



Bioleaching of Iron and Zinc from a galvanized steel waste from automotive industry using *Acidithiobacillus thiooxidans*: a case of study of the bajo area in México

Biolixiviación de hierro y zinc utilizando *Acidithiobacillus thiooxidans* a partir de desechos de la industria automotriz: un caso de estudio del bajo, México

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ABSTRACT

Biohydrometallurgy has been defined as a collection of techniques that involve the use of microbial metabolisms, such as the bacterium *Acidithiobacillus thiooxidans*, for the extraction of metals. Therefore, in the current study, we tested the potential of a pure culture of *A. Thiooxidans* in the removal of zinc and iron from galvanized waste of the automotive industry. Starkey medium was employed and was added 0.5% w/v of solid sample, and incubation took place at 30°C, and 180 rpm for 7 days. The experiment showed a biological removal of 100% of Fe and Zn after 72 hours, as shown with the measurements taken using inductively coupled plasma spectrophotometer (ICP-OES). Additionally, an increase of the concentration of sulphates is reported alongside the process. The present study thus suggests that a fast, efficient, and low energy biological process for the recovery of these metals coming from galvanized steel waste is possible.

Keywords: Bioleaching; *Acidithiobacillus thiooxidans*; Zinc; Iron; waste valorization.

RESUMEN

La biohidrometalurgia se ha definido como un conjunto de técnicas que involucran el uso de metabolismos microbianos para la extracción de metales, como el de la bacteria *Acidithiobacillus thiooxidans*. Por lo tanto, en el presente estudio, evaluamos el potencial *A. Thiooxidans* en la eliminación de zinc y hierro de los desechos galvanizados de la industria automotriz. Para este fin, se utilizó medio de cultivo Starkey y se añadió 0,5% p/v de muestra sólida, incubando el sistema a 30°C y 180 rpm durante 7 días. El experimento mostró una remoción del 100% de Fe y Zn después de 72 horas de tratamiento, evaluado a través de un espectrofotómetro de plasma acoplado inductivamente (ICP-OES). Adicionalmente, se reporta un aumento

de la concentración de sulfatos a lo largo del proceso. Por lo tanto, el presente estudio sugiere que es posible un proceso biológico, ecológico, rápido, eficiente y de baja energía para la recuperación de estos metales como Fe y Zn provenientes de desechos de acero galvanizado.

Palabras claves: Biolixiviación; *Acidithiobacillus thiooxidans*; Zinc; Hierro; Valorización de desechos.

1. STATEMENT OF NOVELTY

Metal recovery from industrial waste through traditional chemical methods is considered to deliver low metal yields and thus may not be a viable option. Therefore, the current study seeks to determine whether metal recovery from galvanized waste derived from the automotive industry with the help of acidophilic bacteria (bioleaching) is feasible and effective. After an in-depth review of the existing literature, the authors state that previous studies have yet to focus on solutions towards galvanized automotive waste.

2. INTRODUCTION

Leaching is a widely known method used to achieve the extraction of a species of interest, such as zinc, iron, and aluminum by means of employing chemical grade sulfuric acid (H_2SO_4) (Abdel-Aal, 2000; Seidel and Zimmels, 1998). Despite the process's efficiency, biotechnological strategies have been explored as early as the 1950s, the discovery in 1947 of the role of microbial activity in iron oxidation of acid mine drainage (AMD) (Ehrlich, 2004), set up a new area of opportunity as commercial alternatives for metal extraction (Brierley and Brierley, 2001) and metal retrieval from mineral waste materials through bioleaching (Bosecker, 2001). Application of these technologies in the mining industry has developed progressively during the last century (Mishra *et al.*, 2005).

Biohydrometallurgy is regarded as a set of technologies and techniques that involve microorganisms for the mobilization and extraction of metals (Anjum, Shahid and Akcil, 2012). Among these bio-processing technologies are biosorption, bioaccumulation, and bioleaching (Mishra *et al.*, 2005). Biosorption is understood as the process through which dissolved metals are absorbed and/or complexed via the chemical interactions with a microbial biomass, consisting of dead and metabolically inactive cells. Volesky and Holan (Volesky and Holan, 1995) describe that the biosorbent behavior of microbial biomass toward metallic ions is dependent on the chemical makeup of the cells it is constituted of. Bioaccumulation, in contrast, is a pollutant removal process that is dependent on the microbial metabolism and resistance to target pollutants (Ahemad and Malik, 2011). Bioleaching, in turn, is the term that refers to the process through which metals are dissolved from their mineral sources by action of microbial metabolism. The resulting chemical transformation of elements from a solid state into a soluble form facilitates their extraction (Anjum, Shahid and Akcil, 2012). It was first noticed by miners, who observed the subtle process that took place in the irrigation of copper-bearing sulfide ore piles, which eventually resulted in the mobilization of copper, then recoverable from the solution (Ehrlich, 2004).

