# An Automatic Method for On-Line Estimation of the Photosynthetic Rate in Open Algal Ponds

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An analytical model for dissolved oxygen concentration in an algal minipond was used to develop a new method for estimating, on-line, the net $O_2$ production rate (OPR) of the biological process. The method was tested experimentally and was found to provide crucial information on the vitality of the biological process and to provide an early warning of a possible forthcoming collapse of the ecosystem. It is suggested that the newly developed model and measurement method could provide investigators with useful tools for optimization of algal cultivation in the laboratory and plant.

## INTRODUCTION

Recently,[1] a computerized system designed for monitoring and control of miniponds was developed and applied as a tool for studying the dynamics of biological processes. One of the potential applications of the developed system is optimization of aquaculture production units by automatically searching with efficient algorithms optimal growth conditions.[2-5] However, before such a far-reaching goal can be pursued, one must develop new measurement methods that will enable a computer to estimate — on-line — the actual rate of growth at any instant. This will allow the system to evaluate whether given growth conditions, such as pH, temperature, or light intensity, are optimal in terms of yield per harvesting cost. It is therefore crucial that the method used for growth rate estimation be automatic and fast to fully utilize the computerized capabilities. The classical methods of growth rate estimation, such as the $C^{14}$ [6] method or those which rely on analytical methods such as increase in dry weight, chlorophyll, or protein, do not provide an ample answer to the problem at hand.[7] More sophisticated methods, either direct ones or indirect ones, must therefore be explored.

In the present study we explored the possibility of applying dissolved oxygen concentration measurements to the estimation of the rate of assimilation in an algal minipond. Following theoretical considerations of the relationship between this parameter and the growth rate, we developed a new on-line procedure from which the desired information can be calculated. The method is based on perturbating the system from its dynamic equilibrium and examining the system's response to the excitation. It was found that by this method one can obtain data with high enough signal-to-noise ratio for estimating the desired parameter, despite the relatively high background noise level.

## THEORETICAL CONSIDERATIONS

The photosynthetic process is usually described by the following conceptual equation:

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

which emphasizes the inherent relationship between $CO_2$ consumption and $O_2$ production rates.[8] The actual $O_2/CO_2$ quotient differs from one and its value depends on the average C:N:P ratio of the algae and the given experimental conditions. Assuming that this quotient does not vary considerably during a given experiment or a set of experiments, the $O_2$ production rate (OPR) can be used as an indicator for the photosynthetic rate. We assume that only one algal species is involved and ignore variations in algae and bacterial respiration. Under these conditions, dissolved oxygen concentration

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in the minipond is governed by the differential equation:

$$\frac{dDO(t)}{dt} = -\frac{D_{O_2}}{z\,H}[\,DO(t) - DO(atm)\,] + F_{ph} \quad (2)$$

where $D_{O_2}$ 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; $F_{ph}$ is the net oxygen evolution rate (OPR); and $H$ is the depth of culture in the minipond.

Equation (2) is based on the assumption that $O_2$ exchange between the pond's solution and the atmosphere can be described by the Film Model.[9] It assumes that the exchange process is controlled by molecular diffusion of $O_2$ through an imaginary stagnant layer (the $z$ layer) that separates the solution from the overlaying atmosphere. The model assumes that the bulk of solution is mixed. The thickness of the $z$ layer is a function of wind velocity and/or stirring rate of the solution.[10] The solution of differential eq. (3) is an exponential function:

$$DO(t) = [DO(0) - A] \exp\left(-\frac{D_{O_2}}{z\,H}t\right) + A \quad (3)$$

$$A = DO(atm) + \frac{z\,H}{D_{O_2}}F_{ph}$$

This equation predicts an exponential increase in DO with a time constant $(D_{O_2}/z\,H)^{-1}$ and a final value $A$. This final value may not be reached if the supersaturation level is high enough to permit gas bubble formation.[1,11] In this case, $O_2$ release will limit the DO level to the critical value at which bubbles are formed. Furthermore, the final value is a function of the oxygen transfer coefficient $(D_{O_2}/z)$ which could vary during an experimental run. Consequently, DO at steady state may not be a good measure of the photosynthetic rate.

If the system is perturbated from steady state by a forced excitation that changes DO from its dynamic equilibrium value, DO will also follow an exponential path when returning to its quasi-steady-state value. This transient response can then be used to evaluate the parameter of interest $(F_{ph})$ by fitting the transient DO data to the model of eq. (2). The equation can be written as a linear equation of the form:

$$y = ax + b \quad (4)$$

where

$$y = \frac{dDO(t)}{dt};\ x = DO(t) - DO\ (atm);\ a = -\frac{D_{O_2}}{zH}$$

and

$$b = F_{ph}$$

The newly defined variables $(x,y)$ can be directly calculated from the DO($t$) data during the transient response, and the parameters $a$ and $b$ can then be estimated by a linear least-square-fitting algorithm.[12] Once the estimates $\hat{a}$ and $\hat{b}$ are obtained, they can be used to derive the estimated values for both $F_{ph}$ and $(D_{O_2}/x)$. It should be noted that the proposed method yields an independent estimate for the gas exchange factor $(D_{O_2}/z)$, which can then be used to calculate the estimated fluxes of both $O_2$ and $CO_2$.[13]

The present proposed method for $F_{ph}$ estimation is similar in many respects to the method for $BOD_5$ prediction suggested earlier.[14] It was shown previously that data noise could be a problem since differentiation considerably amplifies high-frequency noise. It was demonstrated that the signal-to-noise corruption can be reduced by filtering the raw data by a digital filter[15] and by using a numerical differentiation algorithm with an inherent smoothing feature.[14] These precautions must also be taken here to reduce, as much as possible, noise-induced errors.

