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Title: SOME ASPECTS OF THE SULFUR CYCLE IN TIDAL FLAT AREAS, AND THEIR IMPACT ON ESTUARINE WATER QUALITY.

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A conceptual model of the tidal flat system, emphasizing the sulfur cycle was presented. Measurements were made of the vertical distribution within tidal flat deposits of total aerobic and sulfate reducing bacteria, total sulfides, redox potential, volatile solids, and particle size. Variations in dissolved oxygen and free sulfides in the water overlying tidal flat deposits were monitored during a tidal cycle, and profiles within this overlying water obtained. An in situ benthic respirometer was used to measure the rate of free sulfide release to the overlying water.

Laboratory experiments were designed to investigate the mechanism of sulfide production in tidal flat areas. Growth media were prepared from extracts of sediment and algae collected from tidal flats. Rates of sulfide production in these growth media by

mixed cultures of anaerobic bacteria from the same areas were obtained. A mathematical model, based on the common Michaelis-Menton equation, was used to simulate the experiments. A comparison of the simulated and experimental results was presented.

Some Aspects of the Sulfur Cycle in Tidal Flat Areas, And Their Impact on Estuarine Water Quality

by

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INTRODUCTION

Objectives and Scope

The primary objectives of this study were

(1) to explore the general nature of the sulfur cycle within tidal flat sediments and its relationship to the general ecology of selected areas.

(2) to determine the relationship of certain aspects of the sulfur cycle to oxygen uptake by tidal flat sediments.

(3) to investigate the mechanisms of sulfide generation within estuarine deposits.

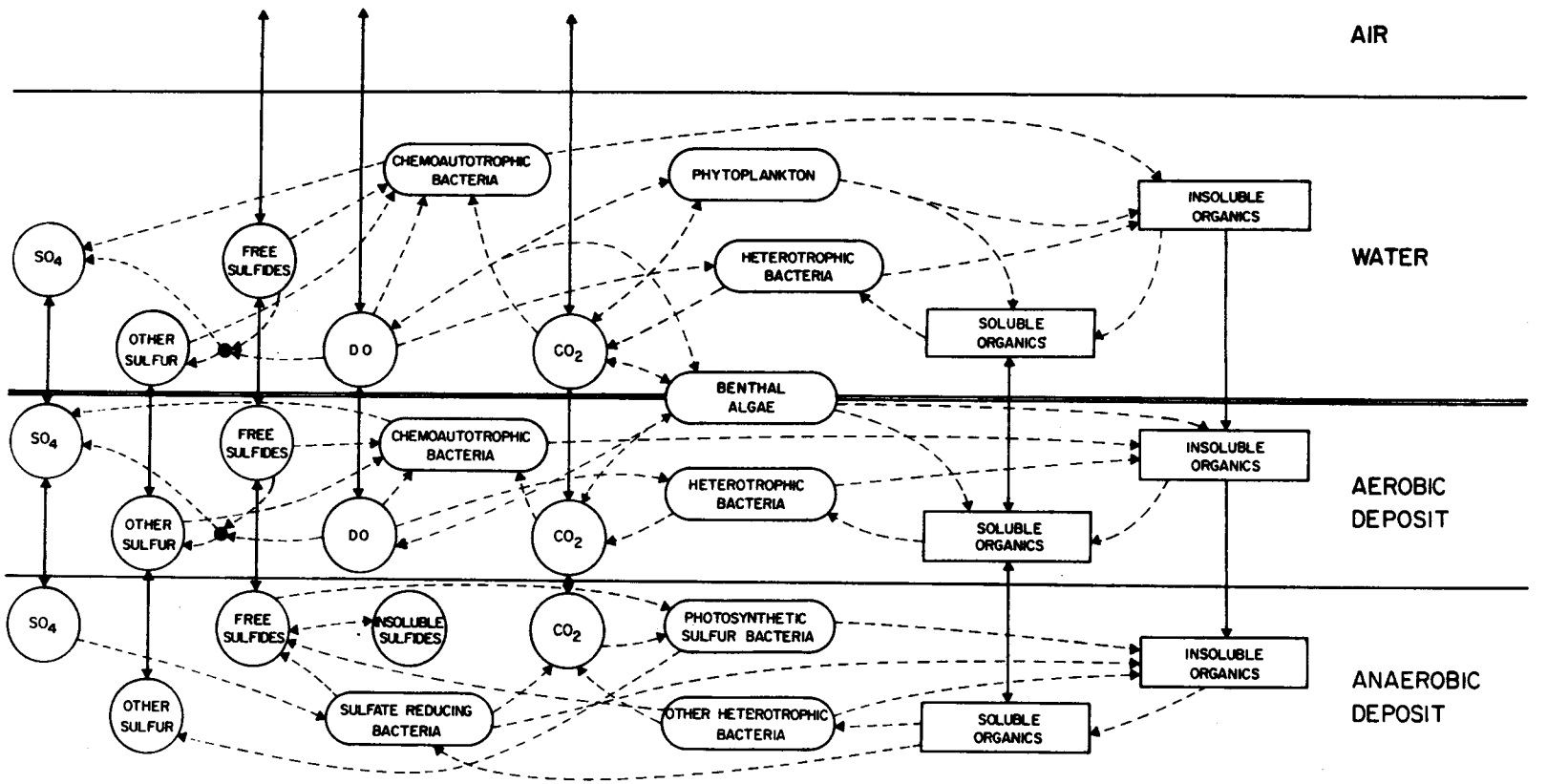
These objectives required a study and analysis of many interrelationships and processes occurring within the tidal flat system. It was realized from the onset of the study that such an analysis would be very broad in scope, and could be approached from any number of levels of resolution. In selecting an appropriate level of resolution from which to model and study any system, it is necessary to trade between detail and perspective (6). Excessive attention to detail increases the number of system components studied, and makes it difficult to define and analyze the relationships between them. To gain

perspective, however, leads to loss of detail and often some useful information.

A conceptual model of the tidal flat system at the order of resolution dealt with in this study is shown in Figure 1 (7). The level of resolution represented was chosen to illustrate those components and processes felt to be particularly relevant to this study. Omission of factors from Figure 1 does not imply they are not important in the ecology of tidal flat areas. Nutrients such as nitrates and phosphates, extracellular metabolites including enzymes, detritus feeders, and fungi are among the many factors that may play significant roles within the tidal flat ecosystem. Yet, for the sake of clarity, and to reasonably limit the scope of this investigation, these and other factors have not been separately included in Figure 1.

Reasons For Studying Tidal Flats

It has been recognized for some time that the tidal flat deposits of estuaries are areas of extreme complexity and activity (4, 79, 80). They serve as both sources and sinks of a nearly infinite variety of compounds and materials, produced by equally as many and varied processes. They are continually in a state of material and energy flux with the surrounding water and air. Consequently any approach to an understanding of them and their effects must involve a study of the biological, chemical and physical factors and processes



(a) VERTICAL LINES REPRESENT PHYSICAL TRANSFER PROCESSES

(b) CHEMICAL REACTION NOTED BY (●)

Figure 1. A conceptual model of the tidal flat system.

comprising the tidal flat ecosystem.

A generalized cross section of a typical estuary is shown in Figure 2 and has been divided into eight zones for purposes of the following discussion. The portion to the left of the vertical line represents the main channel area composed of the main water body (A), a thin layer of water immediately above and in contact with the sediment (B), the aerobic zone of the sediment and its interstitial water (C), and the anaerobic zone and associated interstitial water (D). The portion to the right of the solid vertical line represents that portion of the estuary in which the sediments are periodically exposed by tidal fluctuations - the tidal flat area.

Tidal flats are generally quite extensive and comprise a significant fraction of the total deposit area of estuaries. Within Yaquina Estuary, for example, approximately 1590 acres of tidal flats are exposed at low water, representing more than two-thirds of the benthal deposits (77). In Coos Estuary nearly 7000 acres of tidal flat are similarly exposed (77). Since these regions are so extensive, they should receive appropriate attention.

It has been noted that similar species within ecosystems compete for similar resources such as food, space, and light (28, 33). Despite the tendency for the more efficient species to eliminate the less efficient ones (33), healthy ecosystems usually contain a great variety of species, and species diversity has been considered to be

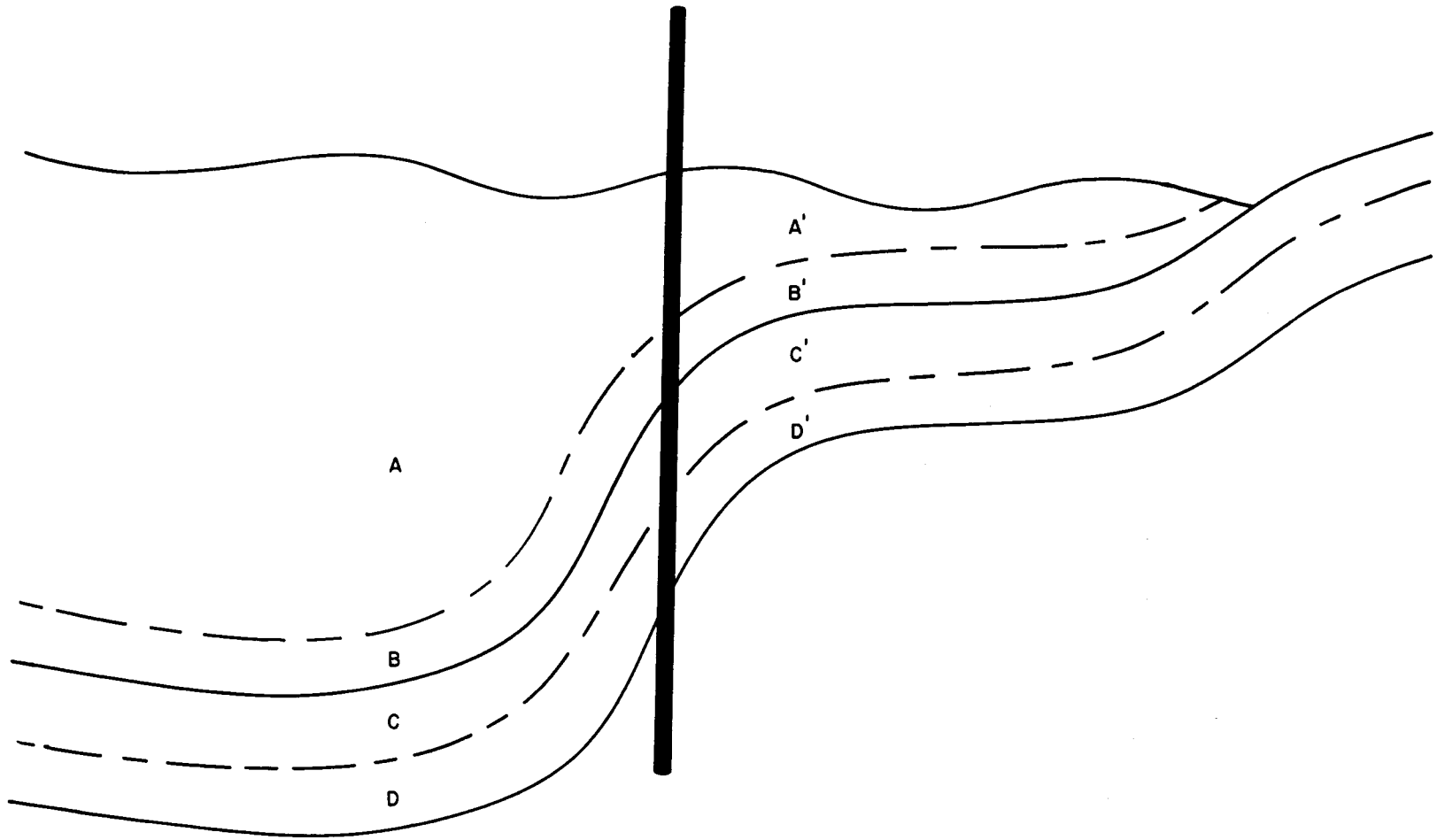


Figure 2. A generalized cross section of a typical estuary.

of considerable importance in the maintenance of ecosystem stability (28). It has been suggested that natural variations in time and space prevent any one species from having an advantage over a wide enough area over a long enough time to displace its competitors (55). If ecosystem stability is desirable, then the variability of conditions which exist within estuaries, and tidal flats in particular, are important in the maintenance of stability. That the unique conditions in estuaries are essential to normal growth and development of many invertebrate species has been established (19).

Within estuaries, the tidal flat deposits display conditions which are often uniquely different from those of deeper water sediments. In particular, they are periodically exposed to the air, and dessication is a condition with which organisms living upon or near the sediment surface must contend. Pollutant materials carried on the water surface may be deposited upon the tidal flats as the tide ebbs.

There exists good evidence that the deposits of estuaries may have a major influence on the ecology of the whole estuary (78). For example, the build-up and release of undesirable gases from the sediments is a common occurrence and may significantly affect water quality (7, 77). In some cases hydrogen sulfide release from sediments may become so great that it escapes into the air becoming a nuisance and producing property damage (59). An understanding of the mechanisms leading to the production and release of gases

is essential to the prevention of such occurrences.

Tidal flat deposits are in general biologically very active and in some cases benthal respiration is a major factor affecting dissolved oxygen concentrations (DO) in the relatively shallow overlying water (20). Through biological degradation, settling,and adsorption to sediment particles organic matter is removed from overlying water. The oxygen utilization resulting from aerobic degradation of organics can significantly deplete the water of DO (20, 43, 62, 78). Reduced byproducts of microbial metabolism formed within anaerobic sediment may also exert a substantial oxygen demand following upward diffusion into aerobic regions (20).

In addition to providing transfer of gases and reduced substances across the sediment-water interface, sediments are also important sites of release of nutrients such as nitrates and phosphates (23, 62). Their importance may increase when overlying waters become nutrient deficient (23, 32, 55).

Bacteria occurring within estuarine sediments have been shown to be of very great importance (79). Because of their high metabolic rates they are capable of rapid turnover of nutrients, and probably serve as vital pathways in the trophic web in the estuarine ecosystem. Their ability to participate in a wide variety of chemical transformations strongly implicates them in the control of the pH and redox potential of the sediment environment (4, 30, 80). They may

be of further importance in production of biologically essential trace compounds such as vitamin B12 (45). They are also known to be responsible for the production of toxic substances such as hydrogen sulfide, and possibly methylmercury, and may thus substantially alter the microecology of certain areas (30, 41). They may well be the major biological factors controlling the ecology of estuarine deposits, and hence the entire estuary (79).

Despite their importance, estuaries, and tidal flats in particular, have been significantly affected by land filling, diking, dredging, and numerous forms of pollution. While estuarine systems have received considerable attention of late, the vast majority of studies or models have been concerned with region A (Figure 2). Furthermore, a review of the sampling procedures used by regulatory agencies indicates that, with few exceptions, the quality of the interstitial and overlying waters of tidal flat sediments has been ignored. It is imperative that the ecology of these regions be understood in order that rational decisions may be made regarding the use and management of estuaries.

The Tidal Flat Area

Physical Factors

Consider a vertical slice of a typical tidal flat at high tide (see

Figure 2). There are two phase boundaries present in this system; the upper boundary (air-water interface), through which gases can pass by transfer processes, and the lower boundary (sediment-water interface), through which soluble compounds may enter and leave the sediment. In addition, two other less distinct boundaries can be defined: the boundary between the upper well-mixed turbulent waters and less-mixed waters adjacent to the sediment surface, and the boundary between the aerobic and anaerobic regions of the bottom deposit.

Within the water phase, energy may enter the system as light, organic, and inorganic compounds, to be used by photoautotrophs, heterotrophs, and chemoautotrophs respectively. Light cannot penetrate more than a few millimeters (mm) into the sediment, however, and thus photosynthesis probably does not occur below 5 mm (30, 75, 80). Chemical energy in the form of organic matter, transported into the tidal flat area or produced through photosynthesis, is imported to the sediments from above by settling to and mixing across the sediment-water interface (Figure 1). This energy may be used directly by detrital feeders, but increasing evidence suggests that bacterial and possibly fungal decomposition of this organic matter is first necessary for complete utilization by other organisms (30, 53, 75).

Within the sediment there are two zones based upon the

availability of oxygen for energy yielding processes: the aerobic zone, and the anaerobic zone. The upper, or aerobic zone, receives dissolved oxygen from the overlying water by means of vertical mixing (30), and the depth of this aerobic zone depends to a large extent on the extent of mixing within the sediment. Vertical mixing within the sediments in turn depends upon a variety of factors. Hydraulic factors, such as tidal variation in water depth, water velocities, wave action, and low tide drainage patterns all contribute to vertical mixing (11, 41). Burrowing and movement of organisms within sediments leads to greater vertical mixing, and studies have shown that the extent of such mixing can be considerable (25, 26, 66). On the other hand, the presence of fine particles within the sediments, as well as certain microorganisms within and upon the deposits, tends to reduce vertical mixing (11, 41). Electrical interaction between particles and interstitial water can lead to a reduction of molecular diffusion, and within biological slimes diffusion can be significantly less than through pure water (12, 24). Consequently vertical mixing can be expected to range from fairly large hydraulic exchanges to values less than that of molecular diffusion in pure water.

Below the aerobic zone lies a region devoid of molecular oxygen. Redox potentials (Eh) within this anaerobic zone are usually quite negative, and are largely determined by microbial processes (80). Although values as low as -500 millivolts (mv) have been

reported, such extremes are rare and probably due to the presence of molecular hydrogen (80). More commonly negative potentials fall between -100 and -250 mv, and it is generally recognized that these are primarily due to the presence of free sulfides (4, 9, 16, 30).

Oxygen

Because of the many processes in which it participates, oxygen is probably among the more important factors affecting the structure and functioning of tidal flat ecosystems. All aerobic life is dependent upon the utilization of oxygen as a hydrogen acceptor, and the vertical zonation of the bottom deposits is a result of the one-way supply of oxygen and light (30).

In addition to DO imported by water movement, oxygen may be added to the overlying water by two principal processes: reaeration, and photosynthesis. Reaeration is a physical process resulting in the transport of oxygen across the air-water interface, and has been studied in detail by many investigators (22, 54, 55). In tidal flat regions, wind and wave action will have a major influence on the air-water transfer of oxygen. This net movement of oxygen may occur either into or out of the water, depending upon the relative partial pressures of oxygen on each side of the interface.

During daylight hours, oxygen is produced photosynthetically by planktonic and benthic green plants through photolysis of water.

The extent of photosynthesis depends upon light, nutrients, temperature, and algal biomass. During the night hours, photosynthesis ceases, but respiration continues, contributing to a nocturnal decrease of DO. Diel DO variations of over eight milligrams per liter have geen reported within tidal flat areas (20).

The DO uptake of benthic deposits has been studied both in the field (20, 39, 47, 51, 55, 62), and in laboratory systems (5, 26, 29, 46, 49, 57, 68). Uptake rates have been shown to be dependent on temperature, water velocity, and the presence and abundance of algae, bacteria, and macrofauna (20, 46). Some investigators believe the uptake to be due mainly to diffusion of oxygen into the sediments, to meet the respiratory requirements of organisms. Others believe it is primarily due to diffusion of oxygen demanding substances out of the sediments (5). Studies employing poisoned systems have shown that one-half to one-third of the oxygen uptake can be non-respiratory (46). The diffusion of oxygen demanding materials (possibly a by-product of anaerobic metabolism) into the aerobic zone of the sediment or into the overlying water, and their subsequent oxidation, could account for the non-respiratory (chemical) oxygen demand (20). In situ studies on oxygen uptake by sediments of Yaquina Estuary have further indicated that such oxygen demanding substances must have a rather high reactivity with DO in order to account for the measured oxygen uptake rates (20).

Because of their widespread abundance in anaerobic deposits, free sulfides have been implicated as a major factor responsible for chemical oxygen uptake (7, 30). The apparent high reactivity between free sulfides and DO in brackish water has contributed to the general assumption that free sulfides released from the anaerobic zone of the deposits will be essentially completely oxidized within the aerobic layer (7). Significant concentrations of free sulfides within waters containing moderate to high DO have generally been considered as a transient condition resulting from dredging, scour, or other similar major benthic disruptions. In general, the continued presence of free sulfides in waters containing DO has been considered improbable (7, 16).

Sulfides

The sulfur cycle has been described in nearly every textbook on ecology. Its importance in intertidal sediment ecosystems has been stressed (4, 30), yet it has received only superficial attention. Various sulfur compounds occur within the water column overlying tidal flat deposits. In most brackish water which is oxygenated, these are usually in an oxidized state, with sulfates being the most abundant form. Those sulfur species which are soluble may diffuse across the sediment-water interface and enter the sediments. Within the water and upper aerobic layer of the deposits, inorganic

sulfur compounds may be utilized by sulfur oxidizing bacteria, such as those of the genus Thiobacillus, which produce sulfates.

Hydrogen sulfide occurs in aqueous solution as part of the pH dependent system

$$H_2S \leftrightharpoons HS^- \leftrightharpoons S^= \qquad (1)$$

At a pH of approximately 7.0 free sulfides are equally divided between H_2S and HS^- with $S^=$ being negligible (59). In the following discussion, components of equation 1 will be referred to as 'free sulfide'. Free sulfide originates within the anaerobic zone of the sediment where it is produced primarily by heterotrophic sulfate reducing bacteria which utilize the sulfate ion as a terminal hydrogen acceptor (4). When adequate sulfate is available, as in marine and brackish waters, stabilization of organic material has been shown to occur via sulfate reduction rather than methane fermentation (31). Free sulfides may also be produced during anaerobic putrefication of sulfur-containing amino acids, but this process is felt to be of lesser importance in the marine environment (16, 30).

The fate of the sulfides will depend upon the physical and chemical characteristics of the sediments. If sufficient amounts of metal ions, such as iron for example, are present, the sulfides will form insoluble precipitates. If removal of these insoluble sulfur compounds does not take place, either by resolubilizing or through

transport out of the area, the concentration of total sulfides, including free, soluble, and insoluble forms, may reach significant proportions in the sediments (7, 30).

If the aerobic layer of the sediment is thin enough to allow light to penetrate to the anaerobic zone, populations of photosynthetic purple and green sulfur bacteria may develop, utilizing the free sulfides as hydrogen donors, and producing free sulfur as a by-product (7). This often occurs below a carpet of blue-green algae, and is possibly due to the lower compensation point for bacterial photoreduction, and to the ability of the photosynthetic bacteria to utilize longer wavelengths of light than can the algae (30, 73).

If the rate of free sulfide production exceeds the rate at which it can be converted to nondiffusible forms, such as ferrous sulfide or insoluble free sulfur, the sulfide may diffuse upward into the aerobic sediment or into the water column itself. Here it will be oxidized to sulfite, thiosulfate, sulfate, or free sulfur (15, 16, 61).

The chemical reaction of free sulfides in aqueous solutions has been studied by many investigators (3, 15, 16, 18, 61, 70). Half lives of free sulfide, in aqueous solutions, ranging from 15 minutes to 70 hours have been reported. Several studies have described the oxidation of free sulfides to occur via second order kinetics (15, 16, 59); however such a description is a simplification of an extremely complex chemical

system (15). These experiments have indicated that pH, temperature, and initial oxygen and sulfide concentrations are all factors affecting the rate of oxidation (15, 16). A recent study using distilled water reported a half life for sulfide of about 50 hours at an oxygen concentration of 25.6 milligrams per liter (mg/l). This study noted that the oxidation is catalyzed by the presence of metallic ions, such as of Ni, Mn, Fe, Ca, and Mg, and is accelerated by some organic substances such as formaldehyde, phenols, and urea. These results suggest that oxidation of free sulfides in estuarine and marine water may be much more rapid than in distilled water due to the presence of catalysts. Within oxygenated sea water the half life of sulfide has been reported to vary from 10 minutes to several hours (8, 16, 61). Since HS^- predominates at the pH of sea water, it has been proposed that the oxidation proceeds by the following reaction (67):

$$2HS^- + 2O_2 = S_2O_3^= + H_2O \qquad (2)$$

Following the above chemical oxidation, the thiosulfate ion is more slowly oxidized to sulfate, probably with the intermediate production of other oxidized forms. Sulfur oxidizing bacteria of the genus Thiobacillus appear to be important in this final oxidation step (40, 71).

The release of sulfides into overlying water can have undesirable effects on water quality. Not only do sulfides exert an oxygen demand, but they have also been shown to be quite toxic at low

concentrations to fish, crustaceans, polychetes, and a variety of benthic microinvertebrates (17, 21, 30, 37, 48, 70). In batch tests assaying for a given toxic effect, it has often been only the initial sulfide concentrations which have been reported as responsible for the effect. Due to the relatively rapid oxidation of sulfides, these reported toxic concentrations may be considerably higher than the average concentrations throughout the test period, and may consequently underestimate the toxicity. One recent study has been made in which relatively constant hydrogen sulfide concentrations below 0.075 mg/l (pH 7.6-8.0) have been found to be significantly harmful to rainbow trout, sucker, and walleye, and particularly to the eggs and fry of these fish (17).

Because of the relatively high toxicity of free sulfides, principally hydrogen sulfide, the presence of free sulfides might often be a more serious water quality problem than the lower DO values resulting from the oxidation of the sulfides.

FIELD STUDIES

Location of Field Work

General Description

The field portion of this research was conducted in the Yaquina and Coos estuaries located on the Oregon coast. Yaquina Estuary, the northern most of the two, is basically a drowned river valley extending approximately 23 miles inland to its head, and covering 2700 acres at mean high water (75). The cities of Newport and Toledo contribute a population of about 5000 to this area. Coos Estuary which lies about 100 miles south, is the larger of the two. It is boarded by the cities of North Bend and Coos Bay, whose combined population is approximately 25, 000. In addition to having a larger population located about it, Coos Estuary is more highly industralized. Wood processing mills are numerous here, and raw effluent is released directly into the estuary in several cases.

Test Site Description

Three primary test sites were chosen for the field studies. The two sites located within Yaquina Estuary are shown in Figure 3. Site one was located on the south side of the estuary immediately to the east of the Oregon State University Marine Science Center. This

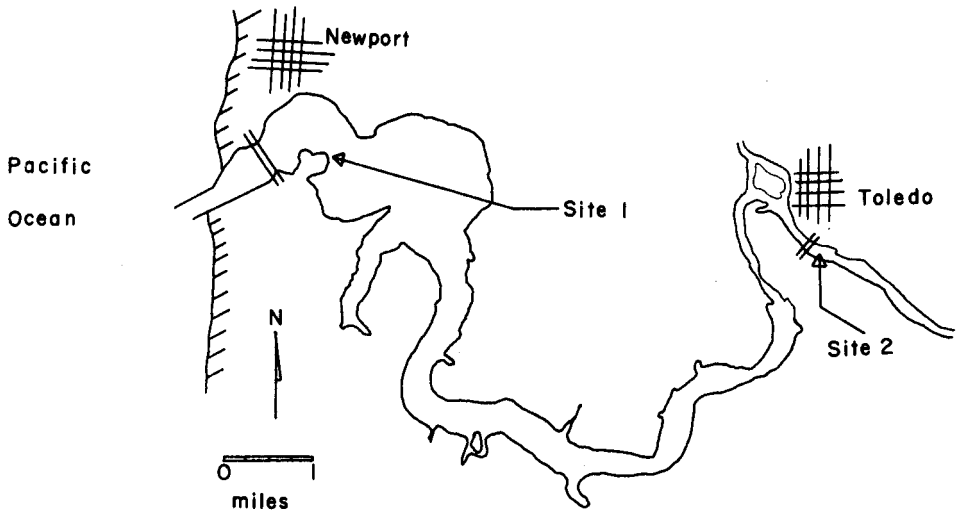


Figure 3. Map of Yaquina Estuary showing the location of sites one and two.

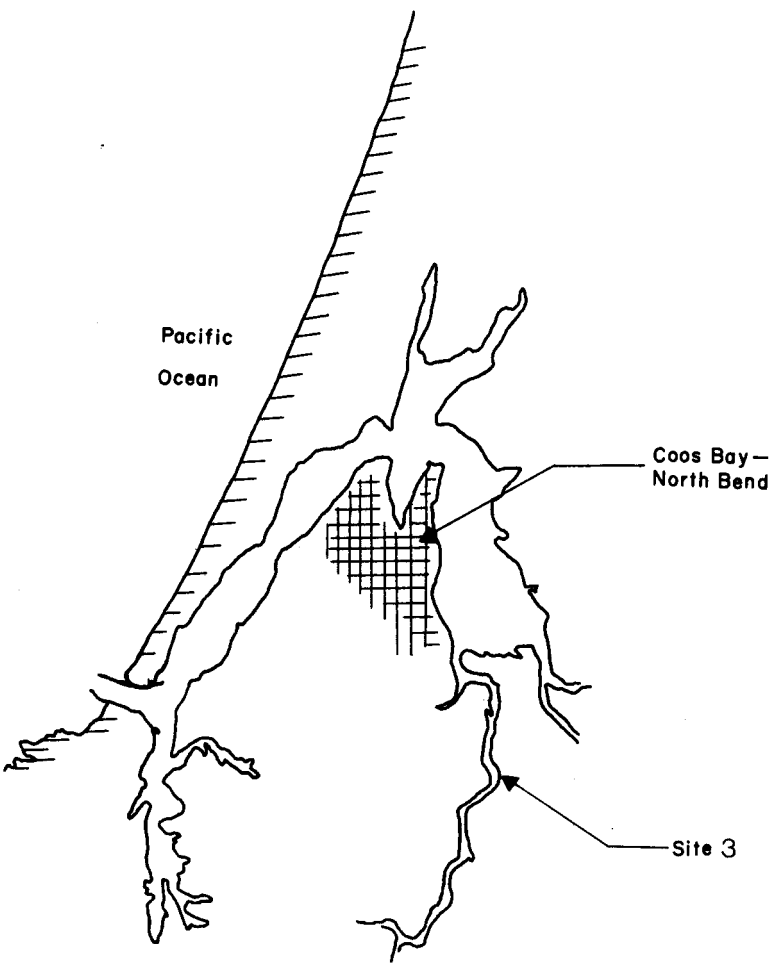


Figure 4. Map of Coos Estuary showing the location of site three.

site lay within approximately one mile of the estuary mouth, and was strongly influenced by marine water. High salinities (33 - 35 parts per thousand), low temperatures (45 - 50° C), and high tidal current velocities (0.6 - 0.8 feet per second) were characteristic here. This area appeared to be fairly remote from any major source of industrial pollution, and no excessive domestic contamination was evident. The sediments here were heavily colonized between +4 and +6 feet above mean low low water (MLLW) by large populations of the mud shrimps Callinassa californiensis and Upogebia pugettensis. They were very active in burrowing and mixing of the sediments at this elevation. There was a distinct lack of attached vegetation in this range, but summer growth of the benthic alga Enteromorpha sp. and of Zostera sp. was extensive below +4 feet MLLW. At approximately +7 feet MLLW the sediment was covered by a thin, but very firm mat of unidentified algae.

Site two was located about 14 miles upstream and 300 feet east of the Yaquina River bridge at Toledo. Unlike site one, this site lay in an industralized area characterized by extensive log rafting and wood processing operations. The effect of fresh water was reflected in the lower salinities (14-20 parts per thousand). Water temperature was higher than at site one, current velocities still fairly high, and the sediment covered by large quantities of bark chips. Burrowing organisms and growths of benthic algae were lacking. This site

had been previously utilized for both in situ and laboratory studies of sediment oxygen uptake (20, 45).

Site three was located on the south side of Isthmus Slough in Coos Estuary (Figure 4). This site was located on a mud flat which was relatively protected from the main channel currents by a dike and log storage area. Tidal velocities were low, temperatures comparable to those at site two, and salinities intermediate between those of sites one and two (28-30 parts per thousand). A number of Sulfite Process wood pulping mills were located nearby. Extensive algal mats primarily of a salt water species of Vaucheria were characteristic here, but burrowing organisms were not evident. A general purplish coloration to the water was very noticeable.

