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Treatment of Low Biodegradability Leachates in a Serial System of Aged Refuse-Filled Bioreactors

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Abstract: This paper presents a technology based on the use of aged refuse that has proven to be highly effective in the treatment of low biodegradability leachates. The tests were developed using two filled bioreactors arranged in series and operated at steady state. The aged refuse used as filling material was extracted from a city located in the southeast of Mexico and characterized by particle size, humidity, volatile solids, and volumetric weight. On the other hand, bacterial characterization made it possible to identify the presence of species related to the degradation and mineralization of organic compounds, as well as to processes of nitrification or reduction of phosphates and Cr (VI). The bioreactor system was operated under four hydraulic loads (10, 20, 35, and 50 L/m^3·d). Maximum removal efficiencies of 85, 86.1, 87.9, 98.6, 97.8, and 97.4% were achieved in COD, BOD$_5$, Color, TP, TN, and N-NH$_3$, respectively, complying with Mexican regulations (NOM-001-SEMARNAT-1996). The system also proved to be stable against shock loads, such as organic load fluctuations in the influent or pH variations. The results of this study show that, in countries such as Mexico, aged refuse extracted from landfills represents a promising option as a sustainable alternative for leachate treatment.

Keywords: biodegradability; bioreactors; leachates; aged refuse

1. Introduction

The growth of commercial and industrial activity, as well as the general tendency of the society towards consumerism, has caused an undeniable increase in the rate of generation of municipal solid waste (MSW). Comparative studies on the various means of elimination of MSW have shown that the most economical way, in terms of operating costs and capital, are landfills [1]. In these sites, harmful liquids are generated as a result of the percolation of rainwater through solid waste, leaching a large amount of polluting materials, and producing very complex residual water, known as sanitary landfill leachates [2]. The contaminants present in the leachates are of both organic and inorganic nature; some of them are refractory and toxic, such as heavy metals. In general, the leachates can be classified as: young (less than 5 years of being generated), intermediate (between 5 and 10 years of being generated), and stabilized or mature (more than 10 years of being generated) [3].

The treatment of leachates has been studied practically with all physical, chemical, and biological processes applied in the field of water treatment, highlighting processes such as adsorption, membrane systems, coagulation-flocculation, chemical oxidation, and biological systems [4]. Physicochemical processes have been applied as the first stage of purification of low biodegradability leachates, usually accompanied by biological processes to improve the efficacy of the treatment [5–7]. On the other hand, biological processes achieve good efficiencies when applied to young leachates, but are less effective in the treatment of mature leachates [8].

In Mexico, leachates have been treated mainly by evaporation and recirculation; however, this method is not advisable because during evaporation water is emitted to the atmosphere as well as volatile organic compounds [9]. In the case of the state of Chiapas, in southeastern Mexico, due to high annual rainfalls, the generation of leachates is abundant, which causes the capacity of evaporation lagoons to be exceeded and, as a consequence, the method of evaporation and recirculation is even more questionable.

A novel approach to leachate treatment consists of the use of MSW extracted from landfills after more than 8 years of being disposed on the site. This material, called stabilized material or aged refuse (AR), is used as packaging in bioreactors, generating a friendly, sustainable, and low-cost operating alternative. Using this innovative technology, several research groups have reported significant removal efficiencies in COD, BOD_5, and TN in ranges of 85.8–96.2, 95.8–99.8, and 60–75%, respectively [10–12]. The high efficiencies in the removal of contaminants shown by this system are attributed mainly to the great microbial activity that exists in the AR [13]. The existence of large and diverse populations of microorganisms that have acclimated to high concentrations of contaminants over the years has resulted in a great capacity to degrade refractory organic matter [10,12].

In addition to allowing the development of a novel and efficient treatment alternative for leachates, the extraction of AR can allow the revaluation of waste and the extension of the useful life of landfills, a very important aspect considering that the search for spaces for the construction of new sanitary landfills is not an easy task and is generally accompanied by serious social problems. Thus, when material deposited for more than 8 years at the final disposal sites is used, space is released for fresh solid wastes, favoring a more sustainable management of MSW.

The AR used as packing material in bioreactors for the treatment of leachates has been reported mainly for intermediate and young leachates [11,14–16], and there are few studies reported with mature leachates [17,18] and none of them with leachates of very low biodegradability. Thus, the objective of this work was to investigate the potential of AR from the landfill of the city of Tuxtla Gutierrez, Mexico, packed in a system of two bioreactors in series, for the treatment of low biodegradability leachate (BI = 0.07) from sanitary landfills. This research provides one of the first results on the revaluation of AR in Latin America, providing information of its potential as an alternative to the treatment of sanitary landfill leachates.

This paper is divided in four sections, Introduction, Materials and Methods, Results and Discussion, and the Conclusions. The background and the state of the art of the use of aged refuse are presented in the Introduction, while the methodology followed to obtain the data that enabled the achievement of the objectives of this project are presented in Materials and Methods. In the Results and Discussion section, the data obtained are shown, as well as their analysis and discussion. In the last section, Conclusions, we highlight the most important achievements of this project.

2. Materials and Methods

2.1. Extraction and Characterization of AR

The AR, aged 8–10 years, was obtained from the landfill in the city of Tuxtla Gutiérrez, located in the southeast of Mexico. The geographic location of the site (Latitude 16°40′7.57″ N and Longitude 93°11′47.36″ W) exposes it to a rainfall and annual temperature of 954.5 mm and 25.8 °C, respectively. To prevent contamination from the cover material, the AR sample was taken at a depth of 1.5 m. A fraction of the extracted material was characterized on a wet basis (WB) within 24 h after the excavation: volumetric weight and the composition (rigid plastics, bags, fine materials, and others) were determined. The rest of the extracted material was spread on tarpaulins and large-volume material (cloth, glass, cardboard, stones, iron, plastics, among others) was removed and discarded. The AR remained extended in the shade and at room temperature for 3 weeks, in order to remove enough moisture to facilitate handling and characterization. During this drying period, the AR was determined through pH, moisture content, and volatile solids. Once dry, the material was subjected to a granulometric analysis, separating it into different particle sizes: >40 mm, between 40 and 15 mm, and <15 mm. In the same way, the volumetric weight and its composition were determined.

