Assessment of the stability of amino acid complexes of heavy metals to biodegradation in the aquatic environment

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Abstract. Sodium salts of amino acids in solution have an alkaline reaction, which makes it possible to effectively bind metals into complex compounds, since in an alkaline medium, under the action of hydroxyl ions, the dissociation of amine groups is suppressed, and carboxyl groups remain dissociated, charging negatively, and behave like an anion.

In our experiments, we used an amino acid-based reagent. When processing various environments (objects) contaminated with heavy metals (HM) with this reagent, HM amino acid complexes are formed. In our experiments, we used an amino acid-based reagent. With processing various environments (objects) contaminated with heavy metals (HM) with this reagent are formed HM amino acid complexes.

Model experiments to assess the effect of microorganisms under aerobic conditions on the stability of amino acid complexes of heavy metals. After biodegradation of solutions with TM complexes, no more than 2-3% of heavy metals pass through the filter, which is ten times less than from untreated with the reagent solution.

1. Introduction

The use of amino acid reagents in practice raises the question of the stability of the amino acid complexes of heavy metals formed during the treatment of sewage sludge. This is especially important when using treated with the sewage sludge reagent as a soil-improving additive.

Chelation technology, one of the new green approaches, has demonstrated its potential for recovering metals from industrial waste [1]. Chelators have strong binding properties that can overcome high adsorption of trace elements on contaminated surfaces and low water solubility. Ethylenediaminetetraacetic acid (EDTA) and diethylenetriaminepentaacetic acid (DTPA) are considered the most commonly used chelating agents. However, the stability of metal chelate complexes and the biodegradability of chelating agents are opposite aspects that must be carefully balanced in order to develop a sustainable metal recovery process.

Although the standard method for determining the stability of constants, a lot of work has been done around the world. However, most of the methods have not yet been developed and require additional and reliable research.

Until now, there are very few data on the stability of amino acid complexes of heavy metals in biologically active environments.
Many amino acids have very similar stability constants, and only two of them, cysteine and histidine, bind the metal much more strongly. Peptides form less strong complexes with metals, but there are more possibilities for binding.

Amino acids form complexes with a large number of metals. Ions of copper, zinc, lead form complexes of ML and ML2 with amino acid anions, and ions of chromium, cobalt, nickel, cadmium form complexes ML, ML2 and ML3. In the complexes, amino acid anions bind the central atom through the donor nitrogen atom of the amino group and the carboxyl group atom \[2\].

\[
\text{where } M = \text{Co}^{2+}, \text{Ni}^{2+}, \text{Cu}^{2+}, \text{Zn}^{2+}, \text{Cd}^{2+}, \text{Pd}^{2+}.
\]

Complexes ML2 and CrL3, which have no charge of the inner sphere, are poorly soluble. According to the values of the stability constants of the ML and ML2 complexes, these complexes are formed in almost 100% yield at the ratio \(M: L = 1: 1 \) and \(1: 2\). Well-known compounds of metals with dissimilar amino acids MLL'. As a rule, MLL' complexes are somewhat more stable than ML2 and ML2'.

According to the literature data \[3,4\], different amino acids have rather close values of the dissociation constants, and the stability constants of metal complexes depend little on the nature of the amino acids.

Therefore, for practical use, it is advisable to use available compositions of sodium salts of amino acids, which can be obtained by hydrolysis of numerous protein-containing wastes \[5\].

Sodium salts of amino acids in solution have an alkaline reaction, which makes it possible to effectively bind metals into complex compounds, since in an alkaline medium, under the action of hydroxyl ions, the dissociation of amine groups is suppressed, and carboxyl groups remain dissociated, charging negatively, and behave like an anion:

\[
\begin{align*}
\text{O} & \quad \text{O}^- \\
\text{C} & \quad \text{C} \\
\text{NH}_3^+ & \quad \text{NH}_2 \\
\text{R} & \quad \text{R} \\
+ \text{OH} & \leftrightarrow + \text{H}_2\text{O} \\
\end{align*}
\]

When metal ions interact with the composition of sodium salts of amino acids, a complex mixture of homogeneous and mixed compounds is formed. Based on this, the composition of amino acids can be represented as a single reagent, the molar content of which corresponds to the sum of the concentrations of all amino acids, and its acid-base properties and complexing ability are characterized by the effective values of the protonization constants, stability constants, and the solubility product of metal complexes \[6\].

