MASS CULTURE OF UNICELLULAR ALGAE USING THE "OPEN CIRCULATION METHOD"

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INTRODUCTION

In 1955 Tamiya (1) reported a new method of mass culture of *Chlorella*, the so-called "open circulation method with a device for intermittent sweeping" which was different in principle from various culture methods so far attempted by other workers (2, 3, 4; see also 5). The principle

Fig. 1. An example of culture ponds of "open circulation system".

Fig. 2. Schematized cross-section of the pond illustrated in Fig. 1.

* The Kyowa Fermentation Industry Co., Ltd.

of this culture method may be brought out by an example of culture unit illustrated in Figs. 1 and 2. In this layout, the main space of the culture unit is a round pond having a small island at the center, and the culture (about 10 cm in depth) is circulated by a pump from one corner of the pond—and through a pipe placed underground—to the two rotatable arms extending from the center to the periphery of the pond. The arms, which are submerged in the culture solution, have many holes which are directed somewhat diagonally downward. By the reaction of water flux from the holes, the arms rotate slowly, and circulate the solution evenly in the pond, and at the same time stir and sweep intermittently the algal cells in the solution. Near the outlet from the pump, a small tube for feeding CO_2 -enriched air is inserted into the circulating pipe, thus the culture is evenly supplied with carbon dioxide during culture. In Fig. 3 is shown another type

Fig. 3. "Polymer-pond" with synchronously rotating sprinkling arms.

of culture pond of the same principle, in which the pond is composed of four round ponds that are, so to speak, polymerized in a row at intervals equal to the radius of each component-pond. Each component-pond is provided with one rotatable sprinkling arm, and by being connected with connecting rods, the arms in the whole culture unit rotate synchronously. Circulation of culture solution and rotation of the arms in the whole culture unit is effected by a single pump.

Until the end of 1955, five of these ponds (see Table 1) were constructed in our Institute, and they were operated under various conditions with the purpose of finding out the most satisfactory way of running the culture.

Table 1. Culture ponds used in the experiments.

Pond	Dimension	H.p. of pump	Illuminated area (m ²)	Volume of culture at 10 cm depth (metric tons)
No. 1 (monomer)	3 m in diameter	$\frac{1}{2}$	7.1	0.71
No. 2 (")	"	$\frac{1}{2}$	"	"
No. 3 (")	5 m in diameter	$\frac{1}{2}$	19.6	1.96
No. 4 (")	10 m in diameter	1	78.5	7.85
No. 5 (tetramer)	4 m $\times$ 10 m	1	35.5	3.55

Described herein are the results obtained during the period of 1956-1957, and several new devices which we have introduced to the system since it was first reported in 1955.

SEQUENCE OF CULTURES

The outline of the procedures adopted in our experiments is shown by the flow sheet presented in Fig. 4. The precultures, which were started from the pure (stock) cultures on agar slants, were run under aseptic conditions using oblong flat flasks of various sizes, the typical shape of which is illustrated in Fig. 5. The features of these flasks are given in Table 2 (for further details see our previous papers, 6, 7). The cultures in these flasks were run indoors at 20°-25° under illumination (in a direction perpendicular to the flat surfaces of the flasks) either with reflector flood lamps or fluorescent lamps. The intensity at the surface of the culture flasks was about 8 kilolux. During the culture the solution was constantly aerated with CO₂-enriched air which was introduced through a glass pipe inserted in the culture solution. The successive cultures in each type of flask lasted 10-14 days. When the cultures in the largest flasks attained the population density of 10-15 ml packed cell volume per liter, they were used as the inoculum for the outdoor cultures in smallest ponds (Nos. 1 and 2, each containing about 0.71 metric tons of culture solution). Inoculum culture used for each pond was about 20

Fig. 4. Flow Sheet of Operation.

Fig. 5. An example of oblong flat flasks used for pre-cultures.

liters in winter and about 10 liters in summer. When the cultures in these ponds attained an appropriate level of population density, for which it took about 30 days in winter and 3 days in summer, they were used as inocula for the cultures in larger ponds (Nos. 3, 4 and 5). The depth of culture solution in these ponds was, on an average, about 10 cm, which fluctuated in the range from 6 to 15 cm under various climatic and operational conditions. The initial population density (packed cell volume per liter) in the larger ponds was about 0.3–0.5 ml in winter and 0.1–0.2 ml in summer. After the population density in these cultures had attained the level of 5 or more ml per liter, the harvesting of algal cells was conducted semi-continuously, *i.e.*, at intervals—varying from 2 to 20 days ac-

Table 2. Flat flasks used for precultures.

