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The Novel Use of Microalgae in the Greening of the Metallurgical Industry

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ABSTRACT

The application of biotechnological methods in the metallurgical industry has the potential to provide an environmentally friendly and cost-effective direction of development. *Thiobacillus ferrooxidans*, a thionic bacterium, and the microalga *Chlamydomonas reinhardtii* TN-72 CH were used as complex reagents for extracting gold from sulfide mineral raw materials. The sorption properties of modal and productive multicomponent gold-bearing solutions were studied. The sorption characteristics of the biosorbents were compared with those of sorbents currently used in gold production – the synthetic resin AM2B and GoldCarb activated carbon. The conducted research studying the sorption properties and survival ability of the microalga *C. reinhardtii* TN-72 CH will make it possible to develop an industrial technology for extracting metal in the hydrometallurgical cycle of gold production. The use of microorganisms in gold hydrometallurgy as an alternative to cyanide methods will reduce the load on the environment while reducing the cost of the technology. In the process of leaching gold-bearing raw materials by microorganisms, even submicroparticles of gold are released, which makes the processing of poor and refractory ores promising. Preliminary biooxidation increases the effectiveness of thiosulfates in terms of both time and gold recovery.

Keywords: greening; gold extraction; microalgae; microorganisms; metallurgical industry; *Thiobacillus ferrooxidans*.

INTRODUCTION

Human industrial activities such as mining have resulted in the release and accumulation of soluble and potentially toxic metals into the environment. These metals, predominantly iron (Fe), copper (Cu), zinc (Zn), nickel (Ni), and cobalt (Co), often exist as elements or compounds that cannot be broken down and are harmful to organisms and ecosystems. The use of environmentally aggressive (EA) reagents in metallurgy and the generation of uncontrolled acid mine effluents (AME) containing large amounts of metal-based environmental pollutants are the most serious global problems arising from these industrial methods [Wang et al., 2022]. Therefore, it is necessary to develop environmentally sustainable technologies and remove (recover) metals from mine effluents or landscapes. The existing methods used to eliminate AME are mostly physicochemical (e.g., adding lime to raise the pH and precipitate out phases containing metals), but due to differences in their selectivity, efficiency and cost, new and improved strategies are needed. In addition, the growing demand for industrially produced modern consumer products based on noble and rare elements means that there is a growing commercial need to recover and reuse these metals from waste streams.

A combined national approach to metallurgical technology, AME recovery and critical material recovery and recycling will have a significant positive impact on the world economy.

Kazakhstan is among the 16 countries with large gold reserves. The significant volumes of gold mining and production have a negative impact on the environment.

The application of biological solutions to gold mining and issues such as soil remediation and AME recovery will contribute to a greener and more sustainable economy.

In the present work, aspects of the use of two bioagents, the bacterium *Thiobacillus ferrooxidans* and the microalga *Chlamydomonas reinhardtii* TN-72 CH, as sorbents for extracting gold during the processing of raw mineral materials are researched.

Bioagents are used in environmental technologies, particularly for bioremediation, to remove or decompose waste contaminants, offering various advantages over physical-chemical methods, including increased safety, efficiency and recovery, as well as reduced operating costs and the use of chemicals. However, certain limitations mean that economically competitive bioremediation methods have not yet been created [Dyo et al., 2018, Willner et al., 2013].

Biosorption is a rather complex mechanism due to the presence of specific functional groups on the outer layer of microorganisms. In the case of using dead microorganisms, only biosorption is carried out. The use of living microorganisms is a more laborious process since it is necessary to provide a system for their life support and reproduction. Desorption of heavy and precious metals from living microorganisms also has several limitations [Kalybekov et al., 2020; Koizhanova et al., 2022] and in such a scenario, it is difficult to maintain the required life support conditions [García-García et al., 2016; Alhasawi et al., 2015].

The use of nonliving microorganisms in technological processes has certain advantages, but the issue of profitability must be decided for each individual case. When choosing the biomass to be used, the main factors considered should be its availability and low cost. The initial biomass can be obtained from industrial waste, for example, from biotechnological production, or from the natural environment, or it can be easily grown under certain conditions.

