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Influence of Nickel and Copper on Photobiological Hydrogen Production and Uptake in *Oscillatoria subbrevis* Strain 111

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In *Oscillatoria subbrevis*, net H_2 production and stimulation of uptake hydrogenase activity were dependent upon the nickel concentration in the medium. Nickel concentration as low as 2 μM inhibited H_2 production and stimulated H_2 uptake activity. H_2 production and uptake activities were decreased in the presence of copper. Chloramphenicol blocked a significant amount of nickel-stimulated uptake hydrogenase activity. EDTA (ethylene-diaminetetraacetic acid)—repressed uptake hydrogenase activity was specifically relieved by nickel.

Key Words: Cyanobacteria, Copper, Nickel, Uptake hydrogenase, EDTA

Introduction

Cyanobacteria are the most abundant photosynthetic prokaryotes found in polluted waters containing a variety of heavy metals (Rai & Raizada 1985). Most of the work on the effect of heavy metals on cyanobacteria is confined only to few parameters like growth, pigment synthesis and nutrient uptake (Raizada et al. 1984). However, little information is available with respect to nitrogen fixation and hydrogen metabolism in cyanobacteria (Daday & Smith 1983).

Trace metals Co^{2+}, Cu^{2+}, Mo^{2+}, Zn^{2+} and Ni^{2+} play a significant role in growth and hydrogen metabolism of photosynthetic microorganisms (Weissner 1962, O'Kelly 1974). It has been shown that Ni^{2+} is essential for the synthesis of active hydrogenase in many bacteria (Graf & Thauer 1981, Freidrich et al. 1982). The work on nitrogen fixing cyanobacteria (Daddy & Smith 1983, Almon & Boger 1984, Xiangkong et al. 1984, Daday et al. 1985) reveals that the uptake hydrogenases of cyanobacteria are Ni^{2+}-containing enzymes. However, the role of Ni^{2+} in filamentous non-nitrogen fixing cyanobacteria is little known and apparently no work has been done. The only report concerning the essentiality of Ni^{2+} for filamentous, non-heterocystous cyanobacteria is that of Van Baalen & O'Donnel (1978), who isolated a Ni^{2+} dependent *Oscillatoria* sp. 3NT. In this communication, effects of Ni^{2+} and Cu^{2+} on growth and hydrogen metabolism in a non-nitrogen fixing *O. subbrevis* are discussed.

Materials and Methods

Oscillatoria subbrevis strain O.U. 111, isolated from fresh water was employed. The strain was characterized as *Oscillatoria subbrevis* following the method of Desikachary (1956) and Rippka et al. (1979). Axenic cultures of *O. subbrevis* were cultivated aerobically in 250 ml Erlenmyer flasks containing 150 ml of BG 11 medium (Rippka et al. 1979), *p*H 8.0, under continuous illumination of 1600-2000 lux for 6 days. Growth was measured by estimating chlorophyll-a in the methanol extract (Mackinney 1941). Protein was estimated by the method Lowry et al. (1951).

Hydrogen evolution and uptake activities were determined according to the method of Howarth and

Codd (1985). 5 ml of BG 11 medium and 5 ml of algal suspension (containing 2-3 μg chl-a/ml) were transferred to 15 × 125 mm test tubes and sealed with suba seals. The head space was evacuated and flushed with argon to create anaerobic conditions. For the assay of hydrogen uptake, 5% H_2 was included at zero time. The *p*H of the medium was adjusted to 7.5. The tubes were incubated at 28 ± 2°C under continuous illumination of 2500 lux. 0.5 ml of the gas phase in the tubes was analyzed with a gas chromatograph (CIC, Baroda) fitted with a Molecular sieve 5A column (2 mm × 1/8" OD SS column, 60-80 mesh) and a thermal conductivity detector. The column temperature was 60°C and nitrogen was the carrier gas (30 ml/min) with 120 mA current.

The methods of Wang et al. (1971) and Jones and Bishop (1976) were used for measuring the photosynthetically evolved oxygen with Hansatech (England) DW_1 oxygen electrode. Two ml of algal suspension (containing 2-3 μg chl-a/ml) was placed in the reaction vessel having oxygen electrode at −0.8 v, with respect to two independent Ag-AgCl reference electrodes. The signals from the electrode were amplified and recorded on a dual pen chart recorder.