Flasks	Size of the main body of the flask (cm)			Volume of culture solution (ml)
	length	width	inner thickness	
Small	15	7	2.4	50
Medium	37	12	2.8	500
Large	52	16	5.8	2000

ording to the season—a certain portion of cultures was taken out, the cells were harvested by centrifugation, and the supernatant was either returned to the ponds or—when the culture lasted rather long—replaced by fresh culture solution; thus the population density of cultures was roughly kept within the range from about 5 to 15 ml per liter. Taking into account the change of mineral composition in the culture, which was caused by the algal growth and rain, the culture medium was occasionally replenished with mineral salts so as to keep the composition roughly constant.* (See the section of “culture medium”.) The semicontinuous cultures could be continued for 100 days in winter and 60 days in summer. Discontinuance of culture and harvesting of the whole algal cells from the pond had to be done on various occasions such as: (i) when some unexpected troubles occurred in the operation system, (ii) when the culture was contaminated by

* In Tokyo the increase of water caused by rain or snow usually exceeds the quantity of water-loss by evaporation. See the section of “climatic conditions”.

other microorganisms (see, however, the section of "prevention of contamination"), (iii) when the removal of accumulated dust became necessary, which occurred at an interval of about 3 months in Tokyo, and (iv) when a large amount of algae was requested from other laboratories for their studies using algal materials.

ALGAL STRAINS USED

Of the numerous algal strains maintained in our Institute, ten strains of *Chlorella* or *Scenedesmus* listed in Table 3 were used under different seasonal and operational conditions. Among those strains, most satisfactory results were obtained with *Scenedesmus* 141 in warmer seasons, and with *Chlorella* 139 in cooler seasons. At moderate temperatures the latter grew somewhat faster than the former. In general, *Scenedesmus* strains withstood contaminations more strongly than did *Chlorella* strains.

Table 3. Strains of algae used in the experiments.

C: *Chlorella* spec.; S: *Scenedesmus* spec.

Month \ Year	1956			1957	
Jan.					C139
Feb.					C139
Mar.					C139
Apr.					C139
May	Cw,	Ce,	S90	C91,	C139
June	Cw,	C145,	S90	C91,	C139, S141
July	C50,	C145,	S90	C91,	C139, S141
Aug.	C50,	C145,	S141	C140,	S141
Sept.		S141		C140,	S141
Oct.	C50,	C91,	C139, S141	C140,	S141
Nov.		C139		C140,	S141
Dec.		C139		C140,	S141

CULTURE MEDIUM

The culture medium used for precultures contained, per liter: 5 g KNO₃, 2.5 g MgSO₄·7H₂O, 1.25 g KH₂PO₄, 5 mg FeSO₄·7H₂O and 1 ml Arnon's "A5" solution (8). For the main cultures run outdoors a culture medium of the following composition was used, per liter: 0.6 g urea or 2 g KNO₃, 0.3 g MgSO₄·7H₂O, 0.3 g KH₂PO₄, 5 mg FeSO₄·7H₂O, 1 ml Arnon's "A5" solution, and 7 mg EDTA (di-sodium salt of ethylenediamine tetraacetic acid.* It has

* In outdoor cultures it is advisable to use urea in concentrations lower than 0.6 g per liter. At higher concentrations, for example at 1.2 g per liter, there occurred, in the long run of culture, partial decomposition of urea resulting in an increase of pH to an undesirable level.

been ascertained by laboratory experiments (using *Chlorella ellipsoidea* as the test organism) that the culture medium used in outdoor cultures was able to support, without being replenished with nutrient salts, the growth of algae in a quantity of as much as 15 ml packed volume per liter. As the nitrogen source, urea was advantageous over nitrate in that its consumption caused no appreciable change of pH of the culture medium. When KNO_3 was used as the nitrogen source, the growth of algae in an amount of about 15 ml (packed cell volume) per liter had caused a shift of pH value from 5.1 to 7.8 (under the condition of sufficient supply of CO_2), although this shift of pH did not affect recognizably the growth of algae.

Table 4. Quantities of main nutrient elements consumed by the growth of algae.

