NITROGEN FIXATION BY BLUE-GREEN ALGAE[a]

P. M. Reddy and P. A. Roger*
Soil Microbiology Department
The International Rice Research Institute
Los Banos, Philippines

a_/Lecture in graduate course Botany 291 "Biology of blue-green algae and their symbiotic forms", 2nd semester (1984-1985), University of the Philippines at Los Banos, Philippines..

*Maitre de Recherches ORSTOM, Visiting Scientist at IRRI.

1) INTRODUCTION

Certain prokaryotes have the ability to fix dinitrogen by converting it into ammonia. Among these, blue-green algae are prominent N2-fixers in terrestrial and aquatic systems. They fix N2 both in free-living state and symbiosis with a wide range of partners. There are more genera of blue-green algae known to fix N2 than there are of heterotrophic and photosynthetic bacteria combined (Stewart, 1973a). Furthermore, it is now generally accepted that the contribution by blue-green algae to the nitrogen status of natural ecosystems is probably much more important than the contribution by heterotrophic microorganisms.

2) METHODS FOR MEASURING N2-FIXATION

N2 fixation can be measured by total nitrogen analysis, measurement of gas ratios, 15N2 gas incorporation, natural isotopic abundance and acetylene reduction assay. The earliest technique was to measure increase in total combined nitrogen by Kjeldahl method when the algae were grown in medium free of combined nitrogen (Fogg, 1942).
This technique is recognized as a standard procedure. Nitrogen analysis by the Kjeldahl technique is also used in field or pot nitrogen balance studies. The use of this method to distinguish between phototrophic N2 fixation by parallel light and dark treatment is suitable only for long-term trials for gross measurements.
Measurement of decrease in the N2/argon ratio by mass spectrometry (Fay and Fogg, 1962) or by manometer (Cox, 1966) was employed to assess N2 fixation by blue-green algae. It is not a common method. The use of 15N2 as tracer (Burris et al., 1943) is a more sensitive method to demonstrate N2-fixation. In this method, the blue-green algae are incubated in the presence of 15N2 enriched atmosphere, and the 15N incorporated in algal proteins is measured by mass spectrometry. 15N techniques are expensive and difficult to use in the field. Nitrogenase reduces a variety of substances including acetylene in addition to elemental nitrogen (Dilworth, 1966; Schollhorn and Burris,1966). Ethylene formed due to reduction of acetylene by nitrogenase can be measured with a gas chromatograph. The acetylene reduction assay was first used with blue-green algae by Stewart et al. (1967, 1968). This technique is a simple and inexpensive rapid-scan method for detecting potential N2-fixing microorganisms. For blue-green algae, the rates of acetylene reduction: nitrogen reduction is frequently near to 3:1 (Stewart et al.,1968). Since this ratio is not constant under all environmental conditions, it was advised that the usage of the technique to quantify N2 fixation in the field should be calibrated, at least

sometimes, with 15N2 measurements (Stewart, 1973a). Measurements of algal acetylene reducing activity in soil are reliable when the incubation is brief, the problem of gas diffusion and greenhouse effects are minimized, and statistically valid sampling methods are adopted. However, the method is generally considered suitable for qualitative estimate only.

3) N2 FIXING BLUE-GREEN ALGAE

Both free-living and symbiotic blue-green are found to fix N2. Three groups of free-living N2-fixing blue-green algae are recognized: heterocystous algae, nonheterocystous filamentous algae and unicellar algae.

31) Heterocystous blue-green algae

These algae fix N2 aerobically and microaerobically. The most common N2-fixing species belong to the genera Anabaena, Aulosira, Calothrix, Cylindrospermum, Nostoc, Scytonema, Tolypothrix, Fischerella, Mastigocladus, and Stigonema. These algae usually differentiate vegetative cells into heterocysts only when grown in the absence of combined nitrogen.

This observation led Fogg (1949) to suggest that heterocysts are the sites of N2 fixation. Subsequent studies have shown that nitrogenase is located in the heterocysts under aerobic growth conditions (Stewart et al., 1969; Fleming and Haselkorn, 1973; Peterson and Wolk, 1978a, b).

Heterocysts appear to be suitable sites for the efficient functioning of an oxygen-sensitive enzyme such as nitrogenase because oxygen evolving Photosystem II is absent in them (Fig. l). There are evidences that vegetative cells of heterocystous forms express nitrogenase activity under anaerobic conditions (Smith and Evans, 1971; Rippka and Stanier, 1978).

32) Nonheterocystous filamentous blue-green algae

Most of these algae fix N2 only under microaerobic conditions.

