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Optimization of copper removal through spherical agglomeration: Effect of pH precipitation and the use of *Agave* spp. leaf extracts as biosurfactants

Optimización de la remoción de cobre mediante aglomeración esférica: Efecto del pH y extractos de material foliar de *Agave* spp. como bio surfactantes

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Abstract

This study investigated the feasibility and efficacy of calcium hydroxide as an alternative to NaOH in the spherical agglomeration process for copper removal in aqueous solutions. This research aimed to evaluate the effectiveness of $Ca(OH)_2$ in precipitation stage by evaluating different pH levels (8.0 and 9.0), surfactant type (Extracts of leaf of *A. tequilana* and *A. lechuguilla*), dosages (0.3, 0.5 and 1.0 g/gCu), and initial copper concentrations (2.0, 5.0 and 10.0 mg/L), on Cu^{2+} removal through spherical agglomeration. The results showed that $Ca(OH)_2$ is an effective alternative to NaOH, achieving high copper removal percentages in various experimental conditions (with an efficiency range of 54.63 to 98.73%). That is particularly relevant in contaminated water treatment applications where cost reduction and sustainability are necessary. The optimal operating conditions were identified (pH_0 9.0, *A. tequilana* leaf extract as biosurfactant and a dose of 0.3g/gCu), such as a specific pH and an adequate dosage of surfactant, which maximized copper removal efficiency (98.73% of copper removal). These results provide valuable guidelines for the wastewater treatment industry and environmental management, where efficient removal of heavy metals is essential.

Keywords: Spherical agglomeration, copper removal, *Agave* spp., surfactant, pH process.

Resumen

Este estudio investigó la viabilidad y eficacia del $Ca(OH)_2$ como alternativa al NaOH en el proceso de aglomeración esférica para la eliminación de cobre en soluciones acuosas. El objetivo de esta investigación fue evaluar la efectividad del hidróxido de calcio en etapa de precipitación evaluando diferentes niveles de pH (8.0 y 9.0), tipo de surfactante (extractos de hoja de *A. tequilana* y *A. lechuguilla*), dosis (0.3, 0.5, y 1.0 g/gCu), y concentraciones iniciales de cobre (2,0, 5,0 y 10,0 mg/L), sobre la remoción de cobre mediante aglomeración esférica. Los resultados demostraron que el $Ca(OH)_2$ es una alternativa eficaz al NaOH, logrando altos porcentajes de remoción de cobre en diversas condiciones experimentales (con eficiencias de 54,63 a 98,73%). Esto es particularmente relevante en aplicaciones de tratamiento de agua contaminada donde la reducción de costos son nesesarios. Se identificaron las condiciones óptimas de operación (pH_0 9,0, extracto de *A. tequilana*, dosis de 0,3 g/gCu), pH específico y dosis apropiadas de surfactante para una máxima eficiencia de eliminación de cobre (98,73 %). Estos resultados son valiosos para el tratamiento de aguas residuales y la gestión ambiental, donde la eliminación eficiente del metal es esencial.

Palabras clave: Aglomeración esférica, remoción de cobre, *Agave* spp., surfactantes, pH del proceso.

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1 Introduction

Only 0.8% of the world's water is available for human consumption, mainly as groundwater. However, due to the over-exploitation of aquifers, the deficit in recharge, pollution by leaching in mines, and natural pollution by the passage of water through the rocks and subsoil results in the presence of heavy metals in water at high concentrations (SEMARNAT, 2007), causing severe health problems, especially in those communities near the mining industry (Conant & Fadem, 2011).

Contamination caused by heavy metals in the environment is a big concern owing to its toxicity and inclination to gather and biomagnify, causing serious ecosystem harm. (Chanthapon *et al*., 2018). Copper is an essential nutrient and a groundwater pollutant widely used in the manufacturing industry. This element is present in drinking water in a broad range of concentrations and the main cause is internal copper pipe corrosion. Water and food are mainly the first contact of exposure to this mineral. Current researches has outlined threshold the impacts of copper present in drinking water in the gastrointestinal tract (WHO, 2017), also causing neurotoxicity, jaundice, and liver toxicity (Liang *et al*., 2011). Due to the problem representing this element in water, it is necessary to pay more attention to effective ways to remove it.

Many processes, such as membrane filtration, ion exchange, precipitation, biosorption and adsorption, have recently been developed to remove heavy metals from water (Asencios *et al*., 2022; Kobielska *et al*., 2018). Although a great variety of techniques and processes are used for this purpose, their disadvantage lies in the high costs and ecological damage they could cause. Therefore, it's important to develop economical and environmentally friendly procedures.

Spherical Agglomeration Technique (SAT) proves to be an effective process for the removal of heavy metals (Proal-Najera, 2011; Gonzalez Valdez, 2013), which has four stages: 1) First, the precipitation of heavy metals is performed by a controlled dosage of a precipitating agent at specific pH range ($Ca(OH)_2$ in this case); 2) Secondly, the hydrophobization step is carried out, which aims to modify the hydrophilic character of the metal surface, using rich natural saponins extracts such as *Agave* durangensis extracts as biosurfactant (Gonzalez Valdez *et al*., 2013). This stage results in the orientation of the long hydrophobic chains of the biosurfactant outwards in the agglomerate sphere (Apfel, 1991); 3) The third stage consists in the step of wetting that is accomplished through the unification of the hydrophobic chains of the biosurfactant using *n*-heptane; and 4) Finally, the fourth agglomeration stage uses an initiator (Ca^{2+}) that changes the nature of the charge of the agglomerate sphere in order to redistribute the loads and allowing the interaction between the free ions in the solution (Proal-Najera *et al*., 1997).

In the first phase of this procedure, as has been explained before, there is a need for a precipitating agent; in most cases, the first choice is NaOH, although the nature of this chemical compound is caustic (Rodrigues *et al*., 2016), causing burns that can lead to deep-liquefactive necrosis on the affected area (Eroglu *et al*., 2012), and according to its safety data sheet, due to its alkalinity, it can have moderate toxicity to aquatic organisms (Elskus *et al*., 2015) being a problem using this chemical during the process of SAT. As well as NaOH, potassium hydroxide (KOH) has disadvantages in precipitation processes, such as the difficulty in controlling the main dosage and the generation of sludge that must be properly treated (Rai *et al*., 2021). It also poses health risks, including allergies, skin lesions, and severe injuries. It can also cause ulcers on contact surfaces and be corrosive to the eyes, skin, and respiratory tract, leading to long-term health problems (Letrell, 2010). However, using $Ca(OH)_2$ in precipitation processes has been found to have environmental benefits compared to sodium hydroxide (Hoyos-Montilla *et al*., 2023). Calcium hydroxide is less toxic, less corrosive, more compatible with materials and equipment, requires lower energy consumption for production, and is obtained from a more abundant and readily available mineral resource. These advantages can lead to a reduced environmental impact and minimized waste generation in precipitation processes.

In the second stage of the SAT, it is necessary to condition the particle by modifying the hydrophilic character of the hydroxide surface formed by adding a hydrophobizing agent (surfactant) whose role is to change the hydrophilic affinity of the colloidal system towards a hydrophobic nature (Bailon Salas *et al*., 2018).The main sources of surfactants are synthetic, industrial and natural. Although the use of synthetic surfactants is wide, the principal disadvantages are their low biodegradability, greater toxicity and environmental risks (de Souza *et al*., 2024; Jahan *et al*., 2020). Synthetic surfactants have disadvantages such as lower biodegradability, greater toxicity and environmental risks. On the contrary, biosurfactants offer several advantages such as greater biodegradability, lower toxicity, resistance to pH and a friendly interaction with the environment, such extracts are made of leaf material, mainly from agavacea, which are distinguished by being rich in saponins (Canul-Chan *et al*., 2023; Rai *et al*., 2018; Tmáková *et al*., 2016). Biosurfactants extracted from *Agave*, present additional benefits compared to other crops that also have saponins, their production is economically competitive in comparison to other plants, since it does not require any controlled fermentation process, in the same way, it has been shown that they have advantages in the metal removal process using the spherical agglomeration technique due to their ability to form stable spherical structures that facilitate the removal of heavy metals (Sharma *et al.*, 2023; Kregiel *et al.*, 2017).

