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Changes of heavy metal forms and chlorinated biphenyls during digestion of pre-hydrolyzed sewage sludge

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ABSTRACT

Two digestion processes were conducted: the mesophilic one (as reference system) and the mesophilic one preceded by thermophilic hydrolysis. The production of biogas (methane), was evaluated, as well as the changes in the content of chlorinated biphenyls with codes: 28, 52, 101, 118, 138, 153, and 180, and the changes of heavy metal forms (Zn, Cu, Ni, Pb, Cd, and Cr) in sewage sludge. Higher biogas production, amounted to $1.15\,\mathrm{L\,g^{-1}}$ of removed dry organic matter was obtained during thermophilic–mesophilic digestion, whereas during mesophilic digestion it amounted to $0.78\,\mathrm{L\,g^{-1}}$; quantity of methane in biogas during both digestions maintained within the range of 61–65%. Sludge hydrolysis accelerated transitions of polychlorinated biphenyls during the mesophilic methane digestion. Twenty-five percent reduction in the indicator polychlorinated biphenyls (PCB) content after the mesophilic digestion was obtained, whereas after digestion preceded by thermophilic hydrolysis—93% decrease in PCB concentration. Lower chlorinated congeners were dominant in fermented sludge. Proceeding sludge digestion promoted the creation of stable chemical forms of heavy metals. Regardless of applied stabilization process, the highest increase in zinc, copper, nickel, cadmium, and chromium content was demonstrated in organic–sulfide fraction, whereas for lead—in the residue fraction.

Keywords: Sewage sludge; Digestion; Thermophilic hydrolysis; Biogas; PCB; Heavy metals

1. Introduction

The final management of sewage sludge generated in wastewater treatment plants in many countries, including Poland, still constitutes a problem for plants operators. One of the factors that may disqualify sewage sludge from further use, besides heavy metals, is the presence of toxic organic substances, e.g. polychlorinated biphenyls (PCB). Most commonly used technology for neutralization of PCB in sewage sludge is aerobic and anaerobic stabilization [1]. During these processes some transformations of PBC take place, which lead to a decrease in PCB content in sludge. In anaerobic conditions reductive de chlorination of PCB proceeds for higher chlorinated congeners (HCB), with the generation of lower chlorinated biphenyls (LCB). During anaerobic degradation

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micro-organisms cause selective elimination of chlorine atoms substituted mostly in meta- and para-positions, therefore, decreasing toxicity of PCB [2–4].

Within the scope of modification of sewage sludge stabilization, dual stage systems are applied: anaerobic–anaerobic and aerobic–anaerobic. Anaerobic–anaerobic stabilization is performed in two chambers connected in line, out of which the first one is highly loaded (acidic digestion), and the other one is low-loaded (acetic and methane digestion). Thanks to this solution, optimal conditions for development of hydrolytic, acidogenic, and methanogenic bacteria are created. Conducting the first stage of digestion in thermophilic range of temperature is favorable in the case of requisite sludge disinfection. Thermophilic–mesophilic digestion also enables obtaining higher amount of methane in biogas [5].

Literature data indicate that in sewage sludge heavy metals can occur in different forms, which determine their bioavailability. Evaluation of the content of various forms is possible by means of metal speciation analysis [6]. Conducting speciation analysis of metals in biochemically stabilized sludge is, especially, important when agricultural application is considered. Familiarity with fraction in which metals occur allows one to evaluate the rate at which metals pass into soil solution. Some of heavy metals in trace amounts (microelements) are essential for plant and animal metabolisms; however, in concentrations above certain level they can demonstrate some toxic effect and pose a threat to plants, animals, and also humans [7].

The aim of this research was to evaluate usefulness of the methane digestion process preceded by thermophilic hydrolysis for reduction of PCB content in sludge, and to demonstrate whether advantageous transformations of heavy metals in sludge occur by means of increasing their concentration in non-mobile fractions.

2. Material and methods

2.1. Materials and anaerobic digestion

As a substrate, preliminary and excess sludge was used, sampled from mechanical–biological wastewater treatment plant that treats sewage in which the amount of industrial sewage is no more than 20%. The capacity of the WWTP is 48,000 m^3/d. Inoculum for digestion was fermented sludge taken from a well-operating mesophilic digester.

To conduct sludge stabilization process, glass bioreactors with volume of 1 L were used. The first hydrolysis process of the mixture of preliminary and excess sludge $(P+E)$ was executed. The hydrolysis stage included single filling of 10 bioreactors with 0.65 L of sludge, and then incubation of hermetically closed reactors in a laboratory incubator at temperature of 55˚C for 4 d. During the second stage, hydrolyzed sludge $(P+E)_H$ was inoculated with mesophilic digestion sludge (D) at volumetric ratio of 1:2. Assumed mix ratio was a result of the necessity to adjust amount of methanogenic microflora to the amount of substrate. Ten bioreactors with 0.65 L of sludge were refilled with obtained sludge mixture $((P+E)_H+D)$. After the removal of air, mesophilic methanogenesis was enforced at temperature of 37˚C for 15 d. Moreover, in 10 bioreactors control culture was proceeded—mesophilic digestion of non-hydrolyzed sludge $(P+E+D)$.

