 ISSN 2522-1841 (Online)
ISSN 0005-2531 (Print)

AMINO ACID AND SYNTHETIC MOLYBDATE (MOO4-2) CHELATES AND THEIR BIOAVAILABILITY TO AZOTOBACTER BACTERIA AT DIFFERENT TEMPERATURES

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Received 12.06.2026
Reviewed 19.07.2026
Accepted 19.08.2026

The efficiency of biological nitrogen fixation by *Azotobacter* bacteria depends on the bioavailability of molybdenum, which is a key microelement of nitrogenase, and varies under conditions of temperature stress in regions with a temperate climate. The study aimed to compare the effectiveness of different chelated forms of molybdenum in supporting the growth, metabolic activity, and nitrogen-fixing ability of *Azotobacter chroococcum* bacteria under temperature stress conditions. The study was conducted *in vitro* on Ashby medium. Five sources of molybdenum were evaluated: unchelated sodium molybdate, amino acid chelates and synthetic chelates. Bioavailability was assessed by growth intensity, nitrogen accumulation in biomass, nitrogenase activity and total protein content. The results showed that amino acid chelates, especially histidine molybdate, provided the highest bioavailability of molybdenum. At optimal temperature, this option showed a 30.7% increase in bacterial growth intensity, a 37.1% increase in nitrogen accumulation, and a 40.2% increase in nitrogenase activity compared to the non-chelated form. Under conditions of temperature stress (18°C), the advantage of amino acid chelates was even more pronounced: the decrease in nitrogen fixation efficiency was only 40.0% for histidine molybdate versus 48.4% for non-chelated molybdate. The study established that amino acid chelates not only improve molybdenum transport into cells but also contribute to the stabilisation of the nitrogenase complex and maintain the overall metabolic status of bacteria under unfavourable conditions. The findings confirmed that amino acid molybdate chelates are promising for use in biological products because they increase the bioavailability of molybdenum and the resistance of nitrogen fixation to temperature stress. The results of the study can be used to develop effective *Azotobacter*-based biological products for regions with a temperate climate, which will help reduce the use of mineral fertilisers.
Keywords: *nitrogenase, biological nitrogen fixation, amino acid complexes, temperature stress, optical density.*

https://doi.org/10.59849/0005-2531.2026.4.22

Introduction

Biological nitrogen fixation is one of the key processes in the global biogeochemical cycle, as it is thanks to this process that reduced nitrogen becomes available to most autotrophic and heterotrophic organisms. In contrast to industrial ammonia synthesis using the Haber-Bosch method, which requires high energy consumption and is accompanied by increased greenhouse gas emissions, microbial nitrogen fixation provides an environmentally friendly and renewable way to enrich ecosystems with nitrogen compounds.

As noted by S.Ya. Kots [1], this process not only maintains the stability of natural biocenoses, but also creates the conditions for sustainable agricultural development, as it reduces dependence on synthetic fertilisers, which is substantial in the context of modern environmental challenges. The study examined in detail the ecological and agronomic factors of this process, justifying its key role in ensuring the sustainable development of agroecosystems.

The paper also addressed the possibilities of reducing dependence on mineral fertilisers by optimising natural nitrogen-fixing systems.

