EFFECT OF LIGHT ON THE PROCESS OF BIOLOGICAL NITROGEN FIXATION IN BLUE-GREEN ALGAE *NOSTOC GELATINOSUM* (SCHOUSB) ELENK.AND *CYLINDROSPERMUM LICHENIFORME* (BORY) KÜTZ. (CYANOPHYTA)

Sergiu DOBROJAN, Irina STRATULAT, Victor ŞALARU, Galina DOBROJAN
Moldova State University, Laboratory of "Algology", str. M. Kogâlniceanu 65A
sergiudobrojan84@yahoo.com

Abstract. Lighting regime directly influences the process of biological nitrogen fixation carried out by algae Nostoc gelatinosum (Schousb) Elenk. and Cylindrospermum licheniforme (Bory) Kutz. The natural light contributes to the intensification of biological fixation of atmospheric nitrogen in these algae, and the dark regime leads to inhibition the biological nitrogen fixation. Biological nitrogen fixation by blue-green algae achieved by Cylindrospermum licheniforme and Nostoc gelatinosum is a reversible self-regulating process.

Key words. biological nitrogen fixation, light regime, blue-green algae.

Introduction

Biological nitrogen fixation (BNF) is one of the most important natural process in biosphere. One of the opportunities for increased the BNF in soil is the application of nitrogen-fixing blue-green algae, that the are beneficial to apply as biofertilizers, because these algae can be grown in mass and requires low amounts of inoculum and a high rate of growth (8-13 g dry weight m^2 day^{-1}) [3, 4].

The BNF process is extremely complicated and dependent on the intensity of biotic and abiotic environmental factors. Light presents the factor that influence both photosynthetic and BNF to algae [6]. Research made by Hee-Mock Oh, J., Maeng, G-Yull Rhee demonstrates, that the process of biological nitrogen fixation in blue-green alga *Anabaena* sp. is directly dependent on the light regime. Thus, the ambient light stimulates both the BNF activity and photosynthesis, which is directly proportional [5]. Therefore, is important to study the effect of light on BNF made by blue-green algae *Nostoc gelatinosum* and *Cylindrospermum licheniforme* to determine the optimal intensity of light that stimulates the BNF. This research has a major interest in the practical use of these algae as soils biofertilizers with low humus concentration.

Materials and Methods

Experimented algae – Nostoc gelatinosum (Schousb) Elenk. and *Cylindrospermum licheniforme* (Bory) Kütz. were selected in pure culture from soil collected in the southern districts of the Republic of Moldova. Nowadays they are part of collection from laboratory of ,,Algology,, Moldova State University

The experiments were carried out in the laboratory at t 24-26˚C, for a period of 12 days. The varied lighting regime was obtained by cultivating at natural light to window of cultivation laboratory (summer, June, 2014), continuous illumination with 3 fluorescent lamps (120W/0,64m2 or 3000 lux) and in dark, in thermostat, with adjustable temperature, model TC-80 MC-2.

The algae *Nostoc gelatinosum* and *Cylindrospermum licheniforme* were cultivated using the periodically method on Drew nutritive medium, sterilized at t 160˚C for 45 min. Alga was inoculated in a dose of 0,4 g/l.

The process of biological nitrogen fixation – was studied by determining the NH_4^+, NO_3^-, NO_2^-, N – in algal biomass, fixed atmospheric nitrogen, total nitrogen and eliminated nitrogen in nutritive medium.

Total nitrogen in algal cells was determined using Nessler reagent – the method is based on measuring the intensity of color of the spectrometer analysis (UVvis + T80) at a wavelength of 420 nm [7]. Ammonia in nutritive medium was determined by spectrometry after Nessler method [1]. Nitrate was determined by spectrometry method as a result of the interaction of nitrate ions with phenol-disulfonic acid obtaining a yellow color [2]. The nitrites were determined spectrometry as a result of the interaction of *Griess-Ilosvay* reagentand nitrites [2]. The nitrogen in nutritive medium was determined by the amount of $N\text{-}NH_4^+ + N\text{-}NO_3^-$.