2.2. Analysis procedure

Manometric measurement of the amount of produced biogas was conducted every 24 h. Biogas composition was verified every 48 h, by means of gas chromatography with thermal conductivity detector (gas chromatograph Agilent 6890N, Agilent Technologies).

Before digestion and on 1st, 3rd, 7th, 10th, and 15th day of the process selected sludge properties were determined: hydration, total and volatile solids. Liquid separated from sludge by means of centrifugation (rotational speed—6,000 rpm, time—10 min), the following parameters were evaluated: pH, alkalinity, volatile fatty acids VFA, total organic carbon TOC according to accepted methods [8], and heavy metal ions concentration (Zn, Cu, Ni, Pb, Cd, and Cr) by means of atomic absorption spectrometry (spectrometer novAA 400, Analytik Jena). Each analysis was repeated three times. Before and after digestion the total content was evaluated as well as heavy metal chemical forms in sludge specified in BCR procedure. In the first stage, in order to extract exchangeable metals, metals bound to carbonates (F1) 0.11 M CH_3COOH was used; in the second stage (extraction of metals bound to hydrated iron and manganese oxides—F2) 0.5 M NH_2OH HCl (pH 2) was used. During the following stage, in order to extract metals bound to organic matter and sulfides (F3), 8.8 M H_2O_2 (pH 2–3) and 1 M·CH_3COONH_4 were used; in the last stage (residual fraction—F4)—HNO_3 (65%) and HCl (37%) were used. Detailed specification of performed procedure was described elsewhere [9]. The correspondence of sum of the metal content in particular fractions, and its total amount estimated independently was verified.

PCB indicator marked with codes: 28, 52, 101, 118, 138, 153, and 180 were evaluated before, during, and after the methane digestion process, according to the procedure described, elsewhere [10], with the use of PCB separation from sludge with hexane and sonication as the extraction method. The extract was condensed in vacuum and then subject to qualitative and quantitative analysis by capillary gas chromatography. The separation was made by a DB-5 column (30 m × 32 mm × 1 μm). The quadrupole mass spectrometer MS 800 working in a selective mode of ion monitoring was used for detection. The analysis was conducted according to programmed temperature values: 40°C—40°C min^{-1}, 120°C—5°C min^{-1}, and 280°C—15 min. The carrier gas flow was set at 70 kPa. The quantification of PCBs was obtained through the single ion monitoring (SIM) m/z. For each PCB, three representative ions were selected. The determination of PCBs was performed for each sample and each four injections of the obtained extract.

3. Results and discussion

3.1. Physicochemical properties of sewage sludge

Values of physicochemical parameters of sludge during mesophilic digestion, and mesophilic digestion preceded by thermophilic hydrolysis are presented in Table 1.

It was demonstrated that the hydrolysis process of substrate conducted at temperature of 55°C, contributed to organic polymers liquefaction into substrate available for micro-organisms. It was confirmed by an increase in TOC content by the factor 7 in a liquid phase of initially hydrolyzed sludge $(P+E)_H$.

Concentration of TOC in liquid sludge $((P+E)_H+D)$ amounted to 1,630 mg L^{-1}. During digestion of sludge $((P+E)_H+\text{D})$ at temperature of 37°C, TOC content initially went up to 1,795 mg L^{-1}, and subsequently it went down to 612 mg L^{-1} on the 7th day of the process, which indicates rapid consumption of easily available organic substrate—Table 1. TOC content on the 15th day of digestion amounted to 452 mg L^{-1}. During processes of hydrolysis and digestion a decrease in organic substance concentration in sludge was obtained, which is equal to 41%. During digestion of non-hydrolyzed sludge $(P+E+D)$, 37% reduction of organic substances was obtained. Therefore, some difference in the process of digestion of initially hydrolyzed sludge and of non-hydrolyzed sludge was demonstrated in higher reduction of organic substance concentration in sludge.

During proceeded digestion, pH values of a liquid phase of sludge were within range of 7.84–7.94, which is acceptable for methane digestion [11]. The amount of produced VFA did not exceed the limit value of 2,000 mg $\text{CH}_3\text{COOH L}^{-1}$, which shows that digestion proceeded correctly.

3.2. Biogas

It was demonstrated that the amount of produced biogas was regularly increasing by significant volumes in the initial days of sludge $(P+E+D)$ digestion, and it amounted to 986 mL L^{-1} of sludge on the 5th day—Fig. 1.

