Scientific Papers. Series A. Agronomy, Vol. LIX, 2016
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

STUDY OF THE BIOLOGICAL NITROGEN FIXATION PROCESS TO COMBINATION OF SOME BLUE-GREEN ALGAE FROM THE GENUS *NOSTOC*

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Abstract

This article exposes the results of studying the process of biological fixation of nitrogen to a combination of nitrogen-fixing algae species belonging to the genus Nostoc. For the achievement of research have been applied the differential method of studying biological fixation of nitrogen. The experimental results showed that combined application of species of nitrogen-fixing blue-green algae, which belong to the genus Nostoc is effective, helping to increase the quantitative amounts of fixed nitrogen, eliminating significant amounts of nitrogen and nitrogen content self-regulation in nutrient medium. Thus, we recommend applying nitrogen-fixing algae combinations that belong to the genus Nostoc in future research in the field.

Key words**:** *blue-green algae, combination, genus Nostoc, nitrogen fixation.*

INTRODUCTION

Biological nitrogen fixation is an important global process carried out by microorganisms that less reactive nitrogen, one of the most abundant components of the Earth's atmosphere, passes in inorganic compounds of nitrogen (Şalaru et al., 2013). Biological nitrogen fixation is carried on symbiotic and non-symbiotic way. Non-symbiotic nitrogen fixation is an important source of nitrogen, especially in ecosystems with a small number of plants capable of association with microorganisms for a symbiotic fixation (Samuel, 2007; Madjar and Davidescu, 2009). The nitrogen fixing blue-green algae are part of non-symbiotic group of organisms.

Many species of blue-green algae are able to fix atmospheric nitrogen, which places these algae among the most complete living organisms (Şalaru et al., 2013). Because of this property, the blue-green algae are widely used as bio-fertilizers in many regions of the world. Using the algal bio-fertilizers on the soils in Tundra region have as the result fixation up to 115 kg N/ha/year, in soils in Norway region - 1-2.5 kg N/ha/year, in Antarctica - 24 kg N/ha/year, in temperate region up to 49 kg N/year, and in tropical region to 90 kg N/ha/year (Grimm and Petrone, 1997).

In addition, application of algal bio-fertilizers contribute to improving the mechanical structure and physico-chemical properties of the soil, increasing the capacity of maintaining of soil water (Громов, 1996; Федоров, 1955), reducing concentrations of toxic elements, such as iron (II) and sulfur (with 16.82 to 24.6%), oxidable organic matter, Al_2O, the increase of organic carbon concentration (1.15 times) and increase crop productivity (Venkataraman, 1981). Algal bio-fertilizers application is of interest and for quantitative reduction of greenhouse gas concentration (such as N_2O).

One of blue-green algae taxon, as a major practical importance is the genus *Nostoc*. Species of genus *Nostoc* have morphological and physiological characteristics that advantage in their practical application as bio-fertilizers. These features are substantiated by the following advantages: the ability of heterocysts forming responsible for nitrogen fixation, filamentous forms of talus**,** vegetative cells undifferentiated functional, mode of reproduction by hormogones and spores which at times stemming from heterocysts, formation of macroscopic colony or less microscopic embedded in gelatinous mass, low humidity and light resistance, the ability to easily accommodate at the different types of soil (Dembitsky and Ezanka, 2005; Еленкин, 1949,

Зенова and Штина, 1990), as well as other increased resistance to adverse environmental conditions. In addition, the algae species from genus *Nostoc* are cosmopolitan, widespread in the regions tropical, temperate and polar terrestrial ecosystems (Potts, 2000). Many species of nitrogen-fixing algae, from genus *Nostoc* form symbiotic relationships with fungi, lichens and many phylogenetic groups of higher plants (Meeks, 1998; Rai et al., 2000), and gelatinous mucilage serves as a living place of many bacteria and unicellular algae (Зенова and Штина, 1990).

