Chapter 24
Role of Algae in Soil Nitrogen Fixation

Anamika Singh

Abstract Microorganisms help to increase the soil nutritional quality, as they are able to decompose organic matter. From soil, plants get nutrition for their growth and development. Along with microorganism, many fungi and algae also perform such functions. Algal cells are natural fertilizer and nowadays it is used worldwide, without any side effect, Algal cells have specific cells called heterocyst and are the site of nitrogen fixation. Algae are able to convert unavailable dinitrogen into bioavailable ammonia. *Anabaena*, *Nostoc*, and many other cyanobacteria (blue green algae, BGA) are able to fix atmospheric nitrogen.

Keywords Nitrogen fixation · Cyanobacteria · Nitrogenase · Nitrogen cycle · Heterocyst

24.1 Introduction

Nitrogen is the most abundant gas in the earth's atmosphere, it is nearly 79%. In atmosphere it is present in the form of di-nitrogen (N_2). It is one of the most important elements for survival of life on Earth. Proteins, nucleic acids and chlorophyll (Ferguson 1998; Smil 2004) are the few most important Nitrogen containing biological molecules. These biological molecules are required for need to build, cytochromes, alkaloids, phytohormones, and many of the vitamins (Bray 1983). The dinitrogen form of nitrogen is highly inaccessible to most organisms, because both the nitrogen atoms (N=N) linked to each other by a triple bonds, that make the molecule almost inert (Jia and Quadrelli 2014; MacKay 2004). About 225 kcal of energy is required to break this triple bond, and it is difficult to achieve. Primary producers are using nitrogen only in the form of ammonia (NH_3), so conversion of dinitrogen gas into ammonia (NH_3) is required. Agronomic, economic, and

A. Singh (✉)
Department of Botany, Maitreyi College, University of Delhi, New Delhi, India
e-mail: asingh@maitreyi.du.ac.in

C. Cruz et al. (eds.), *Soil Nitrogen Ecology*, Soil Biology 62,
https://doi.org/10.1007/978-3-030-71206-8_24

ecological impact of the world depends upon the agriculture production and it is directly linked to availability of fixed nitrogen. Ammonia (NH_3) or nitrate (NO_3^-) (Canfield et al. 2010; Cheng 2008) are the two important fixed forms of nitrogen, utilized by organisms and are continuously sequestered into sediments, rendering them unavailable for metabolism. Nitrification and denitrification are the two combined processes related to nitrogen metabolism and is the conversion of N_2 to NH_3 (Cheng 2008; Thamdrup 2012). The process of conversion of molecular and elemental free nitrogen into nitrogenous compounds, which can be easily absorbed by the plants, is called as nitrogen fixation. It is carried out by physicochemical and biological means. About 10% of natural nitrogen fixation takes place by physicochemical methods and 90% by biological methods. In nature nitrogen fixation is a complex process and takes place by two ways: (1) non-biological nitrogen fixation or physical nitrogen fixation or geochemical processes like lightning (Gruber and Galloway 2008) and (2) biological nitrogen fixation (Burk et al. 1934; McGlynn et al. 2013; Dos Santos et al. 2012).

Non-biological nitrogen fixation mainly includes natural nitrogen fixation and industrial nitrogen fixation (Fig. 24.1). Industrial is through the Haber–Bosch process (Smil 2004; Haber 1992, 1923). Haber–Bosch process is widely used for the production of nitrogen fertilizers and almost half of the human population

