DEPENDENCE OF AZOTOBACTER CHROOCOCCUM CULTURE GROWTH RATE ON SALT CONCENTRATIONS

Yekaterina Astafyeva, Kamila Shalabayeva
M. Auezov South-Kazakhstan State University
astafyeva_sksu@mail.ru

Abstract. Biofertilizers are nutrient availability systems in which biological processes are involved. Being a cost effective, eco-friendly and renewable source of land nutrient and playing a vital role in maintaining a long term soil fertility and sustainability, at the same time reviving the soil health and living on alternate source biofertilizers have become an essential concept, which can be a good supplement for chemical fertilizers. Different factors affect the growth of *Azotobacter chroococcum*, including the temperature, oxygen and salt concentration, etc. During these cultivations, increase of productivity was studied as a bioprocess efficiency parameter compared with different media components ratio. Comparing with the Modified Ashby's medium, which showed $5.1 \cdot 10^8$ cells per ml after 72 hours, in the medium with a higher amount of dipotassium hydrogen phosphate the growth rate of *Azotobacter chroococcum* biomass reached the maximum rate $11.0 \cdot 10^8$ cells per ml after the same period, thereby showing significant acceleration of the process, which may be used to improvelarge scale biofertilizer production technology by reduction of the biomass producing time.

Keywords: *Azotobacter chroococcum*, modified media, inoculation, biofertilizers.

Introduction

Indiscriminate use of chemical pesticides contributed in loss of soil productivity along with addition of salts to the soil [1]. The growing need for supply of agronomic products for food and consumer good processing by the modern society have caused substantial increases in agrarian activities in recent decades. As a result, the need for implementation of methods that allow, among other things, to improve the efficiency of crops, mitigate adverse impacts on the soil, reduce the use of chemical fertilizers, and increase revenues per cultivated area, has been addressed. For this reason, the implementation of conservative agriculture models has been a cornerstone of farming practices globally. The conservative agriculture focuses on reducing adverse impacts on the environment, increasing crop yields and inputs, and implementing sustainable techniques for development of agriculture [2; 3].

Biofertilizers may be an alternative to chemical fertilizers. One of the most prospective biofertilizers increasing the crop productivity are fertilizers based on *Azotobacter chroococcum*, which penetrates into the plant tissue without harming the latter and supports the plant with fixed nitrogen. Furthermore, this microorganism supports plants with phytohormones (auxins), vitamins and phytoncides, which immunize the plant by protecting it from both the infection from the soil and against harmful insects. Fig. 1 shows the view of *Azotobacter chroococcum* cells under electron microscope, magnified 1000 times.

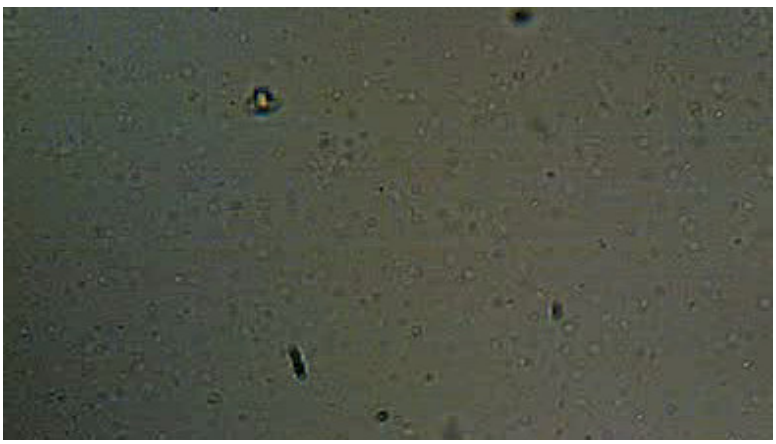

Fig. 1. **Cells of *Azotobacter chroococcum*, ×1000**

Different factors affect the growth of *Azotobacter chroococcum*, including the temperature, oxygen concentration and medium composition, including salt concentration, etc.Previous researches

show that the composition of medium influences the growth of *Azotobacter chroococcum*, which can be used for improvement of large-scale production of biofertilizers. This work was designed to study the influence of different salts on the growth of *Azotobacter chroococcum*and for obtaining the most suitable medium composition for the bacterial biomass production.

