Algal-bacterial interaction within biofilms
by Andreas Rainer Escher

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Civil Engineering
Montana State University
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Abstract:
In a clean, shallow mountain river several parameters of water quality, such as oxygen, pH, alkalinity and others have been observed through day and night. In addition the film itself was observed with SEM. Some of the results were not explicable with simple models.

This observation led to a more detailed investigation of the algal-bacterial aggregates (i.e., films and floes).

The algal-bacterial aggregates are symbiotic communities of microorganisms. The algae produce O2 and soluble organic carbon and consume CO2. Their energy source is sunlight.

Bacteria consume the algal products and produce CO2 for the algae. The exchange of these products takes place with in the film.

The kinetics of these aggregates are not describable with traditional models for algal processes which only predict growth in pure culture, and not organic product formation rate. However, this rate is necessary to describe the interaction and the growth of both algae and bacteria.

Algal cells build their carbon skeleton with organic carbon formed from C02 using light energy. The enzyme ribulose diphosphate (RuBisCO) controls the first step of carbon fixation. RuBisCO has two unique properties: its molecular weight is over 500,000 and it has an active site for both O2 and CO2.

The reaction with water and CO2 (the substrate) yields two phosphoglycerates, one for carbon fixation and one that goes into the Calvin cycle. With O2 as the substrate, the reaction yields one phosphoglycerate that goes into the Calvin cycle and one phosphoglycolate that is released from the cell. Glycolate has recently been identified as a major algal product. The reaction rate of RuBisCO-CO2 and RuBisCO-O2 is controlled by a competitive inhibition or 2CO2 versus O2. Thus the ratio of [Co2/O2] is an important factor influencing the rate of fixation versus the rate of organic carbon release.

Once the rates of carbon fixation and carbon release have been determined, the growth rate of the algal biomass can be predicted.

Traditional kinetic expressions can then predict bacterial growth and respiration (CO2) rate. The resulting integrated model can be used to predict the kinetics of algal-bacterial aggregates.
ALGAL-BACTERIAL INTERACTION
WITHIN BIOFILMS

by
Andreas Rainer Escher

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Civil Engineering

MONTANA STATE UNIVERSITY
Bozeman, Montana
March 1983
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of a thesis submitted by

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This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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ABSTRACT

In a clean, shallow mountain river several parameters of water quality, such as oxygen, pH, alkalinity and others have been observed through day and night. In addition the film itself was observed with SEM. Some of the results were not explicable with simple models.

This observation led to a more detailed investigation of the algal-bacterial aggregates (i.e., films and flocs). The algal-bacterial aggregates are symbiotic communities of microorganisms. The algae produce O₂ and soluble organic carbon and consume CO₂. Their energy source is sunlight.

Bacteria consume the algal products and produce CO₂ for the algae. The exchange of these products takes place within the film.

The kinetics of these aggregates are not describable with traditional models for algal processes which only predict growth in pure culture, and not organic product formation rate. However, this rate is necessary to describe the interaction and the growth of both algae and bacteria.

Algal cells build their carbon skeleton with organic carbon formed from CO₂ using light energy. The enzyme ribulose diphosphate (RuBisCO) controls the first step of carbon fixation. RuBisCO has two unique properties: its molecular weight is over 500,000 and it has an active site for both O₂ and CO₂. The reaction with water and CO₂ (the substrate) yields two phosphoglycerates, one for carbon fixation and one that goes into the Calvin cycle. With O₂ as the substrate, the reaction yields one phosphoglycerate that goes into the Calvin cycle and one phosphoglycolate that is released from the cell. Glycolate has recently been identified as a major algal product. The reaction rate of RuBisCO-CO₂ and RuBisCO-O₂ is controlled by a competitive inhibition of CO₂ versus O₂. Thus the ratio of [CO₂]/[O₂] is an important factor influencing the rate of fixation versus the rate of organic carbon release.

Once the rates of carbon fixation and carbon release have been determined, the growth rate of the algal biomass can be predicted.

Traditional kinetic expressions can then predict bacterial growth and respiration (CO₂) rate. The resulting integrated model can be used to predict the kinetics of algal-bacterial aggregates.
INTRODUCTION

In natural water systems, stagnant and flowing, algae are the first link of the food chain, converting inorganic to organic carbon with energy from light. This carbon fixation provides the aquatic ecosystem with the needed energy for life.

Algal activities can be classified as follows:

**Photosynthesis**: Conversion of light into chemical energy in the photosystems of the algal cells.

**Carbon fixation**: Conversion of inorganic to organic carbon and integration of it in algal cells.

**Photorespiration**: Release of soluble organic carbon, connected with oxygen consumption and energy gained from photosynthesis.

Photorespiration and carbon fixation are parallel processes.

Algal activities vary during the diurnal cycle. The rates of $\text{CO}_2$ uptake and $\text{O}_2$ release change with light intensity and are essentially zero during the night. The rate of photorespiration or release of organic carbon is not constant either. Thus the concentrations of dissolved oxygen (DO), dissolved organic carbon (DOC), pH, and other components, vary during the diurnal cycle. The extent of change increases with the productivity of the system.
The activity of algae is regulated by its micro-environment. A high concentration of CO₂ enables the cell to fix carbon at a high rate whereas a low CO₂ concentration results in a high rate of photorespiration or release of organic carbon.

Bacteria, in close association with algae, consume organic carbon released by algae and respire oxygen, also an algal product. The bacteria transform organic carbon into CO₂, an essential nutrient for algae.

Algae and bacteria thus coexist in symbiosis. This symbiosis is important and can be observed in many natural systems such as rivers, lakes, and ponds.
LITERATURE REVIEW

Recent research on algae is broad and covers many different topics. The goal of this review is to correlate our work to the research trends.

Artificial Systems, Reactors, and Mathematical Models

Only two larger artificial streams are mentioned in the literature. One, in Dorset, U.K. (Marker and Casey, 1982), is a circular channel with recirculation and a controlled inflow and outflow. Marker used this channel for an ecological long-term study. Due to the recirculation, it was possible to assume kinetics of a continuous stirred tank reactor. The length of the channel is 52 meters.

Quite different, however useful, is the system at the Swiss Federal Institute in Zurich. It consists of a series of small parallel concrete channels with trapezoidal cross-section and a length of 200 meters. These channels have no recirculation (plug flow reactors). The parallel channels allow simultaneous experiments with different water compositions for comparable studies.

An interesting and elegant experiment was performed by Edelmann and Wuhrmann (1975) in the Swiss Channel. They used floating plastic foils to measure the generation
or consumption of oxygen. Untreated groundwater, which was most probably oversaturated with CO₂, was the water source.

Literature about laboratory reactors for algal growth is relatively rare and covers chemostats and cyclostats. No description for fixed film reactors could be found. Rhee, Gotham and Chisolm (1981) described extensively the kinetics of chemostats and cyclostats with their limitations. They state that it is difficult to assess the true values of the kinetic parameters by direct measurements. Due to light cycles, growth becomes cyclic and light intensity for a single algal cell is a function of light absorption of the liquid volume (density of suspended biomass) and of the whereabouts of the cell in the reactor. It is not possible to maintain algal biomass over an extended light period. Under such conditions, their metabolic activity becomes very different from their normal behavior.

An interesting approach to formulate the light intensity for suspended algal biomass are the stochastic models for algal photosynthesis by Sheth, Ramakrishna and Fredrickson (1977). These models define the average light intensity for turbulent flow in channels. It should be possible to use these models, with some changes, for chemostats.
Stabilization Lagoons, Field Observations, and Measurements

A large number of publications about algae in stabilization lagoons can be found. Unfortunately, most of these publications are of limited use for basic research on algae. A typical publication is the contribution of Azov and Shelef (1982). Based on work with stabilization lagoons, King (1972) published an interesting study on carbon limitation of algae.

A large group of publications deal with observations, but are often lacking extensive explanations of the observation. Visser and Couture (1981) observed an increased growth of algae in a medium enriched with different concentrations of organics from natural systems. The question is open if this increased growth was due to facultative heterotrophic growth of algae or due to compensation of limitation of macro or micronutrients.

The studies at the Swiss Federal Institute (Wuhrmann and Eichenberger, 1975; Edelmann and Wuhrmann, 1978; Eichenberger and Wuhrmann, 1975; Eichenberger, 1972), tried to elucidate the mechanisms of eutrophication of rivers. They used mixed populations of algae and bacteria in artificial channels of 200 meter length, and demonstrated the effects of inorganic enrichments on rivers. They also built up an energy balance over the systems. However, the ecological studies of Eichenberger
can be misleading. Since he used ground water with an oversaturation of CO$_2$, the relationship of heterotrophic to phototrophic biomass under different additions of waste water does not correspond with reality. In the "clean" water channel, algae were never carbon-limited and, as a result, demonstrated a higher growth rate than observed with an addition of 1% sewage.

King and Ball (1966) studied qualitatively and quantitatively the "Aufwuchs" production in a natural river and compared the heterotrophic and phototrophic growth.

An important ecological phenomenon has been observed by Sondergaard and Schierup (1982). They measured dissolved organic carbon during a bloom of algae in a lake. The highest concentration was 7.2 mg/l as C and 70-90% of dissolved organic carbon had a molecular weight of less than 300 grams per mole. No proportionality between algal biomass and dissolved organic carbon was found, which suggests that dissolved organic carbon production rate by algae is variable and controlled by other parameters besides biomass concentration.

Haack and McFeters (1982) observed and analyzed the relationship between algae and bacteria in an oligotrophic system. They proposed a descriptive model for interaction between algae and bacteria. McFeters, Stuart, and Olson (1978) also compared growth of heterotrophs in
algal growth supernatant with growth in natural water. An increase of heterotrophic growth by two to three orders of magnitude was observed due to the algal products.

Albright et al. (1980) observed in field studies similar to Haack and McPeters inorganic carbon and measured a great decrease of this compound during the peak of dissolved organic carbon. Their results show a relationship between decrease of inorganic carbon and production rate of organic carbon.

Schiefer and Caldwell (1982) finally proved the interaction between phototrophs and heterotrophs. They observed a high stimulation of growth of phototrophs under CO$_2$ limitation and addition of organic carbon. Under CO$_2$ enriched conditions, there was no stimulation. Algal growth was independent of bacterial activity.

Nutrient Uptake, Limiting Nutrients, and Chemical Inhibition.

Raven (1980) published a literature review about nutrient uptake in microalgae which covers the transport mechanisms of all nutrients and also the regulatory mechanisms involved in the transport systems.

In natural systems, there are normally two limiting nutrients: inorganic carbon and/or phosphorous.
Inorganic Carbon

Markel (1977) did an extensive study on CO$_2$ transport and photosynthetic productivity. He found that axenic phototroph cultures are CO$_2$ limited rather by transport of CO$_2$ from air to the cells than by uptake by the cells. Young and King (1973) described a rapid method of quantifying carbon uptake. They assume that the total organic carbon system is in equilibrium and calculate the change of organic carbon with the change of pH. This system of estimation has its limitations: reaeration has to be excluded and the carbonate system has to be in equilibrium. The restrictiveness of these assumptions was shown by Gavis and Ferguson (1975), who described the kinetics of the carbonate system at high pH and the kinetics of CO$_2$ uptake.

All the literature states that algae assimilate CO$_2$. However, Raven cites some evidence for HCO$_3^-$ assimilation and stresses that there is no unequivocal contradicting proof available.

King describes limitations of inorganic carbon in sewage lagoons. He calculates free CO$_2$ from alkalinity and pH, assuming equilibrium. Sources of CO$_2$ for algae are air and heterotrophically degraded organics. This analytical method does not allow complete CO$_2$ analysis, since equilibrium in the carbonate system will not be reached. The important part of this publication is
doubtless the discussion of succession in carbon-limited systems. It appears that bluegreen algae can compete effectively with green algae under CO$_2$-limited conditions. His conclusion concerning prevention of eutrophication by limiting carbon is remarkable.

