Region - Water Conservancy, 2025, Volume8, Issue2
DOI: 10.32629/rwc.v8i2.3742
ISSN Online: 2630-4902

Removal of copper from contaminated waters using rhamnolipids

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Abstract: The use of rhamnolipids constitutes an attractive and eco-compatible alternative in the remediation of contaminated environments with heavy metals. In the present work, the ability of rhamnolipid produced by P. aeruginosa ORA9 to remove copper in contaminated waters of the Blue Lagoon of El Cobre, was evaluated, to control and reduce the human and environmental risk that these waters represent. The influence of the pH of the rhamnolipid solution (4-10), the contact time (2-10 hours) and the concentration of the biosurfactant (40- 120 mg L^{-1}) in the process were studied, through a factorial design Box-Bhenken. An increase in the percentage of copper removal proportional to the contact time and the concentration of the biosurfactant was observed, reaching 48% removal at 10 h and 120 mg L^{-1}. The results obtained indicate that rhamnolipids can be used in the design of a technology to remove copper in polluted contaminated waters.

Key words: rhamnolipid; contaminated water; copper; heavy metals; factorial design

1 Introduction

Contamination with heavy metals is one of the most important problems affecting the environment, due to the toxicity and the damage it causes to human health and to aquatic and terrestrial ecosystems. Due to these chemical naturalness, these compounds are persistent in the environment and do not degrade by biological, chemical or physical methods to more harmless products; rather, they remain in the environment, accumulating as ions or as metal compounds in organisms for long periods of time [1].

The copper, which is essential for the normal functioning of metabolism in humans, its excessive consumption through water or contaminated beverages, can produce nausea, vomiting and/or abdominal pain, hair loss, anemia and renal and hepatic damage [2].

If different techniques have been developed to remove copper from contaminated waters (chemical precipitation, ion exchange, adsorption, membrane filtration, electrochemical treatment techniques, etc.), but the application of these methods is expensive and ineffective in terms of energy consumption and chemical products, it can also generate large amounts of sludge to be treated with difficulty [3].

New technologies that involve microorganisms and their metabolic products are currently being evaluated for the treatment of effluents from industries and mining activity. Within them, the use of biosurfactants has been charged to improve the removal of heavy metals from these effluents [4].

Rhamnolipids (RL) are anionic glycolipid biosurfactants produced mainly by strains of the genus Pseudomonas. Their structure includes a hydrophilic component consisting of rhamnose and a hydrophobic component consisting of saturated and unsaturated fatty acids [5]. They have excellent surface properties comparable to synthetic surfactants, but they also have the advantage of being biodegradable, non-toxic, and can be obtained from renewable raw materials. Several studies have demonstrated the effectiveness of rhamnolipids in removing heavy metals from soils [6], sediments [7], and contaminated waters [8].

The copper mines, located in the town of the same name in Santiago de Cuba, began their exploitation from the 16th century until the end of the 20th century, using open-pit mining. For economic and technological reasons, the mine closed in 2001. More than 4 centuries of exploitation turned the hill into a huge lagoon (Blue Lagoon) that is testimony to the ecological damage that occurred in this area, impacted mainly by copper [9].

Due to the acidic character of the waters of the Blue Lagoon, its high content of copper and other heavy metals [10], and its use by the majority of the population of the place as a pool area, it is necessary to remedy the situation and restore the area. It can have benefits for tourist activity, biodiversity conservation, aquaculture, irrigation and as a source of water supply for other mining activities that are developed, in a landmark where huge efforts are made worldwide for the recovery and protection of vital liquid. There are no reports of the use of ramnolípidos for the removal of copper from contaminated waters from a mine lake.

This research aims to evaluate the potential of a rhamnolipid to extract copper from these contaminated waters, in order to implement a remediation technology and minimize the risk that this aquatic ecosystem represents for human and environmental health.

2 Materials and methods

2.1 Water samples

Samples of contaminated water were taken from the Laguna Azul, which is located at latitudes 20°2’56’’ North and 75°56’49’’ West, has around 4 million m^3 of water, one kilometer at its longest part, around 60 m deep and almost 800 m at its widest part. Considering that the physical-chemical characterization of these waters verified that there is spatial homogeneity between the surface waters and at a depth of 1.5 m[10], samples were taken in an area (20º02’47.8’’N; 75º56’27.1’’E) that is used for recreational purposes (bathing), where the waters are crystal clear and the bottom is not observed due to the great depth. They were collected in the morning in pre-washed 500 mL plastic containers and stored at 4°C until treated in the laboratory.