Among the extracts of leaf material from agavaceae, extracts of *A. lechuguilla* and *A. tequilana* stand out, the former due to the abundance of saponins present in the extracts (Morreeuw *et al.*, 2021) and the latter due to the large amount of leaf material that is thrown into the tequila making process (Perez-Zavala *et al.*, 2020).

The *A. lechuguilla* leaf is traditionally used to obtain ixtle, a natural fiber, of which only 15% is converted into said material, while the remaining 85% is a succulent by-product called guishe that contains saponins, which can be used for various applications (Juarez *et al.*, 2014). It has been found that the leaves of *A. lechuguilla* mainly contain two steroidal saponins, yuccagenin and ruizgenin, utilized as organic chelating agents to eliminate heavy metals present in water (Bermúdez-Bazán *et al.*, 2021).

Furthermore, studies have identified several saponins in *Agave* species, including *A. tequilana*, being the most predominant hecogenin and tigogenia (Sidana *et al.*, 2016). The amount of saponins in *A. tequilana* leaf extracts is significant (Herbert-Doctor *et al.*, 2016; Velázquez *et al.*, 2019), and using these for the manufacture of different products due to its antimicrobial, antifungal, insecticidal, and antifeedant properties (Qasim *et al.*, 2020).

The purpose of this work was to investigate the effectiveness of calcium hydroxide in the precipitation stage of the SAT through the evaluation of the effect on different pH levels (8.0 and 9.0), in addition to evaluating two types of biosurfactants (*A. tequilana* and *A. lechuguilla*) and their different dosages (0.3, 0.5 or 1.0 g of extract per gram of metal present in water), to remove copper in aqueous solutions respectively, giving eco-friendly alternative to the precipitating agent. This study explores the effectiveness of calcium hydroxide as a new alternative to sodium hydroxide for copper removal via spherical agglomeration. Results suggest that $Ca(OH)_2$ can be effectively used to remove these elements from aqueous solutions. The innovative aspect of this work lies in using a precipitating agent different from that applied in other works where SAT was employed, which allows the avoidance of using sodium to remove heavy metals (such as copper and lead).

2 Methodology

2.1 *Reagents and equipment*

A 1000 mg/L copper standard was used for the aqueous solution experiments. (CENAM, DMR-17h México), $Ca(OH)_2$ (J.T. Baker, CAS No. 1305-62-0), *n*-heptane (SIGMA CHEMICAL) and $CaCl_2$ (CAS No. 10043-52-4). For the different copper concentration formulations in aqueous solution, distilled water was utilized (HYCEL, Mexico). An atomic absorption spectrophotometer (AAS) (Perkin Elmer, model 700 AAnalyst; Massachusetts, USA) was used to measure the amount of residual copper.

2.2 *Water design models*

The concentrations of copper in the designed experiments (2.0, 5.0 and 10.0 mg/L, respectively) were selected based on a previous study conducted by Alcazar-Medina *et al.* (2014) and from the permissible concentrations limits for these metals stated in the regulation NOM-127-SSA1-2021 . All experiments were performed in a volume of 250 mL in triplicate, making each determination of all experiments.

2.3 *Spherical Agglomeration Technique (SAT) application*

The copper removal experiments in models were carried out at room temperature (20 ± 2), using 500 mL flasks with deformed walls and 3.5 × 2.5 cm stainless steel propellers, in 250 mL of solution at room temperature (22°C) and maintaining constant agitation (ω) at 600 rpm, during all experiment (Proal *et al.*, 1997).

In a controlled way, the precipitation stage was carried out by adding $Ca(OH)_2$ to the aqueous models until reaching a particular pH of 8.0 and 9.0 (Bailon-Salas *et al.*, 2018). The hydrophobization was carried out without interrupting the reaction, adding the surfactant agent *Agave lechuguilla* Torr. And *Agave tequilana* leaves (0.3, 0.5, or 1.0 g of extract per gram of metal in water). The wetting stage (conditioning of the hydrophobic solids) was carried out by adding a determined volume of *n*-heptane (C_7H_{16}), 6.3 mL per gram of contaminating metal in an aqueous solution. Lastly, the growth stage of the crystalline nuclei was accomplished by adding 10 mL of 1M $CaCl_2$ solution per gram of metal contained in the aqueous solution, stirring without interruption at 600 rpm, at room temperature (22 °C) and for 90 min. (Garcia-Arambula, 2011).

After the specified technique, Whatman #40 filter paper was used to retain the produced agglomerates and measure the residual concentration of copper in the liquid phase using AAS, in accordance with Mexican water quality requirements, NMX-AA-051-SCFI-2001 (Secretaría de economía, 2001), by flame and graphite furnace respectively.

2.4 *Experimental design for the removal of copper in water models*

A Full Factorial arrangement was employed to investigate the effects of two main factors: surfactant type (*A. tequilana* (A) and *A. lechuguilla* (B) respectively) and solution pH (8.0 and 9.0). Each factor was assessed at two different levels. Furthermore, two additional factors, initial metal concentration and surfactant dosage, are already mentioned, with three levels. Conducting all experiment combinations to explore these four factors' interaction and individual effects on the Copper residual concentration response.

2.5 *Water analysis*

The copper analysis in water samples was made by atomic absorption spectrophotometry, following the protocol stated by the SEMARNAT that regulates the water quality in Mexico (NMX-AA-051-SCFI-2001 toxicological concern).

2.6 *Statistical analysis*

The study of the General Linear Model (GLM) that corresponds to the experimental design was carried out using an analysis of variance (ANOVA) while validating the assumptions of normality, independence, and variance homogeneity. (Kolmogorov-Smirnof postulates). The coefficient of determination, Pearson's R-squared (R^2), was estimated to evaluate the model's goodness of fit using the Statistica 7 for Windows program (StatSoft, US, 2004). Subsequently, means were compared using the Least Significant Difference (LSD) Fisher's test to determine the effects of extract dosage, initial Cu^{2+} metal concentration, and the interaction effect between dosage and concentration. Lastly, a quadratic response surface model was performed from the experimental data, representing the relationships between the studied variables. This approach follows the proposed experimentation. It guarantees a comprehensive analysis of the factors under consideration, including using ANOVA to assess their significance and the future application of statistical tests for further insights into their effects.

3 Results and discussion

This research aimed to study the effectiveness of Ca $(OH)_2$ in the precipitation stage through the evaluation of the effect of different pH levels (8.0 and 9.0), two types of surfactants (*A. tequilana* and *A. lechuguilla*) and their different dosages (0.3, 0.5 or 1.0 g of extract per gram of metal present in water), as well as different initial concentrations of copper on its removal through the SAT process. The obtained results were compared with the standards established by the Mexican normative (NOM-001-ECOL-1996, 1997 and NOM-127-SSA1-2021) and provided valuable information on the effectiveness of these factors on the process.

3.1 *Copper removal by SAT*

Through the use of $Ca(OH)_2$, copper removal levels that meet established standards were achieved in this study, highlighting its effectiveness (Table 1). Trends indicate that when *A. tequilana* extract (surfactant A) is used as a surfactant, optimal conditions involve a pH of 9 and a dose of 0.3 g of extract/g of metal, resulting in a removal of 98.61% (Table 1). In the case of using the *A. lechuguilla* extract (surfacant B), a pH 9 with a dose of 0.3g of extract/g of metal, presents an elimination of 98.73% (Table 1). Both surfactants show minimal differences in the most efficient treatments, which positions $Ca(OH)_2$ as a promising alternative to traditional sodium hydroxide in copper remediation.

In the current study, when $Ca(OH)_2$ is incorporated to the system, the OH- ions react with the Cu^{2+} ions of $CuSO_4$, forming slightly soluble copper hydroxide ($Cu(OH)_2$), which precipitates and adheres to the surface of the copper particles, generating the compact layer (LaGrow *et al.*, 2014). The remaining OH^- and Cu^{2+} ions form a diffuse shell, contributing to the electrical double layer and colloidal repulsion; this structure favors agglomeration, improving the efficiency of copper removal (Hong *et al.*, 2002).

