Nitrogen cycle and blue-green algae (2)

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5. Physiological properties of blue green algae which may stimulate their bloom

Whenever there is an enormous increase in algal population in eutrophic inland waters species of the blue-green group are usually involved, and these species dominate the flora. This great population expansion is called 'algal bloom'. In addition to the N-fixing abilities of blue-green algae several other physiological differences between them and other groups may separately or jointly be the cause of the 'bloom' phenomenon which is often a considerable nuisance.

5.1. *Growth in dim light*

Rather low light saturation values (I_k) are reported for blue green algae.
Baker et al (1969) found light saturation for *Oscillatoria agardhii* under field conditions at only 10% on the maximal insolation, while high photosynthetic rates were found even at 4 %. Strong inhibition was found in the surface layers, where light values exceeded 30 % of the total insolation. In a study of the ecology and physiology of Oscillatoria Zimmerman (1969) found an optimal temperature of 14 °C and I_k value of 1000 - 1500 lux. Due to adaption processes the optimum of the culture which was kept at 22 °C shifted to 29 °C. Zimmerman's extensive studies show that either the maximal densities are reached in nature under conditions far from giving optimal growth in cultures or that a rapid adaptation to laboratory conditions may take place. Futhermore the laboratory experiments were performed in a relatively rich culture solution, and perhaps it is unwise to conclude that results would be applicable to field conditions. Besides these low I_k values several species can grow photoheterotrophically although most blue green are obligately photoautotrophic.

Van Baalen et al (1971) found that the two species of blue green algae *Agmenellum quadruplicatum* and *Lyngbya lagerheimii* could utilize glucose at a light intensity too low to support autotrophic growth.
In dim light glucose made a greater contribution to cell constituents (amino acids) than under high light intensity.
The increase in dry weight of his cultures was also higher with glucose in dim light than with normal photosynthesis at high light intensity. Most unicellular blue green algae are obligate photoautotroph.
Two metabolic features perhaps account for this, notably the low permeability (e.g. lack of glucose permease) and the absence of the tricarboxylic acid (TCA) cycle.
Pelroy et al (1972) and Rippka (1972) found that the oxidation of glucose in *Aphanocapsa* (= *Anacystis*) strain 6714, which can grow facultatively chemo-heterotrophically, follows the pentose phosphate pathway as the principal if not the only route; this resembles the pathways occuring in the filamentous blue green algae such as *Anabaena variabilis*. The necessary enzymes such as glucose permease, hexokinase and phosphatases were all found to occur in this strain. The obligate photoautotrophic strain A 6308 is different from 6714 only in its lack of the glucose permease as in neither strain does the TCA cycle occur. Strain A6308 can grow photoheterotrophically with glucose and DCMU, but not in the dark; thus it seems that in the light no permeability barrier is present, which suggests that the importance of the light lies in its suppression of the permeability barrier, not as a source of energy. From Pelroy's metabolic map (see fig. 3) for these blue green algae it can be seen that external glucose may be converted into glucose-6-P, which may either be converted into 6-phosphogluconic acid or enter the pentose-phophate cycle. The enzyme for the first reaction is inhibited by Ribulose-diphosphate (RUDP) a product of the Calvin cycle so that in strong light glucose can not be oxidised to 6-phosphogluconic acid. DCMU which inhibits RUDP formation, removes the RUDP block.
In dim light the RUDP block does not exist either so that external glucose may then be used.
As Pelroy remarks it is noteworthy that it is only in the blue green algae that NADP is used for the electron transport between oxidizable substrate and respiratory chain, whereas in the normal glucose oxidation NAD is used. NADP linked dehydrogenases mediate oxido-reductive steps in biosynthetic pathways, whereas NAD linked enzymes function in dissimilatory (catabolic) pathways. Utilisation of exogenous organic substances by blue green algae under light conditions (photoheterotrophic growth) is reasonably accounted for and described by a pathway such as that shown in fig. 3.

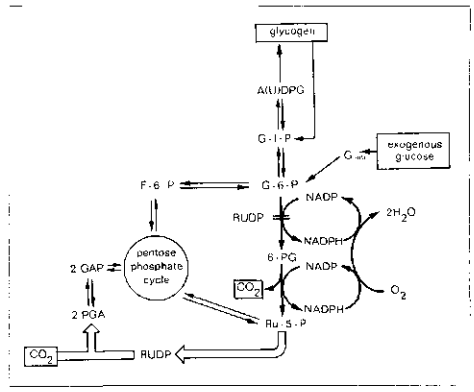


Fig. 3 - Metabolic map of pentose-phosphate cycle (Pelroy, 1972).

