Automatic On-Line Growth Estimation Method for Outdoor Algal Biomass Production

H. Guterman and S. Ben-Yaakov*
Department of Electrical and Computer Engineering, Ben-Gurion University of the Negev, P. O. B. 653, 84105, Beer-Sheva, Israel

A. Vonshak
Microalgal Biotechnology Laboratory, The Jacob Blaustein Institute for Desert Research, Ben-Gurion University of the Negev, Campus at Sde Boker, Israel, 84993

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An on-line measuring procedure for estimating productivity in outdoor algal cultures was developed and tested experimentally. The procedure is based on a previously described method for on-line measuring net O_2 production rate (OPR). The data obtained by this method was found to correlate well with the conventional procedures for estimation productivity by measuring the changes in biomass concentration in the culture. The new procedure seems to be superior to the latter since it can be carried out in an almost continuous way and can give immediate indication on the productivity. OPR could be used to monitor on-line the photosynthetic and/or respiration activity in small research fermentors or in large-scale open systems outdoors.

INTRODUCTION

The potential of algal mass culture for producing a variety of products of industrial importance or for water treatment[1,2] have recently been reviewed. Several industrial examples already exist for single-cell protein production[1,2] and water treatment.[3] Furthermore, recent developments suggest the possibility of extracting a number of chemical products[4] or employing algae as an energy source. However, the feasibility of mass production heavily depends on the optimal utilization of solar energy and of minerals such as carbon, phosphorus, nitrogen, and trace elements. For these optimization studies, an on-line control of growth is essential.[5-9]

In order to build an optimal controller of a process, it is essential to have an update measure or estimation of the state variables involved.[10] That is, an optimal controller for algae biomass production would require on-line information on state variables such as growth rate. Classical methods of growth rate estimation, such as the C^{14} method[11] or those that rely on an increase in biomass concentration, do not provide an answer to the problem since they cannot be performed at a high enough frequency and thus will not provide enough information to update an optimal controller. Furthermore, since each analysis requires considerable time, the data is obtained after a long delay from the sampling time. Hence, these methods provide information about past and not current growth rate. Even optical density (OD), which can be measured on-line, provides only a poor on-line estimate of growth rate as it relies on the calculation of the OD derivative and is thus sensitive to noise and data inaccuracy. Furthermore, it provides information about the past and not the actual or future state of the growth process.

In a recent paper,[12] a new method for on-line estimation of the net O_2 production rate (OPR) in an indoor algal minipond was presented. It was shown that this parameter is useful as a growth rate indicator. The method also provides an estimation of the O_2 and CO_2 exchange rates between an algal culture and the atmosphere.[13,14] Considering the fact that all large-scale algal mass production is carried out in outdoor systems, the next logical step was to explore the possibility of applying this on-line estimation method to an outdoor open algal pond. Utilizing the relationship between the OPR parameter and the growth rate already established in the laboratory,[12] we developed a new on-line measuring method from which the algal growth rate in outdoor ponds can be estimated. The method is based on the analysis of the system's response to a perturbation initiated every 3 h. It was found that the method provides, a good on-line estimate of the growth rate in outdoor ponds of the microalgae *Spirulina platensis*.

THEORETICAL CONSIDERATIONS

Algal growth outdoors is characterized by the daily dark–light cycle. During the day, in the presence of light

* To whom all correspondence should be addressed.

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as an energy source, the photosynthetic process takes place. This process is broadly described by

$$6CO_2 + 12H_2O \xrightarrow[\text{chlorophyll}]{\text{light}} C_6H_{12}O_6 + 6O_2 \qquad (1)$$

which emphasizes the inherent relationship between CO_2 consumption and O_2 production rates.[15] In the dark, respiration processes take place:

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 12H_2O \qquad (2)$$

This reaction emphasizes the relationship between the O_2 consumption and CO_2 production. The actual O_2–CO_2 quotient depends on the average C–N–P ratio of the algae and the given experimental conditions. Assuming that the O_2–CO_2 quotient does not vary considerably during a given set of experiments, the O_2 production–consumption rate (OPR) can be used as an estimator of algal growth rate. The dissolved oxygen concentration (DO) in the system under consideration is governed by the differential equation

$$\frac{d\mathrm{DO}(t)}{dt} = -\frac{\mathrm{DO}_2}{z \cdot H}[\mathrm{DO}(t) - \mathrm{DO(atm)}] + \sum_i \mathrm{Fph}_i - \sum_i \mathrm{Fr}_i \qquad (3)$$

where DO_2 is the molecular diffusion coefficient of O_2, z is the thickness of the solution boundary layer, DO(atm) is the dissolved oxygen concentration at the surface solution film; $\Sigma_i \mathrm{Fph}_i$ is the production rate of all oxygen sources, $\Sigma_i \mathrm{Fr}_i$ is the consumption rate of all oxygen sinks, and H is the depth of culture in the pond.

