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The Effect of Some Chemical Elements on the Growth Process (Biomass) of *Arthrobacter oxydans* 61B

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Abstract

This work studied the influence of Mg, Ca, Na, K, Cu, Zn, Cr, and Si ions on the growth and development of chromium-resistant bacteria ***Arthrobacter oxydans* 61B**. Mg, Ca, Na, and K ions did not affect the growth and biomass of ***Arthrobacter oxydans* 61B,** Mg and Ca ions made a positive contribution to the biomass for 144 hours. Copper and Zinc had a detrimental effect on the logarithmic phase of growth. The addition of Si ions made a significant impact on the development of bacterial biomass. We studied the effect of Si on Zn and Cr(VI) uptake with atomic absorption spectrometry. The given concentration of Si ions (400 μg/ml) on the growth medium significantly increased the uptake of Cr up to 6000 μg/g and up to 240 μg/g for Zn.

Keywords

Arthrobacter oxydans 61B, Na, K, Mg, Ca, Cu, Zn, Cr, Si

Introduction

The influence and importance of chemical elements, particularly metals, on the vital processes of microorganisms and other living organisms during their growth and development is well known (Saikat *et al.*, 2022; Singh et al., 2011). It is also known that magnesium, calcium, sodium, potassium, copper, zinc, and chromium (the elements used in our study) belong to the essential elements (Saikat *et al.*, 2022). For example:

Magnesium ions (Mg^{2+}) are important for all living cells, where they play a major role in the action of biological compounds of polyphosphates, such as ATP, DNA, and RNA (Moomaw and Maguire, 2008).

- Calcium (Ca^{2+}) is essential in the physiology of living organism cells. Calcium ions move in and out of the cytoplasm and function as a signaling ion for many cellular processes like oxidative stress, motility, virulence, and chemotaxis (Njegic et al., 2020; Meyer et al., 2021), It is essential for the Mechanosensation in Escherichia coli (Bruni et al., 2017).
- Most of the sodium in the body is outside the cell (about 15 times more than in the cytoplasm). This difference is provided by the sodium-potassium pump, which pumps sodium that has entered the cell. With

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potassium, sodium performs the following functions: Membrane potential and muscle contraction conditions; Maintaining osmotic concentration in blood; Maintaining the acid-base balance; Normalization of water balance; Ensuring membrane transport: secondary active transport (Shechter, 1986); Activation of many enzymes (Strazzullo and Leclercq, 2014).

- Copper is an important element for living organisms. The connection between copper and vitamins is noteworthy. The relationship between copper and hormones has also been studied. It acts uniquely on hormone functioning, enhancing the action of some and inhibiting others; In addition, copper has a significant effect on tissue respiration, organism growth and development, blood and bone formation, and the course of metabolism (Shabbir et al., 2020; Loutet et al., 2015).
- Zinc is an essential element for the normal functioning of the living organism. Zinc plays a big role in the realization of the hormonal functions of the human organism. Zinc is part of the structural composition of the active center of hundreds of metalloenzymes. It is necessary for the functioning of DNA and RNA polymerases, which carry out the transfer of hereditary information, control protein biosynthesis (formation), and repair processes of the organism (Saleem et al., 2022; Recena et al., 2021; Loutet et al., 2015). Zinc also plays a role in the regulation of microbial virulence and host immune responses (Xia et al., 2021).
- Chromium participates in the metabolism of lipids, proteins, and carbohydrates. This factor determines the interaction of cell receptors with insulin while reducing the body's demand for it. In addition, chromium participates in the regulation of cholesterol metabolism and is an activator of some enzymes (Shahid *et al.*, 2017; Rcheulishvili *et al.*, 2022).
- Silicon is a widely distributed element in nature, but its role in the vital processes of living organisms is less known (Bist *et al.*, 2020).

Certain types of bacteria can assimilate metal ions from the environment (Loutet *et al.*, 2015). The ions that enter the bacteria, participate in various biochemical processes, are transformed, accumulate inside the bacteria, on the bacterial cell wall, or/then expelled outside (Mosulishvili *et al.*, 1980; Tsibakhashvili *et al.*, 2011). Because of this, they promote or, on the contrary, hinder the growth and development of bacteria, as well as various vital processes in them (Loutet *et al.*, 2015; Mosulishvili *et al.*, 1980; Tsibakhashvili et al., 2011). Many microorganisms also cannot adapt to high concentrations of metals (Loutet *et al.*, 2015). Direct isolation of bacteria from metal-contaminated soil, mineral rocks, and water was determined to be the most effective approach (Ishibashi et al., 1990; Wang et al., 1997; Mosulishvili *et al.*, 1980; Tsibakhashvili et al., 2011). *Arthrobacter globiformis* 151B, *Arthrobacter oxydans* 61B belong to this type of bacteria. Arthrobacters are usually soil and basalt-inhabiting bacteria, they have great potential for environmental restoration (Rcheulishvili *et al.*, 2022; Bist *et al.*, 2020; Loutet *et al.*, 2015; Yamada *et al.*, 1975). Most of the Arthrobacter species are resistant to heavy metals (Rcheulishvili *et al.*, 2022; Suzuki and Banfild, 2004; Mosulishvili *et al.*, 1980; Tsibakhashvili *et al.*, 2011).

