The blue-green algae show a degree of development which is unparalleled among prokaryotic organisms. Some filamentous blue-green algae belonging to orders Nostocales and Stigonematales show cellular differentiation in one dimensional pattern. Two kinds of differentiated cells namely the akinetes (spores) and heterocysts, are produced. The transformation of vegetative cells into akinetes in blue-green algae is a morphogenetic process comparable to the phenomenon present in all other multicellular organisms.

Reproduction in blue-green algae is mainly of vegetative type although genetic recombination (Kumar, 1962; Singh and Sinha, 1965; Singh et al., 1966; Singh, 1967; Bazin, 1968) including conjugation (Kumar and Ueda, 1984), transformation (Shestakov and Khyen, 1970) and transduction (Singh and Singh, 1972a) have been reported. Different types of reproductive structures such as resting spores, hormogonia, hormocysts, exospores, endospores, nannocytes and planococci are found in blue-green algae (Table A). Fogg et al. (1973)
<table>
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<tr>
<th>Reproductive structure</th>
<th>Cell wall</th>
<th>Cell envelope</th>
<th>Orders*</th>
<th>Examples</th>
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<td>Akinetes</td>
<td>Parental</td>
<td>Present</td>
<td>Nostocales</td>
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<td>Stigonematales</td>
<td>Hapalosiphon</td>
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*Names of the orders of blue-green algae are adopted from the classification given by Fritsch (1943).
considered the exospores, endospores, nannocytes and planocci as the units of reproduction rather than perennating structures. Resting spores are known as akinetes (Wille, 1887). They are found in Nostocaceae, Rivulariaceae and in some members of Stigonemataceae and they represent usual structures of perennation.

Akinetes in blue-green algae were first observed by Réaumur (1757) and subsequent reports were made by Vaucher (1803), Thuret (1844 and 1857), Carter (1856), De Bary (1863), Rabenhorst (1864), Janczewski (1874), Bornet and Thuret (1880), Sauvageau (1892 and 1897), Beck v. Mannagetta (1898) and Brand (1903).

Structure

Akinetes, under the light microscope are readily distinguishable by their large size, shape, thick wall and modified pigmentation. The development of akinete involves the entire cell including the cell wall during its formation and generally akinetes are termed as spores in case of blue-green algae. However, the formation of other types of spores (endospores and exospores) is clearly distinguishable by the development of new membrane discarding that of the parent (cf. Fritsch, 1935).

During akinete formation, the vegetative cell enlarges and granules appear inside the cell and thick spore coat
develops around the cell. Akinete envelope is continuous around the cell and thus separates it from adjacent cells (cf. Fritsch, 1905). It is in the form of firm envelope usually differentiated into inner investment and cell-sheath. The outer envelope is thick and generally pigmented, and occasionally shows ornamentations such as fibrillar structures in case of akinetes of Cylindrospermum trichotosporum. The akinete envelope sometimes contains more than one layer formed presumably by condensation of the gelatinous sheath of the trichome (cf. Fritsch, 1945).

Many studies were performed employing electron microscope to discern the internal structure of akinetes (Ris and Singh, 1961; Wildon and Mercer, 1963; Leak and Wilson, 1965; Miller and Lang, 1968; Clark and Jensen, 1969; Jensen and Clark, 1969; Wildman et al., 1975; Sutherland et al., 1979 and 1985; Grilli Caiola and de Vecchi, 1980; Grilli Caiola and Favali, 1982a and b). In Cylindrospermum sp (Miller and Lang, 1968; Clark and Jensen, 1969; Jensen and Clark, 1969) and Nostoc 7524 (Sutherland et al., 1979), during akinete formation, enlargement of the vegetative cell was accompanied by condensation of the mucilaginous sheath and deposition of dense fibrillar layer between the condensed sheath and outer layer of the cell wall. The development of fibrous coat was followed by the deposition of electron dense substance within the coat. In both
the organisms, separation of fibrous coat from the cell wall by an electron transparent layer occurred during final stages of akinete development. Further, thickness of intermediate layer of cell wall increased during akinete formation. Regarding the ultrastructure of akinetes of *Cylindrospermum* sp, though many of the findings of Miller and Lang (1968) and Clark and Jensen (1969) and Jensen and Clark (1969) are similar, certain differences are evident pertaining to the development of fibrous coat and the developmental stage at which it separated from akinete cell wall. Miller and Lang (1968) suggested that the fibrous coat resulted from the condensation of mucilagenous sheath of the vegetative cell while Clark and Jensen (1969) and Jensen and Clark (1969) disagreed and suggested that new synthesis occurred. Further, Miller and Lang (1968) suggested that the separation of the cell wall from the akinete coat occurred just before the germination whereas Clark and Jensen (1969) observed that the separation of the fibrous coat from the cell wall by an electron transparent layer took place at an advanced stage during the akinete development itself. According to Sutherland et al. (1979), structural changes during akinete differentiation in *Nostoc 7524* were similar to that in *Cylindrospermum* sp and the findings were more in agreement with the observations of Clark and Jensen (1969) and Jensen and Clark (1969) rather than the views of Miller and Lang (1968). Similar to that in the akinetes of
Cylindrospermum sp and Nostoc 7524, an elaborate multilayered akinete envelope was also found in Anabaena cylindrica (Wildon and Mercer, 1963; Leak and Wilson, 1965), Anabaena variabilis (Braune, 1980) and Nostoc sp (isolated from cycad coralloid roots; Grilli Caiola and de Vecchi, 1980). On the contrary, the envelope of akinetes of Aphanizomenon flos-aquae consists of only two investments; an inner investment enclosing the cell and an outer investment comprising a thin layer of condensed sheath (Wildman et al., 1975).

In akinetes, unlike in vegetative cells, the centroplasm is rather ill-defined and the chromatic substance is less distinct (cf. Fritsch, 1945). However, the granular inclusions are abundant. A marked accumulation of cyanophycin granules was noticed in akinetes (cf. Fritsch, 1945) while polyphosphate granules disappeared (Talpasayi, 1963; Reddy, 1976 and 1983a).

Electron microscopic studies revealed that akinete formation is accompanied by increase in compactness of protoplasts, condensation of photosynthetic thylakoids with decreased intrathylakoidal spaces, disappearance of gas vesicles, increase in number of cyanophycin granules, ribosomes and polyglucon granules and decrease in number of lipid droplets (Ris and Singh, 1961; Clark and Jensen, 1969; Jensen and Clark, 1969). Polyhedral bodies are also retained in akinetes.
During the akinete development from the vegetative cells there was an increase in the cell size and the number of cyanophycin granules, glycogen granules and ribosomes. By contrast, Miller and Lang (1968) did not detect any ribosomes in *Cylindrospermum* akinetes. Lipid granules became less and gas vesicles, common in vegetative cells, were gradually lost. However, Sutherland et al. (1979) did not find any change in polyhedral bodies, lipid granules or polyglucoson granules during akinete differentiation from vegetative cells, although there was an increase in cyanophycin granule and glycogen content.