Phosphorous Limitation

Phosphorous is a major limitation in natural environments. King (1972) proposes in his article about carbon-limitation that phosphorous limitation would be a much better tool to prevent eutrophication. No succession of different species under phosphorous limitation has been found. However, different species have a greater tolerance for phosphorous limitation. Lang and Brown (1981) found that cyanobacteria (bluegreen algae) have a higher tolerance than green algae. A model, similar to other limitations (organic carbon for bacteria, inorganic carbon for algae) has been developed by Auer and Canale (1980) which describes growth of Cladophora near the shoreline of the Great Lakes. The purpose of this model is the evaluation of strategies to prevent eutrophication of the Great Lakes.

Other Nutrients

A remarkable work was published by Azov and Goldman which showed inhibition of photosynthesis by green algae by free ammonia. Since some growth media use NH$_4^+$ as their nitrogen source, it is important to know the
threshold concentration for the inhibition. Batch cultures with ammonia as their nitrogen source are difficult to operate. If they use a high ammonia concentration, the growth will be inhibited. If ammonia is reduced to concentration below toxicity, the culture will be N-limited.

Photosynthesis, Photoinhibition, and Adaptation

Photosynthetic activity consists of two parallel groups of reactions: the light reaction and dark reaction.

The light reaction is a membrane reaction in the photosynthesis I and II. Miller (1979) shows these membranes with SEM of frozen, fractured membranes. He also shows a simplified diagram of electron and proton transport in the photosystems.

The dark reaction is described by Raven (1980) and also by Lorimer et al. (1977). The energy required for the dark reaction is captured in photosystems I and II. The activated enzyme D-Ribulose-1,5-biphosphate carboxylase-oxygenase (RuBisCO) converts CO₂ into organic carbon. This reaction is part of the Calvin cycle. An important characteristic of RuBisCO is its dual substrate affinity. It can react with CO₂ (carbon fixation) or O₂ (photorespiration). The latter substrate yields release of organic carbon of algal cells. The product of photorespiration
glycolic acid or glycolate. CO$_2$ is a competitive inhibitor of the O$_2$ reaction.

An important part of the activation of RuBisCO is CO$_2$ and Mg$^{2+}$.

$$\text{Enzyme} + \text{CO}_2 + \text{Mg}^{2+} \rightarrow \text{enzyme - CO}_2 - \text{Mg}^{2+}$$

This equation shows that RuBisCO cannot be activated in the absence of CO$_2$ or Mg$^{2+}$ and results in the following situation for the reaction:

Under high CO$_2$ concentration, RuBisCO acts only as carboxylase. CO$_2$ activates the enzyme and inhibits O$_2$ reaction. A reduced CO$_2$ concentration still activates RuBisCO but cannot inhibit the O$_2$ reaction or photorespiration. If CO$_2$ is completely absent, RuBisCO cannot be activated. Both CO$_2$ and O$_2$ reactions are inhibited.

It is possible to calculate the ratio of carbon fixation versus photorespiration with the model postulated by Escher and Characklis (1982). They defined the activity of the enzyme RuBisCO as a function of the light reaction. Christeller (1981) measured the half-saturation constants of different cations for the activation and formulated the activity coefficient as a function of CO$_2$ and cations. Calcium does not activate RuBisCO.

Yeoh et al. (1981) measured the variations in kinetic properties of RuBisCO in different plants. They found
that C₃ plants show smaller $K_m(CO_2)$ values (12-25 micromolar) than C₄ plants (28-34 micromolar). Water plants and algae exhibit high values similar to C₄ plants (30-70 micromolar). The variation of $K_m(RBP)$ (for activation) does not correlate to the three groups.

These authors do not mention the temperature dependency of the kinetic parameters. Nakamura and Miyachi (1980) found a great influence of temperature on the inhibiting role of CO₂ for photorespiration. By raising the temperature from 20°C to 36°C during photosynthesis, $^{14}CO_2$ incorporation into glycolate immediately stopped, whereas that into sucrose was greatly enhanced. When the temperature was lowered from 36°C to 20°C, $^{14}C$-glycolate was greatly enhanced, whereas sucrose formation was slowed. No significant change of $^{14}CO_2$ uptake was induced by either temperature change. When α-HPMS was added, glycolate formation increased to 30% of fixed carbon at different temperatures.

Contradictory to the work of Nakamura and Miyachi (1980) is the work of Collins and Boylen (1982) who found the highest rate of photorespiration at high temperatures and low light. (Photorespiration was 40% of total fixed carbon.) Unfortunately, it is not possible to compare the two publications because neither mention growth conditions, especially the CO₂ concentrations.
Not only does CO$_2$ control the activation of RuBisCO but it is also involved in the inhibition of Photosystem II. Khanna et al. (1981) show that CO$_2$ depletion of thylakoid membranes results in a decrease of binding affinity of the Photosystem II inhibitor atrazine. This affects an inactivation of part of the electron transport chains. The CO$_2$ depletion does not produce structural changes in enzyme complexes involved in Photosystem II function of thylakoid membranes, as shown by freeze-fracture studies with SEM. The results also show that as soon as CO$_2$ is available, Photosystem II will be reactivated.

This phenomenon has been observed by Liou and van Eybergen (1982) and Cornic et al. (1978). Liou and van Eybergen (1982) measured a relative decrease in chlorophylls during a longer period of photoinhibition. Cornic et al. (1978) measured the photoinhibition in intact chloroplasts as a function of CO$_2$ concentration. They express the effect of inhibition as percentage inhibition of O$_2$ evolution. Both low CO$_2$ and a high light intensity can cause photoinhibition. A reduction of inhibition was found by addition of Calvin cycle intermediates such as 3-phosphoglycerate.

Summary

Photorespiration is controlled by CO$_2$ as a competitive inhibitor whereas carbon fixation of inorganic carbon only
depends on CO₂.

RuBisCO activation is controlled by cations as 
Ma²⁺, Mg²⁺, and CO₂ (no Ca²⁺ is involved).

Photoinhibition is controlled by light intensity
and CO₂. Intermediates of the Calvin cycle can reduce
photoinhibition.

Adaptation to different light intensities is a very
important process for algae since they are able to optimize
the capacity of the photosystems to the amount of light.
This adaptation is a relatively slow control process
compared to photoinhibition.

Nielsen and Jorgensen (1968) found that algae grown
at 1 klux have about 10 times more chlorophyll a than
algae grown at 21 klux. This observation is important
for studies of algae grown in aggregates, i.e., films
and flocs. Algae in lower layers of films can adapt to
the reduced illumination and fix carbon quite efficiently.

The study of Cornic (1978) shows that photoadaptation
also can be caused by a long term CO₂ limitation. Long-
term experiments with low CO₂ concentrations imposed a
photoinhibition. After this treatment, high CO₂ concen-
trations did not reverse the inhibition.

One can assume the following sequence: After the
first CO₂ limitation Photosystem II was inhibited by
atrazine. After this "short-term" inhibition the
photosystem adapted to a lower level by reducing
chlorophyll a.

Conclusion

It appears that ecological studies between algae and bacteria require a good understanding of the mechanisms controlling photosynthesis. This literature review shows that it is not possible to predict the response of algae to short as well as long-term changes with unstructured models for algae and their photosynthetic activity.
GOALS OF THE RESEARCH

The dynamics of algal systems are receiving increased attention due to their influence on water quality during the diurnal cycle and also their role in conversion of solar energy to biomass and other organic carbon forms. The latter is a new and important topic of research for energy and chemical production. In the last several years, different models have been proposed to describe algal behavior in aquatic systems (Young and King, 1973; King, 1976; Gavis and Ferguson, 1975; Markel, 1977). All the models are important and describe the kinetics of CO₂ uptake for carbon fixation.

The weakness of previous models is their inability to predict the amount of photorespiration, the rate of release of dissolved organic carbon. Photorespiration rate is essential to describe the interaction between algae and bacteria and, therefore, their activity in natural systems. The concentration of dissolved organic carbon controls, together with oxygen, the metabolism of bacteria.

Generally, algae and bacteria occur in close association and form aggregates, i.e., flocs and films. Within aggregates, the mechanisms of algal-bacterial interaction are extremely important. The transport of compounds from
bulk water into aggregates is limited by flux. In contrast, due to the very close association of both algae and bacteria, the transport between the two is not limited. Therefore, the microenvironment for the two microorganisms is mainly determined by their activity.

Figure 1, a scanning electron micrograph, shows a typical film of algae and bacteria. The bacteria are attached to algae (diatoms) and fill parts of the interstitial volume. This sample is taken from Squaw Creek, an oligotrophic mountain stream.

The goal of this research is, first, to develop a model of algal growth and photorespiration and link this model with the traditional kinetic model of bacteria, and second, to derive a mathematical system describing the interaction between algae and bacteria within aggregates.
Figure 1. SEM of algal-bacterial film (bar 10 μm) from Squaw Creek, an oligotrophic mountain stream. Thickness of film was 40–60 μm.
APPLICATION OF THE RESEARCH

The major application of the model for algal-bacterial interaction is prediction of algal-bacterial aggregate behavior under different steady-state and non-steady-state conditions.

Stream and Lake Water Quality

Traditional parameters used to describe water quality are dissolved oxygen, pH, organic carbon, acidity, alkalinity, temperature, and dissolved and suspended solids.

Most of these parameters are not constant and change during diurnal and seasonal cycles, frequently as a direct result of microbiological activity. The relationship of these parameters to the "quality" of the water is not always clear.

This model may help predict the quality of a water system under pressure posed by population growth changes, in farming techniques, and industrial development.

Hydrologists have long been concerned with variability of the flow of rivers. Because of the difficulty of observation, much less attention has been given to the variability of biological activity and physical variability associated with natural variations and cycles in rivers. Many measurements of biological effects are done during low
and summer flows when measurement is easy, organisms often
flourish, and concentrations of various substances in the
flow are high. The effect of winter flow on the growth
of slimes on the bottoms of rivers, for example, and the
influence of periodic floods on flora and fauna are not
well documented. Significantly, however, among the most
common trends in river management is the progressive
regulation of flow through the provision of storage.
Conceivably, regulation rather than pollutants alone may
have the most far-reaching effects on the character of
many river systems. To date, observations have not been
designed to measure these effects.

Wastewater Treatment and Carbon Dynamics

Stabilization ponds are one of the most commonly used
wastewater treatment processes in this country, especially
for small communities and industries. In 1971, over 3,500
stabilization ponds existed in the U.S. for handling
domestic wastewater (McKinney et al, 1971). The stabiliza­tion pond depends primarily on photosynthesis for its oxygen
(stabilization ponds are frequently referred to as oxidation
ponds).

Presumably, pond operation depends on the aerobic
bacterial degradation of organic wastes coupled with photo­
synthesis to supply the necessary oxygen. However, oper­
ating data suggest a more complicated operating regime.
For example, Hendricks and Pote (1974) performed material
balances across an operating pond and found that the removal of total organic carbon was nil. Many investigators have observed this phenomenon before, and attributed the observation to production of algal cells, i.e., conversion of inorganic carbon to soluble and particulate organic carbon.

Hendricks and Pote measured a substantial increase in soluble organic carbon across the pond. The increase could be due to cell lysis or to organic carbon excretion by algal cells. In their study, oxygen was undoubtedly at a supersaturated level and pH was quite high (i.e., low carbon dioxide). Under these conditions a high photosynthesis rate has to be assumed.

The model presented herein determines which parameters must be controlled to improve the performance of the pond. During this research, the influence of \([CO_2]/[O_2]\) ratio on algal-bacterial dynamics will be measured to determine the extent of its influence on bacterial organic carbon degradation, algal CO\(_2\) fixation, and algal organic carbon excretion.

