Biological removal of heavy metal from automotive hazardous paint sludge by semi -continuous bioleaching process

Abstract

Few studies have been conducted on detoxification, metal recovery, and stabilization prior to landfilling automotive paint sludge as hazardous waste. Most studies have focused on the direct use of paint sludge as a sealant and primer, its application in construction materials such as concrete, and the extraction of valuable organic and mineral materials from paint sludge. This study was conducted to remove heavy metals in batch conditions using indigenous *Pseudomonas aeruginosa* bacteria in water-based paint sludge under semi-continuous conditions, utilizing optimal parameters obtained from a previous study with constant parameters of pH 7, a temperature of 32 °C, paint sludge with a 3 mm mesh, a shaker speed of 180 rpm, and variable parameters of aeration(oxygen) and nutrients (glucose) for the removal of zinc and other metals. The results showed that increasing glucose and aeration have linear (direct) effects on the removal process of zinc and other metals, along with a mutual interaction between these two parameters.

Keywords: Heavy Metals; Semi-Continuous Bioleaching; Central Composite Design; Indigenous *Pseudomonas aeruginosa*

1. Introduction

Paint sludge is a waste product of the automotive painting process; approximately 40 to 50 percent of the paint is wasted, and when mixed with water, it produces paint sludge (Ruffino et al., 2023). The major source of hazardous waste in the automotive industry is the painting process and the production of paint sludge with toxicity class 2. There are two types of paints: water-based and solvent-based (Salihoglu et al., 2018), which are classified as hazardous waste due to heavy metals, uncured polymers, and other organic and inorganic contaminants. Each factory produces about 2,555 to 4,380 tons of paint sludge in a year (Khezri et al., 2013). Most methods for removing paint sludge pollution have been based on reducing its volume through filtering, dewatering, incinerating, and landfill in the ground. (Arce et al., 2010 & Khezri et al.,2013). The utilization of this waste in the cement, road construction, activated carbon, repainting, and colored concrete industries is limited (Kulkarni et al., 1975). Paint sludge was used as an additive and filler in the production of modified bitumen by mixing three different types of solvent-based paint sludge (primer, base, and clear coat) with bitumen. The findings showed that with an increasing amount of all three types of paint sludge, ductility and penetration decreased, while the softening point remained almost unchanged. Modified bitumen can be used in waterproofing membranes or sealing applications (Salmani et al., 2025). The synergistic interaction between sewage sludge and paint sludge during the co-pyrolysis process was investigated to optimize the treatment of sewage sludge in a cement kiln system using thermogravimetric analysis (TGA and TGA -mass) (Zhou et al., 2024). Few studies have been conducted on the use of biological methods such as producing compost from melanin resin present in paint sludge (Tian et al., 2018), reducing nickel, chromium, and BTEX metal pollution by composting process (Salihoglu et al., 2018), also reducing chromium and C/N ratio to 0.2 mg/kg and 14.3 units respectively, and reducing BTEX content to standard levels after 90 days by vermi-composting process (Ghomi Avili et al., 2018). In a study, water-based paint sludge was composted in five separate reactors with added sludge as a substrate aid and sunflower stalks as a bulking agent. The proportion of paint sludge added to the compost mixture varied between 40% and 80%. The most efficient composting process was achieved with 60% water-based paint sludge, 20% treated sludge, and 20% sunflower stalks in the reactor (R3). During this process, there was a decrease in organic matter content due to the mineralization of organic matter and a reduction in waste volume, consequently. The composting process can be a beneficial tool for addressing the challenges of paint sludge management (Uçaroğlu et al., 2024). as well as bioleaching method in the batch condition in reducing heavy metals including zinc by Indigenous *P. aeruginosa* and *Acidithiobacillus thiooxidans* on water-based paint sludge and optimizing condition experimental by RSM method (Honarjooy et al., 2020& 2021).

Response Surface Methodology (RSM) is a method for optimizing complex processes using a multivariate approach and a standard method for designing experiments among several different variables, which is used in the fields of biology, chemistry, food, and industry. One type of design is central composite design. CCD is a method for fitting quadratic surface responses and leads to reliable predictions of quadratic and linear interactions of parameters affecting the process (Naseri et al.,2023). The reduction of zinc and heavy metal by Indigenous *P. aeruginosa* using a semi-continuous bioleaching method and its system design by CCD based on the results of batch process (Honarjooy et al., 2020) is the basis of the present study.

2. Materials and Methods

2.1 Combination of Paint Sludge and Microorganisms Used

Water-based paint sludge was air-dried and of sieve, 3 mm crossed. Heavy metals before and after the bioleaching process determined by the EPA 3050B method as described: HNO_3 (65%) ratio of 1:1 at 90-95 °C for 10 to 15 minutes, then was cooled and 5 mL of concentrated HNO_3 was added and refluxed for 30 minutes; again was cooled down, followed by adding 2mL of water and 3mL of H_2O_2 (30%); then, 10mL of HCl (37%) was added to the digest reflux sample for 15 minutes. The digested solution was centrifuged at 2000 rpm filtered through a 0.45 μm filter and diluted to a final volume of 100 mL (Navarro et al., 2011); United States Environmental Protection Agency (USEPA, 2010). The liquid obtained was measured by the absorption spectroscopy model (Spectra Varian AA, 220FS, Australia) and ICP.