To establish effective constants, well-known methods of studying the reactions of protonation and complexation of amino acids were used.

In the aspect of chemical and biological masking, of greatest interest is the transformation of ions and metal compounds into poorly soluble complexes - CrL3, NiL2, CuL2, ZnL2, CdL2, MnL2, PbL2. These compounds are chemically the most stable and stable over a wide pH range. In parallel with the formation of insoluble compounds directly in colloidal particles, the precipitation of metal compounds from the sludge liquid occurs \[7\]. When storing detoxified sediment, the likelihood of the transfer of metal compounds to environmental objects decreases.

For the practical implementation of the method of detoxification of heavy metals with sodium salts of amino acids, a reagent was created - a composition that is obtained by alkaline hydrolysis of protein-containing waste, consists of liquid hydrates of sodium salts of amino acids with an average molecular weight of 125-130 kDa.
2. Research objects
In our experiments, we used an amino acid-based reagent. When processing various environments (objects) contaminated with heavy metals (HM) with this reagent, HM amino acid complexes are formed.

Biologically active environments are distinguished by the presence of bacteria, algae, fungi and their spores [7]. Also, in soil conditions, plants, root exudates, the activity of other soil organisms (worms, etc.) play an important role.

The destructive effect of biological environments means, first of all, the effect of microorganisms in these environments, and it depends on specific conditions. These can be soil conditions - by themselves, having numerous variations, there can be water conditions that differ in pH, salt content, and many other indicators. Biodegradation of the complexes can be carried out in model experiments with the formation of artificial biological media containing certain types of microorganisms or their communities.

The difficulty in assessing the results of biological transformation lies, first of all, in the fact that not only the complexes of heavy metals will undergo changes, but also the environments themselves (soils, reservoirs) in which the transformation will take place.

The aquatic environment is the most suitable for modeling various variants of biological effects affecting the stability of amino acid complexes of HM.

Unlike other media, the aquatic environment has a number of advantages associated with the ability to compare and reproduce the results of experiments.

3. Research methods
3.1. Conditions for conducting experiments in a model environment
The general view of the experiment on the biodegradation of amino acid complexes of HMs in an aqueous medium can be represented as a diagram (Fig. 1).
Then we introduced the microbiological preparation "Baikal-EM1" (a complex of soil microorganisms) - 20 ml / vessel.

The volume of the solution was brought to - 1.5 dm³ / vessel (effective volume of the solution in the vessel). Then the air supply was switched on 0.9 dm³ / vessel. The experiment lasted from 2 to 3 weeks (nickel - 2 weeks, lead, chromium - 3 weeks) During the experiment, the nutrient solution (200 ml each) was added to the vessels 2 times. The nutrient solution was not added for the last week.

During the experiment, the liquid level in the vessels was constantly monitored, and the change in the pH of the solutions was periodically monitored on a pH-millivoltmeter pH-410.

After the end of the experiments, the air supply was stopped. The solutions were allowed to subside for 20 minutes, and then liquid samples were taken, which were filtered through a "blue ribbon" filter. The obtained samples were analyzed for metal content by the atomic absorption method.

### 3.2. Schemes of model experiments on biodegradation

In the experiments, we used solutions of lead salts - Pb(NO₃)₂; nickel - NiCl₂*6H₂O and Ni(NO₃)₂*6H₂O; chromium - Cr(NO₃)₂*9H₂O. Experimental schemes are presented in table 1.

| №  | Characteristic        | Pb, mg/l | Ni, mg/l | Cr, mg/l |
|----|-----------------------|----------|----------|----------|
| 1  | Control               | 0        | 0        | 0        |
| 2  | Metal salt*           | 1000     | -        | 1000     |
| 3  | Metal salt            | 405      | 400      | 500      |
| 4  | Treated metal salt    | 405      | 400      | 500      |
| 5  | Metal salt            | 900      | 1000     | 1000     |
| 6  | Treated metal salt    | 900      | 1000     | 1000     |

* - the nutrient mixture and microbiological preparation were not added

To study the transformation of HM amino acid complexes in an aqueous medium, we assembled a setup (Fig. 2).

Bioreactors - plastic vessels with a volume of 2.5 dm³ were used as containers for the cultivation of microorganisms. Each vessel corresponded to a certain variant (Table 1).