Total nitrogen was calculated by summing the N-total from algal biomass (mgN * algal biomass g) + $N\text{-}NH_4^+ + N\text{-}NO_3^- + N\text{-}NO_2^-$(from the nutrient medium).

The nitrogen eliminated in nutritive medium was calculated using the formula N.el (%) = (Ntm * 100) / (Nt-N0).

Where: Ntm – total nitrogen in nutritive medium, Nt – total nitrogen (algal cells + Ntm); No-total nitrogen from inoculum cells.

Fixed atmospheric nitrogen was determined according to the formula: Nf=(Ntb+Ntm)-N0, where: Ntb – total nitrogen of algal biomass; Ntm – total nitrogen of nutritive medium; No – total nitrogen from inoculum cells.

Mathematical processing of results – The obtained results were processed mathematical, using the program "Microsoft Office Excel 2013", determining the arithmetic mean – X and standard error – x.

Results and discussions

Studying the process of biological nitrogen fixation in nitrogen-fixing blue-green algae, can be carried by the nitrogen from nutritive medium. It is considered that ammonium ions are the form of nitrogen fixed from atmosphere by the algae.

Table 1.

Changes of ammonium ions in nutritive medium at cultivation of nitrogen-fixing algae *Nostoc gelatinosum* and *Cylindrospermum licheniforme*, mg/l

	Variations of lighting	Days of analysis				
		1	3	6	9	12
Cylindrospermum licheniforme	daylight, X±x	0	2,98±0,11	6,11±0,15	6,82±0,21	2,66±0,28
	continuous,X±x	0	2,34±0,11	2,04±0,09	3,3±0,1	2,56±0,18
	dark,X±x	0	5,11±0,18	8,41±0,28	8,94±0,97	10,76±0,1
Nostoc gelatinosum	daylight, X±x	0	2,98±0,11	2,07±0,09	3,85±0,02	2,13±0,1
	continuous,X±x	0	2,87±0,18	2,3±0,13	3,64±0,09	1,74±0,09
	dark,X±x	0	3,09±0,11	4,83±0,14	6,13±0,13	7,24±0,21

As we can see, from the data presented in tab. 1, the concentration of ammonium ions varies depending on the analyzed period and lighting regime. Cultivation the algae in the dark follows with accumulation of the highest amount of ammonium ions that are continuously growing. This, may be due to the alteration of algal biomass and their elimination in medium. In the case of algae cultivation under continuous light and daylight the ammonium ion concentration increases in the first days after follows an obvious decreasing. However, natural daily lighting contribute to setting the highest amounts of ammonium in both studied species.

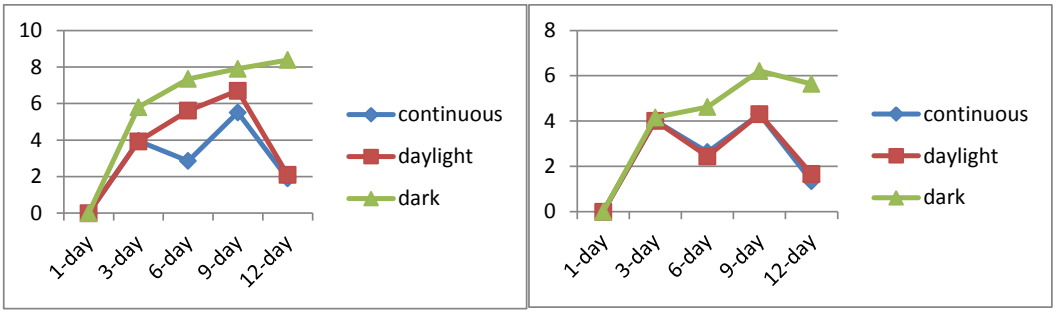
The nitrates result of oxidation process of ammonium ions and are a form of nitrogen accessible to algae.

