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Biological treatment and microbial composition of landfill leachate using a compost process in an airlift bioreactor

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Abstract

Landfill leachate (LL) contains a large amount of toxic compounds and its treatment is currently a matter of concern. In this work, an adapted compost in different airlift bioreactors was used to depurate a complex, toxic and old landfill leachate without treatment (raw leachate) and after pretreatments (coagulation/flocculation; coagulation/flocculation and filtration; coagulation/flocculation, filtration and photo-Fenton process). After the complete pretreatments, a high removal of organic matter was achieved, with a global removal of chemical oxygen demand (COD) and humic acids of 72.4% and 83.4%, respectively; besides, copper and iron in the landfill leachate presented a total removal of 91.1% and 65.8%, respectively. On the other hand, after the contact of raw leachate with compost in the airlift, there was a decrease of COD, copper and iron, while for humic acids there was a slight increase. The same trend was observed when the leachate was submitted to the different pretreatments and subsequently subjected to the biologic process with compost. The respirometric analyses showed that the raw leachate was toxic, although this tendency decreased along the treatments, with inhibition values of 41.8%, 27.5%, 22.0% and 12.1% for raw and pretreated leachate (by coagulation/flocculation, filtration and photo-Fenton processes), respectively. Also, in order to improve our knowledge about the composition of the microbial assemblages in compost samples and their possible role in pollutants removal from LL, we analysed samples from both an adapted and a non-adapted compost before and after being in contact with the raw leachate by applying Illumina sequencing of the 16S rRNA gene. *Bacteroidota, Proteobacteria, Firmicutes, Spirochaetota* and *Deinococcota* were the most abundant phyla in all samples, being present in the 50 most abundant Amplified Sequence Variants (ASVs) of the study, which represented 56.5% of the total analysed sequences and were considered as our *core* community. *Chryseolinea*, *Herbinix*, *Proteiniphilum*, *Pseudomonas* and *Sphaerochaeta* seemed to be the most resilient genera when the compost was in contact to LL. Moreover, our results showed that the metabolisms related to chemoheterotrophy, fermentation and the nitrogen cycle were the most relevant in all samples. In general, the microbial community was able to adapt to adverse conditions and remove pollutants, as heavy metals.

Keywords: Landfill leachate treatment; organic matter; heavy metals; compost, microbial community.

1. Introduction

Currently, with the rapid growth of population, urbanization, industrialization and development of technology, the annual production of municipal solid waste is increasing year after year. The main practice for disposing these wastes is landfill (Miao et al., 2019), but one of the problems of this method is the production of leachates, that is, the percolation of water in the waste layers and the *in situ* occurrence of biological and chemical reactions. The landfill leachate (LL) contains a large amount of refractory organic products, heavy metals, high ammonia and several toxic contaminants (Hassan et al., 2016), as xenobiotic compounds like aromatic hydrocarbons, pesticides, phenols, aliphatic chlorinated and plasticizers. As a consequence, an inadequate disposal or treatment of LL may cause long-term potential harm to the environment (Zhang et al., 2019).

At present, LL is mainly treated by biological and physicochemical processes. Physicochemical processes can remove most of the contaminants, although they are expensive and susceptible to produce secondary pollution (Miao et al., 2019). In contrast, biological methods are the common technique for treating LL due to their simplicity and cost-effectiveness (Bu et al., 2016), and they are applicable when the ratio of biochemical oxygen demand (BOD) to chemical oxygen demand (COD) in LL is higher than 0.4 (De et al., 2019). However, the high concentrations of pollutants present in an old LL may reduce the activity of microorganisms and the efficacy of biological treatment (Liu et al., 2015) due to the presence of nitrogen (Klein et al.,

2017) and organic refractories with high molecular weight and complex structures (Di Iaconi et al., 2006). In this context, it is important to promote an adaptation of the biological system to obtain the depuration of the wastewater that presents problems caused by the low biodegradability and acute toxicity of those pollutants (Bai et al., 2020).

Alternatively, the composting process is a technology for the transformation of organic waste into stable and high-quality agricultural by-products via biochemical reactions, reducing their environmental risk (Li et al., 2012). Besides, the application of composting allows the valorisation of waste. The composting process is driven by the microbial metabolism, and the duration of the composting period depends on factors such as temperature, pH, compost mass bulk density, porosity and aeration, among others (Ren et al., 2018). Furthermore, composting constitutes a bioaugmentation method where thermophilic bacteria participate in the biodegradation of polluting materials (Yanto and Tachibana, 2013), resulting in a feasible bioremediation alternative. Compost has been studied for soil remediation and has been considered a suitable material for *in situ* heavy metal removal (Zhou et al., 2017) and elimination of emerging pollutants (Kuppusamy et al., 2017). The effectiveness of compost use in the treatment of pollutants is either dependent on the adsorption by organic matter or on the degradation by microorganisms and present enzymes (Kuppusamy et al., 2017; Zhou et al., 2017). In general, the compost quality has an impact on the bioavailability and biodegradation of organic pollutants (Cerda et al., 2018; Luo et al., 2018). Unlike conventional biological treatments, this kind of processes are easy and reliable methods for wastewater treatment using very small spaces (Gholami et al., 2020).

Nowadays, several works have reported the use of compost for LL treatment. In the research of Spiniello et al. (2023), the coupling of a hybrid constructed wetland to a solar photo-Fenton process was studied, where the layers of the wetlands included sand, solid compost and carriers, and the removals obtained ranged from 75 to 95% for all the parameters evaluated after LL recirculation. In addition, published the use of compost in the denitrification of LL, observing that it was unable to sustain the denitrification process; a full denitrification was only achieved after the use of augmented substrates. Besides, Liu et al. (2022) evaluated a coagulation and a photo-Fenton process in the treatment of a mature LL using aluminium ions as coagulant agent and, when the conditions were optimal, they obtained a removal efficiency of colour and total organic carbon of 97.6% and 88.1%, respectively.

On the other hand, single bioreactors are often used in wastewater treatment because they offer some benefits such as enhanced organic removal capacity, smaller reactor volume, lower energy consumption, short hydraulic retention time and high organic loading rate (Warmeling et al., 2016). Particularly, airlift bioreactors are single devices that create different oxygen concentration zones (aerobic, anaerobic and anoxic) by embedding the blade as a physical separation, providing a more convenient operation in comparison to other single bioreactors (Asadi et al., 2017). This kind of bioreactor enhances the contact efficiency between gas–solid–liquid phases due to fluid circulation (Mota et al., 2015), providing a very high mixing inside the reactor without the need of a mechanical stirrer (Asadi et al., 2017). Besides, it presents an increased microbial diversity and high removal efficiency of recalcitrant pollutants (Xu et al., 2020). The oxygen bubbles circulate inside the bioreactor thanks to the action of the airlift, increasing the efficiency of oxygen mass transfer, and allowing the growth of microorganisms as well as the removal of organic matter (Mendes and Badino, 2015). Due to all these advantages, in this work, we have focused on the characterization of pollutants, such as COD, iron, humic acids and copper, and heavy metal removal from a LL in an airlift bioreactor packed with a LL preadapted compost. As far as we know, no studies have been done using this kind of setup for LL treatment. Therefore, it is the first research focused on the evaluation of the utilization of an adapted compost to remove pollutants present in a landfill leachate. Microbial communities were further investigated by applying Illumina sequencing of the 16S rRNA gene, a method able to provide thousands of sequence reads.

2. Materials and methods

2.1. Sample collection

Samples (100 L) of LL were obtained from different places of the pond of landfill El Panul, where the municipal solid waste from Coquimbo (Chile, Longitude: -71.3, Latitude: -29.9) is disposed off.

2.2. Pretreatment of the landfill leachate

Due to the fact that it is the first study that considers the use of compost in the depuration of a LL, there is no information available about the effect of the initial characteristics of this wastewater in the biological process. Considering that it is possible to obtain a different response in the removal of pollutants in the biological process due the initial characteristics of the LL, several pre-treatments in the LL and their impact in the final stage were evaluated.