*Nostoc*, isolated from cycas root nodules and cultured in free living conditions, showed two types of morphologically distinct akinetes (Grilli Caiola and de Vecchi, 1980). They were: (a) akinetes with a cytoplasm similar to vegetative cells with thylakoids throughout the whole cell, numerous ribosomes, polyglucoside bodies, small cyanophycin granules and lipid droplets. They differentiated either free in the medium or in chains and were suggested to represent a stage of vegetative cells which have temporary growth difficulties and which can germinate immediately. (b) Akinetes with a cytoplasm containing large and numerous cyanophycin granules, ribosomes, few polyhedral bodies and a large nucleoplasmic area. The thylakoids were peripheral and concentric or branched. These akinetes differentiated within
the filaments and represented mature akinetes capable of survival for a long time under unfavourable conditions.

Chemical composition

The sheath and envelope of akinete of *Cylindrospermum* sp stain readily with ruthenium red and are hence considered to be composed of pectinaceous substance (Miller and Lang, 1968). It was also shown that the dense fibrillar layer did not stain with zinc-chlor-iodide, IKI or phloroglucinol suggesting the absence of cellulose or chitin, but did stain with toluidine-blue indicating the presence of poly-glucoside. Lipids are also absent in fibrillar layer. Dunn and Wolk (1970) analysed the chemical composition of wall fraction of akinetes of *Anabaena cylindrica* and reported 41% carbohydrate, 24% amino compounds, 11% lipid, 2% ash and 2% moisture on dry weight basis. They have also reported that the carbohydrate moieties of akinete wall i.e., glucose, mannose, galactose and xylose are present in the ratio of 76:17:3:4 (cf. vegetative cells 35:50:5:8). The akinete envelope polysaccharide is not only different from that of the vegetative cells but also from the sheath where the ratio of glucose, mannose, galactose and xylose was 47:25:6:21 and which was water soluble in contrast to the akinete polysaccharides which were highly insoluble in water. Thus, these findings lend credence to the views of Clark and Jensen (1969) and Jensen and Clark (1969)
that the new envelope of akinetes is not derived from the sheath but new synthesis is required (Rai et al., 1985). Envelope polysaccharides from akinetes and heterocysts contain about 40% terminal sugars (Cardemil et al., 1974; Cardemil and Wolk, 1976 and 1981a,b). It was found that the percentages of sugars (glucose and mannose) and linkages found in the polysaccharides of the two cell types are essentially identical (Cardemil and Wolk, 1979). The high carbohydrate content and low amino content, similar sugar ratios and identical repeated sequences of sugars in the polysaccharides of heterocysts and akinetes led Cardemil and Wolk (1979) to suggest that there may be identical processes leading to the biosynthesis of heterocyst and akinete polysaccharides.

Fritsch (1945) reported that the granules which do not stain with toluidine-blue or methylene-blue but which stain with carmine or neutral red are cyanophycin granules. Fogg (1951) used the Sakaguchi test and demonstrated that cyanophycin granules contain arginine, an amino acid which occurs in blue-green algal proteins. The cyanophycin granules are absent in young vegetative cells but are present in aged cells (Tischer, 1957). They are abundant in akinetes (Miller and Lang, 1968; Lang and Fisher, 1969). Sutherland et al. (1979) reported 8-fold increase in cyanophycin granule content per cell. The chemical analysis
of isolated cyanophycin granules of *Anabaena cylindrica* by Simon (1971) did not reveal any pigments, lipids or significant quantities of carbohydrates but showed an unusual type of protein consisting of only arginine and aspartic acid amounting to 98% of the dry weight of the granules. The cyanophycin granules serve as reserve materials (cf. Fritsch, 1945). Simon (1971) suggested that these granules serve as a store of combined nitrogen source.

Akinetes retain the polyhedral bodies during formation (Miller and Lang, 1968; Clark and Jensen, 1969; Sutherland et al., 1979) but their function is not known. Edwards et al. (1968) opined that these bodies play a role in storage of ribonucleoprotein in vegetative cells. However, Stewart and Codd (1975) suggested that the polyhedral bodies may contain ribulose 1,5-diphosphate carboxylase (RUDPCase) like carboxysomes of the chemoautotrophic bacterium, *Thiobacillus* because the polyhedral bodies and carboxysomes are structurally similar (Shively, 1974).

There is a dearth of information of lipid composition of akinetes. Yamamoto (1972) reported that in *Anabaena cylindrica* lipid and fatty acid contents of akinetes decreased by about 17% and 28%, respectively, as compared to vegetative cells. It was also found that akinetes contain much less α-linolic acid. Furthermore, the akinetes contained fatty acids with less unsaturation as compared to vegetative
cells. Reddy and Talpasayi (1980) reported that akinetes of *Anabaena fertilissima* and *Anabaenopsis arnoldii* contain an unknown glycolipid which is absent in vegetative cells. A similar glycolipid was detected in laminated layer of heterocyst envelope and suggested to be the main component responsible for resistance of heterocyst cell wall to external adverse conditions (Winkenbach et al., 1972). Among four lipid components associated with photosynthetic lamellar system, only monogalactosyl diglyceride and sulphoquinovosyl diglyceride were found to be present in akinetes while digalactosyl diglyceride and phosphatidyl glycerol were absent (Reddy, 1983b).

Electron microscopic studies have shown that photosynthetic lamellar system is retained in akinetes. In vivo absorption spectra of akinetes and vegetative cells of *Anabaena cylindrica* have shown that pigment composition is similar in both the cell types (Wolk and Simon, 1969). However, subsequent studies have shown that akinete formation is accompanied by changes in the composition of photosynthetic pigments. Fay (1969a) and Reddy (1976) working with *Anabaena cylindrica* and *Anabaena fertilissima*, respectively, found that the contents of xanthophylls (myxoxanthophyll and echinenone) increased and those of $\beta$-carotene, chlorophyll-a and phycocyanin decreased in akinetes as compared to vegetative cells. On the other hand, in *Anabaena*
fertilissima, unlike in Anabaena cylindrica, the total carotenoid content of akinetes was found to be more (Reddy, 1976). Fay (1969a) reported that in akinetes, chlorophyll-a was largely replaced by phaeophytin. In akinetes of Nostoc 7524, the chlorophyll-a content as a percent of dry weight remained constant but doubled per cell (Sutherland et al., 1979). Sutherland et al. (1979) also showed that, on a per cell basis, the phycocyanin content of akinetes of Nostoc 7524 was the same as vegetative cells, although as a percentage of dry weight it had decreased similar to other blue-green algae (Fay, 1969a; Reddy, 1976). The akinetes of Anabaena fertilissima were found to contain, in contrast to low amount of phycocyanin, higher quantity of phycoerythrin (Reddy, 1976; Reddy and Talpasayi, 1976) as compared to vegetative cells. Further, it was observed that phycoerythrin is transformed from C-type in vegetative cells to R-type in akinetes (Reddy, 1983c). Immunological studies showed that the apparent disappearance/reduction in the amount of allophycocyanin/phycocyanin in akinetes is mainly due to the loss of chromophores (bilins) rather than due to the loss of apoproteins (Reddy, 1983c). Björn et al. (1983) reported the presence of allophycocyanin-B in akinetes of Anabaena variabilis. Contrary to the above results, akinetes of Anabaena diliolum were found to be totally lacking in chlorophyll, phycocyanin and phycoerythrin (Rao et al., 1984). It is apparent from the above reports
that at least in the case of Anabaena, akinete formation is accompanied by reduction/disappearance of photosynthetic pigments. However, some of the conflicting reports from various workers may be due to differences in the akinetes of different genera and species, and due to the presence of akinetes belonging to different age groups in the akinete preparations.