These bio-processing technologies are distinguished by the utilization of biocatalysts, such as microorganisms or enzymes, and are generally regarded as more sustainable and eco-friendly approaches of existing processes (Wang *et al.*, 2009). Bioleaching, in particular, has been attributed with advantages such as low cost, easy operation and maintenance, possibility to implement native consortia adapted to a medium containing different metals, and the potential to leach various metals from a polymetallic source (Zazueta-Álvarez *et al.*, 2018). Furthermore, bioleaching allows for greater removal efficiencies in shorter operation times, when compared to traditional hydrometallurgical processes. For instance, a study conducted by Ye *et al.* (Ye *et al.*, 2017), which aimed to evaluate the effects of pH and solid concentration in the bioleaching of heavy metals from lead-zinc mine tailings, demonstrated recovery efficiencies of 3.47%, 3.75% and 26.1% for Fe, Pb and Zn, respectively, in a control group after 50 days of treatment, where the solubilization of metals could be attributed to sulfuric acid in the medium at a pH of 2; in

comparison, in an experimental group with inoculation of *Acidithiobacillus ferrooxidans* and under optimal conditions determined by the authors (Ye *et al.*, 2017), recoveries of 85.45%, 4.12% and 97.85% of Fe, Pb and Zn, respectively, were obtained within the same timeframe of 50 days. Another example is an experiment by Zazueta-Álvarez *et al.* (Zazueta-Álvarez *et al.*, 2018), which demonstrated a removal of 93.49% Mn, 66.45% Zn and >99% Pb from mine tailings at flask level in just 17 days employing native consortia, composed of *Leptospirillum ferriphilum* and *L. ferrooxidans*, in a 9K medium with an initial pH of 2. Due to these characteristics, these processes may contribute in the generation of future technologies, including those related to the treatment of waste, such as that coming from the electronics industry (Wang *et al.*, 2009), and as a plausible option to recover valuable metals from alternative sources in view of their ever growing demand (Zazueta-Álvarez *et al.*, 2018).

One of the most widely used microorganisms for the treatment of waste originating from the mining industry is *Acidithiobacillus thiooxidans*. This is a species of proteobacterium belonging to the class *Acidithiobacillia*, is regarded as a mesophilic and chemolithotrophic organism (Wang *et al.*, 2019; Travisany *et al.*, 2014). This species, along with other members of the genus, possess the ability to oxidize reduced inorganic sulfur compounds, which in turn produces sulfuric acid (Yang *et al.*, 2014) and provides the electrons necessary to fix carbon dioxide (Wang *et al.*, 2019). Having being described for the first time in 1921 by Waksman and Joffe (Travisany *et al.*, 2014), this species has been found to inhabit several sulfur-rich, acidic environments, some of which include, according to Travisany *et al.* (Travisany *et al.*, 2014), mines of coal, copper and uranium, sewage systems, seawater ecosystems and sulfidic caves.

Various reports exist on the applicability of *A. thiooxidans* for the bioleaching of metals from different sources. For instance, the feasibility of recovering Cu from metal slags by using *A. thiooxidans* in a bioleaching process of Zn, Cu and Fe from amorphous slags was explored by Potysz *et al.* (Potysz *et al.*, 2016), and, after 21 days of treatment, 79% of Cu and 76% of Zn was extracted, whereas from crystalline slags 81% of Cu and 79% of Zn was removed (Potysz *et al.*, 2016). Additional authors have explored the use of *A. thiooxidans* in the recovery of metals from residues of the mining industry. An example of this is the work of Nguyen *et al.* (Nguyen *et al.*, 2015) regarding bioleaching of Cu, Mn, Zn and As from mine tailings, and the study conducted by Sethurajan *et al.* (Sethurajan *et al.*, 2016) related to the biorecovery of Zn from zinc plant leach residues. A report also exists on the use of this bacterium species in the recovery of copper, lead and zinc from printed wire boards (Wang *et al.*, 2009), either as a pure culture or as a mixed culture along with *A. ferrooxidans*, showing promising results.