During digestion of pre-hydrolyzed sludge $((P+E)_H+D)$, up until 3rd day of the process, almost stable amount of biogas production was observed—910–940 mL L^{-1} of sludge. The highest quantity was produced on the 4th day of the process—1,180 mL L^{-1}. The total biogas production over mesophilic digestion of sludge $(P+E+D)$ and $((P+E)_H+D)$ amounted to 6,185 and 7,260 mL, respectively. The difference in obtained amount of biogas, especially on the initial days of digestion was due to higher concentration of organic substrate in a liquid phase of initially hydrolyzed sludge. Unitary production of biogas amounted to

Table 1
Chosen physicochemical indexes of sludge before and during mesophilic (sludge $P + E + D$) and thermophilic–mesophilic (sludge $(P + E)_H + D$) digestion

	Digestion of sludge $P + E + D$					Digestion of sludge $(P + E)_H + D$				
Indexes	Before	3rd d	7th d	10th d	15th d	Before	3rd d	7th d	10th d	15th d
pH	7.88	7.91	7.94	7.92	7.88	7.84	7.92	7.90	7.88	7.86
Alkalinity (mgCaCO_3 L^{-1})	2,510	2,720	4,130	4,250	4,380	2,840	3,350	4,090	4,230	4,410
TOC (mg L^{-1})	583	870	533	467	390	1,630	1,795	612	530	452
VFA (mgCH_3COOH L^{-1})	565	930	394	282	256	1,250	1,990	343	274	225
Hydration (%)	96.82	97.11	97.41	97.60	97.71	97.03	97.31	97.46	97.58	97.68
Total solids (g L^{-1})	31.79	28.92	25.90	24.01	22.88	29.68	26.90	25.38	24.20	23.21
Volatile solids (g L^{-1})	21.11	18.39	15.59	14.02	13.22	19.44	16.92	15.01	13.90	13.102
Volatile/total solids (%)	66.4	63.6	60.2	58.4	57.8	65.5	62.9	59.1	57.4	56.4

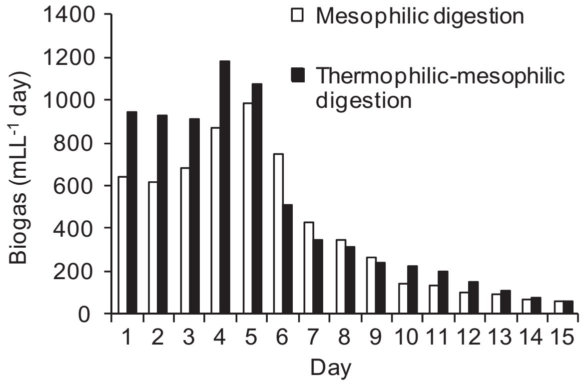

Fig. 1. Production of biogas during mesophilic and thermophilic–mesophilic digestion.

$1.15\,\mathrm{L\,g^{-1}}$ of removed dry organic matter of sludge $((P+E)_{\mathrm{H}}+D)$, and $0.78\,\mathrm{L\,g^{-1}}$ of removed dry organic matter of sludge $(P+E+D)$. Quantity of methane in biogas, apart from 1st day, during both digestions, maintained within the range of 61–65%.

3.3. PCB

In sludge $(P+E)$, before the thermophilic hydrolysis process, the presence of all indicator congeners was observed. Eighty-one percent accounted for higher chlorinated PCB (HCB) containing from 5 to 7 chlorine atoms in particle. After the thermophilic hydrolysis process, 55% decrease in aggregate concentration of PCB was obtained. The highest reduction was demonstrated for PCB 153 and 180, due to a decrease of approximately 70%.

The results of qualitative and quantitative changes of the indicator PCB in sludge $(P+E+D)$ and $((P+E)_{\mathrm{H}}+D)$ are presented in Table 2. The changes in PCB concentration in sludge during methane digestion were calculated based on conducted quality control of the results, and statistic analysis with the assumption that possible difference, in particular PCB congener concentration above 25%, was statistically significant.

In sludge $(P + E + D)$ and $((P + \mathrm{E})_{\mathrm{H}} + \mathrm{D})$ before the digestion process, the presence of all indicator PCB congeners was observed (Table 2). In sludge $(P + E + D)$, HCB concentration was higher that LCB (Fig. 2(A)). In sludge $((P + E)_{\mathrm{H}} + D)$, HCB also dominated (Fig. 2(B)), which was mainly caused by the presence of PCB marked with codes 153 and 118.

On the 7th day of the mesophilic digestion process some significant changes of PCB 118, 153, and 180 were appeared, whose concentrations went down from 31 to more than 50%. LCB content in sludge $(P + E + D)$ increased, whereas HCB contribution decreased. During mesophilic digestion preceded by thermophilic hydrolysis, on the 7th day of the process, 83% decrease of PCB 118 concentration was obtained. In sludge $((P + E)_{\mathrm{H}} + D)$ PCB 138 and 153, which represent HCB did not occur, whereas LCB (PCB 28 and 52) and PCB 101 content went up. Those changes resulted in rising LCB concentration, and significant HCB reduction whose content in sludge went down by 84%.