Another factor that might affect the accuracy of estimation is the quality of the DO data which was obtained, in the present study, with a Clark type membrane-covered electrode.[16–18] This effect can be evaluated by examining the dependence of the model estimation of the electrode's response. The calibration curve of the electrode can be represented by

$$DO = SI + B \quad (5)$$

where $I$ is the output of DO electrode; S is the slope; and B is the offset (or bias).

Assuming that DO (atm) is obtained by registering the electrode's response in air-saturated solution, eq. (2) can be rewritten as a function of current, $I$,

$$S\frac{dI}{dt} = -\frac{D_{O_2}S}{z\,H}[I - I(atm)] + F_{ph} \quad (6)$$

from which it is clear that the accuracy of $F_{ph}$ estimation is linearly dependent on the accuracy of $S$; that is, a 1% error will result in a corresponding 1% error in $\hat{F}_{ph}$. It can be further deduced that the estimate of $D_{O_2}/z\,H$ is not dependent on the accuracy of $S$. The preceeding analysis suggests that an offset error (an error in $B$) in the calibration of the electrode will not affect the accuracy of estimation. This can be intuitively explained by the fact that the model of eq. (2) relates the first derivative of DO to the deviation from equilibrium with respect to the atmosphere. Since both of these terms are independent of $B$, the estimate is insensitive to an error in the electrode's bias.

The error analysis given above assumes a steady (non-time-dependent) error in $S$ and $B$. Real DO electrode exhibits a drift in $B$ and a change in slope $S$ with time. It is thus obvious that the accuracy of the estimated values of $F_{ph}$ and $D_{O_2}/z\,H$ is a function of the stability of the DO sensor used.[18]

## EXPERIMENTAL

### Instrumentation

The present study was carried out by the instrumentation system described in detail elsewhere[1] and, for the sake of brevity, only a general description is given here. The experimental assembly consists of a minipond that is submerged in a thermostatic bath which was in turn regulated by a dip type thermostat. The necessary stirring of the water (to prevent settling of algae) was provided by a motor-driven paddle. An independent level control regulates water height (and volume) by automatically replenishing with deionized water the volume lost by evaporation.

The parameters measured in the present study were pH, dissolved oxygen (DO), optical density (OD), light intensity, and water and air temperatures. The electrode's signal was sent to a microcomputer via a general purpose interface/controller which has been previously described.[19] The interface comprises 16 high-impedance analog inputs, 16 digital input/output (I/O) lines, and 16 control relays. The interface/controller is connected to the microcomputer via one 8-bit I/O port plus an additional edge-sensitive input line. In the present study, we have used a Commodore model CBM3032 microcomputer which includes one free port of a Versatile Interface Adaptor (VIA) type 6522. Analog-to-digital conversion is obtained by first converting the analog signal to a proportional frequency signal and then counting the frequency pulses over a fixed period. The advantages of this conversion method are not only its low cost but also its ability to attenuate interfering noise by the inherent integration operation. The major disadvantage of the method is the low conversion rate, about one sample per second (depending on the required resolution). However, this was not proved to be a problem since the rate of change of the phenomenon under study is rather slow (time constants of hours).

### Algae and Growth Conditions

The algae *Spirulina platensis* was cultivated in a Zarouk[7] solution at ca. 35°C with an average light intensity of ca. 2500 lux. The light source was an array of four fluorescence tubes (Cool White 18 W, Osram). The media was harvested by dilution with fresh Zarouk solutions when an OD of ca. 0.45 was reached. Dilution was carried out by adding the fresh feed solution and discarding the overflow.

The surface area of the minipond was ca. 1000 $cm^2$. The depth of water was ca. 7 cm. The media were gently stirred by a paddle type stirrer with an effective (solution immersed) paddle area of 25 $cm^2$ (for each of the two vanes).

### Analytical Methods

The parameters, dry weight, chlorophyll, $PO_4$, and $NO_3$, were measured on water samples taken from the culture twice a week. Dry weight and chlorophyll were measured as described by Vonshak and Maske.[7] $PO_4$ and $NO_3$ were analyzed spectrophotometrically.[20] Light attenuation of the samples was measured by a Klett–Summerson colorimeter, model 3074-A10, using a green filter. These analyses were performed at the Sde Boker campus of the Ben-Gurion University and required transportation of the samples to a distance of ca. 50 km. Consequently, there was a delay of a few hours, and sometimes a few days between sampling and analysis. The OD was also measured in the laboratory with a spectrophotometer (Spectronic 21, Bausch & Lomb) at 560 nm.

### DO Perturbation

Application of the proposed algorithm for estimation of OPR ($F_{ph}$) was made possible by monitoring the transient in DO concentration following an induced change in DO level. This change was obtained by bubbling air, and in some cases $CO_2$ through the minipond solution and hence accelerating the rate at which $O_2$ was released from the solution to the atmosphere. The bubbling was carried out for ca. 45 min after which normal operation was resumed. Sampling rate during the transient period was one sample every three min.

