

# AN APPROACH TO DIFFERENTIATE NET PHOTOSYNTHETIC AND OTHER BIOCHEMICAL PRODUCTION AND CONSUMPTION OF OXYGEN IN ESTUARINE WATERBODIES AND AQUACULTURE SYSTEMS

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## ABSTRACT

Photosynthetic production in water plays a vital role on the fertility of aquatic ecosystems influencing the dissolved oxygen level and organic productivity. Although there are several methods such as cell count, chlorophyll estimation,  $^{14}\text{C}$  technique and light and dark bottle oxygen technique to assess the photosynthetic production in water, every method has its own merits, demerits and limitations. Moreover, the methods adopted in the open sea water are being applied directly in the shallow estuarine and other shallow backwater systems, which do not always give reliable results due to the influence of other microorganisms. This paper proposes an improvement in the formula to assessment of oxygen which has been tested using a series of data collected from the shallow estuarine waters of the 'Mangalvanam' (mangrove biotope) at Cochin.

**Key words:** Net photosynthetic production, Net bacterial production/consumption, Oxygen, Estuarine waters.

## INTRODUCTION

Estuarine ecosystems are comprising of marshes, swamps, mangroves, mudflats, creeks, canals, bays, lagoons and backwaters. Human interferences in recent years have posed threat to the estuarine environment in several ways resulting in accumulation of sediments and organic pollutants, thereby affecting the ecosystem. 'Mangalvanam' is one of such estuarine mangrove biotopes located at Cochin with high organic load in sediments.

Dissolved oxygen is considered as the most significant parameter in aquaculture system. In a healthy aquatic environment like the open sea, there would be a net gain of oxygen chiefly through photosynthesis, whereas in the shallow estuarine ecosystems the presence of high bacterial load associated with organic decomposition and mineralisation promotes the biochemical oxidation and reduction processes. Turbidity affects the photosynthetic production considerably. As a result, the methods adopted

in the open seawater samples to assess net photosynthetic/primary production do not always give reliable results in the shallow estuarine waters. Therefore an attempt was made to develop a modified formula, to differentiate the oxygen production by photosynthesis from other biochemical processes in the aquatic system.

## MATERIALS AND METHODS

The proposed methodology is based on the estimation of dissolved oxygen in water samples by Winkler's method (Strickland and Parsons, 1972) in the Initial ('I'), Dark ('D') and Light ('L') bottles giving an incubation period of 3 hrs for seawater samples and 2.5 hrs for estuarine water samples in light in the case of 'L' bottles and in darkness in the case of 'D' bottles, while samples in the 'I' bottles are fixed at the beginning of the experiment as adopted in the procedure for primary productivity estimation (Strickland and Parsons, 1972).

The difference in the oxygen values obtained between 'L' and 'I' bottles extrapolated for 12 hrs (L-I) was considered for the net result of biochemical oxygen production by phytoplankton and bacteria together (+value) or consumption (-value) for the day time; and that of 'D' and 'I' bottles (D-I) to indicate the net result of biochemical oxygen production (+ value) or consumption (-value) during night time (Selvaraj, 1997).

Filtered water samples through zooplankton filters (0.4 mm mesh size) were used. Water samples collected from the field were set for the incubation experiments atleast 30 minutes after collection to bring the organisms physiologically stabilized inside the I, D and L bottles, but not later than one hr after sampling. All the 'L' bottles were exposed to uniform light intensities in the laboratory during the course of experiments at normal temperature.

**a. Gross and net photosynthetic production and loss of oxygen by respiration per day:**

Gross photosynthetic production (G.P.P.) of oxygen in water was determined using L-D value extrapolated for 12 hrs of the day since photosynthetic activity is during light hrs of the day alone (12 hrs). Microalgal/ community respiratory loss of oxygen was determined by conducting series of incubation experiments in the laboratory using sterilised glass wares and sterilised (bacteria free) seawater added with essential plant nutrients (Walne's medium). Series of these sterilised seawater samples in one litre conical flasks were inoculated with isolated phytoplankton culture of *Chaetoceros calcitrans*, *Isochrysis galbana*, *Nanochloropsis salina* and *Tetraselmis gracilis* in different concentrations to form a phytoplankton community. Primary productivity experiments (by

L and D 'bottle oxygen technique) were conducted on these inoculated seawater samples after 6, 7, 10, 11 and 20 days to determine the respiratory loss of oxygen (Table 1). Taking the average respiratory rate (15% of G.P.P) into account for 12 hrs of the day and considering the variation in respiratory rate, species composition and physiological stress of the photosynthetic community in the estuarine environment the average respiratory loss for 24 hrs of the day was fixed as 40% of gross production of oxygen, assuming that the respiratory loss is same for the light and dark hours of the day.