In the laboratory, samples were pressure-filtered through 0.45 µm PES (polyethersulfone) membrane filters. Aliquots of water samples for copper analysis were acidified at the time of collection with 1% nitric acid (HNO3, 65% supra pure, Merck) and stored at 4°C until analysis. Copper concentration was determined by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP AES) on an AMETEK spectrometer, Germany. The calibration solution was prepared from a certified monoelemental standard solution (1 mg mL^{-1} copper standard solution as Cu in 2% HNO3, ACROS-ORGANICS®).

2.2 Rhamnolipid solution

The rhamnolipid produced by the bacterium Pseudomonas aeruginosa ORA9 isolated from soils contaminated with oily waste and cultivated in a mineral medium with frying oil as a carbon source was used. [11] It is deposited in the Culture Collection of the Center for Industrial Biotechnology Studies, Faculty of Natural and Exact Sciences, University of

Oriente. The rhamnolipid solution was prepared by dissolving the crude biosurfactant in distilled water for a final concentration of 400 mg L^{-1}.

2.3 Copper removal from contaminated water

The copper removal assay was performed according to the methodology described by Elouzi et al. [12] with modifications. Aliquots of contaminated water and rhamnolipid solution were transferred to centrifuge tubes. Each tube was shaken on a shaker (120 rpm) at room temperature and at the end, and the samples were centrifuged (8,000 rpm, 20 min.) (Heal Force®, China) and filtered through 0.2 µm cellulose acetate filters. The copper concentration was determined by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) on an AMETEK spectrometer, GERMANY, and the percentage of metal removed was calculated by equation (1):

$$\% \mathrm{Re}\,moval = \frac{MCi - MCf}{MCi} x100 \qquad (1)$$

Where

MCi is the initial copper concentration and MCf is the final copper concentration (after treatment with rhamnolipids).

2.4 Experimental design

The effect of three variables: pH of the rhamnolipid solution, contact time, and solution concentration was evaluated using a Box-Behnken response surface experimental design, which consisted of 15 experiments, including three replicates at the center point to assess experimental error. Each independent variable was studied at three levels (-1, 0, +1) (Table 1). The range and levels of the factors studied were selected based on the results of similar studies [11]. The percentage of copper was selected as the response variable.

Table 1. Independent variables and levels studied in the Box-Behnken experimental design for the removal of copper from contaminated water

Variables	Factors	Levels		
		-1	0	1
X1	Tiempo de contacto (h)	2	6	10
X2	pH solución de ramnolípido	4	7	10
X3	Rhamnolipid solution concentration (mg L^{-1})	40	80	120

2.5 Statistical analysis

The results were presented as the mean of three replicates ± standard deviation. For the experimental design, the effect of each factor or independent variable studied, the P-value and the coefficient of determination (r^2) were calculated using Analysis of Variance (ANOVA). A regression analysis was used to determine the mathematical model that describes the behavior of the statistically significant factors on the response variable. The goodness of fit was checked using the correlation graph between the observed values and those predicted by the determined mathematical model. Statistical significance was set at $p \leq 0.05$. The analyses and graphs were performed with the help of the statistical software STATGRAPHICS Centurion XV 15.2 [13].

2.6 Results and discussion

Table 2 shows the average copper removal values obtained in each experimental run, observing percentages of up to 48%.

Although no similar reports were found studying copper extraction from contaminated waters using rhamnolipids, the removal value obtained in this study is similar to those obtained by other authors who evaluated the efficiency of these compounds in the extraction of copper from different contaminated environments. Mulligan [14] reported a maximum copper removal (46%) using a 2% rhamnolipid solution in the washing of a soil contaminated with heavy metals, while Dahrazma and Mulligan [15] managed to remove up to 37% of the copper present in contaminated sediments, when evaluating the efficiency of a rhamnolipid biosurfactant, using a continuous flow configuration for the remediation of contaminated sediments.

