Chapter 15

Phenolic Compounds Removal in Woodwaste Leachate by a Trickling Biofilter

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Additional information is available at the end of the chapter

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Abstract

Woodwaste produces large volumes of leachate, which often contains high concentrations of phenolic compounds. These compounds necessitate appropriate management. Biological methods are efficient, innovative, and economic. In particular, biofiltration process has various advantages compared to CAS and MBR technologies. Two pilot filters, with and without biological activity, were designed for continuous mechanisms to follow. This study supposes that the three mechanisms of volatilization, sorption, and biodegradation are present, confirm these assumptions, and determine the contribution of each mechanism. Good efficiency was obtained in the biofilter and 97–98.2% of COD and BOD removal were observed, respectively. Excellent performances were achieved and reached 99.9% of initial concentrations removal for all the phenolic compounds.

Keywords: phenolic compounds, woodwaste leachate, sorption, volatilization, biofiltration, trickling biofilter

1. Introduction

The industry of paper is one of the principal economic sectors in Quebec. In 2011, 56.4% of total canadian production of paper was achieved by Québec and was evaluated at 14.9% of total world exportations [1]. In this year, approximately 373 million cubic meters were generated as water process and were discharged in the environment after only basic physicochemical treatment.

In 2006, the value of shipments of paper was 10.7 billion or 7.5% of the value of shipments of all Quebec's manufacturing industry. The value of exports in 2005 was estimated at 7.3 billion or 9.7% of all exports of Quebec and contributes to 46.1% of total newspaper production in Canada and 9% of global paper production.

Thus, considerable quantities of wood residue are generated, which produce large volumes of leachate by percolation of water and following industrial processes. Woodwaste leachate contains lignin and phenolic compounds at important concentrations and must be treated before its discharge in the environment.

Phenolic compounds are toxic to humans and ecosystem, persistent, and endocrine disruptor molecules. They are found in woodwaste leachate and pulp and paper industrial effluents. It is necessary to develop new and innovative technologies adapted for these toxic effluents' treatment and phenolic compounds' removal. Several processes were studied for phenol removal such as membrane processes, ozonation [2], advanced oxidation [3], and activated carbon [4]. Ozonation was proven to be effective but it requires energy, and produces gases and causes the formation of not yet identified by-products with an important risk to human health [5–8]. As phenolic compounds are persistent, it might be suitable to combine ozonation with biological treatment [9].

Biological methods are efficient, innovative, and economic. Biological treatment of organic pollutants was investigated in conventional activated sludge treatment plants (CAS) and in membrane bioreactors (MBR). Mailler et al. [10] compared emerging pollutants removal by both biofiltration and conventional activated sludge plants. This classical biological unit (CAS) has already been well documented and compared with membrane bioreactor [11–18]. No significant difference was observed between CAS and MBR when a critical solids retention time (SRT) necessary for nitrification (SRT > 10 days at 10°C) was exceeded and the suspended solids concentration in the effluent was low for CAS plants [19].

Biofiltration process has various advantages compared to CAS and MBR technologies [20, 21]. It is an economical, simple, and innovative solution for water and air pollution control [20, 22, 23].

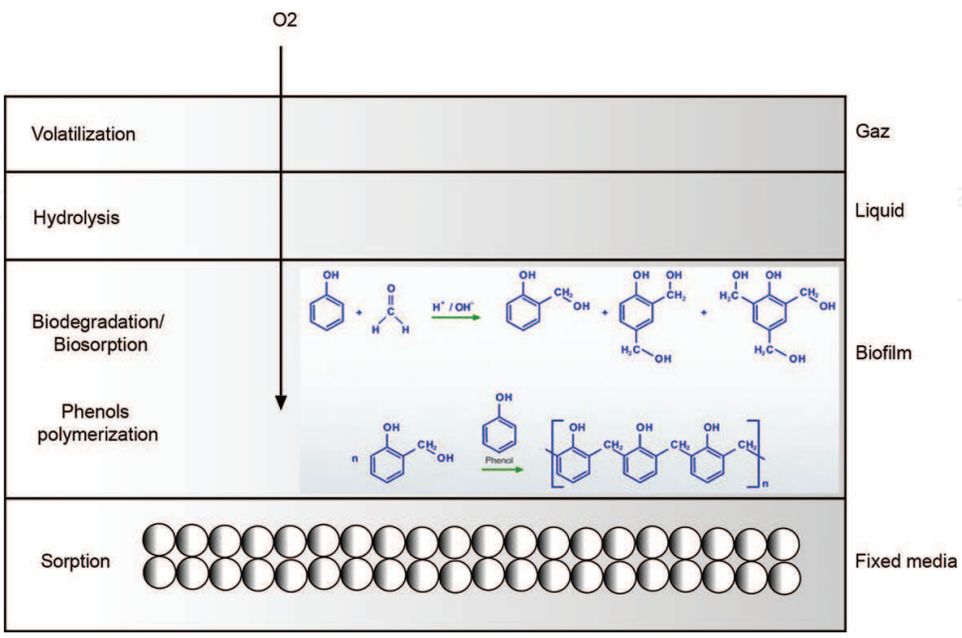

Figure 1. Possible mechanisms in operation during biofiltration process.

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Phenolic compounds removal by biofiltration was investigated by [9, 24] but the involved mechanisms of this removal were not described before.

This work aims to study phenolic compounds removal in woodwaste leachate using a trickling biofilter and, second, to describe the mechanisms responsible for their transformation and removal. In otherwise, we aim to answer the following questions: Are phenolic compounds eliminated by volatilization, biodegradation, sorption, and biosorption or by all of these processes, and what is the contribution of each mechanism?

This study supposes that the mechanisms of volatilization, sorption, and biodegradation operate simultaneously during biofiltration process. We aim at confirming these assumptions and determine the contribution of each mechanism to the overall phenols removal efficiency (**Figure 1**).

2. Material and methods

2.1. Pilot scale unit and experiment set up

Two PVC columns of 0.15 m diameter and 2 m high were packed with a solid support (peat: perlite; 1:9) and used to follow phenols removal in woodwaste leachate. These units (**Figure 2**) are equipped with utilities, which are a storage leachate system composed of two mini-

