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Proposed mechanisms of toluene removal by vermicompost and earthworms *Eisenia fetida*

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ABSTRACT

For potential use in air treatment by biofiltration, a new material composed of vermicompost and earthworms (*Eisenia fetida*) was tested for the removal of a volatile organic compound (VOC), toluene. The removal rate of toluene was measured during batch experiments in presence of vermicompost only, earthworms only and a mixture of both. In the chosen experimental conditions, no mortality of earthworms was recorded and the results showed that the presence of earthworms allowed an increase in toluene removal rate (0.213 $mg.h^{-1}$) compared to vermicompost only (0.084 $mg.h^{-}1$) and earthworms only (0.136 $mg.h^{-1}$). From the experimental data, mechanisms of toluene transfer and adsorption/biodegradation by microorganisms from vermicompost and/or earthworms were proposed.

KEYWORDS

Vermicompost; Vermifiltration; VOC; Mechanisms; Toluene

1. INTRODUCTION

Biofiltration is widely used for the treatment of odorous and/or volatile organic compounds (VOCs). A large number of studies have been published on this process [1-5]. The gaseous effluent passes through a fixed film bioreactor. Pollutants contained within it are transferred to the biofilm present in the packing material and oxidized or converted into biomass by microorganisms [6-8]. The degradation of the pollutants leads to the production of metabolic by-products, carbon dioxide and water. The packing material can be organic or inorganic, natural or synthetic [9,10]. As this material acts as a support for the biomass and a possible adsorbent for the pollutant, it has to be selected carefully [11-13]. Physico-chemical and mechanical properties must be taken into account. Organic materials, like compost, peat, wood mulch, and pine bark, are often used due to their ability to support bacteria and supply nutrient to the biomass [14,15]. However, they have poor mechanical properties and are subject to settling and the generation of preferential pathways [16]. For these various reasons, a mixture of organic and inorganic material is often employed [8,17]. The main role of the inorganic material (pozzolan, porous pebbles, activated carbon, etc.) is to increase the structural stability over time.

Vermiprocesses are emerging techniques used for composting [18-22] and wastewater treatment [23,24]. They have also been studied for the treatment of some industrial effluents such as beverage, distillery or paper mill wastewater [25,26]. These processes are based on the ability of earthworms (*Eisenia fetida*) to convert organic matter into compost (called vermicompost or vermicast) by transit through their gut.

Thus, an original packing material composed of vermicompost and earthworms could be tested for the filtration of waste gas. The physico-chemical properties of vermicompost (surface pH, water hold-up capacity, organic matter content, density, external porosity) seem to be interesting in terms of biomass development [27-31]. The disadvantage of settling could be solved by the addition of earthworms. The mixing of the vermicompost due to earthworm burrowing activity could guarantee good homogenization of the fixed bed, an optimal distribution of the gaseous effluent inside the biofilter, and good oxygen transfer to the biomass. In this case, earthworms would play the role of inorganic material, *i.e.* structural agents. Moreover, they could participate in pollutant degradation and increase the efficiency of the biofilter.

The aim of this experimental work was to propose mechanisms of pollutant transfer and degradation by a VOC removal system packed with a vermicompost colonized by earthworms. The rates of degradation of a pollutant by vermicompost, earthworms, and a mixture of both, respectively, were compared in batch experiments. From the data generated experimentally, potential mechanisms were proposed.

2. MATERIALS AND METHODS

2.1. Vermicompost and earthworms

The earthworms used were *Eisenia fetida*, which were fed on domestic wastes (fruits/ vegetables wastes and coffee grounds). The vermicompost obtained was then matured for about 3 months before being used in the experiments. The physico-chemical and biological characteristics of the matured vermicompost are presented in Table 1. The significant humidity and presence of bacteria in the vermicompost can be noted.

pH	8.9
Humidity (%)	81
Density ($kg.m^{-3}$)	530
Bed porosity (%)	35
Water hold-up capacity ($g.g^{-1}$ dry vermicompost)	7.22
Cell number (UFC. g^{-1} dry vermicompost)	$1.95\ 10^6$
Soluble Total Organic Carbon ($mgC.g^{-1}$ dry vermicompost)	15.10
Soluble Total Nitrogen ($mgN.g^{-1}$ dry vermicompost)	3.27
%C	36.7
%N	5.3
C/N	6.9

Table 1: Physico-chemical properties of vermicompost

2.2 Target volatile organic compound (VOC)

Toluene was chosen as a model of volatile organic compounds (VOCs). It has been widely studied as a model molecule in biofiltration processes [32,33]. Some of its physico-chemical characteristics are: formula: C_7H_8, molecular weight: 92.17 $g.mol^{-1}$, solubility in water (25°C): 0.53 $g.L^{-1}$, density: 0.867 and saturated vapor pressure (20°C): 3 kPa.

