ESTIMATION OF YIELD AND MAINTENANCE PARAMETERS ASSOCIATED WITH SINGLE CELL PROTEIN PRODUCTION ON C-1 COMPOUNDS

by

HYEON YONG LEE

B.S., Yon Sei University, Seoul, Korea, 1981

A MASTER'S THESIS

Submitted in partial fulfillment

of the requirements for the degree

MASTER OF SCIENCE

FOOD SCIENCE

Department of Chemical Engineering

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1984

Approved by:

L E Erickson

Major Professor

LD
2668
T4
1984
.43
c. 2

A11202 957987

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

I would like to dedicate this thesis to my parents who have not been afraid of sacrificing to support my education.

I would like to express my profound gratitude to Professor L.E. Erickson for his sustained efforts to guide, help, and inspire me.

I am really grateful to Dr. S.S. Yang for his invaluable comments and for serving on my committee.

I wish to thank Dr. Daniel Y.C. Fung for reviewing this manuscript and for serving on my committee.

I wish to give my thanks to my best friend, Bumshek Hong, and Mr. M.D. Oner for their help throughout this work.

I also would like to make a grateful acknowledgement to the National Science Foundation for their financial support during the course of this work.

Thanks also to Dr. Y.R. Pyun and Dr. J.H. Yu who are professors in my country for constantly encouraging me during this work.

Last, I devote all the grace to my loving fatherland, Korea.

Chapter 1

INTRODUCTION AND SUMMARY

INTRODUCTION

Utilization of solar energy to produce useful products, such as Single Cell Protein (SCP) is expected to become increasingly important as costs of other sources of energy increase. Long term interest and importance associated with these processes is due to the much higher thermodynamic efficiencies of microbial photosynthesis compared to agricultural plants, which is demonstrated in Table I. Algal cultures can be easily controlled to obtain higher yields. Although photosynthetic microbial processes are of considerable importance and many papers on algal growth experiments have been published, much biochemical engineering process analysis and design research remain to be done with respect to developing methods of estimating photosynthetic growth yields and for achieving high yields in large scale processes. This work is concerned with surveying relevant literature, developing new methods for estimating yield parameters, and applying these methods to literature data. Algal growth on CO_2 and heterotrophic growth on C-1 compounds, such as methanol, are investigated. These processes are related because both involve growth on C-1 compounds. This work is divided into four chapters. Tables, nomenclature, and references are located at the end of each chapter.

Chapter 2 is mainly concerned with summarizing literature on the bioenergetic and food aspects of algal growth. In the bioenergetic section, theoretical bioenergetic considerations, estimation methods for evaluating photosynthetic efficiency, effects of light, carbon dioxide, oxygen, and other nutrients on growth, and experimental methods are discussed in this chapter.

Chapter 3 contains the results of analyzing regularities for photosynthetic microorganisms, whose values are used in estimating true growth yield and maintenance coefficients. Statistical anslysis is used to obtain average values and coefficients of variation from measured values in the literature.

In Chapter 4, estimation methods for yield and maintenance parameters, which have been applied in heterographic growth, are used to analyze continuous cultures of photosynthetic growth. Methods for batch cultivation are also developed and applied to available batch data.

Chapter 5 presents the results of estimating true growth yield and maintenance coefficients for microbial processes grown heterotrophically on C-1 compounds, such as methanol. The analysis process is similar to that used for photosynthetic microbial growth.

REFERENCES

1. S.J. Pirt, Y.K. Lee, R. Amoes, and M.W. Pirt, J. Chem. Tech. Biotech. 30, 25 (1980).

2. S. Aiba, and T. Ogawa, J. Gen. Microbiol. 102, 179 (1977).

3. L. VanLiere, and L.R. Mur, J. Gen. Microbiol. 115, 153 (1979).

4. J. Myers, Proceedings of the International Biological Program/ Primary Productivity, p. 447 (1970).

5. E.C. Wassink, B. Kok, and J.L.P. VanOorschot, Algal Culture from Laboratory to Pilot Plant, J.S. Burlew, (Ed.), p. 55, Carnegie Inst., Washington, DC (1953).

Table 1. Comparison of Efficiencies

	Species	Maximum conversion efficiency (%)	References
Photosynthetic micro-organisms	*Chlorella vulgaris*	*26.7 *39.6++	S.J. Pirt *et al* [1]
	Spirulina platensis	*12.7	S. Aiba *et al* [2]
	Oscillatoria agardhii	*23.0	L. VanLiere *et al* [3]
	Chlorella pyreidosa	*16.43	J. Myers [4]
Agricultural Crop Plants	Onions	0.45	E.C. Wassink *et al* [5]
	Carrots	0.94	
	Sugar cane	1.92	
	Wheat	1.26	
	Potatoes	1.23	

*Results from estimation methods in Chapter 4

++Mixed culture with bacteria

Chapter 2

REVIEW OF LITERATURE

INTRODUCTION

In this chapter, literature on the photosynthetic algal growth process is surveyed with an emphasis on the bioenergetics and process requirements of photosynthesis through light utilization [1-73]. So far the theoretical conversion efficiency is still controversial because the maximum conversion efficiency based on quantum requirements is lower than that value based on some measurements of biomass. Therefore the theoretical considerations and experimental results related to photosynthetic efficiency need to be summarized. The methods of estimating this energetic yield, which have been developed by many investigators, and the process of utilizing light energy are discussed in terms of bioenergetics. Many factors, which can affect algal growth, such as light, temperature, CO_2, and O_2 concentration, etc., are reviewed because they are important in modeling, design, and scaling up systems. Many experimental methods are reviewed, especially the methods for measuring light intensity, and the demonstrated advantages of continuous cultivation. Finally, the possible ways of using algae as food, sometimes called Single Cell Protein, are reviewed.

BIOENERGETICS OF PHOTOSYNTHESIS

1. Theoretical Considerations and Efficiency of Algal Growth

It is important to define qualitatively and quantitatively the thermodynamic and kinetic limits on the photochemical conversion and storage of solar energy when we consider the growth of photosynthetic organisms. Many researchers have dealt with the problem of thermodynamic limits on the conversion of light to chemical energy [1-5]; Ross and Hsiao [5] have recently published a particularly lucid treatment, based on the original treatment by Ross and Calvin [4]. That is, they defined maximum thermodynamic efficiency as a function of wave length and photochemical power yields [4] which they had developed. They have been interested in fuel-generation reactions, such as hydrogen production using photosynthetic reactions. They have shown that a reasonable goal for solar energy storage efficiency in this reaction would be ~ 10 to 13 percent but probably not much higher than 13 percent. Goldman [6] developed another expression for thermodynamic efficiency as a function of wave length. The average value of his calculation for the visible light range which is used for photosynthesis is about 20% as determined from graphical integration. Goldman [6] also considered light utilization efficiency, in his analysis of photosynthetic conversion efficiency. The earliest attempt to quantitatively account for the effect of light saturation was by Burlew [7].

Pirt [10] defines photosynthetic efficiency as the ratio of the free energy of the biomass produced to the amount of light energy absorbed. The free energy of the biomass relative to CO_2 (g), H_2O (ℓ), and aqueous ammonia may be appropriately used when the nitrogen source is ammonia or

urea. When N_2 or nitrate is the nitrogen source, the free energy of combustion of the biomass may be used.

The quantum requirement, which is defined as the number of quanta required for fixing one mole of CO_2 or producing one mole of O_2 photosynthetically, has been used to evaluate the efficiency of light-energy conversion to chemical energy. However, the minimum number of quanta required has been and remains controversial [8,9]. The estimated range of the quantum requirement has varied from three to fourteen. Up to now, quantum requirements of 8-10 quanta per oxygen molecule evolved are widely accepted as yield efficiencies that can be achieved. It is well-known that the Hill and Bendall [71] scheme (the 'Z scheme') for photosynthetic electron transport requires that eight light quanta are utilized per molecule of oxygen evolved. That is,

$$2\ H_2O + 4\ Fd^{+3} \xrightarrow[\text{photons}]{\text{eight}} 4\ H^+ + O_2 + 4\ Fd^{+2} \qquad (1)$$

where Fd refers to ferredoxin.

For higher plants, this scheme is accepted without any particular problem; the CO_2 and H_2O are converted to carbohydrates in the cell, where free energy is stored as chemical energy.

For photosynthetic microorganisms some of the reported quantum requirements [8,10] are smaller than 8. There have been several attempts to calculate the correct quantum requirement for illustrating the exact electron flow system of these organisms. No widely accepted minimum quantum requirement exists at this time. Pirt [10] tried to answer whether the minimum quantum demand is less or greater than 8 $h\nu/O_2$, which is a critical point for present theoretical concepts of the conversion of radiant energy to chemical energy in photosynthesis. For the

biotechnologist the problem is essentially one of determining the maximum growth yield from the available light energy. He interpreted his results very carefully to show that the quantum requirement is less than eight. He justified his value by pointing out that photosystems 1 and 2 may work in parallel, and that the two photosystems may act in series under some conditions where maximum photosynthetic efficiency is not required, e.g., when some factor other than light limits photosynthesis. He also insisted that thermodynamics permits on the average an energy gain of 1.33 eV for each electron excited by a photon at 680 nm which suggests that living cells would have evolved an acceptor to take maximum advantage of this light energy. The selection of an electron shift from water to ferredoxin (ΔE_h = 1.24 eV) would represent near perfection in evolution of photosystem 2. Pirt [10] preferred the measurement of photosynthetic efficiency based on biomass production to those based on short-term O_2 release because algae can use oxygen for respiration as well as produce it. Biomass production measurements are made over a longer period of time and are not as dependent on short term transients which may introduce error into the oxygen release measurements. The thorough, well-controlled application of oxygen measurement by Brackett et al [11] gave a minimum quantum demand of 6.1 $h\nu/O_2 \pm 0.6$ for Chlorella. Brackett also found that the quantum requirement was highly reproducible for a given culture, but it varied from 6 to 13 with different cultures. One possible reason for this variation could be the chlorophyll content of the cultures which varied roughly inversely with the quantum requirement. Williams [12] found that the chlorophyll content of algae can be varied several fold by the growth conditions in continuous culture systems. Even though the measurements based on biomass production have advantages like

those mentioned above, it might be difficult to estimate accurately the light energy flowing to a continuous culture of aglae. However, the use of a continuous flow steady state method can offer methodological advantages which facilitate the mathematical and conceptual analysis of the parameters and variables involved in the relationship between algal growth and a rate limiting factor [13]. The Turbidostatic method proved to be good in reaching steady state conditions rapidly and for maintaining a predetermined concentration of algae in the reactor. The latter is particularly important in assaying waters relatively rich in nutrients in which intensive growth of algae can cause excessive shading.

Even when theoretical data on quantum requirements of specific green plants and/or algal cells are available from the literature, it is difficult from such information per se to precisely answer the question of how much energy is required to harvest a unit amount of cells, unless the absorption spectrum, and a spectrum of the light source are also provided [14]. In order to prevent this difficulty, a growth yield, defined as the grams of dry cells harvested per kJ of light energy absorbed by the cells by Aiba and Ogawa [15], was introduced and used for their work with a dilute supension of Spirulina platensis, a specific blue-green alga [14].

2. Methods to Estimate Energetic Yields

Many researchers [15,18,19,20,55] have been trying to estimate the light conversion efficiency. Ogawa and Aiba [15] estimated the maximum energetic yield as 31.7% based on eight quanta of light required to produce one mole of oxygen, and the average wave length of visible light. They also analyzed the cellular composition of algae for

calculating the energy contained in the cell. However, the theoretical maximum energetic yield based on eight quanta is exceeded by the results of Pirt's estimations [21] which are about 34~42%. Thus, the correct minimum quantum yield is unclear as well as the maximum light conversion efficiency. Aiba [18,54] defined growth yield, Y_{kJ}, as grams of biomass per kJ of light energy input. When Aiba calculated growth yield, he checked the differences in light source on growth yield, and used a wave length distribution given by a halogen lamp with an infrared cut-filter. The wavelength of light source after passing through the filter ranged from 380 to 720 nm. Also, to get the light conversion efficiency, the growth yield was multiplied by the heat of combustion of algal cells, $-\Delta Ha$, kcal/g. According to elemental analysis of Spiralina platensis, $-\Delta Ha$ is estimated as 25.1 kJ/g. Aiba [18] also calculated growth yield based on the measurement of carbon dioxide consumption. Aiba counted the rate of carbon dioxide consumption with radio activity of $C^{14}O_2$, and checked the carbon balance in the culture vessel.

It is interesting to note from Aiba's calculations [54] that the value of Y_{kJ} remains rather unchanged, (3~5) x 10^{-3} g/kJ, despite the difference in algal species and culture conditions as far as autotrophic cultivation of these algae is concerned. Aiba [54] used the specific light energy absorption rate $\xi = \mu/Y_{kJ}$, to calculate the true growth yield with the following relationship,

$$\xi = \frac{\mu}{(Y_{kJ})_G} + m \qquad (2)$$

where

ξ = specific light-energy absorption rate = μ/Y_{kJ}

$(Y_{kJ})_G$ = true growth yield, g/kJ

m = maintenance coefficient, kJ/g/hr

If equation (2) is acceptable for photoautotarophic growth, ξ may be plotted against $\mu(=D)$ to yield a straight line, whose slope and intercept correspond to the reciprocal of $(Y_{kJ})_G$ and m, respectively. Aiba checked the applicability of equation (2), but the linearity between ξ and $D(=\mu)$ was not always satisfactory.

Pirt [19,21] also found the photosynthetic efficiency using the product of the calorific value of the biomass and the growth yield defined as the biomass formed per light absorbed. From the principles of bioenergetics [22,23] which show that the growth yield will be a function of the specific growth rate of the cells, Pirt also found [21] that the light requirement of a growing culture of cells is expected to conform with the relation given by Equation (2).

Pirt mentioned that the maximum growth yield is to be expected only when the energy source is growth-limiting, and showed that Equation (2) may be divided by the specific growth rate, μ, to obtain the relationship

$$\frac{1}{Y_{kJ}} = \frac{1}{(Y_{kJ})_G} + \frac{m}{\mu} \qquad (3)$$

Equation (3) may be treated as a linear relationship by plotting the reciprocal of Y_{kJ} vs that of μ. Thus, the true growth yield $(Y_{kJ})_G$ can be estimated and the photosynthetic efficiency can be found by multiplying

this value by the calorific value of the biomass. Pirt noted that the value of the mass yield should depend on the degree of reduction of the nitrogen source used. Generally nitrate has been used instead of ammonia for algal growth; this will decrease the mass yield with respect to that found using ammonia or urea. This will be discussed quantitatively in the next chapter. Pirt stated that the reduction in photosynthetic efficiency should be of the order of 20% of its value. Also, Pirt estimated the maximum photosynthetic efficiency to be 29%, assuming n = 8 and the mean wavelength of the light is 575 nm; however, his value of biomass growth yield is much higher than that corresponding to a photosynthetic efficiency of 29%. Therefore, Pirt recommended that the quantum requirement should be reconsidered and the assessment of photosynthetic efficiency should be based on biomass growth. Particularly, Pirt showed a way of enhancing the photosynthetic efficiency by symbiotic cultivations with bacteria, Alcaligenes species. The resulting efficiency was higher than for the case of growing algae alone. Also, the photosynthetic efficiency was independent of both the amount of light energy and the intensity of the light incident on the culture surface. Pirt [19,73] also considered product formation; that is, he showed that nitrogen-limited growth of Chlorella in photosynthetic chemostat culture could be used to produce starch. Pirt considered the biomass yield by measuring both the biomass and the total cell dry weight which included the starch. Therefore, Pirt tried to distinguish between a storage product, such as starch, and the functional biomass.

Gons and Mur [20] considered a light-limited system, in which attention was given to the quantity of absorbed light energy, the efficiency of conversion of light energy to chemical energy, and the

maintenance energy requirement of the cells. Gons and Mur [20] developed a model to describe growth in light-limited conditions:

$$(dE/dt) \cdot C = \mu X + \mu_e X \tag{4}$$

where dE/dt is the rate at which light energy is absorbed by a unit volume of algal biomass culture, C is an efficiency factor, X is concentration of organisms, μ is specific growth rate, and μ_e is a maintenance rate constant. Therefore by plotting $(1/X)\cdot(dE/dt)$ vs μ, the efficiency factor, C and maintenance rate constant, μ_e are obtained from a straight line. The efficiency factor, C, should be recognized as the efficiency with which light energy is converted to chemical energy in biomass when the energy required for maintenance is neglected. It is a measure of the photosynthetic efficiency. The maintenance parameter, μ_e, is related to the energetic maintenance coefficient, m_e; that is,

$$m_e = \frac{\mu_e}{C}$$

where m_e is the specific rate of light energy utilization for maintenance with units of hr^{-1} and μ_e is the specific rate of chemical energy utilization for maintenance based on light energy being converted to chemical energy with an efficiency, C.

The equation used by Gons and Mur [20] for parameter estimation is similar to Equation (2); however, the units of the yield parameters are different. Equation (4) may be used with continuous culture data if dE/dt is taken as the rate that light energy is absorbed per unit volume of culture.

Mur and vanLiere [36] reported that estimated values of the efficiency factor, C, ranged from 0.155 to 0.214 while values of μ_e

ranged from 0.015 to 0.062 day^{-1}. Mur and van Liere [24] recognized the difficulties of studying growth yields in light limited batch cultures because of the rapid change in growth conditions and the slow adaptation of microorganisms to these changes.

3. Effects of Light Intensity and Temperature on Algal Growth

The effects of light intensity and temperature on photosynthesis have been extensively reported. At low light intensity a linear relation between intensity and photosynthesis usually has been found [34,54], whereas at high light intensity, the rate of photosynthesis is constant and relatively independent of light intensity. Rabinowitch [25] showed the same general trend in every case of a linear increase of photosynthesis with light intensity up to a saturation point, beyond which higher intensities did not increase photosynthesis but eventually resulted in inhibition, the physiology of which is disucssed by Steemann [26]. At low cell densities, when growth is not light energy limited, an exponential growth phase occurs; at higher densities, growth proceeds linearly with time, while growth decreases gradually at very high cell densities. Mass cultivation of algae has to be designed for the linear range, at which practically all the incident light is absorbed by the culture.

Sorokin and Krauss [27] reported that photosynthetic efficiency must be considered in predicting yields of mass cultures. The density of algal populations often limits the light intensity received by some of the algal cells. According to Kok [28], the complete process of algal growth is more complicated than the photosynthetic conversion of light energy to chemical energy; however, the maximum conversion efficiency of light energy into biomass is of the same order of magnitude

as that observed in photosynthesis experiments using gas exchange measurements.

In growth experiments with mass cultures, Oorschat [29] observed a decrease in the efficiency with increasing light intensities even though the biomass concentration increased at higher intensities. This is connected with the mutual shading of the cells in cultures of high cell density. Oorschat also investigated the functional relationship between the rate of photosynthesis and the light intensity. He considered the theoretical efficiency, assuming that in an algal culture the light intensity decreases with depth and cell density according to Beer's law. The maximum efficiency was about 23%, based on his optional culture conditions.

Frog, Nalewajko, and Watt [30] found that photosynthesis can proceed over a wide range of light intensities, up to ~50k lux. Talling [31] determined maximal photosynthetic rates of about 25 mg O_2 (mg chlorophyll a)$^{-1}h^{-1}$ in experimental exposures of blue-green algae-dominated suspensions in a reservoir on the White Nile, at irradiance level equivalent to 35-40 klux and at a temperature in the range 21~25°C. Talling also showed that the onset of light saturation of photosynthesis, i.e. the light level below which photosynthetic rate becomes light-limited, generally occurred between 6~10 klux. The light requirement of blue-green algae seems generally similar to those of other planktonic algae, although the value for light saturation of photosynthesis is probably lower than that for many other algae. Adaptation of populations to light is also possible during growth, resulting in a further change in the light saturation condition. This may be due to a change in chlorophyll content or a change in the efficiency of photosynthesis.

Myers [32] reported that with higher light intensities, the cells become increasingly larger but they have about the same chlorophyll content per cell. Meffert [33] found optimal growth of planktonic species in culture is often achieved under an incident illumination of 2-3 klux.