Materials and Methods

Establishment of a Transect at Site One

To investigate the variation of a number of parameters with tidal elevation, a transect was established from the high to low water marks at site one. Sampling stations were established and identified by driving painted steel fence posts of eight foot lengths into the sediment. The elevations chosen for the stations were based somewhat on tidal exposure data for the area, and were located at 7.2, 6.4, 5.6, 4.0, 3.1, 1.6, -0.8, -2.3 and -3.6 feet

above (or below) mean low low water (MLLW). Four foot lengths of one-half inch wooden doweling were driven into the deposit two meters from each stake on a line perpendicular to the transect line. This doweling, flush with the deposit surface, served as the center of a two meter diameter circular sampling area from which duplicate cores were taken at random. This rather elaborate procedure, in addition to providing random samples from the sampling area, was used mainly in an effort to prevent disturbance to the sediment by clam diggers, who were apparently particularly attracted to the fence stakes. On several occasions they were seen attempting to pull the fence stakes from the sediment. No transects were established at the other sites, and as these areas were free from human disturbance, samples were taken with considerably less effort.

Obtaining Core Samples

Plastic coring devices were constructed by sharpening one end of a 15 inch long by 2 inch diameter plexiglas cylinder. A rubber stopper for capping each cylinder was fitted with a glass tube and attached rubber tubing which could be clamped off (Figure 5). Cores were collected by pushing the sharpened cylinder into the sediment. Water trapped within the coring device flowed out through the top; the rubber tubing was then clamped off and the core removed from the sediment by pulling the cylinder upward. Following extraction,

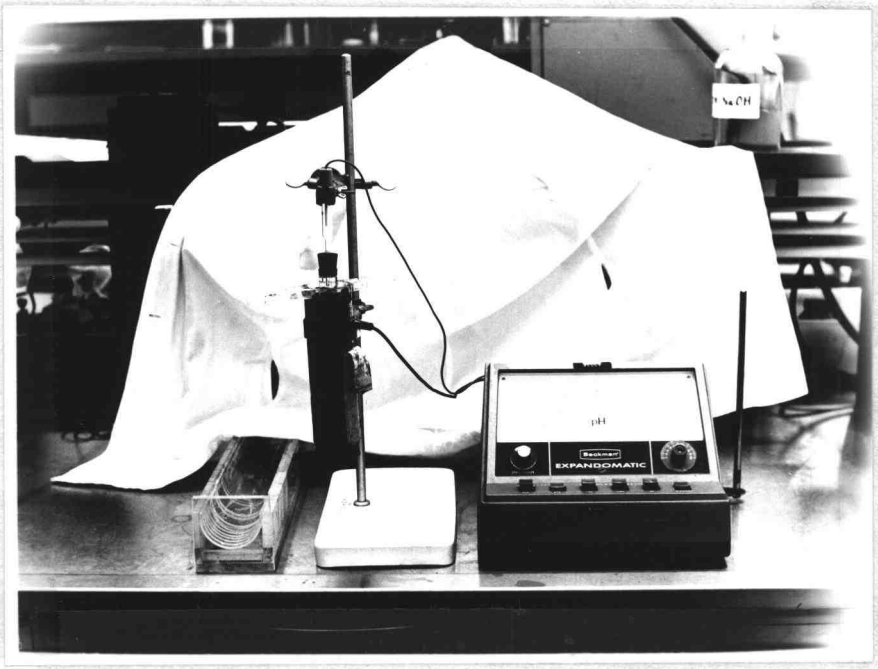


Figure 5. Plexiglas slicing trough and corer with apparatus for determining the Eh of the sediment.

Figure 6. Apparatus for measuring total sulfides.

the base of the coring device was stoppered. Cores appeared to remain intact with little compaction evident. Some negligible distortion was apparent at the boundary between the sides of the corer and the core. Early in the study, the plexiglas corers were modified by drilling small holes at appropriate intervals along their length. Covered during sampling by a strip of masking tape, these holes allowed insertion of a platinum wire for redox measurements at a variety of depths within the intact core. Following extraction of cores, they were immediately placed intact within the corers into a styrofoam cooler and later taken to the laboratory for analysis. This was generally accomplished within one hour following extraction.

Similar corers of aluminum were also built, but were found to cause considerably greater compaction than did the plexiglas ones. An additional advantage of the plexiglas was that their transparency allowed visual examination of the intact cores within the corer.

Core Analysis - Chemical

It was decided that core analysis would be made at the following depths: 0-1 centimeters (cm), 2-3 cm, 4-5 cm, 7-8 cm, 11-12 cm, and 16-17 cm. In some cases longer cores allowed analysis of the 22-23 and 29-30 cm sections. It generally was not possible to obtain good cores of this length, however.

In the laboratory the cores were fixed in a vertical position, and

the cores allowed to slide out of the corers until the top hole in the side of the corer was one-half centimeter below the surface of the core (see Figure 5). Redox potentials were then determined by placing a reference electrode on the sediment surface, and inserting a one mm platinum wire into the core at the predetermined depths. The reference electrode used was a standard fiber junction pH reference electrode modified by attachment of a fine frit Gooch crucible about its tip, and filling the crucible with saturated potassium chloride solution (Figure 5). The large surface area offered by the fritted bottom of the crucible insured proper contact between the probe and sediment. Measurements of potential were made with a Beckman Expandamatic pH meter following prior standardization in a solution of 0.03 Molar (M) potassium ferric cyanide, 0.03 M potassium ferrous cyanide, and 0.1 M potassium chloride, and having a redox potential of +430 mv.

Following measurement of redox potential, cores were extruded by means of a plunger into a specially constructed slicing trough (Figure 5). In this manner cores could be rapidly and easily sliced into the desired one centimeter sections for analysis. One half of each section was placed into dried and tared aluminum pans, reweighed, and dried at 105° C in an oven. Following this drying to a constant weight, samples were again weighed and then ashed at 600° C in a muffle furnace. In this manner it was possible to

determine water and volatile solids content.

The remaining half-section was further divided into three portions for total sulfide, particle size and free sulfide analysis. Particle size determination proceeded by placing one subsection into a 20 milliliter (ml) screw-cap test tube containing five ml of 3.0 N hydrochloric acid. The acid dissolved chitinous and shell material which was present and preserved the sample for future analysis. Samples were washed in distilled water, dispersed in 0.025 Calgon solution, and fine and sand fractions separated by passage through a 74 micron sieve.

Total sulfides (acid-soluble) were determined by a modification of the titrimetric method (2). A weighed subsection of sediment was placed into a 500 ml erlenmeyer flask containing about 200 ml of sparged distilled water, acidified with eight ml concentrated sulfuric acid, and the hydrogen sulfide evolved passed through two zinc acetate traps in a stream of nitrogen or carbon dioxide (Figure 6). Excess iodine was added to the zinc acetate solution and back-titrated with sodium thiosulfate.

Analytical difficulties were encountered with the determination of free sulfides, and this parameter was subsequently discarded.

Core Analysis - Bacterial

A number of cores, in addition to those taken for

chemical-physical analysis, were taken for bacterial analysis. Sampling was at the same depths in most cases as sampling for the other parameters. Inoculum consisted of one or two grams of sediment taken as aseptically as possible from the center of the core. Aerobic and anaerobic plate counts of bacteria were determined by culturing on marine agar 2216, and incubated at 25° C for five and seven days respectively. Numbers of sulfide producing bacteria were determined using the MPN technique on modified SIM or modified halophilic sulfate reducing media, with 16 day incubation at 25° C.

Water Analysis

Water directly above the sediment of the three sites was monitored during a tidal cycle for free sulfides and DO. The sampling apparatus is shown in Figure 7. With the tide out, the vertical rod (A) was driven into the sediment, and the boom arm (B) adjusted so that the two stainless steel sampling tubes (C) were located approximately one cm above the bottom. Samples for free sulfide and DO were obtained by simultaneously drawing water into plastic syringes attached by small bore tubing to the stainless steel sampling tubes. DO was determined on 20 ml water samples within the syringes by a 'micro-winkler' method (46). Free sulfides were determined by immediately fixing 20 ml of water sample inside the syringe with an equal volume of 50 percent antioxidant buffer solution. The standard

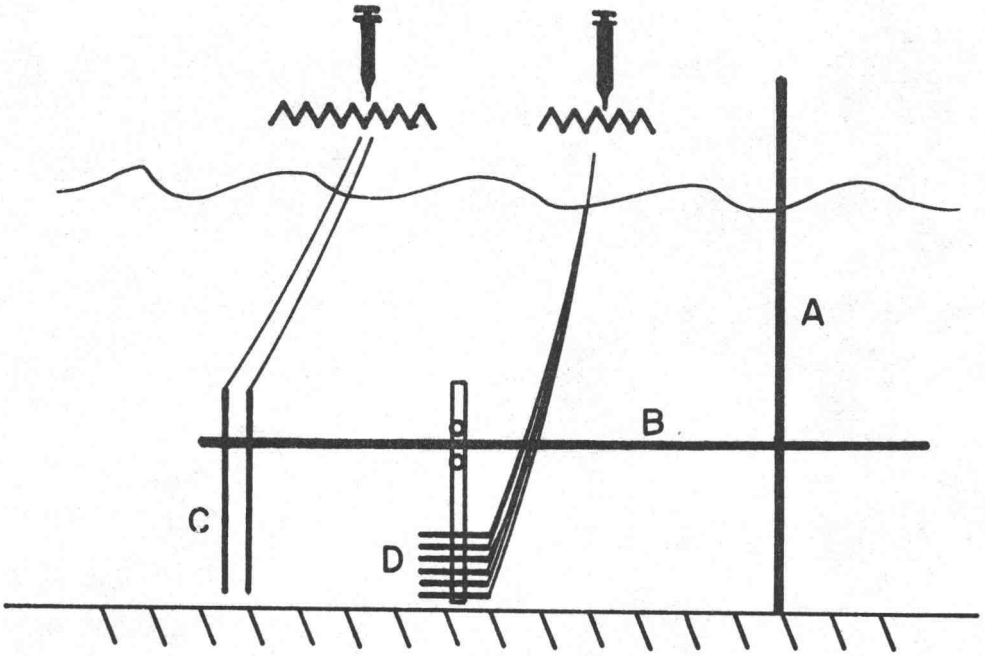


Figure 7. Diagram of apparatus for obtaining multiple samples of water above the sediment and for sampling during a tidal exchange.

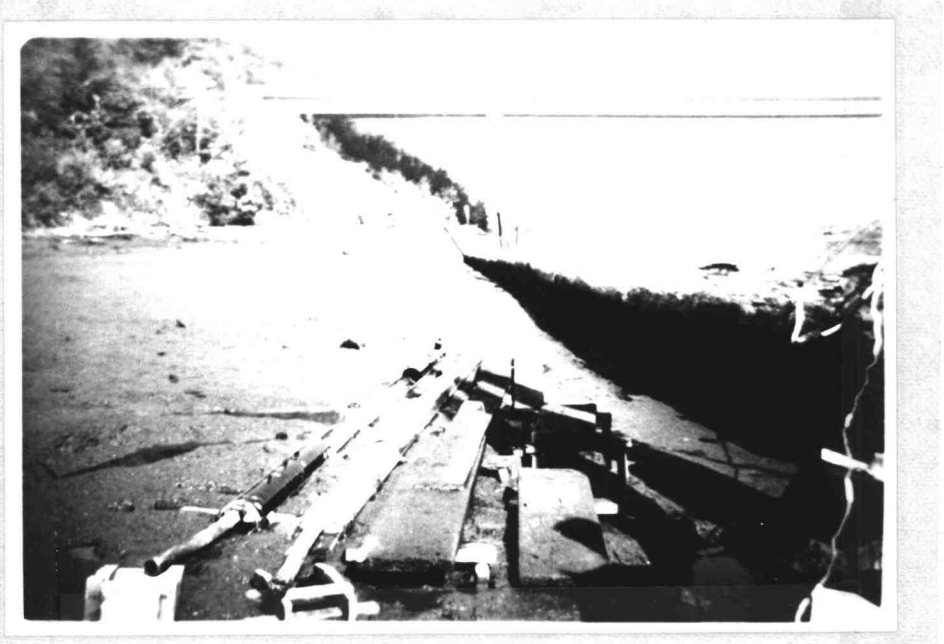


Figure 8. Partly assembled respirometer at site two.

solution, containing 320 grams (gm) of sodium salicylate, 72 gm ascorbic acid, 80 gm sodium hydroxide, and made up to one liter in distilled water, prevented further oxidation of free sulfide and fixed the free sulfide as essentially all free sulfide ion (59). Sulfide content was then obtained by measuring potential on a pH meter equipped with a sulfide selective membrane electrode, which responds to the activity of the free sulfide ion, and reading the free sulfide concentration from a standard curve developed prior to each run (59).

Profiles of free sulfide and DO immediately above the sediment were also occasionally obtained. Six stainless steel tubes were set horizontally at varying heights above the deposit (D - Figure 7). Six plastic syringes, mounted in a sampling head were attached by tygon tubing to the stainless steel tubes and samples drawn simultaneously by pulling on the sampling head. Free sulfides and DO were determined as previously described.

Oxygen uptake and free sulfide release of the sediment at site three was measured using an in situ respirometer, developed by a previous investigator for measurement of in situ oxygen uptake by bottom deposits (20). The respirometer consists of a plexiglas half-cylinder, sealed at both ends, with tubes leading to the surface for removal of samples. It is placed upon the deposit at low tide, and the edges pushed slightly into the sediment to hold it in place (Figure 8).

Results and Discussion

Transect Study - Site 1

Results of measurements obtained along the transect at site one are shown in Figure 9. Reported values of total sulfides and volatile solids are expressed in terms of wet sediment weight. Although the water content of the sediment varied between 19 and 36 percent and showed some tendency to decrease with depth, the majority of cores contained 20 - 25 percent water. The choice of elevations for each station was based upon tidal exposure data for this area (76), since it was felt that tidal exposure might be one important factor influencing the measured parameters. The -0.8 foot elevation is approximately at mean-low-low water (MLLW), 4.0 feet at mean sea level (MSL), 7.2 feet at mean high water (MHW), and -2.3 feet exposed only briefly by the very lowest tides each year.

Volatile solids were chosen as a general indicator of organic material present mainly because of the convenience of determination. It has been suggested that this measure of organic material is not as reliable as others. None of the methods, however, supply information on the availability of the organics to the microorganisms, and this is probably one of the more important features of the organics, especially from an ecological standpoint (14). It was felt

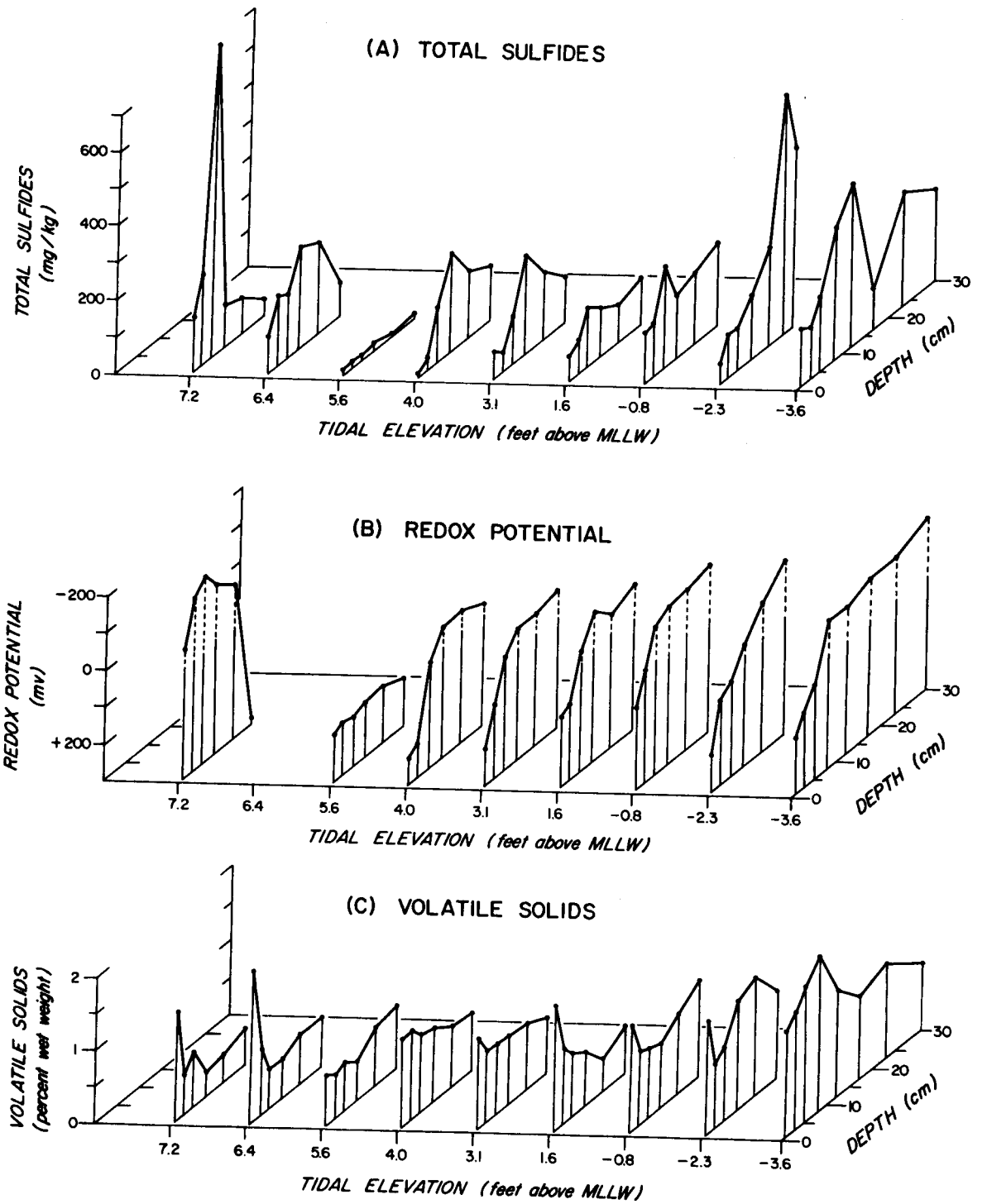


Figure 9. Measurements obtained within the sediments along the transect at site one.

that for a first approximation, the convenience of the volatile solids determination outweighed its disadvantages. The percent volatile solids at the various elevations and depths varied from 0.3 to 2.2. A general trend toward higher volatile solids with depth is apparent. If the organic material were largely associated with the solid phase of the sediment this might explain some of the increase with depth due to decreasing water content. The algae below the +4.0 foot station may contribute significantly to the input of organic material to the sediment, and the algal mat at the high station may have a similar effect.

The value of Redox (Eh) measurements in naturally occurring aquatic systems has been questioned because of the lack of equilibrium conditions, and consequent departure from thermodynamically predictable values (50). The practical value of this parameter, however, as an indicator of the general conditions necessary to the growth of microorganisms, has made it a very useful tool for microbiologists (80). In a very general sense, aerobic environments usually display positive Eh and anaerobic environments negative ones (50). Furthermore, the importance of the Eh to sulfate reduction has been mentioned, as has the reciprocal influence of this process upon the Eh.

At site one the Eh of the deposit was generally positive near the sediment surface, becoming rapidly negative with depth

(Figure 9). The two interesting exceptions to this observation occur at elevations 7.2 and 5.6 feet. At the high station Eh was negative at all depths, reaching a low of -210 mv four to five centimeters below the surface. This low corresponds with the recorded high value for total sulfide. The combined occurrence of these two parameters probably reflects not only the active production of sulfide within this area, but also its retention within the deposit due to lack of mixing. It is known that the presence of certain biological organisms may retard the passage of water through deposits and thus reduce hydraulic mising (7). Where the algal mat was evident it was observed that water collected and did not drain freely into the sediment. The permeability in the top 12 centimeters was only 8×10^{-4} cm/min, whereas in other adjacent deposits lacking the algal mat, permeabilities of $3 - 4 \times 10^{-2}$ cm/min have been measured (8). Thus sulfide appears to be produced and retained within the sediment here, further lowering the Eh, and hence augmenting the anaerobic conditions leading to its production.

At 5.6 feet total sulfides dropped to only one percent of the values recorded at the high station, and no redox potentials below +150 mv were recorded. Hydrological studies have revealed free drainage of water through the sediment at this elevation (8). Furthermore, this area was the site of extensive populations of burrowing organisms, and in particular Callianassa californiensis

and Upogebia pugettensis. Such mud shrimp actively mix the deposits, and a large exchange of water through their burrows, as indicated by dye studies, has been reported (20). It would appear from the observations at site one that production and accumulation of sulfides within such tidal flat deposits is probably closely related to the mixing processes occuring therein.

Comparison of Sites

As previously mentioned, vertical mixing within a tidal flat deposit depends upon a variety of factors. In particular the presence of fine particles tends to reduce vertical mixing. Because of its suspected importance, particle size distribution was determined at each of the sites (Table 1). It is readily noticed that there is a very significant difference in the general particle size among the sites. While site one averaged better than 90 percent sand, site two had a considerably smaller fraction of sand, and site three is primarily silt and clay. Based upon particle size alone, sites two and three would be expected to have reduced mixing within the deposits relative to site one. Lack of apparent large burrowing organisms at sites two and three would also contribute to a reduction of mixing within these sediments. Core analyses of the sediments reflect this reduction in vertical mixing (Table 2). Values reported at each depth for site one are the average of those measurements at all tidal

Table 1. Comparison of particle size at test sites.

Depth (cm)	Site 1		Site 2		Site 3	
	Sand (a)	Silt and clay (b)	Sand	Silt and clay	Sand	Silt and clay
0 - 1	92.2	7.8	15.8	84.2	2.9	97.1
2 - 3	99.3	0.7	14.0	86.0	1.3	98.7
4 - 5	87.6	12.4	8.0	92.0	0.6	99.4
7 - 8	93.1	6.9	9.7	90.3	2.3	97.7
11 - 12	95.3	4.7	11.9	88.1	3.9	96.1

(a) Percent of particles larger than 63 microns.
(b) Percent of particles smaller than 63 microns.

Table 2. Core analysis for total sulfides and volatile solids.

Depth (cm)	Site 1		Site 2		Site 3	
	Total sulfides (a)	Volatile solids (b)	Total sulfides	Volatile solids	Total sulfides	Volatile solids
0 - 1	74	1.5	116	7.0	788	4.0
2 - 3	90	0.9	330	5.3	1614	3.9
4 - 5	222	1.0	1400	5.4	1947	4.2
7 - 8	191	0.9	1200	6.8	870	5.8
11 - 12	190	0.9	1120	7.4	1013	5.9

(a) As mg sulfide per kg sediment.
(b) Percent wet weight of sediment.

elevations. Volatile solids ranged from four to seven percent at sites two and three, approximately five times the values at site one. Total sulfides were significantly higher than at site one with the average values at site three nearly ten-fold greater. In one core taken from the sediment of site three, total sulfide approached 7000 mg/kg sed. Although not shown, redox potentials were quite negative at both sites two and three. Values below -200 mv were recorded within the top 1/2 centimeter of sediment at site three, indicating highly anaerobic conditions, even within the surface layers of the deposit.

As previously mentioned, site three was characterized by extensive growth of an algal mat composed primarily of Vaucheria sp. During the early summer, this algal mat became very extensive and continuous throughout the area. Later in the summer, the algal mat broke up and by the middle of September was very patchy. In addition to the algal mat, the water and deposit surface had a purplish coloration which appeared to be due to large numbers of photosynthetic purple sulfur bacteria. Enrichment cultures, and Winogradsky columns inoculated with water from site three produced large colonies of both purple and green photosynthetic bacteria (63). These bacteria were apparently not present at the other sites.

The abundance of photosynthetic sulfur bacteria in this area suggested an available source of free sulfide. The low redox potentials of the sediments, presence of organic material, and large amounts of

total sulfide suggested that production of free sulfides was taking place Since the salinity of the water at this site reached 30 ppt it was reasonable to suspect that sulfate reduction is actively occurring, and might be the major source of free sulfides. Counts of sulfate reducing bacteria in the top centimeter layer here exceeded 10^5 per gram of wet sediment. This was approximately 10^2 - 10^3 times their numbers at site one (Table 3). While the mere presence of a microorganism in any given environment is not conclusive evidence of its activity, such high counts of sulfate reducers at site three, coupled with the other measured parameters, would strongly lend support to the belief that unusually high sulfate reduction was actively occurring there.

Release of Sulfides to Overlying Waters

A mathematical model has been developed which simulates the downward diffusion of dissolved oxygen into the aerobic zone of the sediment, the upward diffusion of oxygen demanding material from the anaerobic zone, and their subsequent reaction via second order kinetics (7). From this model and one simulating free sulfide concentrations in the water column above the sediment it was found that the free sulfide concentration in the overlying water will vary according to the relationship

$$S = f\left(\frac{1}{D}, \frac{1}{DO}, \frac{1}{Z}\right) \qquad (3)$$

Table 3. Bacterial counts per gram of wet sediment.

Location	Depth (cm)	Total plate count (a)	Sulfate Reducers (b)
Site 1, 3.1 ft. MLLW	0 - 1 (c)	6.3×10^6	3.6×10^2
	2 - 3 (c)	4.7×10^5	7.3×10^2
	4 - 5 (c)	1.5×10^5	3.6×10^2
	10 - 11 (c)	4.5×10^4	300
Site 1, -2.3 ft. MLLW	0 - 1 (c)	5.5×10^6	4.8×10^3
	2 - 3 (c)	1.2×10^6	4.2×10^3
	4 - 5 (c)	1.2 c 10^6	5.7×10^3
Site 2	0 - 1 (d)	3.7×10^6	1.2×10^5
	10 - 11 (d)	2.9×10^5	6.7×10^3
	20 - 21 (d)	1.9×10^5	4.2×10^3
	30 - 31 (e)	2.6×10^5	8.8×10^3
Site 3	0 - 1 (c)	1.4×10^7	2.5×10^5
	2 - 3 (c)	2.5×10^6	3.4×10^4
	4 - 5 (c)	1.4×10^6	4.6×10^4
	7 - 8 (c)	7.9×10^5	8.4×10^3
	11 - 12 (c)	2.2×10^5	6.8×10^3

(a) On marine agar 2216 (numbers per gram of wet sediment).
(b) MPN using modified SIM medium or a modified medium for halophilic sulfate reducing bacteria (36). (numbers per gram wet sediment).
(c) Average of samples taken on one date.
(d) Average of samples taken on three dates.
(e) Average of samples taken on four dates.

where D is the effective vertical mixing coefficient, DO is the dissolved oxygen concentration, and Z is the depth of the water above the sediment. Thus, those areas characterized by low DO in shallow waters overlying deposits in which there was reduced mixing would be prime regions in which to expect release of free sulfides to the overlying water to occur.

Free sulfide and DO profiles measured in the water column during daylight hours at site three are shown in Figure 10. Due to the benthic photosynthetic oxygenation, DO values were highest near the sediment surface, and despite the super-saturated DO values, significant free sulfide concentrations were still measured. The shape of the sulfide gradient strongly suggests that the sulfide input is from the sediment, and not the overlying water, as might be the case in the vicinity of the effluent of a sulfite paper mill.

Results from four sampling runs through tidal cycles at site three are shown in Figure 11. A sag in dissolved oxygen, at least partly due to the nighttime cessation of photosynthesis, is noted in 11b and 11c.

The lower DO values lead to higher free sulfide concentrations, as suggested by equation 3.

Following an initial drop in DO, the free sulfide and DO concentrations shown in Figures 11b and 11d become relatively stable for approximately three hours, with DO concentrations remaining at

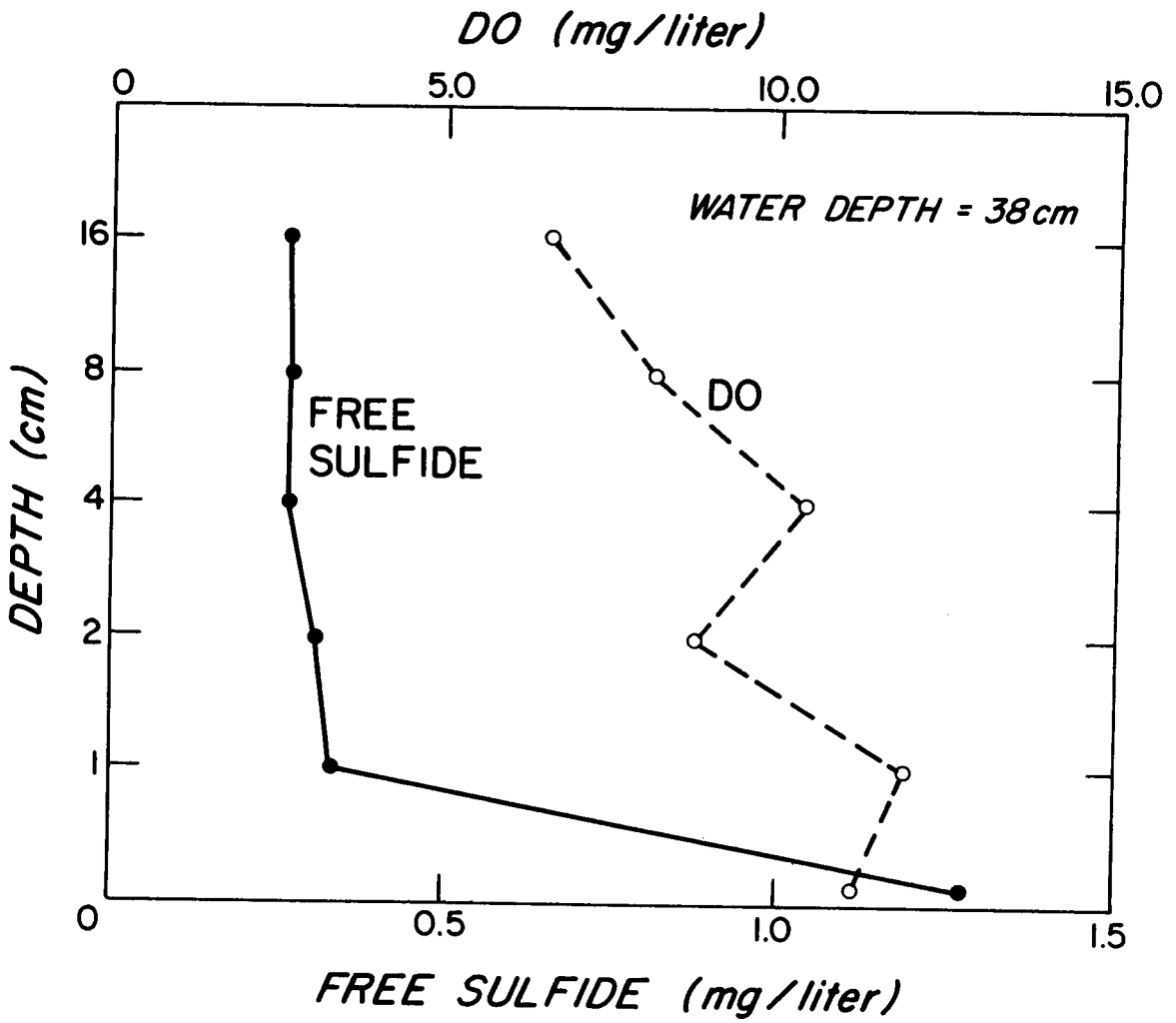


Figure 10. Profiles of DO and free sulfide above the sediment at site three.