According to Nandkar media provide nutrients that enhance the growth and predominance of a particular type of bacterium and do not increase other types of organisms that may be present. It was reported aboutthe requirement of sodium chloride and magnesium sulphate in Jensen's medium, 0.5 $g \cdot l^{-1}$. *Azotobacter* had shown maximum growth at higher concentration of sodium chloride 1.1 $g \cdot l^{-1}$ and at lower concentration of magnesium sulphate observed at 0.3 $g \cdot l^{-1}$ [4].

Juarezobserved that in chemically defined medium containing protocatechuicacid or p-hydroxybenzoic acid, the growth of *Azotobacter chroococcum* was increased according to the concentration of phenolic acid [5].

The growth of *Azotobacter chroococcum*is affected by temperature that has to be in the range of 28 to 32 ºC, while the pH should be kept between 7.0 and 7.5. Dissolved oxygen concentration and medium composition have also considerable impact on the bacterial growth [6]. Some nitrogen containing salts promote bacterial growth by reducing the duration of the lag phase. In the presence of high carbohydrate concentration in broth (>30 $g \cdot l^{-1}$), *Azotobacter chroococcum* accumulates significant quantities of intracellular energy storage material (polyhydroxybutyrate; PHB). PHB synthesis in *Azotobacter chroococcum*is also stimulated by cultivation in oxygen-limited conditions [7-10].

Microorganism, maintenance and growth conditions

*Azotobacter chroococcum*N1 strain (Laboratory of Molecular Biologyand Genetics of Microorganisms, Microbiology Institute of Uzbekistan, Tashkent) was used in these experiments. The culture was maintained on Modified Ashby's medium [11].

*Azotobacter chroococcum*was cultivated in 500 ml Erlenmeyer flasks with200 ml of broth (180 ml of medium and 20 ml of inoculum). In all media, the pH was adjusted to 7.0.The flasks were incubated at 28 ºC for 72 h on a rotary shaker (180 rpm). The media were sterilized at 121 ºC for 20 min. The solutions of salts were sterilized separately and then mixed prior to inoculation. Samples were taken after 72 h.

Modified Ashby'smedium was used ($g \cdot l^{-1}$):

- Medium I, sucrose – 20; $MgSO_4 \cdot 7H_2O$ – 6; $Na_2MoO_4 \cdot 2H_2O$ – 0.005; K_2HPO_4 – 2.4; K_2SO_4 – 0.1; NaCl – 0.2; $FeCl_3$ – 0.01; $CaCO_3$ – 5.
- Medium II, sucrose – 20; $MgSO_4 \cdot 7H_2O$ – 0.2; $Na_2MoO_4 \cdot 2H_2O$ – 0.005; K_2HPO_4 – 6; K_2SO_4 – 0.1; NaCl – 0.2; $FeCl_3$ – 0.01; $CaCO_3$ – 5.
- Medium III, sucrose – 20; $MgSO_4 \cdot 7H_2O$ – 0.2; $Na_2MoO_4 \cdot 2H_2O$ – 0.005; K_2HPO_4 – 2.4; K_2SO_4 – 6; NaCl – 0.2; $FeCl_3$ – 0.01; $CaCO_3$ – 5.

The pH was adjusted to 7.0.The flasks were incubated on a rotary shaker (180 rpm) at 28 ºC for 72 h. The media were sterilized for 20 min at 121ºC. After 72 h samples were taken and the number of *Azotobacter chroococcum*cells was counted with the help of a hemocytometer under the microscope. The counting was provided by calculating the cell concentration per ml based on the numbers obtained from 2 different squares to exclude the sampling error [12].

