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Impact of selected insecticides on the anaerobic stabilization of municipal sewage sludge

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ABSTRACT

The aim of the studies was to indicate whether the insecticides have toxic impact on the course of the process of sewage sludge methane fermentation and their biochemical durability is. The research proved that representatives of insecticides from the group of chlorinated hydrocarbons and phosphoorganic compounds can be toxic to anaerobic biocenosis. The series of insecticide toxicity with respect to anaerobic biocenosis is the following: Chloroorganic compounds > phosphoorganic compounds. The compounds studied can be put into a series with respect to their toxicity as follows: metoxychlor > chlorfenvinphos > fenitrothion > malathion, and for commercial products as follows: Metox > Enolofos > Owadofos > Sadofos. Pesticides from the group of chlorinated hydrocarbons and phosphoorganic compounds can be biodegraded in anaerobic conditions. The degree of decomposition was as follows, for: metoxychlor 99.3–99.9%, chlorfenvinphos 38.1–97.6%, fenitrothion 75.3–99.9%, and malathion, 84.2–99.9%. The study confirms that pesticides (in particular doses), while having a toxic effect on anaerobic biocenosis, are at the same time biologically decomposed in the analyzed conditions of methane digestion. The research has also developed and supplemented the current knowledge on the changes of pesticides in the environment and provides a complete list of compounds toxic to methane fermentation biocenosis.

Keywords: Insecticides; Anaerobic digestion; Toxicity; Biodegradation; Inhibition

1. Introduction

Wastewater treatment plants produce wastewater sludge, which, after having been deposited in the environment needs treatment, that is, the application of methods to change its physical and chemical composition. The basic process used for achieving the biochemical stability of sludge is methane fermentation (anaerobic digestion). In this process, the high-molecular organic substances contained in sludge are decomposed by different groups of bacteria into chemically stabilized simple compounds—mainly to methane and carbon dioxide. Methane digestion is a very complex biochemical process which includes numerous partial reactions which take place at different rates. The rate of the decomposition of organic compounds will then be a result of the partial reactions happening at

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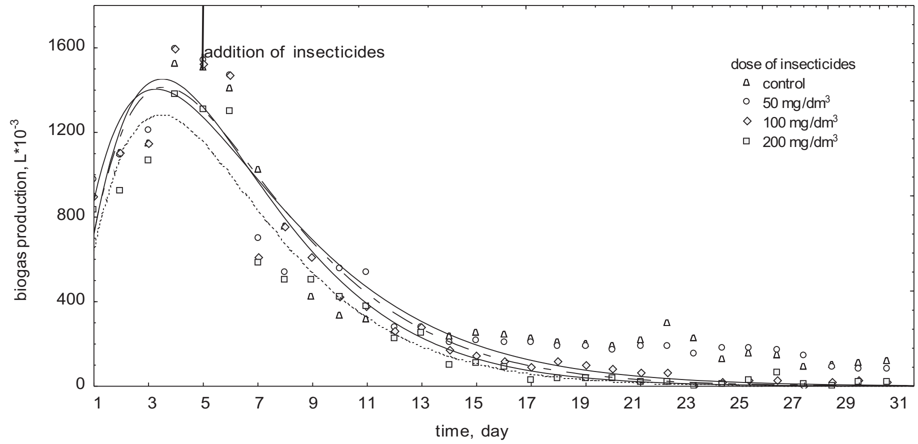


Fig. 1. Gas production during the digestion processes of sludge containing metoxychlor [4,8].