Among nitrogen-fixing microorganisms, free-living bacteria of the genus *Azotobacter* are of particular interest. They are known for their ability to fix atmospheric nitrogen intensively in the soil without entering symbiosis with plants, which makes them an integral component of agroecosystems. As noted by A. Aasfar et al. [2], representatives of this genus act as effective microbial bioinoculants capable of stimulating plant growth through nitrogen fixation, phytohormone synthesis, and increased availability of phosphorus and other nutrients. The study provided evidence of the ability of these microorganisms not only to fix atmospheric nitrogen, but also to synthesise stimulating substances. Similar conclusions are confirmed by J. Nongthombam et al. [3], noting the multifunctionality of *Azotobacter* in the "microbe-plant" system and their potential in biotechnological developments for the intensification of agriculture. The key biocatalyst of the nitrogen fixation process is the nitrogenase enzyme complex, the functioning of which directly depends on the availability of trace elements. One of them is molybdenum, which is part of the FeMo cofactor metal cluster necessary for the reduction of molecular nitrogen. J.S.M. del Campo et al. [4] showed that in *Azotobacter vinelandii*, it is the availability of molybdenum that determines the activity of nitrogenase and the productivity of nitrogen fixation. The study addressed the regulatory mechanisms that control the activity of nitrogenase. The study contains data on the effect of molybdenum on the expression of nitrogen fixation genes. O. Einsle and D.C. Rees [5] emphasised the complexity of the FeMo cofactor architecture, which was formed during a long evolutionary process and requires the coordination of many auxiliary proteins to assemble the active centre. The authors described in detail the architecture of nitrogenase active centres and catalysis mechanisms. As noted by B.E. Smith [6], the integration of molybdenum into the cofactor structure occurs with the participation of specific transport and chelation systems, and any disruption of this process leads to a decrease or complete loss of enzymatic activity. The study analysed the evolutionary aspects of the formation of these enzyme complexes and their adaptation to different environmental conditions, paying attention to the mechanisms of biosynthesis of nitrogenase metallocofactors.

Despite the exceptional significance of molybdenum, its bioavailability in soils is often limited. In alkaline and neutral conditions, molybdenum forms poorly soluble compounds, making it difficult for microorganisms to access. As highlighted in the work of M. Chanderban et al. [7], molybdenum deficiency strictly regulates the expression level of nitrogenase genes and the activity of the corresponding enzymes. Climate fluctuations are an additional limiting factor: temperature stress can complicate the transport and assimilation of micronutrients, exacerbating the problem of effective nitrogen fixation. To overcome molybdenum deficiency, chelate compounds are used in biotechnology. They can maintain high metal solubility and ensuring their targeted delivery to the cell. E.D. Badding [8] emphasised the value of controlled metal transport for the correct assembly of cofactors in metalloenzymes. The study revealed the mechanisms of site-selective labelling of the iron-molybdenum cofactor of nitrogenase by developing methods for studying the structure and functions of this metal cluster. C. Van Stappen et al. [9] noted that an optimal metal-cofactor environment is crucial for maintaining the stability of nitrogenase and preserving its catalytic activity. The study contains data on the stability of the enzyme and its catalytic properties under various conditions. The study proposed new approaches to studying the structure and functions of nitrogenase complexes. S. Burén et al. [10] systematised research of nitrogenase cofactor biosynthesis, emphasising the multi-step nature of this process and the role of numerous chaperone proteins. The authors emphasised that the

incorporation of molybdenum into the FeMo cofactor is strictly controlled and depends on the efficiency of metal transport and intracellular delivery systems. The key thesis of the study is that even minor restrictions in the availability of molybdenum or failures in the assembly of intermediate clusters can lead to a significant decrease in nitrogenase activity.

Despite advances in nitrogenase research, there is a lack of systematic studies comparing the effectiveness of different molybdenum chelate forms under conditions of temperature stress. Most previous studies have focused on synthetic chelates, while the potential of biologically compatible amino acid complexes has remained understudied, especially regarding their effect on the metabolic activity of nitrogen-fixing bacteria at suboptimal temperatures. The lack of such data limits the development of effective biological products for regions with temperate climates, where temperature fluctuations significantly affect microbial activity.

In this context, the study aimed to investigate the bioavailability of amino acids and synthetic molybdate chelates for *Azotobacter chroococcum* under optimal and stressful temperature conditions. The objectives of the study included: a comparative assessment of the effect of amino acid (glycine, histidine) and synthetic (EDTA, DTPA) molybdate chelates on the growth rate and reproduction dynamics of *Azotobacter chroococcum* at temperatures of 28°C and 18°C; to study the effect of various chelated forms of molybdenum on nitrogenase activity and the efficiency of the biological nitrogen fixation process under optimal and stressful temperature conditions; assessment of the ability of amino acid molybdate chelates to maintain the metabolic status of *Azotobacter chroococcum* cells by analysing total protein synthesis and its correlation with nitrogen-fixing activity.

