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A Comparative Study of Primary Production by using the ^{14}C and Oxygen Methods

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^{14}C-法과 酸素法에 依한 一次生産 比較研究

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Abstract

Photometric Winkler titration provides high precision enough to use the oxygen method in moderately productive waters. In short incubation (2-4hr), about 2 μg C/l/h can be differentiated by the photometric titration.

The oxygen and ^{14}C methods resulted in good agreement with each other in the diurnal primary production measurements. Despite small descrepancies the severe underestimation of ^{14}C-technique was not observed in short-term incubation. Size-fractionated production studies indicated the importance of nanoplanktonic production in the coastal water (62-88%).

Bacterial respiration may lead to significant underestimation in estimating net photosynthesis in the oxygen method. In spite of some problems associated with the ecological application of antibiotics, it seems feasible to use Gentamycin in separating planktonic respiration from that of total community.

요약 : Winkler 광적정법을 이용하면 산소법이 연안의 생산량측정에 있어서 충분한 정확성을 갖고 있음이 입증되었다. 2～4시간 정도의 단기 배양을 할 경우에도, 광적정법에 의하여 약 2μg C/l/h의 양까지 측정할 수 있다.

산소법과 ^{14}C-법에 의한 일차생산량 측정에 있어서는 두 방법의 결과가 잘 일치하였으며, 단기 배양에서는 ^{14}C-법의 심한 과소추정치가 관찰되지 않았다. 세포크기별 생산량에서는 연안해역에서 미세 플랑크톤의 일차생산이 62-88%정도로서 상당히 중요한 부분을 차지하였다.

산소법에 의하여 순 광합성량을 추정할때에는 박테리아의 호흡 때문에 상당한 과소추정치를 야기할 수 있다. 항생제를 생태학적으로 적용하는데 있어서 다소의 문제점이 있으나, Gentamycin을 사용하여 전체군집의 호흡량에서 플랑크톤에 의한 호흡량을 분리 측정할 수 있을 것으로 보인다.

INTRODUCTION

Accurate measurement of phytoplankton photosynthesis has been one of the most important concerns in marine ecology, because primary production is essential in understanding the trophic transfer of energy and materials in aquatic ecosystem processes. However, the practical performance of production measurement on natural waters inevitably includes manifold problems of methodology.

Furthermore, ^{14}C-tracer technique first introduced by Steemann Nielsen in 1952 has intrinsic uncertainties as to not only whether the method measures net or gross photosynthesis, or something between the two, but also whether or not there is substantial underes-

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timation from the several error sources (e.g. Ryther 1956, Nalewajko and Lean 1972, McMahon 1973, Pugh 1973, Lean and Burnison 1979, Peterson 1980, and Carpenter and Lively 1980).

In practice, ^{14}C-technique, owing to its high sensitivity sufficiently applicable to the low rates of carbon uptake, has been almost the only standard method for estimating the flux associated with primary production in aquatic environments. But some potential error sources peculiar to ^{14}C method make it complicated to interpret the production data; i) release of dissolved organic matter (Anderson and Zeutschel 1970, Gieskes 1973), ii) high dark uptake possibly due to bacterial or algal dark carbon uptake (Morris et al. 1971) iii) leakage of fixed carbon from algae due to cell rupture during the filtration (Theodorsson and Bjarnason 1975, Silver and Davoll 1978).

Oxygen method, which was already undertaken in the late 1920s and early 1930s, has been almost superseded by the ^{14}C method in the phytoplankton production studies, because of its principal difficulties in the poor sensitivity of oxygen measurements. Using chemical method or oxygen probe, it is nearly impossible to detect the planktonic assimilation rates which have low oxygen change by photosynthesis or respiration. In order to have measurable oxygen differences, oxygen method must suffer from either long incubation or concentrating the samples (Berman 1973). But these may cause the selective changes in populations and physico-chemical conditions in the bottle, or make it difficult to interpret the results due to the changes of natural conditions (Vollenweider 1974).

However, a recently introduced precise Winkler titration method by the sensitive photo-electric end point detector has made it feasible to use the oxygen method in the coastal primary production measurements (Bryan et al. 1976, Harthwig and Michael 1978, Williams and Jerkinson 1982). This means that the oxygen method by the photometric titration will provide more simple and straightforward results of gross and net production, and will be applicable to respiration studies of phytoplankton and bacteria.

In this study, we examined the validity of oxygen method with the photometric titration by evaluating possible error sources. In an effort to separate bacterial respiration, we investigated the ecological application of antibiotics in oxygen method. ^{14}C and oxygen methods were compared through *in situ* primary production measurements, and size-fractionated production studies were also performed by ^{14}C-technique.