different phases of the process [1,2]. The stability of the anaerobic decomposition process, including methane digestion, is guaranteed only by coordinated processes performed by several groups of trophic bacteria. In multi-phase systems, the resultant rate will depend on the reaction taking place at the slowest rate, or, in the case of the presence of toxic substances, on their toxicity [1–3]. The anaerobic sludge digestion process by methane fermentation is a labile process and is thus very sensitive to all possible types of variations in its parameters and is also susceptible to various substances which may prove toxic to the anaerobic biocenosis [5–7]. It is important to determine the factors which inhibit these changes in the reaction rates, owing to the fact that knowledge of the impeding phenomenon is of special significance in the practice of the biological sewage treatment by anaerobic sludge digestion. A toxicity measuring instrument (m) lowers the unit quantity of the digester gas for a particular toxic substance concentration in relation to the control test [1]. Methane bacteria are especially sensitive to changes in the process parameters. A change in the reaction, fluctuations of temperature, incidental inflow of oxygen including substrate, or the presence of toxic substances may inhibit bacteria development, which in turn may lead to a decrease in gas production or even may result in the process inhibition. Toxic reactions may also be shown by the metabolites generated as the result of transformations that organic compounds of the fermentation. These may include [1,7,9]:

(1) organic acids (especially propionic acid) being happening acid phase products,
(2) ammonia, being the product of the mineralization of nitrogen organic combinations,
(3) hydrogen sulfide, being the product of the reduction of sulfates, sulfites, and sulfur organic combinations.

Usually, heavy metals are included in the group of substances which exert a toxic effect on the digestion process, whereas a large groups of organic microelements are omitted, such as: Growth enhancing additives in fodder, disinfectants, insecticidal agents, chemical and therapeutic agents, and antibiotics or crop protection chemical products. These compounds present in sludge subjected to anaerobic stabilization may inhibit the process, or even lead to its collapse. Phosphoorganic insecticides, pyrethroids, carbamates and on a small scale, chlorinated hydrocarbons are used in Europe and in Poland for the chemical protection of crops. Some of them, even though they have been withdrawn from use, are in the environment in the metabolite forms or the trace residues [10–12]. High toxicity as well as the high resistance of the compounds, especially those from the insecticide group causes the accumulated compounds and/or the metabolites of the transformations to have an inevitable impact on the environment. Contrary to the methods used when treating water containing pesticides, which have already been discussed at length to a lesser degree, in the case of wastewater treatment, information concerning the influence of pesticides on the process of sludge digestion after biological wastewater treatment is significantly limited. Studies presented in the literature indicate that xenobiotics and certain pesticides, also those no longer being used, are degraded by pure or a mixed population of microorganisms [13–16]. Also, the role of bacterial strains consortia in the degradation of pesticides is described [11,17–19]. Anaerobic conditions conducive

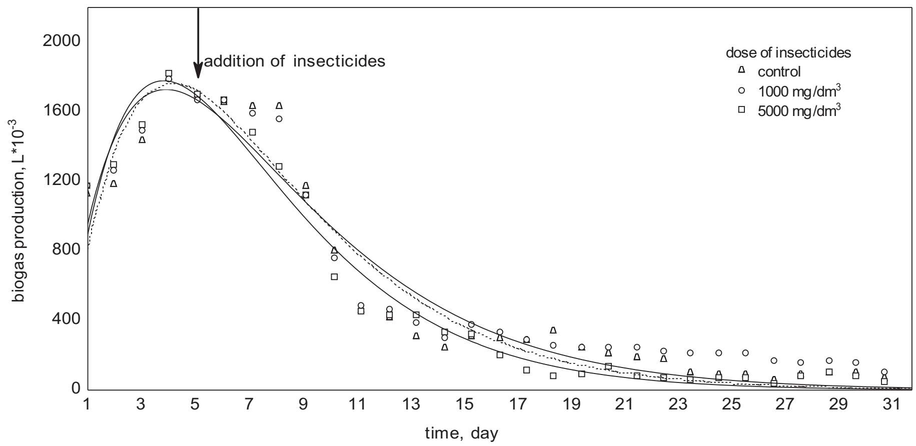


Fig. 2. Gas production during the digestion processes of sludge containing malathion [4,8].

to the degradation of many pesticides include compounds from the group of chlorinated hydrocarbons [20–23]. This is confirmed by the anaerobic degradation of DDT to DDD and DDE, which occurs in soils and sediment [24–28]. Enhanced reductive dechlorination of DDT can be obtained in anaerobic systems in the presence of iron-reducing bacteria [21,29]. The most important reactions in the processes of the reduction of pesticides should be considered: The cleavage of chlorine or halogen (dechlorination and dehalogenation) and the reduction of the nitro or nitroso group to amino [30–33].

