A Novel Laboratory Microcosm for Cocomposting Of Pentachlorophenol Contaminated Soil

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A bench-scale composting system was constructed that relied on heat generation by microbial activity in the material rather than extraneous incubation. The system gave reproducible composting temperatures and eight microcosms could be operated simultaneously. These systems were used to investigate the feasibility of cocomposting pentachlorophenol (PCP) contaminated soil as a bioremediation strategy. Laboratory cocomposting of the contaminated soil successfully reduced PCP concentrations by 80%, from 68 mg/kg to 11 mg/kg in a six-week period. Losses of PCP from compost controls were minimal indicating that removal was due primarily to biotic processes. A comparison of residual PCP levels determined by dichloromethane (DCM) extraction and a methanolic potassium hydroxide (MeOH/KOH) digest prior to DCM extraction suggested that the residual PCP was bound to the compost matrix. Recovery of PCP at the end of a composting experiment using eight vessels simultaneously using the MeOH/KOH method indicated residual concentrations of 8-10 mg/kg.

Introduction

Pentachlorophenol (PCP) is a broad-spectrum biocide, used extensively in the treatment of timber since the 1920s. Though predominantly used as a wood preservative, PCP has also been used as a biocide in oils and paints (Edgehill and Finn 1983, Häggblom and Valo 1995). The extensive use of PCP as a fungicide in saw mills and timber yards has resulted in contamination of surrounding soil and water environments. This contamination has been attributed to accidental spills, stormwater run-off, and leaching from treated timber in service, as well as the treatment processes used (Huling *et al.* 1995, Laine and Jørgensen 1997, Hurst *et al.* 1997).

Approximately 4,000 tonnes of soil contaminated with pentachlorophenol (PCP) and turpentine were excavated from the site of a disused timber yard. PCP concentrations ranging from 50 to >600 mg/kg were detected in the soil. Turpentine used as the organic carrier for the PCP was detected in concentrations of around 100 - 1000 mg/kg in the soil (McClure *et al.* 1997). Representative samples of the soil were used in this study to investigate the feasibility of using the composting process as a bioremediation strategy.

Composting was selected as a remediation strategy due to a number of factors. PCP is not appreciably volatile under alkaline conditions. Losses by volatilization could be expected to be minimal during the composting process (Huling *et al.* 1995, Pfender *et al.* 1997). Previous laboratory and field studies have shown successful use of composting for remediation of PCP contaminated soil (Laine and Jørgensen 1997, Valo and Salkinoja-Salonen 1986, Laine *et al.* 1997). The composting process involves degradation of complex organic compounds including phenols and substituted aromatic rings (Edgehill and Finn 1983). Complex and heavily substituted xenobiotic compounds, such as PCP, appear well suited to this system (Häggblom and Valo 1995). The PCP had persisted at the contaminated site for a number of years after its use had ceased. Persistence may have been due to nutrient limitation or inherent recalcitrance to degradation. Previous studies have indicated that landfarm/biopile type systems

may be ineffective unless augmented with PCP degrading organisms (Warhurst and Fewson 1994, Häggblom and Valo 1995).

The use of laboratory-scale test systems for assessing the efficiency of composting for remediation of pollutants has been previously reported (Ashbolt and Line 1982, Palmisano *et al.* 1993, Tseng *et al.* 1995, Bruns-Nagel *et al.* 1998, Potter *et al.* 1999). Many of these systems have used small amounts of material (<100g) placed in water baths, incubators or modified ovens to reproduce the temperatures attained in natural compost systems (Magalhaes *et al.* 1993, Tseng *et al.* 1995, van Leeuwen *et al.* 1996). A laboratory-scale system was devised which used larger amounts of substrate in a controlled natural composting process. The aim of the system was to provide a microcosm that would be more representative of the environmental conditions that would arise in a full-scale composting process. The development of this microcosm system could then result in more reliable prediction of field scale requirements and outcomes, such as compost composition, initial pollutant concentrations and end-point concentrations.

