

TOMASZ BACZYŃSKI<sup>1</sup>

## INFLUENCE OF PROCESS PARAMETERS ON ANAEROBIC BIODEGRADATION OF DDT IN CONTAMINATED SOIL. PRELIMINARY LAB-SCALE STUDY. PART II. SUBSTRATES AND pH CONTROL

Suitability of various substrates for anaerobic biodegradation of DDT in contaminated soil was tested in lab-scale tests with granular sludge inoculation. Use of carbohydrate-based substances such as starch, sucrose, molasses and whey resulted in acidification, which in extreme cases inhibited DDT removal. A large amount of phosphate buffer prevented pH drop for starch, but not for sucrose. Better results were obtained with calcium carbonate as a buffering agent, which also had little effect on soil salinity. Very good effectiveness of biodegradation was achieved using sodium lactate which, however, caused alkalinisation of the soil, perhaps due to accumulation of sodium carbonate. Alkalinisation did not occur when calcium lactate was used instead. Experiments also demonstrated that Tween 80 surfactant could be successfully used as a substrate, without experiencing problems with pH control.

### 1. INTRODUCTION

The paper presents another part of a study on anaerobic biodegradation of DDT (1,1,1-trichloro-2,2'-bis(4-chlorophenyl)ethane) in polluted soil. In the former part [1], the process enhancement by various doses of surfactant as well as influence of initial contamination level and DDT anaerobic degradation pathways have been examined. The issues addressed in the present paper are the role of additional organic substrate (electron donor), effect of its type and closely related issues of pH control during the treatment.

It is generally agreed that DDT degradation is a co-metabolic process requiring additional organic substrate (electron donor, organic carbon source) to provide energy

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<sup>1</sup>Institute of Water Supply and Environmental Protection, Cracow University of Technology, ul. Warszawska 24, 31-155 Cracow, Poland; e-mail: tomaszb@vistula.wis.pk.edu.pl

(electrons) for the reactions [2]. What is more, addition of an easy biodegradable organic matter stimulates anaerobic, low-redox potential conditions necessary for intensive metabolism of DDT [3]. Substrate type can influence the rate of DDT degradation and production of metabolites. Chiu et al. [4], using liquid-phase tests inoculated with sediment containing DDT-degrading microflora, found that DDT removal was the fastest when yeast extract was used as a substrate, while with glucose it was the slowest one. Concerning formation of DDD (1,1-dichloro-2,2'-bis(4-chlorophenyl)ethane) metabolite, the lowest level was obtained with acetate as an electron donor, whereas the highest one with glucose. Walters [5], for Brij 35 surfactant-amended anaerobic biodegradation tests, stated that use of sodium butyrate in combination with hydrogen resulted in the most effective removal of DDT, with no traces of DDD. Butyrate alone gave lower DDT removal and minimum accumulation of DDD. If hydrogen was used solely as substrate, DDT was hardly degraded.

The main purpose of this study was to assess the suitability of various organic compounds, simple or more complex, as substrates for anaerobic biodegradation of DDT in contaminated soil inoculated with granular sludge. Substrates were chosen from those often used in lab-scale anaerobic biodegradation tests, but also in full-scale bioremediation applications i.e. lactate, starch, molasses, whey and sucrose. An important factor in the selection was wide availability and low cost of the substrates. A problem arising during experiments were pH changes. This involved not only acidification for carbohydrate-based compounds, but also considerable alkalisation in the case of sodium lactate. The latter issue was, to the author's knowledge, not discussed in literature on soil bioremediation studies. Therefore, an explanation had to be found and appropriate countermeasures taken (such as use of buffering agents) to stabilize pH and, consequently, restore the habitat function of remediated soil. An additional and complementary goal was also to determine the influence of substrate dose on the results of the process.

## 2. MATERIALS AND METHODS

*Chemicals, sludge and soil.* Calcium lactate pentahydrate, pure for analysis, was manufactured by the Chempur Company (Piekary Śląskie, PL). The other substrates such as sucrose, potato starch, beet molasses and dried whey were purchased in local groceries. Concentrated phosphate buffer, methanogenic granular sludge and soils: contaminated NIE1 and clean NIE0 were the same or similarly prepared as previously [1].