Elements	Decrease in mg per g of algal growth
N	62-77
P	15-20
K	9-12
Mg	3-5
S	4-7
Fe	0.4-0.6

Using *Chlorella ellipsoidea* as the test organism, the quantities of the main nutrient elements consumed by the algal growth was measured.* The results obtained are summarized in Table 4. Taking these data as well as those reported by Krauss (13) into account, we conducted, in the practice of outdoor cultures, occasional replenishment of nutrients in the culture medium according to the quantity of algae harvested. The quantities of substances added per harvest of 1 kg (dry weight) of algae are given in Table 5.

In running the outdoor cultures continuously, a portion of cultures was taken out at certain intervals, and after harvesting the algae by centrifugation, the supernatant was returned to the culture. On repeating such a procedure for several weeks, the culture solution assumed somewhat yellowish color. It was found that despite such a change, the repeated use of the same culture solution could be continued at least for two months without producing undesirable effects such as retardation of algal growth or causing contamination by other microorganisms.

* Methods of analyses were as follows: for nitrate, Conway's method (9); for phosphate, Allen's method (10); for potassium and magnesium, flame photometry using Beckman's DU spectrophotometer (at 404.6 $\text{m}\mu$ for K, and 371 $\text{m}\mu$ for Mg); for sulfate, magnesium-nitrate method (11); for iron, orthophenanthroline method (12).

Table 5. Quantities of nutrient substances added to the culture medium for replenishment per harvest of 1 kg (dry weight) of algae.

Substance	Quantity
Urea or HNO ₃	160 g 400 ml
KH ₂ PO ₄	53 g
MgSO ₄ ·7H ₂ O	27 g
MgO	2 g
FeSO ₄ ·7H ₂ O	5 g
MnSO ₄ ·4H ₂ O	0.41 g
CuSO ₄ ·5H ₂ O	0.16 g
ZnSO ₄ ·7H ₂ O	0.11 g

PREVENTION OF CONTAMINATION BY OTHER MICROORGANISMS

Unless proper measures were taken, contamination of cultures by other microorganisms could occur during the period from May to October. Most harmful organisms which appeared in our cultures were micro-animals such as *Chilodonella* (a protozoan) and *Diurella* (a rotiferan) and a species of chlorophyll-containing flagellate, *Chromulina*. Extensive search was made for drugs which kill or suppress the growth of these microorganisms without affecting the growth of *Chlorella* or *Scenedesmus*. For each drug available to us, determination* was made of (i) the maximum innocuous concentration (A) for *Chlorella* (at 25°, light intensity: 10 kilolux), and (ii) the minimum lethal concentration (B) for micro-animals (*Chilodonella* and *Diurella*, at 25°) and the ratio (A/B) of these two values was taken as indicating the effectiveness of the drug.** Out of 70 drugs tested, those listed in Table 6 gave A/B ratios of more than 20, indicating that they would suit our purpose. Considering the price and stability of the drugs, we chose for the use in outdoor cultures 2,4-dinitro-6-cyclohexyl phenyl acetate (DN-acetate) in concentrations of 0.3–5 p.p.m., which proved to be effective in preventing the contaminations. This drug is being used (in the concentration of 0.1 percent) for killing mites on various fruit-trees such as tangerine-, apple- and pear-trees. According to Woglum *et al.* (14) its acute (oral) lethal dosis (LD₅₀) for rats is 180 mg/kg. Considering the fact that in our algal cultures it is applied only in minute quantities and also that, in our procedure of harvesting,

* The drugs which were not water-soluble were tested either in the form of emulsion or of wettable powder.

** For each drug tested, almost identical sensitivities were found for *Chlorella* and *Scenedesmus*, on the one hand, and for *Chilodonella* and *Diurella*, on the other.

Table 6. Drugs which were found to be effective in suppressing the growth of micro-animals in algal cultures.*

Drugs	Maximum innocuous concentration for <i>Chlorella</i> (p.p.m.) (A)	Minimum lethal conc. for micro-animals (p.p.m.) (B)	A/B
2,4-dinitro-6-cyclohexyl phenyl acetate	500	2	250
Vitamin K ₃ sodium bisulfite	1000	10	100
"Allethrin" (synthesized pyrethrin)	500	10	50
"Furethrin" (")	1000	25	40
Pentachlorophenyl acetate	50	2	25
Vitamin K ₃ diphosphoric acid ester, tetra sodium salt	250	10	25
Vitamin K ₄	100	5	20

the algae are washed with water after being separated from the culture solution, it may safely be asserted that the food-value of algae will not be affected at all by the use of this substance.

