On-Line Optimization of Biotechnological Processes: I. Application to Open Algal Pond

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A new on-line optimization and control procedure applicable to biotechnological systems for which a precise mathematical model is unavailable has been developed and tested. The proposed approach is based on an on-line search for optimum operating conditions by an automatic system using a modified simplex algorithm to which several features have been added to permit real time operation. The simplex algorithm is the upper level of a hierarchical software package in which the other levels are cost evaluation, control, data acquisition, and signal processing. The optimization method was tested in a laboratory minipond for the cultivation of *Spirulina platensis.* The controlled parameters were light intensity, optical density, pH, and temperature. The proposed optimization method can be applied to other biological processes provided that the pertinent variables can be measured and controlled and the cost function can be defined mathematically.

INTRODUCTION

A common goal of biotechnological research and of commercial production is the definition of optimum conditions for achieving predetermined objectives. This goal is usually translated into the problem of finding the optimum growth conditions that will produce the desired end product at the lowest cost. This quest for optimization is an essential requirement in a variety of biotechnological processes ranging from fermentation to algal and fish production. In all cases, the objective is to provide the appropriate chemical and physical environment required for the growth of the organism while keeping the operating costs at a minimum.[1-5]

Different approaches have been used in the pursuit of this optimization goal. Historically, the first of these was the trial-and-error approach, in which the best combination of operational parameters for a given process is isolated. When this approach is applied manually, it is generally reduced to the search for the optimum conditions by changing one, or at most two, of the variables at a time. For more than two variables, especially when there are interactions between them, other approaches must be tried. Control theory[6-8] provides more sophisticated solutions to this problem, and several researchers have applied optimal control solutions to biotechnological processes. The general method used was to find the optimal growth conditions for a proposed deterministic model of the plant process.[1-4,9,10] For the cases where the system parameters are not constant, the adaptive control theory can offer some solutions, provided that the problem can be reduced to a simple linear model with continuously changing parameters. In such cases, adaptive control can estimate the parameters of the model and "tune up" the controller of the system.[11-13]

Although these approaches are widely used, they both rely on a priori knowledge of the model of the system and its interaction with the environment. However, biological systems are inherently nonlinear, and all the intimate details of their mathematical model are usually unknown. Even in the cases of classical fermentation processes, such as baker's yeast or penicillin production,[10,14-16] optimal conditions are often searched by simulation, which in most cases assume simple Monod or Monod-type models. It should also be emphasized that parameter estimation, which is a necessary step in adaptive control, may not be feasible in many practical cases due to noise, nonlinearity, and time dependence of the process.

Other approaches — like the application of statistical procedures to the control of yeast production[17] or the use of fuzzy control theory to glutamic acid fermentation,[18] response surface techniques,[19] and factorial designs[20] — have been suggested as optimization techniques. However, the basic question of how to find, in a minimum number of experiments, the optimum operating and control conditions of biological systems, when there is only minimum a priori knowledge of the process is still open.

In a recent paper[21] we demonstrated the feasibility of employing on-line a direct optimum search method which does not require a priori knowledge of the mathematical model of the biological system. As this method does not rely on the use of derivatives of the functions, it avoids the mathematical complications of other optimization methods[22] by reducing the critical problem of data noise which is amplified by derivation.[23,24] Several direct search methods, such as the method of Hooke and Jeeves or the pattern search

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method, the Rosenbrock method of rotating coordinates, and the simplex and complex methods, have previously been described.[25,26] The later two have been applied in chemistry and biotechnology. The complex method was used by Saguy[27] to optimize a fermentation process simulated by JERMFERM.[28] Nakai[29] compared different optimization methods in food production and demonstrated the effectiveness of the simplex method in terms of the small number of iterations required. Nyests et al.[30] applied the simplex method developed by Nelder-Mead for on-line optimization of fermentation processes. Other examples of modeling of fermentation processes by factorial analysis, surface methodology, and off-line optimization of processes by simplex or complex methods in wastewater treatment can be found elsewhere.[31,32]

The object of this study was to investigate the feasibility of implementing on-line optimization and control of biological processes by a dedicated hardware–software system. Today, such a system can be cost-effective as a result of the availability of low-cost microcomputers. The proposed system can be used to optimize the operating conditions of a given process by tuning the controlling variables according to a predetermined criterion, while only minimal a priori knowledge of the mathematical model of the system is required.

DESCRIPTION OF THE OPTIMIZATION METHOD

An optimization method to be used in biotechnology should fulfill several conditions in order to be useful in these kinds of systems.

A. The objective function can be defined in simple terms that have a clear meaning to the user. For example, it can be defined as

$$F(\text{cost}) = \frac{\text{cost}}{\text{production}} \quad (1)$$

B. The number of experiments (steps) required to arrive at a minimum or near a minimum of the evaluated function should be small.

C. The objective of the optimization is to arrive as quickly as possible at or near the optimum neighborhood and to stay in that zone as long as possible.

D. The optimization method should be capable of functioning well in a situation of a poor signal-to-noise ratio as might be encountered in real biotechnological systems and should have a certain capability of recovery from erroneous function evaluation.

E. The search method should be capable of solving problems with nonlinear objective functions subject to nonlinear inequality constraints.

F. Variable adjustment may be either continuous or discrete.

Among the direct search methods that can fulfill these conditions, the simplex and complex methods appear to be the most promising.[25–32] However, preliminary convergence trials carried out by us demonstrated that the complex method requires an initial $2n$ experiments vs. $n + 1$ in the simplex method (where n is the number of independent control variables). Furthermore, it was found that the simplex method arrives more quickly at a quasi-optimization zone. It was therefore decided to adopt the simplex method.

