Algal-Bacterial Interactions within Aggregates

INTRODUCTION

The dynamics of combined algal-bacterial aggregates (e.g., flocs or attached films) are receiving increasing attention due to their influence on natural stream quality and also because of their potential to capture solar energy in useful and convenient forms. In the last several years, different models have been proposed to describe their behavior.[1-4] All these models deal only with carbon limitation for carbon fixation and cannot predict the amount of organic carbon released by the cell. Furthermore, these models do not implicitly describe the interaction between algae and bacteria.

This communication presents a model describing an algal-bacterial aggregate, and its behavior during periods of rapidly varying light conditions, i.e., sunrise and sunset. The response of the model will be compared to experimental observations from an oligotrophic (low organic carbon) and eutrophic (high organic carbon) aquatic environment.

BACKGROUND

The organisms comprising the aggregate 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.[5] The overall process occurring in these light systems can be expressed as follows:

$$H_2O \xrightarrow{\text{light}} 2H^+ + 0.5\,O_2 + 2e^- \tag{1}$$

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

Raven[6] states that algae can only fix inorganic carbon in the form of carbon dioxide (CO_2). Other inorganic carbon species (H_2CO_3, HCO_3^-, CO_3^{2-}) 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 inorganic carbon to organic compounds. RuBisCO has active sites for both carbon dioxide and oxygen. RuBisCO has two other notable characteristics: a) Its molecular weight is approximately 5×10^5 which limits the amount of enzyme within the cell volume. As a consequence, a long diffusion path may exist between extracellular CO_2 and the enzyme. b) The enzyme has a relatively slow turnover rate of approximately 10 s^{-1}.

The reactions catalyzed by this enzyme can be expressed as follows

$$\text{RuBisCO-}CO_2 + CO_2 + H_2O \longrightarrow \text{2,3-phosphoglycerate} \tag{2}$$

$$\text{RuBisCO-}O_2 + O_2 \longrightarrow \text{3-phosphoglycerate} + \text{2-phosphoglycolate} \tag{3}$$

Raven[6] 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 photosystem II reactions which are generating O_2. The

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glycolate product in eq. (3) runs counter to the carboxylative trend of photosynthesis, requiring further energy for enzyme synthesis (Fig. 1). 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.[6] 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. Carbon dioxide is released as a product of respiration.

Interaction Between Algae and Bacteria

A high extracellular $[CO_2]:[O_2]$ ratio results in a high intracellular RuBisCO-CO_2:RuBisCO-O_2 ratio and little production of glycolate, an energy-wasting process. In many aquatic environments, carbon fixation is limited by the low $[CO_2]:[O_2]$ ratio in the aqueous phase. However, this ratio may be significantly higher in microenvironments inhabited by respiring bacteria, e.g., algal-bacterial biofilms.[7,8] 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 2. 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 dis-

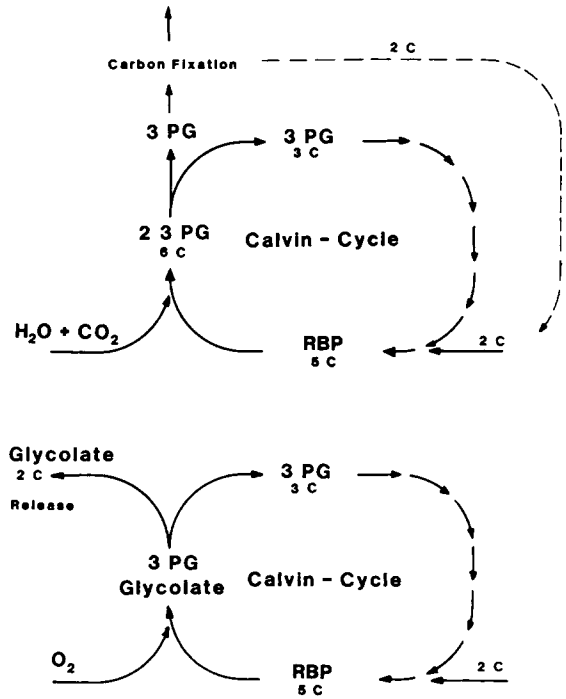

Fig. 1. The Calvin cycle indicating the dual substrate nature of RuBisCO.