### Data Processing

Data processing was carried out off-line on the recorded data. However, there is no objective problem in carrying out most analysis on-line, including the algorithm for $F_{ph}$ estimation. (This technical modification has been included in the software and the calculation is presently performed on-line.) All data processing programs were written in BASIC and the analysis was performed on a microcomputer system similar to the one used for this monitoring operation.[1]

## RESULTS AND DISCUSSION

The experiments of the present study were conducted for a period of 54 days. During that period, performance of the culture was continuously monitored by the computerized system.[1] Oxygen production rate was measured by the proposed method at least once a day, during the investigation period. A typical response of the system to the perturbation, caused by forced air bubbling, is depicted in Figure 1. Air bubbles, which were induced for about 45 min, lowered the degree of $O_2$ saturation from 114% to ca. 109%. Once the bubbling was stopped (at 1 h from time zero), oxygen supersaturation increased exponentially toward a higher degree of supersaturation. This increase in dissolved oxygen concentration was obviously related to oxygen production, through the photosynthesis process but must have been also affected by $O_2$ loss to the at-

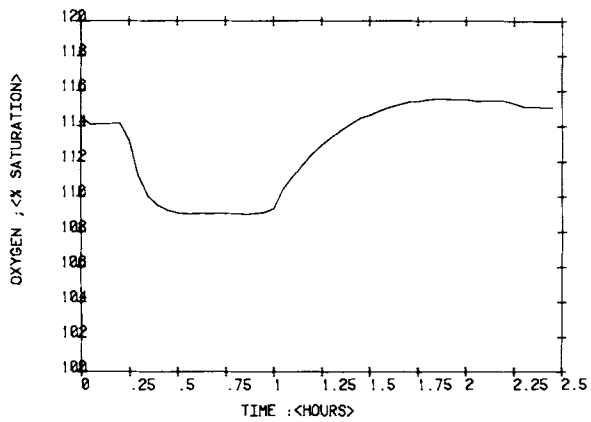


**Figure 1.** Typical DO response in minipond to 45 min of air bubbling.

mosphere. These two parameters, OPR ($F_{ph}$) and the gas exchange coefficient ($D_{O_2}/z$) were evaluated for each air bubbling experiment for the period of investigation. The results of these estimates as well as other pertinent data that was collected during this study are given in Table I and Figures 2 and 3. The correlation coefficient given in Table I provides a measure of goodness of fit of the transient DO data, to the model of eq. (2). It is evident that fitting statistics was good for most experiments, as the majority of the correlation coefficients are above 0.9. The fitting statistics through the $t$ distribution was also used for setting the limits of accuracy for each estimation. On the average, the accuracy of estimation ($\pm$ $2\sigma$) was between 10 and 15%. The estimated $D_{O_2}/z$ values were in the range 0.3–0.6 cm/min, which implies that the stagnant film ($z$) thickness was in the range 21.2–42.4 $\mu$m if we assume that $D_{O_2}$ is ca. $2.12 \times 10^{-5}$ cm$^2$/s.[13]

The behavior of the culture in the minipond system as monitored by the computerized system is depicted in Figure 2. The sharp drops in OD were caused by the harvesting process in which fresh feed solutions were added to the minipond, discarding the over-flow. It should be noted that some abnormal behavior was detected beyond the 45th day of the experiments. [Fig. 2(a)]. During the last 10 days, the OD remained virtually constant, suggesting the onset of a change in the growth process. After the 54th day, the ecosystem collapsed and the algae died. Sample examination under the microscope did not reveal any noticeable change in the appearance of the *Spirulina* until total collapse occurred at the 54th day.

The pH of minipond's solution [Fig. 2(b)] was found to be from pH 9.5 to pH 10. It dropped with each dilution and increased again at about a linear rate. The increase is associated with a reduction of total dissolved $CO_2$ which is consumed during the photosynthetic process.[8] A detailed analysis of the carbonate system in the minipond will be given elsewhere.

The estimated value of $O_2$ exchange coefficient $D_{O_2}/z$ [Fig. 2(d)] was found to fluctuate at ca. 0.4 cm/min. The fluctuation could be a result of measurement inaccuracy or stirring rate fluctuations, although possible dependence on the minipond conditions (such as the concentration of organic surfactants) should not be ruled out. It should be noted, however, that the average value of $D_{O_2}/z$ did not change markedly past the 45th day. This might be an indication that the $O_2$ gas exchange process is controlled by physical parameters such as the stirring rate, and is not affected by the presence of active algae.

The values of the oxygen production rates as obtained by the new method [Fig. 2(e)] seem to provide a very effective means for assessing the vitality of the algae in the minipond. The $O_2$ production rate was found to fluctuate around the value of about 0.4 $\mu$mol/L min from the 1st day to the 35th day of experiments. From then we observed a steady decrease in the OPR toward negative values which are indicative of respiration rather than photosynthesis. None of the other parameters we measured nor the examination of samples from the culture under the microscope revealed at that early stage that anything went wrong with the culture. The first signs of deterioration were observed in the OD date at the 45th day of experiments, about 10 days after the OPR decrease was observed.

Examination of other parameters analyzed by non-automatic methods (Fig. 3) revealed that these variables also failed to show any early warning signs except for chlorophyll [Fig. 3(b)] which seems to exhibit a general trend of decrease. This, however, could be an artifact, stemming from the noncontinuity of the data and the relatively few data points available. At any rate, even this parameter failed to provide any positive indication that the behavior had changed after the 35th day. It was also found that the quality of the data obtained by the automatic system was more reliable than the data of the conventionally analyzed parameters, such as dry weight or chlorophyll. Nonetheless, despite the relatively high scatter,[21] the dry weight, light attenuation, and chlorophyll data were found to be consistent with each other.