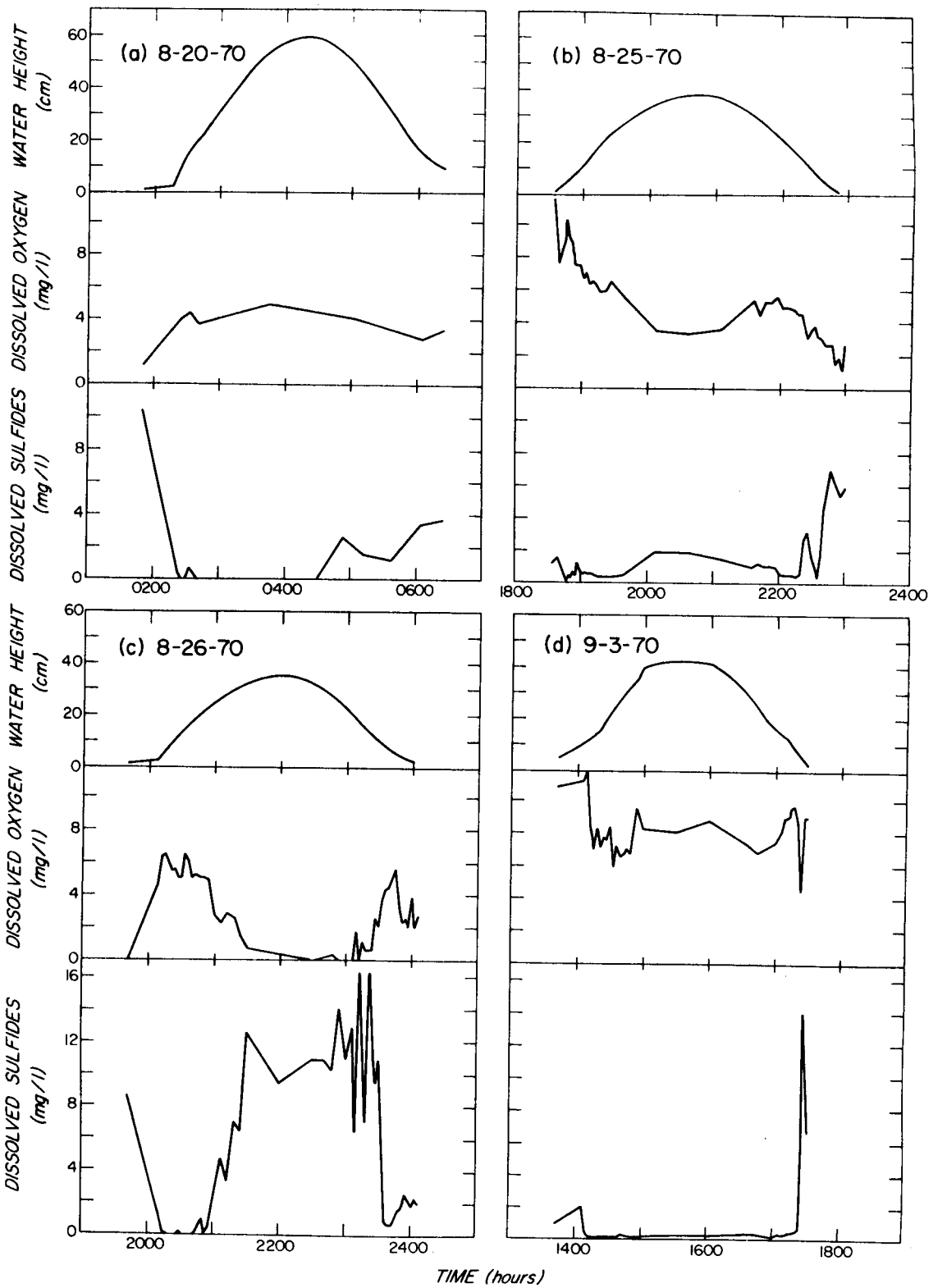


Figure 11. Variations in DO and free sulfides within the overlying water at site three.

approximately four and eight mg/l respectively.

No similar stability is found for the results shown in Figure 11c. Free sulfide concentrations varied considerably, with highest concentrations occurring when DO values were lowest.

The daytime results (Figure 11d) also display significant free sulfide concentrations, particularly during periods of shallow water depth. In fact,in general the results from site three show an increase in free sulfide concentrations as the tide ebbs, especially if DO is coincidently low. These results are also suggested by equation 3. Drainage from higher regions, from beneath the surrounding algal mat, and drainage of some interstitial water may have contributed to this increase as well.

A single respirometer run was conducted at site three using two opaque respirometers having a volume of 11.3 l and enclosing a sediment area of 0.14 m^2. The results of monitoring DO and free sulfide concentrations during the period in which the tide was covering the area are shown in Figure 12. The net rate of sulfide release shown is 1.6 gm/m^2-day, and the oxygen uptake rate 3.2 gm/m^2-day. Assuming oxidation of the sulfide to proceed according to equation 2, and further assuming up to one-half of the oxygen uptake to be due to reaction with the free sulfide then 1.6 gm/m^2-day of the sulfide could be oxidized. Hence the gross rate of sulfide release could approach 3.2 gm/m^2-day.

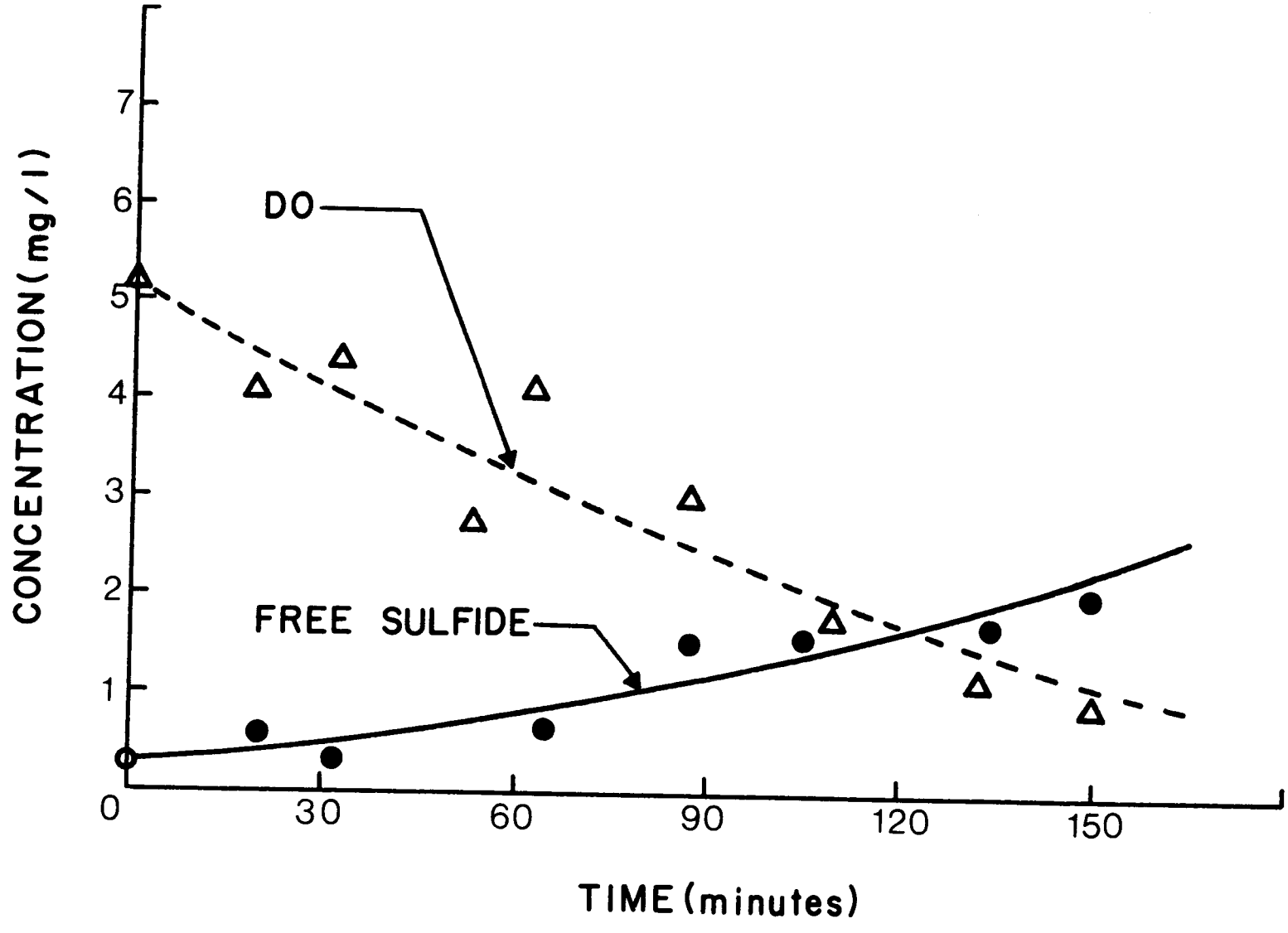


Figure 12. Results of the respirometer run at site three, 9/3/70.

No detectable free sulfides were measured within the overlying waters of site one during the late summer-early fall sampling period. Lower oxygen uptake rates (as determined by respirometer studies), larger particle size, greater hydraulic flushing, lower organic content, and relatively high DO concentration (seldom below 6 mg/l) found at site one, are all conditions which would tend to lead to low free sulfide concentrations.

At site two, fine silt and clay particles within the sediment and a relatively high organic content (wood chips from a nearby pulp mill and saw mill covered the sediments) provide conditions favorable to sulfide release. Water velocities here, however, were relatively high and the steep slope of the deposits prevented pools from forming at low tide. During the period of study, DO concentrations seldom fell below 5 mg/l. Total sulfides and organics within the sediments were relatively high at site two, however, a large fraction of the organic material was wood chips. Such material is largely lignin or other refractory organic, and may not have been available for assimilation by the bacteria.

The presence of free sulfides within the water at site two was detected only during ebbing of the tide where the water intersected the flat as it moved out. The concentrations never exceeded two mg/l, and site two was possibly characterized by a periodic, rather than continual release.

LABORATORY INVESTIGATIONS

General

Purpose of Laboratory Studies

The mathematical model of sulfide release mentioned previously assumes a source of free sulfide within the anaerobic deposits. These sulfides diffuse upward from the sediment into the overlying water. The realism of this model depends upon the existence of such a source, and hence the magnitude and rate of replenishment of this 'pool' of free sulfide is of fundamental importance to an understanding of the phenomenon of sulfide release, and of the system described in Figure 1. It was the purpose of these laboratory studies to investigate the nature of this free sulfide source and the mechanisms leading to its generation.

Studies of Sulfate Reduction

As pointed out earlier the major producers of free sulfides in marine and brackish water appear to be sulfate reducing bacteria. These organisms, belonging chiefly to the genus Desulfovibrio, are ecologically quite versatile, and are ubiquitously distributed in nature (81). They occupy habitats embracing a wide range of pH, salinity, Eh, temperature, and osmotic and hydrostatic pressure.

In general they are regarded as strict anaerobes, requiring redox potentials in the range -100 to -250 mv, and neutral and slightly alkaline pH (81). The relationship between pH and Eh is apparently a complex one. In environments more reducing than -150 mv, sulfate reducers remain active up to pH 9.5, whereas at 0 mv they can function at pH as acidic as 4.2 (64). Most cultures, however, appear to grow best between a pH of 6.2 and 7.9 and an Eh of -50 to -150 mv (81). Sulfate reduction itself tends to lower Eh and raise pH of environments in which it occurs, the magnitude of such effect depending upon the buffering capacity of the medium, and the end-products of the oxidation-reduction process (81).

Based upon salinity tolerance there appear to be two general, although somewhat indistinct, physiological types. Those found within soil, sewage, and fresh water are most active in solutions of less than one percent sodium chloride, and become inhibited at 1.5 - 3.0 percent concentrations. The other group, occurring in marine and brackish waters, appears to require sodium chloride solutions isotonic to sea water or sea water, itself (81).

The trace mineral requirements of these organisms are but imperfectly known and probably quite variable. Ferrous iron is essential, due to the presence of a cytrochrome system in species of Desulfovibrio (64).

Growth of sulfate reducers has been observed at temperatures

ranging from -11° to 104° C, but the majority occur in the ocean floor sediments at temperatures below 5° C. They seem to grow best at 15 - 40° C (81).

Tolerance to hydrostatic pressure is great, and sulfate reducers have been found to actively function at a pressure of 1000 atmospheres (81). As with the other factors, variability among species is great.

In addition to the general physical and chemical conditions mentioned, sulfate reducers require an energy source and available hydrogen acceptors to perform their metabolic activities. These two important inputs are indicated in Figure 1.

The abundance and activity of the sulfate reducers, and hence the production of free sulfide, will depend upon the availability of substances which can be oxidized as energy sources. While the variety of such substances is great, and the ability to utilize them varies among strains, the organic acids as a group (lactate, pyruvate, malate, citrate, proprionate) appear to be the most readily available and preferred energy source (81). In addition fatty acids, simple alcohols, and some mono and disaccahrides are suspect. Complex carbohydrates do not appear to be directly utilizable, but the importance of other microorganisms in the breakdown of these to utilizable forms has been noted (69). In addition to the heterotrophic forms, there are some autotrophs which can utilize molecular hydrogen, but

their abundance and activity is poorly known.

Sulfate ions appear to be by far the most common hydrogen acceptor (64). They are usually in abundant supply in sea water, and there is good evidence that the process of sulfate reduction may have been dominant and had global implications during past eras (65). Within fresh water systems, however, sulfates may become limiting, and in some lakes free sulfide production is linearly dependent upon the sulfate concentration (81).

There is some evidence suggesting that sulfate reduction is not limited until the sulfate concentration drops below 10 mg/l, but it is probable that halophilic strains become limited at much higher concentrations. In estuaries, sulfate reduction in bottom deposits is dependent on both a supply of sulfate and organic material within the interstitial and overlying waters, and sulfate may become limiting at the head, and organic material limiting at the mouth (36).

In addition to sulfates, the use of sulfite, thiosulfate, hydrosulfide, and several other sulfur oxides, as hydrogen acceptors, has been demonstrated (81). These compounds, however, are not generally widely available in nature, and are considered to be of considerably less significance. Since more energy is derived from the more oxidized form (68), it is probable that it would be preferentially utilized when available.

The ability of sulfate reducers to utilize elemental sulfur is

questionable (64, 81). Some autotrophic strains are apparently able to utilize carbon dioxide and the bicarbonate ion as a hydrogen acceptor (81).

In addition to those compounds required by the organisms, and which may be considered to promote the conversion of sulfate to sulfide, there are also compounds which inhibit sulfate reduction. In view of the toxicity of hydrogen sulfide to many organisms, the majority of interest in this regard has centered around the inhibitory nature of the free sulfides themselves. The question of whether the activity of sulfate reducing bacteria in nature and in synthetic media is affected by the hydrogen sulfide (or other free sulfide) produced has been examined by several investigators (27, 64. 81). It appears that the levels of free sulfide which may be tolerated are critically dependent upon pH, available sulfate, the nature of the energy source, and the presence of cations which may form insoluble sulfides (81).

Materials and Methods

Growth Media Preparation

Media containing organic material extracted from sediment or algae were prepared for growing mixed cultures of anaerobic bacteria collected from sites two and three.

Sediment extract media were prepared as follows from sediment collected from the upper five centimeters of deposit at each of the test sites.

A. One liter of distilled water was added to one liter of sediment in a large erlenmeyer flask, thoroughly mixed, and the resulting slurry autoclaved for 45 minutes at 121° C. After removal from the autoclave, the slurry was allowed to cool and the sediment removed by centrifugation. The resulting clear extract was either utilized directly as growth media, or lyophilized to produce a powder. In some cases this powder was added to liquid medium to yield one of higher organic concentration. If desired, the sulfate concentration was increased by addition of sodium sulfate, or decreased by adding barium chloride. The pH was adjusted to near neutral by addition of sodium hydroxide.

B. One liter of sea water was added to one liter of sediment and treated as with the addition of distilled water as described in method A.

C. One liter of either three or ten percent hydrochloric acid was added to one liter of sediment and treated as with the addition of distilled water as described in method A.

Algal Extract Media were prepared from the algal mat collected at site three by the following methods:

D. Two liters of algae and its associated water were placed into a three liter erlenmeyer flask and autoclaved as with sediment media. Following cooling, the algal material was squeezed using a wooden fruit press, and the liquid collected and centrifuged. Sulfate concentrations and pH of the media were adjusted as previously described.

E. Approximately 250 ml of 10 percent hydrochloric acid was added to 500 ml of algae and treated similar to that in method D.

F. Small amounts of liquid were extracted from both sediment (collected from the upper five centimeters at the sites) and algae (site three) by using an hydraulic press and specially designed squeezing cylinder (Figure 13). In addition, approximately one liter of extract was prepared by squeezing by hand algae which had been freshly collected from site three.

Experiment 1 - Addition of Organics

A number of 250 ml bottles were filled with sparged sediment medium (prepared by method B), and inoculated with one ml of a mixed culture containing sulfate reducing bacteria. The culture was supplied from a previous experiment which involved utilization of organic material by mixed cultures of anaerobic bacteria from the sediment of site two (63). The tops of the bottles were closed tightly with a serum cap, and incubated at 25° C in the dark. At

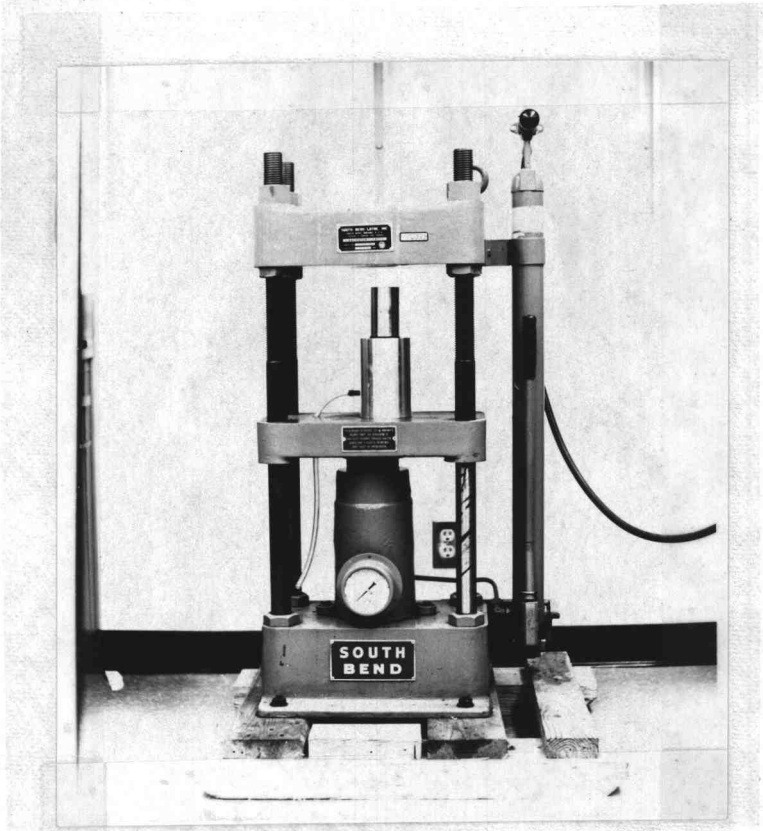


Figure 13. Apparatus for squeezing the interstitial water from sediments and algae.

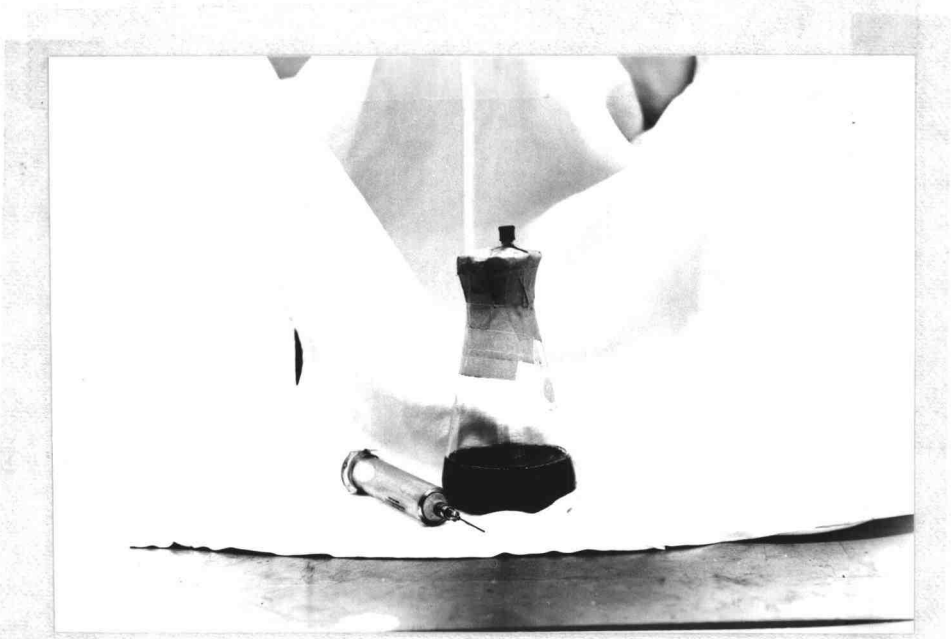


Figure 14. Culture vessels used in studying the rates of sulfide production by mixed cultures of anaerobes.

various intervals during the experiment, lyophilized medium was added to the bottles to observe the effect of the added organic material upon sulfide production. Samples were removed at intervals and analyzed for total sulfide, sulfate, and soluble organic carbon.

Experiment 2 - Rates of Sulfate Reduction

Approximately 600 ml of algal extract medium (prepared according to method D) were placed into each of 12,500 ml erlenmeyer flasks, organic concentrations adjusted by dilution, and desired sulfate levels achieved as previously described. Sodium chloride was added where necessary to adjust the chlorinity of each culture to approximately 20 ppt. In addition, two cultures were similarly prepared using media prepared by hand squeezing algae from site three. Oxygen was initially removed by sparging for 15 minutes with carbon dioxide-free nitrogen. Following sparging, the pH was adjusted to 7.5 - 8.0 by addition of 3.0 N sodium hydroxide, and Eh lowered to approximately -100 mv by addition of a small quantity of sodium sulfide. Each flask was inoculated with two ml of mixed culture (obtained from active cultures of experiment one) and immediately capped by a rubber stopper fitted with a glass tube and serum cap. To prevent leakage, modeling clay was liberally applied around the edges, and the stopper further fastened down by masking tape (Figure 16). Each flask was shaken to disperse the inoculum,

and initial samples were taken for analysis. The flasks were then incubated in the dark at 20° C.

A control was set up by filling several tubes with sterile extract, and capping tightly. At intervals, a tube was opened, the contents removed, and treated in a manner identical to that of the experimental samples.

Sampling procedure

Samples were withdrawn from the flasks at appropriate intervals with a syringe which had been flushed and prefilled with nitrogen. By exchanging the gas for the sample, the flask was maintained anaerobic and development of a negative pressure due to extraction of the samples was avoided. Flasks were shaken thoroughly prior to sampling in order to produce a fairly homogeneous medium, giving a more representative sample. Analyses for sulfides, sulfates, and soluble organic carbon followed as soon as practical. In the event that storage was necessary, carbon samples were frozen in tightly stoppered screw-cap test tubes. Sulfate samples were similarly stored following removal of free sulfides.

Analytical Procedures

Free sulfide was determined on a 10 ml sample by the procedure described previously. Total sulfide was measured by adding

10 ml of sample to a known volume of acidified 0.025 N iodine solution and back-titrating with 0.025 sodium thiosulfate.

Sulfate was determined by a colorimetric procedure using barium chloranilate (10). Samples were passed through a Dowex 50w-x8 20-50 mesh H^+ cation exchange column to remove interfering ions, diluted to 40 ml if necessary, and added to 50 ml of 95 percent ethanol and 10 ml potassium pthalate buffer. Approximately three grams of barium chloranilate were added to precipitate the sulfate. After shaking for ten minutes, the solution was filtered, and the optical density determined on the filtrate with a spectrophotometer. Sulfate concentrations were read from a standard curve.

Soluble organic carbon was used as a measure of the soluble organic material present. Five ml of centrifuged sample were placed into 20 ml screw cap test tubes in an ice bath, and carbonate carbon removed by acidifying to pH 2.0 - 3.0 with three percent phosphoric acid and sparging with carbon dioxide-free nitrogen for 10 minutes. Determination of the remaining soluble organic material was made using a Lira Infrared Analyzer Model 3000.

Sugar and organic acid analyses of the media were made by gas chromatography, Eh measured with a platinum wire electrode, and pH with indicator paper.

Results and Discussion

Growth Media Preparation

The results of preparing media by the various methods of organic extraction are summarized in Table 4. Early attempts at preparing media from sediment (methods A and B) inevitably resulted in media having fairly low organic carbon concentrations (100 - 800 mg/l). Extraction methods with acid resulted in higher concentrations of organic carbon, but a large percentage of this organic carbon precipitated out upon adjustment of the pH with sodium hydroxide to neutral. Hence little was gained in the final media by using acid extraction.

Of the extracts made without the use of acid, the algal extracts contained by far more organics than the others. This is not surprising, as the concentration of organics within the water squeezed from the algal mat were as high as 3000 mg/l, whereas the organic content of the interstitial water of the sediments of sites one, two, and three, were relatively low. It appears that the algal mat may be a potential source of considerable organic material at site three. Concentrations of soluble organic carbon in the water overlying algae retained in a large plastic pan for several days in the warm laboratory rose from 150 mg/l to over 3000 mg/l. These observations,

Table 4. Summary of soluble organic extract procedures.

Sample material	Extraction procedure	Soluble organic carbon (mg/l)[a]	Sulfate[a] (mg/l)
Site 1 - sediment	A[b]	150 - 250	nm.[c]
	C[b]	150 - 300	nm.
	F[b]	50	3200
Site 2 - sediment	B	100 - 150	nm.
	A	100 - 800	10 - 300
	C[b]	3200	nm.
	F[b]	60 - 110	100
Site 3 - sediment	A[b]	200 - 300	200
	C[b]	1600 - 3400	1400
	F[b]	140 - 310	1000 - 1500
Site 3 - algal mat	D	1500 - 3000	3400 - 4000
	E[b]	5400	nm.
	F[b]	2010	4800
	F(hand squeezed)	1125	2150

(a) Approximate.

(b) These extracts not used for media.

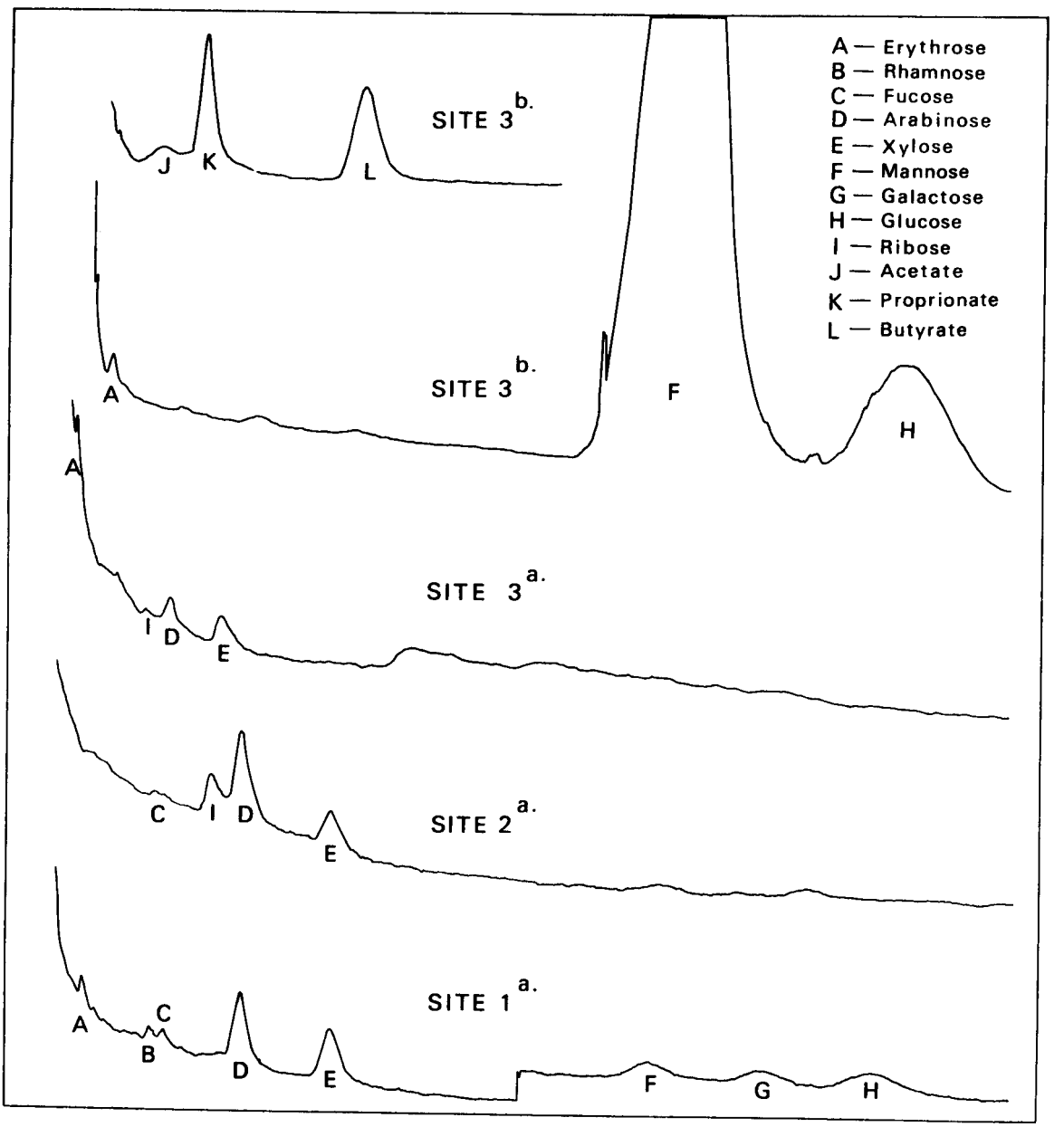
(c) Not measured.

while crude, would suggest that especially large quantities of organics may be released to the water overlying the sediments at site three as the algae decomposes. This release is not entirely surprising in light of other studies which indicate that 38 percent of the gross production of some benthic marine algae may be released directly into the water as soluble organic compounds during growth (43). This organic material would probably be available to sulfate reducing bacteria, and may account for the apparent high sulfide production at site three and under the algal mat at 7.2 ft (MLLW) at site one. Although attempts previously described failed to measure sulfide release at site one, these were made during the early summer. During the late summer and fall the Enteromorpha and Zostera present at site one begin to decompose (76). During this same period, redox potentials dropped dramatically in the upper few centimeters of the sediment, microinvertebrates were observed to migrate from the sediments into the overlying algal material, and the smell of hydrogen sulfide was very noticeable (76). It is likely that sulfide release may occur at site one during this period.

Results of squeezing the water from the algal mat of site three show surprisingly high concentrations of sulfate (Table 4). The salinity of the water overlying the sediments at site three on one occasion was 19.7 parts per thousand (ppt), whereas the salinity of water squeezed from the algal mat was 23.2 ppt. Corresponding

values for sulfate were 1.2 ppt and 4.8 ppt. The enrichment of sulfate within the algal mat (3.6 ppt) would entirely account for the higher salinity occurring there. From this data it appears possible that the algae are concentrating sulfate. Such an ability to concentrate sulfate, if it exists within fresh water algae, has implications concerning sulfate reduction in fresh water systems, where the average sulfate concentrations of the water may be limiting to sulfate reduction.

The results of sugar and organic acid analyses of some of the media are shown in Figure 15. The pentoses (generally considered wood sugars) found in the sediment extract of site two, reflect the input of wood products to this area. Sediment extracts from site one show small amounts of both pentoses and hexoses suggesting that the organic material there may come from more diverse sources. The relatively lower levels at site one reflect the lower organic concentrations measured here. The sugar and organic acid analysis of site three algal extract is rather dramatic. Very large concentrations of mannose and glucose, both hexoses, as well as high levels of proprionate, butyrate, and acetate are present. Although the absolute concentrations of the organic acids cannot be computed with reasonable accuracy (standards were not run), it is estimated that the sugars mannose and glucose account for approximately two-thirds of the organic material in these extracts.



a. Extracts from sediment — method A
b. Extracts from algae — method F

Figure 15. Results of gas chromatography analysis of some of the extracts for sugars and organic acids.

