Carbon and Light Limitation in Mass Algal Culture

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INTRODUCTION

A variety of potential applications for mass algal culture are often proposed. The suggested uses for algal biomass include use as fertilizers, raw material for extracted commercial chemicals, animal or human protein supplements either directly, or indirectly through incorporation into aquaculture systems, as well as energy via conversion to methane gas (1). In spite of the apparent potential usefulness of algal biomass and years of research centered around understanding algal growth in both laboratory and field culture, mass algal culture has not yet been commercially realized to any large extent.

The primary reason for this lack of success lies in the complexity of the process of large scale algal culture. Attempts at modelling the species' specific responses of algal culture has been attempted with varying degrees of success (2-6). The continued development of such models will likely be the only successful means of answering the many questions concerning optimum design and operation of future algae culture.

Most mathematical models describing algal growth revolve around three central sub-models. These sub-models attempt to define the growth of a particular alga as a function of the important environment parameters; nutrient concentration, light levels and temperature. These models attempt to simulate the response of algal cell production as a function of these three factors. Although simple in concept, because of the many possible limiting nutrients, multitudes of possible dominating algal species, combined with interaction between the three central variables, the resultant models become extremely detailed and complex. However, in the situation of high density algae culture, it is often the case that two factors, in particular, become most important in controlling production. The supply of inorganic carbon at a sufficient rate and concentration to meet algal carbon uptake rates and the availability of sufficient light intensity to supply the energy needs of the growing culture, are often suggested as controlling net cell productivities (1,7,11).

For these reasons, attention has been directed at understanding more fully the situation of light and inorganic carbon limitation of algal growth. It is the purpose of this discussion to examine the nature of the carbon limited response of algae and to combine a quantitative model of this behavior with models describing the carbonate equilibrium chemistry and flow-through algal culture. These relationships will be examined under both non-light and light limiting conditions.

PREDICTING THE RESPONSE OF A CARBON LIMITED CONTINUOUS ALGAL CULTURE

I. Algal Response to CO_{2_f}

Over the years considerable controversy has developed over the interpretations of data concerning the uptake of the various forms of inorganic carbon by unicellular algae (4,7,8,9,10,12). Early investigators felt that most algae were capable of using either dissolved carbon dioxide (CO_{2_f}) or bicarbonate (HCO_3^-) as a carbon source.

The basis for the belief in HCO_3^- uptake was centered on observations of algal culture growth to pH values as high as 11.0. Since *in vitro* studies on the K_{SCO_2} of the enzyme Ribulose diphosphate carboxydismutase yielded values as high as 10^{-4} moles/liter, it was felt that the CO_2 concentrations in high pH cultures (10^{-6} - 10^{-8} m/l)

was simply too low to supply to carbon needs of the growing culture (9).

Goldman (12) on the other hand suggested that the interconversion of one carbon form to another was much more rapid than was the carbon uptake rate of a growing algal culture. This led him to conclude that carbon limited response of an algal culture may best be represented as a Monod model of the specific growth rate vs. the total carbon concentration (C_T). He further suggests that this relationship must be modified by effects of culture pH.

Recent work by Brune (15) has, however, led to the continued development of yet another model first proposed by King (7). The basis for this model exists in an array of experiments in which the batch growth of laboratory cultures of various freshwater algae were studied. The typical behavior of these cultures is illustrated in Figures 1-5. It was found that for these cultures growing over a wide range of pH (7-11) the carbon limited growth response could best be modelled as a Monod fit of μ vs. CO_{2_f} (Figure 1). In contrast to this, fits of μ to HCO_3^- (Figure 2) or μ to C_T (Figure 3) yielded plots atypical of what is considered normal microbial response to limiting nutrient levels.

In an attempt to quantify any effects from varying culture pH on this relationship, several cultures were grown in which the initial culture alkalinity was varied. The net result of this modification was the observation of μ at similar CO_{2_f} concentrations but at differing pH values. A sample of the data (Figure 4) did indicate an effect, which was first interpreted as a suppression of growth rate by increased culture pH. However, attempts to relate μ to pH did not prove particularly successful. On the other hand, a strong correlation was discovered between the K_{SCO_2} of the algal response and the ionic strength of the growth medium. It was later discovered that this effect could be reproduced independently of pH by increasing the ionic strength of the growth medium with additions of NaCl (Figure 5).

