SOLID PHASE ANAEROBIC BIOREMEDIATION OF SOIL FROM THE "TOMB" AREA CONTAMINATED WITH CHLORINATED PESTICIDES

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Abstract: The paper presents results of laboratory tests of solid-phase anaerobic bioremediation of soil contaminated with chlorinated pesticides. It was shown that using methanogenic granular sludge as inoculum and lactate as electron donor, it is possible to remove 80% of γ-HCH, 94% of methoxychlor and 93% of DDT against control sample, with DDD accumulation much less than stoichiometric. Pesticides removal was practically completed after 4–6 weeks of incubation at 22°C. Additional application of nonionic surfactant Tween 80 resulted in about one and a half-fold decrease of residual concentrations of some compounds. It also enhanced DDT conversion to some extent, decreasing DDD accumulation and intensifying production of DBP, the terminal metabolite of DDT anaerobic degradation pathway. Use of methanol as electron donor produced effects quite similar to these obtained with lactate, however with reduced results scatter.

INTRODUCTION

Chlorinated pesticides, although banned decades ago, are still considered a serious threat to the environment. Large quantities of these compounds are deposited worldwide on industrial waste repositories or as obsolete pesticides stocks. In Poland these problems are encountered as tenths of thousands of tons of waste from production of γ-HCH (γ-isomer of HCH, known as lindane), DDT and methoxychlor are placed on industrial landfill in Jaworzno, and thousands of tons of obsolete pesticides are stockpiled in so-called "tombs", with large participation of chlorinated compounds.

In many cases severe contamination of surrounding soils occurred. This problem requires particular attention, as even after disposal of pesticide wastes these areas will still be a source of secondary pollution, in addition to restrictions on their use. The usually applied "dig-and-dump" procedure is limited only to the most contaminated areas and small volumes of excavated soil. Moreover, this in fact leads to creation of new hazardous waste dumping sites. The need for efficient remediation methods for soil contaminated with pesticide wastes is therefore evident.

Previous experiments have shown that it is possible to effectively remove pesticides: γ-HCH, methoxychlor and DDT from field contaminated soil in anaerobic process,

using methanogenic granular sludge (self-aggregating biomass from anaerobic reactors treating high-strength organic wastewater) as inoculum and lactate as electron donor [1, 2]. However, these results were obtained for tests using soil slurry in optimized conditions. Although soil slurry reactors are applied for soil bioremediation at full scale, this technology is quite expensive. A question arose whether it was possible to obtain similar efficiency using less expensive "solid-phase" processes, such as biopiles or landfarming. To demonstrate this, suitable bioremediation tests had to be done, with soil water content maintained at level comparable to its water holding capacity in order to facilitate anaerobic conditions. Another interesting issue was whether application of surfactant in these conditions could improve performance of the process, similarly to results found for tests with soil slurry [2].

One of the problems experienced during previous investigations was the large scatter of results, especially for p,p'-DDT, less often for o,p'-DDT and methoxychlor. Within triplicate analyses made there were occasional outliers, i.e. single subsamples with very high concentration of these compounds, suggesting moderate, little or even no removal in opposition to the rest. At the same time, concentrations of formed metabolites were comparable throughout all the subsamples, demonstrating transformation of similar amount of the parent compound. A likely explanation is that part of respective contaminants is present in soil in the form of randomly distributed microaggregates or microcrystals, hardly soluble in water and thus hardly degradable. To overcome this problem use of biodegradable, water-miscible solvent as alternative electron donor was proposed. Such solvent could assumedly dissolve these agglomerates by the effect of co-solvency, thus improving the overall efficiency of the process.

MATERIALS AND METHODS

Chemicals
Hexane, acetone and methanol (Picograde® quality) as well as 99% PCB209 standard used in this study were purchased from LGC Promochem (Łomianki, PL). Anhydrous sodium sulphate (12–60 mesh ultra-resi analyzed) and sodium lactate (60% syrup) were obtained from J.T. Baker (Łódź, PL). Surfactant Tween 80 was from Sigma-Aldrich (Poznań, PL). Chlorinated pesticides and their metabolites standards (purity of 99.6–99.8%): γ-HCH; o,p' and p,p' isomers of DDE, DDD and DDT; methoxychlor and p,p'-DBP (product of DDT degradation) were purchased from the Institute of Industrial Organic Chemistry (Warszawa, PL).

Granular sludge
The granular sludge was taken from the anaerobic reactor treating wastewater from the soft drink factory "Hellena" in Kalisz, PL. Before use, the sludge was rinsed with tap water on a 0.25 mm sieve, to remove products of decay.