**Cooling Water**

A major problem facing the power industry and manufacturing industries in the U.S. is fouling of heat transfer surfaces. Microbial deposits accumulate on heat transfer surfaces reducing heat transfer rate, increasing fluid frictional resistance, and increasing corrosion rates.
Annual energy and material losses are estimated to be in the billions of dollars per year.

In many recirculating cooling tower systems, algal-bacterial mats accumulate on the cooling tower plenum. The algal-bacterial mats are troublesome because when the mats slough periodically, they can clog some of the distributor tubes. Others claim that the mats are responsible, in part, for fouling of the condensers. The algal-bacterial mats presumably contribute organic carbon which is used for energy and carbon for bacterial growth within the condenser tubes. This research will determine if this is a problem within the operating range of pH (pH is controlled in most cooling tower systems) and DO found in recirculating cooling towers. This would be a benefit since various chemical treatments are being used at present because algae are evident, even though it is not known whether they are excreting organic carbon. These treatments are costly and frequently involve non-degradable or toxic materials (e.g., copper sulfate addition).
THEORETICAL MODEL

Figure 2 shows a schematic of a river. It assumes that about 100% of the biomass is attached on the riverbed and forms a biofilm.

Figure 2. Schematic of a turbulent stream, where \( d \) is the depth, \( \text{Th} \) the thickness of film, \( v \) the velocity of bulk water, the concentrations \( c_g \) (air), \( c_b \) (bulk water) and \( c \) within the film. \( E \) is the light energy.
The single algal and bacterial cells of the film are the "reaction sites." The interstitial water fills the porous volume of the film and represents, with its concentrations, the microenvironment of the cells. The laminar boundary layer separates the interstitial from the bulk water.

Figure 3. Schematic of algal-bacterial interaction in aggregates. Arrows indicate the direction of transport.

According to Figure 3 all compounds produced by a cell are released to the interstitial water, where they are taken up by other cells or transported by a chemical
gradient through the laminar boundary layer to the bulk water. Biomass concentration in the bulk water is assumed to be very low, about zero percent of the total system biomass. Therefore, there is no microbiological reaction in the bulk water and all the changes of concentration in the bulk water are due to transport.

The system can be structured into four control volumes, each separated from the other by a permeable boundary (see Figure 3):

1. The Reacting Volume, which consists of a) the algal cells, and b) the bacterial cells. This volume is the reaction site.

2. The Interstitial Water Volume within the film is separated from the cell volume by cell walls and reflects the microenvironment of both algae and bacteria. The Interstitial Water Volume dictates the activities of the algal and bacterial cells and is a "short-term storage" for the products of the cells. Dissolved compounds in the interstitial water are taken up by cells or transported by a chemical concentration gradient to the bulk water.
3. The Bulk Water Volume is separated from the film by the laminar boundary layer and is not in direct contact with the cells. Under the assumption that about 100% of the total biomass is fixed in the film, the bulk becomes a "long-term storage volume." If the bulk water is turbulent, it will be completely mixed and the concentration gradients within the bulk are nil.

4. The Air Volume is separated from the bulk water by the liquid-gas interface. Depending on the partial pressure and the relative concentration, the effects of reaeration cause a flux of oxygen and carbon dioxide through this interface. Generally, the air volume is assumed very large and the flux between bulk water and the air causes no change of the partial pressure in the air overlaying the water.

In this study, measurements were restricted to the bulk water. The sizes of the oxygen and pH probes did not allow direct measurements within the film and the required amount of sample for analysis is too great to examine the concentrations of the interstitial water.

At a future time, this problem may be solved with microelectrodes.
The model must be reduced to a measurable system: the whole film, including algal and bacterial cells as well as the interstitial water becomes the "reaction site" where the flux of compounds through the boundary layer reflects the net rates of consumption and production by algae and bacteria. The net rates do not reflect the single algal or bacterial rates but the sum of both. Thus the measurements of the bulk concentrations give only qualitative interpretations of the individual activities and interactions.

Background

The organisms constituting the aggregate (e.g., attached films and flocs) include algae and bacteria, organisms which differ significantly in genetic, physiological, morphological, and taxonomic characteristics.

Algae

Algae are photosynthetic eucaryotes. The energy derived from light generates the necessary ATP and NADPH for carbon fixation. The electrons for the reduction of NADP to NADPH come from the splitting of water molecules. There are two systems of light reactions, photosystem I and photosystem II, associated with the flow of electrons (Brock, 1979). The overall process occurring in these light systems can be expressed as follows.
\[
\text{H}_2\text{O} \xrightarrow{\text{light}} 2 \text{H}^+ + \frac{1}{2} \text{O}_2 + 2 \text{e}^- \quad (1)
\]

In addition to creating "reducing power," ATP and NADPH are generated and used in carbon fixation and internal metabolism.

Raven (1980) states that algae can only fix inorganic carbon in the form of carbon dioxide (CO\(_2\)). Other inorganic carbon species (H\(_2\)CO\(_3\), HCO\(_3\), CO\(_3^\text{−}\)) cannot be used. The first step in the fixation process is mediated by the enzyme ribulose biphosphate carboxylase-oxygenase, RuBisCO. RuBisCO apparently is present in all photolithotrophs and initiates the reaction sequence leading to net conversion of organic carbon to organic compounds. RuBisCO has active sites for both carbon dioxide and oxygen. RuBisCO has two other notable characteristics: its molecular weight is approximately 500,000 daltons, which limits the amount of enzyme within the cell volume. Further, it has a slow turnover rate of about 10 s\(^{-1}\). These two characteristics are responsible for a low amount of activated enzyme.

The reactions catalyzed by this enzyme can be expressed as follows:

\[
\text{RuBisCO-CO}_2 + \text{CO}_2 + \text{H}_2\text{O} \rightarrow 2 \text{3-phosphoglycerate} \quad (2)
\]

\[
\text{RuBisCO-O}_2 + \text{O}_2 \rightarrow \text{3-phosphoglycerate} + \text{2-phosphoglycolate} \quad (3)
\]
Raven (1980) reports an in vitro RuBisCO-CO\(_2\)/RuBisCO-O\(_2\) ratio of 5 in an air-saturated solution in which the [CO\(_2\)]/[O\(_2\)] ratio was approximately 0.04. In vivo, reactions (2) and (3) are occurring in parallel with photosystem I and II reactions which are generating O\(_2\). The glycolate product in Eq (3) runs counter to the carboxylative trend of photosynthesis, requiring further energy for enzyme synthesis (Figure 4). Glycolate will ultimately be excreted by the algal cell. Production of algal biomass is only possible when carbon uptake is higher than carbon release.

Carbon dioxide and oxygen are transported through the cell wall by diffusion at a rate proportional to the concentration gradient across the cell wall where the active enzymes are located (Raven, 1980). A high flux of CO\(_2\) is necessary to maintain a high RuBisCO-CO\(_2\) reaction rate.

Bacteria

Bacteria are chemosynthetic procaryotes using O\(_2\) (in aerobic environments) to oxidize organic carbon (i.e., heterotrophs) which generates energy for synthesis and other cellular activities. CO\(_2\) is released as a product of respiration.
Interaction Between Algae and Bacteria

A high extracellular \([\text{CO}_2]/[\text{O}_2]\) ratio results in a high intracellular RuBisCO-\text{CO}_2/RuBisCO-\text{O}_2 ratio and little
production of glycolate, an energy-wasting process. In many aquatic environments, carbon fixation is limited by the low \([\text{CO}_2]/[\text{O}_2]\) ratio in the aqueous phase. However, this ratio may be significantly higher in microenvironments inhabited by respiring bacteria, e.g., algal-bacterial biofilms (McFeters et al., 1978; Haack and McFeters, 1982). Apparently, this interaction is symbiotic regardless of whether the bacteria are consuming algal organic excretory products or other organic carbon.

The relationships governing the algal-bacterial interactions within a biofilm are schematically diagrammed in Figure 3. It is evident that significant changes in microbial activity within the biofilm will occur from day to night and will influence pH, inorganic carbon content, and dissolved oxygen within the biofilm which, in turn, may influence these parameters in the overlying water as well.

**Reaction Kinetics: Algal Cell**

The rate of carbon fixation in the algal cells within the biofilm is dependent on two variables:

1. rate of enzyme (RuBisCO) synthesis which is related to energy flow
2. enzyme (RuBisCO) activity which depends on the \([\text{CO}_2]/[\text{O}_2]\) ratio.
Rate of Enzyme Synthesis

The production rate of RuBisCO is proportional to the net ATP and NADPH formation. These two are produced in the photosystem I and II

\[ n \text{ P} + n \text{ ADP} + m \text{ NADP} + m \text{ H}^+ \rightarrow n \text{ ATP} + m \text{ NADPH}^+ \quad (4) \]

and the rate of the reaction is dependent on available light:

\[ r_D = r_{D_{\text{max}}} \cdot \frac{E}{K_E + E} \quad (5) \]

Assuming that all ATP and NADPH produced in the photosystems is available for enzyme synthesis, then

\[ r_R = r_{R_{\text{max}}} \cdot \frac{E}{K_E + E} \quad (6) \]

The differential equation for RuBisCO accumulation within the cell can be written

\[ \frac{d\text{RuBisCO}}{dt} = r_{R_{\text{max}}} \cdot \frac{E}{K_E + E} - r_{RC} - r_{RO} \quad (7) \]

Enzyme Activity

Lorimer et al (1977) investigated and measured the activity of RuBisCO and proposed that the RuBisCO-O₂
reaction is described by a linear competitive inhibition model (Bailey and Ollis, 1977) with CO₂ as the inhibitor. They measured \( K_{O_2} = 200 \) μmole and \( K_{CO_2} = 20 \) μmole.

\[
r_{RO} = [\text{RuBisCO}] \cdot r_{RO_{max}} \cdot \frac{[O_2]}{[O_2] + K_{O_2} \cdot \left(1 + \frac{[CO_2]}{K_{CO_2}} \right)}
\]

\( O_2 \) is not an inhibitor for the RuBisCO-CO₂ reaction. Therefore, the rate for the RuBisCO-CO₂ is

\[
r_{RC} = [\text{RuBisCO}] \cdot r_{RC_{max}} \cdot \frac{[CO_2]}{[CO_2] + K_{CO_2}}
\]

Material Balances Across the Algal Cell

In vivo concentrations of CO₂ and O₂ can be described by the following material balances across the cell boundaries:

\[
\frac{d[CO_2]^0}{dt} = \frac{A_A}{V_A} \cdot k \left( [CO_2] - [CO_2]^0 \right) - r_{RC}
\]

Net rate of accumulation into the cell

Rate of consumption within the cell

\[
\frac{d[O_2]^0}{dt} = \frac{A_A}{V_A} k' \left( [O_2] - [O_2]^0 \right) - r_{RO} + r_P
\]

Net rate of transport into the cell

Rate of photosynthetic production rate within the cell
where $A_A$ = surface of algal cell $(L^2)$

$[\text{CO}_2]^O$, $[\text{O}_2]^O$ = intracellular concentration (mol L$^{-3}$)

$[\text{CO}_2]$, $[\text{O}_2]$ = extracellular concentration (mol L$^{-3}$)

$V_A$ = volume of algal cell $(L^3)$

$k, k'$ = mass transfer coefficient $(L$ t$^{-1})$

Notable features of the model include (1) the importance of the extracellular concentrations of CO$_2$ and O$_2$ and (2) the inclusion of O$_2$ production by photosystems I and II. The model also can account for extracellular and intracellular $[\text{CO}_2]/[\text{O}_2]$ ratios which are important in describing transient conditions (Figure 5). In addition, the model permits flow of oxygen across the cell wall in either direction.