MATERIAL AND METHODS

The study area

The present study was carried out in marine and fresh water. Diurnal productivity was measured for 36 hours in Lake Soyang, the artificial lake of North Han River (Fig. 1). Size-fractionated production was measured at two stations in Gyeonggi Bay of the Yellow Sea (Fig. 1). Two sampling stations in Gyeonggi Bay have comparatively different environmental conditions: station A is relatively unpolluted, but station B receives large untreated domestic and industrial wastewater.

Sampling dates and other physico-chemical conditions were listed in Table 1.

Sampling and incubation

All the experiments of ^{14}C and oxygen

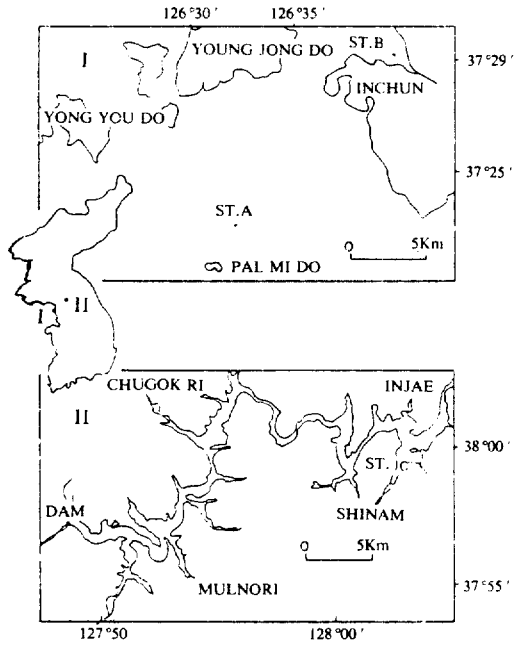

Fig. 1. Map showing sampling stations in Gyeonggi Bay (I) and in Lake Soyang (II).

Table 1. Sampling stations, dates, and physico-chemical conditions

Station	Date	Sampling Depth (m)	Hours Incubated	Water Temp. (°C)	Salinity (‰)	NH_4-N (μg at-N/l)	NO_3-N (μg at-N/l)	PO_4-P (μg at-P/l)	BOD (ppm)
Gyeonggi Bay									
A	15 May	1,5,10	1.8-2.2	12.5-12.8	31.7	N.D.	1.5-8.5	0.21-0.70	12.3-16.4
B	14May	1	2	14.0	30.9	3.80	1.2	0.24	14.6
A	24 July	1,6	2.0-2.2	22.7-23.7	30.1	2.21-4.39	1.1-3.6	0.40-0.41	2.4-6.9
B	25 July	1	3	24.8	27.3	61.70	8.7	0.54	7.6
B	*24 Oct.	0	6	15.0	29.6	16.50	9.8	0.64	9.3
Lake Soyang									
C	25-26 Sept.	1	3.0-24	20.3-24.5	N.D.	0.90-2.50	20.2-38.8	0.18-0.24	N.D.**
C	*28 Oct.	0	9	15.5	N.D.	0.90	30.7	0.28	N.D.

*: laboratory incubation under fluorescent light
**N.D.: not determined

method were conducted by composite sampling. Water samples were filtered through 300 μm Nitex screen in order to remove large zooplankters. Acid-cleaned twelve light bottles and eight dark bottles (ca. 300 ml) were used at a time for duplicate and triplicate analyses. Bottles were laid down on the incubation cartridge (80 × 80 × 100 cm), which could fix the bottles vertical to the incident light (Fig 2).

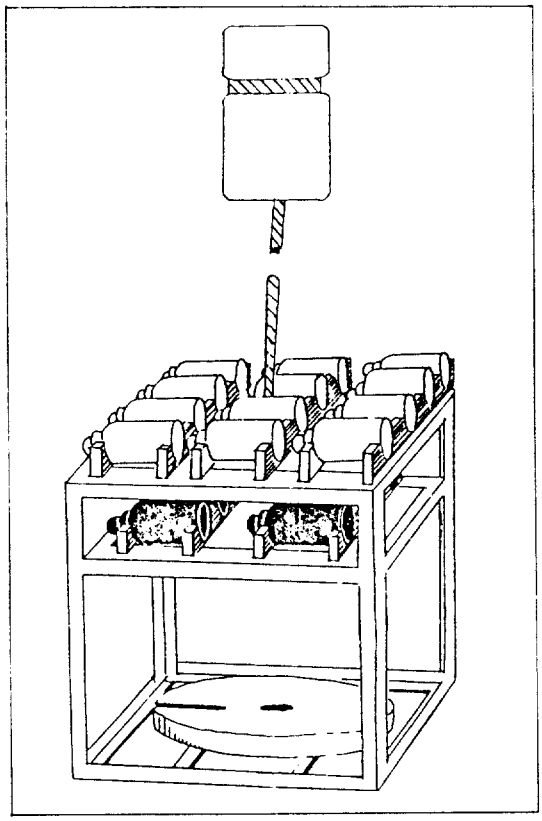

Fig. 2. *In situ* incubation cartridge (80 × 80 × 100 cm)