Equation (3) is based on the assumption that O_2 exchange between the pond and the atmosphere can be described by the film model.[13,16] Assuming that the net consumption or production rate is constant, the solution of differential equation (3) is an exponential function:

$$\mathrm{DO}(t) = [\mathrm{DO}(0) - A]\exp(K_L t) + A \qquad (4)$$

$$A = \mathrm{DO(atm)} + \frac{1}{K_L}\left[\sum_i \mathrm{Fph}_i - \sum_i \mathrm{Fr}_i\right] \qquad K_L = -\frac{\mathrm{DO}_2}{zH}$$

This equation predicts an exponential increase/decrease in DO with a time constant $(\mathrm{DO}_2/zH)^{-1}$ and a final value A. Equations (3) and (4) include two unknown parameters: the mass transfer coefficient (DO_2/zH) and the net oxygen evolution rate (OPR); $(\Sigma_i \mathrm{Fph}_i - \Sigma_i \mathrm{Fr}_i)$. If both are assumed to be constant over the experiment interval, an estimator can be constructed by fitting the data to equation (3). It can be shown that such an estimator is observable for (DO_2/zH) and OPR only if $d\mathrm{DO}(t)/dt \neq 0$ and if DO(atm) is known. This implies that it is impossible to obtain an estimate of these parameters if the system is in steady state. Furthermore, this method can only provide an estimation of the net production–consumption O_2 rate and cannot differentiate between the different species living in the same environment or the different processes taking place in the pond.

To fulfill the condition that $d\mathrm{DO}(t)/dt \neq 0$, the system is perturbated from steady state by a forced excitation that changes DO from its steady-state equilibrium value. DO concentration will follow an exponential path when returning to its quasi-steady-state value. This transient response can then be used to estimate the parameters by building an observer to equation (3). The equation can be written as a linear equation of the form

$$y = ax + b \qquad (5)$$

where

$$y = d\mathrm{DO}(t)/dt \qquad x = \mathrm{DO}(t) - \mathrm{DO(atm)}$$

$$a = -\mathrm{DO}_2/zH = K_L \qquad b = \mathrm{OPR}$$

These variables (x, y) can be directly calculated from the DO(t) data during the transient response, and the parameters can be estimated by a linear least-squares-fitting algorithm.[12] It should be noted that the proposed method also provides an estimate of the gas exchange factor DO_2/z. As already shown,[14] this parameter can be used to estimate the fluxes of both O_2 and CO_2, and can help to build a model of the CO_2 system pond.

The present method for OPR estimation is similar to the on-line estimation of the photosynthetic rate already tested.[12] As was already shown previously, data noise could be a problem because the required data differentiation amplifies high-frequency noise. However, the signal-to-noise ratio can be improved by filtering the raw data with a digital filter and by employing a numerical differentiation algorithm that smooths the data.[17–19] Other factors that can affect the accuracy of the measurement are the quality of the DO data collected in the present study by a Clark-type membrane-covered electrode.[20–22] Since the electrode was temperature compensated,[20] temperature effects of the membrane electrode could be neglected.

As already shown,[12] the DO_2/z estimate does not depend on the accuracy of the electrode calibration. However, OPR estimation is linearly dependent on the accuracy of the slope calibration; that is, a 1% error will result in a corresponding 1% error in OPR. As the estimate relates the first derivative of DO to the deviation from equilibrium with respect to the atmosphere and since both of these terms are independent of the electrodes offset, the estimated parameters are insensitive to an error in the electrode's bias.[12]

Another error source is due to inaccuracy in the determination of DO(atm), which is a function of salinity and temperature of the medium. DO(atm) was calculated in the present study by the Weiss equation.[22] It was found that this approach was sufficient for on-line estimation. However, since the measurements are performed by an automatic control system, automatic recalibration of the electrode can also be implemented.[23] The error analysis discussed in the preceding assumes no drift in the bias nor any change in the slope of the DO electrode with the time. It is obvious that the accuracy of the estimator depends on the stability and/or continuous recalibration of the DO sensor used.[21,22]

EXPERIMENTAL

Instrumentation

The present study was carried out with an automatic data-logging/control system described in detail elsewhere.[24] The experimental assembly was located outdoor in the Sde Boker campus of Ben-Gurion University of the Negev, and consisted of a pond with a surface area of about 2.5 m^2. The necessary stirring (to prevent settling of the algae and facilitate distribution of the solar irradiance as well) was provided by a motor-drive paddle.[2]

The parameters measured during the study were pH, dissolved oxygen (DO), optical density (OD), light intensity, water and air temperature, and during a short period of time, wind velocity. The electrode signals were sent to a microcomputer via a special-purpose interface controller.[24] The interface comprises four channels, each with signal conditioners for DO, light intensity, OD, and temperature and three very high impedance analog inputs and 16 control relays. The interface controller was connected to the microcomputer via two 8-bit input–output ports plus an additional edge-sensitive input line. The microcomputer employed was a Commodore model CMD-64 to which a complex interface adaptor (CIA) has been added. Analog-to-digital conversion was obtained by first converting the analog signal to a proportional frequency signal and then counting the frequency pulses over a determined period of time. The advantages of this conversion method are not only its low cost but also its ability to attenuate interfering noise by the inherent integration method. The major disadvantage of the method is the slow conversion rate (about one sample per second). However, this did not prove to be a problem since the rate of change of the phenomena under study is rather slow (time constant of hours). The system's power supply had a battery backup to ensure uninterrupted operation in case of power failure. The system had graphics capabilities and was programmed to perform automatic self-testing and calibration before every set of measurements. The data was recorded by a tape recorder and later transferred to a floppy disk for further data processing and plotting.

Algae and Growth Conditions

The algae *Spirulina platensis* was cultivated in a Zarouk[4] solution. The surface area of the pond was ~2.5 m^2. The depth of the water was ~12 cm. The media were stirred by a paddle-type stirrer.[2]

Analytical Methods

The parameters dry weight, chlorophyll, PO_4, and NO_3 were measured on water samples taken from the samples. Dry weight and chlorophyll were measured as described by Vonshak.[25] PO_4 and NO_3 were analyzed spectrophotometrically.[2] Light attenuation of the samples was measured by a Klett-Summerson colorimeter, model 3074-A110, using a green filter. These analyses were carried out at the algal biotechnology lab in the desert research institute of Sde Boker.