Indigenous Pseudomonas aeruginosa bacterium was isolated in research from paint sludge and then maintained and applied in the nutrient broth medium used in these studies (Honarjooy et al., 2020).

2.2 Construction and Running of Semi-Continuous System

In continuous systems, the inlet flow rate (Q_{in}) is always equal to the outlet flow (Q_{out}), and the outlet material concentration (C_{out}) is equal to the contents of the reactor tank (C_t). Only the inlet concentration (C_{in}) to the reactor is different (Pathak et al.,2014 & Kim et al.,2005). A 4-liter reactor was designed according to Fig. 1, which is equipped with an aeration system and a propeller stirrer blade (Mäkinen et al.,2020). The components of the reactor in Fig. 2 include: a) an air blower for the system, utilizing an aquarium air pump equipped with a blower hose and an adjustable degree of aeration; the air blower rate was determined by a similar experiment to the Marti bottle with the formula Q = v/t for aeration rate in cubic centimeters. Air was blown at a rate ranging from 20 to 40 cubic centimeters per second at the bottom of the reactor. b) To establish the mixing rate or speed of the shaker, an electric motor with a propeller blade, similar to an air conditioning fan motor, was connected to a shaved metal rod. The assembly (mixer and motor) was connected to a graduated dimmer for adjusting the rpm, and a magnetic stirrer was also utilized to enhance mixing. c) A Lutron model RPM laser meter was employed to calibrate the agitator speed at 180 rpm, achieved by irradiating the impeller with a laser and marking the position on the dimmer. d) To maintain temperatures of 32 to 37 °C, an aquarium thermocouple with a glass cover was installed to withstand acidic conditions. e) An alcohol thermometer that can be integrated into the reactor body was added to read and control system temperature. f) Due to the absence of a peristaltic pump in the laboratory, an outlet valve was created on the system body, and a graduated tank or gallon was positioned above the inlet to inject the culture medium (flow rate) to allow the culture medium to enter the system at designated times. h) The pH adjustment and control in the system were performed using a glass pH electrode connected to a Jenway pH meter. The entire reaction tank, mounted on a magnetic shaker, along with the system components, was washed with 70% alcohol through sterile immersion and then with sterile distilled water to initiate the process.

Semi-continuous system based on optimal conditions from previous research (Honarjooy et al.,2022), with constant parameters of pH 7, a temperature of 32°C, paint sludge of 3 mm size, and a shaker speed of 180 rpm. One liter of fresh nutrient broth culture medium with a pH of 7 and one liter of adapted active bacteria *Pseudomonas aeruginosa* with a cell count with Neobar Lam of 7.5×10^8 cells/mL were added. Initially, the contents of this 2-liter reactor were run in a non-aerated state with a food source (glucose) to achieve stable conditions. The density of waste pulp that was tolerable for bacteria in the reactor environment reached 70 g/l at an optimal temperature of 32 °C, and the number of bacteria reached a steady state from the logarithmic growth phase, similar method studies (Honarjooy et al.,2022 & Bahaloo-Horeh et al.,2018). During this period, pH changes were not noticeable, so there was no need to adjust the pH with sulfuric acid. Therefore, a constant flow rate of the culture medium in the reactor is drawn from the outlet valve at the same rate until the number of microorganisms counted remains constant at a density of 7.5×10^8 cells/ml. This value was obtained in 300 mL of fresh medium at the inlet. Adding an additional 100 ml of culture medium caused a sharp decrease in microorganisms to below 10^7, which is not desirable for bioleaching. Subsequently, one gram of paint sludge was added to the medium sequentially, and bacterial counts were performed along with pH measurements until the level was fixed at 70 g/liter of waste (pulp density). The number of microorganisms counted remained constant at approximately 7.5 × 10^8 cells/ml. In this manner, the reactor became stable and continuous. Based on the growth curve of microorganisms in the bioleaching research, the time interval (T) for adding new culture medium and paint sludge to the reactor contents was adjusted every two days.

2.3 Experimental Design and Method

The experiment was designed using response surface methodology (RSM) and central composite design (CCD) with Design Expert software version 11, selecting two parameters: oxygen (at three levels of 0, 20, and 40 cubic centimeters per second) and the ratio F/M (glucose/bacteria) (0-6-3%), resulting in 13 series of experiments to optimize the process. Then, the DOE software runs were executed, first in the absence of aeration and glucose nutrient conditions for a period of 8 days of hydraulic time, and then the remaining test conditions were executed under continuous conditions to optimize the bioleaching process. The system output was passed through a Whatman No. 4 filter after each run. The zinc content was measured in the effluent and on the solid residue on the filter. The measurements were performed in triplicate, along with a control sample, and the percentage of zinc removal was determined according to (Eq.1) was used to calculate the percentage of heavy metal removal in the leach.

$$\text{Bioleaching} = [(C_s \times V_s) / (C_f \times M_f)] \times 100 \quad \text{(Eq.1)}$$

Where Cs is the concentration of metal in the bioleaching liquid (mg/l), Vs is the volume of bioleaching solution (l), C_f is the amount of metal in the paint sludge waste (mg/kg), and Mf is the weight of the paint sludge waste (kg) and (Eq. 2) shows the metal removal percentage in the solid part of the residue was reported as follows:

$$\text{Absorption} = [(C_i - C_f) / (C_i)] \times 100 \quad \text{(Eq.2)}$$

Where C_i is the metal concentration in the primary paint sludge before digestion and C_f is the metal concentration in the secondary paint sludge after digestion; the residue is in (mg/kg) (Xue et al., 2018).