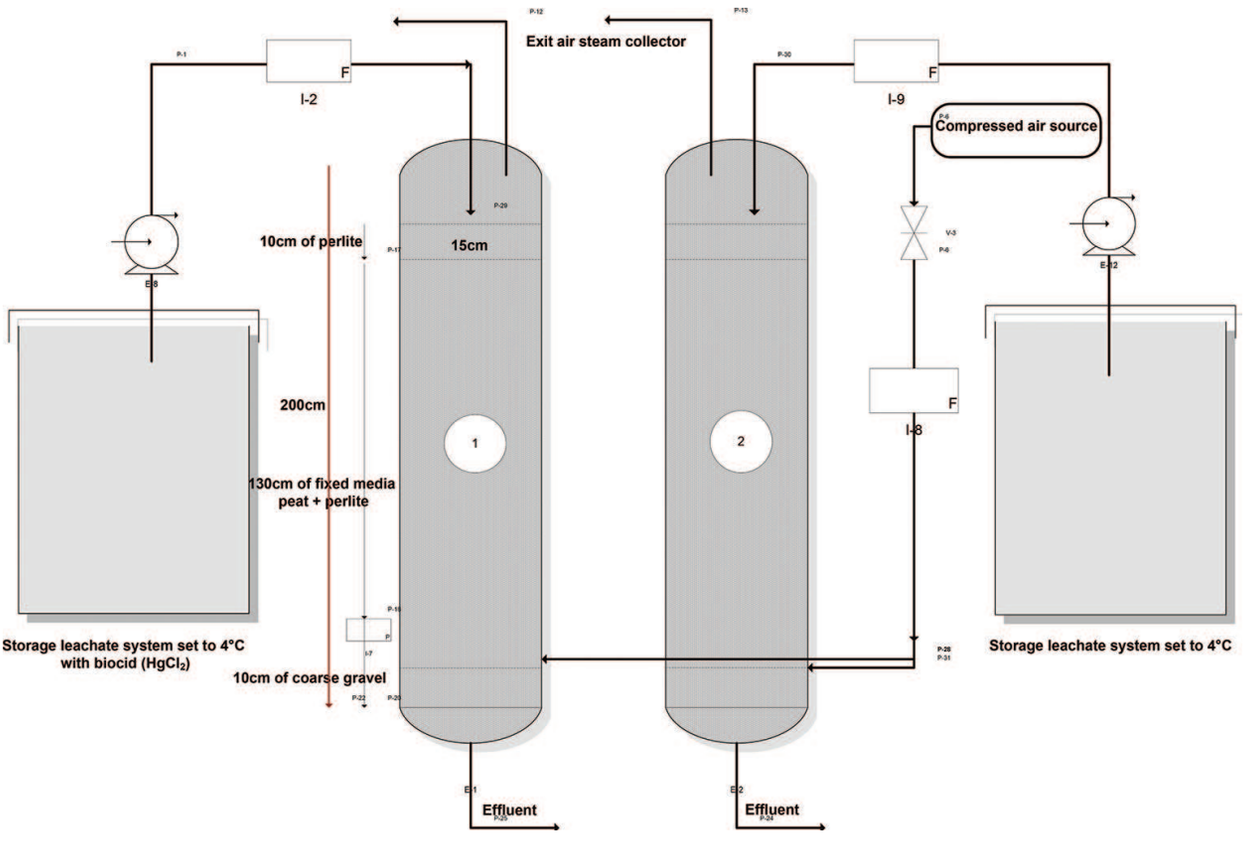

Figure 2. Pilot scale unit for phenolic compounds removal study.

refrigerators set to 4°C and aeration system connected to compressed air source with copper tubes in T-shape and connected to the bottom of each column using rigid tubes after passing in control air flow rate system. Columns are also equipped with exit air steam collector composed of four bottles containing each 100 ml of methanol, connected in series for each column via T-shape connection with the out vacuum evacuation. The output air steam was collected for 4 hours twice a week and if the air steam collection was not in operation, the valve connected to the out vacuum evacuation was opened. Woodwaste leachate was fed continuously to columns via Masterflex tubes at the top using a peristaltic pump, simulating trickling filtration.

2.2. Media composition and preparation

Columns were packed with solid support consisted of a mixture of peat (70% with 0.5 mm) and perlite (4–6 mm) at a 1:9 ratio. After characterization, the peat was dried at 103°C during 48 h to minimize bacterial activity. A layer of coarse gravel with 0.10 m of height was installed first at the bottom of the two columns and then these were packed with the media at a layer height of 1.30 m as described in **Figure 1**. A layer of perlite with 0.10 m of height was installed at the top of the two columns. For the conditioning, columns were fed slowly with tap water (1.5–2 h) in an ascending way to reach a maximum absorption capacity of water by peat [25], a good consistency of media and a resistance to compaction. The conditioning of columns removes also the retained air in the system.

2.3. Laboratory scale unit set up

Two mini-columns with 0.12 m of diameter and 0.20 m of height were set up as described in **Figure 3** to simulate the top of pilot columns and to follow efficiency and parameters variability with time at the top of the pilot scale units.

2.4. Leachate

Woodwaste leachate was collected from a sawmill site in the Quebec City area and characterized. This effluent is a complex matrix with highly variable chemical composition. Concentrations of phenolic compounds depend on the temperature, age, and industrial activities of the region, in particular, those of sawmills and paper industries. Physicochemical characteristics are given in **Table 1**.

2.5. Biomass acclimation

Activated sludge from the sewage treatment station Valcartier in Québec City area was used as a source of microorganisms for biomass acclimation. Volatile fatty acids (VFAs) and phenol were used as the carbon source for microorganisms. The acclimation was conducted progressively to avoid shock to the microorganisms due to the toxicity and to allow the biomass growth. At first, only VFAs were used as carbon source and were replaced gradually by phenol. **Table 2** shows the detailed composition for acclimation process. A synthetic mixture

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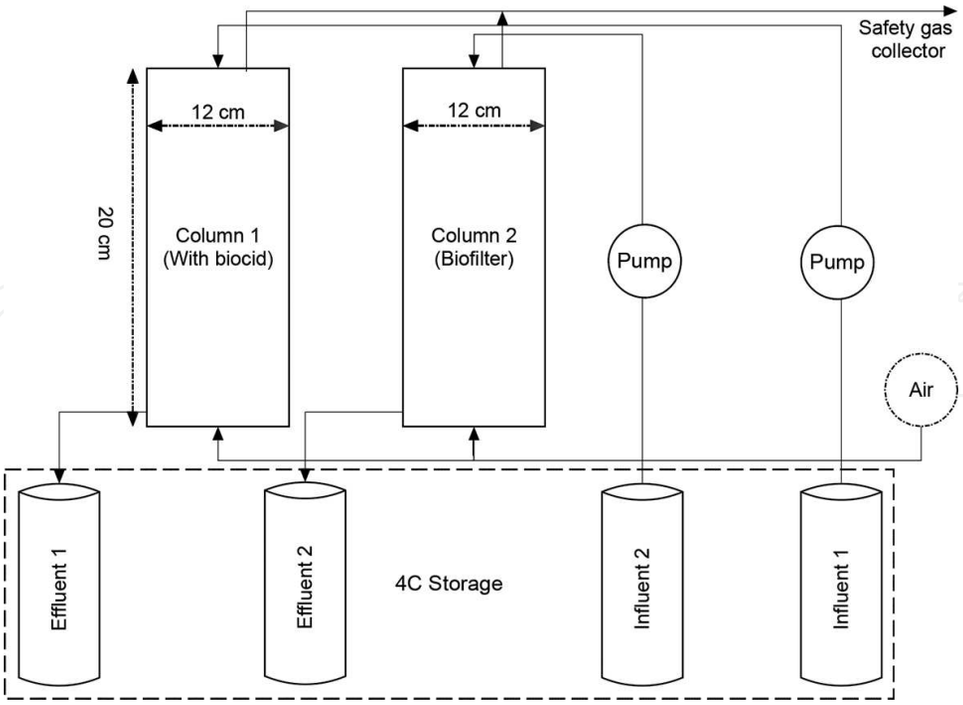

Figure 3. Laboratory scale unit simulating the top of pilot columns to follow phenolic compounds removal.

Parameter (mg L^{-1})	Winter 2006	Summer 2006	Winter 2007	Winter 2008	Autumn 2008	Summer 2009
pH	4.50	4.62	4.48	4.60	4.73	4.45
COD	1400	1100	1600	1700	1100	3792
BOD_5	600	400	750	760	450	1862
MES	415	850	410	480	600	1400
PO4	5.00	5.20	5.10	4.89	5.00	4.30
Total volatile fatty acids	80	50	120	150	40	425
Total phenolics (μg L^{-1})	1946	1625	2010	2080	1540	3810
Phenol	380.86	77.99	450.12	437.25	81.55	80.75
4-Nitrophenol	171.41	160.79	282.05	291.04	158.43	712.25
p, m-Cresol	111.55	95.28	107.38	117.28	88.97	1503.12
o-Cresol	133.86	77.92	141.57	139.50	99.75	801.15
2-Chlorophenol	177.23	175.57	191.35	211.47	178.98	162.17
2,4-Dinitrophenol	1102.03	35.75	78.15	81.75	28.67	576.85
2, 4-Dimethylphenol	3.87	1.35	4.07	5.73	1.55	239.72

Note: Total phenolic compounds were determined by spectrophotometric analysis. Individual phenolic compound values reported in μg L^{-1}.

Table 1. Physicochemical characterization and phenolic compounds quantification of woodwaste leachate.