Copper hydroxides form a compact layer on the surface is caused by the adsorption of hydroxyl ions (OH^-) on the $Cu(OH)_2$ surface (Yoon and Salman, 1971). These OH^- ions carry a negative charge, causing the surface of the $Cu(OH)_2$ to have a net negative charge (Conway *et al.*, 2015), facilitating the formation of a compact layer by attracting and binding positively charged particles in solution, such as copper ions (Cu^{2+}), which contributes to the effective removal of copper in precipitation and coagulation processes (Keleşoğlu, 2007; Ye *et al.*, 2016).

The hydrophobization of colloids is the second stage of the process, which is achieved by adding surfactants with saponins present in the extracts of leaf material from *Agave* Sp. Saponins are amphiphilic compounds made up of a hydrophilic and a hydrophobic part (Rai *et al.*, 2021; Forey *et al.*, 2021; Jia *et al.*, 2022). Therefore, the addition of the saponins contained in the extracts allows the formation of complexes with Ca^{2+} ions, which increases the hydrophobicity of colloids and allows their agglomeration (Bailón Salas *et al.*, 2018; Grzywaczyk *et al.*, 2017), Yu and He (2018) investigated the application of natural surfactants, including saponins, to improve the removal of zinc and copper leaching from different soils, and found that saponins at a concentration of 2 g/L could remove approximately 83% of copper.

Previous studies that used the SAT showed removal levels greater than 95%, which indicates that the SAT is effective for the removal of heavy metals (Alcázar-Medina *et al.*, 2014; González-Valdez *et al.*, 2013). Data obtained under a complete factorial experimental design is presented in Table 1, which evaluates the effect of different pH levels (8 and 9), two types of surfactants (*A. tequilana* and *A. lechuguilla*), and their different dosages (0.3, 0.5 or 1.0 g of extract per gram of metal present in water), as well as different initial concentrations of copper on the removal of this element through SAT.

Table 1. Cu^{2+} removal by SAT under a full factorial design at different biosurfactant types, pH levels, initial copper concentrations and leaf extract dosages.

Surf. type	pH	Initial concentration of Cu^{2+} (mg/L)	Extract dose ††	pH final magnitude	Copper removal (%)
A	9	2	1	7.4 ± 0.1	96.42 ± 1.06
A	9	2	0.5	7.47 ± 0.12	96.93 ± 0.9
A	9	2	1	7.4 ± 0.1	95.92 ± 0.93
A	9	5	0.3	7.29 ± 0.12	97.91 ± 0.31
A	9	5	0.5	7.27 ± 0	96.88 ± 0.38
A	9	5	1	7.63 ± 0.21	97.36 ± 0.21
A	9	10	0.3	7.4 ± 0.17	98.61 ± 0.25
A	9	10	0.5	7.3 ± 0.1	98.54 ± 0.07
A	9	10	1	7.23 ± 0.06	97.58 ± 0.1
A	8	2	0.3	6.81 ± 0.09	85.85 ± 3.68
A	8	2	0.5	6.92 ± 0.08	82.17 ± 3.68
A	8	2	1	6.82 ± 0.09	89.52 ± 0
A	8	5	0.3	6.77 ± 0.06	90.66 ± 0.74
A	8	5	0.5	6.97 ± 0.1	94.71 ± 0.37
A	8	5	1	6.75 ± 0.14	85.88 ± 0.37
A	8	10	0.3	6.85 ± 0.04	95.48 ± 0.17
A	8	10	0.5	6.72 ± 0.07	92.57 ± 0.69
A	8	10	1	6.71 ± 0.03	93.94 ± 1.37
B	9	2	0.3	6.85 ± 0.03	90.3 ± 5.94
B	9	2	0.5	6.63 ± 0.06	88 ± 6.21
B	9	2	1	6.68 ± 0.03	86.52 ± 3.82
B	9	5	0.3	6.34 ± 0.1	94.92 ± 2.48
B	9	5	0.5	6.22 ± 0.11	96.25 ± 0.49
B	9	5	1	6.25 ± 0.03	97.31 ± 0.57
B	9	10	0.3	6.27 ± 0.02	98.73 ± 0.37
B	9	10	0.5	5.92 ± 0.08	98.15 ± 2.12
B	9	10	1	6.01 ± 0.13	96.53 ± 0.37
B	8	2	0.3	6.18 ± 0.13	56.75 ± 7.45
B	8	2	0.5	6.11 ± 0.09	69.78 ± 3.77
B	8	2	1	6.27 ± 0.15	54.63 ± 3.74
B	8	5	0.3	6.37 ± 0.15	80.43 ± 1.59
B	8	5	0.5	6.57 ± 0.06	63.73 ± 5.66
B	8	5	1	6.37 ± 0.47	65.69 ± 4.03
B	8	10	0.3	6.47 ± 0.06	92.79 ± 2.75
B	8	10	0.5	6.43 ± 0.21	93.42 ± 2.42
B	8	10	1	6.9 ± 0.44	96.37 ± 1.12

††Units in g·g^{-1} (mass of extract per gram of lead in water).

Results demonstrated that copper in an aqueous medium is removed thanks to the effective hydrophobization of the copper hydroxide particles formed in the medium. The results also show the effectiveness of both extracts (*A. tequilana* and *A. lechuguilla*) used as biosurfactants by having removal levels similar to chemically synthesized surfactants such as sodium hexadecanoate reported by Barros *et al.* (2018) for the removal of copper in a synthetic effluent through ionic flocculation, in addition to complying with the standards established by Mexican regulations.

These results (Table 1), confirm that the copper precipitates' affinity from hydrophilic to hydrophobic is attributable to the effect of the *Agave* spp. leaf extracts. For all the doses used (0.3, 0.5, 1.0g of extract/g of metal) which demonstrates that the action of both *A. tequilana* and *A. lechuguilla* is comparable to that of *Yucca decipiens* (Bailon-Salas *et al.*, 2018) for the removal of heavy metals. Furthermore, using SAT to extract copper from an aqueous solution permits the copper permissible limits to be achieved. In every case of initial experimental Cu^{2+} concentrations (2, 5, and 10 mg/L) present in the models, the mentioned element residual concentration is significantly below the permissible limit (Total Residual Cu^{2+}<5.0 mg/L), complying with the aforementioned guidelines for the discharge of wastewater into national bodies of water (SEMARNAT, 1997), in the same way, Figures 1 and 2 shows that it was possible to reach residual concentrations as low as those established by drinking water standards for all treatments (SSA, 2000; WHO, 2011), where the lowest residual copper concentrations were achieved under a lower extract dose (0.3 g/gCu).

The extracts of both *A. tequilana* and *A. lechuguilla* act as an effective coating for the precipitated copper particles, creating a hydrophobic affinity in the aqueous system. The wetting agent, *n*-heptane, added with controlled stirring at 600 rpm, promotes kinetic collisions between the particles, facilitating the growth of the nuclei in the third stage of the SAT, which coincides with the mechanism described in the literature, where bridging liquid droplets capture and form agglomeration nuclei (Orlewski *et al.*, 2018).

Achieving complete neutralization of the charge is essential in processes like this since it facilitates the destabilization and agglomeration of the particles (Carolin *et al.*, 2017). Therefore, it is necessary to add $CaCl_2$ during the SAT agglomeration stage to achieve this goal. When Ca^{2+} dissociates, it acts as a bridge between the hydroxyl groups in the surfactant tail, thus joining two saponin molecules in a mechanism comparable to that Grzywaczyk *et al.* (2021) proposed for forming molecular bridges.

