Physical, chemical, and biological treatment of chemical waste from teaching laboratories at Universidad Nacional, Costa Rica

Tratamiento físico, químico y biológico de residuos químicos de los laboratorios de docencia de la Universidad Nacional, Costa Rica

Tratamento físico, químico e biológico de resíduos químicos dos laboratórios de ensino da Universidade Nacional, Costa Rica

Jorengeth Abad Rodríguez-Rodríguez
jorengeth.rodriguez.rodriguez@una.cr
Laboratorio de Biotecnología Microbiana. Escuela de Ciencias Biológicas, Universidad Nacional
Heredia, Costa Rica
https://orcid.org/0000-0001-8452-8256

Seiling Vargas-Villalobos
seiling.vargas.villalobos@una.cr
Instituto Regional de Estudio en Sustancias Tóxicas. Universidad Nacional.
Heredia, Costa Rica
https://orcid.org/0000-0002-8003-6358

Carmen Aparicio-Mora
carmen.mora.aparicio@una.cr
Laboratorio de Gestión de Desechos. Escuela de Química, Universidad Nacional.
Heredia, Costa Rica
https://orcid.org/0000-0002-4405-9480

Nidya Nova-Bustos
nidya.nova.bustos@una.cr
Escuela de Ciencias Biológicas, Universidad Nacional
Heredia, Costa Rica
http://orcid.org/0000-0003-1966-0415

Margaret Pinnock-Branford
margaret.pinnock.branford@una.cr
Laboratorio de Estudios Ecotoxicológicos. Instituto Regional de Estudio en Sustancias Tóxicas. Universidad Nacional.
Heredia, Costa Rica
https://orcid.org/0000-0003-3899-3389

Abstract

Herein, we report the physical, chemical, and biological treatment of wastewater generated in the teaching laboratories at Universidad Nacional, Costa Rica. Initial physicochemical treatment included neutralization and coagulation-flocculation, followed by a biological treatment with fungi (*Aspergillus* sp. and *Penicillium dipodomyicola*) or bacterias, the latter were isolated from the sludge from the campus' wastewater treatment plant and the greywater collection tank at the School of Chemistry. The samples' pH prior to treatment was ≤ 2, while COD ranged between 3000 and 30 000 mg/L. Gas chromatography-mass spectra analysis indicated the presence of 55 organic compounds in the wastewater, some of which reached undetectable concentrations after treatment. The fungi and the bacterial strain removed up to 50% of the substances,

while the toxicity decreased with respect to time of exposure to the treatment. Results support the potential use of these microorganisms as bioremediators. Although the organic compounds were partially removed, the treated wastewater exhibited high toxicity for *Daphnia magna* (water flea). Further experiments with longer treatment times or other strains might be needed for effective removal of pollutants.

Keywords: *Aspergillus* sp.; *Penicillium dipodomyicola*; mass chromatography; toxicity, *Daphnia magna, chemical wastewater, biological treatment.*

Resumen

En este trabajo, se reporta el tratamiento físico, químico y biológico de las aguas residuales generadas en los laboratorios de docencia de la Universidad Nacional, Costa Rica. El tratamiento fisicoquímico inicial incluyó la neutralización y la coagulación-floculación, seguido de un tratamiento biológico con hongos (*Aspergillus* sp. y *Penicillium dipodomyicola*) o bacterias; estos se aislaron del lodo de la planta de tratamiento de aguas residuales del campus y del tanque de recolección de aguas grises en la Escuela de Química. El pH de las muestras antes del tratamiento fue ≤ 2, mientras que la DQO osciló entre 3000 y 30 000 mg /L. El análisis de cromatografía de gases con detector de masa (GC/MS) indicó la presencia de 55 compuestos orgánicos en las aguas residuales, algunos de los cuales alcanzaron concentraciones indetectables después del tratamiento. Los hongos y la cepa bacteriana eliminaron hasta el 50 % de las sustancias, mientras que la toxicidad disminuyó con respecto al tiempo de exposición al tratamiento. Los resultados sugieren el uso potencial de estos microorganismos como biorremediadores. Aunque los compuestos orgánicos se eliminaron parcialmente, las aguas residuales tratadas exhibieron una alta toxicidad para *Daphnia magna* (pulga de agua). Son necesarios más experimentos con tiempos de tratamiento más largos u otras cepas, para la eliminación efectiva de contaminantes.