The $O_2$ production rate parameter (OPR) differs from all other minipond parameters, which are usually used as indicators for biological processes, in at least one major respect. Unlike all other parameters, the OPR gives an indication of the efficiency of the photosynthetic process at the instant of measurement. All other parameters, such as OD, provide a measure of past or potential (not necessarily actual) photosynthetic rates. Hence, growth rate can be deduced from these parameters only if two measurements are taken at a wide enough time space to provide information on the average growth rate. That is, the growth rate can be deduced from chlorophyll concentration or dry weight by differentiating the measured parameter with respect to time. This requirement is a severe limitation for monitoring the performance of algal culture, considering the relatively high noise level in such measurements, the relatively low sampling rate that can be realized

**Table I.** Experimental results.

| Day | $D_{O2}/z$[a] (cm/min) | OPR[a] (μmol/L min) | OD (560 nm) | pH | Correlation coefficient, $R^2$ | Dissolved oxygen Calculated (% saturation) | Dissolved oxygen Measured (% saturation) |
|---|---|---|---|---|---|---|---|
| 1 | 0.427 ± 0.178 | 3.62 ± 1.505 | 0.276 | 9.91 | 0.7238 | 128.56 | 135 |
| 2 | 0.333 ± 0.052 | 3.06 ± 0.446 | 0.292 | 9.83 | 0.8951 | 131 | 133 |
| 3 | 0.353 ± 0.023 | 3.13 ± 0.196 | 0.322 | 9.85 | 0.9791 | 129.96 | 130 |
| 5 | 0.438 ± 0.094 | 3.50 ± 0.725 | 0.39 | 9.92 | 0.8221 | 126.90 | 127 |
| 7 | 0.436 ± 0.077 | 3.40 ± 0.571 | 0.434 | 9.88 | 0.8708 | 126.32 | 128 |
| 8 | 0.315 ± 0.026 | 2.78 ± 0.205 | 0.462 | 9.94 | 0.9848 | 129.71 | 127 |
| 9 | 0.642 ± 0.087 | 4.69 ± 0.594 | 0.46 | 9.91 | 0.9613 | 124.67 | 128 |
| 9[b] | 0.606 ± 0.060 | 4.07 ± 0.376 | 0.28 | 9.32 | 0.9787 | 122.67 | 128 |
| 10 | 0.600 ± 0.070 | 3.40 ± 0.365 | 0.29 | 9.47 | 0.9708 | 119.10 | 125 |
| 11 | 0.605 ± 0.059 | 2.89 ± 0.270 | 0.322 | 9.65 | 0.9588 | 116.11 | 115 |
| 12 | 0.571 ± 0.150 | 5.31 ± 0.437 | 0.35 | 9.73 | 0.7633 | 131.35 | 135 |
| 13 | 0.582 ± 0.051 | 4.74 ± 0.404 | 0.4 | 9.84 | 0.9663 | 127.45 | 130 |
| 14 | 0.396 ± 0.039 | 3.39 ± 0.306 | 0.41 | 9.87 | 0.9793 | 128.90 | 125 |
| 15 | 0.631 ± 0.096 | 4.83 ± 0.745 | 0.35 | 9.57 | 0.9456 | 125.82 | 127 |
| 17 | 0.369 ± 0.093 | 3.50 ± 0.794 | 0.41 | 9.82 | 0.8789 | 131.94 | 130 |
| 18 | 0.585 ± 0.084 | 4.87 ± 0.683 | 0.443 | 9.87 | 0.9112 | 128.03 | 128 |
| 19 | 0.406 ± 0.045 | 3.27 ± 0.341 | 0.305 | 9.43 | 0.9459 | 127.20 | 130 |
| 21 | 0.477 ± 0.113 | 4.38 ± 0.987 | 0.325 | 9.75 | 0.7894 | 130.94 | 133 |
| 23 | 0.474 ± 0.189 | 3.79 ± 1.49 | 0.38 | 9.98 | 0.5697 | 126.96 | 130 |
| 24 | 0.758 ± 0.096 | 5.47 ± 0.685 | 0.385 | 9.96 | 0.9289 | 124.32 | 125 |
| 25 | 0.150 ± 0.055 | 1.45 ± 0.435 | 0.387 | 9.98 | 0.6130 | 132.5 | — |
| 26 | 0.421 ± 0.081 | 3.57 ± 0.628 | 0.275 | 9.6 | 0.9246 | 128.58 | 125 |
| 27 | 0.533 ± 0.132 | 3.68 ± 0.880 | 0.31 | 9.82 | 0.8811 | 123.30 | 125 |
| 28 | 0.536 ± 0.104 | 3.90 ± 0.716 | 0.32 | 9.85 | 0.9490 | 124.57 | — |
| 29 | 0.484 ± 0.087 | 3.42 ± 0.566 | 0.335 | 9.94 | 0.9325 | 123.82 | — |
| 30 | 0.307 ± 0.080 | 2.55 ± 0.635 | 0.15 | 9.52 | 0.7548 | 127.92 | 127 |
| 31 | 0.451 ± 0.075 | 3.76 ± 0.618 | 0.18 | 9.63 | 0.8822 | 128.09 | 127 |
| 33 | 0.483 ± 0.119 | 4.17 ± 1.013 | 0.26 | 9.88 | 0.8809 | 129.11 | 130 |
| 34 | 0.432 ± 0.072 | 3.62 ± 0.586 | 0.29 | 9.98 | 0.9418 | 128.27 | 128 |
| 35 | 0.355 ± 0.047 | 3.13 ± 0.399 | 0.285 | 9.95 | 0.9219 | 129.74 | 130 |
| 36 | 0.382 ± 0.052 | 3.15 ± 0.408 | 0.305 | 10 | 0.9206 | 127.79 | 128 |
| 37 | 0.474 ± 0.022 | 2.37 ± 0.140 | 0.33 | 7.2 | 0.9892 | 117.17 | 120 |
| 38 | 0.230 ± 0.075 | 1.73 ± 0.516 | 0.37 | 9.43 | 0.8673 | 125.33 | 123 |
| 39 | 0.309 ± 0.039 | 1.90 ± 0.223 | 0.41 | 9.68 | 0.9298 | 120.73 | 120 |
| 39[b] | 0.507 ± 0.027 | 1.71 ± 0.086 | 0.2 | 9.27 | 0.9868 | 111.36 | 112 |
| 40 | 0.321 ± 0.041 | 1.51 ± 0.178 | 0.235 | 9.58 | 0.9277 | 115.87 | 115 |
| 42 | 0.307 ± 0.036 | 1.44 ± 0.046 | 0.28 | 9.9 | 0.9962 | 115.80 | 115 |
| 43 | 0.436 ± 0.009 | 2.34 ± 0.144 | 0.31 | 9 | 0.9979 | 118.10 | 118 |
| 44 | 0.656 ± 0.033 | 1.73 ± 0.165 | 0.32 | 9.6 | 0.9834 | 118.90 | 118 |
| 45 | 0.408 ± 0.077 | 2.01 ± 0.352 | 0.345 | 9.8 | 0.8559 | 116.65 | — |
| 47 | 0.416 ± 0.031 | 1.08 ± 0.07 | 0.23 | 9.64 | 0.9743 | 108.77 | 108 |
| 49 | 0.390 ± 0.026 | 1.02 ± 0.059 | 0.255 | 9.48 | 0.9898 | 108.82 | 108 |
| 50 | 0.468 ± 0.023 | 0.91 ± 0.036 | 0.255 | 9.6 | 0.9942 | 106.58 | 106 |
| 51 | 0.194 ± 0.016 | 0.41 ± 0.025 | 0.255 | 9.7 | 0.9696 | 107.19 | — |
| 52 | 0.431 ± 0.111 | 0.14 ± 0.106 | 0.255 | 9.7 | 0.8118 | 101.09 | 101 |
| 54 | 0.516 ± 0.011 | −0.92 ± 0.082 | 0.14 | 9.6 | 0.9978 | 99.5 | 99 |