Materials and Methods

Composting Vessel Design

Forced aeration composting vessels were constructed from modified 30 L wide necked polythene containers. The bottom of the container was filled with 2.5 cm depth of 12 mm coarse gravel. This was to collect leachate from the compost mixture and reduce the tendency for moisture saturation and anaerobic conditions. The gravel was covered by a perforated (5 mm) grill and the compost mixture placed over the grill and filled to the top of the container. A perspex lid was lined with a 8mm neoprene pad which formed an air tight seal in contact with the top of the container. The lid was held in place and the seal maintained using cross bars which screwed down onto the lid.

The outside of the container was insulated with medium thickness foil "anticon" building blanket (1 mm Aluminum foil backed glass fibre, Southern Insulations, Melrose Park, Australia). To allow replicate compost microcosms to be run simultaneously, a multiple vessel system was constructed. Vessels were constructed with identical materials, insulation, and lids, and each fitted with a tap to allow individual variations to air flow rates. A loop of plastic tubing with holes at regular intervals was placed inside the container beneath the gravel layer. This was designed to produce a diffuse flow of air through the compost and reduce the tendency for channeling of the air stream. The eight vessels were connected to a Condor MDR/2 compressor with regulator (1100 KPa) and exhaust air lines passed through a common compost biofilter. Air flow from the compressor to the vessels was balanced to give similar rates to each vessel (1 L min^{-1}). Each vessel was fitted with a PTAT temperature probe linked to a Tain TCS2 multi-channel data logger (Tain Electronics, Melbourne). The thermocouple wire ran through a steel tube sealed with silicone glue, which could be inserted through an airtight port in the vessel lid. Temperature could then be monitored without disturbing the container contents. An additional probe was placed next to the vessels to monitor the ambient temperature. The data logger downloaded data to TCSLOG version 3.8A software (Tain Electronics, Melbourne) installed on a PC.

PCP Extraction Methods

Dichloromethane (DCM) was used to extract PCP directly from the compost mixture. DCM (25 mL) was added to 10 g compost samples in 50 mL centrifuge tubes. The

samples were shaken well and allowed to equilibrate for 30 minutes. After this time the samples were centrifuged at 4500 rpm for 3 minutes using a Beckman CS-15 centrifuge (Beckman Instruments). The solvent layer was retained and placed in fresh centrifuge tubes. The extraction process was repeated and the solvent layers combined to give a total extraction volume of 50 mL. The DCM volume was reduced by evaporation to 1 mL and dried with excess anhydrous sodium sulphate. This procedure gave a ten-fold concentration of the PCP extracted from the original soil sample. There was approximately 89% recovery from compost spiked with 100 mg/kg PCP using this method.

An extraction method was developed to remove PCP bound to the compost matrix modified from the method of van Leeuwen *et al.* (1996). Samples of soil (10 g) were placed in Kjeldahl tubes containing 3 mL of 10 M KOH, and 25 mL of Methanol (MeOH). The samples were refluxed at 70°C for three hours.

After this time, the liquor was decanted into centrifuge tubes and acidified with excess 2 M Sulphuric acid (3 mL) to precipitate PCP from the aqueous phase. To prevent emulsification of the dichloromethane (DCM) solvent with the methanolic aqueous phase, 5 mL of deionised water was added to the tubes. The samples were then extracted twice with 25 mL aliquots of DCM. The DCM extracts were combined and evaporated to a final volume of 1 mL at room temperature under a fume hood. This gave a tenfold concentration of the soil sample. There was approximately 92% recovery from compost spiked with 100 mg/kg PCP using this method.

PCP concentrations were also assessed using continuous solvent (Soxhlet) extraction. Soil or composted material samples were acidified with hydrochloric acid (37%) and 10 g weighed into 28 × 100 mm cellulose extraction thimbles (Whatman International, Maidestone, United Kingdom) and placed in a 100 mL "quickfit" Soxhlet apparatus. Dichloromethane (150 mL) was refluxed over the sample for four hours. This resulted in 8 complete solvent extractions (one per half hour) from the sample. After this time, the extract was concentrated to 1 mL on a rotary evaporator.