Algal biomass is widely used as a biosorbent in scientific and industrial activities. The argument in favor of algae is their flexibility in nutrition and reproduction, which also give economic benefits compared to the use of traditional sorbents such as ion-exchange resins and activated carbon [Lekshmi et al., 2022; Khan et al., 2022].

In open systems, there is always a high risk of contamination by other competing microorganisms. In addition, microorganisms exhibit limited selectivity for metals and a certain accumulation capacity, above which metals can become toxic to the organism itself. Therefore, there is a need to improve existing methods and pave the way for the identification, creation and use of biologically improved organisms. Thus, the work carried out in this study is relevant.

It can be assumed that two mechanisms, binding and adsorption of metals, occur on the surface of the cell wall or plasma membrane through electrostatic interactions between functional groups and metal ions; additionally, the import of metals into the cell occurs through endocytosis or metal transporter proteins. By analogy with *C. reinhardtii TN-72 CH*, metals from gold-bearing solutions can be used by the cell for biological functions, sequestered by metal-binding proteins, or separated into aggregates or membrane-bound structures such as vacuoles as part of a strategy to reduce potential toxicity [Nancharaiah et al., 2016].

Accumulated metals can be isolated from harvested microalgal cultures by drying the biomass and burning the organic matter. With this potential for isolation in mind, one should not lose sight of the commercial potential of microalgae in the reduction of metals [Ahmed et al., 2012; Hilson et al., 2006].

The potential of a new approach for the greening of metallurgical production, the use of the microalga *C. reinhardtii* TN-72 CH, was evaluated by comparing the sorption characteristics of a live algal suspension culture and its lyophilisate with those of known effective sorbents — AM-2B resin and GoldCarb activated carbon. To develop a productive solution, samples of technogenic mineral raw materials were taken with a gold content of 2.48 g/t, iron content of 3.87%, and sulfur content of 0.436%.

Most gold-bearing ores contain oxidized and primary sulfides, as well as oxides of nonferrous metals, such as copper, zinc, lead, and nickel, which easily recovered in the process of leaching.

To determine the location of gold occurrence forms, X-ray fluorescence, X-ray phase and mineralogical analyses were carried out.

Mineralogical analysis of the raw materials was carried out under an optical microscope (AxioScope A1). Gold is defined as finely dispersed and ultrafine and occurs both in the free state and in intergrowths with arsenopyrite and waste rock. The predominance of gold in the free state was determined to be 76.9%. In Figure 1, gold

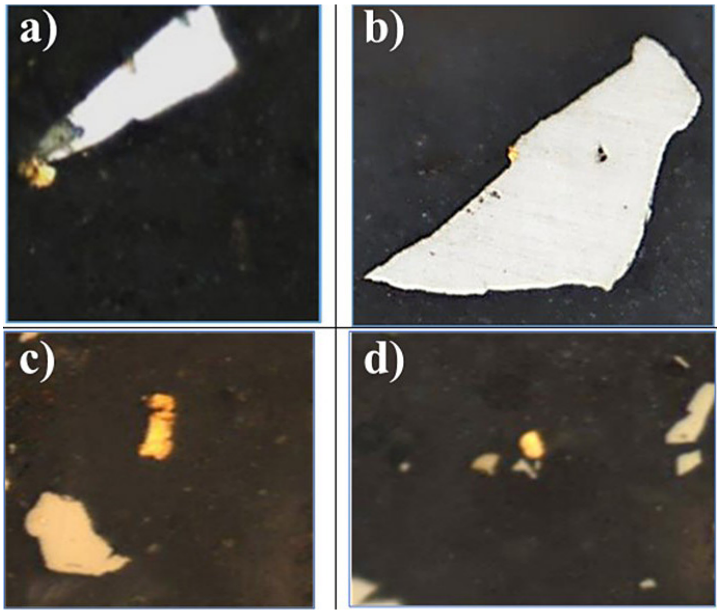

Figure 1 Mineral analysis of raw materials, (a) gold intergrowth with pyrite and waste rock, (b) gold in pyrite, (c) free gold, (d) intergrowth of gold with waste rock

is present in intergrowths with pyrite and empty quartz rock and in a free state.