The present study was undertaken to evaluate the potential of the pure culture of *A. thiooxidans* to remove zinc from galvanized metal waste coming from the automotive industry in Queretaro, México. To our knowledge, this work represents the first report of the recovery of zinc from galvanized metal residues of the automotive industry through biological treatment, and thus serves as an expansion to the current perspectives on the applications of this bacterium for the recovery of economically important metals.

3. MATERIALS AND METHODS

3.1 Microorganisms and culture media

Acidithiobacillus thiooxidans was kindly donated by Dr. Norma G. Avelizapa from CICATA-IPN. The culture medium used was Starkey, with the chemical composition (g L⁻¹) of: KH₂PO₄ (3.0), (NH₄)₂SO₄ (0.2), MgSO₄ · 7H₂O (0.5), CaCl₂ · 2H₂O (0.3), FeSO₄ · 7H₂O (0.01), and 750 mg/L of Na₂MoO₄. The medium was also supplemented with 0.3 g/L of elemental sulfur. Finally, the medium was gradually adjusted to a pH 3 with sulfuric acid.

3.2 Waste sample collection

A representative sample of galvanized metal waste was taken from *Interacero S.A. de C.V.*, a Mexican company invested in the trade of metals and metal residues. The sample was crushed and grounded in a disc mill and sieved in a mesh with cutting edge of 2000 μm to homogenize particle size.

3.3 Waste sample characterization

Residual concentrations of Fe and Zn in galvanized metal waste were determined at the beginning and at the end of microbial treatment by ICP-OES (Varian Model 710-ES). Samples of 100 mg of waste were placed in cylindrical vial of silicon carbide, 6 mL of HNO_3 and 2 mL of HCl were added; samples were digested in a Microwave Reaction System: Multiwave PRO (Anton Paar), using a rotor HF100. Digestion conditions were power 600 W for eight 6 vessels, 40 Bar, temperature 210-240°C, with pRate of 0.3 bar seg-1, ramp 10 min, hold 20 min and cooling 15 min. Afterwards, 20 mL of deionized water was added to cylindrical vial and the supernatant was collected in a 100 mL flask and set with deionized water. Metal concentration of Fe and Zn in galvanized metal waste was calculated based on calibration curve of 0.1-10 ppm using a commercial standard (High-Purity) cat. # ICP-200-7-6. The total amount removed of Fe and Zn from catalyst was calculated by difference in concentration (mg kg⁻¹) between day 0 and 7 and subtracting the amount of Fe and Zn removed in controls.

3.4 Evaluation of sulfur oxidation in presence of the residue

The culture media was enriched with 0.5% (w/v) of solid sample and placed in a 125 mL Erlenmeyer flask containing 30 mL of Starkey medium containing 3×10^8 UFC/mL of *Acidithiobacillus thiooxidans*. The flasks were incubated at 30°C and 180 rpm for 7 days. A Mexican norm NMX-K-436-1977 was used to evaluate the production of sulfates (SO_4^{2-}). The concentration of S_2O_4 was measured using a titration technique. Bromothymol blue was used as a pH indicator and a solution of NaOH 0.5 M as a neutralizing agent.

3.5 Data analyses

Basic statistical parameters and analyses of variance (ANOVA) were performed using the commercial statistical software Minitab 17. Differences with P values of < 0.05 were considered statistically significant.

4. RESULTS

Sample characterization and metal removal.

