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Summary

Sixteen strains of moulds were examined on the point of cerophilic growth and tonophilic.

(1) Cultures were performed on hypertonic growth media containing varied concentrations of sodium chloride. The tested mould strains were divided into three groups, namely "steno-", "eury-" and "non-halophilic" forms.

(2) Cultures were performed under varied atmospheric humidity. The results obtained are shown in Figure 1 and the results of this set of experiments are summarized in Table 2, together with those described in the previous section.

(3) In order to elucidate whether the tonophilic nature of mould depends on its nutritional condition, another series of experiments was performed. The results obtained were shown in Table 3 and 4.

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On the Application of ^{14}C -Method to Measuring Organic Matter Production in the Lake

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市村俊英*・西条八東**：湖沼における物質生産測定への ^{14}C -法の応用について

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Since Steemann Nielsen (1952) used the ^{14}C technique on the Galathea expedition, the ^{14}C -method has been known as the most sensitive technique for measuring primary production in waters. However, many pending questions still remain in this method, though the critical test has been made by several investigators (Steemann Nielsen 1952, 1957, Ryther and Vaccaro 1954, Ryther 1956, 1957, Miyake et al. 1954, Doty 1956,

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1957). Moreover, almost all of these studies were concerned with the sea water and ^{14}C has not yet been employed adequately in lakes. It seems to be indispensable to scrutinize the fitness of this method to measurement of the primary production of lakes.

In the present paper the authors will discuss the application of the ^{14}C -method to the fresh water in comparison with the well known O_2 -method.

Methods

The method used in this paper was similar in principle to Steemann Nielsen's (1952) and Ryther's (1954). Briefly stated, natural water or suspension of pure culture of algae was filled in a clear and a dark glass bottle and then added with 1 ml. of radioactive sodium carbonate solution containing 1 or 4 μC . of ^{14}C . After exposure for about 3 to 6 hours, or in some cases for 24 to 48 hours, the phytoplankton in the bottle was filtrated with a membrane filter, then washed and dried, and its radioactivity was measured with a G. M. counter. The absolute CO_2 assimilation of phytoplankton was calculated as follows;

$$\text{CO}_2 \text{ assim.} = \text{Total CO}_2 \text{ in water} \times \frac{\text{Activity of } ^{14}\text{C} \text{ assim. in phytoplankton}}{\text{Activity of } ^{14}\text{C} \text{ added in water}}$$

Along with ^{14}C -method, O_2 -method was used for determining assimilation and respiration of phytoplankton. Each result was verified with double or triple observations. The amount of total CO_2 in water was measured by Saijō's method (1956), by which even

Table 1. Total CO_2 concentration in surface water of some lakes

Lake type	Lake	Date	Total CO_2 mg./l.
Oligotrophic	Akagi-onuma	July 26, 1955	8.4
	Biwako	Sept. 15, 1955	18.3
	Ashinoko	June 22, 1957	20.0
Mesotrophic	Harunako	Sept. 29, 1957	12.6
	Shinseiko	July 13, 1955	25.9
Eutrophic	Kasumigaura	Sept. 16, 1956	26.6
	Teganuma	March 26, 1957	34.7
	Nakanuma	May 30, 1957	42.0
	Jōnuma	Oct. 27, 1956	71.0

low content of CO_2 was measured in field with an accuracy of 1.5 % only within 15 minutes. The values thus gained in some Japanese lakes are summarized in Table 1. Phytoplankton density was determined by pigment analysis and was indicated as chlorophyll amount. In all cases, O_2 equivalent of CO_2 assimilation was calculated under assumption of $\text{R.Q.}=1$, without consideration on the 6% depression of ^{14}C assimilation in comparison with ^{12}C -assimilation (Steemann Nielsen 1952). Fluctuation of some of results gained with ^{14}C -method is summarized in Table 2, It seems,

Table 2. Fluctuation of count number measured in each sample taken from some waters

Material	Nakanuma raw water		Teganuma raw water		<i>Chlorella</i> sp. pure cult.
Duration hr.	14		2		2
Condition	Laboratory 20°		Laboratory 22°		Laboratory 20°
	12 Klux	Dark	12 Klux	Dark	12 Klux
Assimilation as count/min.	126	6	139	4	329
	128	9	141		352
	131		145		368
	135		156		376
	162				

however, to be mainly caused by heterogeneous distribution of phytoplankton in the suspension rather than by technical error.