Soil
Soil used in the study was collected from a pesticide "tomb" located in Sepno-Radonia (Łódź voievodship, PL). The sampling point was located ca. 3 m below the ground level, close to the one of concrete wells forming the "tomb" structure. The soil was characterized as sandy clay loam (58% sand, 17% silt, 25% clay; pH 3.8; organic matter 1.9%;

water holding capacity (WHC) 41%). Prior to use, soil was air dried, lightly ground using mortar and pestle and finally sieved through a 1 mm sieve. Such preparation was found necessary to improve homogeneity of contaminants distribution. Chlorinated pesticides concentration in mg/kg of dry soil was: γ-HCH 4.3; o,p'-DDT 7.2; p,p'-DDT 26.2; p,p'-DDD 0.9; methoxychlor (p,p' isomer) 5.1. DDT metabolites other than p,p'-DDD were present in traces.

Remediation experiments

80 g of air dried soil was weighed into glass jar of 0.2 dm³ volume, then 0.12 g of CaO was added to neutralize pH (pH measured during incubation was 7.3). Four jars were prepared: one served as control and the remaining three as test samples. To the control sample ("K" label) 36 cm³ of distilled water was added, which corresponded to ca. 110% of the soil WHC. Test samples were amended with 26.7 g of granular sludge (wet mass, solid content 7%) and 27 cm³ of distilled water (the same amount as in control including about 9 cm³ of free water in the sludge added). To the sample "L" 2.3 cm³ of sodium lactate syrup was added as electron donor; the same amount was dosed to the sample "LS" together with 0.4 cm³ of Tween 80 surfactant. The third test sample, "M", was initially spiked with 0.3 cm³ of methanol, and the same amount of the solvent was added to this sample after 2, 4 and 6 weeks of incubation. Such a dosing procedure was adopted as a precaution against possible inhibition of bacteria by high concentration of methanol. All samples were then mixed thoroughly.

Jars were closed to prevent excessive soil drying and then incubated in a temperature controlled chamber at 22°C in the dark. Sampling was performed after 0, 2, 4, 6 and 8 weeks of incubation. During sampling three subsamples of 1–2 g wet mass were collected from each jar, after thorough mixing of its content. The samples were placed on paper filters, weighed and left to dry overnight. Additionally, fourth subsample from each jar was used for determining the soil water content by drying at 105°C.

Analytical procedure

Dried soil was extracted using method described previously in details [1, 2]. Briefly, soil and filter were placed in 40 cm³ amber vial, spiked with surrogate standard (PCB209 solution in toluene) and then heated at 70°C for 4 h with 16 cm³ of 50/50 (v/v) hexane/acetone in the tightly closed vial. The extract was clarified by centrifuging in a test tube containing small amount of anhydrous sodium sulphate and finally diluted with hexane to fit the GC calibration range.

Analyses were performed on MEGA (Carlo Erba) gas chromatograph equipped with ECD detector and capillary column Stx-500 (30 m × 0.25 mm ID, 0.15 μm film) with 5 m guard column. Temperature program: 65°C, increase 15°C/min to 300°C, held for 9 min., carrier gas: hydrogen at 80 kPa. Injection of 1 mm³ extract was splitless (Uniliner, Restek) at 210°C. Measurements included: γ-HCH; o,p' and p,p' isomers of DDE, DDD and DDT; methoxychlor and p,p'-DBP. Calibrations were made daily, using external standards of five different concentrations. Method detection limits were estimated at 0.05 mg/kg for γ-HCH and DDT isomers; 0.1 mg/kg for DDE, DDD and p,p'-DBP; 0.15 mg/kg for methoxychlor. Results were corrected basing on PCB209 recovery factor, determined using appropriately diluted surrogate standard.