Figure 5. Relative algal intracellular and extracellular concentrations of oxygen and carbon dioxide, reflecting the influence of reaction and diffusion.
Assuming negligible accumulation of CO$_2$ and O$_2$ in the cell (i.e., $d/dt = 0$),

$$r_{RC} = \frac{A_A}{V_A} \cdot k \left( [CO_2] - [CO_2]^0 \right) \quad (12)$$

$$r_{RO} = \frac{A_A}{V_A} \cdot k' \left( [O_2] - [O_2]^0 \right) + r_p \quad (13)$$

Inserting the kinetic equations for $r_{RC}$ and $r_{RO}$ (Eq. 8 and 9), the rate of carbon fixation and release of organic carbon can be estimated as follows:

$$\frac{dC}{dt} = (r_{RC} - r_{RO}) \cdot X_A \quad (14)$$

The rate of growth of the algal cells can be related to the rate of carbon accumulation as follows:

$$\mu_A X_A = Y_A \frac{dC}{dt} = Y_A X_A \cdot (r_{RC} - 2r_{RO}) \quad (15)$$

where $Y_A$ is the yield (biomass formed per mass carbon fixed) and $\mu_A$ is the specific growth rate.

The production of algal biomass can be defined as follows:

$$\frac{dX_A}{dt} = \mu_A X_A - d_A X_A \quad (16)$$
where $X_A$ is the algal biomass and $d_A$ is a decay coefficient.

**Bacterial Cell**

Traditional differential equations can be used to describe aerobic bacterial kinetics, such as

$$\frac{dX_B}{dt} = \mu_B X_B - d_B X_B \quad (17)$$

$$\frac{ds}{dt} = -\frac{1}{Y_{BS}} \cdot \mu_B X_B \quad (18)$$

$$\frac{dCO_2}{dt} = \frac{1}{Y_{BC}} \cdot \mu_B X_B \quad (20)$$

$$\frac{dO_2}{dt} = -\frac{1}{Y_{BO}} \cdot \mu_B X_B \quad (19)$$

where $\mu_B$ = specific growth rate (t$^{-1}$)

$x_B$ = bacterial biomass (ML$^{-3}$)

$d_B$ = decay coefficient (t$^{-1}$)

$Y_{Bi}$ = yield (bacterial biomass formed per mass consumed)

$s$ = substrate (organic carbon) (ML$^{-3}$)

The model for the bacteria is a simple unstructured model with Monod kinetics. Their activity is determined by substrate concentration outside the cell.

The growth rate for potential dual substrate limitation is:
\[ \mu_B = \mu_{\text{max} B} \left( \frac{S}{S + K_s} \right) \left( \frac{[O_2]}{[O_2] + K'_O} \right) \]  

Equation 21 reflects the dual substrate limitation of organic carbon and oxygen for bacteria, where \( \mu_{\text{max} B} \) is the maximal growth rate of bacteria.

Most algae assimilate inorganic carbon in the form of \( \text{CO}_2 \). However, some can assimilate \( \text{HCO}_3^- \) as well, either directly or by enzymatic dehydroxylation. Gavis and Ferguson (1975) describe very extensively the inorganic carbon system and the kinetics of carbon dioxide.

The simplified model with no direct measurements in the film does not justify the analysis of the entire inorganic carbon system. At this point, mass balances for inorganic carbon \( C_T \) are more useful than consideration of subspecies \( \text{CO}_2 \).

**Kinetic Model**

Since it is not yet possible to measure directly within the film, the entire biomass and the interstitial water is the reaction volume. The flux from or into the film will be considered as the net reaction rate. The flux is obviously the result of the following condition:
\[
N_b = k \cdot (c - c_b) \quad (22)
\]

- Flux through laminar boundary layer
- Mass transfer coefficient \( k \)
- Chemical concentration gradient
- \( C \): Concentration in interstitial water
- \( C_b \): Concentration in bulk water
- It is assumed that \( c \) is uniform throughout the film.

where \( c \) (concentration in interstitial water) results from the integration of the differential equation.

\[
\frac{dC}{dt} = \alpha_A r_A + \alpha_B r_B - \frac{A_b}{V} N_b \quad (23)
\]

- Accumulation rate within the film
- Rate of algae (consumption or production)
- Rate of bacteria (production or consumption)
- Flux through laminar boundary layer

where \( \alpha_A, \alpha_B \) are proportionality constants, and \( A_b \) the surface area of the bulk water-film interface and \( V \) the volume of the interstitial water.

Therefore, the net reaction rate within the film is equal to the flux

\[
R_{film} = N_b \quad (24)
\]

Assuming a reaction rate \( r \) in the bulk, the following differential equation can be formulated:
where $N_g$ is the flux through the liquid-air interface and $A_g$ the liquid-air interfacial area. Assuming that a river has characteristics of a plug flow reactor, Equation 25 becomes (steady state, $\partial C_i/\partial t = 0$):

$$
\frac{\partial C_i}{\partial t} = \frac{V_x}{\partial x} \frac{\partial C}{\partial x} - \frac{A_b}{V_b} N_b - \frac{A_g}{V_g} N_g - r
$$

(26)

where $N(x)$ is the flux at $x$. Due to dispersion and changes in the flow pattern, the plug flow system may be simplified to a series of CSTR (Continuous Stirred Tank Reactors). This approach is quite common in plug flow studies.

The standard equation for a single CSTR is

$$
V \frac{dc}{dt} = F (C_0 - C) + V r
$$

(27)

Accumulation Flow in and out Reaction of the system

If a short segment (unit length = 1) of the river is treated as reactor $i$ of the series simulating the plug flow of the river, the material balances become:

$$
\frac{dc_i}{dt} = D (C_{i-1} - C_i) + \frac{A_{bi}}{V_{bi}} N_b + \frac{A_{gi}}{V_{gi}} N_g + r
$$

(28)
where \( D \) is the dilution rate (flow rate divided by volume) and \( c_{i-1} \) the inflow concentration.

Equation 28 assumes that the flux over the entire unit length is the same.

Equation 28 also describes a typical continuous stirred tank reactor. If the assumptions for Equation 27 are useful and correct to describe a short segment of a river, then we can also design a reactor which is described by Equation 28.

For this system we can write the following general mass balance:

\[
\frac{V_b \cdot dc}{dt} = F \cdot (c_0 - c) + A_b N_b + A_g N_g + r \tag{29}
\]

accumulation flow in flux from flux from reaction
flow out film into air into rate
in bulk water bulk water within bulk water
By dividing Equation 29 by volume we obtain:

\[
\frac{dc}{dt} = D_b(c_o - c) + \frac{A_b}{V_b} N_b + \frac{A_g}{V_b} N_g + r
\]  

(30)

Equations 28 and 30 are identical. Experiments with a reactor with characteristics of Eq. 29 can be used to simulate a short segment of a river.

The mass balances for different compounds can be written:

**Dissolved oxygen \([O_2]\):**

\[
\frac{d[O_2]}{dt} = D_b([O_2]_o - [O_2]) + \frac{A_b}{V_b} N_{bo} + \frac{A_g}{V_b} N_{go} + r_o
\]  

(31)

**Inorganic carbon \(C_T\):**

\[
\frac{dC_T}{dt} = D_b(C_{To} - C_T) + \frac{A_b}{V_b} N_{bc} + \frac{A_g}{V_b} N_{gc} + r_c
\]  

(32)

Compounds such as dissolved organic carbon and biomass have no exchange with the air and the equations become:

**Dissolved organic carbon, \(s\):**

\[
\frac{ds}{dt} = D_b(s_o - s) + \frac{A_b}{V_b} N_{bs} + r_s
\]  

(33)
Biomass X:

\[ \frac{dX}{dt} = -DX + \frac{A_b}{V_b} N_{bx} + r_x \]  

(34)

**Steady State Conditions**

Under steady state conditions and a dilution rate greater than max. growth rate, \( r_{\text{max}} \) (wash out of suspended biomass), Equation 30 can be rearranged:

\[ \left( \frac{dC}{dt} \right) = 0 ; r_i = 0 \]

\[ D_b (C - C_0) - \frac{A_g}{V_b} N_g = \frac{A_b}{V_b} N_b \]  

(35)

or:

\[ \frac{D_b V_b}{A_b} (C - C_0) - \frac{A_g}{A_b} N_g = N_b \]  

(36)

where:

\[ \frac{D_b V_b}{A_b} = \frac{F_w}{A_b} = L_b \quad [L \cdot t^{-1}] \]  

(37)

\( L_b \) is the hydraulic loading rate for the film surface \( A_b \). Under these conditions, Equations 31 to 34 become:

**Dissolved oxygen \([O_2]\):**

\[ L_b ([O_2] - [O_2]_o) - \frac{A_g}{A_b} N_{go} = N_{bo} \]  

(38)
Inorganic carbon, $C_T$:

$$L_b \left( C_T - C_{T_0} \right) - \frac{A_g}{A_b} N_g C = N_{bc} \quad (39)$$

Dissolved organic carbon, $s$:

$$L_b ( S - S_0 ) = N_{bs} \quad (40)$$

Biomass, $x$:

$$L_b X = N_{bx} \quad (41)$$

At this point, there are four equations for the bulk water phase with six unknowns: $N_{g0}$, $N_g C$, $N_{b0}$, $N_{bc}$, $N_{bo}$, and $N_{bx}$. The first two, the flux of gases through the liquid-gas interface, have to be determined separately.

In natural systems, the flux of $CO_2$ and $O_2$ has to be estimated with Henry's Law and a function of the Reynolds number of the flow of the bulk water.

Under laboratory conditions, the flux can be measured directly (Figure 7). If the gas is in a sealed volume, an additional general equation for the gas phase can be written:

$$\frac{V_g d C_g}{dt} = F_g (C_{g0} - C_g) - A_g N_g \quad (42)$$

Or, after dividing by $V_g$,

$$\frac{dC_g}{dt} = D_g (C_{g0} - C_g) - \frac{A_g N_g}{V_g} \quad (43)$$
Figure 7. Same reactor as Figure 6, but with a controlled gas volume, where $F_g$ is the flow of gas into the reactor, $c_{go}$ the inflow concentration of gas, $c_g$ the gas concentration within gas volume $V_g$ and $N_g$ the flux from gas volume into bulk water.

Under steady state conditions ($\frac{dc}{dt} = 0$) it becomes:

$$D_g(c_{go} - c_g) = \frac{A_g}{V_g} N_g$$

(44)

With the same procedures used in equation 37, equation 44 becomes:

$$L_g(c_{go} - c_g) = N_g$$

(45)

where $L_g$ is the loading rate for the surface $A_g$. 
Now two additional gas phase equations can be written for oxygen and carbon dioxide.

\[ L_g((O_2)_o - (O_2)) = N_{g0} \]  \hspace{1cm} (46)

Inorganic carbon \((C_T)\):

\[ L_g((C_T)_o - (C_T)) = N_{gC} \]  \hspace{1cm} (47)

where \((O_2)\) and \((C_T)\) is mass per volume unit of gas.

With Equation 46 and 47 we can rewrite Equations 38 and 39:

**Oxygen**:

\[ L_b([O_2] - [O_2]_o) - \frac{A_g}{A_b} L_g((O_2)_o - (O_2)) = N_{b0} \]  \hspace{1cm} (48)

**Inorganic carbon**:

\[ L_b(C_T - C_{T0}) - \frac{A_g}{A_b} L_g((C_T)_o - (C_T)) = N_{bC} \]  \hspace{1cm} (49)

**Dissolved organic carbon**:

\[ L_b(S - S_o) = N_{bs} \]  \hspace{1cm} (40)

**Biomass**:

\[ L_bX = N_{bX} \]  \hspace{1cm} (41)
Non Steady State

Under non steady state conditions, the term for accumulation $dc/dt$ does not equal zero. The concentrations are changing with time. Therefore, Equations 40, 41, 48, and 49 are not valid.