GC-FID Analyses

PCP extracts in DCM were dried with anhydrous sodium sulphate and placed in 1.5 mL autosampler vials. 1 uL aliquots were injected onto a 30m DB-5 column (J&W Scientific, Folsom, California, O.32 mm int. dia.) using a Varian 3400CX gas chromatograph (Varian Associates Inc., Walnut Creek, California.) with autosampler (8200CX) and flame ionization detector (FID). The injector temperature was set to 250°C and the detector temperature to 320°C. The oven was programmed to give an initial column temperature of 190°C for 3 mins. The temperature was then increased to 300° C at a rate of 45° C per minute and held at that temperature for 0.2 minutes. This program was designed for isothermal elution of PCP followed by a temperature ramp to remove less volatile components of the organic extracts from the column. PCP concentrations were quantified using an external standard that consisted of 500 mg/kg PCP (Sigma Chemicals, St Louis, Missouri) in dried DCM. PCP concentrations were calculated from peak area relative to that of the external standard used in the run. This standard was included in each run with the inclusion of one standard in every ten samples.

Composting of PCP Contaminated Soil

The multiple vessel composting microcosms described above were used to compare composting of different ratios of the PCP contaminated soil. Duplicate vessels were filled with 1:1, 1:2 and 1:3 ratios (vol:vol) of PCP contaminated soil and green

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waste (mixed grass clippings and woody waste). The ratios were obtained by adding the two components to a rotating drum and mixing thoroughly before addition to the composting vessels. DCM extracts of the three starting mixtures were obtained. Samples were removed from each of the vessels at intervals and DCM extracts obtained for GC-FID analysis of PCP concentrations.

To confirm the reproducibility of the results, the above procedure was repeated using 8 composting vessels filled with a 1:1 mixture of contaminated soil:green waste. Periodically PCP was extracted using DCM extraction and quantified by GC-FID analyses.

To confirm that PCP removal was due to biotic processes, the above process was repeated, but in each of the eight vessels was placed a 250 mL air tight polyethylene bottle. Each bottle contained 70 g of the compost mixture with 1% (W:W) $HgCl_2$ mixed into it to serve as a "killed" control. The systems were sealed and allowed to compost under the conditions described. After 6 weeks 10 g samples of soil were removed from each vessel and each bottle and analyzed for PCP content using both DCM and MeOH/KOH/DCM extraction methods outlined. Mean PCP concentrations at the beginning and end of the composting process were calculated for the test and control samples.

Monitoring of PCP Volatilization From Compost

In three of the compost vessels, activated charcoal tubes (SKC Inc. Pennsylvania) were inserted in the exhaust lines for 5 hours during the thermophilic phase (day 3) of composting. The charcoal tubes were removed after this time. The charcoal was extracted in 1 mL of DCM, and the extract dried with anhydrous sodium sulphate. The samples were analyzed by GC-FID using the method described. At a flow rate of 1 L min^{-1} through the vessels, this represented analysis of volatilization of organic material from 300 L of air passing through the vessels.

Results And Discussion

Composting of Different Contaminated Soil:Green Waste Mixtures

The average temperature profiles and PCP concentrations for the three green waste/soil mixtures are presented in Figures 1 and 2 respectively. The 3:1 mixture of green waste:soil reached a maximum temperature of 50°C after 5 days. The thermophilic phase lasted 6 days after which there was a fairly rapid cooling off period of 4 days. Temperatures fell to ambient values after 10 days. The initial PCP concentrations in the mixture were 58 (mg/kg). This concentration was reduced to 20 mg/kg after the end of the cooling off period and final concentrations of 5 mg/kg were attained after 8 weeks. This equated to >90% reduction in PCP concentrations.

The 2:1 mixture of green waste:soil reached a maximum temperature of 38°C after 5 days. Thermophilic and cooling off phases were of the same duration as in the 3:1 mixture detailed above. Initial PCP concentrations were 84 mg/kg. This concentration was reduced to 11 mg/kg after 8 weeks. This equated to an 85% reduction in PCP concentrations.

The 1:1 mixture of green waste:soil reached a maximum temperature of 32°C after 2 days. Temperatures gradually declined to ambient over the following week. There were no clearly definable thermophilic and cooling off phases. The starting concentration of PCP was 108 mg/kg. This was reduced by greater than 95% to 5 mg/kg at the end of the 8 week period.