Materials and Methods

The study was conducted in 2025 at the National University of Life and Environmental Sciences of Ukraine. The object of the study was a pure culture strain of *Azotobacter chroococcum* VKM B-616 bacteria obtained from the All-Ukrainian Collection of Microorganisms (VKM) at the National Academy of Sciences of Ukraine. The criteria for including this strain were its typical morphology, cultural and physiological properties, in particular its ability to fix atmospheric nitrogen in a nitrogen-free environment, form cysts, and produce exopolysaccharides. The criterion for exclusion was the presence of contaminating microflora.

Microorganisms were cultivated on a liquid nitrogen-free Ashby medium. The composition of the medium was as follows (g/l): mannitol – 15.0; KH_2PO_4 – 0.2; $MgSO_4 \cdot 7H_2O$ – 0.2; NaCl – 0.2; $CaSO_4 \cdot 2H_2O$ – 0.1; $CaCO_3$ – 5.0. The source of molybdenum in the experimental variants was its various forms, which were added to the medium after autoclaving from concentrated solutions sterilised by filtration through membrane filters with a pore size of 0.22 μm. The variants studied were control without molybdenum addition; unchelated sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$); molybdate chelate complexes with glycine (Mo-Glycine); molybdate chelate complexes with histidine (Mo-Histidine); chelate complexes of molybdate with ethylenediaminetetraacetic acid (Mo-EDTA); chelate complexes of molybdate with diethylenetriaminepentaacetic acid (Mo-DTPA). All chelate compounds were prepared *in vitro* by mixing solutions of sodium molybdate and the corresponding chelating agent in a molar ratio of 1:1 and holding at room temperature for 24 hours. The molybdenum concentration in all experimental variants, except for the control, was 1.0 mg/l. All reagents used were manufactured by Sigma-Aldrich (USA).

The medium was inoculated with a suspension of third-generation *Azotobacter chroococcum* cells grown on Ashby medium with optimal molybdate addition. Cultivation was conducted in a thermostatic ELMI (Latvia) shaker cabinet at a rotation speed of 180 rpm for 96 hours at two temperature regimes:

optimal (+28°C) and suboptimal (+18°C). Each experimental variant was represented by four biological replicates.

The growth intensity of the bacterial culture was monitored by measuring the optical density of the culture fluid on a LabAnalyt SP-V1000 spectrophotometer (China) at a wavelength of 600 nm (OD_{600}) every 12 hours. The determination of total nitrogen in biomass, as an integral indicator of the intensity of the nitrogen fixation process, was conducted after the completion of cultivation using the micro-Kjeldahl method on a Velp Scientifica (Italy) device. The activity of the key nitrogen fixation enzyme, nitrogenase, was determined using the acetylene reduction gas chromatography method. For this purpose, 1 ml of acetylene was added to a test tube containing 5 ml of culture fluid and incubated on a shaker at 28°C for 2 hours. Gas samples of 100 µl were taken with a gas syringe and analysed on an Agilent 7890B gas chromatograph (USA) with a flame ionisation detector. The amount of ethylene produced was calculated using a calibration curve and expressed in nmol C_2H_4/hour/mg protein.

The protein concentration in bacterial biomass was determined using the Bradford spectrophotometric method, which is based on the ability of proteins in an acidic environment to bind with Coomassie Brilliant Blue G-250 dye. For the analysis, a ready-made Bradford reagent containing Coomassie Brilliant Blue G-250 dye, phosphoric acid and ethanol, manufactured by Sigma-Aldrich (USA), was used. After culturing the bacteria, the broth was centrifuged at 10,000 rpm for 10 minutes on an Eppendorf 5804 R centrifuge (Germany) to precipitate the cells. The supernatant was removed, and the biomass was resuspended in physiological saline. The cells were lysed using an UD-20 ultrasonic disintegrator (Poland) on ice in a mode of 3 pulses of 30 seconds each. The resulting lysate was centrifuged to remove cell debris, and the clear supernatant was used for analysis. The determination procedure was as follows. 250 µl of Bradford reagent was added to 5 µl of the test sample of protein extract. The mixture was thoroughly mixed on a vortex and incubated at room temperature for 10 minutes for the colour reaction to develop completely. After incubation, the optical density of the resulting solutions was measured on a spectrophotometer at a wavelength of 595 nm. A sample in which 5 µl of distilled water was added instead of the protein sample was used as a control. To construct the calibration curve, serum albumin (BSA) solutions manufactured by Sigma-Aldrich (USA) were used in a concentration range from 0 to 2,000 µg/ml. A BSA solution with a concentration of 1 mg/ml was prepared with distilled water, and standard solutions with the required concentrations were obtained by sequential dilution. The same procedure was performed with each standard solution as with the test samples. Based on the results of measuring the optical density of the standard solutions, a graph of OD_{595} versus protein concentration was constructed. The protein concentration in unknown samples was calculated based on the obtained calibration curve.