Stock standards of metal solution were prepared by dissolving the trace metals ($CuSO_4.5H_2O$ and $NiCl_2.6H_2O$) in metal free deionized distilled water. The required concentration, as indicated in the respective tables and figures were determined by suitable dilutions.

Results

Culture of *O. subbrevis* were grown in BG 11 medium supplemented with different concentrations of Cu^{2+} and Ni^{2+} ranging from 2-10 μM. Ni^{2+} upto 10μM did not show any effect either on growth rate or on biomass yield. However, even low concentrations of Cu^{2+} decreased the biomass yield with simultaneous increase in generation time (table 1).

Addition of Ni^{2+} and Cu^{2+} showed a pronounced effect on net hydrogen production and the rates were decreased in the presence of both metals (figure 1). It was reported earlier (Xiangkong et al. 1984) that Ni^{2+} ions are required for oxygen dependent hydrogen uptake activity (oxyhydrogen reaction). To establish the probable role of Ni^{2+} in oxyhydrogen reaction, oxygen evolution was measured with varying concentrations of Cu^{2+} and Ni^{2+} (figure 2). Addition of Ni^{2+} upto 10 μM showed a stimulation in the oxygen evolution rates, accompanied by a simultaneous increase in hydrogen uptake activity (figure 3). Oxygen evolution and hydrogen uptake activities were inhibited by Cu^{2+}

Table 1 *Effect of Ni^{2+} and Cu^{2+} on biomass yield and generation time in* ***O. subbrevis***

Treatment	Biomass yield (μg chl-a/ml)	Generation time (hours)
Control	3.0 ± 0.2	48 ± 2
Ni(μM)		
2	2.9 ± 0.3	48 ± 2
4	2.9 ± 0.2	48 ± 2
6	3.0 ± 0.1	48 ± 2
8	2.8 ± 0.3	48 ± 2
10	2.9 ± 0.2	47 ± 2
Cu(μM)		
2	2.6 ± 0.2	47 ± 2
4	2.2 ± 0.2	49 ± 2
6	1.2 ± 0.2	58 ± 2
8	1.0 ± 0.1	62 ± 2
10	0.8 ± 0.2	69 ± 2

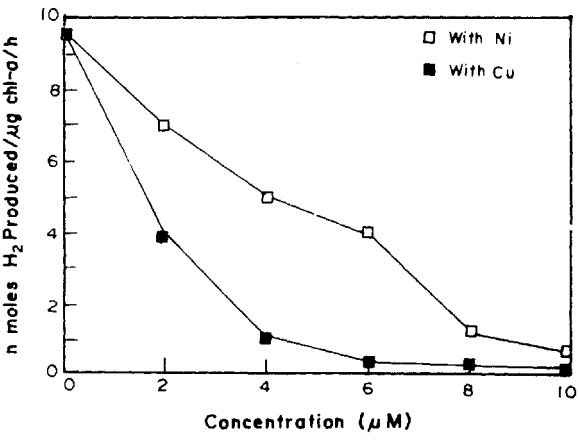

Figure 1 Effect of Cu and Ni on H_2 production in *O. subbrevis* (Cultures were grown for 6 days with indicated Ni and Cu concentrations and assayed for H_2 production)

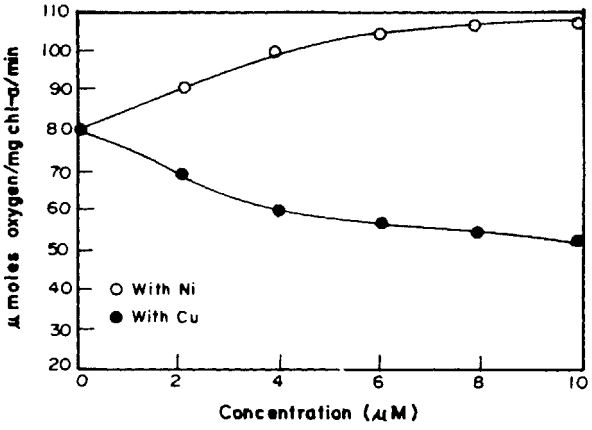

Figure 2 Effect of Ni and Cu on oxygen evolution (Cultures grown in the presence of different concentrations of Ni and Cu were transferred from the growth medium to the electrode chamber and flushed with argon for 5 min)