RESULTS

The concentration changes of individual compounds in relation to incubation time are presented in Figures 1–7. To facilitate comparisons of DDT removal and its metabolites production all data are given as μmol/kg of dry soil. The results for test samples (L, LS, M) were not adjusted for increase in solid content as a result of sludge and other amendments addition, as it was considered negligible (less than 5%).
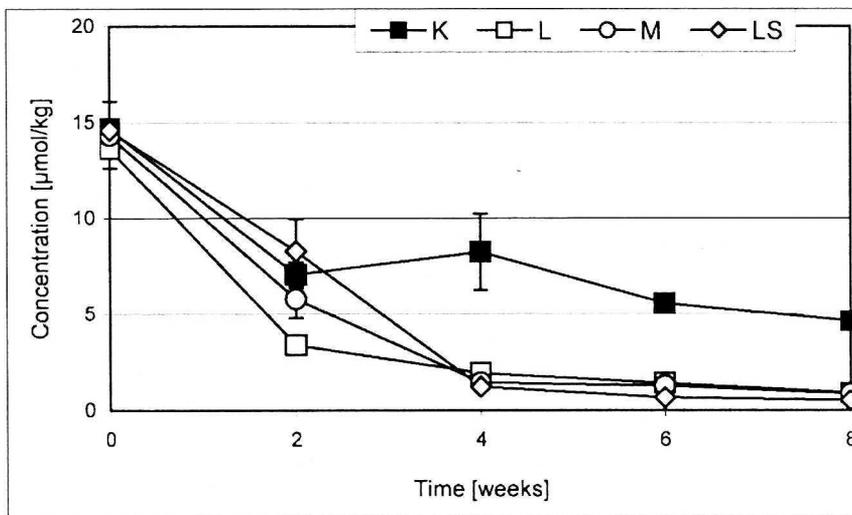

Fig. 1. Concentration of γ-HCH in relation to incubation time
K – control sample, L – sludge + lactate, M – sludge + methanol, LS – sludge + lactate + surfactant; error bars represent standard deviation (some may be covered by symbols)
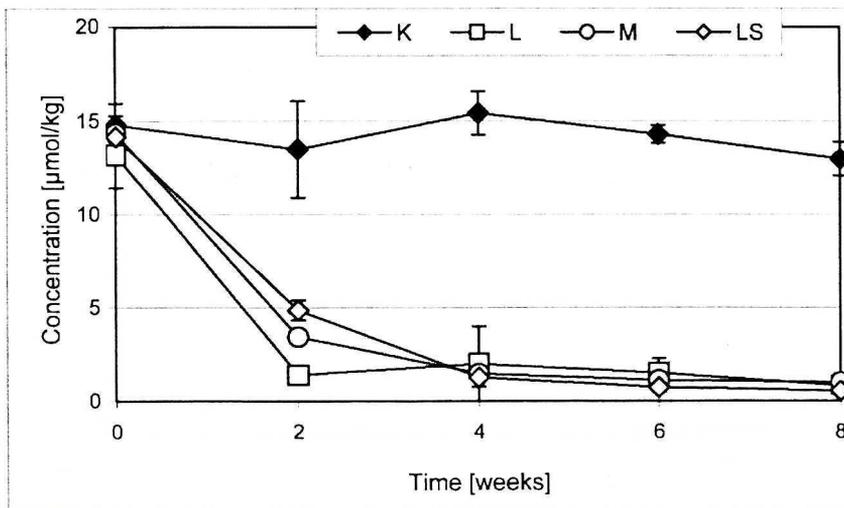

Fig. 2. Concentration of methoxychlor in relation to incubation time
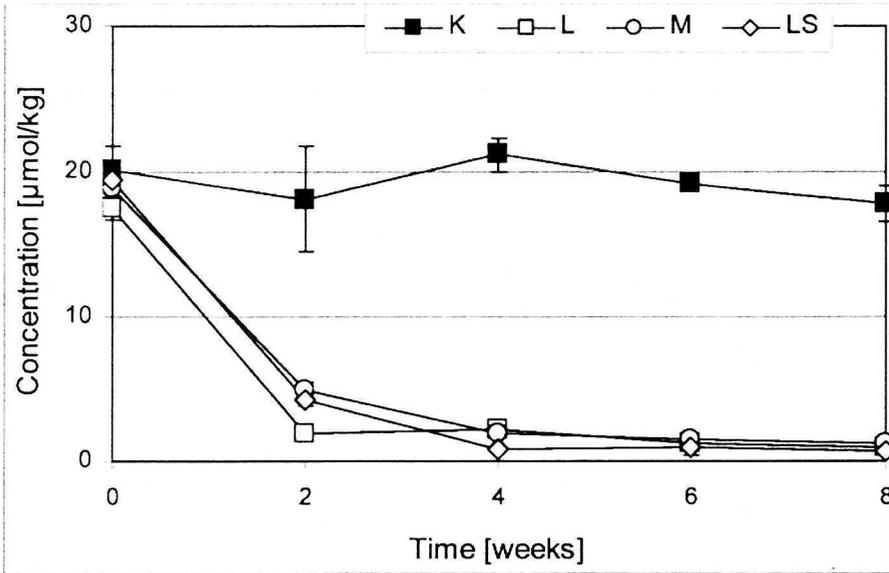
Fig. 3. Concentration of o,p'-DDT in relation to incubation time
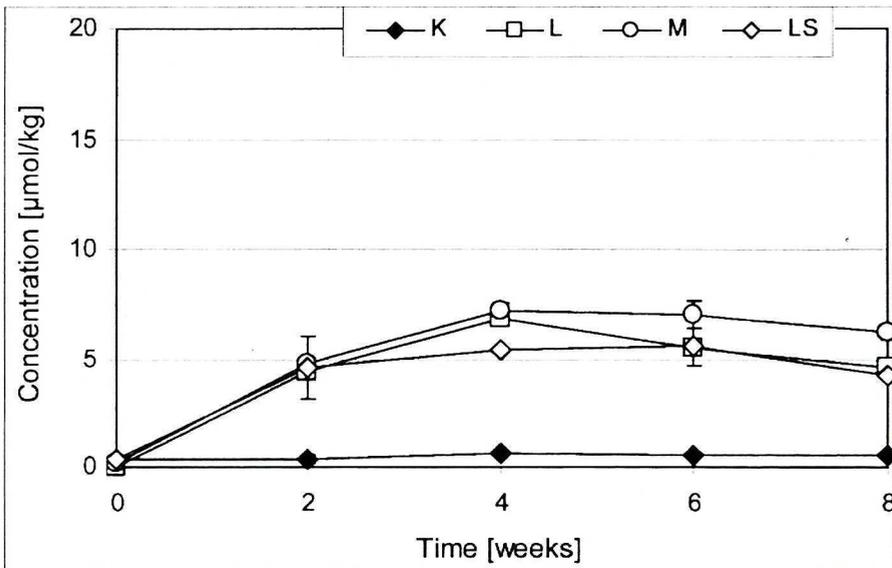

