Environmental mitigation in *Nostoc muscurum* with special reference to nitrogen metabolism during phosphate stress

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Abstract : **Increasing atmospheric carbon dioxide concentration is an important factor in global climate change and the role of other nutrient availability is an important issue regarding microbial responses to increasing carbon dioxide concentration. Studies of changes in cyanobacterial community composition, bloom dynamics, and toxicity in response to increasing temperatures and other environmental factors associated with global warming are of great importance to our understanding of cyanobacterial dynamics. In the current study it was found that phosphate stress inhibited the synthesis of Nitrogen Reductase enzyme. The displacement of an essential metal ion forming the central and functional part of the enzyme protein may be one of the reasons for inhibition of nitrate reductase by heavy metals. NR activity, both in the field and laboratory, are necessary as a contribution, both to understanding algal physiology, as well as to clarify the importance and role of these algae in near-shore biogeochemical cycling.**

Index Terms – **Global warming, cyanobacteria, nitrate redustase, phosphate, climate.**

I. INTRODUCTION

Cyanobacteria have a long evolutionary history, with their first occurrence dating back at least 2.7 billion years ago. Cyanobacteria often dominated the oceans after past mass extinction events. They evolved under anoxic conditions and are well adapted to environmental stress including exposure to UV, high solar radiation and temperatures, scarce and abundant nutrients. These environmental conditions favor the dominance of Cyanobacteria in many aquatic habitats, from freshwater to marine ecosystems. A few studies have examined the ecological consequences of global warming on Cyanobacteria and other phytoplankton over the past decades in freshwater, estuarine, and marine environments, with varying results. The responses of cyanobacteria to changing environmental patterns associated with global climate change are important subjects for future research. Results of this research will have ecological and biogeochemical significance as well as management implications.

Environmental conditions on the early Earth under which cyanobacteria evolved and thrived were very different from today (Kasting and Siefert 2002). Anoxia, higher UV exposure, higher temperatures, and high levels of iron, sulfide and methane were all factors that influenced early life on Earth. Additionally, cyanobacteria experienced little or no grazing pressure or competition from higher organisms. This may explain why cyanobacteria can thrive under conditions of environmental stress and in extreme environments where they are able to out-compete other organisms.

NITROGEN METABOLISM:

Nitrate is the major source of nitrogen for most marine phytoplankton (Parsons 1977, Syrett 1981), and macroalgae (Davison 1984). Nitrate reductase (NR) activity has widely been used as an indicator of nitrate utilisation by phytoplankton and macroalgae (Eppley 1969). The use of NR in an ecological context is particularly relevant for marine environments where nitrogen is often limiting, thereby providing relevant information regarding the physiological nitrogen status of organisms (Hernandez 1993). Nitrate reductase is considered as a key enzyme in nitrogen metabolism, through being, not only the rate-limiting enzyme in inorganic nitrogen assimilation, but also the major regulatory step in nitrogen metabolism (Crawford 1995, Berges 1997, Davison & Stewart 1984, Lartigue& Sherman 2005, Young 2009). Changes in NR activity, both in the field or in laboratory, have been examined in very few macroalgae. Nitrate reductase expression is a complex process regulated by various factors, such as levels of nitrate, CO_2, light, carbon skeletons and nitrogen metabolites (Crawford 1995, Lopes 2002). Furthermore, it is highly regulated in multiple steps, transcriptionally, post-transcriptionally, translationally and post-translationally. These regulatory mechanisms can act individually or synergically, and are correlated to short and long-term NR response. Thus, NR activity can be modified rapidly in response to nitrate availability and other controlling factors.