2.2. Aged Refuse Filled Bioreactors (ARFBs)

The treatability tests were conducted in a system consisting of two ARFBs arranged in series (Figure 1) and of identical dimensions (Ø = 20 cm, h = 230 cm). The 20 cm lower part of each bioreactor was packed with support material (crushed gravel, Ø = 2.5 cm) and 200 cm of packing material (AR of Ø ≤ 40 mm) was placed immediately on top. A 10 cm headspace was left in the upper part of each ARFB. In order to avoid the loss of fine AR, a "75% shade mesh" membrane was arranged between the support material and the packaging material.

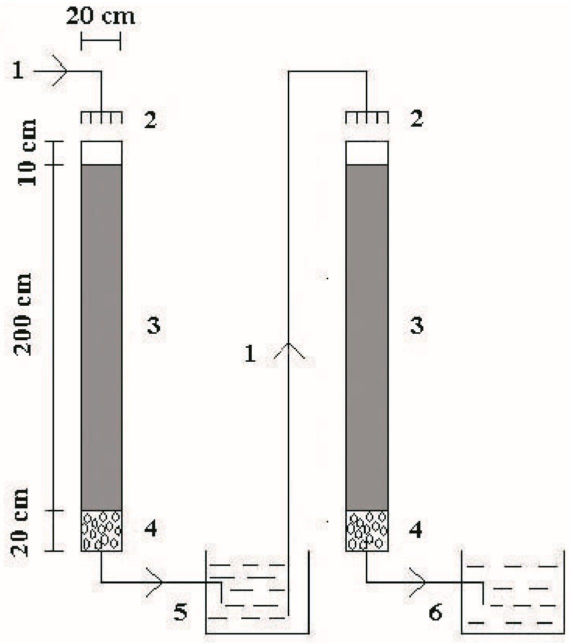

Figure 1. Aged refuse filled bioreactor (ARFB) system used in the treatability tests. (1) Influent; (2) feeding system (sprayer); (3) packing material (AR, Ø ≤ 40 cm); (4) support material (crushed gravel, Ø ≤ 2.5 cm); (5) effluent 1 (E1); (6) effluent 2 (E2).

2.3. Sampling and Leachate Characterization

The leachate sample was collected from the young leachate evaporation pond of the sanitary landfill and kept refrigerated at 4 °C until use. The sample was characterized with the parameters of chemical oxygen demand (COD), biochemical oxygen demand (BOD), color, alkalinity, pH, total suspended solids (TSS), total nitrogen (TN), ammoniacal nitrogen (N-NH_3), total phosphorus (TP), and heavy metals (Cd, Ni, Cr, Pb, and Zn). The COD was quantified using the closed reflux micromethod, digesting the sample at 150° C for 2 h, and subsequently read on a HACH DR-5000 spectrophotometer at 620 nm. The BOD was determined by quantifying the difference between the initial dissolved oxygen concentration and the concentration after five days of incubation at 20 ± 1 °C. Color determination was performed with a HACH DR/890 colorimeter, while alkalinity was determined through the volumetric method, titrating with 0.02 N sulfuric acid. All the analyses were conducted according to standardized methods [19] adapted to the particularities of leachates. Heavy metals were determined using atomic absorption spectrophotometry. In the case of Cd, Cr, Ni, and Pb, a graphite furnace (EPA, Method 7010a) was used, while Zn determination was carried out with flame atomic absorption spectrophotometry (EPA, Method 7000B).

2.4. AR Microbiological Characterization

Microbiological characterization of the aerobic fraction was conducted at the species level. For this purpose, samples were taken from the interior of the bioreactor at three different heights (0.5, 1.0, and 1.5 m). The extracted samples were seeded in nutritious agar; later, microscopic observation was carried out after applying Gram staining. DNA polymerase extraction was performed using the cell lysis-phenol-chloroform method, followed by polymerase chain reaction (PCR). The purification and sequencing was performed by MACROGEN Co, located in Seoul, Korea, on 16S rRNA with comparison to the GenBank database.

2.5. Operation and Monitoring of the ARFB System

The system was operated for eight months in the shade and at room temperature (25.8 ± 5.9). During this period, four hydraulic loads (HLs) (10, 20, 35, and 50 L/m^3·d) were evaluated, each with an operating time of two months. The bioreactors were spray-fed three times a day (9:00, 13:00 and 17:00 h), for 15 min each time, during which the volume corresponding to the HL evaluated was supplied. The parameters monitored during the period of operation of each HL were COD, color, BOD_5, TSS, TN, N-NH_3, and TP, estimating the removal percentage of each parameter through Equation (1). Additionally, pH, alkalinity, and five heavy metal concentrations (Cd, Ni, Cr, Pb, and Zn) were determined. All the analyses were carried out following standardized methods [19].

$$\% \; Removal = \frac{\left(C_{i-}C_f\right)}{C_i} * 100 \tag{1}$$

where C_i is the initial concentration and C_f is the final concentration

2.6. Analysis of Experimental Data

The results obtained were statistically analyzed by one-way analysis of variance (ANOVA), using a significance level of α = 0.05, with the Statistica 7.0 software. Before performing the ANOVA, it was verified that the data fulfilled the statistical assumptions.

3. Results and Discussion

3.1. Excavated Material Characterization

Table 1 shows the main byproducts found in the excavated materials, as well as the particle size distribution of the usable fraction (fine fraction). From Table 1, it is observed that in both WB and dry basis (DB), at least 10% are rigid plastics, higher than the 4.19% reported by Zhao et al. [20]. Similarly, nylon bags represent an important percentage (11.1%) of DB waste. Despite their high presence, both byproducts were easily separated.

Table 1. Composition and distribution of particle size in excavated materials.

Aged Refuse	Plastics and Other Materials in the Excavated Refuse (%)					Particle Distribution of the Fine Fraction (%)			
	Rigid Plastics	Plastic Bags	Others	Fine Fraction	Total	Diameter >40 mm	Diameter 15–40 mm	Diameter <15 mm	Total
WB	10.9	13	14	62.1	100	—	—	—	—
DB	10.2	11.1	21.3	57.4	100	15.05	24.14	60.82	100

WB: wet basis, DB: dry basis.

According to Zhao et al. [21], within the materials with 10 years of being disposed in a sanitary landfill, the usable fraction is one that has a particle size of up to 40 mm. In Table 1, it can be seen that, in our case, approximately 85% had a particle size ≤ 40 mm.