*Biodegradation tests.* The general procedure of preparing biodegradation tests, their incubation and sampling have been presented elsewhere [1]. In experiments II and III, 20 mg of nitrogen per sample were added in the form of an ammonium chloride solution (discarded later as it was found not to be important to the results). Monitoring of pH was done directly in samples, using a CP105 pH-meter (Elmetron, PL),

equipped with a probe customized for measurements in the soil. Experiment I was repeated three times, while the remaining ones used single samples only.

*Gas chromatography (GC) analysis.* Soil sampling, extraction and GC analyses were similar as in [1]. Measurements included p,p' isomers of DDT, DDD, DDE (1,1-dichloro-2,2'-bis(4-chlorophenyl)-ethene) and DBP (4,4'-dichlorobenzophenone), which are given in the text without indication (p,p') of the isomer type.

*Experiment I.* The purpose of this experiment was to study the significance of substrate dose on the effectiveness of biodegradation. Sodium lactate 60% syrup was used here, in doses of 1.45, 0.7, 0.2 and 0 cm<sup>3</sup> (none) per sample. Contaminated soil was used without dilution. No other additives, except sludge, were used, including surfactant, buffer or nutrients.

*Experiment II.* A mixture of contaminated and clean soils was prepared, in proportion of 1 + 3. The suitability of various organic substrates was studied, which included sodium lactate (0.7 cm<sup>3</sup> per sample), potato starch (0.5 g) and sucrose (also 0.5 g). Each substrate was applied in two samples, one with 0.05 cm<sup>3</sup> of phosphate buffer, only as a nutrient source, and the other with 2 dcm<sup>3</sup> of the same buffer for pH control. Additionally, a sample without any substrate was prepared, containing only 150 mg of surfactant (added also to the rest of samples). No control was used in the experiment.

*Experiment III.* A 1 + 3 soil mixture was used to check if calcium ions could be used for pH control in lactate-amended samples. Substrates were 0.7 cm<sup>3</sup> of sodium lactate syrup or 0.7 g of calcium lactate pentahydrate. Samples with sodium lactate were prepared without other additives or with 1 cm<sup>3</sup> of 2.5 M calcium chloride. Calcium lactate was used alone or with 2 cm<sup>3</sup> of phosphate buffer. The control sample was not inhibited with azide.

*Experiment IV.* Contaminated soil diluted in proportion 1 + 9 was used. Suitability of various substrates was studied such as dried whey (0.5 g for sample), beet molasses (0.5 g), sucrose (0.5 g) and starch (0.5 g), each with 1 g of calcium carbonate for pH buffering. Whey was also applied without carbonate addition. Effectiveness of these substrates was evaluated against sole sodium lactate addition (0.7 cm<sup>3</sup> of the syrup).

### 3. RESULTS

Data from experiments I–IV are shown in Figs. 1–4 as concentrations ( $\mu\text{mol}:(\text{kg soil DM (dry mass)})^{-1}$ ) of DDT, DDD and DBP, in relation to the incubation time. Similarly as in the previous part [1] DDE level remained very low, thus minimizing

production of DDD decisive for reduction of the DDX index (the sum of DDT and its primary metabolites DDD and DDE), required by soil quality standards [6]. DBP represents the final product of anaerobic DDT transformations [2]. *o,p'*-DDT, -DDD and -DDE were also monitored, but their changes were similar to *p,p'*-isomers.