Table 1 also shows the final pH of the experiments; it is observed that with the use of the *A. tequilana* extract, the pH at the end of the process complies with the established regulations in all cases; however, with the use of the *A. lechuguilla* extract the final pH decreases significantly. Thereby, it is possible to observe that the effect of the action of the extracts causes the change in the character of the Cu^{2+} particles from hydrophilic to hydrophobic in all doses used (0.3, 0.5, 1.0 g/gCu), with the use of the technique on the Cu^{2+} removal allowable limits are satisfied with standards named by the NOM-127-SSA1-2021 and NOM-001-ECOL-1996 (1997) for wastewater discharges into national waters, in all cases of initial concentration of Cu^{2+} present in the models (2, 5 and 10 mg/L), since the residual concentration of this element (Cu^{2+} residual concentration <2 mg/L), agrees with the results obtained in previous studies in the case of other metals and arsenic (Mireles-Martinez, 2011; García-Arámbula, 2011).

The visual analysis after each experiment showed a correct development of the final stages of SAT process (wetting and agglomeration) since no residues were observed in the flask or the stirring propellers, indicating an adequate hydrophobic interaction between the liquid and the solid; this interaction remained effective at all dosage levels of the extracts in aqueous solution (0.3, 0.5 and 1.0 g/gCu). These results agree with those published in a previous paper from our workgroup, Alcázar-Medina *et al.* (2014), where we were able to achieve a high removal of Cu^{2+} (99%) using NaOH as a precipitation agent, indicating the viability of the use of $Ca(OH)_2$ in the application of SAT.

The best parameters for copper removal in aqueous solutions are those in which *A. tequilana* extract is used as a surfactant, a pH of 9, an initial copper concentration of 10 mg/L, and a surfactant dosage of 0.3 g/gCu. These conditions resulted in removal percentages more significant than 98% and final copper concentrations below 0.2 mg/L.

These results agree with those obtained for alkaline precipitation processes for copper removal (Hu *et al.*, 2017; Vivas *et al.*, 2019). Likewise, the results obtained correspond with those presented in previous works for the removal of Cu^{2+}, Cd^{2+}, Mn^{2+}, and Zn^{2+} in aqueous solutions using SAT and ionic flocculation techniques, using surfactants of industrial origin in the hydrophobization stage (Proal *et al.*, 1997; Barros *et al.*, 2017).

Copper removal by $Ca(OH)_2$ involves complex colloidal systems. Solubility varies with precipitation pH, affecting precipitate formation and removal efficiency (Wen *et al.*, 2019). The point of zero charge influences the particle-particle interaction and aggregation (Li *et al.*, 2023). Chemical coagulation processes with calcium hydroxide favor the agglomeration and sedimentation of particles (Wang *et al.*, 2021). Studies show variability in results due to different methods and conditions. Recent references discuss parameter optimization and the influence of the matrix on removal efficiency.

Chafi *et al.* (2022) reached an 82.6% copper removal efficiency with Oyster Shells (OS) powder as an adsorbent; Darweesh *et al.* (2022) evaluated an adsorption process with Banana leaves employed as an adsorbent and precursor for activated carbon and achieved a removal efficacy of 83% at pH 5; also Mohammmadi-Manesh *et al.*, (2019) obtained 96.6% copper removal through the use of aqueous solutions of manganese ($MnFe_2O_4$) nanoparticles; Bashir *et al.* (2022) obtained 97.5% of copper removal efficiency using sulfide solution for chemical precipitation. The $Cu(OH)_2$ point of zero charge (PZC), or the pH at which the $Cu(OH)_2$ surface does not have net charge, is typically around pH 8. (Yoon and Salman, 1971). The surface has no electrical charge and is less likely to repel charged particles in solution (Abbiw, 2022).

Studies performed highlight the critical influence of pH on copper removal. The solubility of copper varies significantly with pH, being more soluble in acidic media and less so in alkaline media (Vashist *et al.*, 2022). In precipitation and coagulation processes, careful control of pH is essential. Maintaining an alkaline pH favors the formation of insoluble copper hydroxides, which precipitate effectively, optimizing their removal (Wang *et al.*, 2021). This phenomenon is based on the diminution in the solubility of copper ions as the pH rises, which leads to the formation of solids that can be easily separated from water, ensuring the effective removal of the metal (Lupa and Cocheci, 2023).

3.2 *Statistical analysis*

A complete factorial experimental design was used to incorporate the factors mentioned above. The results presented capture the complex interaction between the independent variables and their effects on copper removal by spherical agglomeration. Table 2 provides a comprehensive overview of the experimental combinations, covering the variables mentioned before. Response variability is generally observed, indicating that each combination of factors leads to unique results. However, pH and surfactant dosage are shown to be the most important values for copper removal in aqueous models.

Analysis of the residual of the complete factorial design showed that the Gauss-Markov assumptions are fulfilled, the coefficient of determination for the two experimental series (R^2) was superior to 0.96 for copper removal, which indicates that is a reliable experimental design ($p<0.05$) by demonstrating the efficiency of the spherical agglomeration technique, due to the experimental results obtained are within the normativity established for water quality NOM-001-ECOL-1996 (SEMARNAT, 1996), for residual concentration of Cu^{2+}.

The variance analysis (ANOVA) shown in Table 2, applied to the residual Cu^{2+} concentrations as a dependent variable, presented the existence of significant differences mainly in the type of surfactant, in the treatment pH, and the initial concentration of the metal, which indicates that the parameters themselves, as well as their interactions, are determining factors in the efficiency of Cu^{2+} removal by SAT in aqueous models. The same analysis was also performed with the final pH as the dependent variable. The analyses mainly showed significant effects on the type of surfactant and the initial pH and showed no significant differences in the surfactant dose.

Using *A. tequilana* as a surfactant, Fisher's LSD analysis (Figure 1) showed significant differences in the final copper concentration between the two pH levels used (pH 8.0 and 9.0). The initial concentrations of Cu^{2+}, 2, 5, and 10 mg/L at a pH of 9.0 showed a decrease in the final concentration levels (Figure 1). Likewise, no significant differences were found between the final concentrations of Cu^{2+} for this pH level. The lowest final copper concentration (0.072mg/L) reached 2.0 mg/L Cu_0^{2+} and an extract dose of 0.3 g/gCu.

However, the application of *Agave lechuguilla* extract (Figure 2) presented significant differences between the different pH levels and dosages, having lower final concentrations of Cu^{2+} following the following parameters: pH 9.0, Cu_0^{2+} of 10 mg/L and an extract dose of 0.5 g/gCu. Finally, for an initial pH of 9.0, the best copper removal result was obtained using *A. tequilana* as biosurfactant with a dose of 0.3 g/gCu. Although the percentage of copper removal ranges between 86.5 and 98.4%, in the quadratic fit response surface graphs (figures 3 and 4), the trend demonstrates that with a lower dosage of extract, the lower final concentration of Cu^{2+}.

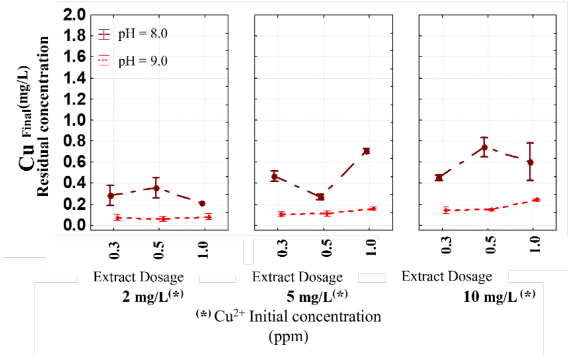

Figure 1. Comparison of means (Fisher LSD) for the residual concentration of Cu^{2+} in aqueous models by SAT for the full factorial design using *A. tequilana* as biosurfactant, plus two pH levels (8.0 and 9.0).

Table 2. Results on the two ANOVAs for residual concentration of Cu^{2+} and final pH level in aqueous models for the full factorial arrangement design.