AR characterization during the drying process allowed us to observe that the average vs. value remained at 19.2% because the materials had reached their highest degree of degradation. On the other hand, the pH presented an average value of 7.54, similar to that reported by Ziyang et al. [22]. These values above neutrality can be explained due to the type of leachate (mature) that percolates through the AR.

3.2. Characterization of Leachate in the Influent

The results of the characterization of the leachates used to feed the ARFB system are presented in Table 2, from which an BI of 0.07, pH values higher than 7 (8.58), and heavy metal concentrations are observed, which are mostly no more than 2 mg/L, characteristic of old leachates [1,23,24]. Considering that the samples were taken from a lagoon destined to accommodate fresh leachates, these results reveal that liquids of different ages are mixed in this lagoon. Thus, the influent used in the ARFB system had, in general, a low biodegradability and can be classified as a mature leachate.

Table 2. Concentration ranges in leachates used.

Parameter	Average	Concentration Range
pH	8.58 ± 0.60	6.3–9.6
Alkalinity (mg $CaCO_3$/L)	2809 ± 1054	1400–5060
COD (mg/L)	4868 ± 509	3874–6486
BOD_5 (mg/L)	347 ± 108	179–477
BOD_5/COD	0.07 ± 0.02	0.04–0.10
Color (Pt-Co)	10,638 ± 1593	7150–13,950
TSS (mg/L)	0.037 ± 0.017	0.024–0.062
TN (mg/L)	725.7 ± 378	377–1104
NH_3-N (mg/L)	636.7 ± 391.8	297–1073
TP (mg/L)	6.9 ± 1.9	4.9–11.2
Cadmium (mg/L)	0.14 ± 0.07	0.1–0.24
Chrome (mg/L)	0.55 ± 0.02	0.53–0.58
Nickel (mg/L)	0.40 ± 0.02	0.38–0.42
Lead (mg/L)	ND	ND
Zinc (mg/L)	ND	ND

ND: Not detectable.

3.3. Bacterial Characterization in the Packing Material

According to the bacterial analysis performed on the packaging material of the bioreactors, the identified bacterial genera were 34.5% Bacillus, 13.8% Pseudomonas, 10.3% Acinetobacter, 6.9% Lysinibacillus, and 3.4% Desulfovibrio and Enterobacter in total samples. *Bacillus sp.* and *Bacillus cereus* were the most abundant species, followed by *Acinetobacter* sp., *Bacillus thuringiensis*, *Pseudomonas* sp., *aeruginosa*, and *mosselii*, *Lysinibacillus* sp. and *sphaericus*, *Desulfovibrio vulgaris* and *Enterobacter* sp. Some of them specialized in the degradation and mineralization of organic compounds. Table 3 shows the main species related to the removal of macronutrients and other reduction processes, for example, *Pseudomonas putida* and *Pseudomonas aeruginosa* participate in nitrification processes, whereas *Lysinibacillus sphaericus*, *Pseudomonas putida*, and *Desulfovibrio vulgaris* are attributed the greatest activity in phosphorus removal. On the other hand, according to the literature [25–27], other identified species, such as *Pseudomonas aeruginosa*, *Lysinibacillus fusiformis*, *Desulfovibrio vulgaris*, and *Pseudomonas putida*, are capable of reducing Cr (VI) to Cr (III). These results reveal the bacterial varieties coexisting in the AR that can promote diverse processes of contaminant removal, such as metal and phosphate nitrification, denitrification, and reduction.

Table 3. Microorganisms identified in the filled material of the ARFB system.

Microorganisms at the Species Level	Function	Reference
Acinetobacter sp.	Possible nitrifier	[28]
Enterobacter sp.	Possible denitrifying and phosphate reduction	[29]
Pseudomonas putida	Nitrifier, denitrifier and Cr (VI) and phosphate reduction.	[27,30]
Bacillus thuringiensis	Biological adsorption of Cr	[31,32]
Desulfovibrio vulgaris	Cr (VI) and phosphate reduction	[26]
Lysinibacillus sphaericus	Phosphate reduction	[33]
Lysinibacillus fusiformis	Reduction of Cr (VI) to Cr (III)	[27]
Pseudomonas aeruginosa	Nitrifier and Cr (VI) reduction	[25]

3.4. Operation and Monitoring of the ARFB System

3.4.1. pH Behavior

The ARFB system was tested with four HLs: 10, 20, 35, and 50 $L/m^3 \cdot d$. Figure 2 shows that the pH values in the influent were around 8.6, highlighting the records close to 9.5 obtained during the feeding period at 20 $L/m^3 \cdot d$; for effluents 1 (E1) and 2 (E2) the pH value decreased to 8.1 on average. These slight reductions are related to biological degradation processes, such as the nitrification processes of organic pollutants that contribute to acidifying the environment. Another reason for the decrease in pH may be related to the hydrolysis of the humus present in the leachates and the AR [34].

3.4.2. Removal of Organic Pollutants (Color and COD)

Figure 3, shows that the color values in the influent were between 7150 and 13,950, and the final effluent between 655 and 2175 Pt-Co units. In general, the color of the influent was black and that of the effluent of the system was light yellow without odor. Regarding the removals achieved by the first bioreactor, similar behaviors are observed in the HL of 10, 35 and 50 $L/m^3 \cdot d$. According to the analysis of variance ($p < 0.05$), with the HL of 20 $L/m^3 \cdot d$, a significant difference is observed with respect to the other three HLs. This can be attributed to the high pH values of the influent (Figure 2), that moved away from the favorable interval (pH 6–8), hindering the biological growth before a greater presence of the ammonia at pH above 8 [35–37]. Considering the global removal, the ARFB system showed a removal between 84 and 88% for the different HLs tested, and according to the ANOVA ($p < 0.05$) there were no significant differences between them, despite the variations in organic load and pH in the influent, which shows that a second bioreactor has the ability to compensate for shock effects that may occur in the first bioreactor. Color removals greater than 80% were also reported by Li et al. [11] and Hassan and Xie [37], who worked with ARFB systems.