The contaminated waters of Laguna Azul have a moderately acidic pH (pH = 4.6 ± 0.2) and high contents of copper (43.6 ± 1.7 mg L^{-1}) and manganese (24.1 ± 1.1 mg L^{-1}), above the acceptable limits for surface water supply sources stipulated by Cuban Standard 1021:2014. [16] It also presents other metals whose concentrations were observed in the following sequence: Al> Zn> Sr> Fe> Co> Cd> Ba> Ni. Trace metals and metalloids such as As, Cr, Mo, Pb, Sb and Se, were found in very low concentrations [10].

Table 2. Box-Behnken experimental design matrix used to evaluate the influence of three factors on copper removal from contaminated waters, using rhamnolipids

Experiment	Contact time (h)	Rhamnolipid solution pH	Rhamnolipid concentration (mg L^{-1})	Removal (%)	
				Experimental	Foretold
1	6	4	120	47,4±4,1	47,9
2	2	4	80	34,0±3,3	33,8
3	6	10	40	36,2±2,5	35,7
4	2	7	40	27,1±1,3	27,1
5	2	10	80	33,0±1,4	33,1
6	6	7	80	40,0±2,5	38,8
7	6	4	40	36,0±2,7	35,7
8	10	7	120	48,0±5,3	47,6
9	10	7	40	37,8±2,2	38,1
10	6	10	120	46,4±4,1	46,6
11	10	4	80	42,3±3,1	42,2
12	6	7	80	41,2±4,4	38.8
13	10	10	80	41,5±3,2	41,7
14	6	7	80	35,2±3,3	38,8
15	2	7	120	41,5±2,2	41,1

Although the presence of multiple metal cations in these waters could have interfered with the removal of copper by rhamnolipid, studies [17] have shown that these compounds form complexes with many elements with different stability constants (from stronger, log K= 10.30 to weaker, log K= 0.96): $Al^{3+} > Cu^{2+} > Pb^{2+} > Cd^{2+} > Zn^{2+} > Fe^{3+} > Hg^{2+} > Ca^{2+} > Co^{2+} > Ni^{2+} > Mn^{2+} > Mg^{2+} > K^{+}$, which indicates that in this case, the formation of the copper-rhamnolipid complex was more prevalent than other metal rhamnolipid complexes that could have formed under the experimental conditions tested.

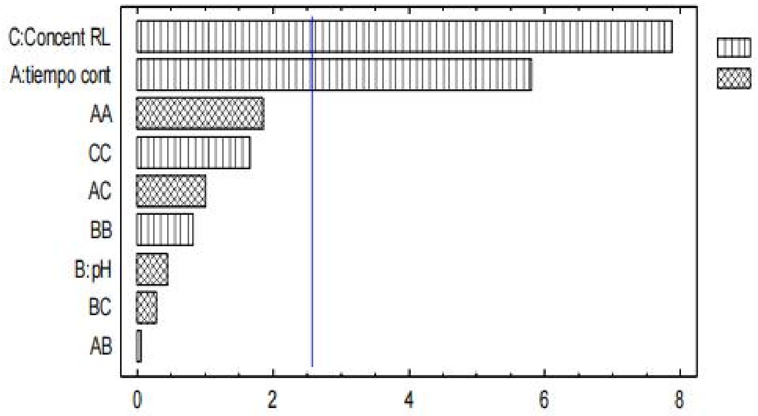
The ability of biosurfactants to form complexes with metals is the main reason why they can be used in the remediation of environments contaminated with heavy metals. Anionic biosurfactants, such as rhamnolipids, form ionic bonds with metals, generating highly stable non-ionic complexes. The presence of the carbonyl group in the carboxyl group exerts an electrowithdrawing effect on the oxygen of the OH group of the carboxyl, causing a low electron density on that oxygen atom. This situation does not occur in the rhamnose hydroxyls, making the union with the cation more favorable. Furthermore, the size and basicity of the donor atoms (OH) in rhamnose favor the formation of stable complexes [18].

Hogan [19] studied the nature of the interactions of mono-rhamnolipid with metals by proton magnetic resonance spectroscopy (H 1 NMR) and Fourier transform infrared spectroscopy (FTIR). He found that the carboxyl group is only weakly involved in the metal complexation, so the high stability of the complexes formed is due to the binding of the metal cation to other parts of the rhamnolipid molecule, such as the hydroxyl groups of the rhamnose molecule, forming a structure known as a binding pocket involving the carboxyl group and the rhamnose sugar.