Thus, to date, the simplest model capable of simulating the carbon limited algal response over the widest possible combinations of environmental conditions appears to be a Monod fit of μ vs. CO_{2_f} modified by increasing K_{SCO_2} with increasing culture ionic strength. For the algae examined thus far, culture pH over a wide range appears to have little, if any, effect on this relationship.

The importance of this model is realized when this biological response is combined with equations describing the carbonate equilibrium chemistry to produce a powerful predictive model of algal culture behavior.

II. Combining the Biological, Physical and Chemical Responses

Given that the specific growth rate (μ) of a carbon limited algal culture can be defined as:

$$\mu = \frac{\mu_{max} (CO_{2_f})}{K_S + (CO_{2_f})} \qquad 1)$$

In addition in a continuous flow algal culture, an algal cell mass balance gives (12);

$$\frac{dx}{dt} = DX_1 - DX_2 + \mu X_2 - K_d X_2 \qquad 2)$$

At steady state ($dx/dt = 0$) and in the case of a rapidly growing culture with the decay rate (K_d) taken as zero, and with the influent cell concentration (X_1) also zero, this equation reduces to:

$$\mu = D = 1/\theta \qquad 3)$$

Therefore, the above relationship suggests that once the dilution rate (D) of a continuous algal culture is fixed and steady state is achieved, the specific growth rate is also fixed. The CO_{2_f} concentration of the effluent can then be obtained from the combination of equations 1 and 3;

$$[CO_2]_2 = \frac{K_{SCO_2}}{\frac{\mu_{max}}{D} - 1} \qquad 4)$$

If the buffering capacity of the culture media is dominated by the CO_2-carbonate-bicarbonate system and if the influent pH and total titratable alkalinity are known, then the cell concentration and pH of the effluent can be obtained by combining equation 4 with the carbonate equilibrium equations given by Stumm;

$$\begin{matrix}\text{algal} \\ \text{biomass}\end{matrix} = X_A = C_{T_1} - C_{T_2} \qquad 5)$$

where: $$C_{T_1} = \frac{[AlK] + [H+] - [OH^-]}{\alpha_1 + 2\alpha_2} \qquad 6)$$

The C_{T_2} concentration (effluent) is determined by μ_{max}, D and K_{SCO_2} and is given by:

$$C_{T_2} = \frac{[CO_2]}{\alpha_o} = \left[\frac{K_{SCO_2}}{(\frac{\mu_{max}}{D} - 1)}\right] \left[\frac{1}{\alpha_o}\right] \qquad 7)$$

therefore:

$$[CO_2]_2 = (\frac{[AlK] + [H+] - [OH^-]}{\alpha_1 + 2\alpha_2})\ (\alpha_o) \qquad 8)$$

Since alkalinity is unaffected by algal growth (except for minor modification; see Brewer 14) and is known, and since $[CO_2]_2$ is determined by D, μ_{max} and K_{SCO_2} and are also known, the effluent culture pH can be obtained. The solution is in the form of a 4th order equation and given by Ricci (17). The predicted culture pH for a hypothetical algae (a composite of pooled data) with a μ_{max} of 0.10 hr^{-1} and K_{SCO_2} ranging from 0.17×10^{-6} m/l to 8.1×10^{-6} m/l (depending on ionic strength) is given in Figure 7.

As can be seen, the tendency toward higher culture pH as a result of increasing alkalinity is eventually overpowered by the decreasing ability of the algae to extract CO_2 to low levels at the increased ionic strength due to higher alkalinity levels. The net result; at high alkalinity, continuous cultures will stabilize at lower pH values at a given detention time. Support for the theoretical model has been obtained in the form of data from actual continuous cultures of the alga Scenedesmus quadricauda. As can be seen (Figure 6) the form of the curve is as predicted.

The important implications of this model are summarized in Figures 8 and 9. If culture pH is allowed to drift without control, a large percentage of total carbon in the influent medium will not be utilized. At the detention time giving optimum production (Figure 8) the carbon utilization will range from only 10 to 30% depending on culture alkalinity. Therefore, attempts to increase carbon supply by alkalinity addition alone (as $NaHCO_3$) will not provide for efficient utilization of inorganic carbon. PH control through acid addition would markedly improve the situation; however, the costs of continuous acid addition combined with dangers of instability produced by destroying the

culture buffering capacity do not favor this technique.