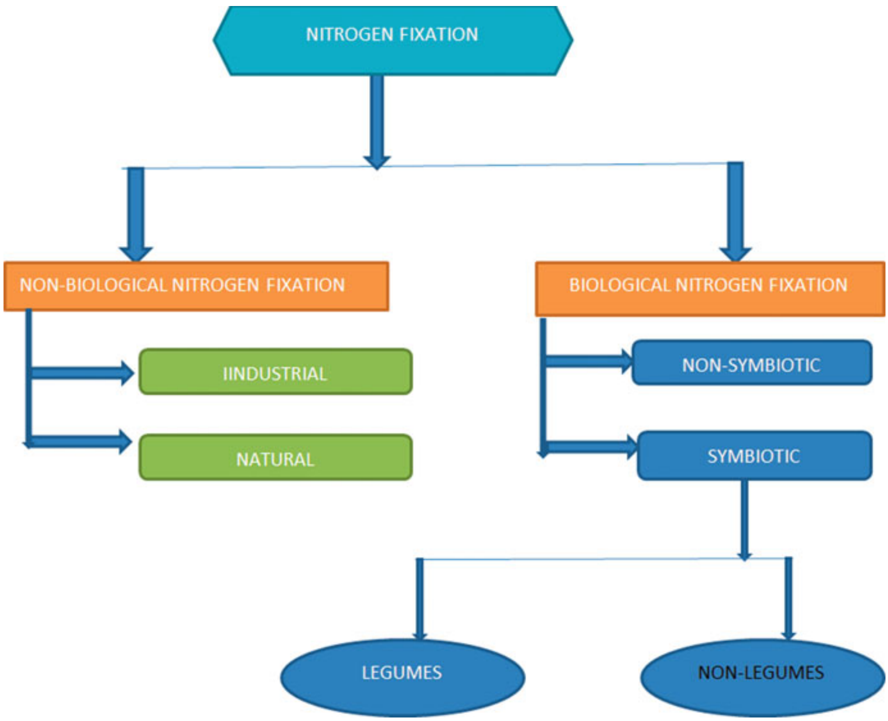

Fig. 24.1 Showing different ways of atmospheric nitrogen fixation

depends upon its applications (Smil 2004; Canfield et al. 2010). In nitrogen cycle few microorganisms like bacteria play important role in the conversion of N_2 to ammonia, other bacteria convert ammonia to nitrate and few nitrates to nitrogen gas.

24.2 Non-biological Nitrogen Fixation or Physical Nitrogen Fixation

It contains natural as well as industrial nitrogen fixation.

24.2.1 Natural Nitrogen Fixation

Under the influence of lightning (i.e., electric discharge in the clouds) and thunder, N_2 and O_2 of the air react to form nitric oxide (NO). The nitric oxides are again oxidized with oxygen to form nitrogen peroxide (NO_2).

The reactions are as follows

$$N_2 + O_2 \text{ Lightning} \rightarrow \text{Thunder } 2NO \text{ (Nitric Oxide)}; 2NO + O_2 \rightarrow 2{NO_2}^{\text{Oxidation}} \text{ (Nitrogen peroxide)}$$

During rains, NO_2 combines with rain water to form nitrous acid (HNO_2) and nitric acid (HNO_3). The acids fall on the soil along with rain water and react with the alkaline radicals to form water soluble nitrates (NO_3-) and nitrites (${NO_2}^-$).

$$2NO_2 + H_2O \rightarrow HNO_2 + HNO_3 \; HNO_3 + \text{Ca or K salts} \rightarrow \text{Ca or K nitrates}$$

The nitrates are soluble in water and are directly absorbed by the roots of the plants.

24.2.2 Industrial Nitrogen Fixation

Ammonia is produced industrially by direct combination of nitrogen with hydrogen (obtained from water) at high temperature and pressure. Later, it is converted into various kinds of fertilizers, such as urea, etc.

24.3 Biological Nitrogen Fixation

Biological nitrogen fixation is a process mediated by living organism, and they are actually fixing the nitrogen into the nitrogenous. It is carried out by two main types of microorganisms: (1) symbiotic microorganisms and (2) non-symbiotic microorganisms. Symbiotic association involves interaction of plants and microorganisms. Biological nitrogen fixation (BNF) involves reduction of atmospheric nitrogen into ammonia.

Diazotrophy

Biological nitrogen fixation through the agency of microorganism occurs in the presence of nitrogenize enzyme. Thiss enzyme is actually a biological catalyst present in few symbiotic microorganisms like *Rhizobium* and *Frankia* or the free-living *Azospirillum* and *Azotobacter* and blue green algae (BGA). These microorganisms are able to reduce inert gaseous dinitrogen (N_2) into ammonia (NH_3). This converted ammonia is easily absorbed by roots of plants and it is known as biological nitrogen fixation or diazotrophy.

It is a complex biochemical reactions and can be summarized as:

$$N_2 + 8H_2 + 16ATP \text{ nitrogenase} \rightarrow 2\,NH_3 + 2H_2 + 16ADP + 16\,P_i$$

In this equation one molecule of nitrogen gas (N_2) combines with eight hydrogen ions (also known as protons) ($8H^+$) to form two molecules of ammonia ($2NH_3$) and two molecules of hydrogen gas ($2H_2$). This reaction occurs in the presence of nitrogenase enzyme. The 16 molecules of ATP (ATP = adenosine triphosphate, an energy storing compound) represent the energy required for the biological nitrogen fixation (BNF) reaction to take place. 16 ATPs are biochemically a large amount and 'expensive' to the plant in terms of energy usage. Energy of sun through photosynthesis is utilized directly. As ammonia (NH_3) is formed in this reaction, it is converted to glutamine. The nitrogen in amino acids can be used by the plant to synthesize proteins for its growth and development.

Nitrogen Fixers

These are mainly bacteria and cyanobacteria (blue green algae, BGA), called as diazotrophs. They are able to fix almost 95% of the total global nitrogen. Diazotrophs may be symbiotic (free living) or symbiotic.