		Acclimation steps				
		1	**2**	**3**	**4**	**5**
	V added (mL)	**3**	**3**	**2**	**1**	**0**
AGV	mg C	109.92	109.92	73.28	36.64	0.00
	(% C)	100.00	96.63	90.54	76.13	0.00
	ppm	0	2	4	6	8
Phenol	mg phenol	0	5	10	15	20
	V added (mL)	0	5	10	15	20
	mg C	0	3.83	7.66	11.49	15.32
	% (C)	0	3.37	9.46	23.87	100
Total	mg C	109.92	113.75	80.94	48.13	15.32

VFAs: 80 g L^{-1} (36.64 g-C/L); phenol: 1 g L^{-1} (0.766 g-C/L); nutrients: 65.86 g L^{-1} (0.052 g-N/L); volume of reactor: 2.5 L.

Table 2. Acclimation of biomass steps.

of the eight studied phenolic compounds was given as substrate when the biomass was acclimated and then the real woodwaste leachate was used for the biomass growth before use in the biofilter. The biomass growth and substrates utilization were followed by respirometric measurements during all the acclimation process.

2.6. Experiments

The two pilot-scale columns were followed for 6 months under continuous flow conditions. The liquid flow rate was maintained at 3 Ld^{-1}. Woodwaste leachate percolated filters with a hydraulic load of 0.169 $m^3\ m^{-2}\ d^{-1}$. Air was applied upward at a flow rate of 5 $m^3\ m^{-2}\ h^{-1}$. Columns were fed with real woodwaste leachate, which presented a pH between 4.5 and 4.7 spiked to 1 μg mL^{-1} with eight phenolic compounds and operated at room temperature (20–25°C). A flow sheet of the pilot scale unit is described in **Figure 2**.

2.6.1. Pilot 1 (with mercuric chloride)

The bacterial activity was inhibited in this column using mercuric chloride ($HgCl_2$) at 0.5 g L^{-1} in the fed leachate. Only physicochemical retention by sorption and volatilization are in operation and responsible for phenolic compounds removal in this column. Liquid influent and effluent were sampled each day for 6 months to establish the parameters variation profiles. pH and COD were determined daily for the first 3 months and each 2 days from the 4th month. Phenolic compounds were quantified each 2 days in the liquid influent and effluent and twice a week in the collected steam during all the operation period. Parameters profile variations are presented in **Figures 4** and **5**.

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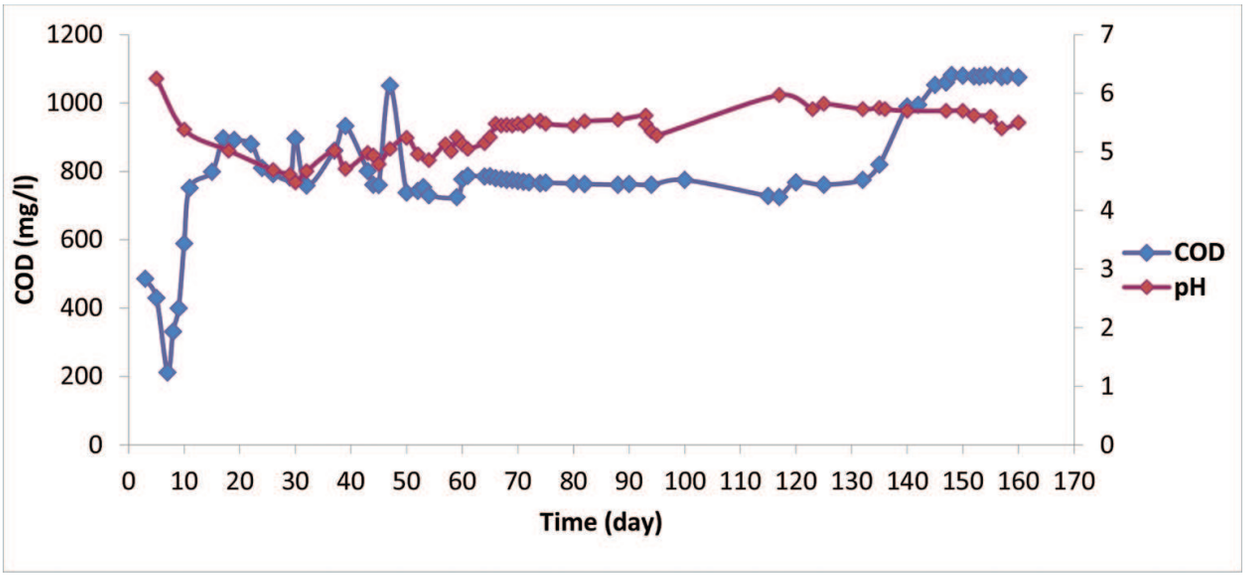

Figure 4. Physicochemical parameters variation in the effluent of column 1 (without bacterial activity).

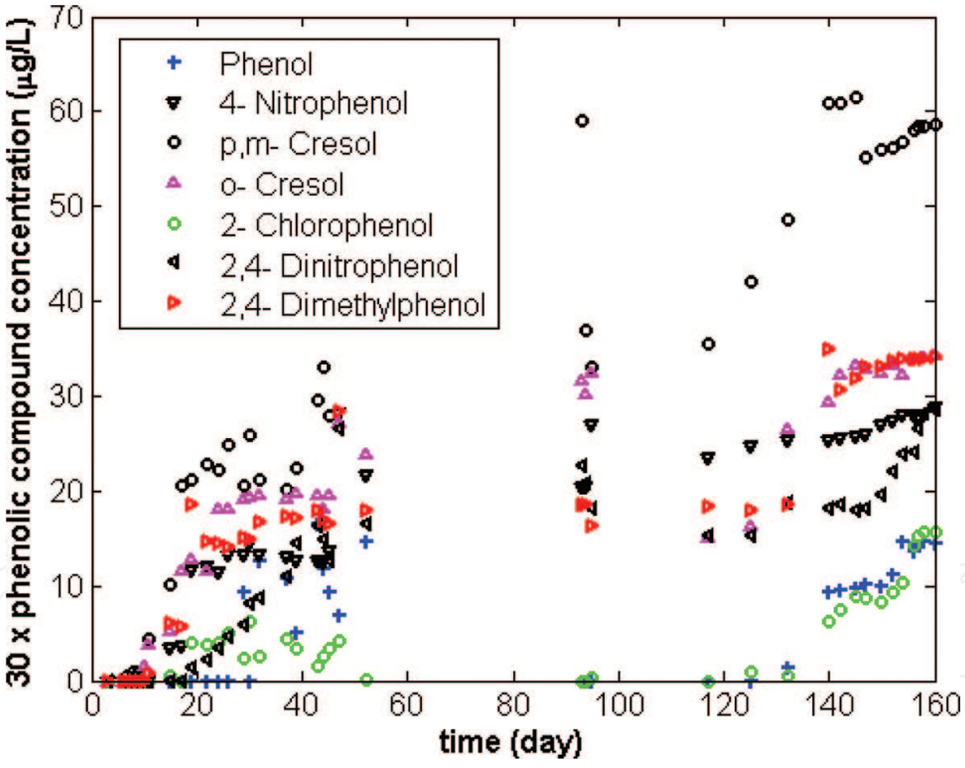

Figure 5. Phenolic compounds profiles variation in the column 1 (without biological activity).

2.6.1.1. Media without biological activity characterization

Both conventional scanning electron microscopy (SEM) and environmental scanning electron microscopy (ESEM) characterized media of pilot 1, with mercuric chloride. The environmental SEM offers all of the performance advantages of a conventional SEM, but allows high resolution and the analysis of wet and environmental samples in their current state without preliminary manipulations. Analysis was performed on JEOL 840-A (MEB) instrument for conventional

SEM and on JSM-6360 instrument at low voltage (30 V of acceleration voltage) and with different vacuum values for ESEM.