Oxygen Method by the Photometric Winkler Titration

The conventional Winkler method for dissolved oxygen determination was improved by the photometric endpoint detection (Bryan et al. 1976). The intense near UV absorption spectrum of tri-iodide ion (350 nm) disappears as liberated iodine is titrated, and the logarithmic increase of photocell signal ceases immediately after the small addition of thiosulfate at the end point (Fig. 4).

The photometric titrator was in principle the same as that described by Bryan et al. (1976). But light sources was improved as fluorescent UV lamp from a fluorometer (F4/T4 UV light lamp), and UV light narrow pass filter at 360 nm (Color # 7-60) was used (Harthwig and Michael 1978). In the light-path, two convex lenses (focal lengh 10 cm) was placed on either side of the sample to spread the beam (Williams and Jerkinson 1982). The driving voltage of the photocell (General Electric 1P39 tube) was reduced to 40 V and provided by a voltage regulator rather than batteries. Another major improvement of this method, the whole bottle titration (Green and Carritt 1966), eliminated the volatilization of liberated iodine due to the transference of acidified solution. Also, Thomas motor-driven automatic burette was used in order to carry out rapid and accurate titration suitable for field and shipboard use. (Fig 3-1, 3-2)

Dissolved oxygen changes between light and dark bottles were measured with photometric titrator described above. In all cases, duplicated or triplicated samples were analyzed and fixed samples were titrated as im-

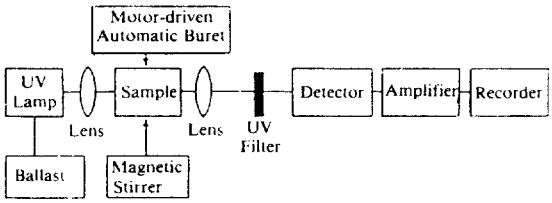

Fig. 3. Block diagram of the Winkler photititrator.

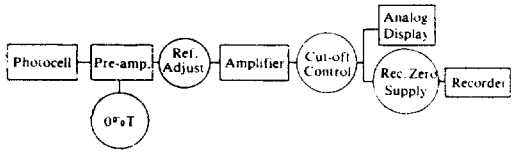

Fig. 3-2. Electronic system of the Winkler photititrator.

mediately as possible.

Respiration correction

We used Gentamycin, broad-spectrum antibiotics with 100μg/ml samples for respiration corrections. Before the incubation, one or more hours of pre-incubation was needed. Plate count was also accompanied in order to estimate the Gentamycin-negative bacteria. Water samples were also pre-filtered with 0.45 μm GF/C, and the oxygen changes of filtrate were measured to compare the Gentamycin correction (Pratt et al. 1959).

We measured ^{3}H-glucose uptake in order to ascertain the bacterial inhibition of Gentamycin. To 300 ml BOD bottle, 3μCi of D-glucose-6-^{3}H (Amersham Corp.) was added with an automatic pipette. Control samples were prepared by adding formalin before the addition of ^{3}H-glucose. Duplicate test and control samples were incubated *in situ* for 2 hr.

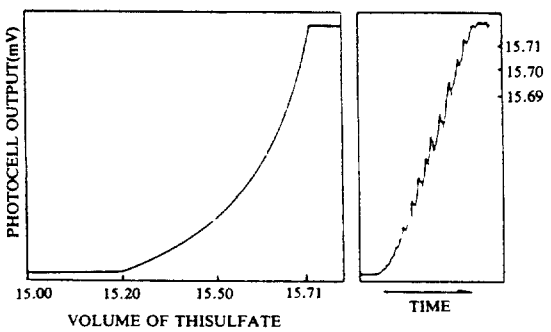

Fig. 4 Diagram showing the photocell output and the trace of pen recorder during the titration of a sample.

They were filtered with 0.2 μm Nuclepore filters under mild vacuum (< 200 mm Hg) and were radioassayed with the counting efficiency of average 26% (Azam and Holm-Hansen 1973).

^{14}C-experiments and size-fractionation

^{14}C-bicarbonate working solution (10 μCi/ml) was prepared from the stock solution (Amersham Corp. 1 mCi/ml). It was oxidized with strong UV light for 12 hours in order to remove organic ^{14}C-labelled impurities (Williams et al. 1972). 5-10 μCi of working solution was added to the different experiments. At the end of an incubation, samples were fixed with Lugol iodide or immediately filtered.