Since a good description of the simplex method and its variants can be found elsewhere,[33] we give here for the sake of clarity only its basic principles and the modifications implemented. An outline of the simplex search method is given in the Appendix. A comparison between the different simplex versions will be given in a future paper.

A simplex is a geometric figure defined by $n + 1$ points, one more than the number of variables or dimensions of the space (n) defined by them. These $n + 1$ points are the vertices of the simplex, i.e., a triangle in a two-dimensional problem or a tetrahedron in a three-dimensional problem. The initial simplex is built by choosing an initial evaluation point and a regular simplex is built later by employing the method proposed by Spendly et al.[22,34] (Fig. 1). The search is carried out by replacing the polygon's vertices in a systematic way which moves the points toward the optimum zone (see Appendix).

In order to improve the convergence of the simplex under our specific operating conditions and to avoid damage to the biological process involved, several modifications were implemented:

1. The initial simplex was built to occupy most of the allowed space. It was found that this markedly enhanced the initial convergence and helped to circumvent the problem of local minima.
2. If a new vertex was found to be outside the permissible boundaries of the independent variables, the point was considered as worse than the worst simplex point (x^h), and no evaluation was performed.
3. As proposed by Morgan and Deming,[26] variables having different units of measurement (pH, temperature,

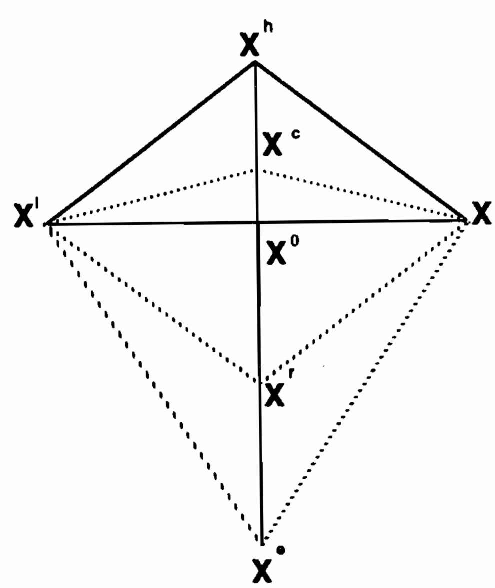

Figure 1. Schematic description of the simplex algorithm in two dimensions.

oxygen, etc.) were normalized to the range 0–1. This was performed by the algorithm in order to obtain an equal-dimensional space.

4. Function evaluation values obtained during the search which were not consistent with a predetermined criterion were assumed to be worse than the current worst value of the simplex.
5. If a point stayed as the best one for $n + 1$ simplex, then a new evaluation of the same point was performed, and the old value was replaced by the new one.
6. The present algorithm allowed us to define a minimum increment for each independent variable.
7. If the evaluated simplex volume was smaller than the volume V_{min} defined by the minimum increment in the independent variables (Δx^i),

$$V_{min} = \prod_{i=1}^{n} \Delta x^i \qquad (2)$$

a new simplex was built.[36,37] The initial evaluation point of the new simplex chosen was the worst previous point, and a regular simplex was built later by employing the method proposed by Spendly et al.[22,34] The initial step was chosen to be between 1 and 3 times the distance (d) determined by the minimum increments of the independent variables:

$$d = \left[\sum_{i=1}^{n} (\Delta x^i)^2 \right]^{1/2} \qquad (3)$$

To eliminate the possibility of oscillations, the initial angle of the simplex is randomly determined. This arrangement helped us to avoid the situation in which the simplex is reduced to a point or to points that are so near that the evaluated points are not distinguishable statistically. This also eliminated the closing off of the simplex while still far from the optimum zone.

8. The preassigned value ε [Eq. (A6)] was given a very low value that practically ensured that the conditions of Eq. (A6) would never meet. Consequently, the optimum condition search would not stop, and instead when it arrived in the optimum zone, the algorithm would continuously look for new possible optimum values and would actually act like a quasi-optimum controller.

EXPERIMENTAL

Instrumentation

The present study was carried out by an instrumentation system similar to the one described earlier.[38] As design details of the present system have already been given, only a general description of the system is presented here. The experimental assembly consisted of a minipond that was submerged in a thermostatic bath operated under computer control. The required stirring of the solution (to prevent settling of the algae) was provided by a motor-driven paddle. An independent level control regulated water height 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, light intensity, and water and air temperatures. The system was built around a low-cost personal computer (Commodore 64), a general purpose interface, and a battery-supported power supply to ensure uninterrupted operation.

Algae and Growth Conditions

The algae *Spirulina platensis* was cultivated in Zarouk's medium,[39] the temperature range was 10–40°C, and the light intensity ranged between 0 and 30 klux. The light was provided either by an array of six fluorescent tubes (Cool White 18W, Osram) or by 6 incandescent lamps (PAR 38, 150W, Tungsrapar, Hungary). Temperature problems due to heating by the incandescent lamps were obviated by using a continuous flowing water filter between the light source and the pond. The algae was harvested by dilution of the medium with fresh Zarouk medium. Dilution was carried out by adding the fresh feed solution and discharging the overflow.