The flux $N_a$ and $N_b$ and the loading rates throughout the system, $L_a (c_o - c)$ and $L_b (c_o - c)$, might not be constant with respect to time. The material balances for the air now become:

$$\frac{V_a dc}{dt} = F_g (c_o - c) - A_g N_g$$

and for the bulk fluid:

$$\frac{V_b dc}{dt} = F_b (c_o - c) + A_g N_g + A_b N_b$$

The number of independent variables increases from four at steady state to six at non steady state. Therefore, six equations are needed to solve the system.
By dividing Equations 50 and 51 by $A_g$, we obtain for the gas:

$$\frac{V_g}{A_g} \frac{dc}{dt} = L_g (c_o - c) - N_g$$

and for the bulk fluid:

$$\frac{V_b}{A_b} \frac{dc}{dt} = L_b (c_o - c) + \frac{A_g}{A_b} N_g + N_b$$

$V = R \frac{A}{A}$

$R$ is the hydraulic radius. In the bulk water, $R_b$ is defined as volume of bulk water divided by wetted surface, i.e., biofilm. In the gas volume, $R_g$ is the volume of gas divided by the gas-water interfacial area. Equations 52 and 53 become:

$$\frac{dc}{dt} = \frac{1}{R_g} \left\{ L_g (c_o - c) - N_g \right\}$$

$$\frac{dc}{dt} = \frac{1}{R_b} \left\{ L_b (c_o - c) + \frac{A_g}{A_b} N_g + N_b \right\}$$
Six continuum equations can now be written for the entire system. For the gas volume:

**Oxygen (O₂):**

\[
\frac{d(O₂)}{dt} = \frac{1}{R_g} \left( L_g((O₂)_o - (O₂)) - N_{gO} \right)
\]

(56)

**Inorganic carbon, (Cₜ):**

\[
\frac{d(Cₜ)}{dt} = \frac{1}{R_g} \left( L_g((Cₜ)_o - (Cₜ)) - N_{gC} \right)
\]

(57)

For the bulk fluid volume:

**Dissolved Oxygen, [O₂]:**

\[
\frac{d[O₂]}{dt} = \frac{1}{R_b} \left( L_b([O₂]_o - [O₂]) + \frac{A_g}{A_b} N_{gO} + N_{bO} \right)
\]

(58)

**Inorganic carbon, Cₜ:**

\[
\frac{dCₜ}{dt} = \frac{1}{R_b} \left( L_b(Cₜ_o - Cₜ) + \frac{A_g}{A_b} N_{gC} + N_{bC} \right)
\]

(59)

**Dissolved organic carbon, s:**

\[
\frac{ds}{dt} = \frac{1}{R_b} \left( L_b(s_o - s) + N_{bs} \right)
\]

(60)

**Biomass, X:**

\[
\frac{dX}{dt} = \frac{1}{R_b} \left( -L_bX + N_{bX} \right)
\]

(61)
Limitations of the Model

The model deals only with concentrations in bulk water and gas volumes. It does not describe the biological activity within the reaction site (i.e., the biofilm) but only the flux from or to the film. Its important limitations are:

- The flux is only the difference between algal production and bacterial consumption rate and vice versa.
- The conditions within the film necessary to solve Equations 10 to 16 for the growth of algae are unknown.
- The biofilm concentration of dissolved organic carbon needed to calculate the growth of bacteria is unknown.

Conclusion

A model has been developed to determine the net rate of algal-bacterial activities with simple measurements in the bulk water and in the overlying air volume. The model assumes that the net flux of compounds into the biofilm is equal to the net rate of algal and bacterial activity. It is also possible to determine the rate of detachment of biomass. However, further work for the verification of the model has to be done.
The response of the theoretical model system will be compared to two sets of observations during the transient period in light intensity occurring at sunrise. One set of observations is from a wastewater lagoon, a eutrophic environment. The other observations were made in an oligotrophic mountain stream. The biotic component in the lagoon consists of algal-bacterial association in flocs suspended in an organic-rich wastewater. In the clear, shallow, turbulent, mountain stream, the biotic activity is restricted to periphytic algal-bacterial associations, i.e., a biofilm.

**Eutrophic Environment**

As sunrise approaches, a wastewater lagoon is rich in CO$_2$ and essentially devoid of dissolved oxygen as a result of bacterial uptake of organic compounds. Just before sunrise, therefore, the extracellular [CO$_2$]/[O$_2$] ratio reaches a maximum and provides optimal conditions for algal carbon fixation (high RuBisCO-CO$_2$/RuBisCO-O$_2$ ratio). King (1976) has observed such a phenomenon (Figure 8). Later, the oxygen consumption of bacteria cannot keep up with the oxygen production of algae. An increase of oxygen up to 40 mg/l is the result. This
Figure 8. Dissolved oxygen concentration and pH during a 24-hour cycle in a stabilization lagoon. (Bear Creek Lagoon, Columbia, MO, 8. 18-19, 1968, after D. King, 1976.

Concentration corresponds to saturation of water with pure oxygen of a partial pressure of 1 atm. The increase of pH indicates a net dehydroxylation of $\text{HCO}_3^-$ to $\text{CO}_2$ and $\text{OH}^-$, where $\text{CO}_2$ is consumed by algae. Due to the constant input of organic carbon in the form of waste and products of photorespiration by algae, the bacterial activity is increased and $\text{CO}_2$ is produced. At this high pH, thermodynamic equilibrium is in favor of $\text{HCO}_3^-$ but conversion rate of $\text{CO}_2$ to $\text{HCO}_3^-$ is relatively slow. Assuming that algae and bacteria form flocs, the transport of $\text{CO}_2$ from bacteria to algae is rapid and $\text{CO}_2$ uptake by algae can compete with formation of $\text{HCO}_3^-$. 
Oligotrophic Environment

During August and early September, measurements of several water-quality parameters were taken in Squaw Creek. This creek, 25 miles southwest of Bozeman, Montana in the Gallatin National Forest, is oligotrophic and contains no wastewater or other stresses posed by population. The values of dissolved organic carbon were less than 3 mg/l and total phosphates less than 1 mg/l. A biofilm consisting of diatoms and Gram negative bacteria covered the riverbed. The film thickness was 40 to 60 μm (thickness

Figure 9. SEM of a biofilm from Squaw Creek, MT. (Bar 10 μm) Small bacteria are attached on diatoms and also reach into interstitial water.
Figure 10. Typical 24-hour cycle of sunlight, water temperature and pH in Squaw Creek, MT, measured in September 1981.
Figure 11. Typical 24-hour cycle of dissolved oxygen at the same period as Figure 10. Solid line represents concentration and broken line relative saturation.

on glass slides exposed for six weeks). A typical set of data for a 24-hour period is shown in Figure 10.

The values for dissolved oxygen showed a remarkable, reproducible, irregularity. At sunrise, both the relative and absolute values of DO are expected to increase due to the algal activity.

However, the values decreased for a short while and started to increase after one hour. This effect has been measured several times and was also observed previously in oligotrophic streams (C. Kaya, pers. comm., 1982).
Explanation of this Effect

In this highly turbulent, shallow, oligotrophic stream, \(O_2\) is not depleted during the night since reaeration introduces \(O_2\) faster than it is removed by bacterial respiration. Bacterial respiration is limited due to the oligotrophic nature of the stream and also by lack of algal organic carbon excretion during the night. The extracellular \([CO_2]/[O_2]\) ratio is controlled by equilibrium conditions with air and is approximately 0.04.

As first sunlight strikes the biofilm, carbon fixation begins at a RuBisCO-CO_2/RuBisCO-O_2 ratio of approximately 5. The sequence of events is shown in Figure 12.

The uptake of carbon dioxide by the RuBisCO-CO_2 reaction is faster than the replacement by reaeration and flux into the film. The activity of bacteria is still zero. [CO_2] concentration approaches a very low value and no longer inhibits the RuBisCO-O_2 reaction, which begins to be the dominant reaction. Total oxygen production rate becomes smaller and dissolved organic carbon is released and accumulates in the film.

Meanwhile [CO_2] reaches zero. Concentration of dissolved oxygen now becomes high enough to instigate aerobic bacterial activity resulting in an additional \(O_2\).
Figure 12. Hypothetical concentrations and rates of oxygen, carbon dioxide, and dissolved organic carbon within the film. Broken line indicates change from dark to daylight.
consumption rate. Total $O_2$ consumption rate is now negative and $[O_2]$ concentration declines. Flux of $O_2$ from the bulk water is now higher than the reaction rate, and $[O_2]$ concentration in the bulk water drops.

Due to bacterial activity, $CO_2$ is formed and total $CO_2$ production rate becomes positive. $CO_2$ starts to accumulate and reaches a level where it partially inhibits the RuBisCO-$O_2$ reaction. Still, dissolved organic carbon is produced and consumed by bacteria. Total $O_2$ consumption rate becomes positive again. The flux of $O_2$ from the bulk water decreases and the bulk water is reaerated. Later, total $O_2$ production increases further as the light increases and $O_2$ is transported from the film to the bulk water.

Finally, the film reaches a "steady state," where both reactions, the carbon fixation (RuBisCO-$CO_2$) and photorespiration (RuBisCO-$O_2$) occur. The net carbon fixation will be proportional to the net $CO_2$ influx.
LABORATORY EXPERIMENTS

Reactor

The development of the flow reactor was a major effort. The purpose of this reactor is to enable observation of algal-bacterial films under controlled conditions. The reactor is a channel with a rectangular profile with total length of 203 cm and 28 cm width. Depth of bulk water, volumetric flow rate, flow velocity, and slope of the channel are variable and can be regulated. The two parallel recycling pumps have a capacity of 160 l/min. Temperature, pH, light, and flow rates can be controlled. The substratum, the surface on which the biofilm grows, can be exchanged for other materials. Due to the airtight hood with built-in light source, all measurements described in the model can be obtained.

High dispersion of turbulent flow and high recycling rate compared to dilution rate justifies mathematical reduction of the reactor from a plug flow to a CSTR. This greatly simplifies analysis and calculation. Therefore, the kinetics of the biofilm can be described with Equations 41, 42, 48, and 49 for steady-state and with Equations 56 to 61 for non-steady-state conditions.
Figure 13. Schematic of Flow Channel
Experimental Conditions

Illumination

In the experiments, a set of fluorescent tubes (G.E. Cool White Deluxe) illuminated the film with a light intensity of 100 microeinstins/m²'s (measured at the surface of the water) and a 14, 10-hour light-dark cycle.

Nutrients

Inorganic, modified Woods Hole medium was used (Appendix A). The modification was an addition of 0.5 mmole ammonia in the final solution for the growth of bacteria. Nutrients were autoclaved and fed into the reactor, controlled by a peristaltic pump and a rotameter.

Dilution Water

Tap water was dechlorinated and polished in a treatment filter (dual medium filter with activated carbon and sand). Turbidity after treatment was less than 1 NTU. The flow of water was controlled with a needle valve and a rotameter.

Gas

Compressed air from the laboratory supply system and CO₂ were continuously mixed and fed into the reactor. The
flow of air and CO₂ were separately controlled with two needle valves and rotameters.

Substratum

Roughened plexiglass was used as a substratum for the film. Additional removable slides were fitted into the surface and were used to sample parts of the film. One slide occupies less than 0.2% of the total reactor surface area.

Growth of the Film

Prior to the experiments, the reactor was inoculated with a mixed culture of algae and bacteria from Squaw Creek. It consisted mainly of diatoms and Gram negative bacteria. During the growth period (5 months), the diatoms disappeared and green algae became dominant.

Analytical Methods

Total organic carbon (TOC) and soluble organic carbon (SOC) were measured with an Oceanography International Carbon Analyzer and a modified method described by Trulear (1983).