1. Free-living nitrogen fixing bacteria
2. Symbiotic nitrogen fixing bacteria
3. Free-living nitrogen fixing algae
4. Symbiotic nitrogen fixing algae

(1) *Free-living nitrogen fixing bacteria*: Free-living nitrogen fixing bacteria adds 10–30 kg of nitrogen/hectare/annum. *Rhodospirillum*, *Chromatium*, *Rhodopseudomonas* are the examples of Photoautotrophic bacterium while *Clostridium* (anaerobic), *Beijerinckia* and *Azotobacter* are saprophytic in nature are able to fix nitrogen. Few chemotrophic bacteria like *Desulphovibrio* are also able to fix nitrogen biologically.
(2) *Symbiotic nitrogen fixing bacteria*: In soil many species of *Rhizobium* are present and only few species are able to fix nitrogen who comes in contact with leguminous planets, as they form a symbiotic association in the form of root nodule. *Rhizobium* is aerobic, gram negative, and symbionts of Papilionaceous roots. Rhizobium is found in roots nodules of *Sesbania rostrata* while *Aerorhizobium* in stem nodules. In non-leguminous plants like *Casuarina* and *Alnus, Frankia* is found in root nodules. Leaves of few family members of Rubiaceae and Myrsinaceae (e.g., *Ardisia*) form symbiotic association with *Xanthomonas* and *Mycobacterium*.
(3) *Free-living nitrogen fixing blue green algae (Cyanobacteria)*: *Anabeaena*, *Nostoc*, *Cylindrospermum*, *Trichodesmium*, and *Aulosira* are the most common blue green algae that help in nitrogen fixation. They almost fix 20–30 kg nitrogen per hectare per annum. *Aulosira fertilissima* is found in rice fields, whereas in sugarcane fields Cylindrospermum is found.

(4) *Symbiotic nitrogen fixing blue green algae (cyanobacteria)*: *Anabaena* and *Nostoc* are the most common algal species found in symbiotic association in lichens (Fig. 24.2). Other important associations are *Cycas* roots with Anthoceros and *Azolla*. A water fern *Azolla pinnata* found in rice fields as *Anabaena azollae* in its fronds helps in nitrogen fixation.

24.4 Cyanophycean Algae and Nitrogen Fixation

Algae is playing an important role in nitrogen fixation especially cyanobacteria (blue green algae, BGA). Cyanobacteria or blue green algae are a diverse group of prokaryotes that mostly form a complex association with bacteria and green algae, and this structure is known as cyanobacterial mats (Rodrigo and Novelo 2007). Cyanophyceae or myxophyceae are algal group having xanthophyll, carotenes, and chlorophyll A and are photosynthetic in nature. They also have phycocyanine, phycoerythrin, and phycobilin pigments. They are known as Cyanophyta (Cyanophyceae, Myxophyta), now popularly called as Cyanobacteria (According to the "Bergey's Manual of Systematic Bacteriology" Vol. 3 1989). There are different types of blue green algae that help differently in soil nitrogen fixation. Cyanobacteria are classified in Bergey's Manual and has five orders (subsections). These five orders (subsections) include three for non-heterocystous types algae and two for heterocystous type algae (Castenholz 2001; Castenholz and Waterbury

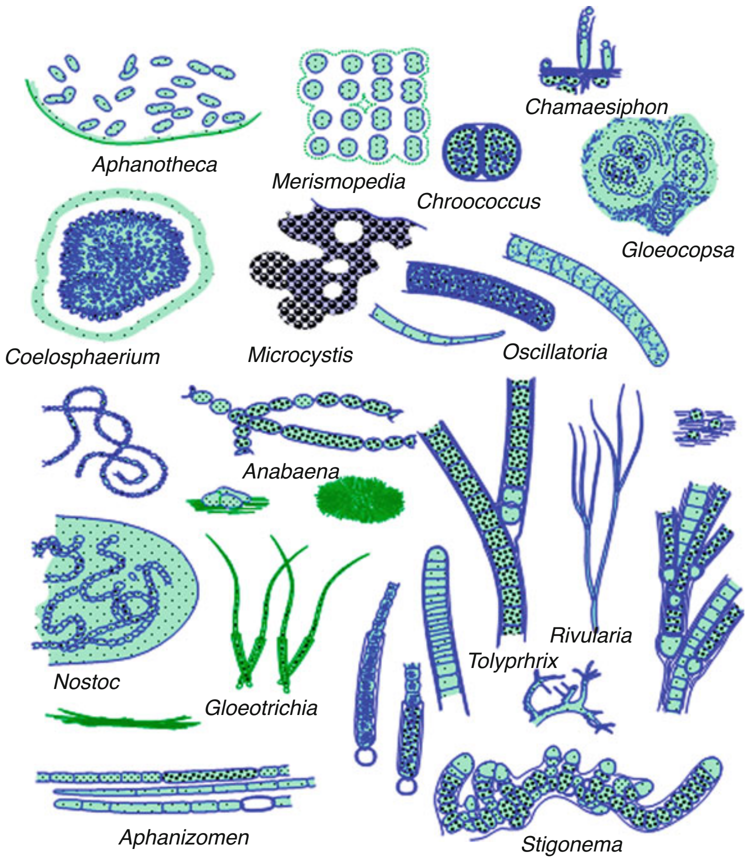

Fig. 24.2 Different blue green algae help in biological nitrogen fixation (Image taken from Issa et al. 1997)