Specifically, the description of the interaction of rhamnolipids with Cu^{2+} ions was studied by Ciésla et al [20], who evaluated mixtures of rhamnolipids in premicellar and aggregated states, in water and in 20 mM KCl solution, at pH 5.5 and 6.0. The biosurfactant revealed the best chelating activity in premicellar solutions. The degree of metal binding decreased with increasing concentration of the system and the presence of K^+ reduced the binding of Cu^{2+} by rhamnolipids, but did not significantly modify the stability of the complex. It was shown that the effectiveness of these compounds depends on the composition of the system and the pH conditions.

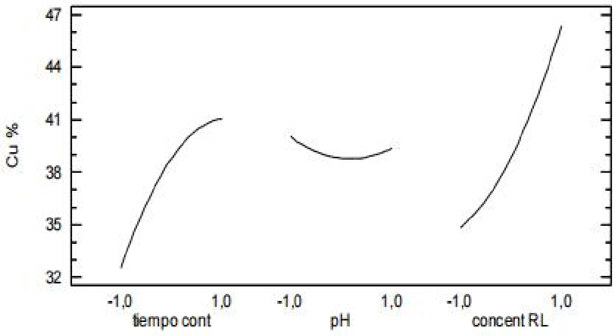
Statistical analysis of these results (Table 2) determined that contact time and concentration of the rhamnolipid solution were the factors with the greatest influence on the metal removal process from the contaminated waters. This was observed when the estimated effects were plotted in decreasing order of importance on a Pareto chart (Figure 1a). The main effects chart shows how the copper removal percentage increased from 32% to 48% as the two main factors with significant effects moved from their lowest to their highest levels (Figure 1b).

Standardized Pareto Chart for Cu



a) Standardized effect

Main Effects Plot for Cu



b)

Figure 1. Pareto (a) and main effects (b) charts obtained in the study of copper removal from contaminated water using rhamnolipid

A longer contact time between the biosurfactant solution and the contaminated water would favor greater interaction of the Cu^{2+} metal cations with the chelating groups of the rhamnolipid, allowing for their subsequent extraction in the form of a rhamnolipid-metal complex. Similarly, a higher concentration of rhamnolipids allows for a greater number of biosurfactant molecules to interact with the dissolved cations, favoring the removal of the metal from the water.

The statistical significance of the variables studied (contact time, concentration, and pH) was verified using an Analysis of Variance (Table 3). It was found that the factors contact time and concentration of the rhamnolipid solution had a P value less than 0.05, with a confidence level of 95.0%.

Table 3. Analysis of the Box-Behnken experimental design used to evaluate the influence of three factors on copper removal from contaminated water using RL

Fountain	Sum of Squares	Gl	Middle Square	Reason-F	P-value
A: t contact.	144,5	1	144,5	33,71	0,0021
B: pH	0,845	1	0,845	0,20	0,6756
C: concentration of RL	266,805	1	266,805	62,25	0,0005
AA	147,692	1	147,692	3,45	0,1225
AB	0,01	1	0,01	0,00	0,9633
AC	4,41	1	4,41	1,03	0,3570
BB	299,077	1	299,077	0,70	0,4416
BC	0,36	1	0,36	0,08	0,7836
CC	119,631	1	119,631	2,79	0,1556
Error total	21,43	5	4,286		
Total (corr.)	470,429	14			

R-squared = 95.44%, R-squared (adjusted for d.f.) = 87.24%

S-Standard error of the est. = 2.07, Mean absolute error = 0.71

T-Durbin-Watson statistic = 2.252 57 (P=0.585 3),

U-Lag 1 residual autocorrelation = -0.127 712

The mathematical model that describes the relationship between the percentage of copper extraction and the two variables with significant influence is given by equation (2):

Cu (%) = 38.8 + 4.25*contact time + 5.775*rhamnolipid concentration (2)

The coefficient of determination (r^2) indicates that this model accounts for 95.4% of the variability obtained in the percentage of metal removal. The goodness of fit obtained by this model was verified with the graph of the predicted versus observed results (Figure 2), which showed a homogeneous distribution in the three variables. The correlation coefficient (r = 0.988 0) obtained indicates a relatively strong relationship between the experimental values and those determined by the fitted model.