In algal fermentation, a non-illuminated part of fermentor, i.e. dark region which is in the center of the vessel, frequently exists. This dark region could affect the growth rate; that is, the specific growth rate may vary with position. The respiration process in the dark region could affect the energetic yield and the maintenance coefficient. The respiration process, which means that the carbohydrates in the cell are consumed by using oxygen to produce carbon dioxide, results in a loss of energy because of the need for this energy for maintenance processes. Ryther [34] made a hypothesis that the ratio of photosynthesis to respiration is 10:1 at light saturation, and that respiration assumed a value of 0.1 relative to maximum photosynthesis because it is not possible to measure the ratio directly in natural populations. Livansky [35] also found that the rate of photosynthesis in algal cells depended on the volume of light and dark region and retention time in these regions. Livansky developed a relationship between growth rate and ratio of non-illuminated volume to total volume. The fraction of volume in the dark region affects the energetic yield. As the ratio of dark region volume is increased, the maintenance coefficient also increased. If the dark volume is controlled, the actual growth rate can be estimated from the equation that Livansky [47] developed. Van Liere, Loagman and Mur [36] stated that the light distribution in the fermenter could be modeled using mean light irradiance at low cell concentrations. Van Liere et al [36] worked with continuous culture at several specific

growth rates with cell concentrations up to 40 mg/l. Under these conditions the specific growth rate is the same at all points in the fermentor.

Lee and Pirt [37] developed a theory to predict the growth rate of photosynthetic microbes in a photobiological reactor with light-sufficient zones and dark zones. The theory predicts that in light-limited cultures the maintenance energy will increase in proportion to the duration of the dark period. Therefore Lee and Pirt [37] found that the maintenance energy was close to zero in the light, but endogenous metabolism of resting cells in the dark corresponded to a large maintenance energy of about 8.8 kJ (g dry wt)$^{-1}h^{-1}$. They pointed out the significance of these results for the design of photobiological reactors.

At very high light intensities, the growth of algae are inhibited. For example, when algae are cultivated outdoors, i.e. in full sun light, the growth yield is much lower than when cultured inside. Even though there are many possible explanations for this, photorespiration, which is defined as the light-dependent, oxygen sensitive, CO_2 evolution in photosynthetic tissues under high light intensities, originating from the metabolism of glycolate, is mostly accepted. Therefore these results may be generally interpreted as light-induced inhibition due to photorespiration. Stewart [38] and Graham [39] explained the mechanism of photorespiration in algae. They showed the evidence of existing photorespiration and inhibitory effect on cell growth. However, Lloyd, Canvin, and Culver [40] reported that photorespiration and formation of glycolate did not exist and dissolved oxygen concentration did not affect apparent photosynthesis in cultivation of several species of fresh water and marine algae. This result is very contradictory to other results. The existence

and effect of photorespiration should be studied further.

Temperature in culturing algae is also an important factor in terms of energetic yield. Livansky [41] found that the growth rate increased with the intensity of illumination and temperature of the suspension to a maximum and then decreased with further increase of temperature. Oorschot [29] observed that the chlorophyll content of the inoculum depended on the temperature, at which the algae had been precultured. Lowest contents were observed at 20°C, while at 40°C somewhat lower contents than at 30°C occurred. Planktonic blue-green algae are widely supposed to have a preference for higher water temperature, but this view is probably an erroneous one based upon the remarkable occurrence of thermophiles thriving in hot-water springs, according to Castenholz [42]. Temperature optima for some of the algae species are much lower, probably in the range 25-35°C. Anabaena flos-aquae began to grow above 5°C, producing its most rapid rate of increase between 10 and 15°C. Microcystis aeruginosa, however, had a wider temperature tolerance, but its increase was most prolific at about 17-18°C. Microcystis did not appear in abundance until the surface temperature had exceeded 15°C, but it is now believed that this temperature is significant only in that its attainment coincides roughly with the onset of thermal stratification. This sort of criticism can be levelled at all correlations between temperature and the time at which populations appear. There is an obvious need for the investigation of temperature optima of specific blue-green algae under controlled conditions.

4. Influence of CO_2 and O_2 Concentration on Photosynthesis

In spite of the important roles of CO_2 and O_2 in photosynthesis, present understanding of the influence of the partial pressure of these gases on photosynthesis is still poor. Warburg [43] first reported that increasing the O_2 partial pressure above 0.02 atm reversibly inhibited photosynthesis as measured by O_2 evolution by resting, that is, non-growing algal cell suspension over a 30 min. period. Björkman [44] found with chlorella that there was no measurable inhibition of CO_2 fixation by O_2. Myers [45] also observed algal photosynthesis is inhibited by an increase in CO_2 partial pressure above 0.05 atm. In addition, CO_2 has been reported as antagonizing the Warburg effect, although this effect seems highly variable. Ogawa et al [51] observed the effect of oxygen on growth yield of chlorella. The result was that in the light-limited environment, the algal specific growth rate deteriorated appreciably with the increase of partial pressure of oxygen.

Pirt and Pirt [46] considered the fact that CO_2 and O_2 are dominant components in the material balance for photosynthesis. They reported that algal growth was inhibited by a step change in O_2 or CO_2 partial pressure of more than about 0.1 atm; however, the cells could be readily adapted to much larger changes in these gases provided the change is made in small steps. The constancy of the maximum growth yield and maintenance energy with an increase in the O_2 partial pressure to 0.8 atm indicates that chlorella does not contain any metabolic site which is made inefficient by oxidation at high O_2 partial pressures.

The required quantities of inorganic carbon for algal growth are more difficult to estimate compared to those of nitrogen and phosphorous. Even though the actual quantities of organic carbon produced via

photosynthesis can be calculated in the same manner as for nitrogen and phosphorous, according to Goldman et al [47], the total amount of inorganic carbon to be supplied is much more difficult to calculate because inorganic carbon is distributed among the chemical species CO_2 (aqueous), H_2CO_2, HCO_3^-, and CO_3^{-2} in an exceedingly complex chemical equilibrium system which is controlled by two parameters, alkalinity and pH. Also Myers [48] pointed out the complexity of effects of CO_2, that is, the carbon dioxide pressure at the surface of the algal cell can never be as great as that in equilibrium with the gas phase since a diffusion gradient must always exist. Goldman et al [47] compared the growth effect between HCO_3^- alkalinity and bubbled CO_2 as carbon sources. The results were that HCO_3^- alkalinity was an excellent source of inorganic carbon below specific pH levels, but chemical precipitation at high pH placed an upper limit on productivity that was far lower than potential light-limiting levels. Also another factor for explaining lower efficiency by using HCO_3^- is that it is conceivable that ATP is consumed in uptake of CO_2 from HCO_3^- at a higher pH value. Therefore, a full understanding of the complex interactions in the aqueous CO_2 - HCO_3^- - CO_2^{-2} system and photosynthetic assimilation of inorganic carbon is necessary to avoid undue wastage of CO_2. Also Goldman et al [49] observed urea is, by far, the most suitable N source for maximizing algal yield when it is supplied in combination with the proper amount of HCO_3^- alkalinity in the growth medium and percent CO_2 in the bubbled gas that will lead to an equilibrium pH near the optimum pH. Finally, Goldman et al [49] concluded that the most effective way to supply inorganic carbon in excess to intensive and continuous microalgal culture was to bubble air enriched with CO_2 into the growth chamber. Briggs and Whittingham [50] considered

the buffer effect by using four strains of chlorella grown in the customary way in 4% carbon dioxide. The rate of photosynthesis in carbonate-bicarbonate buffer solution of low carbon dioxide concentration (0.9×10^{-6} mole/ℓ) increased four-to-five fold during 2 hr. of high illumination (40,000lux) in contrast with a relatively constant rate in a buffer of high carbon dioxide concentration (78.7×10^{-6} mole/ℓ).

5. Summary of Experimental Methods

A. Culture conditions and methods.

It is very difficult to find studies which describe values of specific growth rate observed under well-defined growth conditions with respect to given microorganisms, even though numerous references on microbial photosynthesis have been published. The well-defined conditions should include not only the composition of the culture medium, temperature, and pH of the medium, but also the light source, incident light intensity, size of the bio-photoreactor, and the variation of light intensity with position in the reactor. The light source and measurement methods will be discussed in the next section. The size of the bio-reactor is a very important factor because of the variation of light intensity with position. Little attention has been given to the design of the photoreactor where growth occurs. Few papers have reported fermentation in pilot scale equipment [52,53].

Culture conditions for some species of blue-green, green algae and photosynthetic bacteria are summarized by Aiba [54]. Most of those data are taken in light-limited, continuous cultures either in chemostat or in turbidostat, which have great advantage of being controlled easily to get the best culture condition. Aiba tried to show the specific

growth rate as affected by incident light intensity, temperature and pH, by demonstrating specific growth rate and culture conditions, but it is hard to estimate growth kinetics parameters from his data. His summary gives a sectional view on the microbial growth despite the essential difficulty to handle consistently different divisions, genera and species of the photosynthetic microorganisms.

Outdoor cultivation also should be carefully investigated. Up to now, several workers [55,56] presented their results from outdoor cultivation which have lower efficiency than indoor cultivation. Because the intensity of sun light is difficult to control for optimized cell growth in outdoor cultivation, sometimes photorespiration is important.

In experimental design for photosynthesis, it should be well understood that appropriate attention needs to be given to the kind of cultivation, size and shape of fermentor, indoor or outdoor light conditions, dissolved nutrient culture conditions, and measurement methods for estimating growth parameters and light intensity. Among those factors, measurement of the light intensity and dissolved CO_2 concentration are very important, but not studied very well because light intensity varies with position and both are difficult to be measured. The experimental methods will be summarized in the following sections.

B. Determination of light intensity.

In many older investigations, the influence of light intensity on photosynthesis was studied by illuminating the plants with "white light" (of the sun, or of an incandescent lamp) and introducing gray filters, or altering the distance between light source and plant. Other observers determined the intensity of illumination by visual comparison with a

standard light source. The light source varies from sun light to many different lamps. The characteristics of sun light including maximum and minimum illumination [57] and incandescent lamps [58] are reported by several workers. Of the common photometric devices, only thermoelements and bolometers react uniformly to radiation of all wave lengths. All other instruments — vacuum photocells, barrier layer cells, actinometers - possess a selective spectral sensitivity. Some investigators [54,57] suggested that instruments insensitive to infrared light, e.g., selenium barrier layer cells should be used in preference to thermophiles or bolometers in the measurement of light intensity, in order to avoid measuring the infrared contribution together with visible light. Warburg and Schocken [59] have developed an actinometer based on Gaffron's earlier observations in Warburg's laboratory of sensitized autoxidation of allyl thiourea. Thiourea was substituted for allyl thiourea as oxidation substrate, and pyridine for acetone as solvent; it was found that, with ethyl chlorophyllide (or protoporphyrin) as sensitizer, a quantum yield equal to 1.0 ± 0.1 (molecules oxygen consumed per quantum absorbed) can be obtained over a considerable range of wave lengths and intensities. The convenience of this actinometer is the possibility of using it in conjunction with Warburg reaction vessels in a manometric system. Recently the intensity of light has been measured with a photocell illuminometer or photodiode, and light energy with a temperature-compensating thermopile [54].

Aiba [54] showed evidence that the accurate measurement of light energy absorbed by a dilute suspension of photosynthetic microorganisms in liquid medium, while minimizing the shading effect, is a prerequisite to useful data for estimating the efficiency of light energy conversion

to a cell material. Aiba described several ways of measuring light energy, such as the opalescent plate method, the method of using integrating sphere photometer, and chemical actinometer. The opalescent plate method is highly recommendable, in terms of measuring light energy which passes through the medium; this measurement gives the light energy actually absorbed by the microbial cells. Some workers measured light intensity only at the surface of the fermentor assuming all of it is absorbed. The estimated energetic yield in terms of the energy in the cells divided by the absorbed light energy will depend on how the absorbed light energy is measured if not all energy is absorbed by the cells. The assumption that the light energy absorption is the same throughout the fermentor is frequently made. Most researchers use this assumption for simplifying the analysis, but this problem needs further study. It is important to know what the distribution of light intensity is in the culture vessel for calculating the exact energy absorbed by the cells. This is true when studying the effects of other factors since the light levels should then be neither inhibiting at the surface nor limiting. Van Liere and Walsby [60] measured the light-irradiance profile and from this calculated mean light irradiance in a culture. Also, they designed a system for measuring mean light intensity.

C. Measurement of CO_2 consumption and O_2 evolution.

In measuring CO_2 consumption or O_2 evolution, direct measurement of the gas phase concentrations or partial pressures is routinely done using infrared CO_2 analyzers and paramagnetic oxygen analyzers. Another useful way which was used by Myers [61] is gas chromatography. The results with this method are also quite reliable. However, the dissolved

inorganic carbon is very much affected by the pH of the medium and the quantity dissolved in the medium is large compared to O_2. One should not neglect the carbon dissolved in the liquid broth in any dynamic study of photosynthesis. Van Beusekom et al [62] reported a method for the determination of dissolved carbon dioxide as a function of pH and flow rate. Also Barford and Hall [63] showed a good way of determining CO_2 in the media and cells. Therefore, the measured values from the gas phase analysis should be combined with liquid phase measurements for dynamic processes.

Rabinowitch [57] described methods of measuring oxygen evolution by different chemical or physicochemical methods, either in the liquid phase containing the aquatic plants, or in the gas phase. Because of the low solubility of oxygen in water, methods of the potentiometric determination of the oxygen concentration in solution are suitable only for the measurement of small effects, e.g., for the observation of the photosynthetic activity in the first minutes of illumination. When oxygen production is measured during batch or continuous culture, it is common to neglect the accumulation of oxygen dissolved in the liquid phase.

UTILIZATION OF ALGAE AS A FOOD

Unicellular algae have been used as food since ancient times in some regions of Africa and Mexico [74]. Also nutritional studies carried out by the Institut Francis du Petrole (IFP) showed that Spirulina is one of the richest protein sources ever found. Clement, Ginney and Menzi [64] pointed out too that the use of microalgae for human and animal food predates history. Floating microalgae are sometimes concentrated by the actions of wind or current along the shores of lakes and streams, sufficiently to permit manual collection and use for food and animal feed. According to Faron [65], a Spanish historian, who accompanied Cortez through the street of the Aztec island city of Tenochititlan, wrote of the sale of 'loaves' of a cheeselike material made from a 'slime' collected from nearby Lake Texcoco, near where the blue-green algae Spirulina grows and is harvested today. According to Gross et al [66], Nostoc, another blue-green algae, which forms pea size round modules in water, is reportedly eaten by inhabitants of Northern Thailand and in the Peruvian Andes. Seaweeds are uni- or multi-cellular organisms like plankton that grow in the sea. Seaweeds are also more important economically than is generally realized, since they are used as human and animal food, in medicine and agriculture, and as a source of raw materials for numerous industries [72].

More than three decades ago, Spoehr and Milner [67], following extensive experiments, suggested the mass culturing of algae as a potential solution to the world protein shortage. Microalgae, in common with all other plants, are comprised of protein, carbohydrate, lipids, fiber, and minerals but, unlike most higher plants, are especially rich

in protein. According to Nguyen, Kosaric, and Bergougnou [68], the composition of protein, carbohydrate, fat, moisture, and ash is 63.1%, 16.6%, 1.0% and 13.9% respectively. Moreover, the yield of protein recovery from intact cells was about 66%, which is higher value than any other organism. Clement et al [64] analyzed the amino acid composition of algal protein for nutritional value, and found all the essential amino acids were contained in fairly good amount except that the amounts of the sulphur amino acids, such as cystein and methionine, were low. However, fortunately lysine, tryptophan, and tyrosine, which are sufficient in algal protein, are insufficient in many food products; if food products are supplemented with algal protein, the deficiency of these essential amino acid would be overcome.

For digestibility tests, rats were fed with algal protein and casein [64], and the results (the gain of diet animal weight was measured) showed that there was not much difference between the weight gain of casein diet rats and that of algal protein diet rats. The nutritive value of sewage grown Chlorella and Scenedesmus has been demonstrated in feed trials for poultry, swine, sheep and cattle [69]. These studies suggested that algae are essentially equivalent in nutrient content and digestibility to soybean meal, a common protein supplement for livestock feed.

The most notable algal production in the United States is currently 20 to 40 lb/day of sewage grown algae. This is produced at the University of California, Richmond pilot plant. In contrast, large plants in which algae are grown to produce oxygen have now reached a size of 200 acres, producing as much as 10 tons of oxygen/day [70]. The significant progress toward controlled oxygen production that has been attained over the last 30 years encourages us to believe that, as the need for protein increases,

waste grown algae will become available, perhaps first simply for fertilizer, but, as the need arises, for animal feed. Whether algae production is accomplished in conjunction with or without waste recovery, and whether it is practiced for direct human or for livestock consumption its potential for bettering the lot of mankind seems virtually limitless. It is time, therefore, for industry and government to renew their interest in microalgae technology and to move toward a period when palatable animal protein is more plentiful and less expensive than it is today and when protein itself should no longer be a limiting factor in the development of mankind.

CONCLUSIONS

There still is some uncertainty with respect to the true growth yield for algae, i.e., maximum photosynthetic efficiency because the results of calculating the maximum efficiency based on the observed biomass production sometimes are higher than the maximum value of 29% based on the Z-scheme. The Z-scheme is widely accepted for photosynthesis; in it eight quanta are necessary for fixing one mole of carbon dioxide and producing one mole of oxygen. The effect of maintenance energy, which is an important factor for algal growth, should be considered in estimating true growth yield. Continuous or turbidostat culture is recommended for investigating the true growth yield and maintenance energy requirement. Mass and energy balance equations should be used to check the consistency of the experimental data.

Light intensity, which is a critical factor for growing algae, should be lower than the saturation intensity. Above this intensity the growth is inhibited due to photorespiration. Temperature is an important variable at higher light intensity; the saturation intensity depends on temperature. High partial pressures of CO_2 and O_2 have been found to inhibit cell growth. Several methods of measuring light intensity are described; however, precise measurement of the light absorbed by the culture is difficult.

Algae have been shown to be an excellent protein source, single cell protein (SCP), which has good digestibility and amino acid composition. However, algae have a problem for use as a human food due to different taste and texture, which needs to be studied further. Supplementation with cereal products has been used with better taste and amino acid composition.

Much analysis and design work remains to achieve maximum potential yields in large scale out-door algal cultures. The effect of mixing on yield and system design should be considered, and other variables for controlling light utilizing efficiency, such as cell concentration, flow rate, nutrient concentration and light intensity distribution have to be optimized. Finally, designs for formation of extracellular products from algae, and genetic engineering of algae will be long term goals.

NOMENCLATURE

Q_{CO_2}	Specific rate of carbon dioxide consumption, g mole of CO_2/g cell/hr.
μ	Specific growth rate, hr^{-1}.
ξ	Specific light energy absorption rate, kcal/g cell/hr.
I_a	Total light energy input, kcal/cm^2.
A	Total area, cm^2.
Y_{kJ}	Growth yield, g cell/kcal.
$(Y_{kJ})_G$	"True" growth yield.
m	Maintenance coefficient, kcal/g cell/hr.
X	cell concentration, g/ℓ.
C	Efficiency factor, dimensionless.
q	Specific rate of light utilization, kcal/g cell/hr.

REFERENCES

1. R.T. Ross, J. Chem. Phys. 45, 1, (1966).
2. R.T. Ross, R.J. Anderson, and T.L. Hsiao, Photochem. Photobiol. 24, 267 (1976).
3. M. Almgren, Photochem. Photobiol. 27, 603 (1978).
4. R.T. Ross, and M. Calvin, Biophys. J. 7, 595 (1967).
5. R.T. Ross, and T.L. Hsiao, J. Appl. Phys. 48, 4783 (1977).
6. J.C. Goldman, Wat. Res. 13, 119 (1979).
7. J.S. Burlew, (ed.). Algal Culture from Laboratory to Pilot Plant, Carnegie Institute of Washington Publ. 600, Washington, DC (1953).
8. G. Forti, and S.J. Pirt, Rivista Di Biologia, 74, 347 (1981).
9. R. Emerson and C. Lewis, Am. J. Botany 30, 165, (1943).
10. S.J. Pirt, Biotech. and Bioeng. 25, 1915 (1983).
11. F.S. Brackett, R.A. Olson, and R.G. Crickhard, J. Gen. Physiol. 36, 563, (1953).
12. F.M. Williams, Systems Analysis and Simulation in Ecology, B.C. Patten, ed., p. 197, Academic Press, New York, (1971).
13. G. Shelef, W.J. Oswald and C.C. Golueke, Adv. in Wat. Pollu. Res., vol. 1, p. III-25/1 (1970).
14. T. Ogawa, T. Fujii, and S. Aiba, Biotech. and Bioeng. 20, 1493 (1978).
15. S. Aiba, and T. Ogawa, J. Gen. Microbiol. 102, 179 (1977).
16. G.C. Gerloff, and F. Skoog, Ecology 38, 556 (1957).
17. E.A. Thomas, "The Degree of Eutrophication in Central European Lakes", Int. Symp on Eutrophication, Madison, Wisc. (1967).
18. T. Ogawa, and S. Aiba, J. Appl. Chem. Biotechnal. 28, 515 (1978).
19. M.W. Pirt and S.J. Pirt, J. Appl. Chem. Biotechnal 27, 643 (1977).
20. H.J. Gons, and L.R. Mur, Verh. Internat. Verein. Limnd. 19, 2729 (1975).

