

Treatment system integrated a reactor with sulfate-reducing activity and a constructed wetland for contaminant removal

Julia Mariana Márquez-Reyes*, Celestino García-Gómez, Alejandro Isabel Luna Maldonado, Humberto Rodríguez-Fuentes, Juan Antonio Vidales-Contreras

UANL, Universidad Autónoma de Nuevo León, Facultad de Agronomía. Av. Francisco Villa S/N, Colonia Ex Hacienda El Canadá, General Mariano Escobedo, Nuevo León, México, C.P. 66050, Tel. +52 (81) 13-40-43-99; emails: julia.marquezrys@uanl.edu.mx (J.M. Márquez-Reyes), celestino.garciagm@uanl.edu.mx (C. García-Gómez), alejandro.lunaml@uanl.edu.mx (A.I. Luna Maldonado), humberto.rodriguezfn@uanl.edu.mx (H. Rodríguez-Fuentes), juan.vidalescn@uanl.edu.mx (J.A. Vidales-Contreras)

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ABSTRACT

Industrial activities that discharge pollutants into water effluents cause environmental pollution problems. Reactors and wetlands have proven to be efficient in treating different polluted effluents. This research objective was to construct a hybrid treatment system consisting of an up flow packed reactor with sulfate-reducing activity and a horizontal subsurface flow constructed wetland planted with watercress (*Nasturtium officinale*) for the removal of chromium, lead, nitrates, sulfates, and chemical oxygen demand. The system operated at a recirculation rate of 3.5–3, with a new synthetic solution added every 10 d. Our results show significant variations in the concentration of the monitored compounds on the days following the addition of a new synthetic solution. However, microbial activity remained active; watercress generated shoots and accumulated the highest concentration of metals in roots. The rocky supports used intervened in buffering the abrupt changes in pH and interacted with the contaminants to promote their removal. The system removed 95.7% of chromium, 98.2% of lead, 96% of sulfates, 98% of nitrates, and 95% of chemical oxygen demand. The results suggest that the hybrid system established in this study is a viable alternative for removing various contaminants in water.

Keywords: Sulfate-reducing bacteria; Heavy metal removal; Watercress; Hybrid system

1. Introduction

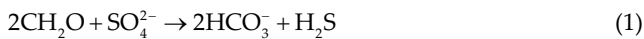
Different systems have been developed to treat water contaminated with heavy metals, sulfates, and organic matter, because these are common pollutants in the discharge water of different industrial sectors such as mining, metal processing, and smelting, among others. Furthermore, due to the complexity of the pollutant mixtures currently found in drains or landfills, it is necessary to develop and implement pollution control and prevention strategies. Therefore,

research on combined aerobic-anaerobic treatment systems has increased in recent years. Up flow anaerobic sludge blanket reactors (UASB) and constructed wetlands (CW) are promising combinations for implementation [1,2].

One adaptation of a UASB is to promote sulfate-reducing activity within the reactor using sulfate-reducing bacteria, which have proven to be efficient in removing heavy metals, metalloids, radioactive compounds, sulfates, explosives, and organic matter [3–7].

* Corresponding author.

In addition, it has been demonstrated that these systems are economically viable due to the vast options of using different carbon sources such as butanol, pyruvate, peat moss, compost, sugarcane vinasse can be used to maintain sulfate-reducing activity [3,8,9]. The reaction mechanism of sulfate-reducing bacteria consists of two stages; in the first stage, organic compounds are oxidized and used as electron acceptors to produce hydrogen sulfide and bicarbonate ions [Eq. (1)]. In the second-stage, the biologically produced hydrogen sulfide reacts with dissolved heavy metal to form insoluble metal sulfide precipitates [Eq. (2)] [6,10].



where CHO_2 represents organic matter, M represents heavy metal, MS represents metals sulfide.

Constructed wetlands (CW) adopt environmentally friendly technology, versatile, easy to maintain, have high pollutant removal efficiency (nitrogen, phosphorus, organic matter, total suspended solids, heavy metals, and emerging pollutants), and low maintenance and operating costs [11–13]. There are many classifications of CW, such as free water (FWS), horizontal subsurface flow (HSSF), vertical flow (VF), and hybrid CW. Hybrid CW effectively reduces different pollutants and can be connected to other types of CW, such as FWS, HSSF, and VF, depending on the characteristics of the influent water [11].