[a] Limits are ± $2\sigma$ based on $t$ distribution.
[b] This is after dilution with Zarouk medium.

and, consequently, the very long time that it might take to obtain a good estimate of the derivative. In the case of the proposed OPR measurement method, the parameter is calculated by a statistical procedure (linear least-square fitting) on many data points (DO measurement) taken during a short transient response of the system. Another major advantage of the method is that it can be fully automated, thereby increasing the reliability of measurement and in most cases reducing the analysis cost.

The OPR parameter can be correlated with other parameters by either differentiating the other parameters or integrating the OPR. Since the former is undesirable for reasons already mentioned, we shall proceed by relating the integral of $O_2$ production rate, i.e. cumulative $O_2$ production, to OD as monitored by the

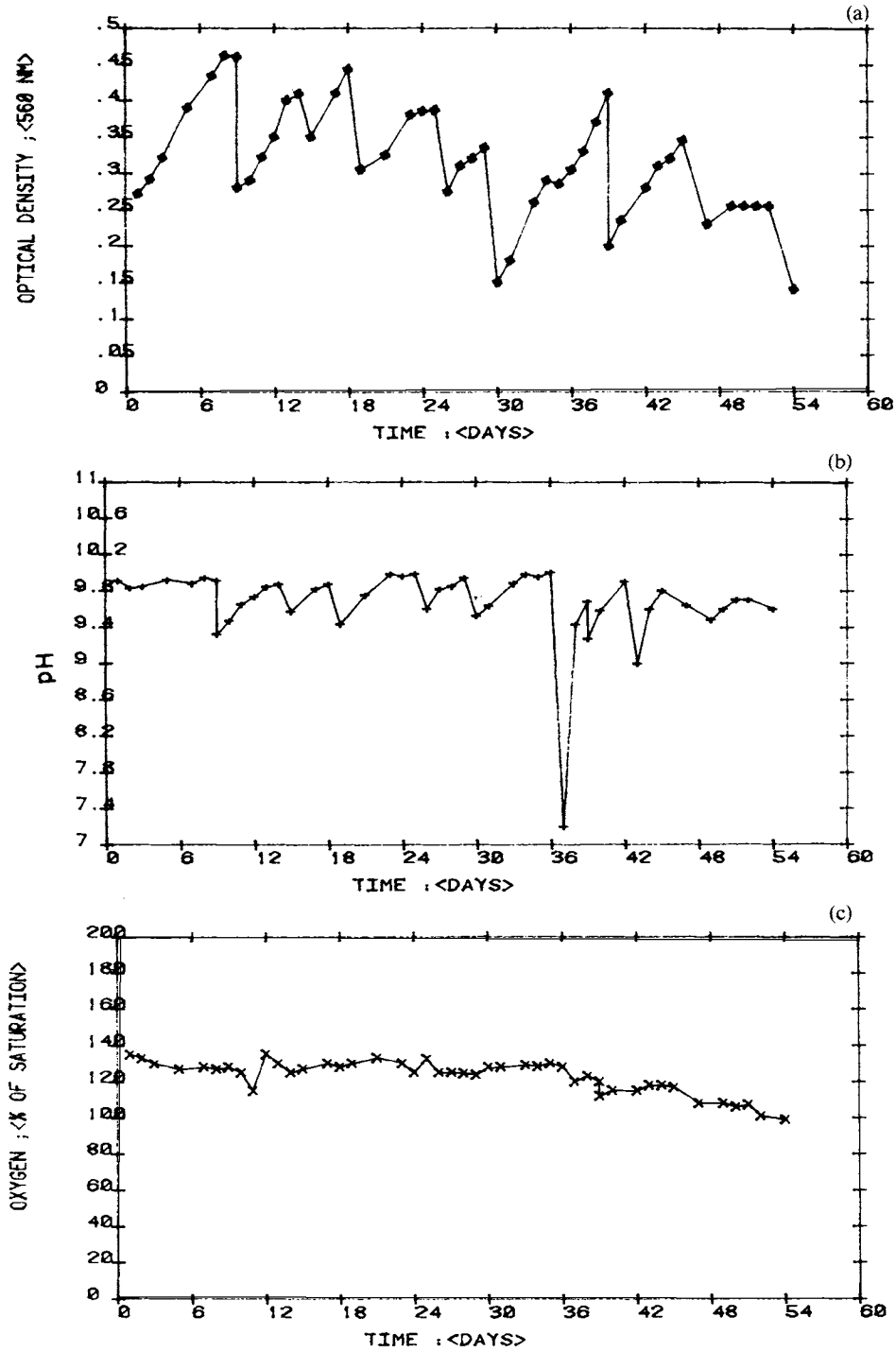


**Figure 2.** Data for (a) optical density, (b) pH, (c) dissolved oxygen, (d) $D_{O_2}/z$, and (e) OPR as measured on-line in algal minipond. The relative large drop in pH at the 37th day was due to a $CO_2$ bubbling experiment.

present computerized system. Assuming a constant growth condition and one algal species, the biomass concentration in the minipond at a given time, $t_i$, should be related to the $O_2$ production rate, $F_{ph}$, by:

$$C(t_i) = K_4 \int_0^{t_i} F_{ph}\, dt \tag{7}$$

where $K_4$ is a constant whose value depends on the average C:N:P ratio of the algae. Assuming that OD $(t)$ is linear with $C(t)$:

$$\mathrm{OD}(t_i) = K_5 \int_0^{t_i} F_{ph}\, dt \tag{8}$$

This functional relationship is plotted in Figure 4 for the period of the present experiments. The plot is composed of segments which exhibit each a linear