21. S.J. Pirt, Y.K. Lee, R. Amos, and M.W. Pirt, J. Chem. Tech. Boitech. 20, 25 (1980).

22. A.H. Stouthamer, In Microbiol Energetics 27th Symposium Soc. General Microbiol. p. 285 (1977).

23. S.J. Pirt, (ed.), Principles of Microbe and Cell Cultivation, Blackwell Scientific Publications, Oxford (1975).

24. L.R. Mur and L. VanLiere, Mitt. Internat. Verein. Limnol. 21, 158 (1978).

25. E. I. Rabinowitch, Photosynthesis and Related Process, Vol. II, Part I, Interscience Publ. Inc., NY (1951).

26. N.E. Steemann, Physiol. Plant. 5, 334 (1952).

27. C. Sorokin, and R.W. Krauss, Plant Physiol. 33, 109 (1958).

28. B. Kok, Acta. Botan. Neerl. 1, 446 (1952).

29. J.L.P. VanOorschot, Med. Van De Land. Te Wag./Nederland, 55, 225 (1955).

30. G.E. Frog, C. Nalewajko, D. Watt, Proc. Royal Soc. London, B162, 517 (1965).

31. J.F. Talling, Proc. Royal Soc. London, B147, 57 (1957).

32. J. Myers, J. Gen. Physiol. 29, 419 (1946).

33. M.E. Meffert, Mitt. der inter. Verein. für theor. ang. Limnol. 19, 189 (1971).

34. J.H. Ryther, Limnol. Oceanol. 1, 61 (1956).

35. K. Livansky, Folia Microbiol. 24, 339 (1979).

36. L. VanLiere, J.G. Loogman, and L.R. Mur, FEMS Microbiol. letters, 3, 161 (1978).

37. Y.K. Lee and S.J. Pirt, J. Gen. Microbiol. 124, 43 (1981).

38. W.D.P. Stewart, (ed.), Algal Physiology and Biochemistry, Univ. of California Press, Berkeley and Los Angeles, (1974).

39. D. Graham, The Biochemistry of Plants, Vol. II, D.D. Davies, ed. p. 525, Academic Press, NY (1980).

40. N.D. H. Lloyd, D.T. Canvin, and D.A. Culver, Plant Physiol. 59, 936 (1977).

41. K. Livansky, J. Theo. Biol. 78, 519 (1979).

42. R.W. Castenholz, Bacteriological Rev. 33, 476 (1969).

43. O. Warburg, Biochemische. Zeitschrift. 103, 188 (1920).

44. O. Bjorkman, Physiologia plantarum 19, 618 (1966).

45. J. Myers, Algal Culture From Laboratory to Pilot Plant, J.S. Burlew. (ed.) p. 37, Carnegie Inst. of Washington Publ. 600, Washington, D.C. (1953).

46. M.W. Pirt, and S.J. Pirt, J. Gen. Microbiol. 119, 321 (1980).

47. J.C. Goldman, M.K. Dennett, and C.B. Riley, Biotechnol. and Bioeng. 23, 995 (1981).

48. J. Myers, Plant Physiol. 19, 579 (1944).

49. J.C. Goldman, M.R. Dennett, and C.R. Riley, Biotechnol. and Bioeng. 24, 619 (1982).

50. G.E. Briggs, and C.P. Whittingham, New Phytologist 51, 236 (1952).

51. T. Ogawa, T. Fujii, and S. Aiba Arch. Microbiol. 127, 25 (1980).

52. D.L. Little, Algal Culture From Laboratory to Pilot Plant, J.S. Burlew. (ed.), p. 235, Carnegie Inst. of Washington Publ. 600, Washington, D.C. (1953).

53. R. Samson, and A. LeDuy, "Multistage Continuous Cultivation of Blue-Green Alga Spirulina maxima in the Flat Tank Photobioreactors with Recycle", presented at the AICHE Meeting, Los Angeles (November, 1982).

54. S. Aiba, Adv. Biochem. Eng. 23, 85 (1982).

55. L. VanLiere, and L.R. Mur, J. Gen. Microbiol. 115, 153 (1979).

56. F. Gummert, M.E. Meffert, and H. Stratmann, Algal Culture From Laboratory to Pilot Plant, J.S. Burlew. (ed.), p. 166, Carnegie Inst. of Washington Publ. 600, Washington, D.C. (1953).

57. E.I. Rabinowitch, (ed.), Photosynthesis And Related Processes, Vol. II (Part I), Interscience Publishers, New York (1951).

58. A.C. Hardy, and F.H. Perrin, The Principles of Optics, McGraw-Hill, New York (1932).

59. O. Warburg, and V. Schocken, Arch. Biochem. 21, 363 (1949).

60. L. VanLiere, and A.E. Walsby, The Biology of Cyanobacteria, N.A. Carr and B.G. Whitton, (Ed.), p. 9, Blackwell Sc. Publ. (1982).

61. J. Myers, Am. J. Bot. 33, 419 (1946).

62. C.N.J. Van Beusekom, H.E. Dekok, and J.A. Roles, Biotechnol. and Bioeng. 23, 2397 (1981).

63. J.P. Braford, and R.J. Hall, Biotechnol. and Bioeng. 21, 609 (1979).

64. G. Clement, C. Ginney, and R. Menzi, J. Sci. Fd. Agric. 18 497 (1967).

65. W.V. Faron, Saturday Rev. 50, 55 (1967).

66. R. Goross, A. Ramirez, L. Cuadra, C. Collazos, N. Gross, and W. Fieldheim, Institutes Nacionales de Salud, Jiron Tizon y Bueno 226 Lima II, Peru (1975).

67. H.A. Spoehr, and H.W. Milner, Plant Physiol. 24, 120 (1949).

68. H.T. Nguyen, N. Kosaric, and M.A. Bergougnou, Can. Inst. Food Sci. Technol. 7, 114 (1974).

69. Anon, "Composition and Nutritional value of the Blue-Gree Algae, Spirulina," NESTLE-IFP paper, 3rd International Congress of Food Science and Technology, Washington, D.C. (1970).

70. W.J. Oswald, Proc. Conf. Ponds as Waste Water Treatment Alternative, Univ. Texas, Water Resources Center, Austin (1976).

71. R. Hill and F. Bendall, Nature 186, 136 (1960).

72. R.R. Colwell, Science 222, No. 4619, p. 19 (7 Oct. 1983).

73. Y.K. Lee and S.J. Pirt, J. Chem. Tech. Biotechnol. 31, 295 (1981).

74. O. Ciferri, Microbiological Review, 47, No. 4, 551 (1983).

Chapter 3

CHARACTERIZATION OF ALGAE USING REGULARITIES

INTRODUCTION

There is considerable interest in the utilization of solar energy for microbial photoautotrophic growth of algae because of the long term potential of photosynthetic processes [1,2,3,4,5]. The regularities of Minkevich and Eroshin [6,7], which are very useful in the analysis of heterotrophic growth processes [8,9,10,11], may also be used in the analysis of photoautotrophic growth processes [3,12]. In this work, values of the weight fraction carbon in algal biomass, σ_b, the reductance degree of algal biomass, γ_b, and the energy content per equivalent of available electrons, Q_o, are reviewed for available literature data. The data in the literature is divided into that for fresh water algae and that for marine algae because of the significant difference in ash content.

ANALYSIS OF DATA

The purpose of this work is to determine the mean and standard deviation of these regularities for algae and to compare the results with those for bacteria and yeasts. Values of the regularities for yeast and bacteria have been examined previously [6,7,13,14]; however, data for algae have not been summarized using the regularities of Minkevich and Eroshin [6,7]. Values of the weight fraction carbon in algae are presented on a dry weight basis and also on an ash free dry weight basis. The elemental composition is used to calculate the values of the biomass reductance degree, γ_b; i.e.,

$$\gamma_b = \frac{\%C(\frac{4}{12}) + \%H - \%O(\frac{2}{16}) - \%N(\frac{3}{14})}{\%C(\frac{1}{12})} \qquad (1)$$

for the valences C = 4, H = 1, O = -2, and N = -3. The above formula is most appropriately used when the nitrogen source has a valence of -3. For N_2 or nitrate as nitrogen source, a valence of N = 0 is employed [3] and the last term in the numerator of Equation (1) is omitted. Values of the reductance degree are calculated for both the valences N = -3 and N = 0.

RESULTS AND DISCUSSION

In Table 1, values of the regularities are presented for the elemental compositions measured by Ketchum and Redfield [15] for *Chlorella pyrenoidosa* and *Chlorella vulgaris*. The average values of weight fraction carbon and reductance degree are similar for those regularities. Additional results for *Chlorella* species are presented in Tables 2 and 3 for the data of Wassink *et al.* [16] and Myers [17,18], respectively. In Table 2, the variation in the weight fraction carbon on an ash-free basis is much smaller than that obtained with ash included. There is considerable variation in ash content in the data in Table 2, and this is reflected in the variation of the values of weight fraction carbon, σ_b. Comparison of Tables 1,2 and 3 shows that values of weight fraction carbon on an ash free basis and reductance degree are very similar for all of the data with *Chlorella*.

In Table 4, values for the regularities for *Spirulina platensis* are presented based on the data of Aiba and Ogawa [19] and Ogawa and Terui [20]. The average values for the weight fraction carbon and reductance degree are slightly lower for *S. platensis* compared to the values of *Chlorella*.

Table 5 summarizes the values of the regularities for other fresh water algae based on elemental composition measurements in the indicated references. While the average values of weight fraction carbon and reductance degree are similar, values of the standard deviation and coefficient of variation are larger in Table 5 compared to the values in Tables 1, 3, and 4.

Table 6 presents values of the regularities from the indicated literature for marine algae. The ash content is larger in these samples because

of the higher salinity of the culture broth. Comparison of the results in Table 1 - 5 shows that the weight fraction carbon and reductance degree are lower for marine algae. This is clearly shown in Table 7 where the average values for fresh water algae and marine algae are compared.

The average values in Table 7 for fresh water algae may be compared with the average values reported by Minkevich and Eroshin [6,7] of $\sigma_b = 0.462$ and $\gamma_b = 4.291$ (for the valence N = -3) for bacteria and yeasts. The weight fraction carbon and reductance degree are about 3% larger for fresh water algae compared to the average values reported by Minkevich and Eroshin. The average values in Table 7 for fresh water algae are slightly smaller than the average values for bacteria of $\sigma_b = 0.482$ and $\gamma_b = 4.423$ which were reported by Minkevich et al. [7].. One can conclude that average values for fresh water algae are similar to values for yeast and bacteria grown heterotrophyically. The values of coefficient of variation reported in Table 7 are similar to those reported for σ_b and γ_b by Minkevich and Eroshin [6,7].

Tables 8 and 9 present some results obtained under conditions of nutritional deficiency. In Table 8 nitrogen and phosphorous deficiencies are considered by examining the data of Ketchum and Redfield [15]. In Table 9, some results from the data of Spoehr and Milner [21] are presented based on the results of their experiments under a wide variety of environmental conditions. Nitrogen deficiency appears to be the primary reason for the significantly larger values of weight fraction carbon and reductance degree in Table 9. Milner [22] has extracted lipids from the samples and found that lipid storage is the reason for the greatly increased values of weight fraction carbon and reductance degree.

Milner [22] also pointed out that the chlorophyll content varied from less than 0.01% to 6% in their experiments [21] and that the weight fraction carbon and reductance degree are not significantly increased because of changes in chlorophyll content.

Spoehr and Milner [21] show in their work that the reductance degree varies with the nitrogen concentration in the culture medium (see Fig. 1 of Spoehr and Milner [21]). Spoehr and Milner [21] point out that the variation of ash content in Table 9 is partly due to the variation in mineral salt composition and partly due to the yield of cells obtained. Lower ash contents were found with higher cell yields.

Spoehr and Milner [21] introduced the concept of degree of reduction in their work. The reductance degree introduced by Minkevich and Eroshin [6] is a modified form which allows one to appropriately consider the valence of nitrogen in making calculations.

The energy content per equivalent of available electrons, Q_o, has not been measured very much for algae; however, one would expect this value to be similar to that for other microorganisms. Based on the experimental results of Pirt et al. [4] and Kok [23], Q_o = 27.1 and 27.7 kcal/eq. of available electrons, respectively. These values are close to the average value of 26.95 kcal/eq. reported by Minkevich and Eroshin [6,7].

CONCLUSIONS

Based on the results presented in this work, it appears that the weight fraction carbon in algae and the reductance degree are relatively constant and that average values may be used as long as the culture is not nutritionally deficient in nitrogen or phosphorous. Thus, for light limited growth conditions, the regularities may be used in the analysis of growth process.

REFERENCES

1. Pirt, S.J., Biotechnol. Bioeng. 25, 1915 (1983).

2. Aiba, S., Adv. Biochem. Eng. 23, 85 (1982).

3. Lee, H. Y., L. E. Erickson, and S. S. Yang, Biotechnol. and Bioeng. In press (1983).

4. Pirt, S.J., Y.K. Lee, R. Amos, and M.W. Pirt, J. Chem. Tech. Biotech. 30, 25 (1980).

5. Shelef, G. and C.J. Soeder, Editors, Algae Biomass Production and Use, Elsevier, New York (1980).

6. Minkevich, I.G. and V.K. Eroshin, Folia Microbiol. 18, 376 (1973).

7. Minkevich, I.G., V.K. Eroshin, T.A. Aleksecva, and A.P. Tereschchenko, Microbiol. Promyshl. 2(144), 1 (1977) (In Russian).

8. Erickson, L.E., I.G. Minkevich, and V.K. Eroshin, Biotechnol. Bioeng. 20, 1595 (1978).

9. Erickson, L.E., Biotechnol. Bioeng. 21, 725 (1979).

10. Oner, M.D., L.E. Erickson, and S.S. Yang, Biotechnol. and Bioeng. 25, 631 (1983).

11. Erickson, L.E. and S.A. Patel, CRC Critical Reviews in Biomedical Engineering 8, 311 (1982).

12. Lee, H.Y., Proceedings of the Thirteenth Annual Biochemical Engineering Symposium, p. 103, Iowa State University, Ames, Iowa (1983).

13. Erickson, L.E., Biotechnol. and Bioeng. 22, 451 (1980).

14. Esener, A.A., J.A. Roels, and N.W.F. Kossen, Biotechnol. and Bioeng. 24, 1445 (1982).

15. Ketchum B.H. and A.C. Redfield, J. Cell and Comp. Physiol. 33, 281 (1949).

16. Wassink, E.C., B.K.K., and J.L.P. Oorschot, Algal Culture, J.S. Burlew ed., Carnegie Inst. of Washington Publ. 600, p. 55 (1953).

17 Myers, J., Primary Productivity in the Sea, P.G. Falkovski ed., p. 1 and p. 447, Plenum Press, NY (1980).

18. Myers, J., "Study of Photosynthetic Gas Exchanger as a Method of Providing for the Respiratory Requirements of the Human in a Sealed Cabin", Report 58-117, School of Aviation Medicine, USAF (1958).

19. Aiba, S. and T. Ogawa, J. Gen. Microbiol. 102, 179 (1977).

20. Ogawa, T. and G. Terui, J. Ferm. Technol. 48, 361 (1970).

21. Spoehr, H.A. and H.W. Milner, Plant Physiol. 24, 120 (1949).

22. Milner, H.W., Algal Culture, J.S. Burlew ed., Carnegie Institution of Washington, Publ. 600, p. 285 (1953).

23. Kok, B., Acta Botanica Neerlandica, 1, 445 (1952)

Table 1. Values of Regularities of Chlorella Species Based on Oata of Ketchum and Redfield [15]

Species	Ash Content (% dry weight)	Weight Fraction Carbon (σ_b)	Weight Fraction Carbon on an Ash-Free Basis	Reductance Oegree (γ_b)*	Reductance Oegree (γ_b)**
	10.98	0.4828	0.5424	4.446	4.813
	10.49	0.4956	0.5537	4.489	4.872
Chlorella	14.02	0.4817	0.5602	4.593	4.912
pyrenoidosa	14.32	0.4769	0.5566	4.503	4.870
	10.50	0.4908	0.5484	4.469	4.821
	13.73	0.4638	0.5376	4.497	4.872
	11.01	0.4870	0.5473	4.499	4.882
	10.73	0.4865	0.5450	4.481	4.834
Average	11.97	0.4831	0.5489	4.497	4.860
Standard Deviation	1.72	0.0097	0.0075	0.403	0.034
Coefficient of variation	0.14	0.0200	0.0137	0.010	0.007
Chlorella vulgaris	12.40	0.4680	0.5337	4.429	4.802
Total Average	12.02	0.4815	0.5472	4.490	4.853
Total Standard deviation	1.61	0.0104	0.0087	0.046	0.040
Total Coefficient of variation	0.13	0.0215	0.0159	0.010	0.008

*Reductance degree based on N = -3.
**Reductance degree based on N = 0.

Table 2. Values of Regularities for Chlorella Species Calculated from Oata of Wassink et al. [16].

Culture Condition	Ash Content (% dry weight)	Weight Fraction Carbon (σ_b)	Weight Fraction Carbon on as Ash-Free Basis	Reductance Oegree (γ_b)*	Reductance Degree (γ_b)**
Outdoor Cultivation	22.4	0.411	0.530	4.37	4.94
	12.8	0.474	0.544	4.35	4.93
	15.4	0.457	0.540	4.42	4.96
Indoor Cultivation	11.2	0.491	0.553	4.43	4.98
	19.2	0.435	0.538	4.44	4.91
	16.2	0.442	0.527	4.32	4.89
Average	16.2	0.452	0.539	4.388	4.935
Standard Deviation	4.114	0.0286	0.0095	0.0488	0.0327
Coefficient of variation	0.2539	0.0634	0.0176	0.0111	0.0066

*Reductance degree based on N = -3.
**Reductance degree based on N = 0.

Table 3. Values of Regularities for Chlorella pyrenoidosa from Oata of Myers' [17].

Nitrogen Source	Ash Content (% dry weight)	Weight Fraction Carbon (σ_b)	Weight Fraction Carbon on an Ash-Free Basis	Reductance Oegree (γ_b)*	Reductance Oegree (γ_b)**
Nitrate	5.68	0.502	0.532	4.423	4.825
	5.87	0.494	0.525	4.357	4.738
	5.40	0.504	0.533	4.579	4.937
	5.39	0.507	0.536	4.414	4.815
Urea	5.19	0.501	0.528	4.380	4.761
	5.22	0.497	0.524	4.380	4.761
	5.22	0.497	0.524	4.387	4.771
	5.61	0.515	0.546	4.418	4.842
	5.64	0.488	0.517	4.511	5.002
	5.64	0.486	0.515	4.434	4.932
Average	5.516	0.499	0.528	4.434	4.847
Standard Oeviation	0.2278	0.0092	0.0096	2.0670	0.0908
Coefficient of Variation	0.0413	0.0184	0.0182	0.0157	0.0187

*Reductance degree based on N = -3.
**Reductance degree based on N = 0.

Table 4. Values of Regularities for Spirulina platensis from data of Aiba and Ogawa [19] and Ogawa and Terui [20].