2.6.2. Pilot 1 (with mercuric chloride)

This pilot simulates the real behavior of a trickling biofilter. The mechanisms of biodegradation, sorption, and volatilization are to be present and in operation during biofiltration. For this column also, phenolic compounds were quantified each 2 days in the liquid influent and effluent and twice a week in the collected steam during all the operation period. The parameters that were monitored in the liquid influent and effluent were BOD, COD, pH, Nitrogen, and phenolic compounds and measured once a week for BOD, and each 2 days for all the other parameters during all the operation period. VFAs were determined sporadically. Parameters profile variations for the biofilter are presented in **Figures 6** and **7**.

2.6.2.1. Biomass characterization

Microbial aspects of fixed biofilm at different biofilter layers were shown by both conventional SEM and ESEM imaging performed on JEOL 840-A (MEB) and JSM-6360 instruments, respectively.

Biological characterization of bacterial diversity in the mixed biofilm was performed by DGGE of the 16 S ribosomal RNA genes molecular technique. PCR-DGGE is a rapid and sensitive method for the detection and investigation of active species in fixed media based on the DNA fragments extraction. Amplicons, with the same length, of dominant microbial organisms are separated in gradient gel based on their differential denaturation process and nucleotide compositions. Two samples were collected by coring the media, during and at the end of experiments. The first coring was done in the middle of the column. The second coring extraction was realized at eight different level heights of the biofilter. Gel preparation and analysis were carried out according to the protocol established by Ercolini [26] and run at Dr Duchaine's research laboratory at Centre de Recherche de l'Institut Universitaire de Cardiologie et de Pneumologie de l' Hôpital Laval (Québec, Canada).

2.6.3. Pilot 1 (with mercuric chloride)

The two laboratory-scale columns were set-up and followed for 4 months under continuous flow conditions. Real woodwaste leachate at pH of 4.5–4.7 and spiked with 1 μg mL^{-1} of eight phenolic compounds was percolated under the same conditions applied to the pilot unit: hydraulic loading of 0.169 $m^3\ m^{-2}\ d^{-1}$ and flow rate aeration of 5 $m^3\ m^{-2}\ h^{-1}$. These laboratory units were followed to reproduce the top of pilot-scale columns and to describe the phenolic compounds removal at the top of these columns. A flow sheet of this mini-columns setup is described in **Figure 3**.

2.7. Parameters analysis

Analyses of phenolic compounds were performed on a Breeze HPLC system (Waters ©) consisting of a 717 plus auto sampler, a 1525 binary pump with internal degasser, and a code

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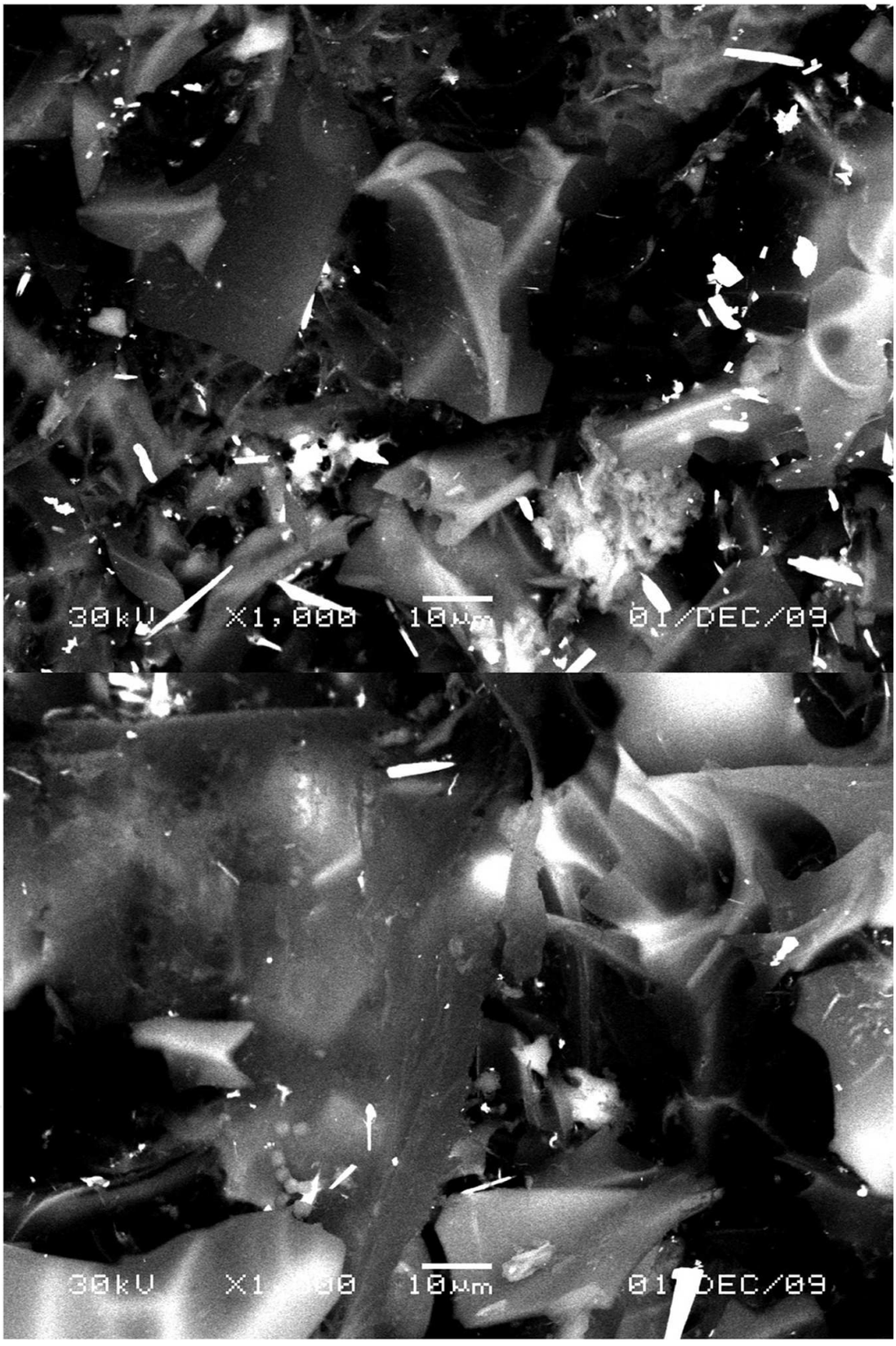

Figure 6. SEM and ESEM imaging of media of pilot 1 (with mercuric chloride).

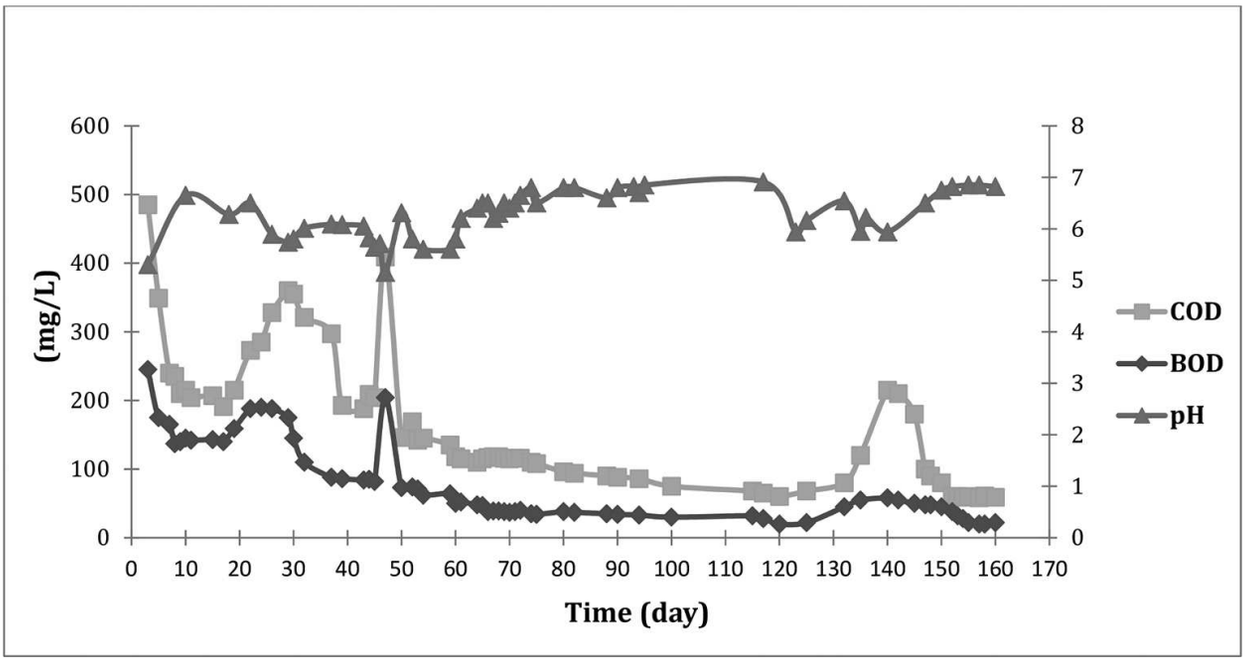

Figure 7. Physicochemical and biological parameters variation in the effluent of column 2 (biofilter).

