CASE 2

Optimization of Small Algae Production by Parameter Design

Abstract: By analyzing a proliferation curve for a production process for small algae on the basis of a dynamic characteristic, we applied an optimal configuration obtained from a small-scale, low-density cultivation experiment to large-scale, high-density cultivation. We had difficulty finding a really optimal configuration because of a time-lapsed change in initial conditions for control factors.

1. Introduction and Generic Function

Our objective in producing small algae was to maximize the volume of algae using the tube cultivation apparatus shown in Figure 1, which is considered promising as a closed-loop continuous cultivation device to solve technical problems such as contamination of bacteria. Since numerous factors affect the proliferation speed of a small alga, we attempted to solve these problems through a small-scale experiment, as an experiment using a large-scale continuous cultivation machine was not regarded as economical.

Ideally, the proliferation curve in the case of producing small algae should have a linear relationship between the logarithmized amount of algae and the cultivation period. However, in actuality, as a cultivation period increases, the proliferation curve's slope decreases, and in the end, the proliferation saturates, as shown in Figure 2. To enhance productivity, we need to cultivate the number of algae as densely as possible while retaining constant proliferation. That is, even in a high-density region, the proliferation curve must be linear. Therefore, considering that a generic function is a linear proliferation between a quantity of sunlight as input energy and the logarithm of the amount of algae (biomass), we studied the conditions for the most ideal proliferation curve. In other words, conditions where both the SN ratio and sensitivity S of the proliferation curve are large are regarded as optimal.

2. Experimental Procedure

The factors affecting the proliferation speed of small algae include environment, cultivation apparatus, bacteria type, or medium. To study medium conditions for obtaining maximum biomass, we need to repeat experiments under the conditions for high-density cultivation. However, since the transparency deteriorates and light energy acts as a constraint factor, we had difficulty performing an experiment on the influence of a medium's ingredients. On the contrary, whereas it is easy to establish an optimal level of medium in the case of low-density cultivation, it is difficult to adopt the results for high-density cultivation. It was expected in this experiment, however, that the results of small-scale low-density cultivation would be applicable for large-scale cultivation.

Since there is a correlation between the amount of small algae produced (biomass, amount of dried cells per liter in the culture solution) and optical density (OD_{680}), the latter was considered as an alternative characteristic, for biomass. There are other substitutive characteristics, such as PCV (volume of cells in culture solution per liter) or cell

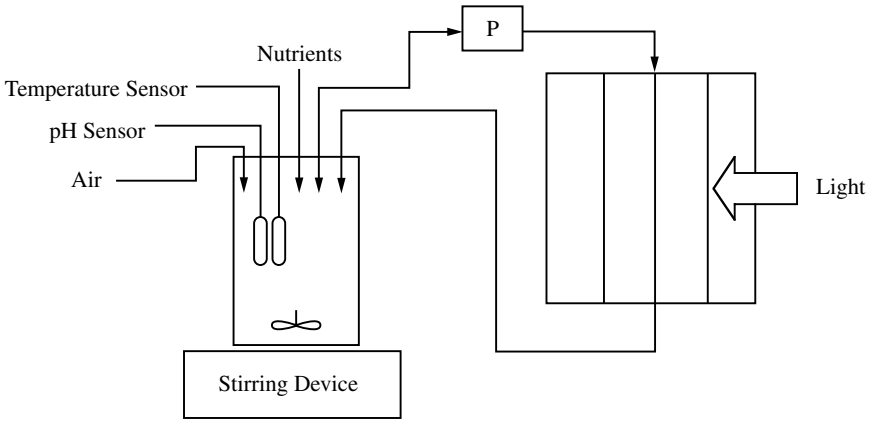

Figure 1
Tube cultivation apparatus

concentration (number of cells in culture solution per milliliter), but light absorption was used for its simplicity, small amount of sample required, and good precision of measurement.

As a signal factor, the number of cultivating days (one to nine days), which is the total amount of light, or input energy, was used. Light absorption at the same time of the day was measured. Artificial illumination with a constant amount of light was used.

As noise factors, environmental conditions (temperature, amount of light, amount of air, etc.) or the shape of apparatus may be considered. But these were not included because of trying to reduce the scale of the experiment and also to minimize the error of algae propagation activity by starting each experiment under the same conditions. It is difficult in biological experimentation to include many noise factors. Therefore, it is important to select carefully both the factors and the scale of the experiment.