Culture Condition	Ash Content (% dry weight)	Weight Fraction Carbon (σ_b)	Weight Fraction Carbon on an Ash-Free Basis	Reductance Degree (γ_b)*	Reductance Degree (γ_b)**
Pure Cultivation	8.2	0.4762	0.5187	4.314	4.907
	8.2	0.4759	0.5184	4.282	4.887
	8.2	0.4668	0.5085	4.177	4.781
	8.2	0.4705	0.5125	4.325	4.291
	8.2	0.4602	0.5013	3.960	4.566
	8.2	0.4561	0.4969	4.064	4.663
Impure Cultivation+	9.7	0.4470	0.4950	4.258	4.822
	8.2	0.4580	0.4989	4.190	4.779
Average	8.39	0.4638	0.5063	4.196	4.791
Standard Deviation	0.530	0.0103	0.0096	0.1284	0.1241
Coefficient of Variation	0.0632	0.0222	0.0189	0.0306	0.0259

*Reductance degree based on N = -3.
**Reductance degree based on N = 0.
+Cultivated with bacteria [20].

Table 5. Summary of Regularities for Other Fresh-water Algae.

Species	Ash Content (% dry weight)	Weight Fraction Carbon (σ_b)	Weight Fraction of Carbon on an As-Free Basis	Reductance Degree (γ_b)*	Reductance Degree (γ_b)**	References
	7.51	0.5106	0.5521	4.396	4.750	
	6.48	0.5160	0.5518	4.541	5.874	
	7.31	0.5140	0.5545	4.530	4.848	
Stichococcus bacillaris	7.06	0.5050	0.5434	4.318	4.638	[15]
	17.21	0.4274	0.5162	4.142	4.438	
	12.08	0.4496	0.5114	4.196	4.468	
	6.50	0.4923	0.5265	4.426	4.912	[22]
	11.24	0.5072	0.5714	5.021	5.183	
Average	9.424	0.4903	0.5409	4.446	4.764	
Standard Deviation	3.8156	0.0333	0.0209	0.2728	0.2469	
Coefficient of Variation	0.4049	0.0679	0.0386	0.0614	0.0518	
Scenedesmus obliquus no. 1.	17.78	0.449	0.5337	4.632	4.986	
Scenedesmus obliquus no. 2.	12.98	0.477	0.5455	4.605	5.010	[15]
Scenedesmus basilensis	14.34	0.470	0.5487	4.652	5.040	
Chlamydomonas sp.	4.74	0.452	0.4746	4.150	4.35	
Anabaenopsis sp.	9.35	0.449	0.4957	4.220	4.60	[22]
Oikomonas termo.	5.08	0.491	0.5176	4.470	4.74	
Total Average	9.976	0.4793	0.5317	4.450	4.774	
Total Standard Deviation	4.3421	0.0298	0.0263	0.2421	0.2496	
Total Coefficient of Variation	0.4352	0.0621	0.0494	0.0544	0.0523	

*Reductance degree based on N = -3.
**Reductance degree based on N = 0.

Table 6. Summary of Regularities for Marine Algae (Sea water Algae).

Species	Ash Content (% dry weight)	Weight Fraction Carbon (σ_b)	Weight Fraction Carbon on an Ash-Free Basis	Reductance Degree (γ_b)*	Reductance Degree (γ_b)**	References
Nitzschia closterium	19.52	0.442	0.550	4.48	4.79	[15]
Gigartina agardhii	17.82	0.353	0.429	3.83	4.18	
Ulva sp.	18.72	0.361	0.444	3.85	4.14	
Amphileura rutilans	46.24	0.250	0.465	4.02	4.34	
Macrocystis pyrifera	37.66	0.287	0.460	4.09	4.46	[22]
Navicula torquatum	35.10	0.314	0.485	4.08	4.55	
Egregia menziesii	31.74	0.341	0.500	4.08	4.36	
Average	29.54	0.335	0.476	4.062	4.402	
Standard Deviation	11.0711	0.0612	0.0403	0.2151	0.2249	
Coefficient of variation	0.3747	0.1824	0.0846	0.0530	0.511	

*Reductance degree based on N = -3.
** Reductance degree based on N = 0.

Table 7. Results of Statistical Analysis of Oata in Table 1 to 6 for Regularities of Algae.

		Ash Content (% dry weight)	Weight Fraction Carbon (σ_b)	Weight Fraction Carbon on an Ash-Free Basis	Reductance Oegree (γ_b)*	Reductance Degree (γ_b)**
Fresh-water Algae (Table 1-5)	Average	10.04	0.4766	0.5306	4.402	4.830
	Standard Deviation	4.274	0.0251	0.0206	0.1763	0.1584
	Coefficient of Variation	0.426	0.0528	0.0388	0.0401	0.0328
Marine Algae (Table 6)	Average	29.54	0.3354	0.476	4.062	4.402
	Standard Deviation	11.07	0.0612	0.0403	0.2151	0.2249
	Coefficient of Variation	0.3747	0.1824	0.0846	0.0538	0.0511

*Reductance degree based on N = -3.
**Reductance degree based on N = 0.

Table 8. Values of Regularities for Chlorella pyrenoidosa Oeficient in Nitrogen and Phosphorous From Data of Ketchum and Redfield [15].

Oeficiency	Ash Content (% dry weight)	Weight Fraction Carbon (σ_b)	Weight Fraction Carbon on an Ash-Free Basis	Reductance Degree (γ_b)*	Reductance Oegree (γ_b)**
Nitrogen and Phosphorous	5.25	0.509	0.538	4.281	4.434
	5.51	0.549	0.581	4.410	4.552
	7.03	0.512	0.551	4.583	4.825
Nitrogen only	8.48	0.516	0.564	4.662	4.765
	9.04	0.503	0.553	4.879	5.004
	---	---	0.565	4.604	4.723
Phosphorous only	4.41	0.514	0.537	4.550	4.966
	5.63	0.525	0.557	4.505	4.896
Recovery+					
In Light	9.94	0.484	0.539	4.401	4.758
In Dark	10.02	0.492	0.547	4.524	4.745

*Reductance degree based on N = -3.
**Reductance degree based on N = 0.
+Culture deficient in nitrogen and phosphorous. Analyzed after recover in the light or in the dark for 24 hours.

Table 9. Effect of Environmental Conditions on Regularities for Chlorella pyrenoidosa from Data of Spoehr and Milner [21].

	Ash Content (% dry weight)	Weight Fraction Carbon (σ_b)	Weight Fraction of Carbon on an Ash-Free Basis	Reductance Degree (γ_b)*	Reductance Oegree (γ_b)**
	3.45	0.478	0.495	4.118	4.601
	3.78	0.491	0.510	4.167	4.717
	20.21	0.412	0.517	4.229	4.932
	5.81	0.514	0.546	4.349	4.838
	8.56	0.501	0.548	4.598	4.861
	7.88	0.503	0.546	4.547	5.053
	2.28	0.547	0.560	4.561	4.922
	2.87	0.580	0.597	4.837	5.044
	1.36	0.629	0.638	4.995	5.119
	5.32	0.619	0.654	5.178	5.242
	4.57	0.651	0.682	5.262	5.310
	3.44	0.668	0.692	5.329	5.373
	3.46	0.667	0.702	5.366	5.419
Average	5.62	0.554	0.591	4.734	5.033
Standard Oeviation	4.850	0.0849	0.0737	0.4560	0.2532
Coefficient of Variation	0.864	0.1533	0.1246	0.0963	0.0503

*Reductance degree based on N = -3.
**Reductance degree based on N = 0.

Chapter 4

ESTIMATION OF YIELD AND MAINTENANCE PARAMETERS FOR PHOTOAUTOTROPHIC GROWTH

INTRODUCTION

There is considerable interest in the estimation of growth yield and maintenance parameters associated with microbial bioenergetics and the efficiency of growth processes [1-41]. Recently Pirt [11] has reviewed some of the research with photosynthetic efficiency. The purpose of this work is to illustrate how some of the methods, which were presented previously for heterotrophic growth [7,8,12-17], may be applied to photoautotrophic growth. The current interest in the growth of algae and photosynthetic bacteria is due to the potential of these organisms to produce renewable chemicals and to convert light energy into useful forms of chemical energy. Microorganisms have a higher photosynthetic efficiency than agricultural plants and growth conditions can be more easily controlled.

Many researchers have developed yield expressions for light energy conversion by photoautotrophic microorganisms [1-5] Pirt [11] has considered the photosynthetic efficiency to be the ratio of the free energy of the biomass produced relative to the amount of light energy absorbed. This concept of photosynthetic efficiency may be used with the true growth yield and maintenance model of Pirt [1,6,11]. Carbon, available electron, nitrogen, and energy balances may be used in photoautotrophic growth to examine data consistency. True growth yield and maintenance parameters may be estimated using all of the experimentally measurable variables simultaneously with the covariate adjustment method. In this work both batch culture and continuous culture data are analyzed.

THEORY

The yield or efficiency of photoautotropic microbial growth is frequently evaluated in terms of the free energy of the biomass divided by the light energy absorbed. When ammonia is the nitrogen source, a chemical balance equation may be written as follows:

$$CO_2 + aNH_3 + c\ H_2O \xrightarrow{\text{light energy}} CH_pO_nN_q + b\ O_2 \qquad (1)$$

where $CH_pO_nN_q$ is the composition of these atoms in the microbial biomass. The method of Minkevich and Eroshin [17] can be used to characterize the biomass in terms of the weight fraction carbon σ_b, reductance degree, γ_b, the energy content per equivalent of available electrons, Q_o, and the free energy content per equivalent of available electrons, g_b.

Equations to examine the consistency of the experimental data may be obtained by employing a carbon balance, an available electron balance, and an energy balance. The carbon balance is simple for this case because the carbon consumed as carbon dioxide should be equal to the carbon in the produced biomass when no extracellular products are formed. For continuous culture with dilution rate, D,

$$Q_{CO_2} X = \frac{DX\sigma_b}{12}$$

or

$$\frac{12\ Q_{CO_2}}{D\ \sigma_b} = 1 \qquad (2)$$

When the valences, C=4, H=1, O=-2, and N=-3, are used, the available electron balance can be written as

$$4Q_{O_2} X = \frac{DX\sigma_b\gamma_b}{12}$$

or

$$\frac{D\sigma_b\gamma_b}{48Q_{O_2}} = 1 \tag{3}$$

where

$$\gamma_b = 4 + p - 2n - 3q$$

A steady state energy balance may be written in the form

Total light energy in	=	Energy incorporated into biomass	+	Energy leaving by radiation, conduction and convection

or

$$I_a A = \frac{FX\sigma_b\gamma_b Q_0}{12} + Q_h$$

which may be written as

$$\frac{FX\sigma_v\gamma_b Q_0}{12I_a A} + \frac{Q_h}{I_a A} = 1 \tag{4}$$

or

$$\eta_{kcal} + \varepsilon_h = 1 \tag{5}$$

where the first term in Equations (4) and (5) is the biomass energetic yield and the second term is the fraction of energy which is lost as heat. The value of the biomass energetic yield

$$\eta_{kcal} = \frac{FX\sigma_b\gamma_b Q_o}{12I_aA} \tag{6}$$

can be estimated directly using microbial biomass and light energy absorption measurements. Indirect estimates of η may also be obtained. The available electron balance and oxygen measurements can be used with light energy absorption measurements to obtain.

$$\eta_{O_2} = \frac{4Q_{O_2}XVQ_O}{I_aA} \tag{7}$$

The carbon balance and CO_2 measurements can be used with light energy absorption measurements to obtain

$$\eta_{CO_2} = \frac{Q_{CO_2}XV\gamma_b Q_O}{I_aA} \tag{8}$$

If the energy losses from the fermentor, Q_h, are measured, these may be used with microbial biomass measurements

$$\eta_Q = \frac{FX\sigma_b\gamma_b Q_O/12}{\frac{FX\sigma_b\gamma_b Q_O}{12} + Q_h} \tag{9}$$

Equations (6), (7), (8) and (9) provide four different estimates of the true growth yield, η. These equations are written in terms of the enthalpy of the biomass relative to ammonia in aqueous solution, CO_2 gas, and H_2O liquid. By substituting the free energy per equivalent of available electrons, g_b, for Q_O, similar expressions can be obtained based on the free energy of the biomass relative to aqueous ammonia, CO_2 gas, and H_2O liquid. The values of Q_O and g_b are similar in magnitude.

Minkevich and Eroshin [17] reported a value of 112. 8 kJ/eq. for g_b. Values of -67.1 and -46.0 kJ/mole of carbon in biomass have been estimated for the free energy of formation of biomass by Roels [26] and Grosz and Stephanopoulos [40], respectively. These values lead to 113 kJ/eq and 117.6 kJ/eq, respectively, for the free energy relative to aqueous ammonia, CO_2 gas, and H_2O liquid.

When nitrate is used as the source of nitrogen, energy is required to convert the nitrogen from the nitrate form to the amino form found in microbial biomass. The free energy change involved in the chemical reaction for the formation of biomass (using -46 kcal/C mole for the free energy of formation of biomass [40])

$$CO_2 + a\ HNO_3 + cH_2O \longrightarrow CH_pO_nN_q + bO_2 \qquad (10)$$

is 557.8 kJ/C mole compared to 559.1 kJ/C mole for the case where aqueous HNO_3 is replaced by N_2 as the nitrogen source. For nitrate as the nitrogen source, it is convenient to consider the free energy and enthalpy relative to CO_2 gas, H_2O liquid, and N_2 gas because the free energy and enthalpy changes in going from aqueous HNO_3 to N_2 and H_2O are very small. In addition the enthalpy becomes that associated with the standard heat of combustion. The average values of Minkevich and Eroshin of p = 1.776, n = 0.495 and q = 0.165 are used in the formula for biomass, $CH_pO_nN_q$; thus, γ = 4.291 for the valences C=4, H=1, O=-2, and N=-3, and γ_b = 4.786 for C=4, H=1, O=-2, and N=0. This gives

$$g_b = \frac{559.1}{4.786} = 116.8 \text{ kJ/eq}$$

for the valence N=0; using Roels' [26] value of -67.1 kJ/C mole for the

free energy of formation of biomass gives g_b = 112.4 kJ/eq. for N=0. The values for Q_0 and g_b are relatively constant and similar for both the valences N=-3 and N=0.

The carbon balance is not affected by the choice of the nitrogen source as long as inorganic nitrogen sources which are free of carbon are selected. Thus, Equation (2) remains valid as the carbon balance associated with Equation (10).

The available electron balance for Equation (1) based on the valences C=4, H=1, O=-2, and N=0 is

$$-5a = \gamma_b - 4b$$

or

$$\gamma_b + 5a = 4b \tag{11}$$

where a=q in Equation (10). If the nitrate consumption is measured, this information may be used in Equation (11); however, when nitrogen consumption is not measured, the elemental composition of the microbial biomass may be used to estimate q for use in Equation (11).

For continuous culture, Equation (11) may be written in the form

$$4Q_{O_2}XV = \frac{DXV\sigma_b\gamma_b}{12} + \frac{5qDXV\sigma_b}{12}$$

or

$$\frac{D\sigma_b\gamma_b}{48Q_{O_2}} + \frac{5qD\sigma_b}{48Q_{O_2}} = 1 \tag{12}$$

Equation (12) is the available electron balance when nitrate is the nitrogen source. The second term accounts for the available electrons required to

convert nitrate to elemental nitrogen.

The energy balance as presented in Equations (4) and (5) may also be used when nitrate is the source of nitrogen provided that the valences C=4, H=1, O=-2, and N=0 are used to obtain the value of γ_b.

For nitrate as the nitrogen source, Equations (6), (8) and (9) may be used to estimate n provided the valence N=0 is used in estimating γ_b. Equation (7) must be modified because of the modified available electron balance. Equations (6) and (12) may be combined to obtain

$$n_{O_2} = \frac{(48Q_{O_2}XV - 5qOXV\sigma_b)Q_O}{12\ I_a\ A} \tag{13}$$

For urea as a source of nitrogen and carbon, the chemical balance equation is of the form

$$a\ N_2H_4CO + cH_2O + d\ CO_2 \longrightarrow CH_pO_nN_q + b\ O_2$$

and the carbon balance becomes

$$a + d = 1.0$$

From the nitrogen balance, we have an expression which may be used to estimate urea consumption when it is not measured directly. The nitrogen balance is

$$2a = q$$

In continuous culture, per unit of liquid volume the carbon balance is

$$\frac{DX\sigma_b q}{24} + Q_{CO_2} X = \frac{DX\sigma_b}{12}$$

or

$$\frac{q}{2} + \frac{12Q_{CO2}}{D\sigma_b} = 1 \tag{15}$$

The appropriate valence for nitrogen is N=-3 when urea is the nitrogen source; energetically aqueous urea is approximately equivalent to aqueous ammonia and CO_2 gas. Equation (8) becomes

$$\eta_{CO_2} = \frac{\frac{DXV\sigma_b q\gamma_b Q_0}{24} + Q_{CO_2} XV\gamma_b Q_0}{I_a A} \tag{16}$$

when urea is used.

ESTIMATION OF YIELD AND MAINTENANCE PARAMETERS

The model of Pirt [1,6] may be used to estimate the true growth yield, η_{max}, and the mainteanance coefficient, m_e, based on experimental yield measurements at several different specific growth rates. This model may be written in the form

$$Y_{1i} = \frac{\mu_i}{\eta_{kcal\ i}} = m_e + \frac{\mu_i}{\eta_{max}} + \text{error} \tag{17}$$

$$Y_{2i} = \frac{\mu_i}{\eta_{O_2 i}} = m_e + \frac{\mu_i}{\eta_{max}} + \text{error} \tag{18}$$

$$Y_{3i} = \frac{\mu_i}{\eta_{CO_2 i}} = m_e + \frac{\mu_i}{\eta_{max}} + \text{error} \tag{19}$$

$$Y_{4i} = \frac{\mu_i}{\eta_{Qi}} = m_e + \frac{\mu_i}{\eta_{max}} + \text{error} \tag{20}$$

where i = 1, ..., n are the n specific growth rates investigated. Two forms of Equations (17) - (20) may be used for parameter estimation. In this paper, Equations (17) - (20) are referred to as Form II.

Form I is obtained by dividing each term by μ. Equations (17) - (20) may each be used individually for parameter estimation. An average of the responses may also be used; that is, let

$$\bar{Y}_{\cdot i} = \frac{\mu_i}{4}\left[\frac{1}{\eta_{kcal\ i}} + \frac{1}{\eta_{O_2 i}} + \frac{i}{\eta_{CO_2 i}} + \frac{1}{\eta_{Qi}}\right] \tag{21}$$

however, then the information in the responses may not be efficiently utilized. A set of covariates, $Z_{1i}, \ldots, Z_{qi}$ $(1 \leq q \leq 3)$, which are

linear combinations of the four dependent variables, may be added to obtain a conditional model (conditional on $Z_{1i},\ldots,Z_{qi}$)

$$\bar{Y}_{.i} = m_e + \frac{\mu_i}{\eta_{max}} + \sum_{K=1}^{q} \alpha_K Z_{Ki} + \text{error}, \quad i = 1,2,\ldots,n \qquad (22)$$

The covariates should be linearly independent and have zero expected value; for example, a full set of covariates is

$$Z_{1i} = Y_{1i} + Y_{2i} - Y_{3i} - Y_{4i}$$

$$Z_{2i} = Y_{1i} + 3Y_{2i} - Y_{3i} - 3Y_{4i}$$

$$Z_{3i} = -Y_{1i} + 3Y_{2i} - 3Y_{3i} + Y_{4i}$$

Methods to efficiently select convariates and to evaluate the reuslts of including one, two, or three covariates are described elsehwere [7, 20,33]. When the maximum number of covariates is included, the maximum likelihood estimate (M.L.E.) is obtained based on all available measurements. Frequently, the estimates with the shortest confidence intervals are obtained when only one or two covariates are included [7,33].

BATCH CULTIVATION

The results of batch growth may also be used to estimate the growth yield. When the growth process is energy limited, a region of linear growth is frequently observed where cell mass increases linearly with time. If the growth rate (dX/dt) is constant for a period of time, the slope may be used to estimate the growth yield in the equation

$$\eta_{kcal} = \frac{(\frac{dX}{dt})V\sigma_b\gamma_b Q_0}{12I_a A} \tag{23}$$

In this region of linear growth, the biomass concentration increases while the specific growth rate decreases. The true growth yield, η_{max}, may be larger than η, but the linear region indicates that η is relatively constant and that the growth yield, η, is a reasonable approximation of the true growth yield, η_{max}.

