



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 flocs).

The algal-bacterial aggregates are symbiotic communities of microorganisms. The algae produce O_2 and soluble organic carbon and consume CO_2 . Their energy source is sunlight.

Bacteria consume the algal products and produce CO_2 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 CO_2 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 O_2 and CO_2 . The reaction with water and CO_2 (the substrate) yields two phosphoglycerates, one for carbon fixation and one that goes into the Calvin cycle. With O_2 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- CO_2 and RuBisCO- O_2 is controlled by a competitive inhibition or $2CO_2$ versus O_2 . Thus the ratio of $[CO_2]/[O_2]$ 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 (CO_2) rate. The resulting integrated model can be used to predict the kinetics of algal-bacterial aggregates.

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WITHIN BIOFILMS

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of

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APPROVAL

of a thesis submitted by

Andreas Rainer Escher

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.

Feb 25, 1983
Date

W. G. Characki
Chairperson, Graduate Committee

Approved for the Major Department

February 25, 1983
Date

Theodore J. Williams
Head, Major Department

Approved for the College of Graduate Studies

2-28-83
Date

Michael P. Malone
Graduate Dean

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TABLE OF CONTENTS

	Page
LIST OF TABLES	vii
LIST OF FIGURES.	viii
ABSTRACT	x
INTRODUCTION	1
LITERATURE REVIEW.	3
Artificial Systems, Reactors, and Mathematical Models.	3
Stabilization Lagoons, Field Observations, and Measurements.	5
Nutrient Uptake, Limiting Nutrients, and Chemical Inhibition	7
Inorganic Carbon	8
Phosphorous Limitation	9
Photosynthesis, Photoinhibition, and Adaptation .	10
Summary.	13
Conclusion.	15
GOALS OF THE RESEARCH.	16
APPLICATION OF THE RESEARCH.	19
Stream and Lake Water Quality.	19
Wastewater Treatment and Carbon Dynamics	20
Cooling Water.	21
THEORETICAL MODEL.	23
Background.	26
Algae.	26
Bacteria	28
Interaction Between Algae and Bacteria	30
Reaction Kinetics	31
Algal Cell	31
Bacterial Cell	36
Kinetic Model	37
Steady State	42
Non Steady State	46
Limitations of the Model.	49
Conclusion.	49

TABLE OF CONTENTS (continued)

	Page
COMPARISON OF THE MODEL TO OBSERVATIONS IN THE FIELD.	50
Eutrophic Environment.	50
Oligotrophic Environment	52
Explanation of the Effects	55
LABORATORY EXPERIMENTS.	58
Reactor.	58
Experimental Conditions.	60
Illumination.	60
Nutrients	60
Dilution Water.	60
Gas	60
Substratum.	61
Growth of Film.	61
Analytical Methods	61
Methods of Calculation	63
Residual Tetracycline	63
Corrected Concentrations of Gas	64
Calculation of Flux	64
Design of Experiment	66
Introduction.	66
Reason for Addition of Tetracycline	67
Duration of Experiments	68
Discussion of Results: Concentrations.	75
pH.	75
Dissolved Oxygen.	75
Inorganic Carbon.	76
Organic Carbon.	77
Biomass	78
Flux from Film into Bulk Water	79
Oxygen.	79
Inorganic Carbon.	79
Organic Carbon.	79
Biomass	80
Limitations Related to Measurements of Flux.	80
Conclusion of Experiments.	80
Further Work	81
SUMMARY	86
BIBLIOGRAPHY.	87
NOMENCLATURE/SYMBOLS.	93
APPENDICES.	98

TABLE OF CONTENTS (continued)

	Page
APPENDIX A: Growth Medium	99
APPENDIX B: Tables of Concentrations and Flux of Experiments I and II	102

LIST OF TABLES

	Page
TABLE I. Constant parameters of Experiment I	69
TABLE II. Constant parameters of Experiment II	71
TABLE III. Concentrations, Experiment I	103
TABLE IV. Corrected concentrations of gas, Experiment I.	104
TABLE V. Flux, Experiment I	105
TABLE VI. Concentrations, Experiment II	106
TABLE VII. Corrected concentrations of gas, Experiment II.	107
TABLE VIII. Flux, Experiment II	108