model 5CH column oven. The separation was carried out on a Symmetry C_{18} (4.6 × 150 mm, 5 μm) column and detection by a 2487 dual wavelength detector. Samples were previously acidified to pH between 1.8 and 2, filtered on 0.45 μm membrane filter, concentrated by SPE on Oasis HLB (60 mg) cartridges and then analyzed by liquid chromatography according to the method described by Kamal et al. [27].

COD and nitrogen were determined according to standard methods using a DR3000 spectrophotometer. BOD was measured using a standard BOD Track system and a polyseed as bacterial consortium.

3. Results and discussion

3.1. Woodwaste leachate characteristics

Table 1 shows the real leachate physicochemical characterization and phenolic compounds quantification from winter of 2006 to summer of 2009. Phenols were found at concentrations varying between 0.38 μg mL^{-1} for phenol and 1.1 μg mL^{-1} for 2,4-dinitrophenol in real woodwaste leachate on winter of 2006.

3.2. Pilot 1 (with mercuric chloride) results

COD drastic decrease was observed in the effluent during the first 10 days of operation. Note that 84.41% of initial COD was removed during this operation period. From the second week,

a removal performance of 41.1% was obtained. A steady state with 43.82% efficiency was then reached and continued until the saturation of the media on the 135th day.

3.2.1. Phenolic compounds profiles variation in the column 1 (without biological activity)

For phenolic compounds also, a significant decrease was observed in the effluent during the first 10 days of operation. A removal performance of 33.33–40.1% was obtained at steady state for all the phenolic compounds except phenol and chlorophenol for which, 40.2–83.33% of initial concentrations was removed. On the other hand, 41.1% of phenol and chlorophenol initial concentrations was found in the collected gas. Thus, in this column, both physicochemical retention and volatilization are responsible for phenolic compounds removal. At the saturation of the media on the 135th day, a maximum of volatilization was obtained and reached 41.1% for more volatile compounds.

3.2.2. Media without biological activity characterization results

Both conventional scanning electron microscopy (SEM) and environmental scanning electron microscopy (ESEM) characterized media of pilot 1, with mercuric chloride. Images are presented in **Figure 6**. No bacterial activity was observed. It was noted that metal spots were present. The X-ray analysis confirmed a presence of mercury (Hg).

3.3. Pilot 2 (biofilter) results

A significant decrease was observed in the effluent during the first 10 days of operation for both COD and BOD. These parameters increased during the second and third week. This can be explained by a resistance and often observed inhibitory effect of organisms during the acclimation process. Good performances of the biofilter were obtained after 30 days. On the 47th day, the system received a high organic load and toxicity due to a periodic increase at the sawmill providing the effluent. However, the biofilter was able to manage this organic shock and fluctuations and to achieve excellent performances at steady state. Indeed, 97–98.2% of COD and BOD removal were observed, respectively. Another increase of the measured parameters was observed on the 135th operation day. This can be explained by the saturation of the media and biological phenomena become a predominant and responsible factor for the treatment. The system, in this situation also, was able to manage the change of physicochemical retention capacities of the media and to achieve the performances of the previous steady state in few days. **Figure 7** shows 59 and 22 mg L^{-1} values for COD and BOD, respectively, with superior performances in reducing organics (97% COD and (98.2% BOD reduction)).

3.3.1. Phenolic compounds profiles variation in the biofilter

Phenolic compounds profile variation, presented in **Figure 8**, followed absolutely COD and BOD variations in the biofilter. Indeed, a significant decrease was observed in the effluent during the first 10 days of operation and performance removal of 99.9% was obtained at steady state for all the phenolic compounds. A maximum of 5–7% of volatilization was observed for phenol and chlorophenol in the biofilter.

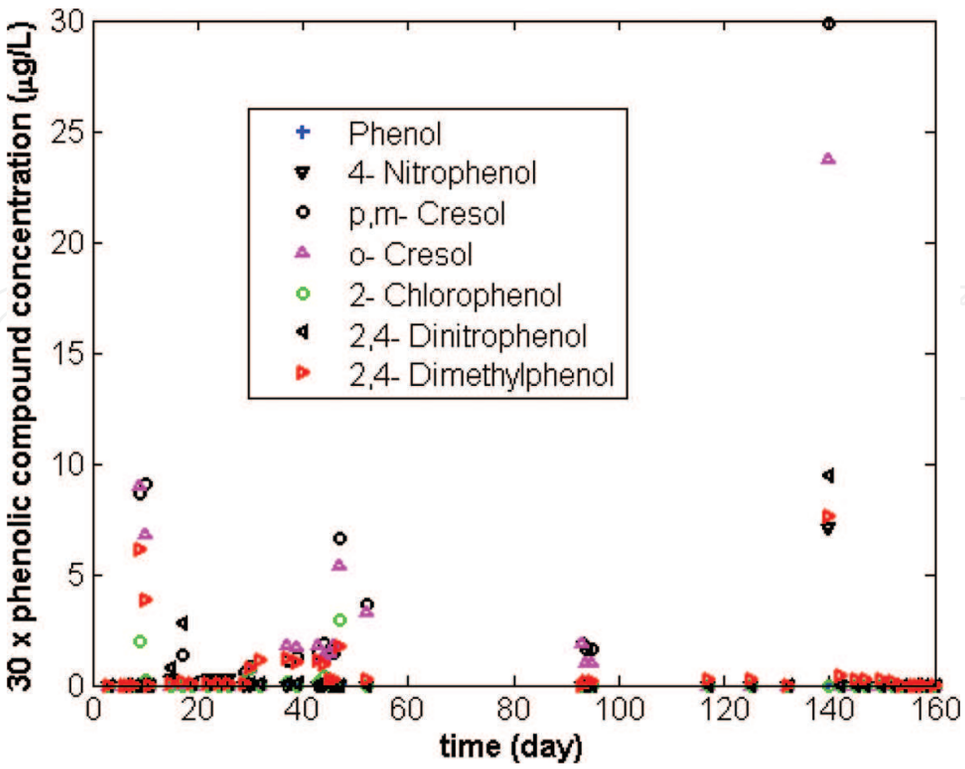

Figure 8. Phenolic compounds profiles variation in the column 2 (biofilter).

3.3.2. Biomass acclimation results

Table 2 shows the detailed steps for acclimation process. Activated sludge from the sewage treatment station Valcartier in Québec City area was used as a source of microorganisms for biomass acclimation. Volatile fatty acids (VFAs) and phenol were used as the carbon source for microorganisms.

3.3.3. Biomass characterization results

Both conventional SEM and ESEM imaging showed microbial aspects of fixed biofilm at different biofilter layers. SEM and ESEM images are given in **Figure 9**.