1989). The non-heterocystous algae belongs to order *Chroococcales*, *Pleurocapsales*, and *Oscillatoriales*, while heterocystous algae belongs to order Nostocales and Stigonematales and they all are able to fix atmospheric (Bergman et al. 1997).

From Table 24.1 it is clear that cyanophycean algae may have heterocyst or may not have but in both the cases they are able to fix atmospheric nitrogen. Totally, more than 150 species of 33 genera have been reported to be able to fix nitrogen (Li et al. 1983). There are many unicellular blue green algae, able to fix nitrogen like Gloeocapsa, almost five different strains have been reported that can fix nitrogen aerobic situation (Wyatt and Silvey 1969; Rippka and Stanier 1978; Rippka et al. 1979). Three strains of Synechococcus perform N_2 fixation under anaerobic conditions. Studies on the nitrogen fixing BGA are in different heads as per the presence and absence of heterocyst in the cell and are known as heterocystous and non-heterocystous algae. Non-heterocystous filamentous blue green algae are able to fix nitrogen under aerobic conditions (Bergman et al. 1997). Non-heterocystous

Table 24.1 Cyanophycean algae and its classifications

Cyanophyceae	Order	Features
Non-heterocystous	1. Chroococcales	Unicellular cyanobacteria and reproduce by binary fission, e.g., Merismopedia Gloeocapsa, *Trichodesmium*, and *Microcystis*
	2. Pleurocapsales	Unicellular cyanobacteria and produces daughter cells smaller than the parent *eg. Pleurocaps*a
	3. Oscillatoriales	They are filaments form of algae and cells are known as trichomes, e.g., *Oscillatoria Phormidium*, *Microcoleus*, *Lyngbya*, and *Planktothrix*
Heterocystous	1. Nostocales	Trichomes present with vegetative cells are also divided into heterocysts. few species have false branching in some species *Anabaena*, *Aphanizomenon*, *Nostoc*, *Calothrix*, and *Tolypothrix*
	2. Stigonematales	Trichomes is present with heterogeneous cellular composition as well as heterocysts and akinetes vegetative cells. Filament multiseriated with true branching, e.g., Stigonema, Mastigocladus, and *Fischerella*

cyanobacteria are able to maintain a nitrogenase level sufficient for photoautotrophic growth at the expense of N_2 under aerobic conditions in both unicellular and multicellular forms. Non-heterocystous algae are having ability to synthesize nitrogenase. *Plectonema boryanum* (Stewart et al. 1969; Lex 1970) can fix atmospheric nitrogen only under microaerobic conditions. Similarly, Phormidium, Raphidiopsis (Singh 1961), Oscillatoria, Lyngbya, and Plectonema were shown to fix N_2 under microaerobic conditions only. Rippka and Stanier (1978) analysed that in strict anaerobic conditions almost 50% non-heterogenous blue green algae are able to synthesis nitrogenase enzyme. Intense anaerobic situations were created by blocking the photosynthetic O2-evolution by 3,4-dichlorophenyl-1,1-dimethylurea (DCMU).

Heterocystous blue green algae are able to fix N_2 aerobically and microaerobically. Among heterocystous *Anabaena*, *Aulosira*, *Calothrix, Cylindrospermum*, *Nostoc*, *Scytonema*, *Tolypothrix*, *Fischerella*, *Mastigocladus*, and *Stigonema* are the most common blue green algae. These algae have specialized cells different from vegetative cell. These special cells are known as heterocyst. Normal vegetative cells change to heterocysts when grown in the absence of combined nitrogen. Fogg (1949) suggested that heterocysts are the actual sites of nitrogen fixation in algae cells. Further it was reported that nitrogenase is located in the heterocyst under aerobic growth conditions (Stewart et al. 1969; Fleming and Haselkorn 1973; Peterson and Wolk 1978a, b). Nitrogenase is an oxygen sensitive enzyme and heterocyst is a suitable site for its function as it lacks photosystem II, which is responsible for oxygen evolution in cells. So heterocysts show nitrogenase activity under anaerobic conditions (Smith and Evans 1971; Rippka and Stanier 1978).