The composting of different mixtures of contaminated soil:green waste resulted in
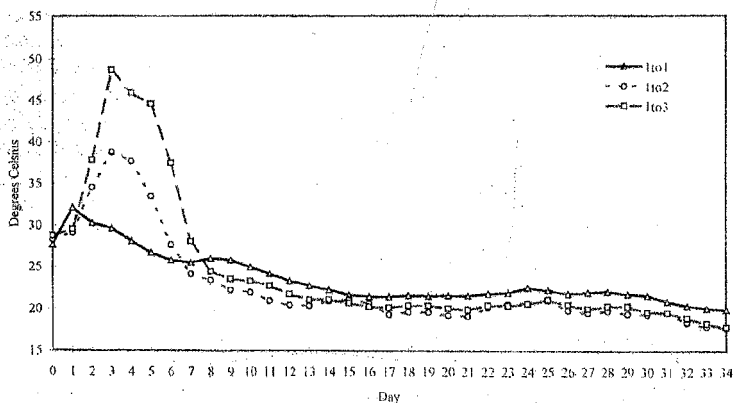
Figure 1. Average compost temperatures for different ratios of contaminated soil:green waste
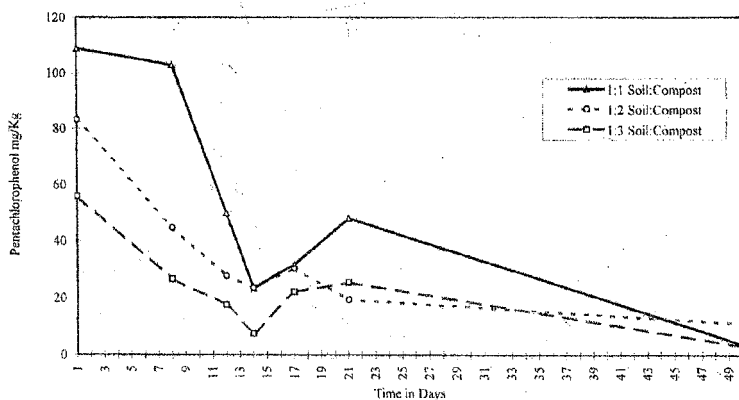

Figure 2. Average PCP concentrations (mg/kg) in compost treatments by GC-FID

similar end-point concentrations of PCP over similar time periods. This result further suggests that the reduction in PCP concentrations was due to microbial action. If the losses were due to binding of the PCP to the organic matrix in the compost, it could be assumed that there would be more binding of the PCP over a shorter time period in the 3:1 green waste:soil mixture (Huling *et al.* 1995). PCP reductions were greatest during the cooling off period. The majority of binding would be expected to occur during the stabilization of the compost when humification and polymerization is most extensive (Mathur 1991).

The temperatures obtained in the 1:1 GW:PCP mixture were lower than the other two mixtures. This may have been due to a lower supply of readily degradable organic material in the system. It may have also have been due to the higher PCP concentration exhibiting an inhibitory effect on microbial activity. The initial lag in degradation of PCP as compared to the other two mixtures may be attributable to initial toxic effects and acclimation of the microbial population or a slower rate of depletion of alternative carbon sources prior to onset of PCP degradation due to lower temperatures.

Temperatures achieved during composting are also a function of the composition of the material. The low temperature achieved in the 1:1 mixture may be related to the age and quality of the green waste as a compost substrate (Brunt, Dean and Patrick 1990, Mathur 1991). Green waste is a diverse mixture of organic materials. Supplementation of the compost with materials such as hard woods with high lignin, aromatic and phenolic content may influence the degradative capabilities of the enriched microorganisms (Laine, Haario and Jørgensen 1997). Of interest for further investigation is the influence of the matrix composition on the degradation end points and rates in the PCP cocomposting process.