Trichodesmium (Taylor et al., 1973) and Microcoleus (Pearson et al., 1979) are exceptions to this general observation, since they were found to fix N2 in aerobic conditions. The observations on the ability of nonheterocystous filamentous algae to fix N2 were under dispute for a long time. Early reports of fixation by species of Lyngbya, Phormidium, Plectonema, Oscillatoria and Trichodesmium (see Stewart, 1971; 1973b) were in doubt either because they were based on studies with non-axenic cultures or else critical tests for nitrogenase activity had not been carried out.

The discovery (Stewart and Lex, 1970) that Plectonema boryanum 594 could fix N2 but only under microaerobic conditions resolved the position to some extent, and pure cultures of various species of Phormidium, Raphidiopsis (Singh, 1972), Oscillatoria, Lyngbya and Plectonema (Kenyon et al., 1972) were shown to fix N2 under microaerobic conditions only.

By creating strict anaerobic conditions, particularly by blocking the photosynthetic O2-evolution by DCMU, Rippka and Stanier (1978) demonstrated that more than 50% of filamentous nonheterocystous forms have the capacity to synthesize nitrogenase.

33) Unicellular blue-green algae

Five strains of Gloeocapsa (= Gloeothece) (Wyatt and Silvey, 1969; Rippka et al., 1971, 1979) and a strain of Aphanothece (Singh, 1973) fix N2 in aerobic conditions while three strains of Synechococcus perform N2 fixation under anaerobiosis. Remarkably, in Gloeocapsa no structural differences exist in cells grown on N2 or on combined nitrogen (Rippka et al., 1971) and there is no evidence of the elaborate membrane systems, as

reported in N2-fixing Azotobacter (Oppenheim and Marcus, 1970) and in heterocysts (Lang and Fay, 1971).

34) Symbiotic blue-green algae

Some heterocystous and unicellular blue-green algae develop in symbiosis. They are found in association with diatoms (Richelia(end- und)/Calothrix in Rhizosolenia), fungi (Nostoc, Calothrix, Scytonema, Fischerella, and Gloeocapsa in lichens), bryophytes (Nostoc in Anthoceros), ferns (Anabaena in Azolla), gymnosperms (Nostoc in Macrozamia and angiosperms (Nostoc in Gunnera) (see Stewart et al., 1980). The importance of Azolla containing Anabaena azollae has been recognized by farmers of the Southeast Asian countries for centuries. Azolla is commonly used as a green manure to improve the nitrogen balance in rice fields. Contribution of blue-green algae of liverworts and lichens to the global nitrogen economy is probably of major importance (Stewart et al., 1980). Some organisms like Cyanophora paradoxa, Glaucocystis geitleri, Glaucocystis nostochinearum, Rhophalodia gibba and Glaucosphaera contain cell inclusions which resemble blue-green algae and these inclusions are referred to as cyanells (Drum and Pankratz, 1965; Schnepf et al., 1966; Robinson and Preston, 1971; Schnepf and Brown, 1971; Kies, 1980). Of all these organisms, only Rhophalodia gibba was found to fix N2 (Drum and Pankratz, 1965; Bothe and Floener, 1980).

4) BIOCHEMISTRY OF N2 FIXATION

41) Nitrogenase

Nitrogenase from bacteria and blue-green algae exhibit almost similar properties. It is a complex enzyme and consists of two iron-sulphur proteins, which, individually, have no detectable activity but together can catalyse the reduction of a variety of substrates (N2, N3-, N2O, HCN, CH3NC, CH3CN, C2H2, H3O+ and Cyclopropene).

The larger protein (= fraction I, protein 1, MoFe-protein or dinitrogenase) of nitrogenase has high molecular mass (200000 - 270000 daltons), contains 18-36 iron, probably the same amount of acid-labile sulphur, one/two molybdenum atoms and consists of four subunits of two different types (structures).

The smaller protein component (= fraction II, protein 2, Fe-protein or dinitrogenase reductase) has relatively low molecular mass (about 60000 daltons), contains a single cluster having four atoms each of iron and sulphur and consists of two subunits which are always identical.

The MgATP2- activated Fe-protein accepts reducing equivalents from ferredoxin/flavodoxin and transfers to MoFe-protein with concomitant hydrolysis of ATP into ADP and inorganic phosphate. The Mo cofactor of MoFe-protein binds N2 and catalyses its reduction with reducing equivalents received from Fe-protein. For every molecule of N2 fixed, about 12-15 molecules of ATP are expended.

42) Requirement for reductant and energy

To reduce N2 into ammonia, the enzyme has to be supplied with reducing equivalents and energy (ATP). The reducing equivalents for N2 fixation may be generated via both Photosystem I (PSI) and Photosystem II (PSII) from water or via PSI alone or via dark reactions while ATP may be provided by cyclic and noncyclic photophosphorylation, oxidative or substrate level phosphorylation (Bothe, 1982). However, experimental evidences have ruled out some of these possibilities.