An important part in implementing the algae as bio-fertilizer is the selection of administrated species. Our previous research showed that the lots combined administration of several species of blue-green algae from genera *Nostoc*, *Cylindrospermum*, *Anabaena* and *Anabaenopsis* in quality of bio-fertilizers in the cultivation of cucumbers, we obtained a larger amount of nitrogen accumulated in the soil and increase harvest vegetables compared to the control where the algalization of soil was achieved only one species (Доброжан et al., 2014).

Selection the species of algal bio-fertilizers that will be combined must be done rationally and well scientifically proven to get the best results in practical application. Therefore, the first researches for combining the species of algae, selected as bio-fertilizers, must been carried out under laboratory conditions on liquid nutrient media.

The research purpose is to study biological nitrogen fixation process in laboratory conditions, at combined administration of algal species of the genus Nostoc.

MATERIALS AND METHODS

The experimented algae - were used three species of the genus *Nostoc* from the collection of laboratory of "Phycology", State University of Moldova.

The experiments were carried out under laboratory conditions at 27-29°C, for a period of 15 days of continuous illumination (120 watts/0.64 m^2). The genus *Nostoc* algae were grown after periodic method on liquid nutrient medium Drew, preventive sterilized using the ultraviolet lamp (Dobrojan et al., 2016). Inoculum consisting of an equal combination to three nitrogen-fixing species and was administered at a dose of 003 g/l recalculated at absolutely dry biomass (BAU).

The process of biological nitrogen fixation was been studied by determining NH_4^+, NO_3^-, total N in algal biomass, fixed atmospheric nitrogen, total nitrogen and nitrogen removed in the nutrient medium. Total nitrogen in algae cells, ammonia and nitrate was been determined by the spectrophotometric method (Воскресенская et al., 2006; Sandu et al., 2010).

Total nitrogen was calculated by summing the total N from biomass (mg, N * algal biomass, g) + $N\text{-}NH_4^+$+$N\text{-}NO_3^-$+$N\text{-}NO_2$- (from nutrient medium). Removed nitrogen was calculated by applying the formula: N. el (%) = (N tm * 100) / (Nt-N0), where: N tm – total nitrogen in nutrient medium, Nt - total nitrogen (N algal cel. + N tm); No- total nitrogen of inoculum. Fixed atmospheric nitrogen was determined according to the formula: Nf = (N tb+N tm)-N0, where: Ntb - total nitrogen in algal biomass; Ntm - total nitrogen in the nutrient medium; No - total nitrogen of inoculant cells.

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

RESULTS AND DISCUSSIONS

One of the forms of accessible nitrogen for higher plants and many microorganisms is ammonium ions. It is been considered that ammonium ions are the form of nitrogen resulting from the biological fixation of atmospheric nitrogen conducted by algae. Therefore, this indicator should have be monitored to assess how the implementation process of biological nitrogen fixation.

Table 1. Changes of ammonia nitrogen ($N\text{-}NH_4^+$) in the nutrient medium for cultivation of algae from genus *Nostoc*, mg/l

Days of analysis	Concentration N- NH_4^+, mg/l (X±x)
1	0
3	1.39±0.12
6	1.20±0.06
9	0.57±0.06
12	1.65±0.12
15	2.08±0.05

As we can see from the presented data in Table 1, ammoniacal nitrogen concentration varies depending on the day of analysis. On the 3rd day showed an increase of ammonia, and then reduce until the 9th day, followed by an increase to the 15th day. This indicates that the algae fix nitrogen up to a limit, after which produce the consumption, the process is continuously repeated.

Ammonium ions from biological nitrogen fixation result oxidizes and turns into nitrates, according to the reaction: $NH_4 + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O$ + Energy. As is well known, oxidation of 1 g of N-NH_4 consumed 4.6 g of O_2 and excessive production of acids (1 mole of N-NH_4 forms 2 moles of H^+) (Belingher and Chimerel, 2011). Doing a theoretical calculation, appears that the genus *Nostoc* species produced quantities of oxygen required to reduce the amount of ammonium ions detected in nutrient medium, ranging from 2.62 to 9,57 mg/l, which shows yet another argument for the practical implementation algal bio-fertilizers - to increase the quantity of oxygen in the soil.