Effect	Degr. of freedom	Cu^{2+} Total Analysis		pH final magnitude Analysis	
		F	*p*	F	*p*
Intercept	1	3959.556	<0.001	225175.3	<0.001
{1} Surf Type	1	418.574	<0.001	696.7	<0.001
{2} pH	1	1508.119	<0.001	101.5	<0.001
{3} Extract Dosage	2	13.121	<0.001	0.8	0.4434
{4} Cu_0^{2+}	2	94.069	<0.001	9.7	<0.001
Surf Type*pH	1	210.52	<0.001	100.4	<0.001
Surf. Type*Extract Dosage	2	0.606	0.5482	13.1	<0.001
pH*Extract Dosage	2	5.514	0.0059	4.2	0.0185
Surf. Type* Cu_0^{2+}	2	131.241	<0.001	1.3	0.2919
pH* Cu_0^{2+}.	2	101.2	<0.001	23.7	<0.001
Extract Dosage* Cu_0^{2+}	4	7.611	<0.001	9.4	<0.001
Surf. Type*pH*Extract Dosage	2	1.772	0.1773	2.8	0.0682
Surf. Type*pH* Cu_0^{2+}	2	101.135	<0.001	22.8	<0.001
Surf. Type*Extract Dosage* Cu_0^{2+}	4	22.676	<0.001	1.6	0.1918
pH*Extract Dosage* Cu_0^{2+}	4	26.582	<0.001	2.5	0.0518
1*2*3*4	4	29.471	<0.001	3.5	<0.001

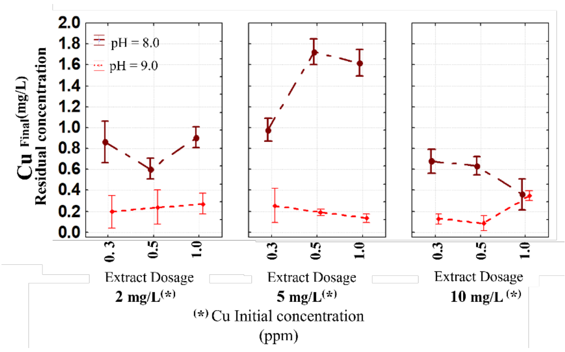

Figure 2. Comparison of means (Fisher LSD) for the residual concentration of Cu^{2+} in aqueous models by SAT for a full factorial design using *A. lechuguilla* as biosurfactant, plus two pH levels (8.0 and 9.0).

In general, higher removal levels are obtained using this biosurfactant, according to the ANOVA carried out. One of the variables with the most significant influence, besides using *A. tequilana* as a biosurfactant, is the initial pH in the SAT process.

The response surface graph with quadratic fit for the residual concentration of Cu, for the use of *A. tequilana* as a biosurfactant and an initial pH 9.0 (Figure 3) is presented by the equation:

$$Cu_{Final} = 0.0904 - 0.2162 * ED + 0.0128 * Cu_0^{2+} + 0.1532ED2 + 0.0168ED * Cu_0^{2+} - 0.0008(Cu_0^{2+})_2 \tag{1}$$

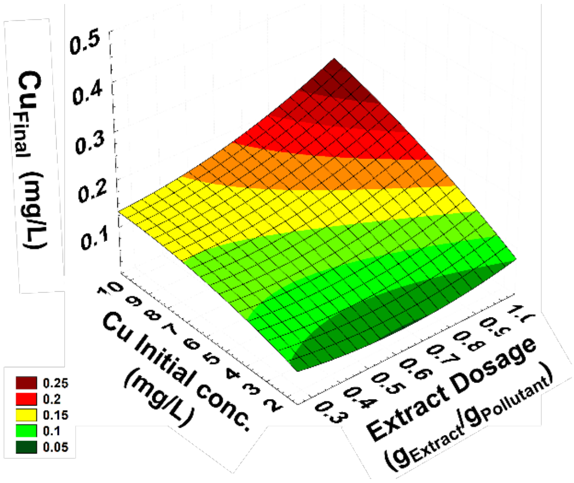

Figure 3. Response surface for the % Removal of Cu^{2+}, using precipitation pH= 9.0 and *A. tequilana* extract as biosurfactant in aqueous models.

It is possible to observe in the corresponding graph that the final copper concentration decreases as Cu_0^{2+} is lower, when the dosage of *A. tequilana* extract is diminished from medium to low. For this case, when a low extract dosage is used (0.3-0.6 g/gCu), the residual copper reaches the lowest concentration, using a minimum initial copper concentration of 2mg/L.

These results also agree with previous reports where a Micellar-Enhanced Ultrafiltration process is used in water samples with a Cu concentration of 100 mg/L, obtaining a removal of 76.46%, where the increase in the surfactant level results in a minor removal (Wolowicz *et al.*, 2022).

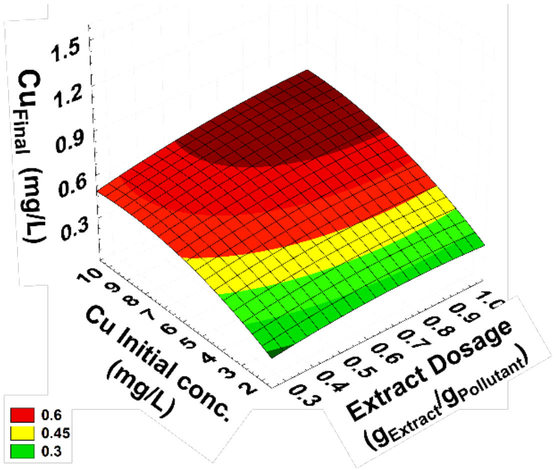

Figure 4. Response surface for the % Removal of Cu^{2+}, using precipitation pH= 8.0 and *A. tequilana* extract as biosurfactant in aqueous models.

Moreover, the response surface graph corresponding to an initial pH 8 (Figure 4) is represented by the following equation:

$$Cu_{Final} = 0.0102 + 0.3278 * ED + 0.0873 * Cu_0^{2+} - 0.237ED^2 + 0.0234ED * Cu_0^{2+} - 0.0051Cu_0^2 \quad (2)$$

A similar behavior is observed when the same extract is used with lower initial concentrations of copper. Also, it is worth mentioning that when using pH 8, the final Cu^{+2} is higher than when the initial pH of the process is elevated.

In the same way, when the extract of *A. lechuguilla* was used for copper removal in aqueous models; the response surface graph obtained (Figure 5) is described by the equation:

$$Cu_{Final} = 0.4883 - 0.6301 * ED + 0.0491 * Cu_0^{2+} - 0.3787ED^2 + 0.0391ED * Cu_0^{2+} - 0.0016(Cu_0^{2+})_2 \quad (3)$$

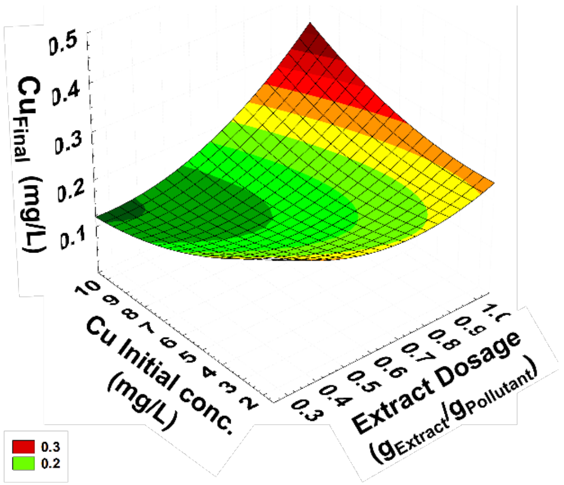

Figure 5. Response surface for the % Removal of Cu^{2+}, using precipitation pH= 9.0 and *A. lechuguilla* extract as biosurfactant in aqueous models.

This graph indicates that, in general, lower final concentrations of copper, when using an initial pH 9, are obtained using a lower dosage of extract, having similar results to those obtained by Schelebush *et al.*, 2023, who found that a low dose of surfactin can float up to 75%, where it is specified that the recovery of the metal is thanks to the formation of hydrophobic complexes between the metal and surfactant.