Since the performance of the photocatalytic processes depends on the transparency of water, and it is needed to avoid the presence of pollutants, such as humic acids, which interfere with the UV transmittance and effectivity (Wang et al., 2023), we carried out a coagulation/flocculation process, that is a physicochemical treatment which allows the removal of organic matter and humic acids (Alfaia et al., 2019). Also, the coagulation/flocculation makes an adequate combination with filtration, since the first one allows to increase the size of the particles through the destabilization and aggregation of colloids and particles, favouring the performance of the second one. Filters reject particles with larger size than its pore diameter so, these particles are retained in the membrane, enhancing the performance of the filtration process (Lee et al., 2020). As a consequence, to improve the removal of pollutants, the raw LL was submitted to a coagulation/flocculation process using $FeCl_3$ with a load of 1 g/L (Merck), stirred at 150 rpm for 2 h (Poblete et al., 2020), then being left to rest for 12 hours. Then the supernatant was collected and submitted to a 5 μm filtration. Subsequently, the filtered LL was treated by a photo-Fenton process in a laboratory scale photoreactor of 1 L capacity, using UV irradiation emitted by an immersion UV lamp of 36 W (Atman Unit) with a maximum emission at a wavelength of 254 nm. The pH of the LL was adjusted to 3 using H_2SO_4 and the reagents used in the photo-Fenton reaction were $FeSO_4 \cdot 7H_2O$ and H_2O_2 in concentrations of 0.3 g/L and 0.67 g/L, respectively. The reagent concentrations were established according to previous results obtained by our research group, aimed to achieve the higher removal of pollutants in a LL treated by a photo-Fenton process (Poblete et al., 2019). The run lasted 1 h. A submerged pump (40 W) recirculated the LL at a flow rate of 8 L/min from a recirculation tank of 5 L useful capacity, which received LL from the photoreactor. Figure 1 shows a schematic diagram of the pretreatments involved.

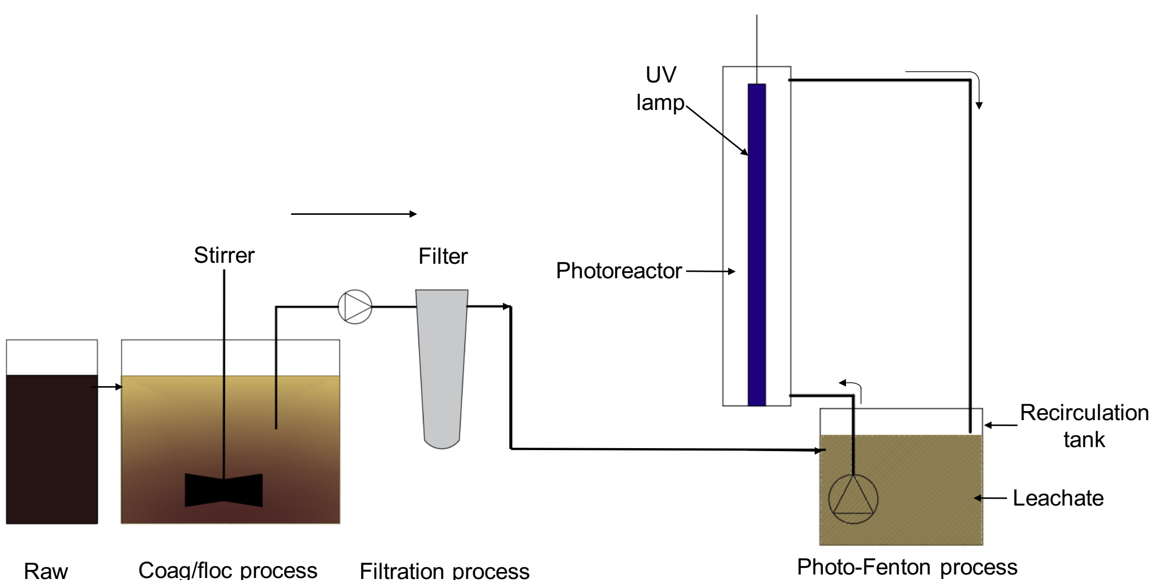


Figure 1. Schematic diagram of the pretreatment of the raw landfill leachate (LL).

2.3. Biological treatment in an airlift packing bioreactor

The removal efficiency of different pollutants present in the LL was evaluated using an airlift packing bioreactor (APBR) at a prototype scale in batch condition. The APBR was inoculated with immature compost that was obtained using organic wastes constituted by 10 kg of granulated solid coffee waste and 10 kg of sawdust, that were placed in a 50-litre cylindrical container. The solid coffee waste used to compost was obtained from a local shop in Coquimbo (Chile). Sawdust was obtained from carpentry stores also in Coquimbo.

With the aim of adapting the microorganisms present in the compost to the complex content of the LL, it was wetted with a 10% concentration of the raw leachate for one week. The following week, the concentration of the leachate was increased again by 10%, and this rise in concentration was maintained until week 10, to finally use a leachate without dilution (pure raw leachate) and maintain the periodically wetted compost with pure raw landfill leachate for a duration of 6 months, in order to allow the microorganisms present in the compost to gradually adapt to the complex and toxic substances of the leachate (Yadav et al., 2020; Tombola et al., 2019).

In the APBR with adapted compost, 1 kg of material was placed inside the reactor as a packed tower of 1 L of volume. A diffusor and a tube of air connected to a 10 W-power air pump were positioned at the bottom of the tower (Figure 2). The air pump provided aeration to allow the oxygenation of the packed tower and the recirculation of the LL.

In the bottom and in the upper parts of the packed tower there was a geotextile membrane to avoid leakage of the compost. The system was filled with 2 L of LL and the air pump was turned on to produce the effect of airlift and the recirculation of the LL in the system with a flow of 0.5 L/min. The packed tower of the APBR was 50 cm high, with an external diameter of 5 cm, connected to a recirculation tube with the same dimensions (Figure 2). In the upper zone of the packed tower a 10 cm extension of the tube was maintained free of LL and compost, which allowed sample collection and the retention of the foam generated during the process.

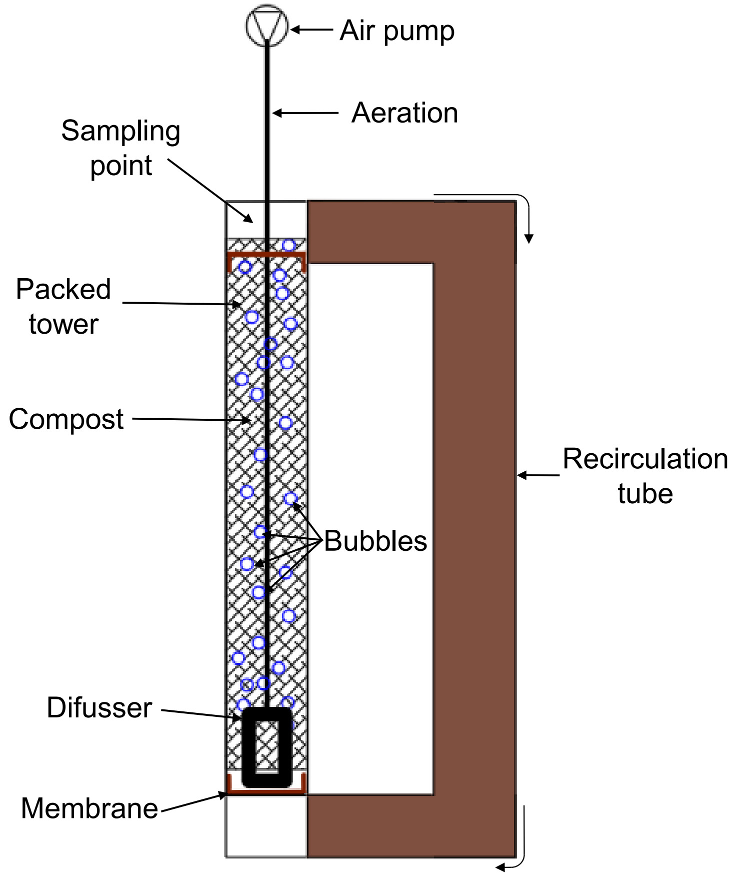


Figure 2. Schematic diagram of the airlift packing bioreactor (APBR).

To evaluate the effect of the pretreatments above described on the biological process, the LL submitted to the different pretreatment stages was treated in the APBRs (Figure 3). The system operated continuously for 10 days for each LL. Thus, the LL added into the bioreactors was: a) raw LL; b) LL submitted to coagulation/flocculation; c) LL submitted to coagulation/flocculation and filtration; and d) LL submitted to coagulation/flocculation, filtration and photo-Fenton process.