Table 2. Changes of N - NO3 in nutrient medium at the cultivation *Nostoc* species, mg/l

Days of analysis	Concentration N - NO_3^-, mg/l (X±x)
1	0
3	0.01±0.001
6	0
9	0
12	0.68±0.03
15	0.40±0.04

As we can see, the concentration of nitrate ions is not in strict dependence on the ammonium ions. The largest quantities of nitrates is been observed during the period when the amount of ammonium is high. Some days of analysis (6'th and 9'th) were not detected nitrates in nutrient medium (Table 2). This can be caused by consumption of nitrate by vegetative cells of algae which can consume both forms of ammonium and nitrates ones.

As we can see, the amount fixed atmospheric nitrogen has the tendency of increasing followed by decrease but the biomass grow exponentially. The highest amount of atmospheric nitrogen fixed by algae attesting at the 9th day reaching 34.67±1.12 mg/l BAU, where biomass increased by 2.5 times (compared to the results obtained from third day of measurement).

This amount of nitrogen at the 9'th day has allowed its consumption by vegetative cells and increase biomass algal until the 12th day, after which insufficient amount of nitrogen and increased demand for nitrogen of vegetative cells had stimulated the initiating process of fixing nitrogen (Figure 1). This allows us to conclude that algae of genus *Nostoc* possesses the property of fixing atmospheric nitrogen but also to consume it for the increase of algal biomass, thus creating a balance of nitrogen concentration in the nutrient medium. Is worth noting that results at combining species of the genus *Nostoc* are higher than using separate specie of *Nostoc flagelliforme*, where the total amount of fixed nitrogen reached maximum 11.52 mg/l (Dobrojan et al., 2014).

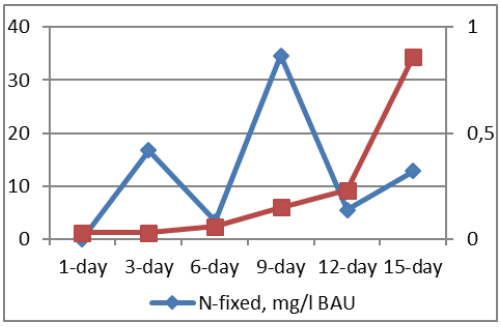

Figure 1. Fixed atmospheric nitrogen and the biomass growth of blue-green algae from genus *Nostoc*

Eliminating activity of nitrogen in nutrient medium, as well as nitrogen fixation, have tends to increase followed by decrease. Nitrogen eliminating occurs when nitrogen fixation activity is lower (at 6'th and 12'th day) (Figure 2), which shows that after nitrogen fixation following the elimination of in nutrient medium. Combined administration of algae from genus *Nostoc* has the effect of eliminating a higher amount of nitrogen (up 42.16%) than the separate administration of *Nostoc gelatinosum* specie (up 12.5%) (Dobrojan et al. 2014).

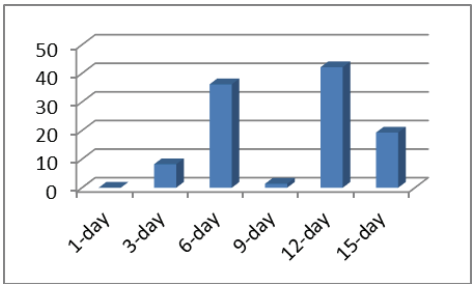

Figure 2. Eliminated nitrogen in nutrient medium for cultivation the algae from genus *Nostoc*, %

CONCLUSIONS

Combined administration of algal species from the genus *Nostoc* contribute to the accumulation of significant amounts of ammonia nitrogen in the nutrient medium and effectively fixes atmospheric nitrogen (34.67 ± 1.12 to mg N/l BAU). The fixed nitrogen is removed in nutrient medium at the algae cultivation, then the process is stopped, and the eliminated nitrogen is used by the vegetative cells growth and increase the biomass, after that the process is initiated again. This indicates that biological nitrogen fixation performed by combinations of algae from genus *Nostoc* is a self-regulating process. We found that conducted research by combinations of algae from the genus *Nostoc* can be applied in future field research.