3.3 Results and Discussion

Table 1 shows the amounts of heavy metals present in the paint sludge using the 3050B digestion method compared to the standard. This indicates that some elements, such as zinc and titanium, are high in the paint sludge. Based on the software design, 13 experiments were conducted with different amounts of oxygen and Glucose under optimal reactor conditions, the results of which are shown in Table 2. To assess the data quality, the R^2, or coefficient of determination, which is the ratio of predicted variance to total variance, is utilized. Values up to 0.8 are considered acceptable (Montgomery, 2017). Therefore, based on the results of the ANOVA in Table 3, the value of 0.97 in this study indicates the model's fit A ratio of predicted to adjusted R^2 less than 0.2 is acceptable. A P-value of less than one indicates that the model is significant. Accordingly, the parameters of glucose and the interaction of glucose with oxygen are effective in bioleaching. The F-value indicates the significance of that parameter in the reaction; therefore, the glucose parameter is of high importance. In the final equation, (A) Oxygen and (B) Glucose of the model, as given in (Eq. 3), show the greatest positive effect of glucose on the bioleaching process. Additionally, these two parameters have a positive reciprocal effect, and their synergy will reduce zinc metal.

$$\text{(Removal of zinc \%)}Y= +13.488 +(0.75\times A) + (6.43\times B) + (0.003958 \times AB) - (0.0188\times A^2) - (1.4148B^2) \quad \text{(Eq.3)}$$

In a similar study on ore oxide in batch bioleaching and removing zinc and copper, the bioleaching process was conducted with *Pseudomonas* at low concentrations of 2%, 4%, 6%, and 8% glucose. It was observed that the best removal was achieved at a glucose level of 6% for copper and zinc metals. Increasing glucose from 2% to 8% enhanced acid production in the environment, subsequently expanding the metal leaching rate (Shabani et al.,2019). Vishwakarma used *Pseudomonas aeruginosa* to isolate lithium from spent batteries due to its strong affinity for Fe^{3+}, ability to form stable complexes with various metals, and siderophore production. The optimal conditions for maximizing siderophore production in sodium succinate (SS) culture medium were pH (5–9) and glucose content (1–5 g/L). Subsequently, the effect of pulp density (1–5 g/L) and culture growth period (48–120 h) on lithium bioleaching was evaluated under constant conditions. The reaction temperature was 35 °C, and the stirring speed was 150 rpm. The results showed that the maximum siderophore production of approximately 52 siderophore units (% siderophore units) was achieved within 48 h, at pH 7 with a glucose content of 1 g/l (Vishwakarma et al.,2024). Very few articles have been dedicated to studying the optimal range of DO concentrations in bioleaching processes, and most reported an inhibitory effect at concentrations above 5 ppm. In his study, Guezennec investigated the effect in a continuous reactor on sulfide tailings with a 20% bacterial consortium at concentrations of 4-17 ppm and observed that it had an increasing effect on sulfide dissolution efficiency from 4-13 ppm (Guezennec et al.,2017). Our results showed that increasing aeration has a positive effect on metal removal. In Rashid's study on paint sludge treatment and organic load reduction, it was observed that by increasing dissolved oxygen to 5.5 ppm, aerobic bacteria performed better and were able to remove more. However, increasing it beyond that point caused a decrease in removal, which could be due to the toxic effect of oxygen on bacteria (Rashidi et al.,2017). The model equation indicated that particle size and temperature exert the largest linear effects, respectively. Temperature and shaker rotation speed have positive quadratic effects. The most negative effect arises from the interaction between temperature and pH parameters, while the most positive effect is attributed to particle size and shaker rotation speed. The optimized utility function in the present study was 0.920. The utility ramp of this process with an aeration rate of 20.4 cm^3/s and a glucose rate of 2.8 g will result in maximum zinc removal, as shown in Fig 3.

The percentage of metal removal in the paint sludge remaining on the filter before and after the bioleaching process under semi-continuous conditions was conducted on the optimized sample using the B3050 digestion method, as shown in Table 4.

The results indicate that despite the reduction of zinc, other toxic pollutants such as chromium, nickel, cadmium, and lead also experienced a significant reduction due to the process, even reaching below the permissible sludge disposal limits indicated in Table 1. The SEM microscopic images in Figure 4 illustrate the texture of the paint sludge and the size of the pores. In Fig a), the paint sludge exhibits a coherent texture with no pores, while in Fig b), after the leaching process, the paint sludge shows more pores and visible bacterial organelles on it. Through quantitative mapping and SEM-EDX analysis of the sample before and after the bioleaching process, the weight values of metals decreased from 18.77% to 11.7% by weight. This decrease in zinc metal from 15.63% by atomic weight to 0.69% by atomic weight is evident, as demonstrated in Fig 5.

4. Conclusion

The results indicate that the semi-continuous bioleaching process is effective in removing zinc, which has the highest metal concentration in water-based paint sludge. This process is also effective for other heavy metals and has reduced the amounts of these metals to the permissible standard for sludge disposal in soil. This study demonstrates that native *P. aeruginosa* can tolerate up to 7 g/l pulp density, with the optimal process time being 8 days. The appropriate time to add paint sludge for the two-stage bioleaching is also 12 hours. Glucose serves as a carbon source for bacteria, and the aeration rate in semi-continuous conditions has both linear (direct) and quadratic effects. Additionally, there is a mutual interaction effect between the parameters of aeration rate and nutrients.