Accordingly, raw materials can be characterized as stubborn and difficult to enrich; for the processing of such raw materials in classical hydrometallurgy, complex production schemes and aggressive chemicals, such as cyanides and sulfuric acid, are used.

To separate gold and transfer it into solution, the mineral raw materials were subjected to biochemical leaching.

To achieve the greening of gold extraction from mineral raw materials, the use of microorganisms capable of functioning under extreme environmental conditions was chosen as the main strategy. The green alga *C. reinhardtii* TN-72 CH, with good potential as a biosorbent, and the bacterium *T. ferrooxidans*, with the potential for biological oxidation of mineral raw materials, were chosen as the most suitable microorganisms. AM-2B resin, GoldCarb, a live cell suspension culture of green microalgae and its lyophilisate were used as sorbents. The distribution of *T. ferrooxidans* bacteria in nature depends on the presence of reduced sulfur compounds used by these bacteria for chemoautotrophic growth. These microorganisms are characterized by the ability to reduce metals and remove sulfur and phosphorus from solutions [Pradhan et al., 2021].

The conducted studies showed the presence of sulfur and iron in the mineral raw materials used. This result suggests that *T. ferrooxidans* can be effectively used in the process of leaching the studied mineral raw materials [Kenzhaliyev et al., 2019; Kaumetova et al., 2022].

MATERIAL AND METHODS

Significant priority may be given to the development of strains with improved metal binding capacity or selectivity that demonstrate significantly increased potential for gold mining technologies. The microbiological, physiological and biochemical properties of the selected microorganisms were studied in accordance with generally accepted methods. Thus, *T. ferrooxidans* was grown on a salt medium with S^0 and Fe^{3+}. To obtain productive solutions, samples of gold-containing raw materials were crushed to 90% of the 0.71 mm class, after which they were oxidized in the presence of *T. ferrooxidans* bacteria. Biooxidation of the raw materials was carried out with bacterial solutions of different concentrations (5, 10, and 20 g/l Fe^{3+}) for 10 days at a temperature of 20 °C. The cultures differed in the rate of Fe^{3+} reduction, and their activity could be assessed by the final result and the end time of the oxidation of Fe^{2+}.

Next, agitation leaching was carried out with a 10 g/l sodium thiosulfate solution. The pulp density during leaching was 40% solids. The pH of the pulp during the tests was maintained at 8.0. The leaching time was 24 hours.

Table 1. Results of X-ray phase analysis of mineral gold-bearing raw materials before and after biooxidation

Compound Name	Formula	S-Q before and after biooxidation	S-Q
Quartz, syn	SiO_2	49.9	55.8
Clinochlore-1MIIb, ferroan	$(Mg,Fe)6(Si,Al)_4O_{10}(OH)_8$	11.2	12.1
Microcline, intermediate	$KAlSi_3O_8$	10.9	11.3
Albite, ordered	$NaAlSi_3O_8$	10.5	6.9
Muscovite	$H_2KAl_3(SiO_4)_3$	4.3	4.5
Actinolite	$Ca_2(Mg,Fe^{2+})_5Si_8O_{22}(OH)_2$	4.2	4.5
Ammoniojarosite, syn	$(NH_4)Fe_3(SO_4)_2(OH)_6$	4.2	2.6
Kaliophilite	$KAlSiO_4$	2.5	2.3
Luogufengite, syn	Fe_2O_3	2.4	55.8

After the concentrate leaching process, productive solutions were obtained containing 0.48 mg/l Au and 0.5 mg/l Au.

According to the results of X-ray phase analysis of the raw materials before and after biooxidation, there was an increase in the contents of quartz and the iron mineral luogufengite and a decrease in the proportion of albite, which is an indicator of the biochemical activity of microorganisms. The results of the analysis are presented in Table 1.