The initial concentration of the Zn and Fe, as well as the percentage of bioleaching by *A. thiooxidans*, were determined in the galvanized steel waste from automotive industry and the experimental treatments. Initially, $930755,0 \pm 30048,5$ mg/kg of Fe and $12965,1 \pm 123,6$ mg/Kg of Zn were detected through ICP-OES in the galvanized steel waste sample. After 24 hours of treatment with *Acidithiobacillus thiooxidans*, there was a removal of 43% and 46% of Fe and Zn respectively. It was observed that after 72 hours, there was a 100% removal of Zn and Fe (Figure 1A). The sulfur oxidation and therefore the production of sulfates can be observed in figure 1C. Therefore, it can be observed that the concentration of sulfates increases with time. The highest value was reached at 168 hours with 20161 mg/L. The abiotic control produced 845 mg/L, so it can be deduced that the medium can oxidize sulfur and therefore produce sulfuric acid, as it is shown in figure 1D. The increase in the concentration though the time is shown. The abiotic control produced a total of 0.005 M, while at 168 hours, there was a production of 0.2 M (Fig. 1D). Decrease in Ph (Figure 1B) is consistent with increase in sulfate (Figure 1C) and sulfuric acid (Figure 1D) concentrations.

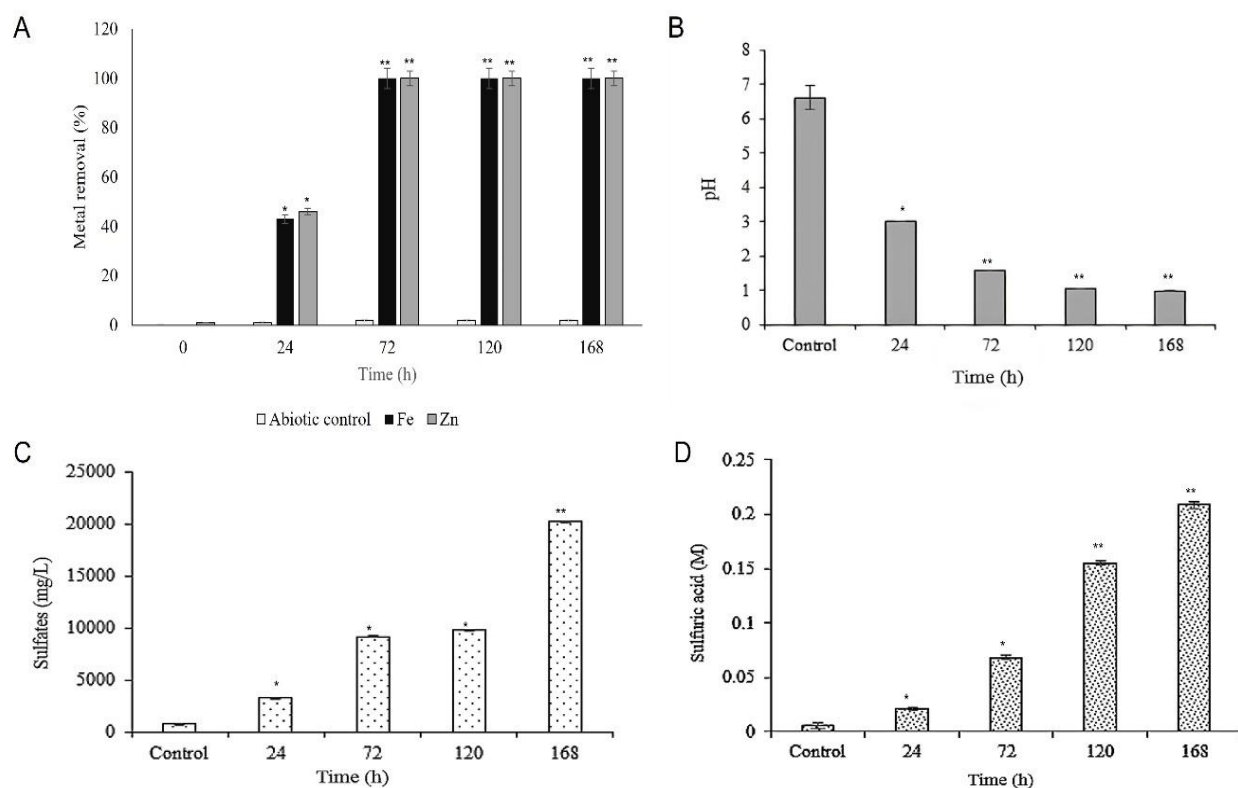


Figure 1 A) Percentage of Fe and Zn removal from the galvanized metal waste during biotreatment with *A. thiooxidans* in Starkey liquid media with $930755,0 \pm 30048,5$ mg/kg of Fe and $12965,1 \pm 123,6$ mg/kg of Zn after 168 h of incubation at 30°C, 180 rpm. B) The pH variation through the time. C) Sulfate concentration (mg/L) increase through time. Measurements were taken in triplicates, following the procedures indicated in the Mexican norm NMX-K-436-1977 for the determination of sulfate ions, and their average is displayed. No further elemental sulfur supplementation was made after the beginning of the experiment. D) Sulfuric acid concentration (M) through time. ** indicate statistically significant difference (one-way ANOVA $P < 0.05$, with Tukey's post hoc analyses).

