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Changes in selected dioxin-like PCB concentration and toxicity in anaerobically stabilized sewage sludge

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ABSTRACT

The process of methane digestion was conducted in two parallel cultures with different sewage sludge. The digestion was conducted three times: the thermophilic one (37°C) for 14 d, the mesophilic one at temperature of 36°C for 15 d and the mesophilic one for 14 d, with a preceding five-d thermophilic hydrolysis. The content of three most detrimental dl-PCB with codes: 77 (3,3´,4,4´-tetrachlorobiphenyl), 126 (3,3´,4,4´,5-pentachlorobiphenyl), and 169 (3,3´,4,4´,5,5´-hexachlorobiphenyl). Changes in toxicity, caused by the presence of coplanar polychlorinated biphenyls (PCB) in sewage sludge, during digestion were evaluated by means of TEQ_{PCB} equivalent. Research results showed that conducted anaerobic stabilization processes of sewage sludge proceeded correctly. It was demonstrated that methane digestion process contributes to some decrease in PCB concentration and in toxicity due to the presence of coplanar PCB in sewage sludge. Intensification of mesophilic digestion by means of thermophilic hydrolysis contributed to some decrease in dl-PCB concentration and toxicity equivalent. The highest reduction in sludge toxicity was obtained during thermophilic digestion. After the process, reduction in WHO_{98}-TEQ_{PCB} equivalent value in sewage sludge by 98% on average was demonstrated.

Keywords: Anaerobic digestion; Coplanar polychlorinated biphenyls; Sewage sludge; Toxic equivalent (TEQ)

1. Introduction

In municipal wastewater treatment plants, stabilization and final neutralization of sewage sludge pose a problem. The amount of sewage sludge which requires treatment is estimated at 1–2% of the sewage flow volume. In the last decade, the amount of sewage sludge increased due to massive implementation of technological solutions based on integrated removal of carbon, nitrogen, and phosphorus compounds [1]. Projections for the nearest future, namely for the year 2020, predict this rising trend to maintain and the production of 13,100000 tons of sewage sludge dry matter in EU countries, including 731,000 tons of dry matter in Poland. Taking that problem into consider-

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ation, it is crucial to employ some technological solutions in order to ensure that the future management of sewage sludge will be completely safe and made in accordance with environmental protection requirements. Direct utilization of sewage sludge in agriculture is limited due to both sanitary considerations (high number of pathogenic bacteria and parasite eggs), and also the occurrence of number of toxic substances, including polychlorinated biphenyls (PCB) [2]. There are many methods of sewage sludge treatment. The most common techniques are aerobic and anaerobic stabilization. The initial stage of sewage sludge treatment is stabilization and hygienization. During hygienization, most of the pathogens and parasite eggs are destroyed. However, toxic substances, such as heavy metals [3], polycyclic aromatic hydrocarbons, or PCB, remain a problem [4]. Therefore, agricultural utilization of stabilized sewage sludge is limited by content of particular contaminants. This fact forces research on intensification of sewage sludge treatment methods aiming at neutralization and removal of toxic substances from sewage sludge. In order to accelerate PCB decomposition, anaerobic sewage sludge stabilization is suggested. During this process, PCB transformation proceeds leading to a decrease in its content and toxicity in sludge [5].