DO Perturbation

The proposed OPR estimation algorithm was applied after monitoring the transient DO concentration transient following a perturbation in DO level. The perturbation was induced by bubbling air through the pond solution and hence changing the DO concentration in the medium. Bubbling was automatically carried out every 3 h (a total of eight experiments per day), for ~15 min after which normal operation was resumed. Data sampling rate during the transient period, following the air bubbling period, was one sample every 3 min. Sampling rate at other times was one sample every 15 min.

Data Processing

Data processing was carried out on-line on the CMD-64 microcomputer. All data processing programs were written in BASIC. Further analysis was carried out on a microcomputer system similar to the one used for this monitoring operation.

RESULTS AND DISCUSSION

The experiments of the present study were carried out during the months of June to November (autumn–summer in Israel) of the years 1984–1986, which are the months of maximum sunlight intensity and day temperature in the Northern Hemisphere, i.e., facilitating maximum growth rate. During that period, performance of the pond was continuously monitored by the computerized system[24] and oxygen evolution rate was measured by the proposed method eight times a day. Typical data collected by the monitoring system is shown in Figure 1. The periodic disturbance in the oxygen curve was produced by the air bubbling, introduced for 15 min every 3 h. During the day, which is characterized by O_2 supersaturation, air bubbling reduces DO concentration. Once the bubbling is stopped, the oxygen supersaturation increases exponentially toward a high level of supersaturation. This increase in DO concentration is obviously related to photosynthetic oxygen production. However, during the dark period, when oxygen concentration is lower than the saturation level, the DO level will increase toward saturation by air bubbling. When air bubbling ceases, it will decrease exponentially toward a lower DO concentration. This decrease is obviously related to oxygen consumption due to respiration. As sun intensity and temperature change (Fig. 1), oxygen saturation level and algal growth rate will change.

The change in dissolved oxygen obviously related to the photosynthesis/respiration processes is affected by the O_2 exchange between the pond and the atmosphere. These two parameters, OPR and the gas exchange coefficient (DO_2/z), were evaluated for each experiment by the method

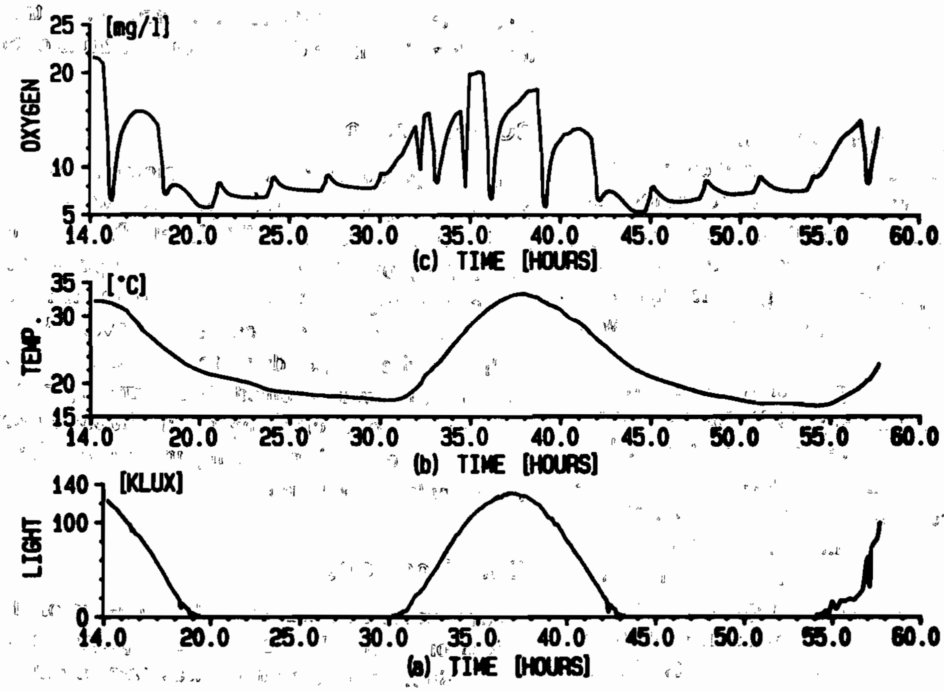

Figure 1. Typical data recorded by automatic monitoring and control systems in outdoor algal pond: (a) light intensity; (b) temperature; (c) dissolved oxygen (DO). Perturbations in DO concentration response are produced by 15 min of air bubbling once every 3 h.

described earlier.[12] The processed data of the experiments of Figure 1 is given in Figure 2. The correlation coefficient R^2 provides a measure of the goodness of fit of the transient data to the equation (3) estimator. It is evident that the best fit is obtained for the experiments in which the OPR and DO_2/z values are almost constant during the measuring interval, i.e., during midday or at night. As OPR is intimately related to light and temperature,[1,2] it is obviously necessary that during the recovery period—used for parameter estimation—the variation in these parameters should not be appreciable. This requirement was not fulfilled during the 6 AM (30-h) experiments (Fig. 2), when the process switched from dark to light conditions. It should also be noted that experimental malfunction can be