Aeration has a positive effect on the biological leaching process of paint sludge under semi-continuous conditions, resulting in bioleaching outcomes that remove more zinc metal than in discontinuous conditions. According to the model equation for zinc metal removal by the native bacteria *Pseudomonas* aeruginosa, it is recommended that future studies on this bacterium be conducted at temperatures below 32 °C, close to ambient temperature, to prevent energy loss and reduce pollution from these wastes before disposal in the environment or incineration in a furnace that forms ash. Moreover, to recycle precious metals such as titanium and reduce pollution, the disposal of ash from fuel should be investigated on a small scale (semi-industrial).

References

Arce, R., Galán, B., Coz, A., Andrés, A., & Viguri, J. R. (2010). Stabilization/

solidification of an alkyd paint waste by carbonation of waste-lime based formulations. *Journal of hazardous materials*. 177(1-3): 428-436.doi: 10.1016/j.jhazmat.2009.12.050

Bahaloo-Horeh, N., Mousavi, S.M. and Baniasadi, M. (2018). Use of adapted metal tolerant *Aspergillus niger* to enhance bioleaching efficiency of valuable metals from spent lithium-ion mobile phone batteries. *Journal of Cleaner Production*, 197, pp.1546-1557). doi.org/10.1016/j.jclepro.2018.06.299

Ghomi Avili, R., Takdastan, A., Atabi, F., & Omrani, G. A. (2018). Feasibility Study of Chromium Removal from Paint Sludge with Biological Sludge, Using Vermicompost by *Eisenia fetida* (Case Study: Saipa Automotive Industry). *Jundishapur Journal of Health Sciences*, 10(3). doi: 10.5812/jjhs.78891

Guezennec, A.G., Joulian, C., Jacob, J., Archane, A., Ibarra, D., De Buyer, R., Bodénan, F. and d'Hugues, P. (2017). Influence of dissolved oxygen on the bioleaching efficiency under oxygen enriched atmosphere. *Minerals Engineering*, 106, pp.64-70. doi.org/10.1016/j.mineng.2016.10.016

Honarjooy Barkusaraey, F., Mafigholami, R., Faezi Ghasemi, M. and Khayati, G. (2022). Zn bio extraction from a zinc rich paint sludge by *indigenous Pseudomonas aeruginosa*. *Chemical Engineering Communications,* 209(10), pp.1397-1412. doi.org/10.1080/00986445.2021.1974410

Honarjooy Barkusaraey, F., Mafigholami, R., Faezi Ghasemi, M., & Khayati, G. (2021). Optimization of zinc bioleaching from paint sludge using *Acidithiobacillus thiooxidans* based on response surface methodology. *Journal of Environmental Science and Health*, *Part A*, 56(11), 1243-1252.doi.org/10.1080/10934529.2021.1979330

Honarjooy Barkusaraey, F., Mafigholami, R., Ghasemi, M. F., & Khayati, G. (2020). Isolation and identification of resistant microorganisms from automotive paint sludge. *Jundishapur Journal of Health Sciences, 12(2)*. doi: 10.5812/jjhs.101226

Kim, I. S., Lee, J. U., & Jang, A. (2005). Bioleaching of heavy metals from dewatered sludge by *Acidithiobacillus ferrooxidans*. Journal of Chemical Technology & Biotechnology: International Research in Process, *Environmental & Clean Technology*, 80(12), 1339-1348. doi.org/10.1002/jctb.1330

Khezri, S. M., Shariat, S. M., & Tabibian, S. (2013). Evaluation of extracting titanium dioxide from water-based paint sludge in auto-manufacturing industries and its application in paint production. *Toxicology and industrial health*, *29*(8), 697-703.

Kulkarni, M., Student, P. G., APCOER, P., Thakare, I. D. S., & Gawande, S. (1975). Recycling of Hazardous Paint Waste in Constructions Material. Cal, 3274, 2500. www.ijeter.everscience.org.

Mäkinen, J., Salo, M., Khoshkhoo, M., Sundkvist, J. E., & Kinnunen, P. (2020). Bioleaching of cobalt from sulfide mining tailings; a mini-pilot study. *Hydrometallurgy, 196, 105418.* doi.org/10.1016/j.hydromet.2020.105418

Montgomery, D. C. (2017). Design and analysis of experiments. *John wiley & sons*.

Mostafavi, M., Mirazimi, S., Rashchi, F., Faraji, F., Mostoufi, N. (2018). Bioleaching and Kinetic Investigation of WPCBs by A. ferrooxidans, *A. thiooxidans* and their Mixtures. *J. Chem. Petrol. Eng*. 52, 81–91. doi:10.22059/jchpe.2018.255842.1227.

Naseri T., Beiki V., Mousavi SM., Farnaud S. (2023). A comprehensive review of bioleaching optimization by statistical approaches: recycling mechanisms, factors affecting, challenges, and sustainability. *RSC Adv*.7;13(34):23570-23589. doi: 10.1039/d3ra03498d.