On the 10th day of mesophilic digestion PCB 52 concentration increased by 52%, while PCB 118, 153, and 180 content decreased by 47, 54, and 65%, respectively. With respect to data obtained before the process LCB concentration in sludge $(P + E + D)$ went up by 42%, HCB—went down by 37%. On that day of the mesophilic digestion process preceded by thermophilic hydrolysis, further decreasing of PCB concentration in sludge $((P + E)_{\mathrm{H}} + D)$ was stated. Some significant changes (with respect to data before the process) were observed for PCB 118 and PCB 180, whose concentration decreased by 91–94%. At the same time PCB 101 content went up by 52%. In comparison to data before process, LCB and HCB concentration went down by 25 and 86%, respectively.

After mesophilic digestion in sludge $(P + E + D)$ still all indicator of PCB congeners were present; however, their aggregated content decreased by 25%. After mesophilic digestion preceded by thermophilic hydrolysis in sludge $((P + E)_{\mathrm{H}} + D)$, the presence of only two PCB congeners, marked with codes 52 and 101 was observed (Table 2). Aggregated PCB concentration in sludge decreased by 93%. In sludge after mesophilic digestion, as before, elevated LCB concentration maintained (Fig. 2(A)). An increase in LCB by 42% and a decrease in HCB by 37% in comparison with the concentration of these compounds before digestion were demonstrated. In sludge $((P + E)_{\mathrm{H}} + D)$ after mesophilic digestion preceded by thermophilic hydrolysis, the concentration of LCB and HCB decreased (Fig. 2(B)) by 88 and 93%, respectively.

After mesophilic digestion preceded by thermophilic hydrolysis, the highest decrease in aggregate concentration of indicator PCB was demonstrated in comparison with results obtained during mesophilic digestion. Lowering of PCB content in sludge was due to complete biodegradation of congeners marked with codes: 28, 118, 138, 153, and 180. The obtained results suggest that thermophilic hydrolysis accelerated PCB transformations in sewage sludge. During mesophilic digestion, significant quantitative changes of LCB and HCB occurred on the 10th day, while during mesophilic digestion preceded by thermophilic hydrolysis those changes were observed on the 7th day of the process, when LCB content was higher than that of

Table 2
Concentration of PCBs (μg kg $^{-1}$ d m) in sludge before and during mesophilic (sludge $P + E + D$) and thermophilic–mesophilic (sludge $(P + E)_H + D$) digestion (mean ± standard deviation, $n = 5$)

	Day of process							
	Before		7th		10th		15th	
Congeners	$P + E + D$	$(P + E)_H + D$	$P + E + D$	$(P + E)_H+D$	$P + E + D$	$(P + E)_H + D$	$P + E + D$	$(P + E)_H + D$
PCB 28	6.4 ± 1.2	0.4 ± 0.1	7.3 ± 1.4	0.6 ± 0.1	9.6 ± 1.6	0.3 ± 0.1	6.1 ± 1.3	nd
PCB 52	4.5 ± 1.6	1.9 ± 0.4	6.8 ± 1.1	2.4 ± 0.2	9.4 ± 2.1	1.2 ± 0.3	6.3 ± 1.4	0.2 ± 0.1
PCB 101	4.6 ± 1.2	0.7 ± 0.3	5.1 ± 1.0	1.6 ± 0.5	4.4 ± 0.8	1.4 ± 0.3	2.9 ± 0.5	1.4 ± 0.3
PCB 118	3.5 ± 1.6	6.7 ± 1.5	2.4 ± 0.9	1.2 ± 0.4	1.9 ± 0.5	0.6 ± 0.4	1.9 ± 0.8	nd
PCB 138	3.3 ± 1.7	1.1 ± 0.4	3.0 ± 1.3	nd	2.4 ± 0.7	nd	2.2 ± 0.6	nd
PCB 153	4.8 ± 1.1	10.4 ±1.7	3.3 ± 0.8	nd	2.2 ± 0.7	nd	1.9 ± 0.5	nd
PCB 180	2.8 ± 1.4	0.5 ± 0.1	1.2 ± 0.7	0.2 ± 0.1	1.0 ± 0.4	0.2 ± 0.1	1.2 ± 0.6	nd
∑PCB	29.9	21.6	29.1	6.0	30.8	3.6	22.5	1.6

Note: nd—not detected.