2.3 Batch experiment procedures

Batch experiments were performed at room temperature (20 ± 2°C) in 1 L conical flasks hermetically sealed with a septum. Several flasks were filled with 100 g of vermicompost (or 10 g of adult earthworms or both). The earthworms used in this study were collected from the laboratory nursery just before the beginning of the experiment, rinsed with distilled water and wiped before being placed in the conical flasks. They were weighed but not counted.

The pollutant, 5 µL (4.33 mg) of toluene (Sigma Aldrich - purity ≥ 99.8%) was injected through the septum. This quantity of toluene in gas phase was chosen in order to easily approach the mechanisms and the impacts of VOC on this biotic system. After injection, the conical flasks were manually stirred.

Some preliminary experiments (data not shown) gave the authors an idea of the kinetic of toluene consumption by vermicompost, earthworms or both. The initial number of flasks and sampling timing were decided according to these preliminary results, as well as the mass of vermicompost and the mass of earthworms used. The mass of earthworms was chosen so that the initial quantity of oxygen in the sealed flask was sufficient to allow their breathing during the entire duration of the experiment. It was verified that no earthworm died during the experiment.

At each sampling time, the toluene concentration in the head space of the conical flask was measured in order to calculate the mass remaining in the gaseous phase (see 2.4). Then, the flask was opened and a random sample of the solid phase (vermicompost and/or earthworms) was collected in order to determine the mass adsorbed. The solid (precisely weighed) was put in a hermetically sealed vial and placed in a stove. After total toluene desorption, the head space of the vial was analyzed (see 2.4). Considering the volume of the head space and the toluene concentration measured by GC FID analysis, the mass of toluene adsorbed on the solid could be calculated. The temperature of the stove, the time of desorption and the amount of solid placed in the vial were previously optimized (data not shown) to ensure the total desorption of the toluene. The optimal conditions were the following: mass to be desorbed: about 2.00 g for vermicompost and about 0.20 g for earthworms, temperature of desorption: 95°C and time of desorption: 5 h.

Then, the mass of toluene removed was calculated according to the mass balance of the VOC in the system (Equation 1).

$$M_{total\ removed} = M_{initial} - M_{gaseousphase} - M_{adsorbed} \qquad \text{Equation 1}$$

where $M_{initial}$ is the mass of toluene initially and manually injected in the system through the septum (4.33 mg), $M_{gaseousphase}$ is the mass of toluene remaining in the gaseous phase of the conical flask and $M_{adsorbed}$ is the mass of toluene absorbed on the solid phase (vermicompost and/or earthworms).

Due to the experimental conditions (experiments performed in hermetically sealed flasks), and assuming that no leak could occurred, the mass of toluene removed ($M_{total\ removed}$) corresponded to the mass of toluene consumed by the microorganisms (and/or earthworms) for their growth (toluene was then the carbon source). The kinetics of toluene removal were performed for different operating conditions in order to investigate the transfer of pollutant into the vermicompost and its biodegradation by microorganisms such as bacteria, fungus… present on the vermicompost surface or earthworms when present in the medium.

2.4 Analyses

Different analysis methods are available to quantify VOC and especially toluene in gas phase [34]. For example, the use of sorbent material of which analysis is carried out by either thermal desorption (TD) or solvent extraction. Considering the concentrations and because only one VOC was used, a simple method was chosen. Analyses of the gaseous phase (head space of the conical flask and head space of the vial) were performed by Gas Chromatography with a FID detector on a Thermo Fisher Focus GC. The column was an Agilent HP-5, 30 m × 0.32 mm, the oven temperature was 100°C, the inlet temperature was 150°C, the detector temperature was 280°C and the carrier gas was N_2. Analyses were triplicate and the mass were calculated with the average concentrations.

3. RESULTS AND DISCUSSION

3.1 Adsorption-biodegradation of toluene by vermicompost

First, experiments were performed using vermicompost as the potential adsorbent material. Chiu *et al.* [35] have shown that toluene vapor can be adsorbed onto natural organic matter and especially compost. Several flasks were filled with vermicompost and then toluene was injected into each of them at the same time. The toluene concentration in the gaseous phase was measured as a function of time and the flask was opened to take a sample for vermicompost desorption procedure. The mass of toluene in the gaseous phase, the mass of toluene adsorbed and the mass of toluene consumed (calculated according to the mass balance in Equation 1) were plotted vs. time (Figure 1).