Chemical oxygen demand was measured with a #1550-L COD Ampule (Oceanography International). The low COD value ampule method recommended by the manufacturer was used.

Total inorganic carbon, C_T, was measured with an Oceanography International Carbon Analyzer. Prior to use, empty ampules were purged for over five minutes with O₂ to
remove CO₂. 1 ml of K₂SO₄ (supersaturated) was added to the ampules to lower the pH of the final mix to less than 3 and purged again for a short time (30 sec.). After 2 ml of sample were added, the ampules were sealed immediately and analyzed. The results were standardized with a series of ampules with a known amount of organic carbon made up and combusted the day before. This method allows accurate measurement of inorganic carbon with a standard error less than 2% (determined from four samples).

Gas Analysis

Gas was analyzed with a gas partitioner (Fisher Hamilton Model 29 B). The results were recorded with a two-channel recorder HP 71000B. Recording was on both channels simultaneously, one with the highest sensitivity for CO₂ and one with a medium sensitivity for O₂ and N₂. The partitioner was standardized with air and additional CO₂. The total air pressure during standardization was 636.26 mm Hg. Oxygen was assumed to be 20.95% of air volume, nitrogen 79%, and CO₂ 0.032%. This assumption led to the partial pressures of O₂ 133.30 mm Hg, of N₂ 502.65, and of CO₂ 0.20 mm. Ten different mixtures of air and CO₂ were measured and calculated. A linear interpolation of both measured and calculated values resulted in r² for CO₂ equal to 0.985; for O₂ 0.952; and N₂ 0.951. Compared to the precision of the flow meters (5%) and partitioner (5%), these r² values are very high.
Dissolved Oxygen

DO was measured with a Yellow Spring dissolved oxygen meter standardized with a Hach digital titrator and Hach chemicals (Winkler method).

Methods of Calculation

Biomass

The organic carbon associated with biomass was obtained by subtracting soluble organic carbon from total organic carbon. This value is the organic carbon of particulate matter, i.e., biomass.

Residual Tetracycline

The entire amount of tetracycline was added at once. The final concentration was calculated with the following equation:

\[ c = c_0 e^{-D(t - t_0)} \]  \hspace{1cm} (62)

where \( t_0 \) is the time of addition of the antibiotic.

Soluble Organic Carbon Produced by Bioactivity

SOC, produced by bioactivity, or substrate S, is the difference of SOC measured minus tetracycline remaining.
Corrected Concentrations of Gas

The development of vapor within the reactor diluted the gas concentrations and reduced their partial pressures. At the same time, flow of gas out of the reactor became greater than flow into it. Under this condition, it was not possible to compare inlet concentrations with reactor concentrations. The effect of dilution by vapor was corrected by measuring the total partial pressure of \( N_2 \), \( O_2 \), and \( CO_2 \) within the reactor and by calculating the ratio of total partial pressure going in to total partial pressure in gas volume. The single concentrations were multiplied by this ratio. The actual flow out of the reactor was calculated by multiplication of inlet flow by this ratio.

Calculation of Flux

Flux from gas into bulk water and from bulk water into biofilm was calculated for non-steady-state conditions. The general Equation 54 for flux from gas to bulk water:

\[
\frac{dc}{dt} = \frac{1}{R_g} \left( L_g (C_0 - C) - N_g \right)
\]

was integrated. \( N_g \) was assumed to be a zero order in concentration. With boundary conditions \( t = 0, c = c_1 \), Equation 62 becomes:
Equations 63 and 64 can be rearranged for $N_g$ as variable and becomes:

$$N_g = L_g \left\{ \frac{c - c_1 e^{- \frac{L_g t}{R_g}} - c_0 (1 - e^{- \frac{L_g t}{R_g}})}{1 - e^{- \frac{L_g t}{R_g}}} \right\}$$

(64)

where $c$, $c_1$, $c_0$, $L_g$, and $R_g$ are known. $L_g$ is the loading rate of inflow and $c$ and $c_1$ are corrected gas concentrations.

Flux from biofilm to bulk water can be calculated in a similar way with Equation 55:

$$\frac{dc}{dt} = \frac{1}{R_b} \left( L_b (c_o - c) + \frac{A_g}{A_b} N_g + N_b \right)$$

(55)

With boundary conditions $t = 0$, $c = c_1$, the integration becomes:

$$c = c_1 e^{- \frac{L_b t}{R_b}} + \left( c_0 + \frac{A_g N_g}{A_b L_b} + \frac{N_b}{L_b} \right) \cdot (1 - e^{- \frac{L_b t}{R_b}})$$

(65)
Rearranged for $N_b$ as variable Equation 65 becomes

$$N_b = L_b \left[ \frac{c - c_1 e^{-\frac{L_b t}{R_b}} - \left( c_o + \frac{A_g N_g}{A_b L_b} \right) \cdot \left( 1 - e^{-\frac{L_b t}{R_b}} \right)}{1 - e^{-\frac{L_b t}{R_b}}} \right]$$

where $c$, $c_0$, $c_1$, $L_b$, and $R_b$ are known. $N_g$ is the result of Equation 64.

Design of Experiments

Introduction

The series of experiments had two major goals. The first goal was to measure the activity of film under different conditions and to compare the results with the model of interaction of algae and bacteria within aggregates.

The second goal, which was more important than the first, was to test the reactor under experimental conditions and to prove adequacy of analytical methods.

The reason for the second goal was the fact that this reactor was a prototype. No literature reference was found for a similar system where both liquid and gas volumes were analyzed.
The constant parameters of the two experiments are shown in Tables 1 and 2. The input concentrations and quantities for both experiments were similar.

The major differences between Experiment I and II were:

- Experiment I had a small enrichment of CO₂ in the gas (0.53 mg/l); Experiment II was air only (0.34 mg/l).
- Experiment II had a high recycling rate and velocity compared to Experiment I (Reynolds number of Experiment I 1200, Reynolds number of Experiment II, 3900).
- Tetracycline added in Experiment II was 2.5 times higher than in Experiment I.

Reason for Addition of Tetracycline

Tetracycline is an antibiotic which does not affect algae but inhibits bacterial ribosome activity (Brock, 1979). Previous work showed a remarkable increase of dissolved oxygen within a very short time after addition of tetracycline to mixed algal-bacterial cultures. It was assumed that this antibiotic inhibits quickly the metabolism of bacteria and, consequently, their oxygen consumption.

If the bacteria in the film were blocked without influencing algal activity, the following sequence of events would presumably occur in the film.
The bacterial activity stops. Oxygen concentration in the film increases and CO\textsubscript{2}, which controls activity of RuBisCO, decreases. Thus, carbon fixation is reduced and photorespiration increases. A higher flux of oxygen and dissolved organic carbon out of the biofilm and inorganic carbon into the film results. If the bulk water is rich in CO\textsubscript{2}, the flux of inorganic carbon will compensate the blocked bacterial activity and photorespiration increases only slightly. If CO\textsubscript{2} in bulk water is low, the flux of inorganic carbon cannot compensate the blocked bacterial activity and photorespiration will be high.

Duration of Experiments

Experiment I lasted seven hours. Light was switched on four hours before starting the experiment. Tetracycline was added one hour after beginning.

Experiment II lasted 10 hours. Illumination was off for nine hours before beginning the experiment. After one hour, it was switched on; four and a half hours later tetracycline was added and after another three hours and twenty minutes, light was switched off.

Figures 14 to 17 show concentrations in bulk water and flux from film into bulk water for different compounds in the two experiments.
<table>
<thead>
<tr>
<th>Table I: Constant Parameters of Experiment I</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Liquid:</strong></td>
</tr>
<tr>
<td>Temperature, $T$: 18.0° C</td>
</tr>
<tr>
<td>Volume bulk water, $V_b$: 18.0 l</td>
</tr>
<tr>
<td>Inflow, $F_w$: 0.167 l/min</td>
</tr>
<tr>
<td>Residence time, $\theta_b$: 108 min</td>
</tr>
<tr>
<td>Dilution rate, $D_b$: $9.28 \cdot 10^{-3}$ min$^{-1}$</td>
</tr>
<tr>
<td>Surface biofilm, $A_b$: 6272 cm$^2$</td>
</tr>
<tr>
<td>Hydraulic radius, $R_b$: 2.87 cm</td>
</tr>
<tr>
<td>Loading rate, $L_b$: $26.6 \cdot 10^{-3}$ cm/min</td>
</tr>
<tr>
<td>Thickness of biofilm, $T_b$: 1.39 mm</td>
</tr>
<tr>
<td>Velocity of bulkwater, $V_b$: 19.6 cm/s</td>
</tr>
<tr>
<td>Reynold's number, $R_e$: 1700</td>
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<tr>
<td>Froude's number, $F_r$: 0.44</td>
</tr>
<tr>
<td>Flow rate recycling, $F_b$: 66.8 l/min</td>
</tr>
<tr>
<td>Ratio $\frac{F_b}{F_w}$: 400</td>
</tr>
</tbody>
</table>

**Input Concentrations:**

- Soluble organic carbon, SOC: 1.24 mg/l
- Total inorganic carbon, $C_t$: 14.81 mg/l
- Dissolved oxygen, DO: 10.4 mg/l

<table>
<thead>
<tr>
<th><strong>Gas:</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume Gas, $V_g$: 277.5 l</td>
</tr>
<tr>
<td>Inflow, $F_g$: 1.5 l/min</td>
</tr>
<tr>
<td>Residence time, $\theta_g$: 186 min</td>
</tr>
<tr>
<td>Dilution rate, $D_g$: $5.38 \cdot 10^{-3}$ min$^{-1}$</td>
</tr>
<tr>
<td>Interface gas-water, $A_g$: 5824 cm$^2$</td>
</tr>
<tr>
<td>Hydraulic radius, $R_g$: 47 cm</td>
</tr>
<tr>
<td>Loading rate, $L_g$: $258 \cdot 10^{-3}$</td>
</tr>
<tr>
<td>Pressure of air, $P_{tot}$: 648.2 mm Hg</td>
</tr>
<tr>
<td>Temperature of tubing to reactor and gas partitioner: 20° C</td>
</tr>
</tbody>
</table>

- $N_2$ : $P_{part}$: 513.6 mm Hg, 788.0 mg/l
- $CO_2$ : $P_{part}$: 0.8 mm Hg, 0.53 mg/l as C
- $O_2$ : $P_{part}$: 133.8 mm Hg, 243.0 mg/l
Table II: Constant Parameters of Experiment II