In all cases, 300 ml aliquots were filtered sequentially through a series of 22 μm Nitex screen and Nuclepore filters of decreasing pore size (3μm, 1μm, 0.4μm, and 0.2μm). After filtration, filters were washed with filtered ambient water and then removed from filter holders and acid-fumed for 20 min.

1ml of 2-methoxyethanol and 0.2 ml of ethanolamine were used to dissolve the filters. Vials were counted in liquid scintillation counter (Packard TRI-CARB 4550) in 10ml scintillation cocktail (2:1 toluene: 2-methoxyethanol plus 5 g of PPO and 0.1 g POPOP per liter).

For the purpose of correcting the errors derived from exudation and cell rupture, dissolved production was measured. The filtrate from the ^{14}C-incubated samples was immediately frozen and later processed in the laboratory. 1N HCl was added to give about pH 2.0 and the solution was bubbled vigorously with air for 2 hr to drive off all ^{14}C-bicarbonate. After the pH of the filtrate was readjusted to 8.4 to 8.5 with NaOH, they were placed in tightly stoppered 500 ml flask, in each of which was suspended a scintillation vial containing 1 ml of Hyamin 10-X. The samples were irrdiated with strong short-wave UV light (Armstrong et al. 1966, Berman and Holm-Hansen 1974) for 6 to 12 hours to oxidize residual dissolved organic matter. They were acidified with HCl, and stirred for 4 hr at room temperature. Evolved $^{14}CO_2$ was trapped by Hyamine (Efficiency 95-98%), and counted with the same method. Counting efficiency was recalibrated with a series of quen-

ched standards containing Hyamine 10-X (Herbland and Bois 1974).

Nutrients and chlorophyll etc.

Ammonia was analyzed in the field station by the method of Solórzano (1969). Other nutrient samples were deep frozen (-20 °C) until analysis (Strickland and Parsons 1972). Chlorophyll-a was also size-fractionated. Nuclepore filters were changed at every 100-300 ml lest the filters should be clogged. Photosynthetically active radiation (PAR) was measured with a LiCor quantum sensor.

RESULTS

Dissolved oxygen measurements by the photometric Winkler titration method

The precision of the photometric Winkler titration was tested by replicate analyses of natural water samples. The normalities of 0.02 N thiosulfate solution were standardized by titrating known equivalents of iodates ($r^2 = 0.998$).

Table 2 shows the results of dissolved oxygen measurements. The range of coefficients of variation (0.03-0.11) implies that the precision at 95% confidence limits is in the range of about 0.005-0.018 ml O_2/l at 6 ml O_2/l level. If the photosynthetic quotient is assumed to be 1.2, this range can be converted to 2.2 to 8.0 μg C/l in the carbon flux.

Although this result means the photometric titration may make it possible to discriminate average 6μg C/l by the oxygen method, it is practically difficult to maintain the precision, because of the natural population inhomogeneities and handling errors of sampling procedure in the production and

Table 2. Precision of the phototitrator in dissolved oxygen measurements

mean oxygen conc. (ml O_2/l)	n	SD	CV
5,440	6	0.005	0.09
5,736	5	0.004	0.07
6.268	5	0.007	0.11
6.514	6	0.006	0.09
6.820	3	0.002	0.03
7.254	4	0.005	0.07
7.857	3	0.004	0.05

n:number of samples
SD: standard deviation CV: coefficient of variation

Table 3. Bottle volume corrections and possible error boundary at 5ml/l level

Bottle type	n	Volume inscribed (ml)	V	Error (ml O_2/l)
A	18	300.0	1.17 ± 1.20	0.021
B	29	299.7 ± 6.08	0.40 ± 0.31	0.007
C	12	308.9 ± 6.28	0.92 ± 0.56	0.017
D	7	299.2 ± 6.42	1.56 ± 0.72	0.028

respiration experiments (Harthwig and Michael 1978). One of the serious error sources was the inaccurate volume inscribed in the BOD bottle (Table 3). Four types of incubation bottles were recalibrated.

Diurnal primary production by the ^{14}C and oxygen methods

Previous studies concerning diurnal patterns of photosynthetic activity suggested the 'afternoon depression' in the photosynthetic rate (Newhouse et al. 1967, McAllister 1961, Tijssen 1979). In order to examine asymmetrical photosynthesis in natural population, time-series *in situ* measurements of primary production were measured in Lake Soyang for about 36 hours. ^{14}C and oxygen methods were simultaneously used. Supplementary incubations for the separation of planktonic respiration from the community respiration were performed by two other methods: i) inhibition of bacterial metabolism by gentamycin, and ii) elimination of phytoplankton by prefiltration before incubation.