Navarro, E. M. G., Tagle, M. E. V., Marín, M. T. L., & Alfonso, M. S. P. (2011). Comparison of USEPA 3050B and ISO 14869-1: 2001. digestion methods for sediment analysis by using FAAS and ICP-OES quantification techniques. *Química Nova*, 34(8), 1443-1449. doi:10.1590/S0100-40422011000800025

Pathak, A., Kothari, R., Dastidar, M. G., Sreekrishnan, T. R., & Kim, D. J. (2014). Comparison of bioleaching of heavy metals from municipal sludge using indigenous sulfur and iron-oxidizing microorganisms: Continuous stirred tank reactor studies. *Journal of Environmental Science and Health, Part A*, 49(1), 93-100. doi:10.1080/10934529.2013.824737

Ruffino, B., Campo, G., Idris, S.S., Salihoğlu, G. and Zanetti, M. (2023). Automotive paint sludge: A review of pretreatments and recovery options. *Resources*, 12(4), p.45. doi:10.3390/resources12040045

Rashidi, S., Sani, A. H., & Razavi, S. (2017). The Effect of Environmental Conditions on the Removal of Organic Compounds from Color Sludge via Bioslurry Method. Biosciences Biotechnology Research Asia, 14(4), 1371-1384. dx.doi.org/10.13005/bbra/2582

Salmani, H.J., Yousefi, M., Sanjarifard, A., Nowee, S.M., Asgari, A., Sedaghat, E. and Lakzian, K.(2025). An environmental friendly procedure for recycling automotive paint sludge. *Recent Progress in Sciences*, 2(1), pp.8-8. doi:10.70462/2025.2.002

Salihoglu N.K., Ucaroglu S., Salihoglu G. (2018). Bioconversion of industrial wastes: paint sludge from automotive manufacturing. *J Mater Cycles Waste Manage*. 20(4):2100–2109. doi:10.1007/s10163-018-0764-z

Shabani, M. A., Irannajad, M., Meshkini, M., & Azadmehr, A. R. (2019). Investigations on bioleaching of copper and zinc oxide ores. *Transactions of the Indian Institute of Metals*, 72(3), 609-611. https://doi.org/10.1007/s12666-018-1509-3

Tian, Y., Chen, L., Gao, L., Michel Jr, F. C., Keener, H. M., Klingman, M., & Dick, W. A. (2012). Composting of waste paint sludge containing melamine resin and the compost's effect on vegetable growth and soil water quality. *Journal of hazardous materials*, 243, 28-36. https://doi.org/10.1016/j.jhazmat.2012.09.013

Uçaroğlu, S. and Gümrah, B.G. (2024). Management of water-based paint sludge originating from the automotive industry via composting. Journal of the Air & Waste Management Association, 74(4), pp.279-289.https://doi.org/10.1080/10962247.2024.2316821

Vishwakarma, A. and Hait, S. (2024). August. Bioleaching of lithium from spent lithium-ion batteries: unveiling the potential of siderophores produced by *Pseudomonas* aeruginosa and kinetic insights. *In 2024 Goldschmidt Conference*. GOLDSCHMIDT.

Xue, C., Qi, P., & Liu, Y. (2018). Adsorption of aquatic Cd^{2+} using a combination of bacteria and modified carbon fiber. *Adsorption Science & Technology*, 36(3-4), 857-871.https://doi.org/10.1177/0263617417724946

Zhou, S., Zhao, Q., Yu, T. and Yao, X. (2024). Co-pyrolysis of Sewage Sludge with Paint Sludge: Kinetics and Thermodynamic Analysis via Iso-conversional Methods. *Journal of Wuhan University of Technology-Mater. Sci.* Ed., 39(3), pp.716-727.doi:10.1007/s11595-024-2930-6

Table 1: Properties of Paint Sludge Compared to Sludge Disposal Standards.

parameters	Sludge disposal standard mg/kg	Heavy metal in primary paint sludge mg/kg
Cr	1200	290
Zn	2800	63860
Ni	420	160
Cd	39	20
Pb	300	230

Table 2: Parameters, Number of Experimental Runs, and Results of Semi-Continuous Bioleaching by *Indigenous P. aeruginosa*.

RUN	A: Oxygen cm3/sec	B: Glucose %	Removal Zn %
1	20	3	32
2	20	6	17
3	20	3	30
4	20	0	20

5	40	3	21.5
6	0	3	21
7	20	3	30
8	0	6	12
9	0	0	14
10	20	3	31
11	40	0	14.25
12	40	6	13.2
13	20	3	32

Table 3: Results of ANOVA Analyses of Bioleaching Paint Sludge by *Endogenous P. aeruginosa*.

Source	Sum of squares	Degree of freedom	Mean squares	F-value	p-value	
Model	721/05	5	144.21	52	< 0.0001	significant
A- Oxygen	0.6337	1	0.6337	0.2285	0.6472	
B-Glucose	6.10	1	6.10	2.20	0.1816	

AB	0.2256	1	0.2256	0.0813	0.7837	
A^2	156.32	1	156.32	56.36	0.0001	
B^2	291.49	1	291.49	105.10	<0.0001	
Residual	19.41	7	2.77			
Lack of fit	15.41	3	5.14	5.14	0.0739	Non-significant
Pure error	4	4	1			
SD	740.46	12				
R^2_{adj} 0.9738, R^2_{Pre}0.9557, $R^2$0.8468, Average 22.15, SD1.67, CV7.52٪						

Table 4: Removal of Heavy Metals in Residual Solids of Paint Sludge on Filters after Bioleaching Processes Using the 3050B Method.

parameter	Amount Heavy Metal(Primary) before Bioleaching Process	Amount Heavy Metal(secondary) after Bioleaching Process	Removal Heavy Metal%
Cr	290	85	72.41%

Zn	63860	40170	37/09%
Ni	160	<5	96.87%
Cd	20	<5	75%
Pb	230	8	96.52%

Figure 1: Semi-Continuous System a) Schematic Plot and b) Factual.

