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INFLUENCE OF PROCESS PARAMETERS ON ANAEROBIC BIODEGRADATION OF DDT IN CONTAMINATED SOIL PRELIMINARY LAB-SCALE STUDY. PART I. SURFACTANT AND INITIAL CONTAMINATION LEVEL

Anaerobic biodegradation of DDT in field-polluted soil was investigated in relation to Tween 80 surfactant dose and initial pollution level. Experiments were carried out as lab-scale tests with flooded soil, inoculated with granular sludge. Higher surfactant doses decreased DDT residual concentration and also reduced DDD metabolite accumulation. However, doses higher than optimum caused DDD production to increase again. Results were also better for lower initial contamination levels – DDD accumulation was smaller whereas formation of terminal metabolite DBP was higher, indicating an enhancement of DDT transformation. Tests with pure compounds spiked in clean soil demonstrated that DDD is degraded slowly. Results point to three possible parallel pathways of anaerobic DDT transformation, not, as commonly reported, only one starting with dechlorination to DDD.

1. INTRODUCTION

DDT (1,1,1-trichloro-2,2'-bis(4-chlorophenyl)ethane) and its derivatives are recognised as endocrine disrupting chemicals and are extremely persistent in the environment [1]. Currently, several sites still exist around the world where soils and sediments are highly polluted with this compound. These places include areas of (former) pesticide manufacturing and formulation [2], water courses used to receive discharges from these factories [3], obsolete pesticide storage locations [4], and others. In Poland, DDT pollution occurs at the former pesticide production site and waste landfill in Jaworzno, as well as at many “tombs” – obsolete pesticide burials, scattered over the whole country (currently still under reclamation) [5]. Previously reported research [6, 7] has demonstrated significant removal of DDT from field-contaminated soil, in

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anaerobic slurry tests with a methanogenic sludge inoculum. Elevated temperature or surfactant amendment enhanced transformation of this pesticide, reducing accumulation of persistent DDD (1,1-dichloro-2,2'-bis(4-chlorophenyl)ethane) metabolite. This is crucial for effective remediation, as soil quality standards [8] demand reduction of the total sum of DDT and its primary metabolites, DDD and DDE (1,1-dichloro-2,2'-bis(4-chlorophenyl)ethene). Another study [9] has shown that similar results could be also obtained in a solid-phase treatment, with soil watered to slightly above its water holding capacity (WHC). Solid phase processes, e.g. biopiles or landfarming, are more convenient for field application than slurry reactors. Thus, the prospect for practical implementation of the suggested technology was opened. However, this would require prior optimization of the process and more profound recognition of its parameters importance. This includes, among others, type and dose of co-metabolic substrate, surfactant, inoculum, etc.

The paper presents part of a larger study, and it discusses aspects of surfactant application, influence of initial pesticide concentration and possible DDT degradation pathways. A separately presented and complementary publication covers the role of additional organic substrates, effect of its type and related issues of pH control during treatment.

In previously reported experiments on anaerobic biodegradation of DDT in contaminated soil slurry [7], DDD accumulation was significantly decreased by addition of Tween 80 surfactant. However, higher doses of this surfactant caused DDD production to rise again. It was hypothesized that the latter effect resulted from exceeding the surfactant critical micelle concentration (CMC) in the water phase. The present research involves more detailed study on relation between Tween 80 doses and biodegradation results. Its effectiveness was also verified in a solid-phase treatment. The other issue addressed in this paper is the influence of initial contamination levels on anaerobic biodegradation effects. Observations made in prior experiments suggested that high pollution of soil could negatively affect DDT transformation. This was studied in an experiment with contaminated soil dilutions using clean sand.

The commonly accepted model of DDT anaerobic biodegradation [10–15] assumes only one “linear” pathway of DDT decomposition. It starts from reductive dechlorination to DDD, which then undergoes succession of transformations leading to DBP (4,4'-dichlorobenzophenone) as a terminal product, not metabolized further in anaerobic conditions [16]. Lower than stoichiometric accumulation of DDD metabolite and large production of DBP, observed in the previous experiments [6, 7], were therefore interpreted as indications of intensive DDD metabolism. However, in the later phase of these tests DDD was not removed further, which contradicts the easy degradability of this metabolite. Sequestration of DDD in the soil, depletion of electron donor or acceptor were excluded as reasons for this persistence [6]. To explain the issue of DDD degradation, additional samples with clean soil spiked with pure DDT and DDD were included in the experimental program.