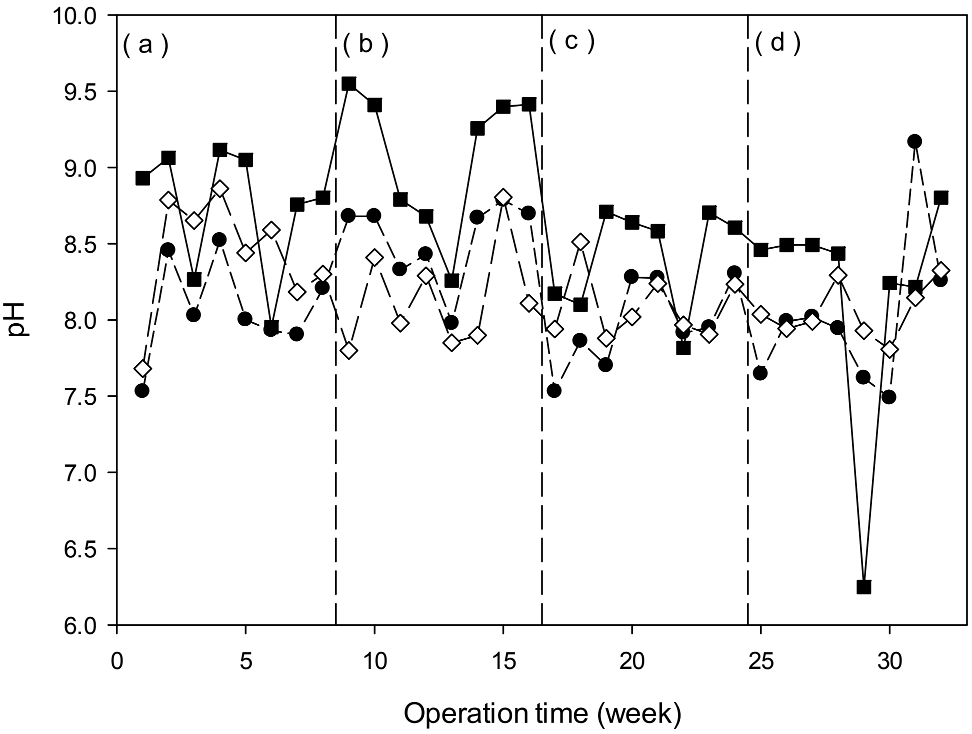

Figure 2. Profile of the pH behavior in the currents of the process; (■) influent, (●) effluent 1, and (◊) effluent 2, under different HLs: (**a**) 10, (**b**) 20, (**c**) 35, and (**d**) 50 L/m^3·d.

Regarding the COD parameter, from Figure 4, the concentration in the influent ranged between 3874 and 6486 mg/L, and in the effluent (E2) between 575 and 1277 mg/L. The average removals achieved in E1, E2, and the total removal considering the higher HL (50 L/m^3·d), were 79.7, 26.1, and 85%, respectively, which indicates that the greatest removal was achieved by the first bioreactor. However, as happened with the color parameter, low efficiencies in the first bioreactor were obtained in the HL of 20 L/m^3·d, which can be attributed to the fact that the leachate of the influent was fed with pH values > 9. The works of Li et al. [10] and Li et al. [11] have documented similar behaviors in the removal of COD when using ARFB systems.

3.4.3. Removal of Biodegradable Matter (BOD) and Alkalinity

Figure 5 shows the behavior profile of the biodegradable fraction, measured as BOD_5, removed by the ARFB system. This parameter, like alkalinity, was monitored on a monthly basis. Figure 5 shows that there is an inverse relationship between the percentage of removal of BOD_5 with respect to the HL fed, where the percentage of removal of the BOD_5 ranged between 85.6 and 98.9%. It is also observed that the behavior profile of alkalinity, under the four HLs studied, is similar to the behavior of BOD_5, which can be explained by the production of intermediate H^+ generated during the degradation processes of organic matter, which reacts with the species responsible for alkalinity. The relationship observed between the BOD_5 and the alkalinity in the effluents of the ARFB systems makes it possible to quickly estimate the BOD_5 value based on the determined alkalinity value.

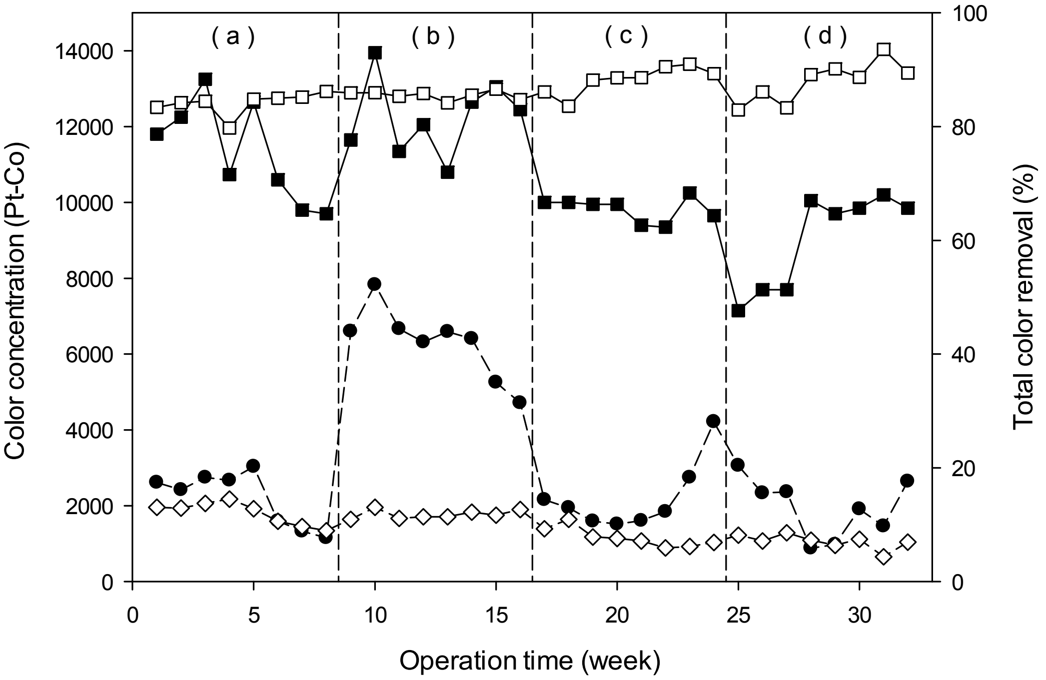

Figure 3. Profile of the color behavior in the currents of the process; (■) influent, (●) effluent 1, (◊) effluent 2, and (□) total color removal, under different HLs: (**a**) 10, (**b**) 20, (**c**) 35, and (**d**) 50 L/m^3·d.