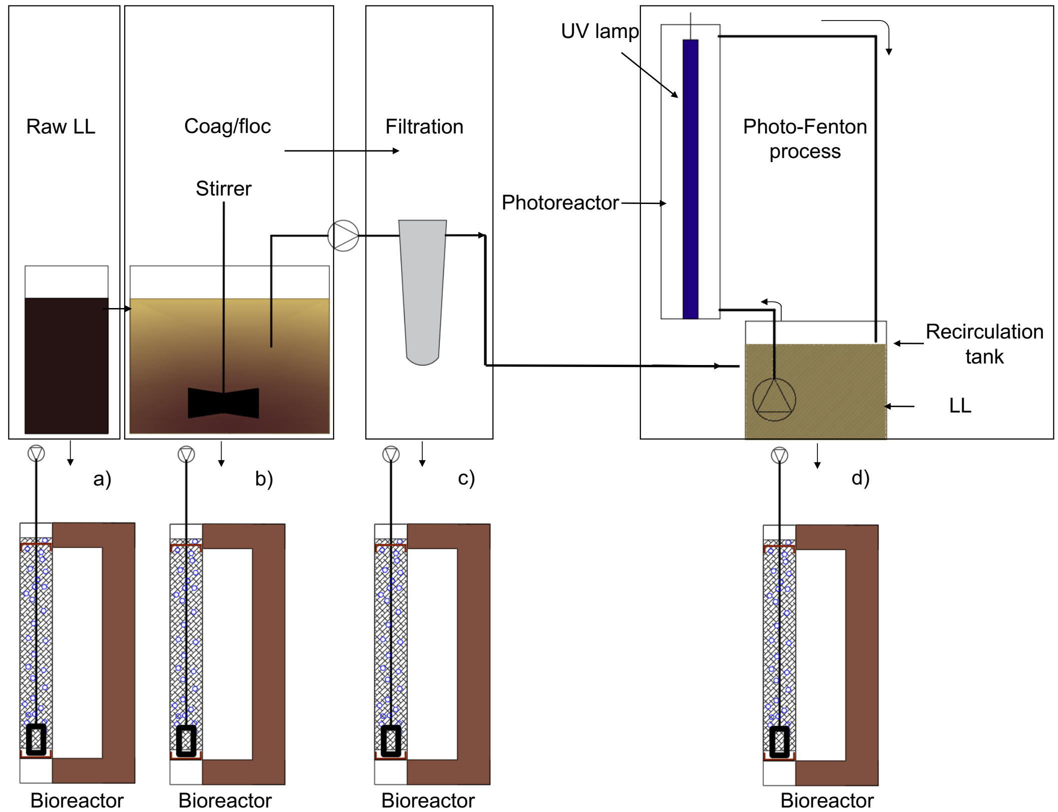


Figure 3. Schematic diagram of the pretreatment processes and final airlift packing bioreactors.

2.4. Determination of pretreatment parameters

The removal efficiency of the pollutants was determined by taking samples of LL in the recirculating system on the sample points and measuring the changes in chemical oxygen demand (COD), humic acids, heavy metals, faecal coliforms and toxicity. These parameters were determined along the different stages of the pretreatment in order to characterize the increase in biodegradability before and after the biological treatment. All the samples were taken by triplicate. The COD of the LL was measured according to the EPA 410.4 methodology and Colorimetric Method, pH was measured using a pH meter (WTW 3150i unit), and humic acids were determined spectrophotometrically in an Optizen Pop spectrophotometer at 254 nm (ABS254). Iron was analysed with the EPA phenantroline method 315B, while total copper was determined using the Bicichoninate Acid Method (HI-93702-01). The Total Organic Carbon (TOC) analysis, was carried out with 50 µL of samples injected in a Shimadzu TOC-L, with an acid-catalysed combustion at 650 °C, using a non-dispersive infrared (NDIR) detector. Faecal coliforms were determined using the NCh 2313/23 method. Heterotrophic plate counts (HPC) microorganisms were measured according to the spread plate method, in terms of Colony-Forming Units (CFU).

To determine the microbial compost stability and activity (Villaseñor et al., 2011), which depends on the biodegradability and toxicity of the mixture of LL+compost in the different APBRs, a respirometric test was carried out based on the oxygen uptake rate (OUR) of a mixture of liquid medium containing nutrients. The aeration and stirring of the suspension allowed oxygen to be adequately dispersed (Scaglia et al., 2011). The test was performed using a BM-T respirometer (Surcis S.L.), constituted by a 1 L capacity vessel, equipped with a thermometer and an oxygen probe (Protos 3400, Knick Elektronische Messgeräte GmbH & v Co. KG). Fifty grams of LL + compost collected from the top of the APBR were shredded and homogenized in a stirrer and then placed in the vessel of the respirometer; 1 L of distilled water was added and the mix was homogenized, the temperature was set at 20°C and the system was continuously aerated and stirred (Scaglia et al., 2007). The reference sample was made up with 50 mL of distilled water with 0.5 g of sodium acetate per gram of volatile suspended solid (as a highly biodegradable compound). Fifty mL of the different samples were added to obtain the OUR. The pH of each sample was previously adjusted to 7 (Lo, 2010). Each assay continued until a plateau in the respiration of the sludge (Rs, mg/L·h) was obtained and the maximum respiration rate (Rs_{max}, mg/L·h) was achieved. The inhibition percentages (%I) were calculated using the following equation:

$$\%I = \left(1 - \frac{Rs}{Rs_{max}}\right) * 100 \quad (1)$$

Anova and Tukey HSD post hoc tests were done to evaluate significant differences between the different treatments, with a p-value of 0.05.

2.5. DNA extraction

We extracted DNA from adapted and non-adapted compost samples, as well as from samples from both types of compost which had been in contact with raw LL for 10 days in order to compare the composition of microbial communities before and after being in contact with LL. DNA extraction was performed using the DNeasy Power Soil Kit (Qiagen) according to the manufacturer's instructions. DNA concentration and purity were measured using a Nanodrop spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE) at 260 nm and 260/280 nm respectively. DNA extracts were conserved at -80ºC for further analyses.

2.6. Amplicon sequencing

DNA was sequenced by RTL Genomics (Lubbock, TX, USA; https://rtlgenomics.com). Two primers were used to amplify bacterial and archaeal 16S rRNA gene: 515F-Y (GTGYCAGCMGCCGCGGTAA) and 926R: (CCGYCAATTYMTTTRAGTTT) (Parada et al., 2016). Illumina MiSeq 2x300 flow cells was used. Sequence data from the MiSeq platform were quality filtered, trimmed, dereplicated, merged and, after a process of chimera removal, clustered into amplicon sequence variants (ASVs) using the DADA2 Pipeline (Callahan et al., 2016). Afterwards, ASVs tabulation, and taxonomy assignment were performed using the Silva taxonomic database (v132). Sequences with unclassified genera or species with the Silva database were taxonomically identified by Blast (NCBI). Finally, the Phyloseq package was used to tabulate relative abundances at various taxonomic levels and to determine the microbial core community (McMurdie and Holmes, 2013). DADA2 and Phyloseq have been run as an R script (in R v.4.2.1) using R packages dada2 v.1.24.0, phyloseq v. 1.40.0 and ggplot2 v.3.3.6. Taxonomic identification was carried out by means of the tool Faprotax (Louca et al., 2016) to predict the functional composition of the microbial communities based on 16S rRNA marker gene profiles.

Sequence statistical analyses were performed using the R statistical software (R Core Team, 2021) and the *vegan* and *venneuler* packages. Alphadiversity analyses were performed using a ASVs abundance table that was previously subsampled down to the minimum number of reads in order to avoid artefacts due to an uneven sequencing effort among samples. For alphadiversity analyses, we calculated the Chao1 index as a measure of richness and the Shannon index as diversity metrics. Sequence data has been deposited in GenBank database with BioProject accession number PRJNA911052.

3. Results and discussion

3.1. Parameters after LL pretreatment

The raw LL was submitted to different pretreatment processes (coagulation/flocculation, filtration and photo-Fenton process) and after each stage, it was subject to a biological process in an APBR system. The main characteristics of the LL samples in the different stages of the pretreatment are presented in Table 1.

Table 1. Landfill leachate (LL) characteristics.

	Stage treatment

	Raw	Coag/Flocc	Filtration	Photo-Fenton
Parameter	Value			
COD (mg/L)	13780	11300	6890	3800
pH	8.85	8.82	8.83	2.95
Total Iron (mg/L)	82.3	94.1	43.5	28.1
Copper (mg/L)	180.6	60.9	17.1	16.8
Humic acids (abs 254 nm)	159.2	101.6	78.0	28.1
TOC (mg/L)	3845.4	3250.6	2801.5	1600.4
Faecal coliforms (MPN)	nd	nd	nd	nd
HPC (CFU)	nd	nd	nd	nd

MPN: most probable number
nd: not detected
The non detection of faecal coliforms and HPC in the raw LL was probably due to the toxicity of this kind of water (Wijekoon et al., 2022).