Nitrates have the same tendency of increasing followed by decreasing. The highest nitrate concentrations is observed at cultivation *Nostoc gelatinosum* (7,97±0,16 mg/L) and *Cylindrospermum licheniforme* (13,07±1,79 mg/l) at continuous illumination (tab. 2). Daylight and darkness regime contributes to the accumulation of low quantities of nitrates in nutritive medium. Nitrites were not detected in nutritive medium, which denotes that, there is no process of denitrification.

Table 2.

Changes of nitrates in nutritive medium at cultivation of nitrogen-fixing blue-green algae *Nostoc gelatinosum* and *Cylindrospermum licheniforme*, mg/l

	Variations of lighting	Days of analysis				
		1	3	6	9	12
Cylindrospermum licheniforme	daylight, X±x	0	7,17±0,11	3,88±0,03	6,27±0,12	0,04±0,004
	continuous,X±x	0	9,45±0,4	5,68±0,75	13,07±1,79	0,04±0,003
	dark,X±x	0	8,13±0,44	3,64±0,12	4,26±0,42	0,17±0,003
Nostoc gelatinosum	daylight, X±x	0	7,55±0,12	3,7±0,09	5,86±0,18	0,034±0,001
	continuous,X±x	0	7,97±0,16	3,75±0,13	6,81±0,41	0,027±0,001
	dark,X±x	0	7,83±0,13	3,9±0,22	6,46±0,25	0,11±0,003



A B

Figure 1. **The influence of illumination on nitrogen in nutritive medium for cultivation of nitrogen-fixing algae *Cylindrospermum licheniforme*(A), and *Nostoc gelatinosum* (B), mg/l**

As we can see (fig.1), the lack of light generates the accumulation the highest quantities of nitrogen in the nutritive medium for cultivation the algae *Cylindrospermum licheniforme* and *Nostoc gelatinosum*. This, may be due to alteration of algal biomass to the 6th day. The daylight contribute to the accumulation of a higher amount of nitrogen in the nutritive medium accordance with the continuous light, especially in the case of cultivation the algae *Cylindrospermum licheniforme* and *Nostoc gelatinosum.*

For determination the potential biological nitrogen fixation, something extremely important is determining the atmospheric nitrogen fixed by alga.

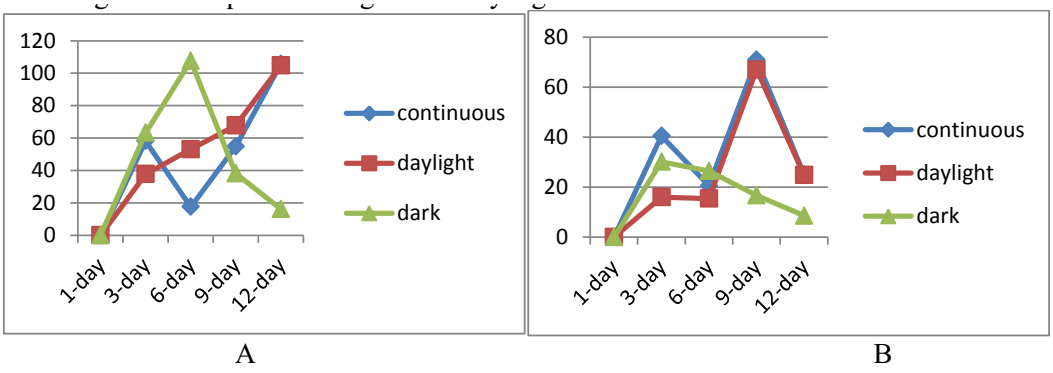


Figure 2. **Influence of illumination on atmospheric nitrogen fixed by algae *Cylindrospermum licheniforme*(A) and *Nostoc gelatinosum* (B), mg/l**

Prolonged exposure the blue-green algae *Nostoc gelatinosum* and *Cylindrospermum licheniforme* in darkness contribute to halting the process of biological nitrogen fixation from the 3rd to 6th day. Natural illumination contribute to fixing a large quantities of atmospheric nitrogen both in algae *Cylindrospermum licheniforme* (105,61±6,3 mg/l) and *Nostoc gelatinosum* (70,95±5,1 mg/l). The quantity of atmospheric nitrogen fixed by the algae *Cylindrospermum licheniforme* and *Nostoc gelatinosum* cultivated at continuous artificial lighting is slightly lower than in daylight.