Palabras clave: *Aspergillus* sp.; *Penicillium dipodomyicola*; cromatografía de masas; toxicidad; *Daphnia magna*, tratamiento biológico, aguas residuales químicas.

Resumo

Neste trabalho, é relatado o tratamento físico, químico e biológico das águas residuais geradas nos laboratórios de ensino da Universidade Nacional da Costa Rica. O tratamento físico-químico inicial incluiu a neutralização e a coagulação-floculação, seguido de tratamento biológico com fungos (*Aspergillus* sp. e *Penicillium dipodomyicola*) ou bactérias; estes foram isolados da lama da estação de tratamento de águas residuais do campus e do tanque de coleta de águas cinzas da Escola de Química. O pH das amostras antes do tratamento foi ≤ 2, enquanto a DQO oscilou entre 3000 e 30 000 mg/L. A análise de espectros de massa por cromatografia gasosa indicou a presença de 55 compostos orgânicos nas águas residuais, alguns dos quais atingiram concentrações indetectáveis após o tratamento. Os fungos e a cepa bacteriana eliminaram até 50% das substâncias, enquanto a toxicidade diminuiu com relação ao tempo de exposição ao tratamento. Os resultados sugerem o uso potencial desses microrganismos como biorremediadores. Embora os compostos orgânicos tenham sido parcialmente eliminados, as águas residuais tratadas exibiram alta toxicidade para *Daphnia magna* (pulga-de-água). São necessários mais experimentos com tempos de tratamento mais longos ou outras cepas para a eliminação eficaz de contaminantes.

Palavras-chave: *Aspergillus* sp.; *Penicillium dipodomyicola*; cromatografia de massas; toxicidade; *Daphnia magna*, tratamento biológico, águas residuais químicas.

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Introduction

Only 2.5% of the water on Earth is freshwater, 70% of this exists in solid form and 30% in liquid form, with less than 1% being usable for human consumption (Valverde *et al.*, 2005). Water is an essential resource for life that is being threatened by pollution. Industrial, domestic, agricultural, commercial, or service-related processes generate wastewater ladened with pollutants, including chemical substances and fecal matter. If left untreated, wastewater can affect environmental and human health (Manahan, 2007).

Wastewater treatment involves physical, chemical and biological procedures, in order to remove pollutants and hazardous characteristics before final release into a body of water, without harming the environment or human health (Aguilar *et al.*, 2002). Initial pretreatment (*e.g.*, grease traps, sand traps, and roughing) consists of a physical process to remove large solids (López & Martin, 2015). The next step is primary treatment, which requires physical and chemical processes, such as decanting, clarification, and neutralization. In this stage of the treatment, the purpose of eliminating solids suspended in residual water (López & Martin, 2015, Manahan, 2007).

Coagulation is a process in which colloidal particles are destabilized through addition of chemicals and agitation, which clarifies wastewater and reduces turbidity, color, and even the concentration of some pathogenic microorganisms. Factors such as pH, turbidity, agitation speed and time, coagulant dose, and the size of colloidal particles directly influence the size of the clot (Fúquene & Yate, 2018).

Secondary or biological wastewater treatment uses the metabolism of microorganisms to reduce pollutant load (Wiesmann *et al.*, 2007). Populations of bacteria, fungi, or other microorganisms in the wastewater use, in an isolated or synergistic manner, the pollutants present as a source of energy, carbon or electrons in their anabolic or catabolic routes (Fritsche & Hofrichter, 2008). However, these populations will also require adequate physical and chemical conditions for their metabolism and the genetic and enzymatic machinery to use these pollutants (Nielsen *et al.*, 2014; Visser *et al.*, 1977).

Finally, the tertiary treatment improves wastewater quality. Depending on its use, different processes can be conducted, such as bleach disinfecting, nutrient reduction, or chemical precipitation (López & Marín, 2017).