Apparently the only effective means of maintaining carbon supply through pH control will be through CO_2 addition. Unfortunately, the low CO_2 content of air makes aeration a very energy intensive means of CO_2 transfer. An alternative method which has been utilized for years in sewage treatment lagoons is the supply of CO_2 from bacterial degradation of waste organics. However, nothing comes free and so it is in this case; the price being the loss of algal productivity by shading of light from the added bacterial biomass.

LIGHT LIMITED ALGAL CULTURE

Once the carbon limitation of the algal culture is removed, the culture will respond by increasing cell density until another factor finally limits cell production. In many cases this factor will be the availability of light. Pipes (18) demonstrated that net algal cell production in a light limited culture is independent of culture detention time. Thus the algal cell density (X) is a linear function of detention time (θ);

$$X \cong \frac{K\theta}{V} \qquad 9)$$

Smith (19) showed that the overall productivity (P) could be related to the biological response of the alga to limiting light and the incipient light levels by the equation;

$$P = \frac{\alpha I}{(1 + (\frac{I}{Pm})^2)^{1/2}} \qquad 10)$$

Using the integrated form of this equation given by Groden (20), with values of the extinction coefficient of algal biomass from Lehman (3) ($\varepsilon = 1.2 \times 10^{-7}$ 1/cell-m) the response of the cell density of a shallow light limited culture of an alga with $P_{max} = 0.10\ hr^{-1}$, $I_o = 5000$ fc, to increasing detention time is given in Figure 10 (computer generated solutions to equation 10). As seen in this figure, cell density responses linearly to hydraulic detention time as predicted by the earlier equation from Pipes. The ideal behavior illustrated in this figure will be modified by many factors; of prime importance will be the additional light shading by the added bacterial biomass and the effects of bacterial CO_2 production. The bacterial biomass present may be predicted from equations describing the decay of influent BOD;

$$\text{where } BOD_E = BOD_I\ 10^{-kt} \qquad 11)$$

The bacteria biomass may be predicted from equations given by Lawrence and McCarty (16);

$$X_B = Y_B \frac{(BOD_I - BOD_E)}{1 + K_b\ \theta} \qquad 12)$$

and the rate of supply of CO_2 from the bacterial decomposition of the incoming BOD;

$$\frac{d[CO_2]}{dt} = -C_1 \frac{d[BOD]}{dt} \qquad 13)$$

Using these equations Figure 10 is modified to account for added bacterial biomass and CO_2 production and the resultant modifications are presented in Figures 12 and

13. Assuming a strong waste influent with a BOD of 500 mg/l, a yield coefficient Y = 0.55, decay rate b = 0.55, C1 = 0.018 milli-moles CO_2/mg BOD oxidized and a coefficient of extinction of light from the bacterial biomass the same as for the algal biomass, the effects of the added bacterial biomass are illustrated in Figure 1[illegible]. Perhaps the most important result of this effect can be seen as the requirement for longer and longer detention time at increasing depth to achieve a stable algal cell population. This effect is due solely to the slower algal growth rate as a result of lowered average light levels per unit of algal biomass.

The final upper limit on cell biomass will again come through carbon limitation and this added effect is combined with the shading effect to produce Figures 11 and 13. These figures illustrate these combined effects on culture pH and algal cell biomass. When bacterial CO_2 production exceeds algal CO_2 fixation the effect will be to drive the pH below the atmospheric equilibrium pH. The lowest level that pH will fall to will depend on the rate at which CO_2 transports across the water surface and exits from the culture. At a steady state pH;

$$\frac{d\ CO_2}{dt} = \overset{net}{CO_2}_{production} = K_{La}\ (CO_{2_S} - CO_2)$$

On the other hand, if algal CO_2 uptake exceeds bacterial CO_2 production, the culture pH will rise according to the carbonate equilibrium chemistry and carbon uptake behavior of the algae as detailed in equation 8. Unfortunately, because of low atmospheric CO_2 levels, CO_2 input (unless aggressively supplied) from surface transport will not usually create a significant pH stabilizing effect as will CO_2 transport out of the solution. The total algal cell biomass will respond by increasing in density with increasing detention time until, as a result of the pH rise, the CO_{2_f} concentration again limits cell production. The effect of either increasing culture depth or increased influent BOD levels will both delay the onset of carbon limitation and increase the detention time for a stable algal biomass population.