2. MATERIALS AND METHODS

Chemicals. Acetone and hexane for residue analysis (Picograde) were purchased from LGC Standards (Lomianki, PL), similarly as 99% decachlorobiphenyl (PCB209) standard. Anhydrous sodium sulphate for residue analysis (crystalline, 12–60 mesh) and 60% solution of sodium lactate (syrup) were from J.T. Baker (Łódź, PL). Standards of DDT, DDD, DDE and DBP (purity 99,6–99,8%) originated from the Institute of Organic Chemistry (Warsaw, PL). Tween 80 surfactant was purchased in Sigma Aldrich (Poznań, PL). Concentrated phosphate buffer was prepared by dissolving 4.34 g of monosodium phosphate and 7.50 g of disodium phosphate in 50 cm³ of distilled water (pH ~ 7). A 10% phosphate buffer was made of monosodium and disodium phosphate (3.3 g and 6.7 g, respectively), dissolved in 100 cm³ of distilled water (pH ~ 7.2)

Methanogenic granular sludge. Granular sludge was taken from an anaerobic reactor treating wastewater from soft drink production (Opatówek, PL). Prior to use, the sludge was rinsed on a 0.25 mm sieve with tap water to remove fine particles and decay products. Solid contents was 5.6% with about 0.9 organic fraction, methanogenic activity ~0.3 (g CH₄-COD)·(g VSS)⁻¹·d⁻¹ at 30 °C.

Soil. Soil sample NIE1 came from a contaminated soil landfill at a pesticide “tomb” at Niedźwiady (Wielkopolska province, PL). To lower inhibition caused by high concentration of pesticides, the soil was mixed with clean sand NIE0. This sand was collected in the vicinity of the “tomb”. Only in experiment I, due to unavailability of NIE0 sand at that time, clean sand MLY0 (of slightly finer granulation) coming from another “tomb” in Mlynów (Poznań voivodeship, PL) was used instead. The main pollution of NIE1 were DDT, p,p'- and o,p'-isomers, and methoxychlor. DDD isomers were present in small quantities, DDE concentration was minimum (Table 1).

Biodegradation tests. A general procedure of preparing samples for biodegradation tests was as follows: tests were performed in 120 cm³ glass jars closed with PTFE-lined screw caps. 50 g of dry soil was mixed with 4 g of wet granular sludge, 0.7 cm³ of sodium lactate syrup as a substrate (electron donor), 150 mg of Tween 80 surfactant (equivalent to 3 mg·(g of soil)⁻¹ dose, and nutrients (nitrogen and phosphorus). Nitrogen, 10–20 mg per sample, was added as ammonium chloride solution; phosphorus doses (as phosphates) differed among individual experiments due to attempts of pH control using phosphate buffers. Samples were mixed and watered with distilled water (about 10 cm³), to slightly above its WHC, thus the soil was flooded with a little water layer above its surface. The control was prepared in a similar manner, without granular sludge inoculation and lactate; biological processes were inhibited with 0.25 g of sodium azide. The initial pH of samples was corrected to 6.5–7.5

using small amounts of calcium oxide powder. Exceptions to this procedure are given in a description of individual experiments below.

Jars were incubated in a temperature controlled cabinet, at 22 °C, in the dark. Previous experiments have shown that a major part of the pesticide is removed within the first two weeks of incubation. In the following time, the progress is not significant and after 4–6 weeks the process could be regarded as practically completed. Due to this, sampling was made at the start of the experiment and after 2 weeks. Next sampling took place after 4–6 weeks of incubation and the final sampling was performed only when long-term effects were monitored, at least after 8 weeks.

Experiment I was done in triplicate, the rest on single samples only. The use of single samples was due to preliminary, screening character of the whole study – large number of process combinations had to be checked in a cost- and time-saving manner. It was also justified by good repeatability obtained for results of experiment I (standard deviation significantly below 10% for most of the data).