In sewage sludge, PCB comes from sewage flowing into wastewater treatment plant, dry and wet deposition from atmosphere, and due to other processes occurring during wastewater treatment. Current legal regulations allow using sewage sludge in farming only if concentrations of heavy metals contained in the sludge do not exceed the permissible level. In 2000, the member states of the European Union passed a new directive called "The Sludge Directive", which, amongst other things, stipulates the permissible level of the total concentration of seven congeners of PCBs marked with codes 28, 52, 101, 118, 138, 153, and 180 in sewage sludge used for agricultural purposes [6]. This Directive does not contain the most detrimental PCB. This group of compounds includes 14 PCB congeners, with 4 coplanar, 8 mono-ortho substituted, and 2 di-ortho PCB. With regard to biochemical activity, previously listed congeners are described as, so called, dioxin-like PCB, abbrev. Dl-PCB. They are similar to dioxins as far as their harmful effect is concerned. Coplanar PCB are analogs of carcinogenic, which is a very strong toxin—2,3,7,8,-tetrachlorodibenzo-p-dioxin (TCDD). Out of all PCBs, three of them: PCB 77, 126, and 169 are the most toxic and biochemically active. The total amount of dioxins and dl-PCB is expressed by means of toxic equivalent (TEQ), which is the total amount of individual congener concentration multiplied by respective toxicity equivalency factor (TEF) [7,8]. TEQ value indicates the degree of toxicity of a particular compound compared to the most toxic human congener, which is TCDD with TEF value of 1. In 1998, WHO introduced a range of TEF values, which was updated in 2005. The toxicity equivalent WHO-TEQ is calculated based on this range [9–12].

The aim of this research was to evaluate the influence of anaerobic stabilization, carried out under thermophilic and mesophilic conditions, on changes in concentrations of three most detrimental dl-PCB with codes: 77, 126, and 169. The changes in toxicity caused by the presence of coplanar PCB in sewage sludge were also analyzed based on WHO_{98}-TEQ_{PCB} equivalent. The conducted research will allow to clarify whether implementation of advanced sewage sludge treatment method (mesophilic digestion preceded by thermophilic hydrolysis) will contribute to minimization of toxic PCB concentration.

2. Materials and methods

2.1. Materials

Sewage sludge was sampled from municipal and mechanical–biological treatment plant of communal wastewater, 20% of which was industrial wastewaters. The treatment process was performed with the use of the activated sludge method, which takes into account nitrification, denitrification, and chemical/biological removal of phosphorus.

2.2. Methane digestion

Methane digestion process was conducted in two parallel cultures with different sewage sludge. Prior to the process, in order to obtain homogenous material, sludge was put through 3-mm mesh sieve. In samples marked with symbol (P + E + F), a mixture of primary and excess sludge (P + E) inoculated with digestion sludge (F) in volumetric ratio of 1:2 was incubated. In samples marked with symbol (P + F), primary sludge (P) was inoculated with digestion sludge (F), also in volumetric ratio of 1:2 [13].

For samples previously prepared in this way, each digestion was conducted three times:

(1) thermophilic; as an inoculum for thermophilic digestion process, micro-organisms which inhabit mesophilic (37˚C) fermentation sludge were used. Inoculum for thermophilic culture was prepared according to European Standard [14], and with the use of Ortega et al. [15]

research experience. Then, the thermophilic digestion process was conducted in bioreactors with volume of 1,5 dm^3, at a temperature of 55 ± 1℃, for 14 d.;
(2) mesophilic at a temperature of 36 ± 1℃ for 15 d; and
(3) mesophilic for 14 d, with previous five-day thermophilic hydrolysis.

The control of the process consisted in manometric measurement of the amount of biogas and, at defined time intervals, analysis of composition of biogas. Before and in the specified intervals for the sludge, the following parameters were determined: humidity, total solids, volatile and non-volatile solids, pH, alkalinity, volatile fatty acids VFA, and total organic carbon TOC. Before, during (on 3rd, 7th, and 10th day), as well as after methane digestion process, the dl-PCBs of the sludge were determined.