A sampling at different column heights was done, by coring of the biofilter in the last week of operation period. **Figure 10** shows the column sampling. Media samples were analyzed by DGGE technique with gel sequence and sequences amplified to determine the composition of the bacterial community. Samples 7 and 8 represent the 10 cm perlite layer at the top of the biofilter. Results of DGGE banding patterns of 16S rDNA fragments presented by **Figure 11** show the presence of generally similar bacterial species. Two intense bands present in perlite layer disappeared in the depth of biofilter to be replaced by other bands. This may be explained by the development of other microorganisms in the media mixture of peat and perlite. The bands representing the different heights of the mixed media are in general similar; this indicates that the same active species are present in all the peat/perlite mixture height of the biofilter and are involved in the phenolic compounds biodegradation process. The list of

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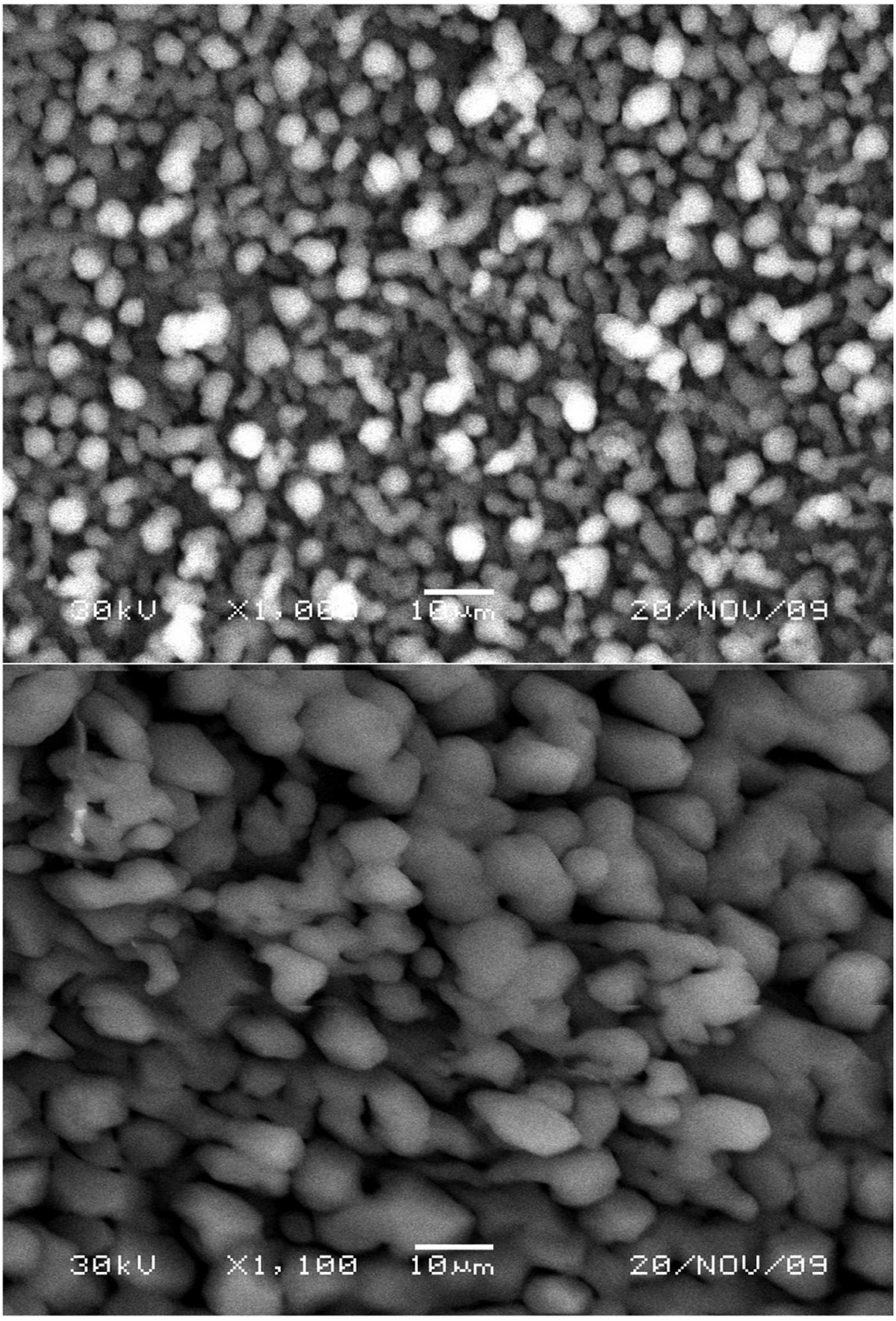

Figure 9. SEM and ESEM imaging of microbial aspects of fixed biofilm at different biofilter layers.

Figure 10. Media samples from different heights of the biofilter.

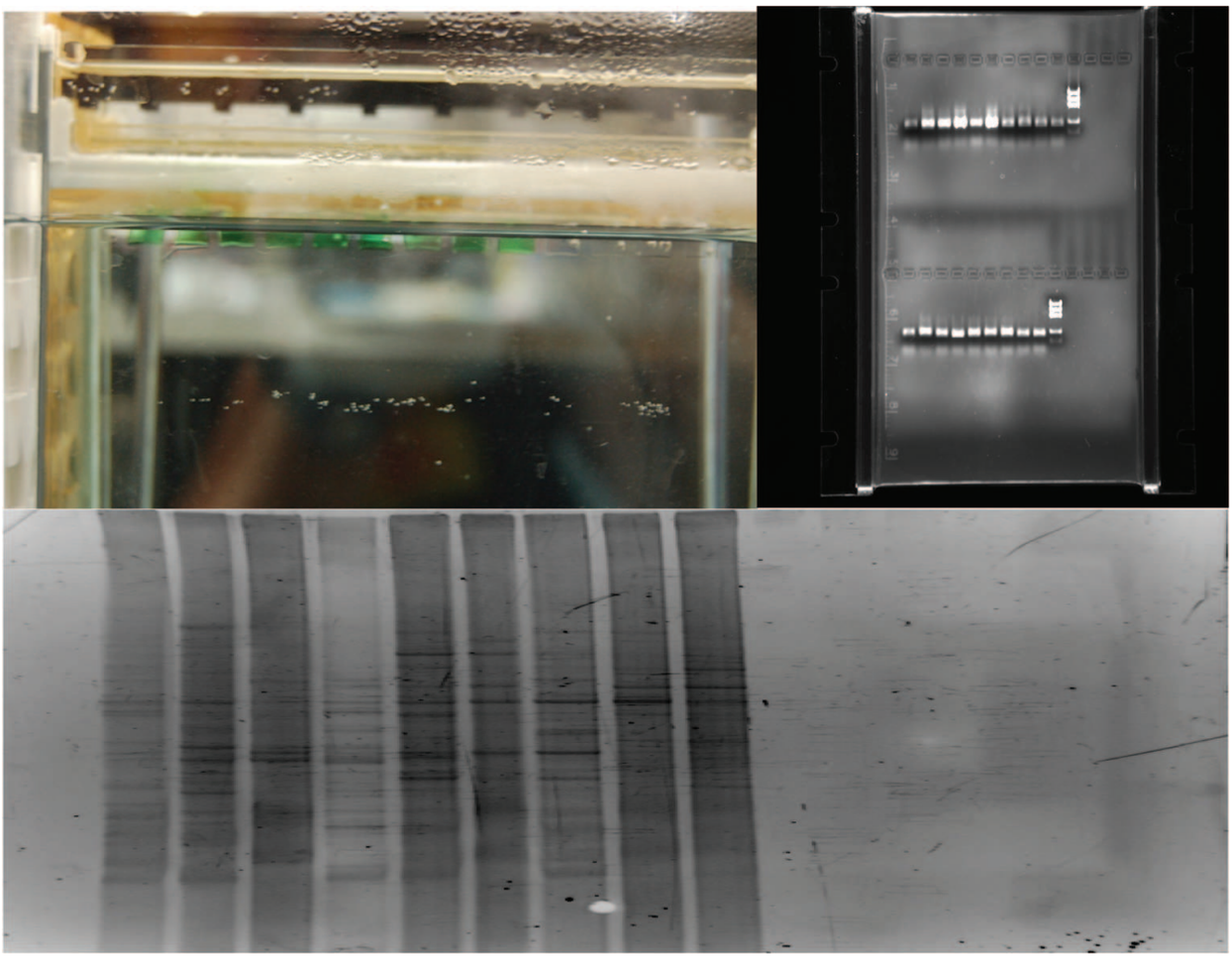

Figure 11. Gel sequence and DGGE banding results of active spices in different biofilter levels. Names of referred bacteria are listed in **Table 3**.

most abundant active species is given in **Table 3**. Both heterotrophic and autotrophic bacterial species are present in the biofilter with a predominance of heterotrophic population.