The obtained digital data were statistically processed using the Statistica 10.0 software package (USA). To assess the reliability of differences between the mean values of the study groups, two-factor analysis of variance (ANOVA) was used, followed by a Tukey comparison for a significance level of $p<0.05$. All results are presented as the arithmetic mean ± standard error of the mean (M±m).

Results and Discussion

3.1. Growth dynamics of *Azotobacter chroococcum* culture under different molybdenum sources and temperature conditions

A detailed analysis of the growth dynamics of *Azotobacter chroococcum* under different temperature conditions and molybdenum sources revealed differences in cell proliferation kinetics. At the optimum temperature of 28°C, a classic S-shaped growth curve was observed for all variants, but with differences in the duration of individual phases and maximum culture density. The shortest lag

phase (8-10 hours) was demonstrated by variants with amino acid chelates, especially Mo-Histidine, where the logarithmic phase began 10 hours after inoculation. In the variant with unchelated molybdate, the lag phase lasted 14-16 hours, and in the control without molybdenum, it lasted more than 24 hours. The maximum OD600 values after 96 hours of incubation showed a clear gradation depending on the form of molybdenum. The Mo-Histidine variant reached a value of 0.98±0.04 units, which was 30.7% higher than the value for non-chelated molybdate (0.75±0.03 units). Mo-Glycine showed a result of 0.92±0.04 units (22.7% higher than the non-chelated form). Artificial chelates occupied an intermediate position: Mo-EDTA – 0.84±0.03 units, Mo-DTPB – 0.81±0.04 units. Under suboptimal temperature conditions of 18°C, a universal prolongation of the lag phase and a decrease in the steepness of the logarithmic growth phase were observed. However, amino acid chelates demonstrated higher adaptability. The lag phase for Mo-Histidine was 16-18 hours, while for non-chelated molybdate it was 24-26 hours. The maximum OD600 values at 18°C also showed the advantage of chelated forms: Mo-Histidine – 0.65±0.03 units, Mo-Glycine – 0.61±0.03 units, which exceeded the indicator of non-chelated molybdate (0.45±0.02 units) by 44.4% and 35.6%, respectively. Artificial chelates showed values of 0.48±0.03 units for Mo-EDTA and 0.42±0.02 units for Mo-DTPB (Table 1).

Table 1. Kinetic parameters of *Azotobacter chroococcum* growth under different molybdenum sources

Parameter	Source of molybdenum	28°C	18°C	Change at 18°C (%)
Duration of the lag phase (hours)	Control	26.5±1.2	42.3±2.1	+59.6
	Na_2MoO_4	15.2±0.8	25.8±1.3	69.7
	Mo-Glycine	10.8±0.6	18.5±0.9	71.3
	Mo-Histidine	9.5±0.5	16.2±0.8	+70.5
	Mo-EDTA	13.4±0.7	22.7±1.1	+69.4
	Mo-DTPA	14.1±0.7	24.3±1.2	+72.3
Maximum growth rate (ΔOD/hour)	Na_2MoO_4	0.032±0.002	0.018±0.001	-43.8
	Mo-Glycine	0.041±0.002	0.025±0.001	-39.0
	Mo-Histidine	0.045±0.002	0.028±0.001	-37.8
	Mo-EDTA	0.035±0.002	0.020±0.001	-42.9
	Mo-DTPA	0.033±0.002	0.019±0.001	-42.4
Time to reach steady state (hours)	Na_2MoO_4	68.5±3.2	92.5±4.3	35.0
	Mo-Glycine	60.2±2.8	80.3±3.7	+33.4
	Mo-Histidine	56.8±2.6	76.5±3.5	34.7
	Mo-EDTA	65.3±3.0	88.7±4.1	35.8
	Mo-DTPA	67.1±3.1	90.2±4.2	+34.4