Figure 5 demostrated changes in photosyn-

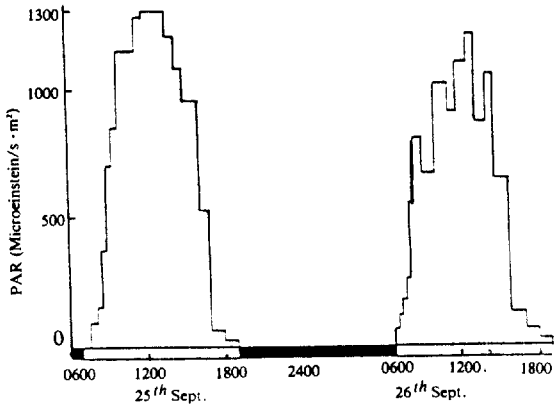

Fig. 5. Incident photosynthetically active quantum flux over the experimental period 25-26 Sept. 1984. Surface reflection was corrected (PAR: photosynthetically active radiation).

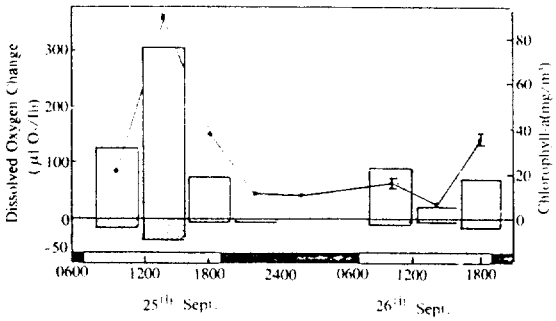

Fig. 6. Dissolved oxygen changes and chlorophyll-a concentrations (The hatched portion is the corrected respiration of phytoplankton and open is net production. The line connecting solid circles represents the changes of chlorophyll-a concentration).

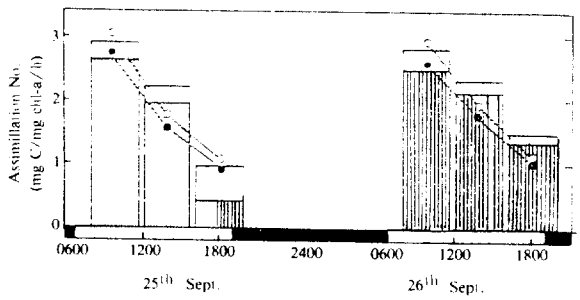

Fig. 7. ^{14}C- and oxygen-derived primary productivity per unit chlorophyll-a concentration (Histogram refers to the ^{14}C-fixation: hatched portion is particulate production and open is soluble production. Upper line connecting open circles is gross production converted from O_2 method, and lower line connecting solid circles is net production calculated by correction method).

thetically active quantum flux over the experimental period 25-26 September.

Time-series measurements of *in vitro* dissolved oxygen changes (Fig. 6) represented marked fluctuations during the experimental period, and the highest afternoon peak of the first day experiment was observed, which had not been expected. If three- to four-fold diurnal oscillations of pigment contents cannot be practically allowed, close relation between oxygen evolution and chlorophyll concentrations means the inequality of sampling at an interval of 4 hours. One of the possible explanations was the patch distribution of natural phytoplankton. As all these samples for the oxygen and ^{14}C incubations were not taken within the enclosed water column trapped on the large-volume bag (McAllister et al. 1961), it may be possible for small patch derived by wind or water movement to intrude on the sampling station located in the vale. Also these assumptions were supported by the observation of dense patch of *Peridinium* sp.

^{14}C-and oxygen-derived rates of photosynthesis were normalized by chlorophyll-a. Fig. 7 shows the diurnal changes of the photosynthetic rates per unit of chlorophyll-a. The results of assimilation number (mg C/mg chl-a/h) indicates the obvious asymmetrical and periodic pattern of photosynthesis. Although the ^{14}C data were quite closely tracking gross oxygen production in the morning, there were small discrepancies between the two sets of experiments.

Oxygen-derived net and gross production was corrected by gentamycin and prefiltration methods. Oxygen consumption of dark bottle was generally large, which indicated the possible underestimation of net production without correction method (Table 4).