Gas chromatography (GC). 2–3 g of wet soil was taken from the sample and placed on a paper filter. Then it was left for 1–2 days to dry. Simultaneously, a similar amount of soil was used for determination of moisture content after drying. Dry soil, together with a filter, was weighted and spiked with a surrogate standard. Next, the soil and filter were extracted with 16 cm³ of hexane/acetone in 40 cm³ amber vials, tightly sealed with a PTFE-lined screw cap. Vials were heated for 4 h at 70 °C. More details of this procedure, its development and verification are given elsewhere [6]. After cooling, 5 cm³ of extract was centrifuged with 0.8 g of anhydrous sodium sulphate (5 min, 7000 rpm) to remove fine particles. As extract was subsequently largely diluted with hexane to fit the GC calibration range, an additional purification was not necessary – compound peaks on chromatograms were well resolved from any interference.

Surrogate standard was 0.5% PCB209 solution in toluene. In each sampling series, blank GC samples were also prepared by diluting the surrogate standard with 16 cm³ of hexane/acetone. Blanks were analyzed concurrently with soil extracts, and results for PCB209 recovery factor were used for correcting results for pesticides.

Analyses were performed on MEGA 5300 gas chromatograph (Carlo Erba), equipped with ECD detector. Injection of 1 µl was splitless (“Uniliner” insert with glass wool plug, Restek). Separation was made on 30 m Stx-500 capillary column (0.25 mm ID, 0.15 µm film) with a 5 m deactivated guard column. This phase gave good resolution of *o,p'*-DDT and *p,p'*-DDD, usually co-eluting on other more commonly used phases, like Rtx-5 or equivalent. Temperature was 120 °C, increasing 12 °C/min to 300 °C, hold for 6 min. Carrier gas was hydrogen, 80 kPa. Calibration was multipoint, before each series of analyses. In the text, results for measurements of *p,p'*-isomers of DDT, DDD, DDE and DBP are given, without *p,p'* indication of the isomer type.

During verification of the above described GC procedure, the pesticide recoveries from spiked soil samples were: DDE $105 \pm 3\%$, DDD $100 \pm 1\%$, DDT $103 \pm 7\%$ (average \pm standard deviation). Detection limits depended on dilution of the extract, e.g. for most often applied 1 + 3 soil mixture DDT and its metabolites could be detected at about $0.3 \mu\text{mol}\cdot(\text{kg soil DM})^{-1}$.

Experiment I. The purpose of the experiment was to investigate the influence of the surfactant dose on the effectiveness of pesticide removal. Four doses were used: 0.5, 1, 2.5 and 5 $\text{mg}\cdot(\text{g of soil DM})^{-1}$. Controls were not amended with surfactant. Sodium lactate was added in double amount (1.4 cm^3). Sampling was made after 0, 2, 5 and 8 weeks of incubation. One part of contaminated soil NIE1 was mixed with three parts of MLY0 clean sand (1 + 3). Phosphorus, 2 mg per sample, was added as dipotassium phosphate solution.

Experiment II. The experiment was aimed at the impact of higher surfactant doses: 3, 6, 9, 12 and 15 $\text{mg}\cdot(\text{g soil DM})^{-1}$ (150, 300, 450, 600 and 750 mg for sample). In this case, the control also contained sodium lactate. Soil mixture was prepared using one part of NIE1 and three parts of NIE0 clean sand. Phosphorus was added as 2 cm^3 of concentrated phosphate buffer in an attempt to stabilize pH during the biodegradation process.

Experiment III. The aim of this experiment was providing more insight into the DDT degradation process by using pure compounds, DDT and DDD, and studying the effect of pesticide concentration on biodegradation effectiveness. For the first, two samples of NIE0 sand, one spiked with DDT and another with DDD, were prepared. Spiking was made by adding stock standards in hexane, diluted to 10 cm^3 with acetone. Soil was soaked with this solution and then left to evaporate the solvents; test samples were prepared as usual. For the second, a series of dilutions were prepared using NIE1 and NIE0: 1 + 0, 1 + 1, 1 + 3, 1 + 9 and 1 + 19 parts on weight basis. The control sample was made with 1 + 3 soils mixture. Phosphorus was added as 0.3 cm^3 10% phosphate buffer.