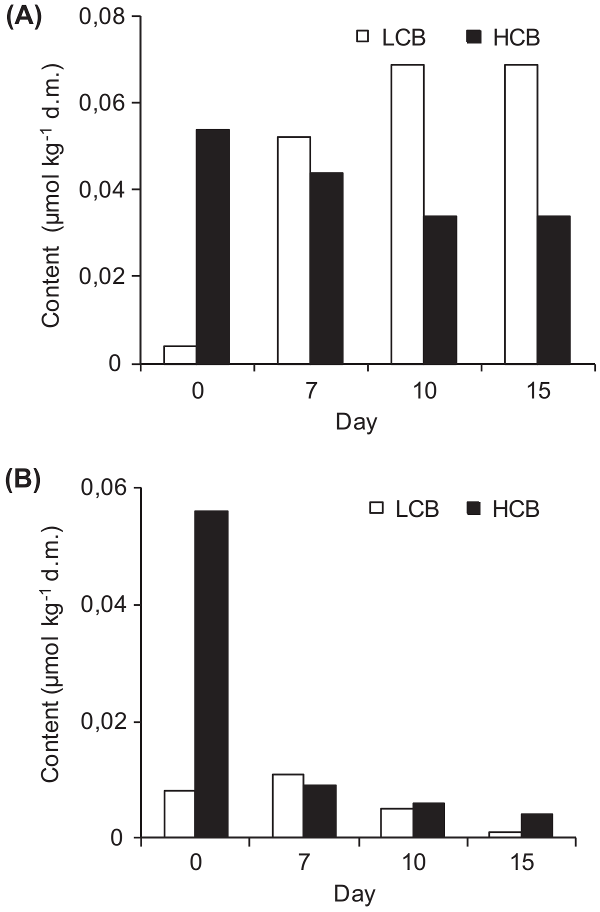

Fig. 2. LCB and HCB content before and during: (A) mesophilic digestion of sewage sludge ($P + E + D$); (B) thermophilic–mesophilic digestion of sewage sludge $(P + E)_H + D$.

HCB. Literature data confirm that after the end of mesophilic digestion process, a decrease in PCB concentration in sludge can be obtained [1,12,13]. In previously mentioned research, after anaerobic sludge stabilization process, authors obtained a decrease in PCB content by 40% [1], and by 47% [13].

Research results confirmed literature data regarding biodegradation of HCB to LCB in anaerobic conditions. However, it must be emphasized that the periodic increase in lower chlorinated PCB concentration not only due to biodegradation of HCB to LCB [1,12,14], but also their desorption, indicated by degradation of organic matter in the digestion process during which it is possible to obtain the elevated amount of PCB, which was previously adsorbed [15]. According to some literature references, HCB show high values of *n*-octanol/water partition coefficient K_{ow} and *n*-octanol/carbon K_{oc} partition coefficient [16]. Due to these properties PCBs with higher number of chlorine atoms in a molecule are strongly adsorbed by the organic fraction of sewage sludge *w* comparatively LCB. Research confirmed that in a liquid phase of sludge with increased PCB content, up until the 14th day of mesophilic digestion process, a gradual increase in amount and sorts of indicator PCB congeners was observed. LCB were released into sludge liquid first [17].

The low content of HCB in sludge after methane digestion can be explained by their capability to bioaccumulation, which the stronger is the more chlorine atoms are in biphenyl molecule. The results showed a significant decrease in PCB 180 concentration in sludge, which can be explained by, previously suggested in literature, biodegradation of heptachlorobiphenyl to tri- and tetrachlorobiphenyl [18,19].

3.4. Heavy metals

Over hydrolysis, zinc concentration in liquid sludge $(P + E)_{\text{H}}$ increased by the factor 5 in comparison with its initial content equal to $0.41\ \text{mg L}^{-1}$, whereas for copper—by the factor 4 (from approx. 0.06 up to $0.22\ \text{mg L}^{-1}$). In the case of other metals changes the concentration values were not that high. The concentration of nickel and chromium went up by the factor 1.8, lead—1.5.

During digestion of preliminary hydrolyzed sludge $((P + E)_{\text{H}} + D)$, it was determined that heavy metal concentration in a liquid phase of sludge was decreasing, zinc and chromium until 10th day, nickel, copper, and cadmium until 7th day of digestion (Table 3). During the further process, concentration remained at nearly constant level. In the case of zinc concentration amounted to approx. $0.8\ \text{mg L}^{-1}$, copper, nickel, and chromium approx. $0.12\ \text{mg L}^{-1}$, lead $0.08\ \text{mg L}^{-1}$, cadmium $0.02\ \text{mg L}^{-1}$. Within digestion of non-hydrolyzed sludge $(P + E + D)$, zinc and copper concentrations were slightly lower than in a liquid phase of hydrolyzed sludge, while other metal content was at a comparable level (Table 3).

An average content of examined heavy metals in chemical fractions of sludge $((P + E)_{\text{H}} + D)$ and $(P + E + D)$, before and after digestion are presented in Table 4. The aggregate amount of metal concentration in four analyzed fractions was equal to 91–108% of its total quantity in sludge determined without fractionation.

After sludge digestion, heavy metal concentration in exchangeable and carbonate fractions remained at the level comparable to values prior to digestion (in the case of nickel, lead, and cadmium), or it was slightly (copper and chromium) or highly (zinc) reduced. The highest increase in lead concentration, after stabilization both in $((P + E)_{\text{H}} + D)$ and $(P + E + D)$ sludge was obtained in the residue fraction. The lead content in this fraction amounted to 88–89% of its total quantity in stabilized sludge. This was confirmed by [20,21]. With respect to zinc, copper, nickel, cadmium, and chromium, their concentrations increased mainly in organic–sulfide fraction. Thereby after the digestion process, percentage proportion of this fraction in bonding of zinc, copper, nickel, cadmium, and chromium was, respectively, as follows: 71; 84; 46; 46; and 78% for $((P + E)_{\text{H}} + D)$ sludge. A similar percentage of metal content in this fraction was obtained after digestion of $(P + E + D)$ sludge—72; 84; 49; 43; and 81%. The presence of zinc, copper, and chromium, mainly in an organic–sulfide fraction was confirmed in analyses of other sludge stabilized by means of mesophilic digestion conducted by [20–22]. A dominant role of an organic–sulfide fraction in binding chromium was also demonstrated by [23]. While, in the case of cadmium [24] its occurrence was confirmed mainly in the residue fraction.