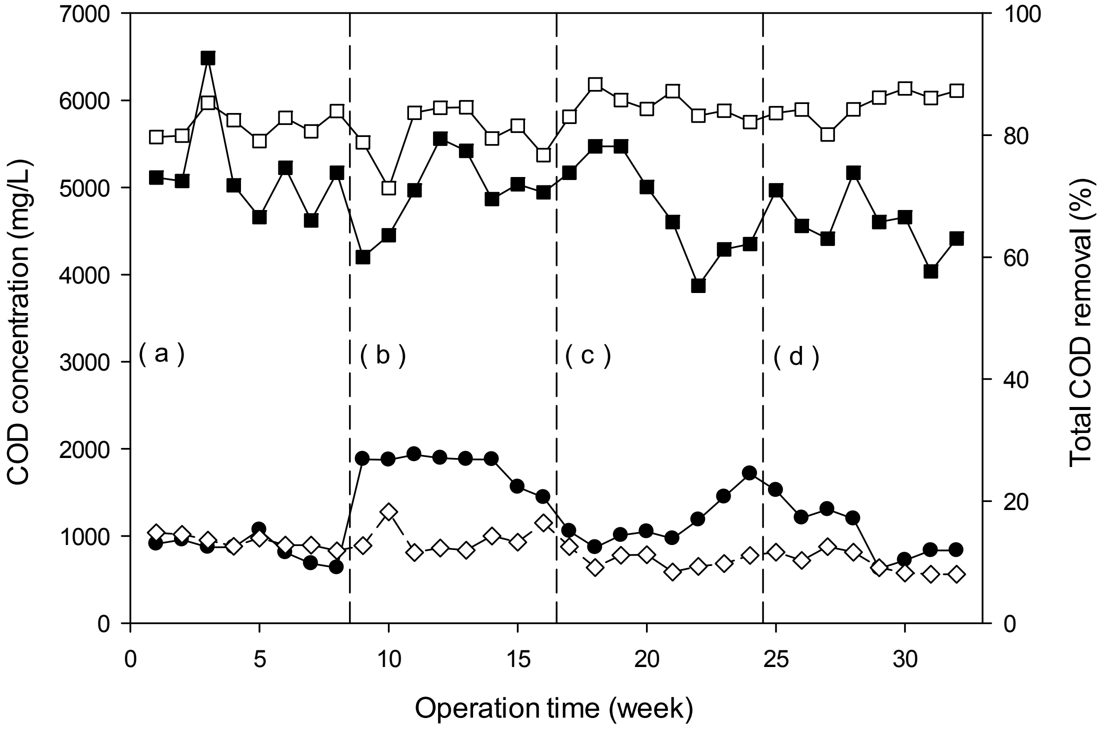

Figure 4. Profile of the chemical oxygen demand (COD) behavior in the currents of the process; (■) influent, (●) effluent 1, (◊) effluent 2, and (□) total COD removal, under different HLs: (**a**) 10, (**b**) 20, (**c**) 35 , and (**d**) 50 L/m^3·d.

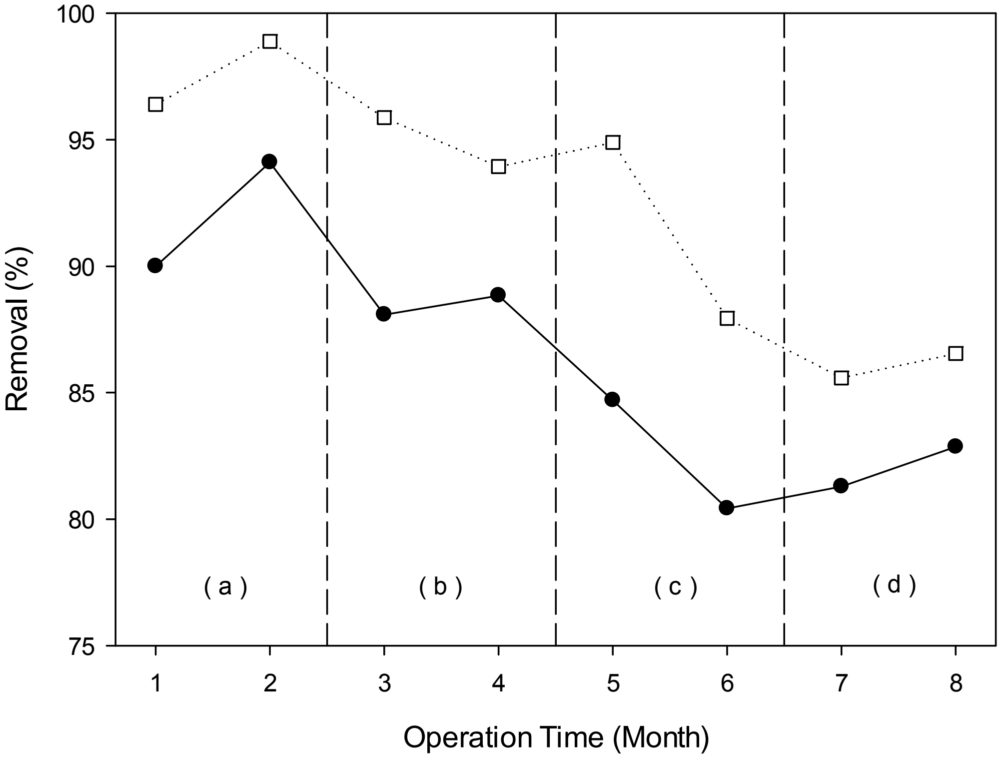

Figure 5. Global biochemical oxygen demand (BOD_5) removal and profile of the alkalinity behavior in the currents of the process: (**a**) 10, (**b**) 20, (**c**) 35, and (**d**) 50 L/m^3·d. Where: (●) Total alkalinity and (□) BOD_5.