Composting of Replicate Contaminated Soil:Green Waste Mixtures

PCP concentrations in the composting vessels decreased from 100% (68 ± 23 mg/kg) to 10% (7 ± 3.5 mg/kg) within a six week period (Figure 3). After this time no further decreases in PCP concentrations were detected. Compost temperatures reached a maximum of 50°C after 5 days, temperatures dropped to ambient levels within 10 days. The maximum PCP degradation rate was observed during the cooling off period. 95 percent of the PCP losses were recorded in the first 4 weeks. It was noticeable that the variation in sampling results decreased throughout the course of the experiment. This was probably due to the mixing of the compost during successive samples, and to the increasing homogeneity of the mixture as the composting process progressed.
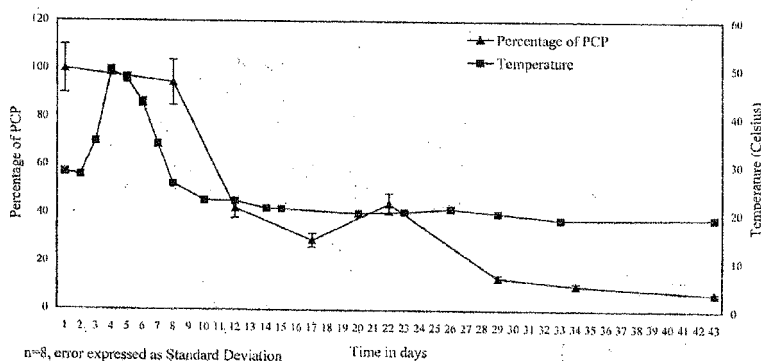

Figure 3. Mean percentage PCP recovery (dichloromethane extraction) and mean compost temperatures in eight microcosms

Composting of "Killed" Contaminated Soil:Green Waste Mixtures

There was a slight but not significant decrease in PCP concentrations, from 68 ± 23 mg/kg to 42 ± 12 mg/kg in the killed control samples placed in the vessels (Figure 4). This may have been due to binding and incomplete extraction from the compost. The mercuric chloride addition to control samples did not sterilize the soil and so some loss of PCP, at a reduced rate, due to microbial activity was possible. Composted soil showed reductions of PCP concentrations from 68 ± 23 mg/kg to 12 ± 9.8 mg/kg in 15 weeks. Temperature profiles and PCP losses were similar to those recorded in the previous batches over the same time periods. The majority of PCP degradation occurred in the cooling off phase.
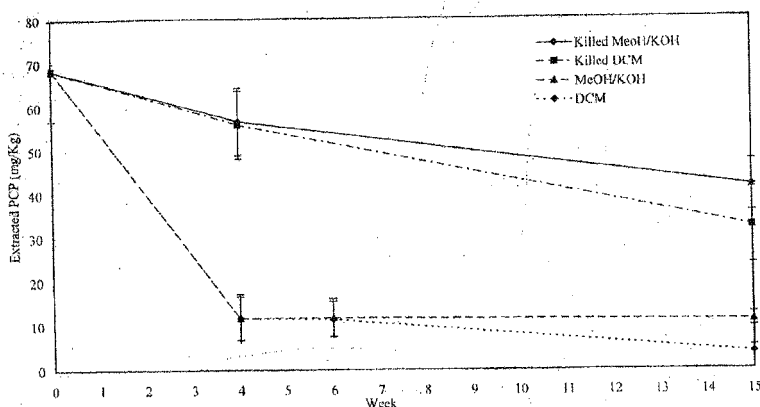
Figure 4 Comparison of MeOH/KOH and straight DCM extractions from test and control composts

The results from "killed" control samples in the laboratory system demonstrated that the removal of PCP was due primarily to biological activity. Control samples were heterogenous, looking similar to the starting mixture and did not show visible evidence of composting. It was interesting to note that PCP degradation occurred during the cooling off phase, and not in the thermophilic phase. Valo and Salkinoja-Salonen (1986) reported that the majority of degradation of PCP occurred in the early stages of composting but whether this was during or after the thermophilic stage was not stated. Temperatures attained in their system were relatively low (38°C) but showed similar degradation rates and endpoints to those reported in this study (Valo and Sakinoja-Salonen 1986).

Volatilization of PCP During Composting

GC-FID analysis showed no detectable PCP from three separate samples of 300 L of air that passed through the vessels during the composting process. This result agrees with previous reports regarding volatilization of PCP from soil systems (Huling *et al.* 1995, Pfender *et al.* 1997). As the compost mixture remained alkaline, between pH 7.5 and 8.5, throughout the process the PCP could be expected to exist as the more polar, less volatile pentachlorophenolate ion (Huling *et al.* 1995).

