Charles University in Prague, Faculty of Science
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Summary of the Ph.D. Thesis

Application of composting for bioremediation of solid materials contaminated with selected organic pollutants and micropollutants

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The presented thesis is based on the following publications:

Publication 1: Němcová, K., Lhotský, O., Stavělová, M., Komárek, M., Semerád, J., Filipová, A., Najmanová, P., Cajthaml, T. Effects of different organic substrate compositions on the decontamination of aged PAH-polluted soils through outdoor co-composting. *Chemosphere* 2024, *362*, 142580.

Publication 2: Němcová, K., Linhartová, L., Boháčková, J., Cajthaml T. Antropogenní mikropolutanty. Academia, Prague (chapter in book Environmentální hydrologie a hydrochemie. The book is in preparation for review.)

Publication 3: Hanč, A., Dume, B., Hřebečková, T., Michal, P., Hrčka, M., Němcová, K., Grasserová, A., Cajthaml, T. The fate of pharmaceuticals and personal care products during composting of sewage sludge. *Sustainable Chemistry and Pharmacy* 2024, *38*, 101498.

Publication 4: Semerád, J., Lhotský, O., Filipová, A., Urban, O., Širová, K., Boháčková, J., Komárek, M., Cajthaml, T. Remedial trial of sequential anoxic/oxic chemico-biological treatment for decontamination of extreme hexachlorocyclohexane concentrations in polluted soil. *Journal of Hazardous Materials* 2023, *443*, 130199.

Abstract

Composting is not only an established technology for the treatment of organic waste but also a bioremediation method with great potential. The microorganisms responsible for the composting process can break down not only organic material but also a lot of pollutants. The bioremediation efficiency of composting depends on many process parameters, such as the type and amount of contaminated matrix and added organic material, moisture, nutrient ratio, etc., in addition to the kind and bioavailability of the pollutants themselves. To correctly assess the applicability of composting in specific cases and to set appropriate conditions, it is important to understand the influence of the individual parameters.

Soil contamination with polycyclic aromatic hydrocarbons (PAHs) and other aromatic hydrocarbons remains a problem in many locations. Composting can be a suitable remediation method for this type of contaminated material supposing PAHs are sufficiently bioavailable. In this dissertation thesis, the effect of the organic substrate composition on the removal efficiency of PAHs from historically contaminated soils was studied. The results indicate that if optimal composting conditions (moisture, aeration, etc.) are maintained and the maturation phase lasts at least one year, the final extent of PAH loss is not significantly dependent on the composition of the substrate. However, it is possible to influence the rate of PAH loss in the initial active phase to some extent. The effect of the ratio of contaminated matrix to residual material on the degradation of selected micropollutants was investigated in the case of sewage sludge composting. The sludge contains a lot of nutrients which makes it a suitable fertilizer, but at the same time, it may pose a risk due to the presence of some impurities. The results show that 75 % or more of sludge has a negative effect on the composting process. At the same time, it implies that sludge composting can effectively reduce the concentration of some drugs, while others are relatively resistant to degradation. In the case of substances particularly resistant to biodegradation, e.g. hexachlorocyclohexanes (HCHs), the solution may be based on a combination of biological and chemical methods. Composting has been proven in this work to be a suitable final step in the remediation of HCH-contaminated soil. Its main purpose was to reduce the ecotoxicity of the remediated material caused by the various degradation products produced during the previous biological-chemical phases.

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1 Introduction

Composting is understood as a process of aerobic conversion of a mixture of organic substrates into stabilized organic matter – compost [1]. Traditionally, it has been used to process a wide variety of organic wastes. However, due to the degradation potential of the microorganisms present and the relative affordability and technological availability, in recent decades, it has also proven to be a promising bioremediation method for solid materials contaminated with various organic pollutants [2]. These include soils, sludges, and sediments contaminated with polycyclic aromatic hydrocarbons (PAHs), petroleum hydrocarbons, hormones, some pesticides, pharmaceuticals, etc.

A typical example is the bioremediation of soils contaminated with PAHs by composting in a mixture with suitable organic material (substrate), which serves as a source of nutrients as well as microorganisms and helps to set up suitable composting conditions [3]. The substrate is one of the important factors that influence the composting process and thus the efficiency of bioremediation. Other parameters that need to be considered are for instance the ratio of a substrate to a contaminated matrix, moisture, bioavailability of pollutants present, or other potential toxic impurities (e.g. heavy metals), etc. A sufficient understanding of the influence of each of these factors on the bioremediation efficiency of composting is essential to minimize the risk of remediation failure, which would then lead to the production of an even bigger amount of contaminated material.