LIST OF FIGURES

	Page
Figure 1. SEM of algal-bacterial film from Squaw Creek, an oligotrophic mountain stream	18
Figure 2. Schematic of a turbulent stream	23
Figure 3. Schematic of algal-bacterial interaction within aggregates	24
Figure 4. Simplified schematic of Calvin cycle, indicating the dual substrate nature of RuBisCO	30
Figure 5. Relative algal intracellular and extracellular concentrations of oxygen and carbon dioxide, reflecting the influence of reaction and diffusion	34
Figure 6. Schematic of a continuous stirred tank reactor with film growth	40
Figure 7. Same reactor as Figure 6, but with a controlled gas volume	44
Figure 8. Dissolved oxygen concentration and pH during a 24-hour cycle in a stabilization lagoon	51
Figure 9. SEM of a biofilm from Squaw Creek	52
Figure 10. Typical 24-hour cycle of sunlight, water temperature and pH in Squaw Creek	53
Figure 11. Typical 24-hour cycle of dissolved oxygen at the same period as Figure 10	54
Figure 12. Estimated concentrations and rates of oxygen, carbon dioxide, and dissolved organic carbon within the film	55
Figure 13. Schematic of Flow Channel	59

LIST OF FIGURES (continued)

	Page
Figure 14. Concentrations of compounds, measured in bulk water during Experiment I. . . .	71
Figure 15. Concentrations of compounds, measured in bulk water during Experiment II. . . .	72
Figure 16. Flux from biofilm into bulk water during Experiment I.	73
Figure 17. Flux from biofilm into bulk water during Experiment II.	74
Figure 18. Oxygen profile through an algal film, illuminated with 60 W tungsten light..	82
Figure 19. Oxygen profile through an algal film, illuminated with direct sunlight. . . .	82
Figure 20. Schematic of measurement of net oxygen production of algae within aggregates.	84
Figure 21. Transient response of oxygen concentration to changes of light, measured with microelectrodes within biofilms.	85

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 O_2 and soluble organic carbon and consume CO_2 . Their energy source is sunlight.

Bacteria consume the algal products and produce CO_2 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 CO_2 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 O_2 and CO_2 . The reaction with water and CO_2 (the substrate) yields two phosphoglycerates, one for carbon fixation and one that goes into the Calvin cycle. With O_2 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- CO_2 and RuBisCO- O_2 is controlled by a competitive inhibition of CO_2 versus O_2 . Thus the ratio of $[CO_2]/[O_2]$ 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 (CO_2) 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 CO_2 uptake and 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 CO_2 enables the cell to fix carbon at a high rate whereas a low CO_2 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 CO_2 , 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 Edelman 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 CO_2 , 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 McFeters 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 CO₂ limitation and addition of organic carbon. Under CO₂ 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

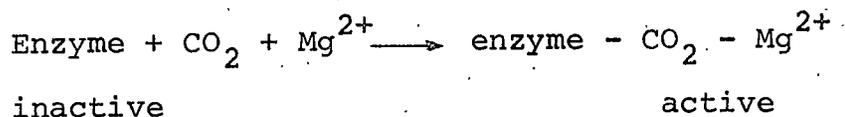
Photosynthetic activity consists of two parallel groups of reactions: the light reaction and dark reaction.

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 CO_2 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 CO_2 (carbon fixation) or O_2 (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+} .



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 C_3 plants show smaller $K_m(\text{CO}_2)$ values (12-25 micromolar) than C_4 plants (28-34 micromolar). Water plants and algae exhibit high values similar to C_4 plants (30-70 micromolar). The variation of $K_m(\text{RBP})$ (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 CO_2 for photorespiration. By raising the temperature from 20°C to 36°C during photosynthesis, $^{14}\text{CO}_2$ incorporation into glycolate immediately stopped, whereas that into sucrose was greatly enhanced. When the temperature was lowered from 36° to 20°C , ^{14}C -glycolate was greatly enhanced, whereas sucrose formation was slowed. No significant change of $^{14}\text{CO}_2$ 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 CO_2 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-frac-ture 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 chlorophylla 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 CO_2 .

RuBisCO activation is controlled by cations as Mn^{2+} , Mg^{2+} , and CO_2 (no Ca^{2+} is involved).

Photoinhibition is controlled by light intensity and CO_2 . 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 CO_2 limitation. Long-term experiments with low CO_2 concentrations imposed a photoinhibition. After this treatment, high CO_2 concentrations did not reverse the inhibition.

One can assume the following sequence: After the first CO_2 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.