PCP Extraction Methods

The improved recovery of PCP from the compost using the MeOH/KOH extraction method demonstrated that it was binding to the compost matrix. It was noticeable that the amount of binding was most apparent towards the end of the experiment. This indicates that binding is a relatively slow process and may be linked to soil humification processes (Brunt, Dean and Patrick 1990). Recoveries of PCP from spiked soil were similar for both DCM and MeOH/KOH/DCM extraction methods (data not shown). Weathering or humification of the contaminant may account for the difference in observed recoveries from the test samples (Alexander 1994). It can be assumed from these results that weathered PCP contamination may become increasingly more difficult to remediate (McAllister, Lee and Trevors 1996). MeOH/KOH extracts at the end of the

composting of the 1:1 soil:green waste experiment with "killed" controls recovered 8 - 10 mg/kg PCP as compared to 3 - 5 mg/kg from DCM extracts. It is possible that the degradation of PCP in the systems ended once the molecule was no longer bioavailable or reduced below concentrations sufficient for enzyme induction (Fetzner and Lingens 1994). The MeOH/KOH extraction was rigorous, modified from a method used for extraction of chlorophenols from lake sediments. The difference in concentrations between the DCM and MeOH/KOH extracts may be that fraction not available for microbial degradation. Valo and Salkinoja-Salonen (1986) reported residual concentrations of 15 mg/kg PCP after composting of chlorophenol wastes from a wood treatment site. Laine and Jørgensen (1997) reported final PCP concentrations of less than 10 mg/kg from pilot scale composting of chlorophenol contaminated soil. These observations agree well with the results of the composting experiments described in this investigation. Laine *et al.* (1997) demonstrated that binding and polymerization of PCP to the compost matrix was minimal by monitoring changes in molecular weight distribution of organic halogenated compounds. They concluded that polymerization was not a predominant PCP fate during composting.

The binding of PCP to the organic matrix in the compost would reduce bioavailability and toxicity of the residual pollutant (Alexander 1994, Bouwer and Zehnder 1993). In this case the residual concentrations of PCP in the composted material could be expected to be persistent. Binding may be attributable to chemical humification and sorption between microbial cells and humic substances (van Leeuwen *et al.* 1996, Agnihotri and Barooah 1994, Fujimura, Kuwatsuka and Katayama 1996). Slow degradation of the bound PCP fraction may occur and this may explain the gradual decreases in extracted PCP observed after the end of the cooling off period of composting (Agnihotri and Barooah 1994). The humification and binding processes at the end of composting result in the immobilization of the pollutant (Miller 1993, Agnihotri and Barooah 1994, Fujimura, Kuwatsuka and Katayama 1996). The buffering capacity of the compost (Miller 1993) and the nature of humification results in non-availability and therefore bioremediation of the soil (Fujimura, Kuwatsuka and Katayama 1996). The MeOH/KOH extraction method demonstrated that the residual PCP may not have been bioavailable. Any release of PCP from the compost matrix would be slow and probably linked to degradation (Agnithotri an Barooah 1994). The demonstrated losses of PCP in this study do not take into account the presence or concentrations of PCDDs (polychlorinated-p-dibenzodioxins) or PCDFs (polychlorinated-p-dibenzofurans). These compounds have been demonstrated to be persistent in compost though less mobile than chlorophenols (Laine *et al.* 1997). Composting has been shown to mobilize these compounds in soil and they may also be formed by the action of fungi on chlorophenols. Due to the high lipophilicity and demonstrated accumulation in mammals of PCDD and PCDF, caution should be exercised in the disposal of composted chlorophenols (Laine *et al.* 1997).

Composting Vessel Design

Composting is best suited to the remediation of less volatile compounds, as more volatile compounds may pose problems with the containment of emissions and possible environmental impacts (Martin and Bardos 1995). The provision of aerobic, anaerobic, oxidizing and reducing and mesophilic and thermophilic environments during composting allows for a much larger range of chemical and biochemical transformations to occur (Tseng *et al.* 1995, Miller 1993, Mathur 1991). The multiple vessel laboratory composting systems have the potential for bioremediation feasibility testing for a

range of less readily degradable pollutants including PAHs, pesticides, and heavy oils. This system offers rapid, reproducible results under controlled conditions using small amounts of material that duplicate many of the constraints of a larger scale system.