Thus, while considering 20% of the G.P.P. as the respiratory loss by photosynthetic organisms for the 12 light hrs of the day (photosynthetic period), 80% of G.P.P., i.e. 0.8 (L-D) per 12 hrs would give the net photosynthetic (primary) production (N.P.P) of organic matter per day.

**b. Net production of oxygen by photosynthetic community in 24 hrs):**

The difference between the oxygen values obtained from G.P.P. per day and the respiratory loss for 24 hrs of the day would give the net photosynthetic production of oxygen (ml/l) in 24 hrs of the day, i.e.  $[(L-D) - 0.4(L-D)]$  per 24 hrs, which would give the net value of 0.6 (L-D) oxygen in ml per day (24 hrs).

**c. Other biochemical production of oxygen in 24 hours of the day (hereinafter referred as the bacterial production of oxygen) :**

For the assessment of bacterial production of oxygen during day time (12 hrs), positive oxygen values obtained from the formula, L-I value per 12 hrs minus N.P.P. value i.e.,  $[(L-I) - 0.8(L-D)]$  per 12 hours were considered. For the production rate at night time (12 hrs), positive values obtained from the formula, D-I value per 12 hrs

plus loss by respiration per 12 hrs, i.e.,  $[(D-I)+2(L-D)]$  per 12 hrs or, the values obtained for the day time, whichever is more was considered.

Computation of the day and night oxygen values thus obtained would give the bacterial production value of oxygen (ml/l) per 24 hrs of the day.

**d. Other biochemical consumption of oxygen in 24 hrs of the day** (hereinafter referred as the bacterial consumption of oxygen)

Consumption of oxygen by bacterial action at night hours could be estimated using the formula, I-D value per 12 hrs minus loss by respiration in ml O<sub>2</sub>/l per 12 hrs, i.e.  $[(I-D)-0.2(L-D)]$  per 12 hrs. Consumption of oxygen at day time could be assessed by the formula,  $[I-D]-0.2(L-D)]$  per 12 hrs plus positive oxygen value (if any) obtained from  $[(I-L)-(I-D)]$  per 12hrs. Computation of these positive oxygen values obtained for the 12 hrs light and 12 hrs dark periods would give the bacterial consumption value of oxygen in 24 hrs of the day. This could be summarised as follows:

$2[(I-D)-0.2(L-D)]$  per 12 hrs +  $[(I-L)-(I-D)]$  per 12 hrs (if any).

**e. Net bacterial production/consumption of oxygen in 24 hrs of the day :**

Computation of the oxygen values thus obtained from the estimated production and consumption of oxygen by bacterial action (Items c + d) would give the net bacterial production (+value) or consumption (-value) of oxygen per 24 hrs of the day.

**f. Net gain/loss of oxygen by phytoplankton and bacteria together in 24 hrs of the day :**

Computation of the values obtained from items 'b' and 'e' would give the net gain (+ value) or loss (-value) of oxygen in the ecosystem per 24

hrs of the day, which would be equal  $[(L-I)+(D-I)]$  per 12 hrs.

For illustration, the following methodology (items 1-12) has been applied on the basic data (ml O<sub>2</sub>/l) obtained from a series of incubation experiments (2.5 hrs of incubation) by L and D bottle oxygen technique during 8th September - 11th November 1998 from two stations fixed in the shallow estuarine waters of the 'Mangalvanam' having an average depth of one metre. All values are expressed in ml O<sub>2</sub>/l.

1. Gross photosynthetic production (G.P.P.) :  
 $(L-D)_{12 \text{ hrs}} = (L-D)_{24 \text{ hrs}}$
2. Net photosynthetic (primary) production (N.P.P.) per 12 light hours of the day:  
 $0.8(L-D)_{12 \text{ hrs}} = 0.8(L-D)_{24 \text{ hrs}}$
3. Loss of oxygen by respiration :  
 $0.2(L-D)_{12 \text{ hrs}} = 0.4(L-D)_{24 \text{ hrs}}$
4. Net photosynthetic production of oxygen in 24 hrs of the day :  
(Items 1-3) =  $0.6(L-D)$  per 24 hrs.
5. Bacterial production of oxygen at day time :  
 $[(L-I)-0.8(L-D)]$  per 12 hrs
6. Bacterial production of oxygen at night time :  
(+ values)  
a)  $[0.2(L-D)+(D-I)]$  per 12 hrs, if D-I value is positive;  
b)  $[0.2(L-D)-(I-D)]$  per 12 hrs, if D-I value is negative;  
c)  $[0.2(L-D)]$  per 12 hrs; if D-I value is zero.
7. Bacterial production of oxygen in 24 hrs of the day (items 5+6)
8. Bacterial consumption of oxygen at day time :  
(+ values)  
 $[0.8(L-D)-(L-I)]$  per 12 hrs
9. Bacterial consumption of oxygen at night time :  
 $[(I-D)-0.2(L-D)]$  per 12 hrs. (+ values)
10. Bacterial consumption of oxygen in 24 hours of the day : (Items 8+9)
11. Net bacterial production/consumption of oxygen in 24 hrs of the day: (Items 7-10).  
(+ = production, - = consumption)