REFERENCES

Belingher M.L., Chimerel M.E., 2011. Sursele de azot şi bazele procesului de nitrificare - denitrificare. Analele Universităţii "Constantin Brâncuşi" din Târgu Jiu, Seria Inginerie, Nr. 2: 196-203.

Dembitsky V.M., Ezanka T., 2005. Metabolites produced by nitrogen-fixing *Nostoc* species. Folia Microbiol. 50 (5): 363-391.

Dobrojan S., Stratulat I., Şalaru V., 2014. The influence of light on biological nitrogen fixation to alga *Nostoc flagelliforme*. 2nd International Conference Microbial Biotehnology - scientointensiv domain of modern knowledge, p. 41-45.

Dobrojan S., Stratulat I., Şalaru V., Dobrojan G., 2014. Effect of light on the process of biological nitrogen fixation in blue-green algae *Nostoc gelatinosum* (Schousb) Elenk.and *Cylindrospermum licheniforme* (Bory) KÜTZ. (Cyanophyta). V International Conference Actual Problems in Modern Phycology, p. 45-49.

Dobrojan S., Şalaru V., Şalaru V., Melnic V., Dobrojan G., 2016. Cultivarea algelor. CEP USM, Chişinău.

Grimm N.B., Petrone K. C., 1997. Nitrogen fixation in a desert stream ecosystem. Biogeochemistry Vol. 37, No. 1:33-61.

Madjar R., Davidescu V., 2009. Agrochimie. Bucureşti.

Meeks J.C., 1998. Symbiosis between nitrogen-fixing cyanobacteria and plants. BioScience, 48: 266-276.

Potts M., 2000. *Nostoc* in the ecology of cyanobacteria: their success in time and space. Klu-wer Academic Publishers, Dordrecht: 465-504.

Rai A.N., Söderbäck E., Bergman B., 2000. Cyanobacterium–plant symbioses. New Phytol,147: 449-481.

Samuil C., 2007. Tehnologii de agricultură ecologică. Iaşi.

Sandu M., Lozan R., Tărîţă A., Ropot V., 2010. Metode de instruire privind controlul calităţii apei. Ericon SRL, Chişinău.

Şalaru V., Bulimaga V., Şalaru V., Trofim A., Zosim L., Pisov M., 2013. Rolul unor alge cianofite azotfixatoare în rezolvarea problemei alimentare. Studia universitatis moldaviae, nr.6(66): 33-41.

Venkataraman G.S., 1981. Blue-green algae for rice production-a manual for its promotion. Italy, FAO, Rome.

Воскресенская О.Л., Алябышева Е.А., Половникова М.Г., 2006. Большой практикум по биоэкологии. Ч. 1: учеб. Пособие. Марийский государственный университет..

Громов Б., 1996. Цианобактерии в биосфере. Соросовский оброзовательный журнал. nr. 9: 33-39.

Доброжан С.,Шалару В.В., Шалару В.М., Стратулат И., Семенюк Е., 2014. Использование некоторых видов сине-зелёных азотфиксирующих водорослей в качестве биологического удобрения при культивировании огурцов. Algologia, 24 (3): 418-421.

Еленкин А.А., 1949. Синезеленые водоросли СССР. Специальная часть 2. АИСССР, Москва-Ленинград.

Зенова Г.М., Штина Э.А., 1990. Почвенные водоросли. Изд-во МГУ, Москва.

Федоров М., 1955. Микробиология. Государственное издательство сельскохозяйственной литературы, Москв.