Results and Discussion

1. Relation of the photosynthesis to the light intensity

Pure culture of *Chlorella* sp. was suspended in 1/5 concentration of Detmer's solution and the suspensions were exposed to various light intensities at 22° in 100 ml. glass bottles. As light source, a 500 W flood lamp was used. The initial total CO₂ in the suspension was 20 mg./l. and the chlorophyll concentration was 0.052 mg./l. Similar values were observed in natural eutrophic lakes in Japan (Ichimura 1956). Photosynthesis was measured after 2 hour exposure to light, during which the bottles were rotated at twenty minute intervals in order to prevent the algae from sedimentation.

Between the photosynthetic rates which were determined with ¹⁴C- and O₂-methods good agreement was proved in higher illumination (Fig. 1), though in lower illumination there was a discrepancy which might be caused by CO₂ dark fixation. This result coincides fairly well with Ryther's (1956).

Fig. 1. Photosynthesis measured by O₂(○)- and ¹⁴C(●)-methods in a pure culture of *Chlorella* sp.

The influence of CO₂ concentration on photosynthesis was investigated with both the methods. Various amount of sodium bicarbonate was added into 1/5 Detmer's solution. The concentration of total CO₂ was 5.5, 11, 22, and 44 mg./l., respectively; these fall in the range of natural total CO₂ concentration in Japanese lakes. Fig. 2 shows the results obtained at 20° and in 12 kilolux illumination. The CO₂ saturation in photosynthesis

occurred at about 5 mg. CO_2 per litre, afterwards increase of CO_2 concentration caused only slight increase of photosynthesis. No special difference was detectable between the values obtained with ^{14}C - and O_2 -methods.

Further comparison of both methods was carried out on raw water sampled from 1 m. depth of lakes. The experiments were started under laboratory condition within 2-6 hours after sampling. Exposure time was varied depending on the phytoplankton concentration; it was usually about 2 hours for eutrophic lakes samples and 6 hours for oligotrophic ones. Fig. 3 indicates the light assimilation curves obtained with the samples collected from Lake Teganuma (eutrophic) and Lake Haruna (mesotrophic). Dominant phytoplankters in the former were *Fragillaria crotonensis* and *Tabelaria fenestrata*, and in the latter they were *Melosira italica*, *Eudorina elegans*, etc. A fairly close agreement with the result obtained in *Chlorella* suspension was found in the case of Lake Teganuma. The data at Lake Haruna also showed similar trend, though it is not so clear as at Lake Teganuma. In Table 3 the main features obtained with both methods are summarized.

Fig. 2. Relation between photosynthesis and total CO_2 concentration measured by O_2 (○)- and ^{14}C (●)-methods in a pure culture of *Chlorella* sp.

Fig. 3. Photosynthesis measured by O_2 (○)- and ^{14}C (●)-method in the sample waters taken from Lake Teganuma (eutrophic) and Lake Haruna (mesotrophic).

Table 3. Main photosynthetic features obtained by ¹⁴C- and O₂-methods in sample waters taken from Lake Teganuma and Lake Harunako. Figures in brackets denote mg. oxygen per mg. chlorophyll per hour

Lake	Total CO ₂ mg./l.	¹⁴ C-method mg. O ₂ /l./hr.		O ₂ -method mg. O ₂ /l./hr.	
		Light	Light-Dark	Gross.	Net.
Teganuma	34.7	0.112 (6.6)	0.106 (6.2)	0.123 (7.2)	0.099 (5.8)
Harunako	12.6	0.036 (4.8)	0.031 (4.1)	0.041 (5.5)	0.035 (4.7)

2. Determination of photosynthetic rate under field condition.

The general procedure was similar to that described previously. Photosynthesis was measured with so-called “in situ” method. Namely water samples taken up from various depths of lakes were suspended, after filling the 100 ml. or 200 ml. clear and dark bottles, to the respective depths where they had been sampled. In parallel with this, light intensity, temperature, chlorophyll and nutrient salt contents (PO₄-P, NH₄-N, NO₃-N) in the water were determined by usual methods.