The systems provided a reproducible, self heating system which stimulated a microbial response linked directly to the composition of the material provided. This system could be used to provide a valid and realistic evaluation of the likely outcomes of composting material at a larger scale. The system allowed replicate experiments to be performed under controlled conditions.

Composting has been used as a remediation process for a number of pollutants including PAHs, hydrocarbons and chlorophenols (Laine and Jørgensen 1997, Joshua, Macauley and Hudson 1994, Riggle 1995, Martin and Bardos 1995). Composting has had more limited commercial application than conventional treatments such as landfarms and bioreactors (Martin and Bardos 1995). Laboratory based feasibility studies of composting have used systems in which heat in the compost mixture was generated and maintained by incubators or water jackets. These systems permit reproducibility and control of the composting process (Ashbolt and Line 1982, Tseng *et al.* 1995, Huling *et al.* 1995, Palmisano *et al.* 1993). The laboratory composting system presented in this investigation relied on microbial activity to heat the compost. The system allowed reproducible natural composting with good control.

References

Agnihotri, N.P. and A.K. Barooah. 1994. Bound residues of pesticides in soil and plant – a review. *Journal of Scientific and Industrial Research*, 53:850-61.

Alexander, M. 1994. *Biodegradation and Bioremediation*. Academic Press, Ithaca, New York. ISBN 0-12-049860-X.

Ashbolt, N.J. and M.A. Line. 1982. A bench-scale system to study the composting of organic wastes. *Journal of Environmental Quality*, 11:3:405-8.

Biddlestone, A.J. Gray, K.R. and C.A. Day. 1987. Composting and straw decomposition. In: Forster, C.F. and D.A.J. Wase (eds) *Environmental Biotechnology*. Ellis Horwood, Chichester. ISBN 0-85312-838-3. pp135-75.

Bouwer, E.J. and A.J.B. Zehnder. 1993. Bioremediation of organic compounds – putting microbial metabolism to work. *Trends in Biotechnology*, 11:360-7.

Bruns-Nagel, D. Drzyzga, O. Steinbach, K. Schmidt, T.C. Von L?w, E. Gorontzy, T. Blotevogel, K. –H. and D. Gemsa 1998. *Environmental Science and Technology*, 32:1676-79

Brunt, L.P. Dean, R.B. and P.K. Patrick. 1990. Composting. In: Suess M.J. (ed.). *Solid waste management*. W.H.O. pp37-77.

Edgehill, R.U and R.K. Finn. 1983. Microbial treatment of soil to remove pentachlorophenol. *Applied and Environmental Microbiology*, 45:3:1122–5.

Fetzner, S. and F. Lingens. 1994. Bacterial dehalogenases: biochemistry, genetics and biotechnological applications. *Microbiology Reviews*, 58:4: 641-85.

Fujimura, Y. Kuwatsuka, S. and A. Katayama. 1996. Bioavailability and biodegradation rate of DDT by Bacillus sp. B75 in the presence of dissolved humic substances. *Soil Science and Plant Nutrition*, 42:2:375-81.

Häggblom, M.M. and R.J. Valo. 1995. Bioremediation of chlorophenol wastes In: Young, L. Y. and C.E. Cerniglia, (eds.). *Microbial transformation and degradation of toxic organic chemicals*. Wiley-Liss. 11:389-434.

Huling, S. G. Pope, D. F. Matthews, J. E. Sims, J. L. and D.L. Sorenson. 1995. In: Hinchee, R. E. Hoeppel, R.E. and D.B. Anderson, (eds.). *Bioremediation of recalcitrant organics*. Battelle Press. Columbus Ohio. 3:7:101- 109.

Hurst, C. J. Sims, R. C. Sims, J. L. Sorenson, D. L. McLean, J. E. and S. Huling. 1997. Soil gas oxygen tension and pentachlorophenol degradation. *Journal of Environmental Engineering*, 123:4:364-70.