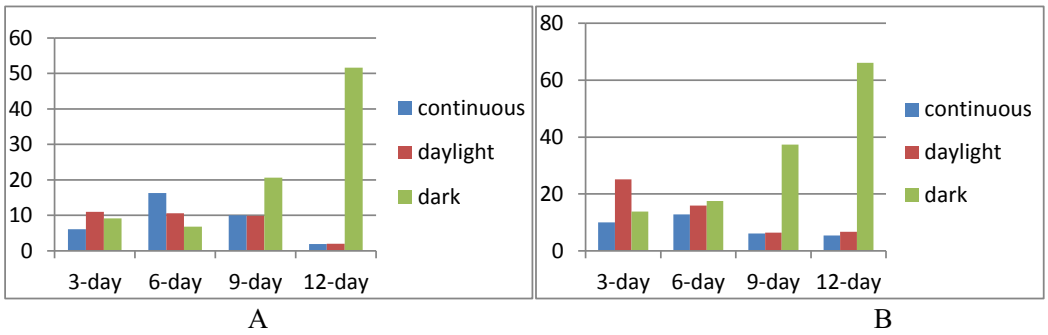


Figure 3. **Influence of illumination on the atmospheric nitrogen eliminated in nutritive medium by algae *Cylindrospermum licheniforme*(A), and *Nostoc gelatinosum* (B),%**

The highest amounts of atmospheric nitrogen eliminated in nutritive medium by *Cylindrospermum licheniforme* (51,62±1,1%) and *Nostoc gelatinosum* (66,13±1,5%) was certifies at cultivation in the dark at the 9th and 12th day. This is due to algal biomass alteration and elimination of cellular nitrogen in the nutritive medium. Natural lighting regime helps to eliminate the nitrogen quantities

slightly higher than the cultivation of algae in continuous illumination, which does not exceed 25,12±1,12% (fig. 3).

Conclusions

Lighting regime directly influences the process of biological nitrogen fixation achieved by blue-green algae *Cylindrospermum licheniforme* (Bory) Kütz. and *Nostoc gelatinosum* (Schousb) Elenk.. The natural illumination contribute to intensification of biological fixation of atmospheric nitrogen in the these blue-green algae and it accumulation in algal cells and eliminating into nutritive medium. Prolonged exposure the blue-green algae in darkness contribute to reducing the process of biological nitrogen fixation and nitrogen reduction in algal cells. Biological nitrogen fixation in blue-green algae *Cylindrospermum licheniforme* and *Nostoc gelatinosum* is a reversible self-regulating process.

References

1. Grigheli Gh., Şalaru V., Jigău Gh., Stasiev G., GalburăO. Analiza chimică a calităţii apei. Chişinău. 2006. 113 p.
2. Sandu M., Lozan R., Tărîţă A., Ropot V. Metode de instruire privind controlul calităţii apei. Chişinău. 2010. 173 p.
3. Boussiba S. (1991). Nitrogen fixing eyanobaeteria potential uses. J. Plant and Soil 137, p. 177-180.
4. Fontes A. G., Vargas M. A., Moreno J., Guerero M. G., Losada M. Factors affecting the production of biomass by a nitrogen-fixing blue-green alga in outdoor culture. Biomass 13, 1987, p. 33-43.
5. Hee-Mock Oh, J. Maeng, G-Yull Rhee. Nitrogen and carbon fixation byAnabaena sp. isolated from a rice paddy and grown under P and light limitations. Journal of Applied Phycology 3, 1991, p. 335-343.
6. Lewis WM Jr, Levine SN. The light response of nitrogen fixation in Lake Valencia, Venezuela. Limnol. Oceanogr., 1984, 29, p. 894-900.
7. Воскресенская О.Л., Алябышева Е.А., Половникова М.Г. Большой практикум по биоэкологии. Ч. 1: учеб. Пособие. Москва. 2006. 107 с.