3. RESULTS

Data from experiments I and II are shown in Figs. 1 and 2, as concentrations of DDT, DDD and DBP (in $[\mu\text{mol}\cdot(\text{kg soil DM})^{-1}]$) in relation to incubation time. This presents disappearance of the parent compound, formation of its primary and terminal metabolite in anaerobic conditions [12], respectively. As DDE remained very low throughout the experiments, minimizing production of DDD was essential, as soil quality standards refer to sum of DDT, DDD and DDE (below termed DDX). Amount

of produced DBP demonstrates completeness of anaerobic DDT transformations. Changes of *o,p'*-isomers of DDT, DDD and DDE were similar to *p,p'*-isomers.

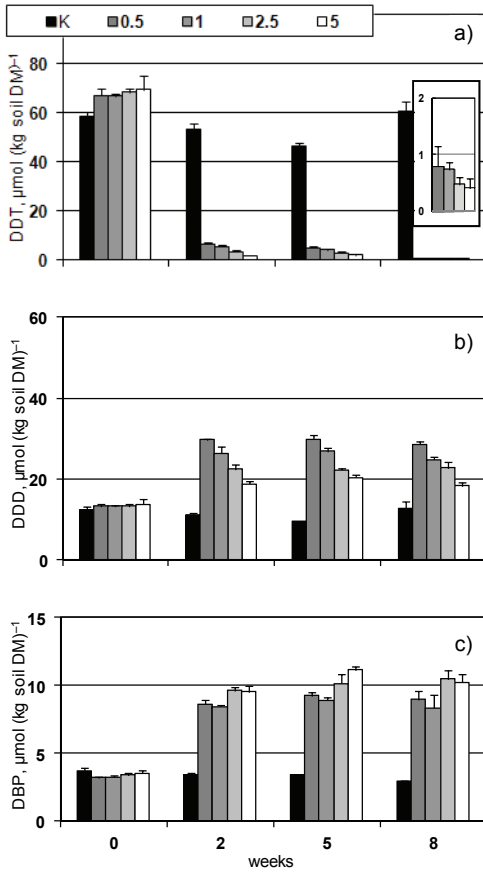


Fig. 1. Changes of DDT (a), DDD (b) and DBP (c) in experiment I. Insert in Fig. 1a shows residual concentration of DDT after 8 weeks in surfactant-amended samples. Bars represent standard deviation; K – control, surfactant doses in the figure are given in $\text{mg} \cdot (\text{g of soil DM})^{-1}$

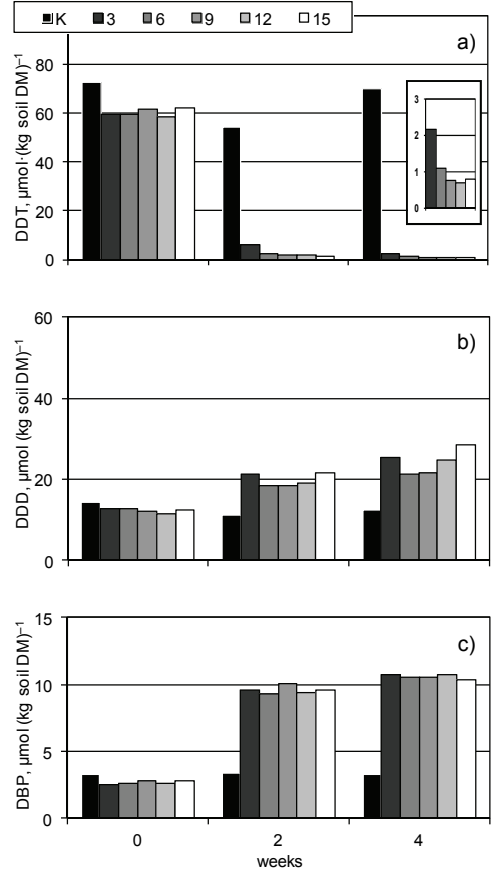


Fig. 2. Changes of DDT (a), DDD (b) and DBP (c) in experiment II. Insert in Fig. 2a shows residual concentration of DDT after 4 weeks in surfactant-amended samples. K – control, surfactant doses in the figure are given in $\text{mg} \cdot (\text{g of soil DM})^{-1}$