Number	Active species	GenBank	Group	Accession number
1	Alpha proteobacterium	AF256029.1	–	–
2	Gamma proteobacterium clone	EU665097.1	–	–
3	Gamma proteobacterium clone	EU665097.1	–	–
4	Bacillus sp.	GU935303.1		
5	Oxalobacteraceae bacterium	GU473126.1	Herbaspirillum sp	GQ355286.1
6	Oxalobacteraceae bacterium	GU473092.1	Herbaspirillum sp	GQ355286.1
7	Rhizobiales bacterium	FN794225.1		
8	Aucune corespondance	–	–	–
9	Aucune corespondance	–	–	–
10	Alpha proteobacterium	EF665816.1	Uncultured Rhizobiales bacterium	FJ475425.1
11	Alpha proteobacterium	EU051984.1	–	–
12	Séquence double	–	–	–
13	Alpha proteobacterium	GU553130.1	Methylosinus sp	GU556372.1
14	Alpha proteobacterium	–	–	–
15	Séquence double	–	–	–
16	Aucune corespondance	–	–	–
17	Aucune corespondance	–	–	–
18	Acidobacteriaceae bacterium	EU449736.1	–	–
19	Nitrobacter sp.	GU556337.1	–	–

Table 3. Most abundant active species and results of PCR re-amplified and sequenced 16S rDNA DGGE bands.

3.3.4. Toxicity evaluation

Toxicity evaluation of leachate before and after treatment was performed in the Centre d'Expertise en Analyses Environnementales de Québec using the Microtox test on two species susceptible to the toxicity, which are daphnia and algae. Results are presented in **Table 4**. Toxicity evaluation confirmed that effluent was not toxic for algae and daphnia. It was confirmed also that algae were stimulated in the presence of diluted (5.6% V/V) effluent.

3.4. Pilot unit profiles comparison

Figures 12 and **13** illustrate the profiles performance comparison. COD (feed) was the influent COD and COD (Out) was the effluent COD in column 1 without bacterial activity. The variation of BOD in the woodwaste influent and in the biofilter effluent showed high biofilter performances.

The pH distribution in column 1 (without bacterial activity) and in column 2 (biofilter) is presented in **Figure 14**.

Concentration % V/V	Influent			Effluent		
	Daphnia		Algae	Daphnia		Algae
	% Mortality	% Immobility	% Inhibition	% Mortality	% Immobility	% Inhibition
56	100	100	99	0	1	0
42	100	100	99	0	0	0
24	99	100	99	0	0	0
18	30	40	99	0	0	0
13.5	25	30	99	0	0	0
10	25	30	99	0	0	0
7.5	20	20	99	0	0	0
5.6	10	5	80	0	0	−1.7
Control	0	0	0	0	0	0

Table 4. Toxicity evaluation of leachate before and after treatment.

3.5. Mini-columns COD profile comparison

The mini-columns COD decreased in the first 10 days of operation from 1896 mg L^{-1} as a fed COD influent to 968 mg L^{-1} for the first column (without bacterial activity) and 737 mg L^{-1} for

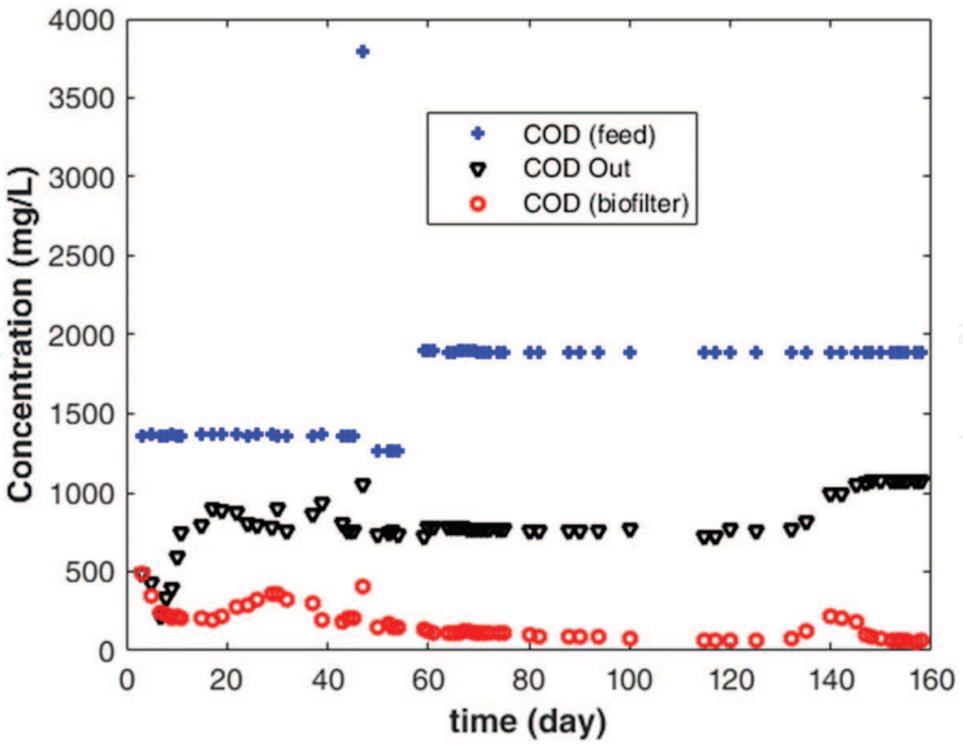

Figure 12. COD profiles comparison in woodwate leachate influent and in both columns with and without bacterial activity.

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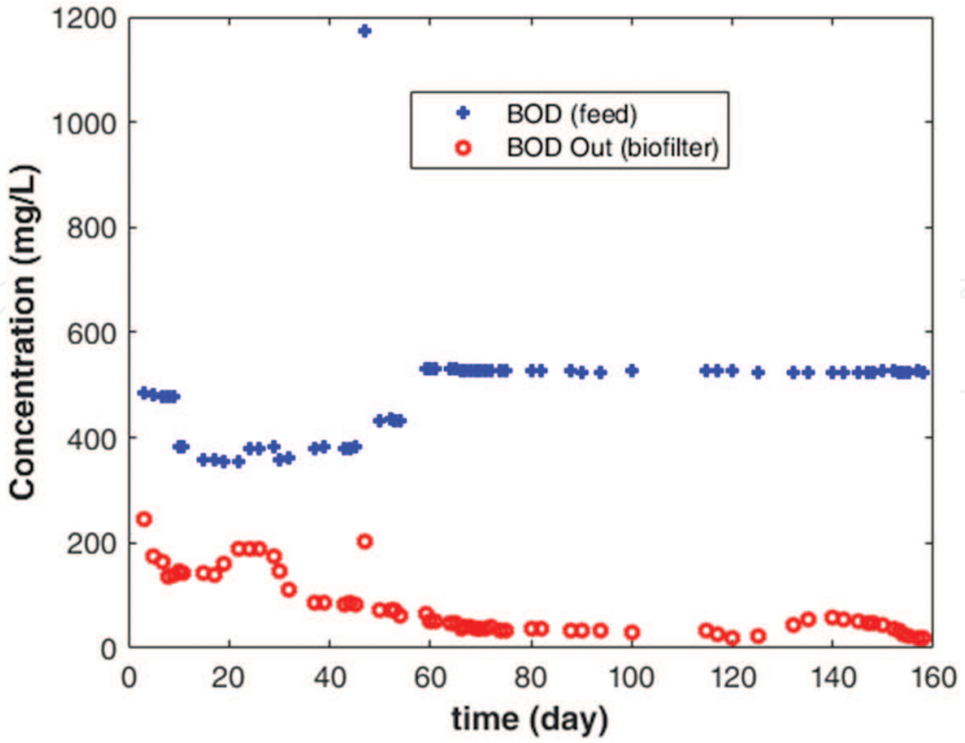

Figure 13. Treatment efficiency and BOD performance in the biofilter.