Photochromic pigment analysis of akinetes of Anabaena variabilis revealed the presence of phycochromes b and d, possible photoreceptors for initiating germination processes, in them (Björn et al., 1983).

Nucleic acid analysis of akinetes of different blue-green algae showed large variations in DNA and RNA contents. DNA content of akinetes was found to be more in Anabaena baltica (Ueda, 1971) and Cylindrospermum sp (Ueda and Sawada, 1972) than in vegetative cells while it remained approximately equal in both the cell types in Anabaena cylindrica (Simon, 1977a), and Mostoc 7524 (Sutherland et al., 1979). It was also reported that RNA:DNA ratio increased from 13 in vegetative cell to 54 in akinete of Anabaena cylindrica (Simon, 1977a) whereas the ratio did not change and was found to be approximately 20 in vegetative cells as well as in akinetes of Mostoc 7524 (Sutherland et al., 1979). It is not clear whether such variations in nucleic acid contents is a reflection of
true inter-species variation, or difference in the methods employed to estimate DNA and RNA (Nichols and Adams, 1982). However, it should be mentioned here that the relative concentration of DNA and RNA depends on the stage of growth of the organism (Leach et al., 1971; Mann and Carr, 1974). Further, it was evidenced that the development of akinetes in *Aphanizomenon flos-aquae* was accompanied by an increase in ribosome and polyribosome content (Wildman et al., 1975) and as such one should expect at least an increase in the RNA level.

Proteins of akinetes of *Anabaena fertilissima* when analysed by polyacrylamide gel electrophoresis showed certain differences in protein profiles on gels compared to those of vegetative cells (Reddy, 1976; Reddy and Talpasayi, 1979). Immunological studies clearly established the presence of at least one protein component specific to akinetes of *Anabaena fertilissima* (Reddy and Talpasayi, 1978). Protein content of akinetes was found to be three-fold higher than in vegetative cells in *Anabaena cylindrica* (Simon, 1977a).

Nitrogen content of akinetes of *Anabaena cylindrica* was less (4.8%) as compared to vegetative cells (7.7%) (Fay, 1969b). In spite of the presence of large number of cyanophycin granules in akinetes which are rich in nitrogen content as arginine and aspartic acid (Fogg, 1951 and Simon, 1971), the low total nitrogen content indicates that akinete
development is accompanied by general reduction in nitrogenous components. One of the reasons for such a reduction may be due to decrease in the amounts of amino compounds in wall fraction of akinetes (Dunn and Wolk, 1970). In Nostoc 7524, on per cell basis, nitrogen content in akinetes increased by 17% (Sutherland et al., 1979).

Glycogen accumulation in cells undergoing akinete formation has been demonstrated in Anabaena sp (Sarma and Kanta, 1979, 1980; Kanta and Sarma, 1980) and Nostoc 7524 (Sutherland et al., 1979), and it was suggested that it serves as an endogenous carbon reserve.

Metabolism

Information on metabolic activities of akinetes is limited because of the difficulty in obtaining akinetes of homogeneous age (Nichols and Adams, 1982).

Fay (1969b) studied the metabolic activities of isolated akinetes of Anabaena cylindrica. During the formation of akinetes, photosynthesis continues at a gradually decreasing rate which results in several fold increase in dry weight probably due to the increase in reserve materials. The low rate of photosynthesis or its complete absence in akinetes was suggested to be due to elimination of phycocyanin (Fay, 1969a) and digalactosyl diglyceride
(Reddy, 1983b) in these cells. Nitrogenase activity was also found to be absent in akinetes (Fay, 1969b). Further, it was shown that the akinetes of *Anabaena cylindrica* evolve carbon dioxide at a higher rate in dark. It was suggested that increased rate of respiration might be due to enhanced enzymatic activity which occurs in developing and germinating akinetes rather than in mature akinetes.

Thiel and Wolk (1983) have shown that the akinetes of *Nostoc spongiaeforme* are metabolically active performing protein synthesis, respiration and photosynthesis. These akinetes have been shown to incorporate $^{35}$S from Na$_2$$^{35}$SO$_4$ into protein and lipids and to evolve and consume oxygen at a rate, approximately, 7% of that found in vegetative cells. The oxygen evolution process was DCMU-sensitive. Thus, although the reduced rate of photosynthetic oxygen evolution by *Nostoc spongiaeforme* is consistent with the findings of Fay (1969b) on *Anabaena cylindrica* akinetes, the reduced rate of respiratory oxygen consumption reported by Thiel and Wolk (1983) contradicts Fay's (1969b) observations. This could be due to different metabolic nature of these akinetes, isolated from different algae. However, it should be noted that in cultures where akinete development occurs in a non-synchronous manner, some isolated akinetes may be entering the germination stage (Rai et al., 1985). In fact, Fay (1969b) suggested that the increase in endogenous
respiration may have been due to reorganization of akinete enzymatic activities prior to germination.

The difficulty in obtaining preparations of pure akinetes of homogenous age has probably restricted our knowledge of akinete metabolism. However, studies with *Nostoc* 7524 (Sutherland et al., 1979) and *Anabaena variabilis* (Braune, 1980) have shown that akinete differentiation can be synchronized and this should greatly facilitate metabolic studies of both resting and germinating akinetes. Further, various methods have been described for preparing akinetes free from vegetative cells (Fay 1969a,b; Reddy, 1976; Braune, 1979; Sutherland et al., 1979; Chauvat and Joset-Espardellier, 1981; Thiel and Wolk, 1983).

Rao et al. (1984), using akinetes of *Anabaena doliolum*, have studied photosynthesis and respiration. The respiration rate in the akinetes was 48 nmol O₂ consumed min⁻¹ mg⁻¹ protein while that for the vegetative cells was 79 nmol O₂ consumed min⁻¹ mg⁻¹ protein. Photosynthetic oxygen evolution was totally absent in akinetes whereas vegetative filaments showed a photosynthetic oxygen evolution rate of 343 nmol O₂ evolved min⁻¹ mg⁻¹ protein. The finding that respiratory activity is reduced in akinetes is consistent with the reports of Chauvat et al. (1982) for *Nostoc* 7524 and of Thiel and Wolk (1983) for *Nostoc spongiaeforme*
akinetes. However, Chauvat et al. (1982) and Thiel and Wolk (1983) reported low (7 and 10%, respectively) rates of photosynthetic oxygen evolution which may reflect differences in the metabolism of akinetes of *Anabaena doliolum* from those of *Nostoc* 7524 and *Nostoc spongiaeformae*.