Source: *compiled by the authors.*

Statistical analysis using two-factor ANOVA revealed a highly significant effect of both temperature ($F=125.6$; $p<0.001$) and molybdenum form ($F=89.3$; $p<0.001$) on all growth parameters studied. The Tukey post-hoc analysis showed that, at both temperatures,

variants with amino acid chelates differed significantly from the other groups ($p<0.01$). The ratio of growth efficiency at 18°C to that at 28°C was highest for Mo-Histidine (66.3%), compared with 60.0% for non-chelated molybdate. The higher growth efficiency of the amino acid chelates is consistent with evidence that inorganic molybdenum compounds may have limited biological availability, whereas organic ligands can improve molybdenum solubility and stabilise molybdenum-containing complexes [11, 12].

3.2. Accumulation of total nitrogen in biomass as an indicator of nitrogen fixation intensity

The determination of total nitrogen in biomass after completion of the 96-hour cultivation period served as a key integral indicator of the efficiency of the biological nitrogen fixation process in *Azotobacter chroococcum*. The results obtained demonstrate a clear dependence of nitrogen fixation intensity on the form of molybdenum supply and cultivation temperature conditions. At an optimal temperature of 28°C, a statistically significant ($p<0.05$) increase in nitrogen accumulation was observed in all variants with molybdenum addition compared to the control without molybdenum (0.9±0.1 mg/l). The maximum nitrogen accumulation was found in the biomass of bacteria cultivated on a medium with Mo-Histidine (8.5±0.3 mg/l), which was 37.1% higher than the variant with unchelated molybdate (6.2±0.2 mg/l). The Mo-Glycine variant also showed high results (7.9±0.3 mg/l), yielding only 7.1% to Mo-Histidine. Artificial chelates showed slightly lower but still statistically significant results: Mo-EDTA (7.0±0.3 mg/l) and Mo-DPPA (6.8±0.2 mg/l), which exceeded the non-chelated molybdate by 12.9% and 9.7%, respectively. At a temperature of 18°C, a regular decrease in nitrogen fixation intensity was observed in all experimental groups, which is associated with the suppression of bacterial metabolic activity and a decrease in the efficiency of the nitrogenase complex. However, the relative advantage of amino acid chelates remained. The highest nitrogen content was recorded in the Mo-Histidine variant (5.1±0.2 mg/l), which was 59.4% higher than that of the non-chelated molybdate variant (3.2±0.1 mg/l). The nitrogen fixation efficiency at 18°C in the Mo-Histidine variant was 60.0% of its efficiency at 28°C, while for non-chelated molybdate this figure was significantly lower at 51.6% (Table 2). This indicates the ability of amino acid chelates to partially compensate for the negative effect of low temperature on the nitrogen fixation process.

Table 2. Accumulation of total nitrogen in *Azotobacter chroococcum* biomass under different molybdenum sources and cultivation temperatures

Source of molybdenum	Temperature of 28°C (mg/l)	Temperature of 18°C (mg/l)	Reduction at 18°C (%)
Control (without Mo)	0.9±0.1	0.8±0.1	11.1
Na_2MoO_4	6.2±0.2	3.2±0.1	48.4
Mo-Glycine	7.9±0.3	4.3±0.2	45.6
Mo-Histidine	8.5±0.3	5.1±0.2	40.0
Mo-EDTA	7.0±0.3	3.8±0.2	45.7
Mo-DTPA	6.8±0.2	3.6±0.1	47.1

