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Research Article

Growth response of Cyanobacterium: *Nostoc calcicola* Breb In the presence of Nickel (Ni^{+2}) ions

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ABSTRACT

A cyanobacteria strain Nostoc calcicola Breb was exposed to different concentrations (5, 10, 15 and 20μM) of Nickel (Ni+2) ions combinations. Specific growth rate constant and protein content were determined at different exposure periods (0, 2, 4, 8 and 10) days as indicate the growth response. Results showed that growth response of cyanobacterium Nostoc calcicola Breb to nickel was depending on the nickel ions concentration and population density of cyanobacteria. The 20μM of nickel ions concentration showed complete inhibition of growth of Nostoc calcicola Breb. The specific growth rate showed its progressive decline growth as increses the nickel concentration in medium and at 11 μM of nickel it was found to cause 50% decrease in specific growth rate of the cyanobacterium. The effect of nickel on growth of cyanobacterial at varying culture density showed that higher population size having 90 μg ml-1 of protein, cyanobacterial growth was better throughout than the two conditions (30 or 60 μg protein ml-1).

KEY WORDS: Cynobacterium, nickel, growth

Introduction

Pollution of the biosphere with toxic metal has accelerated dramatically since the beginning of the industrial revolution. The primary source of pollution are mining, burning of fossil fuels, smelting of the metallicferous ores, fertilizers, pesticides, municipal waste, and sewage [1]. The release of heavy metals from industries, agriculture and sewage into the environment has created many problems for both human health and aquatic ecosystems. [2], [3], [4]

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Accumulation of these heavy metal pollutant can alter macro and microbiological communities and in plants causes physiological and biochemical changes.[5],[6],[7]. Some of heavy metals like Nickel and Copper at low concentrations have an important role in microbiological activity but at high concentration it has a toxic effect. [8], [9],[10], [11]

Conventional chemical methods for heavy metals removal from wastewater Precipitation, Filtration, ion-exchange and reduction-oxidation are expensive and ineffective, particularly when metal concentration is low.

Presently phytoremediation is most desirable technology which uses microorganism for removal of environmental pollutants or

detoxification to make them harmless.[12] Microalgae remove heavy metals directly from polluted water by using two major mechanisms; the first is bioaccumulation by the cells at low concentrations and the second is biosorption.

Therefore, this is regular recent trend for removal of trace amounts of toxic metal from aqueous waste. Spirulina platensis biomass used as adsorbent for Copper removal from water solution and copper was removed by 12.5-81.8% from wastewater by using cyanobacterial cultures of Nostoc muscorum and Anabaena subcylindrica. [13], [14].

Nickel is an essential heavy metal that plays an important role in health of eukaryotes and prokaryotes. Nickel used as a necessary cofactor for enzymatic function in prokaryotes and nickel-associated enzymes play an important role in maintaining the global cycle for nitrogen, carbon, and oxygen. [15] Nickel is categorized as a potential carcinogen, at higher concentrations it is potentially toxic and cause detrimental damage to lung tissue. [16],[17]. In human cell lines, nickel accumulates intracellularly and effects DNA methylation and iron-uptake systems resulting in iron deficiency.

The unique nature of cyanobacteria to grow in a wide range of environmental conditions makes it an ideal model for experiment. Cyanobacteria and other prokaryotes can directly be damaged by nickel. Nickel can target or interfere with important enzymes involved in photosynthesis. [18],[19]. The damaging effects of nickel have allowed prokaryotes to develop a wide range of survival methods.

The present study was conducted to find out the effect of nickel on growth pattern of Nostoc calcicola Breb with the help of measuring the effect of nickel on specific growth rate constant and protein contentof cyanobacterial.

Material and Methods

Microorganisms

A strain of cyanobacterium Nostoc calcicola Breb was used in this study obtained from Algal Research Laboratory, Centre of Advance Study in Botany, Banaras Hindu University, Varanasi (Uttar Pradesh) India, in the form of mass cultured in a capacity of 500 ml Erlen-Mayer flask containing 200ml Allen – Arnons combined nitrogen free suspension medium. The cyanobacterium were sub cultured in 500 ml Erlen-Mayer flask containing 200ml Allen-Arnons combined nitrogen free medium and incubated photoautotrophically under the air conditioned room which was illuminated with white fluorescent lights (intensity 14.4 watt m-2 on the surface of culture flask) with 18/6 light/dark cycle. All cultures flasks were routinely shaken with the help of rotator flask shakers at 130rpm for 20-30 minutes to obtain homogenous suspension in liquid medium.