Figure 4 shows the changes in the concentration of copper, humic acids (ABS_{254}) and iron in the raw LL submitted to subsequent coagulation/flocculation, filtration and photo-Fenton processes before the biological treatment. A high removal of organic matter was observed in terms of COD and humic acids along the pretreatment process, with COD decreasing from 13780 to 11300 mg/L after coagulation/flocculation, from 11300 to 6890 mg/L after filtration, and from 6890 to 3800 mg/L due to photo-Fenton, achieving a global removal of a 72.4%. In the case of humic acids, the trend was similar, going from 159.5 to 101.7 mg/L, from 101.7 to 78.0 mg/L and from 78.0 to 26.4 mg/L, due to coagulation/flocculation, filtration and photo-Fenton processes respectively, reaching a global removal of 83.4%.

We have to take into consideration that the UV-quenching substances of LL are constituted by a hydrophilic fraction and also hydrophobic humic substances such as humic acids and fulvic acids, and each has distinct behaviours during its treatment (Iskander et al., 2018). Since these kind of substances can attract heavy metals and organic matter, allowing their transportation into surface water of groundwater (Reshadi et al., 2020), their removal is crucial for environmental protection.

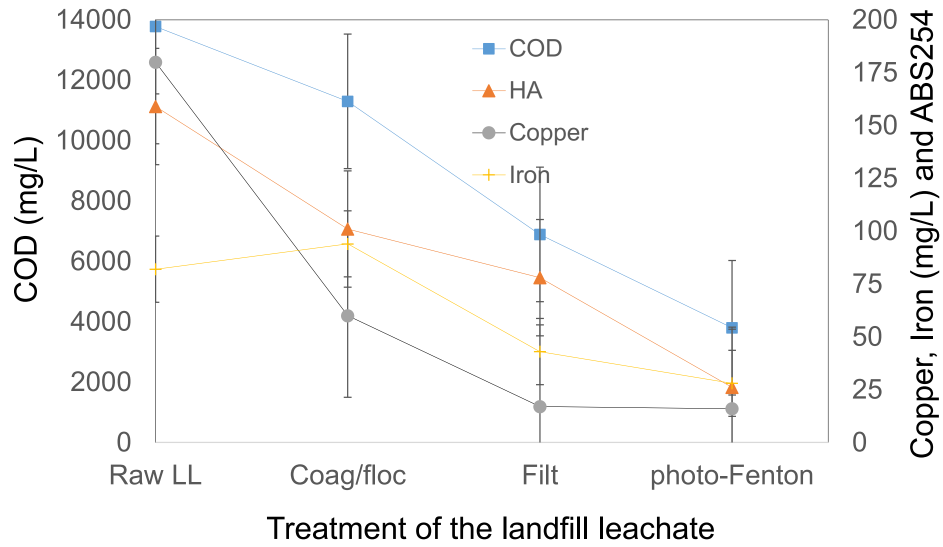


Figure 4. Change in the concentration of COD, copper, humic acids (ABS_{254}) and iron in the different stages of LL pretreatment. Error bars represent the standard deviations for three replicated measures (n = 3).

The removal of organic matter due to coagulation/flocculation could be explained by the agglomeration of particles in the LL suspension, including organic colloids (Cheng et al., 2020; Ibrahim and Yaser, 2019). The suspension is destabilized by the coagulant, and the flocculation occurs when compact flocs are formed and settled (Zahrim et al., 2018). The flocs that do not settle can be trapped in the filter, reducing the concentration of organic matter. On the other hand, the decrease of organic matter in the photo-Fenton process can be due to the generation of the highly reactive hydroxyl radicals, that are non-selectively oxidant (Espinoza-Quiñones et al., 2019), as shown by equations 2 and 3 which allow the oxidation of the organic matter present in the LL and enhance its biodegradability, as previously reported (Poblete and Pérez, 2020).

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + \cdot OH \quad (2)$$

$$Fe^{3+} + H_2O + hv \rightarrow Fe^{2+} + H^+ + \cdot OH \quad (3)$$

Concerning the heavy metals analysed, the copper decreased from 180.3 to 60.5 mg/L after coagulation/flocculation, from 60.5 to 17.8 mg/L after filtration and from 17.5 to 16.8 mg/L due to the photo-Fenton process, obtaining a global removal of 91.1%. In the case of iron, its concentration changed from 82.0 to 94.1 mg/L, from 94.1 to 43.5 mg/L and from 43.5 to 28.1 mg/L, due to coagulation/flocculation, filtration and photo-Fenton processes, achieving a global removal of 65.8%. The increase of the concentration of iron after the coagulation/flocculation process is due to the addition of this metal in the process, concentration that was reduced after the filtration process. The removal of copper in the LL achieved by the coagulation/flocculation process, that used Fe(III), demonstrated the high performance of Fe(III), being in agreement with the data reported by Chu et al., (2020), who published removal rates of dissolved organic carbon, Ni and As, of 84.1%, 73.1% and 96.9%, respectively.

As can be observed in Table 1, TOC underwent a high removal, especially in the photo-Fenton process, where an elimination of 42.8% was achieved, mostly caused by the removal of organic carbon as aromatic compounds, leading to the generation of hydroxyl radicals in the oxidation process (Welter et al., 2018). Nonetheless, the subtraction of TOC was lower than that obtained by Liu et al., (2022) who reached a 76.4% removal of this parameter, probably due to the fact that they spent longer time in the photocatalytic process. On the other hand, we did not detect the presence of faecal coliforms nor heterotrophic plate counts, probably due to the toxicity of this kind of water.

3.2. Parameters during LL biological treatment

The raw LL and the LL submitted to different pretreatments were placed in four different APBR with the aim of promoting their contact with the compost throughout the 10 day experiment, and to evaluate the removal of pollutants as well as the effect of the different pretreatment stages. As can be observed in Figure 5, there is a difference in the removal of the pollutants due to the biological process in the APBR, depending on the stage of the pretreatments carried out. When LL without any pretreatment (raw LL) was fed to the biological process (Figure 5a), the change in COD varied from 13780 to 11870 mg/L, while copper decreased from 180.6 to 100 mg/L, and iron from 82.3 to 32 mg/L; in the case of humic acids, there was a slight increase from 159.5 to 163 ABS_{254}. When the LL was subjected to coagulation/flocculation and to the biologic process (Figure 5b), the COD diminished from 11300 to 9500 mg/L, while copper declined from 60.9 to 20.4 mg/L, and iron varied from 94.1 to 75.6 mg/L; humic acids increased again from 101.6 to 162.0 ABS_{254}. When the LL was submitted to coagulation/flocculation and filtration processes in the bioreactor (Figure 5c), the change in COD was from 6890 to 4800 mg/L, for copper from 17.1 to 8.3 mg/L, for iron from 43.5 to 35.4 mg/L and, in the case of humic acids from 78.8 to 205.6 ABS_{254}. When the LL was submitted to the whole pretreatment (coagulation/flocculation, filtration plus photo-Fenton), decreases in COD from 3800 to 1520 mg/L, for copper from 16.8 to 8.37 mg/L, and for iron from 28.1 to 15.4 mg/L were obtained in the biological process (Figure 5d), while for humic acids the value increased from 26.1 to 35.5 ABS_{254}.