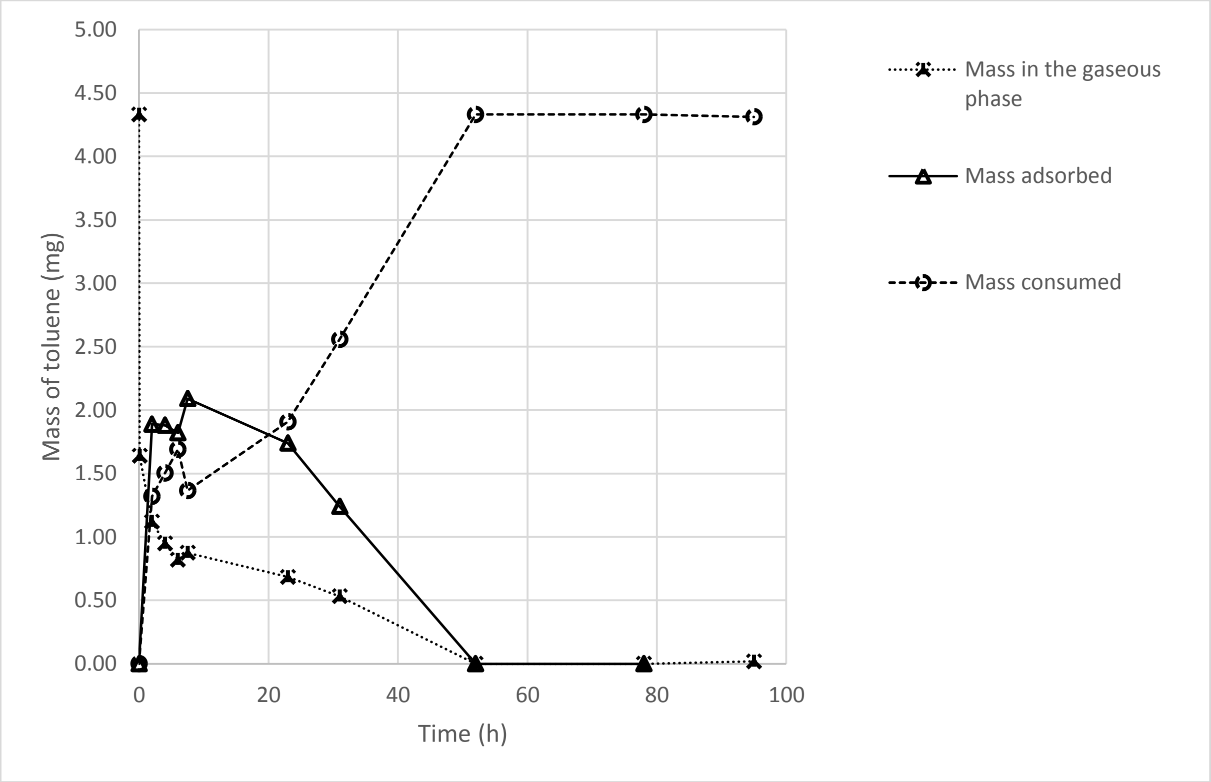


Figure 1: Evolution (chain reactions) of mass of toluene in the gaseous phase, mass adsorbed and mass consumed by 100 g of vermicompost as a function of time.

Figure 1 shows the evolution (chain reactions) of toluene during the successive steps in the reactor. First, there was a rapid decrease in the toluene concentration in the gaseous phase. This behavior was due to both adsorption on the surface of the solid material and consumption by the bacteria present in the vermicompost during the first 10 h. Equation 2 represents the phenomena occurring during this first phase.

$$T + (C + X) \longrightarrow T_1C + (X + \Delta X_1) + B + T_2 \quad \text{Equation 2}$$

where T is the initial mass of toluene, C is the compost mass and X is the biomass (microorganisms from the vermicompost). A part of toluene (T_1) is adsorbed on the vermicompost ($T_1 < T$). Another part is consumed by microorganisms and leads to an increase in the biomass (ΔX_1) and by-products (B). The remaining mass of toluene in the gaseous phase is called T_2.

After 20 h of experiment, the mass adsorbed and the mass in the gaseous phase both decreased. At the same time, the mass removed by the vermicompost increased linearly as a function of time. After 50 h, the toluene was fully consumed by the vermicompost. Equation 3 represents the phenomena occurring during the second phase.

$$T_1C + (X + \Delta X_1) + B + T_2 \longrightarrow (X + \Delta X_1 + \Delta X_2) + B_1 + C \quad \text{Equation 3}$$

The adsorbed toluene (T_1) is consumed by microorganisms and the biomass increases (ΔX_2) as do the by-products ($B_1 > B$). As soon as T_1 is consumed, the gaseous toluene T_2 can be adsorbed and then consumed. At the end of the experiment, the toluene was totally consumed and the vermicompost (C) was free of toluene.