Preparation of the microalga *Chlamydomonas reinhardtii* TN-72 CH

A live concentrated algal suspension was obtained by recultivation in fresh TAP culture medium with an alternative carbon source for mixotrophy and with exposure to light. After 8 days of cultivation on a solid nutrient medium, TAP was transferred to a liquid nutrient medium in 50 ml flasks. The flasks were placed under constant illumination and stirred on a shaker. Upon reaching the desired cell usage efficiency on the 10th day of cultivation, they were subcultured into 200 ml flasks. After 12 days of cultivation in the 200 ml flasks, the suspension culture was concentrated by centrifugation to a volume of 50 ml and used for the experiment.

Lyophilized biomass of the microalga *C. reinhardtii TN-72 CH* was obtained by gentle drying without temperature adjustment to preserve the preparation in the absence of volatile solvents. Buffer solutions and other chemicals were not used to protect the microalgal cells during lyophilization. The resulting lyophilisate was kept under moderate cooling conditions (approximately 0 °C). During preparation, the lyophilisate was moistened with TAP nutrient medium until a liquid inactivated suspension was obtained.

Experimental comparison of sorption characteristics

Laboratory tests were carried out for the selected sorbents, AM-2B resin, GoldCarb activated carbon, and a live concentrate of algae biomass in dynamic mode, in a chromatographic sorption column with an inner diameter of d = 16 mm. A 5 g mass of sorbent or 50 ml volume of algal biomass was introduced into the sorption column, and 500 ml of a productive solution with a gold content of 0.48 mg/l was flowed into the column at a rate of 1 ml/min. The conditions were kept consistent over all tests. The sorbent was prepared according to a standard procedure.

Table 2 shows the analytical results for the saturated gold content in the sorbents AM-2B, GoldCarb activated carbon and *C. reinhardtii* TN-72 CH algae obtained during sorption from productive leaching solutions. All sorbents showed a high efficiency in extracting gold. The best results were an efficiency of 87.3% for GoldCarb activated carbon and 87% for the microalgae. It can be assumed that, in addition to having a high efficiency in gold sorption and selectivity, the biosorbents are environmentally friendly. When choosing the most effective sorbent, the kinetic factors affecting the process of gold sorption from complex multicomponent solutions were determined.

The effect of temperature on the degree of gold sorption from gold-bearing solutions was studied; it was found that increasing the temperature to 30 °C significantly increased the rate of gold sorption when using an inactivated liquid suspension of lyophilized biomass of the microalga *C. reinhardtii* TN-72 CH as a sorbent. The tests were carried out in a thermostat for 10 days. A productive gold solution with an Au content of 0.5 mg/L was used. The maximum rate of gold sorption was 87%. The results of experiments on

Table 2. Chemical analysis of solutions after the sorption process

Name	AM-2B	GoldCarb	*Chlamydomonas reinhardtii* TN-72 CH
Gold content in the initial solution, mg/l	0.48	0.48	0.48
Sorbent weight, g	5	5	5
Gold content in the solution after sorption, mg/l	0.024	0.013	0.015
Sorption time, h	20	20	240
Recovery, %	85	87.3	87.0

Table 3. Results of leaching extraction of gold from productive solutions under various temperature conditions

The name of indicators	Results		
	Test 1	Test 2	Test 3
Gold content in the initial solution, mg/l	0.5	0.5	0.5
Temperature, °C	20	25	30
Gold content in solution after sorption, mg/l	0.03	0.027	0.012
Sorption time, days	10	10	10
Recovery, %	84.0	84.6	87.0

the biosorption parameters of microalgal biomass in gold-containing solutions under various temperature conditions are shown in Table 3.

Effect of microalgal biomass concentration on gold recovery

The tests were carried out in agitation mode at low stirrer speeds (50 rpm). A certain amount of the alga *C. reinhardtii* TN-72 CH was introduced into the solution with a measuring cylinder, and a productive solution with a gold content of Au 0.5 mg/l was used. With an increase in the concentration of microalgae, the efficiency of sorption increased to 87.6%. Table 4 shows the test results.