The use of hybrid systems (reactors – wetlands) has proven to be a viable alternative to removing the efficiency of 16 pharmaceuticals and personal care products, volatile fatty acids, volatile suspended solids, and heavy metals (Hg, Cr, Zn, Al, As, Cd, Co, Cu, Pb, and Ni), chemical oxygen demand, total nitrogen and total phosphorus [1,2,14,15]. However, the combination of microbial activity and plant species that can be used in a hybrid system is extensive, as they can be chosen based on the specific needs of a contaminated site. Therefore, the objective of the present study was to construct a hybrid treatment system (HTS) consisting of a packed up flow reactor with sulfate-reducing activity and a constructed horizontal subsurface flow wetland planted with watercress (*Nasturtium officinale*) to evaluate the removal of nitrates, sulfates, chemical oxygen demand, lead and chromium in synthetic water.

2. Material and methods

2.1. Sulfate-reducing consortium

The sulfate-reducing consortium used in this study was obtained from a site impacted by mining activity in Monterrey, Nuevo Leon, Mexico. Maintenance and biomass production was performed in 120 mL serological bottles, which contained saline medium at pH5 (NH_4Cl (1 g/L), KH_2PO_4 (0.5 g/L), Na_2SO_4 (4.5 g/L), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (0.04 g/L), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.06 g/L), $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (0.004 g/L) and were maintained at 25°C. Peat moss was used as an electron donor, having a chemical oxygen demand (COD)/ SO_4^{2-} ratio equal to 0.71 and a substrate affinity constant of 740 mg/L.

2.2. Reactor

The procedures were performed in a glass upflow-packed bioreactor with dimensions 60 cm high, 4.6 cm in diameter, and three sampling ports. The packing material was kept in 50–50 (W: W) ratios and gravel and volcanic rock, with an approximate diameter of 1–2 cm, were used. The reactor was inoculated with the isolated sulfate-reducing consortium at a concentration of 2×10^8 cells/mL and 12 g of peat moss. The reactor was conditioned for 60 d with a constant flow of saline medium and maintained a hydraulic retention time of 0.86 h.

2.3. Plant characteristic

Watercress is a perennial aquatic herb native to Europe and naturalized in America. It is a free-floating species due to adventitious roots that develop underwater and grow in moist soils and riverbanks. It is tolerant to a wide range of light and shade conditions, requires nutrients in high concentrations, and grows in a pH range between 4.3 to 8.3 and temperatures between 20°C to 28°C. Bioremediation studies have demonstrated its ability to accumulate metals (Cr, Cu, Cd, Co, Zn, and Ni), metalloids (As), and radioactive compounds [16,17]. It has been reported that the higher the metal concentration in the solution, the greater the accumulation in the plant [18]. Roots and stems accumulate metals when combined with other plant species or solid substrates [19,20].

For the present study, watercress seeds were purchased from a specialized store. The seeds were washed, disinfected, and sown in germination trays containing peat moss. The trays were kept inside a climatic chamber (Biotronette Mark III) under controlled conditions of illumination ($270 \mu\text{mol}/\text{m}^2 \cdot \text{s}^2$), photoperiod (16 h light: 8 h dark), and temperature (25°C) for 15 d. Then, the seedlings obtained were washed with tap water and planted in the wetland.

2.4. Constructed wetlands (CWs)

In this study, we worked with a horizontal subsurface flow CW. It was constructed in hard plastic with dimensions of 85 cm long, 47 cm wide, 23 cm high, and a slope of 1°. The efficient volume was 0.09188 m^3 . The support substrate used was gravel with a diameter of 2–4 cm and a porosity of 24%. The hydraulic retention time was 0.406 h^{-1} , with a flow rate of 1.65 L/h, and the hydraulic loading rate was maintained at 99.12 mm/d.

Table 1
Accumulation of heavy metals in watercress

| | Lead (mg/kg) | Chromium (mg/kg) |
|-------|---------------------------|--------------------------|
| Leaf | 33.4 ± 14 ^{ab} | 25.2 ± 7.1 ^a |
| Stem | 6.5 ± 2.5 ^a | 25.2 ± 17.1 ^a |
| Root | 368 ± 59 ^b | 289.1 ± 69 ^b |
| Shoot | 1,795.5 ± 42 ^c | 235.7 ± 8.7 ^b |

Values shown are means ± standard deviation. Letters represent mean comparisons between treatments for similar plant parts ($p < 0.05$).