Results and discussion

During these cultivations, increase of productivity by the cell growth rate was studied as a bioprocess efficiency parameter compared with different media components ratio.*Azotobacter chroococcum* cells were counted with the help of a hemocytometer under the microscope.

The dependence ofthe growth rateof *Azotobacter chroococcum* microorganism on salt concentrations is shown in Fig. 2.

According to the obtained results, which are shown in the given table, the cultivation with a higher K_2HPO_4 salt content was the most suitable for the bacterial biomass production.

Medium composition defining suitable for *Azotobacter chroococcum* biomass producing at large scale was the main goal of this investigation. Modified Ashby's medium and complex media were used for *Azotobacter chroococcum* cultivation to define the benefits of the media. All media contained sucrose as a carbon source and inorganic salts as a source of microelements that are important for the growth of *Azotobacter chroococcum*. In preliminary researches, *Azotobacter chroococcum*was cultivated on chemically defined media with different sucrose concentration (9.5 to 38.1 $g \cdot l^{-1}$). On the basis of these results, the medium with 20 $g \cdot l^{-1}$ of sucrose was selected for further experiments in which the effect of the concentration of inorganic salts on the growth was examined [13-16].

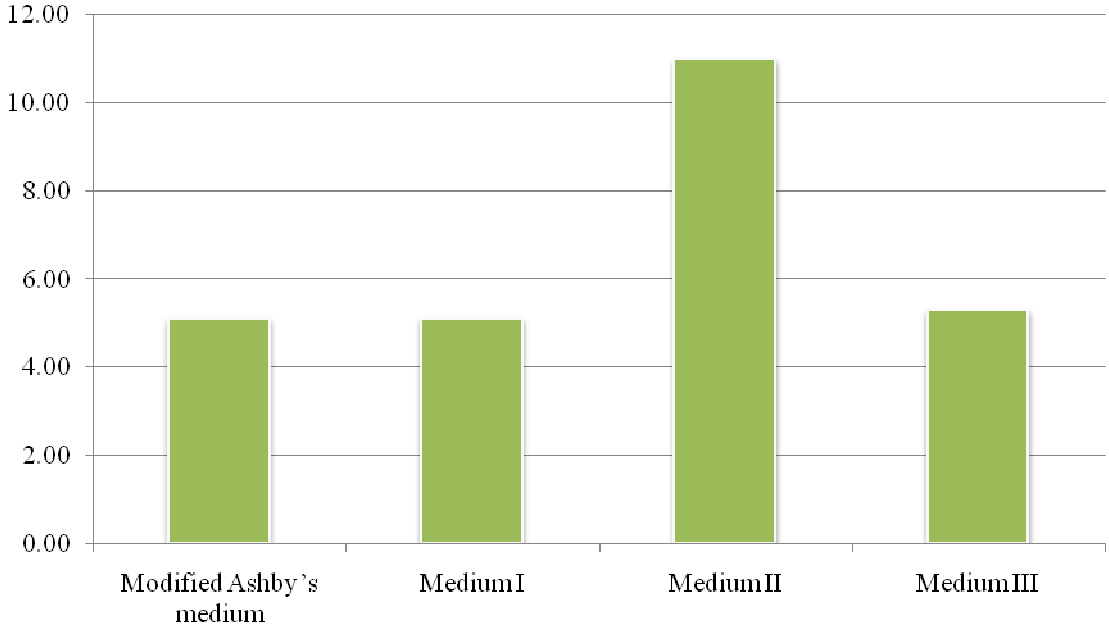

Fig. 2. **Dependence of the growth rate on salt concentrations**

In previous researches the growth of the organism was maximal at 2.0 $g \cdot l^{-1}$ K_2HPO_4 [17]. Similar results have been reported by Borah,etal., 2002 where they found that the growth of the organism was maximal at 2.0 $g \cdot l^{-1}$ K_2HPO_4[18]. These results are in good conformity with the finding of the Medium II as the most suitable for *Azotobacter chroococcum* cultivation, including, sucrose – 20; $MgSO_4 \cdot 7H_2O$ – 0.2; $Na_2MoO_4 \cdot 2H_2O$ – 0.005; K_2HPO_4 – 6; K_2SO_4 – 0.1; NaCl – 0,2; $FeCl_3$ – 0.01; $CaCO_3$ – 5.