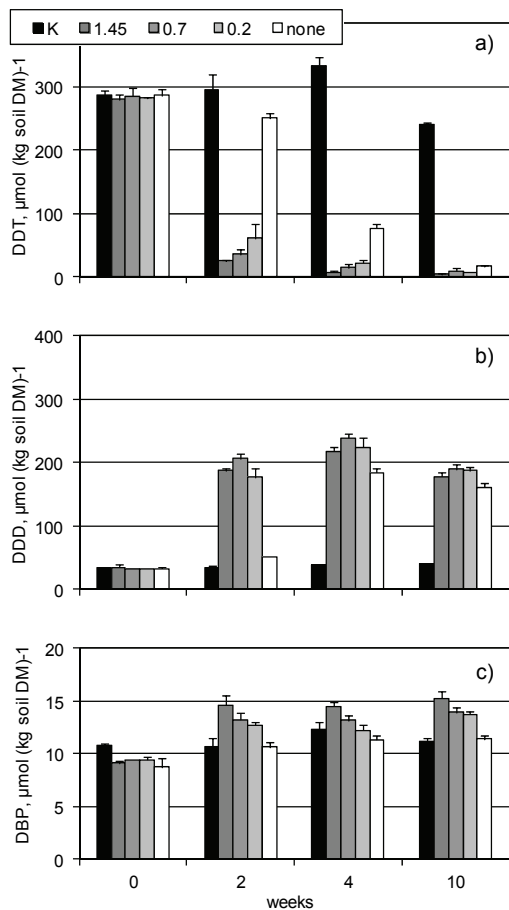


Fig. 1. Concentration changes of DDT (a), DDD (b) and DBP (c) in experiment I. K – control, 1.45 etc. – sodium lactate in  $\text{cm}^3$  per sample. Bars denote standard deviation

With regard to experiment I (Fig. 1), it is visible that lowering the substrate (lactate) dose resulted in lower initial rates of DDT decomposition, from 271 to 235  $\mu\text{mol} \cdot (\text{kg soil DM})^{-1}$  removed in the first 2 weeks, for samples with 1.45 and 0.2  $\text{cm}^3$  of lactate, respectively. However, this effect was not very noticeable, thus the results in terms of per cent removal after 10 weeks differed only slightly (96–98%; final concentrations of 5.1–9.8  $\mu\text{mol} \cdot (\text{kg soil DM})^{-1}$  against 241  $\mu\text{mol} \cdot (\text{kg soil DM})^{-1}$  remaining in the control). For 1.45  $\text{cm}^3$  lactate the stoichiometric ratio of DDD production to removed DDT was a little lower than for other doses, 58% versus 63–64% at the end of the test. DDD concentrations increased to 178  $\mu\text{mol} \cdot (\text{kg soil DM})^{-1}$  versus 188–190  $\mu\text{mol} \cdot (\text{kg soil DM})^{-1}$  for lower

doses and  $41 \mu\text{mol}\cdot(\text{kg soil DM})^{-1}$  as background in the control. DBP formation was higher for the highest dose of lactate; concentration increased to  $15.3 \mu\text{mol}\cdot(\text{kg soil DM})^{-1}$  against  $11.2 \mu\text{mol}\cdot(\text{kg soil DM})^{-1}$  in the control, that corresponded 1.7% conversion of DDT. At the same time, in samples with lower doses DBP reached  $13.7\text{--}14.0 \mu\text{mol}\cdot(\text{kg soil DM})^{-1}$ , equivalent to 1.1–1.2% transformation. Where lactate was absent (“none”) DDT decline was slow, only  $45 \mu\text{mol}\cdot(\text{kg soil DM})^{-1}$  removed in the first 2 weeks. However after 10 weeks, the removal effectiveness of 93% (concentration  $17.3 \mu\text{mol}\cdot(\text{kg soil DM})^{-1}$ ) was not much inferior to the other samples. Accumulation of DDD was even lower than for samples amended with substrate ( $161 \mu\text{mol}\cdot(\text{kg soil DM})^{-1}$ , 53% conversion of DDT), however, there was no noticeable production of DBP (final level of  $11.4 \mu\text{mol}\cdot(\text{kg soil DM})^{-1}$ ). For all samples with addition of lactate, the pH increased continuously during the incubation, from the initial 7.4–7.5 to 9.0–9.5 after 10 weeks. Without any substrate, pH was rather stable, ranging from 6.9 to 7.2.