CIRCULATION OF CULTURE SOLUTION

In our culture system, the circulation of culture has three-fold purpose: (i) distribution of carbon dioxide in the whole area of culture pond, (ii) mixing of the culture, which is necessary on such occasions as seeding of fresh algal cells, and the regulation of composition or acidity of culture solution, and (iii) intermittent agitation of algal suspension and sweeping of precipitating cells from the bottom of culture pond. To achieve these purposes effectively, the following conditions have to be fulfilled: (a) the velocity of rotation of sprinkling arms should be adequate, (b) the depth of the culture solution and the clearance between the arms and the bottom of the pond should be adequate, and (c) the culture solution should be effectively ejected from the holes of the sprinkling arms.

* The following drugs gave the A/B ratios of 1-5, but were found to be unusable in outdoor cultures: invert soaps, DDT, γ -BHC, chlordan, aldrin, dieldrin, pentachlorophenol, pentachlorophenoxy acetic acid, 2,4-dinitrophenyl thiocyanate, 2-methyl-4-chlorophenoxy acetic acid, pentachlorophenyl benzoate, phenylsalicylate, β -naphthol, 2,4-dinitro-6-cyclohexyl phenol, p-aminosalicylic acid sodium salt, 2,4-dichlorophenoxyacetic acid sodium salt, dehydroacetic acid, parathion, "Malathion", tetraethylpyrophosphate, "Strobane" (chlorinated camphene), pyrethrin, tetramethyl thiuram disulfide, tetraethyl thiuram disulfide, maleic hydrazide, phenothiazine, rotenone, ascaridol, nicotine, nicotine sulfate, vitamin K₃, acriflavine, acrinol, eosin.

The following drugs gave A/B ratios smaller than unity: phygon, chloranil, p-chlorophenyl-p-chlorobenzene sulfoester, p-chlorophenol, o-phenyl phenol, 4-chloro-o-cresol, 4,6-dinitro-o-cresol, 2,4,5-trichlorophenoxy acetic acid, salicylic acid, methyl-, ethyl- and butylesters of p-oxybenzoic acid, thiourea.

In our first attempt of the present culture method (*cf.* Figs. 1 and 2), the rotating arms (1 inch in diameter) were provided, at intervals of 4.5 cm, with many holes (3.5 mm in diameter) which were directed diagonally downward (45°). The culture solution ejected from these holes served both as the sweeper of precipitating algal cells, and as the driver of rotating arms. The depth of culture solution was 6–15 cm and the clearance between the arms and the bottom of pond was 4–5 cm. In this system it often occurred that—since the culture solution was ejected from the arms at a right angle to the arms—the algal cells that precipitated in the region between the loci of the holes were not satisfactorily swept by the jet of culture solution. To improve this point, we adopted the following method. Five holes located at the end of the arms remained the same as before, and the remainder of arms was provided with the jets which ejected the solution diagonally downward in the direction toward the periphery (or toward the center) of the pond as illustrated in Fig. 6. The solution ejected from the simple holes at the end of the arms gave the driving force for the rotation of arms, while the solution ejected from the jets shown in the figure swept effectively the precipitating cells in considerably wider areas than before.

In another pond (the tetramer pond), somewhat different system was adopted using the same type of sweeping system. In this case, the rotating arms were provided, over the whole length, with the jets shown in Fig. 6,

Fig. 6. New type of the jets attached to the sprinkling arms.

and the rotation of arms was effected—independently of the system of water circulation—by a mechanical means, details of which will be described in

a later section. Using these equipments, the sweeping effects of the jets of various calibers (4, 5 and 7 mm) were compared at various rates of water circulation. It was found that the sweeping effect was most satisfactory when (i) the jets of 5 mm caliber were marshalled at intervals of 15 cm on the arms which had a clearance of 5 cm or less from the bottom of the pond, and (ii) from each jet the solution was ejected at a rate of 4 liters per minute. In the case of the tetramer pond, equipped with the mechanical rotation system*, the above mentioned rate of water ejection was realized by circulating the solution with a pump of 1 h.p. and 280 lit./min. capacity (the water pressure at the outlet of pump being 4.8 lb.).**

As regards the rate of rotation of the arms (effected either by the mechanical means or by the reaction of water flux from the arms), it was found that a rate of approximately 120° per minute (0.33 r.p.m.) was sufficient for obtaining good results in CO₂-feeding as well as in sweeping of algal cells.