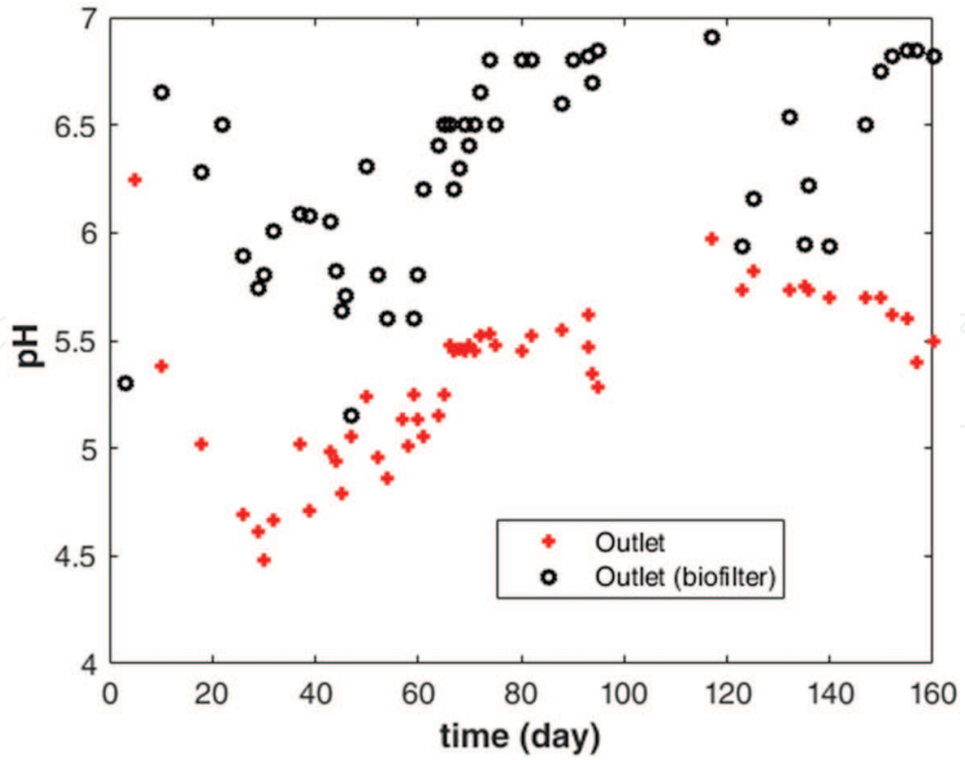

Figure 14. pH distribution in column 1 (without bacterial activity) and in column 2 (biofilter).

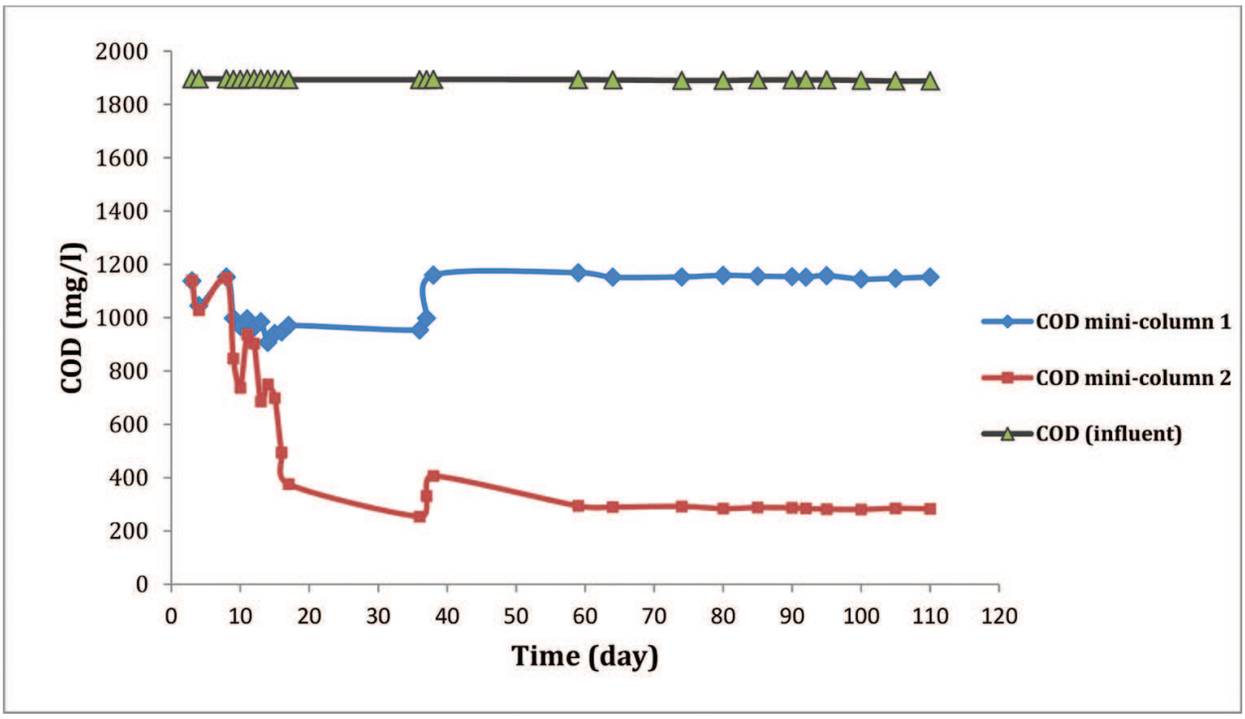

Figure 15. Mini-columns COD profiles comparison.

the second one (mini-biofilter). After 10 days of operation, the mini-biofilter COD fluctuated due to the biomass adaptation and then decrease in time at 254 mg L^{-1} (**Figure 15**). COD concentration of the two mini-columns increased at the 37th day of operation. This increase may due to the media saturation. COD of mini-column without bacterial activity was set at COD concentration influent while that of the mini-biofilter decreased to reach 285 mg L^{-1} values at the steady state with 84.9% of COD removal.

4. Conclusion

Two pilot filters, with and without biological activity, were designed for continuous mechanisms to follow. It has been shown that mechanisms of volatilization, sorption, and biodegradation operate simultaneously during the biofiltration process. For, column 1, in which bacterial activity was inhibited by mercuric chloride addition, a removal efficiency of 33.33–40.1% was obtained at steady state for all the phenolic compounds except phenol and chlorophenol for which 40.2–83.33% of initial concentrations was removed. On the other hand, 41.1% of phenol and chlorophenol initial concentrations were found in the collected gas. Thus, in this column, both physicochemical retention and volatilization are responsible for phenolic compounds removal. At the saturation of the media on the 135th day, a maximum of volatilization was obtained and reached 41.1% for more volatile compounds.

Good treatment efficiency was obtained in the biofilter (column 2). Note that 97–98.2% of COD and BOD removal were observed, respectively. Excellent performances were achieved and

reached 99.9% of initial concentrations removal for all the phenolic compounds. Volatilization did not exceed 5–7% at the steady state for the more volatile compounds in this column simulating the real biofilter operation.

Woodwaste leachate was treated with an excellent efficiency by biofiltration process on the mixed media peat: perlite, although a slight problem with color due probably to peat. This is why the authors recommend improving aspect effluent quality by a final treatment if necessary. Toxicity evaluation confirmed that effluent was not toxic for algae and daphnia. It was confirmed also that algae were stimulated in the presence of diluted effluent.

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