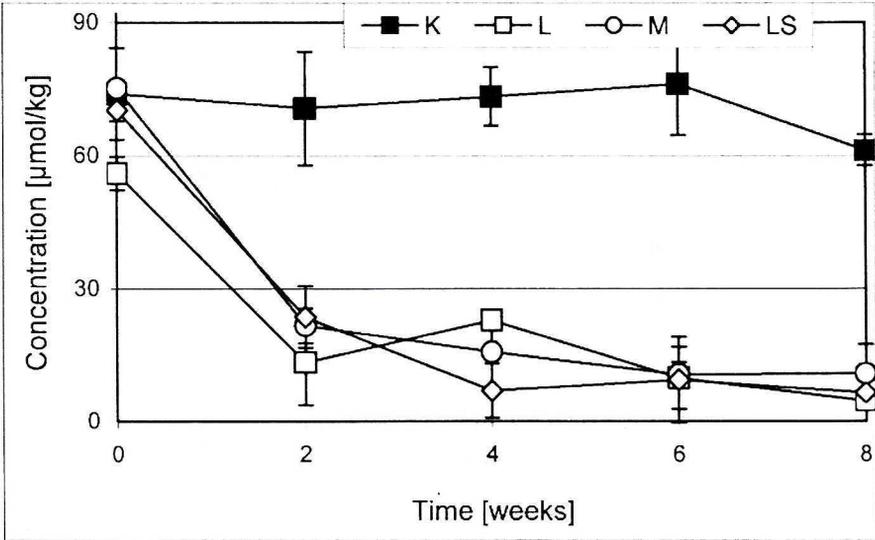Fig. 4. Concentration of o,p'-DDD in relation to incubation time

Fig. 5. Concentration of p,p'-DDT in relation to incubation time
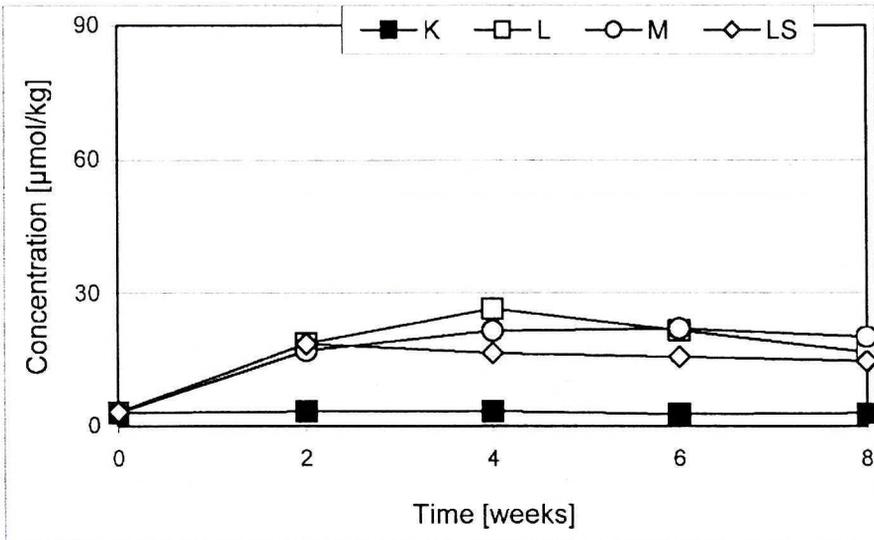