SUPPLY OF CARBON DIOXIDE TO THE CULTURE

Carbon dioxide supplied to the culture was generated by burning coal gas using a round gas burner (12 inches in diameter) with two rings of jets, which was placed in a housing illustrated in Fig. 7. According to the Tokyo Gas Company, the composition of the gas they supply has the following composition, in percent: H₂ 30.1, N₂ 23.0, CO 18.5, CH₄ 15.2, CO₂ 6.4, O₂ 3.8, C₃H₈ 3.0; and the complete combustion of this gas with a minimum amount of ordinary air is to yield a gas mixture consisting of 10.5% CO₂, 20.7% H₂O and 68.8% N₂. The gas mixture produced from our CO₂-generating system contained—after it was freed from water vapor by cooling—about 2–3% CO₂ in air. By the aid of an air-compressor (7½ h.p. with a capacity of 50 m³/hr.) this gas mixture was sent to each pond at a rate of 3–5 lit./m²-min. (the area referring to the illuminated surface of ponds). The content of CO₂ in the gas mixture was occasionally checked by using a Rauter CO₂-meter MR-1 (Mitaka Kogyo Co., Ltd., Tokyo). Sufficient supply of CO₂ to the pond was necessary not only for enabling algae to photosynthesize rapidly but also for keeping the pH value of culture medium at an adequate level (especially when nitrate was used as the nitrogen source).

SYNCHRONIZATION OF ROTATION OF THE ARMS IN THE POLYMER POND

In the polymer pond we attempted two methods of synchronized rotation of arms. In one method, each center island of component-pond had one arm

* Each component-pond had two rotating arms, and each arm had 5–10 jets.

** The capacity of this pump was somewhat in excess for the operation of our tetramer pond (4 m × 10 m). It is expected that the pump of this capacity can well be used for operating a pond of 5 m × 12.5 m in dimension.

which moved by the reaction of water flux, and all arms were connected with connecting rods. When only one series of rods was used—as it was the case in the layout shown in Fig. 3—disturbances of synchronous movement happened to occur at the moment when the supporting points of the rods passed the line connecting the centers of the component-ponds. To improve this point, two series of connecting rods as illustrated schematically in Fig. 8 were used

Fig. 7. CO₂-generator, a housing for a gas-burner. The upper part is a water tank for utilizing the produced heat.

in later experiments. The purpose of using two series of rods was to ensure the smooth running of synchronous movement of the arms.

The method adopted in another device of polymer pond, in which the movement of arms and its synchronization were effected by a mechanical means, is illustrated in Figs. 9 and 10. In this case the polymer pond was equipped, along its longitudinal axis, with a rod which was supported by two stays and connected to the centers of rotating arms by means of bevel-gears.

Fig. 8. Schematized representation of the connecting rods used for polymer-pond.

Each component-pond had two sprinkling arms. The rod was rotated slowly by being driven by a motor (fitted with a two-fold rate reducer consisting of worm-gears and a pulley) which was placed outside the pond. The bevel-gears of the rod were connected to the axes of component-ponds alternately in opposite directions, so that the arms of the adjacent component-ponds rotated alternately in opposite directions. Since the arms of successive

Fig. 9. The polymer-pond, in which the sprinkling arms were rotated by mechanical means.

component-ponds were directed in the manner as illustrated in Fig. 10, all arms in the pond rotated smoothly without touching with each other. The rate of rotation of arms was about 0.7 r.p.m. Thus far the most satisfactory results of culture have been obtained with this system of mechanical rotation and by adopting to it the jet system as illustrated in Fig. 6.

Fig. 10. The polymer-pond, in which the sprinkling arms were rotated by mechanical means. The arrows show the direction of the movement of arms, which rotated smoothly without touching with each other.

HARVESTING AND PROCESSING

Algal cells were separated from the culture solution by using a Sharples centrifuge of 3 h.p. (capacity: 500 lit. per hr.). The centrifuged cells were washed with 50–100 volumes of water and separated again by centrifugation. Further dehydration of cells was carried out by the following methods.

