

***De novo* formation of hepta- and octachlorodibenzo-*p*-dioxins from pentachlorophenol in municipal sewage sludge**

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Introduction

Polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) in sewage sludge have recently become a subject of public and scientific interest. Substantial amounts of PCDD/Fs, especially the higher chlorinated dioxins, have been found^{1,2}. In studies attempting to calculate a PCDD/F mass balance for sewage treatment plants, it has not been possible to account for all the OCDD found in the sewage sludge^{3,4}. Is sewage sludge a *primary source* of dioxins and dibenzofurans?

Earlier, we found that peroxidases oxidise chlorophenols to PCDD/Fs *in vitro*⁵. Last year, we reported on the peroxidase-catalysed oxidation of tetrachlorophenols and pentachlorophenol (PeCP)⁶, where considerable amounts of higher chlorinated PCDD/Fs, especially HpCDDs and OCDD, were formed. Pentachlorophenol, was oxidised to 100 ppm OCDD, and the PCDD/F isomeric pattern was a typical "sewage sludge pattern," not a "pentachlorophenol pattern." Quite recently, we analysed human faeces⁷ and waste water from dish washers and washing machines⁸. The results imply a possible formation of PCDD/Fs in sewage sludge. Here, we present analyses of municipal sewage sludge, showing *de novo* formation of PCDDs from pentachlorophenol, and possible biotransformation.

Experimental

The sewage sludge was collected from the Umeå sewage treatment plant, which serves a population of approximately 90000. The capacity of the sewage treatment plant is currently 85000 person equivalents. Iron sulphate is added before the sewage reaches the activated sludge pool. There, the heavily stirred and aerated activated sludge have a residence time of four hours; The sedimented sludge is aerated too, with a residence time of three hours. The

Table 1. Sample description.

ACT-CP	activated sludge	0.6% dw	¹³ C-PeCP	22°C incubation
ACT	activated sludge	0.6% dw		22°C incubation
SED22-CP	sedimented sludge	4% dw	¹³ C-PeCP	22°C incubation
SED22	sedimented sludge	4% dw		22°C incubation
SED10-CP	sedimented sludge	4% dw	¹³ C-PeCP	10°C incubation
SED10	sedimented sludge	4% dw		10°C incubation
ACT-raw	thick activated sludge	2% dw		no incubation
SED-raw	sedimented sludge	4% dw		no incubation

total processing time is less than 24 hours, during which time 40 tons of centrifuged sludge (22% dry weight) is produced.

Temperature of the sludge was 7-9 °C at collection. Each sample (Table 1) consisted of approximately 1 kg of sludge. ¹³C-PeCP (Cambridge Isotope Laboratories) dissolved in toluene was extracted into 1.6 mM KOH, and added to samples at a final concentration of 10 μM (2 ppm) ¹³C-PeCP. The same kind of sludge, supplemented with only 1.6 mM KOH instead of ¹³C-PeCP, served as experimental controls (Table 1). The sludge samples were handled in UV-protected environment and incubated in the dark, aerated, and thermostated.

Then they were dried, ground, extracted, cleaned-up, and analysed on HRGC-HRMS according to reported methods^{1,7,8}.

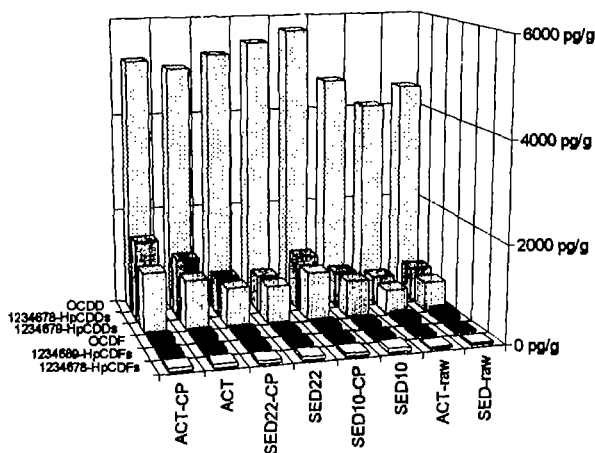


Figure 1. ¹²C-PCDD/Fs found in municipal sewage sludge. Abbreviations in Table 1.