During the linear phase (calculated between 23h and 52h), a consumption rate of 0.084 $mg.h^{-1}$ was recorded, corresponding to 4.4 $\mu g.g^{-1}$ dry weight.h^{-1}. As vermicompost presents variable physico-chemical characteristics depending on the raw material used, it is very difficult to compare these results with those found in the literature. However, some discussions and comparisons with previous studies can be made. Vermicompost has previously been used in a mixture with rice paddy soil for methane removal [36], in a mixture with powdered activated carbon for methane removal [37] or for ethylene removal [38] but never for toluene removal. Park *et al.* [37] recorded a methane oxidation rate of 9.7 g.m^{-3}.h^{-1} while Moon *et al.* [36] obtained 17.9 g.m^{-3}.h^{-1}. Moreover, the authors proved that CO_2 and H_2O were the end-products and that the amount of CO_2 produced was less than the amount of methane oxidized, contributing to reducing greenhouse gas production. In the case of ethylene, Fu *et al.* [38] demonstrated that the pollutant removal was due to microbial activity and not to physico-chemical properties. A maximum rate of 2.6 µg.g^{-1} dry weight.h^{-1} was recorded during batch tests performed at 30°C. Rates were higher at 20 and 25°C and lower at 10 and 40°C. It should be noted that the rates obtained for methane and ethylene removal were calculated after a lag phase, which was not the case for toluene removal in the present study.

Removal of gaseous toluene has been widely studied over the past years and it is known that many bacteria (*Pseudomonas sp., Rhodoccocus, Achromobacter*) can degrade toluene by a heterotrophic pathway. The use of a bacterial consortium has enabled good performances in different kinds of bioprocess. Concerning biofiltration, several packing materials inoculated with activated sludge [39-42] have produced elimination rates ranging from 95 to 100% for loadings ranging from 50 to 200 g.m^{-3}.h^{-1}. Cheng *et al.* [43] demonstrated that a fungal/bacterial biofilter showed better removal (142 g.m^{-3}.h^{-1} with an Empty Bed Retention Time of 24 s) than a fungal or bacterial one. Moreover, it produced less CO_2 than a bacterial one due to the presence of fungi. As the vermicompost used in the present study contained many microorganisms (1.95 10^6 UFC.g^{-1} dry vermicompost), it was neither inoculated nor acclimated before experiments. This could explain the relatively low elimination capacity (about 1 g.m^{-3}.h^{-1}) recorded for vermicompost. However, some preliminary tests (data not shown) indicated that better performances could be obtained after an acclimation period.

3.2 Removal of toluene by earthworms

The second experiment was performed with 10 g of earthworms. The same experimental procedure as previously described (several flasks) was used. At the end of the experiment, we noted that all earthworms were still alive. The LD50 or LC50 data for *Eisenia fetida* were not found in the literature. LD50 and LC50 are the Lethal Dose (amount of a material which causes the death of 50% of a group of test animals) and the Lethal Concentration (concentration of the chemical in air that kills 50% of the test animals during the observation period (4h)), respectively.

But we note that the acute inhalation toxicity for the rat (LC50) for 4 h was more than 20 mg/L. This value has to be compared to the concentration of toluene in this present work which was 4.33 mg/L. Figure 2 shows the results obtained.