It was recognized from the onset that the extraction procedures would possibly affect the organic material present in the sediment or algae. While it was realized that treatment with either heat or acid might produce hydrolysis of some of the sugars, denaturation of some of the protein, and loss of some volatile compounds, this was the only method available of obtaining sufficient quantities of extract of high enough organic content to use for growth media. Whether this alteration in the organic content would seriously affect the ability of the sulfate reducing bacteria to utilize it, and consequently lead to sulfide production rates which were not representative of those occurring in the field, could not be determined at this early date.

Early Experimental Efforts

In the early phase of the laboratory studies a preliminary experiment was conducted using methods and materials similar to those used in experiment two. While little quantitative information was derived due to some errors in experimental design, sampling, and frequent instrument malfunction, the results were valuable in the design of the subsequent experiments. Initial concentrations of soluble organic carbon in the media used in this early experiment (prepared by methods A and B) never exceeded 800 mg/l, and in the cultures having the higher sulfate levels, never exceeded 400 mg/l. The initial conditions in the cultures were characterized by slightly

acidic pH and Eh ranging from +100 to +200 mv. In most cases, by the time suitable conditions of Eh and pH for the growth of the sulfate reducing bacteria were reached, the organic carbon concentrations had dropped to only half the initial values. It was assumed that other bacteria present in the inoculum, consisting of several grams of sediment from site two, had utilized this organic material.

In the few cultures which eventually did show some sulfide production, the maximum rates of production were much lower than anticipated, and never exceeded 30 milligrams of sulfide per liter per day (mg/l-day). It was felt that organic deficiency was limiting production. Hence experiment one was developed to determine the effect of an input of organic material after proper conditions of pH and Eh existed.

Results of Experiment 1

Results of the organic addition studies are plotted in Figures 16-21. Vertical dashed lines locate dates on which lyophilized medium was added to the cultures. Numbers along the curves indicate the rates of sulfide production in milligrams per liter per day (mg/l-day) measured during the period(s) immediately following addition of the organic material. Data was not collected between approximately the 35th and 60th days of the experiment because of instrument failure.

In general, the rates of sulfide production increased with

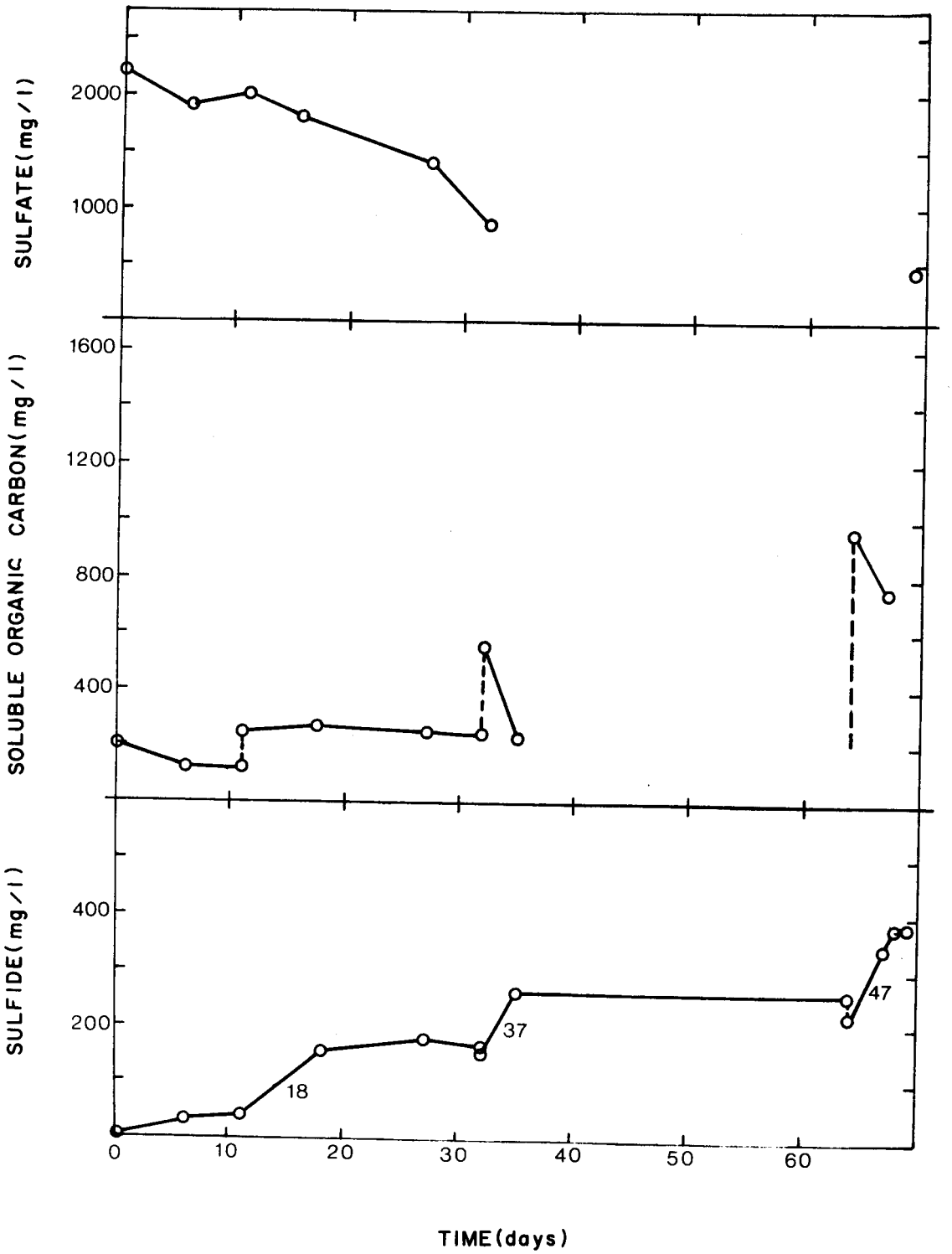


Figure 16. Results of experiment 1. Culture a.

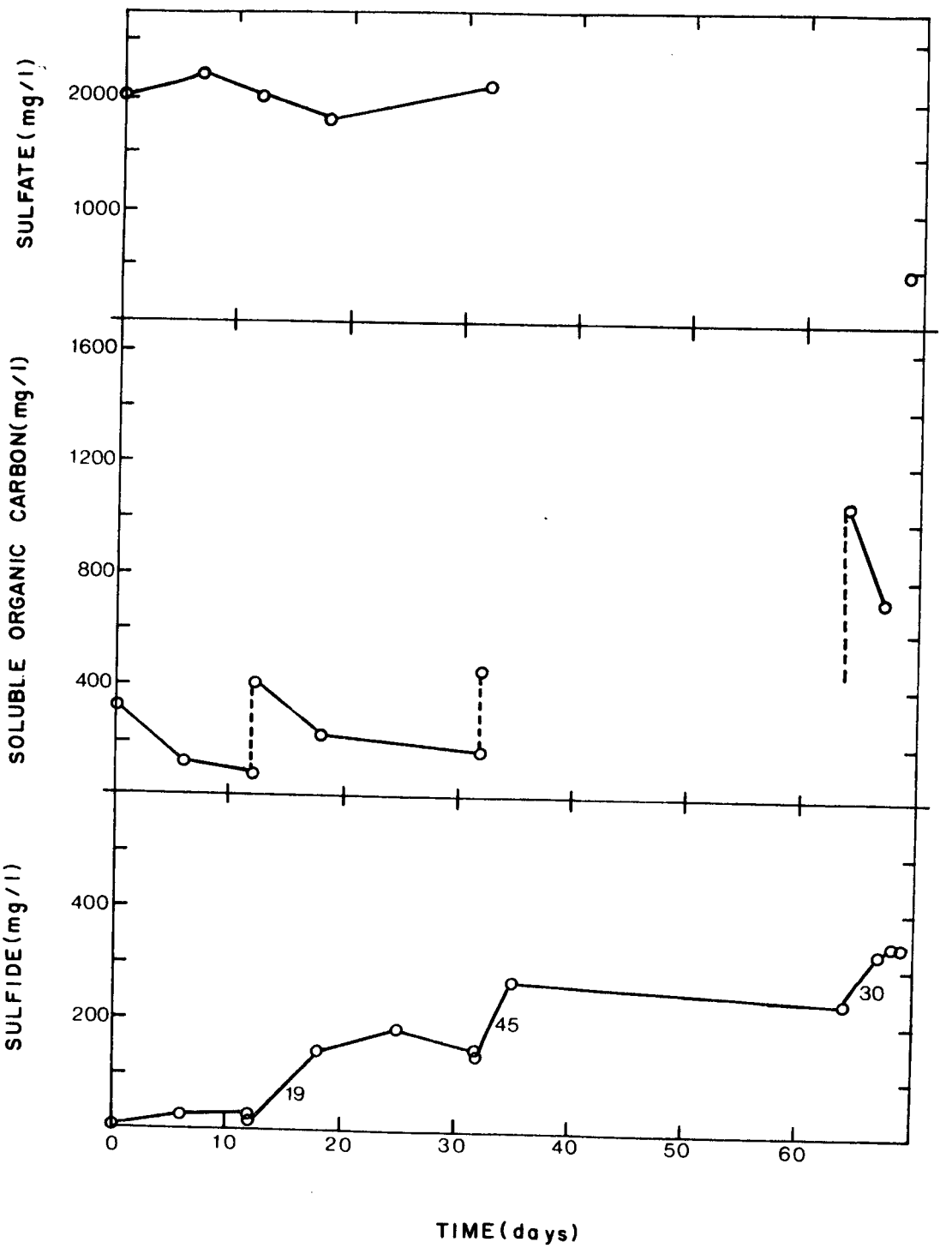


Figure 17. Results of experiment 1. Culture b.

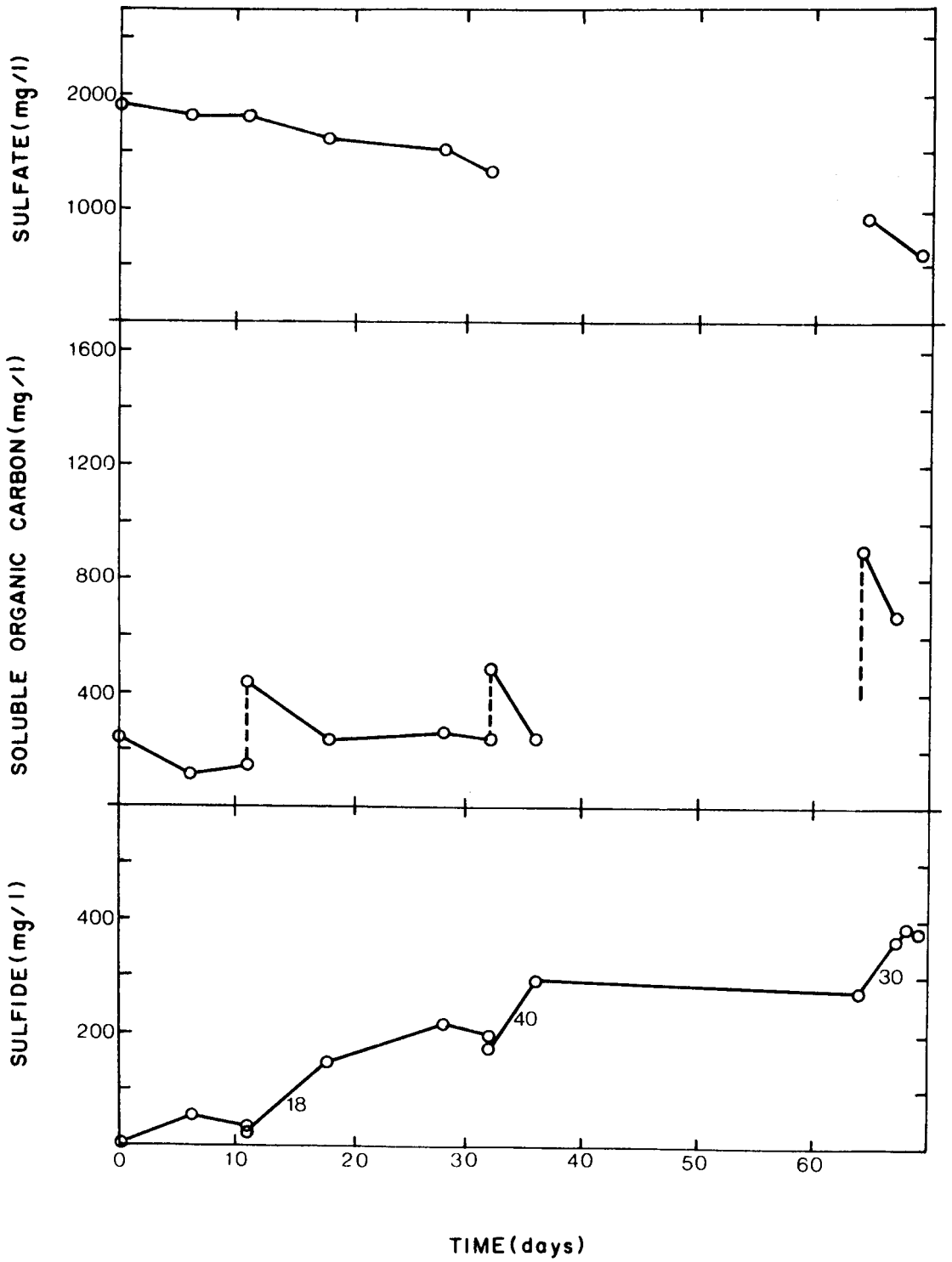


Figure 18. Results of experiment 1. Culture c.

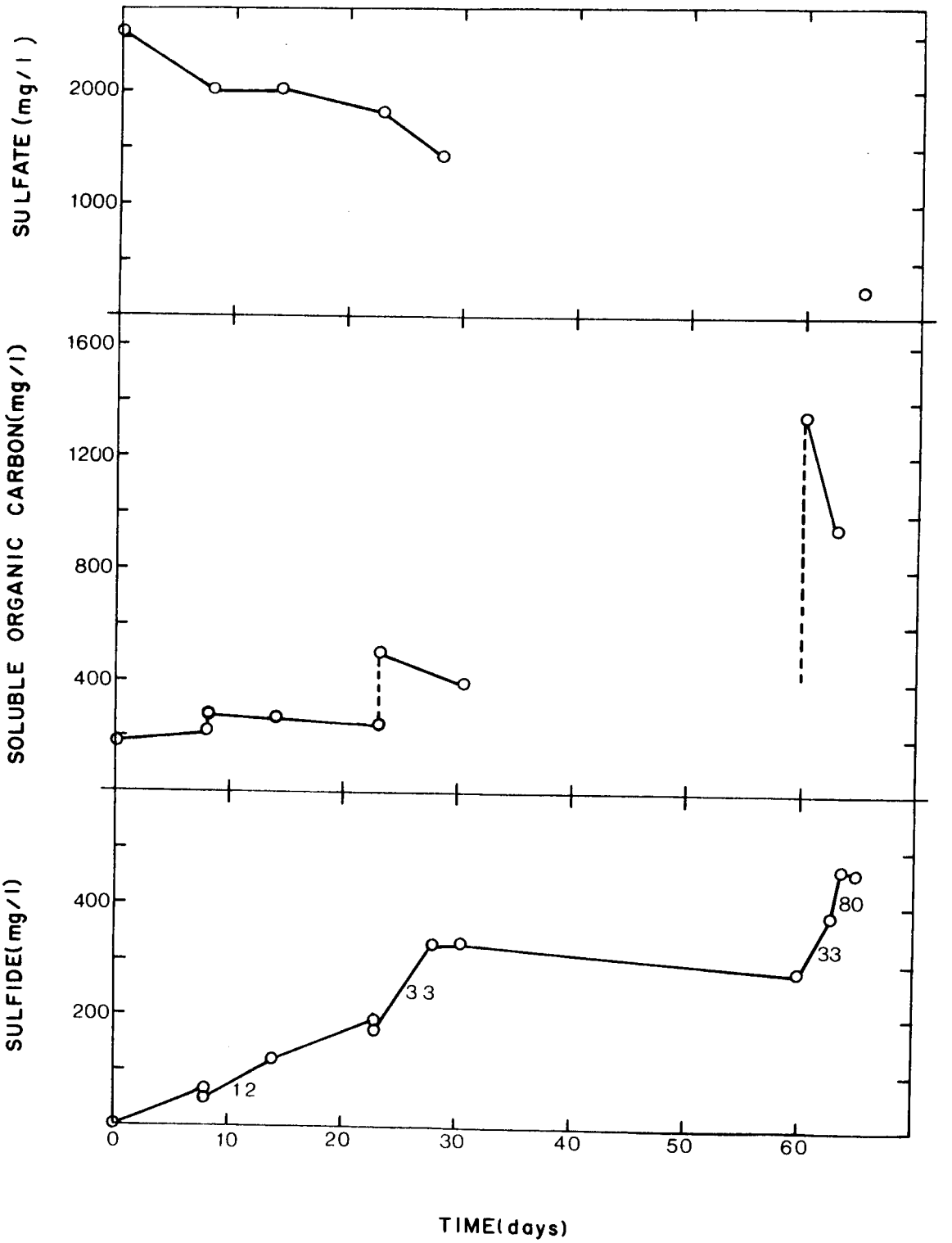


Figure 19. Results of experiment 1. Culture d.

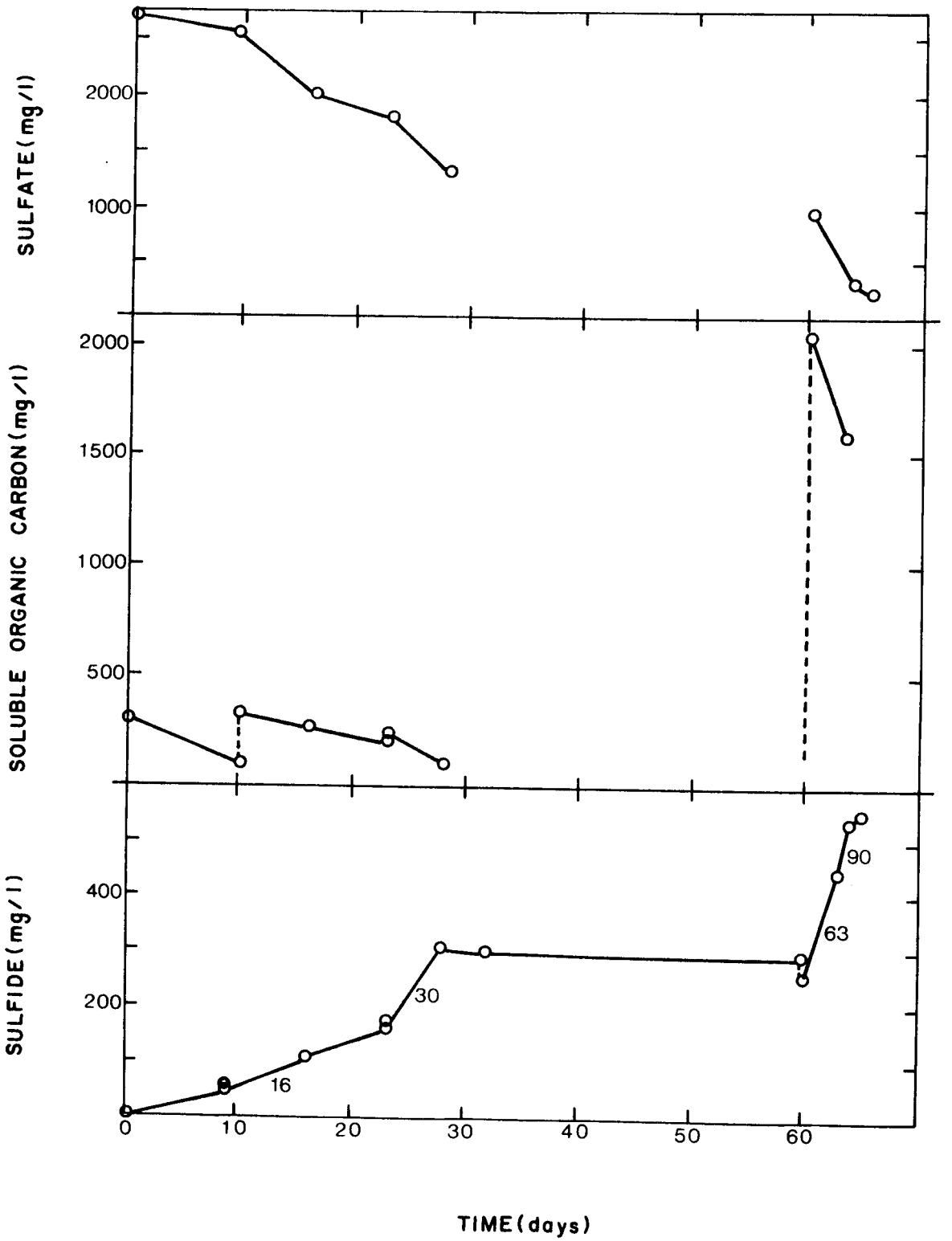


Figure 20. Results of experiment 1. Culture e.

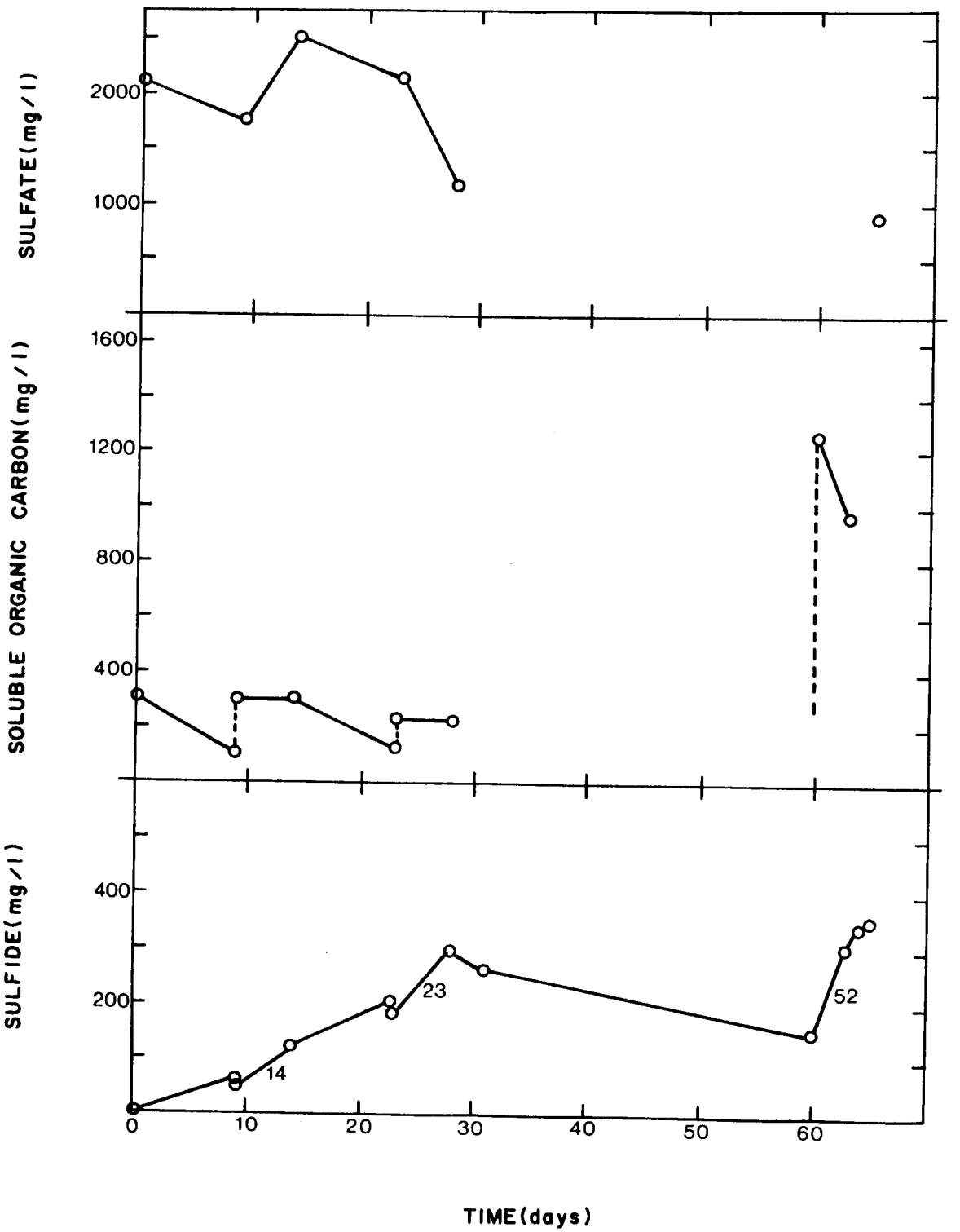


Figure 21. Results of experiment 1. Culture f.

subsequent addition of organics through the experiment. It is possible that this could result from a gradual acclimatization of the organisms to their environment, or to a development of more optimal conditions for the production of sulfide as the runs progressed. It is doubtful, however, that such changes would take such a long time to occur, and it is more likely that the increased rates are the result of other factors.

In most cases, the larger the amount of organic material added, the greater the rate of sulfide production following such addition. This is clearly the case in cultures a, d, e and f. The final measurements taken in d and f further suggest that a lag in sulfide production, following organic addition, took place. This would explain the contrary results in cultures b and c. If a lag time of one to two days were present following addition on the 64th day, then rates of 30 mg/l-day are an underestimation of the actual rates. A larger lag-time would be expected following this final addition, since the cultures had been inactive (as indicated by lack of sulfide production) for 30 days prior to it. Rates are probably underestimated in all cases where sampling intervals exceed one day.

Evidence from the literature suggests that the vast majority of sulfide produced when adequate quantities of sulfate are present, is a result of dissimilatory sulfate reduction (30, 80). While it is possible that some of the sulfide produced may be a result of

putrefication of bound organic sulfur, this is probably a minor fraction. Consider, for example, culture 2, days 60 to 63, and estimate that the organic material present contains one mg of bound sulfur for every 100 mg organic carbon. This is a realistic estimate based on studies of the mineral content of plants, phytoplankton, and benthic algae (1, 67, 72). If sulfide were released by putrefication as organic carbon were utilized, then the utilization of 450 mg/l of organic carbon during days 60 to 63 would correspond to a production of 4.5 mg/l of sulfide. During this period, however, 190 mg/l of sulfide were produced, and thus little more than two percent may be attributable to putrefication.

In sulfate reduction, three mg of sulfate is utilized in the production of one mg of sulfide. This relationship is independent of the energy source metabolized (41, 81). Production of 190 mg/l of total sulfide should theoretically account for a utilization of 570 mg/l of sulfate. The 625 mg/l decrease in sulfate during this interval is close to the theoretical amount utilized in sulfate reduction considering the errors inherent in this method of sulfate determination.

In addition, proteolytic bacteria, were added to a sterile flask containing this growth medium, and no measurable sulfide was produced. This evidence further substantiates the assumption that sulfate reduction is the dominant mechanism leading to sulfide production in this experiment.

Examination of the relationship between only sulfate concentration and the rate of sulfide production implies that higher rates of production occur at lower sulfate concentrations. In view of the evidence advanced suggesting that sulfate reduction is the main mechanism leading to sulfide production in this experiment, this inverse relationship is probably an artifact produced by the combined circumstances of gradually decreasing sulfate throughout the run, and higher inputs of organic material at successive additions.

The highest rate of sulfide production measured (90 mg/l-day) occurred in culture e when the sulfate concentration was below 500 mg/l. When the sulfate level dropped to 250 - 300 mg/l, however, the sulfide production was observed to drop off dramatically. Since an estimated 1200 - 1300 mg/l of soluble organic carbon still remained on the 65th day (estimated by extrapolation of data), it is most probable that the sulfate, and not the organic, levels limited production. A similar situation probably occurred in culture d. A rate of 80 mg/l-day occurred here at organic carbon concentrations several hundred mg/l lower than the maximum rate measured in culture e. Limitation by sulfate deficiency appeared to develop here as well. Although the exact concentration at which it occurred is uncertain, it appears to have been at about the same level as that in culture e.

If only about 50 percent of the organic material added were

utilizable by the sulfate reducing bacteria, then the possibility does exist that organic limitation may have been responsible for the reduced rate of sulfide production at the termination of the experiment. This is considered unlikely, however, since the percent utilization of added organic carbon in cultures A (32nd-35th day) and B (32nd-36th day) was considerably greater than 50 percent.

Eh and pH were measured in the cultures at the termination of the experiment. In all cases the Eh was -180 to -220 mv., and the pH 7.5 - 8.2. These ranges are in agreement with the measured values of these parameters in sediments where active sulfide production was occurring (Section II), and are characteristic of conditions necessary for and resulting from the growth of sulfate reducers (64, 80).

Results of Experiment 2

General. The results of experiment 2 are plotted in Figures 22 through 35. Cultures 1 through 12 contained media prepared from autoclaved algae (method E), whereas cultures 13 and 14 contained media prepared from squeezed unautoclaved algae (method F). In cultures 1 through 6 the initial concentrations of soluble organic carbon were at a uniformly high level, while the sulfate concentrations were successively lower. In cultures 7 through 12 sulfate was initially high while soluble organic carbon was successively lower.

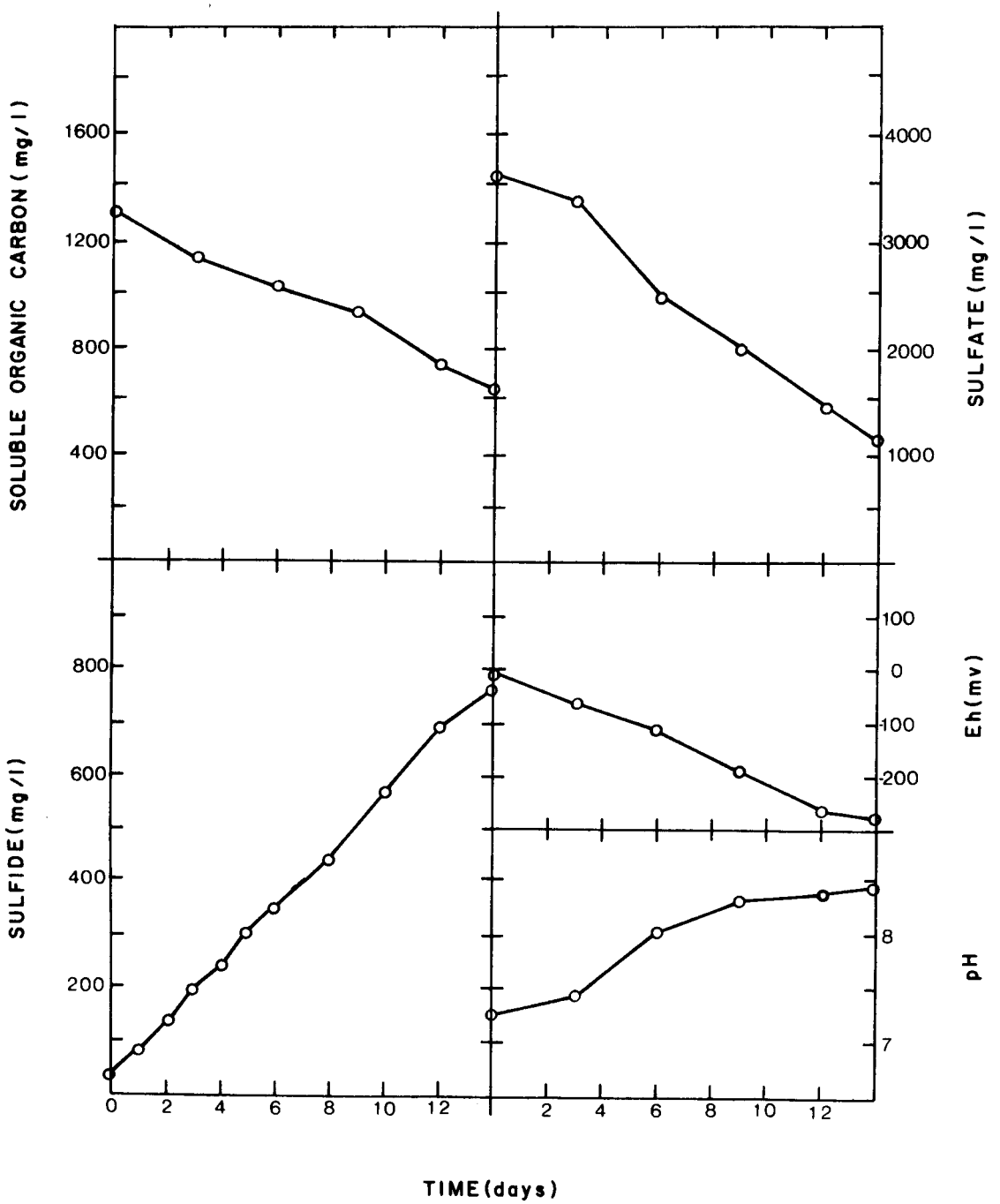


Figure 22. Results of experiment 2. Culture 1.