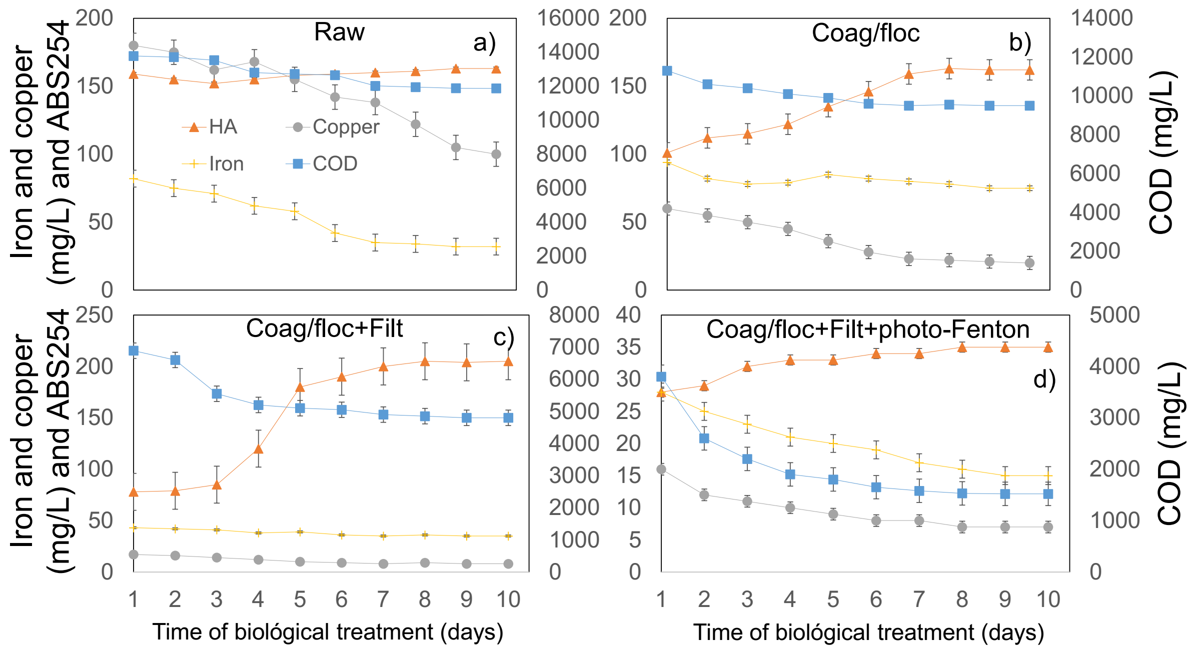


Figure 5. Change in the concentration of different pollutants present in the LL submitted to the biological process without pretreatment (a) or after the different pretreatments (b: coagulation/flocculation, c: coagulation/flocculation + filtration, d: coagulation/flocculation + filtration + photo-Fenton). Error bars represent the standard deviation of the results (n = 3).

In general, there was an enhancement in the concentration of humic acids throughout time in the different bioreactors due to the action of the compost. Humic acids are organic substances rich in amino, hydroxyl and carboxyl groups, present in the compost due to the high concentration of lignin (Wang et al., 2022). They participate in the control of nutrients for microbial consortia and they protect microorganisms from the action of hazardous compounds (Sun et al., 2022), being also their presence an indicator of fertility in soils (Liu et al., 2023). Therefore, their presence in the compost promotes the removal of toxic material from the LL.

Concerning the removal of COD, the results showed significant differences, with F values higher than the tabulated number (440.3>0.005) (Table 2). A Tukey test (Table 3) indicated that there were significant differences between all the treatments evaluated. Also, significant differences were observed in the removal of humic acids, with F values higher than the tabulated result (36.9>0.00004). The Tukey test showed that there were significant differences between the photo-Fenton treatment and all the rest of the treatments, including raw LL.

Table 2. Analysis of variance (ANOVA) for COD removal

	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	3	6.78E+08	2.26E+08	440.30	0.005
Error	36	1.85E+07	513510.6		
Total	39	6.97E+08			

Table 3. Tukey tests for COD removal.

	Mean Diff	SEM	q Value	Prob	Alpha	Sig
Coag/floc Raw	-2696	320.4	11.89721	0	0.05	1
Filt Raw	-7317	320.4	32.28928	0	0.05	1
Filt Coag/floc	-4621	320.4	20.39207	0	0.05	1
Photo-Fenton Raw	-10681	320.4	47.13432	0	0.05	1
Photo-Fenton Coag/floc	-7985	320.4	35.23711	0	0.05	1
Photo-Fenton Filt	-3364	320.4	14.84504	2.55E-07	0.05	1

Sig equal 1 indicates that the means difference is significant at the 0.05 level.

In the case of the variation of cooper in the bioreactor after the different treatments, significant differences were obtained in this parameter, with F values higher than the tabulated number (154.6>2.86). The Tukey test showed that there were meaningful differences between all the treatments except for the photo-Fenton and filtration process.

Taking into consideration the change on the concentration of iron in the LL, the anova test shows that there were significant differences in this parameter, with F values higher than the tabulated number ($59.8>4.64*10^{-14}$). The Tukey test showed that there were meaningful differences between all the treatments evaluated.

The percentage of removal of pollutants such as COD, copper and iron present in the LL after the biological treatment is showed in Figure 6. The biological treatment that reached the highest removal of COD was the one that underwent coagulation/flocculation plus filtration plus photo-Fenton pretreatment processes, with an elimination value of 60.0%. A similar trend was observed with iron, which achieved a removal of 46.5%. In contrast, for copper, the highest elimination was accomplished with LL pretreated with the coagulation/flocculation process, with a value of 56.3%.

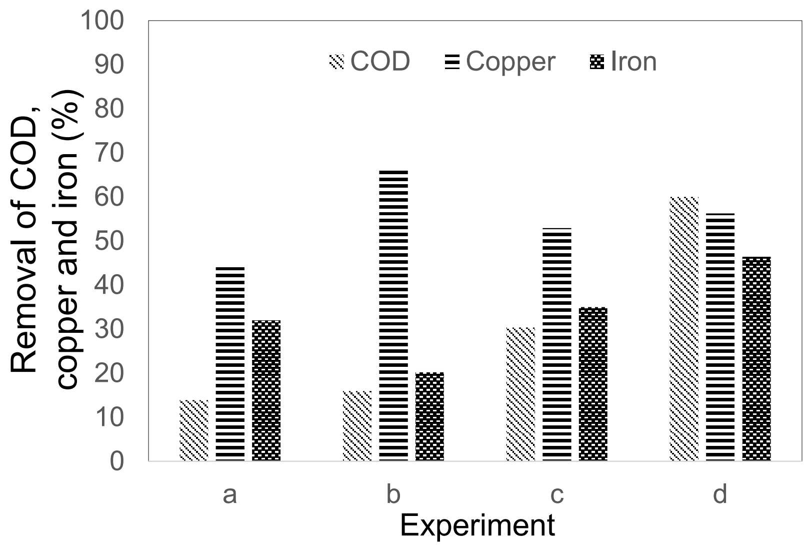


Figure 6. Removal of pollutants (%) present in LL treated in the APBRs, using a) raw LL, or different pretreatment processes: b) Coag/Floc, c) filtration and d) photo-Fenton).

According to the respirometric tests carried out using sludges, the raw LL was toxic and although toxicity decreased along the pretreatments of LL. Inhibition values of 41.8%, 27.5%, 22.0% and 12.1% were obtained for raw, coagulation/flocculation, filtration and photo-Fenton processes respectively. These results agree with previous findings from our research group (Poblete and Pérez, 2020), where a decrease in the toxicity throughout the LL treatments was reported. This reduction in the inhibition of sludge in the presence of the LL + compost mixture is due to the pretreatment and the biological process, which promoted the removal of toxic compounds from the wastewater, such as the adsorption of copper. Up to now, a 95% removal of this heavy metal has been previously reported (Pennanen et al., 2020), as well as the oxidation of complex organic matter by hydroxyl radicals, with a removal of an 80% of COD of a LL using a photo-Fenton process (Carbajo et al., 2021). Additionally, the use of UV light and a photocatalyst, enhanced the biodegradability of LL by 89% (da Costa et al., 2018). Table 4 shows a comparison on the pollutants removal obtained in this work and in other ones where different treatments were applied.

Table 4. Comparison on the pollutants removal obtained in this work and in other ones where different treatments were applied.

Pollutant	Treatment method	% of removal In this manuscript	% of removal In other works	Reference
COD	Coag/Flocc	14	35	(Reddy et al., 2022)
	Filtration	16	33	(Rohers et al., 2021)
	Photo-Fenton	60	43	(Ghanbarzadeh Lak et al., 2018)
	Biological process	60	60	(Tripathy and Kumar, 2022)
Copper	Coag/Flocc	67	87	(Ma et al., 2023)
	Filtration	53	28	(Bremner et al., 2020)
	Biological process	56	59	(Genethliou et al., 2023)
Humic acids	Coag/Flocc	36	20	(Kong et al., 2021)
TOC	Coag/Flocc	15	15	(Righetto et al., 2021)
	Filtration	14	24	(Agabo-García et al., 2023)
	Photo-Fenton	43	32	(Kanafin et al., 2023)

3.3. Operational costs

Table 5 shows the operational costs of the evaluated treatment, considering the expenses of the reagents as well as the costs of the electric power consumption by the equipment used in the different experiments. It should be highlighted that experiment a (raw LL) corresponds to less reagent and total costs, as expected, since there is not a pretreatment, which led to the lowest removal of pollutants (Figure 6). In contrast, experiment *d,* which considers all the pretreatments, has the highest values of operational costs, especially due to the use of reagents, although it achieves the best levels of iron and copper removal, allowing less inhibition of the sludge in the respirometric test. It is quite an interesting result, because copper present in LL is considered an harmful substance, hazardous to the aquatic environment (Wdowczyk and Szymańska-Pulikowska, 2021) and with negative effects on microorganisms (Kwak et al., 2020).