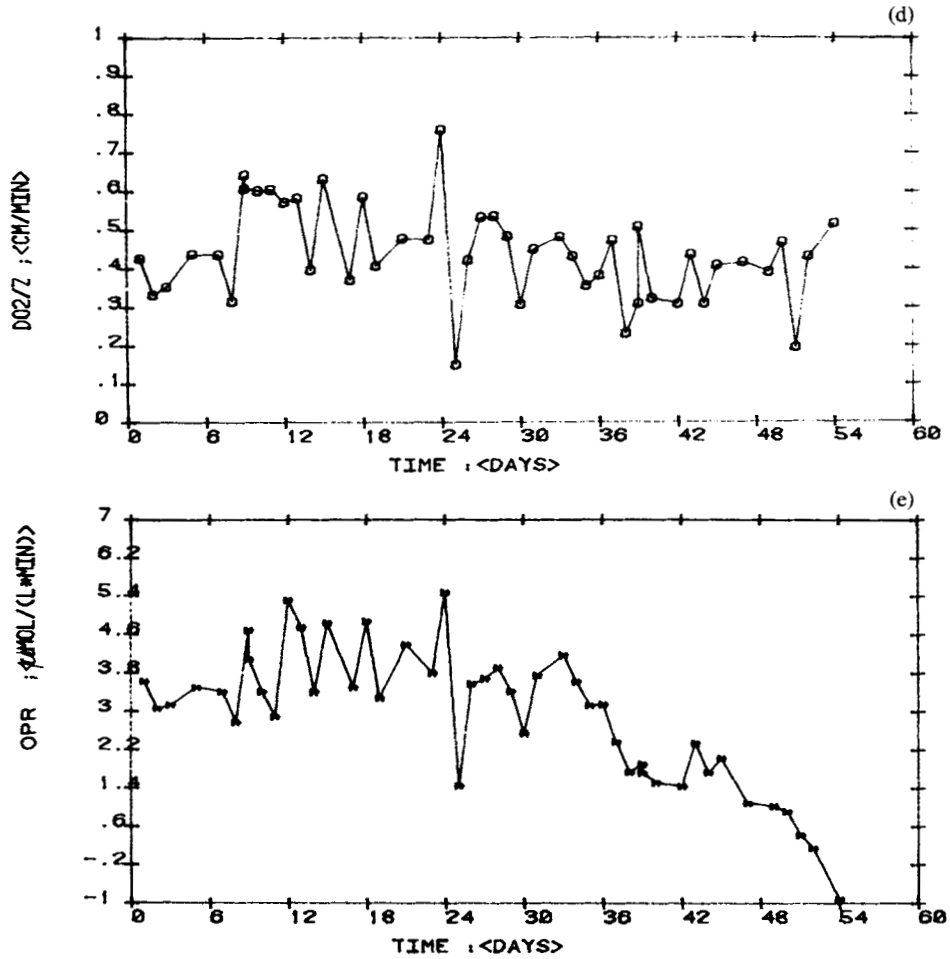


**Figure 2.** Continued.

trend, as expected. The segmentation is caused by the harvesting episodes which cause a sudden dilution, and hence a sudden decrease in OD. Eliminating these drops in OD and plotting the segments as a continuous curve (Fig. 5), an uninterrupted curve, which is simpler for visual examination, is produced. The relationship for the present experimental period is found not to be constant. Although the expected linear relationship is observed for most of the period, a markedly different behavior is detected from day 35 and on. This change in regularity is well correlated with the period at which a deterioration process commenced [Fig. 2(d)]. It is

