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# NITROGEN FIXATION BY BLUEGREEN ALGAE IN PORTONOVO MARINE ENVIRONMENTS

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ABSTRACT

Several bluegreen algae capable of fixing nitrogen were identified and isolated from Portonovo marine environments. The most common forms are *Anabaena* sp., *Aphanocapsa* sp., *Nodularia* sp. and *Trichodesmium* sp. For planktonic bluegreen algae the estimated nitrogen fixation rates were 0.504 mg N/m$^3$/d in estuary, 0.238 mg N/m$^3$/d in coastal waters and 0.336 mg N/m$^3$/d in mangrove waters. This could contribute 3.5%, 1.6% and 2.2% of nitrogen requirements of phytoplankton of the respective environments (assuming an average daily productive rate of 100 mg C/m$^3$/d and a C : N ratio of 6). Nitrogen fixation by benthic bluegreen algae were 3.22 and 3.32 mg N/m$^2$/d for estuarine and mangrove environments. The contribution of benthic bluegreen algae to the nitrogen requirement of benthic macroalgae could be about 0.72% on the basis of a net production value of 2700 mg C/m$^2$/d. The epiphytic algae could fix about 0.968 mg N/m$^2$/d in estuary and 1.347 mg N/m$^2$/d in mangrove waters. The contribution by epiphytic algae to the nitrogen demands of macroalgae was only 0.2% and 0.3% in the estuary and mangrove waters.

## INTRODUCTION

IN THE MARINE environment nitrogen is generally considered to be a major limiting nutrient for the growth of phytoplankton (Ryther and Dunstan, 1971). The importance of biological nitrogen fixation in productivity and its substantial contribution to nitrogen budget of the marine and related environments has been studied extensively and documented by Mague *et al.* (1974), Carpenter and Mc Carthy (1975) and Marsho *et al.* (1975).

Burris (1976), Bohlool and Wiebe (1978), Gundersen (1981) and Fogg (1978) reported nitrogen fixation in the oceans and its possible contribution to the nitrogen budget. The reported estimates of nitrogen fixation and their subsequent contribution vary widely. For instance the estimated rates of nitrogen fixation for the Baltic sea were $2 \times 10^6$ tonnes/year (Ostrom, 1976); $1.5 \times 10^4$ tonnes/year (Lindahl *et al.*, 1977) and $1.0 \times 10^3$ tonnes/year (Rinne *et al.*, 1977). Based on the existing reports and the possible fixation rates, Fogg (1978) proposed a factor of $5 \times 10^6$ tonnes per year for world Oceans.

The estimated contribution of fixed nitrogen to the nitrogen requirement of phytoplankton also varies widely. While Gundersen *et al.* (1976) reported nitrogen fixation rates of *Trichodesmium* to be equal to 0.69 g $N_2$/m$^2$/yr which could supply 3.8% of the requirement of the plankton, Fogg (1978) from the estimates of Mague *et al.* (1974) suggested that the fixed nitrogen could meet from 5% to

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30% of the daily nitrogen requirements of the standing crop of phytoplankton. However, most of these estimates were only for nitrogen fixation associated with planktonic blue green algae. Additional nitrogen fixed by the benthic algal community might also be of significant importance. Substantial nitrogen fixing activity associated with epiphytes (Carpenter, 1972 ; Goering and Parker, 1972 ; Patriquin and Knowless, 1972 ; McRoy *et al.*, 1973 and Capone *et al.* 1977), Coral reef community (Wiebe *et al.*, 1975) and benthic algae (Burris, 1976) were also reported. Its contribution to the nitrogen economy and productivity of the associated community was found to be very significant.

Inthe present study an attempt was made to estimate the nitrogen fixation associated with the three groups of (viz. planktonic, benthic and epiphytic) algae of the local marine environs and their significance in the nitrogen budget.

## Materials and Methods

### *Planktonic blue green algae*

Surface water samples (25 l) were collected from three different stations representing the three different study areas *viz.* estuary, coastal and mangrove environs and filtered through a fine mesh sieve (5 $\mu$ size). The filtered samples were concentrated by centrifugation (4000 $\times$ g) and the concentrates were then made up to 25 ml with enriched sterile sea water. Then 5 ml portions of the algal suspension were placed in 20 ml serum bottles and subjected to acetylene reduction assay as described below, 1 ml of the sample was used for the determination of the total population of the blue green algae. Based on acetylene reduction rates the nitrogen fixed/m$^3$/day was calculated. The experiments were carried out in triplicate and the average value taken for calculations.

