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STUDIES ON GROWTH RATE, FERRICYANIDE REDUCTION AND BIOANODIC PROPERTIES IN ANABAENA SP. AND NOSTOC SP.

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Introduction

Cyanobacteria are a diverse group of Gram – negative prokaryotes carrying out oxygenic – and anoxygenic photosynthesis, aerobic respiration or fermentation thus being able to growth in light or in darkness, either in aerobiosis or in anaerobiosis, in media with only N_2 as nitrogen source (Rippka et al. 1979; Zarnea 1983; Fay 1992). The most studied processes are photosynthesis, nitrogen fixation and, in the last decade after a silent period of about 30 years, aerobic respiration together with their relationship (Scherer et al. 1988).

Special emphasis was done on the use of dark/light incubation periods in order to manage the ability of cyanobacteria to reduce ferricyanide; these experiments are important for better understanding respiration – photosynthesis relationship and for the design of biosensors useful for environmental monitoring (Rawson et al 1987, Kreysa et al. 1990, Ardelean et al. 1992).

The general ability of bacteria to donate electrons to extracellular added redox carriers, namely bioanodic property (Bennetto 1984), because it is the basis for the ability of bacteria to act as biocatalysts in the anodic compartment of a bioelectrochemcal fuel cell (BECFC) (Allen 1972, Bennetto 1984, Ardelean and Zarnea 1990). In BECFC the electrons pass from the anodic compartment through the external circuit to the cathode compartment. The electric current thus generated is dependent on the metabolic activity from which the electrons are withdrawn (Bennetto 1984; Ardeleann et al. 1992). The magnitude of the curent being directly proportional to the metabolic status of the biocatalyst (Ardelean et al. 1983), any disturbance affecting respiratory or photosynthetic activity can be rapidly detected (Rawson and Gaisford 1990, Ardelean et al. 1992).

Here we present our preliminary results on the growth rate, ferricyanide reduction and bioanodic property of Anabaena sp. and Nostoc sp. grown with either N_2 or ammonium as sole nitrogen source.

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Materials and methods

Isolation and cultivation of cyanobacteria: cyanobacteria strains used in this study were isolated from soil and cultivated in BG_{11} with molecular nitrogen (nitrogen fixing conditions–NFC) or ammonium (nitrogen nonfixing conditions–NNFC) at 25°C under fluorescent white light of 1000 lux.

Purification and identification: selected strains are heterocystous cyanobacteria, with trichomes covered by a thick polysacharidic sheath in which there are heterotrophic bacteria. Axenic cultures were obtained following the method of Wieringa (1968) based on the heat resistance of ackinetes as compared with vegetative cells of heterotrophic unsporulated bacteria, which are normally found within the sheath of cyanobacteria (Wieringa 1968). The method involves the incubation of cyanobacterial suspension having ackinetes at 48–49°C for 60–75 minutes, followed by an inoculation in BG_{11} under NNFC. The cultures thus obtained are bacteria – free as demonstrated by inoculating them in nutrient broth supplemented with glucose (50 mM). This method is not useful to separate cyanobacteria from sporulated bacteria but it also works to separate heterocystous cyanobacteria from other cyanobacteria, unicellular or filamentous. The identification of selected strains was made following Rippka et al. (1979), only at the genus level, as being Anabaena sp. and Nostoc sp.

Determination of biomass was made using the gravimetric method (Malette 1969). 10 ml of bacterial culture centrifugated 10 minutes at 4000 × g were washed and the sediment resuspendent in distilled water in special vials and then dried at 105°C until they were no more variations of the dried biomass (around 20 hours).

The calculation of growth rate done according to the following equation: $\mu = \ln (OD_{i+1}/OD_i)(t_{i+1} - t_i)$ where t or OD indicates time and optical density (or corresponding biomass density) at the corresponding times of analysis (i or i + 1) (Hornsten 1992).