In Costa Rica, 70% of households use septic tanks, while 14.4% use a centralized treatment system (AyA & MINAE, 2017). The most common centralized treatment strategies in the country use activated sludges with pre-, primary, and secondary treatments. In a secondary treatment, microorganisms are used to purify wastewater generated at houses, offices, and public establishments, among others (Ruiz, 2012).

Unlike ordinary wastewater, special or industrial wastewater shows a composition that depends on the productive activity. Such waste usually contains high organic loads and substances that are toxic to the environment and the microorganisms in a conventional treatment system. These chemicals alter the microorganism's metabolism, decreasing the efficiency of the activated sludge plant (Díaz, 2018).

The composition of laboratory waste from academic institutions is complex and varies constantly depending on the activities and courses offered throughout the year, such as general, analytical, organic,

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bio-, and physical chemistry (Bertini & Cicerone, 2009; de Souza & Tenuta, 2010). These residues contain important hazardous materials such as metals, solvents, acids, bases, and oxidants. Their toxicity as a mixture is unknown.

Special chemical waste is cataloged as a dangerous product due to its explosive, corrosive, flammable, or toxic properties. The wastewater generally reaches bodies of water, either by runoff or inadequate management, thus creating a risk to human and environmental health (Carrillo, 2003). Consequently, it is important to monitor the physical, chemical, microbiological and toxicological characteristics of special wastewater after treatment and prior to its disposal.

Bioassays help evaluate the effects of pollutants on organisms. Toxicity tests are useful tools to diagnose the effects of dissolved pollutants on biota, since they evaluate alterations in model organisms. Acute or chronic exposure under standardized laboratory conditions provides information on mortality, inhibition of growth, and effects on the reproduction of organisms. For instance, *Daphnia magna* (water flea) is an ideal organism for toxicological tests due to its small size, wide geographic distribution, rapid population growth rate, ease of handling, and sensitivity to a variety of substances dissolved in water (Suhett *et al.*, 2015).

Wastewater generated at the Omar Dengo Campus of Universidad Nacional (UNA) is discharged into an activated sludge treatment plant. However, the presence of toxic substances in the influents from teaching laboratories has hindered sludge generation and decreased the treatment efficiency of the plant. Herein, we report the characterization and the effects of physical (coagulation-flocculation), chemical (neutralization), and biological (bioremediation) treatment

of laboratory wastewater, followed by the evaluation of leftover toxicity using the *D. magna* bioassay. Bioremediation was conducted using microorganisms isolated after bioprospection with wastewater as the sole energy source. Furthermore, identification of organic substances after bioremediation was performed using gas chromatography, thus generating an inventory of pollutants still present in the wastewater.

Methodology

2.1. Sample collection

The waste used in this study was collected at the School of Chemistry at the Omar Dengo Campus of the Universidad Nacional in Heredia, Costa Rica, in 2017. We collected the aqueous chemical waste generated at the laboratories of General Chemistry, Organic Chemistry, and Physical Chemistry, as well as the Laboratory for Preparation of Reagents. 40 samples, which accounted for 305 L in total, were collected throughout the year. These samples had different composition based on the experiments being performed at a particular time of the semester.

2.2. Physical and chemical treatment

The chemical treatment consisted in addition of calcium oxide to increase pH and precipitate heavy metals. The resulting mixture was decanted, followed by coagulation-flocculation using 1 min of rapid agitation (200 rpm) with coagulant, 1 min of rapid agitation (200 rpm) with flocculant, 3 minutes of slow agitation (50 rpm), and 10 minutes of sedimentation (Aguilar *et al.*, 2002). Due to the difference in chemical composition between samples, each sample was jar tested to select the best coagulants

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and flocculants, their concentration and dose. Polyaluminum chloride (PAC) and Ferrocryl 8751 were identified as the optimal coagulant and flocculant, respectively, and used in all treatments. COD analyses were conducted before and after treatment (American Public Health Association, 2005; Fúquene & Yate, 2018).