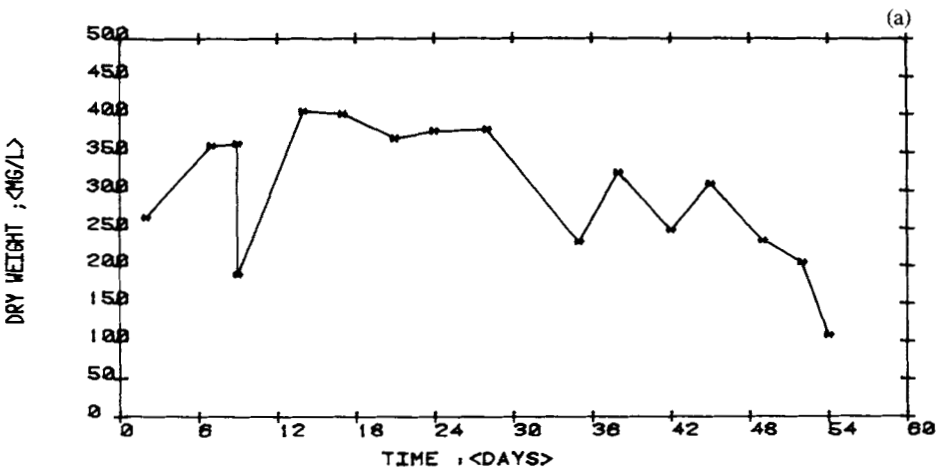


**Figure 3.** Data for (a) dry weight, (b) chlorophyll, (c) nitrate, and (d) phosphate in algal minipond samples.

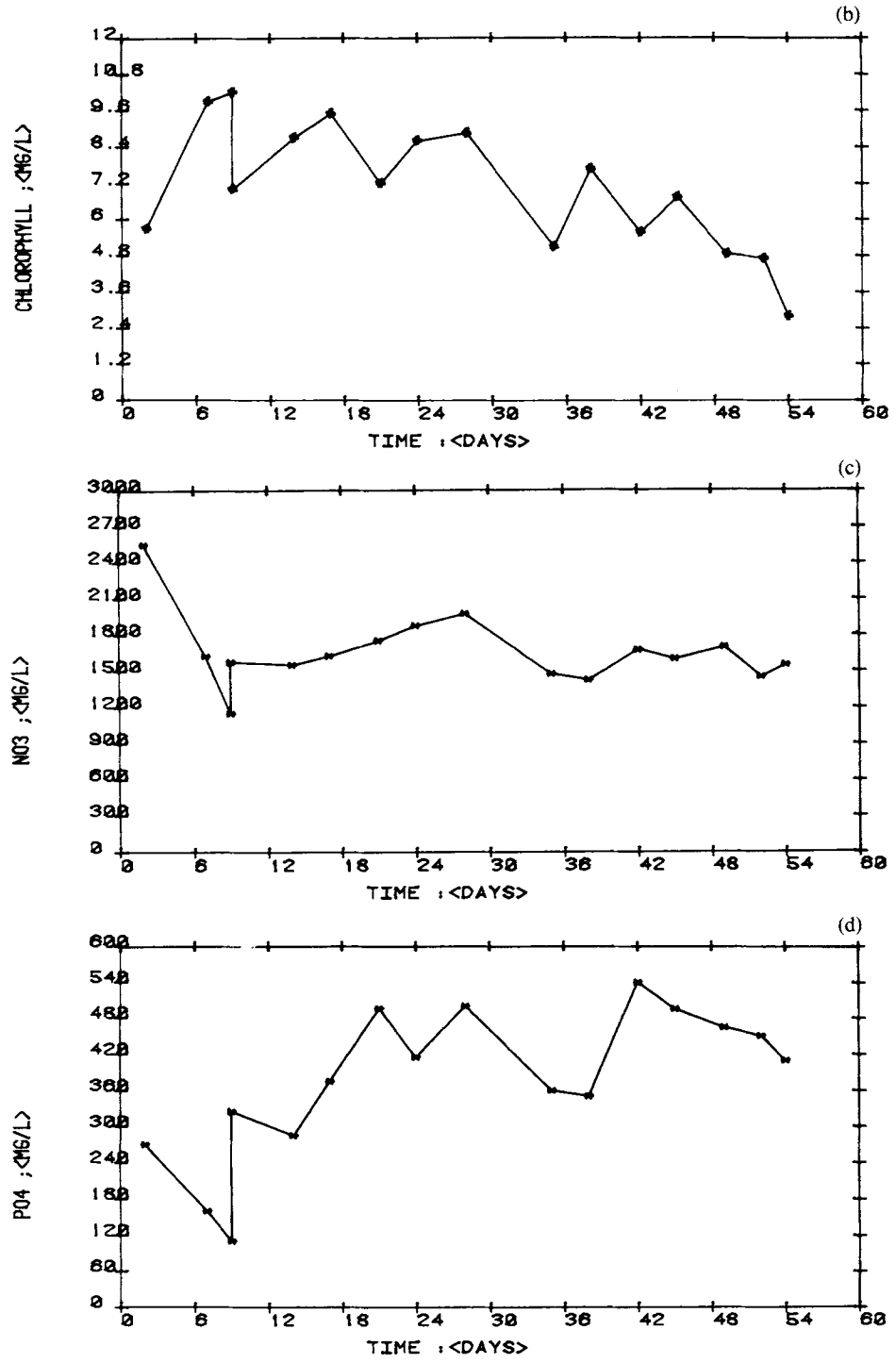


**Figure 3.** Continued.

of interest to note that the average increase in OD per mole of $O_2$ production (or per a given amount of $CO_2$ assimilation) is higher beyond that point. This relatively high increase rate in OD is obviously not associated with an accelerated increase in algal biomass, since the culture was on the verge of a collapse. Rather, the faster OD increase was probably due to some factor that either changed the optical characteristic of the algae or perhaps of the solution, such as bacterial blooming. This question, however, will have to remain open until more experiments are performed to elucidate deterioration process in continuous algal culture maintained for several weeks at a time.

The theoretical analyses presented here and supported by the experimental results of this study may be applied in a number of ways. For monitoring and data acquisition, the models provide the theoretical basis for evaluating the performance of the algal culture and estimating its vitality through the OPR parameter. The models, however, can be used not only for ex-

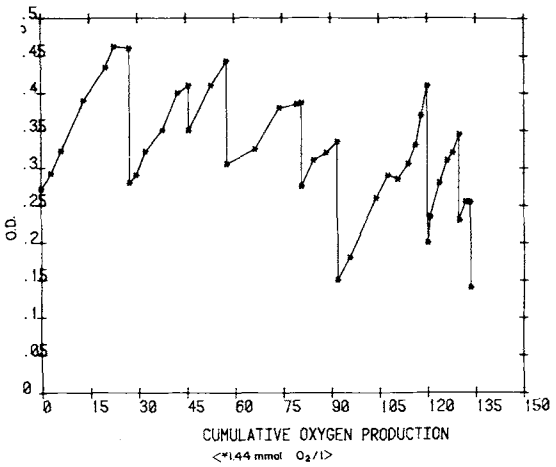


**Figure 4.** Optical density vs. integral of OPR in minipond for the period of investigation.

amining the operation of a culture at any given instance but also to predict its future performance. This could have been accomplished by first estimating the basic parameters of the system such as $F_{ph}$ or $D_{O_2}/z$ [eq. (2)] and then running the computer simulation to predict future performance. Using this approach the model of eq. (3) can be used to predict the expected degree of $O_2$ supersaturation in the minipond. The agreement between the predicted and measured values (Table I) is good, well within the experimental accuracy. The agreement should not be as good if an appreciable amount of $O_2$ is released to the atmosphere through the mechanism of bubble formation.[9,11]