Size-fractionated productivity and biomass

Table 5 shows size-fractionated primary productivity and biomass in Gyeonggi Bay and

Table 4. Respiratio corrections by the gentamycin and prefiltration methods in Lake Soyang

Time	Pg	Rc	Pn	Gentamycin		Prefiltration	
	(ml O_2/l/h)			Pn'	Pn'/R'	Pn''	Pn''/R''
25 Sept.							
8-12	0.14	0.113	0.027	0.125	8.3	0.042	0.4
12-4	0.345	0.058	0.288	0.305	7.6	0.313	9.8
4-8	0.091	0.029	0.062	0.074	4.4	0.080	7.3
26 Sept.							
8-12	0.101	0.056	0.045	0.090	8.9	0.091	9.1

(Pg: gross photosynthesis, Rc: community respiration, Pn: uncorrected photosynthesis, Pn': net photosynthesis corrected by gentamycin, and R': true respiration of phytoplankton corrected by gentamycin)

Table 5. Size-fractionated primary productivity and chlorophyll-a concentration in Gyeonggi Bay and Lake Soyang

Station	Date	Depth (m)	Total chl-a(mg/m³) P(mgC/ m³/h)		% of Total				Nano/Total (%)
					22μ >	22-3μ	3-1μ	1-0.4μ	
A	*15 May	1	chl-a	4.26	17	69	7	7	83
			P	3.81	38	44	6	7	62
		5	chl-a	3.80	12	51	24	14	88
			P	1.10	29	36	14	16	71
		10	chl-a	9.11	58	33	5	4	42
			P	0.36	—	—	—	—	—
	24 July	1	chl-a	1.11	12	64	14	10	88
			P	0.92	22	44	24	10	78
		6	chl-a	1.29	23	36	23	18	77
			P	0.43	25	36	30	9	75
B	24 May	1	chl-a	5.56	36	46	11	7	64
			P	1.39	26	64	7	3	74
	25 July	1	chl-a	2.00	3	62	25	10	97
			p	0.72	12	56	25	7	88
C	26 Sept.	1	chl-a	30.10	90	5	—	5	10
			P	75.55	87	8	3	1	13

Lake Soyang. Size-fractionated productivity in Gyeonggi Bay indicated that small nanoplankton species were responsible for most of the carbon fixation in the phytoplankton assemblage. Nanoplankton production comprised 62-88% of total phytoplankton production. The contribution of the $< 3\mu$m cells was variable et each season and station.

As noted in the carbon fixation rate, nanoplankton chlorophyll concentrations comprised the major fraction of total chlorophyll in this area.

In Lake Soyang the contribution of net phytoplankton production reached average 87%. But this may be overestimated owing to the abundant colonized microflagellates (3-7μ).

Planktonic v.s. bacterial respiration

Bacterial respiration is one of the most significant problems in estimating net oxygen evolution of phytoplankton. If additional respiration is severe, the calculation of net photosynthesis ($DO_{light} - DO_{control}$) will be erroneously low by the 'same' amount.

Practically *in vitro* measurements (i.e. bottle incubation) have the possibility that there can be a larger bacterial growth on the bottle wall than the surrounding water mass. Pratt et al. (1959) proposed the correction procedure by prefiltration of phytoplankton before incubation, but this was blind to the bacterial attached to the particles larger than filter pore size.

So, if it is possible to apply antibiotics gentamycin as the inhibitor of bacterial population, it seemed that oxygen method could give us better informations about the rate of carbon or energy flux in the ecosystem as well as about the net planktonic production.

Although vial counts indicated the over 95% reduction of bacterial colonies, these results do not mean the immediate inhibition in short-term experiments but the percentage of gentamycin-sensitive strain. Also short-term uptake experiments of ^{3}H-glucose after 2 hr preincubation represented average 61% inhibition of bacterial metabolism (Table 6).

Therefore in order to apply the bacterial inhibitor in the short-term production measurements, the verification by the time-course

Table 6. ^{3}H-glucose turnover time, turnover rates and percentage inhibition of Gentamycin in Gyeonggi Bay on May, 1984

Station	Depth (m)	^{3}H-glucose turnover time (day)	^{3}H-glucose turnover rate (%/hr)		% Inhibition
			total	Gentamycin	
A	1	2.2	1.94	0.61	69
	5	5.6	0.75	0.31	59
	10	10.7	0.39	0.17	56
B	1	1.0	4.34	1.69	61
Southern California Bight, USA (Azam, March 1979)		0.9-29.6	0.141-4.87		

*S/v = 5/f (Azam and Holm-Hansen 1973)
S: substrate concentration, v: uptake rate, t: incubation time, and f: fraction of the added tracer taken up in time t (S/v = turnover time).

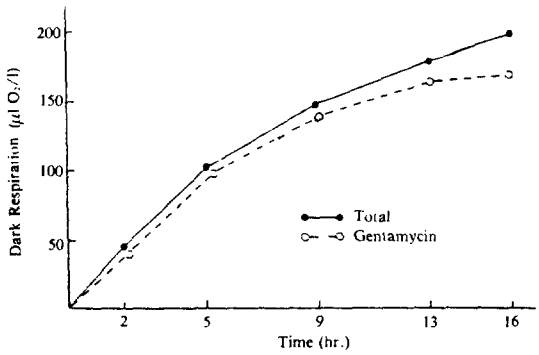

Fig. 8. Time-course experiment of gentamycin test to the samples of St. B, October (Gentamycin-treated samples were preincubated for 2 hours).

incubation was needed (Fig. 8, 9). Figure 8, however, does not shows linear respiration rate in the original sample. Figure 9 indicated another time-course inhibition test accompanied by ^{3}H-glucose uptake experiments. It appeared that gentamycin could not block the bacterial respiration in early incubation periods.