The watercress seedlings were conditioned for 2 weeks by recirculating nutrient solution at pH5: KNO₃ (101.0 g/L); KH₂PO₄ (27.2 g/L); MgSO₄·7H₂O (0.06 g/L); Ca(NO₃)₂·4H₂O (2.95 g/L); NH₄Cl (1.0 g/L); Na₂SO₄ (4.5 g/L); CaCl₂ (0.06 g/L); FeSO₄·7H₂O (1.3 g/L); ZnSO₄·7H₂O (0.2 g/L); CuSO₄·5H₂O (0.08 g/L). The distance between the three rows was 10 cm, while the distance between each plant was 7 cm, having a density of 82 plants/m².

2.5. Hybrid treatment system (HTS)

The HTS was structured by placing the reactor in a series followed by the wetland. Between the junctions of the two systems, the influent had a drop of 8 cm to promote an increase in dissolved oxygen concentration within the CW (Fig. 1). The HTS was maintained for 15 d in conditioning with the synthetic working solution, which consisted of the nutrient solution (described in the previous section) plus 150 mg Pb/L and 15 mg Cr(VI)/L. The ratio of recirculation flow rate to influent was set at 3.5–3. A new synthetic solution was added every 10 d and continued for 50 d. Samples were collected from the effluent to determine pH, oxidation–reduction potential (ORP), SO₄²⁻, NO₃⁻, COD, and total concentration of Pb and Cr. At the end of the experiment, plants were removed from the CW to quantify the total concentration of Pb and Cr in roots, stems, leaves, and shoots. While the rocky substrates were recovered in the reactor, the adhered material was removed by washing with tap water. The weight of the bacterial biomass that developed on the surface was determined.

The Cr and Pb removal efficiency of HTS was calculated according to Eq. (3)

$$RE = \frac{C_{in} - C_{out}}{C_{in}} \times 100 \tag{3}$$

where RE is the Pb and Cr removal efficiency (%), while C_{in} and C_{out} represent the inlet and outlet metals concentrations, respectively (mg/L).

2.6. Analytical methods

The pH and the redox potential were determined with specific electrodes. Sulfate and nitrate concentration was measured by turbidimetric methods using a UV-Vis spectrophotometer [21]. The closed reflux technique determined COD, and the quantification of microbial biomass was determined by the volatile suspended solids method [21]. Plants were washed, weighed, fractionated (roots, stem, leaves, and shoots), and dried for subsequent analysis (40°C). From each fraction, 200 mg of plant material (n = 10) were taken for acid digestion with H₂O₂/HNO₃ (3:10) at 85°C for 10 min to quantify metals by atomic absorption spectroscopy (AAS). The effluent samples were filtered, acidified, and kept refrigerated for subsequent analysis by AAS.

2.7. Statistical analysis

The obtained concentrations of Pb and Cr in watercress were subjected to a one-way analysis of variance and a mean comparison test (Tukey) when significance was found between treatments (P < 0.05). Contaminant removal results were analyzed using the statistical package SPSS 17.0 (Inc., USA) for Windows, and graphs were produced in Origin 8.0.

3. Results and discussion

3.1. pH

The pH behavior was similar in both systems during the operation time. With each addition of a new synthetic working solution, the pH values decreased. The lowest quantified values were determined on days 18 (4.2) in the reactor