5. DISCUSION

Results from this study indicate a 100% removal of iron and zinc from milled galvanized steel waste at 72 hours post inoculation of *A. thiooxidans* under the previously described conditions. This suggests that a relatively fast, efficient and with low energy consumption requirements for the recovery of these metals from galvanized steel residues is possible. Travisany et al. (Travisany *et al.*, 2014) have previously mentioned that this species of bacterium has been considered of special biotechnological interest in the field of bioleaching due to its role as a sulfur oxidizer, and in the keeping of acidic conditions due to the production of sulfuric acid and in the removal of surface level sulfur from minerals. Additionally, this bacterial species, along with sister species *A. ferrooxidans* and *A. caldus*, has been reported to be of use in the treatment of wastes of electronic origin containing heavy metals, such as batteries, circuit boards (Wang *et al.*, 2009; Wang *et al.*, 2019), and printed wire boards (Wang *et al.*, 2009), which may be, in turn, an interesting, and more economic, alternative to pyrometallurgical and hydrometallurgical processes for the retrieval of solid metals (Wang *et al.*, 2009). The results of this study may thus represent an expansion to the current known applications of *A. thiooxidans*.

Potysz et al. (Potysz *et al.*, 2016) mention that the effectiveness of metal bioleaching is dependent on the bacterial ability to produce sulfuric acid; the presence of acid is also mentioned to favor metal extraction by promoting proton replacement. The comparison of the removal rates of iron and zinc between the abiotic controls and the experimental treatment, as indicated in Fig. 1, suggests that bioleaching of zinc and iron was due to the action of microbial metabolism and not a result of the used culture medium, considering that no significant removal of metals from the sample was found in the abiotic control during the period of study. This is further supported by the increase of sulfuric acid concentration and decrease in pH through time, as shown in Figures 3 and 4, respectively.

Results from a previous work related to the bioleaching of zinc from metallurgical leach residues by Sethurajan et al. (Sethurajan *et al.*, 2016) indicate that the dissolution of zinc from zinc plant leach residues is dependent on the concentration of sulfuric acid, and also provided evidence that high sulfur concentrations in medium lead to higher bioleaching efficiencies. *A. thiooxidans* has been reported (Potysz *et al.*, 2016; Wang *et al.*, 2019) to metabolize elemental sulfur for the obtention of energy and the production of sulfuric acid, through a process represented by the following reaction (Potysz *et al.*, 2016): $S + 1.5O_2 + H_2O \rightarrow H_2SO_4 \rightarrow SO_4^{2-} + 2H^+$. Furthermore, in an experiment related to the recovery of metals from steel slag, Hocheng et al. (Hocheng, Su and Jadhav, 2014) reported higher metal removal efficiencies in a system employing a pure culture of *A. thiooxidans*, when compared to systems employing cultures of *A. ferrooxidans* and *Aspergillus niger*, which suggests that the sulfuric acid generation of *A. thiooxidans* may facilitate metal leaching. For future research, elucidation of the effect of sulfur concentration on the leaching of zinc from automotive residues, and the optimization of such concentration, such as done by Sethurajan et al. (Sethurajan *et al.*, 2016) through a response surface methodology, is recommended.

Compared to other residues, the present study showed that bioleaching of metals from galvanized automotive waste can be achieved with little sample pretreatment, other than grinding and milling, when compared to that applied to the steel slag as reported by Hocheng et al. (Hocheng, Su and Jadhav, 2014). These results indicate a possible advantage of the process of treating galvanized waste through biological methods and could thus attract the industry's attention.