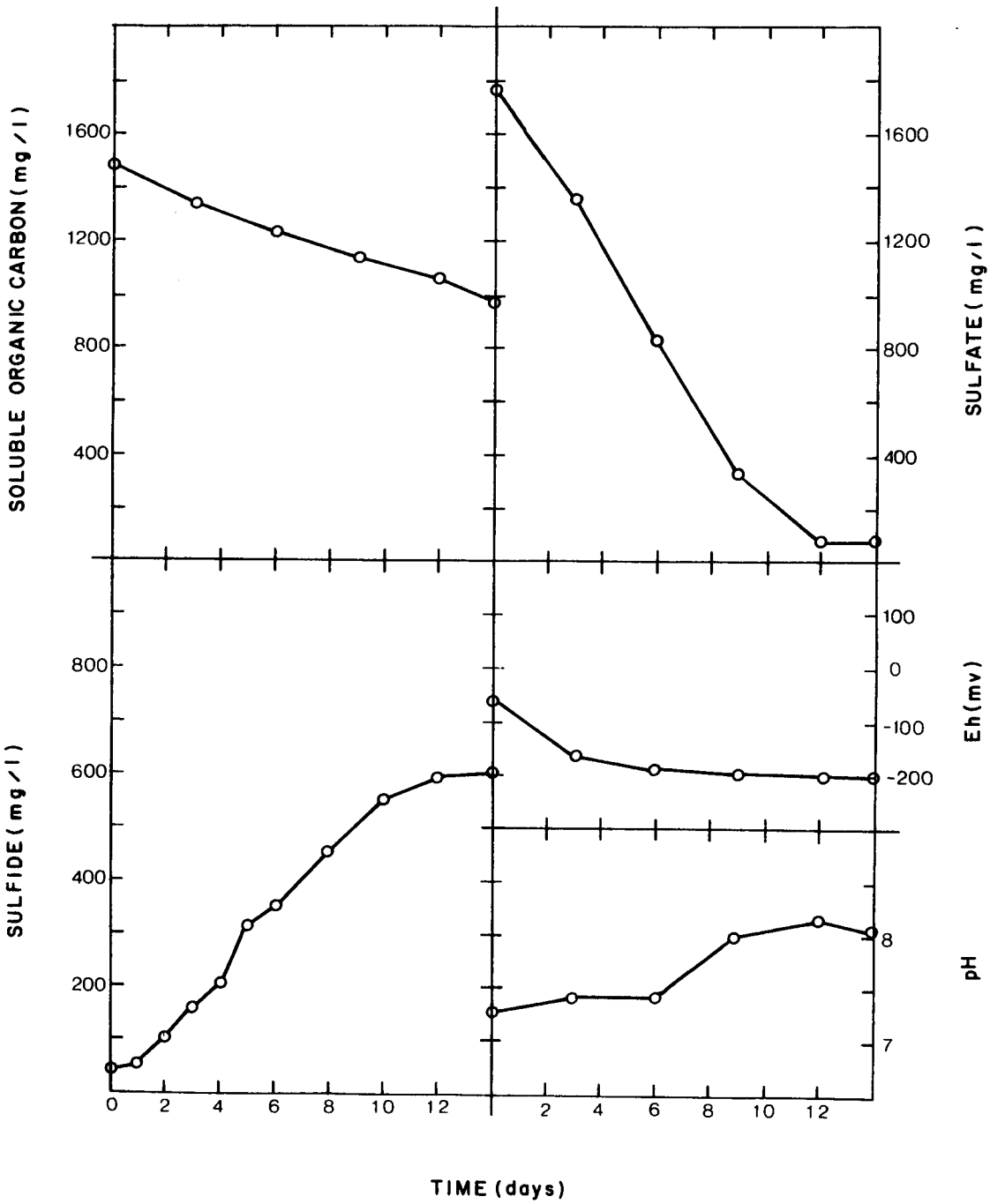


Figure 23. Results of experiment 2. Culture 2.

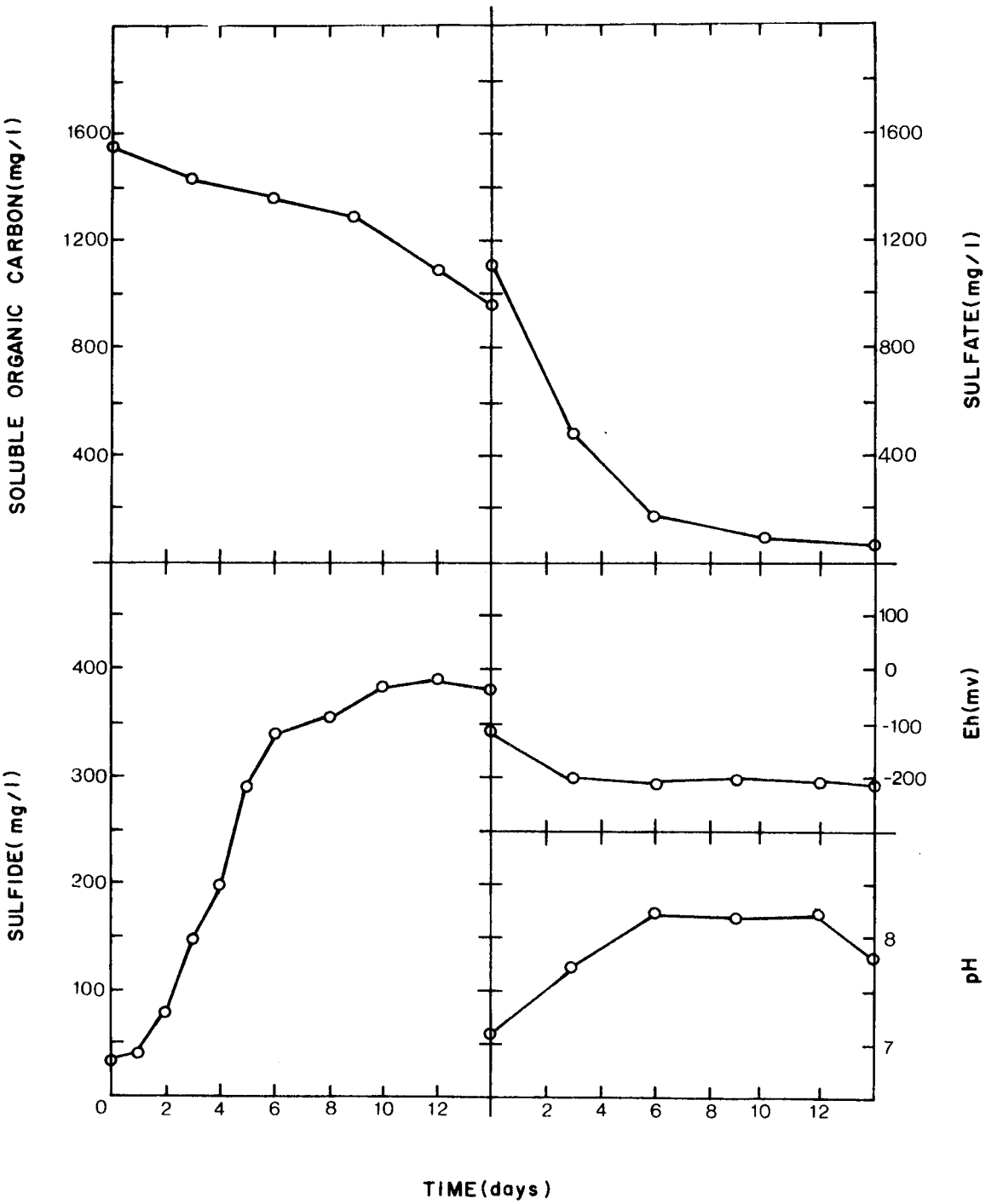


Figure 24. Results of experiment 2. Culture 3.

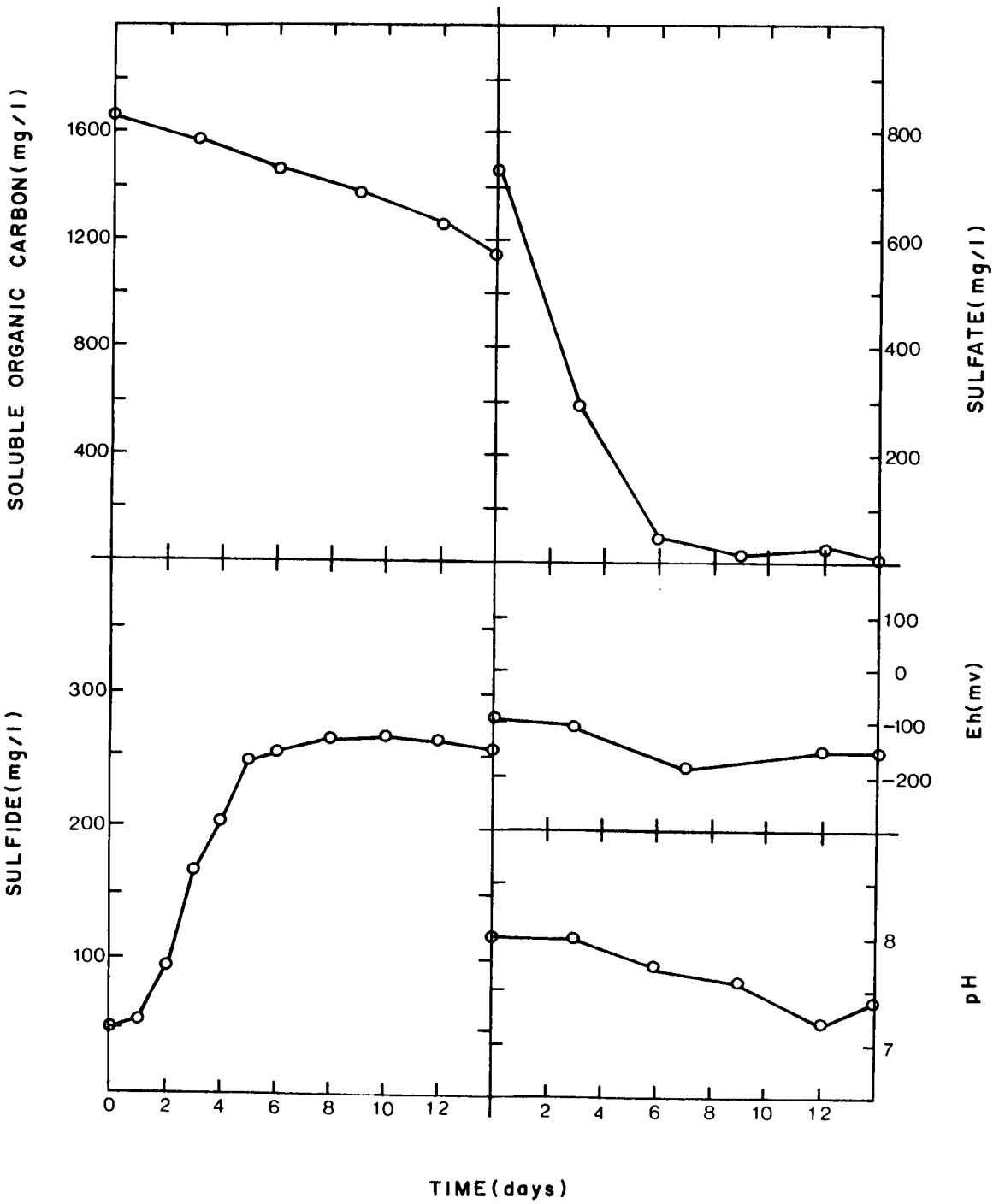


Figure 25. Results of experiment 2. Culture 4.

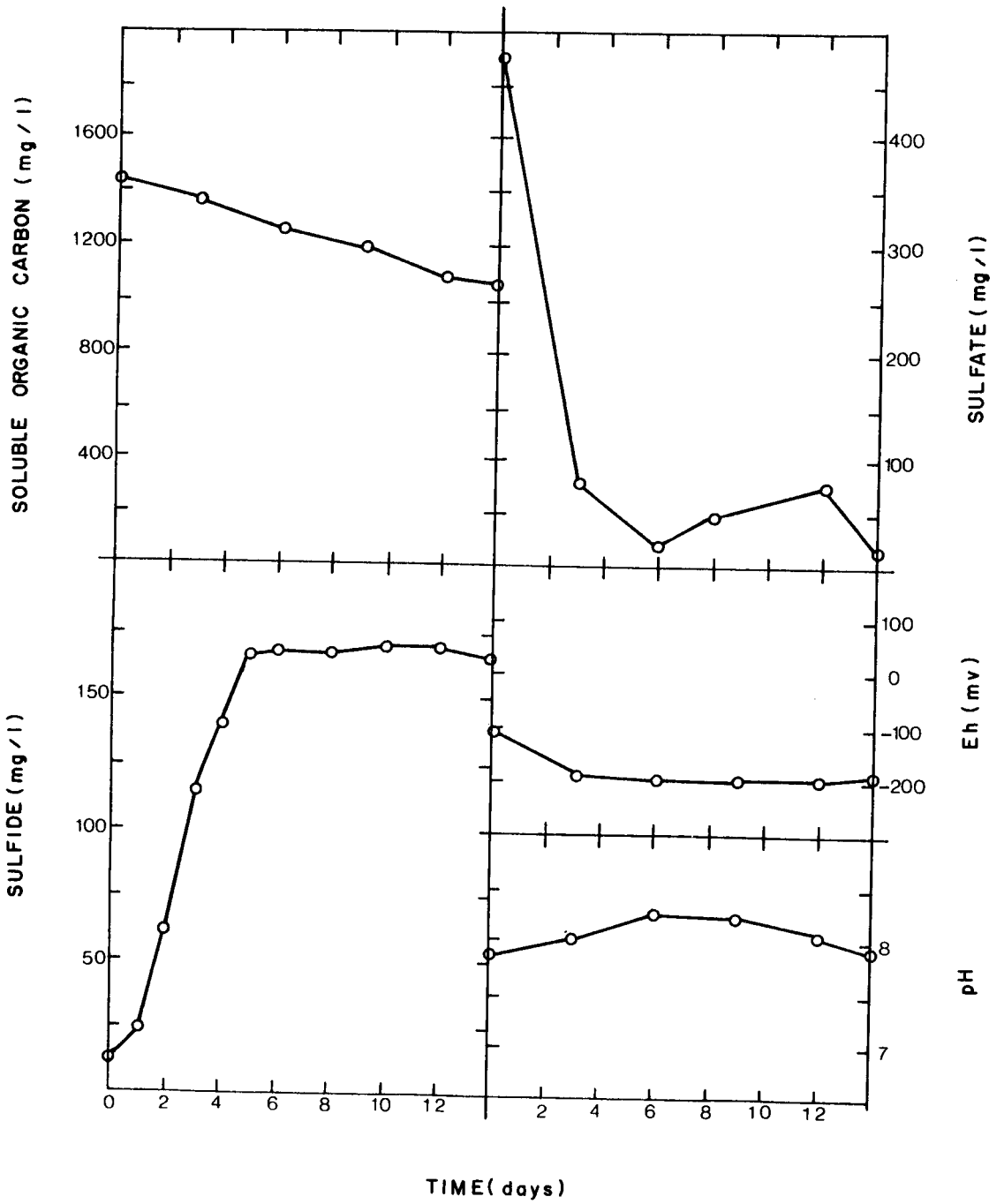


Figure 26. Results of experiment 2. Culture 5.

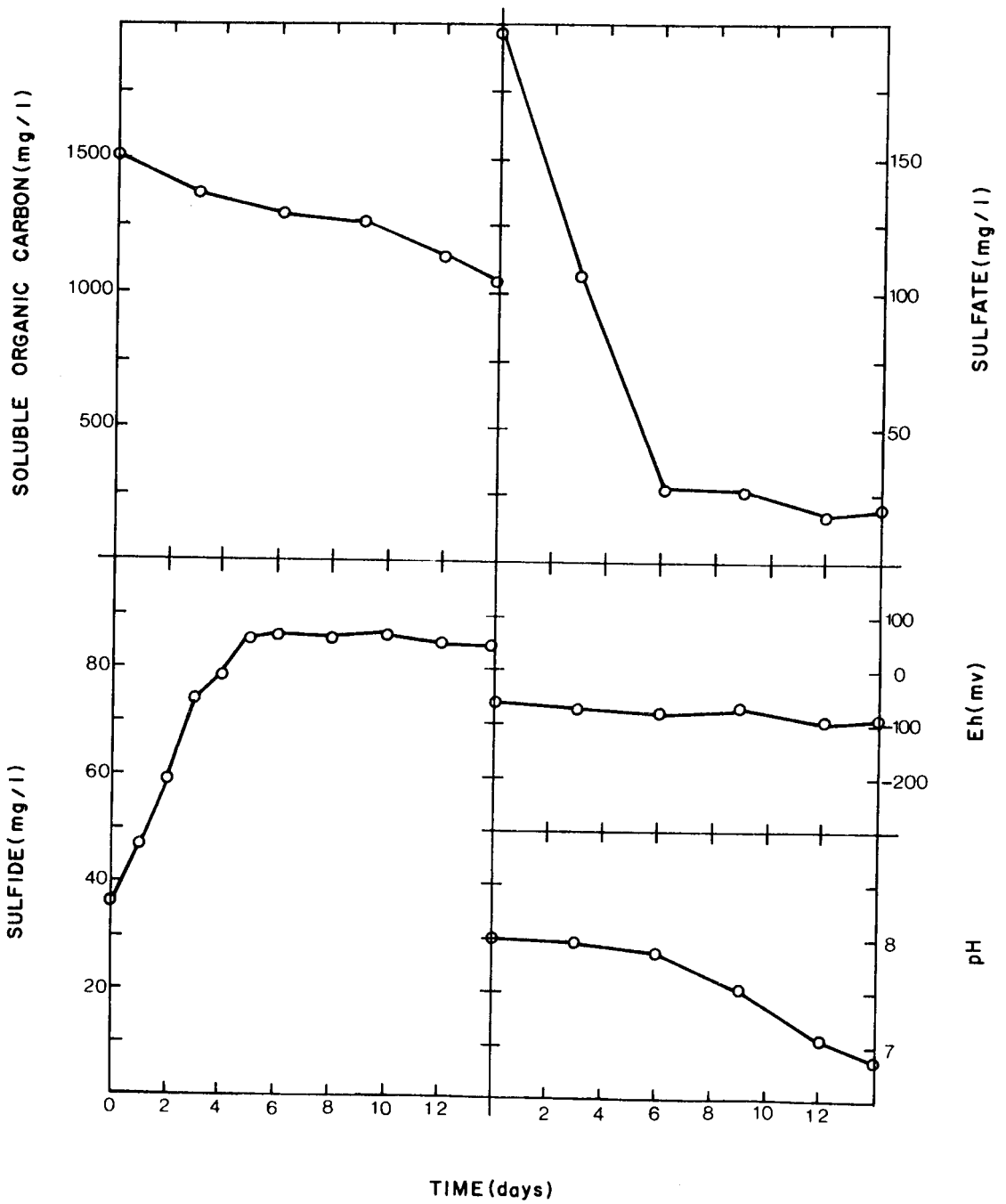


Figure 27. Results of experiment 2. Culture 6.

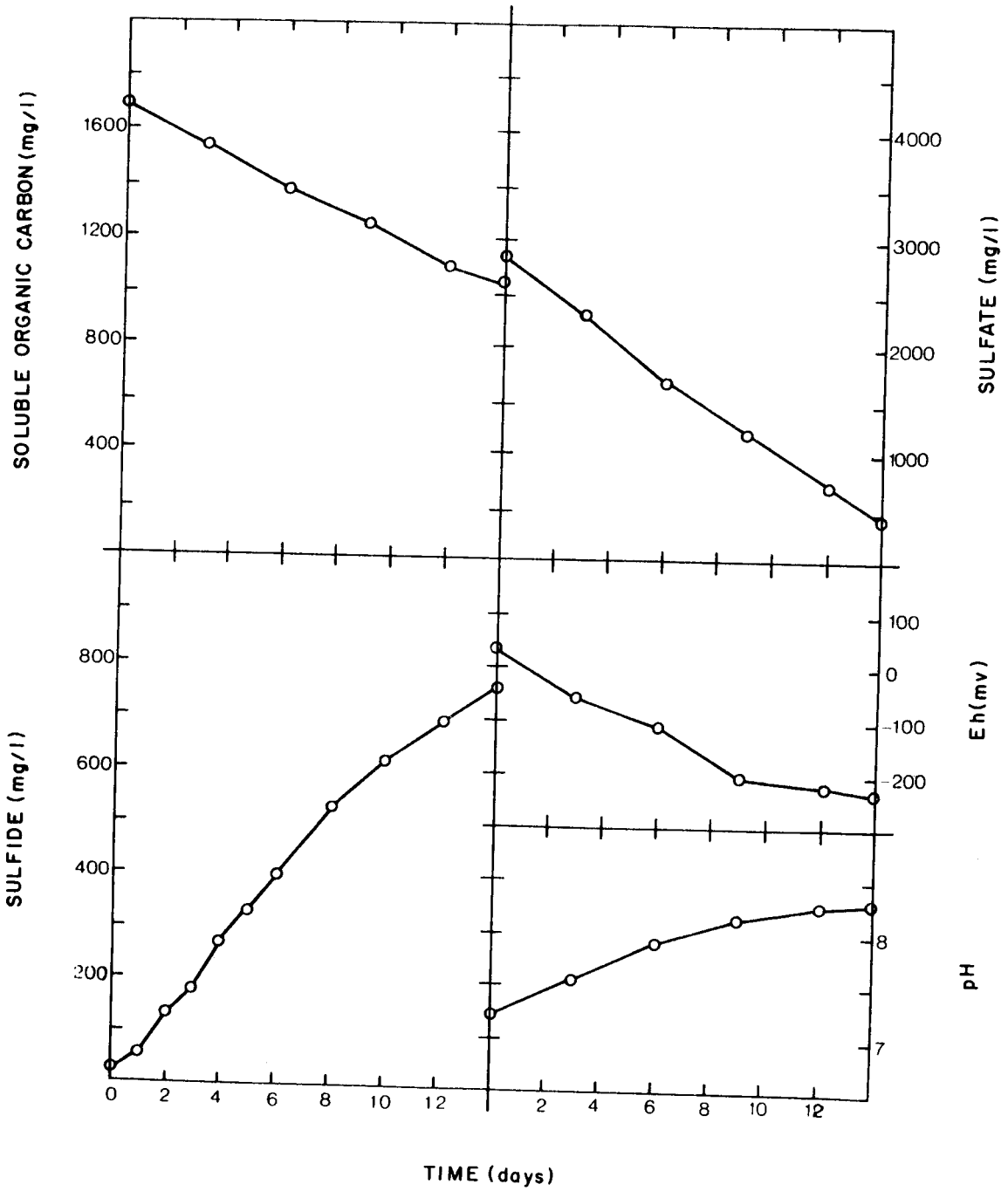


Figure 28. Results of experiment 2. Culture 7.

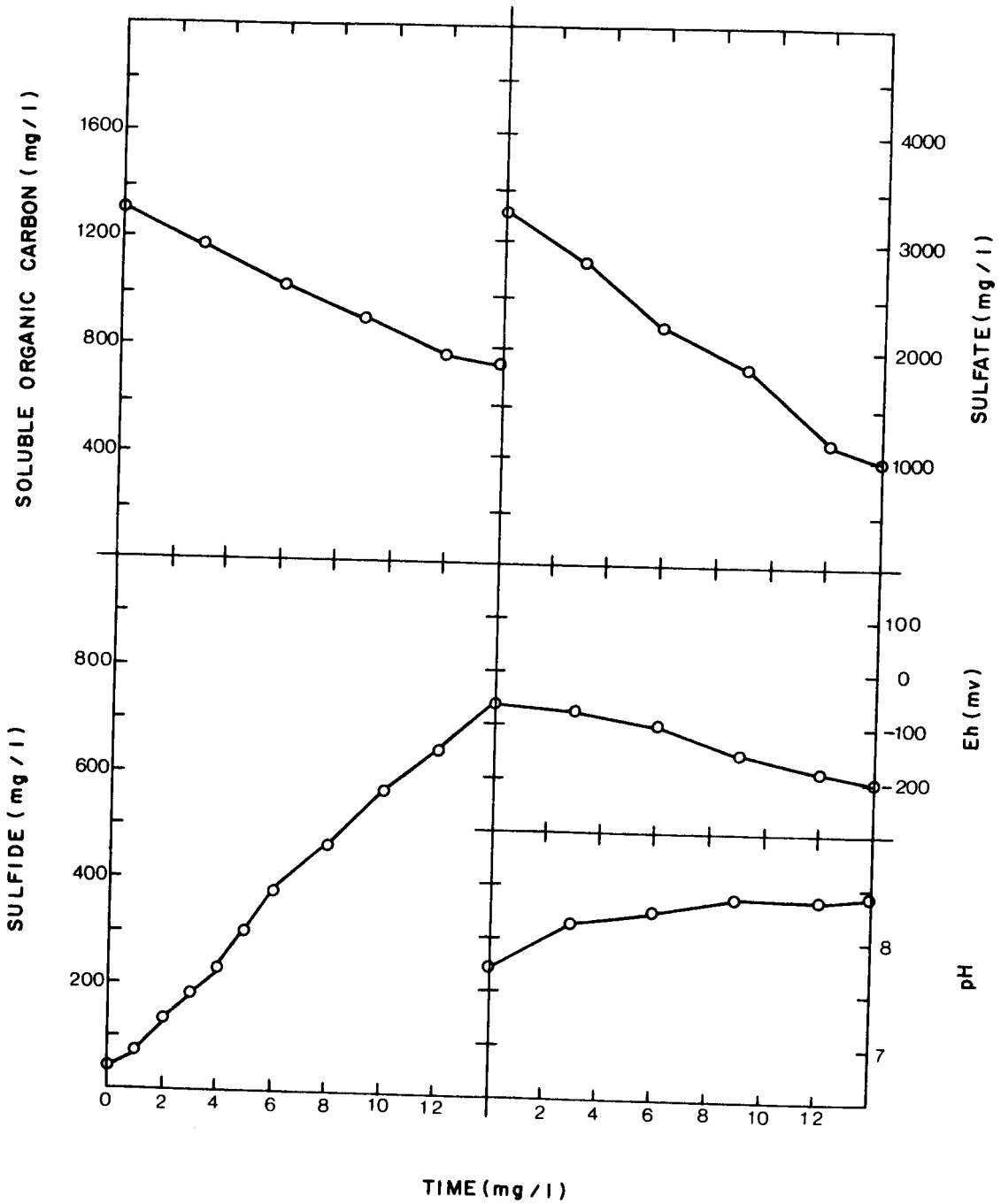


Figure 29. Results of experiment 2. Culture 8.

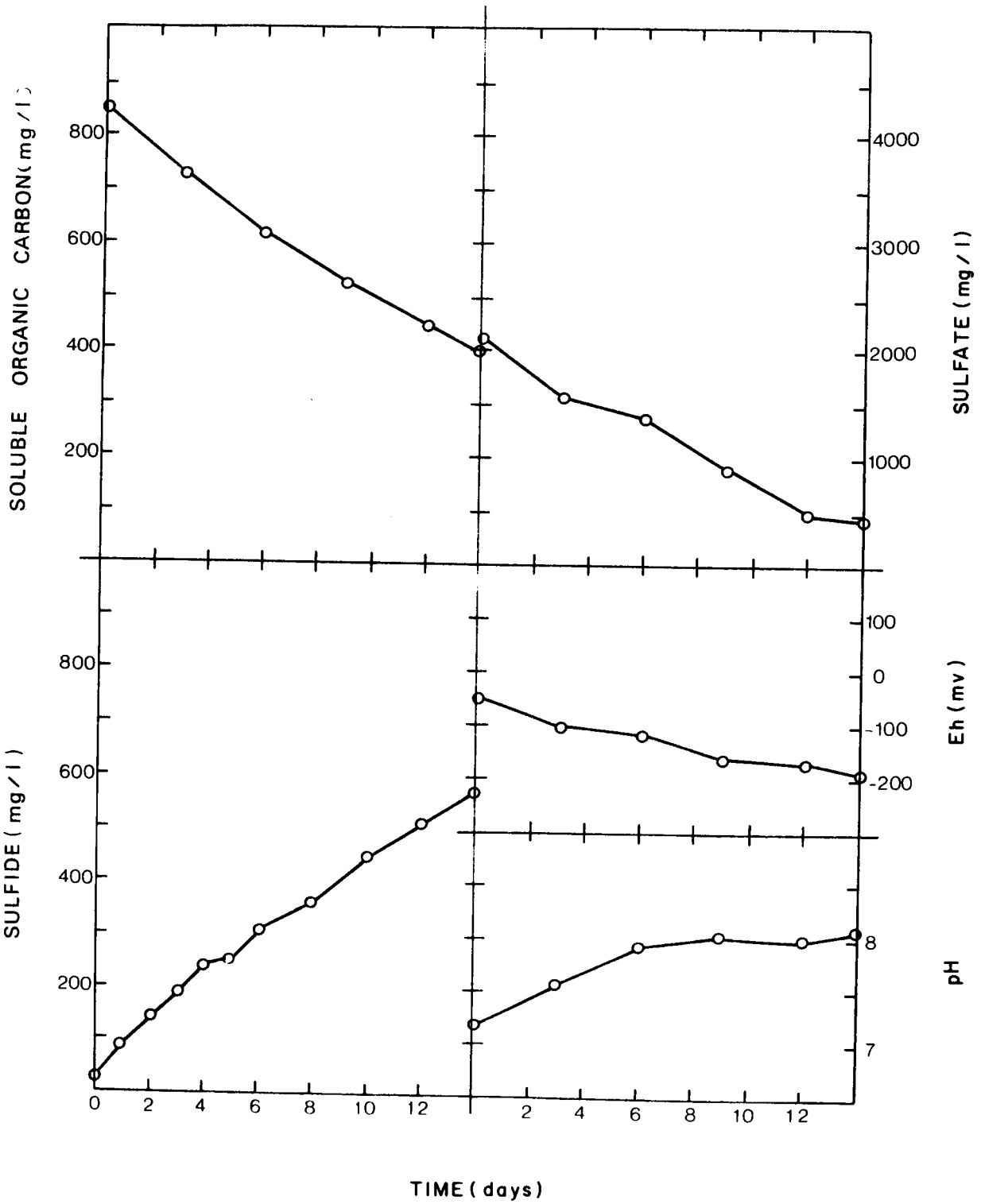


Figure 30. Results of experiment 2. Culture 9.