LIGHT AND CO_2 MODELS AS A PREDICTIVE TOOL

The model presented here considers only the case of carbon and light limited growth of algal culture. It is, of course, an over-simplification of complex algal-bacterial culture; rather, this model is viewed as a starting point for a more comprehensive model which will be developed to include the important modifiers of the relationships presented here. Of particular importance will be additions to the chemical model to account for various non-carbonate buffers such as ammonia, phosphates, borates, etc. Field determination of the many empirical constants must be made, as well as an assessment of the validity of applying the laboratory derived kinetic data to field situations.

Although the model may be simplistic in nature, the power of a simple carbon and light limitation model in predicting, in general, responses of field algal culture should not be dismissed. Observations of algal cell production from a recent pilot study (21) indicate that the theoretical behavior describes reasonably well the actual culture responses (Figure 14). Although complicated by changing influent BOD loading rates used in this study, the observations of cell density compare well with predicted light and carbon limited values. The culture pH, which was observed to rise to 10 in shallow cultures, and level off at 8.0-9.0 in deeper cultures, while dropping to 7.6 in the bacterial cultures, behaves as predicted by the CO_{2_f} limitation model. A simple yet often unappreciated corollary of the carbon model suggests that whenever culture pH rises above the atmospheric equilibrium value, external carbon is not being supplied at a rate fast enough to meet the algal carbon fixation rate, thus the culture obtains the needed carbon by extracting it from the carbonate system. Unless this situation is carefully controlled, the pH may stabilize at values which yield CO_{2_f} concentrations that will limit algal grow rates.

EXTRACTING ENERGY FROM ALGAL CULTURE; RECYCLING CARBON

An attempt has been made to quantitatively show the importance of CO_{2_f} concentration in controlling algal cell production. One promising method of CO_{2_f} - pH control is through careful section of detention time, influent BOD, and depth of a combined algal-bacterial culture.

Even though bacterial decomposition of organics to CO_2, followed by CO_2 fixation by algae, does not represent a net organic carbon fixation, it can be used to obtain a net energy fixation. This may be particularly applicable to the situation in which algal biomass is converted to methane gas via anaerobic digestion. The resulting CO_2 from gas combustion and the low energy short chain organics in the digester effluent represent a recyclable carbon supply to be returned to the algal ponds. In this situation the importance of proper balancing of algal CO_2 uptake against bacterial CO_2 production cannot be over-emphasized. An imbalance in either direction will result in a loss of efficiency in carbon utilization. Proper selection of the control parameters will likely come through continued development and refinement of models such as presented here.

SUMMARY

The carbon limited kinetic responses of various fast growing algal species have been summarized. These results suggest that the growth responses of many algae used in mass culture may best be represented as a Monod fit the specific growth rate (μ) to the free carbon dioxide concentration (CO_{2_f}). The environmental modifiers of primary importance appear to be light levels, temperature and the ionic strength of the growth media.

The various mathematical models describing the algal biological response to limiting CO_{2_f} concentration, the carbonate equilibrium chemistry and the physical configuration of a flow-through microbial culture are combined to yield equations which predict the pH, total carbon concentration (C_T) and algal cell concentration of a continuous algal culture, given a μ_{max} and K_{SCO_2} for the alga of interest. This model is further used to illustrate the under-utilization of inorganic carbon in mass algal cultures in which the pH is uncontrolled.

One method of pH control in such cultures involves the utilization of CO_2 supply from bacterial degradation of waste organics in the influent culture medium. In such a situation both the culture pH and algal cell production will often be governed by either carbon or light limitation depending primarily on the influent BOD loading, detention time and culture depth. An example is given in which the light dependent response of a particular alga is combined with equations describing the bacterial cell and CO_2 production as a function of influent BOD. The resultant calculations are used to explain why algal populations in combined algal-bacterial culture are often observed to be unstable at detention times considerably longer than theoretical minimum detention time based on laboratory culture data. The effect of increasing culture depth is shown to amplify this effect.

In spite of the obvious over-simplification of considering only light and carbon limits in describing the behavior of mass algal culture, comparisons to actual field data suggest that these two parameters will be of paramount importance in controlling net algal cell production rates.