Both their low I_k value and their facultative photoheterotrophy may be the reason why blue green algae are often found below the thermocline (Gorlenko and Kusnetsov 1972). In these regions the light intensity is low and the mineralization processes there result in release of organic substances or molecules.
Saunders (1972) presented evidence for a heterotrophic uptake of glucose and acetate by *O.agardhii* and other *Oscillatoria* species at substrate concentrations, known to occur naturally. Other species of blue green algae — e.g. *Aphanizomenon flos aquae* — were unable to utilize glucose or acetate.
No comparison was made with photoheterotrophic uptake which might occur in the lake at the depth from where samples were taken. Saunders observed that *O.agardhii* occurred in winter in Frains Lake in deep water, where the ligth intensity was $< 10^{-3}$ lux. When anaerobic conditions developed in the hypolimnion in late spring, *O.agardhii* appeared in the lowest layers of water and migrated upward as anaerobiosis intensified. This species was even found in H_2S containing waters.
Saunders suggested that when the algae reach shallower water photolithotrophy develops, the heterotrophic mechanism being repressed.
This flexibility of assimilation methods might be related to the somewhat bacterial character of the blue green algae. It certainly confers upon them the ability to grow in two completely different niches of the lake habitat.

5.2. *Presence of gas vacuoles*

Various species of blue green algae contain so-called gas vacuoles which can be seen under the light-microscope as highly refractive bodies with an irregular outline.
They are not merely passive bubbles produced by some process, but have a definite structure.
The units are surrounded by selferecting three dimensional membranes of about 2-3 nm thickness and have a diameter of about 70 nm and a lenght between 100-300 nm. Since the membranes are freely permeable to gases, the spaces might be initiated in the protoplasm and then be filled passively with gas. They may be destroyed by sudden pressure if insufficient time is given for the gas to equilibrate.
Walsby (1969) found that they are produced more abundantly at low light intensities than at high ones. The gas vacuoles enhance the buoyancy. Thus Klebak (1922) measured an increase of specific gravity from 1.0065 to 1.0085 after destruction of the gas vacuoles whereafter

the algae sink. He destroyed the gas vacuoles easily by applying a sharp blow to the cork of a completely filled blue-green algal culture bottle. The algae sank quickly to form a sediment. By varying the number of these gas-vacuoles the blue green algae are able to migrate between the zone of optimal light conditions and the dark, more nutrient rich, layers. The influence of low and high light intensities favours this movement. The migration movements increase the diffusion of nutrients near the plants since microlayers may develop around algal cells deprived of nutrients. The cells may also hover at depths where dim light occurs and where the heterotrophic photoassimilation described earlier may take place, although there is no real proof for the occurrence of this phenomenon in nature.

Very little real evidence for the occurrence of algal movements exists although diurnal migrations are frequently referred to in the literature (Walsby 1969, 1970, 1971, 1972, 1973, Fogg + Walsby 1971, Fogg 1961, Reynolds, 1972).

Reynolds (1972) studying a natural population of *Anabaena circinalis* found that gas vacuole volume is determined primarily by the rate of increase of the alga. It decreased from 6 % to 1 % of the cell volume if the doubling time decreased from > 50 to about 4 days. Reynolds also showed that in vitro vacuole volume is influenced by cell turgor pressure, as on transfer to favourable light condition buoyancy — measured as flotation rate — is reduced to 10 %. After the population was returned to darkness the effect was reversed. Reynolds method of studying the cell turgor pressure was by applying pressure with compressed N_2, while the cells were suspended in different concentrations of sucrose. This is quite ingenious and can easily be used even in field conditions. Reynolds suggests that buoyancy of the dense surface layers is caused by an inhibition of photosynthesis resulting in an increased relative vacuole volume. This may be increased further in darkness or when growth is arrested by a limiting factor. (P.conc. decreased from 1.6 % to 0.5 %). The bloom is thus the result of a redistribution of an existing population. Finally it is noteworthy to report Reynolds figures for the ratio heterocysts over vegetative cells. It was greater than 1 : 1000 generally in May with a maximal value of 1 : 13,000 on 18 May when inorganic nitrogen was still present but changed to 1 : 100-300 in August during the second period of blooming.