Table 5. Operational costs of the different treatments carried out.

Exp	Operational costs ($US/m^3) Reagent	Operational costs ($US/m^3) Energy	Operational costs ($US/m^3) Total
a (raw LL and biologic)	3.8	22.2	26.0
b (coag/floc and biologic)	34.0	22.3	56.3
c (coag/floc, filtr and biologic)	34.0	22.3	56.3
d (coag/floc, filtr, photo-Fenton and biologic)	79.8	23.7	103.5

Also, it is possible to observe that operational costs associated to energy consumption were similar for all the experiments, since these runs were carried out in the APBR, that used aeration for 10 days. Although the power of the air pump was small (10 W), its prolonged use over time, necessary to maintain a sufficient amount of gas/liquid phases mass recirculation, implied a relatively high energy consumption which generated higher operational costs (Chen et al., 2021; Yen and Liu, 2014). In fact, the energy spent by the aeration process was more than 50% of the total energy consumed in the biological treatment (McCarty et al., 2011), lower than that used by Hu et al., (2020), who used an air compressor with a very high power. On the other hand, Wei et al., (2023) published a cost of 113,9 €/m^3 of oxypirolysis of LL using $Fe_2O3@SiO_2\text{-}Al_2O_3$ as a catalyst.

The whole treatment, that includes coagulation-flocculation, filtration, photo-Fenton reaction and biological process, has higher operational cost than others, as reported by Turan et al., (2023) who published a cost of 50 \$US/m^3 using $FeCl_3$ as coagulant for LL treatment. However, it has to be taken into consideration that the non-biodegradable and toxic behaviour of the mature LL studied in this work required complex treatments (Yan et al., 2022; Gautam and Kumar, 2021).

It has been reported that aeration allows the enrichment of heterotrophic denitrifying bacteria and the removal of nitrogen and organic matter (Song et al., 2020). Also, the utilization of the APBR allows an effective suspension of solids, high mass transfer, low shear stress to microorganisms suitable for biological processes, and the absence of stagnant volumes into this reactor (Mirghorayshi et al., 2020).

3.4. Microbial community structure and predicted metabolisms in adapted and non-adapted compost samples

In order to improve our knowledge about the composition of the microbial assemblages in compost samples and their possible role in pollutants removal from LL, we analysed samples from both an adapted and a non-adapted compost before and after being in contact with the raw leachate on the basis of the 16S rRNA gene sequence. After a rigorous quality control (see Materials and Methods), a total of 443,640 sequences passed quality filtering. On average, 73,940 sequences were obtained per sample (ranging from 44,031 to 105,511). At this sequencing depth, rarefaction curves revealed a reasonable coverage of bacterial richness (Figure S1). Observed richness (number of Amplicon Sequence Variants or ASVs) varied between 749 and 1,901 (Table 6). These values were similar to those obtained from the richness estimate index (Chao1), which ranged from 953 to 2072. Despite variation in ASVs number, no significant differences were found between samples in richness indices (Kruskal-Wallis and Wilcoxon's test with Bonferroni correction, $p > 0.05$). Shannon's diversity index varied from 3.33 to 5.95, showing usual diversity values for microbial communities (Feranchuk et al., 2018). In this case, significant differences were found between samples, showing lower diversity values without preadaptation and without contact to the LL than those which were previously adapted or exposed to the LL (one-way ANOVA and Tukey post-hoc test, $p < 0.05$).

Table 6. Alphadiversity from the microbial communities of the studied compost samples.

Sample	**Observed**	**Chao1**	**Shannon**
Adapted & No LL	1647	1960.84	5.59
Adapted & LL	1327	1791.55	4.69
Non- adapted & No LL	749	952.55	3.33*
Non-adapted & LL	1901	2072.23	5.95

* significant differences in Shannon index ($p < 0.05$)

Bacteroidota, Proteobacteria, Firmicutes, Spirochaetota and *Deinococcota* were the most abundant phyla in the studied samples (Figure 7). These five phyla were found in all samples, being present in the 50 most abundant ASVs of the study, which represented 56.5% of the total analysed sequences and were considered as our *core* community (Neu et al., 2021).

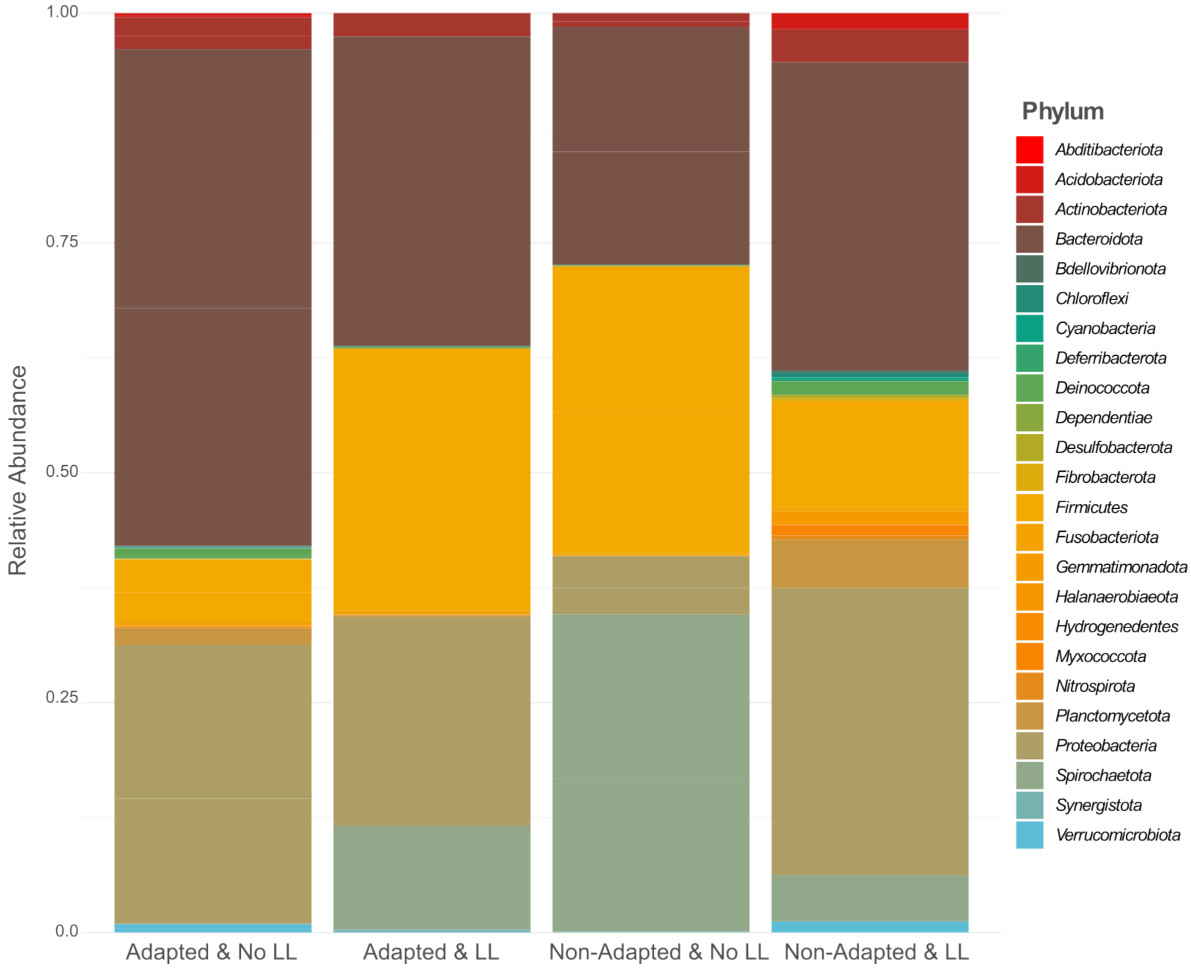
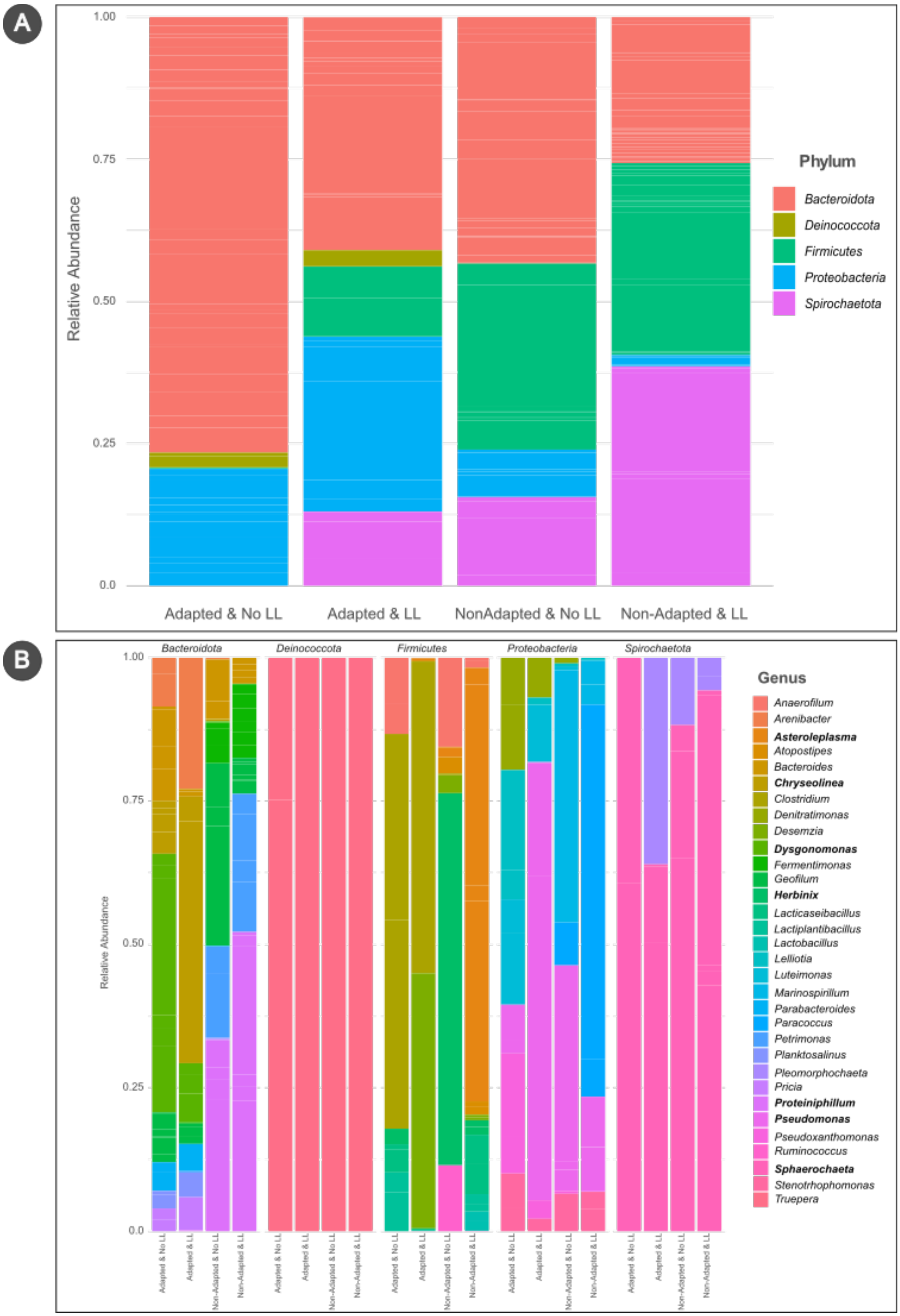