2.3. Isolation of microorganisms:

Isolation of microorganisms was performed following the procedures described by Meerbergen *et al.* (2018) and Sanders (2012). Sampling was conducted in two locations: the activated sludge in the wastewater treatment plant and sludge from the distribution box that collects wastewater from the teaching laboratories of the Department of Chemistry, both at the Omar Dengo Campus of Universidad Nacional.

The microorganisms collected were placed on a selective agar plate composed of 15 g/L of Agar-Agar and pretreated wastewater (i.e., wastewater after the coagulation-flocculation process). After a 15-day incubation at room temperature, the strains capable of growing in the presence of the pollutants were isolated and identified using the VITEK®2 automated system from BioMérieux (France) or by DNA sequencing (vide infra).

2.4. Removal tests with strains isolated from the treatment plant:

Removal tests were conducted using a methodology adapted from Kim & Lee (2007), Meckenstock *et al.* (2002) and Wiesel *et al.* (1993). Materials and media were autoclaved at 121°C and 15 psi for 15 minutes. Erlenmeyer flasks containing the chemical water after coagulation-flocculation were inoculated at 10% of the work volume with a bacterial suspension in sterile saline solution, at a concentration of 1 McFarland Standard. Treatments were mounted on an orbital shaker at 150 rpm or an aerating static mixer for 15 days and ran at room temperature in duplicate.

2.5. Removal tests with strains isolated from the distribution box:

Biological treatment systems were prepared with 10 L of pretreated wastewater and processed as in assay 2.4. Each system was inoculated with one of the isolated microorganisms at 100 000 spores/mL or 100 000 CFU/mL and ran by duplicate. One system was not inoculated and served as control. The systems were connected to a continuous aeration setup (bubbling rate of 21.5 L/min) for 30 days. Samples were collected prior to inoculation (day 0) and weekly thereafter. Chemical substances present in the samples were identified at the Laboratory of Pesticide Residue Analysis (LAREP), whereas their toxicity was evaluated using the *D. magna* bioassay at the the Laboratory of Ecotoxicological Studies (ECOTOX).

2.6. DNA extraction, PCR, and sequencing of microorganisms:

200 mg spores were taken from the axenic samples isolated from the distribution box sludge, placed in a sterile Eppendorf tube, and frozen at -20°C. Genomic DNA was extracted at the Laboratory of Plant Biotechnology (LBP) at UNA using a protocol modified from Stewart (1997). Cells were lysed with CTAB, followed by extraction with chloroform:octanol and precipitation with ethanol.

Regions were amplified for molecular identification using ITS1 and ITS4 primers, based on White *et al.* (1990). The PCR

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reaction was performed using the Thermo Scientific DreamTaq PCR Master Mix (USA), following the manufacturer's recommendations with 0.5 µM of each primer and 100 ng of genomic DNA. PCR products were purified and sequenced bidirectionally in Macrogen, Inc. (South Korea) using the Sanger method (Sambrook *et al.*, 2001). Bioinformatic analysis consisted of manual sequence editing and a BLAST (Altschul *et al.*, 1990) analysis of similarity, both done with the Geneious 9.1.8 software (Biomatters, Ltd. New Zealand).

2.7. Chemical analysis of wastewater samples:

Samples were analyzed prior to pretreatment (n=5), after pretreatment and prior to inoculation (n=6), and after biological treatment (n=24). 100 mL of each sample were treated with 20 g of NaCl and extracted with 25 mL of dichloromethane. The organic phase was concentrated until obtaining a final extract of 2 mL in isooctane. The final extract was analyzed an Agilent gas chromatograph 7890 C coupled to a mass spectrometer 5975 C (GC-MS). 1 µL of sample was injected using a split ratio of 2:1, CTC Analytics, PAL System injector and interface were set at 230 and 280 °C, respectively, and a SGE BPX35 column (30 m long, 0.25 mm diameter, and 0.25 µm film thickness) was used. Oven temperature started at 60°C (1 min) and was increased at 10°C/min 300°C (5 min). MS detection was performed using scan mode (total ion current, TIC) with a column pressure of 109.42 kPa.