### *Benthic Algae*

Benthic algal mats (10 cm$^2$ area) were collected from the estuarine and mangrove areas. These mats were repeatedly washed in sterile sea water to remove mud and then made in to a uniform suspension by breaking with a glass rod. The volume was made up to 25 ml of which 5 ml were incubated for acetylene reduction assay.

### *Epiphytic algae*

Submerged seaweeds were collected from the intertidal regions of the Vellar estuary and the mongrove environment. The colonized epiphytic algae were carefully removed by repeatedly washing the algae and by agitating them in a rotary shaker with filtered sterile habitat water. The washed water was then concentrated by centrifugation and the volume was made up to 25 ml. 5 ml portions of the suspension were incubated for acetylene reduction assay. The expriments were carried out in triplicates. The macroalgae from which the colonized algae removed, were dried and weighed. The extent of nitrogen fixation per m$^2$ of these algae in the respective environments was calculated and presented.

### *Acetylene Reduction assay*

The acetylene reduction assay as a measure of nitrogen fixation activity is being used extensively in recent years, owing to its simplicity, sensitivity, reliability and rapidity (Dilworth, 1966 ; Schollhorn and Burris, 1976).

The algal samples for the assay were placed in serum bottles (20 ml volume) and capped with a serum stopper. The rims were further wrapped with scotch tapes to prevent any air leaking. 2 ml of tank acetylene (Matheson Co., USA) was injected into the bottles at zero time. The bottles were then shaken thoroughly and exposed for 1 hour in sunlight in a shallow tray with flowing tap water. Water temperature was maintained at 28 $\pm$

1°C. After 1 hour the reduction of acetylene to ethylene was terminated by adding 3 ml of saturated cupric sulphate solution (Mague *et al.*, 1974) through the serum stopper with a hypodermic syringe. Control vessels containing filtered sea water alone were also run simultaneously. Samples (0.5 ml) were analysed promptly for ethylene by Gas Chromatograph with a FID detector (Column temperature 60°C, Detector 110°C and Injection port 120°C). Flow of hydrogen and nitrogen were kept constant. The unit was standardized with ethylene samples diluted with air in the range of $10^{-5}$ to $10^{-11}$ moles ethylene per 0.5 ml gas system.

Stainless steel column (6' long, 1/6" diameter) packed with Porapak R served as gas chromatographic column. Ethylene was generated in the lab by heating a mixture of ethyl alcohol and sulphuric acid (50% v/v) to 170°C. The gas evolved was washed with dilute potassium hydroxide solution to remove sulphur dioxide and/or carbon dioxide and the ethylene was collected over water. A theoretical conversion ratio (based on electrons transferred) of 3 moles of acetylene reduced to 1 mole of nitrogen fixed (Hardy, 1968 and Lipscultz *et al.*, 1979) was assumed for all calculations.

## Results and Discussion

### *Nitrogen fixation associated with Planktonic blue green algae*

The planktonic samples revealed the presence of *Anabaena* sp., *Nodularia* sp., *Oscillatoria* sp., *Phormidium* sp. and *Trichodesmium* sp. Among these *Trichodesmium* dominated the rest of the algae. The population size was 400-420 filaments per litre for the estuarine environment; 220-230 filaments per litre for the coastal water and 350-370 plaments per litre for the mangrove water. Among these algae *Trichodesmium* has been recognised widely as a nitrogen fixer in recentl years. Nirtogen fixing *Nodularia* sp. and *Anabaena* sp. were earlier reported by Stewart (1971).

The detailed calculations for the estimation of nitrogen fixation rates for the three environments and the contribution of fixed nitrogen to the nitrogen requirement of phytoplankton are given in Table 1. The estimated rates were 36 $\mu$ moles $N_2/m^3$/day (or 0.184 g $N_2/m^3$/yr) for estuary; 17 $\mu$ moles $N_2/m^3$/day (or 0.087 gm $N_2/m^3$/yr) for coastal water and 24 $\mu$ moles $N_2/m^3$/day (or 0.123 g $N_2/m^3$/yr) for mangrove environment. Their contribution to nitrogen requirement of phytoplankton were 3.5%, 1.5% and 2.25% respectively. Since these estimations were made in the month of July (1980) during which the populations were not so significant, these may not represent the real contribution of the blue green algae to the nitrogen budget of the waters. Based on these estimates and on the previous seasonal variation studies the nitrogen fixation by blue green algae during the summer months (when their population was about 5 to 8 times of the present size) could be as much as 10-20% of the total nitrogen requirement of phytoplankton. But during other months (*i.e.* from August to December) during which period their population was insignificant the contribution may not be appreciable. However considering their average population size based on the recorded population (for 1978 and 1979) of 545, 475, and 340 filaments/l respectively for the estuarine, coastal, and mangrove waters these estimates seemed to be rather practical and probable. The estimates by Gundersen *et al.* (1976) 0.69 g $N_2/m^2$/yr and Lindahl *et al.* (1977) 0.6 g $N_2/m^2$/yr (observed when the blue green algae were in blooms) for Pacific Ocean and Baltic sea are in agreement with the present estimates, if calculated for the bloom populations.