Our research aims to determine how some chemical elements (Mg, Ca, Na, K, Cu, Zn, Cr, and Si) affect the biomass of *Arthrobacter oxydans* 61B bacteria at different stages of its

growth and development.

We studied the effect of sodium, potassium, calcium, and zinc on the biomass of *Arthrobacter oxydans* 151B at different stages of its growth and development. We introduced the listed chemical elements into the nutrient medium at different concentrations and observed the growth and development of the bacteria (Rcheulishvili *et al.*, 2019; Rcheulishvili *et al.*, 2019; Rcheulishvili *et al.*, 2020; Tugushi *et al.*, 2021).

Materials and Methods

Materials that are used in this study: A bacterium of the genus Arthrobacter was selected as the object of study. *Arthrobacter oxydans* 61B, which was isolated from basalts taken in the Kazreti region contaminated with heavy metals (Rcheulishvili *et al.*, 2020).

Metals- Mg^{2+}, Ca^{2+}, Na^{+}, K^{+}, Cu^{2+}, Zn^{2+}, $Cr^{6+,}$ and Si^{4+} ions ($MgCl_2$, $CaCl_2$, NaCl, KCl, $CuCl_2$, $ZnCl_2$, $K_2Cr_2O_7$ and Na_2SiO_3 soluble salts respectively) with concentration as - 400 μg/ml. The concentration ensures that the metal ions remain soluble in the medium and bioavailable for interaction with bacterial cells.

Bacteria life cycle characteristics

Colonies of *Arthrobacter oxydans* 61B are colorless, white, water-colored, smooth, round, with a convex surface, shiny. The culture does not produce pigment. It grows well on simple synthetic and complex organic nutrient media. The phases of culture development are coccus-rod-coccus. When grown on solid nutrient media, a 15-17 hour culture develops rods, which then form different shaped formations. In the period of 18-24 hours, the structures formed by it gradually fragment into cocci. Cell size ranges from 0.8-1.0×2-3 μm. They do not form spores and are non-motile. The cell wall contains the amino acid lysine and the monosaccharide galactose. The culture is gram-positive, and not resistant to acid.

The study aimed to determine how different chemical elements (Mg, Ca, Na, K, Cu, Zn, Cr, and Si) affect the biomass of *Arthrobacter oxydans* 61B bacteria at different stages of its growth and development.

To achieve the research goal, we conducted the following experiment: Starting the experiment, we transferred *Arthrobacter oxydans* 61B culture from solid medium (TSA agar) into 100 mL liquid medium (TSB) in 500 ml Erlenmeyer flasks. The samples were placed in a thermostat on a shaker. After 24 hours of cultivation, 10-10 ml of cultural fluid (suspension) was transferred to flasks prepared for the experiment, where there was 90 ml of TSB (i.e. in total there is 90+10=100 ml of TSB). Thus, cultivation was carried out in 500 ml Erlenmeyer flasks in 100 ml TSB at 28°C. In one flask we had only a bacterial sample (control). In 8 flasks, we added Mg, Ca, Na, K, Cu, Zn, Cr, Si solutions separately to the bacterial samples, so their concentration in the nutrient medium made up 400 μg/ml. Cultivation of bacteria was carried out for 17, 24, 48, 96, and 144 hours. After cultivation, we precipitated the cells by centrifugation (3000 rpm, 10 min, 0°C). We poured off the supernatant and washed the remaining bacterial pellet with sterile, distilled water (the samples were washed three times and re-centrifuged).

We dried the obtained biomasses using a low-temperature lyophilizer (Rcheulishvili *et al.*, 2022; Mosulishvili *et al.*, 1980) as follows: the wet biomass was transferred to the working chamber of an adsorption-condensation lyophilizer. At this point, the cylindrical chamber was filled with regenerated SiO_2 granules, which ensured the removal of moisture from the wet bacterial biomass. Immersing the cylindrical chamber containing SiO_2 granules in a liquid nitrogen Dewar vessel ensures the cooling of the granules and lowering of the temperature in the working chamber in a short time.