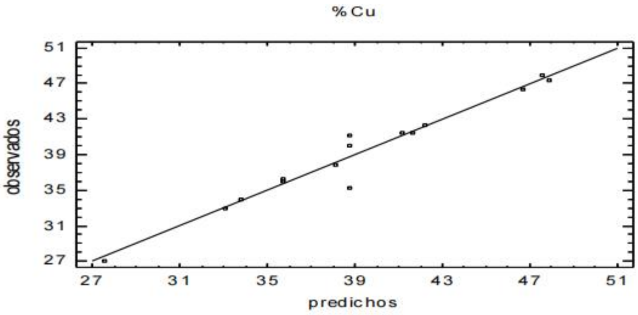


Figure 2. Graph of values predicted by the mathematical model and those observed experimentally in the removal of copper from contaminated water using rhamnolipid

Currently, efforts to achieve effective technologies for the remediation of waters contaminated with heavy metals have focused on the use of rhamnolipids combined with other physical-chemical or biological techniques used for these purposes, with the aim of increasing their effectiveness, such as micellar membrane ultrafiltration [21], precipitation by flotation [8] and phytoremediation [22].

3 Conclusions

Rhamnolipids are an eco-friendly option for removing heavy metals from contaminated waters. This research demonstrated the potential of rhamnolipids produced by Pseudomonas aeruginosa ORA9 for removing copper from the contaminated waters of Laguna Azul del Cobre and the influence of contact time and biosurfactant concentration on this removal process. The mathematical model that explains the metal removal process was also determined. This research constitutes a necessary background for establishing an appropriate technology.

Acknowledgments

The authors of this work would like to thank the University of Oriente, the Agence Universitaire de la Francophonie (AUF), the Caribbean Bureau, and the French Embassy in Cuba for funding the projects that supported the research. They also thank the Geominera Oriente Sur company for their collaboration in obtaining the results.

Conflicts of interest

The authors declare that there are no conflicts of interest in the submitted manuscript.

References

[1] HASHIM, M., et al. Remediation technologies for heavy metal contaminated groundwater. *Journal of Environmental Management*. 2011, 92, 2355-2388. doi:10.1016/j.jenvman.2011.06.009

[2] CAROLIN, C., et al. Efficient techniques for the removal of toxic heavy metals from aquatic environment: A review. *Journal of Environmental Chemical Engineering*. 2017, 5, 2782–2799. doi:10.1016/j.jece.2017.05.029

[3] FU, F., WANG, Q. Removal of heavy metal ions from wastewaters: A review. *Journal of Environmental Management*. 2011, 92, 3, 407-418. doi:10.1016/j.jenvman.2010.11.011

[4] WU, M., et al. Decontamination of multiple heavy metals-containing effluents through microbial biotechnology. *Journal of Hazardous Materials*. 2017, 337, 189–197. doi: 10.1016/j.jhazmat.2017.05.00

[5] DESAI, J., BANAT, I. Microbial production of surfactants and their commercial potential. *Microbiology and Molecular Biology Reviews*. 1997, 61, 1, 47-64. doi:10.1128/.61.1.47-64.1997

[6] MULLIGAN, C., YONG, R., GIBBS, B. Surfactant-enhanced remediation of contaminated soil: a review. *Engineering Geology*. 2001, 60, 371-380. doi:10.1016/S0013-7952(00)00117-4

[7] CHEN, W., et al. Heavy metal (Cu, Cd, Pb, Cr) washing from river sediment using biosurfactant rhamnolipid. *Environ SciPollut Res*. 2017, 24, 16344-16350. doi:10.1007/s11356-017-9272-2

[8] ABYANEH, A., FAZAELIPOOR, M. Evaluation of rhamnolipid (RL) as a biosurfactant for the removal of chromium from aqueous solutions by precipitate flotation. *Journal of Environmental Management*. 2016, 165, 184-187. doi:10.1016/j.jenvman.2015.09.034

[9] VILASÓ, J. E. Laguna azul del Cobre: bella pero... [en línea]. (Consultado: 5/09/2020). Disponible en: https://www.elchago.com/2020/07/28/laguna-azul-del-cobre-santiago-de-cuba/

[10] RODRÍGUEZ, O., et al. Assessment of water quality from the Blue Lagoon of El Cobre mine in Santiago de Cuba: a preliminary study for water reuse. *Environmental Science and Pollution Research*, 2019, 26, (16), 16366-16377. doi:10.1007/s11356-019-05030-311.