In *vivo* fluorescence measurements of photosynthetic pigment systems in *Anabaena variabilis* have shown that energy transfer from phycocyanin to allophycocyanin is decoupled in akinetes (Björn et al., 1983). Further, it was also found that the delayed light emission from the pigments was weak in akinetes compared to vegetative cells. From the study it was suggested that akinetes lack an active photosystem II.

Rao et al. (1984) measured activities of various nitrogen metabolizing enzymes alanine dehydrogenase, aspartate dehydrogenase, glutamate dehydrogenase (both NADH and NADPH-dependent), glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, glutamine synthetase (both biosynthetic and transferase), nitrate reductase and nitrogenase in the akinetes of *Anabaena doliolum*. The glutamine synthetase (both transferase and biosynthetic), aspartate dehydrogenase and nitrate reductase and nitrogenase activities were undetectable in akinetes, although whole filaments showed normal activities, suggesting that the enzymes have
been lost during akinete development. Glutamate dehydrogenase, alanine dehydrogenase and glutamate pyruvate transaminase activities of akinetes were, respectively, only 13%, 27% and 36% of that in vegetative filaments. However, glutamate oxaloacetate transaminase activity was similar in both the types of cells. Rao et al. (1984) suggested that glutamine synthetase activity could be used as a marker of akinete development and maturity.

**Factors governing akinete development**

Formation of akinetes is controlled by many factors. In a variety of blue-green algae akinete development occurred after cessation of exponential growth (Fay, 1969b; Roelofs and Oglesby, 1970; Reddy 1976; Simon 1977b; Sutherland et al., 1979; Nichols et al., 1980). In contrast to these findings, akinete development in *Cylindrospermum licheniforme* (Hirosawa and Wolk, 1979a) and *Anabaena torulosa* (Fernandes and Thomas, 1982) was found to occur in exponentially growing cultures. Nichols and Adams (1982) opined that such an akinete development in logarithmically growing cultures might have resulted from a small number of cells which are not growing exponentially.

Nutrient depleted medium was shown to enhance akinete formation in *Cylindrospermum* sp (Glade, 1914). Dilute media (with respect to salt concentration) stimulates rapid akinete
Phosphate concentration in medium is an important factor in regulating akinete formation. A deficiency of phosphate has been reported to induce akinete formation in *Cylindrospermum* sp (Glade, 1914 and Reddy, 1976), *Anabaena cylindrica* (Wolk, 1965), *Aphanizomenon flos-aquae* (Gentile and Maloney, 1969), *Anabaena torulosa* (Fernandes and Thomas, 1982), *Anabaena variabilis* and *Nostoc linckia* (Reddy, 1983d). *Anabaena cylindrica* (Wolk, 1965), *Anabaena variabilis*, *Nostoc linckia* and *Cylindrospermum trichotosporum* (Reddy, 1983d) formed akinettes in the medium devoid of phosphate but only when larger amounts of inoculum was used. Reason for akinete production apparently being dependent upon inoculum quantity is not known and requires further investigation. On the other hand, phosphate starvation did not stimulate akinete production in planktonic blue-green algae (Rother and Fay, 1977) and *Nostoc 7524* (Sutherland et al., 1979), *Anabaena fertilissima* and *Anabaenopsis arnoldii* (Reddy, 1983d). Kaushik et al. (1971) found that the optimum concentration of phosphate in the medium aids in quicker development of akinetes in *Anabaena doliolum* and *Fischereilla muscicola*. They also reported that the concentration of phosphate beyond a particular level inhibits akinete formation. Since polyphosphate granules disappear during akinete development irrespective of akinete formation in the presence
or absence of phosphate in the medium, Reddy (1983a) suggested that loss of polyphosphate from a vegetative cell initiates akinete formation in blue-green algae.

A deficiency of combined nitrogen in the medium has been reported to induce akinete formation (Glade, 1914; Harder, 1917a; Demeter, 1956). This observation gained support from recent findings which showed inhibition of akinete development when nitrate, nitrite or ammonia were supplied (Singh and Srivastava, 1968; Tyagi, 1974 and 1978). On the other hand, induction of akinete formation in Anabaena sp (Canabaeus, 1929), Anabaena fertilissima and Anabaenopsis arnoldii (Reddy, 1976) could take place only when sufficient concentrations of nitrate was maintained in the medium. Studies of Singh (1973) on physiology and genetics of akinete formation in Anabaena doliolium suggest that inorganic nitrogen sources are not only involved in physiological control of akinete formation but also regulate the mutation frequency to loss of akinete formation (non-sporulation) in this blue-green alga.

Many different chemicals were used to study their influence on akinete differentiation. Harder (1917a) and Sutherland \textit{et al.} (1979) reported inhibition of akinete formation by sucrose. Kaushik \textit{et al.} (1971) observed that in Anabaena doliolium akinete formation was inhibited by glucose while Tyagi (1974 and 1978) reported a stimulation
with glucose. Canabaeus (1929) observed that *Anabaena lara* produced akinetes extensively when high concentration (0.8% to 1.2%) of sodium chloride was maintained in the medium. Canabaeus (1929) further reported that in media containing very high concentration of sulphate, almost all the vegetative cells in five *Anabaena* spp examined were transformed into akinetes. Demeter (1956) reported stimulation of akinete development in *Anabaena oscillarioides* Bory when 0.1% sodium glutamate was included in the medium. In *Anabaena cylindrica*, a deficiency of iron increased akinete frequency while deficiencies in other trace ions either decreased their frequency (magnesium and calcium) or had no effect (molebdenum and sulphate) (Sinclair and Whitton, 1977). However, in *Gloeotrichia ghosei*, a deficiency in iron decreased akinete frequency. Elimination of calcium chloride, sodium chloride or magnesium sulphate did not stimulate akinete formation in *Anabaena variabilis* and *Nostoc linckia* (Reddy, 1983d). Wolk (1965) reported that some factors such as the presence of alanylglucose or alanyl-alanine as a buffer, inclusion of sodium acetate and of a relatively high concentration of calcium glucuronate and exposure to an optimal light intensity ensured rapid akinete formation in *Anabaena cylindrica*. Presence of calcium glucuronate in the medium also increased akinete production in *Cylindrospermum licheniforme* (Hirosawa and Wolk, 1979a).
In *Cylindrospermum majus*, it was found that exogenous supply of growth promoting substances, namely, indoleacetic acid, gibberellin and adenine enhanced vegetative growth (Singh et al., 1965). However, sporulation period was hastened by only gibberellin and adenine. On the other hand, 2,4-dichlorophenoxy acetic acid delayed sporulation in *Anabaena doliolum* (Srivastava and Tiwari, 1985).

Amino acids such as tryptophan, Aspartic acid, phenylalanin, proline and isoleucine stimulated akinete production in *Cylindrospermum licheniforme* (Hirosawa and Wolk, 1979a) while amino acid analogues like canavanine and cyanoalanine (analogues of arginine) increased akinete formation in *Anabaena cylindrica* (Nichols et al., 1980).