Bacteria immobilization and gross dehydrogenase activity (ferricyanide reduction by whole cells) were determined as previously described (Ardelean and Canja 1991) and BECFC design and operation were as previously reported (Ardelean et al. 1983).

Results and discussions

In figure 1 is presented the time–evolution of biomass density of Anabaena sp. grown with either N_2 or ammonium as sole nitrogen source.

In figure 2 is presented the results from a similar experiment carried out with Nostoc sp., the calculated growth rates being 0.039 h^{-1} and 0.0442 h^{-1} for NFN(·) and NNFC(×), respectively.

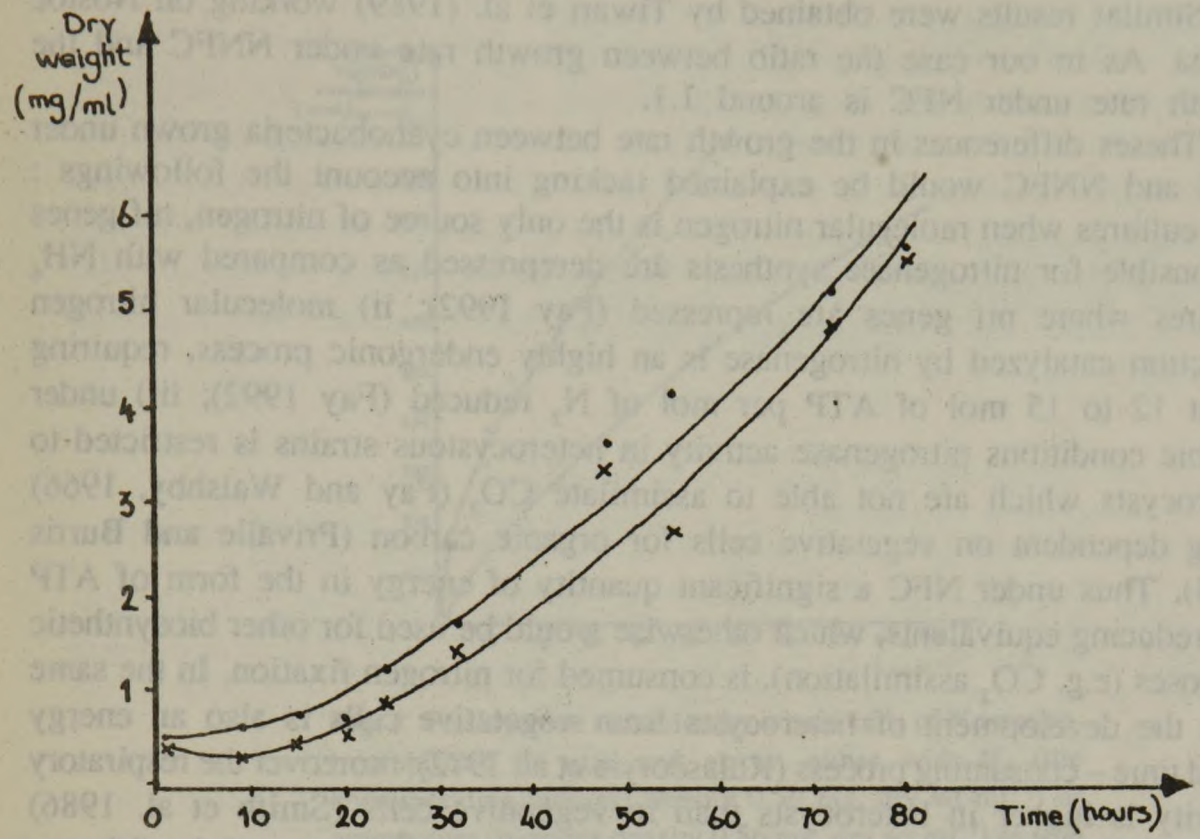

Fig. 1. The growth of Anabaena sp. with N_2 (·) or ammonium as sole nitrogen source (×).