Other estimates of the growth yield, η, may be obtained by following the CO_2 consumption, oxygen production, and heat generation; that is, Equation (8) may be used to estimate the growth yield in the region where the rate of CO_2 consumption, $Q_{CO_2}X$, is constant. When the O_2 production rate, $Q_{O_2}X$, is constant, the growth yield may be used with nitrate provided the slope of the batch curve dX/dt is used in place of DX in Equation (13). Similarly,

$$\eta_Q = \frac{\frac{dX}{dt} V\sigma_b\gamma_b Q_0/12}{\frac{dX}{dt}\frac{V\sigma_b\gamma_b Q_0}{12} + Q_h} \tag{24}$$

may be obtained from data in the region where the growth rate and heat evolution rate are constant.

As batch growth continues, a region is encountered in which the growth rate decreases and then approaches zero. The stationary phase where $dX/dt = 0$ may be used to estimate the maintenance coefficient, m_e, where

$$m_e = \frac{12 I_a A}{X V \sigma_b \gamma_b Q_0} \tag{25}$$

This estimate of the maintenance coefficient often exceeds the actual value because the stationary condition may be due to reasons other than energy limitation. That is, only a portion of the energy may be required for maintenance purposes.

In the process of estimating the parameters from the batch experimental data, the average values of $\sigma_b = 0.462$ and $Q_o = 26.95$ kcal/eq. of available electrons have been used. When the nitrogen source has a valence $N = -3$, $\gamma_b = 4.291$ has been used; when N_2 or nitrate is used as a nitrogen source, $\gamma_b = 4.773$ has been used.

RESULTS AND DISCUSSION

The experimental data of Aiba and Ogawa [2,3] from continuous culture of Spirulina platensis is shown in Table 1. These investigators measured dilution rate, liquid volume, cell dry weight, carbon dioxide consumption rate, and light energy absorption at several different dilution rates. The biomass energetic yield is estimated in Table 1 using Equation (6) with biomass concentration, light energy absorption and dilution rate measurements to obtain η_{kcal} and using Equation (8) with CO_2 consumption and light energy absorption measurements to calculate η_{CO_2}. The last column in Table 1 is a measure of the consistency of the experimental data as measured using the carbon balance, Equation (2). The consistency of the data appears to be better at higher dilution rates. The values of the weight fraction carbon, σ_b and the reductance degree, γ_b, which are used in Tables 1 and 2 are based on the biomass elemental compositions reported by Aiba and Ogawa [2,3]. The valance, N=0, is used to estimate γ_b because nitrate was used as the source of nitrogen.

Table 2 contains the estimates of the true biomass energetic yield, η_{max}, and the maintenance coefficient, m_e, for the data of Aiba and Ogawa [2,3] which is presented in Table I. Results are presented for both Form I and Form II of Equations (17) and (19). The point and 95% confidence interval estimates, which are presented in Table 2, include some negative values; however, only positive values are physically possible. Thus, the interval of interest is limited to values greater than zero.

The covariate adjustment method with Equations (21) and (22) for this data set involves at most one covariate because only Equations (17) and (19) may be used with the available data. The average results, which

are obtained when no covariates are included in Equation (22), gave better estimates than when one covariate was included. This appears to be partly due to the limited number of dilution rates where were considered and the undesirability of introducing a third unknown parameter when so few data points are being analyzed. The 95% confidence intervals for the case where all of the data are utilized appear to be very realistic; that is, one would expect $0.071 \leq \eta_{max} \leq 0.29$ and $0 \leq m_e \leq 0.18$. In Table 2, the confidence intervals are large because of the small number of data points. The shortest 95% confidence intervals for η_{max} are obtained when Form 2 is used while the shortest interval estimates for m_e are obtained with Form I.

Point and 95% confidence interval estimates for the true biomass energetic yield, η_{max}, and the maintenance coefficient, m_e, are presented for the data of Pirt et al. [1] in Table 3. The results presented by Pirt et al. [1] are compared to estimates which are obtained using their data and the average values of the regularities ($\sigma_b = 0.462$, $\gamma_b = 4.291$, and $Q_o = 26.95$ kcal/eq. of available elections) for urea as nitrogen source (N=-3). Pirt et al. [1] reported that $\sigma_b = 0.486$, that the elemental biomass composition was $CH_{1.8}O_{0.432}N_{0.143}$ ($\gamma_b = 4.51$) and that the heat of combustion was 22.7 kJ/g dry weight.

The true growth yield estimates of Pirt are larger than one would expect based on the experimental evidence that the maximum value of η_{max} should be about 0.29 [11,22,23,24]. Recalculation of Pirt's data using the average values of the regularities still results in a 95% confidence interval for η_{max} which exceeds the widely accepted maximum value of 0.29 for the entire interval when mixed cultures are considered.

The true growth yield estimates with mixed cultures are higher than for Chlorella by itself. The symbiotic growth of bacteria and algae may be a promising method for converting solar energy to chemical energy [25].

The experimental results of Myers [5,35] with Chlorella pyrenoidosa in continuous culture are analyzed in Tables 4 and 5. Myers measured dilution rate, cell dry weight, oxygen production, carbon dioxide consumption, and incident light energy. The carbon and available electron balances are used in Table 4 to check the consistency of the experimental data. For the first eleven data points in Table 4, nitrate was the nitrogen source; for the last two data points, urea was the nitrogen source. The appropriate available electron balance, Equation (12) with nitrate and Equation (3) with urea, was used to check the consistency of the data. For the consistency check using the carbon balance, Equation (2) was used with the nitrate data, and Equation (15) was used with the urea data. The consistency analysis of the experimental data shows that for all cases the carbon and available electron content of the biomass is less than that estimated from CO_2 and oxygen measurements. If one compares the carbon balance results with those in Table 1, the same trend is observed because the measured CO_2 consumption exceeds the carbon incorporated into biomass for all except one of the data points in Table 1.

Values of the biomass energetic yield are estimated in Table 4 from the available measured values at each dilution rate. For nitrate as the nitrogen source, the valence N = 0 was used, and thus, $\gamma_b = 4.849$ and $\sigma_b = 0.4870$ based on Myers data [5,35]. Values of the biomass energetic yield in Table 4 are in good agreement with the values reported by Myers

[5,35].

Table 5 shows the results of parameter estimation using the data in Table 4 together with the covariate adjustment method to obtain point and 95% confidence interval estimates for the true growth yield, η_{max}, and the maintenance coefficient, m_e. The maximum liklihood estimate (MLE) is obtained by including two covariates in Equation (22). Results are also shown in Table 5 for the cases where each of the covariates is included separately. The average value is the result obtained when all data are analyzed and no covariates are included. The point and interval estimates for the true growth yield, η_{max}, are in good agreement; that is, the results with Form I and Form II are similar and the effect of including covariates appears to be relatively small. When covariate adjustment is used here, all of the point estimates are within all of the 95% confidence intervals irrespective of the form and the number of covariates included.

The results of analysis using each data set individually is also shown in Table 1. The point estimates of η_{max} vary from 0.1405 to 0.1778 in this portion of the table. There is also greater variation in the estimates of the maintenance coefficient when the individual data sets are analyzed.

The results for Form II are shown graphically in Figure 1. Curve A is the maximum likelihood estimate when two covariates are included; Curve B is the average of the three individual estimates which is obtained when no covariates are included in Equation (22); Curve C is the individual estimate based on biomass and light measurements. The results for Form II appear to be better than those in Form I in that the point estimates of m_e are positive when Form II is used.

Table 6 contains the results of estimating the growth yield and maintenance parameters from the batch culture data of Winokur [9]. Equation (23) is used to estimate the growth yield, η, and Equation (25) is used to estimate the maintenance coefficient, m_e. The estimates of the maintenance coefficient may be significantly larger than the actual value if batch growth stopped for reasons other than energy limitation.

The effect of agitation is shown in Table 6; significantly larger growth yields are observed when the culture is agitated. The maintenance coefficients are smaller if agitation is used. These results indicate that more of the light energy reaches the locations where it can be effectively utilized when the culture is agitated. The results show that the growth yield is higher for a light intensity of 200 meter candles than it is for 6000 meter candles. This suggests that some of the light energy is not effectively used at the higher light intensity even when the culture is agitated. The estimated value for the maintenance coefficient is generally smaller for a light intensity of 2000 meter candles than it is for 6000; this result also suggests that the distribution of light is less efficient at the higher intensity.

Some other growth yields from batch culture studies are presented In table 7. The results of Kosaric and coworkers [10] with Spirulina maxima show that much larger yields are obtained with synthetic media as compared to those obtained using the effluent from secondary municipal wastewater treatment. The limited quantity of nitrogen in the effluent from secondary treatment appeared to limit growth of the algae. Batch cultivation under conditions where the nitrogen supply was exhausted also gave a yield of about $\eta = 0.1$ in the work of Leduy and Therien [37] which was carried out in an annular pilot scale reactor. In steady state continuous culture, the growth yield was found to be 0.15 and 0.17

at dilution rates of 0.24 day^{-1} and 0.54 day^{-1} respectively.

The effect of mixing was also investigated by Persoone and coworkers [32] who used airlift pumps when they cultured Chlorella saccharophila in 100 liter tanks containing sea water and culture medium. While the yields are relatively small in this work, it is clear that yields are larger under conditions of better mixing.

Analysis of the results of Allen and Arnon [36] for the growth of Anabaena cylindrica indicates that growth yields (η) are relatively high under good nutritional conditions. (See Table 7). This research was carried out with 5% CO_2 in air passing over 250 mℓ of liquid in Roux bottles on an illuminated shaker. Pirt et al. [1] also used CO_2 in air in their research with high growth yields. Allen and Arnon [36] clearly show that mineral limitations can be very important in algal growth and that calcium is essential for growth.

Ogawa and Terui [38] cultured Spirulina platensis in Roux bottles using aeration to provide agitation. Analysis of their batch culture results (See Table 7) shows that the growth yield, η, is in relatively good agreement with the true growth yield, η_{max}, from the data of Aiba and Ogawa [2,3] which is reported in Table II.

Table 8 contains a summary of continuous culture yields for photoautotrophic microbial growth. In these studies the maintenance coefficient appears to be larger when the true growth yield is smaller. This may be because of experimental designs in which some of the light energy is not effectively utilized. The yield of outdoor cultivation is much smaller than that of indoor cultivation.

The results in Tables 6 and 7 and the results of Prokop and Ricica [41] in Table 8 depend on the value of the conversion factor used to

convert luminous light intensity to radiant energy. The conversion factor at maximum luminescence at 550 nm (680 lumens/watt) was used where it was necessary to convert luminous measurements to energetic measurements. Since there is not sufficient wavelength information to estimate the correct value, the parameter values reported should only be used to appreciate the relative effects of nutritional and operational conditions. Since the actual conversion factor will be less than 680 lumens/watt, the actual values of growth yield in Tables 6 and 7 and for Prokop and Ricica [41] in Table 8 will be smaller than those presented.

Some values of the true growth yield exceed the maximum value of about 0.29 associated with the widely accepted Z-scheme of photosynthesis [11, 22]. In Tables 6 and 7 and for the data of Prokop and Ricica [41] in Table 8, this may be due to the fact that luminous intensity was measured and reported, and that no accurate estimate of radiant energy is available. It appears consistency of the data can be examined in order to obtain more reliable estimates of the true growth yield and maintenance coefficient. The measurement of light energy with a non-selective detector should be included in future work. It may also be desirable to use selective detectors and instruments which measure luminous intensity; however, for estimating the photosynthetic efficiency, it is important to know the light energy which enters the photoreactor.

CONCLUSIONS

Methods are presented for examining the consistency of experimental measurements associated with the estimation of yield and maintenance parameters for photoautotrophic growth. The covariate adjustment method is used to estimate true growth yield and maintenance parameters for photoautotrophic growth. Values of true growth yield and maintenance parameters are presented for several sets of literature data. Values of the true growth yield appear to depend on mixing intensity, availability of nutrients, incident light intensity, and the geometry of the fermentor.

NOMENCLATURE

A	Surface area, cm^2
a	Stoichiometric coefficient
b	Stoichiometric coefficient
c	Stoichiometric coefficient
D	Dilution rate, $time^{-1}$
F	Volumetric flow rate, liters/hr
g_b	Free energy of biomass per equivalent of available electrons
I_a	Intensity of light, $kcal/cm^2(hr)$
m_e	Maintenance coefficient, $time^{-1}$
Q_{CO_2}	Specific consumption rate of CO_2, g moles CO_2/g cell (hr)
Q_h	Rate of energy loss from fermentor, kcal/hr
Q_o	Energy of biomass per equivalent of available electrons
Q_{O_2}	Specific rate of production of O_2,g moles O_2/g cell (hr)
V	Liquid volume, liters
X	Biomass concentration, g/liter
Y	Response function; see Equations (17-21)
Z	Covariate in Equation (22)
α	Coefficient in Equation (22)
γ_b	Reductance degree of biomass, equivalents of available electrons per g atom carbon
η_{CO_2}	Biomass energetic yield based on CO_2 and light measurements
η_{kcal}	Biomass energetic yield based on biomass and light measurements
η_{max}	True growth energetic yield parameter, dimensionless
η_{O_2}	Biomass energetic yield based on oxygen and light measurements
η_Q	Biomass energetic yield based on biomass and energy measurements
μ	Specific growth rate, $time^{-1}$
σ_b	Weight fraction carbon in biomass

REFERENCES

1. Pirt, S.J., Lee, Y.K., Amos, R., and Pirt, M.W., J. Chem. Tech. Biotech. 30, 25 (1980).

2. Ogawa, T., and Aiba, S., J. Appl. Chem. Biotechnol. 28, 515 (1978).

3. Aiba, S., and Ogawa, T., J. Gen. Microbiol. 102, 1979 (1977).

4. Aiba, S., Adv. Biochem. Eng. 23, 85 (1982).

5. J. Myers, Primary Productivity in the Sea, P. G. Falkowski ed., p. 1 and p. 447. Plenum Press, N.Y. (1980).

6 Pirt, S.J., Principles of Microbe and Cell Cultivation, Blackwell Scientific, London (1975).

7. Solomon, B.O., S.S. Yang, and L.E. Erickson, American Chemical Society National Meeting, Kansas City (September, 1982); Biotech. and Bioeng. In Press (1983).

8. L.E. Erickson, Biotech. and Bioeng. 22, 1929 (1980).

9. M. Winokur, Am. J. Bot. 35, 118 (1948).

10. N. Kosaric, H. T. Nguyen, and N.A. Bergougnou, Biotech. and Bioeng. 16, 881 (1974).

11. Pirt, S.J., Biotech. and Bioeng. 25, 1915 (1983).

12. Erickson, L.E., Selga, S.E., and Viesturs, U.E., Biotech. and Bioeng. 20, 1623 (1978).

13. Erickson, L.E., Minkevich, I.G., and Eroshin, V.K., Biotech. and Bioeng. 20, 1595 (1978).

14. Erickson, L.E., Biotech. and Bioeng., 21, 725 (1979).

15. Erickson, L.E., Annals N.Y. Academy of Science, 326, 73 (1979).

16. Ferrer, A., and Erickson, L.E., Biotech. and Bioeng. 21, 2203 (1979).

17. Minkevich. I.G., and Eroshin, V.K., Folia Microbiol. 18, 376 (1973).

18. E. C. Wassink, Algal Culture, Burlew, J.S. ed., p. 55, Carnegie Institution of Washington Publication 600, Washington, D.C. (1953).

19. Kratz, W., and Myers, J. Am. J. Bot. 42, 282 (1955).

20. Yang, S.S., B.O. Solomon, M.D. Oner, and L.E. Erickson, Technometrics (1982); submitted for publication.

21. Lee, H.Y., Proceedings of the Thirteenth Annual Biochemical Engineering Symposium, p. 103, Iowa State University, Ames (1983).

22. Erickson, L.E., and Patel, S.A., CRC Critical Reviews in Biomedical Engineering 8, 311 (1982).

23. Stewart, W.D.P., Algal Physiology and Biochemistry, University of California Press, Berkeley and Los Angeles (1974).

24. K.S. Ng, and J.A. Bassham, Biochem. Biophys. Acta. 162, 254 (1968).

25. Bushell, M.E., and Slater, J.H., Mixed Culture Fermentation, Academic Press, London and New York (1981).

26. Roels, J.A., Energetics and Kinetics in Biotechnology, Elsevier, N.Y. (1983).

27. Van Liere, L., and Mur, L.R., J. Gen. Microbiol. 115, 153 (1979).

28. Gons, H.J., and Mur, L.R., Mitteilungen der Internationalen Vereinigung fur Theorctische und Angewandte Limnologie 21, 125 (1978).

29. Oswald, W.J., Proceedings of the International Biological Program/ Primary Productivity, pp. 473-488 (1970).

30. Burlew, J.S., Algal Culture from Laboratory to Pilot Plant, Carnegie Institution 600, Washington, O.C. (1953).

31. Goldman, J.C., Water Res. 13, 1 (1979).

32. Persoone, G., Morales, Verlet, H., and De Pauw, N, Algal Biomass, Shelef, G., and Soedr, C.J., Ed.pp. 505, Elsevier, New York (1980).

33. Solomon, B.O., M.O. Oner, L.E. Erickson, and S.S. Yang, Estimation of Parameters where Dependent Observations are Related by Equality Constraints, A.I.Ch.E. Journal. In Press (1983).

34. Stouthamer, A.H., International Review of Biochemistry 21, 1 (1979).

35. Myers, J., "Study of a Photosynthetic Gas Exchanger as a Method of Providing for the Respiratory Requirements of the Human in a Sealed Cabin," Report 58-117, School of Aviation Medicine, USAF (1958).

36. Allen, M.B., and Arnon, O.I., Plant Physiol., 38, 366 (1955).

37. Leduy, A., and Therien, N., Can. J. Chem. Eng., 57, 489 (1979).

38. Ogawa, T., and Terui, G., J. Ferment. Technol. 48, 361 (1970).

39. Minkevich, I.G., Limitation and Inhibition of Microbial Processes (V.K. Eroshin, Editor), p. 55, Academy of Sciences Scientific Center for Biological Research, Pushchino, Moscow Region, USSR (1980). (In Russian).

40. Grosz, R. and G. Stephanopoulos, Biotechnol. and Bioeng. 25, 2149 (1983).

41. Prokop, A. and J. Ricica, Folia Microbiol. 13, 353 (1968).

42. Myers, J., Proceedings of the International Biological Program/ Primary Productivity, pp. 447-455 (1970).

Table 1. Experimental results, biomass energetic yields, and results of data consistency analysis with carbon balance for data of Aiba and Ogawa [2,3] for Spirulina platensis.

Dilution Rate $D\ (hr^{-1})$	Cell dry Weight $X(g/\ell)$	Q_{CO_2} $\frac{\text{m mole}}{\text{g-cell} \cdot \text{hr}}$	Intensity $I_a \times 10^3$ $(kcal/cm^2 hr)$	η_{kcal}	η_{CO_2}	$\frac{12\ Q_{CO_2}}{D\sigma_b}$
0.014	0.272	0.768	1.5	0.0721	0.0900	1.25
0.022	0.246	1.88	1.44	0.101	0.130	1.29
0.023	0.177	---	1.25	0.0917	---	--
0.029	0.169	1.38	1.21	0.108	0.125	1.15
0.034	0.122	1.48	1.05	0.105	0.111	1.05
0.038	0.057	1.62	0.61	0.0982	0.0978	0.996

Table 2. Average and individual estimates of true growth energetic yield, η_{max}, and maintenance coefficient, m_e, for Spirulina platensis for data in Table 1.

Data Used	Form	η_{max}		m_e, hr^{-1}	
		Point	Interval	Point	Interval
all	I	0.138	(0.0831, 0.414)*	0.0638	(-0.0443, 0.172)*
	II	0.115	(0.0711, 0.294)	0.0255	(-0.128, 0.179)
η_{kcal}	I	0.150	(0.0955, 0.346)*	0.0929	(0.00078, 0.178)*
	II	0.127	(0.0822, 0.282)	0.0627	(-0.061, 0.186)
η_{CO_2}	I	0.129	(0.0734, 0.519)*	0.0346	(-0.0966, 0.166)*
	II	0.105	(0.0626, 0.318)	-0.0118	(-0.196, 0.173)

*Results with shortest 95% confidence interval for each case.