Source: *compiled by the authors.*

Statistical analysis using one-way analysis of variance confirmed significant differences among the experimental variants ($F=45.8$; $p<0.001$). At 28°C, the Mo-Histidine and Mo-Glycine variants differed significantly from all other groups ($p<0.05$), whereas the difference

between Mo-EDTA and Mo-DTPA was not significant ($p>0.05$). At 18°C, both amino acid chelates maintained a significant advantage over the other molybdenum forms ($p<0.05$). The smaller reduction in nitrogen accumulation observed with Mo-Histidine indicates that amino acid complexation maintained greater molybdenum availability under temperature stress. This interpretation is consistent with evidence that trace-metal availability limits nitrogen fixation by Azotobacter and that organic ligands can act as molybdenum buffers in nitrogen-fixing bacterial cultures [13, 14]. The ability of Azotobacter to utilise amino acids as carbon and nitrogen sources may also have contributed to this effect [15].

3.3. Nitrogenase activity under different cultivation conditions

A detailed study of the activity of the key nitrogen fixation enzyme, the nitrogenase complex, was conducted using a highly sensitive gas chromatographic method of acetylene reduction. At an optimal temperature of 28°C, the maximum specific activity of nitrogenase was observed at the end of the logarithmic growth phase (48-60 hours). The highest values were recorded in the Mo-Histidine variant, where the activity reached 152.4±6.8 nmol C_2H_4/hour/mg protein. This significantly exceeded the activity in the variant with unchelated molybdate (108.7±5.2 nmol C_2H_4/hour/mg protein) by 40.2%. The Mo-Glycine variant showed an activity of 142.1±6.5 nmol C_2H_4/hour/mg protein, which is 30.7% higher than the control with unchelated molybdenum.

Table 3. Kinetic parameters of nitrogenase activity in *Azotobacter chroococcum*

	Source of molybdenum	28°C	18°C	Reduction at 18°C (%)	Temperature sensitivity coefficient (Q10)
Maximum activity (nmol C_2H_4/hour/mg protein)	Control	15.2±1.1	5.8±0.4	61.8	2.62
	Na_2MoO_4	108.7±5.2	41.2±2.1	62.1	2.64
	Mo-Glycine	142.1±6.5	61.5±2.9	56.7	2.31
	Mo-Histidine	152.4±6.8	68.3±3.1	44.8	2.23
	Mo-EDTA	125.3±5.9	52.7±2.5	57.9	2.38
	Mo-DTPA	118.6±5.4	48.9±2.3	58.8	2.42
Time to reach maximum activity (hours)	Na_2MoO_4	54.2±2.6	68.5±3.2	+26.4	-
	Mo-Glycine	50.8±2.4	62.3±2.9	22.6	-
	Mo-Histidine	48.5±2.3	60.2±2.8	24.1	-
	Mo-EDTA	52.7±2.5	66.8±3.1	+26.8	-
	Mo-DTPA	53.5±2.5	67.3±3.1	25.8	-
Duration of maximum activity support (hours)	Na_2MoO_4	18.3±0.9	12.5±0.6	-31.7	-
	Mo-Glycine	22.7±1.1	16.8±0.8	-26.0	-
	Mo-Histidine	24.2±1.2	18.3±0.9	-24.4	-
	Mo-EDTA	19.8±1.0	13.7±0.7	-30.8	-

	Source of molybdenum	28°C	18°C	Reduction at 18°C (%)	Temperature sensitivity coefficient (Q10)
	Mo-DTPA	18.9±0.9	12.9±0.6	-31.7	-