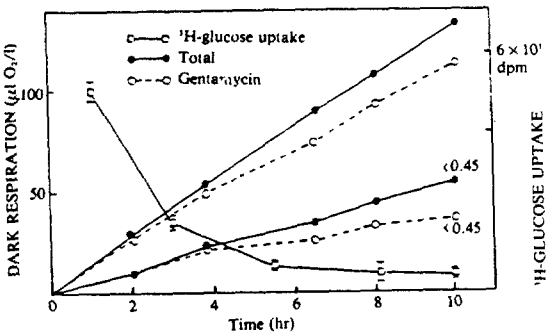

Fig. 9. Time-course experiment of gentamycin test to the pond water (Lower two lines represented the dark respiration of samples filtered with glass fiber filter (GF/C). Tritium glucose uptake experiment was carried out in the filtered water).

DISCUSSION

Some problems of production measurement by the oxygen method

Although the Winkler method has been subject to some criticism and modification (Carpenter 1965, Carrit and Carpenter 1966) it has been most commonly used in the determination of dissolved oxygen in natural water because of its precision superior to oxygen electrode. Furthermore, the introduction of the photometric titration made it possible to quantify small oxygen concentration change.

There are, however, some problems in using the oxygen method in the production and respiration measurements. Incubation time is an important factor which controls the precision of the method. If 24 hour incubations are permissible without errors, then the precision will be average 7 μl O_2/l/day. But this precision is inadequate for oligotrophic water, and practically such long exposure are more susceptible to a variability of possible errors. If short incubation (2-4 hr) is used, about 2 μg C/l/h can be only differentiated by the photometric titration.

Furthermore, oxygen method has two basic uncertainties: bacterial respiration and photorespiration. Bacterial respiration correction was attempted in this study, but photorespiration cannot be solved, though ^{14}C method is also blind to the photorespiration.

In order to attain maximum performance in the production measurements, much attention should be paid to handling of the samples rather than titration procedure. The volume of all the bottles should be determined to 0.01 ml, if possible (Williams and Jerkinson 1982). Much care must be given to composite sampling in order to make same oxygen concentrations. The presence of reducing and oxydizing agents if hormogeneously dispersed, may not affect the changes due to photosynthesis and respiration through the constant under- or overestimation of the true oxygen concentration. But it seemed that the interference from the oxidation of organic matters could be only minimized by immediate analysis (phillips 1973).

Serveral error sources found in the experiments are described below.

i) Intrinsic problems of the routine Winkler method

1) air bubbles trapped in the bottle during the sampling procedure
2) iodine volatilization during the titration
3) interfering substances including oxidizing and reducing agents which are naturally occurring (e.g. NO_2^-, Fe (II), Fe (III), Cu(II), Cr(VI), OCl^-, H_2S, hydrazine, thiosulfate, thiourea, and organic matters acting reducing agents such as glucose, peptone etc.) (Phillips

1973).

ii) Problems in the photometric and whole-bottle titration

1) inaccurate volume of oxygen fixatives (manganous sulfate and alkaline iodide: 1.00 ± 0.05 ml)
2) possible volume inequalities from the temperature differences of samples (Williams and Jerkinson 1982)
3) transparency shift due to the optical inconsistency of bottles or sample turbidity
4) interference in the near UV absorption band such as Hg (Carpenter 1965).

Diurnal primary productivity by the ^{14}C and oxygen methods

Many authors suggested that ^{14}C-technique may underestimate primary production in the oligotrophic lake and open ocean water (Steemann Nielsen 1955, Gieskes et al. 1979). But these discrepancies have not been fully understood. Although ^{14}C and oxygen methods were compared under a wide variety of culture and field conditions (Ryther 1956, McAllister 1961, Antia et al. 1963, Linderboom and De Bree 1982, Davis and Williams 1984), their results were often contrary to each other. The major discrepancies between the ^{14}C- and oxygen results seems to be that the excreted early products of photosynthesis were not measured in the routine ^{14}C experiment. Another basic difficulties in comparing ^{14}C and oxygen methods were the variability of photosynthetic quotient (PQ) (Williams et al. 1979) as well as the several problems caused by long incubation.