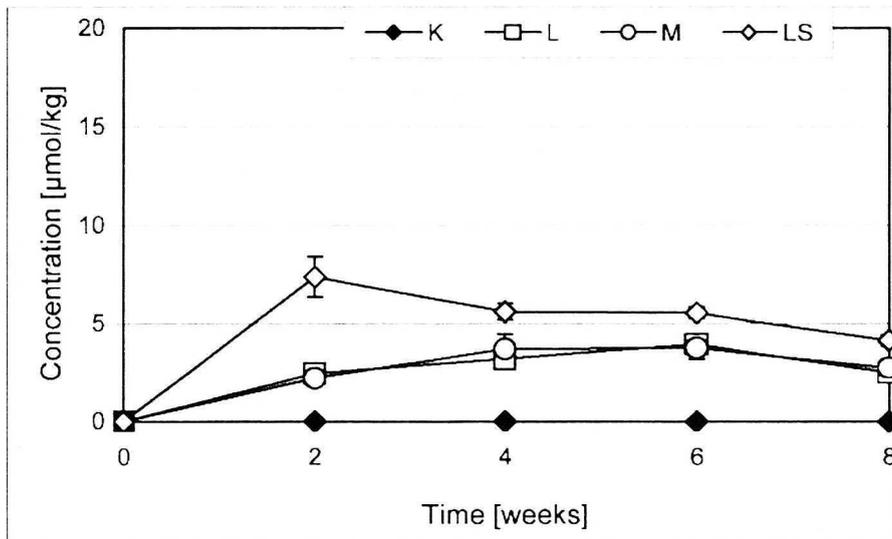Fig. 6. Concentration of p,p'-DDD in relation to incubation time

Fig. 7. Concentration of p,p'-DBP in relation to incubation time

There were no changes of contaminants concentrations in the control sample, apart from γ-HCH, whose amount was reduced by 68% over the whole 8-week incubation (Fig. 1). As this compound is very prone to volatilization from soil [10], this was the most likely cause of this loss.

In contrast to that, in the test sample L inoculated with granular sludge concentrations of all pesticides decreased rapidly and appreciably within first 2–6 weeks. Further incubation produced only minimal or even no changes. γ-HCH loss was substantially higher than in control sample, especially on week 4 onwards. The final removal efficiency for this compound reached 80% in relation to the control. Decrease in DDT isomers was accompanied by formation of respective DDD isomers and p,p'-DBP. These metabolites accumulated during first 4 weeks of experiment, simultaneously with considerable DDT removal. Thereafter, their concentrations remain stable or were slightly reduced towards the end of the experiment. DDE level stayed minimal throughout the incubation. What should be stressed, the amount of formed DDD was much smaller than that resulting solely from simple transformation of DDT. For example, p,p'-DDD concentration during the last two samplings (week 6 and 8) corresponded stoichiometrically to only 30–33% of removed p,p'-DDT (similarly for o,p'-isomers). This, together with formation of p,p'-DBP, points to occurrence of further DDD transformation during bioremediation.

A large scatter of p,p'-DDT removal efficiency within the analyzed triplicate sets of subsamples was also found in this treatment, similarly to previous tests with soil slurry (see „Introduction"). This is clearly visible from the disturbance of the course of p,p-DDT concentration changes (Fig 5, week 4) and considerable standard deviations of results for this compound throughout the experiment. Even more, a few measurements were excluded from the graphs for all DDT-related compounds as they were obvious outliers, with p,p'-DDT concentration (and sometimes o,p'- as well) in these individual subsamples changed little or even exceeded the initial value (2 out of 3 subsamples on the week

4, 1 out of 3 on the week 6). A partial explanation for this variability could be generally heterogenic distribution of this compound in the investigated soil, indicated by the considerable standard deviations for control sample. However, this explanation is not sufficient in the case of these extreme outliers, being caused rather by the supposed random presence of non-degradable aggregates.

Surfactant addition caused delay in pesticides removal in the very first period of incubation. However, beginning from the week 4 it became comparable or even greater than that for the sample L, which suggests that this problem was probably caused by adaptation of biomass to more complex conditions in the presence of surfactant. The problem of p,p'-DDT outliers was also experienced for this sample (1 subsample excluded on week 4).