Lastly, the response surface graph for an initial pH 8 and the use of *A. lechuguilla* as a biosurfactant can be represented by the equation:

$$Cu_{Final} = -0.9277 + 2.1627 * ED + 0.6155 * Cu_0^{2+} - 1.117ED^2 - 0.0948ED * Cu_0^{2+} - 0.049(Cu_0^{2+})_2 \quad (4)$$

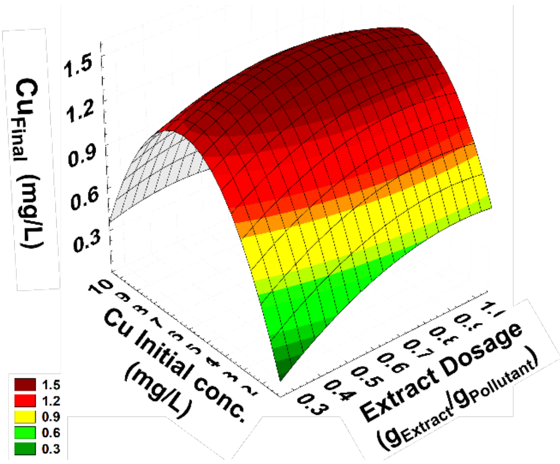

Figure 6. Response surface for the % Removal of Cu^{2+}, using precipitation pH= 8.0 and *A. lechuguilla* extract as biosurfactant in aqueous models.

This graph exhibits that with lower dosages of surfactant agent (0.3g/gCu), the copper concentration, in general, is lower. It should be noted that although lower removal levels were obtained in previous studies (Alcazar-Medina *et al.*, 2014), where NaOH was used as a precipitating agent and *Agave lechuguilla* to replace sodium oleate as a surfactant, the residual concentration of Cu^{+2} complies with the regulations established for drinking water throughout the experimental design used, which also demonstrates the efficiency of $Ca(OH)_2$ as a copper precipitation agent.

In this research, a similar effect is observed, since the authors suggest that adequate pH control is necessary in addition to the correct dosage of surfactant is critical to the removal of metals present in water, which also coincides when hydroxide precipitation processes are used in the treatment of simulated electroplating waters, where the degree of copper removal depends significantly on the pH (Quiton *et al.*, 2022). This reaffirms the effectiveness of the SAT application since high copper removal percentages were obtained, and its residual concentration correctly satisfy the permissible limits established for drinking water quality in all the experimental cases.

Conclusions

- The results obtained reveal the effectiveness of calcium hydroxide as an alternative to sodium hydroxide in copper removal. Where a consistent trend of high removal percentages and final concentrations below the limits established by regulations for drinking water is observed in all cases, highlighting its viability as a precipitation agent for the removal of heavy metals present in water.
- A significant trend is observed where, at higher pH levels, lower final copper concentrations are achieved (0.072 - 0.240mg/L), demonstrating the critical influence of pH on removal efficiency. Furthermore, it is identified that the dosage of the *A. tequilana* extract in concentrations in the medium to low range improves copper elimination.
- Removals of up to 98.73% of copper were attained in aqueous solutions. The optimal doses of *A. tequilana* extract in aqueous solutions were 0.3-0.5 g/gCu. The experiments at pH 9 generally obtained removals above 85% for all the initial copper concentrations used (2, 5, and 10 mg/L).
- By using leaf material extracts from both *A. lechuguilla* and *A. tequilana*, comparable levels of copper removal were obtained. A particular benefit of using *Agave tequilana* is that it is widely used in a variety of industrial uses, which can make its extracts more available and economically viable for use in real-world applications.
- The ANOVA carried out indicated that the parameters type of extract, pH, initial concentration of copper, dose of extract, as well as the interactions between them, have a significant effect ($p<0.05$), highlighting the complexity of the SAT process and the need to consider multiple factors to achieve optimal removal of both copper and other heavy metals.
- A coefficient of determination (R^2) of more than 0.90 indicated that the Full Factorial Design experimental design was a reliable method for assessing all the factors in copper removal and allowed a thorough investigation of the interactions between the independent variables stated above.
- The optimization and reduction of contamination by heavy metals present in water when using calcium hydroxide as a substitute for sodium hydroxide as an agent are essential takeaways from these results, which have implications for removing metal contaminants through SAT. That will lower operating costs. Additionally, the findings offer helpful recommendations for refining copper removal procedures, emphasizing the need to consider pH and surfactant dose in future studies.

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Nomenclature

AAS	Atomic absorption spectrophotometer
ED	Extract dose
ANOVA	Analyzes of variance
Cu^{2+}	Divalent copper ions
Cu_0^{2+}	Initial concentration of copper (mg/L)
Cu_{Final}	Final concentration of copper (mg/L)
R^2	Pearson determination coefficient
SAT	Spherical agglomeration technique
WHO	World Health Organization

References

Abbiw, E. (2021). Removing Heavy Metals from Mine-Polluted Waters Using Agricultural Waste Materials. Master Thesis in Program of Environmental Studies at George V. Voinovich School of Leadership and Public Service of Ohio University.

Alcázar-Medina, F., Proal-Nájera, J., Gallardo-Velázquez, T., Cháirez-Hernández, I., Antileo-Hernández, C., and Alvarado-De La Peña, A. (2014). Aplicación de extractos de lechuguilla (*Agave lechuguilla Torr.*) en la remoción de Cobre (II) en modelos de agua por aglomeración esférica. *Revista Mexicana de Ingeniería Química 13*, 605-617.

Apfel, H. (1992). Untersuchungen zum Anwendbarkeit der Umbenetzungsagglomeration zur Reinigung Schwermetallhaltiger Abwasser. Diplomarbeit. Institut für Mechanische Verfahrenstechnik der Universität Karlsruhe (TH), Germany.

Armenante, P. M. (1997). *Precipitation of Heavy Metals from Wastewaters*. Ed. New Jersey Institute of Technology, USA.

Asencios, Y. J. O., Parreira, L. M., Perpetuo, E. A., & Rotta, A. L. (2022). Characterization of seaweeds collected from Baixada Santista litoral, and their potential uses as biosorbents of heavy metal cations. *Revista Mexicana de Ingeniería Química 21*, IA2600. https://doi.org/10.24275/rmiq/IA2600

Bailón-Salas, A. M., Ordaz-Díaz, L. A., Cháirez-Hernández, I., Alvarado-de la Peña, A., and Proal-Nájera, J. B. (2018). Lead and copper removal from groundwater by spherical agglomeration using a biosurfactant extracted from *Yucca decipiens Trel*. *Chemosphere 207*, 278-284. https://doi.org/10.1016/j.chemosphere.2018.05.103

Barros, G. K. G. C., Melo, R. P. F., and de Barros Neto, E. L. (2018). Removal of copper ions using sodium hexadecanoate by ionic flocculation. *Separation and Purification Technology 200*, 294-299. https://doi.org/10.1016/j.seppur.2018.01.062

Bashir, M., Mohan, C., Tyagi, S., and Annachhatre, A. (2022). Copper removal from aqueous solution using chemical precipitation and adsorption by Himalayan pine forest residue as biochar. *Water Science and Technology 86*, 530-554. https://doi.org/10.2166/wst.2022.222

Bermúdez-Bazán, M., Castillo-Herrera, G. A., Urias-Silvas, J. E., Escobedo-Reyes, A., and Estarrón-Espinosa, M. (2021). Hunting bioactive molecules from the *Agave* genus: An update on extraction and biological potential. *Molecules 26*, 6789. https://doi.org/10.3390/molecules26226789

Canul-Chan, M., Rodas-Junco, B. A., Uribe-Riestra, E., & Houbron, E. (2023). Biodegradation of crude oil present in wastewaters: evaluation of biosurfactant production and catechol 2, 3 dioxygenase activity. *Revista Mexicana de Ingeniería Química 22*, 2932. https://doi.org/10.24275/rmiq/Bio2932

Carolin, C. F., Kumar, P. S., Saravanan, A., Joshiba, G. J., and Naushad, M. (2017). Efficient techniques for the removal of toxic heavy metals from the aquatic environment: A review. *Journal of Environmental Chemical Engineering 5*, 2782-2799. https://doi.org/10.1016/j.jece.2017.05.029

Chafi, M., Byadi, S., Barhoumi, A., Limouni, W., Tizliouine, A., Jama, C., and Omari, L. E. H. (2022). Study of copper removal by modified biomaterials using the response surface methodology, DFT Calculation, and molecular dynamic simulation. *Journal of Molecular Liquids 363*, 119799. https://doi.org/10.1016/j.molliq.2022.119799

Chanthapon, N., Sarkar, S., Kidkhunthod, P., and Padungthon, S. (2018). Lead removal by a reusable gel cation exchange resin containing nano-scale zero valent iron. *Chemical Engineering Journal 331*, 545-555. https://doi.org/10.1016/j.cej.2017.08.133

Conant, J., and Fadem, P. (2011). Guía comunitaria para la salud ambiental. Hesperian.