Symbiotic blue green algae are heterocystous and few are unicellular blue green algae able to develop symbiosis. Different groups of cyanophycean algae were found which can fix nitrogen (Table 24.2). Blue green algae are having vast host range and

Table 24.2 Different Cyanophycean algae and its groups

S. No	Algal group	Features
1.	Unicellular group	Unicellular strains successfully grow on BG II medium in the absence of nitrogen (broth is a universal medium for cultivation of blue green algae)
2.	*Anabaena* group	Heterocystous algae having thin sheath, no branches, non-mucilaginous colonies with definite shape *(Anabaena, Nodularia, Cylindrospermum, Anabaenopsis,* etc.)
3.	Nostoc group	Heterocystous mucilaginous strains without branching, but thick sheath and well-defined shape *(Nostoc)*
4.	*Aulosira* group	Heterocyst containing strains, having thick sheath, without branching, does not form diffuse colonies on agar medium *(Aulosira)*
5.	*Scytoneina* group	False branched algae with heterocyst, no polarity. Velvet like patched colonies found when grown in agar medium *(Scytonema)*
6.	*Calothrix* group	Same as Scytonema group *(Calothrix, Tolypothrix, Hassalia*)
7.	*Gloeotrichia* group	Definite shape mucilaginous colony with heterocyst, with polarity *(Gloeotrichia, Rivularia*)
8.	*Fischerella* group	True branching with heterocyst *(Fischerella, Westiellopsis, Stigonema*)

found in different parts of plant. They are found in close association with diatoms, lichens, fungi, bryophytes, gymnosperms, and angiosperms. Two most important characteristics of blue green algae are presence of heterocyst and formation of short motile fragment known as hormogonia. This hormogonium makes algal cells motile otherwise immotile. *Nostoc*, *Calothrix*, *Scytonema*, *Fischerella*, and *Gloeocapsa* are commonly found in symbiosis with fungi and form lichens. In bryophytes *Azolla* and *Anthoceros* are symbiotically associated with *Anabaena* and *Nostoc*, respectively. Macrozamia (gymnosperms) is symbiotically found with Nostoc and Gunnera (angiosperms) is found with Nostoc (Stewart et al. 1980). *Azolla* mainly used as green manure to improve the nitrogen balance in rice fields. Global nitrogen fixation is highly influenced by blue green algae of lichens and liverworts (Stewart et al. 1980). Different algal groups of blue green algae are summarized in Table 24.2.

24.5 Heterocyst and Nitrogen Fixation

Heterocysts (Gr. Hetero = different; Cyst = swollen and encapsulated cell) are found in many species of filamentous blue-green algae (Fay and Fogg 1962). Heterocysts are anaerobic factories for nitrogen fixation. These cells are slightly larger size as compared to vegetative cells, and it also develops from vegetative cells and may be solitary, or in pairs, or several in a row (Fogg 1949). It is a colourless, enlarged, thick walled cell without chlorophyll (Fig. 24.3). It is the site for nitrogen fixation, and it has nitrogenase enzyme (Stewart et al. 1980). The cell is maintained in its anoxic condition as it lacks photosynthetic activity (produce oxygen) and thick

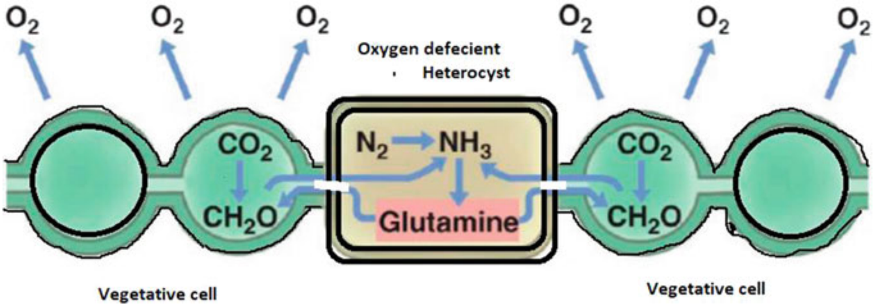

Fig. 24.3 Algal cells showing vegetative cell and heterocyst cells

wall, these conditions are essential for the proper functioning of nitrogenase. Heterocyst contains only photosystem1 for ATP production, Photosystem II is completely absent. Heterocysts cells are linked with other cells through plasmodesmata and get nutrients from surrounding cells. The shape of the heterocysts and its position in the trichomes are determined genetically. The number of heterocysts in trichomes of any algal populations depends on the nitrogen supply in the environment. As the level of NH_4^+ or NO_3^- increases, the number of heterocyst decreases within a trichome.