The aim of the studies was to demonstrate whether insecticides have a toxic impact on the course of the methane fermentation process of sewage sludge and what the biochemical resilience of the selected compounds in anaerobic conditions is.

2. Experimental procedure

2.1. Sludge characteristics

The input substrate for methane fermentation was excess sludge taken from the municipal wastewater treatment plant in western Poland. The sludge was inoculated at a ratio of (3 + 1 by volume) with the digested sludge collected from the fermentation chambers. The sludge mixture was characterized by the content of dry matter from 50 to 55 kg/m^3 (including 64–75% dry organic matter) and a pH of 6.8–7.4. Appropriate doses of pesticides were added to the sludge mixture starting on the 4–6th day from the beginning of the process.

2.2. Insecticides used in the tests

The following active substances were used in the tests [4,8]:

(1) from the group of chlorinated hydrocarbons—metoxychlor,
(2) from the group of phosphoorganic compounds:

  (a) chlorfenvinphos—a phosphoric acid derivative,
  (b) fenitrothion—a thionic phosphoric acid derivative,
  (c) malathion—a dithio phosphates derivative.

Crop protection agents used in agriculture are a mixture of active substances combined with auxiliary substances of various types such as dissolvents, emulsifiers, carriers, and substances enhancing adherence. The following agents were chosen from a great number of commercial products:

(a) Metox 30—containing 24% chemically pure metoxychlor as a representative of chlorinated hydrocarbons;
(b) Enolofos 50—containing 44% chemically pure chlorfenvinphos; Owadofos—containing 50% chemically pure fenitrothion; Sadofos 30—containing 26% chemically pure malathion as a representative of the group of phosphoorganic compounds, assuming on the one hand that they are the most commonly spread in our country, and due to their content and chemical composition, the most appropriate representatives of their particular group, on the other.

The characteristics of the insecticides used in the tests are presented below:

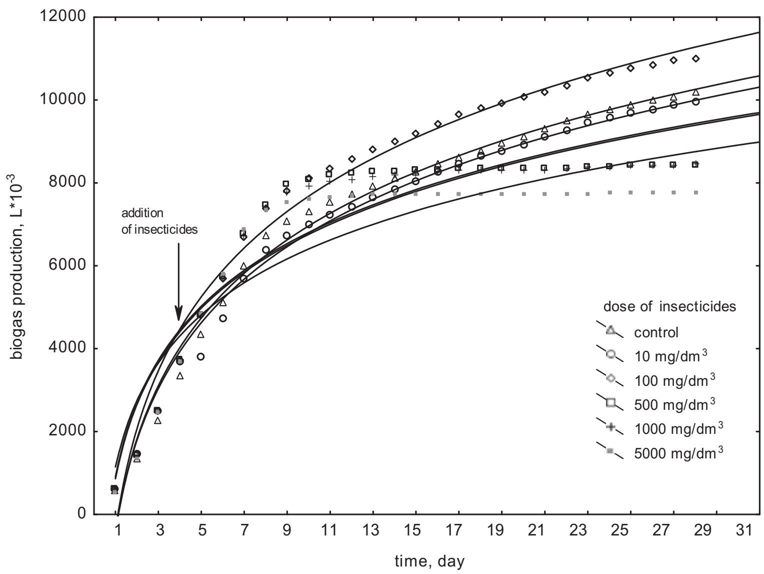
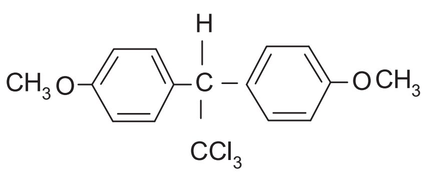


Fig. 3. Production of gas during the digestion process of sludge containing chlorfenvinphos [4,8].