5.3. *Organic substances including toxins*

Blue green algae often occur simultaneously with high concentrations of organic matter, often organic pollutant. Pearsall (1932) found that a correlation between blue green algal blooms and organic Nitrogen concentration occurred in the previous month, but this correlation does not always hold. Results of work by Singh (1955), Brook (1959) and Vance (1965) support a general impression that organic pollution and myxophycean blooms are related, although most planctonic algae are less successful than bacteria in competing for organic substances. The problem seems to be that the presence of organic matter is an effect of and not the cause of the algal bloom, since many blue green algae excrete considerable amounts of organic compounds. Fogg (1952, 1966 and 1971) has found that sometimes more than 50 % of the assimilated nitrogen can be excreted mostly as (poly) amino acids. These compounds are end-products of the algal metabolism, however, and unlike the production of glycollate cannot be considered as substances that can promote growth.

It is unlikely that these compounds can act as a substrate either for photoassimilation or for heterotrophic growth, at least in their original form.

Many of the excreted products (e.g. for Microcystis) are potent toxins, which may cause death of animals (Shilo 1964, 1967, Gorham 1964, Hughes et al 1958, Heany 1971, and the impressive review list of Schwimmer and Schwimmer (1967) in wich mass deaths of fish and cattle and waterfowl has been described). Some of these toxins have been identified as cyclic polypeptides, (Mol. weight about 1200). Gorham et al (1964) showed that the ability to produce toxins is not nesessarly a property of the whole species, but only of certain strains. As blooms may contain an association of several strains only some of which may produce toxins it is easy to understand why not all blooms are equally toxic or why some are not toxic al all.

It should also be realised that a toxin for one animal species will not automatically be toxic for another one. Heaney (1971) described samples of *Microcystis aeruginosa* from the Metropolitan Water Board which were toxic to mice but produced no abnormal mortalities of fish or birds. Hughes et al isolated a unialgal culture of *M. aeruginosa* which produced symptoms similar to those described for waterblooms. Two distinct factors were present in the culture, a fast and a slow death factor. The fast one is probably an endotoxin which is detected when cells become leaky (autolysis) or disintegrated. Although the toxicity of the excretion products of the blue green algae is well documented — certainly for cattle and waterfowl — its ecological significance is difficult to establish. There is no evidence to suggest an influence of blue green algae on other algae and the described events should mostly be attributed to other causes. Supposed competitive effects of blue green algae against other species are quite often mentioned hypothetically, but are rarely proved actually to occur e.g. Vance (1965). If they do occur it is more likely that a competition for nutrients is involved rather than a poisonous inhibitor.

Nutrient supply and depletion should always be assessed and controlled, in experiments studying competition. Production of toxins is not restricted to blue green algae. Shilo who reviewed the literature (1964) and suggested a model for the formation and mode of action of algal toxins (1967) showed that an ichthyotoxin was produced by the flagellate *Prymnesium parvum*. Shilo showed that a large number of physiological and environmental factors are concomitantly involved in the process of toxin formation and excretion. Synthesis of the toxins is greatest during the late stages of the logarithme phase of growth. In the stationary growth phase P-depletion enhances toxin production even before affecting growth. Activation of the excreted products is a second essential step, depending perhaps on presence of chelators, favourable pH etc.

Light is essential for toxin production, although light in the 400 to 510 mμ and UV ranges removes activity. Other factors inactivating the toxins are adsorption on colloids and changes of the pH.

Thus not only biosynthesis but also extracellular stability of the toxin are affected by environmental conditions. Blue green algae seem to be less grazed upon by zooplankton or even fish than other algae, which could cause their dominance. This inhibition of grazing might be caused by their toxins but evidence for this is scarce.

The question of whether or not toxin production by blue green algae does stimulate their blooms under natural conditions is still open to doubt.

The influence on fish is also difficult to prove. The blooms in which fish deaths occur are often very dense and the resultant O_2 depletion should been taken into account. Enormous fish deaths in Lake George for example seemed to be related more to the anaerobic conditions caused by excessive respiration and mineralization rates than to any other factor. Such anaerobic conditions may favour development of *Clostridium botulinum*, a very toxic species of bacteria.

