (µM)
140
120
100
80
60
40
20
0
0
50
100
150
200
250
300
Time (seconds)
Darkness
Light

FIGURE 6B

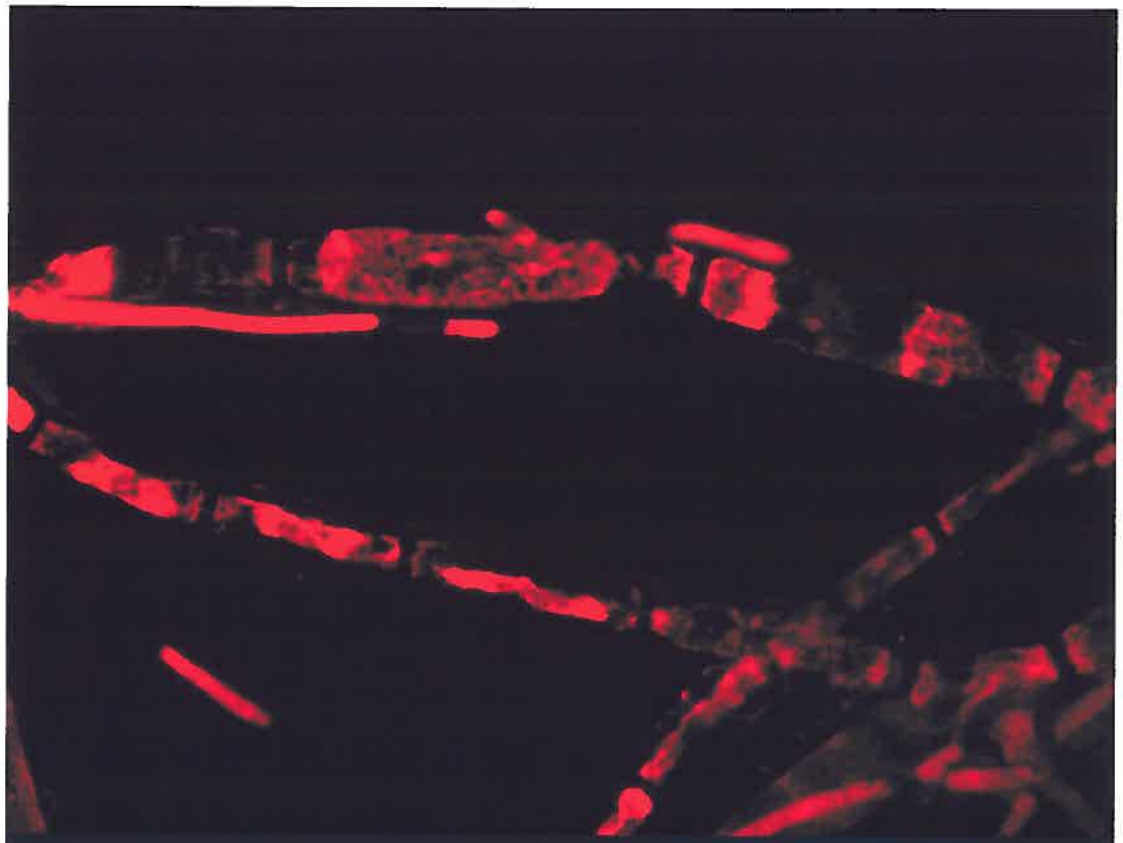


Phase

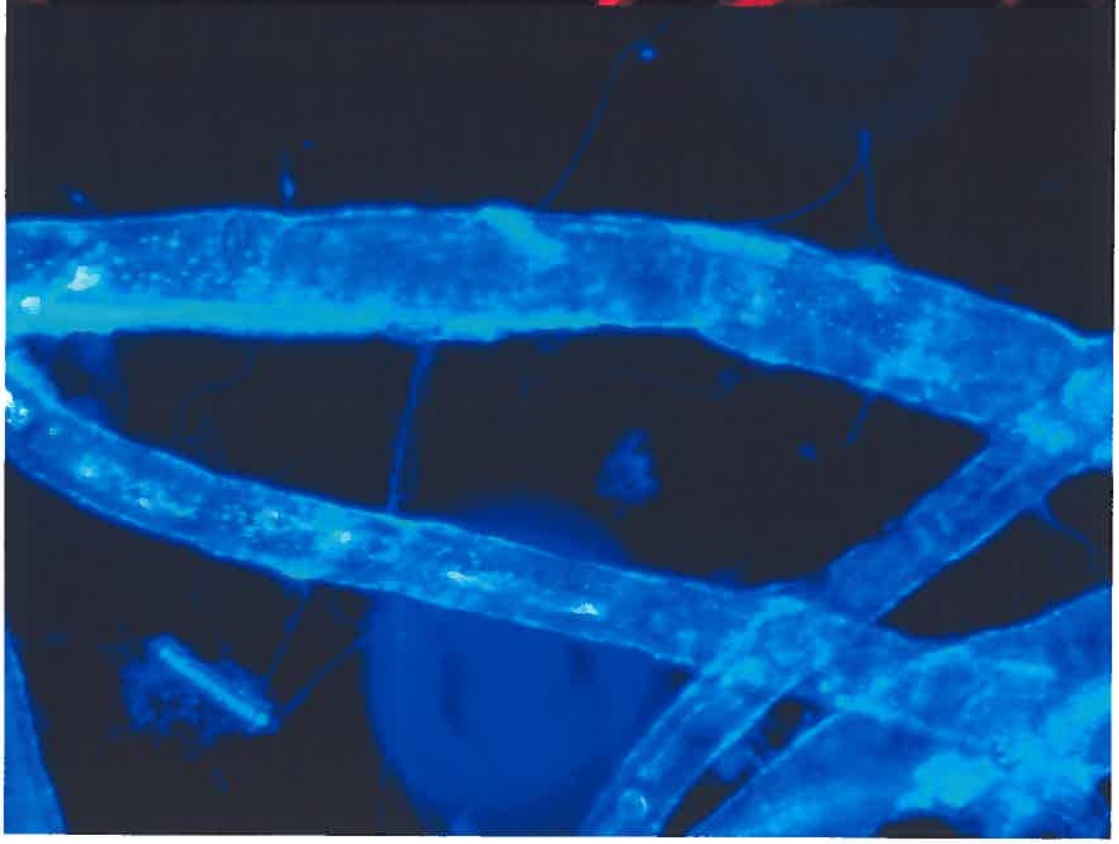
Gamma

DAPI

FIGURE 7A



Delta



DAPI

FIGURE 7B