2.3. Analytical methods

The content of three coplanar PCB: 3,3´,4,4´-tetrachlorobiphenyl (PCB 77), 3,3´,4,4´,5-pentachlorobiphenyl (PCB 126), and 3,3´,4,4´,5,5´-hexachlorobiphenyl (PCB 169) was detected in sludge. Ten grams sample of sludge was placed in a tube and 5 mL of 2 M KOH in methanol was poured in. The tube was hermetically closed and heated in water bath at temperature 70℃ for 2 h. Next, the sample was extracted with 5 mL of hexane:methanol mixture (1:1, v/v). Nonpolar phase was concentrated to the volume of 1 mL and purified on preconditioned BAKERBOND spe SiO_2/Al_2O_3 columns. The first fraction of PCBs was washed from column sorbent with hexane. The obtained solution was treated by passing through preconditioned BAKERBOND spe Florosil 500 columns. Next, *ortho*-PCBs (fraction II) were washed from sorbent with hexane and coplanar PCBs (fraction III) with hexane: dichloromethane (9:1, v/v) mixture. Extracts were concentrated to the volume of 1 mL using BAKER SPE-12G vacuum pump. The extract was compacted in a vacuum and then subjected to qualitative and quantitative analysis by means of gas chromatography (CGC). Separation was made on a DB-5 column (30 m x 32 mm x 1 mm). A quadrupole mass spectrometer, MS 800, working in a selective mode of ion monitoring was used for detection. Determination was performed for each sample and for each of the four injections of the obtained extract.

In order to check the procedure for determination of PCBs in sewage sludge, the recovery values were also determined. Sewage sludge was enriched with Ehrenstorfer's PCB MIX 13 (PCB77, PCB 126, and PCB169) at the concentration of 10 μg/mL. Then, the determination of biphenyls was carried out using the above-mentioned procedure. The recovery rate for particular congeners amounted to 65% (PCB 77), 73% (PCB 126), and 85% (PCB 169), and was within the range typical of the references [16]. The precision of the determination was expressed by means of the values of standard deviation.

3. Results and discussion

Research results showed that conducted anaerobic stabilization processes of sewage sludge proceeded correctly. During the process of mesophilic and thermophilic methane digestion, recommended temperature was maintained, at 36 and 55℃ accordingly, and pH value ranged from 6.8 to 7.4. The amount of volatile fatty acids (FVA) produced during digestion process (1071–1189 mg CH_3COOH/L) did not exceed the limit value of 2000 mg CH_3COOH/L, furthermore, the quotient of VFA and alkalinity was lower than 0.3, which confirmed that digestion processes had proceeded without any disturbance.

Changes in toxicity, caused by presence of coplanar PCB in sewage sludge during digestion, were evaluated by means of TEQ_{PCB} equivalent. TEQ_{PCB} parameter was calculated according to following formula:

$$TEQ = \sum (m_i \, TEF_i) \quad (1)$$

where m_i—weight of single congener and TEF_i—equivalent toxicity coefficient, according to WHO recommendation from 1998, for i-th PCB congener, with regard to TCDD.

The presence and concentration of PCB 126 have a decisive impact on the value of PCB toxicity equivalent. This is due to the fact that TEQ_{PCB} equivalent value is calculated as total of PCB 77, PCB 126, and PCB 169 concentration, multiplied by standard TEF factors. The highest TEF value (0.1) is given for PCB 126, whereas for PCB 169 and PCB 77, TEF value is, respectively, 10 and 100 times lower [7,11].

Changes of coplanar PCB concentration in sewage sludge during digestion are presented in Tables 1 and 2.

Prior to thermophilic digestion, PCB 169 was dominant in sludge (P + E + F), and its concentration amounted to 23.5 ng/g dm PCB 126 concentration was over twofold lower (Table 1).

Table 1
Changes in coplanar PCBs concentration in (P + E + F) sewage sludge during methane digestion, ng/g dm