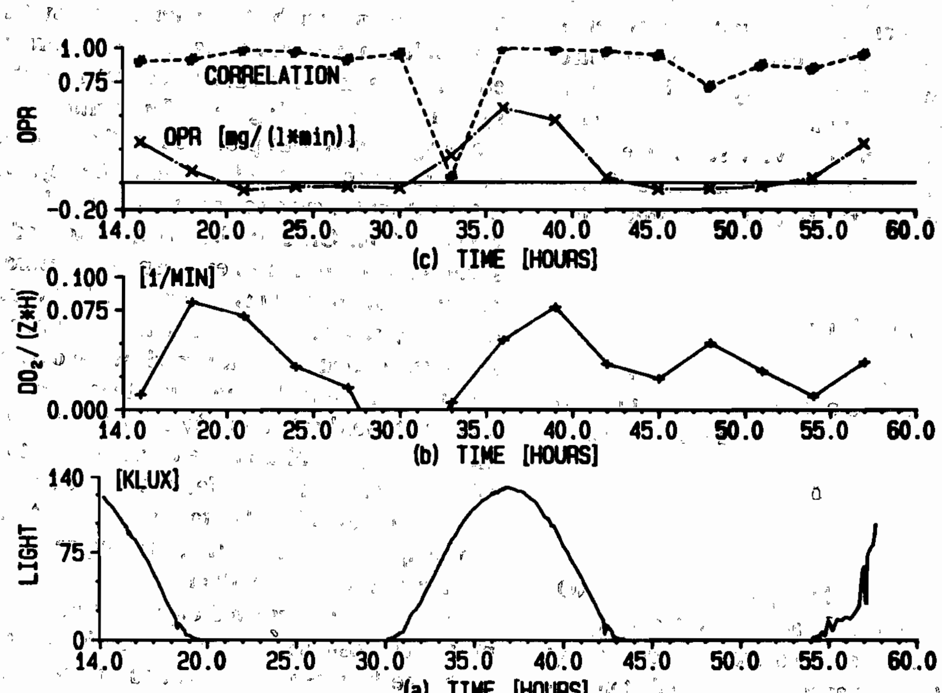

Figure 2. Results of OPR and DO_2/zH estimation of data given in Figure 1: (a) light intensity; (b) DO_2/zH; (c) OPR (x) and correlation coefficient of curve fitting (#).

detected by monitoring the correlation coefficient R^2 (see, e.g., the last two experiments in Figs. 1 and 2).

The necessary condition for obtaining a good parameter estimation is a constant OPR during the experiment interval. This condition cannot be met in the rigorous sense since OPR varies diurnally. The possible effect of OPR variations on the estimation method was investigated by computer simulation. Two general models that reflect the time dependence of OPR were employed. The first model assumes a linear OPR time function to simulate the biological system response during night when the respiration process that is a function of the temperature and biomass concentration is the dominant one. To a first approximation the respiration rate during the night decreases with time due to the gradual drop in the ponds temperature. In this case the DO concentration can be described by the differential equation

$$\frac{d\mathrm{DO}(t)}{dt} = -\frac{\mathrm{DO}_2}{zH}[\mathrm{DO}(t) - \mathrm{DO(atm)}] + \mathrm{OPR}(t) \qquad (6)$$

where

$$\mathrm{OPR}(t) = a \cdot t + b$$

The solution of the differential equation (6) is

$$\mathrm{DO}(t) = [\mathrm{DO}(t) - B]\exp\left(\frac{-\mathrm{DO}_2}{zH}t\right) + B + A \cdot t \qquad (7)$$

where

$$B = \frac{K_L \cdot \mathrm{DO(atm)} + b}{K_L} + \frac{a}{K_L^2}$$

$$A = \frac{a}{K_L} \qquad K_L = -\frac{\mathrm{DO}_2}{zH}$$

The second model assumes that the OPR(t) changes in a sinusoidal form. This simulates the system response during day when the photosynthetic process takes place and is mainly a function of light intensity. In this case OPR(t) [eq. (6)] would be

$$\mathrm{OPR}(t) = A_m \sin(\omega t) \qquad (8)$$

The solution of the differential equation is now

$$\mathrm{DO}(t) = [\mathrm{DO}(0) + G]\exp(-K_L t) + D\cos(\omega t + \Theta) + \mathrm{DO(atm)} \qquad (9)$$

where

$$G = \frac{A_m \omega}{[\omega^2 + K_L^2]} - \mathrm{DO(atm)}$$

$$D = \frac{A_m}{[\omega^2 + K_L^2]^{1/2}} \qquad \Theta = \arctan\left(\frac{K_L}{\omega}\right)$$

Results of the simulation for the first case (linear OPR change) is given in Figure 3. The OPR(t) (Fig. 3a) represents the respiration process in the algal pond as a time function. It can be seen (Fig. 3b) that the K_l relative estimation error is constant during the dark period and is a direct function of the OPR changes that in this model are reflected by the slope a [eq. (5)]. The OPR estimation error (Fig. 3b) relative to the OPR mean value during the measuring interval will increase as the mean OPR value decreases. This relative estimation error will be more significant (about −50%) in the last dark hours as the respira-