N2 fixation in blue-green algae is light dependent. It was found that C2H2 reduction takes place in far red light and in the presence of DCMU which inhibited CO2 fixation by blocking PSII (Cox, 1966; Bothe and Loose, 1972). Further, action spectrum of C2H2 reduction was similar to that of PSI (Fay, 1970; Thomas, 1970) and C2H2 reduction, unlike photosynthetic CO2 fixation, did not show an Emerson-enhancement effect (Lyne

and Stewart, 1973). Based on these evidences Lex and Stewart (1973) suggested that reducing equivalents for N2 fixation came from a pool of reductants composed of fixed carbon compounds and the duration of N2 fixation depends on the size of this reductant pool which has to be replenished by photosynthetic electron transport.
Thus the dependence of N2 fixation on photosynthetic water photolysis is indirect (cf. Bothe, 1982). Since heterocysts lack the photosynthetic water-splitting reaction and ribulose - 1, 5-biphosphate carboxylase (Tel-Or and Stewart, 1976), they depend on vegetative cells for the supply of carbohydrates (Fig. 1). The light stimulated C2H2 reduction may be dependent on ATP generated in cyclic photosphosphorylation. In addition, light could also be necessary to supply the electrons to nitrogenase in a PSI dependent reaction. Alternatively, the electrons could be generated in the dark. These later two possibilities are difficult to rule out, since an inhibitor specific to PSI is not available. In Gloeocapsa, generation of electrons depends on the operation of both photosystems (Gallon, 1980).
In the dark, oxidative phosphorylation may also provide ATP for nitrogenase operation. Substrate level phosphorylation was demonstrated for Anabaena cylindrica but is believed to be of rather limited importance in providing ATP to nitrogenase (Bottomley and Stewart, 1976). The immediate electron carrier to nitrogenase is ferredoxin (Bothe et al., 1983). Blue-green algae contain a soluble, typical plant-type ferredoxin which mediate C2H2 reduction by nitrogenase (Bothe, 1970). When Fe deficiency limits the biosynthesis of ferredoxin, several microorganisms form flavodoxin (Bothe, 1977) which takes over the function of supplementing electrons to nitrogenase (Bothe, 1969). Since ferredoxins or flavodoxins are continuously transferring electrons to nitrogenase, they must be reduced by electron donors supplied from cell metabolism. The most important electron donor is probably NADPH. The formation of NADPH seems to be via hexosemonophosphate shunt where glucose-6-phosphate is degraded by glucose-6-phosphate dehydrogenase (Bothe, 1982). The sources, other than glucose-6-phosphate, which may reduce ferredoxin are isocitrate (Bothe et al., 1980), pyruvate (Leach and Carr, 1971; Bothe et al., 1974), glycollate, malate and succinate (Murai and Katoh, 1975). The transfer of electrons from all these donors to nitrogenase proceeds either in dark or in a PSI dependent reaction. PSI is definitely involved when H2 is the electron donor. In fact, H2 gave the highest activity among all electron donors in C2H2 reduction experiments with isolatd heterocysts (Eisbrenner et al., 1978; Peterson and Burris, 1978).

43) Strategies to protect nitrogenase from damage by oxygen

Since nitrogenase is highly sensitive to oxygen, N2-fixing blue-green algae have evolved different mechanisms to protect nitrogenase from oxygen.

431) Compartmentation
Heterocysts function as compartments. These specialized cells are specially suited for activity of nitrogenase since they eliminate oxygen from them and are strongly reducing environments. They lack O2-evolvi ng PSII. Further, respiratory oxygen consumption may remove the gas from nitrogenase site since the respiratory activity of heterocysts is higher than in vegetative cells (cf. Bradley and Carr, 1966). Hydrogen gas evolved during N2 fixation by nitrogenase also seem to protect the enzyme from oxygen since C2H2 reduction was found to be more stable against O2 addition in presence of hydrogen (Bothe et al., 1978; Wolk, 1979). With regard to the O2 of air, a special function in binding O2 was suggested for four unique glycolipids found in laminated layer of the heterocyst envelope (Lambein and Wolk, 1973). Gloeothece possesses an elaborate system of internal membranes which may represent some intracellular protective compartment (Postgate, 1982). Gallon et al. (1975) suggested that it protects nitrogenase by a temporal separation of N2 fixation and photosynthesis, by building up a reserve of

fixed nitrogen with little photosynthesis early in growth and later photosynthesizing without fixing N2.