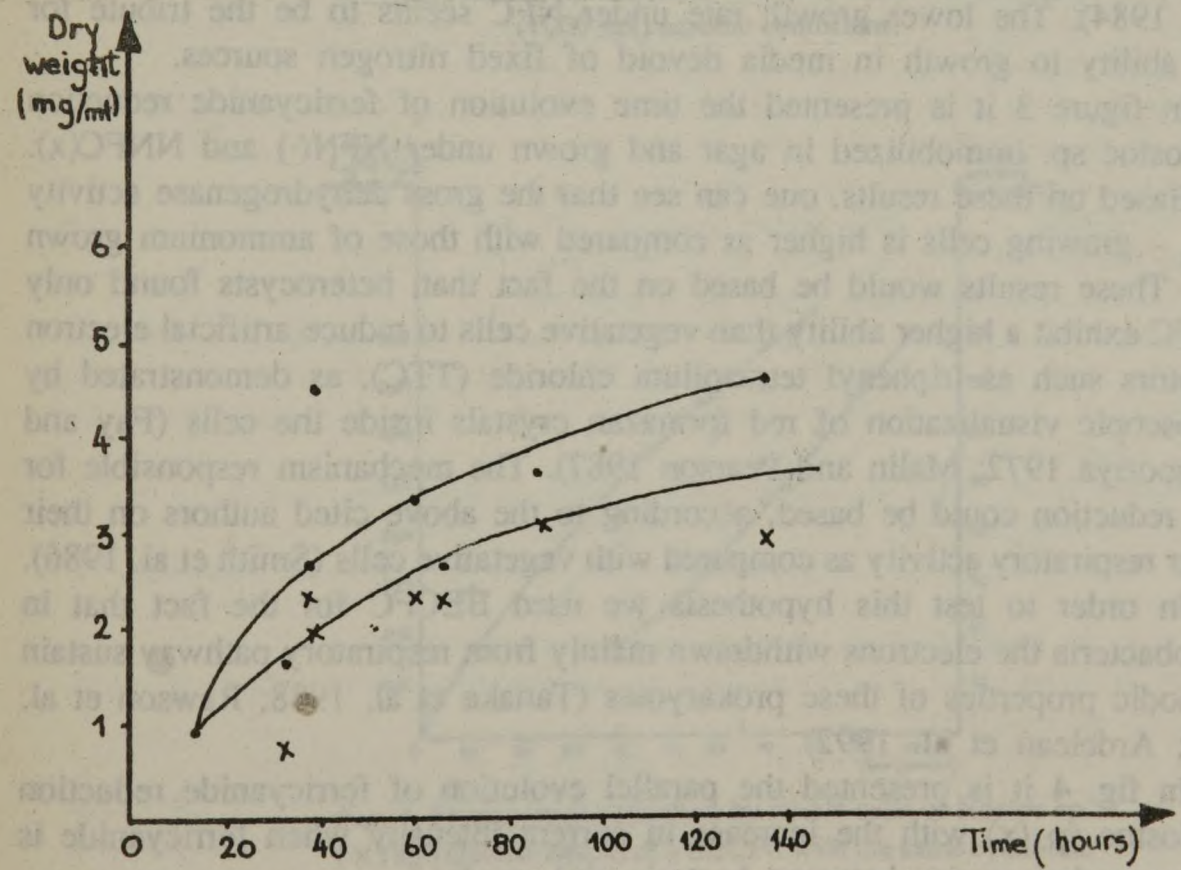

Fig. 2. The growth of Nostoc sp. with N_2 (·) or ammonium as sole nitrogen source (×).

Similar results were obtained by Tiwari et al. (1989) working on Nostoc linckia. As in our case the ratio between growth rate under NNFC and the growth rate under NFC is around 1.1.