In an exchangeable-carbonate fraction, the highest content of nickel was confirmed (30–31%). With respect to other metals, zinc, copper, lead, cadmium, and chromium, their concentrations in this fraction amounted, respectively, to: 1.9; 0.2; 4.2; 10.3; and 0.6% for $(P + E + D)$ sludge, and 2.1; 0.3; 4.5; 9.2; and 0.7% for $((P + E)_{\text{H}} + D)$ sludge. High nickel concentration in an exchangeable-carbonate fraction of sludge stabilized by mesophilic digestion was stated by [23,25]. Nickel enrichment in all fractions during the digestion process was observed by [26].

After digestion of both $((P + E)_{\text{H}} + D)$ and $(P + E + D)$ sludge the following distribution of metals in the fraction was determined: Zn: organic/sulfides > reducible ~ residual > exchangeable/carbonates; Cu: organic/sulfides > residual > reducible ~ exchangeable/carbonates; Ni: organic/sulfides > exchangeable/carbonates > reducible ~ residual; Pb: residual > organic/sulfides ~ exchangeable/carbonates > reducible; Cd:

Table 3
Concentration of heavy metal ions (mg L^{-1}) in sludge liquid before and during mesophilic (sludge $P + E + D$) and thermophilic–mesophilic (sludge $(P + E)_{\text{H}} + D$) digestion

Metal	In liquid phase of sludge during digestion											
	$P + E + D$						$(P + \text{E})_{\text{H}} + \text{D}$					
	Before	1st d	3rd d	7th d	10th d	15th d	Before	1st d	3rd d	7th d	10th d	15th d
Zn	0.51	0.61	0.63	0.63	0.68	0.71	1.29	0.99	0.97	0.84	0.76	0.77
Cu	0.09	0.09	0.09	0.09	0.10	0.11	0.16	0.14	0.13	0.12	0.12	0.12
Ni	0.16	0.14	0.13	0.13	0.12	0.12	0.23	0.15	0.14	0.12	0.12	0.12
Pb	0.08	0.08	0.09	0.08	0.09	0.08	0.10	0.09	0.09	0.08	0.08	0.08
Cd	0.03	0.03	0.03	0.03	0.02	0.02	0.04	0.03	0.03	0.02	0.02	0.02
Cr	0.21	0.22	0.17	0.18	0.15	0.13	0.25	0.22	0.21	0.16	0.12	0.12

Table 4
Chemical fractionation of heavy metals (mg kg^{-1} d m) in sludge before and after mesophilic and thermophilic–mesophilic digestion (mean ± standard deviation, $n = 5$)

		Sewage sludge			
		Before digestion		After digestion	
Metal	Fraction	$P + E + D$	$(P + E)_H + D$	$P + E + D$	$(P + E)_H + D$
Zn	F1	385 ± 8	206 ± 4	65 ± 4	67 ± 2
	F2	555 ± 11	596 ± 6	476 ± 7	454 ± 6
	F3	1,436 ± 17	1,516 ± 19	2,498 ± 12	2,208 ± 16
	F4	211 ± 4	96 ± 4	410 ± 6	390 ± 14
	Sum	2,587	2,414	3,449	3,119
Cu	F1	6.2 ± 0.2	5.8 ± 0.4	0.8 ± 0.2	1.2 ± 0.1
	F2	2.7 ± 0.3	1.6 ± 0.1	0.7 ± 0.1	0.8 ± 0.1
	F3	246 ± 3.0	250 ± 1.6	301 ± 3.6	316 ± 2.2
	F4	41.2 ± 1.7	37.4 ± 0.4	55.0 ± 0.9	56.9 ± 0.3
	Sum	296.1	294.8	357.5	374.9
Ni	F1	50.1 ± 0.8	46.3 ± 0.2	51.2 ± 0.4	54.5 ± 0.9
	F2	16.3 ± 1.1	14.6 ± 0.4	18.9 ± 0.6	22.5 ± 0.1
	F3	52.0 ± 1.8	50.9 ± 1.1	84.5 ± 1.5	81.8 ± 0.5
	F4	13.5 ± 0.5	10.8 ± 0.2	18.4 ± 0.7	17.7 ± 0.3
	Sum	131.9	122.6	173.0	176.5
Pb	F1	4.0 ± 0.2	3.9 ± 0.5	3.8 ± 0.3	4.1 ± 0.3
	F2	3.6 ± 0.2	3.6 ± 0.5	2.3 ± 0.2	2.7 ± 0.2
	F3	4.3 ± 0.8	5.0 ± 0.4	4.2 ± 0.3	4.2 ± 0.2
	F4	55.1 ± 2.5	54.9 ± 1.4	80.7 ± 2.5	80.1 ± 0.6
Cd	F1	0.31 ± 0.05	0.49 ± 0.07	0.60 ± 0.06	0.46 ± 0.05
	F2	0.82 ± 0.07	0.97 ± 0.04	1.20 ± 0.05	0.72 ± 0.06
	F3	1.58 ± 0.11	1.45 ± 0.12	2.49 ± 0.14	2.33 ± 0.18
	F4	0.95 ± 0.10	1.11 ± 0.10	1.56 ± 0.10	1.52 ± 0.06
	Sum	3.66	4.02	5.85	5.03
Cr	F1	7.9 ± 1.1	3.5 ± 0.3	2.4 ± 0.7	3.1 ± 0.3
	F2	2.9 ± 0.7	2.5 ± 0.3	2.2 ± 0.2	2.0 ± 0.3
	F3	263 ± 8	287 ± 3	336 ± 8	326 ± 7
	F4	45.2 ± 2.5	26.5 ± 0.4	76.2 ± 3.6	88.2 ± 1.1
	Sum	319.0	319.5	416.8	419.3