3.4.4. Behavior of the Biodegradability Index (BI)

An important relation in the monitoring of biological systems is the biodegradability index (BI). Figure 6 shows that the average initial BI (BI_0) of the ARFB system evaluated was 0.07, which suggests that the leachate to be treated is low in biodegradable compounds and high in refractory organic compounds, such as humic substances [14]. According to Table 4, most of the studies in ARFB systems report BI_0 between 0.2 and 0.7 (leachates from intermediate to young) and with final BI between 0.03 and 0.05. In the present study, the average BI_0 was lower (0.07), even than the one reported by Xie et al. [17]. Despite this, removal efficiencies in biodegradable organic matter were high (>85%), with an average final BI of 0.03. These results show an additional advantage of the ARFB systems, which can also be efficient in leachates with very low biodegradability, which is not the case with other biological systems.

Table 4. Initial and final biodegradability index reported in ARB.

Leachate	BI_o	BI_f	Applied System	Reference
Young	0.5	0.03	ARB in series	[15]
Young Young	0.5 0.7	0.03 0.05	ARB (aerobic) ARB (anaerobic)	[14]
Intermediate	0.2	0.03	ARB in series	[11]
Mature	0.1	0.04	ARB-Fenton	[17]

BI_o: Initial biodegradability index, BI_f: Final biodegradability index after the treatment.

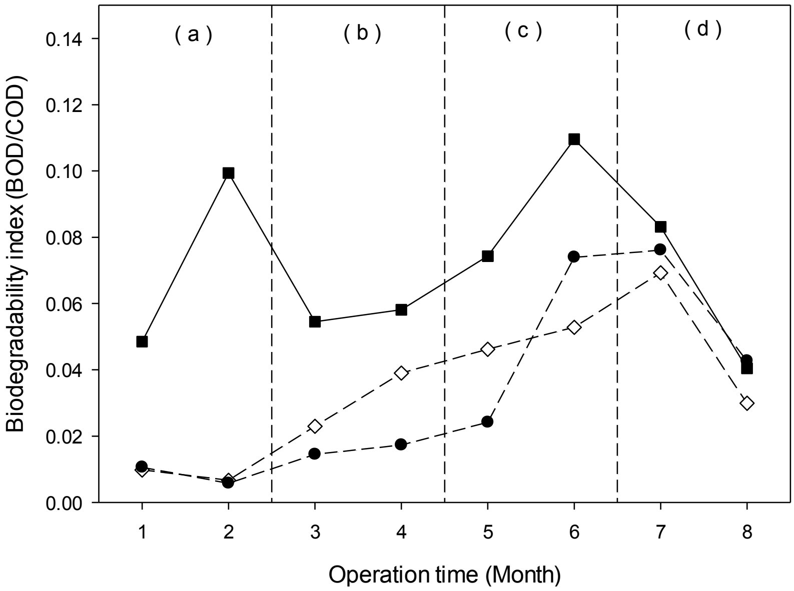

Figure 6. Profile of the biodegradability index (BI) behavior in the currents of the process; (■) influent, (●) effluent 1, and (◊) effluent 2 under different HLs: (**a**) 10, (**b**) 20, (**c**) 35, and (**d**) 50 L/m^3·d.

3.4.5. Total Phosphorus Removal

Figure 7 shows the behavior profile of the total phosphorus removal (TP) efficiency in the raw leachates reached by the system under study. As shown in this figure, the ARFB system achieved a total average removal of 99%, independent of the fluctuations of the TP concentration present in the influent (4.9–11.2 mg/L TP). It can also be seen that, despite HL feed increase, TP removal rates greater than 98% are reached in the first reactor alone. These rates are higher than the 96% reported in other studies [12,17]. The high TP removal rate can be explained by the presence of microbial consortiums, such as *Pseudomonas putida*, *Desulfovibrio vulgaris*, and *Lysinibacillus sphaericus*, found in the AR used in the present study.

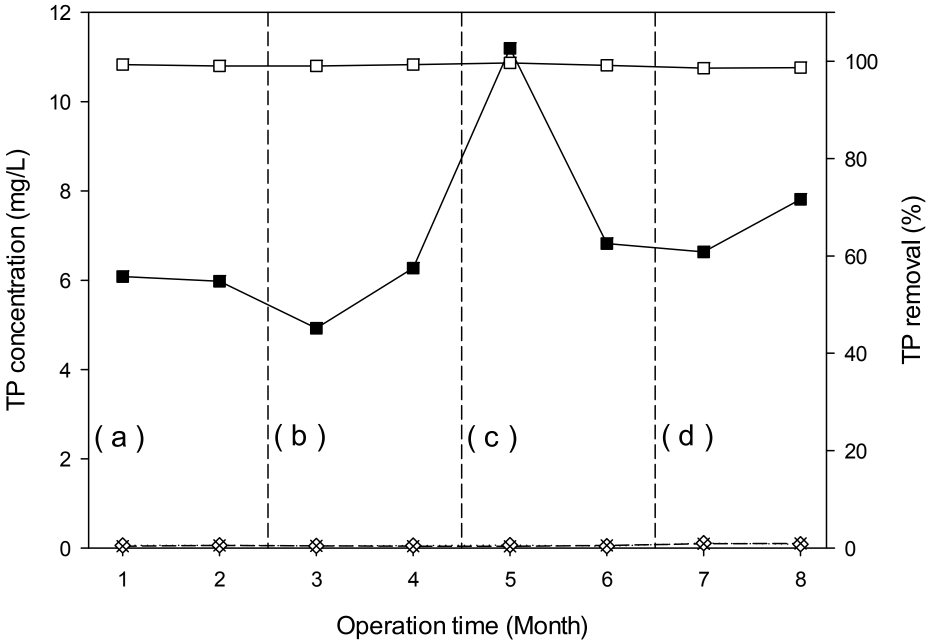

Figure 7. Profile of the total phosphorus (TP) behavior in the currents of the process; (■) influent, (◊) effluent 1, (×) effluent 2, and (□) total removal of TP under different HLs: (**a**) 10, (**b**) 20, (**c**) 35, and (**d**) 50 L/m^3·d.

3.4.6. Removal of TN and N-NH_3.

Ammonia concentrations in the influent ranged between 297 and 1073 mg/L (Figure 8), while, in the case of effluents E1 and E2, it ranged between 9 and 31 mg/L and 8 and 13 mg/L, respectively, corresponding to an average global removal rate of 97%. Again, the greater E1 effluent, the greater contaminant removal rate (95%) is achieved in the E1 effluent. According to Li et al. [11], ARFB systems in series show strong nitrification capacity, where ammonium is first adsorbed before being removed by nitrifying microbial populations. This synergy allows the restoration of the adsorption capacity of the AR [38], which explains the stability of the ARFB system in series in the removal processes for long periods of operation.