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

Oxygen Production Rate Measurement

The rate of growth (yield) of the algae was determined indirectly by measuring on-line the oxygen production rate (OPR) by a previously developed method.[40] The method is based on perturbing the system from its dynamic equilibrium and examining its response to the excitation. In the present case, application of the method for estimation of OPR was made possible by monitoring the transient in the dissolved oxygen (DO) concentration following an induced change in DO level. This change was initiated by bubbling air through the pond solution and hence accelerating the rate at which oxygen was released from the solution to the atmosphere. The bubbling was carried out for 15 min, after which normal operation was resumed. Sampling rate during the transient period was one sample every 3 min. The transient data were then fitted to the model[40] by a linear least-squares fitting from which the OPR and the DO exchange rate between the medium and the atmosphere were obtained. The advantages and reliability of this method as well as its correlation with other analytical or physical methods such as optical density (OD) or dry weight have been discussed earlier.[40,41] It should be noted that while conventional methods provide a measure of the past or potential (not necessarily actual) photosynthetic rates, the OPR provides an indication of the efficiency of the photosynthetic process at the instant of measurement,[40,41] and this is the characteristic that makes it useful in control and optimization applications.

Computer Program

The monitoring, control, and optimization operation was carried out under the supervision of a hierarchical program that consists of four levels (Fig. 2). In the upper level, the simplex algorithm performed the task of data evaluation and made decisions regarding future steps. As was already noted, the simplex algorithm is independent of the nature of the process that it optimizes. In other words, the same algorithm can be used without any changes for other processes. The lower levels of the program carried out the basic data acquisition and control operation, i.e., sampling, filtering, estimation, PID or on–off control, etc. The intermediate level evaluated the cost function from the data received by the lower levels. A priori knowledge about the process can be added at this level. For example, if there already exists a partial or complete model of the plant or if some operating conditions are dangerous to the microorganism, the "cost" value can be modified accordingly. This can improve the convergence of the simplex algorithm.

RESULTS AND DISCUSSION

Data from a typical optimization run are presented in Figure 3. In this run two variables, water temperature and light intensity, were controlled. The light source was a cluster of six fluorescent tubes connected in such a way as to provide six optional levels of light intensity. In order to provide the simplex algorithm with a greater number of control levels and at the same time to test its performance in the presence of local minima, the algorithm did not change the light intensity directly. Rather, it set a digital number that was translated into a light control signal. Light intensity and the number of tubes connected as a function of the digital control are shown in Figure 4. The function obtained with this method is discontinuous with several local minima.

The production yield was estimated by the method of OPR given in units of milligrams of oxygen produced per liter per minute.[40,41] The cost function was defined as

$$F(\text{cost}) = \frac{[L \cdot K_L + \mathrm{T} \cdot K_T]}{(\mathrm{OPR} \cdot K_S)} \qquad (4)$$

where $K_L = 10^4$ and $K_T = 10^3$ are the light and temperature costs, respectively, and $K_S = 10^2$ is the sale price. The objective here was to reduce operational cost per production unit. Since the constant K_S does not affect the location of the minimum, its only contribution is as a scale factor. In this experiment, the simplex algorithm approached the optimum zone (defined as a zone around 20% of the absolute optimum point), in about five steps of function evaluations, i.e., in about $2n + 1$ function evaluations, where n is the number of independent variables. Computer simulation runs performed on Monod or Monod-type models[10,14–16] demonstrated that the present algorithm has a mean convergence rate of about $2n + 1$ to $3n + 1$ steps to approach a zone around 10–15% of the absolute optimum point (Fig. 3). During an optimization run of this study, the simplex algorithm determined, at each point, the next values of the control variables (in the present case, water temperature and light intensity). The lower level of the computer program then shifted the system to the new working conditions. Once the system arrived at the new operating conditions, it was left there to stabilize the biological process for about 2 h and only then was an OPR evaluation performed. The time required for the evaluation was about half an hour.[40] Hence, it took about 12 h to arrive at the optimum neighborhood ($2n + 1$ steps) and about 2 days to reach the optimum point. Long-term reliability and stability of the instrumentation is therefore a prerequisite for successful optimization runs of this type. The required time delay between evaluations is inherent in the process of algal growth. The delay is a func-

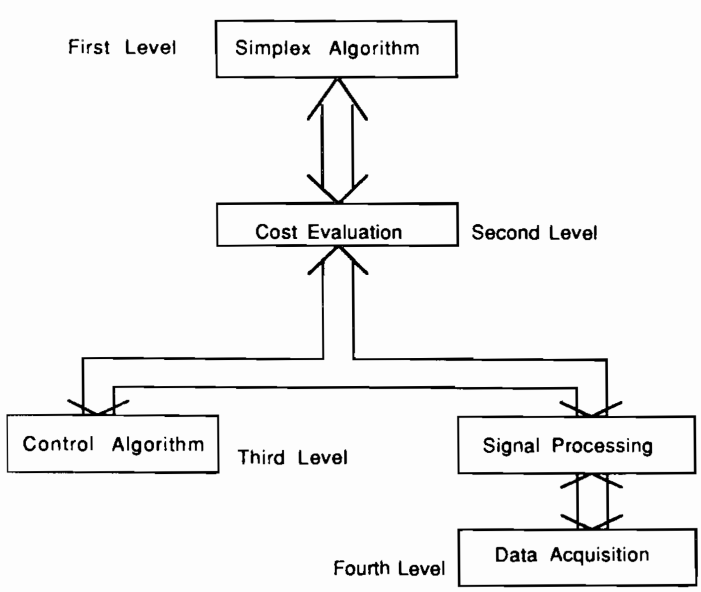

Figure 2. Hierarchical arrangement of the computer program.