Composting is also a widespread treatment technology for sewage sludge [4]. As a by-product of wastewater treatment, it contains a large amount of valuable nutrients and can therefore be used in agriculture. However, amending the soil with sludge poses the risk of environmental contamination, as sludge may contain various pollutants (pharmaceuticals and personal care products, endocrine disruptors, PAHs, etc.) in addition to beneficial substances [5]. Sludge composting prior to the application can help reduce the risk of transferring undesirable substances into the soil. However, according to the published literature, it is evident that the effect of composting on individual substances from the group of pharmaceuticals and personal care products (PPCPs) varies considerably depending on the characteristics of the specific substance and the process conditions. Therefore, it is important to understand the influence of individual process parameters on the removal of these micropollutants and the fate of individual substances during composting.

Hence, this dissertation thesis deals mainly with the influence of selected process parameters on the efficiency of composting in terms of the removal of selected pollutants. The influence of organic substrate composition was investigated in the

bioremediation of PAH-polluted soils under composting conditions with long-term maturation. Furthermore, the effect of the ratio of the polluted matrix (in this case, sewage sludge) and the organic substrate on the final concentration of selected PPCPs was investigated in the composting of sewage sludge. In addition, the application of composting as a final step in the chemical-biological remediation of soil contaminated with hexachlorocyclohexanes (HCHs) was investigated.

2 Aims of the study

Composting is a promising bioremediation method for a range of differently contaminated solid matrices due to the degradation potential of the microorganisms present and its relative affordability and technological availability. It can be used as the main technology or in combination with other remediation methods. It is an established method for the further treatment of sewage sludge, in which case it can help to reduce the risk of micropollutant transfer to agricultural soil. This dissertation aimed to assess the influence of selected process conditions on the bioremediation efficiency of composting and to verify its applicability for ecotoxicity reduction when it is used as a complement to other remediation methods.

The sub-objectives of this thesis were:

1. to assess the effect of composting substrate composition on the bioremediation efficiency of composting used for remediation of soils contaminated with polycyclic aromatic hydrocarbons (Publication 1);

2. to provide an up-to-date comprehensive overview of the main groups of organic pollutants with emphasis on substances frequently found in sewage sludge (Publication 2);

3. to assess the influence of composting with different proportions of sewage sludge in the starting mixture on selected micropollutants from the PPCP group present in sludge (Publication 3);

4. to verify the effectiveness of composting as a final step in chemical-biological remediation of soil contaminated with hexachlorocyclohexanes (Publication 4).

3 Material and methods

The experimental design, methods, and materials are described in detail in the corresponding publications. Here is a brief overview of the main methods used:

3.1 Pollutant extraction and analyses

The solid samples were dried (or mixed with Na2SO4 for extraction), homogenized, and extracted using an accelerated solvent extractor (ASE 200, Dionex). In the case of PAHs, the hexane/acetone (3:1) extracts were evaporated and reconstituted in acetonitrile, centrifuged, and analyzed by liquid chromatography with fluorescence detection (LC–FLD) (Waters Alliance 2695 system, Waters 2475 detector, Waters; LiChroCart column filled with LiChrosphere® 100 RP-18, Merck). Centrifuged methanol extracts were used for the analysis of PPCPs by LC-MS (Agilent Infinity 1260 with Agilent 6470 Triple Quadrupole, Agilent; Poroshell 120 EC-C19 column with pre-column, Agilent). HCHs were extracted using a mixture of hexane and acetone (1:1). Extracts were concentrated and processed through several cleaning steps including gel permeation chromatography. Purified liquid samples were analyzed by gas chromatography-mass spectrometry (GC-MS) (Scion SQ, Bruker with CP8400 autosampler, Agilent; DB5MS column, Agilent). Benzene and chlorobenzenes were analyzed using GC-MS and GC coupled with a flame ionization detector.

The supercritical fluid extraction and kinetic desorption curves were used to predict a bioavailable fraction of the PAHs or HCHs in the contaminated soils.

Details regarding extraction and detection methods can be found in the following publications: Covino et al. [6] and Němcová et al. [7] – Publication 1 (PAHs); Ashfaq et al. [8], Grasserová et al. [9] and Hanč et al. [10] – Publication 3; Semerád et al. [11] – Publication 4 (HCHs).