During experiment I (Fig. 1a–c) there was a clear trend of enhancing the process effectiveness by a higher dose of surfactant. The DDT residual concentration (after 8 weeks of incubation) decreased from 0.79 to 0.40 $\mu\text{mol} \cdot (\text{kg soil DM})^{-1}$ upon increase of Tween 80 dose (see Fig 1a insert), against 60.9 $\mu\text{mol} \cdot (\text{kg soil DM})^{-1}$ remaining in the control. Simultaneously, final concentration of DDD also decreased at the end of the experiment where it was 28.6, 24.8, 22.7 and 18.5 $\mu\text{mol} \cdot (\text{kg soil DM})^{-1}$ for the lowest to

highest amount of surfactant. Taking into account the $12.7 \mu\text{mol}\cdot(\text{kg soil DM})^{-1}$ concentration of this metabolite in the control sample, formed DDD corresponded only 27, 20, 17 and 10% of removed DDT. As a result, the DDX removal efficiency increased from 60 to 65, 69 and 74%, respectively (on stoichiometric basis). The DBP final concentration was $8.4\text{--}9.0 \mu\text{mol}\cdot(\text{kg soil DM})^{-1}$ for $0.5\text{--}1.0 \text{ mg}\cdot(\text{g soil DM})^{-1}$ Tween 80 and $10.3\text{--}10.5 \mu\text{mol}\cdot(\text{kg soil DM})^{-1}$ for $2.5\text{--}5.0 \text{ mg}\cdot(\text{g soil DM})^{-1}$ Tween 80. For the control, it was $2.9 \mu\text{mol}\cdot(\text{kg soil DM})^{-1}$, thus the production of this metabolite was equivalent to 9–10% of removed DDT for lower and about 12% for higher doses.

Similarly, in experiment II (Fig 2a–c) increases of surfactant dose led to smaller residual concentrations of DDT. For samples incubated for 4 weeks, these residues decreased from $2.14 \mu\text{mol}\cdot(\text{kg soil DM})^{-1}$ for the $3 \text{ mg}\cdot(\text{g soil DM})^{-1}$ Tween 80 to about $0.7 \mu\text{mol}\cdot(\text{kg soil DM})^{-1}$ for $9\text{--}15 \text{ mg}\cdot(\text{g soil DM})^{-1}$ doses (insert in Fig 2a). In the control sample it was $69.9 \mu\text{mol}\cdot(\text{kg soil DM})^{-1}$. Regarding the DDD final level, it was noticeable that the smallest concentration occurred at $6\text{--}9 \text{ mg}\cdot(\text{g soil DM})^{-1}$ surfactant: $21.5\text{--}21.7 \mu\text{mol}\cdot(\text{kg soil DM})^{-1}$. Subtracting the $12.6 \mu\text{mol}\cdot(\text{kg soil DM})^{-1}$ in the control sample, this corresponds to about 13% formation of DDD in relation to DDT removed. Higher doses of 12 and $15 \text{ mg}\cdot(\text{g soil DM})^{-1}$ caused the final DDD concentration to rise to 24.8 and $28.7 \mu\text{mol}\cdot(\text{kg soil DM})^{-1}$, respectively, increasing its accumulation to 18 and 23%. This resulted in lowering the DDX removal from 73% for $6\text{--}9 \text{ mg}\cdot(\text{g soil DM})^{-1}$ surfactant to 69% and 64% for 12 and $15 \text{ mg}\cdot(\text{g soil DM})^{-1}$. Final concentration of DBP ($10.4\text{--}10.8 \mu\text{mol}\cdot(\text{kg soil DM})^{-1}$) seemed independent of Tween 80 dose. Taking into consideration $3.3 \mu\text{mol}\cdot(\text{kg soil DM})^{-1}$ of this compound in the control, production of DBP stoichiometrically corresponded to 10–11% of removed DDT.