Source: *compiled by the authors.*

Artificial chelates showed intermediate results: Mo-EDTA – 125.3±5.9 nmol C_2H_4/hour/mg protein (15.3% higher than the control), Mo-DTPB – 118.6±5.4 nmol C_2H_4/hour/mg protein (9.1% higher than the control). The control variant without molybdenum showed minimal nitrogenase activity – 15.2±1.1 nmol C_2H_4/hour/mg protein. At a temperature of 18°C, a sharp decrease in nitrogenase activity was observed in all experimental variants, which is associated with the thermosensitivity of this enzyme complex. Maximum activity was achieved later, at 60-72 hours of cultivation. The highest values were again demonstrated by amino acid chelates: Mo-Histidine – 68.3±3.1 nmol C_2H_4/hour/mg protein, Mo-Glycine – 61.5±2.9 nmol C_2H_4/hour/mg protein (Table 3)Statistical analysis using two-factor ANOVA revealed highly significant effects of both temperature ($F=215.8$; $p<0.001$) and molybdenum form ($F=98.7$; $p<0.001$) on nitrogenase activity. The temperature sensitivity coefficient was lowest for the amino acid chelates ($Q_{10}=2.23–2.31$). At 18°C, Mo-Histidine retained 44.8% of the nitrogenase activity recorded at 28°C, whereas non-chelated molybdate retained 37.9%. Nitrogenase activity was strongly correlated with total nitrogen accumulation in biomass at both 28°C ($r=0.94$) and 18°C ($r=0.91$). These findings show that the chemical form of molybdenum influenced its availability for nitrogenase-related processes, in agreement with studies of molybdenum availability, organic ligand buffering, and molybdate-dependent metabolism in Azotobacter [13, 14, 16].

3.4. Total protein content in bacterial cells

At an optimal temperature of 28°C, a clear differentiation in protein content was observed depending on the form of molybdenum. The maximum values were recorded in the Mo-Histidine variant (0.38±0.02 mg/ml), which was 31.0% higher than the value for unchelated molybdate (0.29±0.01 mg/ml). The Mo-Glycine variant showed a result of 0.35±0.02 mg/ml (20.7% higher than the control). Artificial chelates occupied an intermediate position: Mo-EDTA – 0.32±0.02 mg/ml, Mo-DTPB – 0.31±0.01 mg/ml. At a temperature of 18°C, a general decrease in protein content was observed in all variants, which is associated with the suppression of metabolic activity and protein synthesis. However, amino acid chelates showed a relative advantage: Mo-Histidine – 0.24±0.01 mg/ml, Mo-Glycine – 0.22±0.01 mg/ml, which exceeded the indicator of non-chelated molybdate (0.17±0.01 mg/ml) by 41.2% and 29.4%, respectively.

Statistical analysis using two-factor ANOVA revealed highly significant effects of both temperature ($F=156.3$; $p<0.001$) and molybdenum form ($F=87.9$; $p<0.001$) on total protein content. The protein synthesis coefficient was highest in the variants containing amino acid chelates. Total protein content was strongly correlated with nitrogenase activity at 28°C ($r=0.89$) and 18°C ($r=0.86$), as well as with nitrogen accumulation at 28°C ($r=0.91$) and 18°C ($r=0.88$). Thus, the treatments that supported higher nitrogenase activity also maintained higher protein accumulation under temperature stress. The broader biological relevance of metal complexes with organic ligands is supported by Ismayilova et al. [17], who reported effects of Mn(II) and Zn(II) complexes with organic acids on physiological and biochemical processes in wheat seedlings. Together with the established potential of Azotobacter in sustainable agriculture [18], these findings support further

evaluation of Mo-Histidine as a component of microbial biological products.

Conclusions

A comprehensive study of the effect of various chelated forms of molybdate on the bioavailability of molybdenum for *Azotobacter chroococcum* bacteria under different temperature conditions has led to several fundamental and applied conclusions. The main result of the study is the proof of the superiority of amino acid molybdate chelates over traditionally used artificial chelates and non-chelated forms. The molybdenum complex with histidine demonstrated maximum efficiency, providing an optical density of the culture at 0.98±0.04 units at an optimal temperature of 28°C, which was 30.7% higher than that of non-chelated molybdate. This advantage is based on the ability of amino acid chelates to perform several functions simultaneously: serve as an effective source of molybdenum, provide additional nitrogen and carbon supply, and maintain the structural integrity of enzyme complexes.

The ability of amino acid chelates to significantly mitigate the negative impact of temperature stress on the nitrogen fixation process was also demonstrated. At a temperature of 18°C, the decrease in nitrogenase activity in the Mo-Histidine variant was 44.8% (i.e., 55.2% of the level at 28°C was preserved), while for non-chelated molybdate, the decrease was 62.1% (only 37.9% was preserved). This effect is associated with the ability of amino acid chelates to stabilise the conformation of the nitrogenase complex and maintain optimal redox potential in the cell under adverse conditions. Another notable aspect is the positive effect of amino acid chelates on the overall metabolic status of cells. The study showed that cells cultured with Mo-Histidine contained 31.0% more total protein compared to the control with unchelated molybdenum. This indicates not only the activation of nitrogenase synthesis but also the overall stimulation of biosynthetic processes in the cell.