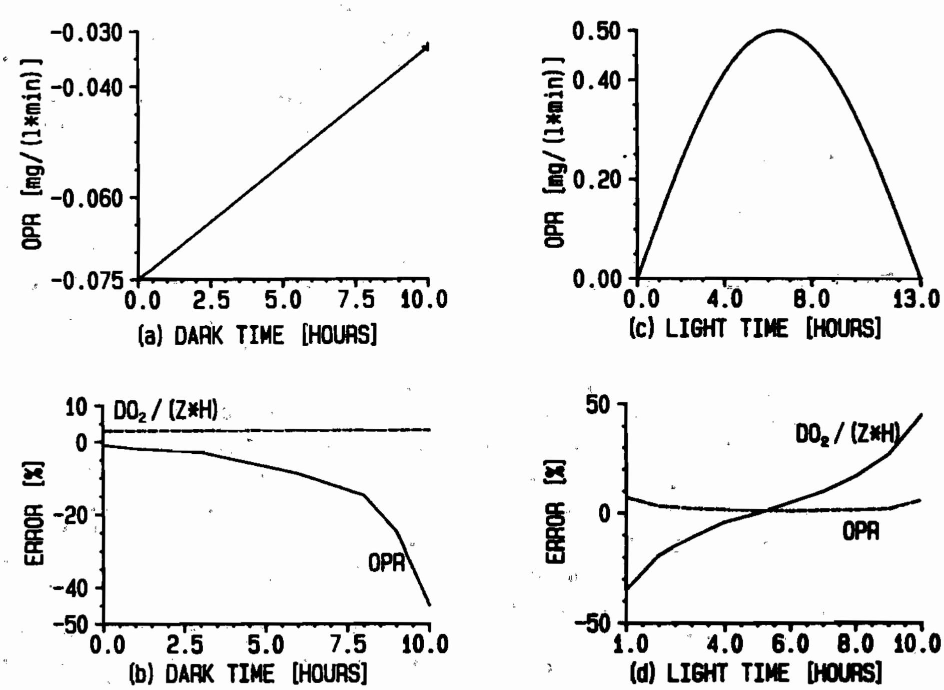

Figure 3. (a) Simulated linear OPR response as function of dark time. (b) Estimation error for DO_2/zH and OPR as function of dark time. (c) Simulated linear OPR response as function of light time. (d) Estimation error for DO_2/zH and OPR as function of light time.

tion process reduces its activity as a consequence of a lower temperature.

During the day [eq. (9)], the exchange coefficient (K_L) estimation error could reach $\pm 50\%$ (Fig. 3d) in the first and last light hours. However, the OPR relative estimation error does not exceed 7% even for those hours that are of low photosynthetic activity. The total mean OPR estimation error during the day relative to the total OPR activity (sinusoidal variation) is about 3% against an approximately -5% error estimation during the night relative to the total respiration activity. Taking into account the fact that the photosynthetic activity is greater than that of respiration, the estimation error for net diurnal biomass production (light plus dark period) should be insignificant, not more than about 1–2%.

Considering the preceding, it is thus of importance to investigate the possibility of reducing the estimation errors by using a more sophisticated estimator, which takes into account the nonconstancy of OPR. This question was studied by applying estimators that assume a varying OPR during each experiment. The general model of the estimators used in these investigations was

$$\mathrm{DO}_i = A \cdot \mathrm{DO}_{i-1} + \phi(i, p, q, \cdots) \qquad (10)$$

which is the difference equation form of the basic state equation (3).[10] Here DO at the sampling instant i is expressed as a function of DO at $i - 1$ and a consumption–production function ϕ that is a function of time i and possibly other parameters $(p, q, \ldots)$ such as light intensity, temperature, etc. The explicit solution of this incremental model for the constant OPR case is

$$\mathrm{DO}_i = \mathrm{DO}_{i-1} \exp\left(-\frac{\mathrm{DO}_2}{zH}\Delta t\right) + \phi \qquad (11)$$

where

$$A = \exp\left(-\frac{\mathrm{DO}_2}{zH}\Delta t\right)$$

$$\phi = (1 - A)K$$

$$K = \mathrm{DO(atm)} + \frac{zH}{\mathrm{DO}_2}\left[\sum_i F_p - \sum_i F_r\right]$$

$$\Delta t = t_i - t_{i-1}$$

The main difference between this approach and our original numerical method is the way by which the state derivative ($d\mathrm{DO}/dt$) is treated. Our original estimation method[12] employs a preliminary numerical step in which the state derivative is evaluated for each sample by a numerical differentiation algorithm that smooths the data.[17–19] On the other hand, the equation (11) estimator already includes the differential relationship so no extra calculation step is required. Computer simulation showed no difference between the results when using either of these estimators. However, when noise was added to the synthetic data, the parameter estimation errors were larger when using the estimator of equation (11). This can be attributed to the smoothing effect of the numerical differentiation used in which fits the data is fitted to a second-order polynomial.[18] Computer simulations have also shown that the equation (10) estimator can be successfully used in nonconstant OPR models. For example, the general form of the equation (10) estimator for the case of a linear time dependence of OPR is

$$\mathrm{DO}_i = A \cdot \mathrm{DO}_{i-1} + B \cdot i + C \qquad (12)$$

The constant parameters A, B, and C can be estimated by linear least-squares fitting of the transient DO data to the model of equation (5). It was found indeed that the parameters estimated for synthetic data yielded accurate estimates for both OPR and DO_2/zH. However, attempts to apply this estimator to measured data yielded inconsistent and unacceptable results [such as negative diffusion coefficients (DO_2)]. Similarly, attempts to fit the data to nonconstant OPR models of the form

$$\mathrm{DO}_i = A\mathrm{DO}_{i-1} + BP_i + Cq_i^2 + C \qquad (13)$$

where p and q are time (i), temperature T, light intensity L or their combination also failed to yield meaningful results.