Conway, J. R., Adeleye, A. S., Gardea-Torresdey, J., and Keller, A. A. (2015). Aggregation, dissolution, and transformation of copper nanoparticles in natural waters. *Environmental Science and Technology 49*, 2749-2756. https://doi.org/10.1021/es504918q

Darweesh, M. A., Elgendy, M. Y., Ayad, M. I., Ahmed, A. M., Elsayed, N. K., and Hammad, W. (2022). Adsorption isotherm, kinetic, and optimization studies for copper (II) removal from aqueous solutions by banana leaves and derived activated carbon. *South African Journal of Chemical Engineering 40*, 10-20. https://doi.org/10.1016/j.sajce.2022.01.002

de Souza, A. C., Silva, M. S., Simões, L. A., Fernandes, N. A. T., Schwan, R. F., & Dias, D. R. (2024). Advantages of biosurfactants over petroleum-based surfactants. *Industrial Applications of Biosurfactants and Microorganisms*, 371-393. https://doi.org/10.1016/B978-0-443-13288-9.00002-4

Elskus, A. A., Ingersoll, C. G., Kemble, N. E., Echols, K. R., Brumbaugh, W. G., Henquinet, J. W., and Watten, B. J. (2015). An evaluation of the residual toxicity and chemistry of a sodium hydroxide-based ballast water treatment system for freshwater ships. *Environmental Toxicology and Chemistry 34*, 1405-1416. https://doi.org/10.1002/etc.2943

Eroglu, M., Mutluoglu, M., Uzun, G., and Ay, H. (2012). Caustic skin burn caused by sodium hydroxide. *BMJCase Reports 2012*, 1. http://dx.doi.org/10.1136/bcr-2012-007103

Forey, N., Atteia, O., Omari, A., and Bertin, H. (2021). Use of saponin foam reinforced with colloidal particles as an application to soil remediation: Experiments in a 2D tank. *Journal of Contaminant Hydrology 238*, 103761. https://doi.org/10.1016/j.jconhyd.2020.103761

García Arámbula, C. G. (2011). Aplicación de sapogenina como agente surfactante, en la remoción de metales Cd y Ni en modelos de agua. Tesis de Maestría en Ciencias en Gestion Ambiental, Instituto Politécnico Nacional CIIDIR Unidad Durango, México.

Gonzalez-Valdez, L. S., Almaraz-Abarca, N., Proal-Nájera, J. B., Robles-Martinez, F., Valencia-Del-Toro, G., and Quintos-Escalante, M. (2013). Surfactant properties of the saponins of *Agave durangensis*, application on arsenic removal. *International Journal of Engineering 4*, 8269.

Grzywaczyk, A., Smułek, W., Smułek, G., Ślachciński, M., and Kaczorek, E. (2021). Application of natural surfactants for improving the leaching of zinc and copper from different soils. *Environmental Technology and Innovation 24*, 101926. https://doi.org/10.1016/j.eti.2021.101926

Herbert-Doctor, L. A., Saavedra-Aguilar, M., Villarreal, M. L., Cardoso-Taketa, A., and Vite-Vallejo, O. (2016). Insecticidal and Nematicidal effects of *Agave tequilana* juice against *Bemisia tabacil* and *Panagrellus redivivus2*. *Southwestern Entomologist 41*, 27-40. https://doi.org/10.3958/059.041.0105

Hernández, R., Lugo, E. C., Díaz, L., and Villanueva, S. (2005). Extracción y cuantificación indirecta de las saponinas de *Agave lechuguilla* Torrey. *e-Gnosis 3*, 1-9

Hong, K.-J., Tokunaga, S., and Kajiuchi, T. (2002). Evaluation of remediation process with plant-derived biosurfactant for recovery of heavy metals from contaminated soils. *Chemosphere 49*, 379-387. https://doi.org/10.1016/S0045-6535(02)00321-1

Hoyos-Montilla, A. A., Tobón, J. I., and Puertas, F. (2023). Role of calcium hydroxide in the alkaline activation of coal fly ash. *Cement and Concrete Composites 137*, 104925. https://doi.org/10.1016/j.cemconcomp.2022.104925

Hu, H., Li, X., Huang, P., Zhang, Q., and Yuan, W. (2017). Efficient removal of copper from wastewater by using mechanically activated calcium carbonate. *Journal of Environmental Management 203*, 1-7. https://doi.org/10.1016/j.jenvman.2017.07.066

Jahan, R., Bodratti, A. M., Tsianou, M., & Alexandridis, P. (2020). Biosurfactants, natural alternatives to synthetic surfactants: Physicochemical properties and applications. *Advances in Colloid and Interface Science 275*, 102061. https://doi.org/10.1016/j.cis.2019.102061

Jia, K., Yi, Y., Ma, W., Cao, Y., Li, G., Liu, S., An, N. (2022). Ion flotation of heavy metal ions by using biodegradable biosurfactant as collector: Application and removal mechanism. *Minerals Engineering 176*, 107338. https://doi.org/10.1016/j.mineng.2021.107338

Juarez, D. S., Flores, L., Sánchez-Robles, J. H., De la Cerda, S., Rodríguez, C., and Jiménez, D. (2014). Variability of saponins concentration in guishe collected in different geographical areas and weather conditions. *Journal of Chemistry 2*, 105-115. http://dx.doi.org/10.15640/jcb.v2n2a5

Keleşoğlu, S. (2007). Comparative adsorption studies of heavy metal ions on chitin and chitosan biopolymers. Master Thesis in Chemistry, Izmir Institute of Technology, Turkía.

Kregiel, D., Berlowska, J., Witonska, I., Antolak, H., Proestos, C., Babic, M., Zhang, B. (2017). Saponin-based, biological-active surfactants from plants. *Application and Characterization of Surfactants 6*, 184-205.

LaGrow, A. P., Sinatra, L., Elshewy, A., Huang, K.-W., Katsiev, K., Kirmani, A. R., Bakr, O. M. (2014). Synthesis of copper hydroxide branched nanocages and their transformation to copper oxide. *The Journal of Physical Chemistry C 118*, 19374-19379. https://doi.org/10.1021/jp503612k

Li, Z., Cheng, H., Fu, Y., Zuo, K., Gao, P., and Han, Y. (2023). Dissolution property of serpentine surface and the effect on particle-particle interaction behavior in solution. *Minerals 13*, 799. https://doi.org/10.3390/min13060799

Liang, W., Zhan, L., Piao, L., and Rüssel, C. (2011). Lead and copper removal from aqueous solutions by porous glass derived calcium hydroxyapatite. *Materials Science and Engineering: B 176*, 1010-1014. https://doi.org/10.1016/j.mseb.2011.05.036

Lupa, L., and Cocheci, L. (2023). Heavy metals removal from water and wastewater. *IntechOpen*. https://doi.org/10.5772/intechopen.110228

Luttrell, W. E. (2010). Toxic tips: Potassium hydroxide. *Journal of Chemical Health and Safety 17*, 54-55. https://doi.org/10.1016/j.jchas.2009.11.005

Mireles Martínez, A. (2011). Determinación de la eficiencia de extractos de *Agave* durangensis en la remoción de arsénico en agua. Tesis de Maestría en Ciencias en Gestión Ambiental, Instituto Politécnico Nacional CIIDIR Unidad Durango, México.