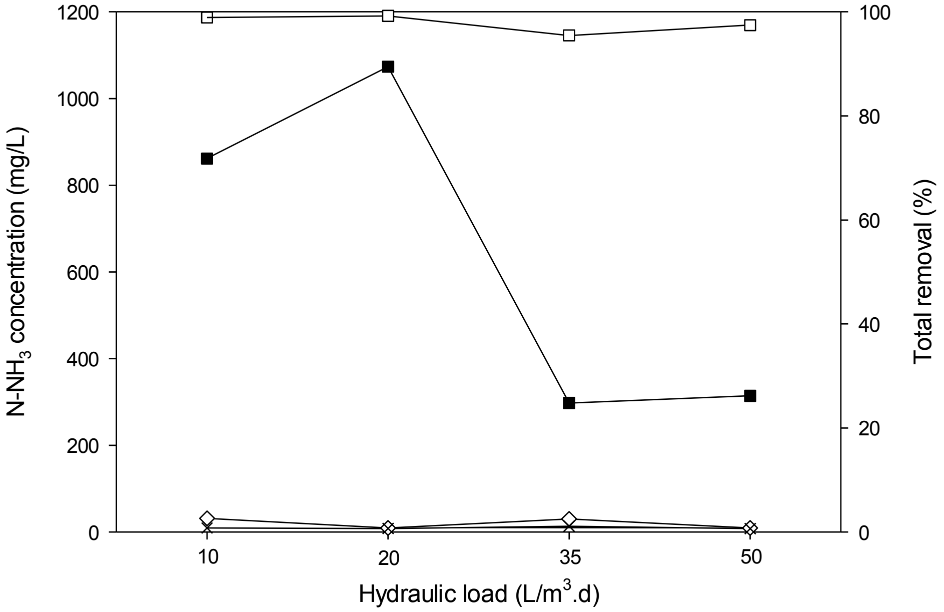

Figure 8. Concentration of ammoniacal nitrogen in the currents of the process and its removal in the different HLs; (■) influent, (◊) effluent 1, (×) effluent 2, and (□) total removal.

Figure 9 shows the elimination of total nitrogen (TN) in the raw leachates, where an average total removal of 97% was observed, independently of the fluctuations of the influent (377–1104 mg/L). According to Chen et al. [39], TN removal rate is favored when a source of high organic carbon is available. Regarding the present study, the high efficiencies achieved in TN removal rates can be attributed to the good COD/TN ratio, approximately 6.7, compared to other studies where this relationship was ≤2 and with lower TN removal rates (49–63%) [11,12]. Moreover, the presence of microbial consortiums, such as *Pseudomonas putida* and *Pseudomonas aeruginosa*, found in the AR of the present study can also explain the high removals achieved, as they relate to nitrification and denitrification processes [25,27,30].

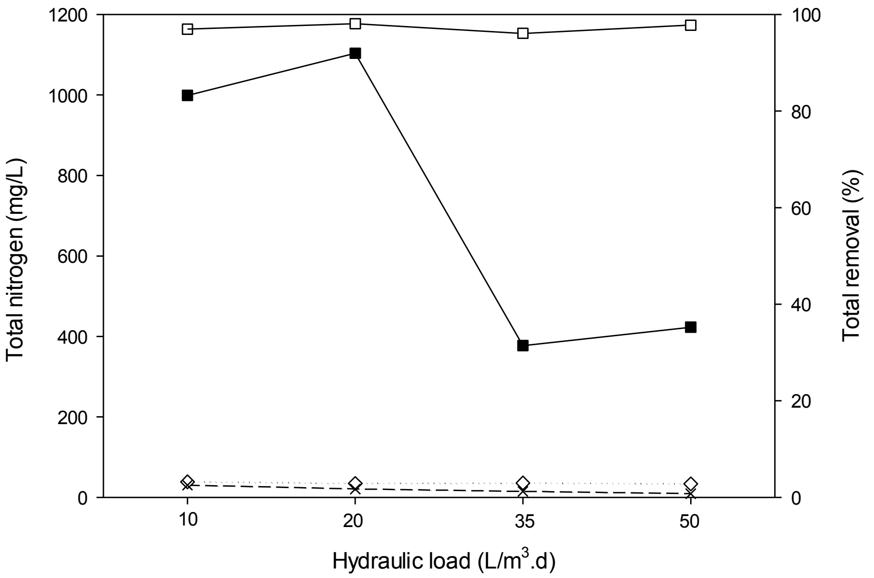

Figure 9. Concentration of total nitrogen (TN) in the currents of the process and its removal in the different HLs; (■) influent, (◊) effluent 1, (×) effluent 2 and (□) total removal.

3.4.7. Physicochemical Quality of the Final Effluent

The physicochemical parameters contemplated in the Mexican regulations (NOM-001-SEMARNAT-1996) [40], such as BOD_5, TSS, TP, and TN concentration, were all foundto be below the maximum permissible limits in the final effluent (Table 5).

Table 5. Physicochemical quality of the final effluent.

Parameter	Units	Final Effluent Concentration	Mexican Regulations
BOD_5	mg/L	39	150
TSS	mg/L	<1	150
TP	mg/L	0.1	20
TN	mg/L	9.4	40

3.4.8. Behavior of the ARFB System in Series with Higher HL

In order to test a higher HL, an additional experiment was performed with an HL of 100 L/m^3·d and compared with the initial HLs (10, 20, 35, and 50 L/m^3·d), monitoring the color and COD parameters. The results shown in Figure 10 show that for the initial HL, the removal efficiencies in both parameters were stable and higher than 80%, with no significant differences between them according to ANOVA. However, for the HL of 100 L/m^3·d, the efficiency of the system decreased significantly, by up to 14 and 38% in COD and color removal, respectively.