Nickel

Nickel used in study was procured in the form of Nickel Chloride (Nickel II chloride 6-hydrate Anala R) from BDH Reagents and Chemicals UK. Nickel (Ni+2) ions as working solutions (5, 10, 15 and 20µ M) were prepared by dissolved nickel chloride in deionised distilled water.

Growth experiments were carried out in 500 ml Erlen-Mayer flask. The one flask containing 200ml Allen-Arnons combined nitrogen free medium supplied with one concentration of metal. Another flask which containing no metal, served as control. Experiments were carried out in triplicate. Each flask was inoculated with 6 - 8 day old exponentially growing cyanobacterial cells and initial cell density was adjusted to 30 µg protein /ml culture (absorbance, 0.02). The all experimental flasks were incubated under the experimental conditions and 3.0 ml sample of each treatment was taken at different exposure periods (0, 2, 4, 8 and 10 days) for finding the nickel effect on growth pattern. The effect of nickel on cyanobacterial growth find out with the help of measurement of specific growth rate constant and Protein content measurements. [20],[21].

Results

The statistical analysis (ANOVA test) revealed a significant ($P<0.05$) growth response that caused by the different concentrations of Nickel ions on the growth of a strain of cyanobacterium Nostoc calcicola Breb.

The effect of Nickel ion (Ni+2) on specific growth rate constant:

Nostoc calcicola Breb grew well photoautotrophically Allen-Arnons combined nitrogen free medium. Growth rate was measured as a time dependent increase in optical density of the cyanobacterial culture at 650 nm. At this wavelength, photosynthetic light is mainly absorbed by chlorophyll a. Since chlorophyll a is the main pigment of photosynthesis, its use as an index of photoautotrophic growth is quite justified. The effect of nickel on the cyanobacterial growth was examined and results are shown as in Figure 1. We compared cyanobacterial growth response to nickel in term of changes in specific growth rate (K). Growth of cyanobacterium decreased with increasing Ni concentrations, and this decrease was reflected both in terms of lag in growth and decrease in specific growth rate. A nickel concentration of 20µM resulted in complete inhibition of cyanobacterial growth. We have also find out the interrelation between growth rate (k) and nickel concentration as shown in Figure 2. The specific growth rate showed its progressive decline growth ultimately approaching zero at 20 µM of nickel and at 11 µM of nickel it was found to cause 50% decrease in specific growth rate of the cyanobacterium.

The effect of Nickel ion (Ni+2) on Protein:

The effect of nickel on the cyanobacterial growth was examined with the help of protein estimation We compared cyanobacterial growth response to nickel in term of changes in protein content. Protein content decreased with increasing nickel concentrations. A 90% and 50% protein content decreased at 20µM and 13 µM of nickel concentration respectively. A progressive decline in protein content occurs as the nickel concentration increases in the medium.

The effect of Nickel ion (Ni+2) on varying population density:

We have also examined the effect of varying population density on the growth inhibitory action of nickel subsequently and results are shown as in Figure 3. As already shown, growth never occurred in 20 µM nickel concentration at the culture density maintained at 30 µg protein ml-1. However, when this initial cell density was doubled from 30 to 60 µg protein ml-1 in similar nickel containing medium, growth of cyanobacterial occurs immediately without any lag. When the same experiment was repeated with still higher population size having 90 µg ml-1 of protein, cyanobacterial growth was better throughout than the two conditions (30 or 60 µg protein ml-1). These results suggest that growth toxicity of nickel is a function of inoculums density