Table 3. Estimates of true biomass energetic yield and maintenance coefficient for data of Pirt [1].

Case Considered	Method*	η_{max}		m_e, hr^{-1}	
		Point	Interval	Point	Interval
Chlorella	P	0.347		0.00088	
	R	0.267	(0.214, 0.355)	-0.0150	(-0.122, 0.0922)
Mixed culture of algae and bacteria	P	0.468	(0.427, 0.515)	0.00132	(0, 0.0141)
	R	0.396	(0.327, 0.501)	0.00293	(-o.0315, 0.0388)
Mixed culture (algae only)	P	0.468		0.0184	
	R	0.389	(0.318, 0.503)	0.0275	(-0.0112, 0.0662)

*P refers to Pirt's original results [1]; R refers to results obtained with data of Pirt [1] and parameter estimates based on Form II and σ_b = 0.462, γ_b = 4.291, and Q_o = 26.95 kcal/eq. of available electrons. The interval results are 95% confidence intervals.

Table 4. Experimental values, results of data consistency analysis, and biomass energetic yield for data of Myers [5,35] for Chlorella pyrenoidosa.

Dilution Rate $D(day^{-1})$	Cell dry Weight X(g/)	$Q_{O_2}XV$ (m mole/day)	$Q_{CO_2}XV$ (m mole/day)	Light Intensity I_aA(kcal/day)	Carbon Balance	Available Electron Balance	η_{kcal}	η_{O_2}	η_{CO_2}
0.44	0.61	1.91	1.335	1.066	0.932	0.885	0.1532	0.1726	0.1644
0.73	0.87	4.36	3.110	2.670	0.894	0.924	0.1434	0.1565	0.1519
0.57	0.59	2.54	1.714	1.319	0.909	0.848	0.1551	0.1868	0.1706
0.29	0.51	1.05	7.540	0.555	0.909	0.901	0.1621	0.1822	0.1783
0.61	0.48	2.36	1.700	1.304	0.755	0.789	0.1354	0.1766	0.1701
0.50	0.74	2.66	2.00	1.690	0.811	0.884	0.1320	0.1511	0.1544
0.26	0.57	--	--	0.700	--	--	0.1277	--	--
0.31	0.42	0.95	6.920	0.541	0.868	0.864	0.1438	0.1696	0.1657
0.97	0.44	3.16	2.230	1.690	0.883	0.868	0.1509	0.1806	0.1710
0.70	0.39	2.13	1.590	1.143	0.752	0.815	0.1440	0.1812	0.1815
0.60	0.52	2.19	1.630	1.331	0.839_{+}	0.905_{**}	0.1414	0.1581_{***}	0.1598
0.63*	0.58	2.13	1.760	1.281	0.861_{+}	0.855_{**}	0.1529	0.1789_{***}	0.177++
0.72*	0.52	2.19	1.870	1.380	0.832_{+}	0.852	0.1455	0.1708	0.1746++

*Nitrogen source is urea; nitrate is used in all other runs.

**Equation (3) is used with urea; Equation (12) is used with nitrate.

***Equation (7) is used with urea; Equation (13) is used with nitrate.

+Equation (15) is used with urea; Equation (2) is used with nitrate. Tabular values are reciprocals of values calculated from these equations.

++Equation (16) is used with urea; Equation (8) is used with nitrate
σ_b = 0.5043 for nitrate and 0.4870 for urea.
γ_b = 4.849 with N = 0 for nitrate and 4.465 with N = -3 for urea.

Table 5. Estimates of true growth energetic yield, η_{max}, and maintenance coefficient, m_e, for data in Table 4.

Data Used	Covariates Included	Form	η_{max} Point	η_{max} Interval	m_e, day^{-1} Point	m_e, day^{-1} Interval
All	Z_1, Z_2	I	0.1508	(0.1282, 0.1831)	-0.0837	(-0.4262, 0.2588)
		II	0.1514	(0.1296, 0.1821)	0.0181	(-0.4348, 0.4709)
All	Z_1	I	0.1475	(0.1277, 0.1744)	-0.1027	(-0.4277, 0.2223)
		II	0.1506	(0.1308, 0.1776)	0.0204	(-0.4006, 0.4414)
All	Z_2	I	0.1593	(0.1364, 0.1914)	-0.0622	(-0.4144, 0.2900)
		II	0.1615	(0.1394, 0.1919)	0.0719	(-0.3987, 0.5425)
All	None	I	0.1576	(0.1430, 0.1757)	-0.0688	(-0.3897, 0.2521)
		II	0.1643	(0.1470, 0.1863)	0.0754	(-0.3675, 0.5183)
η_{kcal}	None	I	0.1405	(0.1272, 0.1571)	-0.1422	(-0.5112, 0.2268)
		II	0.1471	(0.1322, 0.1658)	0.0365	(-0.4357, 0.5087)
η_{O_2}	None	I	0.1695	(0.1497, 0.1951)	0.0346	(-0.4318, 0.6747)
		II	0.1778	(0.1534, 0.2116)	0.1214	(-0.4318, 0.6747)
η_{CO_2}	None	I	0.1663	(0.1499, 0.1867)	-0.0296	(-0.3534, 0.2941)
		II	0.1713	(0.1507, 0.1983)	0.0683	(-0.4221, 0.5586)

The interval results are 95% confidence intervals.

Table 6. Estimates of the growth energetic yield, η, and the maintenance coefficients, m_e, from the batch culture data of Winokur [9].

Organism	Light Intensity, Meter Candles	Agitation of Culture	Growth Yield,	Maintenance Coefficient m_e, day^{-1}
Chlorella vulgaris var. viridis	6000	Yes	0.1745	0.3024
	6000	No	0.0083	0.6755
	2000	Yes	0.1548	0.2452
	700	Yes	0.0610	0.2889
Chlorella pyrenoidosa	6000	Yes	0.1129	0.4389
	6000	No	0.0846	0.6108
	2000	Yes	0.1522	0.2988
	700	Yes	0.0702	0.3710
Chlorella luteoviridis	6000	Yes	0.0909	0.3953
	6000	No	0.0440	1.0610
	2000	Yes	0.1200	0.2239
	700	Yes	0.0469	0.2548
Chlorella saccharophila	6000	Yes	0.0875	0.4956
	6000	No	0.0732	0.8783
	2000	Yes	0.1048	0.4862
Chlorella luteoviridis var. lutescens	6000	Yes	0.0794	0.3762
	6000	No	0.0458	1.010
	2000	Yes	0.1182	0.2480
	700	Yes	0.0518	0.1425

Nitrogen source is nitrate; $\sigma_b = 0.462$; $\gamma_b = 4.773$

Table 7. Estimates of the growth energetic yield, n, and the maintenance coefficient, m_e, from batch culture data for several species of algae.

Species	Light Intensity, klux	Culture Conditions	Biomass Energetic Yield, n	Maintenance Coefficient, m_e, day-1	Refer-
Spirulina maxima	1.6	lake water	0.0861	+	[10]
	1.6	synthetic media	0.2121	+	
	2.7	lake water	0.1117	+	
	2.7	synthetic media	0.2153	+	
	4.0	lake water	0.1263	0.8368	
	4.0	synthetic media	0.2734	+	
	5.5	lake water	0.1120	1.226	
	5.5	synthetic media	0.2340	+	
Spirulina maxima	14.2	pilot scale annular reactor	0.103	+	[37]
Chlorella Saccharophila	2.85	pilot scale with airlift mixing (12 to 14 ℓ/min) natural seawater	0.0632	+	[32
	2.85	without mixing	0.0421	1.430	
	2.85	mixing at 14 ℓ/min	0.0666	0.717	
	2.85	mixing at 7 ℓ/min	0.0475	0.949	
	2.85	CO_2 enriched air mixing at 14 ℓ/min	0.0833	+	
	2.85	CO_2 enriched air without mixing	0.0521	+	
Anabaena cylindrica	7.5	N_2 and complete medium, CO_2 enriched gas and shaking	0.2575	0.8383	[36]
	7.5	nitrate instead of N_2	0.2138	0.9151	
	7.5	N_2 and 1 ppm calcium	0.2484	1.1637	
	7.5	N_2 and 10 ppm calcium	0.2682	+	
	7.5	N_2 and 20 ppm calcium	0.3167	0.601	
	7.5	nitrate and no calcium	0.0603	+	
	7.5	nitrate and 1 ppm calcium	0.1088	1.159	
	7.5	nitrate and 10 ppm calcium	0.1478	+	
	7.5	nitrate and 20 ppm calcium	0.2113	+	
	1.8	N_2 and complete medium	0.2126	1.115	
	1.6	N_2 and complete medium	0.3797	+	
Spirulina platensis	10	nitrate; pure culture	0.1490	1.727	[38]
	10	nitrate; culture contaminated with bacteria	0.1227	+	

+Maintenance coefficient could not be estimated.

Table 8. Comparison of the true biomass energetic yield and maintenance coefficient for photosynthetic organisms.

Species	η_{max}	m_e, hr^{-1}	References
Chlorella**	0.27	-0.015	Pirt [1]
Chlorella,** mixed culture with bacteria	0.40	0.0029	
Chlorella pyrenoidosa	0.64 0.35	0.030 0.016	Prokop and Ricica [41]
Spirulina platensis	0.115	0.0255	Aiba and Ogawa [3]
Oscillatoria agardhii	0.23	0.00438	VanLiere and Mur [27]
O. agardhii	0.19	0.0211	
O. agardhii	0.15	0.00667	
Scenedesmus protuberans**	0.18	0.0444	Gons & Mur [27,28]
S. protuberans**	0.13	0.0692	
S. obliquus	0.04	0.225	Oswald [27,29]
Chlorella***	0.08	0.187	Myers [42]

**Nitrogen source, urea.
***Light source, approximate full sunlight.

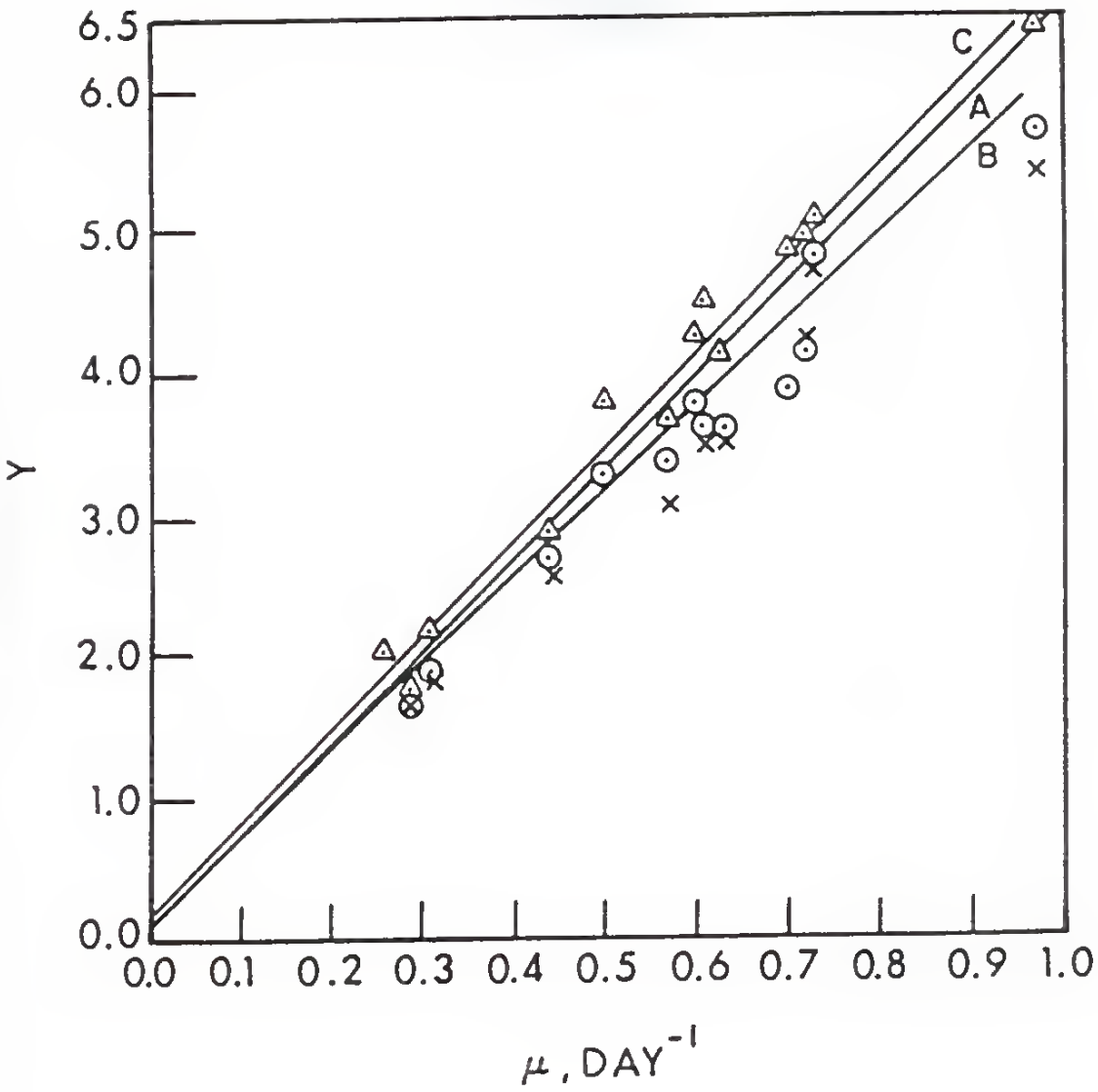

Fig. 1. Comparison of parameter estimation results using Form II and data in Table 4; A. maximum likelihood estimate; B. average result from all data and no covariates; C. result using only η_{kcal}; Δ is from η_{kcal}; Θ is from η_{CO_2}; X is from η_{O_2}.

Chapter 5

HETEROTROPHIC MICROBIAL GROWTH ON C-1 COMPOUNDS

INTRODUCTION

Long considered a most promising solution for the world protein shortage, large scale productions of protein from single-cell organisms is currently attracting industrial interest [1]. The process of photosynthesis in which light energy is converted into useful protein was fairly well surveyed in earlier parts of this thesis. In photosynthesis, microogranisms utilize carbon dioxide as a carbon source, which means photosynthetic microorganisms grow on C-1 compounds. Therefore, it is worthwhile considering the energetics of microorganisms which can grow heterotrophically on other C-1 compounds, especially methanol.

Large scale production of protein by growing single-cell organisms continues to be of considerable industrial interest [1]. In the early seventies, many studies were conducted to develop single-cell protein (SCP) technology based on petroleum feedstocks such as n-paraffin hydrocarbons and gas oil. However, the advantages of using a low-cost, water soluble feedstock, such as alcohol, led to the successful use of methanol for producing SCP [1]. Since these SCP products are free from hydrocarbon residue, they are more acceptable as feed supplements than those produced from hydrocarbons. Other advantages of alcohol-based processes are the comparatively lower cooling costs and oxygen requirements. In addition, methanol can be produced from a variety of raw materials (coal, wood, petroleum, and natural gas). Because of this, there is long term interest in the production of SCP from methanol.

Some nutritional considerations of SCP from methanol utilization were reported [1]. The results are that the products have been proven safe with rats, pigs, chickens, ducks, fish, and ruminants. In all these

studies, no signs of pathologic, neurologic, or other adverse or toxic reactions have been observed in the test animals. By mild alkaline treatment, the nucleic acid content of SCP from methanol utilization can be reduced to less than 2% (from 5~7%), making it suitable for even human consumption. Also Protein Efficiency Ratio (PER) tests have shown that it requires 1.4 pounds of soybean meal to equal the crude protein contained in one pound of SCP from utilizing methanol. The product can be substituted for soybean meal to a high proportion in diets with a high level of feed efficiency [1].

Van Dijken and Harder [2] and Harrison et al. [3] studied the theoretical yields on methanol and compared them with experimentally obtained yields. Van Dijken and Harder assumed a value of Y_{ATP} of 10.5 g cells/g mole ATP on 3-phosphoglycerate, and calculated yields based on two different pathways; one is the serine pathway, the other is the ribulose phosphate pathway. The calculated theoretical maximum cell yield on methanol was 0.73 g cells/ g substrate, which can be converted into a growth yield, n, of 0.643 using available electron units The observed experimental yields have been smaller than this value.

In SCP production more than 50% of the operating cost is frequently associated with the cost of the carbon substrate. Because of the economic importance of yield in SCP production, the literature on growth yield is reviewed. In order to compare yields on the C-1 compounds methanol, formaldehyde, and formate, the available electron yield, n, is used. Losses of methanol due to evaporation are considered by checking the consistency of the data with carbon and available electron balances where this is possible. The true growth yield, n_{max}, and maintenance parameter,

m_e, in Pirt's model [4] are estimated using all of the data simultaneously with the covariate adjustment method [5].

THEORY

The efficiency or yield of heterotrophic microbial growth can be expressed in either mass or energetic terms. Material and energy balances and regularities associated with microbial biomass may be used in the yield analysis [6,7,8,9,10]. The chemical balance equation for growth with no product formation based on methanol utilization with ammonia as nitrogen source is

$$CH_3OH + aNH_3 + b\ O_2 \longrightarrow y_c\ CH_pO_nN_q + c\ H_2O + dCO_2 \qquad (1)$$

where $CH_pO_nN_q$ is the composition of these atoms in the biomass, and a, b, c, d, and y_c are stoichiometric coefficients. Minkevich and Eroshin [11] have found that the weight fraction carbon in the biomass, σ_b, the reductance degree, γ_b, and the energy content per equivalent of available electrons in biomass, Q_o, are relatively constant. The carbon balance based on equation (1) is

$$y_c + d = 1.0 \qquad (2)$$

where y_c is the fraction of substrate carbon incorporated into biomass, and d is the fraction evolved as carbon dioxide. The available electron balance is

$$\eta + \varepsilon = 1.0 \qquad (3)$$

where $\eta = y_c\gamma_b/\gamma_s$ is the fraction of available electrons incorporated into biomass and $\varepsilon = 4b/\gamma_s$ is the fraction of available electrons transferred to oxygen [8,9,10,11]. Equation (3) may also be viewed as an approximate energy balance; however for very small molecules the regularity identified by Thornton [12] and Kharasch [13,14] shows womewhat larger deviations [15].

Table 1 gives the heat of combustion and free energy of combustion for methanol, formaldehyde, and formic acid as well as the average value for microbial biomass. Table 1 shows that the values for these small molecules deviate significantly from the regularity value.

By using Pirt's yield model [4] and the available electron concept Erickson [16] derived the following equation:

$$\frac{1}{\eta} = \frac{1}{\eta_{max}} + \frac{m_e}{\mu} \tag{4}$$

Two related equations are obtained by using the equality constraints from equation (2) and (3) as follows [5,16]:

$$\frac{(y_c+d)}{\eta} = \frac{1}{\eta_{max}} + \frac{m_e}{\mu} \tag{5}$$

$$\frac{(\eta+\varepsilon)}{\eta} = \frac{1}{\eta_{max}} + \frac{m_e}{\mu} \tag{6}$$

Two forms of equation (4-6) are used for parameter estimation. In this work, equations (4-6) are referred to as Form I. Form II is obtained by multiplying each term in equations (4-6) by μ.