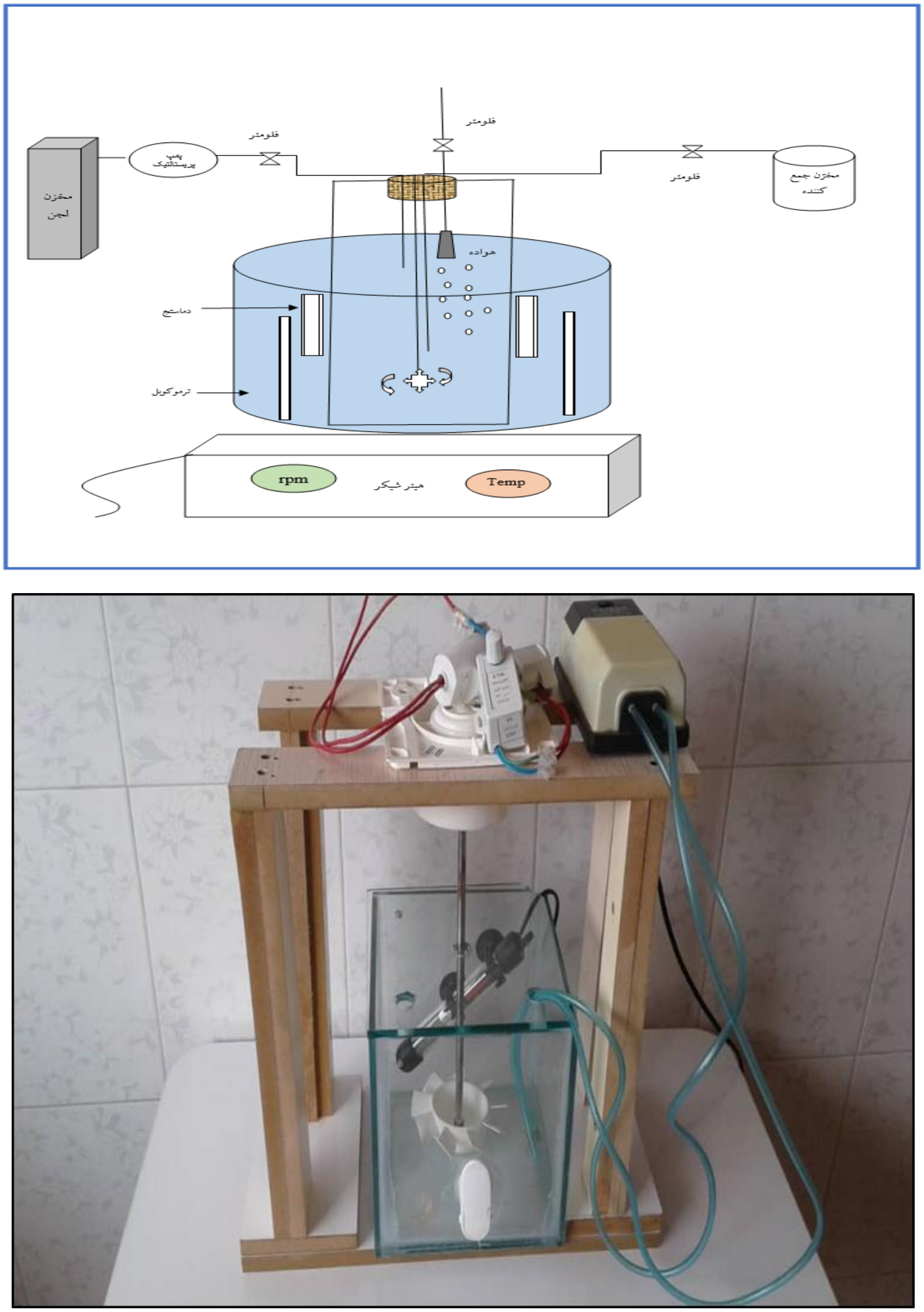
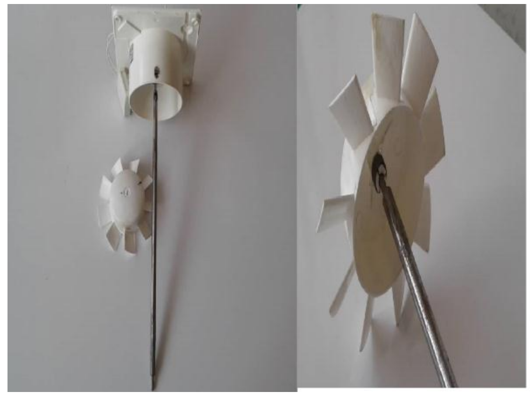
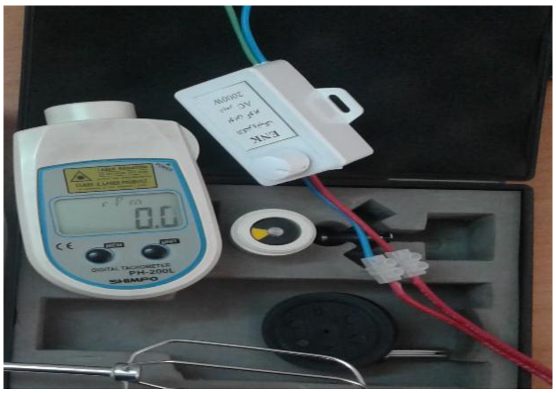


Figure 2: Some Components that Comprise the Semi-Continuous System.