There were some noticeable benefits of this amendment use. The first was about one and a half-fold decrease of final, residual concentrations of pesticides γ-HCH; o,p'-DDT and methoxychlor. Considerable scatter of results for p,p'-DDT could obscure occurrence of similar effect for this compound. The second was slight enhancement of DDT conversion, resulting in lesser DDD accumulation and increased amount of formed p,p'-DBP. For comparison, p,p'-DDD concentration during last two samplings (week 6 and 8) in LS sample corresponded to 23–27% of removed p,p'-DDT only (Fig. 6). As for p,p'-DBP formed, the respective result was 7.5–8.3% against 4.4–6.0% for L sample (Fig. 7).

Use of methanol as electron donor produced results generally comparable to these of the L treatment. The only differences were: delay of removal in the first period (similar as in LS sample) and somewhat higher DDD level at the end of the experiment. There were no outliers for DDT; however, deviations of removal efficiency for this compound were still significant.

DISCUSSION

The results obtained clearly confirm feasibility of the solid-phase bioremediation using granular sludge as inoculum, which was the main objective of the study. The process performance was comparable to that found in slurry tests with the same soil, with the little lower rate of γ-HCH removal during the initial period of incubation, perhaps resulting from slower adaptation of respective degrading microorganisms. Nevertheless, the finally achieved concentrations were similar.

Formation of DDD and DBP confirms DDT degradation in this study, as these compounds are respectively the first and the terminal metabolite in the DDT anaerobic biodegradation pathway (Fig. 8) [7]. What is interesting, there were no observable differences in removal efficiency for o,p' and p,p' isomers, which is contrary to reports of greater persistence of o,p'-DDT and its metabolites [8]. However, this could be possibly explained by involvement of different degrading microbial community. As for γ-HCH, the results of studies on this and other HCH isomers degradation by granular sludge [4, 13] indicate that most probably it also underwent successive dehalogenation, with chlorobenzene and benzene as final products. Methoxychlor structure resembles that of DDT, so also similar degradation pathway is expected, with additional demethylation reactions occurring on its aryl methoxy groups replacing persistent chlorines of DDT [11].
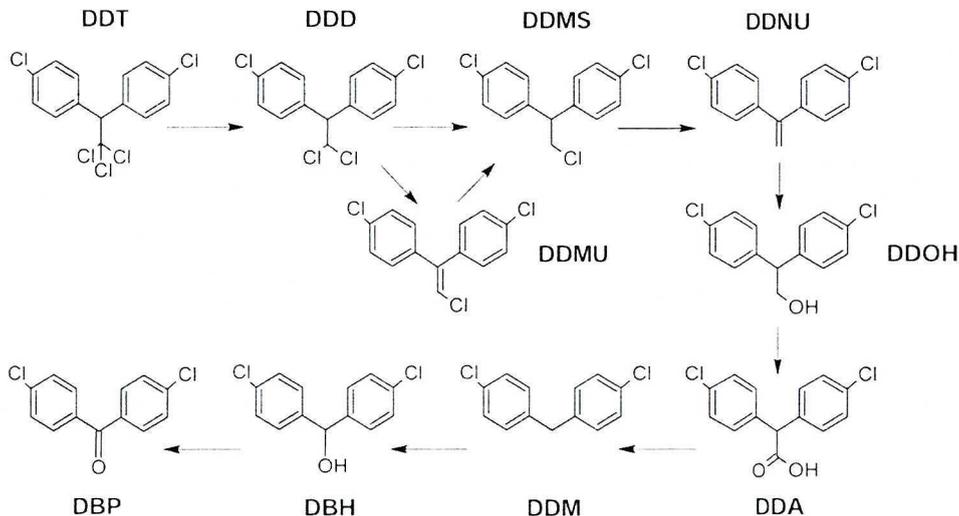
Fig. 8. Pathway for anaerobic biodegradation of p,p'-DDT [7]

The character of the mentioned, putative products of anaerobic bioremediation points to the necessity of post-treatment. Chlorobenzene and benzene can be degraded in the presence of oxygen, even at a little concentration, with nitrate enhancing this process [9]. Also DBP is transformed under aerobic conditions [5]. Application of aerobic conditions after anaerobic incubation multiplied emission of $^{14}CO_2$ from soil spiked with [^{14}C]-labeled methoxychlor, indicating intense mineralization of this compound [6]. Thus, the next and final phase of bioremediation should involve aerobic processes.