An insight on the relative importance of including the various variables in the model of equation (13) was obtained by multivariable analysis. In these analyses we applied the F-test, partial-F-test, and t-distribution to estimate the statistical validity of various models and the relative importance of the variables in each model.[26,27] The results of these analyses (Table I) reveal that the contribution of light intensity L and temperature T to the model is insignificant. The contribution of time t to the power of 2 (t^2) is extremely high and in, fact, (DO_i) can be fitted extremely well to a second-order polynomial in i. This fact by itself is not surprising since a second-order polynomial in i can be considered a serious expansion of the real (exponential) function. Surprising, however, is the fact that the F-test results are highest for a model that includes only t and t^2, higher than the model that includes DO (Table I). One would have assumed that the "real" model based on the state equation (3) and therefore must include DO_{i-1} should yield a better fit. This puzzle was resolved by computer simulation in which the estimation algorithm and statistical analyses were run on data synthesized for some state equation models. It was found that the multivariable regression analysis fails to recognize the importance of variables to the regression when the data is corrupted by noise (Table II). For example, for a constant OPR model, the partial F-test probability strongly supports the assumption that the contribution of time t to the estimator is insignificant. (Table II, first line.) However, when noise is added, the partial F-test suggests an apparent significance to time t. When the data is synthesized by linear time-dependent OPR (Table III), the partial F-test clearly reveals the importance of DO and t for noiseless data. When noise is added, the apparent relative importance of t increases. The statistical analyses can therefore not be used to determine the relative importance of the variables in the regression if the data is noisy (com-

Table I. Analysis of variance of measured data.

Variables in regression[a]	F-test	Variables added	Partial F-test	F-probability (%)
DO, t, t^2	2891.18	DO	0.1371	27.06
		t	9.6703	97.91
		t^2	11.9627	98.65
DO, L, L^2	967.44	DO	139.2019	99.99
		L	1.1523	67.56
		L^2	1.1097	66.73
DO, T, T^2	1176.21	DO	194.7742	99.99
		T	0.3789	43.92
		T^2	0.5195	50.18
DO, T, L	1184.63	DO	215.2651	99.99
		T	0.5659	51.96
		L	2.7126	84.93
DO, L	1426.56	DO	428.6350	99.99
		L	0.6717	56.05
DO, T	1894.09	DO	275.8480	99.99
		T	3.1850	88.25
DO, t	1683.50	DO	84.4500	99.99
		t	2.0500	80.46
DO, t^2	1935.89	DO	690.8290	99.99
		t^2	3.4040	89.24
t, t^2	4946.53	t	1774.0490	99.99
		t^2	261.3207	99.99
L, T	52.81	L	1.3942	72.37
		T	10.1570	98.46
DO	2974.53			
t	287.11			
T	99.34			
L	44.52			
t^2	36.47			
T^2	101.16			
L^2	47.14			

[a] Symbols: t, time; L, light intensity; T, temperature.

pare Table II to Table III). Addition of noise to actual field data increases the masking effect on the regression analysis (Table IV). It can thus be concluded that the interference of the present noise level of our experimental data undermines the use of a nonconstant OPR model at present. The more sophisticated models would be applicable only if the quality of data can be improved.

The O_2 production rate parameter (OPR) differs from all other growth parameters (dry weight, chlorophyll, OD), which are usually used as indicators for biological processes, by the fact that it gives a clear indication of the photosynthesis or respiration rate at the measurement moment. Other methods provide a measure of the past net photosynthetic activity. In order to deduce the growth rate from these methods, it is necessary to perform two measurements at a wide enough time span to provide information on the growth rate. For example, the growth rate in an algae pond is usually estimated by measuring dry weight twice a day and dividing the difference by the sampling time difference. Such a coarse estimate is a serious limitation when monitoring the performance of algal culture, not

Table II. Analysis of variance: synthetic data, constant OPR model [eq. (3)] for different noise levels.[a]

Noise level (%)	F-test	Variable added	Partial probability (%)
0	215.10^9	DO	99.99
		t	0.00
1	399.41	DO	98.87
		t	84.77
3	55.35	DO	64.87
		t	89.70
5	22.02	DO	49.15
		t	87.70
10	7.05	DO	43.97
		t	83.36

[a] Variables in regression: DO, t.

Table III. Analysis of variance: Synthetic data, variable OPR model [eq. (5)] for different noise levels.[a]

Noise level (%)	F-test	Variable added	Partial probability (%)
0	175.10^9	DO	99.99
		t	99.99
1	413.56	DO	99.65
		t	78.16
3	58.53	DO	54.48
		t	87.37
5	23.57	DO	54.48
		t	87.37
10	7.46	DO	44.31
		t	83.46

[a] Variables in regression: DO, t.

Table IV. Analysis of variance: Measured data, different noise levels.[a]

Noise level (%)	F-test	Variable added	Partial probability (%)
0	1683.50	DO	99.99
		t	80.46
1	499.75	DO	98.88
		t	36.13
3	147.69	DO	52.63
		t	92.01
5	66.36	DO	51.3
		t	93.11
10	21.31	DO	48.95
		t	89.65

[a] Variables in regression: DO, t.

to mention the almost impossible task of performing an optimal control of the growth process. In the case of the OPR measurement method, the parameter is calculated on-line by a fully automated system, increasing thereby the reliability of the measurement and lowering the time of analysis and its cost.

The OPR parameter can be correlated to the increase in dry weight by integrating the OPR.[12] If we assume a constant relationship between O_2 and CO_2 and biomass production–consumption and by one algal species, the biomass in the pond at a given time t_i should be related to the O_2 production rate by

$$C(t_i) = K\int_0^{t_i} \mathrm{OPR}\,dt + C(0) \qquad (14)$$

where K is a constant whose value depends on the average C–N–P ratio of the algae, and $C(0)$ is the initial biomass concentration.