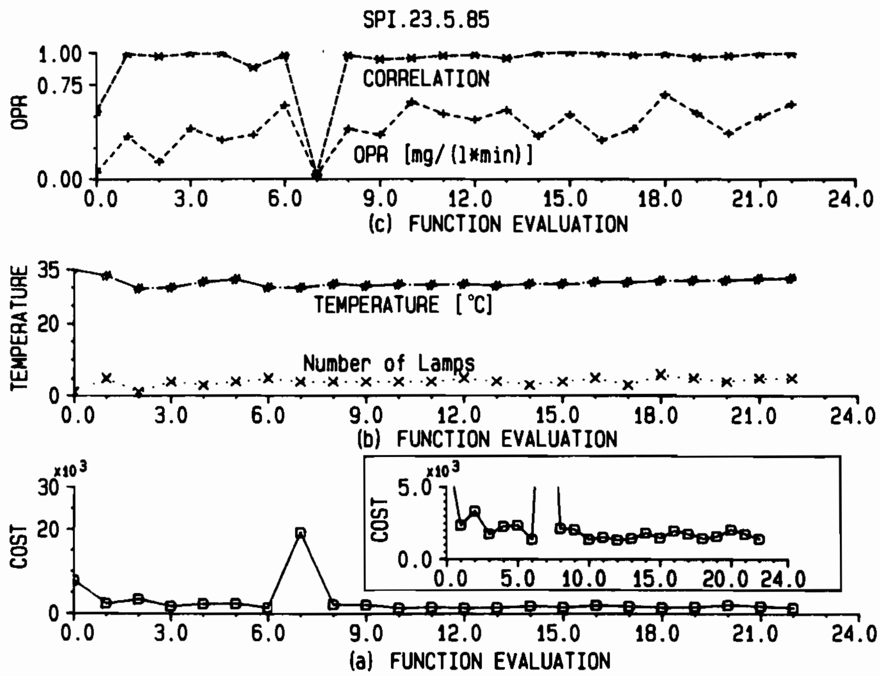

Figure 3. Optimization of a two-dimensional simplex run. (a) Cost function defined by Eq. (4). (b) Control variables: light intensity (as number of tubes operating) and water temperature. (c) OPR is the measure of growth rate; the correlation coefficient provides a measure of the goodness of fit.

tion of the system's dynamics and is a function of the type of organisms involved. Experience has shown that about 2 h recovery time is required, in the present case. Consequently, changing more than two variables in each optimization experiment was found prohibitive as a result of the very long time involved which might cause dramatic changes in the algae system.

The robustness of the present modified simplex algorithm to noise spikes can be appreciated by examining the simplex run of Figure 3. The system approached optimum operating conditions (lowest cost) after about four or five steps. From that point on, the algorithm was actually functioning as a quasi-optimal controller. A noise spike at the seventh step resulted in an erroneous value of the estimated

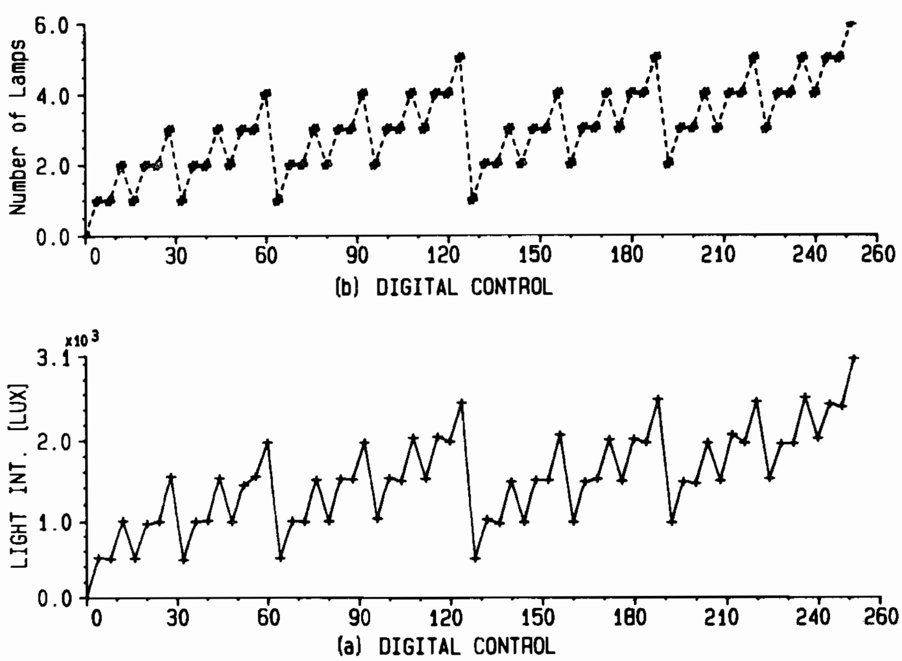

Figure 4. Light control. The light intensity (a) and the number of tubes operating (b) as a function of the digital control.

OPR (as indicated by the low correlation coefficient value, Fig. 3), which in turn caused the cost function to be extremely high. This erroneous function evaluation was automatically discarded by the present simplex algorithm since previous cost function values were already lower.