Higher NR activities are also associated with parts with active metabolic rates, and probably, to high levels of photosynthetic activity. In macroalgae with apical growth, NR activity in the tips is higher than in the basal parts (Chow 2004), thereby implying post-translational regulation. Nevertheless, NR protein content in the basal parts is the highest, possibly indicating that a large part of NR is in an active form, compared to the basal part of the thallus. Post-translational regulatory mechanisms are common in NR enzymes, especially during short-term response. This mechanism includes phosphorylation (Huber 1992) involving specific protein kinases, protein phosphatases and a protein inativator (MacKintosh 1995; Glaab& Kaiser 1996). In most studies of macroalgae NR activity, it is possible to establish the same trend of physiological response, i.e., the pronounced dependence on the external and internal pool of available nitrate, light stimulation and low or constitutive dark activity, temperature range of action according to the natural habitat of the seaweed, and correlations with carbon and ATP availability from photosynthesis and respiration. The slight differences in NR behavior can be attributed to species-specific response depending on particular environmental conditions, and may reflect special turnover of NR activity, as a product of acclimation and adaptation response to the extremely changeable intertidal environment. Nitrate reductase, through being the first enzyme in the nitrogen assimilatory pathway, assumes the responsibility for controlling the nitrate assimilatory rate in all algal cells. Thus, due to its importance in the general metabolism connected to N and C pathways, there is a constantly growing interest in studying the molecular and catalytic properties of NR enzymes and physiological responses to environmental stressing conditions and intracellular factors. The constant accumulation of

knowledge on NR activities, together with studies of nutrient uptake, will facilitate the collection of tools for:

(1) identifying limiting, defective and saturating levels of growth-nutrients;

(2) regulating pathways of nitrogen assimilation and incorporation;

(3) providing environmental indicators for monitoring;

(4) identifyingmacroalgae with high nitrate-reduction potential for biofilter application in eutrophized environments and increasing the standing crop in polycultures;

(5) optimizing culture systems by regulating the reduction rate of nitrate with optimal nitrogen uptake and reduction;

(6) developing management strategies for the culture of economically important algae;

(7) understanding evaluative patterns that support the adaptation of macroalgae to their environment. Further studies of NR activity, both in the field and laboratory, are necessary as a contribution, both to understanding seaweed physiology, as well as to clarify the importance and role of these algae in near-shore biogeochemical cycling. Moreover, the constant changes in the coastal environment, brought about by anthropic action (artificial eutrophication), and variations arising from global climate change, will undoubtedly influence the ranges of tolerance and acclimation of algae, whereby the necessity for monitoring changes in coastal environments and communities.

II-MATERIALS AND METHODS

The methods for isolation and protocols of cyanobacterial cultures employed throughout the course of study were as follows.

1.1.Organisms used: *Nostoc muscorum*

The cyanobacteria *Nostoc muscorum* is a fresh water, filamentous and diazotrophic and is capable of oxygenic photosynthesis. Its vegetative cells are the site for oxygenic photosynthesis, while nitrogen fixation occurs in specialized cells known as heterocyst.

1.2.Culture media and growth conditions:

In modified Chu No. 10 medium (Gerloff et al., 1950) wild type strain of the cyanobacteria *N. muscorum* would be grown for routine as well as for experimental purposes.

1.3. Composition of Chu 10 media

5 cm^3 each of the macronutrients and 1 cm^3 of the micronutrients mixture solution would be taken in a final volume of 1000 cm^3 double distilled water and its pH would be adjusted to 7.5 prior to autoclaving. The cultures would be maintained at a temperature of 25-30 +/-1ºC illuminated by white cool florescent tubes to receive a light intensity of approximately 100µ $E/m^2/s$

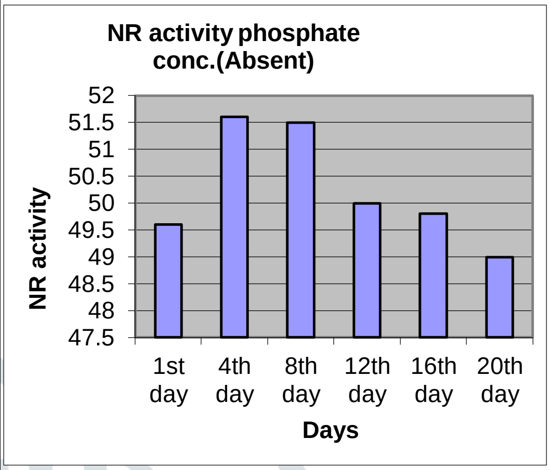
1.4. Measurement of Nitrate reductase (NR) activity

NR activity was measured by the method as described by Manzano et al. (1976). NR activity was calculated in terms of nmol NO_2- produced mg^{-1} protein min^{-1}.