Mass spectra of the peaks with relative areas greater than 0.5% of the total integrated area were compared with the NIST 17 Mass Spectral Library (NIST/EPA/NIH Mass Spectral Library, 2017).

Peaks were identified based on a spectral similarity greater than 85% compared to the library spectrum.

2.8. Acute toxicity bioassay:

Acute toxicity bioassays were conducted using *D. magna* (Water Flea) based on the protocols of Canada's Environmental Protection Agency Series (EPS, 1990). Daphnia less than 24 hours old were exposed to 25 mL of undiluted samples (100%) and 6 dilutions (50, 25, 12.5, 6.25, 3.125, and 1.56%) of the untreated (n=5) and biologically treated (n=15) samples, in addition to a negative control (hard reconstituted water) and a positive control (0.10 µg Cr^{+6}/mL). Each assay contained 10 Daphnia and was performed by triplicate.

Results

Physicochemical treatment: 305 liters of chemical waste were collected during 2017, between the months of February and November. Variations in properties such as color, odor, pH, and turbidity were observed in the wastewater collected from the laboratories. Values of pH ranged between 0 and 2, while COD ranged between 3000 mg/L and 30 000 mg/L. In all cases, waste was neutralized prior to treatment.

Jar testing was used to determine the best coagulant and flocculant for the chemical and physical treatment of wastewater. Polyvinyl chloride (PAC) and Ferrocryl 8751 allowed the most efficient agglomeration of suspended material, resulting in evident precipitation. The optimal dose was determined as 5.00 mL of PAC at 10% and 3.00 mL of Ferrocryl 8751 at 0.2%.

Isolation of microorganisms: A total of 51 bacterial strains were obtained from the

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sludge collected at the treatment plant, including 29 Gram-negative, 20 Gram-positive, and 2 Gram-variable strains. Only 3 strains grew in the laboratory wastewater-enriched media, which were identified as possible *Bacillus cereus, Bacillus thuringensis,* or *Bacillus mycoides*, with a 94% probability according to the report generated by VITEK®2 system. These 3 strains did not survive the first biological treatment assay, under any agitation method at 150 rpm or direct aeration.

Two fungi and one bacterial strain were isolated from the distribution box sludge. An amplicon of 800 pb was obtained for each strain, which were identified as *Aspergillus* sp. and *Penicillium dipodomyicola* (IIF7SW-F4) with identity percentages of 99.6% and 99.8%, respectively, using BLAST Sequence Similarity Analysis. We were unable to identify the bacteria by VITEK®2 or DNA sequencing.

Chemical analysis: GC-MS analysis of the samples during the 30-day test showed the presence of 55 compounds. Table 1 shows

a selection of 15 substances, these were selected for being present from the beginning of the assay until the end, while others likely arise from the metabolic processes of the microorganisms, thus appearing on days 8, 15, and 22. Phenol, 1-indanone, benzothiazole, benzenesulfonamide, caffeine, and 2-methyl benzimidazole were detected throughout the treatment period (Table 1). The peak areas of benzenesulfonamide and 1-indanone increased with time, while the peak areas of phenol showed the opposite trend with fungi, and bacteria. Triethyl citrate, benzoic acid, and 4-chloro-benzenesulfonamide were identified exclusively in the control treatment at day 30.