Searching by the simplex algorithm is performed by reducing the value of the objective function [Eq. (1)] until the standard error[35] defined by the termination criterion (see Appendix) is below a preset value. Then the algorithm's convergence rate can be described by the mean value of the cost function (M_C),

$$M_C = \frac{1}{n+1} \sum_{i=1}^{n+1} F(x^i) \tag{5}$$

and its standard deviation for every simplex,

$$\sigma_C = \frac{1}{n} \sum_{i=1}^{n+1} [F(x^i) - M_C] \tag{6}$$

The cost function mean value (M_C) as a function of the number of steps or function evaluation is shown in Figure 5a. At every new step, the objective function mean value was calculated [Eq. (5)]. When there was no improvement (the mean value did not change), the algorithm tried a new step. The first n points (steps 0–1) are given in their real values since M_C and σ_C cannot be evaluated for the initial steps.

The upper graph (Fig. 5b) shows the search improvement expressed in percentage relative to the mean value of the best (final) simplex (100%) and the initial (0%). This parameter clearly shows that by the sixth step the simplex algorithm was already inside the zone around 15% of the absolute optimum point, and by the twelfth step it had already arrived at the optimum zone; practically no further improvement was obtained later on. As the algorithm converged toward the optimum zone, the vertices that define the successive simplex approached each other as well as their cost function values, reducing the uncertainty of the optimum location. This convergence was reflected by a decrease in the value of the cost standard deviation defined by Eq. (6) (Fig. 5b).

The production rate improvement obtained along the optimization search is presented in Figures 5c and d. Although the OPR convergence toward the optimum value was slower than the cost convergence (about 12 steps to arrive at a zone 15% around the optimum), the production rate was seen to improve from an OPR mean value of 0.1 mg/L · min to more than 0.6 mg/L · min, an improvement in the production rate of about 500%. Furthermore, the OPR standard deviation, which is a measure of the uncertainty in the determination of the optimum production rate, was also improved (Fig. 5c). It should be emphasized that although the function to be optimized exhibited several local minima (Fig. 4), the algorithm successfully converged to the optimum zone.

One of the most important variables in aquatic biological systems is pH. The most common method of determining the optimum pH is to prepare a large number of samples of different pH values and to grow them under constant temperature and light conditions. This kind of arrangement involves the preparation and measurement of a large number of samples. Factorial design methods and response surface methodology[19,20] have also been applied to find the opti-

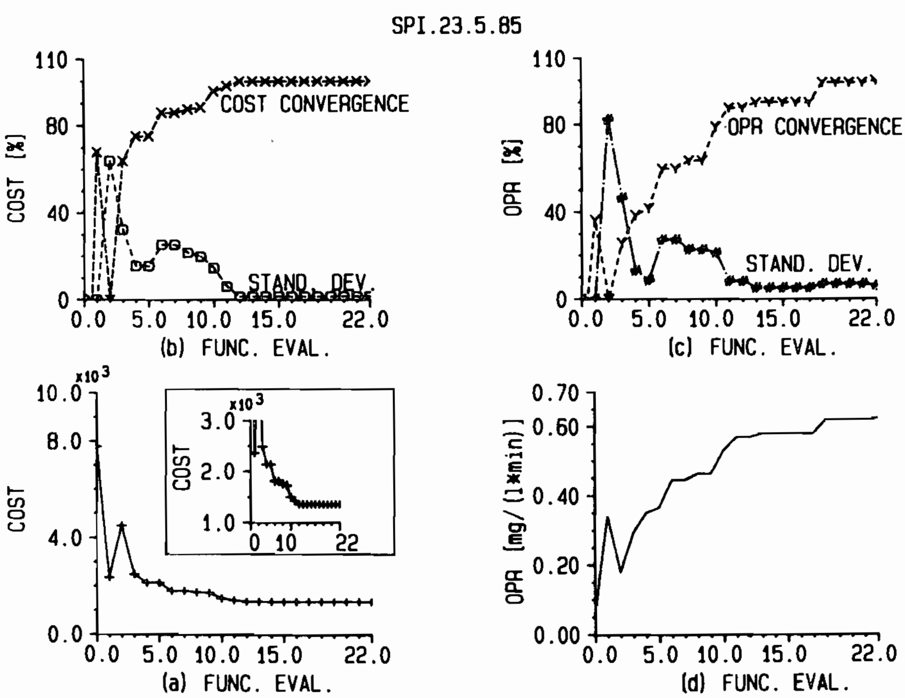

Figure 5. Convergence analysis of on-line optimization run by the simplex algorithm. Conditions as in Fig. 3. (a) Mean value of simplex [Eq. (5)]. (b) Convergence of algorithm and standard deviation in %. (c) OPR convergence and standard deviation in %. (d) Mean value of OPR (mg/L · min).

mum pH conditions of several microorganisms. Nakai[29] and Morgan and Deming[26] have shown the advantages of employing the simplex algorithm instead of more traditional approaches. We have examined the problem of the pH optimization in the present culture by on-line simplex runs, in which both pH and light intensity were the control variables.

The alkalinity of the Zarouk medium was reduced 10-fold to 20 meq/L (its minimum safe level) in order to reduce the effect of its high buffer capacity, which makes it difficult to obtain changes in pH. Light intensity was controlled as described above (Fig. 4). The pH was lowered by bubbling CO_2 through the medium. As it was found difficult to reach high pH levels by simply bubbling N_2 through the medium, the inherent algal CO_2 consumption capability via photosynthesis was used to facilitate pH increase. When needed, maximum light intensity was applied, and the consequent increase in algal mass led to an increase in the pH as a result of accelerated consumption of dissolved carbon dioxide in the medium.[42] It was found that this method shortened the delay. The increase in biomass was controlled by dilution of the culture with Zarouk's medium, through which nitrogen was continuously bubbled to maintain a high pH. Figures 6 and 7 show the results of the optimization run that used the above described pH control scheme. The objective function was defined as

$$F(\text{cost}) = \frac{K}{\text{OPR}} \tag{7}$$

where K is an arbitrary scaling factor. The objective here was to find the optimum operational point (pH and light). The water temperature was maintained constant at 33°C during the experiment to allow intensive growth. It is well known that the growth pH range of *S. platensis* is between 8.5 and 11,[42,43] but it can survive (without growth) at pH as low as 7. Therefore, the allowed pH range was taken as 7–11. The minimum pH increment was set as 0.1 for practical considerations. The OD range was kept between 0.3