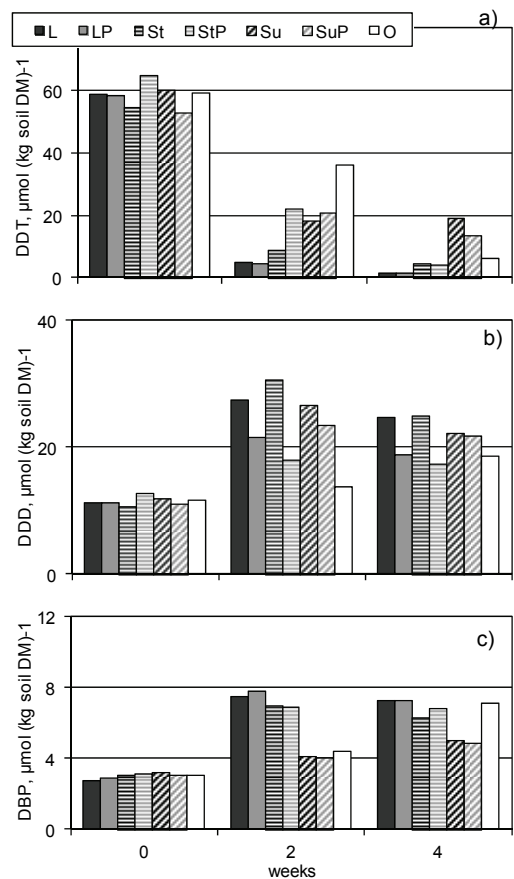


Fig. 2. Concentration changes of DDT (a), DDD (b) and DBP (c) in experiment II. L – sodium lactate, LP – sodium lactate and phosphate, St – starch, StP – starch and phosphate, Su – sucrose, SuP – sucrose and phosphate, O – surfactant only

Presence of phosphate buffer did not prevent increase of pH to a final level of 9.0 in samples with sodium lactate (experiment II, Fig. 2). However, high-phosphate dosed sample (LP) exhibited low DDD accumulation, and its concentration increased from initial  $11.2$  to  $18.9 \mu\text{mol}\cdot(\text{kg soil DM})^{-1}$  after 4 weeks of incubation. This was equivalent to 13% conversion of DDT removed ( $58.6$  to  $1.41 \mu\text{mol}\cdot(\text{kg soil DM})^{-1}$ ). For comparison, in sample L with minimum phosphate addition, DDD level increased from  $11.2$  to  $24.8 \mu\text{mol}\cdot(\text{kg soil DM})^{-1}$ , which corresponded to 24% of  $57.1 \mu\text{mol}\cdot(\text{kg soil DM})^{-1}$  removed DDT. Use of starch as a substrate (St) produced gradual acidification of the soil, pH decreased from 6.7 to 6.2 after the first week, 5.5 after the second and 5.2 after the fourth. The result was slightly lower removal of DDT than that for sodium lactate. Newly produced DDD was similar to that of L, with little lower formation of DBP. Phosphate buffering (StP) maintained pH at the level of 6.6–6.8. Accumulation of DDD was diminished here to  $17.4 \mu\text{mol}\cdot(\text{kg soil DM})^{-1}$  at the end of the experiment ( $12.6 \mu\text{mol}\cdot(\text{kg soil DM})^{-1}$  at the start), corresponding to only 11% transformation of DDT removed ( $60.7 \mu\text{mol}\cdot(\text{kg soil DM})^{-1}$ ). Samples with sucrose Su underwent quick and deep acidification to pH 3.6; phosphate buffer (SuP) prevented that only minimally (pH 4.8–4.9). Such a low pH was probably responsible for poor DDT removal, from  $60.2$  to  $18.9 \mu\text{mol}\cdot(\text{kg soil DM})^{-1}$  and from  $52.9$  to  $13.6 \mu\text{mol}\cdot(\text{kg soil DM})^{-1}$  only (68 and 77% in 4 weeks), respectively for Su and SuP. Accumulation of DDD was appreciable, to  $22.2$  and  $21.7 \mu\text{mol}\cdot(\text{kg soil DM})^{-1}$  (corresponding 23–27% conversion of DDT) while rise of DBP concentration was small (from 3.1–3.2 to 4.8–5.0  $\mu\text{mol}\cdot(\text{kg soil DM})^{-1}$ ). Interestingly, in sample O, with no organic substrate added (but with addition of the surfactant, as the others) the process effectiveness was noteworthy. Admittedly, DDT decomposition was slower but after 4 weeks the effect (89% removal, final concentration of  $6.3 \mu\text{mol}\cdot(\text{kg soil DM})^{-1}$ ) was only slightly lesser than for both sodium lactate added samples and similar to that with starch. DDD concentration increased from  $11.6$  to  $18.6 \mu\text{mol}\cdot(\text{kg soil DM})^{-1}$ , corresponding 14% of removed DDT. DBP production reached  $7.1 \mu\text{mol}\cdot(\text{kg soil DM})^{-1}$  from initial  $3.1 \mu\text{mol}\cdot(\text{kg soil DM})^{-1}$ , equivalent to 8% transformation of DDT – similar as for samples with lactate, the best in this respect. That pointed to intensive DDT transformations. Also pH remained reasonably stable.