432) Aggregation
Clustering as an oxygen-restricting process has been reported in Trichodesmium (Carpenter and Price, 1976). The filaments of Trichodesmium form bundles, on the outside of which photosynthesis occurs and within which N2 fixation takes place (cf. Bryceson and Fay, 1979).

433) Mucilage
Massive amount of mucilage produced by colony forming blue-green algae may arrest the diffusion of oxygen thereby protecting nitrogenase.

44) Nitrogenase and hydrogenase relationship
H2 formation by blue-green algae takes place only under N2 fixing conditions (cf. Bothe, 1982). The formation of H2 is largely stimulated by light, but can also proceed in the dark, provided low levels of oxygen are present to allow respiration. These observations indicate that H2 must be generated by ATP-dependent reduction of H3O+ catalyzed by nitrogenase. H2 evolved by nitrogenase is not necessarily loss to blue-green algae.
It can be utilized by two different pathways, both of which are catalyzed by hydrogenases (Both et al. 1977; Eisbrenner et al. 1978; Peterson and Burris, 1978; Peterson and Wolk, 1978; Tel-Or et al., 1978; Antarikanonda et al., 1980).
In the major pathway, H2 is consumed in an oxygen dependent reaction in respiratory chain and supplies organism with extra ATP.
In the second pathway, H2 is utilized in a strictly light-requiring reaction. In this pathway, the transfer of electrons from H2 to the substrates has to proceed via PSI (Both, 1982).

45) Regulation of nitrogense
The regulation of nitrogenase biosynthesis has been extensively investigated in Klebsiella pneumoniae, where studies have established that glutamine synthetase is key control-molecule. This enzyme turns into adenylylated form due to feed back inhibition by glutamine and stops nitrogenase synthesis. The de-adenylylated form of the enzyme is formed when this inhibition is removed and it is believed to promote nitrogense biosynthesis at the transcriptional level. However, in Anabaena, nitrogenase was not controlled by adenylylation. Furthermore, Rowell et al. (1977) observed poor correlations between the activities of nitrogenase and glutamine synthetase. However, they found an inverse correlation between the intracellular pools of glutamine and aspertate, and therefore concluded that glutamine, or a derived product of glutamine, rather than glutamine synthetase itself, regulates nitrogenase biosynthesis in Anabaena. In Klebsiella (Brill, 1975; Yates, 1980), O2 not only destroys nitrogenase activity but also represses enzymes biosynthesis. Regulation by O2, light and other factors such as temperature, pH or nutrient supply may also occur in blue-green algae. Further, molybdenum deficiency severely decreased nitrogenase activity (Fay and de Vasconcelos, 1974) probably because of the production of inactive form of MoFe-protein under Mo starvation conditions (Nagatani and Haselkorn, 1978).

ECOLOGICAL AND AGRONOMICAL SIGNIFICANCE OF N2 FIXATION

Biological N2 fixation is a crucial step in the global nitrogen cycle in that it returns to the biosphere in combined forms, nitrogen that has been lost in the atmosphere mainly through denitrification and ammonia volatilization. Biological N2 fixation is the major factor determining the productivity of major natural ecoystems and all but most advanced

agricultural areas of the earth.The following is a brief summarization of the nitrogen contribution by N2-fixing blue-green algae in major ecosystems (Table 1).

51) Cold dominated ecosystem
The importance of N2 fixation by free-living and symbiotic (lichens and moss-algal associations) blue-green algae in cold ecosystem is well documented (see Metting, 1981). Nostoc commune is probably the most widespread N2 fixer in tundra and antartic soils either as free-living or as phycobiont in lichens. In a review on N2 fixation by blue-green algae in cold dominated ecosystems, Alexander (1975) listed estimated values for nitrogen input into soils. Estimate based an in vitro acetylene reduction ranged from a few grams/ha per year in some tundra soils to l15 kg/ha per year for a mire, in Sweden, dominated by various vascular species. Estimates for soils of diverse habitats in Norway ranged from 1 to 2.5 kg N/ha per year. A value of 24 g N/ha per year was reported from Antarctica (see Metting, 1981).

52) Temperate soils
In temperate soils, blue-green algae have been reported to fix up to 51 (Henriksson, 1971) or 94 (Granhall, 1975) kg N/ha per year. However most estimates are based on quantifications made during the growing season. The best evidence for the importance of asymbiotic N2 fixation in temperate soils has been provided by the Broadbalk experiment at the Rothamsted Experimental Station in England. The upper 20 cm of soil in stubbed (woody plants continuously cut back) and in wooded plots has been monitored for total nitrogen since 1882. Due in part to algal fixation and in part to bacterial fixation in the soil and the rhizosphere soils exhibited an average increment of 39 kg N/year for the stubbed plot and 49 kg N/year for the wooded plot.(Witty and Dart, 1977).
Whether cultivated or uncultivated temperate soils support more active populations of N2-fixing algae has not been firmly established. Number of heterocystous blue-green algae were reported to decrease in cultivated soils of eastern Washington while the number of N2-fixing blue-green algae in temperate soils in the Soviet Union were reported to increase upon cultivation (see Metting, 1981).