An extracellular compound excreted into the medium by healthy vegetative cells stimulated akinete production in *Cylindrospermum licheniforme* (Fisher and Wolk, 1976), *Nodularia spumigena* (Pandey and Talpasayi, 1980) and *Anabaena torulosa* (Fernandes and Thomas, 1982). The akinete-stimulating substance from culture-filtrates has been purified and its chemical formula determined to be \( C_7H_5OSN \) (Hirosawa and Wolk, 1979b). Contrary to these findings, culture-filtrates of akinete containing *Nostoc* 7524 (Sutherland et al., 1979) and *Anabaena cylindrica* (Nichols and Adams, 1982) failed to stimulate akinete formation in actively growing cultures.
Increase of pH higher than required for optimum growth resulted early akinete formation in *Nostoc linckia*, *Anabaena variabilis* and *Anabaena fertilissima* (Reddy, 1983d). Both *Nostoc linckia* and *Anabaena variabilis* (which grow better at pH 7.3) formed akinetes faster at pH 7.6 as compared to akinete formation at pH 7.3. Further, *Anabaena fertilissima* (which grows better at pH 7.0) formed akinetes in pH range 7.0 to 10.5 and it was observed that the rapidity of induction of akinetes increased with increase in pH from 7.0 to 8.5 and thereafter it decreased steadily up to 10.5. But *Anabaenopsis arnoldii* did not show any signs of early akinete development when pH was increased in the medium (Reddy, 1983d). Early akinete development at high pH conditions might have resulted due to non-availability of certain chemicals like phosphorus which get gradually precipitated or fixed as pH increases.

Incipient desiccated conditions induced akinete formation in *Nostoc* (Janczewski, 1874). Induction of akinete formation in *Anabaena variabilis* and *Nostoc linckia* was quicker on agar media as compared with liquid media (Reddy, 1983d). Rapid formation of akinetes on agar media was suggested to be due to incipient desiccated conditions prevailing on agar surface than due to higher aeration. This assumption was inferred from the observation on aerated liquid cultures, in which number of akinetes formed for
a similar period was found to be very less. On the other hand, such desiccated conditions did not stimulate rapid akinete development in *Anabaena fertilissima* and *Anabaenopsis arnoldii* (Reddy, 1983d).

Lemmermann (1900) indicated that akinete development in *Aphanizomenon flos-aquae* in the Zwischenahner lake, Germany, was seasonal i.e., during the months of September through January. Smith (1920) found that the akinete formation of this alga in Wisconsin lakes is restricted to winter months. Rose (1934) reported that in Silver lake, Iowa, the akinete development in *Aphanizomenon flos-aquae* occurs in winter as well as in summer. Overwinter is largely responsible for production of akinetes in *Aphanizomenon flos-aquae* in temperate region lakes (Chernousova et al., 1968). However, in a tropical lake such as in Sambhar salt lake in India, development of akinetes in *Anabaenopsis arnoldii* starts by the end of winter (February) while *Anabaena fertilissima* starts forming akinetes in late summer (June to July) (Reddy, 1984a). Under laboratory conditions, akinete formation in *Anabaena variabilis* (Reddy, 1983d) as well as *Anabaena fertilissima* was hastened by higher temperature (35°C) while low temperature (28°C) brought about rapid akinete development in *Anabaenopsis arnoldii* (P.M. Reddy, unpublished data). In *Anabaena torulosa*, higher temperatures (40°C) induced early sporulation (Fernandes and Thomas,
1982). It was suggested that low temperatures are not always essential for the production of akinetes and its influence varies depending on organisms or strains of organisms (Reddy, 1983d).

Light seems to have a considerable influence on akinete formation. In *Nostoc linckia* and *Anabaena variabilis*, low light intensities hastened akinete formation (P.M. Reddy, unpublished data). Further, Reddy also observed that red light was most effective in bringing about early akinete formation while green light exhibited the least influence on akinete development. Since low light intensity as well as red light support only very low rates of photosynthesis (cf. Fogg et al., 1973), the amount of photosynthetic products produced under these circumstances may not be sufficient to support the metabolic activities of the vegetative cells and hence, such a stress condition may result in early akinete development. In *Anabaena cylindrica*, decreasing light intensity resulted in akinete formation at a lower cell density and vice versa (Nichols et al., 1980). Sutherland et al. (1979) found that addition of exogenous sucrose prolonged the exponential growth phase and concurrently delayed akinete differentiation in *Nostoc 7524*. Thus, limitation of energy supply, whether in the form of light or sucrose, suggested to be a major factor in the induction of akinete differentiation (Nichols and
Adams, 1982).

Studies with *Nodularia spumigena* have shown that continuous illumination encourages sporulation better than light-dark cycles (Pandey and Talpasayi, 1980).

Variations in enzyme levels during sporulation in *Anabaena torulosa* was studied and it was found that the activities of catalase, and acid and alkaline phosphatases increased while that of ATPase and glutamine synthetase decreased as the akinetes matured (Sarma and Kanta, 1982).

**Akinete pattern formation**

The distribution of akinetes along the algal filament varies according to species. In algae such as *Gloeotrichia* and *Cylindrospermum*, akinetes always develop adjacent to the basal or terminal heterocysts, respectively. In some other blue-green algae, akinetes may occur singly or in chains which originate next to (*Anabaena cylindrica*) the heterocysts. In certain species all the vegetative cells may develop into akinetes. Based on such observations several workers suggested that heterocysts are involved in akinete development (Carter, 1856; Fritsch, 1904 and 1951; Bharadwaja, 1933; Singh, 1942; Wolk, 1966; Tyagi, 1974). On the contrary, akinetes were also observed to develop in the absence of heterocysts (Eberly, 1966; Hill, 1970; Sutherland et al., 1979) thereby indicating the
non-involvement of heterocysts in their development. It was suggested that though the presence of heterocysts is not required for akinete development, they do maintain spacial regularity of akinetes in filaments (cf. Nichols and Adams, 1982).

Fritsch (1951) suggested that heterocysts when aged may secrete substances which stimulate akinete formation. On the contrary, Wolk (1965 and 1966) proposed that heterocysts in *Anabaena cylindrica* (in which akinetes develop centripetally) exert an inhibitory influence on akinete development and that akinete formation can only commence when this inhibitory effect is diminished. Tyagi (1974) working with *Anabaena doliolum* (in which akinetes develop centrifugally) reported that akinete differentiation is controlled by critical levels of nitrogen fixed by heterocyst. He suggested that nitrogen which is inhibitory to akinete development diffuses from the heterocyst into the vegetative cells in nitrogen-fixing cultures; a concentration gradient of nitrogen develops along the trichome and its endogenous level is presumably minimum at the site of differentiation of the first akinete. However, the fixed nitrogen may not be acting as an inhibitory substance since akinetes could differentiate in non-nitrogen fixing conditions such as in the presence of ammonia or nitrate (Sutherland et al., 1979). Under such non-nitrogen fixing conditions, because
nitrate or ammonia is assimilated equally by all vegetative cells, no gradients of fixed nitrogen should exist (Nichols and Adams, 1982). Nevertheless, spacial regularity of akinetes with respect to position of heterocysts suggests that gradient of some compound is involved in akinete differentiation when heterocysts are present. Stimulation of akinete formation in *Cylindrospermum licheniforme* by hydrogen gas (in the presence of oxygen and in the presence or absence of nitrogen gas) and detection of localized activity of uptake hydrogenase in heterocysts led to the suggestion that akinete formation is controlled by some substance (other than nitrogen) which was reduced by this hydrogenase (Hirosawa and Wolk, 1979a).