At low intensity of light and at high concentration of phosphate in medium, the number of heterocyst in filament increases in algae (Fay and Fogg 1962). Heterocyst's fixes atmospheric nitrogen under anaerobic condition due to the presence of nitrogenase, which is maintained within the heterocyst. Heterocyst can be at the tip of trichome, i.e., terminal (*Cylindrospermum*), intercalary (*Anabaena*) or metameric, i.e., roughly at regular distances from one another, or in pairs (*Anabaenopsis*). During development, the thylakoid apparatus degrades and specific DNA rearrangements (e.g., *nif* genes) occur.

Nif Gene

The atmospheric nitrogen fixation is an energy intensive process. If there is no nitrogen fixation by cell all the enzymes must be tightly controlled and not to fix nitrogen at any cost. So the genetic control of nitrogen fixation in bacteria must have turn off and turn on mechanism whenever required. These are a specific gene working for nitrogen fixation in cells. The nif gene codes proteins that are actually able to fix atmospheric nitrogen. It is also found in few nitrogen fixing bacteria (free-living nitrogen fixing bacteria and in symbiotic bacteria) and cyanobacteria. The nif genes are genes encoding enzymes involved in the fixation of atmospheric nitrogen along with this it also encodes few regulatory proteins involved in nitrogen fixation. Primarily nif genes encode nitrogenase complex, which can convert atmospheric nitrogen to ammonia like nitrogen forms.

(continued)

Nitrogen fixation is regulated by nif regulon, which has both positive and negative regulators and a set of seven operons which includes 17 nif genes. Nif genes have both positive and negative regulators. Some nif genes are: Nif A,D, L,K, F,H,S,U,Y,W,Z.

Nitrogen sensitive NifA protein activates nif genes transcription. NtrC, which is an RNA polymerase is used by plants when there is not enough fixed amount of nitrogen factor, and it triggers NifA's expression. Further NifA activates the transcription for the nif genes. NifL protein gets activated whenever there is sufficient amount of reduced nitrogen or oxygen present in the cell. In such situation NifL inhibits activity of NifA activity that causes inhibition of nitrogenase formation in cell. Then NifL is regulated by other proteins, and this protein is sensitive for O_2 and ammonium concentration of the surrounding environment. The nif genes are also present in bacterial cells and are associated with plasmids along with few other genes responsible for nitrogen fixation in bacterial cell.

Nitrogenase Nitrogenase term was given by Burk 1934 for the enzyme that catalyses the conversion of atmospheric unavailable nitrogen to a bio-accessible nitrogen (Burk et al. 1934). Nitrogenase is playing very vital and meaningful role in heterocyst. Nitrogenase is two soluble protein components: (1) Component I or Mo-Fe protein also known as dinitrogenase consists of 2 Mo atoms, 28–34 Fe atoms, and 26–28 acid-labile sulphides, also known as iron-molybdenum cofactor (FeMoco). Component I has two copies each of two subunits α and β (Fig. 24.4). (2) Component II or electron-transfer Fe protein, this unit is known as dinitrogenase reductase. Component II contains two copies of a single subunit (Fig. 24.4). This protein has four non-heme Fe atoms and four acid-labile sulphides (4Fe-4S). A reducing source and catalysis MgATP is required. Substrate binding and reduction takes place on component I, which binds to ATP and ferredoxin or flavodoxin proteins (Fdx or Fld). The hydrolysis of ATP supplies the energy for the reaction, while the Fdx/Fld proteins supply the electrons and this reduction reaction electron must be added to

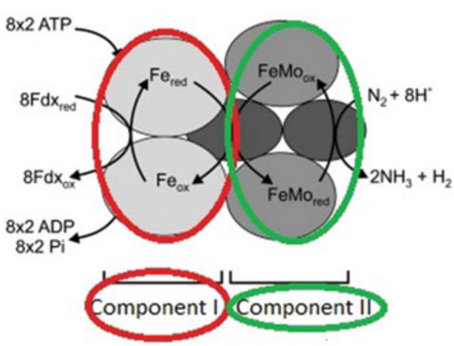

Fig. 24.4 Structure of nitrogenase enzyme components I dinitrogenase and component II dinitrogenase reductase

the nitrogen (N_2) to reduce it to NH_4. In short component II simply supplies electrons to component I, one at a time. ATP is not hydrolysed to ADP until component II transfers an electron to component I. 21–25 ATPs are required for each N_2 to be fixed.

Damage Control Mechanism of Nitrogenase

Enzyme nitrogenase is oxygen and cold sensitive, so cannot perform functions in the presence of O_2. Photosystem II is also absent in heterocysts, which is responsible for water photolysis and generates oxygen, this is the reason why PSII is absent. PSI helps to generate ATP, i.e., assimilatory power helps in nitrogen fixation. Absence of PSII maintains oxygen free environment and hydrogen rich inside the cell and nitrogenase acts perfectly in this condition. Oxidase enzymes is a very importanat enzymes and it is found in heterocystous cells, by chance if oxygen molecules enters inside heterocyst through polar plug, It converts it into water molecule as hygrogen gas is already in cells. Due to this it mainatins a reducing and non-oxygenating environment within heterocystous cells.