## CONCLUSIONS

Among the possible methods for estimating growth in an algal culture, the proposed $O_2$ producion rate (OPR) measurement was found to be the most reliable, accurate, and versatile. The method can be easily automated and is therefore useful for continuous monitoring and control operation. The method could be invaluable not only in the commercial plant but also in the research laboratory, providing the investigator with a tool for assessing on-line the general performance of the system. Integrating of this method into an optimization algorithm could markedly aid a search for optimal growth condition of many photosynthetic organisms. This could eventually lead to the development of "smart" controllers with a built in ability to seek the most economical growth conditions.

Application of the proposed computerized system[1] and models can provide investigators with a new insight into the biological process of algal cultures. The proposed model and measurement method could be used to estimate a number of parameters which influence and control growth and development. For example, the model can be used to estimate the $O_2$ exchange between solution and atmosphere as well as $CO_2$ exchange between the two phases.[13,22] This information could then be used to calculate the amount of carbon that is contributed by diffusion from the atmosphere. It can thus be concluded that the proposed hardware and software approach has high potentialities in the study and control of biotechnological systems.

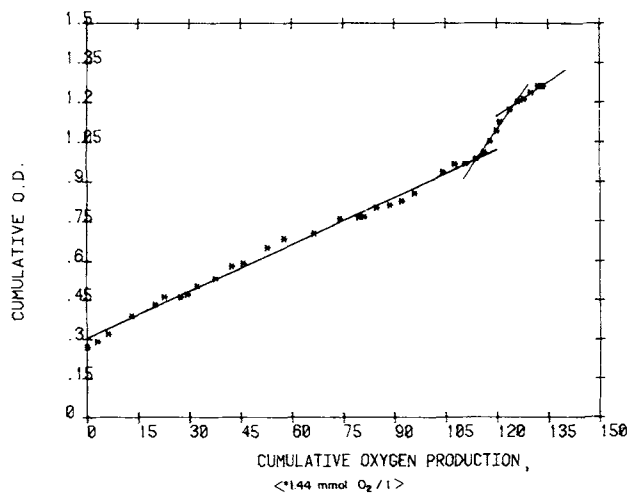


**Figure 5.** Same as Figure 4 but corrected for decrease in OD due to harvesting dilution.

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