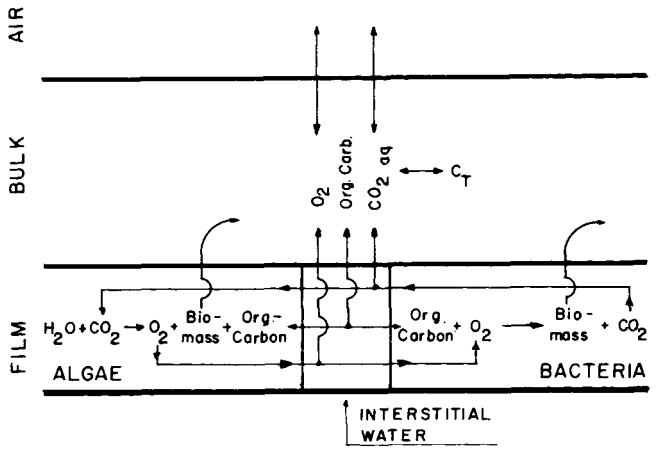

Fig. 2. Schematic diagram of algal-bacterial interactions within a biofilm.

solved oxygen within the biofilm which may influence these parameters in the overlying water as well.

THE MODEL

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 $[CO_2]:[O_2]$ ratio.

Rate of Enzyme Synthesis

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

$$nP + n\text{ADP} + m\text{NADP} \xrightarrow{E} n\text{ATP} + m\text{NADPH}^{+} \tag{4}$$

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

$$r_p = r_{p_{\max}}\left(\frac{E}{K_E + E}\right) \tag{5}$$

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

$$r_R = r_{R_{\max}}\left(\frac{E}{K_E + E}\right) \tag{6}$$

The differential equation for RuBisCO accumulation can be written

$$\frac{d\,\text{RuBisCO}}{dt} = r_{R_{\max}}\left(\frac{E}{K_E + E}\right) - (r_{RC} + r_{RO}) \tag{7}$$

(accumulation in the algal cell) $\neq$ (synthesis of enzyme) $\neq$ (consumption of enzyme)

Enzyme Activity

Lorimer, Badger, and Andrews[9] investigated and measured the activity of RuBisCO and proposed that the RuBisCO-O_2 reaction is described by a linear competitive inhibition model[10] with CO_2 as the inhibitor. They measured $K_{(O_2)} = 200$ μmol and $K_{(CO_2)} = 20$ μmol:

$$r_{RO} = [\text{RuBisCO}]\,(r_{RO_{max}})\,\frac{[O_2]}{[O_2] + K_{(O_2)}[1 + ([CO_2]/K_{(CO_2)})]} \tag{8}$$

Note that O_2 is not an inhibitor for the RuBisCO-CO_2 reaction. Therefore, the rate for the RuBisCO-CO_2 is

$$r_{RC} = [\text{RuBisCO}]\,(r_{RC_{max}})\,\frac{[CO_2]}{[CO_2] + K_{(CO_2)}} \tag{9}$$

Material Balances across the Algal Cell

In vivo concentrations of CO_2 and O_2 can be described by the following material balances across the cell boundaries:

$$\underset{\substack{\text{(net rate of}\\ \text{accumulation}\\ \text{in the cell)}}}{d[CO_2]^0/dt} = \underset{\substack{\text{(net rate of trans-}\\ \text{port into the cell)}}}{k([CO_2] - [CO_2]^0)} - \underset{\substack{\text{(rate of consumption}\\ \text{within the cell)}}}{r_{RC}} \tag{10}$$

$$\underset{\substack{\text{(net rate of}\\ \text{accumulation}\\ \text{in the cell)}}}{d[O_2]^0/dt} = \underset{\substack{\text{(net rate of}\\ \text{transport}\\ \text{into the cell)}}}{k'([O_2] - [O_2]^0)} - \underset{\substack{\text{(rate of consumption}\\ \text{within the cell)}}}{r_{RO}} + \underset{\substack{\text{(photosynthetic}\\ \text{production rate}\\ \text{within the cell)}}}{r_p} \tag{11}$$

where $[CO_2]^0$ and $[O_2]^0$ are the intracellular concentrations (mol/L^3); $[CO_2]$ and $[O_2]$ are the extracellular concentrations (mol/L^3); and k and k' are mass transfer coefficients (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 $[CO_2]$:$[O_2]$ ratios which are important in describing transient conditions (Fig. 3). In addition, the model permits flow of oxygen across the cell wall in either direction.