Mechanism of Protection of Nitrogenase

Blue green algae is having very important enzymes that helps in nitrogen fixation within algala cell. This enzyme is Nitrogenase and it is sensitive to oxygen. As it is oxygen sensitive so blue green algae have evolved different mechanism of protection. Among them first one is the presence of thick mucilaginous coating around blue green algae and it protects algal cells from oxygen diffusion. Other important modification is that PS II is absent and there is no oxygen evolved during photosynthesis. Heterocyst cells act as a separate compartment. Respiratory activity is higher in heterocyst as compared to vegetative cells. Oxygen from air binds to special glycolipids found in laminated layer of heterocyst envelope. In case of Gloeothece an intensive internal protective compartment is found. The cells manage to perform little photosynthesis, reserve of fixed nitrogen, and later more photosynthesis without nitrogen fixation activity. This mechanism generates an intensive system of internal membranes which actually represent intracellular protective compartment. In case of *Trichodesmium* (Carpenter and Price 1976), it filament forms a bundle like structure and outsider filaments perform photosynthesis while the filaments placed at inner side perform nitrogen fixation.

Under nitrogen fixing conditions hydrogenase produces along with nitrogenase and it helps in formation of H_2 in blue green algae. Hydrogen formation stimulated in light and proceeds in dark, and low concentration of oxygen present for respiration. ATP-dependent H_3O^+-reduction catalysed by nitrogenase enzymes and finally production of Hydrogen occurs. This hydrogen is used by cell in two different ways and both ways were catalysed by hydrogenases. First it is used as oxygen dependent reaction in respiratory chain and helps in supply of extra ATP production. Second it may be used in light-requiring reaction.

24.6 Significance of Algal Nitrogen Fixation

Globally fixed nitrogen ultimately affects the productivity of the major ecosystems, especially agriculture fields are highly affected. Throughout world there are different regions and variety of cyanophycean algae is able to fix atmospheric nitrogen. These regions are different due to climatic conditions and difference in soil texture. It has been observed that in temperate soil heterocystous blue green algae increases nitrogen content in soil. Reports show that blue green algae fixes nitrogen up to 51 or 94 kg N/ha per year. In cold dominant ecosystem, blue green algae fixes atmospheric nitrogen in the form of Lichens and algal moss association, while few free-living algae were also reported. Nostoc commune is the most common specie reported in tundra and Antarctic soils either as free-living or as photobiont in lichens. Blue green algae are widely found in tropical and temperate region but most abundant in tropics, highly active in submerged soil. In weed free maize fields *Cylindrospermum licheniforme* grows successfully and fix atmospheric nitrogen. In freshwater environment nitrogen fixation is very critical due to uneven distribution of algae in water (Fogg 1949), while in marine system blue green algae are less common. Trichodesmium (Oscillatoriaceae) forms large aggregated biomass in the form of bundles of filaments. These aggregates are able to fix nitrogen and can develop large biomass and fix N_2 (Fogg 1949). Few species of Calothrix were found to colonize large areas of sand (P. Roger unpub.). *Anabaena cylindrica* was isolated from an aquatic habitat and there is no report of its presence in freshwater. Anabaena few species are found in freshwater and considered as important nitrogen fixer. In modern agriculture system cyanobacteria (BGA) is widely used as biofertilizer.

24.7 Conclusion

Algae play important roles in many fields like medicine, food, agriculture, and in biological research. Cyanobacteria are ubiquitous in the world soils and are primary photosynthetic agents of the soil, play important role in soil ecology like soil fertility and reclamation. Cyanobacteria have special feature of nitrogen fixation and make them an important biofertilizer. As the continuous use of chemical fertilizer leads to soil health degradation so cyanophycean algae is a boom for soil and environment. Cyanobacterial (BGA) biofertilizers lead to soil enrichment and are compatible with long-term sustainability. Most interestingly it is eco-friendly and not at all dangerous to the environment. Other important feature is its adaptation for extreme environments.

References

Bergman B, Gallon JR, Rai AN, Stal LJ (1997) N_2 fixation by non-heterocystous cyanobacteria. FEMS Microbiol Rev 19:139–185

Bray CM (1983) Nitrogen metabolism in plants. Longman Group Ltd, Harlow

Burk D, Lineweaver H, Horner CKJ (1934) The specific influence of acidity on the mechanism of nitrogen fixation by azotobacter. Bacteriology 27:325

Canfield DE, Glazer AN, Falkowski PG (2010) The evolution and future of earth's nitrogen cycle. Science 330:192

Carpenter EJ, Price CC (1976) Marine Oscillatoria (Trichodesmium): explanation for aerobic nitrogen fixation without heterocysts. Science 191(4233):1278–1280