Figure 7. Relative abundance of each phylum in the microbial community from adapted and non-adapted compost samples before (no LL) and after (LL) being exposed to raw LL

Interestingly, the most representative phylum in samples which had been in contact with leachate in some moment (i.e. preadaptation or treatment of LL) was *Bacteroidota* (Figure 8). This phylum utilizes complex organic substrates in aquatic environments, being able to degrade recalcitrant compounds, an activity previously reported in studies from LL (Gabarró et al., 2013; Wang et al., 2022). Nevertheless, in the compost sample not preadapted to leachate, *Spirochaetota* and *Firmicutes* were more abundant than *Bacteroidota.* Both phyla, *Spirochaetota* and *Firmicutes*, were previously described in microbial communities from compost of organic waste or from rice straw (Tanahashi et al., 2005; Galitskaya et al., 2017). Recent studies have shown the possible role of these phyla in decontaminating metals from different environments. On the one hand, some genera from *Bacteroidota*, *Spirochaetota*, and *Firmicutes,* are involved in the sulfur cycle and can use iron in their metabolisms (Astorch-Cardona et al., 2023; Hashemi et al., 2022). Furthermore, these three phyla have also been found in artificial copper-contaminated samples from MFCs and even in gut microbiota (Gao et al., 2023; Wu et al., 2022). In this sense, it showed the relevance of these phyla in the *core* community, taking into account that all of them could contribute to the decontamination of LL.


A
1.00
0.75
0.50
0.25
0.0
Relative Abundance
Adapted & No LL
Adapted & LL
NonAdapted & No LL
Non-Adapted & LL
Phylum
Bacteroidota
Deinococcota
Firmicutes
Proteobacteria
Spirochaetota
B
Bacteroidota
Deinococcota
Firmicutes
Proteobacteria
Spirochaetota
Relative Abundance
Genus
Anaerofilum
Arenibacter
Asteroleplasma
Atopostipes
Bacteroides
Chryseolinea
Clostridium
Denitratimonas
Desemzia
Dysgonomonas
Fermentimonas
Geofilum
Herbinix
Lacticaseibacillus
Lactiplantibacillus
Lactobacillus
Lelliotia
Luteimonas
Marinospirillum
Parabacteroides
Paracoccus
Petrimonas
Planktosalinus
Pleomorphochaeta
Pricia
Proteiniphillum
Pseudomonas
Pseudoxanthomonas
Ruminococcus
Sphaerochaeta
Stenotrhophomonas
Truepera

552

Figure 8. Taxonomy of the *core* community. Data refer to the different microbial communities from adapted and non-adapted compost samples before (no LL) and after (LL) being exposed to raw LL. a) Relative abundance of each phylum in the most abundant 50 ASVs b) Relative abundance of genus belonging to different phyla from the main 50 ASVs. Bold letters label the most representative genera from the microbial community.

At genus level, the relative abundance of some ASVs presented differences between samples, and the final composition of the microbial community appeared to depend on the preadaptation or not of the compost samples. In the case of the preadapted compost to LL, the main genus was *Dysgomonas* (34% of sequences of *core* community). However, when the sample was exposed to raw LL, *Dysgomonas* became less abundant (until 0.1% of sequences) and another genus that was not predominant at the beginning turned into the representative bacteria of the community. Other genera that underwent significant changes in abundance before and after being exposed to LL were *Herbinix* (from < 0.1% of sequences before the contact of raw LL to 21% of sequences after the treatment), *Proteiniphilum* and *Sphaerochaeta* (Figure 8). *Herbinix,* and concretely *Herbinix luporum* was described as a cellulose degrading bacterium able to metabolize lignocellulose to acetate, ethanol, and propionic acid in different biogas reactors (Koeck et al., 2016; Liu et al., 2017). In these kind of reactors, previous to the digestion process of the substrate, pollutants were frequently found (i.e. nitrogen compounds and heavy metals) (Rahman et al., 2021). Moreover, *Proteiniphilum* sp. was also usually detected in biogas reactors. This genus has been reported as strict anaerobes that use several macromolecular organic molecules as carbon and energy sources and produce acetic acid (Dang et al., 2013). *Proteiniphilum* sp. has been recently isolated from anaerobic digesters treating brewery wastewater or maize silage and wheat straw, as well as from municipal solid waste landfill (Orellana et al., 2022; Wang et al., 2022). Thus, the most representative genera of our microbial community after being preadapted and exposed to raw LL, showed a tolerance to different complex substrates, being involved in the decrease of COD and metals concentration of the raw LL.