Because akinetes in *Anabaena cylindrica* developed in random positions in the presence of canavanine (an analogue of arginine) in addition to the normal position adjacent to heterocysts, Nichols et al. (1980) suggested that the analogue is acting on vegetative cells rather than heterocysts. If the latter was the case only the formation of chains of akinetes adjacent to heterocysts would have taken place. They proposed that under exponential growth conditions some aspect of the intracellular physiology of vegetative cells suppresses their development into akinetes and this inhibition in neighbouring vegetative cells is removed or negated by heterocysts, rather than heterocysts directly stimulate akinete formation.
The tryptophan analogue, 7-azatryptophan, has been shown to induce a change in heterocyst pattern (Mitchison and Wilcox, 1973; Sutherland et al., 1979) as well as akinete pattern (Sutherland et al., 1979) suggesting that the control of heterocyst and akinete spatial patterns may be related (Sutherland et al., 1979).

In *Anabaena doliolum*, since heterocyst formation is sequential, while akinete formation is simultaneous, it was suggested that akinete and heterocyst differentiation are two independent morphogenetic stages and heterocysts play no part either in initiating or control of akinete formation (Singh and Srivastava, 1968).

**Germination**

Réaumur (1757) and Vaucher (1803) were probably the first investigators who described the formation of trichomes from akinetes (graines). Thuret (1857) and De Bary (1863) described the germination of akinetes of *Cylindrospermum* and *Rivularia*, respectively. Later, Janczieszski (1874) gave a detailed account of germination in several *Nostoc* spp.

Akinetes start germinating under favourable conditions thereby implying that the process is triggered by external conditions such as light, temperature, nutrients, etc. During germination the akinetes turn green, elongate and
enlarge. The enlargement is accompanied by the appearance of cytoplasmic granules. Later the germling emerges out of akinete coat.

Fritsch (1945) and Stulp and Stam (1982) described modes of germination in *Anabaena* spp. In some cases the growing protoplast extrudes out through a pore in akinete envelope, while in other the cell division takes place within expanding akinete coat until rapture or dissolution of the coat which results in the liberation of a germling. In general, during the germination a part of akinete envelope gelatinizes and the germling grows out through the gelatinized area as in *Anabaena cylindrica*. However, in case of *Nostoc ellipsosporum*, the entire akinete coat gelatinizes (cf. Fogg et al., 1973). Similar changes in akinete envelope such as pore formation or occurrence of complete gelatinization during emergence of germling were observed in *Cylindrospermum* sp (Miller and Lang, 1968), *Nostoc* sp (Grilli Caiola and de Vecchi, 1980) and *Anabaena variabilis* (Braune, 1980). The germlings of *Anabaena fertilissima* and *Anabaenopsis arnoldii*, either single celled or 2 to 4 celled, emerge out of akinete envelope (Reddy, 1976). However, it is not clear whether they emerge out through a pore or gelatinized part of the akinete coat. Further, the akinete coats in case of *Anabaenopsis arnoldii* remain intact with germlings for a very long time, while those of *Anabaena fertilissima*
disappear by 120 h after germination. The germination of akinetes of *Cylindrospermum trichotosporum* follows a different pattern (Reddy, 1976). In this case, protoplast emerges out through a pore by pushing off a lid-like structure which is the former site of heterocyst attachment. Such a mode of germination was never observed in case of *Anabaena fertilissima* and *Anabaenopsis arnoldii*. It is not yet known about the structural modifications in weak spots in the akinete envelope through which the new filaments emerge out.

In *Nostoc* 7524, during germination, a laminated layer, similar in structure and position to that found in the heterocyst envelope, appeared in the akinete envelope (Sutherland et al., 1985). It is yet to be seen whether such a layer appears during germination of akinetes of other blue-green algae.

During germination, the developing germling gets separated from akinete envelope due to shrinkage or secretion of mucilagenous substance between the wall of germling and envelope or dissolution of inner layers of akinete (Miller and Lang, 1968; Grilli Caiola and de Vecchi, 1980; Braune, 1980). In germinating akinete, cytoplasm becomes densely packed with glycogen granules, lipid droplets and gas vesicles. Further, the thylakoids expand due to fusion of small vesicles while cyanophycin granules gradually

Akinetes of blue-green algae germinate in response to favourable conditions without undergoing any dormancy period. The only exception to this rule seems to be the akinetes of *Aphanizomenon flos-aquae* (Wildman et al., 1975) collected from mud sediments. These akinetes of *Aphanizomenon flos-aquae* which were collected in November failed to germinate immediately in the laboratory when the favourable conditions were provided, but germinated only in May which coincided with germination of akinets in nature.

It was suggested that in nature, akinetes of *Anabaena circinalis* (Reynold, 1972) and *Gloeotrichia echinulata* (Roelof and Oglesby, 1970) germinated in response to increased solar radiation and increased day length which occurs generally between May and July. Reddy (1984a) reported that in Sambhar salt lake, though the germination of akinetes of *Anabaena fertilissima* and *Anabaenopsis arnoldii* takes place in June, it occurs only after monsoon has set in. Hence, it was suggested that the fresh nutrients and changed pH conditions brought about in the lake by inflow of waters during rains also play an important role in the regulation of akinete germination. Thiel and Wolk (1982) reported that the cultures of *Nostoc spongiforme* at sporulation
stage excrete certain substances into the medium which inhibit the germination of akinetes. On the contrary, Rother and Fay (1977) observed in *Anabaena circinalis* and *Aphanizomenon flos-aquae* that akinetes germinate immediately after their maturation.

Exogenous supply of phosphate and nitrate plays an important role in the promotion of germination of blue-green algal akinetes (Reddy, 1976 and 1984b). It was observed that only 30 to 40% of akinetes of *Anabaena fertilissima* and *Anabaenopsis arnoldii* germinated in phosphate or nitrate deficient medium while the remaining akinetes just turned green without showing any further development indicating the initiation of the process of germination only. This type of differential behaviour of akinetes in set conditions, was suggested to be due to their developmental differences, particularly of certain intrinsic differences in the amounts of phosphorus and nitrogen reserves in them. Reddy (1984b) also suggested that the akinetes which contain sufficient amount of reserve materials to meet needs of high rate of metabolic activities during germination (cf. Fay, 1969) complete all the process of germination in nutrient deficient conditions. Further, the initiation or completion of process of germination (in phosphate deficient medium) by akinetes which lacked polyphosphate (Reddy, 1983a) indicated that the phosphorus reserves in akinetes are present in a form
other than polyphosphate material (Reddy, 1984b). Electron microscopic studies revealed that cyanophycin granules (store-houses of reserve nitrogen) are abundant in akinetes. However, based on the results pertaining to the failure of some akinetes to germinate in the medium devoid of nitrate, Reddy (1984b) suggested that the akinetes are either unable to utilize the nitrogen reserve of cyanophycin granules or the reserve nitrogen is insufficient for the completion of all the processes of germination. More biochemical data is required on the nature and content of phosphorus and nitrogen reserves in matured akinetes to understand their exact role in germination.