The reduction of final concentrations of some pesticides in the sample amended with surfactant can be possibly explained by the partial release of desorption-resistant fractions of these compounds. It was demonstrated previously that pesticides removal is closely linked to their desorbability. Only easily desorbing contaminants were degraded, whereas their desorption-resistant fractions persisted, forming residues not removed even after considerably prolonged incubation. An existence of such residues, however small, could be also noted in the present study (Figs 1–3). According to Pignatello and Xing [12], such hardly-desorbing fractions develop during long residence of hydrophobic organic contaminants in soil as a result of sorption in glassy (condensed) domains of soil organic matter and/or entrapment in nanopores of soil particles. Surfactants are known to make soil organic matter swell, thus being able to release these bound contaminants at least partially [14]. Similar effects were also reported by Cuypers et al. [3] for bioremediation of PAHs contaminated sediments.

Another effect of surfactant, which was the decrease in amount of formed DDD metabolite, confirmed results previously obtained by You et al. [16] and also by Walters and Aitken [15]. This issue is important as soil quality standards usually refer to the collective sum of DDT and its intermediates DDD and DDE. Although DDD accumulation was much lower than stoichiometric (see "Results"), it was still high enough to significantly affect DDT/DDD/DDE removal, which amounted to 54–67% and 50–53% for the last two samplings in L and M sample, respectively (sum of both isomers). Surfactant application increased this to 62–68%. However, even this result should be regarded as rather

moderate. Certainly, more research is needed to find methods of intensifying further DDD transformation.

There were no excessive deviations of DDT removal efficiency in sample M dosed with methanol, in contrast to other treatments. This would have implied that use of solvent could indeed improve the overall result by dissolving this pesticide's aggregates. However, taking into account the limited scale of the presented experiment, this conclusion should be considered tentative. Additional confirmation in a larger number of tests is required, especially because some apparent drawbacks of methanol application were noticed, like higher DDD level towards the end of the experiment, in spite of generally similar performance to the lactate-amended test.

CONCLUSIONS

Tests confirmed effectiveness of solid-phase anaerobic bioremediation of soil contaminated with chlorinated pesticides. Use of granular sludge as inoculum and lactate as electron donor allows for high removal of γ-HCH, methoxychlor and DDT, with reduced accumulation of DDD. Anionic surfactant application enhances the process performance, lowering residual concentrations of some compounds and causing additional decrease of DDD accumulation. Use of methanol as electron donor resulted in a smaller scatter of pesticides removal efficiency results; however, this effect needs to be verified for a larger number of samples.

SYMBOLS USED

HCH	hexachlorocyclohexane,
methoxychlor	1,1,1-trichloro-2,2-bis-(4-methoxyphenyl)ethane,
o,p'-DDT	1,1,1-trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane,
PAH	polycyclic aromatic hydrocarbon,
PCB209	decachlorobiphenyl,
p,p'-DBH	4,4'-dichlorobenzhydrol,
p,p'-DBP	4,4'-dichlorobenzophenone,
p,p'-DDA	2,2-bis-(4-chlorophenyl)acetic acid,
p,p'-DDD	1,1-dichloro-2,2-bis-(4-chlorophenyl)ethane,
p,p'-DDE	1,1-dichloro-2,2-bis-(4-chlorophenyl)ethene,
p,p'-DDM	bis-(4-chlorophenyl)methane,
p,p'-DDMS	1-chloro-2,2-bis-(4-chlorophenyl)ethane,
p,p'-DDMU	1-chloro-2,2-bis-(4-chlorophenyl)ethene,
p,p'-DDNU	2,2-bis-(4-chlorophenyl)ethene,
p,p'-DDOH	2,2-bis-(4-chlorophenyl)ethanol,
p,p'-DDT	1,1,1-trichloro-2,2-bis-(4-chlorophenyl)ethane.

REFERENCES

[1]   Baczyński T.: *Możliwości bioremediacji gruntów zanieczyszczonych pestycydami chloroorganicznymi*, [in:] Materiały konferencji PZiTS Rekultywacja i rewitalizacja terenów zdegradowanych, Poznań – Puck 2007, pp. 103–112.

[2]   Baczyński T., M. Kryłów, A. Małachowska-Jutsz, T. Stobiecki: *Efektywność różnych dodatków w biore-*

mediacji gruntu skażonego chloroorganicznymi środkami ochrony roślin, Biotechnologia, **81**, 162–173 (2008).

[3] Cuypers C., T. Pancras, T. Grotenhuis, W. Rulkens: *The estimation of PAH bioavailability in contaminated sediments using hydroxypropyl-beta-cyclodextrin and Triton X-100 extraction techniques*, Chemosphere, **46**, 1235–1245 (2002).