Castenholz RW (2001) Cyanobacteria. In: Boone DR, Castenholz RW (eds) Bergey's manual of systematic bacteriology, vol 1, 2nd edn. Springer, New York, pp 473–487

Castenholz RW, Waterbury JB (1989) Group I. Cyanobacteria. In: Krieg NR, Holt JG (eds) Bergey's manual of systematic bacteriology, vol 3. Williams & Wilkins, Baltimore, pp 1710–1728

Cheng QJ (2008) Perspectives in biological nitrogen fixation resesarch. Integr Plant Biol 50:786

Dos Santos PC, Fang Z, Mason SW, Setubal JC (2012) Dixon R. Distribution of nitrogen fixation and nitrogenase-like sequences amongst microbial genomes. BMC Genomics 13:162

Fay P, Fogg GE (1962) Studies on nitrogen fixation by blue-green algae. III. Growth and nitrogen fixation in Chlorogloea fritschii Mitra. Arch Mikrobiol 42:310–321

Ferguson S (1998) Nitrogen cycle enzymology. J Curr Opin Chem Biol 2:182

Fleming H, Haselkorn R (1973) Differentiation in Nostoc muscorum: nitrogenase is synthesized in heterocysts. Proc Natl Acad Sci U S A 70:2727–2731

Fogg GE (1949) Growth and heterocyst production in Anabaena cylindrica Lemm. II. In relation to carbon and nitrogen metabolism. Ann Bot 13:241–259

Gruber N, Galloway JN (2008) An earth-system perspective of the global nitrogen cycle. Nature 451:293

Haber F (1923) Bemerkung zu vorstehender Notiz. Naturwissenschaften 11:339–340

Haber F (1992) About the representation of ammonia from nitrogen and hydrogen. Nat Sci 10:1041

Issa AA, Alla MHA, Ohyama T (1997) Nitrogen fixing cyanobacteria: future prospects, 2014. In: Advances in biology and ecology of nitrogen fixation. InTech Open, London

Jia H-P, Quadrelli E (2014) Mechanistic aspects of dinitrogen cleavage and hydrogenation to produce ammonia in catalysis and organometallic chemistry: relevance of metal hydride bonds and dihydrogen. Chem Soc Rev 43:547

Lex M (1970) Nitrogenase activity in the blue-green alga *Plectonema boryanum* strain 594. Arch Mikrobiol 73:250–260

Li S, Ley SH, Qianlin W (1983) Nitrogen fixing blue-green algae, a source of biofertilizer. In: Tseng CK (ed) Proc of the joint China-US phycology symposium. Science Press, Beijing, p 479

MacKay BA (2004) Dinitrogen coordination chemistry: on the biomimetic borderlands. Chem Rev 104:385

McGlynn SE, Boyd ES, Peters JW, Orphan VJ (2013) Classifying the metal dependence of uncharacterized nitrogenases. Front Microbiol 3:419

Peterson RB, Wolk CP (1978a) Localization of an uptake hydrogenase in Anabaena. Physiology 61:49

Peterson RB, Wolk CP (1978b) High recovery of nitrogenase activity and of 55Fe-labeled nitrogenase in heterocysts isolated from Anabaena variabilis. Proc Natl Acad Sci U S A 75:6271–6275

Rippka R, Stanier RY (1978) The effect of anaerobiosis on nitrogenase synthesis and heterocyst development by nostocacean cyanobacteria. J Gen Microbiol 105:83–94

Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY (1979) Generic assignments, strain histories, and properties of pure cultures of cyanobacteria. J Gen Microbial 111:l61

Rodrigo V, Novelo E (2007) Seasonal changes in periphyton nitrogen fixation in a protected tropical wetland. Biol Fertil Soils 43:367–372

Singh RN (1961) Role of blue-green algae in nitrogen economy. In Indian Council Agric. Res. New Delhi, India

Smil V (2004) Enriching the earth. In: Haber F, Bosch C (eds) The transformation of world food production. MIT Press, Cambridge

Smith RV, Evans MCW (1971) Nitrogenase activity in cell free extracts of the blue-green alga Anabaena cylindrica. J Bacteriol 105:913–917

Stewart WDP, Haystead A, Pearson HW (1969) Nitrogenase activity in heterocysts of blue-green algae. Nature 224:226–228

Stewart WDP, Rowell P, Rai AN (1980) Symbiotic nitrogen-fixing cyanobacteria. In: Stewart WDP, Gallon JR (eds) Nitrogen fixation. Academic, London, pp 239–277

Thamdrup B (2012) New pathways and processes in global nitrogen cycle. Annu Rev Ecol Evol Syst 43:407

Wyatt JT, Silvey JKGS (1969) Nitrogen fixation by Gloeocapsa. Science 165:908–909