Among amino acids, carboxylic acids, calcium chloride and potassium iodide, only acetate accelerated the germination of akinetes of *Anabaena cylindrica* to a significant extent (Yamamoto, 1976). Respiratory and photosynthetic rates of both akinetes and vegetative cells were not affected by the addition of acetate and its role on germination was not clear. Glycine, alanine and isoleucine were found to inhibit germination while other compounds tested did not show any effect.

Bai et al. (1981) reported that cytokinins and gibberellic acid promoted germination in *Anabaena azollae* at lower light intensity but they could not completely
In Anabaena cylindrica, germination was about five times greater in aerobic conditions than in the presence of nitrate in anaerobic conditions, indicating obligate requirement for oxygen (Yamamoto, 1976).

The pH of the medium is one of the important factors which governs akinete germination (Reddy, 1976 and 1984a; Yamamoto, 1976). The range of pH for maximum germination was wide in Anabaena fertilissima (pH 7.0 to 10.5; Reddy, 1976) while it was narrow in Anabaenopsis arnoldii (pH 7.0 to 8.5; Reddy, 1976) and Anabaena cylindrica (pH 7.0 to 8.0; Yamamoto, 1976). The pH optimum for germination as well as vegetative growth was found to be same in Anabaena cylindrica (Yamamoto, 1976). On the other hand, Anabaena fertilissima showed good growth at pH 7.0 and akinetes rapidly germinated at pH 8.6 to 10.5, whereas Anabaenopsis arnoldii grew well at pH 8.5 and the akinetes germinated faster at pH 7.0 (Reddy, 1976). Such a differential behaviour in Anabaena fertilissima and Anabaenopsis arnoldii was attributed to their ecological adaptations (Reddy, 1984a). In natural conditions, Anabaenopsis arnoldii is dominant and forms bloom. In the lake, rapid akinete formation and germination in Anabaena fertilissima in highly alkaline conditions may be a strategy adopted by the alga to overcome the competition offered by Anabaenopsis arnoldii and...
continue to exist in the lake from one year to another. It is still not known whether rapid germination of akinetes of various blue-green algae at different pH conditions is due to increased permeability of akinete wall caused by pH changes in the environment or enhancement of the activity of the germinants (stimulants of germination) at optimal pH conditions or existence of different germination systems in akinetes of various algae which have different pH optima for different enzymes involved (Reddy, 1984a).

Akinetes of *Anabaena cylindrica* did not survive when they were pretreated with temperatures higher than 55°C (Yamamoto, 1976), though the ability of akinete to survive below 27°C (culture room temperature) was not tested. In the case of *Anabaena fertilissima*, akinetes resisted high (47°C) and low (0°C) temperature pretreatments effectively (Reddy, 1976, 1983e). Further, pretreatment of akinetes with higher temperature, 37°C and 47°C, stimulated germination in *Anabaena fertilissima*. The resistance/stimulation of germination of akinetes pretreated with higher temperatures suggests the presence of mechanism which confers thermo-resistance/thermostimulation to the akinetes (Dipicolinic acid which offers heat resistance to bacterial spores was found to be absent in the akinetes of blue-green algae; Reddy, 1983e). On the contrary, in *Anabaenopsis arnoldii*, about 95 to 97% akinetes lost viability when subjected
to high as well as low temperature treatments (Reddy, 1983e). However, the akinetes of *Anabaenopsis arnoldii* which were collected during summer (50°C) from the surface of the dried lake sediments, when tested for viability, showed about 50% germination. Reason for the loss of ability of akinetes (of *Anabaenopsis arnoldii*) collected from cultures to survive at elevated temperatures is not known. Löwenstein (1903) found that the blue-green alga *Mastigocladus laminosus*, which grows in nature at 52°C, lost its capacity to survive at such temperatures on prolonged culture at room temperature. It was suggested that the inability to survive at high temperatures is due to impairment of particular biosynthetic system. Huber (1984) reported that akinetes of *Modularia* collected from the sediments of Peel-Harvey Estuary, Western Australia, survived refrigeration at 4°C for several months.

Prolonged desiccation at 27°C promoted loss of viability of akinetes (Reddy, 1976, 1983e) more rapidly than at lower temperatures (Yamamoto, 1975). Further Yamamoto (1975) reported that akinetes of *Anabaena cylindrica* desiccated in dark retain better capacity to germinate than those desiccated in light. Reddy (1983e) found that akinetes formed in natural conditions can survive desiccated conditions better than the akinetes formed in cultures. Although akinetes, unlike vegetative cells, show high resistance to desiccation, it is not yet clearly understood whether their resistance
to desiccation is due to morphological features or physiological properties (Yamamoto, 1975).

Aging of akinetes resulted in gradual loss of viability (Reddy, 1983e). Reddy (1983e) also found that akinetes of \textit{Anabaena fertilissima} retain viability better than the akinetes of \textit{Anabaenopsis arnoldii} when stored for long duration thereby indicating differential capacities of akinetes of different algae to survive prolonged storage. There are reports mentioning the retention of viability by the dried akinetes for prolonged durations from 5 to 107 years (Table B). It seems that akinetes collected from soil retain viability for longer periods than those obtained from cultures.

The influence of light on germination of akinetes has been investigated by Harder (1918) who reported that akinetes of \textit{Nostoc punctiforme} could germinate equally well in red, blue or white lights. Kaushik and Kumar (1970) also obtained similar results with \textit{Anabaena doliolum} and \textit{Fischerella muscicola} and concluded that the akinetes of these blue-green algae required non-photosynthetic light for germination, since green, blue, yellow, red and white lights supported germination, while vegetative growth occurred only in photosynthetic (yellow, red or white) light. However, Reddy et al. (1975) working with \textit{Anabaena fertilissima} and \textit{Anabaenopsis arnoldii} and Yamamoto (1976) with \textit{Anabaena}
Table B. Retention of viability of blue-green algal akinetes for prolonged periods

<table>
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<tr>
<th>BGA</th>
<th>Spores Source</th>
<th>Age (years)</th>
<th>Reference</th>
</tr>
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<tr>
<td>Nostoc commune</td>
<td>Herbarium</td>
<td>107</td>
<td>Cameron and Blank (1966)</td>
</tr>
<tr>
<td>Anabaena</td>
<td>Sediments</td>
<td>64</td>
<td>Livingstone and Jaworski (1980)</td>
</tr>
<tr>
<td>Aphanizomenon</td>
<td>Sediments</td>
<td>18</td>
<td>Livingstone and Jaworski (1980)</td>
</tr>
<tr>
<td>Anabaena cylindrica*</td>
<td>Culture</td>
<td>5</td>
<td>Yamamoto (1975)</td>
</tr>
</tbody>
</table>