Mohammmadi-Manesh, H., Shahidizandi, S., and Loghavi, M. M. (2023). Chemical co-precipitation synthesis of manganese ferrite ($MnFe_2O_4$) and evaluation of its applications in copper removal from aqueous solutions. *Journal of Nanostructures 13*, 16-28. https://doi.org/10.22052/JNS.2023.01.003

Morreeuw, Z. P., Castillo-Quiroz, D., Ríos-González, L. J., Martínez-Rincón, R., Estrada, N., Melchor-Martínez, E. M.,Reyes, A. G. (2021). High throughput profiling of flavonoid abundance in *Agave lechuguilla* residue-valorizing under explored Mexican plant. *Plants 10*, 695. https://doi.org/10.3390/plants10040695

Orlewski, P. M., Ahn, B., and Mazzotti, M. (2018). Tuning the particle sizes in spherical agglomeration. *Crystal Growth and Design 18*, 6257-6265. https://doi.org/10.1021/acs.cgd.8b01134

Pérez-Zavala, M. d. L., Hernández-Arzaba, J. C., Bideshi, D. K., and Barboza-Corona, J. E. (2020). *Agave*: a natural renewable resource with multiple applications. *Journal of the Science of Food and Agriculture 100*, 5324-5333.

Proal-Najera J. B. Patent IMPI MX/E/2011/053602, 2011

Proal-Nájera, J. B., Tabche, L. M., and Mueller, M. (1997). Estudio sobre el tratamiento de aguas residuales industriales altamente concentradas en metales pesados bajo aglomeración esférica. *Journal of the Mexican Chemical Society 41*, 51-56.

Qasim, M., Islam, W., Ashraf, H. J., Ali, I., and Wang, L. (2020). Saponins in insect pest control. *Co-evolution of Secondary Metabolites*, 897-924. https://doi.org/10.1007/978-3-319-96397-6_39

Quiton, K. G. N., Huang, Y. H., and Lu, M. C. (2022). Recovery of cobalt and copper from single-and co-contaminated simulated electroplating wastewater via carbonate and hydroxide precipitation. *Sustainable Environment Research 32*, 1-22. https://doi.org/10.1186/s42834-022-00140-z

Rai, A. K., Singh, S. P., Pandey, A., Larroche, C., & Soccol, C. R. (Eds.). (2021). *Current Developments in Biotechnology and Bioengineering: Technologies for Production of Nutraceuticals and Functional Food Products*. Editorial Elsevier, Netherlands. https://doi.org/10.1007/978-3-031-21682-4_23

Rai, S., Acharya-Siwakoti, E., Kafle, A., Devkota, H. P., and Bhattarai, A. (2021). Plant-derived saponins: a review of their surfactant properties and applications. *Science 3*, 44. https://doi.org/10.3390/sci3040044

Reyes-Agüero, J. A., Aguirre-Rivera, J. R., and Peña-Valdivia, C. B. (2000). Biología y aprovechamiento de *Agave lechuguilla* Torrey. *Botanical Sciences 67*, 75-88. https://doi.org/10.17129/botsci.1626

Rodrigues, C. I. S., Jackson, J. J., and Montross, M. D. (2016). A molar basis comparison of calcium hydroxide, sodium hydroxide, and potassium hydroxide on the pretreatment of switchgrass and miscanthus under high solids conditions. *Industrial Crops and Products 92*, 165-173. https://doi.org/10.1016/j.indcrop.2016.08.010

Schlebusch, I., Pott, R. W. M., and Tadie, M. (2023). The ion flotation of copper, nickel, and cobalt using the biosurfactant surfactin. *Discover Chemical Engineering 3*, 7. https://doi.org/10.1007/s43938-023-00023-8

Secretaría de Economía. (2001). NMX-AA-051-SCFI-2001. Análisis de agua -determinación de metales por absorción atómica en aguas naturales, potables, residuales y residuales tratadas - Método de prueba. Diario Oficial de la Federación.

SEMARNAT, M. (2007). *¿Y el medio ambiente? Problemas en México y el mundo*. SEMARNAT. Mexico.

SEMARNAT. (1997). NOM-001-ECOL-1996. Límites máximos permisibles de contaminantes en las descargas de aguas residuales en aguas y bienes nacionales. Diario Oficial de la Federación.

Sharma, J., Sundar, D., & Srivastava, P. (2023). Advantages and Disadvantages of Biosurfactants over Other Synthetic Surfactants. In: *Advancements in Biosurfactants Research*, (Aslam, R., Mobin, M., Aslam, J., Zehra, S, eds) Pp. 505-523. Springer Cham. `https://doi.org/10.1007/978-3-031-21682-4_23`

Sidana, J., Singh, B., and Sharma, O. P. (2016). Saponins of *Agave*: Chemistry and bioactivity. *Phytochemistry 130*, 22-46. `https://doi.org/10.1016/j.phytochem.2016.06.010`

SSA. (2021). Modificación a la norma oficial Mexicana NOM-127-SSA1-2021. Salud ambiental, agua para uso y consumo humano. Límites permisibles de calidad y tratamientos a que debe someterse el agua para su potabilización. Diario Oficial de la Federación.

Vashist, U., Sadri, F., Choi, Y., and Ghahreman, A. (2022). Systematic study of critical parameters on magnesium saturated thiosulfate gold leaching process; part A: Effect of ammonia and lime for pH adjustments and concentration of magnesium and copper (II). *Hydrometallurgy 213*, 105943. `https://doi.org/10.1016/j.hydromet.2022.105943`

Velázquez Ríos, I. O., González-García, G., MelladoMojica, E., Veloz-García, R. A., Dzul-Cauich, J. G., López, M. G., and García-Vieyra, M. I. (2019). Phytochemical profiles and classification of *Agave* syrups using 1H-NMR and chemometrics. *Food Science and Nutrition 7*, 3-13. `https://doi.org/10.1002/fsn3.755`

Vivas, E. L., Alfafara, C. G., Migo, V. P., Cho, K., Detras, M. C. M., Trinidad, L. C., ... and Lee, S. (2019). Comparative evaluation of alkali precipitation and electrodeposition for copper removal in artisanal gold smelting wastewater in the Philippines. *Desalination and Water Treatment 150*, 396-405. . `https://doi.org/10.5004/dwt.2019.23790`

Wang, L. K., Wang, M. H. S., Shammas, N. K., and Hahn, H. H. (2021). Physicochemical treatment consisting of chemical coagulation, precipitation, sedimentation, and flotation. *Integrated Natural Resources Research*, 265-397. `https://doi.org/10.1007/978-3-030-61002-9_6`

Wen, T., Zhao, Y., Zhang, T., Xiong, B., Hu, H., Zhang, Q., and Song, S. (2019). Effect of anions species on copper removal from wastewater by using mechanically activated calcium carbonate. *Chemosphere 230*, 127-135. `https://doi.org/10.1016/j.chemosphere.2019.04.213`

World Health Organization. (2011). *Guidelines for Drinking-Water Quality*, Editorial WHO Press, Switzerland.

Wołowicz, A., Staszak, K., and Hubicki, Z. (2023). Effect of anionic surfactants on the heavy metal ions removal by adsorption onto ion exchangers-batch and column studies. *Journal of Water Process Engineering 53*, 103792. `https://doi.org/10.1016/j.jwpe.2023.103792`

Ye, L., Chai, L., Li, Q., Yan, X., Wang, Q., and Liu, H. (2016). Chemical precipitation granular sludge (CPGS) formation for copper removal from wastewater. *RSC Advances 6*, 114405-114411. `https://doi.org/10.1039/C6RA11165C`

Yoon, R. H., and Salman, T. (1971). Zero point of charge of cupric hydroxide and surface area determination by dye adsorption. *Canadian Metallurgical Quarterly 10*, 171-177. `https://doi.org/10.1179/cmq.1971.10.3.171`

Yu, X. L., and He, Y. (2018). Tea saponins: Effective natural surfactants beneficial for soil remediation, from preparation to application. *RSC Advances 8*, 24312-24321. `https://doi.org/10.1039/C8RA02859`