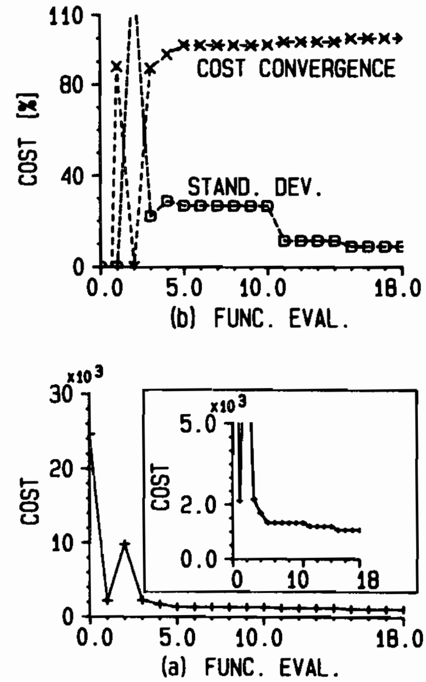

Figure 7. Convergence analysis of on-line optimization run by the simplex algorithm. Conditions as in Fig. 6. (a) Mean value of simplex [Eq. (5)]. (b) Convergence of algorithm and standard deviation in %.

and 0.35. When the OD was greater than 0.35, dilution was activated. It took between 15 and 25 min to reduce the pH value by bubbling CO_2 through the medium. However, raising the pH from 7 to 11 took more than 7 h. Consequently, 15 h were required to build up the initial simplex, and more than 24 h to arrive at the optimum zone (less than 15% to the optimum point) after five steps (Fig. 7). From there, the time between experiments was reduced to no more than 3 h as the pH changes were not so extreme. It took more than $2\frac{1}{2}$ days to carry out the 18 steps of the optimization run.

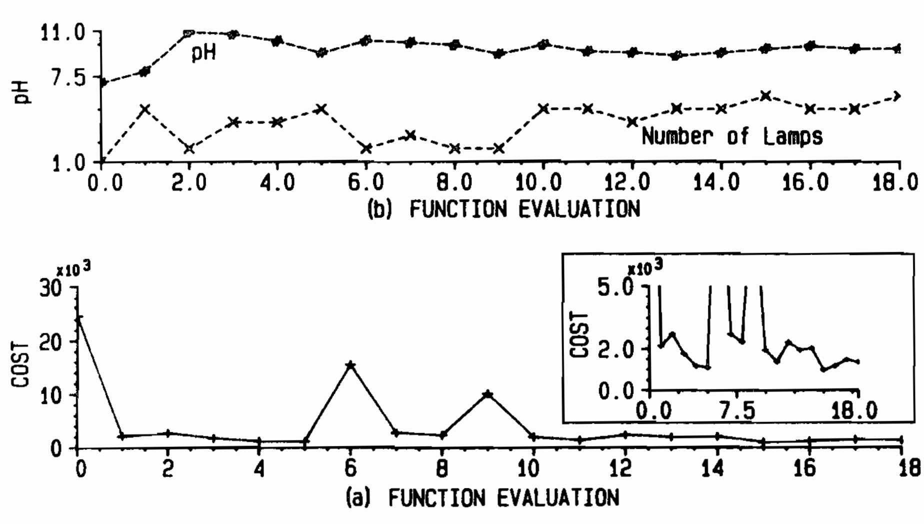

Figure 6. Optimization of a two-dimensional simplex run. (a) Cost function defined by Eq. (7). (b) Control variables: light intensity (as number of tubes operating) and pH. Optical density 0.3–0.35.

Results of a similar optimization run, in which OD was kept in the range of 0.8–0.85, are given in Figures 8 and 9. Since it was known that the algae *S. platensis* prefers high pH, the initial simplex was built up to occupy only half of the allowed space (Fig. 8). This helped to reduce the delay between steps. At steps 1 and 4 (Fig. 8) the ability of the algorithm to deal with unexpected operating conditions can be seen. As a consequence of the high OD, the light intensity provided by two tubes was insufficient to supply the necessary energy to produce a net photosynthetic process. There was no increase of biomass, as the OPR measurement (Fig. 8) revealed a respiration process. The low correlation coefficient of the estimation was apparently due to the small signal-to-noise ratio due to the small actual difference between the DO and the oxygen saturation levels (less than 3%).[40] Once the OPR values indicated respiration rather than a photosynthetic process, the cost function was assumed to be worse than the current worst value of the simplex, and the algorithm automatically discarded it since previous values were better. The latter two experiments suggest that the algae *S. platensis* can grow without serious constraint at pH 9–11, although it clearly prefers high pH levels (pH 10.5–11). This is an important conclusion since by operating the system at high pH one can prevent the waste of inorganic carbon caused by CO_2 diffusion from the solution to the atmosphere and obtain a CO_2 contribution (albeit a small one) from the atmosphere.[42]