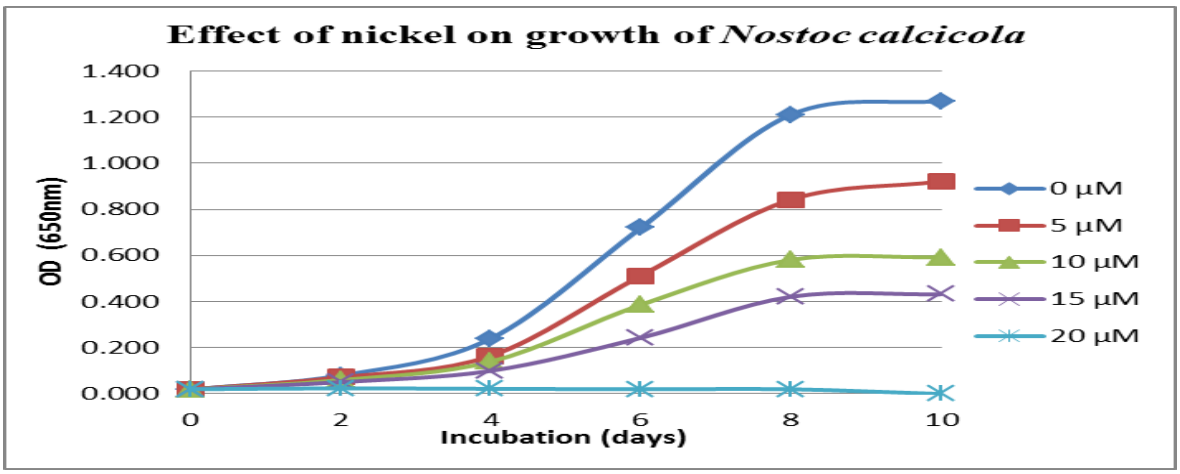

Figure 1. Growth of Nostoc calcicola cells exposed to different concentration of nickel

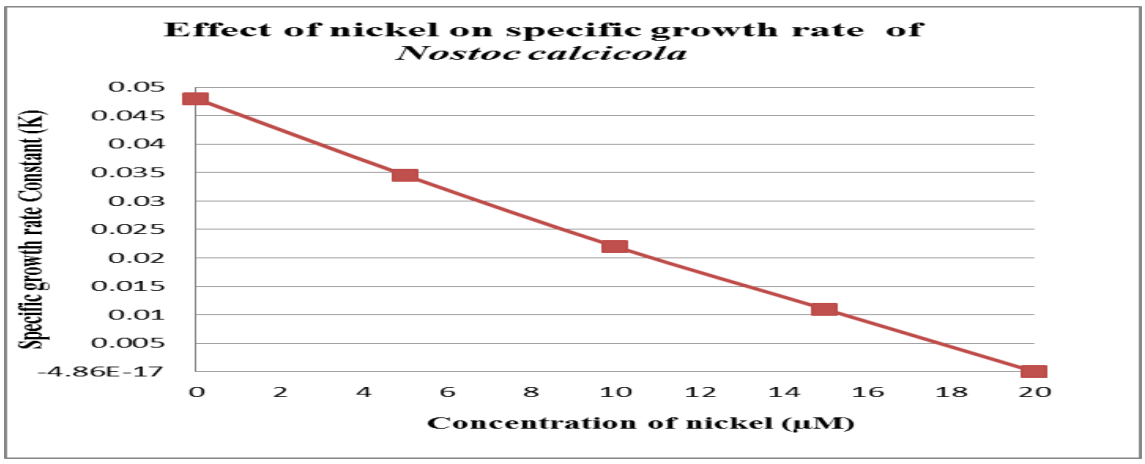

Figure 2: Specific growth rate constant (K) of *N. calcicola* cells at different concentration of nickel