Several different estimates of the parameters from equations (4-6) can be obtained. By treating the three equations as a multivariate linear model with common parameters, a method of analysis of growth curves which utilizes the technique of analysis of covariance may be applied [17,18,19]. To apply this method equations (4-6) are linearized as follows:

for Form I

$$Y_{ji} = \beta + m_e X_i \qquad \begin{matrix} j = 1, 2, 3 \\ i = 1, 2, \ldots, N \end{matrix} \tag{7}$$

for Form II

$$Y_{ji} = m_e + \beta\mu_i \qquad \begin{matrix} j = 4, 5, 6 \\ i = 1, 2, \ldots, N \end{matrix} \tag{8}$$

where

$$Y_{1i} = \frac{1}{n_i}, \quad Y_{2i} = \frac{(y_{ci}+d_i)}{n_i}, \quad Y_{3i} = \frac{(n_i+\varepsilon_i)}{n_i},$$

$$Y_{4i} = \frac{\mu_i}{n_i}, \quad Y_{5i} = \frac{\mu_i(y_{ci}+d_i)}{n_i}, \quad Y_{6i} = \frac{\mu_i(n_i+\varepsilon_i)}{n_i},$$

$$\beta = \frac{1}{n_{max}}, \quad X_i = \frac{1}{\mu_i}$$

and N is the number of observations for each variable. The three responses Y_{1i}, Y_{2i}, and Y_{3i} contain information from the experimental measurements. The average of these values for Form I

$$\overline{Y}_{.i} = \frac{1}{3} \sum_{j=1}^{3} Y_{ji} \tag{9}$$

may be used to estimate the parameters m_e and β; that is

$$\overline{Y}_{.i} = \beta + m_e X_i + \text{error} \tag{10}$$

However, a conditional model, which includes the error structure of the equality constraints,

$$\overline{Y}_{.i} = \beta + m_e X_i + \sum_{k=1}^{q} \alpha_k Z_{ki} + \text{error}, \quad (1 \leq q \leq 2) \tag{11}$$

may provide better estimates. In equation (11) Z_{ki} are covariates which are selected such that they have zero expected value. For example,

$$Z_{1i} = Y_{1i} - 2\,Y_{2i} + Y_{3i} \tag{12}$$

$$Z_{2i} = Y_{1i} - Y_{3i} \tag{13}$$

provide a set of covariates with zero expected value. Methods for selecting covariates have been discussed in detail elsewhere [5,18,19]. The coefficients, α_k, are estimated in the regression analysis. The maximum likelihood estimate is obtained when the maximum number of covariates is included (2 in this case with two equality constraints). Since an additional degree of freedom is introduced with each covariate that is introduced, the parameter estimates with shortest confidence interval may arise from using zero, one or two covariates. Each case may be considered and the results may be compared using the shortness of the confidence interval as one of the measures of the quality of the estimate [5,18,19].

An analogous procedure is used with Form II. By using multiple linear regression methods (in this work SAS [20] was used) estimates of $\beta = 1/\eta_{max}$ and m_e may be obtained for both Form I and Form II. Equations (4), (5), and (6) may also be considered individually. The 95% confidence intervals for the parameters can be obtained by using the estimated standard errors of the parameters, $\hat{\sigma}_\beta$ and $\hat{\sigma}_{m_e}$, which are directly obtained from the SAS computer output. The confidence interval for β is

$$\hat{\beta} \pm t_{0.025}\,(N - p - q)\,\hat{\sigma}_\beta \tag{14}$$

where p is the number of parameters (p = 2) and q is the number of covariates (q = 0, 1, or 2). Generally, smaller confidence intervals for the

parameters may result when the number of covariates is decreased without sacrificing an appreciable amount of information. More detail on this is presented elsewhere [5,18,19].

The 95% confidence interval for m_e is found using an equation which is analogous to equation (14). The confidence interval reported for $\eta_{max} = 1/\beta$ is found by taking the reciprocal values after applying equation (14) to find the confidence interval.

The numerical values of $\sigma_b = 0.462$ and $\gamma_b = 4.291$ based on the average values of Minkevich and Eroshin [11] were used in this work to estimate the parameters, η_{max} and m_e.

RESULTS AND DISCUSSION

Several sets of experimental data in the published literature are analyzed using the carbon balance and available electron balance to examine data consistency, and the covariate adjustment method to estimate the true growth yield and maintenance parameters, η_{max} and m_e, respectively. The data of Held et al. [23] is presented in Table 2 and 3 together with an analysis of data consistency using carbon and available electron balances. Methanol, formaldehyde and formate concentrations were measured; however, formaldehyde and formate concentrations, respectively, were less than 250 μ moles/liter and 100 μ moles/liter. Since these quantities are less than 0.1% of the substrate supplied, formaldehyde and formate are not included in the carbon and available electron balances. Figure 1 provides a graphical analysis of the results of data consistency calculations for the data sets in Tables 2 and 3. Note that for both sets of data, the failure to account for all the carbon and available electrons tends to be greater at higher dilution rates. It is not clear why the recovery of carbon and available electrons is low, but evaporation of methanol and formaldehyde is one possible explanation. For the data in Table 2 measurable quantities of methanol and formaldehyde are observed in the culture broth at dilution rates of 0.143 hr^{-1} and higher; this also corresponds to the region of poorest recovery. With the higher feed concentration of 30 g/ℓ of methanol in Table 3, measurable quantities of methanol and formaldehyde are reported at dilution rates of 0.06 hr^{-1} and higher. Estimates of vapor losses based on the measured concentrations of methanol and formaldehyde in the culture broth and the respective vapor pressures indicate that methanol losses should be greater than formaldehyde losses

and that the fraction which is lost is almost of the same order of magnitude as the missing fraction in Tables 2 and 3. The maximum estimated value of the losses due to volatility occurs at the largest specific growth rate in Table 3; 2% of the methanol which enters is lost as vapor. Since only 40% of the methanol is consumed, the vapor loss accounts for about 5% of the carbon and available electrons in the balances. Phase equilibrium and ideal solution behavior are assumed in making these estimates.

Tables 4 and 5 provide estimates of the true growth yield, η_{max}, and maintenance parameter, m_e for the data in Tables 2 and 3, respectively. In these tables, the results of parameter estimation with the covariate adjustment method (equation 11) with zero, one, and two covariates are reported. In addition, the results of using each of equations(4), (5), and (6) separately with the appropriate data are also presented. Both Form I and II are used with covariate adjustment and with the individual equations to obtain point and 95% confidence interval estimates of true growth yield, η_{max}, and maintenance parameter, m_e.

The first two rows of Tables 4 and 5 contain the maximum likelihood results which are obtained by using two covariates. In both Tables 4 and 5 larger estimates of η_{max} are obtained when covariates are included compared to the average results where covariate adjustment is used without any covariates. In Table 4 the shortest confidence intervals with covariate adjustment are for the cases with no covariates and one covariate, Z_2. In Table 4 larger estimates of the true growth yield parameter, η_{max}, are obtained from equations(5) and (6) than from equation (4). This is expected because in equations (5) and (6) the sum from the available electron and carbon balances, respectively, is used as the measure of consumed substrate.

The results in Table 5 are generally lower than those in Table 4. Held et al. [23] point out that much of the data in Table 3 for use in Table 5 was collected under oxygen limited conditions while most of the data at the lower feed concentration (Tables 2 and 4) was obtained under methanol limited growth conditions.

The values of the maintenance parameter, m_e, should be positive; however, the 95% confidence interval frequently includes a negative region because the values of this parameter are frequently not significantly different from zero.

Data consistency results are presented in Table 6 for the data of Dostalek and Molin [24] who cultured Methylomonas methanolica on methanol in continuous culture. The data consistency results are best at higher dilution rates and poorest at lower dilution rates. The reason for the failure to recover all of the carbon and available electrons is not clear; however, there is good agreement of the carbon and available electron balances with each other. Evaporation of methanol is consistent with the nearly identical recoveries of the carbon and available electron balances; however, since the residual methanol concentration is low the predicted rate of evaporation is less than 0.1%.

Table 7 displays the results of parameter estimation using covariate adjustment, and also the results from each equation individually. The shortest confidence intervals for true growth yield with covariate adjustment occur when convariate Z_2 is included. For the estimation of the maintenance coefficient, the confidence intervals are slightly shorter for the average value where no convariates are included. Figure 2 shows how each of these two cases fit the data using Form II. The results using the covariate Z_2 do not fit the data as well as the average value where no

covariates are included; however, the consistent deviation of the carbon and available electron balances from one is appropriately included in the analysis when covariate Z_2 is included. Note that the deviation of the two models is greatest at small specific growth rates where the deviations of the balances from one is greatest.

The estimates of true growth yield in Table 7 are significantly greater than those found in Table 4 and 5. All of the point and interval estimates using covariate adjustment are less than the theoretical value of η_{max} = 0.643 based on the analysis of van Dijken and Harder [2] for Y_{ATP} 10.5 g cells/mole ATP.

Recently Tsuchiya et al. [25] have examined growth yields for Pseudomonas AM-1 growing on methanol, formaldehyde, and formate. The consistency of the data is examined and reported to be relatively good [25]. The results of parameter estimation using the covariate adjustment method are reported in Table 8. Because of the small number of data points, the shortest confidence intervals are those obtained when no covariates are included in equation (11).

Comparison of the results in Table 8 shows that yields based on free energy, η_{th}^{max}, are lower than the yields based on available electrons, η_{max}, because of the higher free energy of the substrate relative to that of the microbial biomass as shown in Table 1.

The true growth yield based on free energy is similar for formaldehyde and methanol in Table 8. The true growth yield based on available electrons, η_{max}, is about the same for methanol and formate. The somewhat lower true growth yield based on free energy, when the substrate is formate, is not surprising; while formate has a greater free energy per equivalent of available electrons, not all of that energy is effectively used in the

conversion process.

Values of true growth yield, η_{max} and maintenance parameter, m_e, are presented for several sets of literature data in Table 9. Most of these studies were conducted with Hansenula polymorpha. Sufficient data to check the consistency of the experimental measurements using mass balances was available for only one case [26]. The highest true growth yields in Table 9 are similar for Pichia pastoris CBM 10 and Hansenula polymorpha DL - 1.

Values of the true growth yield and maintenance coefficient have been estimated for Paracoccus denitrificans by van Verseveld [30,31]. His results are presented in available electron units in Table 10. Values of true growth yield based on methanol as substrate are similar to several of the values reported in the other tables. The true growth yields on formate are lower in Table 10 than they are in Table 8.

In the work of Held et al. [23] and the work of Allais and Baratti [27] better yields were obtained at lower methanol feed concentrations. Held et al. [23] attributed the decreased yield at higher methanol feed concentrations to oxygen transfer limitations. Since the true growth yield is economically very important in SCP production [32], growth conditions which maximize the yield should be maintained.

Recently, Amano et al. [33] have reported their results with Methylomonas methanolovorans growing on methanol in batch and continuous culture. The consistency of their data is relatively good. Conversion of their estimates of true growth yield and maintenance coefficient to available electron units gives $\eta_{max} = 0.44$ and $m_e = 0.029\ hr^{-1}$. This value of the true growth yield is similar to that reported in Table 7 for Methylomonas methanolica for the data of Dostalek and Molin [24].

CONCLUSIONS

Values of the true growth yield and maintenance parameter are presented for several sets of literature data for microbial growth on methanol, formaldehyde, and formate. The estimated values of the true growth yield for Methylomonas methanolica were found to be the largest of all those which were considered. The results indicate that the interval $0.395 < \eta_{max} < 0.596$ probably includes the true growth yield for this organism. This interval is less than the theoretically estimated maximum yield of 0.643 based on the analysis of van Dijken and Harder.

The covariate adjustment method allows all of the experimental measurements to be used simultaneously in the estimation of the parameters. The carbon balance and available electron balance have been used to examine the consistency of the data. One of the advantages of the covariate adjustment method is that covariates are introduced based on the expected values of the carbon and available electron balances. The covariate adjustment method also considers the error structure of the experimental data.

The volatility of methanol and formaldehyde was examined and estimated to be significant in some of the experimental results.

The methods and results presented in this work should be helpful to those who are working to develop strains with significantly improved yields.

NOMENCLATURE

a	Moles of ammonia per quantity of organic substrate containing one g atom carbon, g mole/g atom carbon.
b	Moles of oxygen per quantity of organic substrate containing one g atom carbon, g mole/g atom carbon.
c	Moles of water per quantity of organic substrate containing one g atom carbon, g mole/g atom carbon.
d	Moles of carbon dioxide per quantity of organic substrate containing one g atom carbon, g mole/g atom carbon.
m_e	Maintenance coefficient, hr^{-1} (available electron units).
m_{th}	Maintenance coefficient, hr^{-1} (free energy units).
Q_{CO_2}	Specific rate of carbon dioxide evolution, mole CO_2/g cell/hr.
Q_{O_2}	Specific rate of oxygen consumption, mole O_2/g cell/hr.
P	Number of parameters, η_{max} and m_e.
q	Number of added covariates to original equation.
$t_{0.025}^{(N-p-q)}$	Student's distribution with (N-p-q) degrees of freedom and 0.95 probability (for one tailed table).
y_c	Fraction of organic substrate carbon in biomass, dimensionless.
Z_k	Covariate
β	Yield parameter; $1/\eta_{max}$, dimensionless.
γ_b	Reductance degree, equivalents of available electrons per gram atom carbon.
ϵ	Fraction of available electrons in organic substrate which are transferred to oxygen, dimensionless.
η	Fraction of available electrons in organic substrate which is converted to biomass, dimensionless.
η_{max}	"True" biomass available electron yield, dimensionless.
η_{th}^{max}	"True" biomass free energy yield, dimensionless.
μ	Specific growth rate, hr^{-1}.
$\hat{\sigma}_{\beta_k}$	Estimate of variance of k^{th} parameter.

REFERENCES

1. G.H. Wegner, Chemical Engineering, 90 (18), 56 (1983).

2. J.P. Van Dijken and W. Harder, Biotechnol. and Bioeng. 26, 15 (1975).

3. D.E.F. Harrison, H.H. Topiwala, and G.Hamer, Proc. 4th Int. Fermentation Symp., G. Terui, (ed.), p. 491, Soc. Ferment. Technol., Japan (1972).

4. S.J. Pert, (Ed.), Principles of Microbe and Cell Cultivation. Blackwell Scientific, London (1975).

5. B.O. Solomon, S.S. Yang, and L.E. Erickson, American Chemical Society National Meeting, Kansas City (September, 1982); Biotech. and Bioeng. 25, 2683 (1983).

6. A. Ferrer and L.E. Erickson, Biotech. and Bioeng. 21, 2203 (1979).

7. L.E. Erickson, Annals N.Y. Academy of Science, 326, 73 (1979).

8. L.E. Erickson, I.G. Minkevich, and V.K. Eroshin, Biotech. and Bioeng. 20, 1595 (1978).

9. L.E. Erickson, S.E. Selga, and U.E. Viesturs, Biotech. and Bioeng. 20, 1623 (1978).

10. L.E. Erickson, Biotech. and Bioeng. 21, 725 (1979).

11. I.G. Minkevich and V.K. Eroshin, Folia Microbiol. 18, 276 (1973).

12. W.M. Thornton, Philos. Mag. 33, 196 (1917).

13. M.S. Kharasch, Bur. Stand. J. Res. 2, 359 (1929).

14. M.S. Kharasch and 8. Sher, J. Phys. Chem. 29, 625 (1925).

15. S.A. Patel and L.E. Erickson, Biotechnol. and Bioeng. 23, 2051 (1981).

16. L.E. Erickson, I.G. Minkevich, and V.K. Eroshin, Biotechnol. and Bioeng. 21, 575 (1979).

17. J.E. Grizzle and D.M. Allen, Biometrics 25, 357 (1969).

18. S.S. Yang, B.O. Solomon, M.D. Oner, and L.E. Erickson, Technometrics submitted for publication (1983).

19. 8.O. Solomon, M.D. Oner, L.E. Erickson, and S.S. Yang, AIChE Journal In Press (1983).

20. SAS User's Guide: Basics (Statistical Analysis Systems Institute, Inc. Cary, NC, 1982).

21. J.A. Roels, Biotechnol. and Bioeng. 22, 2457 (1980).

22. R.C. Weast, Handbook of Chemistry and Physics, 56th Edition, CRC Press, Boca Raton, Florida (1975).

23. W. Held, G. Schlanderer, J. Reimann, and H. Dellweg, Eur. J. Appl. Microbiol. Biotechnol. 6, 127 (1978).

24. M. Dostálek and N. Molin, Single-Cell Protein II, S.R. Tannenbaum, and D.I.C. Wang, (Ed.), p. 385, the MIT Press, Massachusetts (1975).

25. Y. Tsuchiya, N. Nishio, H.M. Roldán, and S. Nagai, J. Fer. Tech. 60, 333 (1982).

26. L.E. Erickson, V.D. Kuvshinnikov, I.G. Minkevich, and V.K. Eroshin, J. Fer. Tech. 56, 523 (1978).

27. J.J. Allais, and J. Baratti, J. Fer. Tech. 61, 339 (1983).

28. P. Jara, J.J. Allais, and J. Baratti, Eur. J. Appl. Microbiol. Biotechnol. 17, 19 (1983).

29. C.L. Cooney and N. Makiguchi, Biotech. and Bioeng. Symp. No. 7, 65 (1977).

30. H.W. van Verseveld, Influence of Environmental Factors on the Efficiency of Energy Conservation in Paracoccus denitrifications, Ph.D. Dissertation, Free University, Amsterdam, The Netherlands, (1979).

31. H.W. van Verseveld and A.H. Stouthamer, Arch. Microbiol. 118, 21 (1978).

32. S.R.L. Smith, In Microbial Growth on C_1 Compounds, Howard Dalton, ed., p. 342, Heydon, London (1981).

33. Y. Amano, N. Takada, H. Sawada, H. Sakuma, and G. Terui, Biotechnol. and Bioeng. 25, 2735 (1983).

Table 1. Heat of combustion and free energy of combustion in kcal per equivalent of available electrons.

Substance	$-\Delta H_c$	$-\Delta G_c$
Methanol	28.9	28.0
Formaldehyde	32.2	31.2
Formic acid	32.2	32.9
Biomass	27.0	27.0

Based on data from Roels [2] and CRC Handbook of Chemistry and Physics [22].

Table 2. Data Consistency Check with Carbon and Available Electron Balance from Continuous Culture of Candida boidinii at S_o = 10 g methanol/ℓ [23].

μ	Q_s	Q_{O_2}	Q_{CO_2}	Carbon Balance			Available Electron Balance		
				y_c	d	$y_c + d$	η	ε	$\eta + \varepsilon$
0.019	0.08	3.0	1.3	0.2926	0.5200	0.8126	0.2093	0.8000	1.0093
0.043	0.12	4.1	2.0	0.4415	0.5330	0.9748	0.3157	0.7289	1.0446
0.061	0.20	5.2	2.4	0.3758	0.3840	0.7598	0.2688	0.5547	0.8235
0.085	0.26	7.2	3.7	0.4028	0.4603	0.8631	0.2881	0.5908	0.8789
0.091	0.29	8.0	4.0	0.3853	0.4399	0.8252	0.2756	0.5856	0.8621
0.122	0.37	9.6	4.9	0.4118	0.4287	0.8405	0.2945	0.5611	0.8556
0.143	0.50	12.5	6.5	0.3560	0.4202	**0.7762	0.2546	0.5387	**0.7933
0.152	0.57	13.0	6.9	0.3280	0.3861	**0.7141	0.2346	0.4857	**0.7203
0.156	0.63	13.9	7.5	0.3051	0.3810	**0.6861	0.2182	0.4703	**0.6885

**Formaldehyde produced as a product.

Table 3. Data Consistency Check with Carbon and Available Electron Balances from Continuous Culture of Candida boidinii at S_o = 30 g methanol/ℓ [23].

μ	Q_s	Q_{O_2}	Q_{CO_2}	Carbon Balance			Available Electron Balance		
				y_c	d	$y_c + d$	η	ε	$\eta + \varepsilon$
0.018	0.090	2.1	1.1	0.2464	0.3911	0.6375	0.1762	0.4978	0.6740
0.042	0.104	4.1	2.0	0.4975	0.6185	1.1160	0.3558	0.8410	1.1968
0.060	0.080	6.5	3.2	0.4107	0.5689	0.9796	0.2937	0.7704	1.0641
0.072	0.200	5.0	2.4	0.4441	0.3840	0.8281	0.3176	0.5333	0.8509
0.076	0.250	7.0	3.5	0.3770	0.4480	0.8250	0.2696	0.5973	0.8669
0.078	0.280	7.0	3.7	0.3432	0.4229	0.7661	0.2454	0.5973	0.8427
0.080	0.312	7.0	3.9	0.3159	0.4000	0.7159	0.2259	0.5973	0.8232
0.082	0.290	6.6	3.6	0.3484	0.3972	0.7456	0.2492	0.4833	0.7325
0.090	0.348	7.9	4.0	0.3186	0.3678	0.6864	0.2270	0.4843	0.7122
0.096	0.341	7.6	4.3	0.3468	0.3988	0.7456	0.2480	0.4755	0.7235
0.104	0.396	8.4	4.2	0.2941	0.3394	0.6335	0.2103	0.4525	0.6628
0.111	0.385	10.3	4.9	0.3552	0.4073	0.7625	0.2540	0.5707	0.8247
0.121	0.41	11.2	5.2	0.3636	0.4059	0.7695	0.2600	0.5828	0.8428
0.123	0.45	13.7	6.8	0.3367	0.4800	0.8167	0.2408	0.6495	0.8903
0.130	0.51	13.9	7.1	0.3140	0.4455	0.7595	0.2246	0.5823	0.8069
0.146	0.59	15.0	7.8	0.3049	0.4231	0.7280	0.2181	0.5424	0.7605
0.148	0.61	18.5	9.4	0.2989	0.4931	0.7920	0.2138	0.6484	0.8622

Table 4. Estimates of True Growth Yield, η_{max}, and Maintenance Coefficient, m_e, for Data in Table 2.