Prospects for further research include studying the molecular mechanisms of absorption of molybdenum amino acid chelates and their effect on the expression of nitrogenase genes, optimising the formulas of biological products by combining them with other microelements to achieve a synergistic effect with subsequent field trials, as well as developing environmentally safe methods for synthesising these compounds and studying their long-term impact on soil microbial communities.

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AMİN TURŞUSU VƏ SİNTETİK MOLİBDAT (MoO_4^{2-}) XELATLARI VƏ MÜXTƏLİF TEMPERATURLARDA *AZOTOBACTER* BAKTERİYALARI ÜÇÜN ONLARIN BİOƏLÇATANLIĞI

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Azotobacter bakteriyaları tərəfindən həyata keçirilən bioloji azot fiksasiyasının səmərəliliyi azotazanın əsas mikroelementi olan molibdenin bioloji əlçatanlığından asılıdır və mülayim iqlimli bölgələrdə temperatur stresi şəraitində dəyişikliyə məruz qalır. Tədqiqatın məqsədi temperatur stresi şəraitində *Azotobacter chroococcum* bakteriyalarının böyüməsini, metabolik aktivliyini və azotfiksasiya qabiliyyətini dəstəkləməkdə molibdenin müxtəlif xelat formalarının effektivliyini müqayisə etmək idi. Tədqiqat *in vitro* şəraitdə, Eşbi (Ashby) mühitində aparılmışdır. Molibdenin beş mənbəyi qiymətləndirilmişdir: xelatlaşdırılmamış natrium-molibdat, amin turşusu xelatları və sintetik xelatlar. Bioloji əlçatanlıq böyümə intensivliyi, biokütlədə azotun toplanması, azotaza aktivliyi və ümumi zülal miqdarı əsasında qiymətləndirilmişdir. Nəticələr göstərmişdir ki, amin turşusu xelatları, xüsusilə də histidin-molibdat, molibdenin ən yüksək bioloji əlçatanlığını təmin etmişdir. Optimal temperaturda bu variant, xelatlaşdırılmamış formaya nisbətən bakteriyaların böyümə intensivliyində 30,7%, azotun toplanmasında 37,1% və azotaza aktivliyində 40,2% artım nümayiş etdirmişdir. Temperatur stresi şəraitində (18°C) amin turşusu xelatlarının üstünlüyü daha qabarıq şəkildə müşahidə olunmuşdur: azotfiksasiya səmərəliliyindəki azalma xelatlaşdırılmamış molibdat üçün 48,4% təşkil etdiyi halda, histidin-molibdat üçün bu göstərici cəmi 40,0% olmuşdur. Tədqiqat müəyyən etmişdir ki, amin turşusu xelatları təkcə molibdenin hüceyrələrə daşınmasını yaxşılaşdırmır, həm də azotaza kompleksinin sabitləşməsinə töhfə verir və əlverişsiz şəraitdə bakteriyaların ümumi metabolik vəziyyətini qoruyub saxlayır. Əldə edilən nəticələr təsdiqləyir ki, amin turşusu-molibdat xelatları bioloji məhsullarda istifadə üçün perspektivlidir, çünki onlar molibdenin bioloji əlçatanlığını və azotfiksasiya prosesinin temperatur stressinə qarşı davamlılığını artırır. Tədqiqatın nəticələri mülayim iqlimli bölgələr üçün effektiv *Azotobacter* əsaslı bioloji məhsulların hazırlanmasında istifadə oluna bilər ki, bu da mineral gübrələrin istifadəsini azaltmağa kömək edəcəkdir.

Açar sözlər: azotaza, bioloji azot fiksasiyası, amin turşusu kompleksləri, temperatur stressi, optik sıxlıq.