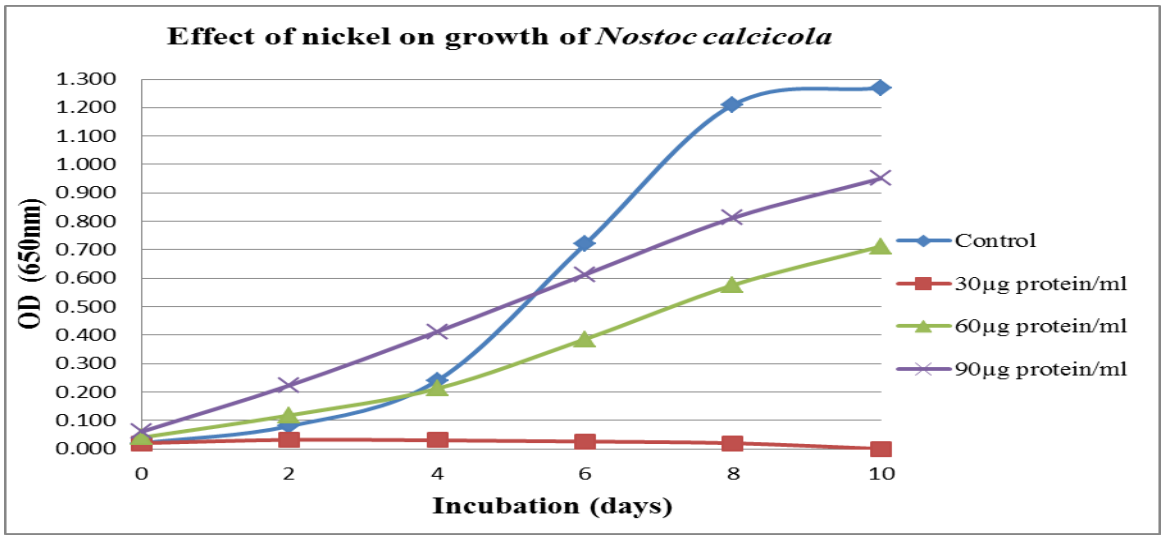

Figure- 3: Growth behavior of N. calcicola exposed to 20µM nickel concentration at different initial cell densities populations (30 µg protein ml-1, 60µg protein ml-1 and 90µg protein ml-1).

Reduction in growth, photosynthetic pigments, nitrate reductase, alkaline phosphatase, and uptake of nutrients or damage of cell membrane occurs under heavy metals stress. [22], [23], [24], [25]. Survival and motility of algae were affected at (1-200 ppm) concentrations of heavy metals (Ni, Cu, Zn, Co, Fe and Hg) [26].

At lower concentration the copper and nickel has been found to enhance plant growth and act as a micronutrient, but at higher concentration copper caused 50% inhibition of photosynthesis in 118 isolates of algae and nickel has been found to be phyto-toxic. [27] This may be due to the retention of nickel in the plant system. Enzyme activity which is directly responsible for plant growth may be directly inhibited due to nickel accumulation. Severe cases of acute nickel toxicity have been found to divert the metabolic pathway and may even cause death of the plant.

Like other cations, nickel is also known to be accumulated by algae and to inhibit algal growth. [28],[29], [30],[31],[32]. Anabaena inaequalis cells

exposed to nickel (approx. 30μM) responded in terms of increase in the lag phase of growth, culture doubling time and a faster retardation of growth, possibly due to poisoning of intracellular enzyme systems by nickel.[33]. The toxicity response by Anacystis nidulans also came within the same range with a slight tolerance (38μM).[34]. In a separate study significant reduction in growth of Anabaena flosaquae and Anabaena cylindrical was observed by nickel amounting to 10.2 μM. [35]. In the present study nickel is also significantly inhibited photoautotrophic growth of the cyanobacterium Nostoc calcicola Breb at 20 μM. The average values (based on K) indicate that 11μM nickel was the 50% inhibitory concentration under the prevailing initial population size (30 μg protein ml-1) and culture conditions.

Different cell densities were employed to establish the possible correlation between the cell surface availability and the metal cations prevailing in the medium. Initially 20 μM nickel concentration is invariably lethal to the Nostoc calcicola Breb at the culture density of 30 μg protein ml-1 but at higher cell mass i.e., 60 or 90 μg protein ml-1, survival of the cyanobacterial was occurs. Such a differential behavior of the three initial cell densities clearly demonstrated that algal cell mass was the determining factor in nickel toxicity to Nostoc calcicola Breb. Such observations can be explained on the basis of the availability of lesser nickel ions on per cell basis at higher cell density compared to those in lower cell density population. Similar results were also obtained for reduced phenyl mercuric acetate toxicity with increase in cell density of Chlamydomonas variabilis and for Cu, Cd or Zn toxicity to marine algae. [36], [37]. Significance of such an observation is that the population density can alone serve as a source of nickel detoxification mechanism for a given concentration of nickel.

Conclusions

In the present investigation, it is concluded that nickel treatments produced toxic impact on growth of Nostoc calcicola Breb as compared to control. Decrease in growth occurs as increase the nickel concentration in the medium. Therefore there is a need to implement certain rules that help in the reduction of metal level from a wide range of sources such as from the metal processing industries and power generation plants. The vegetable crops have the ability to uptake the heavy metals through their roots and transport them to the edible portion of the plant that are consumed by people or fed to animals and their increased concentrations in human food chain over a long time can cause damage to health.

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