<table>
<thead>
<tr>
<th>Liquid:</th>
<th>Gas:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Temperature, ( T ):</strong> 18.0° C</td>
<td><strong>Volume gas, ( V_g ):</strong> 274 l</td>
</tr>
<tr>
<td><strong>Volume bulk water, ( V_b ):</strong> 21.5 l/min</td>
<td><strong>Inflow, ( F_g ):</strong> 1.5 l/min</td>
</tr>
<tr>
<td><strong>Inflow, ( F_w ):</strong> 0.167 l/min</td>
<td><strong>Residence time, ( \theta_g ):</strong> 180 min</td>
</tr>
<tr>
<td><strong>Residence time, ( \theta_b ):</strong> 130 min</td>
<td><strong>Dilution rate, ( D_g ):</strong> 5.48 ( \times ) ( 10^{-3} ) min(^{-1} )</td>
</tr>
<tr>
<td><strong>Dilution rate, ( D_b ):</strong> 7.77 ( \times ) ( 10^{-3} ) min(^{-1} )</td>
<td><strong>Interface gas-water: ( A_g ):</strong> 5824 cm(^2 )</td>
</tr>
<tr>
<td><strong>Surface biofilm, ( A_b ):</strong> 6272 cm(^2 )</td>
<td><strong>Hydraulic radius, ( R_g ):</strong> 47 cm</td>
</tr>
<tr>
<td><strong>Hydraulic radius, ( R_b ):</strong> 3.34 cm</td>
<td><strong>Loading rate, ( L_g ):</strong> 258 ( \times ) ( 10^{-3} ) cm/min</td>
</tr>
<tr>
<td><strong>Loading rate, ( L_b ):</strong> 26.6 ( \times ) ( 10^{-3} ) cm(^{-1} )</td>
<td><strong>Pressure of air, ( P_{tot} ):</strong> 624.78 mm Hg</td>
</tr>
<tr>
<td><strong>Thickness of film, ( Th ):</strong> 1.72 mm</td>
<td>Temperature of tubing to reactor and gas partitioner: 20° C</td>
</tr>
<tr>
<td><strong>Velocity of bulk water, ( v_b ):</strong> 38 cm/s</td>
<td></td>
</tr>
<tr>
<td><strong>Reynold's number, ( R_e ):</strong> 3930</td>
<td></td>
</tr>
<tr>
<td><strong>Fruode's number, ( F_r ):</strong> 0.82</td>
<td></td>
</tr>
<tr>
<td><strong>Flow rate recycling, ( F_r ):</strong> 140 l/min</td>
<td></td>
</tr>
<tr>
<td><strong>Ratio ( \frac{F_b}{F_w} ):</strong> 840</td>
<td></td>
</tr>
</tbody>
</table>

**Input Concentrations:**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Soluble organic carbon, SOC: 1.35 mg/l</td>
<td>( N_2 ): ( p_{part} ): 496.3 mm Hg, 761.15 mg/l</td>
</tr>
<tr>
<td>Total inorganic carbon, ( C_t ): 14.74 mg/l</td>
<td>( CO_2 ): ( p_{part} ): 0.5 mm Hg, 0.34 mg/l as C</td>
</tr>
<tr>
<td>Dissolved oxygen, DO: 9.0 mg/l</td>
<td>( O_2 ): ( p_{part} ): 128.1 mm Hg, 224.07 mg/l</td>
</tr>
</tbody>
</table>
Figure 14. Concentrations of compounds, measured in bulk water during Experiment I.
Figure 15. Concentrations of compounds, measured in bulk water during Experiment II.
Figure 16. Flux from biofilm into bulk water in μg/cm²·min during Experiment I. Positive values are for flux from film into bulk water and negative for flux from bulk water into film.
Figure 17. Flux from biofilm into bulk water in μg/cm²·min during Experiment II. Positive values are for flux from film into bulk water and negative for flux from bulk water into film.
Discussion of the Results: Concentrations

pH

Tetracycline acts as a weak acid and drops pH in bulk water. In Experiment I, pH did not drop after addition of tetracycline as it did in Experiment II, suggesting that the pH probe was fouled and tetracycline did not penetrate the film within a short time. Later, pH increased and reached 9.2, compared to 9 at the time before addition, indicating a higher algal consumption of CO₂ from the C_T pool as compensation for reduced bacterial activity.

At the beginning of Experiment II pH was 8.4 and increased to 9.2 after light was turned on. This value was higher than that of Experiment I.

Due to the addition of tetracycline, pH decreased sharply to 8 and within three hours reached a value of 9. After the light was switched off, pH dropped and reached values typical of dark cycles.

Dissolved Oxygen (DO)

DO in Experiment I increased after addition of tetracycline from 11.4 mg/l to 11.7 mg/l and declined later to 11.0 mg/l. To a certain degree, this decline may be partially attributed to fouling of the oxygen probe.
DO in Experiment II is lower than in Experiment I because of the higher velocity in Experiment I. In Experiment II, DO started at about 8.5 mg/l and increased after the light was switched on to about 9.2 mg/l. After tetracycline was added it increased further to 9.5 and started to drift between 9.2 and 9.4 with an interval of two hours. After deprivation from light, it declined to 7.9 and rose again to 8.4. This "overshoot" is remarkable and indicates a higher net oxygen consumption at the switch-off of light than one hour later.

Inorganic Carbon, $C_T$

All inorganic carbon values in Experiment I were higher than those of Experiment II. This was due to the higher $CO_2$ concentration of the inflow of gas. In Experiment I, inorganic carbon increased significantly during the period of sloughing. An explanation for this effect could be that a larger part of film broke off and was "homogenized," i.e., broken down to single cells by the recycling pump. $CO_2$ formed by bacteria in suspension was converted to $HCO_3^-$ rather than consumed by algae. The longer transport distance favored the $CO_2$ to $HCO_3^-$ conversion. A similar effect was observed in Experiment II at 21.40 hours. The increase of both sloughing and inorganic carbon was, in this case, not as high as it was in Experiment I. The increase, after light was turned off, was expected. Carbon fixation by algae stopped but
bacterial metabolism declined slowly.

Organic Carbon, S

Throughout Experiment I, organic carbon was between 1.5 and 2 mg/l. It is not possible to see any increasing or decreasing trend during the entire experiment. Two explanations are possible. Carbon limitation of algae was never high enough to trigger an increased rate of photorespiration, or the amount of tetracycline was not high enough to stop activity of bacteria. The second explanation is not probable. The increase of oxygen after addition of tetracycline indicates a reduction of bacterial activity. It seems more probable that algae were never CO₂-limited. If there was a change in pH, perhaps not recorded due to a fouled probe, the carbon equilibria were shifted in favor of CO₂ and the fast decrease of Cₜ after the high peak at 15.15 hrs down to 11.4 mg/l indicates a high CO₂ uptake. It appears that during Experiment I, algae were not CO₂-limited.

In Experiment II, conditions were completely different. During the dark period, organic carbon was about 30% higher than after light was switched on. The concentration was even higher than the input concentration of liquid. After light is switched on, organic carbon was relatively constant in the range of 1.4 to 1.5 mg/l. After addition of tetracycline, it increased first to
2.3 mg/l and one hour later to 2.85 mg/l. The first increase was parallel with an increase of DO and a drop in pH. It can be assumed that this was due to the reduced activity of bacteria. The second step increase, parallel with a decrease of DO and an increase of pH, indicates a higher rate of photorespiration. After light was switched off, organic carbon decreased again to low levels.

Biomass

Due to the low shear force of the water (low bulk velocity), biomass tended to break off in larger parts (sloughing). The increase of suspended biomass at 15.00 hr is due to sloughing and it increased further after addition of tetracycline. Then, the decrease of suspended biomass was extremely fast so that a high reattachment rate has to be assumed.

The only significant detachment in Experiment II was observed after addition of tetracycline. Again the reattachment was rapid. A smaller detachment was observed after the change from light to dark with a rapid reattachment.

The higher detachment rate in Experiment II can be explained by the higher fluid shear force.
Flux from Film to Bulk Water

Oxygen

The calculation of oxygen flux is extremely sensitive to errors in gas volume measurement. Flux of oxygen is not consistent with bulk water oxygen concentration. Due to the low solubility of oxygen (10 mg/l in water and 245 mg/l in air at the same time), an error of 1% in the gas analysis can cause an error in flux which is far greater than 100%.

Inorganic Carbon

This calculation is less sensitive to error than the flux of oxygen. In Experiment I, \( N_{bc} \) is quite consistent with \( C_T \) in bulk water. In Experiment II, \( N_{bc} \) gives relatively good values also. But at the end of the experiment the flux does not correspond well with concentrations.

Organic Carbon

Initially, \( N_{bs} \) in Experiment I is not stable. Due to possible small measurement errors (precision of DOC measurements ± 0.2 mg/l), \( N_{bs} \) oscillated between positive and negative values. With more stable concentration measurements, \( N_{bs} \) becomes stable and interpretation is possible.

From 17:30 to 21:00, \( N_{pc} \) and \( N_{bs} \) are inversely
proportional. Consequently, smaller flux of carbon into the film is equivalent to a CO₂ limitation for the algae and higher photorespiration. This is also true for Experiment II.

Biomass

In both experiments, Nₙₓ gives relatively consistent values for detachment and reattachment.

Limitations Related to Measurement of Fluxes

There are two major limitations related to measurement of flux.

1.) Measurement intervals must be reduced to at least 15 min. The relatively low precision of analytical methods at low concentrations causes the flux to be a random number more than a systematic value.

2.) Gas analysis, especially oxygen, must be improved. The precision of the gas partitioner (5%) is unsatisfactory. Calculation of dilution by vapor in the gas volume also causes problems with gas analysis.

Conclusion of the Experiments

The experiments and their analysis have proven that the flow reactor and the simplified model are useful tools for studying algal-bacterial aggregates. The experiments have also demonstrated problems in analytical methods which must be solved. If simultaneous flux of oxygen, inorganic carbon, and organic carbon are to be compared,
photorespiration, the link of algal-bacterial interaction, can be quantitatively evaluated.

**Further Work**

So far, all measurements were made in the bulk water and in the gas volume but not within the biofilm. Measurement within the biofilm, the "new dimension," would be possible with the use of microelectrodes. pH and O₂ concentrations could be measured directly within the film, yielding a concentration profile with biofilm depth. Figures 18 and 19 show such a profile through an algal film grown on agar.

Another goal of further experimentation would be the measurement of oxygen production of the photo systems, correlated to light. This measurement is based on the following relationship:

\[
\frac{d O_2}{dt} = - K_B + K_A
\]

(67)

**Accumulation**  **Consumption**  **Net production**

in film  bacteria by algae  by algae

where

\[
K_A = k_1 - k_2
\]

(68)

**Net production**  **Primary production**  **Enzymatic O₂ consumption**

by algae  in photo  system I and II  RuBisCO-O₂
Figure 18. Oxygen profile through an algal film, illuminated with 60 W tungsten light. This measurement was made with an oxygen micro probe, typ size 10 μm.

Figure 19. Oxygen profile through same film as in Figure 18, but under direct sun light. The remarkable increase of O₂ concentration is due to the higher light intensity.
Based on technique developed by N. P. Reversbrech (pers communication, 1982), it is possible to measure $K_A$ directly with an $O_2$ sensitive microelectrode.

The values of $K_A$ are measured in transient situations after a steady state. The $O_2$ production is directly related to the light energy (without intermediates), so the response to changes in light is very fast. Equation 67 can be modified in the following way:

During dark periods, $K_A = 0$ and, therefore

$$\frac{dO_2}{dt} = -K_B$$

(69)

During light periods, $K_A > 0$ and, therefore

$$\frac{dO_2}{dt} = -K_B + K_A$$

(67)

At steady state, $\frac{dO_2}{dt} = 0$ and $K_A = K_B$

Hence, upon instantaneously changing from a steady state

$$\frac{dO_2}{dt} = -K_A$$

(70)

This relationship is only valid during the first seconds after the change but is easily measurable with a fast response $O_2$ - microprobe within the biofilm.
Figure 20. Schematic of measurement of net O₂ production of algae within aggregates. The slope of the curve dO₂/dt after the change from light to dark represents -K₁, the net O₂ production rate.

This experiment with K₁ will be repeated with different O₂ and CO₂ interstitial water concentrations to estimate k₁ and k₂ of equation 68 which most probably depend on O₂ and CO₂ concentrations and diffusional resistances.

Figure 21 shows a series of transient responses of O₂, measured with microelectrodes.
Figure 21. Transient response of $O_2$ to changes of light. Measurements were made in the bulk water, within the film and in the substratum (agar). $K_A$ values were calculated for the first two seconds after change of light intensity.
SUMMARY

A theoretical model has been developed which describes the nature of interaction between algae and bacteria within aggregates. The model of interaction is based on the CO₂ concentration of the interstitial water, since this concentration controls the rate of carbon fixation versus the rate of photorespiration, producing dissolved organic carbon which subsequently controls bacterial activity. The model has been compared to observations from two contrasting aquatic systems.