A similar trend in the core microbial community was observed between compost samples without preadaptation before and after the contact to raw LL. In this case, *Sphaerochaeta* (37% of sequences of *core* community) and *Asteroleplasma* (26% of sequences) were the dominant genera in the microbial assemblage of the non-adapted compost before being in contact with LL. After the treatment of compost with landfill leachate, the genera *Pseudomonas* (from 0.3% of to 23%) and *Chryseolinea* (from <0.1% of to 19%), underwent a substantial abundance increase. *Asteroleplasma* almost disappeared after the treatment, while *Sphaerochaeta*, though decreased its relative abundance, remained one of the 5 most abundant genus of the community (Figure 8). *Pseudomonas* strains are frequently reported as exhibiting a great metabolic versatility and could survive in different ecological niches including contaminated environments (Rojo, 2010). *Pseudomonas* sp. is a microorganism that can naturally produce extracellular polysaccharide (EPS). EPS structures support the sequestration of metal ions and prevent them from penetrating into the cell surface or from being released in the environment (Imron et al., 2021). *Pseudomonas putida* has been previously showed to have heavy metal resistance genes and the ability to biodegrade phenolic LL (Paliwal et al., 2014; Michalska et al., 2020). *Pseudomonas parafulva* and *Pseudomonas putida*, which are closely related between them, were the main species in the compost sample in contact to LL. On the other hand, *Chryseolinea* has been reported to be an aerobic chemotrophic bacterium (Kim et al., 2013) that could remove nutrients from water. Bacteria belonging to this genus were found in different reactors treating landfill leachate and were mainly related to nitrogen and phosphorous cycles (Xu et al., 2018; Mu et al., 2022), suggesting a great ability to adapt to complex environments.

Finally, *Sphaerochaeta* was a genus present in all samples after the contact with raw LL. *Sphaerochaeta* is a strictly fermentative genus which produces volatile fatty acids, ethanol, hydrogen, and CO_2 by fermenting complex organic compounds (Zhou et al., 2017). Moreover, *Sphaerochaeta* has been previously reported as the predominant bacteria in landfill leachate (Wong et al., 2019; Zhao et al., 2021; Nimonkar et al., 2022), showing high resilience after the exposure to adverse conditions.

On the other hand, on the basis of our 16S rRNA sequences, we utilized the Faprotax tool in order to perforym a prediction of the main potential metabolisms ruling the microbial communities of adapted and non-adapted compost samples before and after their exposition to raw LL. Our results showed that the metabolisms related to chemoheterotrophy, fermentation and the nitrogen cycle were the most relevant in all samples (Table S2). Furthermore, in samples exposed to raw LL from adapted and non-adapted composts, the most abundant metabolisms were related to anaerobic pathways, which might play important roles in degrading complex organic molecules into easily degradable substances (Zhao et al., 2021). Particularly, samples from non-adapted composts were characterized by routes related to nitrogen and methane metabolisms (Figure 9), a trend that has already been observed in composting processes (Hoang et al., 2022). However, when these samples were exposed to raw LL, those metabolisms were lost and enriched with routes related to sulfur cycle, which has been previously associated to the presence of iron (Long et al., 2016), as well as to plastic, hydrocarbon and aromatic compounds degradation, and ligninolysis and cellullolysis metabolisms, implying high activities of carbon-related metabolisms and the biotransformation of organic substances (Toledo et al., 2017; Zhao et al., 2021). In contrast, in the preadapted compost sample, all these last metabolisms were found from the very beginning and once it was in contact with raw LL, the prevailing pathways related to hydrocarbon degradation. In summary, both phylogenetic and metabolic results showed that, after being exposed to raw LL, microbial communities were enriched with groups able to use recalcitrant material indifferently if the biological sample was preadapted to LL or not, thus the DOC removal and the degradation of complex compounds and heavy metals were optimized with a minimal cost. The presence of *Pseudomonas*, *Chryseolinea*, and *Sphaerochaeta* as the dominant genera in the microbial community was found to have the best removal effect, considering the community diversity and ability to recover after exposure to raw landfill leachate. When these three genera were predominant, the most prevalent metabolisms in the microbial community were related to the degradation of complex organic compounds found in the LL.

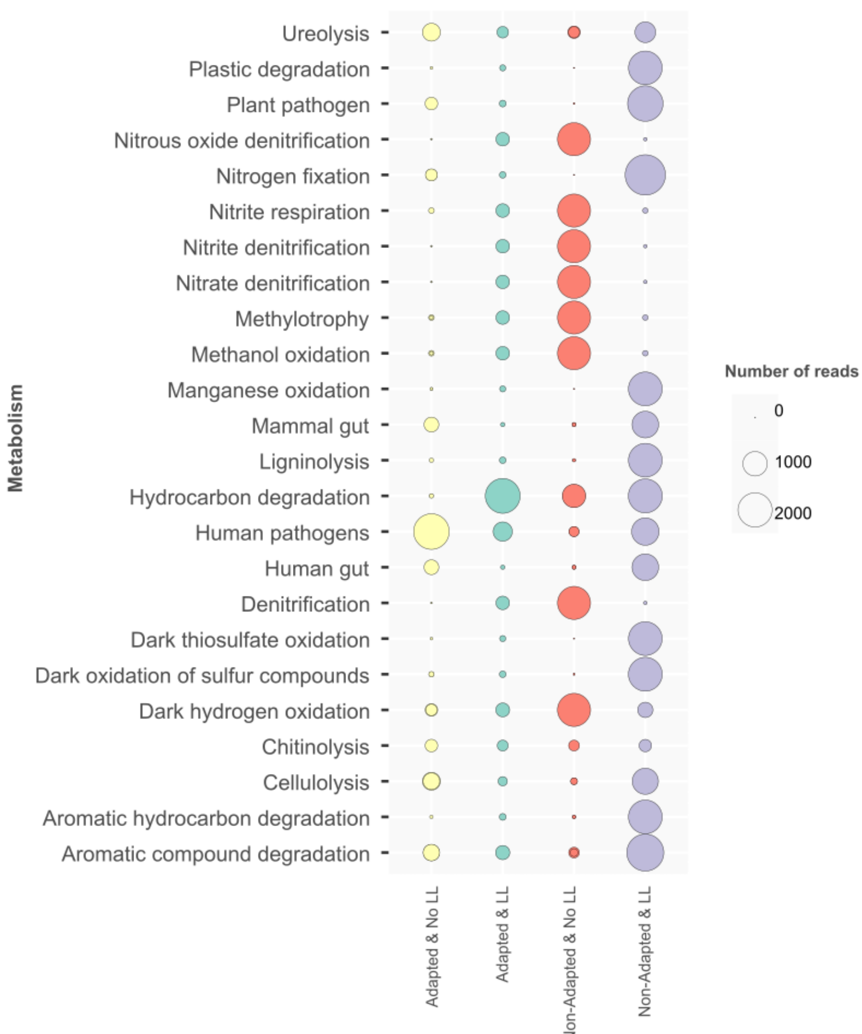


Figure 9. Main representative metabolisms in microbial communities from adapted and non-adapted compost samples before (no LL) and after (LL) being exposed to raw LL. The diameter of the circles represents the prevalence of each metabolism in the sample.

4. Conclusions

The different pretreatments carried out allowed a high removal of organic matter and heavy metals in the LL. The removal of organic matter was observed along all the pretreatments, although in the case of copper and iron, the removal was especially due to the coagulation/flocculation and filtration processes. The contact of LL with the compost produced an enhancement of humic acids throughout time in the bioreactors, which stimulates the removal of toxic material in this polluted water. The biological treatment with compost reached a higher removal of COD and iron when coagulation/flocculation plus filtration plus photo-Fenton as pretreatment processes were performed. On the other hand, the highest copper removal was obtained when the LL was pretreated by coagulation/flocculation before the biological process. In summary, the toxicity of the raw LL was reduced by the action of the pretreatments and the biological treatment carried out, due to the removal of toxic compounds, such as copper and recalcitrant organic matter.

The biological process applied to raw LL led to the lowest costs, but when this process was applied to a LL submitted to all the pretreatments, the operational costs raised due to the increase of reagents. Otherwise, the energy consumption was similar for all the experiments, since all of them included an aerated bioreactor.

Concerning the microbial community, it was able to adapt to raw LL, showing a high tolerance and resilience to toxic conditions, both in the compost previously in contact with LL or not, though this item could affect the species composition of the population. However, the genus *Sphaerochaeta* was able to hold up in all the studied conditions. The prediction of microbial metabolisms also showed a remarkable ability from microorganisms to remove and metabolize different complex and toxic substances.

Thus, we can conclude that the application of this airlift bioreactor allows an effective removal of pollutants in the LL with a minimal cost.

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