As shown in Figure 1, the number of substances present in the control treatment increased after day 15, from 10 to 13 substances. The number of substances present in the samples treated with the bacteria decreased up to 58%, while those treated with *Aspergillus* sp. and *Penicilliun dipodomycola* were removed up to 50%.
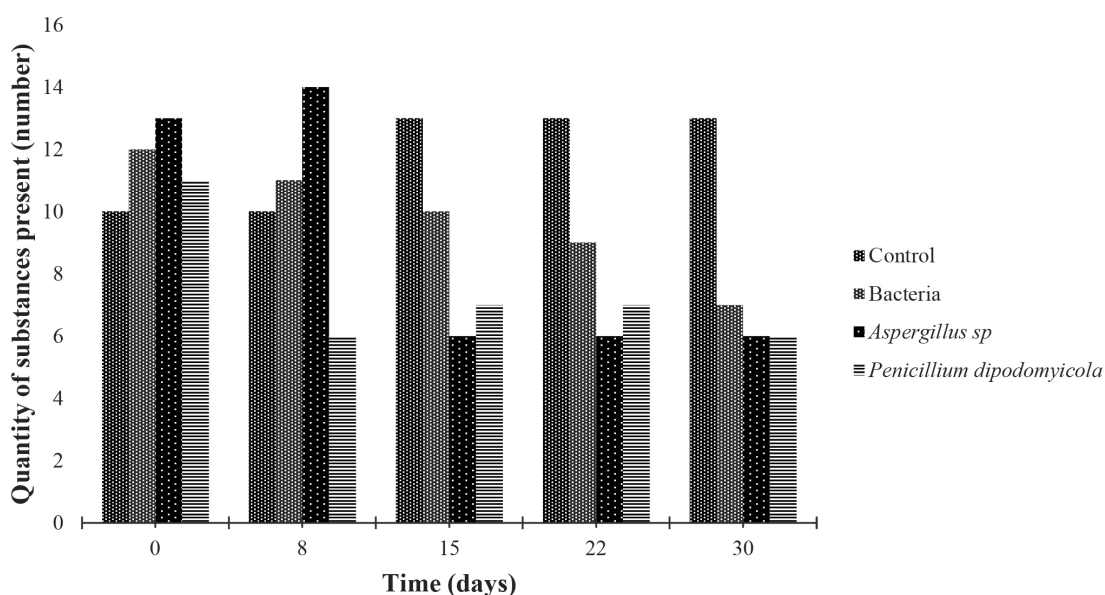

Fig. 1. Trend of the number of substances in treatments with *Aspergillus* sp., *Penicillium dipodomyicola* (IIF7SW-F4), bacteria strains, and control during the 30-day testing period.

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Figure 2 shows biomass formation around the air diffusers used in aerobic treatments inoculated with fungal strains.

Fig. 2. Air diffusers at the end of removal test No. 2. From left to right: *Aspergillus* sp., control, and *Penicillium dipodomyicola* strain IIF7SW-F4.

A total of 55 substances were detected, whereas ***Table 1*** shows those substances that were present consistently during the biological treatment. Some substances, such as octanoic acid, were degraded by microorganisms from day 0. Other compounds, including vainillin, were not present at the beginning, but appeared during the treatment, and thus, were likely produced by microorganisms. The concentration of other substances, such as phenol, decreased over time both in the treated samples and control, and were probably lost due to microorganisms and evaporation.

Table 1. Peak areas (present as 10^6) of 15 compounds, detected with constant presence throughout of the biological treatment, in samples analyzed by GC-MS.