Change of the initial contamination level did not influence the effectiveness of DDT removal (experiment III, Table 1, samples from 1 + 0 to 1 + 19), reaching 97–98% after 4 weeks of incubation. However, initial concentration was important for production of both DDD and DBP. For the non-diluted sample, 1 + 0, DDT decrease from 288 to $10 \mu\text{mol}\cdot(\text{kg soil DM})^{-1}$ after 4 weeks was accompanied by DDD and DBP increase from 36.4 to $120 \mu\text{mol}\cdot(\text{kg soil DM})^{-1}$ and from 13.2 to $21.1 \mu\text{mol}\cdot(\text{kg soil DM})^{-1}$, respectively. This was equivalent to 30% formation of DDD and only 3% production of DBP, in comparison to removed DDT. For less contaminated samples, the relative amount of produced DDD decreased, while this of DBP increased. Concerning the most diluted 1 + 19 sample, DDD accumulation (from 1.45 to $2.78 \mu\text{mol}\cdot(\text{kg soil DM})^{-1}$) corresponded 12% of removed DDT (11.5 to $0.35 \mu\text{mol}\cdot(\text{kg soil DM})^{-1}$). At the same time DBP formation (increase from 0.48 to $1.70 \mu\text{mol}\cdot(\text{kg soil DM})^{-1}$) was equivalent to 11% of the removed parent compound. The effectiveness of DDX removal increased from 60 to 76%, from the most to the least polluted soil mixture. The results obtained for soil spiked with DDT were even more pronounced than those received for field-polluted soil dilution of comparable pesticide concentration: DDD formation ($4.89 \mu\text{mol}\cdot(\text{kg soil DM})^{-1}$) was lower (13% of removed DDT, from initial 46.1 to

7.10 $\mu\text{mol}\cdot(\text{kg soil DM})^{-1}$) whereas DBP production (4.82 $\mu\text{mol}\cdot(\text{kg soil DM})^{-1}$) was higher (12%). Spiked DDD disappeared very slowly, and only 22% was removed after 4 weeks. Based on this data, its half-life time was estimated at about 11 weeks (first order rate constant of -0.0088 d^{-1}). Slow DDD degradation was coupled with a little increase of DBP concentration (1.17 $\mu\text{mol}\cdot(\text{kg soil DM})^{-1}$, corresponding 6% of removed DDD). Chromatographic analyses (data not shown) showed also formation of traces of DDMU (1-chloro-2,2'-bis(4-chlorophenyl)ethene), an intermediate of DDD degradation pathway [12], but this compound was not quantified.

Table 1

Concentrations of DBP, DDE, DDD, DDT in test samples [$\mu\text{mol}\cdot(\text{kg soil DM})^{-1}$]^a

Week	Compound	Control	Dilution of soil with clean sand					DDT	DDD
			1 + 0	1 + 1	1 + 3	1 + 9	1 + 19		
0	DBP	3.32	13.2	5.93	2.70	0.94	0.48	BDL	BDL
	DDE	3.31	3.59	1.71	0.61	0.22	0.11	0.25	BDL
	DDD	8.83	36.4	17.8	7.60	2.88	1.45	BDL	93.8
	DDT	64.5	288	140	60.6	23.5	11.5	46.1	BDL
2	DBP	3.52	25.2	11.3	7.35	3.75	2.25	7.10	0.30
	DDE	2.53	1.85	0.73	0.38	0.14	0.07	0.09	BDL
	DDD	8.34	124	43.9	19.6	7.01	3.42	6.17	84.2
	DDT	62.12	20.3	8.67	3.54	1.77	0.78	3.20	BDL
4	DBP	3.27	21.1	10.3	6.03	2.93	1.70	4.82	1.17
	DDE	4.07	1.37	0.70	0.39	0.12	0.05	0.19	BDL
	DDD	7.28	120	50.1	19.4	5.49	2.78	4.89	73.0
	DDT	56.1	10.0	4.44	1.85	0.57	0.35	7.10	BDL

^aDDT and DDD are pesticide spiked samples, bdl means below detection limit.

4. DISCUSSION

4.1. EFFECTS OF SURFACTANT

The results of the presented experiments confirmed previous conclusions [7] that surfactant application results in lowering production of DDD metabolite, and because of that, it enhances the DDX removal efficiency. It also decreases the residual concentration of DDT, remaining after biodegradation. However, while the last effect intensifies with increase of surfactant dose, the DDD accumulation in relation to surfactant amount has a distinctive minimum. Exceeding the optimum surfactant dose range causes DDD production to increase again.

Enhancement of DDT transformation and decrease of DDD amount as a result of surfactant application during anaerobic biodegradation in soil were already addressed

in the reports by You et al. [10] and Walters and Aitken [17]. You et al. [10] stated also that Triton X-114 intensified formation of DBP. However, the reported production of this compound – 3.1% in relation to removed DDT – was much lower than found in the present research. It is possible that this resulted from a much higher concentration of DDT, $2500 \text{ mg}\cdot(\text{kg soil})^{-1}$ ($\sim 7000 \text{ }\mu\text{mol}\cdot(\text{kg soil})^{-1}$) in soil used by these authors, though their tests were carried out in diluted, 5% soil slurry.