As a first-order approximation it will be assumed that the OPR value is approximately constant between the measuring points. This permits us to approximate equation (14) by the discrete form

$$C(t_n) = K\sum_{i=0}^{n} \mathrm{OPR}_i\,\Delta t + C(0) \qquad (15)$$

where OPR_i are the OPR values obtained from n experiments and Δt the time delay between experiments (in our case 3 h). The biomass increment between two dry-weight determinations can be calculated from equation (15):

$$C(t_n) - C(0) = K\sum_{i=0}^{n} \mathrm{OPR}_i\,\Delta t = \Delta C \qquad (16)$$

The results of dry weight measured by the conventional analytical methods and the dry weight estimated by equation (16) are given in Table V and Figure 4, which exhibit a good correlation between the two. However, as was already stated, the dry-weight increase calculated from the analytical data only reflects the average diurnal increase and not the trajectory or growth dynamics along the day. Furthermore, the OPR method also provides data about the net photosynthesis and respiration process. This can be judged by examining Table V, from which it can be inferred that a marked biomass decrease prevails during the dark period. In order to eliminate the possibility that these results were not caused by artifacts in the OPR estimation method or by an inherent error in the measurements, several experiments were performed where the sampling rate for dry-weight determination was increased to about the same rate as the OPR experiments (one every 3 h). The results of one set of these experiments is given in Figure 5. It was found that the OPR results are in good correlation with the manual dry-weight determination. This implies that some of the biomass increase during the day due to the photosynthetic activity is lost during the dark periods due to the respiration process. Our data suggest a biomass loss between 10 and 50% relative to the biomass production during the day (Table V). This biomass consumption heavily depends on the temperature and biomass concentration, while the limiting factor for biomass production appears to be light intensity or temperature.[28] The large variability of the OPR values obtained can perhaps be attributed to some other variable in the pond such as bacterial contamination, age of culture, or the organic load. These questions, however, will have to remain open until a more exhaustive

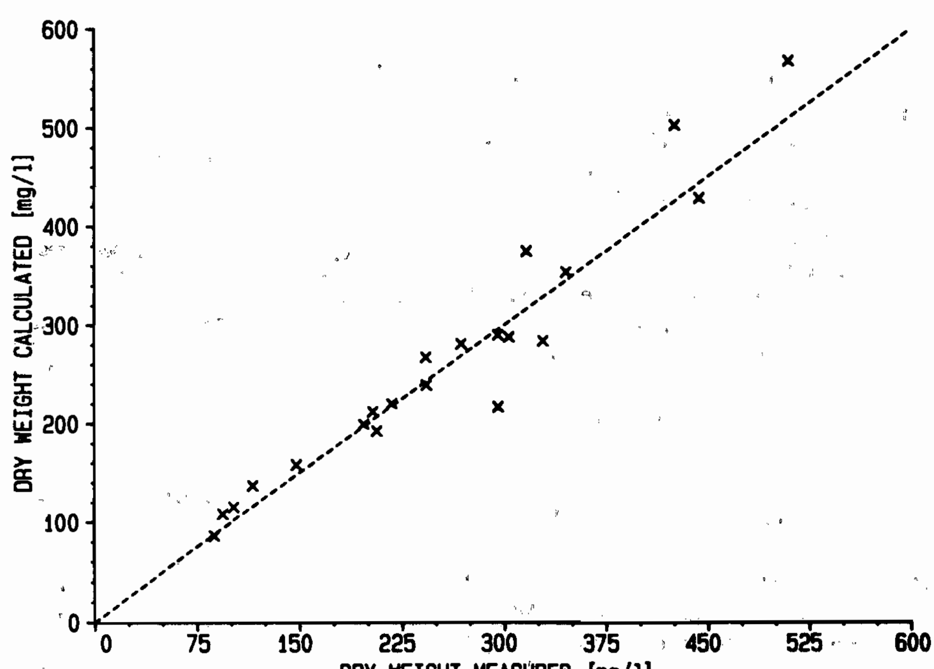

Figure 4. Biomass dry weight measured by conventional analytical; methods vs. dry weight calculated from OPR estimation methods.

Table V. Experimental results.

Date	Dry weight (mg/L Measured)	Biomass increment (mg/L) From Dry weight	From OPR	Deviation (%)	Photosynthesis (mg/L)	Respiration[a] mg/L	%
7/20/84	1545	—	—	—	—	—	—
7/22/84	1789	243.5	239	−1.64	259	−20	7.81
7/22/84[b]	1585	—	—	—	—	—	—
7/22/84	2029	444	429	3.19	584	−154	26.41
7/25/84	1330	—	—	—	—	—	—
7/26/84	1626	296	217	−26.38	379	−161	42.52
7/28/84	1833	207	192	−7.21	402	−210	52.32
7/28/84[b]	1530	—	—	—	—	—	—
7/30/84	1678	148	158	6.87	278	−119	42.95
7/30/84[b]	1163	—	—	—	—	—	—
7/31/84	1360	197	199	1.41	265	−65	24.78
8/1/84	1689	329	284	−13.68	327	−43	13.20
8/1/84[b]	1321	—	—	—	—	—	—
8/3/84	1638	317	375	20.42	449	−73	16.26
8/14/84	1201	—	—	—	—	—	—
8/15/84	1303	102	115	13.28	268	−153	57.02
8/16/84	1517	204	212	3.94	264	−52	19.75
8/21/84	1542	—	—	—	—	—	—
8/22/84	1658	116	137	18.56	185	−47	75.70
8/28/84	530	—	—	—	—	—	—
8/29/84	773	243	267	10.12	327	−60	18.40
8/30/84	991	218	220	1.22	270	−49	18.34
9/2/84	1417	426	503	18.11	604	−101	16.80
9/3/84	1586	—	—	—	—	—	—
9/4/84	1680	94	108	15.33	161	−55	34.03
9/5/84	1768	88	86	−1.65	147	−61	41.34
9/10/84	440	—	—	—	—	—	—
9/11/84	709	269	281	4.51	358	−77	21.52
9/13/84	610	—	—	—	—	—	—
9/14/84	956	346	354	2.37	390	−36	9.34
7/14/85	640	—	—	—	—	—	—
7/15/85	944	304	288	−4.96	329	−41	12.44
8/27/85	620	—	—	—	—	—	—
8/29/85	1130	510	568	11.52	658	−45	6.88
9/8/85	428	—	—	—	—	—	—
9/10/85	726	296	290	−1.96	416	−125	30.30