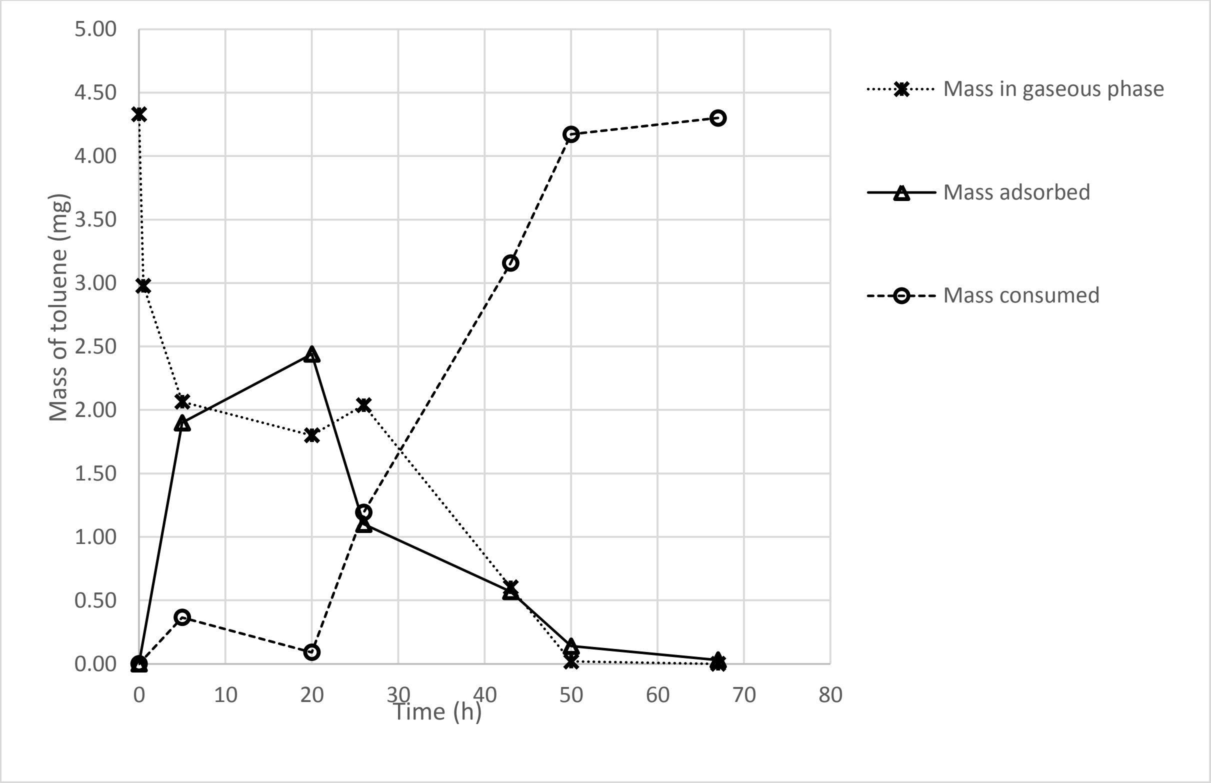


Figure 2: Evolution (chain reactions) of mass of toluene in the gaseous phase, mass adsorbed and mass consumed by 10 g of adult earthworms as a function of time.

During the first 20 h, the mass of toluene in the gaseous phase decreased, due to adsorption only. No consumption was recorded during this lag phase. During this phase (Equation 4), a part of toluene (T_1) was adsorbed onto the earthworm (W) surface and the other part (T_2) remains in the gaseous phase ($T = T_1 + T_2$) .

$T + W \longrightarrow (WT_1) + T_2$ Equation 4

Between 20 h and 50 h, the mass of toluene consumed increased linearly with time. At the same time, a decrease in the mass adsorbed was observed. After 50 h, the toluene was fully consumed by earthworms. Equation 5 represents the phenomena occurring during the second phase.

$(WT_1) + T_2 \longrightarrow (W + \Delta W) + D$ Equation 5

The toluene adsorbed (T_1) was consumed by earthworms. Then T_2 could be adsorbed and consumed. The toluene degradation led to an increase in earthworm weight (ΔW) and by-product formation (D).

During the linear phase (between 20h and 50h), a consumption rate of 0.136 mg of toluene per hour (13.6 $\mu g.g^{-1}$ fresh earthworm.h^{-1}) was recorded. Wen *et al.* [44] studied the accumulation and elimination kinetics of ciprofloxacin in earthworms *Eisenia fetida* and showed an increase in the concentration during the first 7 days followed by a decrease until 30 days, as it was the case for toluene. On the other hand, only an accumulation phase was observed by Nyholm *et al.* [45] during a 30-day exposure of earthworms to several brominated chemicals (flame retardants).

3.3 Toluene removal by a mixture of vermicompost and earthworms

A third experiment was performed with 100 g of vermicompost and 10 g of adult earthworms. The results are presented in Figure 3.