Results

Consistent patterns and amounts of ¹²C-PCDD/Fs are found in the eight sewage samples (Fig. 1, Table 2). Comparing the population averages for non-incubated (ACT-raw, SED-raw) and incubated sewage sludge shows significant differences (Table 2), e.g., suggesting *de novo* formation of ¹²C-PCDDs in the six incubated samples.

Significant amounts of ¹³C-HpCDDs and ¹³C-OCDD were found in the ¹³C-PeCP spiked samples (ACT-CP, SED22-CP, SED10-CP; Figs. 2 and 3, Table 3). The formation corre-

Table 2. ¹²C-PCDD/Fs (pg/g dry weight) found in municipal sewage sludge. See Table 1 for abbreviations.

SAMPLE:	1234678 -HpCDFs	1234689 -HpCDFs	SUM HpCDFs	OCDF	1234679 -HpCDDs	1234678 -HpCDDs	SUM HpCDDs	OCDD	SUM PCDD/Fs
ACT-CP	73	60	134	86	1215	1647	2862	5185	8267
ACT	78	58	136	92	1005	1230	2235	5011	7475
SED22-CP	68	63	131	87	787	779	1566	5271	7055
SED22	71	60	132	55	763	749	1513	5500	7199
SED10-CP	70	63	133	97	985	1118	2103	5730	8062
SED10	86	60	149	86	748	714	1462	4647	6340
ACT-raw	36	30	66	125	490	563	1053	4081	5325
SED-raw	44	33	77	163	608	704	1312	4468	6020
AVERAGE: incubated	74	61	136	84	917	1039	1957	5224	7400
S.D.	6	2	6	13	169	334	502	345	643
AVERAGE: raw sludge	40	31	71	144	549	634	1183	4274	5672
S.D.	4	2	6	19	59	71	130	193	347

Table 3. ^{13}C -PCDDs (total pg) found in sludge samples spiked with ^{13}C -PeCP, and proportion of ^{13}C -PeCP converted to ^{13}C -PCDDs. Abbreviations according to Table 1.

Sample	^{13}C -1234679- HpCDD	^{13}C -1234678- HpCDD	Tot ^{13}C -OCDD	Tot ^{13}C -PCDDs	^{13}C -PCDDs: ^{13}C -PeCP
ACT-CP	274	3726	2188	6187	3.5 ppm
SED22-CP	310	1395	2597	4302	2.4 ppm
SED10-CP	403	2494	3017	5913	3.4 ppm

spends to 2-4 ppm of converted ^{13}C -PeCP (Table 3). No ^{13}C -PCDDs were found in the controls at levels of 0.5% of those found in the ^{13}C -PeCP spiked samples. No ^{13}C -PCDD/Fs could be detected in the aqueous ^{13}C -PeCP spiking solution (Fig. 3), nor in ^{13}C -PeCP crystals (CIL) dissolved in toluene. No contamination of laboratory blanks was detected.

The total formation of ^{13}C -PCDDs was similar in the three samples. However, analysing the dioxin formation in detail (Fig. 2) suggests that specific metabolic events occur. Moreover, the relative abundance of ^{12}C - and ^{13}C -PCDDs in these samples (Tables 2 and 3) reveals a similar isomer distribution specific to each kind of sludge.