53) Tropical soils
Blue-green algae are widely distributed in tropical and temperate areas but seems to be more abundant in the tropics. They are especially abundant and active in submerged soils, which explains why wetland rice can be grown on the same land year after year without N fertilizer and can produce low but constant yield.
The average of 38 quantitative evaluations of N2 fixation in rice fields recorded in the literature was 27 kg N/ha per crop and the highest value was 50-80 kg N/ha per crop (Roger and Kulasooriya, 1980). (To be compared with values recorded in temperate soils, values expressed in kg N/ha per crop have to be multiplied by three which lead to an average value of about 90 kg N/ha per year).
BGA may also significantly contribute to the nitrogen economy of tropical dryland soils. Singh (1961) reported a profuse growth of N2 fixing blue-green algae in sugar cane fields in India from beginning of the rainy season. Among the strains, Cylindrospermum licheniforme was found to add about 90 kg N/ha in about 75 days. The same species was reported to colonize very succesfully the clean soil surface of weed-free maize fields.

54) Freshwater environments
Bloom of N2-fixing species have been reported from freshwater bodies in many countries (see Fogg et al., 1973). Estimates of N2 fixation in lakes are difficult because of the uneven distribution of the algae in the water. Estimate of N2 fixation rates in the surface waters of lakes range from 0 to 125 ug/liter and per day (Fogg et al., 1973).

55) Marine environments

N2-fixing blue-green algae are less common in marine environements than in freshwaters. The most studied N2-fixing marine blue-green alga is Trichodesmium (Oscillatoriaceae) which can develop large biomass and fix N2 when aggregated in bundles. It can occur at depths of 20 to 40 m (see Fogg et al., 1973).

In littoral fringe of West Africa, Calothrix sp. was found to colonize large areas of sand (P. Roger unpub.). Annual fixation rates of marine blue-green algae are still very poorly documented. The recent book by Humm and Wicks (1980) do not give any estimates.

REFERENCES CITED

1. Alexander, V. A. 1975. Nitrogen fixation in polar and subpolar regions. In W. D. P. Stewart, ed. Nitrogen fixation by free-living microorganisms. IBP programme 6. Cambridge Univ. Press, Cambridge.

2. Antarikanonda, P., H. Berndt, F. Mayer, and H. Lorenzen. 1980. Molecular hydrogen: a new inhibitor of photosynthesis in the blue-green alga (Cyanobacterium), Anabaena sp. TA1. Arch. Microbiol. 126:1-10.

3. Bothe, H. 1969. The role of phytoflavin in photosynthetic reactions. Pages 1483-1491 in H. Metzen, ed. Progress in photosynthetic research Vol. III. Univ. Tubingen, Germany.

4. Bothe, H. 1970. Photosynthetische stickstoffixierung mit einem zellfreien Extrakt aus der Blaualge Anabaena cylindrica. Ber. dt. bot. Ges. 83:421-432.

5. Bothe, H. 1977. Flavodoxin. Pages 217-221 in A. Threbst and M. Avron, ed. Encyclopedia of Plant Physiology New Series Vol. 5. Springer Verlag, Berlin.

6. Bothe, H. 1982. Nitrogen fixation. Pages 87-104 in N.G. Carr and B.A. Whitton, ed. The biology of cyanobacteria. Blackwell Scientific Publications, Oxford.

7. Bothe, H., E. Distler, and G. Eisbrenner. 1978. Hydrogen metabolism in blue-green algae. Biochimie 60:277-289.

8. Bothe, H., B. Falkenberg, and U. Nolteernsting. 1974. Properties and function of the pruvate: ferredoxin oxidoreductase from the blue-green alga Anabaena cylindrica. Arch. Microbiol. 96:291-304.

9. Bothe, H., and L. Floener. 1980. Nitrogen fixation in Rhopalodia gibba, a diatom containing blue-greenish inclusions symbiotically. Pages 541-552 in W. Schwemmler and H.E.A. Schenk, ed. Endosymbiosis and cell biology, Vol. I. Walter de Gruyter, Berlin.

10. Bothe, H., and E. Loose. 1972. Effect of far red light and inhibitors on nitrogen fixation and photosynthesis in the blue-green alga Anabaena cylindrica. Arch. Mikrobiol. 86:241-254.