Congener	Thermophilic digestion (d)					Mesophilic digestion (d)					Mesophilic digestion with thermophilic hydrolysis stage (d)			
	Before	3rd	7th	10th	14th	Before	3rd	7th	10th	15th	Before	7th	10th	15th
PCB 77	1.4 ± 0.5	1.5 ± 0.6	1.3 ± 0.5	2.1 ± 0.7	0.9 ± 0.4	1.8 ± 0.5	1.8 ± 0.6	2.4 ± 0.7	4.3 ± 0.6	2.5 ± 0.7	1.0 ± 0.3	2.1 ± 0.6	3.5 ± 0.7	4.9 ± 1.5
PCB 126	9.9 ± 1.4	10.4 ± 1.6	1.0 ± 0.3	0.5 ± 0.2	0.4 ± 0.1	11.2 ± 1.1	10.8 ± 1.00	14.5 ± 1.3	10.2 ± 1.2	6.6 ± 1.0	7.6 ± 0.9	6.9 ± 0.9	6.1 ± 1.3	5.9 ± 1.3
PCB 169	23.5 ± 1.8	23.2 ± 1.8	1.6 ± 0.5	1.2 ± 0.6	0.9 ± 0.6	23.4 ± 1.7	23.1 ± 1.8	19.5 ± 1.9	10.3 ± 2.0	5.1 ± 1.3	14.6 ± 2.3	14.0 ± 2.3	14.3 ± 3.0	nd[a]
∑PCB	34.8	35.1	3.9	3.8	2.2	36.4	35.7	36.4	24.8	14.2	23.2	23.0	23.9	10.8

[a]nd—not detected.

Table 2
Changes in coplanar PCBs concentration in (P + F) sewage sludge during methane digestion, ng/g dm

Congener	Termophilic digestion (d)					Mesophilic digestion (d)					Mesophilic digestion with thermophilic hydrolysis stage (d)			
	Before	3rd	7th	10th	14th	Before	3rd	7th	10th	15th	Before	7th	10th	15th
PCB 77	1.3 ± 0.5	1.4 ± 0.5	1.3 ± 0.7	1.9 ± 0.8	nd	1.6 ± 0.5	1.6 ± 0.6	2.2 ± 0.9	4.7 ± 0.9	2.8 ± 0.9	0.9 ± 0.2	3.8 ± 0.3	3.4 ± 0.3	nd
PCB 126	11.9 ± 1.9	11.4 ± 1.6	0.8 ± 0.2	0.5 ± 0.1	0.3 ± 0.1	4.7 ± 1.6	5.6 ± 0.9	4.4 ± 0.8	7.2 ± 1.3	2.7 ± 0.8	7.0 ± 1.3	5.7 ± 1.7	2.7 ± 0.4	0.6 ± 0.3
PCB 169	8.0 ± 1.7	7.7 ± 1.3	4.4 ± 1.7	2.7 ± 1.0	nd	8.2 ± 2.6	7.0 ± 1.6	5.6 ± 1.7	3.6 ± 1.6	1.6 ± 0.7	13.3 ± 2.4	10.7 ± 2.7	nd	nd
∑PCB	21.2	20.5	6.5	5.1	0.3	14.5	14.2	12.2	15.5	7.1	21.2	20.2	6.1	0.6