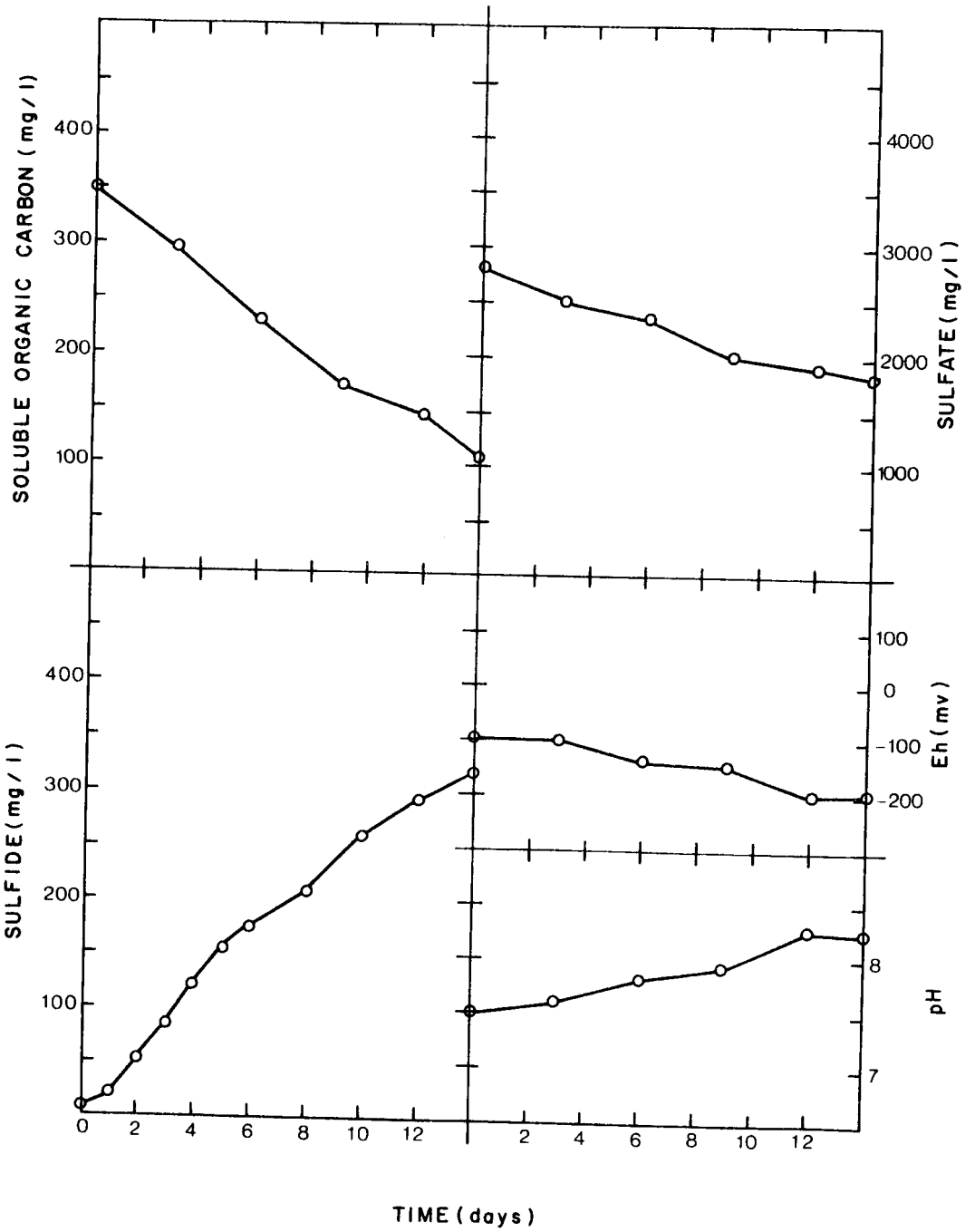


Figure 31. Results of experiment 2. Culture 10.

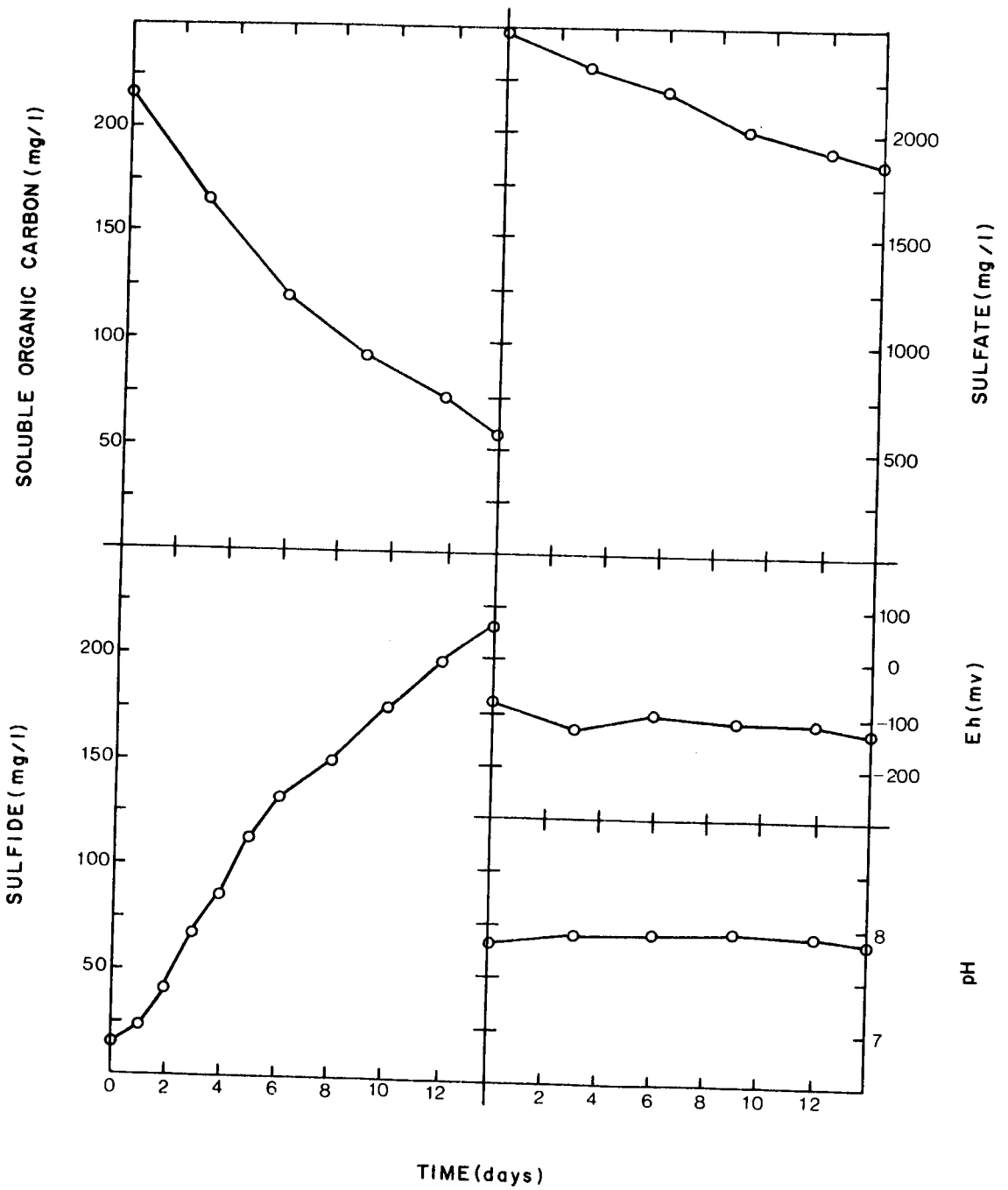


Figure 32. Results of experiment 2. Culture 11.

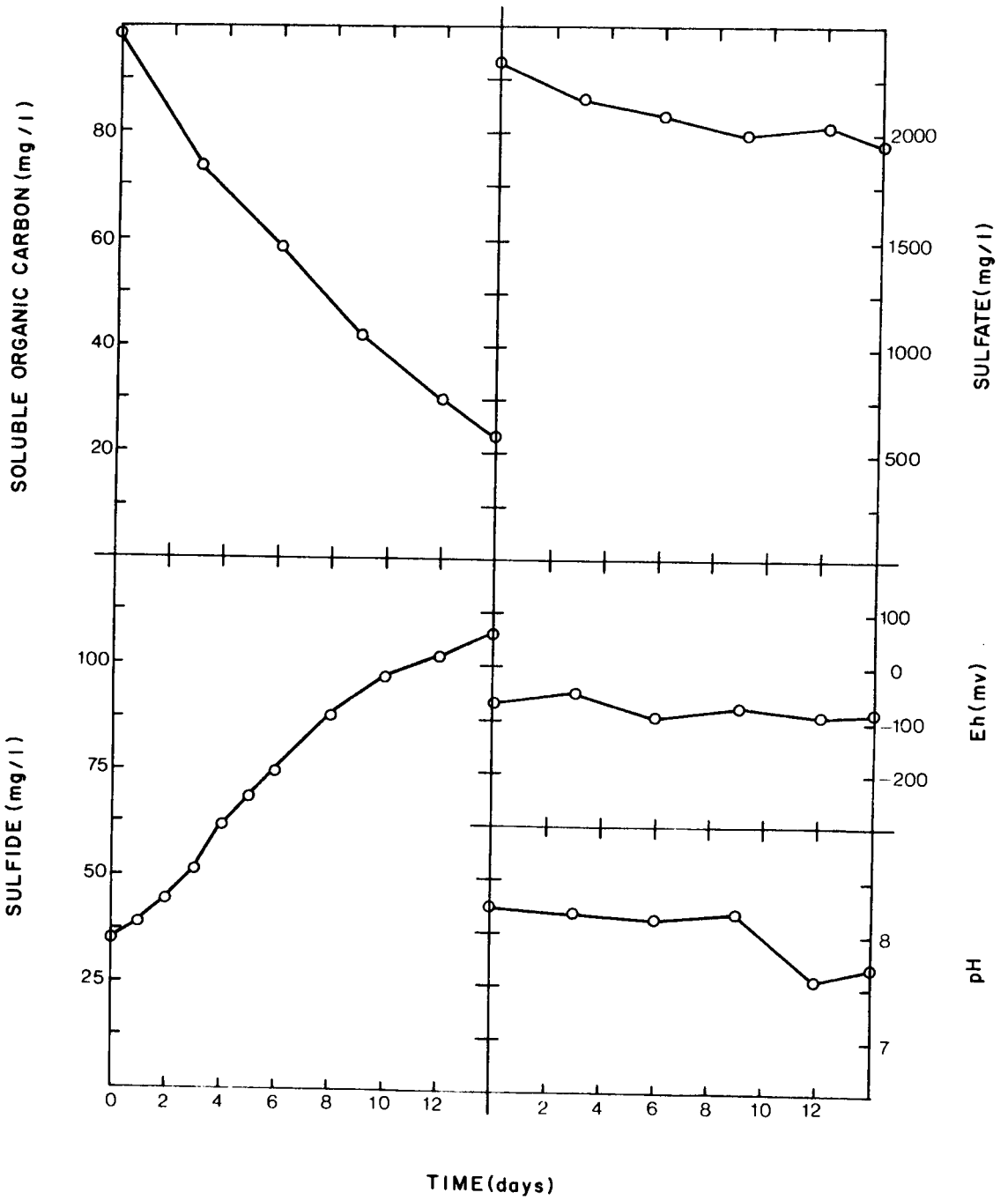


Figure 33. Results of experiment 2. Culture 12.

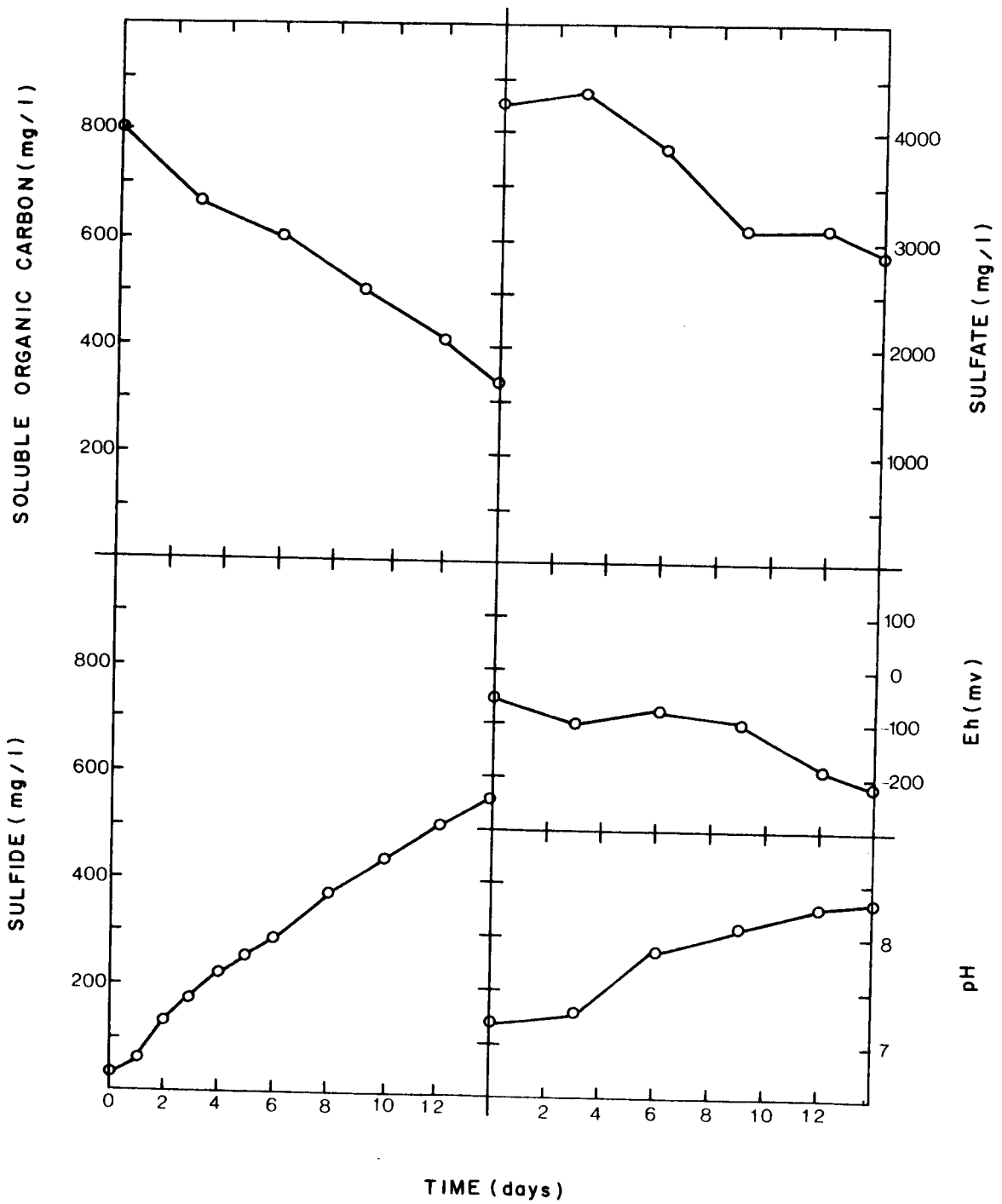


Figure 34. Results of experiment 2. Culture 13.

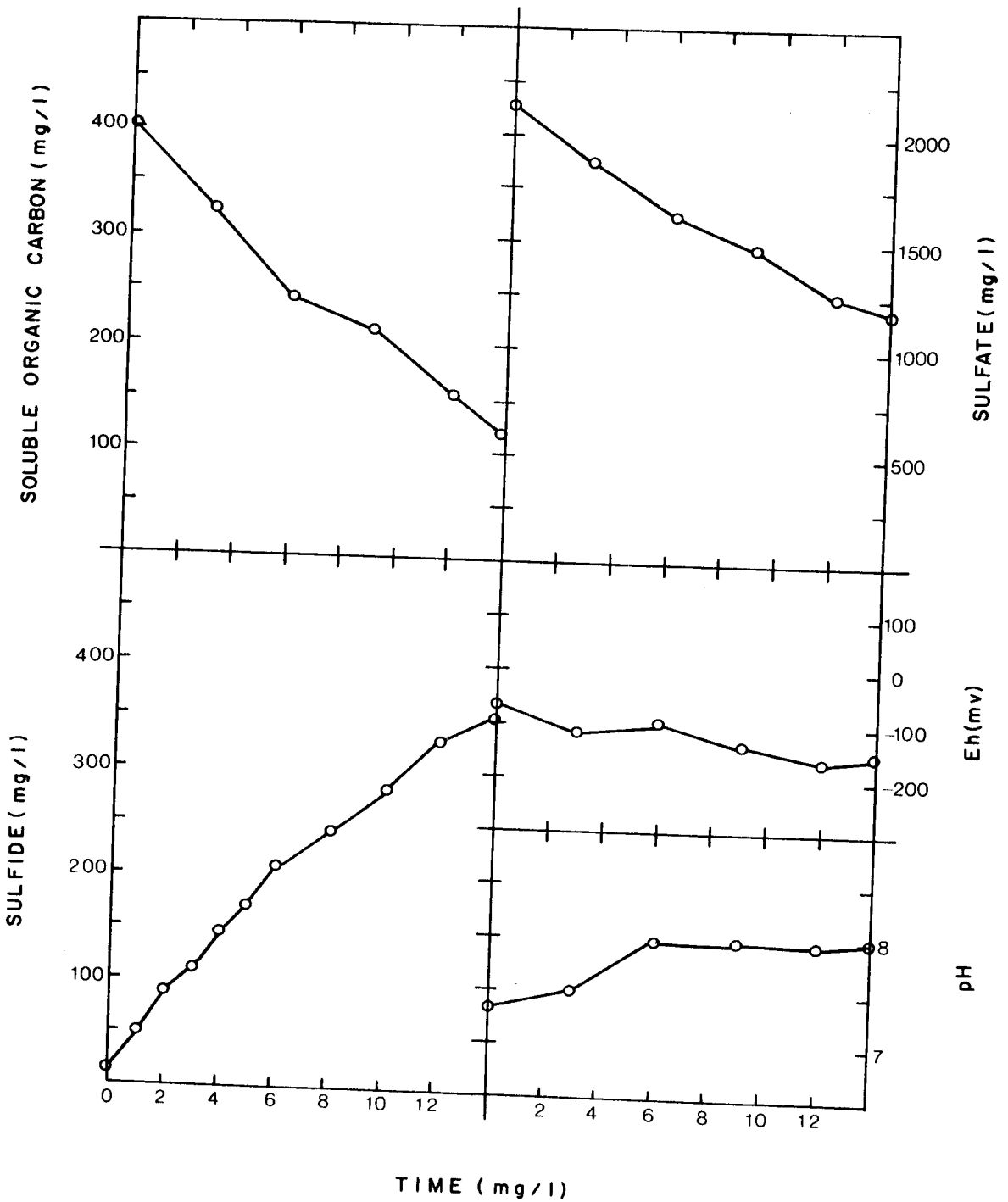


Figure 35. Results of experiment 2. Culture 14.

The medium in culture 14 was a 50 percent dilution of that in 13.

The recording of data, as indicated by day 0 in figures, began after stability of pH and Eh was achieved. This occurred three to five days following inoculation. Although the initial conditions were adjusted so that pH was slightly alkaline and Eh negative, considerable difficulty in maintaining such conditions was experienced. The pH decreased in all cultures, dropping as low as 4 in several, accompanied by an increase in Eh to positive values. The reasons for this are not entirely clear, but it is possible that the high concentration of simple sugars in these media (Figure 15) may have provided for the rapid growth of organic acid producing bacteria. The organic acids resulting from such growth could account for the drop in the pH. This possibility seems reasonable since the lowest pH was achieved in those cultures having the highest organic carbon concentrations, and hence the highest sugar content. A concurrent decrease in soluble organic carbon was observed, probably due to incorporation of carbon into bacterial structure and conversion of some of the carbon to carbon dioxide and carbonates. Initial concentrations of sulfate dropped only slightly, but were accompanied by no measurable increase in sulfide.

To overcome this utilization of organic carbon, and to maintain conditions conducive to the growth of sulfate reducers, concentrated sodium hydroxide was added to the cultures at frequent

intervals. After several days, stable conditions of pH and Eh were achieved, further addition of base became unnecessary, and active sulfate reduction began to occur.

A definite lag in sulfide production is evident in many of the cultures during day 0 to 1, and may reflect both acclimatization and growth of the sulfate reducing bacteria, as well as the growth of other bacteria. Experimental studies of the growth of sulfate reducers in both synthetic and organic extract media have indicated that in batch culture the populations of the bacteria reach a maximum of 10^7 - 10^8 cells per ml (27, 63). Since the generation time of halophilic strains of Desulfovibrio has been determined to be approximately 2 to 2 1/2 hours (27), it is estimated that the populations of sulfate reducing bacteria present in these cultures reached maximum stable levels no later than day 2.

In all cultures in which high production of sulfide occurred, pH remained at initial values or increased to 8.0 - 8.5, and Eh dropped to -150 to -220 mv (see Figures 22 through 35). These results are in general agreement with those of other studies, and are the result of sulfide production (27, 81). In cultures exhibiting lower production, the effect on pH and Eh was reduced, and where relatively little sulfide was produced (cultures 6 and 14), Eh remained relatively stable and the pH decreased. It is again suggested that this drop in pH may have been due to production of carbon

dioxide and possibly some organic acids.

No significant change in any of the parameters was measured in the control series.

Relationship Between Sulfide, Sulfate, and Organic Carbon. Dissimilatory sulfate reduction requires an organic source for energy, and sulfate as a hydrogen acceptor (40, 64). The stoichiometric relationship for sulfate reduction is given as (40)

$$\underset{-(12)}{SO_4^{=}} + \underset{-(3)}{2C_{organic}} \longrightarrow \underset{+(4)}{S^{=}} + 2CO_2 \qquad (4)$$

where the numbers below the chemical formulas indicate the reaction on a weight basis. Thus during sulfate reduction, a production of 1.0 mg of sulfide will theoretically require 3.0 mg of sulfate and 0.75 mg of organic carbon. These 'yield ratios' have been calculated for the mixed cultures over the 14 days of experiment two, and are presented in Table 5 where

$$Y_{sul} = \frac{\text{mg/l of sulfate consumed}}{\text{mg/l of sulfide produced}} \qquad (5)$$

and

$$Y_{soc} = \frac{\text{mg/l of soluble organic carbon consumed}}{\text{mg/l of sulfide produced}} \qquad (6).$$

It is important to recognize that these yield ratios reflect the activity of all the bacteria within the mixed cultures of experiment

Table 5. Summary of yield ratios and maximum rates of sulfide production for each culture from experiment two (a).

Culture No.	Y_{sul}(b)	Y_{soc}(c)	V_{max}(d)
1	3.2	0.82	54
2	3.0	0.89	60
3	3.0	1.60	54
4	3.2	2.00	48
5	3.0	2.30	35
6	3.4	6.05	10
7	3.1	0.86	70
8	3.2	0.86	62
9	3.0	0.91	51
10	3.2	0.80	30
11	3.0	0.88	20
12	3.1	1.10	10
13	3.1	0.94	54
14	2.9	0.84	34
Theoretical	3.0	0.75	--

(a) Yield ratios (Y_{sul} and Y_{soc}) calculated over the 14 days of the experiment.

(b) (mg/l of sulfate utilized)/(mg/l of sulfide produced).

(c) (mg/l of soluble organic carbon utilized)/(mg/l of sulfide produced).

(d) The maximum rate of sulfide production (mg/l-day) as measured over three day intervals, during the 14 days of the experiment.

two, and not only that of the sulfate reducers. As indicated by experiment one, however, the only significant source of sulfide in such cultures is from the dissimilatory reduction of sulfate.

Agreement between the theoretical value of Y_{sul} for sulfate reduction and the values of Y_{sul} determined from the experiment was very good. The average value for cultures 1 through 12 was 3.1, less than five percent in excess of the theoretical. This excess may be due in part to assimilatory reduction of sulfate during initial growth of all of the bacteria present in the cultures.

Y_{soc} was much more variable than Y_{sul} and exceeded the theoretical value of 0.75 in every culture. Since these mixed cultures contain bacteria other than sulfate reducers, which are capable of utilizing the organic carbon, this result is not surprising. That these other bacteria are capable of such utilization was apparent from the drop in soluble organic carbon in the absence of measurable sulfate reduction prior to day 0. In general, where sulfide production became curtailed by deficiency of sulfate (this is evident in cultures 3 through 6), the value of Y_{soc} increased well above the theoretical. In culture 6 sulfate limitation occurred early and Y_{soc} became quite large. It is likely in this culture that much of the organic utilization was due to processes other than sulfate reduction.

Rates of Sulfate Reduction. The yield ratios for sulfate calculated from the culture data indicate that sulfate reduction is responsible for the sulfide production measured. The maximum rate at which this sulfate reduction occurred (expressed as mg. of sulfide/l-day) in each culture of experiment two is shown (V_{max}) in Table 5. Rates have been estimated over a three day interval in each culture in which the maximum rate appeared to be occurring. It was felt that smoothing over a three day interval would tend to eliminate erroneously high or low rates resulting from variation in sampling time from day to day. It can be readily seen from Table 5 and Figures 22 through 35 that the lower the initial concentration of sulfate (cultures 1 through 6 or soluble organic carbon (cultures 7 through 12), the lower the maximum rate of sulfate reduction. These results strongly suggest that the rate of sulfate reduction, at least under the conditions existing in experiment two, is directly dependent upon the transient concentrations of sulfate and organic carbon.

Results obtained in cultures 9 and 10 correspond closely to those of culture 13 and 14 respectively. (see Figures 31, 32, 35, 36, and Table 5). This close agreement indicates that the effect upon sulfide production of autoclaving the algae used in the preparation of cultures 1 through 12 was not significant. The media for cultures 13 and 14 were obtained by no more drastic a procedure

than hand squeezing the algal mat found in abundance at site three. It is suggested that the fundamental composition of the media was consequently the same as the soluble material released by these algae during growth and following death to the surface sediments and water at site three. Only the absolute concentrations of substances were altered during media preparation to obtain cultures having varying concentrations of sulfate and soluble organic carbon.

The maximum rates of sulfate reduction measured during this study are compared to those reported by other investigators (Table 6). Maximum rates in the study by Edwards (27) were higher than those measured in this study. The higher incubation temperature (30° C), and use of sufficient lactate (a completely utilizable carbon source) to produce soluble organic concentrations well above those in the author's media would likely account for these higher rates. The maximum rates measured by Nakai and Jensen (51) were about half those reported here. The use of different procedures and experimental design prevents further comparison. Measurements by Ivanov (40) and Sorokin (71) of sulfate reduction in lake muds using labled sodium sulfate ($NA_2S^{35}O_4$) resulted in rates lower than those determined by the studies in this report. The difference in units used to report the rates makes comparison difficult. If, however, it is assumed that their mud samples were roughly 50 to 75 percent water, then the reported rates would range from about 15 to 40 mg sulfide

Table 6. Comparison of the rates of sulfide production measured in this study with those of previous investigators.

Investigator	Sulfide production rates [a]	Comments
Author	3.2 [b]	In situ measurement of sulfide release, respirometer
Author	17-90 [c]	Experiment 1
Author	10-70 [c]	Experiment 2
Edwards (27)	200-250 [c]	In lab, pure batch cultures of D. desulfuricans on MacPherson's medium, growing populations
Edwards (27)	100-150 [c]	In lab, pure batch cultures of D. desulfuricans on MacPherson's medium, stable populations
Ivanov (40)	0.5 - 1.5 [d]	Field measurements in 10 cm mud cores from deepest part of lake, determined with S^{35}
Ivanov (40)	12-19 [d]	Field measurements in 10 cm mud cores from slope of lake, determined with S^{35}
Nakai and Jensen (51)	10-45 [c]	In lab, mixed cultures containing sulfate reducing bacteria, cultures consisted of 30 ml sea water and 65 ml wet sediment
Sorokin (71)	0.1-0.2 [c]	Field measurements in lake water using S^{35}
Sorokin (71)	10-15 [d]	Field measurements in muds collected from slope of lake near river mouth, S^{35} used

(a) approximate range
(b) mg (S)/m^2 - day
(c) mg(S)/l-day
(d) mg(S)/Kg wet sediment-day

per liter per day. In the study, 10 cm mud samples were utilized and the rates reported based on production of sulfide over this depth. If the production were occurring within only the top few cm, however, then the rates reported might underestimate the actual production occurring within this active upper region. Accounting for these factors would produce approximate agreement with the results of the author's studies.

It is interesting to compare the maximum rates of sulfide production measured in the laboratory experiments with the estimated rate of sulfide release obtained with the respirometer during the field studies. Experiment one indicated that rates of sulfide production of at least 90 mg/l-day are possible when the mixed cultures, containing sulfate reducing bacteria, were given an adequate input of organic material. To obtain production of 3.2 gm/m^2-day of sulfide (rate of release measured at site three) would require production of 90 mg/l-day to occur within the sediments to a depth of three to four cm. This assumes that all of the sulfide produced is being released from the sediments as free sulfide. Measurements of sulfate concentration within the interstitial water of site three sediments obtained during July, 1971, indicated a rapid decrease within the top five centimeters (63). In one sample no measurable sulfate was detected below three to four centimeters. This data indicates that the maximum rates of sulfide production

measured in the laboratory in this study may approximate the rates of sulfide production that occur at site three.

Mathematical Model

General

To further explain and analyze the results of experiment two, a mathematical model will be used which relates the production of sulfide to the utilization of sulfate and soluble organic carbon. Results of experiment two indicated that the sulfide production rate increased as the concentration of sulfate and/or organic carbon increased. This effect, however, was most pronounced at lower levels of sulfate and carbon, and became relatively small at higher concentrations. An equation which has been used to describe such a saturation effect at high substrate concentrations is the common Michaelis-Menton equation, which is most often used to describe enzyme catalyzed reactions (8). The basic form of the equation is

$$\frac{dP}{dT} = R_{max} \left(\frac{N}{K_n + N} \right) \qquad (7)$$

where P is the concentration of the product, R_{max} the maximum rate of product formation, N the substrate concentration, and K_s the substrate concentration at which $dP/dT = 1/2\ R_{max}$. At high concentrations of N the term in parenthesis approaches 1,

and dP/dT approaches R_{max}, while at low concentrations of N, dP/dT approaches a linear variation with N. Modifications of this equation have been used commonly to simulate growth of microorganism populations, including the growth of halophilic sulfate reducing bacteria (27).

With sulfate and soluble organic carbon serving as substrates, the rate of sulfide production may be expressed as

$$\frac{dS}{dt} = R_{max} \left(\frac{SUL}{K_{sul} + SUL} \right) \left(\frac{SOC}{K_{soc} + SOC} \right) \qquad (8)$$

where SUL is the sulfate concentration, SOC the soluble organic carbon concentration, and K_{sul} and K_{soc} the Michaelis coefficients for SUL and SOC respectively. Bacterial populations are assumed to be relatively high and stable, and the effect of multiple substrate limitation is given by the product of their individual effects as described in equation 7. At high concentrations of the substrates, the rate of sulfide production approaches R_{max}. If either SUL or SOC become low dS/dT is reduced, while at low concentrations of both, dS/dT becomes even smaller.

Since sulfate and soluble organic carbon are being consumed in the production of sulfide, their rate of utilization may be expressed as

$$\frac{d(SUL)}{dt} = -Y_{sul} \left(\frac{dS}{dT} \right) \qquad (9)$$

and

$$\frac{d(SOC)}{dt} = -Y_{soc}\left(\frac{dS}{dt}\right) \qquad (10)$$

A system of equations similar to 8, 9, and 10 has been suggested for use in describing sulfate reduction in estuarine benthic deposits (8).

It is important to realize that in the mixed cultures of experiment two, the yield coefficients for sulfate and soluble organic carbon reflect utilization by the complete microbial system including sulfate reducers and other anaerobic bacteria. Hence their departure from the theoretically derived yield coefficients as previously described.

Estimation of Parameters

The value for Y_{sul} for use in the model was obtained by averaging the measurements of this yield coefficient for cultures 1 through 12 (Table 5). Y_{soc} was similarly obtained, but by weighting most heavily those values of Y_{soc} from cultures which were not markedly sulfate limited during the experiment. Measurements from cultures 13 and 14 were not included in the average since these cultures were prepared with unautoclaved media.