Substance/Day	Aspergillus sp					Penicillium dipodomyicola					Bacteria				
	0	8	15	22	30	0	8	15	22	30	0	8	15	22	30
1-Bromine naphthalene	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.5×10^6	n.d	n.d	n.d	n.d	$6. \times 10^6$	n.d
1-Indanone	n.d	2.5×10^6	0.9×10^6	0.9×10^6	0.6×10^6	n.d	0.6×10^6	0.9×10^6	0.8×10^6	0.7×10^6	n.d	7.6×10^6	1.1×10^6	1.1×10^6	9.8×10^6
2-methyl benzimidazole	n.d	1.7×10^6	0.4×10^6	0.4×10^6	0.3×10^6	n.d	n.d	0.4×10^6	0.4×10^6	0.4×10^6	n.d	0.6×10^6	0.3×10^6	0.5×10^6	5.3×10^6
3-hydroxy-4-methoxybenzaldehy	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
4-chloro-benzene-sulfonamide	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Benzoic acid	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Dodecanoic acid	2.8×10^6	n.d	n.d	n.d	n.d	1.1×10^6	n.d	n.d	n.d	n.d	1.4×10^6	n.d	n.d	n.d	n.d
Octanoic acid	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.9×10^6	0.3×10^6	n.d	n.d
Benzenesulfamide	3.8×10^6	24×10^6	5.9×10^6	6.8×10^6	5.1×10^6	3.7×10^6	5.5×10^6	6.8×10^6	6.7×10^6	5.2×10^6	4.0×10^6	5.1×10^6	5.9×10^6	$7. \times 10^6$	6.4×10^6
Benzothiazole	n.d	1.6×10^6	0.5×10^6	0.4×10^6	0.3×10^6	n.d	0.5×10^6	0.6×10^6	0.5×10^6	0.4×10^6	n.d	0.5×10^6	0.3×10^6	$4. \times 10^6$	3.8×10^6
Caffeine	0.4×10^6	2.8×10^6	0.6×10^6	0.7×10^6	0.6×10^6	4.5×10^6	0.6×10^6	0.7×10^6	0.7×10^6	0.8×10^6	0.4×10^6	0.5×10^6	0.6×10^6	7.0×10^6	7.0×10^6
Phenol	7.4×10^6	2.2×10^6	9.3×10^6	5.6×10^6	2.1×10^6	9.7×10^6	9.1×10^6	8.7×10^6	6.2×10^6	3.2×10^6	10×10^6	8.7×10^6	8.9×10^6	6.0×10^6	3.4×10^6
N-phenyl acetamide	n.d	n.d	0.4×10^6	0.4×10^6	n.d	n.d	n.d	0.4×10^6	0.4×10^6	n.d	n.d	n.d	0.6×10^6	0.6×10^6	5.6×10^6
Triethylcitrate	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Vanillin	n.d	1.3×10^6	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.3×10^6	n.d	n.d	n.d

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Table 2. Results of acute toxicity bioassays with *Daphnia magna* exposed to wastewater diluted 98 times.

Treatment	Day 0		Day 8		Day 15		Day 21		Day 30	
	Percentage of mortality in 24 and 48 h assays									
	24	48	24	48	24	48	24	48	24	48
Aspergillus sp.	27	70	60	83	3	90	33	47	20	43
P. dipodomyicola	3	23	23	63	10	70	17	27	33	50
Bacteria	23	83	50	83	0	3	10	20	7	67
Control	20	80	50	73	0	10	63	80	33	100

Acute toxicity bioassay: Untreated and biologically treated samples were toxic to *D. magna*. All organisms died before 24 hours of exposure. Table 2 shows the death rate of water fleas exposed to the lowest dilution tested (1:98) for 48 hours.

Discussion

Although teaching laboratories generate a lower volume of waste than industrial laboratories, they present similar risks (Bertini & Cicerone, 2009; de Souza & Tenuta, 2010). The maximum COD value obtained in the samples from the Universidad Nacional was 30 000 mg/L, which exceeds the average values reported in different industrial sources, such as the meat (7000 mg/L) and brewing (5000 mg/L) sectors, or landfill leachates (16 000 mg/L) (Houbron *et al.*, 2002; López *et al.*, 2017).

The 55 chemical substances detected in the wastewater samples belong to different groups, including fatty acids, aromatic organic compounds, reducing agents, strong oxidants, and amides, among others. Organic chemistry laboratories generate a wide variety of compounds, such as phenols, alcohols, ketones, and esters. For instance, we identified the presence of vanillin, a phenolic aldehyde considered to be antioxidant, antimicrobial and anti-inflammatory, inhibits the growth of Gram-positive and negative bacteria, as well as fungi and yeast (Arroyo, 2016). On the other hand, heavy metal residues are common in analytical laboratories, due to technical and economical constrains, heavy metals could not be quantified.

The presence of aromatic organic compounds in the chemically treated wastewater can explains why 94% of the strains isolated from the treatment plant did not grow, as well as the high mortality of *D. magna* observed in the toxicity tests (Ghosal *et al.*, 2016; Musa *et al.*, 2017; Gami *et al.*, 2014). Cyclic aromatic compounds, such as phenol, are toxic, mutagenic, genotoxic, and carcinogenic. Only certain microorganisms have the potential to survive and degrade them. Degradation depends on the growth of microorganisms, which highly depends on the degree of acidity and incubation temperature (Gami *et al.*, 2014). It also depends on the disposition of nutrients and the chemical properties of the compound. Having these elements as a reference, it can be inferred that the remaining 6% of the strains have the potential to be used in the secondary treatment. Authors like Sakthipriya *et al.*, 2015; Saleem *et al.*, 2014; Wassie *et al.*, 2017, have shown the potential of bacteria of the genus *Bacillus* spp. as bioremediators. It is also important to consider the possible presence of heavy metals in the sample. They were detected in the sludge resulting from the primary treatment but were not monitored in the chemical waste samples.