The lowest concentration was demonstrated for PCB 77. In sludge (P + F), the highest concentration determined for PCB was 126 (11.9 ng/g dm) (Table 2). Toxicity level in both sludges did not exceed the value of 1.3 ng/g dm (Figs. 1 and 2). During the 3rd day of the process, changes of coplanar congener concentration in both sludges were not statistically relevant. Value of toxicity equivalent WHO_{98}-TEQ_{PCB} was comparable to prior process data. On the 7th day of digestion, a significant decrease in PCB 126 and PCB 169 concentration in sludge (P + E + F) was obtained, by 90 and 93%, respectively. PCB 77 concentration in sludge was maintained at the same level as it was before the digestion process. Reduction of PCB 126 and 169 concentrations resulted in decrease in toxicity equivalent by 91%to 0.11 ng/g dm. In sludge (P + F), the biggest changes occurred for the most toxic PCB 126, whose concentration decreased by 93%. PCB 169 concentration was lowered by 45%. Reduction in PCB 126 and 169 concentrations caused a significant decrease in value of toxicity equivalent, down to level of 0.12 ng/g dm. On the 10th day of digestion, PCB 126 and 169 concentrations in both sludges decreased further. This caused successive reduction in WHO_{98}-TEQ_{PCB} equivalent value. In sludge, a 30% increase in PCB 77 content was demonstrated, in comparison with the results obtained before the digestion process. After completion of the process, differences between initial and final concentration of coplanar PCB in sludge were statistically significant. The highest decrease in concentration was demonstrated for PCB 126 and 169. Their concentration in sludge (P + E + F) was reduced by 96%, whereas PCB 77 concentration was decreased by 40%. Those changes generated crucial reduction in sludge toxicity level by 97%. Value of WHO_{98}-TEQ_{PCB} equivalent for sludge (P + E + F) after digestion process amounted to 0.04 ng/g dm (Fig. 1). In sludge (P + F), after digestion no presence of congeners 77 and 169 was demonstrated. Concentration of the most toxic PCB 126 was reduced by 97%. A decrease in toxicity of sludge (P + F) by 98% was obtained. WHO_{98}-TEQ_{PCB} equivalent value for sludge (P + F) after the process amounted to 0.03 ng/g dm (Fig. 2).

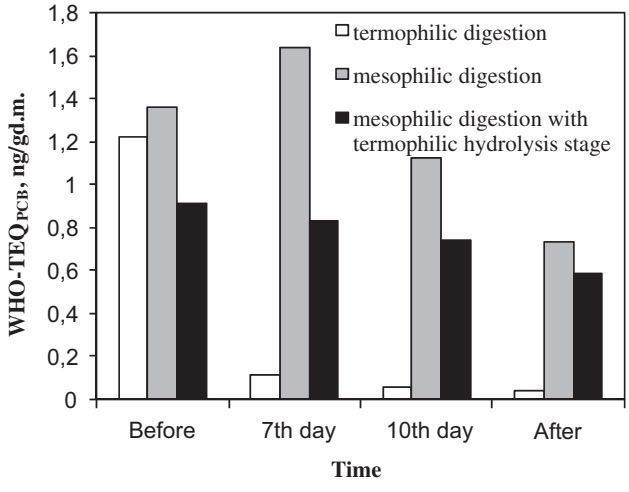

Fig. 1. Changes in value of WHO_{98}-TEQ_{PCB} during methane digestion of sewage sludge (P + E + F).

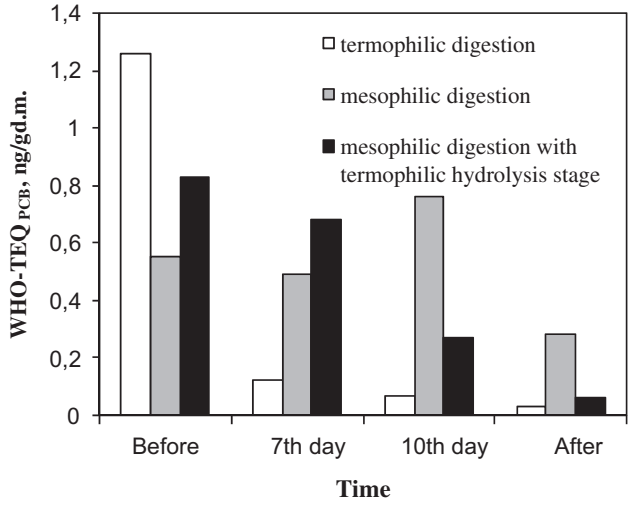

Fig. 2. Changes in value of WHO_{98}-TEQ_{PCB} during methane digestion of sewage sludge (P + F).

Typical value of *t*-Student distribution was assumed depending on PCB analysis performed in parallel. The number defining a latitude rate for results executed in three repetitions amounted to 4. Values lower than critical value $t_{0.05} = 2.776$ indicated that the change in average concentration of particular PCB, before and after the digestion, is not statistically significant. Remaining average values differed from each other in a statistically substantial way. In sludge after digestion *t*-Student value, marked for measurements between an average initial PCB content and the one after 14 d of process, was lower than 2.776.