At the end of the process, the presence of living cells in the biomass was analyzed by

Table 4. Indicators of gold sorption from productive solutions depending on the biomass concentration of *Chlamydomonas reinhardtii* TN-72 CH

Indicators	Results			
	Test 1	Test 2	Test 3	Test 4
Gold content in the initial solution, mg/l	0.5	0.5	0.5	0.5
Sorbent volume, ml	25	50	75	100

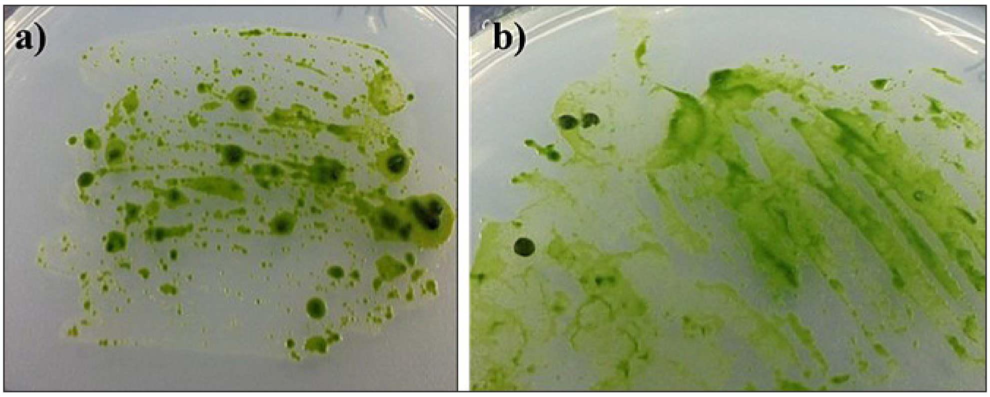

Figure 2. Culture of the microalga *Chlamydomonas reinhardtii* TN-72 CH; a) Culture on the 15th day of cultivation in laboratory conditions after inoculation of the working solution with a microbiological loop using the streak plate method on solid TAP medium; b) culture on the 3rd day of cultivation under laboratory conditions after inoculation of live algal cell concentrate solution with a microbiological loop using the streak plate method on solid TAP medium

inoculation of the working solution with a microbiological loop using the exhaustive streak method on a solid TAP medium and subsequent cultivation under continuous illumination with moderate shading for 15 days.

As a control, live algal concentrate was inoculated on solid TAP medium with a microbiological loop using the streak plate method before it was added to the working solution. The control was cultured under constant illumination with moderate shading (Figure 2).

RESULTS AND DISCUSSION

The presence of living cells at the end of the sorption process indicates the ability of the strain *C. reinhardtii* TN-72 CH to survive under the extreme conditions of the thiosulfate solution.

Analysis of the solutions showed a gold content of 0.1 mg/l, which allows us to conclude that the algal biomass concentrate has a high sorption capacity in vivo.

When using a liquid inactivated suspension of lyophilized microalgae biomass as a biosorbent, the following results were obtained.

The growth dynamics of *C. reinhardtii* TN-72 CH cells introduced into gold-containing solution in the form of a live algal cell concentrate were negative, as expected when planning this experiment (Figure 3).

However, the presence of living cells at the end of the biosorption process, confirmed after 10 days by inoculation with a microbiological loop by the streak plate method on TAP solid nutrient medium (Figure 4), suggests that this strain has the potential for adaptation to the extreme conditions of the thiosulfate solution.

Since the possibility of using various species and strains of microalgae as biosorbents and biooxidants differs significantly depending on their specific characteristics, such as the rate of