NOMENCLATURE

BOD_I = Influent BOD_5
BOD_E = Effluent BOD_5
P_m = Light saturated photosynthetic rate
P = Average photosynthetic rate

CO_{2f} = Free carbon dioxide concentration
$[CO_2]_2$ = Effluent CO_{2f}
C_{T_1} = Influent total carbon concentration
C_{T_2} = Effluent total carbon concentration
$[CO_2]_S$ = Atmospheric equilibrium CO_{2f} concentration
C_1 = Moles CO_2 produced per mg BOD_5 oxidized
D = Dilution rate
I = Effective light level
k = BOD decay coefficient
K_b = Bacterial decay coefficient
K_d = Algal decay coefficient
K = Overall algal productivity (from Pipes, 18)
K_{La} = CO_2 transfer coefficient
K_{SCO_2} = CO_{2f} concentration at which $\mu = 1/2\ \mu_{max}$
t = Time
V = Reactor volume
X_1 = Influent algal cell concentration
X_2 = Effluent algal cell concentration
X_A = Algal cell concentration
X_B = Bacterial cell concentration
Y_B = Bacterial yield coefficient
α = Slope of photosynthetic rate vs. light intensity curve
α_o = CO_{2f} fraction of C_T
α_1 = HCO_3^- fraction of C_T
α_2 = CO_3^- fraction of C_T
μ = Specific growth rate
μ_{max} = Maximum specific growth rate
θ = Hydraulic detention time = 1/D

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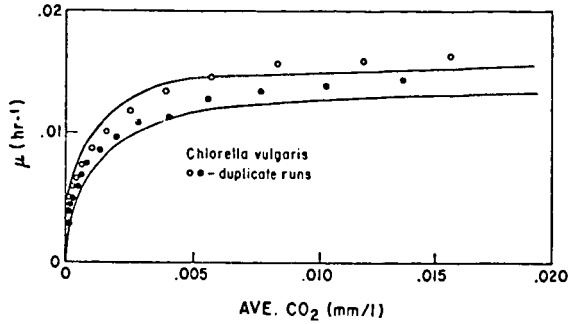

Figure 1: 95% confidence intervals around a Monod fit of μ vs. CO_{2f} for Chlorella vulgaris grown at 15°C and saturated light levels.

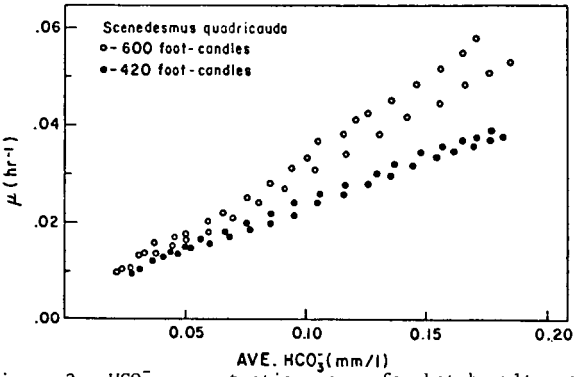

Figure 2. HCO_3^- concentration vs. μ for batch cultures of Scenedesmus quadricauda grown at 27°C and two light levels.

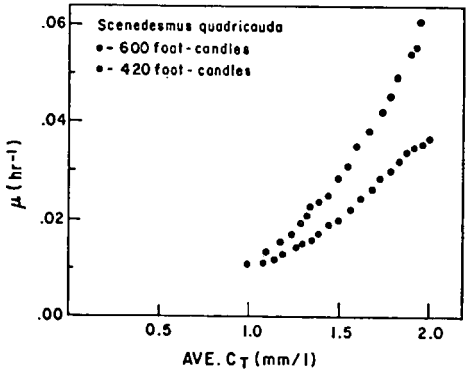

Figure 3. C_T concentration vs. μ for batch cultures of Scenedesmus quadricauda grown at 27°C and two light levels.

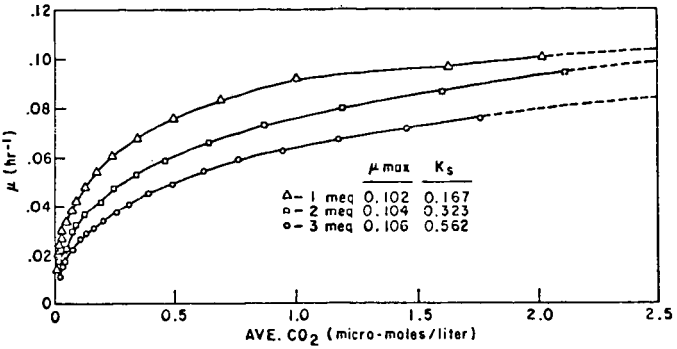

Figure 4. μ vs. CO_{2_f} for batch cultures of Chlorella vulgaris grown in a medium of varying initial alkalinity.