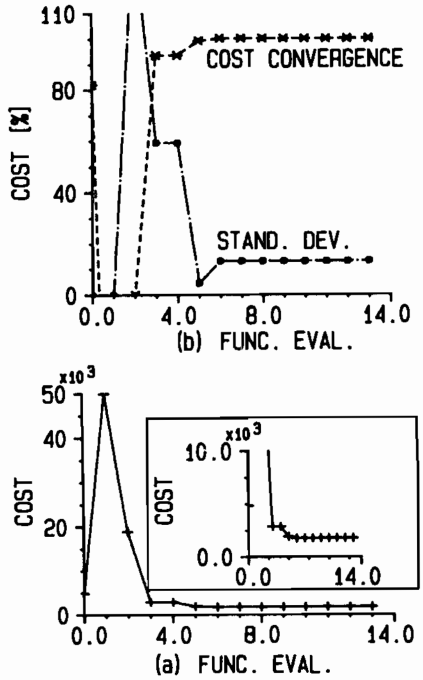

Figure 9. Convergence analysis of on-line optimization run by the simplex algorithm. Conditions as in Fig. 8. (a) Mean value of simplex [Eq. (5)]. (b) Convergence of algorithm and standard deviation in %.

One of the most commonly used operational modes of bioreactors is the turbidostat mode, in which the biomass concentration is maintained constant by dilution. The turbidostat arrangement allows us to study a culture under no substrate limitations. In the following simplex run example,

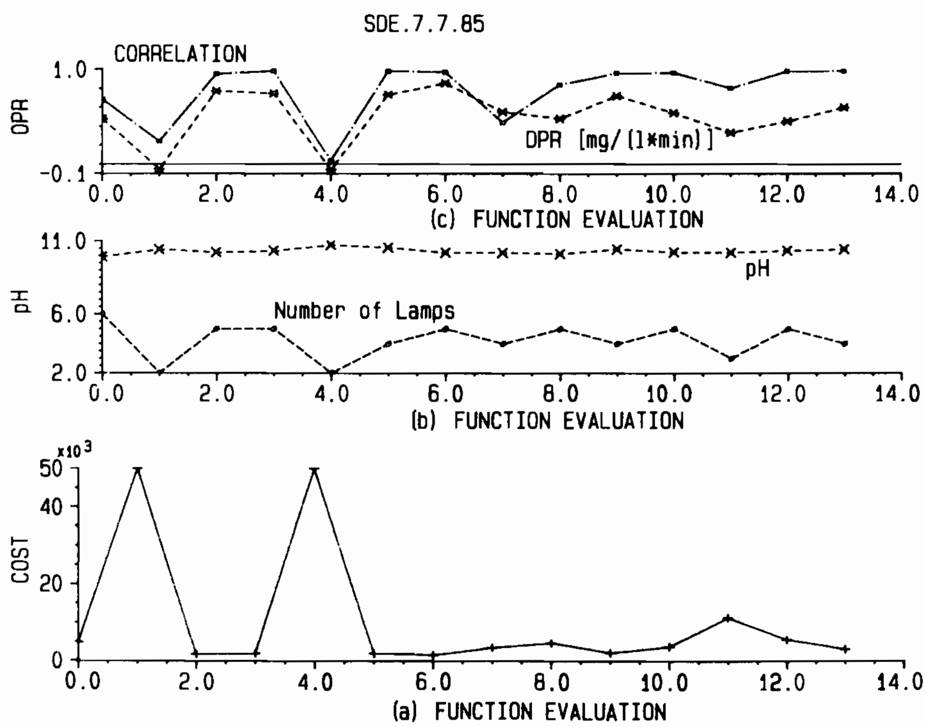

Figure 8. Optimization of a two-dimensional simplex run. (a) Cost Function defined by Eq. (7). (b) Control variables: light intensity (as number of tubes operating) and pH. (c) OPR is the measure of growth rate; the correlation coefficient provides a measure of the goodness of fit. Optical density 0.8–0.85.

the minipond was forced by the computerized system to behave as a turbidostat. The objective function was defined as

$$F(\text{cost}) = \frac{K}{(\text{OPR})^2} \quad (8)$$

where K is an arbitrary scaling factor. The quadratic term in Eq. (14) was employed to ensure that all the F(cost) values would be positive. In addition, as the simplex algorithm is essentially a steepest descent method,[34] the quadratic form of the objective function would accelerate the convergence rate of the simplex algorithm.[22,34] However, no significant improvement in the algorithm's convergence rate was detected. This can be explained by the fact that the initial simplex was built to occupy most of the allowed space, which markedly enhances the initial convergence of the algorithm. In the turbidostat mode water temperature and light intensity were the controlled variables. The light source comprised an array of six incandescent lamps controlled by a dimmer. The computerized system controlled the dimmer's conduction angle through a digital-to-analog (D/A) converter to simulate a diurnal light intensity function. The OD was maintained at a constant level by dilution with Zarouk's medium. It took about 3 h to evaluate each operating condition (this includes the time to shift the system to the new operating conditions, stabilizing the system, and OPR evaluation) (Figs. 10 and 11). The optimum growth temperature was found to be about 33°C, a value which is in good agreement with previous studies.[43] As the light intensity was relatively low, the algal growth was light limited, and no light saturation effect was observed.