11. Bothe, H., G. Neuer, I. Kalbe, and G. Eisbrenner. 1980. Electron donors and hydrogenase in nitrogen fixing microorganisms. Pages 83-112 in W.D.P. Stewart and J.R. Gallon, ed. Nitrogen fixation. Academic Press, N.Y.

12. Bothe, H., J. Tennigkeit, and G. Eisbrenner. 1977. The utilization of molecular hydrogen by the blue-green alga Anabaena cylindrica. Arch. Microbiol. 114:43-49.

13. Bothe, H., M.G. Yates, and F.E. Cannon. 1983. Physiology, biochemistry and genetics of dinitrogen fixation. Pages 241-285 in A. Lauchli and R. H. Bieleski, ed. Inorganic plant nutrition (Encylopedia of Plant Physiology New Series, Vol. 15A). Springer Verlag. Berlin.

14. Bottomley, P.J., and W.D.P. Stewart. 1976. ATP pools and transients in the blue-green alga Anabaena cylindrica. Arch. Microbiol. 108:249-258.

15. Bradley, S., and N.G. Carr. 1976. Heterocyst and nitrogenase development in Anabaena cylindrica. J. Gen. Microbiol. 96:175-184.

16. Brill, W.J. 1975. Regulation and genetics of nitrogen fixation. A. Rev. Microbiol. 29:109-129.

17. Bryceson, I., and P. Fay. 1979. Nitrogen fixation associated with surface blooms of Oscillatoria (Trichodesmium) erythraea in the coastal waters of Dar Es Salaam. Page D2 in J.M. Nichols, ed. Abstracts of the third international symposium on photosynthetic prokaryotes. Univ. Liverpool, England.
18. Burris, R.H., F.J. Eppling, H.B. Wahlin, and P.W. Wilson. 1943. Detection of nitrogen fixation with isotopic nitrogen. J. Biol. Chem. 148:349-357.
19. Carpenter, E.J., and C.C. Price IV. 1976. Marine Oscillatoria (Trichodesmium): explanation for aerobic nitrogen fixation without heterocysts. Science, N.Y. 191:1278-1280.
20. Cox, R.M. 1966. Physiological studies on nitrogen fixation in the blue-green alga Anabaena cylindrica. Arch. Mikrobiol. 53:263-276.
21. Dilworth, M.J. 1966. Acetylene reduction by nitrogen-fixing preparations from Clostridium pasteurianum. Biochim. Biophys. Acta 127:285-294.
22. Drum, R.W., and S. Pankratz. 1965. Fine structure of an unusual cytoplasmic inclusion in the diatom genus, Rhopalodia. Protoplasma (wien.) 60:141-149.
23. Eisbrenner, G., E. Distler., L. Floener, and H. Bothe. 1978. The occurrence of the hydrogenase in some blue-green algae. Arch. Microbiol. 118:177-184.
24. Fay, P. 1970. Photostimulation of nitrogen fixation in Anabaena cylindrica. Biochim. Biophys. Acta 216:353-356.
25. Fay, P., and L. de Vasconcelos. 1974. Nitrogen metabolism and ultrastructure in Anabaena cylindrica. II. The effect of molybdenum and vanadium. Arch. Microbiol. 99:221-230.
26. Fay, P., and G.E. Fogg. 1962. Studies on nitrogen fixation by blue-green algae. III. Growth and nitrogen fixation in Chlorogloea fritschii Mitra. Arch. Mikrobiol. 42:310-321.
27. Fleming, H., and R. Haselkorn. 1973. Differentiation in Nostoc muscorum: nitrogenase is synthesized in heterocysts. Proc. Nat. Acad. Sci. U.S.A. 70:2727-2731.
28. Fogg, G. E. 1942. Studies on nitrogen fixation by blue-green algae. I. Nitrogen fixation by Anabaena cylindrica Lemm. J. Exp. Bot. 19:78-87.
29. Fogg, G.E. 1949. Growth and heterocyst production in Anabaena cylindrica Lemm. II. In relation to carbon and nitrogen metabolism. Ann. Bot. 13:241-259.
30. Fogg, G. E., W. D. P. Stewart, P. Fay, and A. E. Walsby. 1973. The blue-green algae. Academic Press, Lond, N.Y.
31. Gallon, J.R. 1980. Nitrogen fixation by photoautotrophs. Pages 197-238. in W.D.P. Stewart, ed. Nitrogen fixation. Academic Press, N.Y.
32. Gallon, J.R., T.A. La Rue, and W.G.W. Kurz. 1975. Photosynthesis and nitrogenase activity in the blue-green alga Gloeocapsa. Can. J. Microbiol. 20:1633-1637.
33. Granhall, U. 1975. Nitrogen fixation by blue-green algae in temperate soils. In W. D. P. Stewart ed. Nitrogen fixation by free-living microorganisms. IBP programme 6, Cambridge Univ. Press, Cambridge.
34. Henriksson, E. 1971. Algal nitrogen fixation in temperate regions. In T. A. Lie and E. G. Mueder, ed. Biological nitrogen fixation in natural and agricultural habitats. Plant and Soil Special Volume.
35. Kenyon, C.N., R.Rippka, and R.Y. Stanier. 1972. Fatty acid composition and physiological properties of some filamentous blue-green algae. Arch. Microbiol. 83:216-236.
36. Kies, L. 1980. Morphology and systematic position of some endocyanomes. Pages 7-19 in W. Schwemmler and H.E.A. Schenk. ed. Endosymbiosis and Cell Biology. Walter de Gruyter, Berlin.
37. Lambein, F., and C.P. Wolk. 1973. Structural studies on the glycolipids from the envelope of the heterocysts of Anabaena cylindrica. Biochemistry, N.Y. 12:791-798.
38. Lang, N.J., and P. Fay. 1971. The heterocysts of blue-green algae. II. Details of ultrastructure. Proc. R. Soc. Lond. B178:193-203. `