Regarding the contribution to the nitrogen requirement of phytoplankton Gundersen *et al.* (1976) reported 3.8% whereas Mague *et al.* (1974) reported 4%. These values seemed

TABLE 1. *Nitrogen fixation estimates for planktonic blue green algae and its contribution to nitrogen requirement*

*I. Estuarine environment*

| | |
|---|---|
| Ethylene reduced for samples | 9 n moles/hr/1 |
| Hence nitrogen fixed/1/hr | 3 n moles |
| ,, /m³/hr | 3 μ moles |
| ,, /m³/day | 36 μ moles = 0.504 mg |

Assuming the average daily production rate of 100 mg C per day per m³ and a C : N ratio of 6 the nitrogen requirement will be 15.2 mg/day and the contribution will be @ 3.5%.

*II. Coastal waters*

Similarly for the coastal waters wherein the estimated rates of nitrogen fixation was 17μ moles/m³/day (0.238 mg) the contribution will be @ 1.6%.

*III. Mangrove waters*

The estimated rate of nitrogen fixation was 24μ moles per m³ per day (0.336 mg) for mangrove waters and hence the contribution will be @ 2.25%.

to agree well with the observed values of the present study. Mague *et al.* (1974) suggested that nitrogen fixation may be of greater significance than this value since fixed nitrogen can be repeatedly recycled within the euphotic zone by processes of active excretion, cell lysis, zooplankton grazing etc. They also suggested that it would not be possible to estimate the ecological importance of this nitrogen fixation in regard to primary productivity until a knowledge of their subsequent fate in the aquatic environments was clearly established.

*Nitrogen fixation associated with benthic blue green algae*

The benthic blue green algal populations consisted of *Calothrix, Nodularia, Oscillatoria Phormidium* and *Spirulina.*

The extent of combined nitrogen level in the waters overyling the benthic algae was between 20 and 40 μ g/1. These levels were not known to inhibit either the synthesis or activity of nitrogenase. The nitrogen fixation rates estimated are given in Table 2. No significant variations were observed between the benthic algae from the estuarine and mangrove environments. The estimated rates were 3.22 and 3.32 mg/m²/day for estuarine and mangrove environments respectively. The nitrogen fixation rates per hectare per year (assuming their existence for 9 months except during the monsoon season *i.e.* October to December) were 8.8 and 9.09 kg. Burris (1976) reported values ranging from 6.8 kg/ha/yr to 30.6 kg/ha/yr (for 12 months period) for blue green algae attached to rocks in the Lizard area of the Great Barrier reef. In the present study though the amount of nitrogen fixed by the benthic algae was much higher than that of the planktonic forms, their contribution to the nitrogen requirements of benthic algal community were very low. The net benthic macro algal production in these waters was about 2700 mg C/m²/day (Ramachandran *et al.*, 1979). Considering this value the contribution to the total nitrogen requirement of the benthic macro algae (450 mg N/m²/day—assuming a C : N ratio of 6) by benthic blue green algae would be about 0.72%. However, since the areas of distribution of the benthic blue green algae and macro algae were different, the nitrogen fixed by the benthic algae may be readily available for the planktonic forms than for the benthic algae. In such cases

TABLE 2. *Nitrogen fixation estimates for benthic blue green algae*

*I. Estuarine Environment*

| | |
|---|---|
| Ethylene reduced/5 ml sample/hr | 114.9 n moles |
| Nitrogen fixed/5 ml sample/hr | 0.0383 μ moles |
| (Since algae collected from 10 $cm^2$ area was made up to 25 ml) | |
| Nitrogen fixed/10 $cm^2$/hr | 0.1915 μ moles |
| Nitrogen fixed/$m^2$/hr | 19.15 μ moles |
| | = 268.1 μg |
| Nitrogen fixed/$m^2$/day | 268.1 × 12 |
| | = 3217.2 μg |
| | = 3.22 mg |

*II. Mangrove Environment*

Similarly for the mangrove environment where in the estimated rates of nitrogen fixed for 5 ml sample was 0.0396 μ moles/hr, the calculated nitrogen fixation rate was 3.32 mg/$m^2$/day.