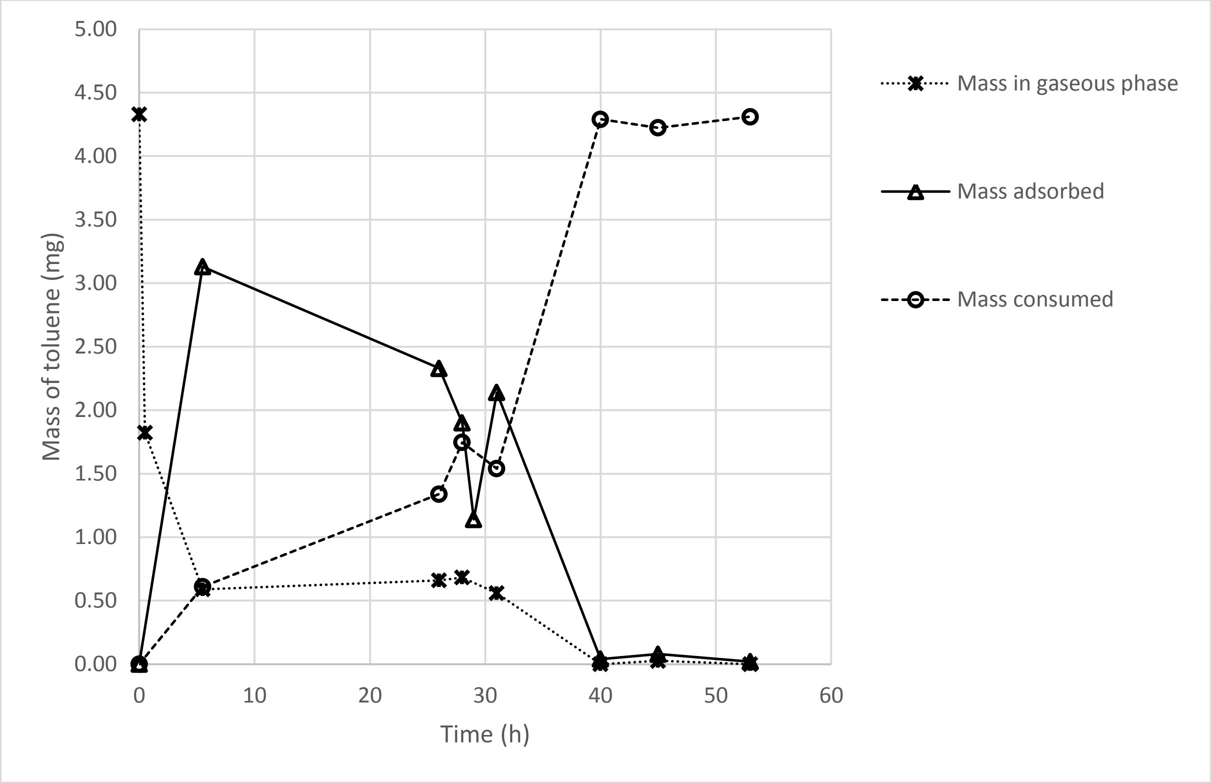


Figure 3: Evolution (chain reactions) of mass of toluene in the gaseous phase, mass adsorbed and mass consumed by 100 g of vermicompost + 10 g of adult earthworms as a function of time.

It was observed that the result of this experiment was the addition of the two previous ones i.e. adsorption of about 1.5 mg of toluene during the first phase (20 h), followed by a linear consumption phase. The consumption rate calculated for this experiment (0.213 mg of toluene per hour between 26h and 40h) corresponded to the sum of the consumption rates by the vermicompost (first experiment) and the earthworms (second experiment). After 40 h, the toluene was fully consumed, showing that the contribution of both vermicompost and earthworms led to a shorter degradation time.

$T + (C+X) + W \longrightarrow T_1C + T_2W + T_3 + (X+\Delta X1) + B$ Equation 6

During the first phase (Equation 6), a part of toluene (T_1) was adsorbed on the vermicompost, a second part (T_2) was adsorbed on the earthworm surface, a third part (T_3) remained in the gaseous phase and the last part ($T-T_1-T_2-T_3$) was consumed by the microorganisms from the vermicompost and leads to an increase in the biomass (ΔX_1) and formation of by-products (B).

$$T_1C + T_2W + T_3 + (X+\Delta X_1) + B \longrightarrow C + (W+\Delta W_1) + (X+\Delta X_1+\Delta X_2) + B_1 + D \quad \text{Equation 7}$$

In the second phase (Equation 7), the adsorbed toluene was consumed by both earthworms and biomass leading to an increase in weight, (ΔW_1) and (ΔX_2) respectively, and the formation of by-products, (D) and (B_1) respectively, ($B_1>B$).

Hartenstein [46] studied the effect of aromatic compounds on the growth of earthworm *Eisenia Fetida* and showed that a concentration of 1% (w/w dry weight) of toluene in activated sludge affected the growth of earthworms and that 4% of toluene led to 100 % of mortality in 6 weeks. During our experiments, the concentration of toluene did not exceed 3.5 mg/100g of vermicompost, i.e. 18 mg/100g of dry vermicompost (0.018%). Moreover, the duration of the exposure of earthworms to polluted vermicompost did not exceed 40h. So, it was not surprising, all the earthworms were alive and the end of the experiment.