During experiment III (Fig. 3) alkalisation was prevented partially by addition of calcium chloride (LCa) or eliminated completely by replacement of sodium salt of lactate by the calcium one (CL) as electron donor. However, if calcium lactate was used simultaneously with phosphate addition (CLP) pH increase was comparable to that occurring in the sample with sodium lactate alone. It should be noted that amount of phosphate added was in stoichiometrical excess of calcium contained in calcium lactate. It is thus very likely that, from the very start, calcium was completely precipitated as insoluble phosphates. Samples with calcium remaining in a soluble form, LCa and CL, exhibited the highest accumulation of DDD (concentration  $33.9$  and  $31.6 \mu\text{mol}\cdot(\text{kg soil DM})^{-1}$  at week 8) and slightly worse DDT removal. Unfortunately,

perhaps due to accidental contamination with sludge during sampling and lack of azide inhibitor, slow degradation of DDT occurred in the control sample from week 4. This was coupled with an almost equivalent increase of DDD concentration. Also, LCa subsample, taken at the start, was destroyed during extraction, thus this data point is missing.

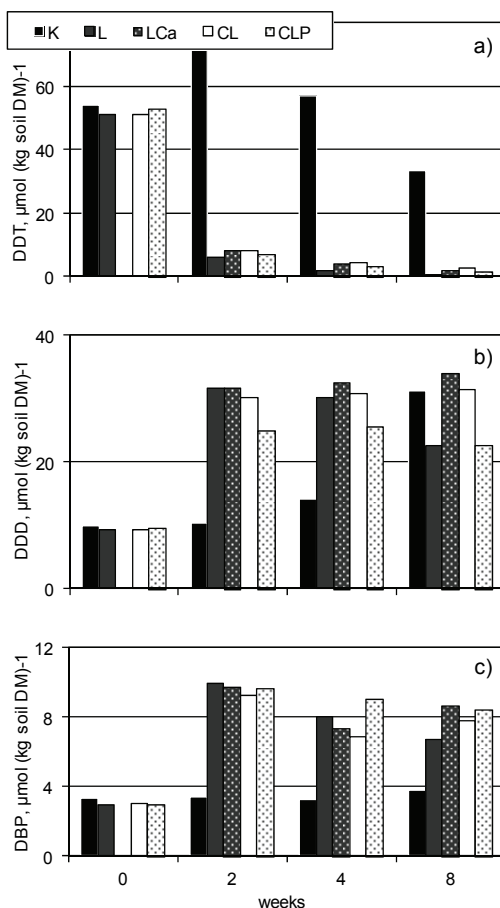


Fig. 3. Concentration changes of DDT (a), DDD (b) and DBP (c) in experiment III. K – control, L – sodium lactate, LCa – sodium lactate and  $\text{CaCl}_2$ , CL – calcium lactate, CLP – calcium lactate and phosphate

In experiment IV (Fig. 4) addition of calcium carbonate together with easy-fermentable substrates (whey WhC, molasses MC, sucrose SuC and starch StC) resulted in no acidification and kept pH at almost constant level (except for a short-term decrease to 5.7–5.8 for the first three substances). For the sole whey application (Wh), an initial acidification occurred, even to pH 4.6, but after 2 weeks pH started to increase. After 4 weeks it returned to 6.8, almost the initial value. Samples with carbohydrate substrates and carbonate exhibited higher formation of DDD:  $8.46\text{--}9.44 \mu\text{mol} \cdot (\text{kg soil DM})^{-1}$  against  $2.85 \mu\text{mol} \cdot (\text{kg soil DM})^{-1}$  background in the control, corresponding to 28–33% of DDT removed. Yield of DBP was lower – 2.61–3.14

$\mu\text{mol}\cdot(\text{kg soil DM})^{-1}$  with  $1.69 \mu\text{mol}\cdot(\text{kg soil DM})^{-1}$  remaining in the control. This was equivalent to 5–6% transformation of removed DDT. For the sample with lactate, the final concentrations of DDD and DBP were  $7.73$  and  $4.25 \mu\text{mol}\cdot(\text{kg soil DM})^{-1}$ . This corresponded to 24% and 13% of DDT removed, respectively. Use of whey alone produced quite similar DDD accumulation but significantly lower transformation into DBP, comparing to the lactate sample.