A flow channel was built to test the model under laboratory conditions. The design allows measurements within bulk water and gas volume to determine the flux from film into bulk water. This flux is the net rate of activity of algae and bacteria within films. The dimension of the reactor allows the use of microelectrodes for measurements within the film. Preliminary studies with microelectrodes were successful, proving that it is possible to measure not only profiles of concentration but also photosynthetic activity of algae within aggregates.

The combination of measurements within the film and complete mass balances over the entire system allow a direct test of the model and its interpretation.
BIBLIOGRAPHY


NOMENCLATURE/SYMBOLS

\( A_A \)  
surface algal cell \((L^2)\)

\( A_B \)  
interface biofilm bulk water \((L^2)\)

\( A_g \)  
interface bulk water gas volume \((L^2)\)

ADP  
adenosine diphosphate

ATP  
adenosine triphosphate

c  
general concentration in interstitial water \((ML^{-3})\)

c_b  
general concentration in bulk water \((ML^{-3})\)

c_g  
general concentration in gas volume \((ML^{-3})\)

c_o  
general concentration in inflow \((ML^{-3})\)

C_T  
total inorganic carbon \((ML^{-3})\)

d  
depth of bulk water \((L)\)

d_A  
decay coefficient of algae \((t^{-1})\)

d_B  
decay coefficient of bacteria \((t^{-1})\)

D_B  
dilution rate bulk water \((t^{-1})\)

D_g  
dilution rate gas volume \((t^{-1})\)

DO  
dissolved oxygen \((ML^{-3})\)

DOC  
dissolved organic carbon \((ML^{-3})\)

E  
light energy \((EL^{-2})\)

F_b  
flow rate recycling \((L^3t^{-1})\)

F_g  
flow rate gas \((L^3t^{-1})\)

F_w  
flow rate into reactor \((L^2t^{-1})\)

k  
mass transfer coefficient of CO_2 through algal cell wall \((L^{-2}t^{-1})\)
\( k' \) mass transfer coefficient of \( O_2 \) through algal cell wall \((L^{-2}t^{-1})\)

\( k_1 \) overall primary production rate of Photosystem II \((ML^{-3}t^{-1})\)

\( k_2 \) overall enzymatic \( O_2 \) consumption rate \((ML^{-3}t^{-1})\)

\( K_A \) overall net production rate of algae \((ML^{-3}t^{-1})\)

\( K_B \) overall net consumption rate of bacteria \((ML^{-3}t^{-1})\)

\( K_E \) half saturation constant of light reaction \((EL^{-2})\)

\( K_{(O_2)} \) half saturation constant of \( O_2 \) of dark reaction \((mole L^{-3})\)

\( K_{(CO_2)} \) half saturation constant of \( CO_2 \) of dark reaction \((mole L^{-3})\)

\( K_S \) half saturation constant of bacterial substrate uptake \((ML^{-3})\)

\( K'_{O_2} \) half saturation constant of bacterial oxygen uptake \((ML^{-3})\)

\( L_b \) hydraulic loading rate of bulk water for film surface \((Lt^{-1})\)

\( L_g \) hydraulic loading rate of gas volume for interface gas bulk water \((Lt^{-1})\)

\( \text{NADP} \) nicotinamide adenine dinucleotide phosphate

\( N_b \) flux from biofilm into bulk water \((ML^{-2}t^{-1})\)

\( N_g \) flux from gas volume into bulk water \((ML^{-2}t^{-1})\)

\( P_{\text{part}} \) partial pressure of gas \((\text{mm Hg})\)

\( 3\text{-pg} \) 3-phosphoglycerate

\( \text{RBP} \) ribulose biphosphate
RuBisCO ribulose biphosphate carboxilase oxygenase
RuBisCO-\text{CO}_2 ribulose biphosphate carboxilase
RuBisCO-\text{O}_2 Ribulose biphosphate oxygenase

$R_b$ hydraulic radius bulk water \((L)\)

$R_g$ hydraulic radius gas volume \((L)\)

$r$ overall reaction rate in bulk water \((ML^{-3}t^{-1})\)

$r_A$ overall reaction rate of algae \((ML^{-3}t^{-1})\)

$r_B$ overall reaction rate of bacteria \((ML^{-3}t^{-1})\)

$r_P$ rate of photosynthesis \((Mt^{-1})\)

$r_R$ rate of RuBisCO formation \((Mt^{-1})\)

$r_{RC}$ rate of RuBisCO-\text{CO}_2 reaction \((Mt^{-1})\)

$r_{RO}$ rate of RuBisCO-\text{O}_2 reaction \((Mt^{-1})\)

$s$ concentration of DOC in interstitial water \((ML^{-3})\)

$s_b$ concentration of DOC in bulk water \((ML^{-3})\)

$s_o$ concentration of DOC in inflow \((ML^{-3})\)

$Th$ thickness of biofilm \((L)\)

$v$ velocity of bulk water \((Lt^{-1})\)

$V$ volume of interstitial water \((L^3)\)

$V_b$ volume of bulk water \((L^3)\)

$V_g$ volume of gas \((L^3)\)

$X$ total biomass, algae and bacteria \((ML^{-3})\)

$X_A$ algal biomass \((ML^{-3})\)

$X_B$ bacterial biomass \((ML^{-3})\)

$Y_A$ yield: algal biomass formed from inorganic carbon fixed \((M \text{ mole}^{-1})\)
\( Y_B \) yield: bacterial biomass formed ÷ organic carbon consumed \( (\text{MM}^{-1}) \)

\( \alpha_A \) proportionality coefficient algae \( (-) \)

\( \alpha_B \) proportionality coefficient bacteria \( (-) \)

\( \delta \) thickness boundary layer \( (\text{L}) \)

\( \mu_A \) specific growth rate algae \( (t^{-1}) \)

\( \mu_B \) specific growth rate bacteria \( (t^{-1}) \)
APPENDICES
APPENDIX A

GROWTH MEDIUM
Stock solutions of each macronutrient are prepared at 1000-fold concentration thus 1 ml of each stock is added to 1 l medium. Stock solution of micronutrients is prepared at 1000-fold concentration. The tris solution, which is also prepared as a stock solution, should have pH adjusted to 7.2.

Macronutrients, 1 ml each l

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaCl$_2$•2H$_2$O</td>
<td>36.76</td>
</tr>
<tr>
<td>MgSO$_4$•7H$_2$O</td>
<td>36.97</td>
</tr>
<tr>
<td>NaHCO$_3$</td>
<td>12.60</td>
</tr>
<tr>
<td>K$_2$HPO$_4$</td>
<td>8.71</td>
</tr>
<tr>
<td>NaNO$_3$</td>
<td>85.01</td>
</tr>
<tr>
<td>NaSiO$_3$</td>
<td>28.42</td>
</tr>
</tbody>
</table>

Micronutrients, 1 ml each l

<table>
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<th>Concentration (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na$_2$EDTA</td>
<td>4.36</td>
</tr>
<tr>
<td>FeCl$_3$•6H$_2$O</td>
<td>3.15</td>
</tr>
<tr>
<td>CuSO$_4$•5H$_2$O</td>
<td>0.01</td>
</tr>
<tr>
<td>ZnSO$_4$•7H$_2$O</td>
<td>0.022</td>
</tr>
<tr>
<td>CoCl$_2$•6H$_2$O</td>
<td>0.01</td>
</tr>
<tr>
<td>MnCl$_2$•4H$_2$O</td>
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</tr>
<tr>
<td>Na$_2$MoO$_4$•2H$_2$O</td>
<td>0.006</td>
</tr>
</tbody>
</table>
101

Vitamins

Thiamine. HCL 0.1 mg/l
Biotin 0.5 μg/l
Cyanocobalamin 0.5 μg/l

Tris, 2ml each l

Tris(hydroxymethyl)-aminomethane 50g/200ml

Adjust pH to 7.2 with HCl
APPENDIX B

TABLES OF CONCENTRATIONS AND FLUX

OF EXPERIMENTS I AND II
Table III. Concentrations during Experiment I.

<table>
<thead>
<tr>
<th>Time</th>
<th>PH</th>
<th>DO</th>
<th>SOC</th>
<th>TOC</th>
<th>TOC-SOC</th>
<th>Tetrac.</th>
<th>SOC-Tetrac.</th>
<th>C_T</th>
</tr>
</thead>
<tbody>
<tr>
<td>hrs.</td>
<td></td>
<td>mg/l</td>
<td>mg/l</td>
<td>mg/l</td>
<td>mg/l</td>
<td>mg/l</td>
<td>mg/l</td>
<td>mg/l</td>
</tr>
<tr>
<td>14.00</td>
<td>9.0</td>
<td>11.48</td>
<td>1.92</td>
<td>2.01</td>
<td>0.09</td>
<td>-</td>
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<td>9.0</td>
<td>11.40</td>
<td>1.53</td>
<td>2.16</td>
<td>0.63</td>
<td>-</td>
<td>1.53</td>
<td>12.35</td>
</tr>
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<td>11.40</td>
<td>1.63</td>
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<td>-</td>
<td>1.63</td>
<td>16.91</td>
</tr>
<tr>
<td>15.10</td>
<td></td>
<td></td>
<td>Tetracycline 20 mg/l</td>
<td></td>
<td>6.21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15.15</td>
<td>9.0</td>
<td>11.60</td>
<td>7.10</td>
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<td>12.35</td>
<td>5.93</td>
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<td>9.0</td>
<td>11.70</td>
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<td>1.73</td>
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<td>11.60</td>
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<td>2.96</td>
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<td>16.50</td>
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<td>11.60</td>
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<td>4.50</td>
<td>0.49</td>
<td>2.46</td>
<td>1.55</td>
<td>11.99</td>
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<td>4.07</td>
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<td>1.86</td>
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<td>12.95</td>
</tr>
<tr>
<td>17.45</td>
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<td>11.50</td>
<td>3.15</td>
<td>3.41</td>
<td>0.26</td>
<td>1.47</td>
<td>1.68</td>
<td>11.89</td>
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<tr>
<td>18.30</td>
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<td>11.30</td>
<td>2.94</td>
<td>3.78</td>
<td>0.84</td>
<td>0.97</td>
<td>1.97</td>
<td>13.43</td>
</tr>
<tr>
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<td>0.24</td>
<td>2.27</td>
<td>11.94</td>
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</table>
Table IV. Corrected concentrations of gas during Experiment I.

<table>
<thead>
<tr>
<th>Time (hrs.)</th>
<th>Lg (cm/min)</th>
<th>O₂ (mg/l)</th>
<th>C_T (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14.15</td>
<td>0.261</td>
<td>243.36</td>
<td>0.38</td>
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<tr>
<td>14.25</td>
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<tr>
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<td>0.264</td>
<td>244.58</td>
<td>0.20</td>
</tr>
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</table>
Table V. Flux during Experiment I.

<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>$N_{gO}$</th>
<th>$N_{bO}$</th>
<th>$N_{gC}$</th>
<th>$N_{bC}$</th>
<th>$N_{bS}$</th>
<th>$N_{bX}$</th>
<th>$N_{b\text{tot. carb.}}$</th>
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</thead>
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<tr>
<td>14.00</td>
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Table VI. Concentrations during Experiment II.

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<td>mg/l</td>
<td>mg/l</td>
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Table VII. Corrected concentrations of gas during Experiment II.

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Table VIII. Flux during Experiment II.

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<th>( N_{GC} )</th>
<th>( N_{bC} )</th>
<th>( N_{bS} )</th>
<th>( N_{bX} )</th>
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<td>( \mu g/cm^2\text{min} )</td>
<td>( \mu g/cm^2\text{min} )</td>
<td>( \mu g/cm^2\text{min} )</td>
<td>( \mu g/cm^2\text{min} )</td>
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Algal-bacterial interaction within biofilms.