TABLE 3. *Nitrogen fixation estimates for epiphytic blue green algae*

*I. Estuarine Environment*

| | |
|---|---|
| Ethylene reduced/5 ml sample/hr | 0.145 μ moles |
| Nitrogen fixed/sample/hr | 0.048 μ moles |

Since the total volume of epiphytes were 25 ml and the dry wt. of the macro algae from which the epiphytes were collected was 2.183 g.

| | |
|---|---|
| Nitrogen fixed/2.183 g/hr | 0.2415 μ moles |
| The dry wt. of macro algae collected from | 1 $m^2$ area was 52.11 g |
| Nitrogen fixed/$m^2$/hr | 5.7648 μ moles |
| Nitrogen fixed/$m^2$/day | 69.18 μ moles |
| | = 0.968 mg |

*II. Mangrove Environment*

| | |
|---|---|
| Ethylene reduced/5 ml sample/hr | 0.1724 μ moles |
| Nitrogen fixed/5 ml sample/hr | 0.0575 μ moles |
| The dry wt. of algae was 2.273 g | |
| Nitrogen fixed/2.273 g/hr | 0.2875 μ moles |
| The dry wt. of macro algae collected from 1 $m^2$ area was 63.4 g | |
| Hence Nitrogen fixed/$m^2$/hr | 8.010 μ moles |
| Nitrogen fixed/$m^2$/day | 1.347 mg |

together with the plank tonic forms, the supply of nitrogen by nitrogen fixing algae in these waters are substantial and these forms play a vital role in the nitrogen budget and productivity of the local waters.

*Nitrogen fixation associated with epiphytic algae*

The epiphytic blue green algae were represented by *Aphanocapsa* sp., *Oscillatoria* sp., *Lyngbya* sp. and *Stigonema* sp. in abundance along with some quantity of *Calothrix* sp. and *Nodularia* sp.

The estimated nitrogen fixation rates associated with these epiphytes were 0.968 mg/$m^2$/day (or 69.18 μ moles) and 1.347 mg/$m^2$/day (or 96.23 μ moles) for estuarine and mangrove environments respectively (Table 3). The macro algal species with which the epiphytes associated were *Padina* sp., *Enteromorpha* sp. *Chaetomorpha* sp., and *Hypnea* sp. The extent of nitrogen fixation associated with *Enteromorpha* sp., as estimated by Bohlool and Wiebe (1978) was between 22 and 158 μ moles per $m^2$ per day. The observed values in the present study were well within this range, The nitrogen fixed by epiphytes associated with 1 gm dry wt. of macro algae/hour was 1.55 $\mu$g for estuarine algae and 1.77 $\mu$g for mangrove algae. High rates of nitrogen fixation associated with epiphytes for *Microdictyan* sp. (3 $\mu$g/g dry wt./hr ; Capone *et al.*, 1977) for decaying leaves of red mangroves (11 $\mu$g/g dry wt./hr and epiphytes associated with phyllosphere of *Thalassia testudinum* (4-5 mg/ $m^2$/day ; Capone and Taylor, 1977) were also reported.

The importance of submerged vegetations in aquatic productivity especially in shallow water ecosystems has been widely recognised (Blinks, 1955 ; Thayer *et al.*, 1975 and Ramachandran *et al.*, 1979). As nitrogenous nutrients often limit the productivity in natural waters (Patriquin, 1972), the nitrogenous nutrient source and uptake mechanisms received more attention. Harlim (1975) suggested that epiphytes may provide additional nitrogenous nutrients to the associated macrophytes. Though it was not evident that transfer of nitrogen occurs between the epiphytes and their hosts, Stewart (1971) showed the ability of macro algae to utilize the exuded nitrogen from *Calothrix*. Epiphytes were also shown to be an important source of nitrogen for submerged vegetation by Capone and Taylor (1977). Capone and Taylor (1977) estimated that in summer nitrogen fixation associated with the phyllosphere of *Thalassia testudinum* could provide from 4 to 23% of its nitrogen requirement. However, according to estimates of the present study the contribution by epiphytes to the nitrogen demand of macro algae was only 0.2 and 0.3% in the estuary and mangroves, based on the requirement of 450 mg N/$m^2$/day for the production of 2700 mg C/$m^2$/day (C : N ratio 6) observed for the waters (Ramachandran *et al.*, 1979).

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