(a)



(b)



(c)

(d)

(e)

(f)

Figure 3: Numerical Optimization Ramp with Adjusted Software on Maximum Response based on Recommendation DOE between Parameters.

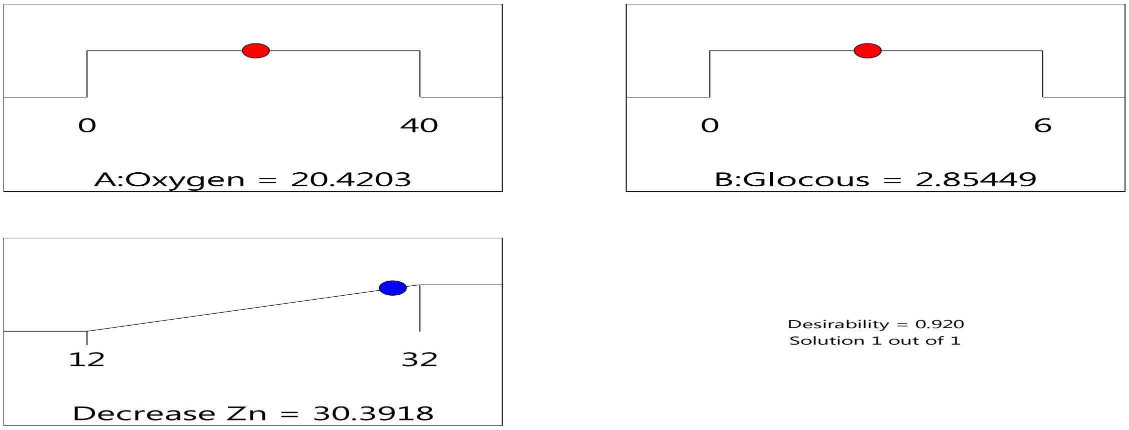


Figure 4: Morphological Changes of the Surfaces at 500 Magnification Scale (a) Before, (b) After the Bioleaching Process by *Endogenous P. aeruginosa*, after 7 days under the following conditions: Temperature 32°C, RMP 180, Particle Size 3, Pulp Density 7, and Optimized Response for Oxygen Dissolution 20.4 cm³ /sec, Crocuses 2. 8 g.