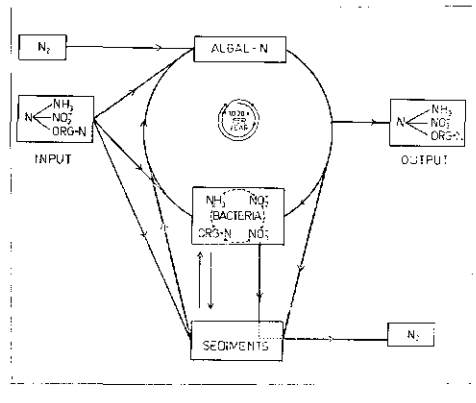
6. Concluding remarks

Low soluble phophate and nitrogen concentrations are sometimes reported to accompany blue green algal blooms. (e.g. Reynolds, 1972). These may be the effects of the bloom rather than its cause. There is no evidence that the blue green algal cells have a lower than average N or P content, and it is not correct to consider the remaining P- and N-compounds in solution. From the shallow Dutch lakes there is evidence that Part. P. and Part. N are as high as during blooms of other algae.
It is likely that the real cause is a combination of several factors. Quantitative effects of algal blooms on grazing are difficult to ascertain and more work on this is desirable, but predation as a factor controlling the size of algal populations must certainly be considered (Golterman 1970).
It is clear that although local remedies are possible the only real basis for control is to decrease the nutrient input especially of phosphate into lakes and rivers. As a possible controlling factor disturbance of stratification should be mentioned since this may interrupt migration of the algae. This method was introduced by the Metropolitan Water Board in Britain as a general means of algal control but it seems in fact to be effective especially against blue green algae (Fogg (1968)),

7. The N-cycle

A nitrogen (fig. 4) cycle can be composed in a similar form to that already described for summarizing phosphate processes. (Golterman 1973).
Inorganic sources for algae are NH_3, NO_3^-, and also N_2 in cases, where N-fixation occurs. The uptake of inorganic nitrogen is dependent on C-sources, either from active photosynthesis or from heterotrophic growth from carbon accumulated during photosynthesis under N-starvation. Algal nitrogen content will be up to 10 % of the dry weight and after the death of the algae a rapid mineralization will occur, mostly by bacterial action. By this process nitrogen is liberated mainly as NH_3 although small amounts of organic nitrogen compounds will escape to the water. Probably a larger part than for phosphate will be converted into refractory compounds. Humic acids contain about 4 - 5 % N. In dissolved organic matter a C:N ratio of 5 is found, a value which is close to that occurring in living material. Some of these refractory compounds will sediment, and mineralization will occur slowly. Nitrogen may be lost from sediments by denitrification as N_2. Very little is known of the turnover time of nitrogen, which is much more difficult to estimate than that of phosphate. Besides the accumulation of nitrogen compounds as refractory material in the sediments, adsorption and desorption of ammonia occurs, although in shallow lakes the mud will not remove as much NH_3 as it does phosphate.
Although most if not all reactions in the N-cycle are well known a quantitative model is still difficult to assess. Thomann et al (1971) suggested a mathematical model for nitrification and algal cycles in estuaries.
Analysis of the nitrification phenomenon can be carried out using a generalized steady state multidimensional feedback model. Any configuration of reaction systems seemed to be permissible provided first order reaction kinetics and linearity are maintained. For more complex algal growth situations non-linear equations are necessary to describe the phytoplankton dynamics such as growth, death and predation which are described as functions of factors such as temperature, sunlight and nutrient concentrations. The formulae can only be used with the aid of a sophisticated computer and apply to situations where one has to assume that initial microbial mass is large relative to initial substrate concentrations, while throughout the whole study nitrogen is assumed to be a limiting factor and the influence of phosphate is never mentioned.
Filter feeding rates for zooplankton are measured and included, but many other data are taken from the literature. Use of a computer makes it possible to test different trial models until a good fit is obtained between predicted and actual values. The formula which provides the best conformity between predicted and actual values is not necessarily to only one which can do so, and since not all possible nitrogen reactions are even included in the scheme the significance of the obtained results by Thomann et al is difficult to evaluate.

Fig. 4 - Nitrogen cycle with in- and output in freshwater.



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