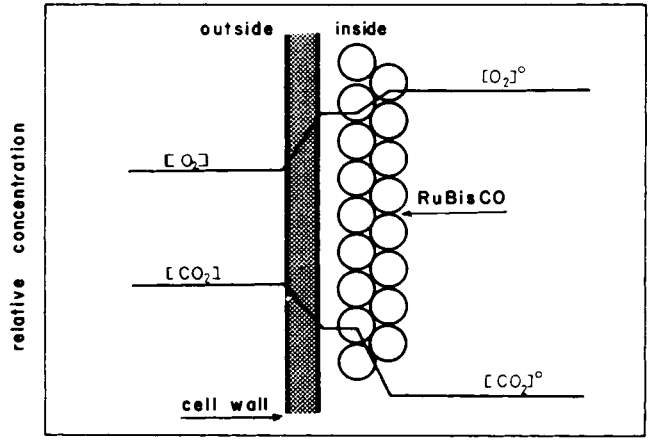

Fig. 3. Relative algal intracellular and extracellular concentrations of oxygen and carbon dioxide reflecting the influence of reaction and diffusion.

Assuming negligible accumulation of CO_2 and O_2 in the cell (i.e., $d/dt = 0$),

$$r_{RC} = k([CO_2] - [CO_2]^0) \tag{12}$$

$$r_{RO} = k'([O_2] - [O_2]^0) + r_p \tag{13}$$

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

$$\underset{\text{(accumulation in cell)}}{dC/dt} = \underset{\text{(carbon fixation)}}{(r_{RC}} - \underset{\text{(carbon release)}}{2r_{RO})} \underset{\text{(algal biomass)}}{X_a} \tag{14}$$

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

$$\mu_a X_a = Y_a(dC/dt) = Y_a X_a (r_{RC} - 2r_{RO}) \tag{15}$$

where Y_a is the yield (biomass formed per mass carbon fixed) and μ_a is the specific growth rate.

The production of algal biomass can be defined as follows

$$dX_a/dt = \mu_a X_a - k_a X_a \tag{16}$$

where X_a is the algal biomass and k_a is a decay coefficient.

Material Balances across the Bacterial Cell

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

$$dX_b/dt = \mu_b X_b - k_b X_b \tag{17}$$

$$dS/dt = -(1/Y_{sb})\,\mu_b X_b \tag{18}$$

$$dO_2/dt = -(1/Y_{ob})\,\mu_b X_b \tag{19}$$

$$dCO_2/dt = (1/Y_{cb})\,\mu_b X_b \tag{20}$$

where the substrate (S) is the source of organic carbon and energy.

COMPARISON OF MODEL TO OBSERVATIONS

The response of the 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[2] has observed such a phenomenon (Fig. 4).

Oligotrophic Environment

In a 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 the air and is approximately 0.04.

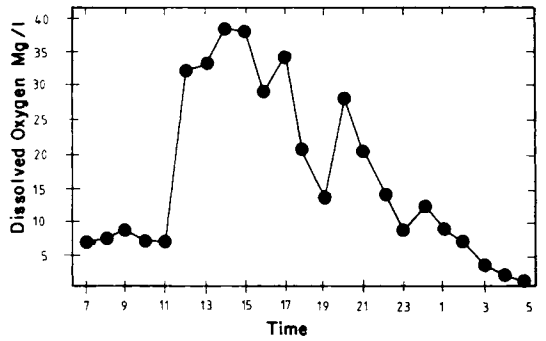

Fig. 4. Diurnal dissolved oxygen concentration in eutrophic wastewater treatment lagoon (ref. 2).