Initial comparison of experimental results with calculated results of equations 8, 9, and 10 demonstrated that production of sulfides responded more sharply to changes in sulfate concentrations

at low sulfate concentrations than is described by the traditional Michaelis-Menton equation. This sharper response could be expressed within the model by raising the sulfate concentration in equation 8 to higher powers (see Figure 36). Based on simulation of cultures 3 through 6, it was decided to raise the sulfate concentration to the 1.3 power, thus replacing equation 8 by

$$\frac{dS}{dt} = R_{max} \left(\frac{SUL^{1.3}}{K_{sul} + SUL^{1.3}} \right) \left(\frac{SOC}{K_{soc} + SOC} \right) \quad (11)$$

Estimates of R_{max}, K_{sul} and K_{soc} were obtained using multiple non-linear regression analysis to fit the combined data of cultures 1 through 12 to equation 11. Since the model assumes no lag in sulfide production, data for the first one to two days of those cultures in which an obvious lag occurred was not included in the analysis.

The result of the regression indicated a maximum rate of sulfide production (R_{max}) of 77 mg/l-day. The values of K_{sul} and K_{soc} were 320 mg/l and 650 mg/l respectively. A value of 3.1 was used for Y_{sul} and 1.0 for Y_{soc}. The resulting equations with the fitted parameter estimates are shown below:

$$\frac{dS}{dt} = 77 \left(\frac{SUL^{1.3}}{320 + SUL^{1.3}} \right) \left(\frac{SOC}{650 + SOC} \right) \quad (12)$$

$$\frac{d(SUL)}{dt} = -3.1 \left(\frac{dS}{dt} \right) \quad (13)$$

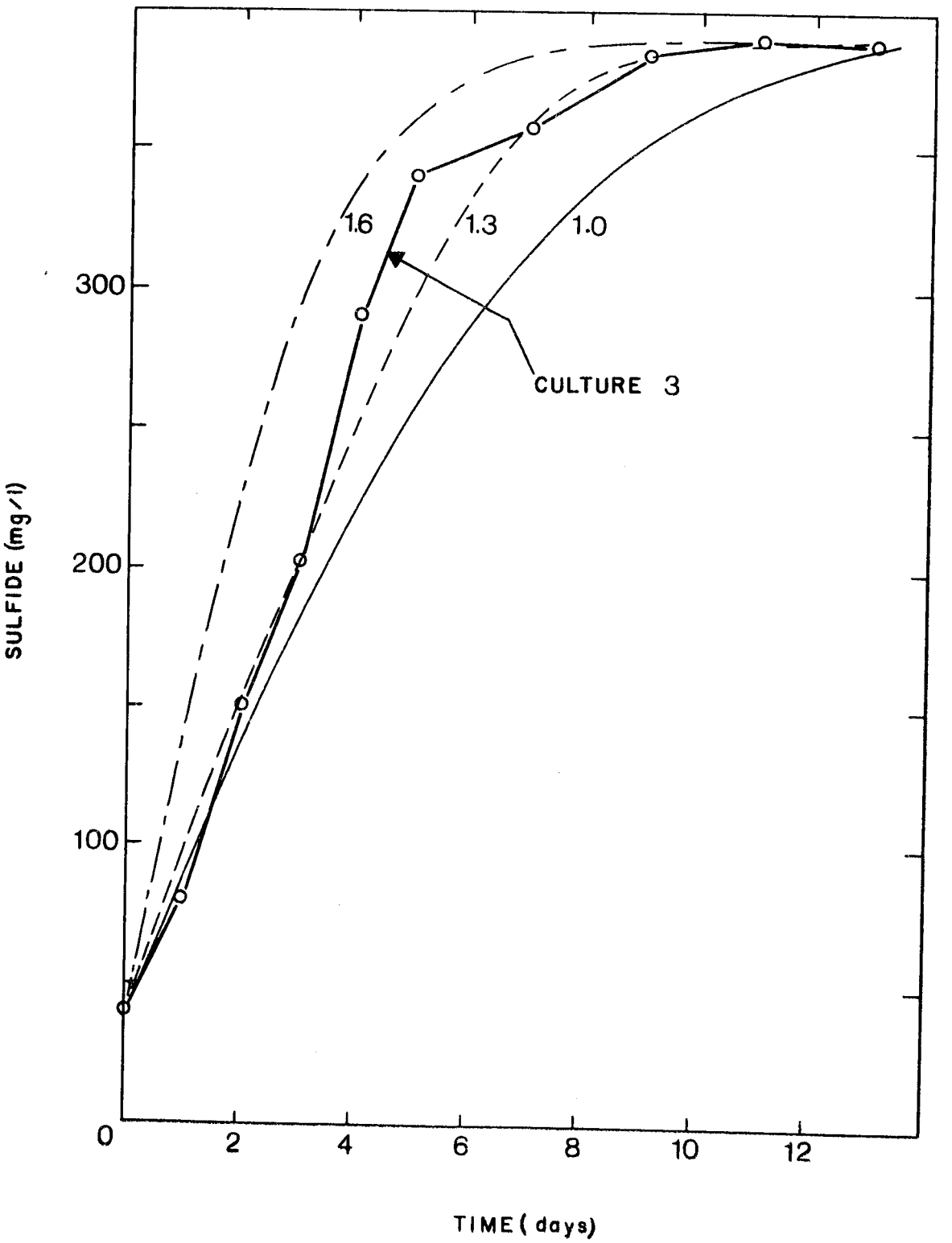


Figure 36. Resultant simulations of culture three obtained by raising SUL to three different exponents.

$$\frac{d(SOC)}{dt} = -1.0 \left(\frac{dS}{dt} \right) \qquad (14)$$

Simulation of Experiment 2

Approximations to the simultaneous solutions of equations 12, 13, and 14 were obtained by digital computer using a fourth order Runge - Kutte finite difference method. The simulations of cultures 1 through 12 are shown in Figures 37 through 48. Solid lines represent the values of sulfide, sulfate, and soluble organic carbon calculated by the mathematical model. Circles are the data taken from the respective cultures of the experiment. The values of pH and Eh indicated are from the experiment.

Agreement between simulated and measured results are especially good for those cultures in which the rates of sulfide production were highest. Agreement was in general best for sulfide and sulfate, while the calculated concentrations of soluble organic carbon deviated more from the experimental measurements. The selection of a Y_{soc} of 1.0 for the simulation model is reflected in these results. This value produced higher rates of organic utilization than experimentally determined in all cultures except 3 through 6. In these cultures, the calculated results underestimated the organic carbon utilization. The accuracy of the simulation of sulfide production was also reduced for those cases in which sulfate limitation

occurred, and for culture 12, the culture having the highest soluble organic carbon concentration. For cultures 1 and 2, and 7 through 11, the simulation was generally within 10 percent of the experimental results, and within five percent in a number of these. Part of the difference between the simulation and experimental results can be attributed to the lag period which occurred in many of the cultures from day zero to one. It will be recalled that the model assumes no such lag, and that data for this lag was not included in the regression analysis. Excluding the lag period from Figures 37 through 50 by considering day one the beginning of the experiment, would result in a much closer agreement of simulated with measured results in most cultures. This was the method used in plotting the experimental data in Figure 36.

Equations 12, 13, and 14 were used to simulate production of sulfide and consumption of sulfate and soluble organic carbon in cultures 13 and 14 (see Figures 49 and 50). Simulated sulfide production was within 10 percent of the actual production, a projected result which lends justification to the use of this form of model for microbial systems such as those of experiment two. Soluble organic carbon consumption was underestimated as with simulations of all cultures having a Y_{soc} less than 1.0.

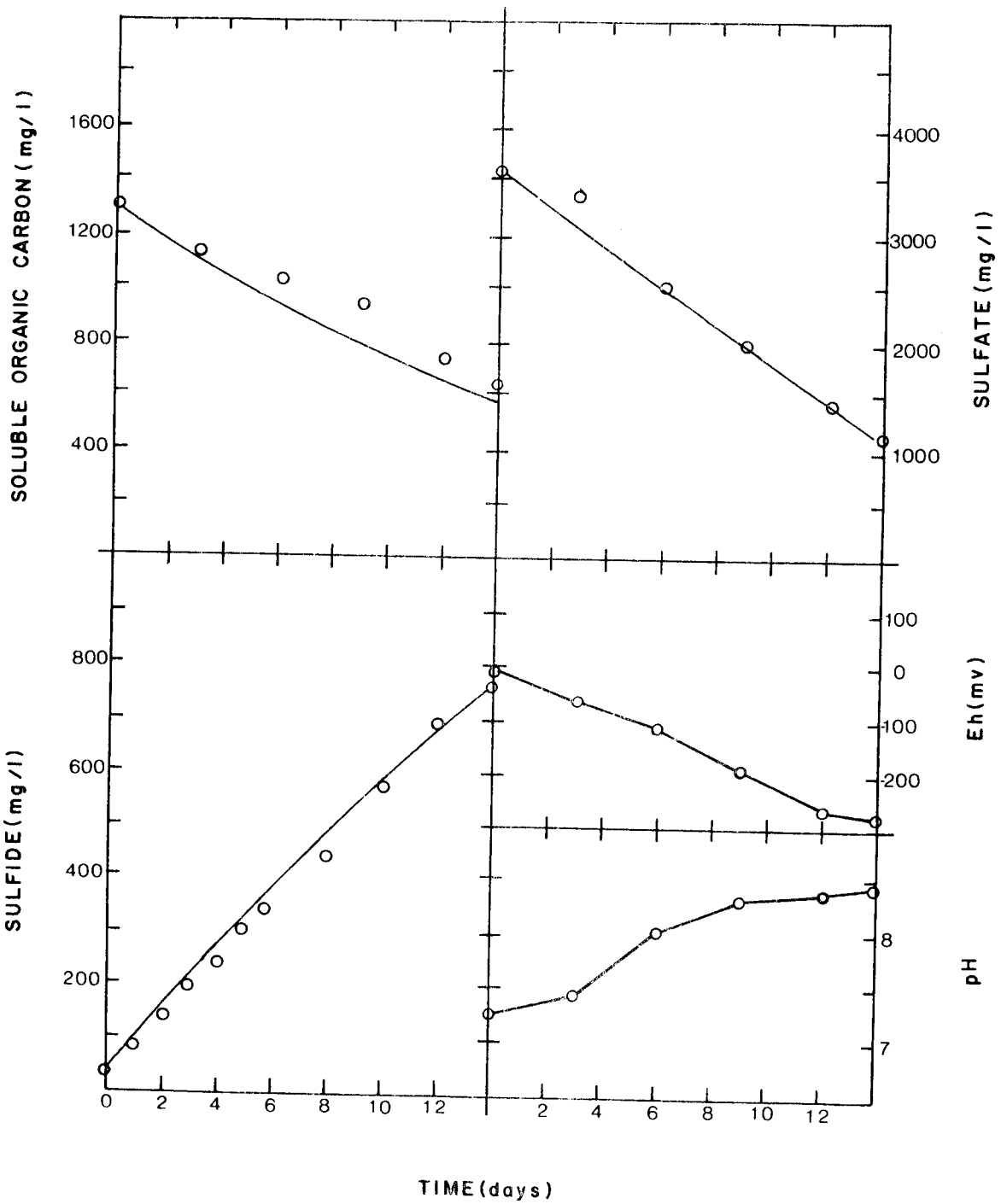


Figure 37. Comparison of the simulation of culture 1 with the experimentally determined results.

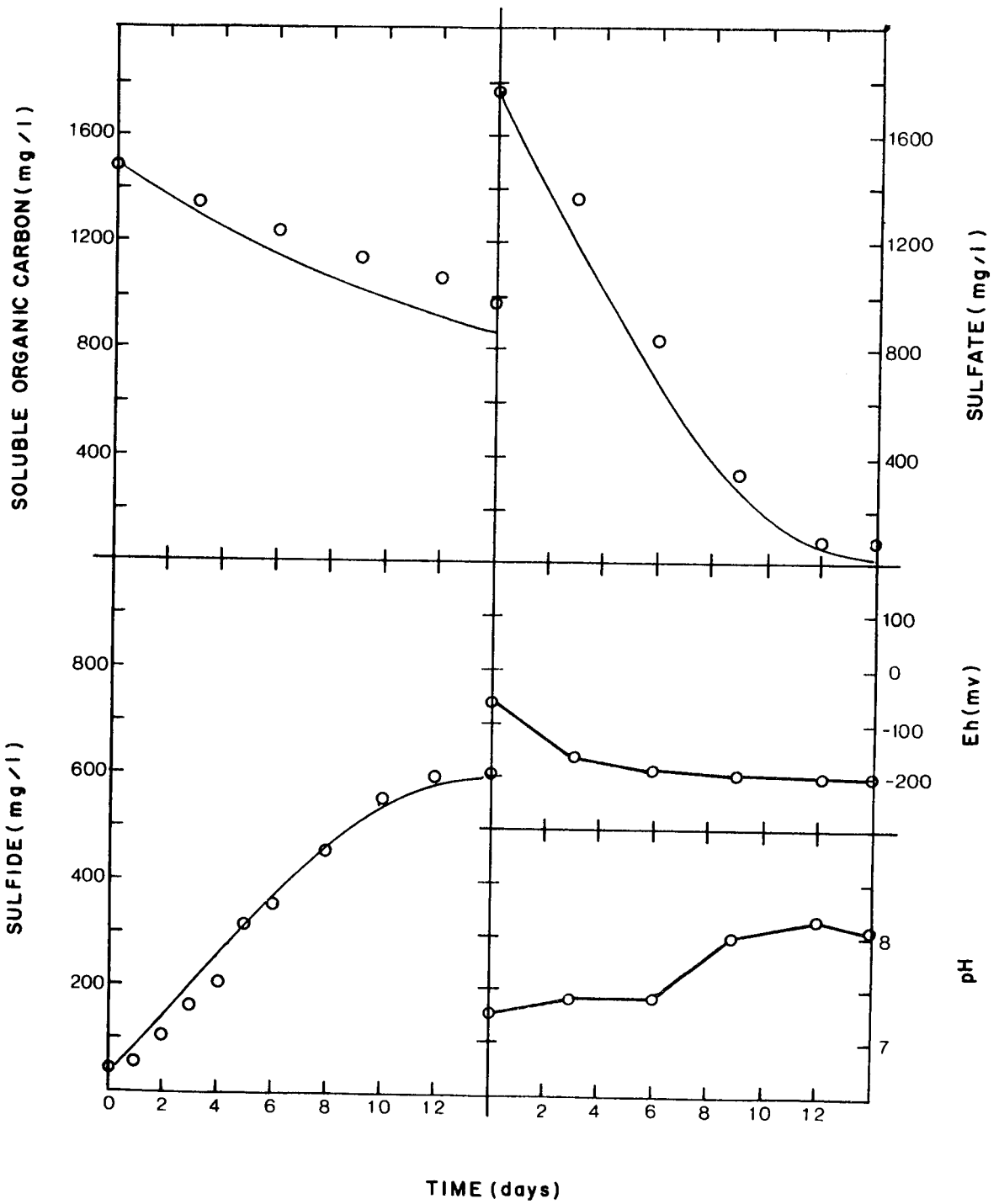


Figure 38. Comparison of the simulation of culture 2 with the experimentally determined results.

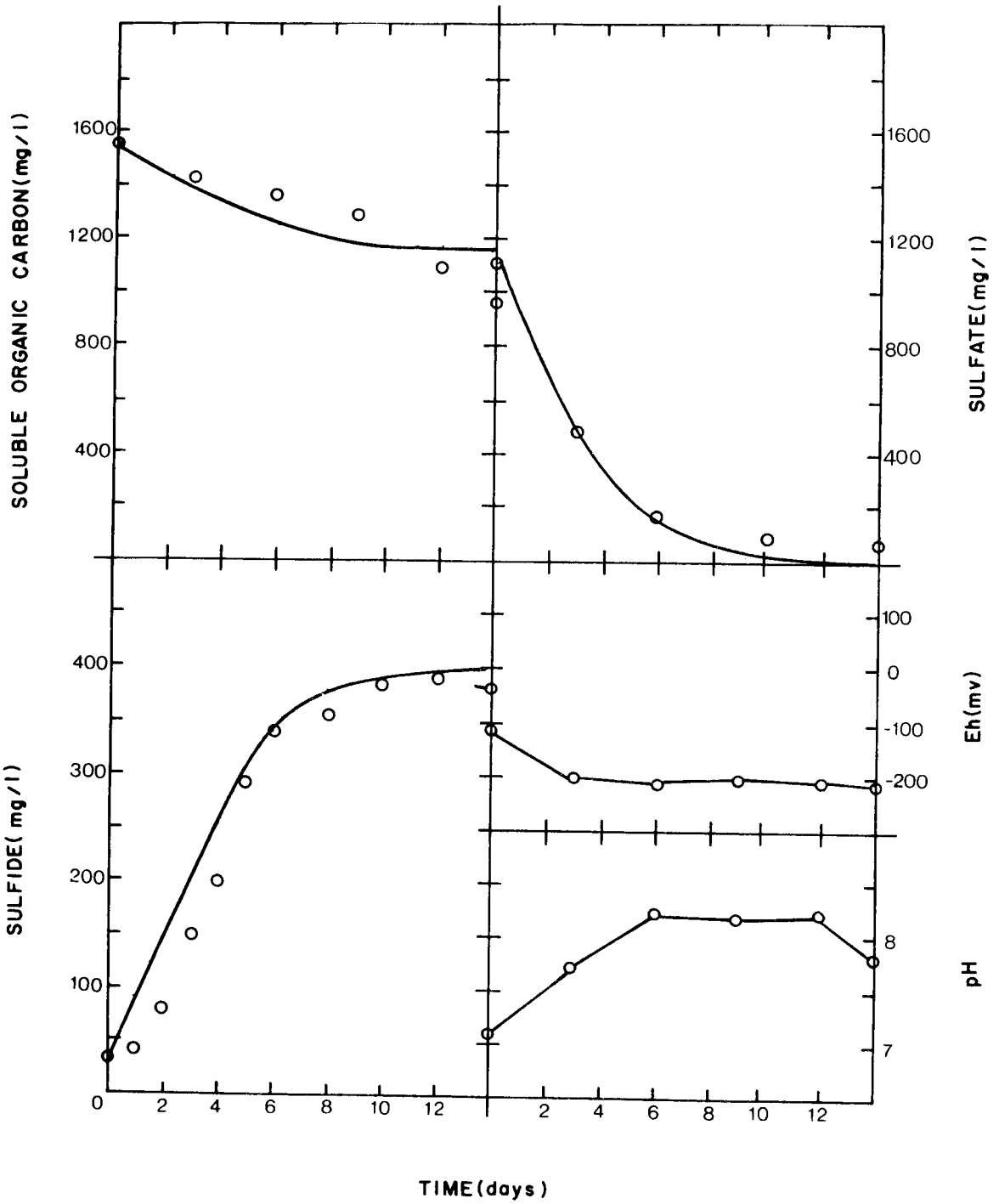


Figure 39. Comparison of the simulation of culture 3 with the experimentally determined results.

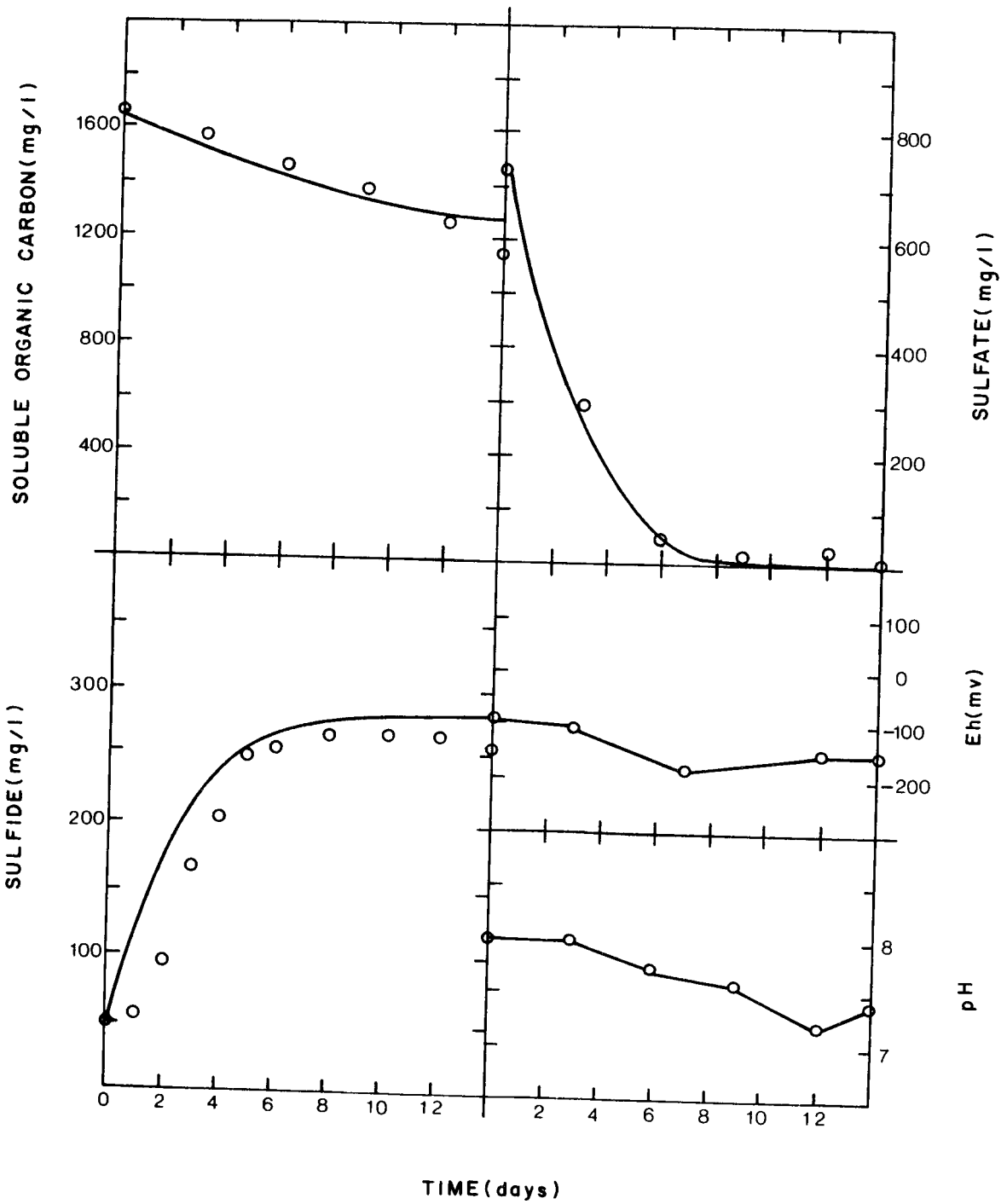


Figure 40. Comparison of the simulation of culture 4 with the experimentally determined results.

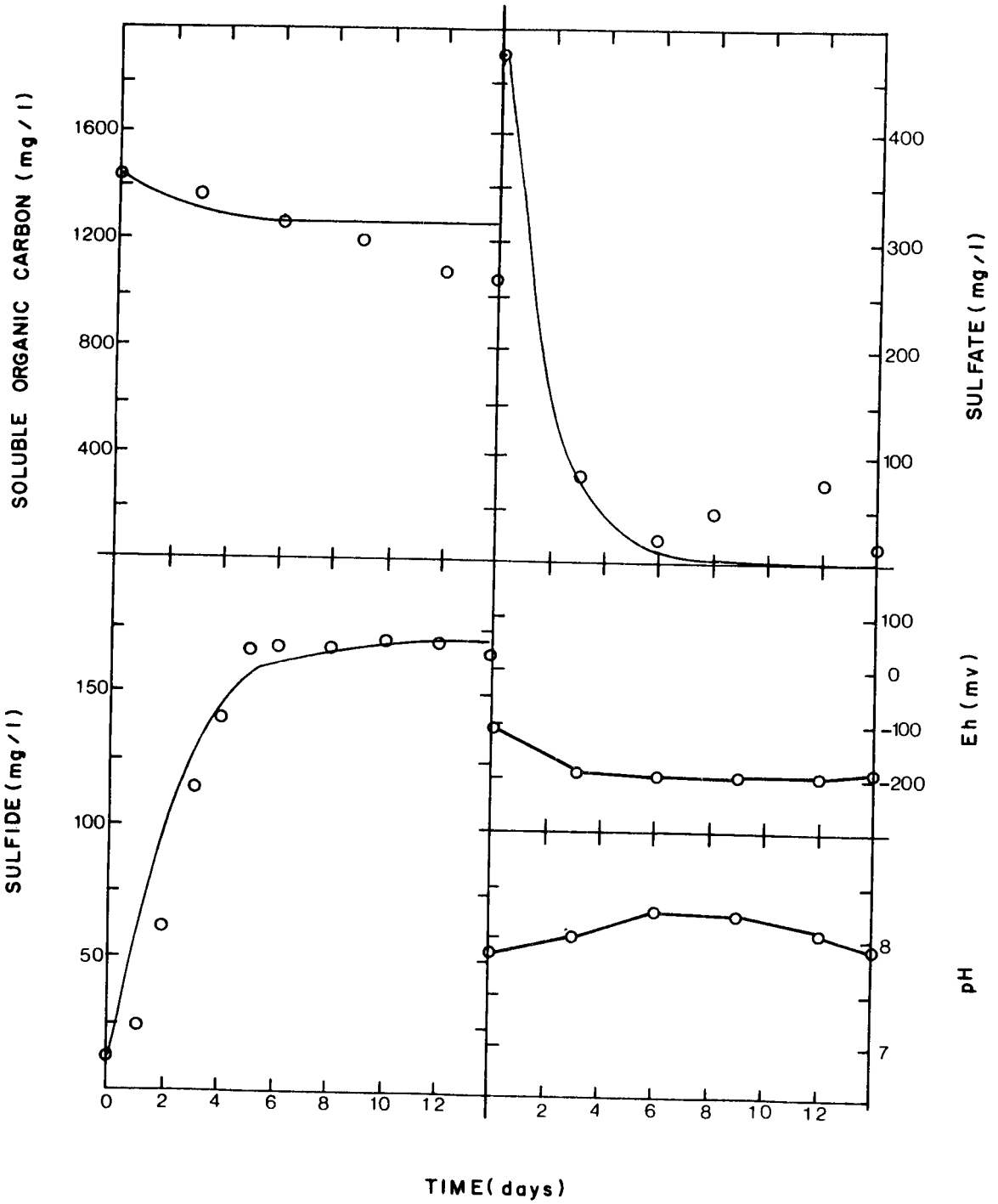


Figure 41. Comparison of the simulation of culture 5 with the experimentally determined results.

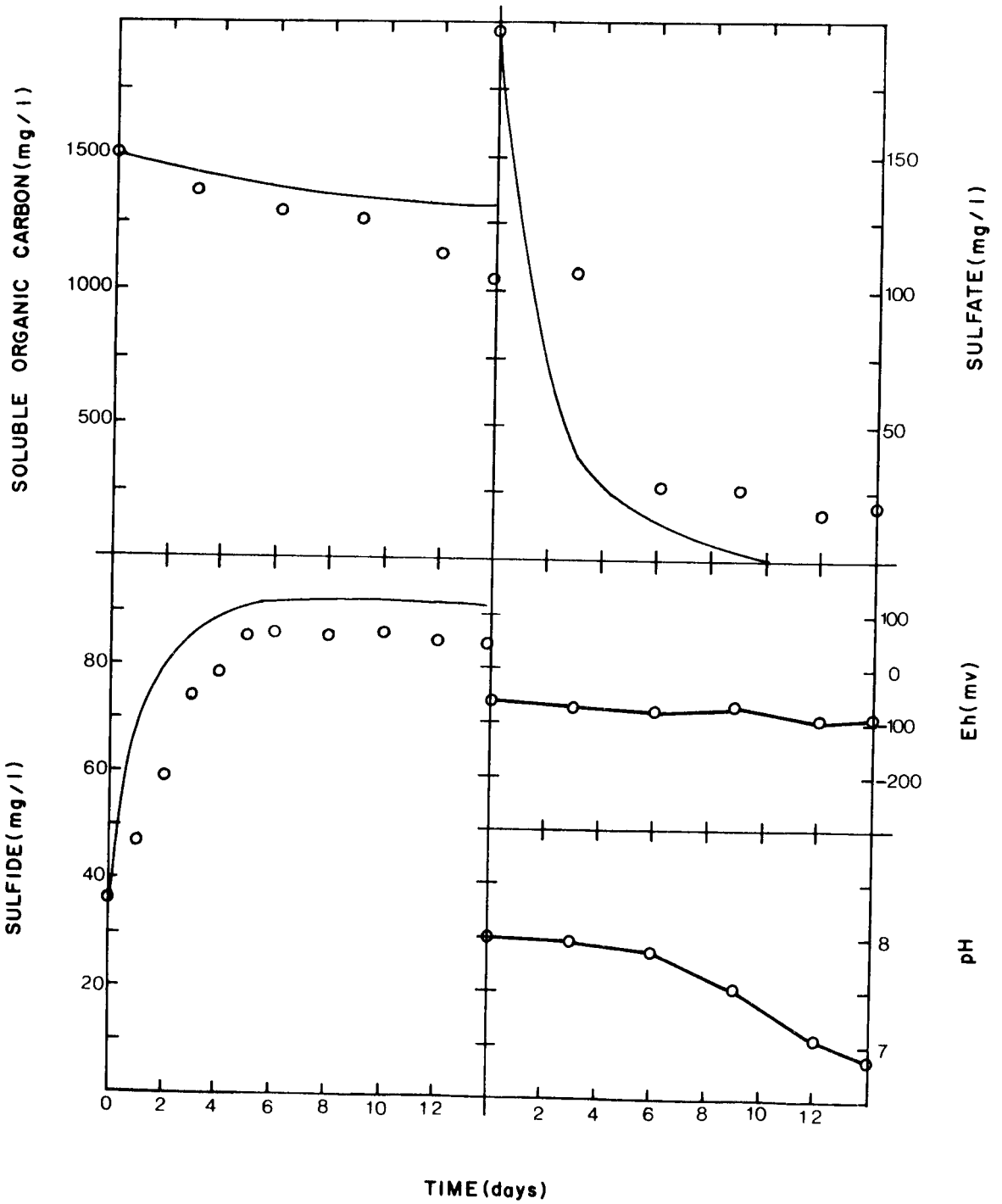


Figure 42. Comparison of the simulation of culture 6 with the experimentally determined results.