3.2 Microbial community analysis

Phospholipid-derived fatty acids (PLFAs) were extracted by a mixture of chloroform, methanol, and phosphate buffer (1:2:0.8; v/v/v). Extracts were purified and subjected to mild alkaline hydrolysis. The resulting free methyl esters of PLFAs were analyzed by GC-MS (450–GC, 240–MS, Varian). Further information about the analysis and data interpretation of PLFA bioindicators can be found in the work of Šnajdr et al. [12]. More thorough microbial community profiling was achieved by DNA extraction and 16S rRNA gene sequencing which is described in Publications 1 [7] and 4 [11].

4 Results and discussion

4.1 Effects of different organic substrate compositions on the decontamination of aged PAH-polluted soils through outdoor co-composting (Publication 1)

To assess the effects of organic substrate composition on the efficiency of bioremediation of PAH-polluted soils by composting, an experiment was conducted with four different substrates consisting of a mixture of organic wastes. The main components of each mixture were: garden waste, sewage sludge, poultry litter, and fruit and vegetable waste. Two aged PAH-polluted soils were chosen for composting, which differed both in total PAH concentration and the type of predominant PAHs (sum of 16 US EPA PAHs in soil A 5926 mg kg^{-1}, in soil B 369 mg kg^{-1}). The experiment was carried out in outdoor composters with a volume of 750 L (aerated by periodic mixing) and lasted more than 680 days to ensure sufficient maturation.

In all composters, more than 95% removal of the monitored PAHs was achieved in both soils, while in the controls containing only soil without substrate, PAH loss was insignificant (soil A) or reached 39% (soil B). Moreover, in contrast to the soils alone, the resulting composts showed practically no ecotoxicity by the chosen tests. According to the established rate constants, the fastest PAH loss was observed in the first active phase in the composters with garden waste. From these results, it can be concluded that if the conditions for composting are suitable (sufficient air supply, nutrient and moisture content, thermophilic phase, maturity, etc.) and the contaminants are sufficiently biodegradable, the composition of the substrate does not have a major influence on the final extent of biodegradation but may accelerate it in some cases. It should also be noted that loss of PAHs with more than 4 rings has been observed even after 300 days, so a long maturation phase seems crucial for this group.

4.2 Anthropogenic micropollutants (Publication 2)

Publication 2 provides an overview of organic pollutants and micropollutants frequently found in the environment and their physicochemical and ecotoxicological properties. It focuses primarily on substances that enter the environment from wastewater treatment systems, particularly through land application of sewage sludge.

4.3 The fate of pharmaceuticals and personal care products during composting of sewage sludge (Publication 3)

The concentrations of 12 micropollutants from the PPCP group were monitored during composting of sewage sludge with different amounts of straw pellets. In addition to the concentrations of the selected contaminants, some physicochemical parameters of the composts were also evaluated after 4 months of composting. The sludge content in the individual actively aerated composters was (% w/w) 100, 75, 50, 25, 0. The results show that in terms of contaminant removal, composting had a positive effect on caffeine, citalopram, diclofenac, mirtazapine, venlafaxine, and partially sulfapyridine. For the rest of the PPCPs investigated, composting had no effect and, in many cases, even an increase in concentration was observed, probably due to a reduction in the total compost mass. From the analysis of the data, it appears that the degradation of PPCPs is not significantly affected by pH and total nitrogen content (in the range tested). It can also be concluded from the data that the loss of the above-mentioned substances is caused by bacteria and not by fungi. The effect of composting on the individual substances also depended on the sludge content in the starting mixture; according to the data, 25% sludge and 75% straw pellets seem to be the best composition. In the composters where the sludge content was 75% or 100% the highest recorded temperature was below 40 °C. This indicates that such a high sludge content in the chosen arrangement is unlikely to promote proper composting.