There are also other differences in the present experiments, relevant to the other of the mentioned papers. In the experiments by Walters and Aitken [17] (also by Walters [13]) decrease of DDD concentration was supposedly caused by degradation of this compound taking place only in the later phase of the long, 30-week biodegradation test. Contrary to that, in the present experiments lower accumulation of DDD was noticed already at their early stage. Moreover, these authors assumed that enhancement of DDT transformation would require considerable increase of solubility of this compound, implicating its better bioavailability. This was achieved by application of Brij 30 surfactant in a dose high enough for exceeding CMC in the water phase. However, a rough estimation of surface tension changes (by capillary rise method) in the present research indicated that CMC in the water phase was exceeded only at Tween 80 dose of $2.5 \text{ mg}\cdot(\text{g soil DM})^{-1}$ in experiment I, and $3 \text{ mg}\cdot(\text{g soil DM})^{-1}$ in experiment II. That suggests that advantageous effect of surfactant application occurred even before CMC was reached (experiment I). Therefore, increasing DDT solubility in the water phase may be not so important. It can be expected that below surfactant CMC solubility of hydrophobic organic contaminants could even be reduced due to their partitioning between the water phase and soil-sorbed surfactant [18]. This issue is however more complicated in the case of extremely hydrophobic compounds, like DDT, as surfactant monomers present in the water phase already before CMC is achieved could enhance their solubility to a certain degree [19]. This matter cannot be decided based on the present data and more research is necessary. Certainly, CMC determination should be verified using more accurate methods of surface tension measurement. However, small volume of free liquid available in biodegradation tests makes use of such methods problematic. Effective surfactant performance at doses not exceeding CMC is important because of lower risk of contaminant mobilization in the soil [18]. It should be also noted that during treatment surfactant concentration might decline as Tween 80 was perhaps partially biodegraded.

In the presented experiments, one of the likely effects of surfactant appears enhancement of mass transport from the soil to the water phase. This would explain observed gradual reduction of residual fraction of pesticide remaining after the process. Probable responsible mechanisms are acceleration of diffusion in soil pores [18] and swelling of organic matter [20]. Supposedly, as suggested by You et al. [10], intensification of DDT transformation may be related to increase of permeability of microorganisms' cell membranes to chemicals, caused by the surfactant.

In the previous publication [7] it had been speculated that poorer effectiveness of DDT biodegradation (higher production of DDD) might have resulted from exceeding Tween 80 CMC in the water phase. The present study seems to confirm that. However, this detrimental effect manifested only when surfactant doses were 3–4 times higher than the dose necessary for reaching CMC. Therefore, adverse influence of surfactant could be produced by its high concentration, inhibiting activity of microorganisms, rather than by specific action of micelles. Different effects of Tween 80, dose dependent, were also shown by van Hoof and Javfert [21]. Biological dechlorination of hexachlorobenzene was stimulated below and slightly above CMC, but it was inhibited at surfactant concentrations considerably exceeding CMC.

4.2. EFFECT OF INITIAL POLLUTION LEVEL

There was no reference found in the available literature with respect to observed influence of initial contamination level on the production of metabolites DDD and DBP. The only information by Chiu et al. [22] concerned the effect of DDT concentration on disappearance of this parent compound. Although only graphical data were given by these authors, it is evident that increase of DDT concentration from 0.5 to 10 mg dm⁻³ was accompanied by decrease of its removal rate. At 100 mg dm⁻³ inhibition appeared complete. Contrary to that, in the present experiments even at the highest DDT level of 288 μmol·(kg soil DM)⁻¹ (102 mg·(kg soil DM)⁻¹) no decrease of its removal effectiveness was noticed. The only changes manifested in relative production of metabolites. This disparity may result from dissimilar experimental conditions. Chiu et al. [22] performed their tests in a liquid medium with small (2.5%) inoculation with river sediments containing cultures of microorganisms capable of DDT degradation.

It seems convincing that differences in metabolite accumulation may be produced by soil toxicity. Formation of DBP appears especially sensitive to that.