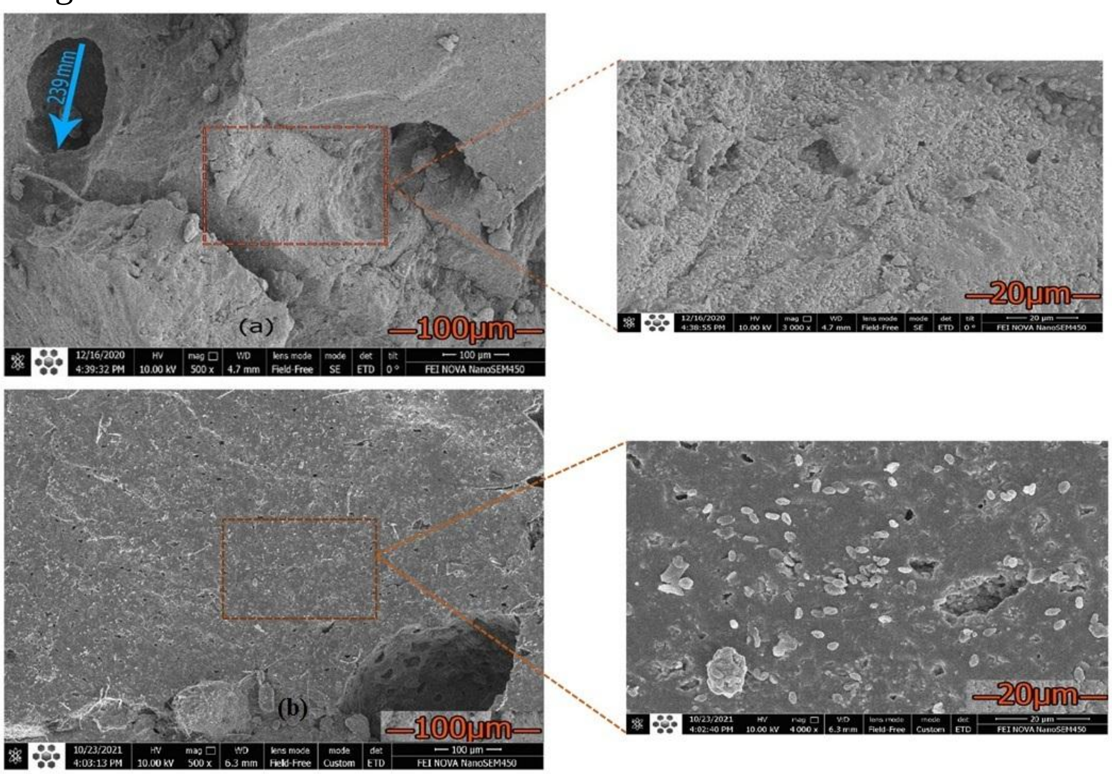
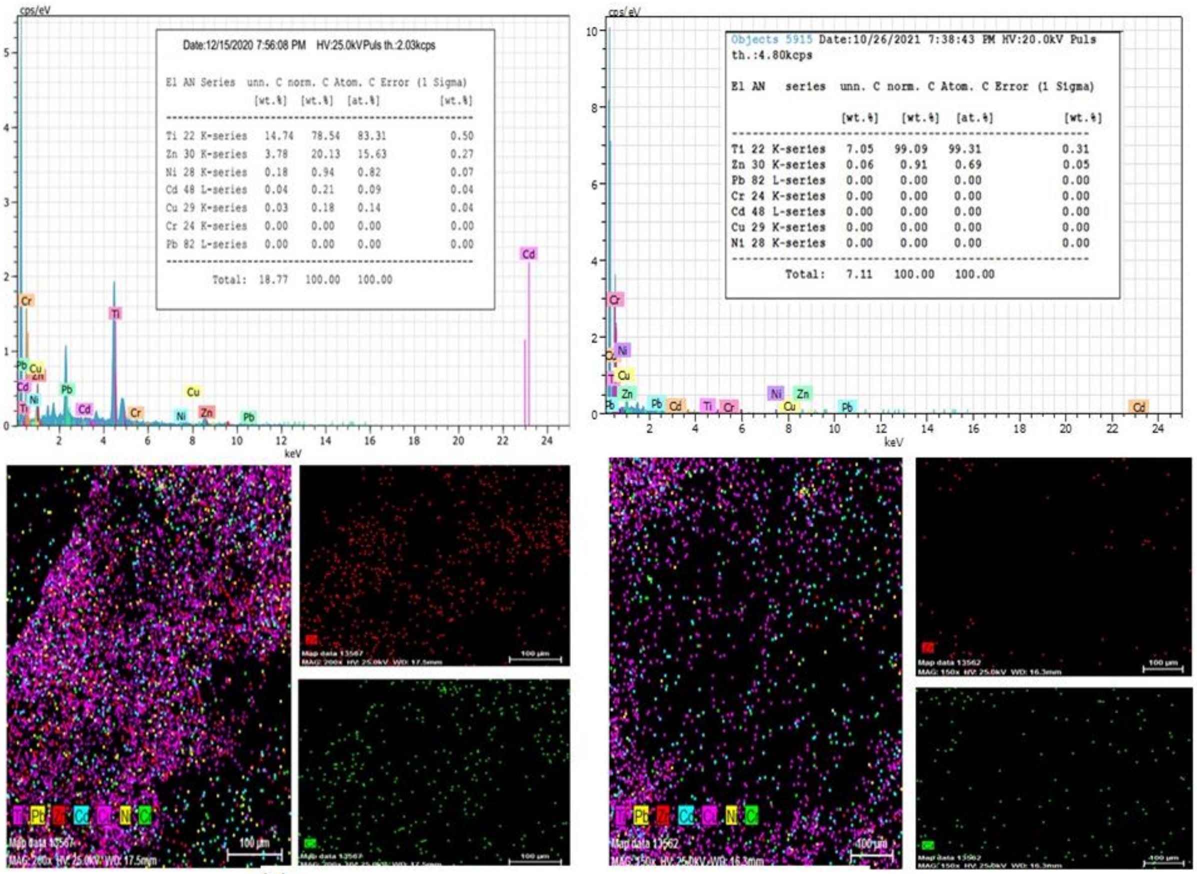


Figure 5: EDX Analysis and Mapping (a) Before the Paint Sludge Bioleaching Process, (b) After the Bioleaching Process with *Endogenous P. aeruginosa* from the Residual Sludge Sample on the Filter.


cps/eV
Date:12/15/2020 7:56:08 PM HV:25.0kVPuls th.:2.03kcps
El AN Series unn. C norm. C Atom. C Error (1 Sigma)
[wt.%] [wt.%] [at.%] [wt.%]
Ti 22 K-series 14.74 78.54 83.31 0.50
Zn 30 K-series 3.78 20.13 15.63 0.27
Ni 28 K-series 0.18 0.94 0.82 0.07
Cd 48 L-series 0.04 0.21 0.09 0.04
Cu 29 K-series 0.03 0.18 0.14 0.04
Cr 24 K-series 0.00 0.00 0.00 0.00
Pb 82 L-series 0.00 0.00 0.00 0.00
Total: 18.77 100.00 100.00
keV
cps/eV
Objects 5915 Date:10/26/2021 7:38:43 PM HV:20.0kV Puls th.:4.80kcps
El AN series unn. C norm. C Atom. C Error (1 Sigma)
[wt.%] [wt.%] [at.%] [wt.%]
Ti 22 K-series 7.05 99.09 99.31 0.31
Zn 30 K-series 0.06 0.91 0.69 0.05
Pb 82 L-series 0.00 0.00 0.00 0.00
Cr 24 K-series 0.00 0.00 0.00 0.00
Cd 48 L-series 0.00 0.00 0.00 0.00
Cu 29 K-series 0.00 0.00 0.00 0.00
Ni 28 K-series 0.00 0.00 0.00 0.00
Total: 7.11 100.00 100.00
keV

(a) (b)