12. Net gain/Loss of oxygen by photosynthesis, respiration and bacterial action in 24 hrs of the day : (Items 4+11)

OR, [(L-I)+(D-I)] per 12 hrs

(+ = gain/production, - = loss/consumption)

## RESULTS AND DISCUSSION

Biochemical reactions involving photosynthesis, respiration and other microbial oxidation processes influence much on the rate of production and consumption of oxygen in the shallow estuarine waters. Respiration is the basic parameter for the determination of primary production and energy transfer in the aquatic ecosystems. But, due to the influence of bacterial processes, it is very difficult to measure the loss of oxygen by respiration in the natural environment. As a result, measurement of oxygen in primary productivity and respiration experiments by means of monometry, oxygen electrode or iodometry (Winkler's method) never provides satisfactory results in such estuarine waters. Laboratory experiments alone might give reliable results close to the reality.

The results of the laboratory experiments conducted in the present study using isolated phytoplankton culture in sterilised (bacteria free) seawater confirm that the <sup>loss by respiration</sup> average  $\lambda$  during photosynthetic period (12 light hrs of the day) is 15% of G.P.P. (Table 1). Considering these results, changes in the respiratory rate, species composition and physiological stress in the estuarine environment, the average respiratory loss of oxygen has been fixed as 20% of G.P.P. (ml O<sub>2</sub>/l) during the light hours of the day (12 hrs) : and 40% of gross production of oxygen during 24 hrs of the day assuming that the respiration rate is same for the light and dark hours of the day in the present methodology. Nair *et al.*, (1973) have estimated the potential fishery resources based on the mean primary productivity potential

for the inshore waters (within 50m depth zone) of the Indian coast assuming that 40% of gross production is utilised for respiration during photosynthetic phase (12 light hours) of the day as compared to the experimental results obtained in the present study (Table-1).

The coastal water bodies tend to show irregularities on the production and consumption of oxygen in the light and dark bottle incubation experiments. While L-I oxygen values (N.P.P.) are always lower than L-D values (G.P.P.) in the offshore seawater samples during primary productivity experiments, the L-I oxygen values in the estuarine water samples often exceed the G.P.P. values. In such cases, L-I values do not indicate the real picture of the net photosynthetic production because of the considerable involvement of bacterial action on the release of oxygen by other biochemical reduction processes in the estuarine waters alongwith the photosynthetic release of oxygen (Selvaraj, 1997) both of which together boost the L-I values and at times to the extent of exceeding the L-D values. The L-D values always indicate the gross photosynthetic production only. So also, when the water samples are in the oxidising state by other biochemical processes, L-I often shows very low or even nil values masking the net photosynthetic production of oxygen when the G.P.P. is showing positive values.

Whenever the bacterial production of oxygen increases beyond the respiratory loss in the estuarine water samples, the 'L' and 'D' values exceed the 'I' values thereby masking the respiratory loss (Table2). In such cases, 80% of L-D (in the proposed methodology) would give a genuine picture of the N.P.P. which would be much closer to the reality and 0.2(L-D) for respiration per 12 hrs of the day (Table 2).

**Table 1. Experimental results to determine respiratory rate (ml O<sub>2</sub>/l) in primary productivity experiments using isolated phytoplankton culture in sterilised seawater (Incubation: 3 hours)**