[a] Percentage is respiration relative to photosynthesis.
[b] After dilution with Zarouk medium.

statistical analysis of the data to correlate the different parameters involved in the biological system (temperature, light, biomass, etc.) is carried out to further elucidate the biomass production–consumption process.

The theoretical analyses presented here and supported by experimental data could be employed in a number of different ways. For monitoring and data acquisition, the method provides the tool for the determination of the real state of the growth process and the actual vitality of the pond. It also provides information about the gas exchange[14] rate between the pond and atmosphere for on-line control. Furthermore, the suggested OPR method provides an accurate and almost immediate estimate of the growth rate opening thereby the possibility of realizing on-line optimal control.

CONCLUSIONS

Among the different methods for estimating growth rate in an outdoor algal culture, the O_2 production rate (OPR) estimation method appears to be reliable, accurate, and the most versatile. The method can be incorporated in a continuous automatic monitoring and control operation. It could be useful not only for biomass production plants but also in the research laboratory, providing the researcher with a tool that can give a better insight into the behavior of the biological system under study. The use of this estimation method in conjunction with an optimization algorithm[9] can be employed in the search for optimum growth conditions. This can be used to devise "smart" controllers that can help to find the optimal growth conditions. It can

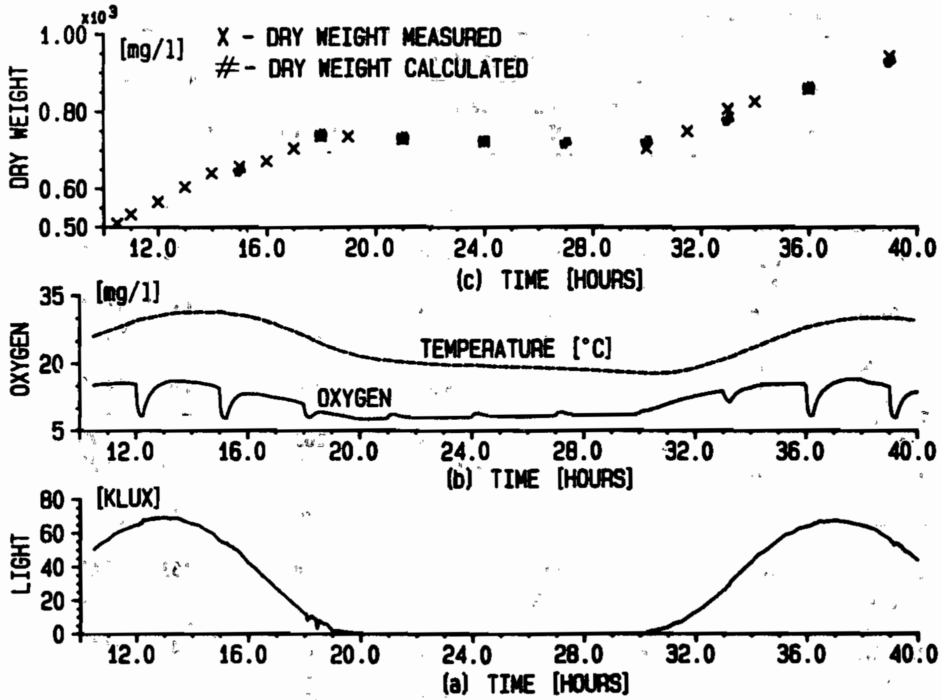

Figure 5. Typical data recorded by automatic monitoring and control systems in outdoor algal pond. Sampling dry-weight determination was increased to about same rate as OPR experiments (one every 3 h): (a) light intensity and (b) dissolved oxygen (——); temperature (------) (perturbations in DO concentration response produced by 15 min air bubbling once every 3 h); (c) biomass dry weight measured and calculated from OPR estimation method.

also reduce the costs of operation as it does not require the use of highly specialized personnel in order to monitor the system, reducing thereby the cost per sample and hence the total production cost.

The proposed measurement method could be used to estimate the effect of different parameters such as temperature and light on the growth rate in the outdoor pond, allowing the researcher to develop models of the pond and to test various hypotheses. This capability has already been proved in a minipond,[13,14] where the present exchange model was used to estimate the O_2 exchange between solution and the atmosphere and the CO_2 exchange between the two phases and to calculate the total carbon evolution in the pond. The suggested tool could also help to shed light on pending questions such as the maximum biomass production attainable. Hence, the proposed on-line estimation method might have great potential in future commercial applications and in theoretical studies of biological systems.

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