[11] RODRÍGUEZ, O., et al. Screening and Characterization of Biosurfactant-Producing Bacteria Isolated from Contaminated Soils with Oily Wastes. *J. Environ. Treat. Tech*., 2017, 5 (1), 5-11. ISSN: 2309-1185.

[12] ELOUZI, A, et al. Removal of heavy metals contamination by bio-Surfactants (Rhamnolipids). *Journal of Chemical and Pharmaceutical Research*. 2012, 4, 90: 4337-4341. ISSN: 0975-7384.

[13] Statgraphics Centurion, X. V. I. Statpoint Technologies. INC. version, 16, 17. 2009.

[14] MULLIGAN, C. Recent advances in the environmental applications of biosurfactants. *Current Opinion in Colloid & Interface Science*, 2009, 14, 372-378. doi:10.1016/j.cocis.2009.06.005.

[15] DAHRAZMA, B., MULLIGAN, C. Investigation of the removal of heavy metals from sediments using rhamnolipid in a continuous flow configuration. *Chemosphere*, 2007, 69, 705-711. doi:10.1016/j.chemosphere.2007.05.037

[16] NORMA CUBANA 1021 (2014) Higiene comunal. Fuentes de abastecimiento de agua. Calidad y protección sanitaria.

[17] OCHOA-LOZA, F. J., ARTIOLA, J. F., MAIER, R. M. Stability constants for the complexation of various metals with a rhamnolipid biosurfactant. *J. Environ. Qual.* 2001, 30, 479-485. doi:10.2134/jeq2001.302479x

[18] FRANZETTI, A., GANDOLFI, I., FRACCHIA, L. Biosurfactant Use in Heavy Metal Removal from Industrial Efflents and Contaminated Sites. En: KOSARIC, N., SUKAN, F. V (Eds) Biosurfactants: *Production and Utilization - Processes, Technologies and Economics*. Taylor & Francis, 2014, pp 361-369, ISBN 9781466596696

[19] HOGAN, D. E. *Biosurfactant (monorhamnolipid) complexation of metals and Applications for aqueous metalliferous waste remediation*. Dissertation Submitted For the Degree of Doctor Of Philosophy, University Of Arizona, 2016. Disponible en: https://repository.arizona.edu/handle/10150/623071.

[20] CIÉSLA, J., KOCZANSKA, M, BIEGANOWSKI, A. An Interaction of Rhamnolipids with Cu^{2+} Ions. Molecules 2018, 23, 488, 2-12. doi:10.3390/molecules23020488

[21] KOWALSKA, I., KLIMONDA, A. Removal of copper ions from aqueous solutions by means of micellar-enhanced ultrafiltration. E3S Web of Conferences 22, 00087. 2017. doi: 10.1051/e3sconf/20172200087

[22] LIDUINO, V. S., SERVULO, E. F., OLIVEIRA, F. J. Biosurfactant-assisted phytoremediation of multi-contaminated industrial soil using sunflower (Helianthus annuus L.). J Environ Sci Health A Tox Hazard Subst Environ Eng. 2018, 53, 7, 609-616. ISSN: 1093-4529.

Authors' contribution

Odalys Rodríguez Gámez: Participated in the acquisition, interpretation, and analysis of experimental data. She participated in the evaluation of results and the writing of the manuscript.

Rocio Pérez Macías: Participated in the acquisition, interpretation, and analysis of experimental data. She participated in the evaluation of the results and the writing of the manuscript.

Isabel Aguilera Rodríguez: Participated in the chemical analysis and interpretation of the experimental data.

Rosa María Pérez Silva: Participated in the design and supervision of the research, as well as in the discussion of the results, review and final approval of the manuscript.

Arelis Abalos Rodríguez: Participated in the design and supervision of the research, as well as in the discussion of the results, review and final approval of the manuscript.