39. Leach, C.K., and N.G. Carr. 1971. Phyruvate: ferredoxin oxidoreductase and its activation by ATP in the blue-green alga Anabaena variabilis. Biochim. Biophys. Acta 245:165-174.
40. Lex, M., and W.D.P. Stewart. 1973. Algal nitrogenase, reductase pools and photosystem I activity. Biochim. Biophys. Acta 292:436-443.
41. Lyne, R.L., and W.D.P. Stewart. 1973. Emerson enhancement of carbon fixation but not of acetylene reduction (nitrogenase activity) in Anabaena cylindrica. Planta. (Berlin) 109:27-38.
42. Metting, B. 1981. The systematics and ecology of soil algae. Bot. Rev. 47(2):195-312.
43. Murai, T., and T. Katoh. 1975. Photosystem I - dependent oxidation of organic acids in the blue-green alga Anabaena variabilis. Pl. Cell Physiol. Tokyo 16:759-797.
44. Nagatani, H. H., and R. Haselkorn. 1978. Molybdenum independence of nitrogenase component synthesis in the non-heterocystous cyanobacterium Plectonema. J. Bact. 134:597-605.
45. Oppenheim, J., and L. Marcus. 1970. Correlation of ultrastructure in Azotobacter vinelandii with nitrogen source for growth. J. Bact. 101:286-291.
46. Pearson, H. W., R. Howsley, C.K. Kjeldsen, and A.E. Walsby. 1979. Aerobic nitrogenase activity associated with a non-heterocystous filamentous cyanobacterium. FEMS Microbiol. Lett. 5:163-167.
47. Peterson, R.B., and R.H. Burris. 1978. Hydrogen metabolism in isolated heterocysts of Anabaena 7120. Arch Microbiol. 116:125-132.
48. Peterson, R.B., and C.P. Wolk. 1978a. Localization of an uptake hydrogenase in Anabaena. Pl. Physiol. Lancaster 61:688-691.
49. Peterson, R.B., and C.P. Wolk. 1978b. High recovery of nitrogenase activity and of 55Fe-labeled nitrogenase in heterocysts isolated from Anabaena variabilis. Proc. Nat. Acad. Sci. U.S.A. 75:6271-6275.
50. Postgate, J.R. 1982. The fundamentals of nitrogen fixation. pp. 252. Cambridge University Press, Cambridge.
51. Rippka, R., J. Deruelles, J.B. Waterbury, M. Herdman, and R.Y. Stanier. 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J. Gen. Microbiol. 111:1-61.
52. Rippka, R., A. Neilson, R. Kunisawa, and G. Cohen-Bazire. 1971. Nitrogen fixation by unicellular blue-green algae. Arch. Mikrobiol. 76:341-348.
53. Rippka, R., and R.Y. Stanier. 1978. The effects of anaerobiosis on nitrogenase synthesis and heterocyst development by nostocacean cyanobacteria. J. Gen. Microbiol. 105:83-94.
54. Robinson, D.G., and R.D. Preston. 1971. Studies on the fine structure of Glaucocystis nostochinearum Itzigs. J. Exp. Bot. 22:635-643.
55. Roger, P. A., and S. A. Kulasooriya. 1980. Blue-green algae and rice. The International Rice ResearchInstitute. Los Banos, Philipipnes.
56. Rowell, P., S. Enticott, and W.D.P. Stewart. 1977. Glutamine synthetase and nitrogenase activity in the blue-green alga Anabaena cylindrica. New Phytol. 79:41-54.
57. Schnepf, E., and R.M. Brown, Jr. 1971. Pages 299-322 in H. Reinert and H. Ursprung, ed. Origin and continuity of cell organelles. Springer Verlag, Berlin.
58. Schnepf, E., W. Koch, and G. Deichgraber. 1966. Zur cytologie und taxonomischer Einordnung von Glaucocystis. Arch. Mikrobiol. 55:149-174.
59. Schollhorn, R., and R.H. Burris. 1966. Study of intermediates in nitrogen fixation. Fedn. Proc. Fedn. Am. Socs. Exp. Biol. 25:710.
60. Singh, P.K. 1973. Nitrogen fixation by the unicellular blue-green alga Aphanothece. Arch. Microbiol. 92:59-62.
61. Singh, R. N. 1961. Role of blue-green algae in nitrogen economy. In Indian Council Agric. Res. New Delhi, India.