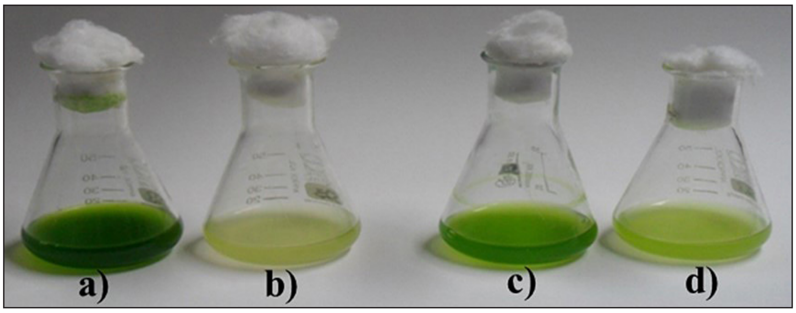

Figure 3. *Reinhardtii TN-72 CH* microalgae on the 5th day after addition to the working solution; a) Live algal cell concentrate of *Chlamydomonas reinhardtii* TN-72 CH before addition to the working solution; b) live algal cell concentrate of *C. reinhardtii* TN-72 CH on the 5th day after addition to the working solution; c) inactivated liquid suspension of lyophilized biomass of *C. reinhardtii* TN-72 CH before addition to the working solution; d) inactivated liquid suspension of lyophilized biomass of *C*

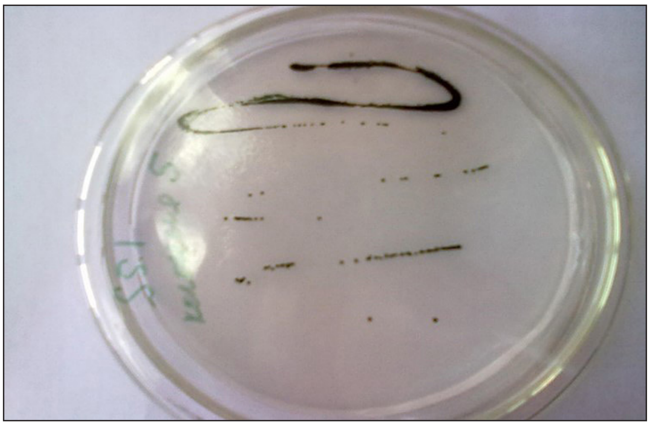

Figure 4. Solid TAP medium after microbiological loop inoculation by the streak plate method of 10-day-old working solution containing live *Chlamydomonas reinhardtii* TN-72 CH algal cell concentrate on the 8th day of exposure

biomass growth; the requirements for the composition of the nutrient medium, lighting, and mixing; and even contamination of the suspension with various microbiological agents, a necessary condition for choosing the most promising representatives is the establishment of experimentally confirmed data.

Study of the sorption capacity of microalgae to assess their potential for use as a biosorbent in the extraction of gold from complex solutions

The most important physicochemical characteristic of a sorbent is its capacity, which is understood as a quantitative measure of its ability to absorb gold ions. The amount of sorbent required to absorb ions from a given volume of solution depends on its capacity. Biomass of *C. reinhardtii* TN-72 CH was used in gold-bearing solutions to study the sorption capacity when used as a biological sorbent.

The experiment was carried out in static mode at a temperature of 25 °C. To a 10-l container with a model gold-containing solution with a gold content of 2 mg/l, an inactivated liquid suspension of lyophilized microalgae biomass and live algal cell concentrate were introduced with a volume of 50 ml. The contact time of microalgae with the solution was 10 days.

During the experiment, it was found that the sorption capacity of the live algal cell concentrate of *C. reinhardtii* TN-72 CH for gold was 10 kg of gold per 1000 kg of dry matter. However, it is premature to discuss the full sorption capacity, since the limit was not reached.

The study showed the promising biosorption potential of the microalga *C. reinhardtii* TN-72 CH, which is determined by its stability, growth rate and gold sorption rate of up to 87.6% at 30 °C, with a sorption capacity of 10 kg of gold per 1000 kg of dry matter.

The use of microorganisms in gold hydrometallurgy as an alternative to cyanide methods will reduce the load on the environment while reducing the cost of the technology. In the process of leaching gold-bearing raw materials by microorganisms, even submicroparticles of gold are released, which makes the processing of poor and refractory ores promising. Preliminary biooxidation increases the effectiveness of thiosulfates in terms of both time and gold recovery. The environmental friendliness of thiosulfate leaching techniques opens up additional opportunities for the use of mine waste residues in agriculture as fertilizers.

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