4.4 Remedial trial of sequential anoxic/oxic chemical-biological treatment for decontamination of extreme hexachlorocyclohexane concentrations in polluted soil (Publication 4)

Publication 4 describes a chemical-biological method for remediation of soil contaminated with high concentrations of HCHs from historical lindane production. The method was based on a sequence of anaerobic and aerobic phases, promoting both reductive dechlorination under anaerobic conditions and oxidation and aerobic degradation of HCHs and their degradation products formed in the anaerobic phase. Different combinations of nano- and microzerovalent iron (nZVI/mZVI) and different oxygen sources (H_2O_2, MgO_2, and CaO_2 in different forms) were used to initiate, maintain, and alternate these phases. Grass was added with nZVI/mZVI (1st and 2nd anoxic phase), or alone (3rd and 4th anoxic phase) as a carbon source for the present bacteria. The chemical-biological process started and finished with an anaerobic phase. After the last, fourth, one, composting with straw in outdoor rotary composters was included in the procedure (127 days). The purpose of this final step was to remove the

remaining HCH degradation products and to reduce the overall ecotoxicity of the material, most likely caused by products of HCH degradation as well as grass decomposition. From the results of the described experiment, it can be concluded that although no further degradation of residual HCH occurred during composting, its inclusion significantly reduced the ecotoxicity of the remediated soil compared to the status after the last anoxic phase. Successful bioremediation of the soil was achieved by the chosen procedure, as the resulting material can be disposed of in a standard landfill according to the legislation in force.

5 Conclusions

This dissertation thesis is focused on composting as a bioremediation technology and especially the various process conditions that affect its efficiency in contaminant removal. Two aged PAH-contaminated soils were treated by composting with four different organic substrates to study the influence of substrate composition. The final PAH removal was 95% or higher after more than 680 days of composting with all the tested organic substrates. This indicates that it is possible to use a lot of different organic materials without negatively affecting the final extent of PAH loss, although some of them can support slightly faster removal. The ratio of treated material to a composting substrate can be crucial as was demonstrated by composting of sewage sludge with straw pellets. With 75% or more of sludge in the starting mixture, the process development was altered. This can manifest for example in a lower removal of contaminants as well as in achieving a maximum temperature in the mesophilic range instead of the thermophilic. Composting enhanced the removal of six of the monitored PPCPs, while it did not affect the other six. It can be concluded that further research is needed to optimize the composting of sewage sludge and to better understand the fate of individual substances. Composting has also proved to be an effective final step in sequential anoxic/oxic chemical-biological remediation of HCH-contaminated soil because it can reduce the content of degradation products and lower the overall ecotoxicity of the treated material.

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List of scientific publications and conference contributions

Publications:

Němcová, K., Lhotský, O., Stavělová, M., Komárek, M., Semerád, J., Filipová, A., Najmanová, P., Cajthaml, T. Effects of different organic substrate compositions on the decontamination of aged PAH-polluted soils through outdoor co-composting. *Chemosphere* 2024, *362*, 142580.

Hanč, A., Dume, B., Hřebečková, T., Michal, P., Hrčka, M., **Němcová, K.**, Grasserová, A., Cajthaml, T. The fate of pharmaceuticals and personal care products during composting of sewage sludge. *Sustainable Chemistry and Pharmacy* 2024, *38*, 101498.

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Sredlova, K. **Sirova, K.**, Stella, K., Cajthaml, T. Degradation Products of Polychlorinated Biphenyls and Their In Vitro Transformation by Ligninolytic Fungi. *Toxics* 2021, 9, 81, doi: 10.3390/toxics9040081.

Conference contributions:

- 7th International Symposium on Biosorption and Biodegradation/Bioremediation, Prague, Czech Republic, June 16–20 2024
  Poster presentation: Effects of different organic substrate compositions and soil-to-substrate ratios on the decontamination of aged PAH-polluted soils through outdoor co-composting

- 15th PGS Conference, Vysočina, Czech Republic, April 18–19 2024
  Oral presentation: Composting of PAH-polluted soils – Composition and amount of organic substrate

- 14th PGS Conference, Vysočina, Czech Republic, April 24–25 2023
  Oral presentation: Indirect analysis of conjugated endocrine disruptors and their presence in sewage sludge

- Životní prostředí – Prostředí pro život 2022, Prague, Czech Republic, September 12–14 2022
  Oral presentation: Degradace vybraných endokrinních disruptorů během kompostování čistírenského kalu

- 8th European Bioremediation Conference, Chania, Greece, June 12–17 2022
  Oral presentation: Degradation of selected endocrine disruptors during sewage sludge composting

- 13th PGS Conference, Vysočina, Czech Republic, April 25–26 2022
  Oral presentation: Degradation of selected endocrine disruptors during sewage sludge composting

- 12th PGS Conference, Prague, Czech Republic, September 27–29 2021
  Short oral presentation: Fate of organic pollutants during composting