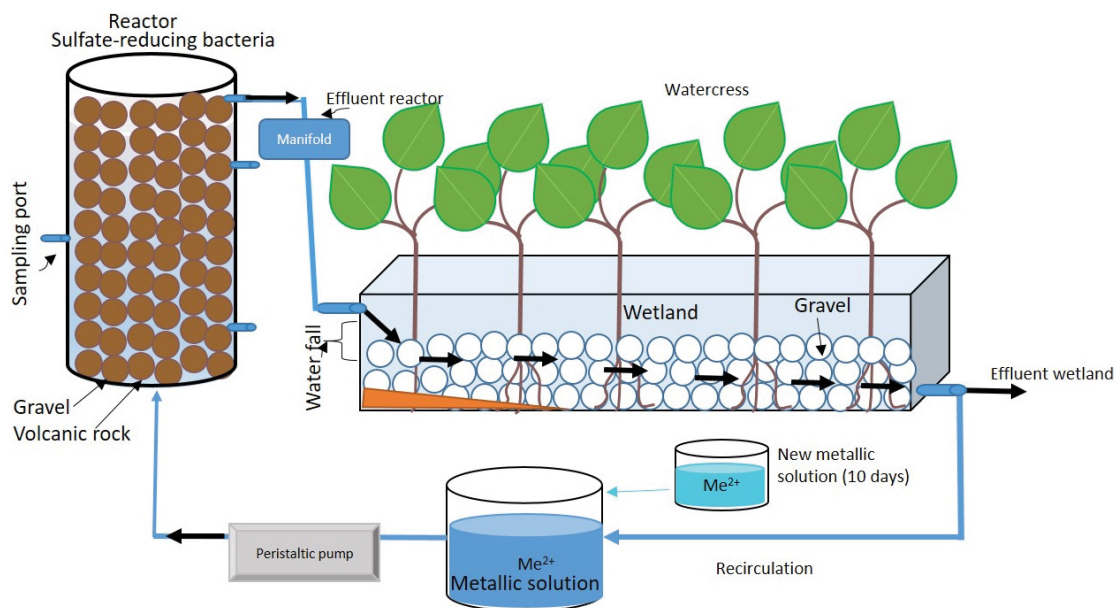


Fig. 1. Schematic diagram of the hybrid system reactor-wetland.

and days 21 and 39 (5.4) in the CW. On the remaining days, the values ranged from 4.6 to 6.7. Sulfate-reducing bacteria produce carbonate ions which react with acid compounds favoring their neutralization [22]. In addition, the calcareous rock material functioned as a buffering substrate for changes within the systems [23]. The pH fluctuations did not inhibit the sulfate-reducing activity, which can develop in acidic conditions and have a behavior that promotes the elimination of heavy metals [24]. The above shows that the established bacterial consortium reduced sulfate and produced hydrogen sulfide, which was used to precipitate chromium and lead inside the reactor (Fig. 2A). While, pH changes within the system also favored the formation of metal precipitates [25,26].

3.2. Oxidation–reduction potential

The ORP fluctuations were quantified each time a new solution was added. Within the reactor, the values ranged

from -90 to 180 mV. ORP values between -400 to -200 mV have been estimated to be optimal for reductive activity [27]. However, design parameters in reactors can favor microbial conditions to make their operation efficient, such as hydraulic retention time (HRT), reactor configuration, and carbon source used [28,29]. The ORP behavior indicates that the design and operation of the reactor were adequate since the sulfate-reducing activity was obtained; however, improvements can be made to increase microbial activity (Fig. 2A).

In the CW, ORP values ranged from 141 to 223 mV (Fig. 2B), which promoted the vegetative development of the watercress as new shoots were observed to form. The increase in ORP is mainly attributed to the CW design since it had a water drop between the reactor effluent and the CW influent. However, due to the anaerobic conditions of the reactor, the ORP values are not the expected optimum. Values below 250 mV are related to decreased nutrient availability and mobility to plants, as well as dissolved oxygen concentration, which can alter the nutrition of plant species [30]. Since the reactor had no design configuration to prevent leakage of microorganisms, entrainment of sulfate-reducing bacteria to the CW, which could have been established due to the pH and ORP values quantified, is not ruled out. In addition, anaerobic microbial communities have been demonstrated in horizontal wetlands, which are also actively involved in pollutant removal [31,32].

3.3. Sulfate

The sulfate concentrations at the reactor outlet increased with adding a new synthetic solution (Fig. 3A). This behavior indicates that there was no direct relationship with the production of biogenic sulfide, which is explained by the pH and ORP variations, which were not optimal to promote the increase of sulfate-reducing activity. However, bacterial activity was not inhibited either, since 75% removal percentages were reached in the first 10 d of operation and an overall average removal rate of 82.5% was maintained considering a volumetric load of sulfate of $4,000$ mg·SO₄²⁻/L added every 10 d.

In the wetland, sulfate removal remained at 15% for the first 13 d. However, it was observed that from day 14, the concentration increased (from $1,700$ to $3,000$ mg/L). This behavior could be due to chemical changes in sulfur speciation due to the pH and ORP fluctuations quantified, which again favored the presence of sulfates in the medium. At pH 5.5, destabilization of sulfide chemical phases within wetlands has been reported [32]. In the last days of wetland operation, the removal percentage increased (50%), suggesting that some sulfate-reducing bacteria were already established in the anoxic parts of the wetland, which favored the removal of sulfate. In its 50 d of operation, the HST removed 96% of the sulfate concentration from entering the system.