Data Used	COVARIATES Included	Form	η_{max}		m_e (hr^{-1})	
			point estimate	95% confidence interval	point estimate	95% confidence interval
All	Z_1, Z_2	I	0.407	0.314, 0.578	0.034	-0.034, 0.101
		II	0.378	0.321, 0.460	0.018	-0.036, 0.072
All	Z_1	I	0.438	0.368, 0.541	0.054	0.036, 0.072
		II	0.390	0.335, 0.466	0.037	0.010, 0.065
All	Z_2	I	0.396	0.353, 0.450	0.026	0.018, 0.034
		II	0.378	0.335, 0.435	0.018	-0.002, 0.201
All	None	I	0.327	0.294, 0.369	0.023	0.007, 0.040
		II	0.290	0.260, 0.330	-0.009	-0.053, 0.036
$\frac{1}{\eta}$	None	I	0.273	0.231, 0.332	0.013	-0.018, 0.045
		II	0.229	0.192, 0.282	-0.044	-0.134, 0.046
$\frac{(\eta + \varepsilon)}{\eta}$	None	I	0.371	0.342, 0.406	0.038	0.027, 0.048
		II	0.340	0.316, 0.369	0.018	-0.007, 0.042
$\frac{(y_c + d)}{\eta}$	None	I	0.356	0.332, 0.384	0.018	0.009, 0.028
		II	0.331	0.308, 0.357	0.001	-0.023, 0.025

Table 5. Estimates of True Growth Yield, η_{max}, and Maintenance Coefficient, m_e, for Data in Table 3.

Data Used	COVARIATES Included	Form	η_{max}		m_e, (hr^{-1})	
			point estimate	95% confidence interval	point estimate	95% confidence interval
ALL	Z_1, Z_2	I	0.355	0.316, 0.406	0.006	-0.006, 0.018
		II	0.303	0.246, 0.393	-0.026	-0.006, 0.014
ALL	Z_1	I	0.302	0.275, 0.333	0.009	-0.008, 0.026
		II	0.246	0.218, 0.282	-0.042	-0.086, 0.003
ALL	Z_2	I	0.324	0.292, 0.365	0.007	-0.008, 0.021
		II	0.265	0.222, 0.331	-0.035	-0.081, 0.010
ALL	None	I	0.295	0.271, 0.323	0.011	-0.006, 0.028
		II	0.249	0.225, 0.279	-0.041	-0.083, 0.002
$\frac{1}{\eta}$	None	I	0.259	0.227, 0.301	0.019	-0.011, 0.049
		II	0.209	0.186, 0.239	-0.057	-0.116, 0.001
$\frac{(\eta + \epsilon)}{\eta}$	None	I	0.307	0.281, 0.337	0.007	-0.009, 0.009
		II	0.266	0.234, 0.308	-0.035	-0.085, 0.016
$\frac{(y_c + d)}{\eta}$	None	I	0.326	0.305, 0.351	0.007	-0.005, 0.002
		II	0.286	0.257, 0.321	-0.030	-0.068, 0.009

Table 6. Data Consistency Check with Carbon and Available Electron Balance from Continuous Culture of Methylomonas methanolica at S_0 =7.96 g methanol/ℓ and 30°C [24].

$\mu(hr^{-1})$	Carbon Balance			Available Electron Balance		
	y_c	d	y_c+d	η	ε	η + ε
0.10	0.3065	0.5187	0.852	0.2192	0.6067	0.8259
0.17	0.3405	0.4876	0.8381	0.2435	0.6134	0.8369
0.22	0.4025	0.4895	0.8920	0.2878	0.5933	0.8811
0.25	0.4180	0.4732	0.8912	0.2989	0.6067	0.9056
0.26	0.4490	0.4929	0.9419	0.3211	0.6200	0.9411
0.29	0.4644	0.4692	0.9336	0.3322	0.6133	0.9455
0.35	0.5032	0.4706	0.9738	0.3600	0.6033	0.9633
0.45	0.5734	0.4500	1.0234	0.4101	0.5933	1.0034
0.47	0.6050	0.4312	1.0362	0.4326	0.5867	1.0193
0.50	0.5439	0.4244	0.9683	0.3890	0.5833	0.9723

Table 7. Estimates of True Growth Yield, η_{max}, and Maintenance Coefficient, m_e for Data in Table 6.

Data Used	COVARIATES Included	Form	η_{max}		m_e (hr^{-1})	
			point estimate	95% confidence interval	point estimate	95% confidence interval
ALL	Z_1, Z_2	I	0.416	0.385, 0.453	0.054	-0.045, 0.154
		II	0.448	0.400, 0.508	0.115	-0.017, 0.246
ALL	Z_1	I	0.423	0.366, 0.443	0.062	-0.127, 0.252
		II	0.462	0.395, 0.556	0.127	-0.059, 0.312
ALL	Z_2	I	0.419	0.395, 0.446	0.066	-0.007, 0.125
		II	0.452	0.413, 0.498	0.128	0.041, 0.215
All	None	I	0.471	0.419, 0.537	0.213	0.157, 0.269
		II	0.527	0.473, 0.596	0.275	0.203, 0.347
$\frac{1}{\eta}$	None	I	0.501	0.424, 0.612	0.287	0.209, 0.364
		II	0.584	0.495, 0.713	0.364	0.261, 0.467
$\frac{(\eta + \varepsilon)}{\eta}$	None	I	0.459	0.413, 0.517	0.180	0.121, 0.239
		II	0.509	0.462, 0.567	0.238	0.172, 0.304
$\frac{(y_c + d)}{\eta}$	None	I	0.454	0.418, 0.497	0.173	0.132, 0.213
		II	0.496	0.460, 0.538	0.223	0.171, 0.275

Table 8. Comparison of True Growth Yield Based on Available Electrons, η_{max}, and Free Energy, η_{th}^{max}, and Maintenance Coefficient Based on Available Electrons, m_e, and Free Energy, m_{th}, for Pseudomonas AM-1 Growing on Different C-1 Compound Substrates; Methanol, Formaldehyde, and Formate [25].

Substrate	Form	η_{max}		η_{th}^{max}		m_e (hr^{-1})		m_{th} (hr^{-1})	
		Point estimate	95% Confidence Interval	Point estimate	95% Confidence Interval	Point estimate	95% Confidence Interval	Point estimate	95% Confidence Interval
Methanol	I	0.3011	0.2693, 0.3390	0.2904	0.2597, 0.3269	-0.0153	-0.0575, 0.0268	-0.0148	-0.0554, 0.0258
	II	0.2990	0.2601, 0.3508	0.2883	0.2508, 0.3383	-0.0168	-0.0802, 0.0465	-0.0162	-0.0773, 0.0448
Formaldehyde	I	0.3375	0.2770, 0.3970	0.3919	0.2401, 03436	0.0058	-0.0314, 0.0421	0.0050	-0.0272, 0.0364
	II	0.3334	0.2794, 0.4135	0.2885	0.2418, 0.3578	0.0036	-0.0382, 0.0454	0.0031	-0.0331, 0.0393
Formate	I	0.3110	0.2353, 0.4588	0.2552	0.1930, 0.3765	0.0309	-0.0735, 0.1352	0.0248	-0.0603, 0.1110
	II	0.3060	0.2416, 0.4173	0.2511	0.1983, 0.3425	0.0253	-0.0730, 0.1236	0.0208	-0.0599, 0.1014

Table 9. True Growth Yield, η_{max}, and Maintenance Coefficient, m_e, for Several Methanol Utilizing Yeasts.

Species	Data Used	Covariate Invluded	Form	η_{max}		m_e (hr^{-1})		Ref.
				Point estimate	95% Confidence Interval	Point estimate	95% Confidence Interval	
H. polymorpha CBM 11	$\frac{1}{\eta}$	None	I	0.3295	0.2442, 0.5062	0.0213	-0.0873, 0.1296	[27]
			II	0.2796	0.1828, 0.5962	-0.0530	-0.3553, 0.2493	
H. polymorpha DL-1	All	Z_1	I	0.3770	0.2865, 0.5510	0.0138	-0.0962, 0.1238	[26]
			II	0.3703	0.2735, 0.5732	0.0077	-0.1221, 0.1373	
P. pastrois CBM 10	$\frac{1}{\eta}$	None	I	0.3742	0.3669, 0.3817	0.0099	0.0068, 0.0130	[28]
			II	0.3744	0.3628, 0.3868	0.0100	0.0012, 0.0178	
H. polymorpha DL-1	$\frac{1}{\eta}$	None	I	0.2587	0.2314, 0.2934	-0.0194	-0.0774, 0.0387	[29]
			II	0.2536	0.2202, 0.2988	-0.0303	-0.1204, 0.0600	

Table 10. Estimates of true growth yield, η_{max}, and maintenance parameter, m_e, based on parameter estimates of van Verseveld [30,31] for Paracoccus denitrificans growing on methanol and formate.*

Substrate	Data Used	η_{max} point estimate	η_{max} 95% confidence interval	m_e, hr^{-1} estimate	m_e, hr^{-1} 95% confidence interval
Methanol	$\frac{1}{\eta}$	0.339	0.306, 0.379	0.0673	0.0277, 0.107
	$\frac{\eta + \varepsilon}{\eta}$	0.370	0.338, 0.408	0.0712	0.0396, 0.100
	$\frac{y_c + d}{\eta}$	0.326	0.308, 0.347	0.0119	-0.0079, 0.0317
Formate	$\frac{1}{\eta}$	0.220	0.197, 0.250	0.0092	-0.0132, 0.0317
	$\frac{\eta + \varepsilon}{\eta}$	0.285	0.235, 0.366	0.0501	0.0185, 0.0844

*Results are based on Form II and the cell composition $C_6 H_{10.8} N_{1.5} O_{.9}$ reported by van Verseveld [30].

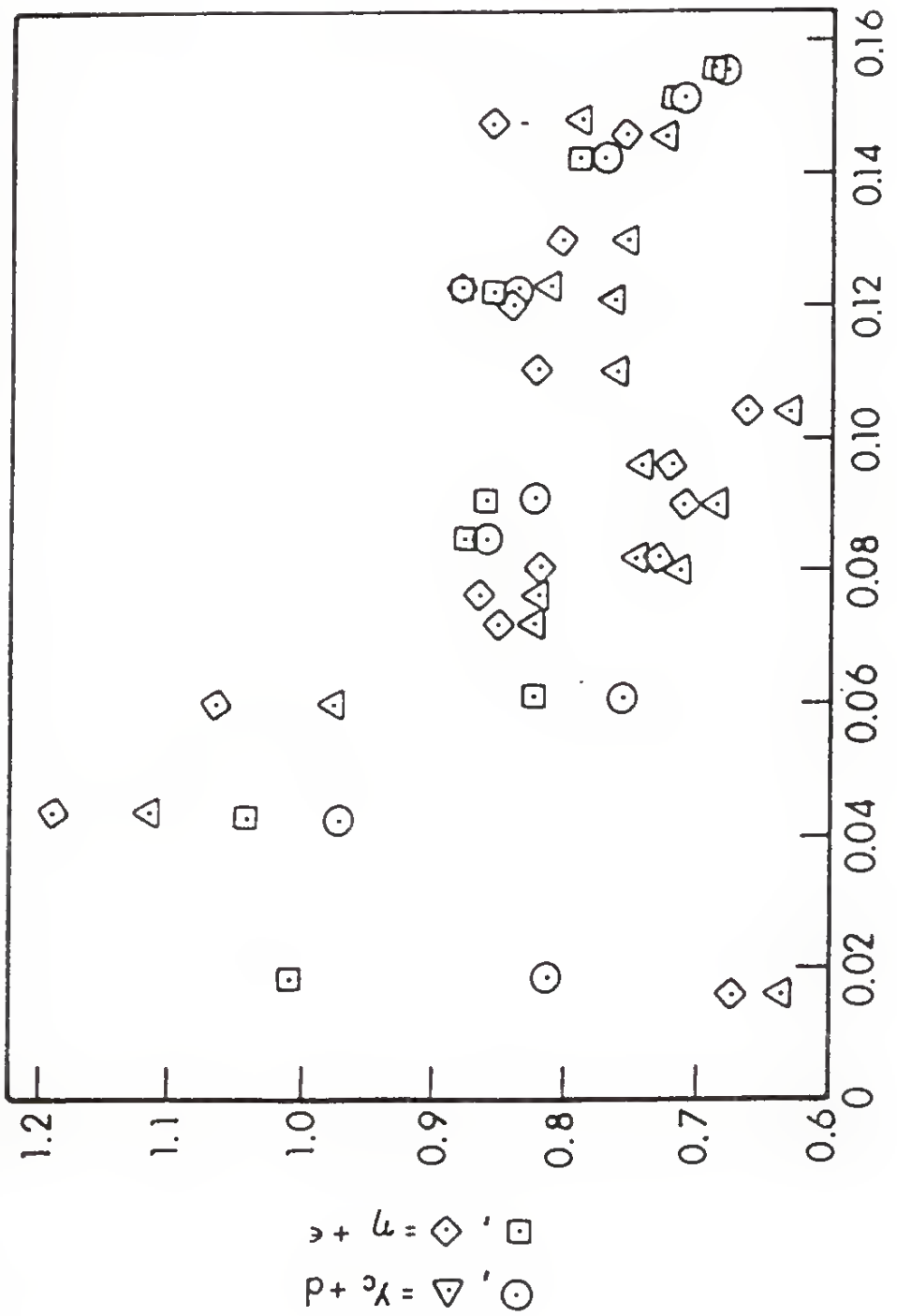

Fig. 1. The effect of specific growth rate (dilution rate) on the closure of the carbon and available electron balances; the circles and squares refer to the carbon and available electron balance, respectively, for S_o = 10 g/ℓ while the triangles and diamonds refer to the carbon and available electron balances, respectively, for S_o = 30 g/ℓ. Data of Held et al. [23].

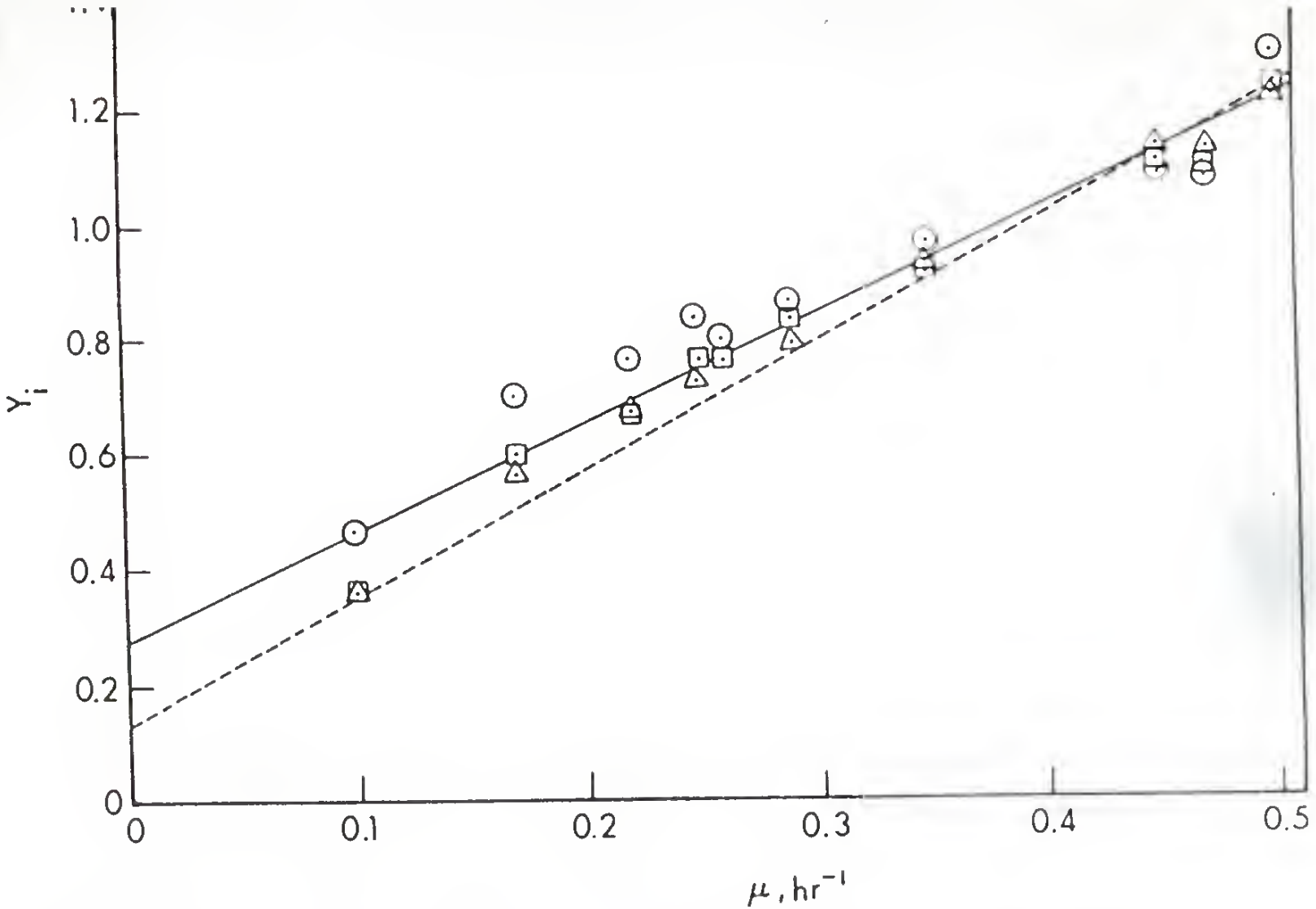

Fig. 2. Comparison of the parameter estimation results for Form II using covariate adjustment with one covariate (dashed line) and the average value (no covariates) (solid line); circles refer to Y_5, and squares refer to Y_6. Data of Dostalek and Molin [24].

ESTIMATION OF YIELD AND MAINTENANCE PARAMETERS ASSOCIATED WITH SINGLE CELL PROTEIN PRODUCTION ON C-1 COMPOUNDS

by

HYEON YONG LEE

B.S., Yon Sei University, Seoul, Korea, 1981

AN ABSTRACT OF A MASTER'S THESIS

Submitted in partial fulfillment of

the requirements for the degree

MASTER OF SCIENCE

FOOD SCIENCE

Department of Chemical Engineering

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1984

The photoautotrophic growth of algae and other microorganisms is an important consideration when long term solutions to food and energy needs are examined. In this work the bioenergetics of algal growth are examined. Literature on the utilization of algae as a food source is reviewed.

Methods are presented for examining the consistency of experimental data of microbial growth where light energy is converted to chemical energy through photosynthesis. Methods of parameter estimation are presented which allow all of the measured variables to be used simultaneously for parameter estimation. The results show that a wide range of values have been found for the true growth yield and maintenance parameters. Values of the true growth yield range from 0.04 to values above those predicted by the Z-scheme model for photosynthesis.

The weight fraction carbon and reductance degree of algae are reviewed using literature data for a wide range of growth conditions. The results show that the standard deviation and coefficient of variation are small as long as the algae are grown under adequate nutritional conditions. For nitrogen deficient growth conditions, the storage of lipids has been observed; this results in values of weight fraction carbon and reductance degree which are larger than the average values.

The growth of methanol ultilizing organisms, which is related to photosynthesis in terms of using C-1 compounds, is analyzed to estimate and compare values of the true growth yield and maintenance coefficient. A covariate adjustment method is presented and used to analyze all of the experimental data simultaneously for each set of literature data. The consistency of the data is examined using carbon and available electron

balances. True growth yield estimates are presented and compared for growth on methanal, formaldehyde, and formate.