62. Singh, R.N. 1972. Physiology and biochemistry of nitrogen fixation by blue-green algae (Final Technical Report). 1967-1972. Dept. Bot., Banaras Hindu University (India), 66 pp.
63. Smith, R.V., and M.C.W. Evans. 1971. Nitrogenase activity in cell free extracts of the blue-green alga Anabaena cylindrica. J. Bact. 105:913-917.
64. Stewart, W.D.P. 1967. Nitrogen turnover in marine and brackish habitats. II. Use of 15N in measuring nitrogen fixation in the field. Ann. Bot. 31:385-407.
65. Stewart, W.D.P. 1968. Nitrogen input into aquatic ecosystems. Pages 53-72 in D.F. Jackson, ed. Algae, Man and Environment. University Press, Syracuse, N.Y.
66. Stewart, W.D.P. 1971. Physiological studies on nitrogen-fixing blue-green algae. Pages 377-391 in T.A. Lie and E.G. Mulder, ed. Biological nitrogen fixation in natural and agricultural habitats. Plant and Soil, Special Volume.
67. Stewart, W.D.P. 1973a. Nitrogen fixation. Pages 260-278 in N.G. Carr and B. A. Whitton, ed. The biology of blue-green algae. Blackwell Scientific Publications, England.
68. Stewart, W.D.P. 1973b. Nitrogen fixation by photosynthetic microorganisms. A. Rev. Microbiol. 27:283-316.
69. Stewart, W.D.P., G.P. Fitzgerald, and R.H. Burris. 1968. Acetylene reduction by nitrogen-fixing blue-green algae. Arch. Mkrobiol. 62:336-348.
70. Stewart, W.D.P., A. Haystead, and H.W. Pearson. 1969. Nitrogenase activity in heterocysts of blue-green algae. Nature, London 224:226-228.
71. Stewart, W.D.P., and M. Lex. 1970. Nitrogenase activity in the blue-green alga, Plectonema boryanum strain 594. Arch. Mikrobiol. 73:250-260.
72. Stewart, W.D.P., P. Rowell, and A.N. Rai. 1980. Symbiotic nitrogen-fixing cyanobacteria. Pages 239-277 in W.D.P. Stewart and J.R. Gallon, ed. Nitrogen fixation. Academic Press, London.
73. Taylor, B.F., C.C. Lee, and J.S. Bunt. 1973. Nitrogen-fixation associated with the marine blue-green alga, Trichodesmium as measured by the acetylene-reduction technique. Arch. Microbiol. 88:205-212.
74. Tel-Or, E., L.W. Luijk, and L. Packer. 1978. Hydrogenase in N2-fixing cyanobacteria. Arch. Biochem. Biophys. 185:185-194.
75. Tel-Or, E., and W.D.P. Stewart. 1976. Photosynthetic electron transport, ATP synthesis and nitrogenase activity in isolated heterocysts of Anabaena cylindrica. Biochim. Biophys. Acta. 423:189-195.
76. Thomas, J. 1970. Absence of the pigments of photosystem II of photosynthesis in heterocysts of a blue-green alga. Nature, London 228:181-183.
77. Witty, J. F., and J. M. Dart. 1977. The nitrogen economy of the Broadbalk experiments. II. Biological nitrogen fixation. Rothamsted Exp. Station Rep. Part 2 1976:lll-l18.
78. Wolk, C.P. 1979. Intercellular interactions and pattern formation in filamentous cyanobacteria. Pages 247-266 in S. Subtelny and I.R. Konigsberg, ed. Determinants of spatial organization, 37th Symp. Soc. Developmental Biol. Academic Press, New York.
79. Wyatt, J.T., and J.K.G. Silvey. 1969. Nitrogen fixation by Gloeocapsa. Science 165:908-909. 80. Yates, M.G. 1980. The biochemistry of nitrogen fixation. Pages 1-64 in B.J. Miflin,