3.4. Nitrate

The nitrate concentration inside the reactor decreased steadily, with removal rates of 99% during the last experimental days (Fig. 2A). The decrease in nitrate concentration

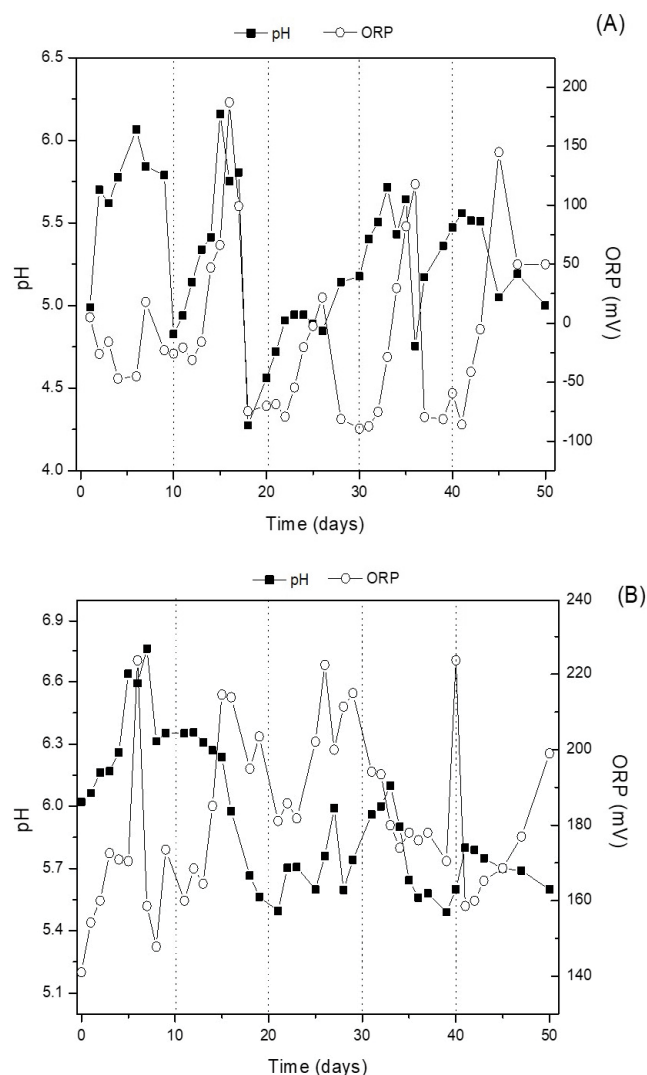


Fig. 2. Reactor (A) and wetland (B) effluent pH and ORP measurements.

could be due to its use as an electron acceptor or donor by the microbial communities inside the reactor [33]. While in the wetland, the nitrate concentration (Fig. 3B), oscillating between 2,500 to 650 mg/L, presented the maximum concentration at 25 d, behavior similar to that observed in the quantification of sulfates. This behavior can be attributed to the generation of nitrites due to the degradation of the cress roots by the ORP conditions presented, which generate scarce dissolved oxygen, promoting the decrease of nitrification processes and the elimination of total nitrogen [34,35]. In its 50 d of operation, the HST removed 98% of the nitrate concentration entering the system.

3.5. Watercress

The watercress reached an average length of 29 cm ($N = 30$), with a fresh weight of 6 g and a dry weight of 1.2 g. No changes in the pigmentation of leaves and stems were observed in the first 30 experimental days. Subsequently, small chlorotic spots on leaves and softening of stems were observed. The observations in leaves and stems are related to heavy metal toxicity [36]. However, shoots were present, indicating the establishment and growth of watercress within the CW. The shoots were processes like the rest of the watercress to be considered in quantifying metals.