Date of Expt.	'I' value	'D' value	'L' value	G.P.P. (L-D) per 12 hrs (ml O <sub>2</sub> /l)	Respiration (I-D) per 12hrs (ml O <sub>2</sub> /l)	(%ofGPP)
10.12.98 (after 6 days)	3.252	2.944	6.070	12.504	1.232	9.85
"	3.298	2.827	5.807	11.920	1.884	15.80
"	4.023	3.751	5.640	7.556	1.088	14.40
"	4.050	3.805	5.314	6.036	0.980	16.24
"	4.023	3.805	5.273	5.872	0.872	14.85
"	4.240	3.995	5.463	5.872	0.980	16.69
"	4.240	3.995	5.409	5.656	0.980	17.33
11.12.98 (after 7 days)	2.419	2.337	2.935	2.392	0.328	13.71
"	2.365	2.283	3.044	3.044	0.328	10.77
"	2.446	2.365	2.935	2.280	0.324	14.21
"	2.392	2.310	3.071	3.044	0.328	10.77
14.12.98 (after 10 days)	5.028	4.621	7.148	10.108	1.628	16.11
"	5.055	4.566	7.230	10.656	1.956	18.36
15.12.98 (after 11 days)	4.230	3.716	7.058	13.368	2.056	15.38
24.12.98 (after 20 days)	2.717	2.079	5.821	14.968	2.552	17.05
Average						15%

**Table 2. Water samples showing Dark ('D') values higher than Initial ('I') values (ml O<sub>2</sub>/l) in primary productivity experiments at 'Mangalvanam' during September - November 1998.**

Date of expt.	'I' value	'D' value	'L' value (Inc: 2.5 hours)	(D-I) (Oxygen values per 12 hours)	(L-D)	0.2 (L-D)
14.9.98	1.0340	1.0880	1.1424	0.2592	0.2611	0.0522
30.9.98	1.0774	1.1648	1.1648	0.4195	0.0	0.0
26.10.98	1.1849	1.3200	1.2926	0.6485	Nil	Nil
29.10.98	1.0532	1.1565	1.1822	0.4958	0.1234	0.0247
31.10.98	0.6682	0.7325	0.5783	0.4019	Nil	Nil
6.11.98	1.4274	1.4549	1.5372	0.1320	0.3950	0.0790

Table-3. Experimental results to indicate photosynthetic production, respiration and other biochemical (bacterial) production and consumption of oxygen in 'Mangalvanam' waters during Sept-Nov. 1998 (Inc, time: 2.5 hrs; all values in ml O<sub>2</sub>/l)

Date :	25/9	14/9	28/9	8/9	9/9	6/11	15/9	31/10	21/10	26/10	10/9	30/9	
water quality	L>D	L>D	L>D	L>D	L>D	D>L	D>L	D>L	D>L	D>L	L=D	L=D	
	I>D	D>I	I=D	I>D	I>D	D>I	I>D	D>I	I=D	D>I	I>D	D>I	
'I' value	1.310	1.034	1.252	2.584	1.768	1.612	1.849	0.668	1.239	1.185	1.632	1.077	Average
'D' value	1.252	1.088	1.252	2.258	1.741	1.618	1.632	0.735	1.239	1.320	1.333	1.165	
'L' value	1.485	1.142	1.281	2.475	1.768	1.612	1.550	0.574	1.185	1.293	1.333	1.165	
1. G.P.P. per day	1.118	0.259	0.139	1.042	0.130	-	-	-	-	-	0.0	0.0	0.224
2. N.P.P. per day	0.894	0.207	0.111	0.834	0.104	-	-	-	-	-	0.0	0.0	0.179
3. Respiration per day (24 hrs)	0.447	0.104	0.056	0.417	0.052	-	-	-	-	-	0.0	0.0	0.090
4. Net gain of oxygen by photosynthesis per day (Items 1-3)	+0.67	+0.16	+0.08	+0.62	+0.08	-	-	-	-	-	0.0	0.0	+0.134
5. Bact. prod. of oxygen during light hours (12 hrs)	-	0.311	0.028	-	-	-	-	-	-	0.518	-	0.422	0.107
6. Bact.prod. of oxygen during night (12 hrs)	-	0.311	0.028	-	-	0.029	-	0.322	-	0.648	-	0.422	0.146
7. Bact. prod. of oxygen in 24 hrs (Items 5+6)	-	0.622	0.056	-	-	0.029	-	0.322	-	1.166	-	0.844	0.0253
8. Bact.consump. of oxygen during light hours (12 hours)	0.054	-	-	1.357	0.104	-	1.435	0.451	0.259	-	1.435	-	0.424
9. Bact. consump. of oxygen during night (12 hours)	0.054	-	-	1.357	0.104	-	1.042	-	-	-	1.435	-	1.333
10. Bact.consump.of oxygen in 24 hrs (Items 8+9)	0.108	-	-	2.714	0.208	-	2.477	0.451	0.259	-	2.870	-	0.757
11. Net bact. prod./ consump. of oxygen in 24 hrs (Items 7-10) +=prod; -=consump.	-0.11	+0.62	+0.06	-2.71	-0.21	+0.03	-2.48	-0.13	-0.26	+1.17	-2.87	+0.84	-0.504
12. Net gain/loss of oxygen in 24 hrs													