As the first sunlight strikes the biofilm, carbon fixation begins at RuBisCO-CO_2: RuBisCO-O_2 ratio of approximately 5. The following sequence of events occurs (Fig. 5):

1) The $[CO_2]$:$[O_2]$ ratio decreases because of O_2 production by photosystems I and II and use of CO_2, without replacement by bacterial respiration.
2) The RuBisCO-O_2 reaction is dominant and is consuming $[O_2]$ and producing glycolate which is excreted. Glycolate excretion increases bacterial respiration which contributes to further depletion of $[O_2]$. The O_2 in the bulk water is now diffusing into the film with a higher rate than the reaeration rate.
3) Decreasing $[O_2]$ parallels bacterial CO_2 production as long as the $[CO_2]$:$[O_2]$ ratio approaches a value favorable for the RuBisCO-CO_2 reaction. However, the RuBisCO-O_2 reaction is still appreciable and provides the bacteria with glycolate for continued respiration. Because of the close association of the algae and bacteria in the biofilm, the O_2 and CO_2 exchange between organisms is immediate and rapid and does not necessarily influence $[O_2]$ and $[CO_2]$ in the bulk, overlying water.
4) Subsequently, $[O_2]$ increases in the bulk water due to reaeration and O_2 production by photosystems I and II.

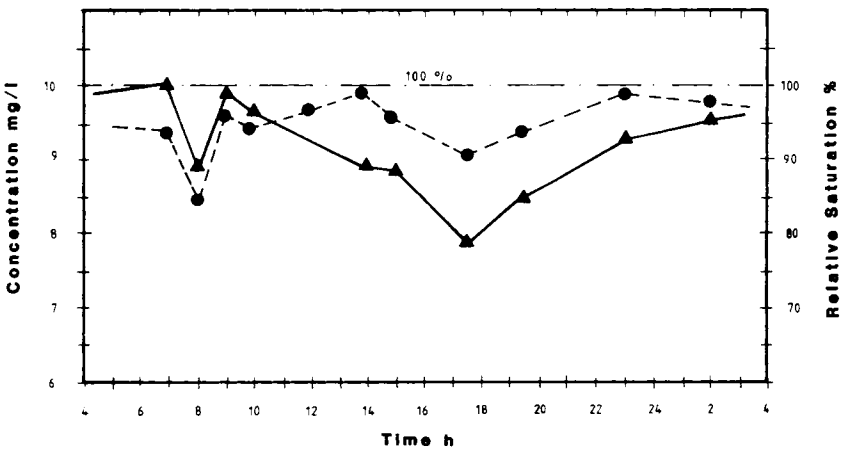

Fig. 5. Diurnal dissolved oxygen concentration in an oligotrophic mountain stream: (▲) concentration and (●) saturation.

5) The $[CO_2]:[O_2]$ ratio increases due to increased bacterial CO_2 production and the RuBisCO-CO_2 reaction rate increases.

Figure 5 presents data from Squaw Creek, a mountain stream in southwestern Montana and is consistent with the sequence of events described above.

CONCLUSIONS

A model has been presented which describes the nature of the interaction between algae and bacteria in aquatic environments. The model has been compared favorably to observations from two contrasting aquatic systems.

Nomenclature

E	light energy (E/L^2)
k, k'	mass transfer coefficients (t^{-1})
k_a	decay algae (t^{-1})
k_b	decay bacteria (t^{-1})
K_E	half-saturation constant of light (E/L^2)
$K_{(CO_2)}$	half-saturation constant of CO_2 (mol/L^3)
$K_{(O_2)}$	half-saturation constant of O_2 (mol/L^3)
RBP	ribulose biphosphate
RuBisCO	ribulose biphosphate carboxylase oxygenase
RuBisCO-CO_2	ribulose biphosphate carboxylase
RuBisCO-O_2	ribulose biphosphate oxygenase
r_p	rate of photosynthesis (M/t)
r_R	rate of RuBisCO formation (M/t)
r_{RC}	rate of RuBisCO-CO_2 reaction (M/t)
r_{RO}	rate of RuBisCO-O_2 reaction (M/t)
s	substrate concentration (M/L^3)
Y_a	yield (biomass algae formed/carbon fixed)
Y_{sb}	yield (biomass bacteria formed/substrate consumed)
Y_{ob}	yield (biomass bacteria formed/O_2 consumed)
Y_{cb}	yield (biomass bacteria formed/CO_2 released)
X_a	algal biomass (M/L^3)
X_b	bacterial biomass (M/L^3)
$[CO_2]^0$, $[O_2]^0$	intracellular concentration (mol/L^3)
$[CO_2]$, $[O_2]$	extracellular concentration (mol/L^3)
μ_a	specific growth rate algae (t^{-1})
μ_b	specific growth rate bacteria (t^{-1})

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