Quantification of metals in the different plant fractions indicates that watercress can accumulate Pb (368 mg/kg DW) and Cr (289.1 mg/kg DW) in roots, leaves (33.4 mg-Pb/kg DW, 25.2 mg-Cr/kg DW) and stems (6.5 mg-Pb/kg DW, 25.2 mg-Cr/kg DW) (). The highest concentration of both metals was found in the roots, indicating that it is a mainly stabilizing and rhizofiltering plant [16,37,38]. However, the highest concentration of Pb (1,795.5 mg/kg DW) and the second highest concentration of Cr (235.7 mg-Cr/kg DW) were quantified in new shoots. Due to the high concentrations to which the plants were exposed, the presence of new shoots was not expected, indicating that the plant presents mechanisms that help it to counteract the effects of metal toxicity, which are suggested to be studied in future experiments.

3.6. Removal of Pb, Cr, and COD

The removal of heavy metals in the reactor presented the same behavior pattern as that observed for pH and ORP. Each time a new synthetic solution was fed to the system, the removal percentage decreased to 15% for Cr and 60% for Pb. However, as time elapsed, the pH and ORP conditions favored the stabilization of the system to improve its performance reaching removal percentages of 97% and 70% for Pb and Cr, respectively (Fig. 4A). A decrease in the Cr removal percentage was observed compared to that quantified for Pb with each new addition of metal solution, and this behavior could be due to the reactor's operating conditions (pH, ORP, microbial activity), which mainly favored the removal of lead. It is not excluded that the accumulated chromium concentration may have decreased the sulfate-reducing activity due to its toxicity. Similar studies have reported a decrease in sulfate-reducing activity due to the presence of chromium [39]. It should be noted that using peat moss as a

carbon source promotes the formation of stable complexes with metals due to its heterogenic conformation, consisting of numerous oxygen-containing functional groups and fractions (humic acids, fulvic acids, and humin) with different molecular weights. Under operating conditions, the reactor can remove 80% and 57% of lead and chromium, respectively.

The biotic and abiotic components that made up the CW removed 91% of Pb and 66.6% of Cr (Fig. 4B). The synergistic substrate-plant-microorganism relationship favored the removal of the studied metals, as reported in other studies with constructed wetlands [23,40]. Considering the concentrations of the compounds monitored during the 50 d of HTS operation and taking into account the cumulative concentration, removal percentages of 98.2% of Pb, 95.7% of Cr, and 95% of COD were estimated.

The concentration of COD during the 50 d of operation of the HST was constant in the effluents, quantifying removal percentages of 95%. This behavior indicates that the sulfate-reducing activity present in the reactor used the COD as an electron donor. While in the CW, the rocky

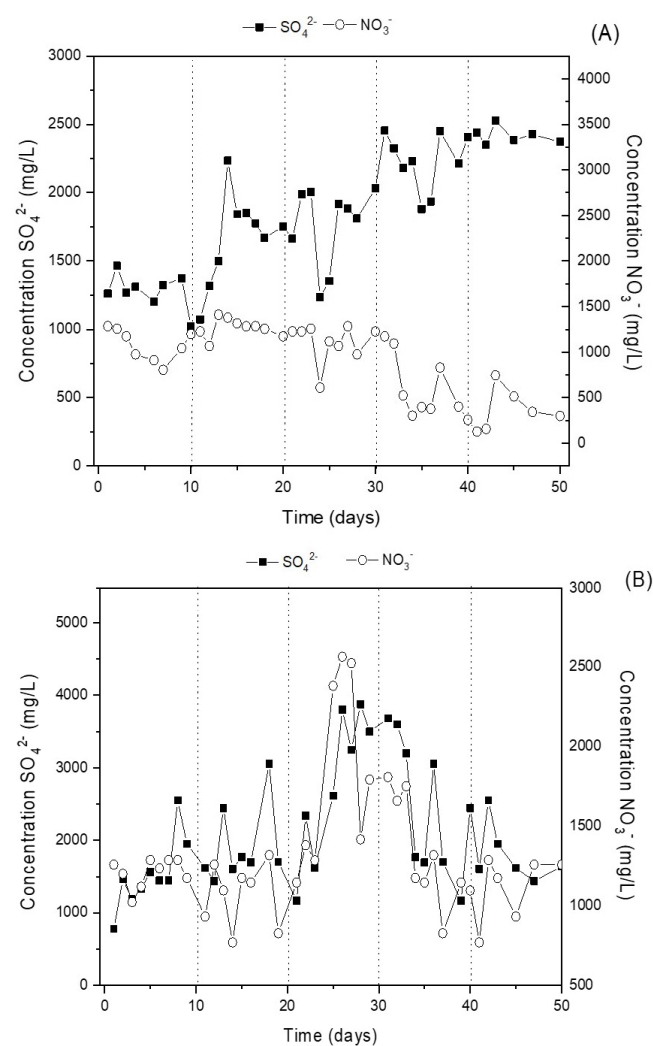


Fig. 3. Reactor (A) and wetland (B) effluent SO_4^{2-} and NO_3^- concentrations.

substrate contained the organic load. Therefore, the microorganisms and the watercress could have been used as a source of nutrients.