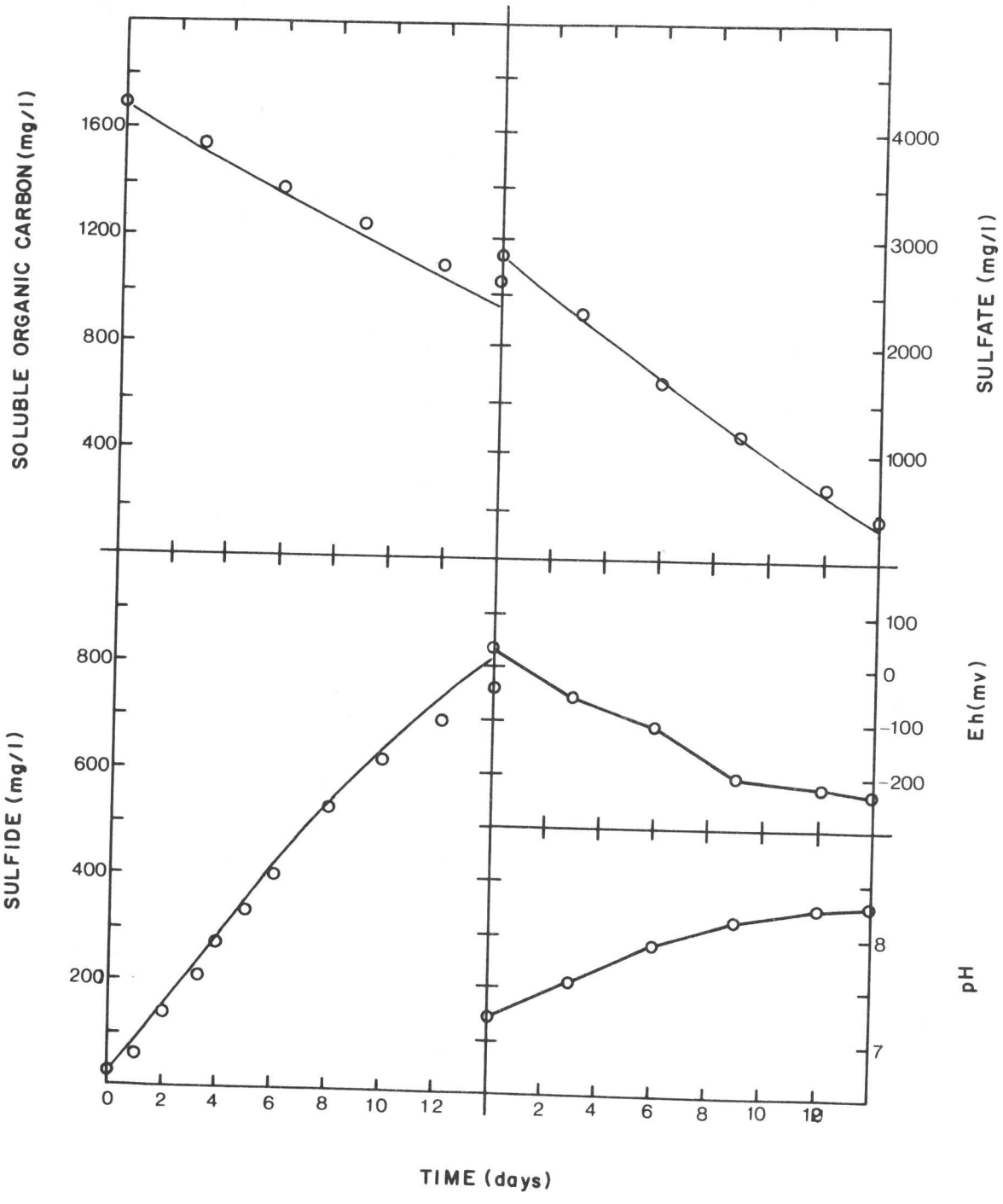


Figure 43. Comparison of the simulation of culture 7 with the experimentally determined results.

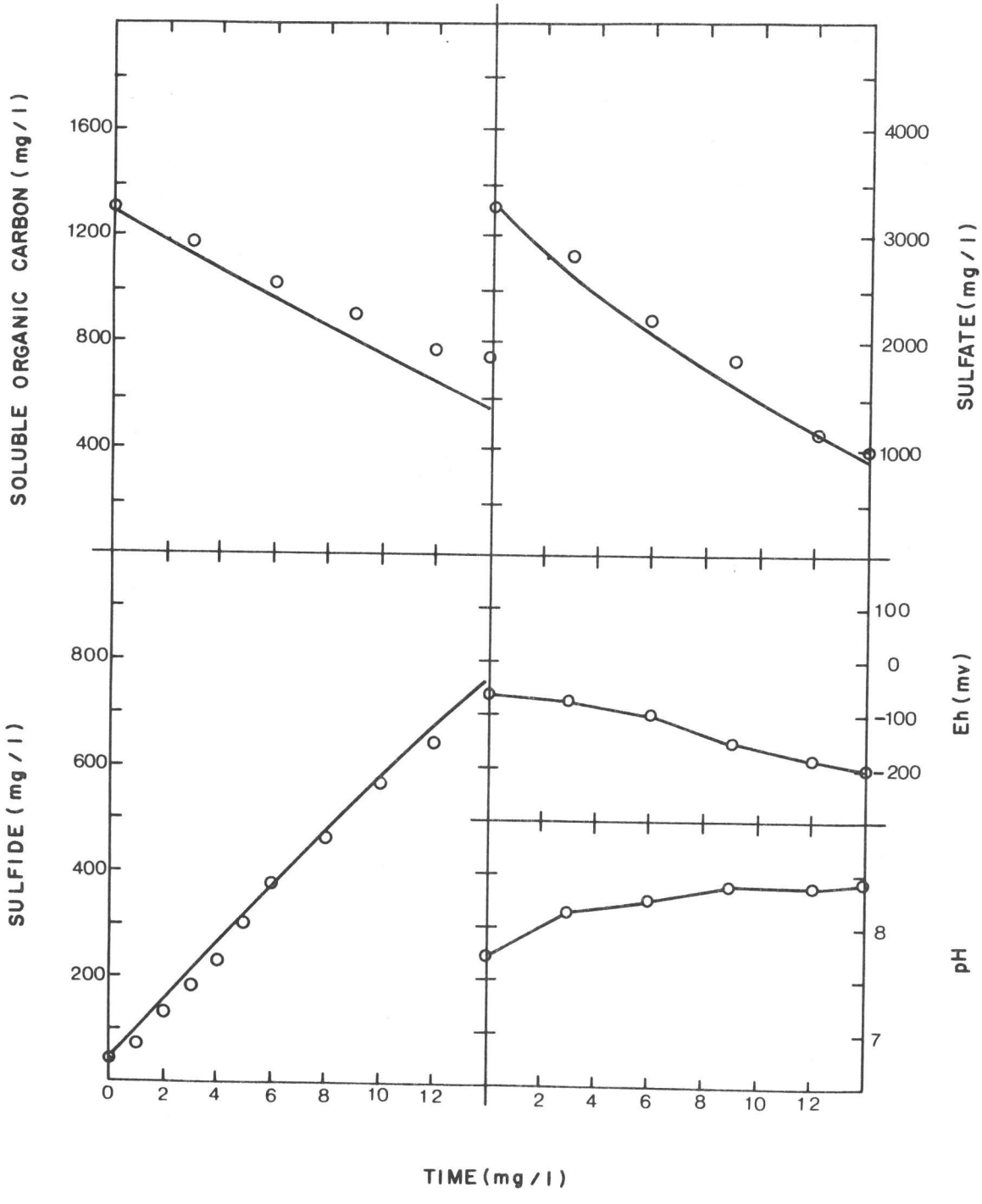


Figure 44. Comparison of the simulation of culture 8 with the experimentally determined results.

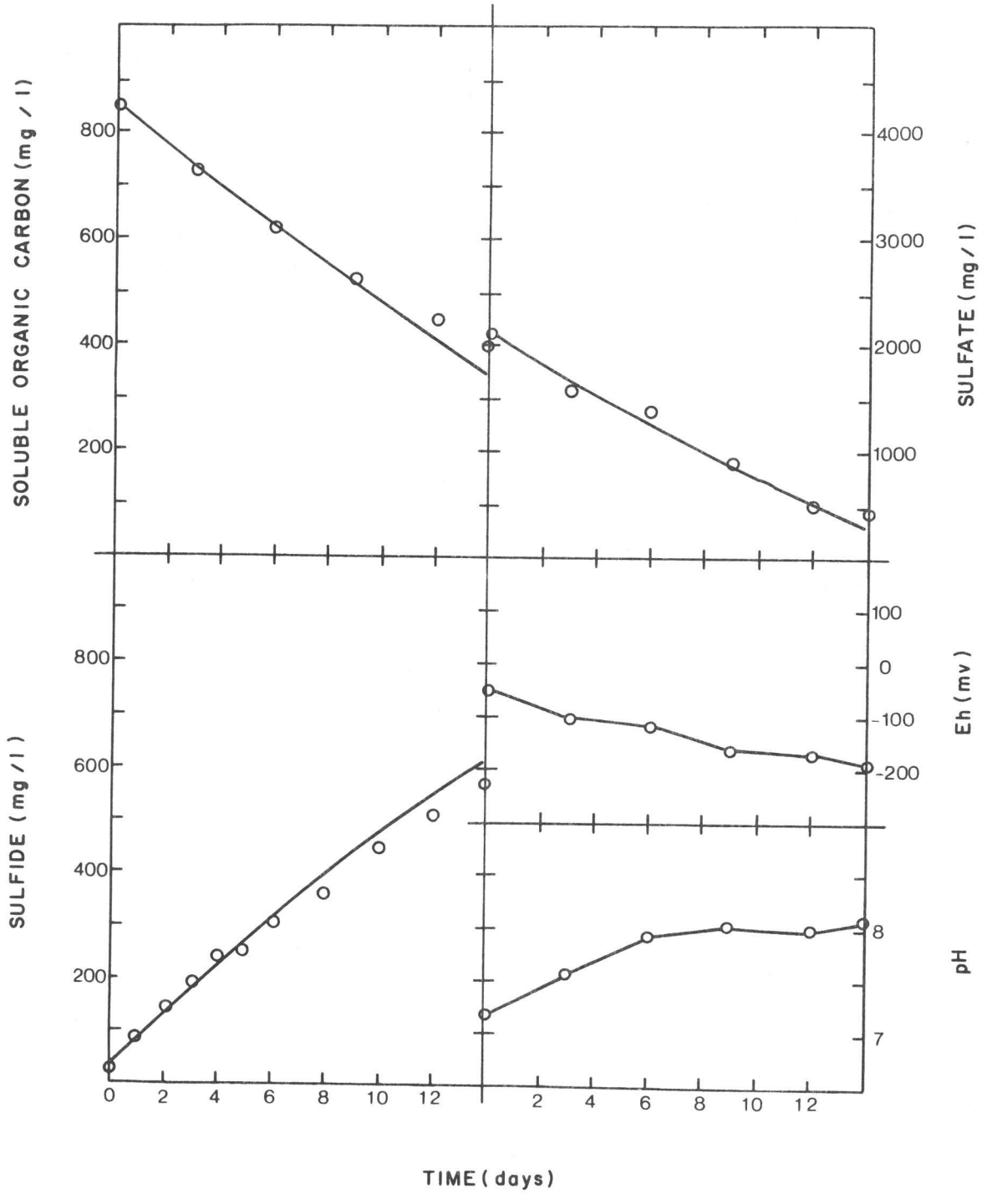


Figure 45. Comparison of the simulation of culture 9 with the experimentally determined results.

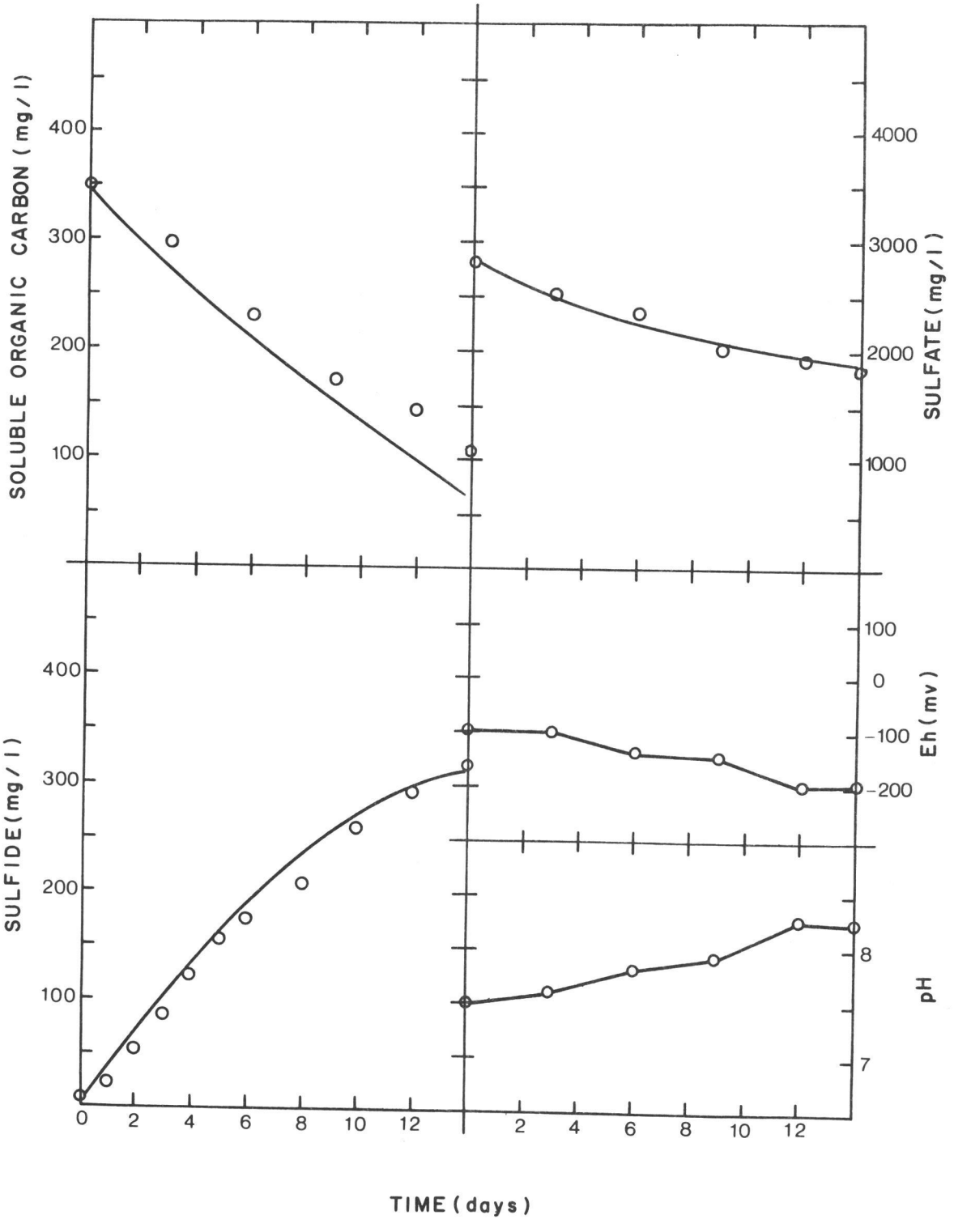


Figure 46. Comparison of the simulation of culture 10 with the experimentally determined results.

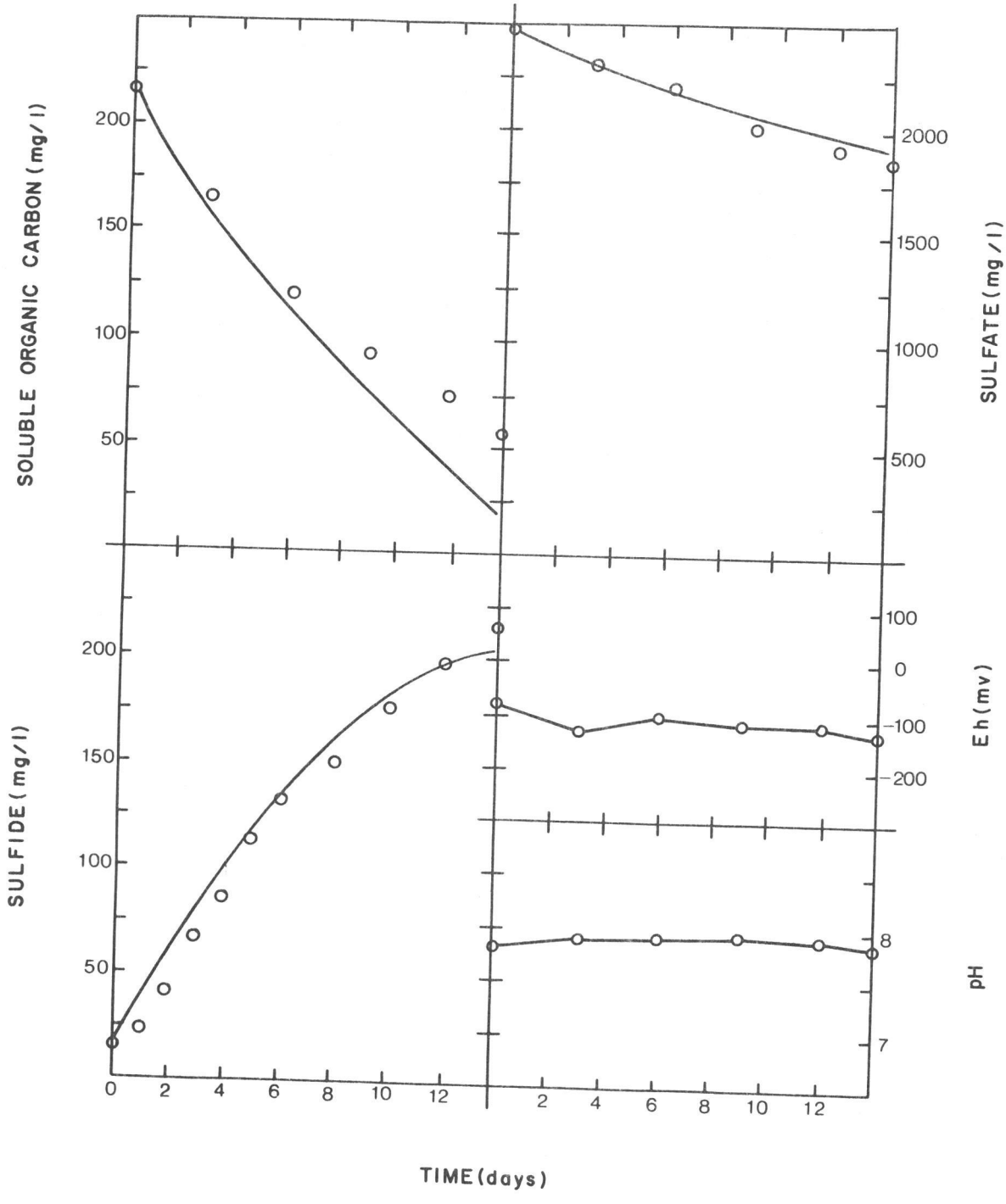


Figure 47. Comparison of the simulation of culture 11 with the experimentally determined results.

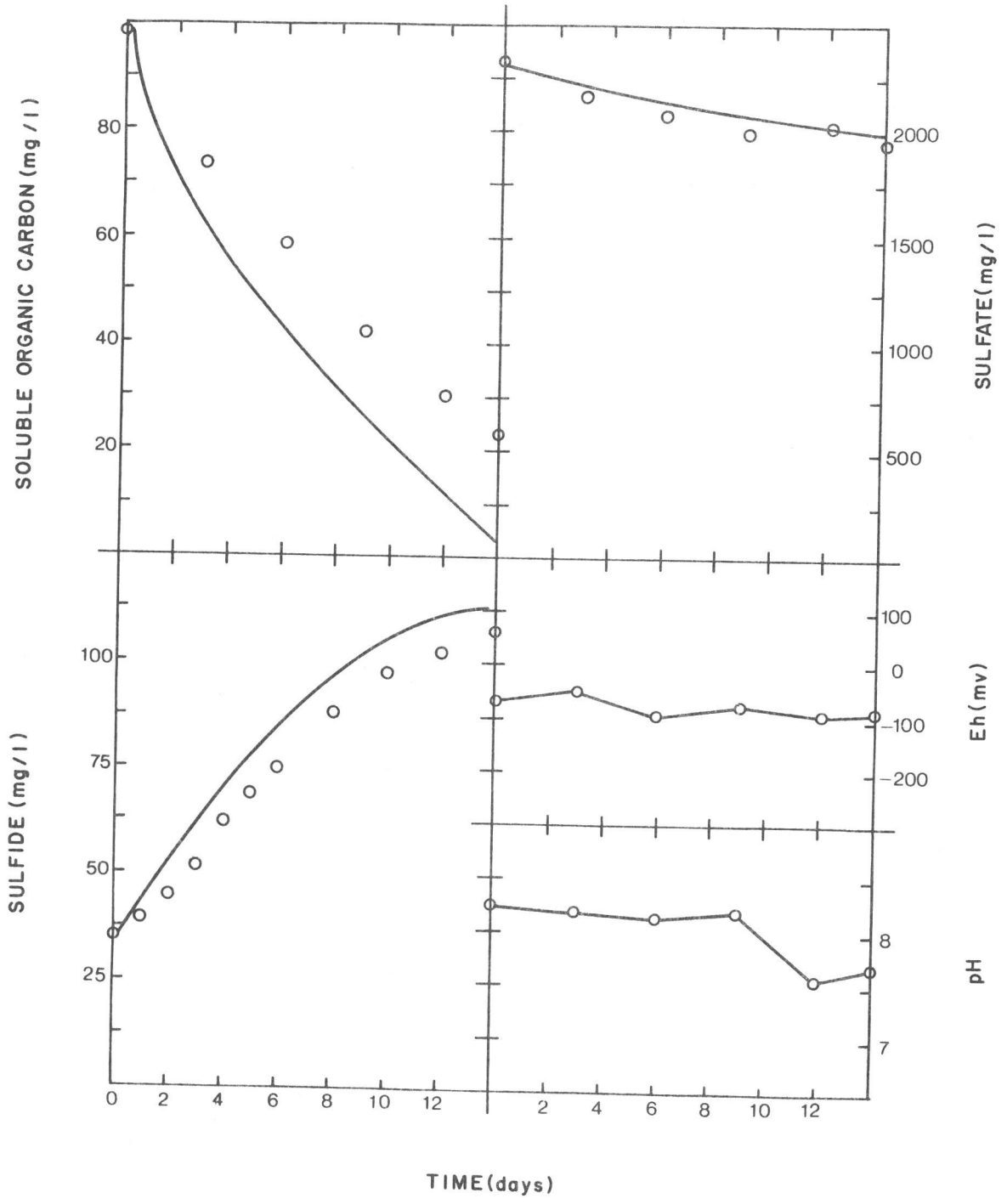


Figure 48. Comparison of the simulation of culture 12 with the experimentally determined results.

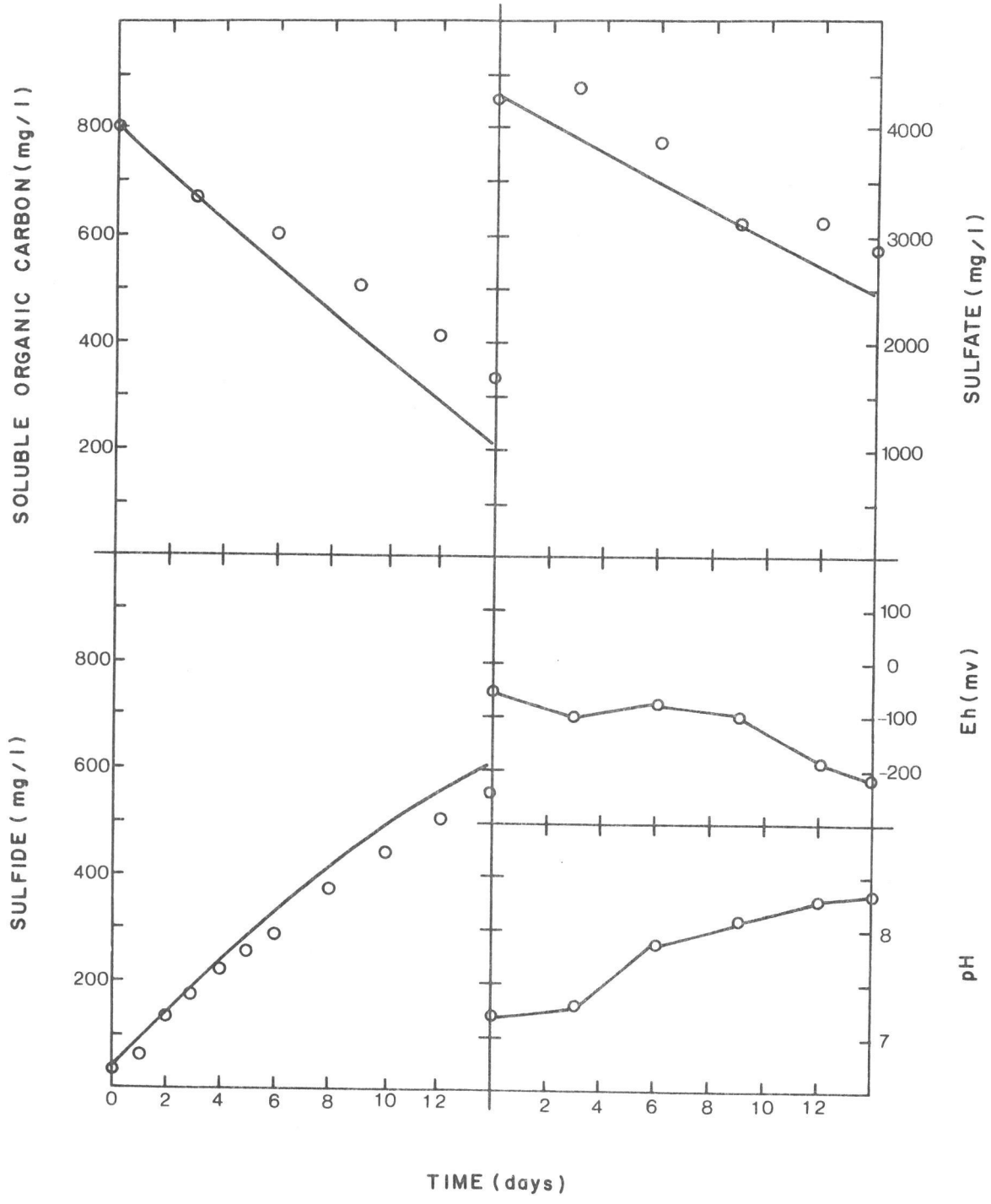


Figure 49. Comparison of the simulation of culture 13 with the experimentally determined results.

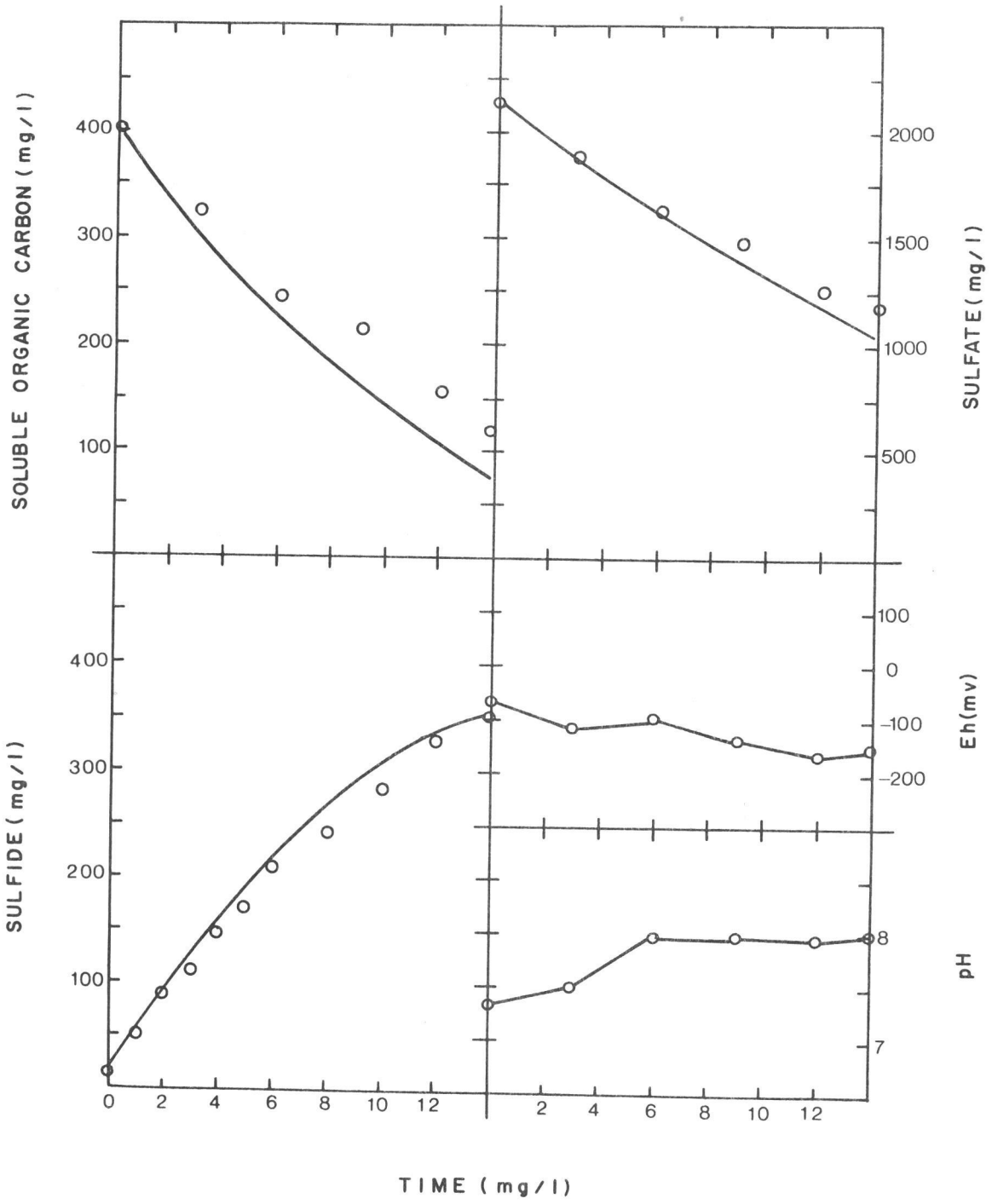


Figure 50. Comparison of the simulation of culture 14 with the experimentally determined results.

SUMMARY AND CONCLUSIONS

Studies have indicated that tidal flat areas may exert substantial oxygen demand on the overlying water. The impact of the sulfur cycle on the oxygen demand was considered important. The distribution in selected tidal flat deposits of total aerobic and sulfate reducing bacteria, total sulfides, redox potentials, volatile solids, and particle size was determined. The results of these measurements indicated that in general the finer sediments had higher concentration of total sulfides, a higher percent of volatile solids, and lower redox potentials. There was also a trend toward larger numbers of sulfate reducing bacteria in the finer deposits.

Measurements were made of the free sulfide concentrations in the water overlying tidal flat deposits having high concentrations of volatile solids, low redox potentials, and relatively large number of sulfate reducing bacteria. Free sulfide concentrations of approximately one mg/l were obtained, even in water containing four mg/l or more of dissolved oxygen. The literature indicated that such free sulfide levels could be quite toxic to a wide variety of aquatic organisms. At lower DO concentrations, free sulfides reached 16 mg/l; approximately 50 percent was estimated to be in the form of hydrogen sulfide.

Profiles of free sulfides immediately above these deposits

indicated higher concentrations near the sediment-water interface, suggesting production within and release of free sulfides from the sediments.

Laboratory studies were undertaken to further investigate the mechanism (s) leading to the production of free sulfides within tidal flat deposits. Mixed cultures of anaerobic bacteria were obtained from tidal flat sediments, and grown in media prepared from extracts of sediment and algae obtained from tidal flat areas.

Sulfide production rates as high as 90 mg(S)/l-day were measured following addition of lyophilized media to established liquid cultures of the anaerobic bacteria. The concentrations of sulfate and soluble organic carbon were observed to decrease in these cultures. The results indicated that the activity of sulfate reducing bacteria present in the mixed cultures was responsible for the sulfide production.

In media having varying initial concentrations of sulfate and soluble organic carbon, a maximum rate of 70 mg(S)/l-day was measured. This rate occurred at relatively high concentrations of both sulfate and soluble organic carbon, while at low concentrations of either, the rate was considerably reduced. The effect of changes in sulfate and soluble organic carbon concentrations upon the rate of sulfide production was much greater at low concentrations of either.

To further analyze and explain these relationships, a

mathematical model, based on the common Michaelis-Menton equation was developed. Data from experimental runs was used to fit estimates of parameters to the mathematical model. The results of simulation of cultures in which sulfate deficiency was strongly limiting sulfide production, indicated a sharper response of sulfide production to changes in sulfate concentration than expressed by standard Michaelis-Menton kinetics. By further modifying the model, close agreement was obtained between the experimental and simulated results of sulfide production.

Agreement was best for higher rates of sulfide production. The mathematical model results indicated a maximum attainable rate of sulfide production by stable populations of sulfate reducing bacteria growing in the mixed cultures of 77 mg(S) 1-day.

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