Theses differences in the growth rate between cyanobacteria grown under NFN and NNFC would be explained tacking into account the followings : i) in cultures when molecular nitrogen is the only source of nitrogen, nif genes responsible for nitrogenase synthesis are derepressed as compared with NH_4 cultures where nif genes are repressed (Fay 1992); ii) molecular nitrogen reduction catalyzed by nitrogenase is an highly endergonic process, requiring about 12 to 15 mol of ATP per mol of N_2 reduced (Fay 1992); iii) under aerobic conditions nitrogenase activity in heterocystous strains is restricted to heterocysts which are not able to assimilate CO_2 (Fay and Walshby, 1966) being dependent on vegetative cells for organic carbon (Privalle and Burris 1984). Thus under NFC a significant quantity of energy in the form of ATP and reducing equivalents, which otherwise would be used for other biosynthetic purposes (e.g. CO_2 assimilation), is consumed for nitrogen fixation. In the same time the development of heterocysts from vegetative cells is also an energy – and time – consuming process (Kulasooryia et al. 1972); moreover the respiratory activity is higher in heterocysts than in vegetative cells (Smith et al. 1986) thus lowering the oxygen tension in order to promote nitrogenase activity (Murray et al. 1984). The lower growth rate under NFC seems to be the tribute for their ability to growth in media devoid of fixed nitrogen sources.

In figure 3 it is presented the time evolution of ferricyanide reduction by Nostoc sp. immobilized in agar and grown under NFN(·) and NNFC(x).

Based on these results, one can see that the gross dehydrogenase activity in N_2 – growing cells is higher as compared with those of ammonium grown cells. These results would be based on the fact that, heterocysts found only in NFC exhibit a higher ability than vegetative cells to reduce artificial electron acceptors such as triphenyl tetrazolium chloride (TTC), as demonstrated by microscopic visualization of red formazan crystals inside the cells (Fay and Kulasooriya 1972; Malin and Pearson 1987). The mechanism responsible for TTC reduction could be based, according to the above cited authors on their higher respiratory activity as compared with vegetative cells (Smith et al. 1986).

In order to test this hypothesis we used BECFC for the fact that in cyanobacteria the electrons withdrawn mainly from respiratory pathway sustain bioanodic properties of these prokaryotes (Tanaka et al. 1988; Rawson et al. 1987; Ardelean et al. 1992).

In fig. 4 it is presented the parallel evolution of ferricyanide reduction by Nostoc sp (x) with the increase in current intensity when ferricyanide is used as redox carrier between bacteria and anode(·).

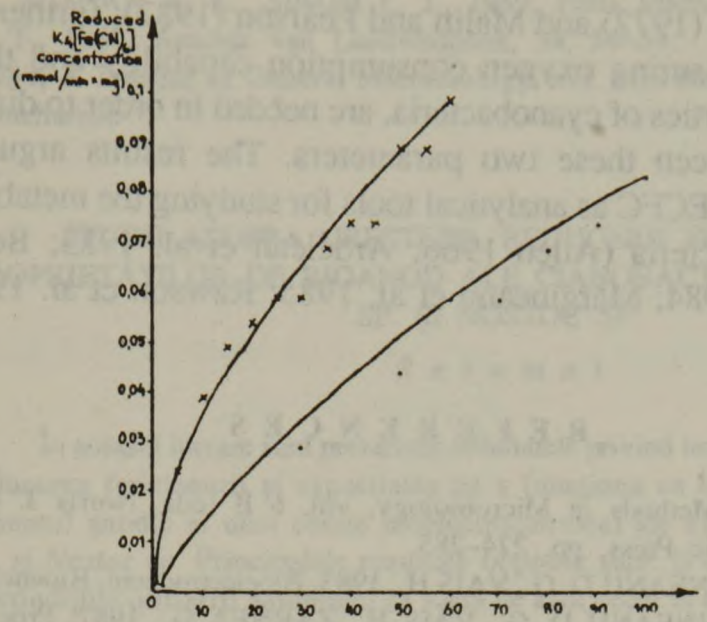

Fig. 3. Ferricyanide reduction by whole cells of Nostoc sp. immobilized in agar and grown either with N_2 (the corresponding biomass density 0,50 mg. dry wt ml^{-1}) or ammonium (biomass density 0,56 mg. dry wt ml^{-1}) as sole nitrogen source. The reaction was performed under light (1000 lux) aerobic conditions.