3.4 Potential mechanisms

Mechanisms of toluene removal are proposed according to the knowledge on mass transfer and the experimental results of the present study. In the case of the vermicompost alone (Figure 4), the first step was the diffusion of toluene from the gaseous phase to the gas/liquid interface followed by absorption in the biofilm and/or adsorption onto the solid surface. As consumption was recorded simultaneously with the beginning of the experiment, it is supposed that some of the microorganisms, initially present in the vermicompost, could degrade the pollutant without an acclimation period. After the acclimation phase (about 20 h), more microorganisms were able to degrade the toluene and the biodegradation rate increased. After toluene degradation (totally or not), by-products could be transferred to the gaseous phase. During experiments, no by-product was identified in the gaseous phase. Three hypotheses could explain this fact: (i) the concentration of end-products was under the detection threshold, (ii) oxidation of toluene was total and produced only CO_2 and H_2O, (iii) by-products were not released into the gaseous phase.

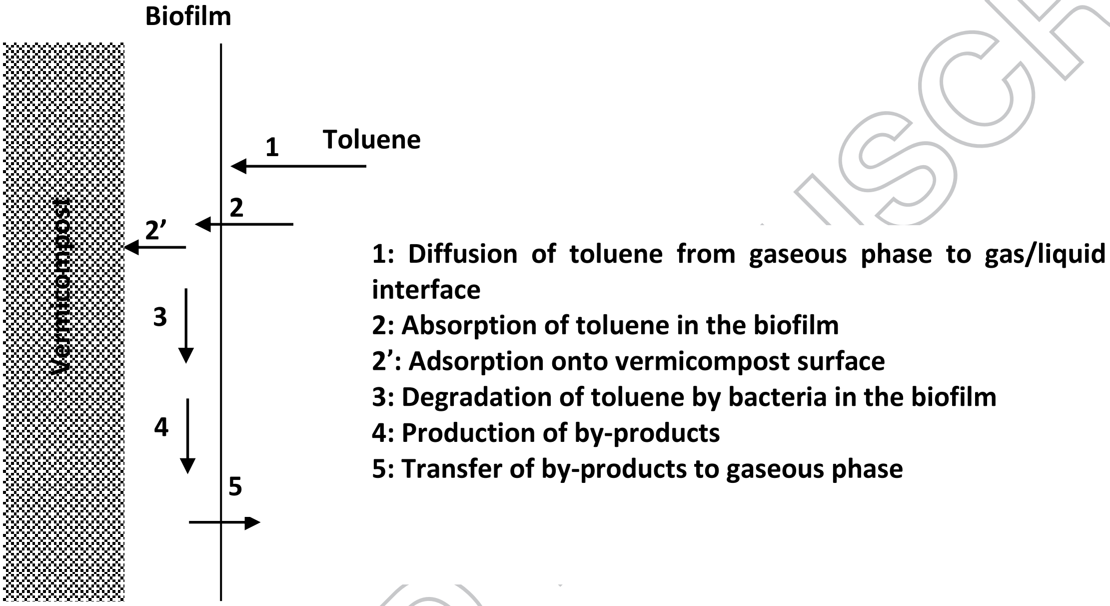
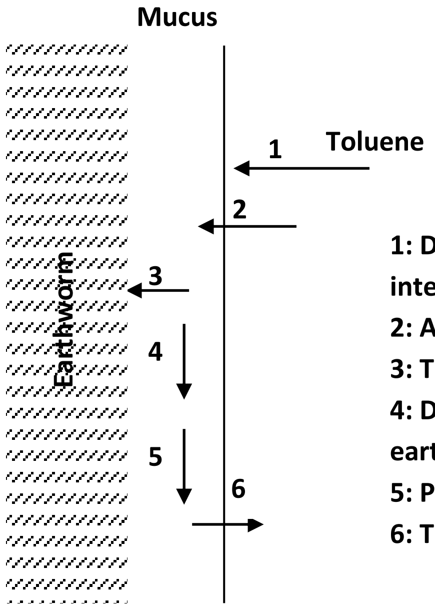