Notes: Fraction: F1—exchangeable/carbonates, F2—Fe/Mn oxides, F3—organic matter/sulfides, and F4—residual.

organic/sulfides > residual > reducible > exchangeable/carbonates; and Cr: organic/sulfides > residual > exchangeable/carbonates ~ reducible.

The residue fraction is considered to be chemically stable and biologically inactive. It contains metals incorporated in crystal lattice of primary and secondary minerals which constitute sludge. It consists mostly of metals of silicate and aluminum silicate minerals. In natural conditions, metals in this fraction can be considered as permanently immobilized. As more mobile, therefore, they easily pass into soil solution and are collected by plants. They are considered exchangeable and bounded to carbonates metal forms. The digestion process, for both pre-hydrolyzed and non-hydrolyzed sludge, did not result in an increase in the metal content in this mobile fraction.

4. Conclusions

Based on the conducted experiment the following conclusions can be drawn:

(1) Subjecting the sludge to temperature of 55˚C, accelerated hydrolysis of organic substrate, which enabled obtaining higher biogas amount during mesophilic digestion, in comparison with digestion of non-hydrolyzed sludge (1.15 L and 0.78 L g^{-1} of removed dry organic matter of sludge, respectively).

(2) Sludge hydrolysis accelerated transitions of PCB during mesophilic methane digestion. Twenty-five percent reduction of the indicator PCB content after mesophilic digestion was

obtained, whereas after digestion preceded by thermophilic hydrolysis—93% decrease in PCB concentration.

(3) During the methane digestion process, some intensive quantitative changes of higher and lower chlorinated PCB congeners were demonstrated. LCB were dominant in fermented sludge.

(4) Proceeding sludge digestion promoted creation of stable chemical forms of heavy metals. Regardless of the applied stabilization process, the highest increase in zinc, copper, nickel, cadmium, and chromium content was demonstrated in an organic-sulfide fraction, whereas for lead—in the residue fraction.

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References

[1] D. Patureau, E. Trably, Impact of anaerobic and aerobic processes on polychlorobiphenyl removal in contaminated sewage sludge, Biodegradation 17 (2006) 9–17.

[2] J. Borja, J.A. Taleon, J. Auresenia, S. Gallardo, Polychlorinated biphenyls and their biodegradation, Process Biochem. 40 (2005) 1999–2013.

[3] F. Fava, G. Zanaroli, L.Y. Young, Microbial reductive dechlorination of pre-existing PCBs and spiked 2,3,4,5,6-pentachlorobiphenyl in anaerobic slurries of a contaminated sediment of Venice Lagoon (Italy), FEMS Microbiol. Ecol. 44 (2003) 309–318.

[4] E.R. Master, V.W. Lai, B. Kuipers, W.R. Cullen, W.W. Mohn, Sequential anaerobic–aerobic treatment of soil contaminated by weathered Aroclor 1260, Environ. Sci. Technol. 36 (2002) 100–103.

[5] H. Ge, P.D. Jensen, D.J. Batstone, Pre-treatment mechanisms during thermophilic-mesophilic temperature phased anaerobic digestion of primary sludge, Water Res. 44 (2010) 123–130.

[6] C.R.M. Rao, A. Sahuquillo, J.F. Lopez Sanchez, A review of the different methods applied in environmental geochemistry for single and sequential extraction of trace elements in soils and related materials, Water Air Soil Poll. 189 (2008) 291–333.

[7] P.C. Nagajyoti, K.D. Lee, T.V.M. Sreekanth, Heavy metals, occurrence and toxicity for plants: A review, Environ. Chem. Lett. 8 (2010) 199–216.