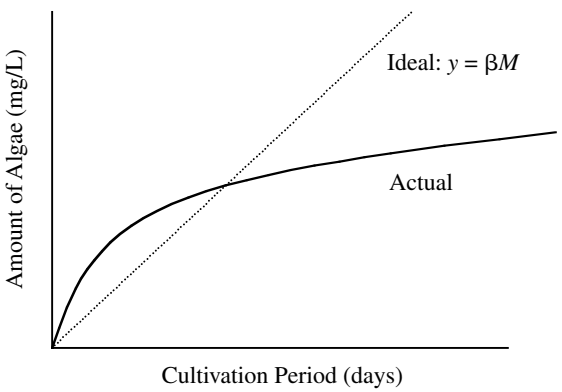

Figure 2
Proliferation curve

3. SN Ratio

To analyze the measurements of optical density using a linear relationship passing through the origin (zero-point proportional equation), we include their logarithmized values in Table 1. Now x_0 and x indicate optical density (OD_{680}) and y represents a logarithmized value.

$$y = 1000[\log(1000x) - \log(1000x_0)] \qquad (1)$$

For a logarithmized value (y), considering that a zero-point proportional $y = \beta M$ is ideal, we compute the SN ratio and sensitivity based on a dynamic characteristics as below. Next, we detail an example for experiment 1 (Table 1).

Total variation:

$$S_T = 298.20^2 + 577.40^2 + \cdots + 1318.98^2$$
$$= 8{,}434{,}868.8523 \qquad (f = 8) \qquad (2)$$

Table 1
Logarithmized measurement of optical density

	Day								
No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	0.0	298.2	577.4	833.7	1002.8	1127.6	1265.2	1303.8	1319.0
2	0.0	306.6	604.6	900.3	1089.9	1198.1	1285.8	1235.7	668.6
⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮
17	0.0	443.9	781.9	1020.9	1213.5	1314.4	1419.1	1497.5	1518.2
18	0.0	443.9	800.1	1089.9	1259.6	1291.6	1301.9	1340.6	1115.7

Effective divider:

$$\gamma = 1^2 + 2^2 + \cdots + 9^2 = 249 \tag{3}$$

Variation of proportional term:

$$S_\beta = \frac{[(1)(298.20) + (2)(577.40) + \cdots + (9)(1318.98)]^2}{249}$$

$$= 8{,}046{,}441.5662 \qquad (f = 1) \tag{4}$$

Error variation:

$$S_e = 8{,}434{,}868.8523 - 8{,}046{,}441.5662$$

$$= 388{,}427.2861 \qquad (f = 7) \tag{5}$$

Error variance:

$$V_e = \frac{388{,}427.2861}{7} = 55{,}489.6123 \tag{6}$$

SN ratio:

$$\eta = 10 \log \frac{(1/249)\ (8{,}046{,}441.5662 - 55{,}489.6123)}{55{,}489.6123}$$

$$= -2.38 \text{ dB} \tag{7}$$

Sensitivity:

$$S = 10 \log \frac{1}{249}(8{,}046{,}441.5662 - 55{,}489.6123)$$

$$= 45.06 \text{ dB} \tag{8}$$

4. Results of Experiment

Table 2 shows the control factors. Adding as inoculants small algae (*Nannnochrolopsis*) in 18 culture

Table 2
Control factors and levels

	Level		
Control Factor	**1**	**2**	**3**
A: illuminance (lx)	2000	4000	—
B: concentration of added seawater (%)	30	0	70
C: nitrogen coefficient	0.385	0.585	0.785
D: phosphorus coefficient	0.001	0.002	0.002
E: carbonic acid coefficient	0.010	0.016	0.022
F: coefficient of small-quantity ingredient	0.014	0.022	0.030
G: coefficient of added fertilizer	1.90	2.04	2.18

flasks that contain 300 mL of each type of medium assigned to the L_{18} orthogonal array, we began to cultivate them with two types of illuminance. The amount of air is fixed for all conditions. By sampling a small amount of culture solution at the same time of the day, we measured an optical density (OD_{680}). Based on measurements of an optical density, we computed the number of algae and added a required nutrient equivalent daily to that multiplied by each coefficient of the aforementioned nutrient-related control factors.

Figures 3 shows the response graphs of the SN ratio and sensitivity. The more the slope of a logarithmic proliferation inclines, the larger the dynamic sensitivity S becomes. The more linear and less varied the slope of a logarithmic proliferation becomes, the larger the dynamic SN ratio becomes. Therefore, picking up levels with a large sensitivity and SN ratio according to the response graphs, we determined an optimal configuration.

As a result, the optimal configuration selected from the response graphs was $A_2B_2C_1D_1E_3F_3G_1$. It was