*Vegetative cells did not survive more than 10 days when dried.
*cylindrica* found that akinetes germinated only in red, yellow and white lights but not in green and blue lights. Although akinete germination was red light (600-660 nm) dependent, it could take place even in dark when stimulated by brief spells of red illumination thereby suggesting the non-photosynthetic light reaction (Reddy, 1978; Reddy and Talpasayi, 1981). Indeed it was evidenced that two non-photosynthetic pigments, viz. red/far red (Reddy et al., 1975; Reddy and Talpasayi, 1981) and red/green (Reddy, 1984c) light-absorbing photo-reversible pigments, act as photoreceptors in the regulation of akinete germination in *Anabaena fertilissima* and *Anabaenopsis arnoldii*. Pandey and Talpasayi (1981) also evidenced the presence of red/green light-absorbing photoreversible pigment acting as photoreceptor during akinete germination in *Modularia spumigena*. The action of red/far red photoreversible pigment in *Anabaena fertilissima* and *Anabaenopsis arnoldii* is comparable to phytochrome action in higher plants (Reddy et al., 1975; Reddy and Talpasayi, 1981). In *Anabaena variabilis*, although increased dry matter content (which takes place due to photosynthetic activity) of germinating akinetes is not a precondition for outgrowth (Braune and Snake, 1979), C-phycocyanin pigment was shown to be the main photoreceptor in light dependent germination process (Braune, 1979). Braune (1979) suggested that C-phycocyanin might be acting as a photoreceptor for the germination process in two ways.
One was via its role in the energy yielding process of photosystem I (cyclic photophosphorylation), by transferring its absorbed light energy. The other was by a regulative function of the pigment, comparable with phytochrome action in higher plants, there being a close chemical relationship between the phytochrome of plants and the photochromic phycobiliproteins which could be responsible for photo-convertible photomorphogenesis in blue-green algae (Bogorad, 1975). Bai et al. (1985) based on their results pertaining to action spectra of germination in *Anabaena azollae* suggested that stimulation of germination at 385 and 615 nm may be due to absorption of light by photoreceptors such as flavin and phytochrome, respectively. Since DCMU, at a level which reduced carbon incorporation to 5%, only reduced germination by 50%, Braune (1979) concluded that formation of photosynthetic products was not necessary for akinete germination. Akinetes of *Nostoc punctiforme* (Harder, 1918), *Anabaena doliolium*, *Fischerella muscicola* (Kaushik and Kumar, 1970), *Anabaena fertilissima* and *Anabaenopsis arnoldii* (Reddy, 1984c) could germinate equally well under low as well as high light intensities. Harder (1917b and 1918) reported that germination of akinetes of *Nostoc punctiforme* occurred in complete darkness if glucose, sucrose or asparagine were present. In contrast, akinetes of *Anabaena doliolium* and *Fischerella muscicola* were unable to germinate in the
dark, even under heterotrophic conditions (Kaushik and Kumar, 1970). Obligate requirement for light for the germination of akinetes of *Nostoc ellipsosporum* (Ahluwalia and Kumar, 1980) and *Anabaena vaginicola* (Rai and Pandey, 1981) was also reported.

Biochemical aspects of akinete germination have been mainly studied in *Anabaena doliolum* and *Nostoc 7524*. In *Anabaena doliolum*, during the induction of germination, the RNA synthesis started 4 h after transfer of akinetes from dark to light and continued until 20 h while DNA synthesis started after 24 h (Singh and Sunita, 1974). In *Anabaena* sp, RNA and DNA synthesis during akinete germination took place after 30 min and 2 h, respectively (Grilli Caiola and Favali, 1982a,b). Sutherland et al. (1985), on the other hand, reported that in *Nostoc 7524*, RNA synthesis occurred without lag while DNA synthesis commenced only after 80 min. Singh and Sunita (1974) found that protein synthesis during 0 to 4 h was essential for RNA, chlorophyll, phycocyanine and DNA synthesis. Sutherland et al. (1985) observed that cyanophycin in the akinetes of *Nostoc 7524* was degraded after 6 h of commencement of germination. However, it was found that nitrogen reserves were not required for protein synthesis, which was initiated immediately after the induction of germination in *Nostoc 7524*. Rai (1980) reported that in *Anabaena doliolum*, protein synthesized
during the first 24 h of incubation was critical for the initiation of germination.

In *Anabaena doliolum*, during the induction of germination, chlorophyll-a synthesis started after 8 h and phyco-cyanin after 12 h (Singh and Sunita, 1974). Singh and Sunita (1974) also reported that photosynthetic light was not essential for the formation of proteins involved in the synthesis, however, possibility of a photoactivation was not ruled out. During germination of *Nostoc* 7524 akinetes, respiratory and photosynthetic activities increased markedly during the first 9 to 10 h (Chauvat et al., 1982). Resumption of photosynthetic activity in *Nostoc* 7524 required RNA and protein synthesis while respiratory activity developed even in the absence of de novo metabolism. Further, Chauvat et al. (1982) reported that cyclic phosphorylation or photosystem I could not efficiently fulfill the energy requirements of akinete germination. In the presence of photosystem I and respiratory activity, only 21% of the akinetes germinated thereby indicating that the endogenous reserves are inadequate to support the completion of process of germination. Rapid and complete germination was observed when both photosystems operated (Chauvat et al., 1982). Sutherland et al. (1985) observed that in *Nostoc* 7524, the large glycogen reserves of the akinete were not necessary for germination.
since CO₂ fixation commenced immediately after the induction of the process.

Changes in protease activity commencing from germination till sporulation was studied in *Anabaena* SP 310 (Reddy and Sarada, 1985). It was found that starting from the initiation of germination, the enzyme exhibited a gradual increase in its activity and reached a maximum at sporulation stage. Partially purified protease showed that it is stable up to a temperature of 40°C and its activity was found to be maximum at pH 9. More work is needed to elucidate the enzymology of akinetes of blue-green algae.

**Conclusion**

Blue-green algae are widely distributed in nature and form a substantial fraction of the biomass in several important habitats of which the most important being flooded rice fields. A number of blue-green algae belonging to orders Nostocales and Stigonematales fix atmospheric nitrogen and make a moderate contribution to nitrogen fertility of rice fields. Following the work of De (1939), who showed nitrogen-fixing blue-green algae to be abundant in Indian rice fields, it became apparent that they could be used as a source of biofertilizers to enhance rice production. For increasing the growth of blue-green algae in water logged rice fields, dried vegetative filaments are being
used as inoculum. However, as the vegetative cells are sensitive to drying, the counts of effective propagules decrease during the preparation of inoculum and prolonged storage. Further, the vegetative propagules are also sensitive to pathogens. In fact, dried vegetative cell inoculants were reported to have limited potential for blue-green algal population enhancement (Tiedman et al., 1980). To overcome this problem it may be advantageous to use akinetes as inoculum since they are more resistant to adverse environmental conditions as well as viral, bacterial and fungal attacks. Since many of the nitrogen fixing blue-green algae are sporulating (akinetes producing) forms, their akinetes can be used as inoculum by producing them on mass scale. To achieve this end, the information on akinetes derived from a wide range of investigations may be useful.