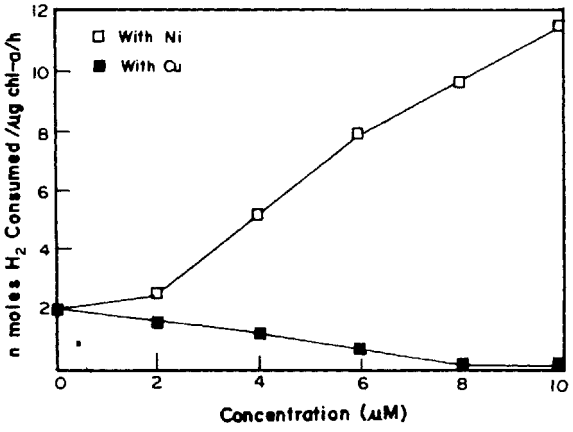

Figure 3 Effect of Cu and Ni on H_2 uptake activity in *O. subbrevis*

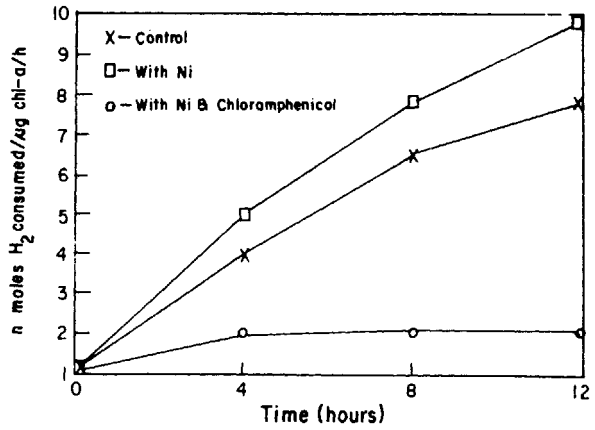

Figure 4 Time course of H_2 uptake activity in the presence of Ni (10 μM Ni was added to the assay medium at zero time)

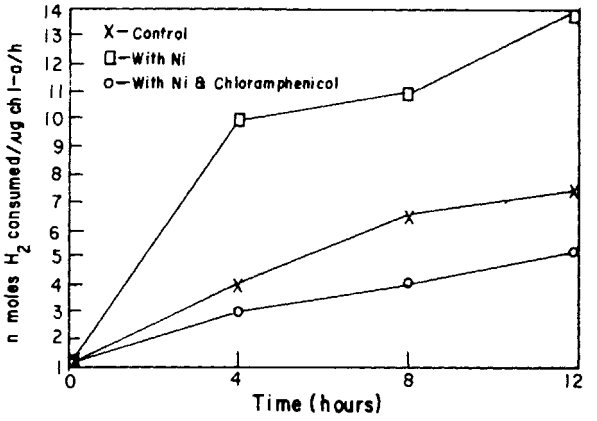

Figure 5 Time course of H_2 uptake activity in the cells grown under hydrogen atmosphere (Cultures were grown under H_2 atmosphere for 6 days and estimated for the uptake hydrogenase activity. 10 μM Ni and chloramphenicol were added at zero hour during assay conditions)

To determine whether Ni^{2+} ions are required for induction of H_2-uptake enzyme, the protein synthesis inhibitor chloroamphenicol (20 μg/ml) was added to the medium during assay conditions (figure 4). Chloramphenicol sharply reduced uptake hydrogenase activity, during the transition from Ni^{2+} deficiency to Ni^{2+} sufficiency. Since chloramphenicol was added during assay conditions (for a short duration of 4-12 hr), total protein content was not decreased significantly (results not shown). Cultures were also grown under hydrogen atmosphere with added Ni^{2+} and measured for the uptake hydrogenase activity, since growth under hydrogen atmosphere stimulates the uptake hydrogenase activity (Houchins 1984). Ni^{2+}-stimulated uptake hydrogenase activity was severely inhibited by chloramphenicol (figure 5). It appears that in general, the uptake hydrogenase is a Ni^{2+} containing enzyme but not the reversible hydrogenase. To substantiate the role of Ni^{2+} in the biosynthesis of the enzyme(s) involved in H_2 metabolism of nonitrogen fixing *O. subbrevis*, the cultures were grown for 6 days in the presence of 25 μM EDTA, which has been shown to effectively repress the synthesis