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Variation in the chemical composition of chemical waste affected the survival capacity of the three strains of *Bacillus*, possibly due to their lack of enzymatic mechanisms or adaptations at the membrane level to allow them to make use of these toxic compounds (Murínová & Dercová, 2013). This does not rule out their usefulness to treat other types of contaminated soil or water.

According to Gami *et al.*, 2014, microbial degradation is a useful strategy for treating wastewater and eliminating organic compounds such as phenol, since yeast, bacteria, and fungi use these compounds as a source of carbon and energy. Other authors such as Bosso & Cristinzio, 2014; Kim & Lee, 2007; Leitão, 2009 have reported that strains of the genus *Penicillium* spp. can degrade xenobiotic compounds such as hydrocarbons and aromatics, some of which are present in the samples. Authors like Gulzar *et al.*, 2017; Jurkovic & Simonovicova, 2013; Mukherjee, 2016 report that *Aspergillus* can be used for the treatment of contaminated soil and water, since it can also metabolize these types of molecules. This is achieved by the enzymatic reactions of oxidation and peroxidation, which adds functional groups to the rings through monooxygenases and peroxidases characteristic of their metabolism (Leitão, 2009). This explains the capacity of the strain *Aspergillus* sp. and the strain *P. dipodomyicola* IIF7SW-F4 isolated in this investigation of removing up to 50% of the pollutants in the samples.

Although the samples were toxic for *D. magna* in all the treatments, at the end of the experiment the percentage of mortality decreased at 48 hours between the control treatment (100%) and the bacterial (67%), *P. dipodomyicola* (50%), and *Aspergillus* sp. (43%) treatments. The number of substances in the samples also decreased at the end of the treatments. Toxicity in mixed chemical substances depends on the interaction between the components, and the resulting effect can be additive, synergistic or antagonistic. Although the combined toxic effect of the chemical substances was not studied here, results suggest that it could be greater than that of each of them individually, since mortality was high (40-100%) in dilutions lower than 2%. Some of the substances detected in the samples are toxic to aquatic invertebrates and vertebrates: octanoic acid (EC50, 48 h *D. magna* 13.4 mg/L), phenol (EC50; 48 h *D. carinata* 7mg/L, *Simocephalus vetulus* 37 mg/L, *D. magna* 27.9 mg/L and LC50, 96 h *Oncorhynchus mykiss* 13.0 mg/L), triethyl citrate (LC50; 48 h *Leuciscus idus* 450 mg/L and vanillin (EC50; 48 h *D. magna* 36.79 mg/L, and LC50; 96h *Pimephales promelas* 57mg/L) (Vainillina 7887, 2015; Triffault-Bouchet *et al.*, 2005).

The aromatic organic compounds detected in the samples and the possible presence heavy metals could be the cause of the mortality of *Daphnia magna*. Phenol was present in all samples until the end of the experiment. Authors such as Musa *et al.*, 2017 and Gami *et al.*, 2014 claim that organic compounds such as phenol in water at very low concentrations are toxic to aquatic species such as fish, algae, and microcrustaceans and that toxicity is associated with the ability to alter the cell membrane structure and its barrier, which produces an imbalance in the cell environment and eventually death.

Conclusions

Although the results of this research paper highlight the potential of microorganisms as bioremediators, further experiments are required for longer periods of time with these and other strains in aerobic and anaerobic conditions.

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It is important to reduce or eliminate the use of toxic substances in laboratory practices in order to avoid the environmental problems caused by the waste generated in teaching activities.

Chemical and biological treatments of wastewater should be accompanied by ecotoxicological assessment to ensure the protection of aquatic biota.

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