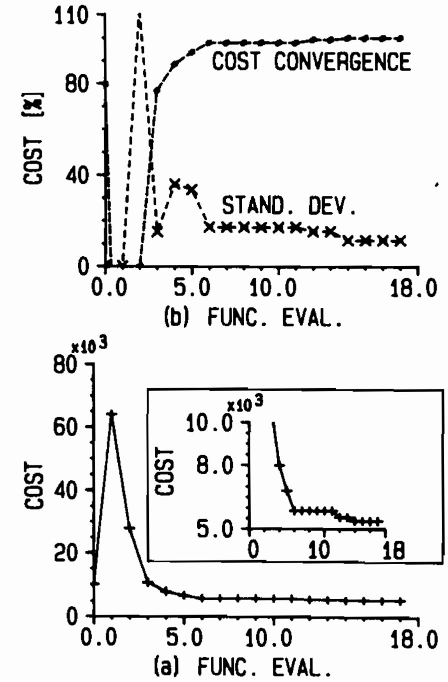

Figure 11. Convergence analysis of on-line optimization run by the simplex algorithm. Conditions as in Fig. 10. (a) Mean value of simplex [Eq. (5)]. (b) Convergence of algorithm and standard deviation in %.

CONCLUSIONS

The optimization and control methodology studied here could be a viable solution when there is not sufficient knowledge of a system for a more classical approach. Although the present study was concerned with a specific process (open algal ponds), it demonstrated the feasibility of solving on-line optimization control problems in biological systems. Since the present approach does not rely on any a priori knowledge of a mathematical model, it is applicable to a multitude of cases within and outside the biotechnological field. The only prerequisites are the ability to measure and control the pertinent variables and the ability to define a cost function. This is not always an easy task as in order to

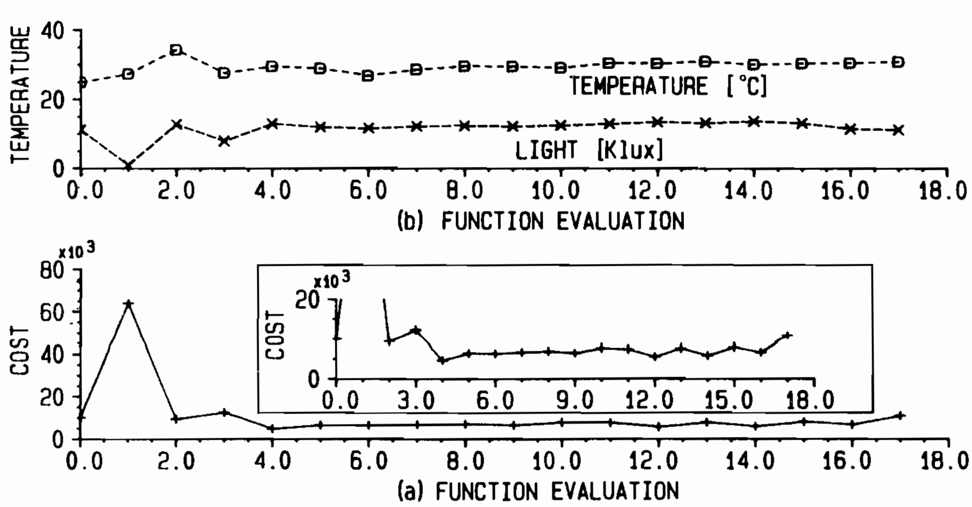

Figure 10. Optimization of a two-dimensional simplex run. The algal pond as a turbidostat. (a) Cost function defined by Eq. (8). (b) Control variables: light intensity and temperature. (a) Optical density 0.32.

define a cost function it is necessary to have a good general knowledge of the biological organism and its interaction with the environment. It should be noted that the data obtained during the optimization runs can also be used for mathematical modeling of the biological process and that there are no objective problems in changing the experimental design of the proposed procedure to suit any given process.

As the availability of low-cost powerful microcomputers increases, the possibility of applying the proposed algorithm is becoming economical not only for industrial applications but also for small-scale production or laboratory research. Its application can provide investigators with a powerful tool that can help to bring new insight to biotechnological processes and can be an initial step in the development of more sophisticated control and optimization strategies. The present approach is applicable to systems with constant or slow changing parameters. Further research is needed to investigate on-line optimization methods that would be satisfactory for handling systems with fast parameter change.

APPENDIX: THE SIMPLEX SEARCH

The "cost" function is evaluated at each of the vertices of the simplex. The worst response in the simplex is found (x^h), and the point is geometrically reflected through the line defined by the worst point and the centroid defined by the other vertices (x^0):

$$x^0 = \frac{1}{n} \sum_{\substack{i=1 \\ i \neq h}}^{n+1} x^i \tag{A1}$$

The reflection operation can be written as

$$x^r = (1 + \alpha)x^0 - \alpha x^h \qquad \alpha > 0 \tag{A2}$$

If further improvement along the line is expected, an expansion is performed:

$$x^e = \gamma x^r + (1 - \gamma)x^0 \qquad \gamma > 1 \tag{A3}$$

If the reflected point does not give any improvement, a contraction is performed:

$$x^c = \beta x^n + (1 - \beta)x^0 \qquad 0 < \beta < 1 \tag{A4}$$

In the present work the values proposed by Nelder and Mead ($\alpha = 1$, $\gamma = 2$, $\beta = 0.5$) were used.[22,35]

In the case that no improvement is obtained, the entire simplex is shrunk around the best point (x^1):

$$x^i = \tfrac{1}{2}(x^i + x^1) \tag{A5}$$

The search is terminated when the improvement of the objective function is below a preassigned value (ε):

$$\Delta_c = \frac{1}{n}\left(\sum_{i=1}^{n+1} [f(x^i) - f(x^n)]^2\right)^{1/2} < \varepsilon \tag{A6}$$

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