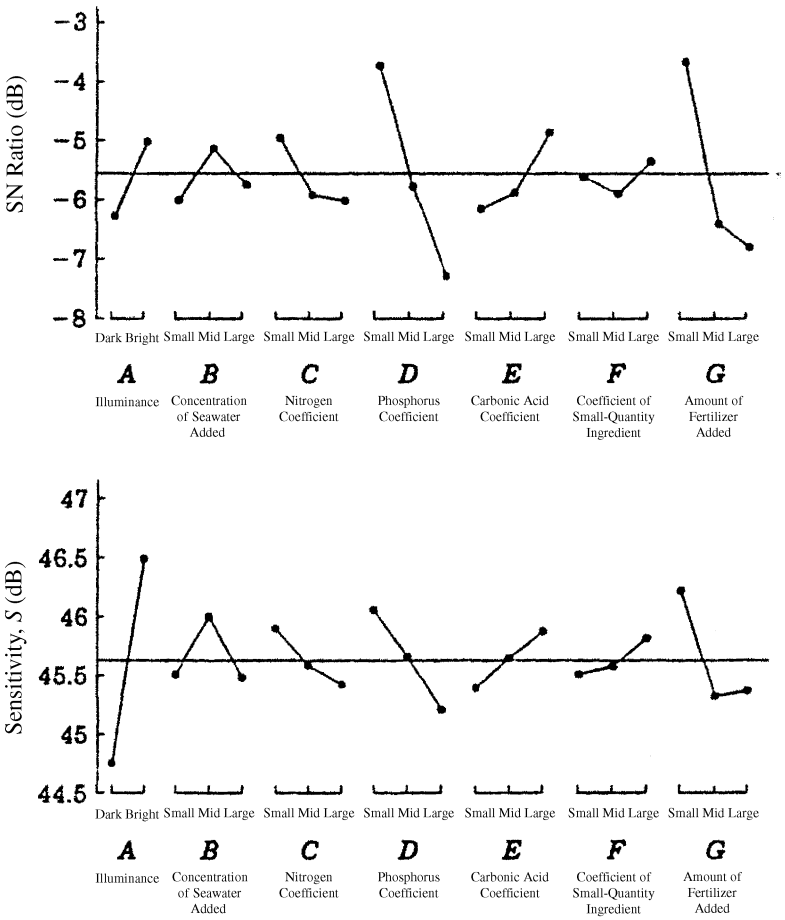

Figure 3
Response graphs for proliferation

found that the factor effects of phosphorus coefficient D and added fertilizer coefficient G were significant among all control factors. For factor F, because of its small effect on the SN ratio and sensitivity, we can select F_1, which requires only a small amount of chemical.

As the next step, to estimate the process average under the optimal configuration selected, $A_2B_2C_1D_1E_3F_3G_1$ we calculated the SN ratio and sensitivity, S.

SN ratio:

$$\eta = -5.00 - 5.12 - 4.97 - 3.75 - 4.90 - 5.39 - 3.67 - (6)(-5.65) = 1.10 \quad (9)$$

Sensitivity:

$$S = 46.49 + 46.00 + 45.88 + 46.03 + 45.86 + 45.81 + 46.20 - (6)(45.62) = 48.55 \quad (10)$$

On the other hand, the process average under the current configuration, $A_2B_2C_2D_2E_2F_2G_2$, is as follows.

SN ratio:

$$\eta = -5.00 - 5.12 - 5.94 - 5.82 - 5.90 - 5.96 - 6.44 - (6)(-5.65) = -6.28 \quad (11)$$

Sensitivity:

$$S = 46.49 + 46.00 + 45.57 + 45.64 + 45.63 + 45.56 + 45.31 - (6)45.62 = 46.48 \quad (12)$$

Table 3 compares the confirmatory experimental result and the estimate.

As a result of this confirmatory experiment, although there is little improvement in confirmatory experiment 1, fairly good reproducibility and significant improvement in the SN ratio and sensitivity were gained in confirmatory experiment 2, resting on a tube cultivation apparatus. In this case, the gains in SN ratio and sensitivity are 5.80 and 4.35 dB, respectively, which are quite consistent with the estimations. Consequently, our experiment implies that by analyzing a proliferation curve for a production process of small algae on the basis of a dynamic characteristic, we could apply an optimal configuration obtained from a small-scale, low-density cultivation experiment to large-scale, high-density cultivation.

In an experiment on a production process for algae, such as our study, even for the simple purpose of determining proper conditions for a medium, we had difficulty finding a really optimal configuration because of a time-lapsed change in initial conditions for control factors. For instance, focusing on illuminance selected as a control factor, we can see that the quantity of light that algae receives decreases gradually, as less external light reaches the inside of a culture solution, due to the proliferation of organisms. In addition, each nutrient diminishes accordingly, whereas the number of algae increases.

Two major solutions for this problem are (1) to devise a control system of maintaining these control factor conditions at constant levels, and (2) to determine an optimal configuration for the system by changing these control factors to noise factors.

Table 3
Estimation and confirmation of process average (dB)

	SN Ratio			Sensitivity S		
		Confirmation			Confirmation	
Configuration	Estimation	1	2	Estimation	1	2
Optimal	1.10	−2.81	2.72	48.55	43.85	47.37
Initial	−6.28	−3.08	−3.08	46.48	43.02	43.02
Gain	7.38	0.27	5.80	2.07	0.83	4.35

Although it is possible to maintain the conditions of control factors using current technology, it is costly and difficult to expect perfect control. Therefore, in the future, by attempting to use the control system to retain the same conditions to some extent, we might consider the control factors as noise factors in an economical study of an optimal configuration for biomass.

Reference

Makoto Kubonaga and Eri Fujimoto, 1993. Optimization of small algae production process by parameter design. *Quality Engineering*, Vol. 1, No. 5.

This case study is contributed by Makoto Kubonaga.