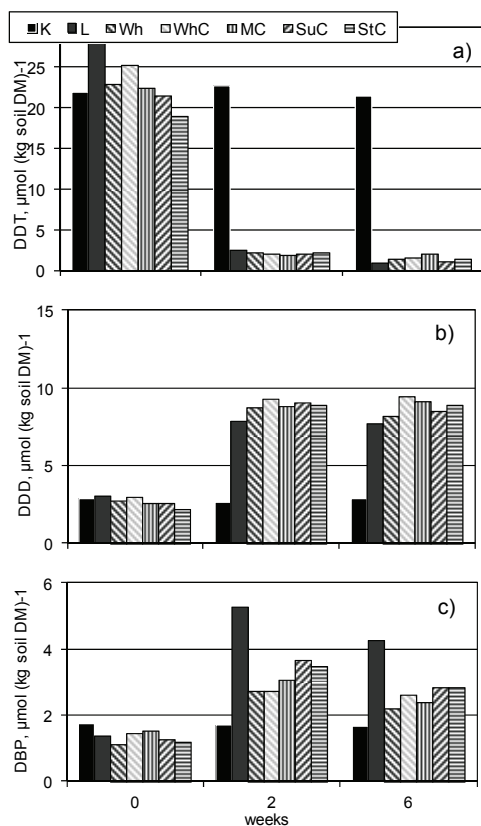


Fig. 4. Concentration changes of DDT (a), DDD (b) and DBP (c) in experiment IV. K – control, L – sodium lactate, Wh – whey, WhC – whey and  $\text{CaCO}_3$ , MC – molasses and  $\text{CaCO}_3$ , SuC – sucrose and  $\text{CaCO}_3$ , StC – starch and  $\text{CaCO}_3$

## 4. DISCUSSION

### 4.1. SIGNIFICANCE OF SUBSTRATE DOSE

Experiment I confirmed the importance of addition of easy-biodegradable substrate for the effective anaerobic biodegradation of DDT. This was particularly clear for samples with no electron donor added. Not only decomposition of DDT was slow there, but also its transformation was a little advanced, as shown by lack of DBP ter-



minal metabolite production. Supposedly, microorganisms could use only internal sources of carbon and energy, storage materials, products of cell decay, etc. Even a little amount of additional substrate (lactate) caused notable improvement, both with respect to faster DDT removal and advanced decomposition process (indicated by intensity of DBP formation). Although increasing the lactate dose produced even better results, this further enhancement was not so great. Therefore, taking into account the cost related to higher amounts of substrate in possible full-scale application, it was found that an optimum and reference dose of sodium lactate syrup should be  $0.7 \text{ cm}^3$  for 50 g of soil.

More intensive conversion into DDD and much lower formation of DBP in experiment I probably resulted from a higher initial level of contamination, as well as from lack of surfactant application.

#### 4.2. MULTI-ASPECT INFLUENCE OF SUBSTRATE TYPE

All substrates used in this study produced, in optimum conditions, similar removal of DDT, thus their effects can be compared only with respect to stoichiometric ratios of DDD accumulation (decisive for reduction of DDX index) and production of DBP (indicating completeness of anaerobic DDT transformation). With regard to that, the best results were obtained applying lactate. What is also important, this compound did not cause acidification, which eliminates the necessity of buffering.

However, adding lactate in the form of sodium salt resulted in considerable alkalisation of the soil ( $\text{pH} \geq 9$ ). A probable reason for this phenomenon was forming of sodium carbonate species as a product of degradation of sodium lactate. Li et al. [7] stated that during degradation of salts of organic acids in soil, respective carbonate salts accumulate as a result of combination of produced carbonate ions with released cations. This leads to large pH increase, also due to proton consumption during decarboxylation of salts of organic acids, although this being of minor importance. The key factor is presence of basic cations. If respective organic acids are applied, with no basic cations, alkalisation does not occur, pH decreases initially, and then returns back to the starting level. In the reported research, occurrence of soluble carbonates was indicated by immediate pH drop after addition of calcium salt to incubated samples with sodium lactate (results not shown here; supposed precipitation of calcium carbonate).