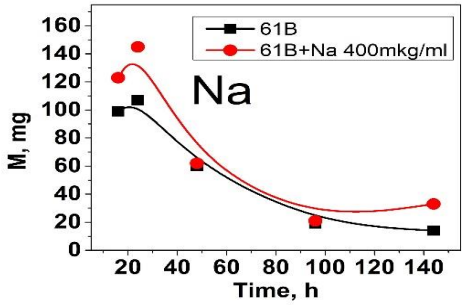
Investigation of Zn^{2+}, Cr^{6+} Uptake by bacteria

In the second set of experiments, the uptake of Zn^{2+}, and Cr^{6+} by bacteria was studied when the

growth medium contained 400 µg/ml of (A) silicone and zinc and (B) silicone and chromium. Bacterial biomasses were harvested after 17, 24, 48, 96, and 144 hours of cultivation. The Zn^{2+} and Cr^{6+} ions concentration in bacterial strain samples was determined as follows: the samples were centrifuged at 3000 g for 30 minutes, and the pellet was resuspended in bidistilled water. This washing procedure was repeated three times. The samples were dried by lyophilization (Marthur and Paul, 1967) weighed, and diluted with nitric acid, and the total concentration of chromium and zinc was measured using an atomic absorption spectrometer (Analyst 800, Perkin Elmer) with an acetylene-air flame. The detection was carried out at 357.9 nm for chromium and 213.8 nm for zinc.

Results and Discussion

After the measurement of dried biomasses of *Arthrobacter oxydans* 61B cells, it was noticed that sodium and potassium ions have no impact on the biomass of bacteria. The biomass of control cells and cells harvested from 400 µg/ml sodium and potassium mediums exhibited a similar growth pattern (fig. 1 and 2). Both elements are present in most of the growth mediums like TSB (5g/L NaCl and 2.5 g/L K_2HPO_4). The excess ions had no effect on the normal growth process. The effects of sodium (Na) and potassium (K) on the growth and development of *Arthrobacter oxydans* 61B showed an initial promotion of bacterial growth within the first 24 hours. However, in the later stages, the presence of these elements inhibited the growth and development of the bacteria. The amount of biomass was minimum in the 96-hour sample. Bacterial growth was observed again during subsequent cultivation stage.



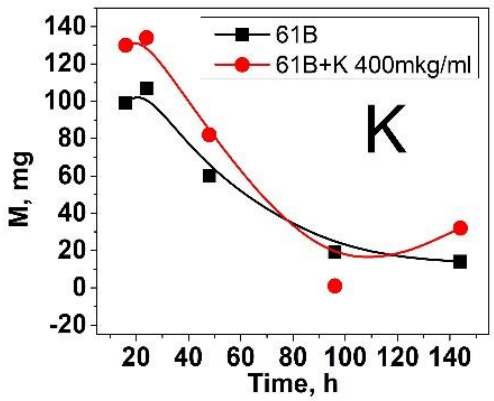
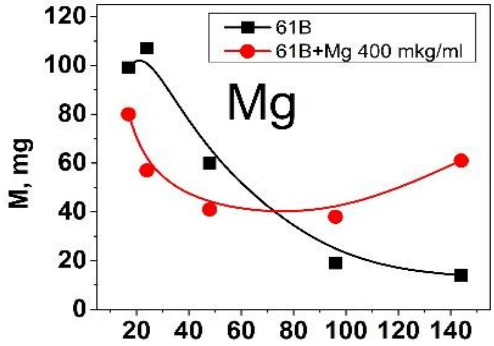


Fig. 1, 2. The effect of **Na** and **K** on ***Arthrobacter oxydans*** **61B**.

The effect of Magnesium and Calcium, both have the same tendency on the biomass and growth process of *Arthrobacter oxydans* 61B. As we can see from Figures 3 and, 4 magnesium (Mg) reduces the biomass growth of *Arthrobacter oxydans* 61B during the first 48 hours, while in 96- and 144-hour samples, the bacterial biomass increases. The same relationship is seen in the case of calcium (Ca) influence. Under calcium influence, the 96-hour sample yielded the lowest biomass.



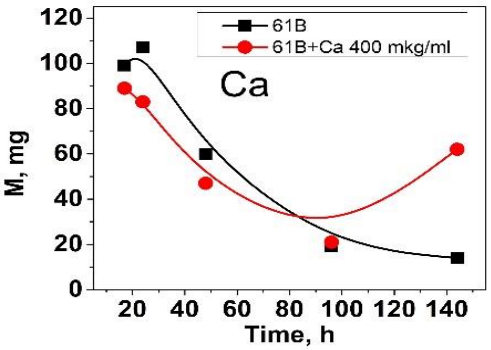


Fig. 3, 4. The effect **Mg** and **Ca** on ***Arthrobacter oxydans*** **61B.**

As the studies show (Fig. 5, 6) copper inhibits the growth and development of bacteria. We see the same picture in the case of zinc (Zn) influence. The graph shows that the growth and development of *Arthrobacter oxydans* 61B is decreasing (insignificant).