Prior to mesophilic digestion process, in examined sludge (P + E + F) and (P + F), PCB 169 was dominant, and its concentration amounted to 23.4 and 8.2 ng/g dm, respectively. The lowest concentration was determined for congener marked with code 77. In WHO_{98}-TEQ_{PCB}, TEQ value amounted to 1.36 and 0.55 ng/g dm, respectively (Figs. 1 and 2). On the 3rd day of process, in both the sludges, no significant variation in coplanar congener concentration was found, and as a result the value of WHO_{98}-TEQ_{PCB} equivalent was comparable to the level prior to the process. On the 7th day of process, in sludge (P + E + F) an increase in

PCB 126 concentration by 23% was observed, which caused sludge toxicity level to be the highest on that day, and it amounted to 1.64 ng/g dm (Table 1). In sludge (P + F), a decrease in PCB 169 concentration by 32% was stated. Simultaneously, concentration of PCB 77 went up by 29%. PCB 126 concentration was comparable to initial concentration. The toxicity level of sludge (P + F) was amounted to 0.49 ng/g dm (Fig. 2). On the 10th day of digestion, PCB 169 concentration in sludge (P + E + F) was reduced by 56%, which caused a decrease in toxicity level down to 1.12 ng/g dm. At the same time, PCB 77 concentration increased by over 58% in comparison with the initial value. In sludge (P + F), as well as in sludge (P + E + F), PCB 169 concentration was decreased by 56%, whereas PCB 126 content went up by 35%, which affected a rise in sludge toxicity up to 0.76 ng/g dm. After digestion, coplanar PCB 126 and 169 concentrations in sludge fell down. In sludge (P + E + F), PCB 169 concentration decreased by 78% with respect to data obtained prior to the process. The toxicity level was reduced by 46% to 0.73 ng/g dm (Fig. 1). In sludge (P + F), the highest reduction, by 81%, was observed also for PCB 169, whereas PCB 126 concentration decreased by 43%. As a result of those variations, toxicity in sludge (P + F) decreased by 49% to 0.28 ng/g dm. In both sludge, differences between initial and final concentration of coplanar PCB were statistically significant. In sludge (P + E + F) t-Student value, marked for measurements between an average initial PCB 126 content and the content after 15 d of process, was lower than 2.776, which is the limit value accepted according to t-Student test.

Before mesophilic digestion process was preceded by thermophilic hydrolysis in sludge (P + E + F) and (P + F), the highest concentration was shown for PCB 169, which amounted to 14.6 and 13.3 ng/g dm, respectively (Tables 1 and 2). PCB 126 concentration in both sludges was twofold lower. Even lower concentration was obtained for PCB 77. WHO_{98}-TEQ_{PCB} sludge toxicity equivalent did not exceed 1 ng/g dm. On the 7th day of the process, in sludge (P + E + F), no statistically significant variation in coplanar congener concentration was observed, consequently, WHO_{98}-TEQ_{PCB} value was comparable to prior process data (Fig. 1). In sludge (P + F), over fourfold increase in PCB 77 concentration was observed (Table 2). The toxicity equivalent went down to 0.68 ng/g dm (Fig. 2). On the 10th day of digestion in sludge (P + E + F), variations in PCB 126 and PCB 169 concentrations were not statistically significant. However, an increase caused by factor 3 in PCB 77 concentration was stated. This did not result in any increase in toxicity caused by coplanar PCB presence in sludge, as TEF factor for this congener is the lowest—0.0001. In sludge (P + F), a decrease in PCB 126 concentration by 61% was demonstrated. PCB 77 content was comparable to data obtained on the 7th day of the process. PCB 169 content was not detected in sludge. With respect to the results prior to the process, the toxicity equivalent went down by 67% to 0.27 ng/g dm. After mesophilic digestion process was preceded by hydrolysis, in sludge (P + E + F), a further increase in PCB 77 was shown, while the presence of PCB 169 was not observed. Toxicity equivalent value decreased by 35% to 0.59 ng/g dm. In sludge (P + F), presence of PCB 77 and 169 was not detected. PCB 126 concentration was reduced by 91%. A decrease in WHO_{98}-TEQ_{PCB} toxicity equivalent by 93% was stated, which amounted to 0.06 ng/g dm after the process.