Alkalisation hinders restoration of habitat functions of remediated soil. What is more, high concentrations of sodium carbonate change soil characteristics to that of an alkaline-sodic one with high pH and poor, impermeable structure [8]. This last feature is a result of clay dispersion by sodium ions. Plant growth on such soils is very difficult.

Such alkalisation was prevented by initial dosing of calcium ions, either as calcium chloride or within calcium salt of lactate. Produced carbonate was then precipi-

tated as hardly soluble calcium carbonate. Use of calcium lactate was definitely more beneficial as it minimally affected the overall salinity of remediated soil. It should be noted, however, that effectiveness of the process with respect to amount of accumulating DDD was not as high as for sodium lactate.

Application of starch or sucrose as substrates caused pH decrease, apparently resulting from generation of a large amount of organic acids during acid fermentation. This significant decrease of pH should be held responsible for inhibition of DDT removal. This was most visible for sucrose, which due to simpler structure was more susceptible to fast acid fermentation. In the study by Huang et al. [9], anaerobic DDT biodegradation completely stopped at pH 5.9, whereas its rise to only 6.7 provided the best conditions for its removal. The process studied here was, in fact, not so sensitive. Despite lower pH values in samples with starch (minimum of 5.2–5.5) and whey (minimum of 4.6) significant DDT disappearance was still observed, however, DBP production was lower than for lactate-dosed soil (especially for whey). It is likely that slow progress of pH decrease (starch) or its transient occurrence (whey) limited the inhibitory effect of this factor. The subsequent increase of pH for whey could be caused by decomposition of generated organic acids but also by release of ammonium due to degradation of proteins (13% of whey mass, according to the manufacturer). This cation, of basic character, could also contribute to some accumulation of carbonates.

Easy fermentability of substrates such as starch, sucrose, molasses or whey makes simultaneous addition of buffering agent necessary. From the tested ones, good results were obtained for phosphates with starch – they were similar to those obtained for lactate used together with phosphates. However, such a large amount of phosphates (ca. 1% of soil by weight) enormously increased the soil salinity. In this respect, use of calcium carbonate was more favourable. However, with such buffered substrates, the results of biodegradation in terms of production of DDD and DBP were inferior to those obtained for lactate.

A significant observation was good effectiveness of biodegradation achieved in experiment II with the sample containing no substrates but Tween 80 surfactant. Although decomposition of DDT was slower in the initial phase of treatment, the percentage removal of this compound after 4 weeks was not much worse than for samples with lactate or starch. The conclusion is that Tween 80 could be also used as electron donor. Yeh et al. [10] demonstrated that Tween 80 (polyoxyethylenesorbitan monooleate) was partially biodegraded during methane fermentation. In the later report [11] a putative anaerobic degradation pathway of Tween surfactant was presented. It suggests that Tween hydrophobic moiety is subject to degradation, with intermediates such as ethanol, lactate, acetate, formate and hydrogen. Walters [5] implied that Brij 30 surfactant could be used as an electron donor for DDT anaerobic dechlorination, also being degraded in its hydrophobic part. Yeh and Pavlostathis [12] used

Tween 60, 61 and 65 as substrates for hexachlorobenzene dechlorination, after adaptation of mixed cultures of methanogenic microorganisms.

## 5. CONCLUSIONS

Selection of electron donor, necessary for anaerobic biodegradation of DDT in contaminated soil, proved to be a complex issue. It does not only influence degradation of DDT and production of metabolites, but affects pH and soil salinity as well. Carbohydrate-based substrates are prone to acidification, which in some instances could be detrimental for DDT removal. A buffering agent must therefore be used but it should not raise the soil salinity considerably, as it would damage habitat functioning of the remediated soil. Calcium carbonate seems a good choice here. Lactate appeared to be the best substrate in terms of effectiveness of DDT removal but it cannot be used in the form of acid because of its low pH. Use of sodium salt of lactate is also not recommended. The reason is that excessive amount of basic sodium cations leads to alkalisation and salinisation of soil during degradation of organic acids, due to accumulation of respective soluble sodium carbonate. Calcium lactate is much better as evolving carbonates are precipitated as calcium salts. It seems also that Tween surfactant, originally applied only for additional stimulation of DDT transformation, could be used in the function of an electron donor as well. Tween 80 sole application, without other substrates, provided good results of DDT degradation, without a noticeable alteration to pH level.

## ACKNOWLEDGEMENTS

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