The photosynthetic capacity of water alters with light intensity, and the density of phytoplankton varies with water depth. Fig. 4-A shows the vertical change of the

photosynthetic capacity of water at Lake Nakanuma, which was determined at the deepest part of the lake in May. The bottles were kept in water for two days (a cloudy and a rainy). In the surface layer the value determined with ¹⁴C-method agreed closely with the net assimilation measured with O₂-method, but in low light intensity at or below the compensation depth, the former value was greater than that of O₂-method as expected from the results mentioned already. Fig. 4-B indicates the results obtained at Lake Kasumigaura on a cloudy day of November 1956. Samples were suspended in the lake from noon to next noon. Prevailing dominant phytoplankton was *Microsistis* sp. In case of measuring productivity under field condition, besides the photosynthesis itself, dark fixation of CO₂ should be taken into consideration. Intensity of dark fixation in lake water, so far as we know, was usually only few per cent of that of photosynthesis under optimal light condition, and it seems that the dark fixation is generally not so im-

Fig. 3. Vertical changes of photosynthetic rate measured by O₂- and ¹⁴C-methods in Lake Nakanuma and Lake Kasumigaura. O₂-method (—●—, net-photosynthesis; ...●..., respiration) ¹⁴C-method (—●—, photosynthesis; ---●---, dark fixation)

portant in the primary production of the lake as for that of the sea. However, occasionally high values of dark fixation have also been measured in the authors experiments. It is therefore desirable to determine CO₂ dark fixation in each case in parallel with the measurement of photosynthesis. The authors will treat this problem in other paper in detail.

3. Relation of duration time to the photosynthetic rate

It has been said that phytoplankton enclosed in a bottle cannot keep its normal photosynthetic activity under the unnatural condition for a long time. As the duration time, 2-6 hours are generally adopted (Verduin 1954). Effect of long duration on photosynthetic rate should be examined with the ¹⁴C-method, too. Fig. 5-A shows the change of photosynthetic rate with time course, in continuous illumination of 12 kilolux at 22°. Water was sampled from Lake Nakanuma in April 1956. During the first six hours, photosynthesis proceeded actively and lineary correlated with the time elapsed, but afterwards activity declined gradually. Same trend was also observed in an experiment with sea water (Fig. 5-B). The dark fixation of CO₂ was extremely low in both the cases. Similar experiment was done at Lake Ashinoko (oligotrophic) with the “in

Fig. 5. Effect of duration time on photosynthesis in continuous illumination measured by O₂- and ¹⁴C-methods in natural waters. O₂-method (○-gross-photosynthesis: ● net-photosynthesis). ¹⁴C-method (⊙ photosynthesis)

Table 4. Relation of duration time to photosynthetic rate obtained by O₂-and ¹⁴C-methods in Lake Ashinoko in July. First two days were rainy and last day was fine

Duration time	¹⁴ C-method mg. O ₂ /l.		O ₂ -method mg. O ₂ /l.		
	Light	Dark	Net	Resp.	Gross
6 hours (noon to sunset)	0.007	0.002	0.008	0.002	0.010
24 " (noon to next noon)	0.017	0.003	0.017	0.005	0.022
48 " "	0.028	—	0.037	—	—

situ" method on a cloudy day of July. The sample water was collected from 1 m. depth and some bottles were suspended at the same depth from noon to sunset, and the others were suspended from noon to next noon. The results are summarized in Table 4. The total photosynthesis throughout a day was almost the double of a half day value.

Concerning this, other experiment was carried out with O_2 -method at Lake Kasumigaura in June. Photosynthesis during 12 hours was measured in two ways, i.e. one was summing of the photosynthesis measured every two hours, and the other was determining photosynthesis at sunset after a continuous 12 hour exposure. It was sunny during the experiments. Real assimilation of 0.85 mg. O_2 /l. was calculated in the former and 0.67 mg. O_2 /l. was obtained in the latter. The authors confirmed that the duration of exposure should not exceed half a day, because the continuous exposure from sunrise to sunset gave a depressed productivity. However, when a longer exposure is inevitable, it is recommended that the experiment will be started at noon and finished at the next noon.

Summary

In this paper the authors tried some fundamental investigations on the ^{14}C -method for measuring primary production in the lake in comparison with the O_2 -method.

From the results obtained it was confirmed that the ^{14}C -method is useful for the measurement of the net photosynthesis and the exposure to light for the measurement should not exceed half a day even though in case of the ^{14}C -method.

In general, dark fixation of CO_2 in lake is low, but occasionally high abnormal values were measured in some waters. It is therefore desirable to determine the dark fixation simultaneously along with the photosynthesis.

Since the ^{14}C -method is not fit for measuring respiration, proper allowances for respiration should be made in order to calculate the gross photosynthesis.

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