Our results show some similarities to those reported by Wang et al. (Wang *et al.*, 2009): in their experiment, around 100% zinc from printed wire boards was solubilized by day 5 employing a pure culture of *A. thiooxidans* in the liquid medium 9K with an initial pH of 2.5 when adding a concentration of 7.8 g/l⁻¹ PWBs and particle size of <0.35 mm is used; in addition, the same study showed that mixed cultures of *A. thiooxidans* and *Acidithiobacillus ferrooxidans* were effective in solubilizing copper and lead from printed wire boards, in addition to zinc. Yang et al. (Yang *et al.*, 2014) report an 83.8% Zinc removal when after 72 h with *A. ferrooxidans* under the following conditions: 198 mL 4.5 K medium, 2 mL inoculum, 15 g/L of PWBs with a particle size 20~40 mm and a constant pH of 2.25 by constant addition of H₂SO₄. Yang et al. (Yang *et al.*, 2014) report a negative relationship between concentration of sample and metal leaching; this could explain the success of the bioleaching treatment addressed within this study, since the concentration that was implemented in this experiment was of 0.5% (w/v). It is also important to consider that *A. thiooxidans* has been reported to acidify the medium on its own, as part of its metabolism. Therefore the media wouldn't need to be acidified, and resources as well as energy would be saved in the process. In consequence, *A. thiooxidans* proves to be a viable biotechnological option for metal removal from waste (Matias *et al.*, 2019).

Something that is to be contrasted with the results published by Wang et al. (Wang *et al.*, 2009) is the particle size. The authors report a removal of Zn of around 50% at day 5 for the particle sizes ranging from 1 to 3 mm employing a pure culture of *A. thiooxidans*, whereas the removal of Zn within this experiment for a particle size of 2 mm is of 100% at day 3. These fluctuations may be due to different bacterial counts in the used inoculums; Yang et al. (Yang *et al.*, 2009) report a higher mobilization rate of copper in the process of treating PWBs with *A. ferrooxidans* when employing greater amounts of inoculum.

Thus, considering the variations that may arise when employing different experimental conditions, we suggest doing additional experiments to better understand the bioleaching behavior of the strain under different conditions of sieve fraction and inoculum. Further research based on DOE methodologies for the optimization of conditions to maximize iron and zinc removal, remains a strong interest of our research group. Additionally, migrating the experiment to a bioreactor system and upscaling are other focuses that will be explored.

6. CONCLUSION

Through the current work, the bioleaching process with *A. thiooxidans* was proved to be a fast, efficient, and low-cost metal recovery method for Zn and Fe from galvanized residues from the automotive industry, and thus could serve as an option for the industry, since a 100% of the Zn present in the sample was successfully removed within 72 hours. Moreover, some aspects such as the influence of the particle size, sample concentration, elemental sulfur addition and modification of the acidification step are yet to be explored, thus leaving an open door to further research work.

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SEMBLANZA DE LOS AUTORES



Francisco Armando Granados-Contreras nació en Querétaro, Querétaro, México, en donde cursa el último año de la carrera de Ingeniería en Biotecnología en el Tecnológico de Monterrey. Interesado por la innovación que trae consigo esta área de estudio, ha colaborado en equipos de investigación relacionados con biorremediación, bioinformática y más recientemente inmunología, de manera nacional e internacional.

Además de su interés por la investigación científica, Francisco ha colaborado con instituciones que abogan por mejorar el acceso a la educación, entre ellas como tutor de química general en la preparatoria virtual de carácter social del Tecnológico de Monterrey y como tutor de matemáticas en iniciativas como “Jóvenes ayudando a niñas y niños”.

Con su graduación aproximándose, Francisco busca continuar su educación con un posgrado para seguir involucrándose en la aplicación de la biotecnología.



Grisel Fierros-Romero: Actualmente es Investigadora Asociada en Florida A&M University. Se desempeña como directora de carrera de ingeniería en Biotecnología y profesora investigadora en el Tecnológico de Monterrey, campus Querétaro. Pertenece al Sistema Nacional de Investigadores, nivel 1. Su investigación se desarrolla en el área de biotecnología ambiental, estudiando la resistencia microbiana a metales pesados con fines de biorremediación, interacciones de bacterias depredadoras de origen marino con bacterias del género *Vibrio*, virus y protistas con potencial biotecnológico, síntesis y uso de nanopartículas en áreas como medicina, agricultura y ambiente, y genómica microbiana ambiental. Obtuvo el grado de doctorado con mención honorífica en Tecnología Avanzada con enfoque en Biotecnología Ambiental en el Instituto Politécnico Nacional. Es autora de artículos internacionales, y capítulos de libros.