4.3. HYPOTHETICAL PATHWAY OF DDT DEGRADATION BY GRANULAR SLUDGE

Slow rate of DDD disappearance excludes that degradation of this metabolite may be the main reason for its much lower than stoichiometric accumulation during DDT degradation. The only explanation of this phenomenon is that DDT must be simultaneously transformed into other compound(s) as well, and that “DDD pathway” is not at all dominant. This concept was yet not considered in studies on anaerobic bioremediation of soil polluted with this pesticide [10–15].

One such possible “alternative” transformation pathway was described in the 1970s. Albone et al. [23] and Jensen et al. [24] found that products of DDT degradation by digested sewage sludge were not only DDD and its further metabolites but also

2,2'-bis(4-chlorophenyl)acetonitrile (DDCN). However, in later studies on DDT-contaminated soil bioremediation, the issue of transformation to DDCN was not discussed. Only at the turn of the century it drew some attention in research on sediment contamination by DDT and its derivatives [3, 25]. The question raised already by the first researchers, whether DDCN is formed directly from DDT or is rather a product of degradation of DDA (2,2'-bis(4-chlorophenyl)acetic acid), one of the intermediates of the "DDD pathway", was not answered so far. In the present study, preliminary GCMS analyses (data not shown) confirmed occurrence of DDCN in DDT-spiked sample after biodegradation. Slow rate of DDD decline, and consequently small amounts of formed DDA, argues for direct DDCN production from DDT.

Likewise, high formation of DBP cannot be explained only as a result of DDD transformation due to its slow rate. Therefore it seems that DBP could also be a terminal product of DDCN degradation and/or it is formed in another pathway directly from DDT. Supposition about existence of the latter process was assumed by Schulze et al. [26] in the scheme describing DDT metabolism in sediments.

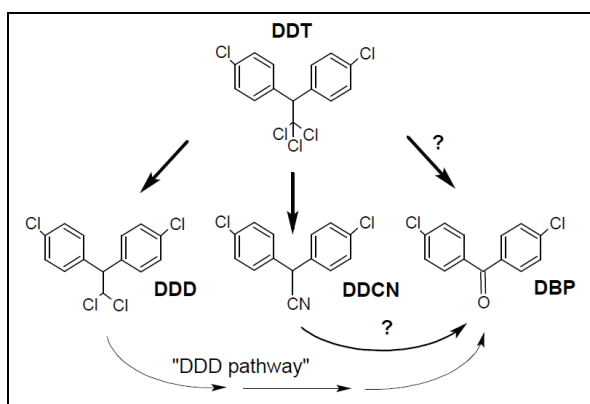


Fig. 3. Hypothetical pathway of DDT anaerobic degradation by granular sludge

Hypothetical pathways of DDT degradation by methanogenic granular sludge, summarizing above considerations, are presented in Fig. 3. Certainly, its highly speculative character must be stressed. Its confirmation and elucidation would require more detailed and rigorous study. The most important is quantification of DDCN production and explanation of its degradation products, which requires authentic standard of this compound, currently not available commercially.

5. CONCLUSIONS

The experiments have shown that application of Tween 80 surfactant in optimum doses caused considerable enhancement of anaerobic biodegradation of DDT in con-

taminated soil. This included reduction of residual concentration of this pesticide, remaining after the process, and decrease of DDD metabolite accumulation. All this leads to higher removal of DDX (sum of DDT, DDD and DDE, used as a measure of soil quality). However, higher doses, above this optimum, were detrimental in respect of DDD production minimisation. This stresses the necessity to find optimum surfactant amount for each application. Initial soil contamination level was also very important. Lower pollution resulted in better effects, smaller production of DDD and more intensive transformation of intermediates, manifesting in higher amounts of terminal metabolite DBP. Therefore, the investigated process could be best suited to applications to low-contaminated soils. This is strengthened by the fact that Polish soil quality standards are very strict in terms of acceptable DDX content. For example, with respect to surface layers in industrial areas, which potentially could be places to deposit remediated soil, the maximum allowable value is $0.25 \text{ mg} \cdot (\text{kg soil DM})^{-1}$. As the effectiveness of DDX removal reaches not much above 70%, the starting DDT concentration should not exceed $0.9\text{--}1.0 \text{ mg} \cdot (\text{kg soil DM})^{-1}$. Initial presence of excessive amounts of DDD metabolite would be disadvantageous, due to slow rate of its degradation.

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