Under anaerobic conditions, micro-organisms cause elimination of chlorine atoms from higher chlorinated PCB, with the production of lower chlorinated biphenyls [17], which explains a recurring increase in PCB 77 concentration (3,3,4,4-tertrachlorobiphenyl) during methane digestion. Particularly, subject to anaerobic biodegradation are compounds with chlorine atoms in meta and para positions, and the decomposition process contributes to some decrease in PCB toxicity. For PCB 77, there are typical substitutions—2 in para and 2 in meta positions, for PCB 126–2 substitutions in para and 3 in meta positions, and for PCB 169–2 in para and 4 in meta positions [5,8–12,18]. Ho and Liu [19] confirmed anaerobic biodegradation of three coplanar PCB. The authors demonstrated that biodegradation took place for PCB 81 and PCB 126, which represent tetra- and pentachlorobiphenyls, respectively. For PCB 169 (3,3,4,4,5,5-hexachlorobiphenyl), biodegradation was not demonstrated. The authors confirmed that dechlorination of higher chlorinated biphenyls proceeded faster than that of lower chlorinated PCB, which explains a decrease in PCB 126 and 169 concentration during last days of digestion process. Velocity, range, and the ways of PCB dechlorination depend on environmental conditions, such as: availability of organic compounds, presence of electron acceptors and donors (other that PCB), temperature, and pH. Wu et al. [18] demonstrated that temperature has a large effect on velocity, biodegradation trend, and products of PCB dechlorination. Results confirmed that during methane digestion conducted under thermophilic conditions, the highest (98%) decrease in toxicity was obtained. According to Wu et al. [18] and Wiegel and Wu [20], stereospecific dechlorination is affected by pH of the system, and the elimination of chlorine atoms in meta positions proceeds with pH in range of 5.0–8.0, whereas in ortho positions—6.0–7.5.

Limiting pH values of sludge liquid during digestion were within the range of 6.8–7.4, therefore, favorable conditions for dechlorination in meta and para positions were maintained.

Some decrease in PCB 126 and 169 concentrations in the last days of digestion can be explained by biodegradation and/or biosorption of PCB. Those processes determine a decrease in sludge toxicity. It was demonstrated that in sludge (P + E + F), PCB 169 content after thermophilic digestion decreased, whereas in sludge (P + F), presence of this compound was not observed. This confirms the possibility of biodegradation of PCB 169, as certain bacterial cultures in sewage sludge can cause a decrease in PCB concentration by 66% just after 200 min [21].

4. Conclusions

It was demonstrated that methane digestion process contributes to some decrease in PCB concentration and in toxicity, caused by presence of coplanar PCB, in sewage sludge. The highest reduction in sludge toxicity was obtained during thermophilic digestion. After the process, reduction in WHO_{98}-TEQ_{PCB} equivalent value in sewage sludge by 98% on average was demonstrated. Significant changes in coplanar PCB concentration during methane digestion were observed on the 7th and 10th day of the process. After mesophilic digestion, the obtained decrease in PCB toxicity was significantly lower (46–49%). Intensification of mesophilic digestion by means of thermophilic hydrolysis, increased the efficiency of PCB toxicity reduction only for sludge (P + F), with respect to results obtained after mesophilic digestion, as WHO_{98}-TEQ_{PCB} equivalent decreased by 93%. For one of the examined sludge efficiency of the process was not satisfactory, as PCB toxicity was reduced only by 35%.

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