Joshua, R.S. Macauley, B.J. and C.R. Hudson. 1994. The development of an environmentally controlled composting system for the recycling of grease trap sludges. *BioCycle*, 35:12:46-7.

Laine, M.M. and K.S. Jørgensen. 1997. Effective and safe composting of chlorophenol-contaminated soil in pilot scale. *Environmental Science and Technology*, 31:371-378.

Laine, M.M. Haario, H. and K.S. Jørgensen. 1997. Microbial functional activity during composting of chlorophenol-contaminated sawmill soil. *Journal of Microbiological Methods*, 30:21-32.

Laine, M.M. Ahtianen, J. Wägman, N. Öberg, L.G. and K.S. Jørgensen. 1997. Fate and toxicity of chlorophenols, polychlorinated dibenzo-p-dioxins, and dibenzofurans during composting of sawmill soil. *Environmental Science and Technology*, 31:3244-50.

Magalhaes, A.M.T. Shea, P.J. Jawson, M.D. Wicklund, E.A. and D.W. Nelson. 1993. Practical simulation of composting in the laboratory. *Waste Management Research*, 11:143-54.

Martin, I. and P. Bardos. 1995. *A review of full scale treatment technologies for the remediation of contaminated soil*. EPP Publications, Surrey, UK. ISBN 1-900995-00-X.

Mathur, S.P. 1991. Composting processes. *In:* Martin, A.M (ed.). *Bioconversion of waste materials to industrial products*. Elsevier Applied Science, London. 5:147-83.

McAllister, K. A. Lee, H. and J.T. Trevors. 1996. Microbial degradation of pentachlorophenol. *Biodegradation*, 7:1:1-40.

McClure, N.C., Dandie, C., Bentham, R.H., Franco, C., and I. Singleton. 1997. Establishment of a bioremediation facility in South Australia – research and commercial potential. *Australian Biotechnology*, 7:6:345-9.

Miller, F.C. 1993. Composting as a process based on the control of ecologically selective factors. *In:* Metting, B jnr. (ed.). *Soil Microbial Ecology*, 18:515-44.

Miller, F.C. 1996. Heat evolution during composting of sewage sludge. *In:* de Bertoldi, M., Sequi, P. Lemmes, B. and T. Papi. (eds.). *The Science of Composting*, Blackie Academic & Professional, Glasgow. ISBN 0-7514-0383-0 pp106-15.

Palmisano, A.C. Maruscik, D.A. Ritchie, C.J. Schwab, B.S. Harper, S.R. and Rapaport, R.A. 1993. A novel bioreactor simulating composting of municipal solid waste. *Journal of Microbiological Methods*, 18:99-112.

Pfender, W. F. Maggard, S. P. Gander, L. K. and L.S. Watrud. 1997. Comparison of three bioremediation agents for mineralization and transformation of pentachlorophenol in soil. *Bulletin of Environmental Contamination and Toxicology*, 59:230-7.

Potter, C.L. Glaser, J.A. Chang, L.W. Meier, J.R. Dosani, M.A. and R.F. Herrmann 1999. Degradation of polynuclear aromatic hydrocarbons under bench-scale compost conditions. *Environmental Science and Technology*, 33:1717-25.

Riggle, D. 1995. Successful bioremediation with compost. *BioCycle*, 36:2:57-9.

Tseng, D.Y. Chalmers, J.J. Tuovinen, O.H. and H.A. Hoitink. 1995. Characterisation of a bench-scale system for studying the biodegradation of organic solid wastes. *Biotechnology Progress*, 11:443-51.

Valo, R and Salkinoja-Salonen. 1986. Bioreclamation of chlorophenol-contaminated soil by composting. *Applied Microbiology and Biotechnology*, 25:68-75.

van Leeuwen, J.A. Nicholson, B. C. Hayes, K.P. and D.E. Mulcahy. 1996. Resistance of bound chloroguaiacols and AOX from pulp mill effluent to degradation by Trichoderma harzianum isolated from Lake Bonney, South-eastern South Australia. *Marine and Freshwater Research*, 47:961-9.

Warhurst, A. M. and C.A. Fewson. 1994. Biotransformations catalyzed by the Genus Rhodococcus. *Critical Reviews in Biotechnology*, 14:1:29-73.