ANALYZING THE USE OF *PSEUDOMONAS AERUGINOSA* TO BIOSORB NIOBIUM FROM SYNTHECTIC SOLUTIONS AT ACIDIC CONDITIONS – AN INTRODUCTORY STUDY

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ABSTRACT

Niobium (Nb) has become one of the main metals used in the production of metal alloys and its application towards green energy production. As industrial and energetic demand increases, so does the mining of the metal and the impacts generated by it. Bioprocesses are becoming a way to mitigate the damages caused by common means of mining, extraction and purification with the use of biocomponents and microorganisms. One of the said bioprocesses, biosorption, was the chosen for this work to explore the potential of dry biomass of the bacteria *Pseudomonas aeruginosa* as a biosorbent. The aim was to also test the conditions in which the adsorption of Nb would be possible regarding pH, metal and dry biomass concentrations, and lastly to study the functional groups that would be influencing the process.

Keywords: Bioprocess. Biosorption. *Pseudomonas aeruginosa*. Niobium.

1 INTRODUCTION

Niobium (Nb) is a transition metal discovered in 1802 by Charles Hatchett, it became a critical metal to world development as its mainly used for alloy stabilization in the iron and steel industries, as superconductor materials, for its capability to alter metal properties and as a photocatalyst towards solar energy production.[1,2]

The world's reserve of Nb is currently found in Brazil with 94% of it reserves found in its national territory.[3] While recurrent in Brazil's soil the concentration of Nb worldwide is considerably low (24ppm).[4] The most common form in which the metal is found are in the form of pyrochlore, a pure form of Nb oxides, and columbite which contains a mix of Tantalum, Uranium, Thorium and other minerals.[5] Since the metal is sparsely distributed and current methods of extraction in Brazil are lacking the technology to extract the metal effectively there's the possibility that eventual shortages may happen. The more common techniques used for Nb extraction are harmful and aggressive due to the use of strong acids to leach the metal such as hydrofluoric acid (HF) or ammonium fluoride (NH_4F) solutions, both reagents are extremely toxic to life.[6]

To allow for a more environmentally safe approach and towards a better flow of Nb towards the global market new processes are being developed, among these are bioprocesses that apply microorganisms for metal extraction and recovery. Biosorption is one of the aforementioned process, it use live or dead biomass as adsorbent agents as the cellular wall contain can contain lipids, proteins and functional groups that allow for metal adsorption. It has been shown to be renewable due to continuous microbial growth and less damaging when compared to acid leaching with HF. For this work the dry biomass of the bacteria *P. aeruginosa* has been selected as the biosorbent and synthetic solutions will be used to test the biomass capabilities to adsorb Nb without interference of other factors like metals and pH influencing factors.

2 MATERIAL & METHODS

An isolated *P. aeruginosa* culture was grown for 2-3 days in Luria-Bertani (LB) agar solid medium at 30ºC at an incubator. The bacteria were then harvested and identified utilizing a matrix-assisted laser desorption ionization time-of-light mass spectrometer (MALDI-TOF) and, subsequently inoculated in liquid LB media in an orbital shaker at 30ºC and 150rpm. Its growth was analyzed utilizing a UV-VIS spectrophotometer at a wavelength of 600nm in a quartz cuvette hourly, 0 to 8h or until the stationary phase was achieved.

To collect the biomass the cultures were grown until the stationary phase was achieved and the max number of cells were encountered, then they were autoclaved at 121ºC for 30 minutes to obtain dead biomass. After being autoclaved, the cultures were centrifuged for 10 minutes at 4000rpm, and the resulting supernatant discarded while the biomass was retained at the end of the centrifuge tube. It was then washed three times and resuspended with a saline solution (0,9% NaCl). Later, the biomass was frozen at -80ºC in an ultra-freezer and lyophilized at -50ºC under vacuum until it was completely dry. The dry biomass was analyzed in a Fourier-Transform Infrared Spectroscopy (FTIR) equipment to study the functional groups present.