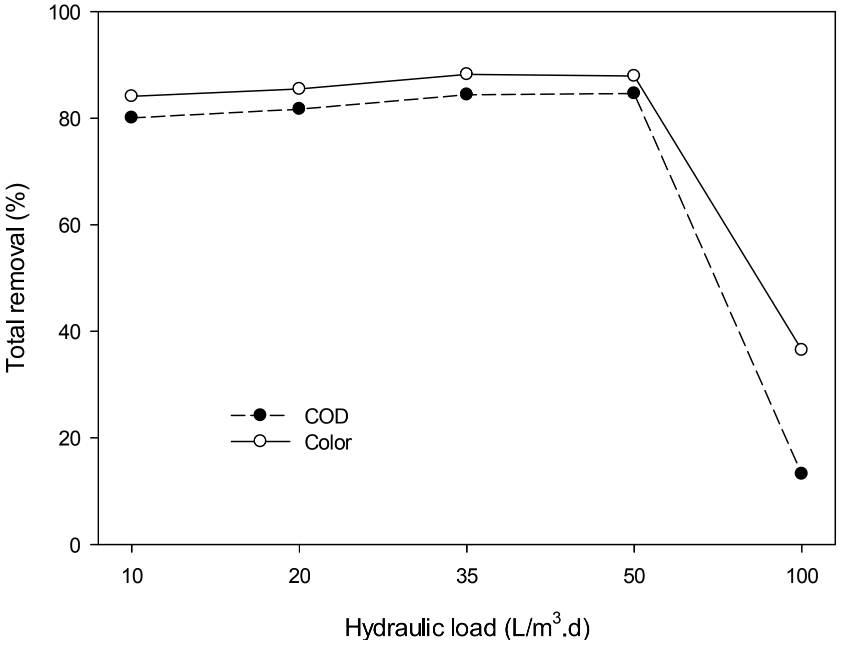

Figure 10. COD and color removal profile at different HLs.

3.4.9. Heavy Metal Removal

Elements such as Cd, Ni, Cr, Pb, and Zn were determined in the study and the values found are shown in Table 6. The concentration in the influent was low, typical of a mature leachate (<2 mg/L), and the values found in E1 and E2 varied very little with respect to the initial concentration, and elements such as Cd and Zn were below the maximum permissible limits according to Mexican regulations (NOM-001-SEMARNAT-1996) [40], except for Cr in the E2 stream and HL of 20 L/m^3·d, (0.79 mg/L) which was slightly above the value of 0.5 mg/L set by the standard. According to Wang et al. [41], in ARFB systems for the treatment of wastewater, the Cr can be removed by processes of adsorption and reducing bacterial action from Cr (VI) to Cr (III); this observed slight increase could be due to a lower adsorption capacity of the system.

Table 6. Concentrations of heavy metals in the currents of the process and their removal in the different HLs.

HL	Sample	Cadmium (mg/L)	Chromium (mg/L)	Nickel (mg/L)	Lead (mg/L)	Zinc (mg/L)
10 L/m^3·d	*Inf	<LOQ	0.55	0.38	<LOQ	<LOQ
	*E1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	*E2	<LOQ	0.54	0.38	<LOQ	<LOQ
20 L/m^3·d	Inf	0.11	0.53	0.41	<LOQ	<LOQ
	E1	<LOQ	0.13	0.40	<LOQ	<LOQ
	E2	<LOQ	0.79	0.38	<LOQ	<LOQ
35 L/m^3·d	Inf	<LOQ	0.55	0.42	<LOQ	<LOQ
	E1	<LOQ	0.27	0.43	<LOQ	<LOQ
	E2	<LOQ	0.16	0.41	<LOQ	<LOQ
50 L/m^3·d	Inf	0.24	0.58	0.42	<LOQ	<LOQ
	E1	<LOQ	<LOQ	0.40	<LOQ	<LOQ
	E2	0.21	<LOQ	0.44	<LOQ	<LOQ
Limit of Quantification (LOQ)		0.10	0.12	0.31	0.5	5
Maximum permissible limit mg/L (NOM-001-SEMARNAT-1996)		0.1	0.5	2	0.2	10

*Inf: Influent, E1: Effluent from bioreactor 1, E2: Effluent from bioreactor 2.

On the other hand, although the oxidation states of Cr were not quantified, reduction processes in the system could take place, considering that species such as *Bacillus thuringiensis*, *Desulfovibrio culgaris*, *Lysinibacillus fusiformis*, *Pseudomonas aeruginosa*, and *Streptomyces* sp. were identified inside the reactor, which are able to reduce Cr [25–27,42].

4. Conclusions

The analysis of the obtained results demonstrates the ARFB system as a promising alternative for the treatment of leachates of very low biodegradability (BI = 0.07). This paper represents one of the first successful studies of a biological process used in the treatment of leachates that are difficult to degrade.

According to the ANOVA test performed on the obtained results, no significant differences were observed between the treatments. For example, for the highest HL tested (50 $L/m^3 \cdot d$), the ARFB system showed average COD, BOD_5, and color removals of 85, 86.1, and 87.9%, respectively.

Other parameters, such as TP, TN, and $N\text{-}NH_3$, were also evaluated, reaching average removals of 98.6, 97.8, and 97.4%, respectively. The high efficiencies in TP were related to the presence of microbial consortiums such as *Pseudomonas putida*, *Desulfovibrio vulgaris*, and *Lysinibacillus sphaericus* within the bioreactors; while for TN, species identified as *Pseudomonas putida* and *Pseudomonas aeruginosa*, in addition to the high COD/TN ratio (6.7), favored their removal.

The physicochemical quality (BOD_5, TSS, TN, and TP) of the final effluent complies with the Mexican regulations (NOM-001-SEMARNAT-1996). This is also the case for the heavy metals evaluated (Cd, Ni, Cr, Pb, and Zn), but not for Cr in one of the HLs tested.

On the other hand, the ARFB system in series proved to be stable against possible shock loads, such as the fluctuations in organic load in the influent (COD from 3874 to 6485 mg/L and color from 7150 to 13,950 Pt-Co units), or variations in pH (6.25 to 9.55).

Thus, these results encourage further testing with compounds that are refractory to conventional biological processes, and, in general, with low biodegradability wastewater.

Finally, considering the little attention that has been given in Latin America to the treatment of leachates from sanitary landfills, the use of AR for the treatment of these liquids provides a window of opportunity for the revaluation of MSW disposed in landfills or any other place of final disposal whose age exceeds at least 8 years.

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