[4] Eekert M.H.A. van, N.J.P. van Ras, G.H. Mentink, H.H.M. Rijnaarts, A.J.M. Stams, J.A. Field, G. Schraa: *Anaerobic biotransformation of β-hexachlorocyclohexane by methanogenic granular sludge and soil microflora*, Environ. Sci. Technol., **32**, 3299–3304 (1998).

[5] Focht D.D., M. Alexander: *DDT metabolites and analogues: ring fission by Hydrogenomonas*, Science, **170**, 91–92 (1970).

[6] Fogel S., R.L. Lancione, A.E. Sewall: *Enhanced biodegradation of methoxychlor in soil under sequential environmental conditions*, Appl. Environ. Microbiol., **44**, 113–120 (1982).

[7] Foght J., T. April, K. Biggar, J. Aislabie: *Bioremediation of DDT-contaminated soils: a review*, Bioremediation Journal, **5**, 225–246 (2001).

[8] Huang H.J., S.M. Liu, C.E. Kuo: *Anaerobic biodegradation of DDT residues (DDT, DDD and DDE) in estuarine sediment*, J. Environ. Sci. Health, Part B, **36**, 273–288 (2001).

[9] Langenhoff A.A.M., J.J.M. Staps, C. Pijls, A. Alphenaar, G. Zwiep, H.H.M. Rijnaarts: *Intrinsic and stimulated in situ biodegradation of hexachlorocyclohexane (HCH)*, Water, Air and Soil Pollution, Focus, **2**, 171–181 (2002).

[10] Lichtenstein E.P., K.R. Schulz: *Volatilization of insecticides from various substrates*, J. Agric. Food Chem., **18**, 814–818 (1970).

[11] Muir D.C.G., A.L. Yarechewski: *Degradation of methoxychlor in sediments under various redox conditions*, J. Environ. Sci. Health, Part B, **19**, 271–296 (1984).

[12] Pignatello J.J., B. Xing: *Mechanisms of slow sorption of organic chemicals to natural particles*, Environ. Sci. Technol., **30**, 1–11 (1996).

[13] Quintero J.C., M.T. Moreira, J.M. Lema, G. Feijoo: *An anaerobic bioreactor allows the efficient degradation of HCH isomers in soil slurry*, Chemosphere, **63**, 1005–1013 (2006).

[14] Volkering F., A.M. Breure, W.H. Rulkens: *Microbiological aspects of surfactant use for biological soil remediation*, Biodegradation, **8**, 401–417 (1998).

[15] Walters G.W., M.D. Aitken: *Surfactant enhanced solubilization and anaerobic biodegradation of 1,1,1-trichloro-2,2-bis-(p-chlorophenyl)-ethane (DDT)*, Wat. Env. Res., **73**, 15–23 (2001).

[16] You G., G.D. Sayles, M.J. Kupferle, I.S. Kim, P.L. Bishop: *Anaerobic DDT transformation: enhancement of surfactants and low oxidation reduction potential*, Chemosphere, **32**, 2269–2284 (1996).

BEZTLENOWA BIOREMEDIACJA W FAZIE STAŁEJ GRUNTU ZANIECZYSZCZONEGO
PESTYCYDAMI CHLOROWANYMI POCHODZĄCEGO Z TERENU MOGILNIKA

Artykuł przedstawia wyniki badań laboratoryjnych nad beztlenową bioremediacją gruntu zanieczyszczonego pestycydami chlorowanymi, prowadzoną w fazie stałej. Stwierdzono, że przy zaszczepieniu metanogennym osadem granulowanym oraz użyciu mleczanów jako donora elektronów możliwe jest usunięcie 80% γ-HCH, 94% metoksychloru i 93% DDT w odniesieniu do próby kontrolnej, przy akumulacji DDD znacząco mniejszej od stechiometrycznej. Usuwanie pestycydów było praktycznie zakończone po 4–6 tygodniach inkubacji w temperaturze 22°C. Zastosowanie dodatku niejonowego środka powierzchniowo czynnego Tween 80 skutkowało około półtorakrotnym obniżeniem stężeń resztkowych niektórych związków. Spowodowało ono także pewną intensyfikację przemian DDT, przejawiającą się obniżeniem akumulacji DDD oraz zwiększeniem produkcji DBP, będącego ostatecznym produktem beztlenowej degradacji DDT. Wykorzystanie metanolu w roli donora elektronów przyniosło rezultaty zasadniczo podobne jak w przypadku mleczanu, jednakże przy zmniejszonym rozrzucie wyników.