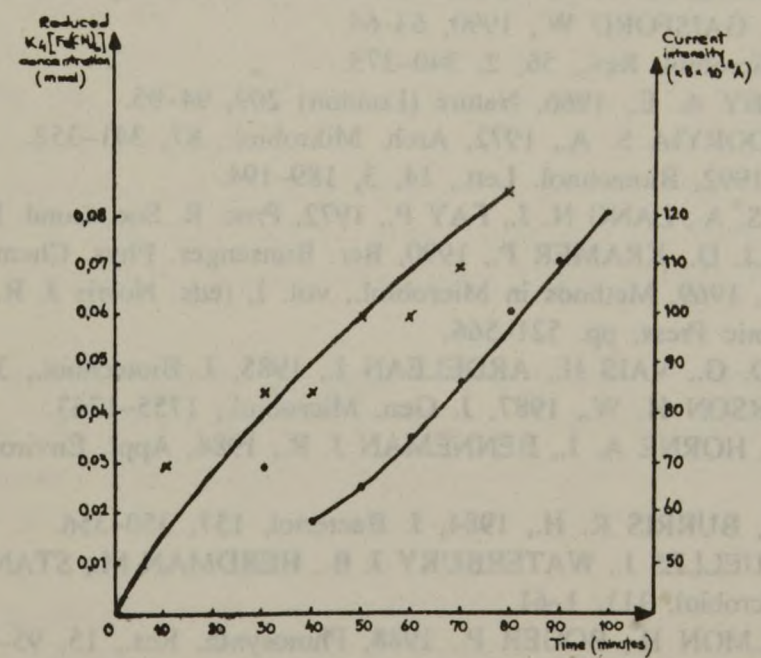

Fig. 4. Ferricyanide reduction by whole cells of Nostoc sp. (×) and electric output of a BECFC with the same cyanobacteria as biocatalyst in the anodic compartment (·).

These preliminary results are consistent with the hypothesis proposed by Fay and Kulasooriya (1972) and Malin and Pearson (1987); further experiments, especially those measuring oxygen consumption capability and the corresponding bioanodic properties of cyanobacteria, are needed in order to directly quantify the correlation between these two parameters. The results argue once again the potentialities of BECFC as analytical tools for studying the metabolic processes occurring within bacteria (Allen 1966; Ardelean et al. 1983; Bennetto 1984; Aston and Turner 1984, Mărgineanu et al. 1985; Rawson et al. 1987, Ardelean et al., 1987).

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STUDII ASUPRA CREŞTERII, REDUCERII FERICIANURII ŞI PROPRIETĂŢILOR DE BIOANOD ALE CIANOBACTERIILOR ANABAENA SP. ŞI NOSTOC SP.

Rezumat

În această lucrare sunt prezentate rezultatele privind unele caracteristici (creştere, reducerea fericianurii şi capacitatea de a funcţiona ca biocatalizator în compartimentul anodic al unei celule bioelectrochimice) ale cianobacteriilor *Anabaena* sp. şi *Nostoc* sp. Principalele rezultate obţinute sunt: i) creşterea este mai rapidă în condiţiile utilizării amoniului ca sursă de azot decât în condiţiile fixării azotului; ii) activitatea dehidrogenazică globală (reducerea fericianurii de către celulele intacte) este mai ridicată în culturile crescute în condiţiile fixării azotului molecular şi iii) la *Nostoc* sp. proprietatea de bioanod este determinată de către activitatea dehidrogenazică globală. Rezultatele, interpretate pe baza datelor din literatură, deschid posibilitatea modelării proprietăţii de bioanod a cianobacteriilor capabile de a diferenţia heterocisti, prin cultivarea lor cu N_2 ca unică sursă de azot.