For the biosorption experiments synthetic solutions containing Nb were produced, and the following parameters were tested to better understand the behavior between biomass and the solution: first was the pH, second was dry biomass concentration and third the time of the process. For the pH studies four synthetic solutions containing 10ppm of Nb were prepared and had their pH adjusted to 3, 4, 5 and 6 utilizing a 1M NaOH solution. Lastly, 0,1 g/L of dry biomass were added, and the flasks taken to an orbital shaker at 30ºC and 150 rpm. Aliquots were taken after 1, 5, 10, 15, 20, 30 and 60 minutes had passed, this time pattern was used throughout all the study. The aliquots were filtered with a 0,22µm syringe filter and stored for analysis.

After the most efficient pH was selected, the biosorption experiments were initiated with solutions containing 10, 25, 50, 100ppm of Nb. These solutions were prepared with 50ml of ultra-pure water and the metal was added from a pattern solution containing 10.000ppm of Nb and 1M NaOH was used to correct the pH. The dry biomass was added in two different concentrations of 0,1 and 0,5g/L, but maintaining the metal concentration, time and pH. Aliquots were collected from each experiment in the specified timeframes and filtered as previously mentioned.

The filtered aliquots from all experiments were diluted 20x in 10mL volumetric flasks, and two analysis curves were prepared in the following intervals: 0,1; 0,25; 0,40; 0,55; 0,70; 0,85; 1,00ppm and 1,0; 2,5; 4,0; 5,5; 7,0; 8,5; 10,0ppm of Nb. All dilutions, including the analysis curve, were diluted utilizing 3% HNO_3. They were analyzed in an inductively coupled plasma optical emission spectroscopy (ICP-OES). All testes were done in triplicates and blank solutions of each of the Nb concentrations and parameters were used as control solutions. The collected results from the ICP-OES were analyzed regarding the Nb removal rate (R%). In equation 1, C_i is the initial concentration of Nb and C_f is the final concentration found.

$$R\% = \frac{C_i - C_f}{C_i} \times 100 \quad (1)$$

3 RESULTS & DISCUSSION

The bacteria grown in LB media and analyzed by MALDI-TOF was identified, as expected, as the gram-negative *P. aeruginosa* (ATCC 27853 THL) with a score of 2,196 by the equipment, which demonstrates a reliable result since ≥2,000 indicates a certain analysis. The bacterial growth measured in the UV-VIS spectrophotometer, illustrated in figure 1a., did not demonstrate any anomalies and was constant throughout the study showing the characteristic green color the species is known for. The FTIR analysis of the dry biomass demonstrated the predominance of the following functional groups: –NH, –OH, –CH, –C=O, –CN and –SO_3 stretching bands, figure 1b., the bands are compatible with available literature.[7]