3.7. Substrates

Gravel and volcanic rock promoted the development of microbial activity within the HTS, with higher biofilm growth in volcanic rock with 5.5 mg SSV/g rock mainly due to its porosity compared to gravel (1.5 mg SSV/g rock). The support substrates must provide surface area and be inert [41]. However, several studies show that substrates participate in physical, chemical, and biological reactions during the immobilization of heavy metals through adsorption, sedimentation, or filtration mechanisms [42–46], which coincides with what was observed in the present study.

4. Conclusions

The system evaluated in the present study was an anaerobic reactor with sulfate-reducing activity and a constructed wetland planted with watercress, which removed 96% of sulfates, 98% of nitrates, 98.2% of Pb, 95.7% of Cr, and 95% of COD. The system's operating conditions were able to maintain the sulfate-reducing activity and promote the vegetative development of the watercress. As a result, watercress can accumulate Pb in root > shoot > leaf > stem and Cr in root > shoot > leaf = stem, classifying it as a rhizofiltering plant since it accumulates metals mainly in the root.

Hybrid treatment systems are efficient, tolerant to changes in the water composition to be treated, and promote microbial activity, which favors the removal of contaminants. Therefore, the system evaluated is a viable, economical, and environmentally friendly alternative to eliminate contaminants in water.

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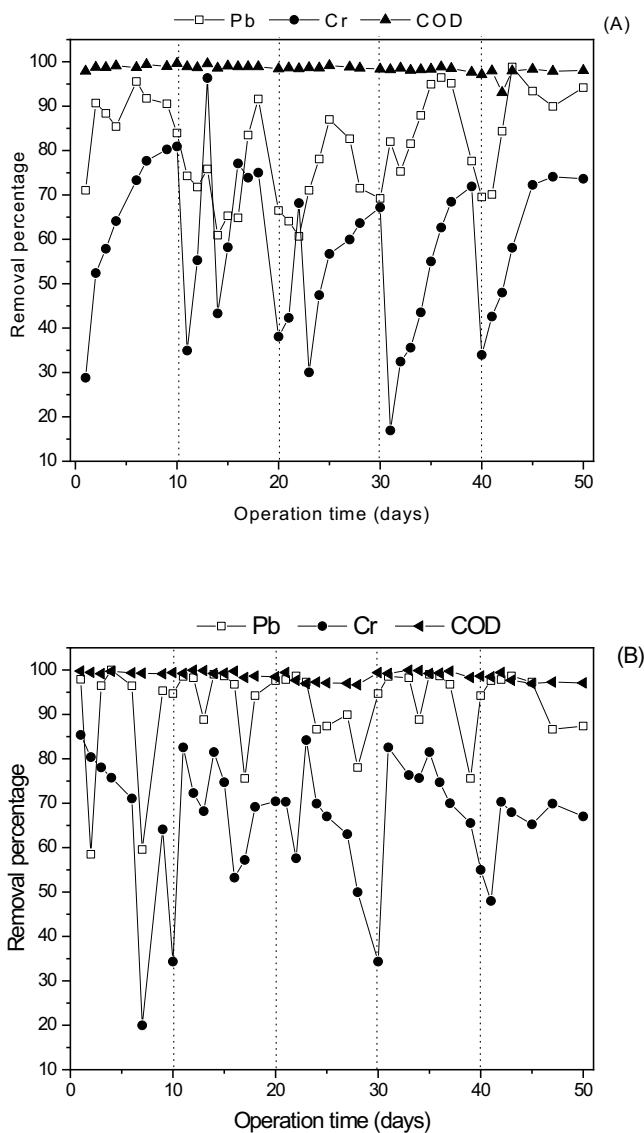


Fig. 4. Removal percentages of Cr, Pb and COD in the effluent of the reactor (A) and wetland (B).

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