Increasing of $MgSO_4 \cdot 7H_2O$ and K_2SO_4 salts did not influence the increase of the growth rate of *Azotobacter chroococcum* microorganism. Therefore, this concentration of$MgSO_4 \cdot 7H_2O$ and K_2SO_4 salts (0.2 $g \cdot l^{-1}$ and 0.1 $g \cdot l^{-1}$) which proved to be the optimum from earlier investigations, was used in the following experiments [19].

The growth rate of *Azotobacter chroococcum*reached $11 \cdot 10^8$ cells per ml using the medium II, while the media I and III showed the results $5.1 \cdot 10^8$ and $5.3 \cdot 10^8$ cells per ml. Comparing with the Modified Ashby's medium, which showed $5.1 \cdot 10^8$ cells per ml, in the medium II the growth rateof *Azotobacter chroococcum* biomass reached the maximum rate$11 \cdot 10^8$ cells per ml, that can be used at large scale production of biofertilizers for acceleration of the process.

Furthermore, the activity and viability of the microorganisms in creased that had been tested by seed treatment with biological fertilizer, which included *Azotobacter chroococcum* as a basis. The experiments were provided on the basis of the South Kazakhstan State University and microbiological company "BioZherKushi". The results of this scientific work were patented by the Ministry of Justice of the Republic of Kazakhstan and were approbated in industrial scale at microbiological company "BioZherKushi" [20; 21].

According to Bandhu, inoculation with *Azotobacter* significantly increases the plant height, grain and stover yield of maize. Only inoculation of *Azotobacter* increased the maize grain yield up to 35 % over non inoculated treatment. The benefit of *Azotobacter* inoculation was higher when the chemical fertilizer was not used. Therefore, it was concluded that *Azotobacter* could be one of the biofertilizer

options for sustainable and environmental eco-friendly maize production where the chemical fertilizer is limited [22].

Abd El-Lattiefobserved that the use of biofertilizers became ineludible to minimize the environmental pollution, caused by the chemical ones, and to improve the yield quality of various crops needed at the time being. Although 25 or 50 % of mineral nitrogen was replaced by biofertilizers (double-inoculation of *Azotobacter* and *Azospirillum*), the yield and its components of wheat increased compared to that obtained with the recommended dose of mineral nitrogen. Finally, biofertilizers of efficient strains could save 25 or 50 % of the recommended dose of mineral nitrogen [23].

The influence of biofertilizer application on the yield contributing characters of plants was also studied by Wayase and Bhalekar. According to the researches *Azotobacter* application significantly enhances the seed, straw and biological yield [24].

Conclusions

Dissolved oxygen concentration and medium composition have considerable impact on the bacterial growth. In this work *Azotobacter chroococcum*was cultivated using different media components ratio in order to optimize the biomass obtaining for large scale production of biofertilizer. Comparing with the Modified Ashby's medium, which showed $5.1 \cdot 10^8$ cells per ml after 72 hours, in the medium with a higher amount of dipotassium hydrogen phosphate the growth rate of *Azotobacter chroococcum* biomass reached the maximum rate $11.0 \cdot 10^8$ cells per ml after the same period of time, thereby showing significant acceleration of the process tending to increase the growth rate of biomass production, which might play a significant role in producing good quality planting stock. It could be concluded that medium composition have profound effects on the microbial growth of *Azotobacter chroococcum*, however, there is still a need to investigate the effects of the salt concentration on the microorganism ability to fix atmospheric nitrogen.

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