Although the two methods measure different processes (carbon flux vs. energy flux), our *in situ* data represented good agreement between the two methods of measuring plankton photosynthesis. We could not find the severe underestimation of ^{14}C-technique in short-term incubation. These may implies that conventional ^{14}C-technique provides fairly accurate results of organic production.

Diurnal productivity experiments by the two methods showed that ^{14}C method measured the approximate gross production in the morning, but there were small discrepancies between the two in the afternoon. The observation that more severe afternoon depression was in the oxygen method may suggest i) the uncertainty in adopting a PQ of 1.2, or ii) active photorespiration at high light intensity and high O_2, low CO_2 conditions.

Diurnal changes in the rate of photosynthesis seems to be responsible for the endogeneous factors which affect the overall rate of photosynthesis per cell (Yentsh and Ryther 1957). The presence of phased cell division in semi-synchronous natural population or photorespiration and photoinhibition (Harris 1980) may be one possible explanation of the afternoon depression.

Size-fractionated productivity and biomass

Many ecological studies emphasized the relative importance of different size fractions of phytoplankton on the ground that species succession in marine phytoplankton can be influenced by the several biological differences related with cell size (e.g. nutrient requirement and uptake kinetics, photosynthesis and respiration rates, sinking rate, and susceptabilities as food for grazer: Durbin et al. 1975). It has been reported that a variable but significant portion of primary production in lakes and oceans is contributed by the nanoplankton (e.g. Malone 1971, Kalff 1972, Seller 1983).

Our results also support the dominance of nonoplanktonic production in the coastal water. In seems that small cells generally have shorter generation times and higher growth rates in a given environment than do larger cells. In addition, the observation of higher percentage in nano-fraction at the polluted area (St. B) suggests that rather selective effects of certain pollutants may enhance the survival of small cells because nanoplankton may persist in situation where pollution has resulted in the loss of netplankton (Eppley and Weiler 1979). Low assimilation rate in Station B seems to be due to toxic effects of domestic and industrial wastewater.

Low productivity on 15 May resulted from low light intensity, but low assimilation rate in summer was ascribed to small biomass. In summer, we observed a large bloom of *Noctiluca sintillans,* a non-photosynthetic dinoflagellate. The decline of photoplankton biomass may be ascribed to the active grazing of zooplankters, and the increase of nano-fraction in summer implies high grazing pressure on the net-fraction of the phyto-

plankton species assemblage.

Assessment of bacterial respiration

Ecological application of antibiotics as metabolic inhibitors of bacterial population includes many methodological problems. The one is the interfering substance contained in the commercial gentamycin sulfate. Gentamycin which consists of several complexes (C_1, C_{1a}, C_2, A etc.) is a kind of aminoglycoside antibiotics inhibiting bacterial protein synthesis. Also gentamycin is non-reducing sugar, which does not interfere the oxidation and reduction reactions in the Winkler method. But small amount of sodium bisulfite contained in the commercial gentamycin sulfate acts as a reductant. The oxidation of sodium bisulfite consequently leads the apparent decrease of dissolved oxygen concentration. If reagent grade is not used, gentamycin should be added to the samples before subsampling and thoroughly mixed.

Another problem is that we have no evidence that gentamycin only blocks bacterial metabolism and that of eukaryotes and blue-green algae remains relatively unaffected.

Also there are some uncertainty about gentamycin-resistant strain with the aminoglycoside-inactivating enzyme (Davies 1975). And the most important problem is whether the effect of gentamycin leads a substantial decrease in oxygen uptake in short-term experiments. Time-course experiments shows how many hours of preincubation will be required to depress bacterial respiration. The results respresented that during the early incubation period (1-4 hr), susceptible cells showed relatively small respiration inhibition.

The respiratory inhibition by antibiotics would be greatest during early log-phase, but late-log or stationary phase bacteria would not be readily affected, because the production and activity of respiratory enzymes in early log-phase cell are greatest and then interference with protein synthesis would have maximum effect (Yetka and Wiebe 1974). Therefore our results suggest that at least 4-6 hr of preincubation should be required to the complete suppression.

CONCLUSION

The introduction of the photometric end point detection to conventional Winkler method made it possible to measure small dissolved oxygen changes from photosynthesis and respiration. Although sensitivity is still unable to overtake that of ^{14}C-tracer technique, this method seems to be comparable to ^{14}C-inethod for the production measurements and the related respiration studies.

Bacterial respiration is one of the most significant problems in estimating net oxygen evolution of phytoplankton. Practically the ecological application of antibiotics has some problems, but it seems that bacterial respiration must be corrected in case of high microbial activity.

Our in situ data represented good agreement between ^{14}C and oxygen methods, and the severe underestimation of ^{14}C-technique was not observed in short-term incubation. These result may suggest that conventional ^{14}C-method provide fairly accurate estimates of organic production in short-term incubation.

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