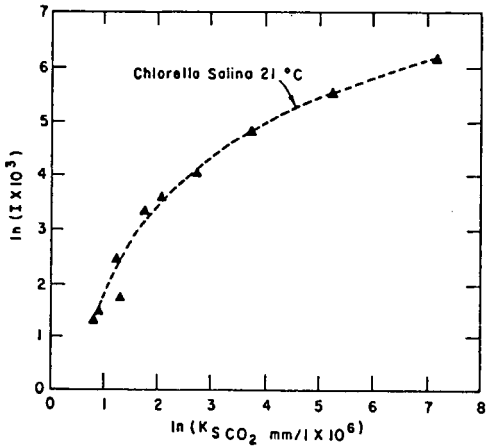

Figure 5. Observed relationship between K_{SCO_2} and the culture ionic strength for Chlorella salina.

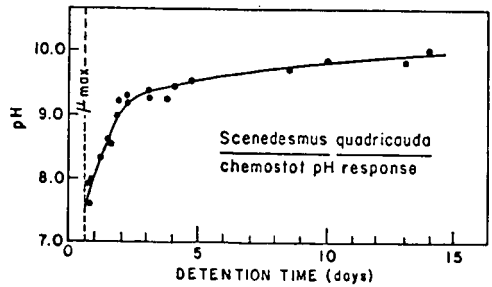

Figure 6. Continuous culture pH response of Scenedesmus quadricauda with influent alkalinity of 2.5 meq/liter.

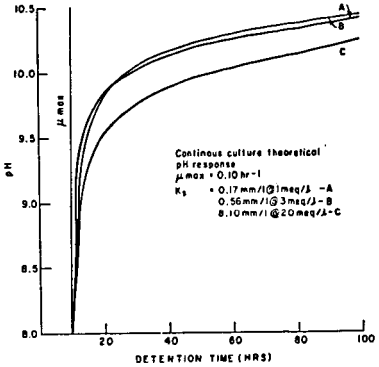

Figure 7. Theoretical pH response of continuous algal culture at varying influent alkalinity.

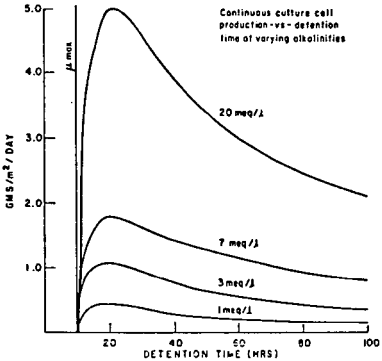

Figure 8. Theoretical production (in gms carbon/m^2) from a 10 cm deep carbon limited continuous algal culture.

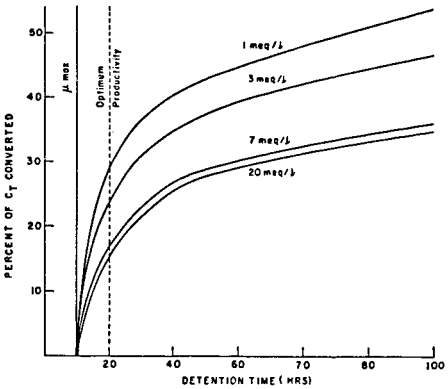

Figure 9. Percent of total carbon in influent medium converted to algal biomass in a carbon-limited continuous culture.

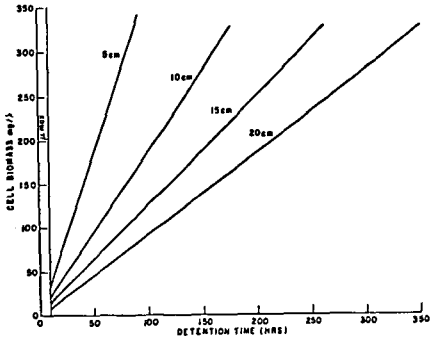

Figure 10. Theoretical algal cell biomass vs. detention time in a light limited continuous culture.