(1) Freeze-drying. Use was made of a R-L-500 freeze-dryer of Kyowa Applied Physics Co., Ltd., Tokyo. About 2 kg of the cake of algal cells (water content: about 75%) could be freeze-dried in 20 hours. The powder obtained was brilliantly green-colored and had more grassy flavor and taste than the samples dried at higher temperatures.

(2) Spray drying. A spray dryer of Happo Seiki Co., Ltd., Tokyo, was used. (Fig. 11). The centrifuged algal cake was thoroughly mixed with water to make a thick suspension containing about 100 ml (packed cell volume) per liter. The cell suspension was introduced to the “atomizer” at a rate

Fig. 11. Spray dryer used for drying harvested algal cells.

of about 30 ml per minute. The temperature of drying was estimated to be about 100° . The powder obtained was dark-green in color and showed rather strong odor and taste characteristic of unicellular green algae.

(3) Treatment with methanol. Using an extraction apparatus (Soxhlet type), 50 liters in capacity, about 3 kg of algal cake (water content: about 75%) was extracted with 20 liters of methanol. The filter cloth used was "Nylon 9A-100" of Toyo Rayon Co., Ltd., Tokyo. After 9 hours of extraction (at about 60°) during which time about 80 liters of methanol passed through the mass of algal cells, most of the pigments and grassy-odored substances went into the methanol fraction. The algal powder obtained after driving off methanol by heat was light green in color and deprived considerably of flavor and taste characteristic of algae. When the process of drying (removal of methanol) was carried out in air, the powder assumed a somewhat grayish color, which seemed to be due to the oxidation of some fatty substance. This did not occur and an almost colorless and tasteless powder was obtained when the methanol-treated algae were further extracted with ether.

CLIMATIC CONDITIONS

All the outdoor cultures were run under natural sunlight and without regulating the temperature. In Table 7 are presented statistical data, collected by the Central Meteorological Observatory, Tokyo, on the average solar radiation, average atmospheric temperature and average amounts of precipitation and evaporation in each month in Tokyo.

Generally speaking, the main factor which determines the yield of algae is the temperature in winter and the solar radiation in summer. The temperature of culture, which was mainly dependent on the atmospheric temperature and the amount of solar radiation, varied in a wide range both diurnally and seasonally. In Table 8 are given the highest and lowest values of culture temperature which were observed in each month of the year 1956.

Table 7. Average solar radiation (including infra-red light), atmospheric temperature, and the amounts of precipitation and evaporation in each month in Tokyo.

Month	Solar radiation* in gram-cal./cm ² -day	Atmospheric temperature** (C)	Precipitation in mm/month** (a)	Evaporation in mm/month** (b)	a-b
Jan.	267	3.2	40.7	53.1	-12.4
Feb.	316	3.9	77.2	58.7	18.5
Mar.	376	7.0	95.3	83.0	12.3
Apr.	455	12.8	136.1	103.0	33.1
May	477	17.2	138.0	116.6	21.4
June	428	20.8	176.6	109.1	67.5
July	477	25.1	146.5	135.2	11.3
Aug.	452	26.4	148.0	144.5	3.5
Sept.	342	22.6	228.9	97.0	131.9
Oct.	281	16.4	226.2	69.1	157.1
Nov.	239	11.0	96.7	54.6	41.8
Dec.	220	5.7	57.7	46.2	11.5

* Data collected during the period from 1935 to 1952.

** Data collected during the period from 1921 to 1950.

YIELD OF ALGAE

Since the outdoor cultures in the present study were run under various operational conditions using various strains of algae with the purpose of finding out suitable procedures and favorable conditions, the yield obtained in each series of experiment were not always as good as they were expected for optimum conditions in respective seasons. From numerous data collected during the period of the present study, those for the two representative strains, *Chlorella* 139 and *Scenedesmus* 141, which were grown from January

Table 8. The highest and lowest values of culture temperature which were observed in each month of the year 1956.

Month	Temperature (C)	
	minimum	maximum
Jan.	-0.8	12.0
Feb.	-1.0	19.0
Mar.	-1.5	24.0
Apr.	2.5	26.2
May	9.0	32.5
June	13.0	36.5
July	15.0	37.5
Aug.	20.0	40.0
Sept.	15.0	37.0
Oct.	10.0	35.0
Nov.	5.0	21.0
Dec.	0.0	13.0

Table 9. Yield of algae, *Chlorella* 139 and *Scenedesmus* 141, obtained during the period from January to December 1957.