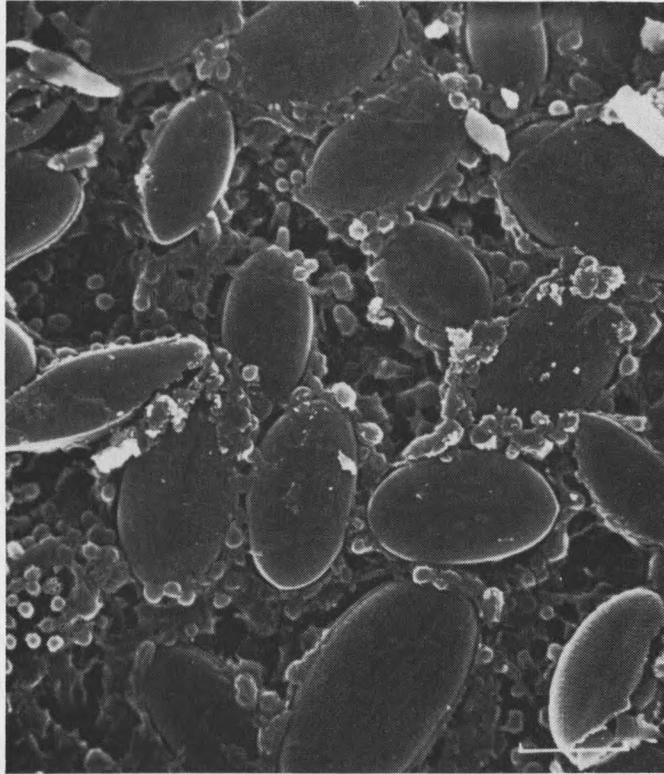


Figure 1. SEM of algal - bacterial film (bar 10 μm) from Squaw Creek, an oligotrophic mountain stream. Thickness of film was 40 - 60 μm .

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 photosynthesis to supply the necessary oxygen. However, operating 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 photo-respiration 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.

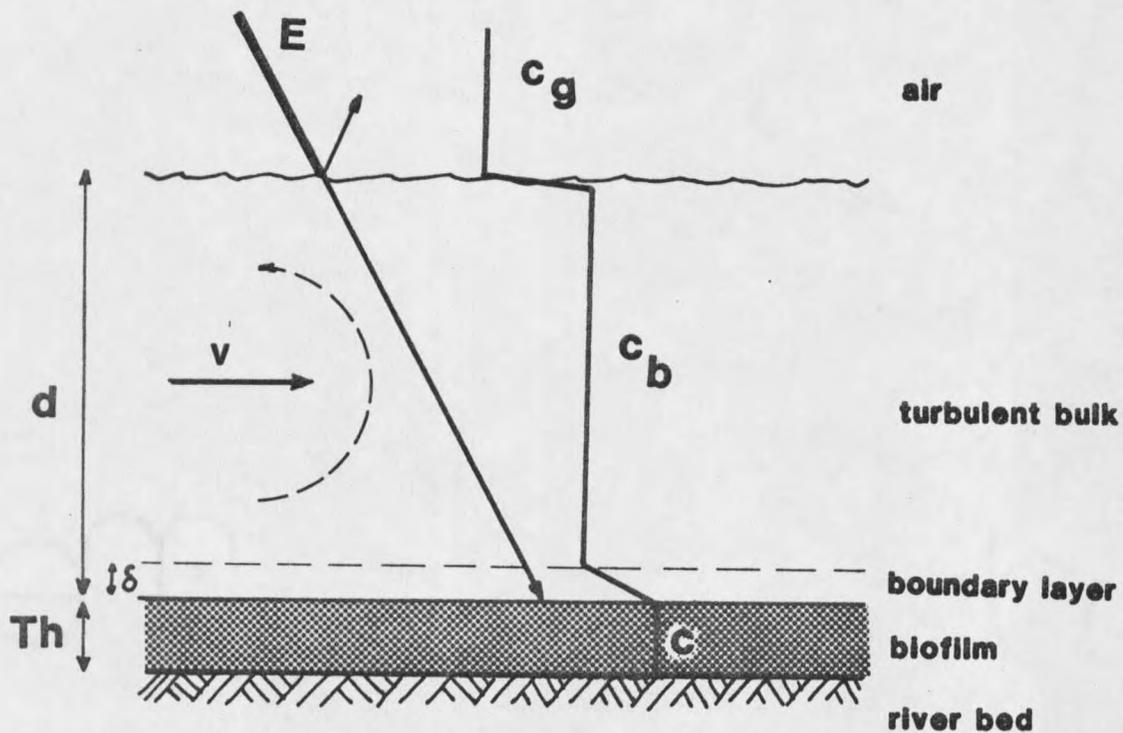


Figure 2. Schematic of a turbulent stream, where d is the depth, Th the thickness of film, v the velocity of bulk water, the concentrations c_g (air), c_b (bulk water) and c within the film. E is the light energy.