Conclusions

^{13}C -PCDDs are formed *de novo* in municipal sewage sludge spiked with ^{13}C -PeCP. The findings are consistent with biological activity as a cause of the transformation. Though at this stage, abiotic factors cannot be ruled out. We think that chemical (auto)oxidation or photooxidation is less likely to have occurred, since the three types of sludge show different patterns of isomer formation. The lab is equipped with all UV-filter coating. No ^{13}C -PCDFs were found, which agrees with the low amounts of ^{12}C -PCDFs often found in sewage sludge.

^{13}C -PeCP was converted into ^{13}C -PCDDs at a rate of 2-4 ppm counted on ^{13}C -PeCP. Probably, ^{13}C -PeCP undergoes other reactions simultaneously. One might have expected a pronounced

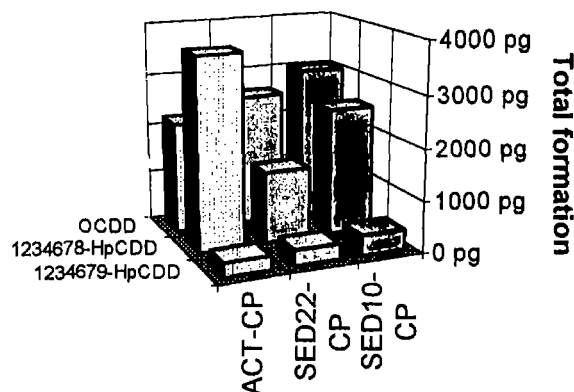


Figure 2. Total ^{13}C -PCDDs found in sludge samples spiked with ^{13}C -PeCP. Abbreviations in Table 1.

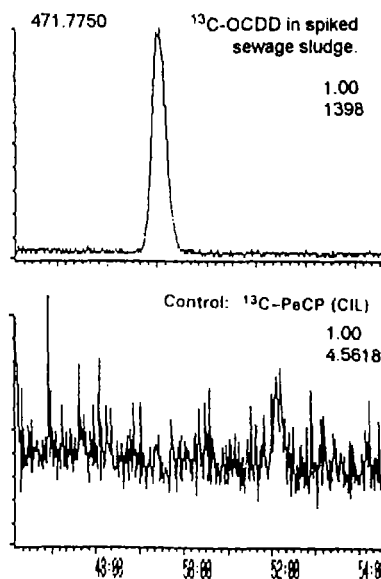


Figure 3. ^{13}C -OCDD is only found in the spiked sewage sludge, not in the ^{13}C -PeCP.

difference in total amount of formation of ^{13}C -PCDDs between activated sewage sludge and sedimented sludge, if the sedimented sludge was nearly anaerobic. This is not so in the sewage water treatment process at the Umeå plant. This speaks for similar microbial conditions in the three sludges at the beginning of the experiment.

The total formation of ^{12}C -PCDFs is similar in the three types of sludge. However, the average amount of ^{12}C -PCDD/Fs of incubated sludge is significantly different from the non-incubated sludge. Data point to a concomitant *de novo* formation of ^{12}C -PCDDs in incubated sludge, along with the formation of ^{13}C -PCDDs from ^{13}C -PeCP. The extent and quality of formation are strikingly similar for ^{12}C - and ^{13}C -PCDDs, which suggests that there are ^{12}C -chlorophenolic precursors present in the sludge. This agrees with published data⁹. We have observed a similar increase of ^{12}C -PCDDs in garden compost (Öberg, L.G., personal communication). Differences in average amount of formation of ^{12}C -PCDFs is also observed, although the values are low. The behaviour of ^{12}C -OCDF warrants further studies with ^{13}C -OCDF spiked sewage sludge. The ACT-CP, SED22-CP, and SED10-CP samples differ at the isomeric level, suggesting specific metabolic events and microbial conditions. According Table 2, 20 g of HpCDDs and OCDD leave the Umeå sewage treatment plant each year. amount may be higher, when all other PCDD/Fs are considered.

The *de novo* formation of PCDDs in sewage sludge has not yet been proven to belong to a specific enzymatic activity, but investigating the role of peroxidases is a good bet.

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