[8] L.S. Clesceri, A.E. Greenberg, A.D. Eaton (Eds.), Standard Methods for the Examination of Water and Wastewater, twentieth ed., American Public Health Association, Washington, DC, 1998.

[9] G. Rauret, J.F. Lopez-Sanchez, A. Sahuquillo, E. Barahona, M. Lachica, A.M. Ure, C.M. Davidson, A. Gomez, D. Lück, J. Bacon, M. Yli-Halla, H. Muntau, Ph. Quevauviller, Application of a modified BCR sequential extracion (three-step) procedure for the determination of extractable trace metal contents in a sewage sludge amended soil reference material (CRM 483), complemented by a three-year stability study of acetic acid and EDTA extractable metal content, J. Environ. Monitor. 2 (2000) 228–233.

[10] L. Dąbrowska, A. Rosińska, Change of PCBs and forms of heavy metals in sewage sludge during thermophilic anaerobic digestion, Chemosphere 88 (2012) 168–173.

[11] L. Appels, J. Baeyens, J. Degreve, R. Dewil, Principles and potential of the anaerobic digestion of waste-activated sludge, Prog. Energy Combust. Sci. 34 (2008) 755–781.

[12] T. Benabdallah El-Hadj, J. Dosta, R. Torres, J. Mata-Alvarez, PCB and AOX removal in mesophilic and thermophilic sewage sludge digestion, Biochem. Eng. J. 36 (2007) 281–287.

[13] L. Bertin, S. Capodicasa, S. Fedi, D. Zannoni, L. Marchetti, F. Fava, Biotransformation of highly chlorinated PCB mixture in an activated sludge collected from a membrane biological reactor (MBR) subjected to anaerobic digestion, J. Hazard. Mater. 186 (2011) 2060–2067.

[14] J. Wiegel, Q. Wu, Microbial reductive dehalogenation of polychlorinated biphenyls, FEMS Microbiol. Ecol. 32 (2000) 1–15.

[15] M.K. Durjava, T.L. Laak, J.L.M. Hermens, J. Struijs, Distribution of PAHs and PCBs to dissolved organic matter: High distribution coefficients with consequences for environment al fate modeling, Chemosphere 67 (2007) 990–997.

[16] C.C. Hung, G.C. Gong, K.T. Jiann, K.M. Yeager, P.H. Santschi, T.L. Wade, J.L. Sericano, H.L. Hsieh, Relationship between carbonaceous materials and polychlorinated biphenyls (PCBs) in the sediments of Danshui river and adjacent coastal areas Taiwan, Chemosphere 65 (2006) 1452–1461.

[17] A. Rosińska, L. Dąbrowska, Sewage sludge digestion at increased micropollutant content, Chem. Eng. Res. Des. 92 (2014) 752–757.

[18] M. Barret, H. Carrère, L. Delgadillo, D. Patureau, PAH fate during the anaerobic digestion of contaminated sludge: Do bioavailability and/or cometabolism limit their biodegradation? Water Res. 44 (2010) 3797–3806.

[19] H. Nollet, I. Van de Putte, L. Raskin, W. Verstraete, Carbon/electron source dependence of polychlorinated biphenyls dechlorination pathways for anaerobic granules, Chemosphere 58 (2005) 299–310.

[20] E. Alonso Álvarez, M. Callejon Mochon, J.C. Jimenez Sanchez, M. Ternero Rodriguez, Heavy metal extractable forms in sewage sludge from wastewater treatment plants, Chemosphere 47 (2002) 765–775.

[21] A. Fuentes, M. Lloréns, J. Sáez, M.I. Isabel Aguilar, J.F. Ortuño, V.F. Meseguer, Comparative study of six different sludges by sequential speciation of heavy metals, Bioresource Technol. 99 (2008) 517–525.

[22] A. Pathak, M.G. Dastidar, T.R. Sreekrishnan, Bioleaching of heavy metals from sewage sludge by indigenous iron-oxidizing microorganisms using ammonium ferrous sulfate and ferrous sulfate as energy sources: A comparative study, J. Hazard. Mater. 171 (2009) 273–278.

[23] M.K. Jamali, T.G. Kazi, M.B. Arain, H. Afridi, N. Jalbani, G.A. Kandhro, A.Q. Shah, J.A. Baig, Speciation of heavy metals in untreated sewage sludge by using microwave assisted sequential extration procedure, J. Hazard. Mater. 63 (2009) 1157–1164.

[24] Özge Hanay, H. Hasar, N.N. Kocer, S. Aslan, Evaluation for agricultural usage with speciation of heavy metals in a municipal sewage sludge, B. Environ. Contam. Tox. 81 (2008) 42–46.

[25] I. Walter, F. Martinez, V. Cala, Heavy metal speciation and phytotoxic effects of three representative sewage sludges for agricultural uses, Environ. Pollut. 139 (2006) 507–514.

[26] A.A. Zorpas, V.J. Inglezakis, M. Loizidou, Heavy metals fractionation before, during and after composting of sewage sludge with natural zeolite, Waste Manage. 28 (2008) 2054–2060.