The vessels were filled with solutions of saline metals, treated and not treated with the reagent. Metal salt solutions were treated with the reagent to pH 9.
Figure 2. Installation for modeling the microbiological effect on amino acid complexes of HM under aerobic conditions.

1 - bioreactors (vessels) with solutions; 2 - glass comb; 3 - distribution pipes for air supply; 4 - air supply (adjusted to supply 0.9 dm³/min for one vessel)

Nutrient solution 200 ml/vial was added. The nutrient solution was taken for Pseudomonas fluorescens, a gram-negative rod-shaped bacterium belonging to the Pseudomonaceae family, which is very widespread in soil and water and can use an extremely large number of different organic compounds as a source of carbon and energy. NaCl was taken 1 g instead of 2 g according to the method.

Minimal environment for bacteria. Dissolve glucose 3.0 g in 100 ml of distilled water; NaCl - 1.0 g dissolve 150 ml dist. water; KCl - 1.0 g dissolve in 100 ml dist. water; K2HPO4 - Dissolve 0.5 g in 100 ml dist. water; MgSO4 - 7H2O - 0.2 g dissolve - 150 ml dist. water; NH4Cl - 1.0 g dissolve in 100 ml of diets, water; CaCl 2H2O - 0.02 g dissolve in 200 ml dist. water. The solutions are mixed, controlled at pH = 7.60 and, if necessary, adjusted with dilute HCl or NaOH. Then, using distilled water, it is brought to 1000 ml and sterile filtered. The medium is stored at 4 °C in the refrigerator.

4. Results
4.1. Results on biodegradation of lead amino acid complexes
The experiment on the biodegradation of lead amino acid complexes was carried out according to the scheme.

Figure 3. Concentrations of lead in the filtrates after the biodegradation test. A - Pb - initially 500 mg/l; B - Pb - initially 1000 mg/l
From the data of analyzes of samples of filtered extracts from reactors, it can be seen that there is a decrease in the content of lead in the samples under study (Fig. 3). Moreover, in the variants where the investigated metal solutions were treated with the reagent, there is a sharp decrease in the metal content. In variant 4, there is a 10.8-fold decrease in lead concentration compared to variant 3, which was not treated with the reagent before the start of the experiment. In variant 6, the concentration is 26.4 times less than in variant 5.

4.2. Results on biodegradation of amino acid complexes of nickel

In the first attempt nickel chloride (NiCl2 * 6H2O) was used in the experiment. As a result, it was determined that only a small part of the nickel passed through the filter (Fig. 3). Nevertheless, it can be noted that the concentration in the filtrate in the variants with treated nickel is 3.8 (out. 400 mg/l) and 5.4 times (out. 1000 mg/l) lower than in the variants with untreated solutions.

**Figure 4.** Concentrations of nickel in the filtrates after the biodegradation experiment. A - Ni - initially 400 mg/l; B - Ni - initially 1000 mg/l

4.3. Results on biodegradation of chromium amino acid complexes

A feature of the experiments on the biotransformation of chromium complexes was the presence in the studied solutions of an increased content of NO3- ions, since the experiment used a chromium nitrate salt.
It can be seen from the obtained data that in the variants where the investigated metal salt solutions were treated with the reagent, there is a significant decrease in the gross metal content than in the variants where the treatment was not carried out (Fig. 5).

In variant 4, there is a 300-fold decrease in the chromium concentration compared to variant 3. In variant 6, the concentration is 660 times less than in variant 5.

5. Conclusions
Model experiments to assess the effect of microorganisms under aerobic conditions on the stability of amino acid complexes of heavy metals made it possible to draw the following conclusions:
• After biodegradation of solutions with TM complexes, no more than 2-3% of heavy metals pass through the filter, which is ten times less than from untreated with the reagent solution;
• The decrease in the concentration of metals after the end of the experiments is: lead 11 times - 12.4 mg/l, nickel 4 times - 0.21 mg/l, chromium 5 times - 1.56 mg/l.
• Experiments with the composition of sodium salts of amino acids have demonstrated their potential for metal extraction. In the obtained results, it was shown that sodium salts of amino acids have strong binding properties, and low solubility in water, and also, which is very important, are stable to biodegradation;
• However, the stability of metal chelate complexes and biodegradability need further research in order to develop a sustainable metal extraction process.

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