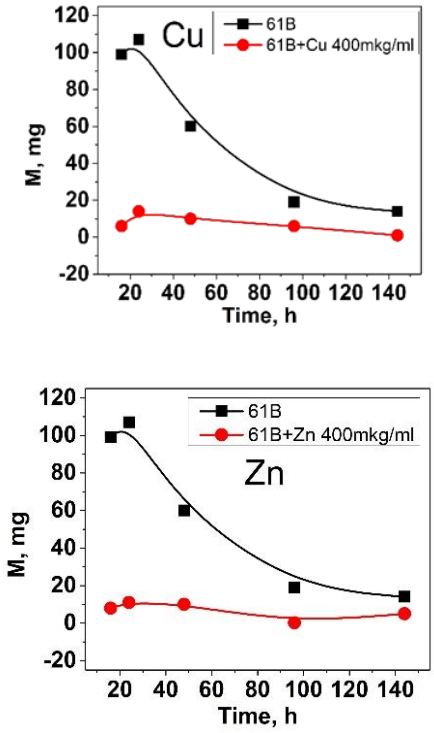


Fig. 5, 6. The effect **Cu** and **Zn** on ***Arthrobacter oxydans*** **61B**.

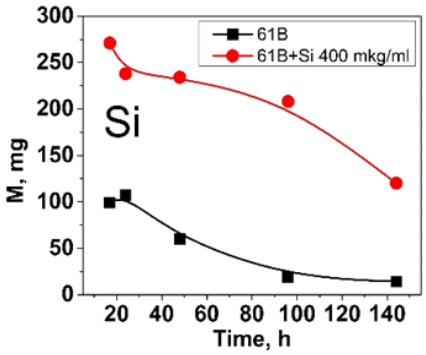


Fig. 7. The effect **Si** on ***Arthrobacter oxydans*** growth process.

Figure 7 demonstrates that silicon (Si) significantly enhances the growth of *Arthrobacter oxydans* 61B. Compared to the control, Si consistently increased bacterial biomass at all measured time points.

Another element on which the experiment was conducted is chromium (Cr). The addition of Cr proved to be an inhibitory effect for bacteria. Bacteria stopped growing and developing when the chromium concentration in the nutrient medium reached 400 μg/ml. As the addition of Si ions made a significant impact on the growth of bacterial biomass, we studied the effect of Si on the uptake of Zn and Cr(VI) with atomic absorption spectrometry. The given concentration of Si ions (400 μg/ml) on the growth medium significantly increased the uptake of Cr up to 6000 μg/g and up to 240 μg/g for Zn.

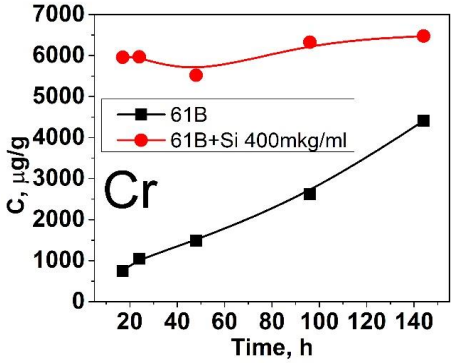


Fig. 8. Effect of Si on the uptake of Cr by *Arthrobacter oxydans.*

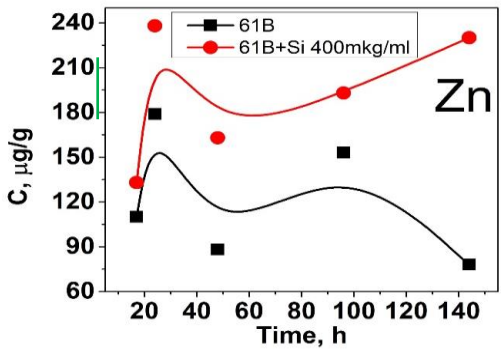


Fig. 9. Effect of Si on the uptake of Zn by *Arthrobacter*.

Conclusion

Based on the results of the experiments conducted, we summarise that among the listed elements-Mg, Ca, Na, K, Cu, Zn, Cr, and Si-silicon is the only element that promotes the growth and development of *Arthrobacter oxydans* sp. 61B at all stages. Silicon enhances the uptake of Cr and Zinc ions by *Arthrobacter oxydans* 61B from the growth medium.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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