Algae	Month	Days of operation	Yield (in g/m ² -day) from		
			Pond 3	Pond 4	Pond 5
C. 139	Jan.	29	1.61	—	—
	Feb.	28	2.20	—	—
	Mar.	24	6.70	4.97	4.95
	Apr.	16	9.87	13.16	—
	May	25	16.47	—	19.39
	June	21	—	13.56	14.15
S. 141	July	28	—	—	9.1
	Aug.	31	13.40	—	—
	Sept.	16	10.25	13.52	13.11
	Oct.	17	8.02	9.13	9.66
	Nov.	29	3.48	6.29	5.67
	Dec.	10	2.43	1.89	2.06

to June, and from July to December 1957, respectively, are summarized in Table 9. In this table the average yield in each month is given in terms of dry weight (g) of cells per square meter per day.

Based on these data, the average daily yield throughout the year is calculated to be 8.64 g/m²-day which corresponds to a yield of 13 metric tons

per acre per year. Earlier outdoor experiments (using the medium-sized flat flasks) performed by Sasa *et al.* (15), who cultured *Chlorella ellipsoidea* and a thermophilic strain of *Chlorella* throughout the year, have shown that the combined use of these two strains in different seasons would give an average daily yield of 12.4 g/m²-day or 18 metric tons per acre per year. The figures we have obtained from our mass cultures were about 70 percent of this expected value.

SUMMARY

Summary report was made on the results of mass culturing of unicellular algae using the method of "open circulation system", which was conducted during the period from 1956 to 1957. Various attempts were made in the device of culture units and the procedure of their operation, and the methods which were found to give satisfactory results were described in detail. Cultures run throughout the year, using certain strains of *Chlorella* and *Scenedesmus* gave an average daily yield of 8.6 g/m²-day, which corresponds to a yield of 13 metric tons (dry weight) per acre per year.

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REFERENCES

- (1) H. TAMIYA: Proceedings of the World Symposium on Applied Solar Energy, 231 (1955), Phoenix, Arizona, November 1-5, 1955. Publ. by Stanford Research Institute, Menlo Park, California.
- (2) Algal Culture from Laboratory to Pilot Plant. Edit. John S. BURLEW, Carnegie Inst. of Washington Publ. 600 (1953), Washington, D.C. pp. 357.
- (3) F. GUMMERT, M.-E. MEFFERT and H. STRATMANN: Carnegie Inst. Wash. Publ. 600, 166 (1953).
- (4) A. MITUYA, T. NYUNOYA and H. TAMIYA: Carnegie Inst. Wash. Publ. 600, 273 (1953).
- (5) H. TAMIYA: Ann. Review of Plant Physiol., **8**, 309 (1957).
- (6) H. TAMIYA, E. HASE, K. SHIBATA, A. MITUYA, T. IWAMURA, T. NIHEI and T. SASA: Carnegie Inst. Wash. Publ. 600 204 (1953).
- (7) H. TAMIYA, T. IWAMURA, K. SHIBATA, E. HASE and T. NIHEI: Bioch. et Bioph. Acta, **12**, 23 (1953).
- (8) D. I. ARNON: Amer. J. Bot., **25**, 322 (1938).
- (9) E. J. CONWAY: Microdiffusion Analysis and Volumetric Error. Crosby Lockwood & Son, Ltd., London, 1950.

- (10) B. J. L. ALLEN: *Bioch. J.*, **34**, 858 (1940).
- (11) Official Methods of Analysis of the Official Agricultural Chemists. p. 104. Assoc. Offic. Agric. Chemists, Washington, D.C., 1950.
- (12) E. B. SANDELL: *Colorimetric Determination of Traces of Metals*, Chemical Anal., **3** (1950), Intersci. Publishers, Inc., N. Y.
- (13) R. W. KRAUSS: *Nutritional Requirements and Yields of Algae in Mass Culture*. A paper presented at the Conf. Solar Energy: the Scientific Basis, Tucson, Arizona, October 31 and November 1, 1955.
- (14) R. S. WOGLUM, J. R. LAFOLLETTE, W. E. LANDON and H. C. LEWIS: *J. Econ. Ent.*, **40**, 818 (1947).
- (15) T. SASA, Y. MORIMURA and H. TAMIYA: *J. Gen. & Appl. Microbiol.*, (Japan), **1**, 183 (1955).