III-RESULTS

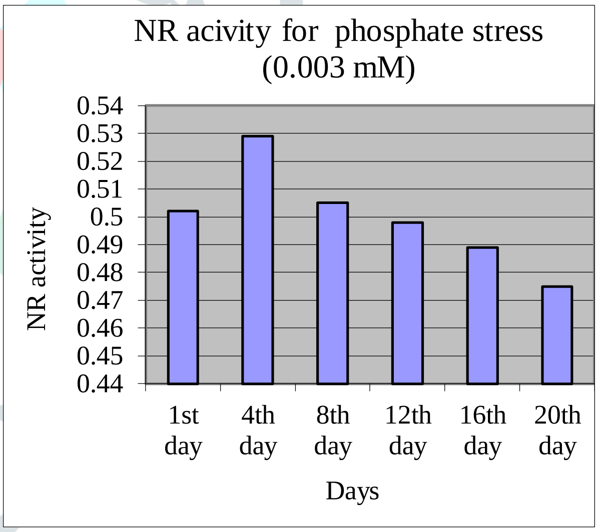
1. **NR activity in phosphate absent culture medium:-**

S.No	Days	520nm	NR activity
1	1st day	0.496	49.6
2	4th day	0.526	51.6
3	8th day	0.515	51.5
4	12th day	0.500	50
5	16th day	0.498	49.8
6	20th day	0.490	49



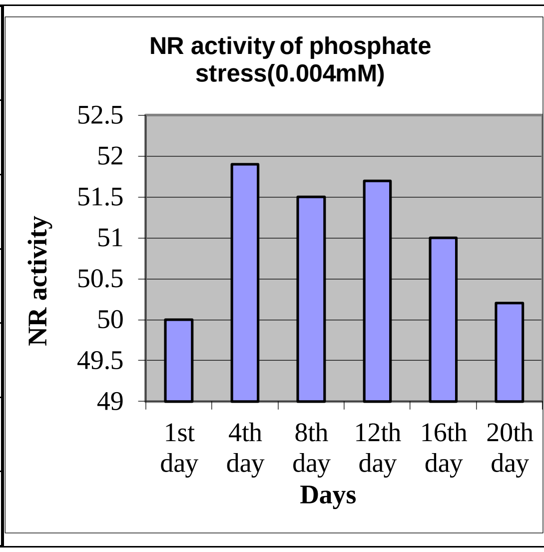
2. NR activity in culture medium with phosphate concentration at 0.003mM

S.No	Days	520nm	NR activity
1	1st day	0.502	50.2
2	4th day	0.529	51.9
3	8th day	0.505	50.5
4	12th day	0.498	50.4
5	16th day	0.489	49.9
6	20th day	0.475	49.3



3. NR activity in culture medium with phosphate concentration at 0.004mM

S.No	Days	520nm	NR activity
1	1st day	0.500	50
2	4th day	0.528	51.9
3	8th day	0.520	51.5
4	12th day	0.523	51.7
5	16th day	0.510	51
6	20th day	0.503	50.2



Experiments on NR activity revealed that Phosphate stress inhibited the synthesis of NR. The displacement of an essential metal ion forming the central and functional part of the enzyme protein may be one of the reasons for inhibition of nitrate reductase by heavy metals.

CONCLUSION AND FUTURE PROSPECTS

In natural ecosystems cyanobacteria are exposed to multiple environmental conditions. Therefore, it is necessary to find out responses to environmental changes on the physiological as well as biochemical properties of the organism. Increasing atmospheric carbon dioxide concentration is an important factor in global climate change and the role of other nutrient availability is an important issue regarding microbial responses to increasing carbon dioxide concentration. The present investigation will also help in evolving integrating nutrient specific pathways and their roles in the survival strategy under constantly changing environmental conditions. Examination of the evolutionary history of cyanobacteria, studies of their ecophysiology, and recent investigations of phytoplankton dynamics and community structure in response to global climate change all suggest that cyanobacteria will probably thrive under environmental conditions associated with global warming. Most studies addressing cyanobacterial responses to changes in irradiance, temperature and nutrients have been done in the laboratory; few studies have addressed these important topics under field conditions. Clearly, studies of changes in cyanobacterial community composition, bloom dynamics, and toxicity in response to increasing temperatures and other environmental factors associated with global warming are of great importance to our understanding of cyanobacterial dynamics. Interactions among anthropogenic effects, including eutrophication and food web alterations, and changing environmental conditions are likely to be complex. A much better understanding of these issues is urgently needed.

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