Figure 4: Mechanism of toluene degradation by vermicompost

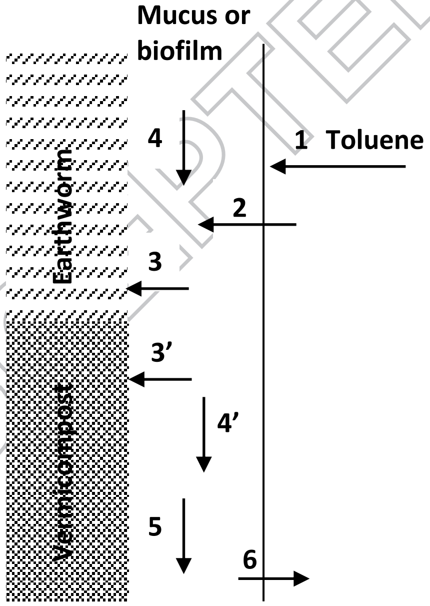
In the case of earthworms only (Figure 5), the first step was the diffusion of toluene from the gaseous phase to the mucus that totally covers the skin of the earthworm. As the mucus contains microorganisms, these could degrade the toluene like in the vermicompost. The earthworms used in this study were collected from the laboratory nursery and rinsed before being placed in the conical flask. They were in contact with the vermicompost, so it is supposed that some microorganisms from the vermicompost still remained in the mucus. Moreover, Huang *et al.* [47] studied the role of earthworm mucus in a vermicomposting system and concluded that the mucus significantly stimulated the microbial activity and bacterial abundance. A second degradation route by diffusion through the mucus and transfer to the earthworm body could be considered [48]. Then, the degradation of toluene would occur inside the earthworm. Several hundred bacterial strains belonging to different taxa have been isolated and identified from the digestive tracts of soil and compost earthworms [49]. These authors showed that bacteria associated with earthworm intestines were capable of growth on humic acids as a sole carbon source and that intestinal isolates had elevated activities of proteases and dehydrogenases. These properties suggest that toluene could be oxidized in the earthworm gut. After toluene degradation, in mucus or in the earthworm gut, by-products could be transferred to the gaseous phase. Lanno *et al.* [50] studied the bioavailability of chemicals in soil for earthworms. According to these authors, during movements through the soil, earthworms encounter and interact with only a specific portion of environmentally available chemicals. Interaction may be through either direct dermal contact with chemicals in the soil solution or soil atmosphere or ingestion of soil or specific fractions of soil. Thus, in the absence of vermicompost, direct dermal contact with the atmosphere loaded with toluene should be taken into account. It is not yet known whether dermal or intestinal uptake is the predominant mechanism for contaminant uptake in earthworms. Pan *et al.* [51] studied the interaction between earthworm's mucus and the pesticide imidacloprid. They concluded that there is a high affinity of mucus for the organic pollutant but could not confirm whether the mucus serves as a protective barrier against toxic pollutants or promotes the entry of pollutants into the earthworm. Therefore, the degradation of toluene could occur in both the mucus and the gut of the earthworm.



1: Diffusion of toluene from gaseous phase to gas/mucus interface
2: Absorption of toluene in mucus
3: Transfer from mucus to earthworm body
4: Degradation by microorganisms in the mucus or earthworm gut
5: Production of by-products
6: Transfer of by-products to gaseous phase

Figure 5: Mechanism of toluene degradation by earthworms

In the case of both vermicompost and earthworms, the same types of degradation might occur. One more could be added: ingestion of toluene by earthworms through vermicompost ingestion. In this case, toluene could be degraded in the earthworm gut. Figure 6 proposes a combined mechanism of degradation.



1: Diffusion of toluene from gaseous phase to gas/mucus interface
2: Absorption of toluene in mucus or biofilm
3: Transfer from mucus to earthworm body
3': Transfer onto vermicompost
4: Degradation by microorganisms in the mucus or earthworm gut
4': Degradation by microorganisms in biofilm
5: Production of by-products
6: Transfer of by-products to gaseous phase

Figure 6: Mechanism of toluene degradation by the combination of vermicompost and earthworms

4. CONCLUSION

The results of the batch experiments showed that vermicompost and earthworms were both able to remove toluene at low concentration (5µL in 1L hermetically sealed conical flask). During the batch experiments, no mortality of earthworms was recorded. In the case where the conical flask was filled with a mixture of 100g of vermicompost and 10 g of earthworms, the removal rate (0.213 mg of toluene per hour between 26h and 40h of experiment) corresponded to the sum of the consumption rate by 100g of vermicompost only and the consumption rate by 10g of earthworms only. According to the knowledge on mass transfer and the experimental results, mechanisms of toluene removal have been proposed.

In the case of vermicompost only, it was assumed that the toluene was adsorbed in vermicompost and degraded by the biofilm. In the case of earthworms only, it was assumed that the toluene was absorbed in mucus, then transferred from mucus to earthworms body and that the removal of toluene occurred by microorganisms presents in the mucus and the gut. In the case of both vermicompost and earthworms, all these steps were added together, resulting in an increase of the removal rate.

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