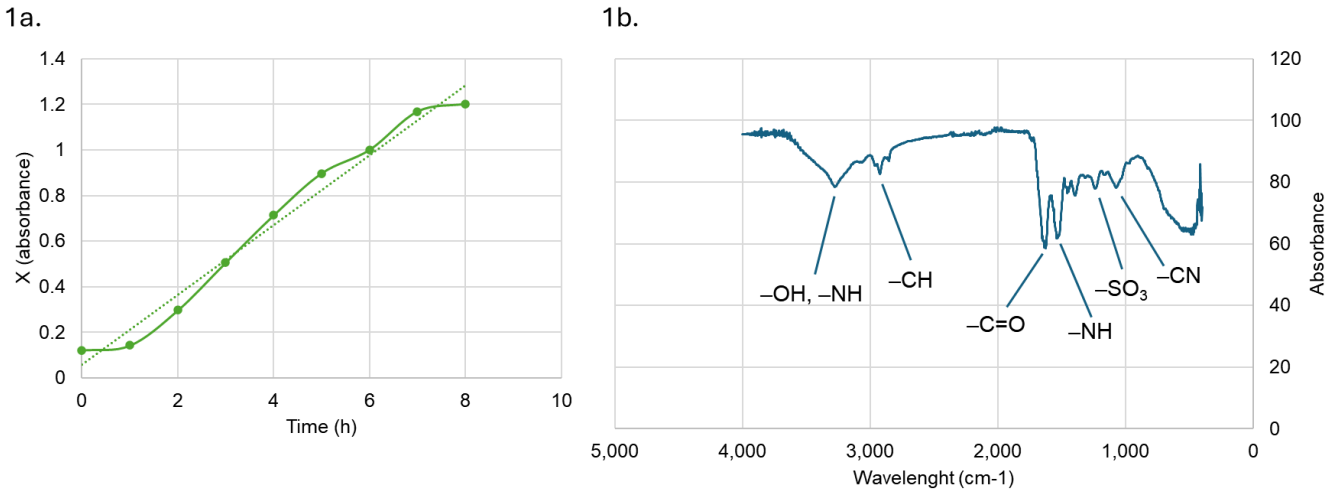

Figure 1 The figure indicates the characteristics of the bacteria: 1a. Growth curve of the P. aeruginosa bacteria (1a.) and the FTIR spectrum of the dry biomass with the respective functional groups depicted (1b.).

The hydrogen potential (pH) influence was a determinant factor to the biosorption analysis since Nb tends to precipitate as pH increases. The experimental parameters of 0,1g/L of dry biomass and 10ppm of Nb were fixed throughout the experiments where pH varied with pH 4 being the adsorption point, 5 being an intermediary test point and 6 a probable precipitation point for the metal. Resulting R% for the pH tests demonstrated that pH 4 had the highest R% with 42% followed by 21% at pH 5 and 30% at pH 6. The discrepancy between pH 5 and 6 can be due to Nb precipitating since, according to available literature, Nb tends to precipitate at high pH values and, therefore, cannot be adsorbed to the biomass.[8]

The adopted pH was then set as 4 which was maintained for all following biosorption experiments. A possible reason for this pH being the most optimal one can be assimilated not only to low Nb precipitation, but due to the action of specific functional groups that were more prevalent in said pH, those being: –NH and –OH. These groups tend to protonate at low pH which electrostatic attracts Nb ions to the biomass. In addition, gram-negative bacteria have lipopolysaccharides, peptidoglycans and phospholipids that allow for more anionic characteristic and, therefore, the ability to retain metals.[9]

With the pH set at 4 the biosorption experiments were conducted with the overall results being expressed in table 1. Dry biomass tend to have a high impact in the biosorption process as it defines how much surface area is available (contact zones) for the metals to attach to, as well as the amount of functional groups and lipids available. Usually, the higher the biomass the higher the R%. Focusing solely on dry biomass concentrations, the best result was encountered when 0,5g/L of dry biomass were added with 73% of the Nb present being adsorbed, most probably due to higher availability of contact zones. While higher dry biomass concentrations made more contact zones available, Nb concentration also affected the overall process. All R% decreased as Nb concentrations increased, the phenomena can be due to metal saturation as contact zones were occupied as the process progressed as can be observed in other experiments.[10]

Table 1 The table represents the results obtained from the biosorption experiments at pH 4 with varying dry biomass and Nb concentrations.

Dry biomass conc. (g/L)	Nb conc. (ppm)	R%
0,1	10	37,612
	25	27,042
	50	20,449
	100	10,042
0,5	10	73,329
	25	56,280
	50	20,697
	100	7,585

4 CONCLUSION

Very little has been studied regarding Nb extraction via biological pathways and, as observed by the results obtained, it is possible to extract the metal utilizing the *P. aeruginosa* bacteria biomass. It is possible to affirm that the process will be entirely reliant on the type of biomass used as it will influence the functional groups, lipids and saccharides available. The pH will also heavily influence the process as metals can precipitate at different intervals as well as it will impact the type of functional groups that will be more active. The biomass used in this study showed potential to biosorb Nb at acidic conditions, pH 4, obtaining considerably high R% of 73,329% at low Nb concentration and up to 56,280% at intermediate metal concentration. For further advances on the field of Nb extraction via biosorption it is important to study the technique with real world samples, as they will contain different and more complex minerals which will impact the parameters studied in this work drastically. Working with bioprocesses can be seen as low cost and environmentally safe when compared to the most common routes, therefore, making its study a viable alternative for metal extraction or recovery.

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