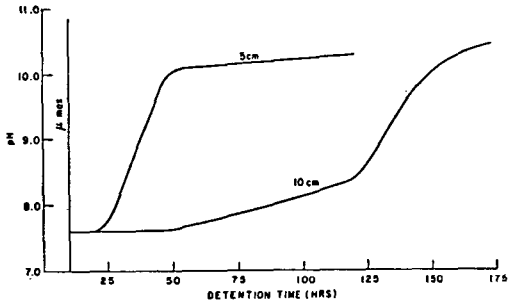

Figure 11. Prediction of culture pH based on algal response to CO_{2f} and bacterial CO_2 production with influent BOD_5 = 500 mg/ℓ.

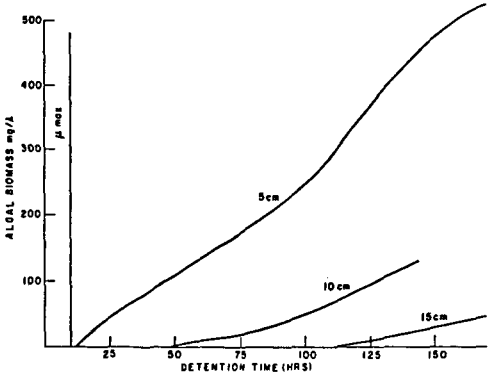

Figure 12. Effect of bacterial biomass production from BOD degradation on algal biomass density.

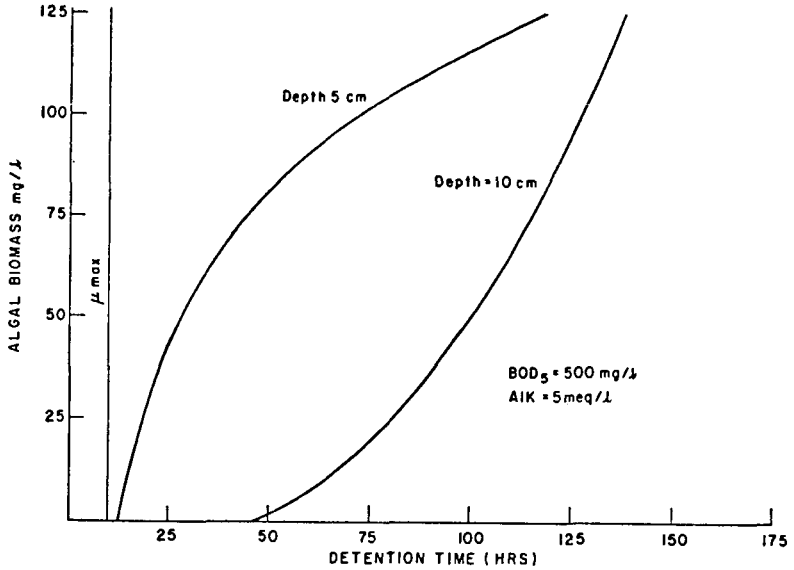

Figure 13. Combined effects of carbon limitation and bacterial biomass shading on algal biomass at different culture depths.

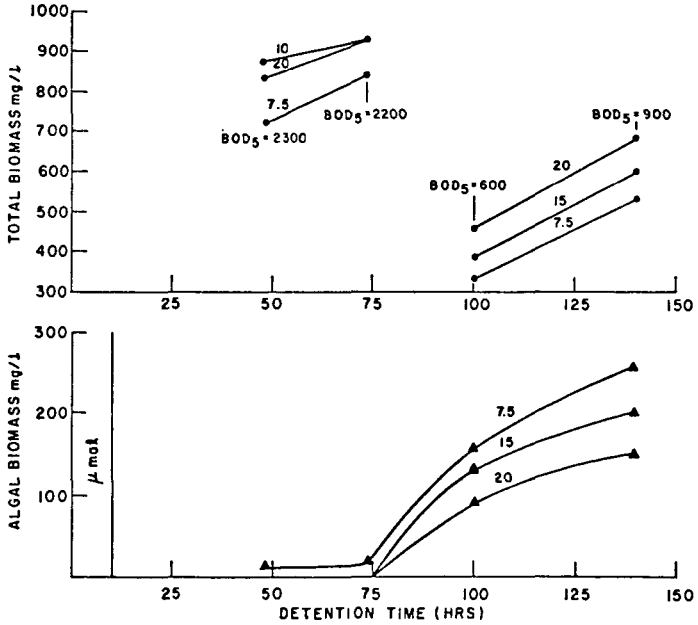

Figure 14. Data from pilot scale algal-bacterial culture (from Boersma, et al.). Total and algal biomass at culture depths of 7.5, 15 and 20 cm.