Table 2 *Influence of Ni^{2+} on H_2 production and H_2 uptake activities in* ***O. subbrevis***

Treatment		H_2 production (% activity)	H_2 uptake (% activity)
EDTA (25 μM)	Nickel (10 μM)		
—	—	100	100
+	—	58	59
+	—	75	98

100% (control without EDTA and nickel) = 320 n moles H_2/mg protein/hr(for H_2 evolution) and 462 n moles H_2/mg protein/hr (for H_2 uptake).
Cultures were grown for 6 days with 25 μM EDTA and 10 μM Ni^{2+} and assayed for hydrogenase activity.

of hydrogenase in several microorganisms (Pedrosa & Yates 1983). EDTA repressed the specific activity of both hydrogen uptake and hydrogen evolution by about 40%. Addition of Ni^{2+} to the medium relieved the repression of hydrogenase activity (table 2). Remarkably, with 10 μM Ni^{2+} in the medium, H_2 uptake was higher than the H_2 production. The total protein content was not affected by the addition of EDTA as reported earlier (Papen et al. 1986).

Discussion

Trace metals are absolutely essential for the healthy growth and proper functioning of biosynthetic pathways in photosynthetic microbes (Weissner 1962, O'Kelly 1974) at lower concentrations. Addition of Cu^{2+} to the medium inhibited the biomass yield and hydrogen production as well as hydrogen uptake. Though heavy metals, including Cu^{2+} are required for cyanobacteria, there are suggestions that Cu^{2+} predominantly inhibits the photosynthetic activity and photosynthetic pigment synthesis at about 4-8 μM concentration (McBrien & Hassel 1967, Gupta 1988).

Oxygen evolution and hydrogen uptake rates were more with increasing concentrations of Ni^{2+} (figures 2 & 3). Since uptake hydrogenase functions in the presence of oxygen via cytochrome containing electron transport system (Houchins 1984), this observation suggests that uptake hydrogenase gets activated in the presence of photosynthetically evolved oxygen. During H_2 formation, invariably oxygen would be evolved as a consequence of photolysis of water. However, the net hydrogen production is dependent on the local tensions of the oxygen which may regulate the hydrogenase activity either in the reversible mode or uptake mode. We found that not only did nickel ions decrease the hydrogen formation but also increase the degree of hydrogen uptake due to oxygen accumulation in the closed system.

Low levels of reversible hydrogenase activity is constitutively present in cyanobacteria (Houchins 1984), but not the uptake hydrogenase. Uptake hydrogenase activity can be induced by growing the cultures under hydrogen atmosphere. However, in contrast to the induction of uptake hydrogenase activity, growth under hydrogen atmosphere does not exhibit additional reversible hydrogenase activity (Houchins & Burris 1984). That hydrogen uptake was induced in the presence of Ni^{2+} and hydrogen atmosphere (figure 5) and was inhibited by chloramphenicol, suggests that Ni^{2+} ions are required for the induction of uptake hydrogenase activity.

Uptake hydrogenases of several bacteria have been shown to be dependent on the availability of Ni^{2+} in the growth medium (Graf & Thauer 1981, Freidrich et al. 1982). Though controversies exist regarding the requirement of Ni^{2+} ions for hydrogenase induction (Daday & Smith 1983), the present study shows that Ni^{2+} ions are required for an active uptake hydrogenase activity, since chloramphenicol reduced a signifiant amount of uptake hydrogenase activity (figure 4) and the repression of hydrogenase activity by EDTA was specifically relieved by Ni^{2+} (table 2). However it is not known, how the availability of Ni^{2+} in the growth medium controls the induction of active uptake hydrogenase or any other factor which may in turn activate the uptake hydrogenase. Though Ni^{2+} ions have been found to be essential for the synthesis of active hydrogenase in various bacterial sp., studies on *Anabaena cylindrica* (Daday & Smith 1983, Daday et al. 1985) suggests that Ni^{2+} ions are required for

hydrogen consumption. Since, Ni^{2+} stimulates uptake hydrogenase activity in cyanobacteria, it can be used as a probe in establishing the occurrence of uptake hydrogenase activity.

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