Metoxychlor (DMDT) (decommissioned in 1997) is a DDT analog and has the molecular formula of $C_{16}H_{11}O_2Cl_3$ and the structural formula (1):

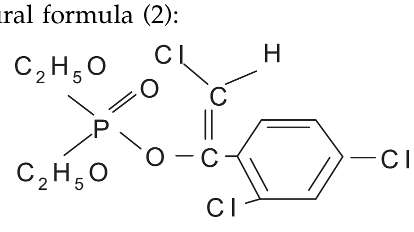


(1)

DMDT belonged to the class III for toxicity [34]. The concentrations of metoxychlor used in the tests were within the range of 0.002–1,000 g/m^3, which conformed with the range of: 0.1×10^{-6}–7.0×10^{-2} g as/g d.o.m (g active substance/g dry organic matter).

Metox 30 is a usable, unary metoxychlor formulation. It contains 24% chemically pure metoxychlor and the remaining 66% are emulsifiers. Because of the acute toxicity (*per os*) to mammals, Metox 30 was classified as class IV toxicity [34]. The influence of Metox 30 on the process of methane digestion was analyzed, taking into account the concentrations of 10–500 g/m^3, which conformed with the range of 0.15×10^{-3} to 0.012 g as/g d.o.m.

Chlorfenvinphos is a representative of phosphoorganic insecticides, a phosphoric acid derivative with the molecular formula of $C_{12}H_{14}Cl_3O_4P$ and the structural formula (2):

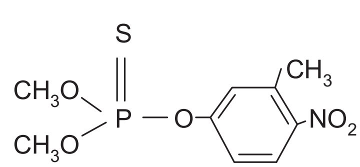


(2)

The fact that in the structure of chlorfenvinphos molecule, there are two ethyl radicals and one oxygen atom means that this compound has a very high sharp toxicity and is classified as belonging to the I b class of toxicity. LD50 for rats (*per os*) is 31 mg/kg [34]. The influence of chlorfenvinphos on the progress of the methane digestion process of the sludge, as well as the persistence of this compound in the conditions of anaerobic biocenosis were both analyzed taking into account a wide range of doses from 0.5 to 10,000 g/m^3. This conformed with the concentrations of 4×10^{-5}–0.85 g as/g d.o.m.

Enolofos 50 is a technical product containing, according to the information furnished by its producer, 44% chemically pure chlorfenvinphos, the other components are xylene and the emulsifiers P_1, P_2, P_3. This formulation is classified as belonging to the Ib class of toxicity [34]. The effect of Enolofos 50 on the progress of the methane digestion process of the sludge, as well as the persistence of this compound in the conditions of anaerobic biocenosis were both analyzed taking into account the doses of 100–500 g/m^3 which conformed with the concentrations of 4.5×10^{-3}–1.5×10^{-2} g as/g d.o.m.

Fenitrothion is a derivative of thionic–phosphoric acid with the molecular formula of $C_9H_{12}NO_5PS$ and the structural formula (3):



(3)

Table 1
Parameters of sludge digestion depending on added different dose of insecticides

Insecticides	Sample Control dose (g/m^3)	unit	Total biogas production, (calculated from the moment the pesticide has been added) ($L\ 10^{-3}$)	Average gas yield L/kg d.o.m.	VFA g $CH_3COOH/(m^3)$	pH –	Insecticides: Supernatants (g/m^3)	Insecticides: Insludge	Biodegradation of insecticides (%)
Metoxychlor	Control		6,718	369.0	34.2	7.0	–	–	–
	50		6,201	364.6	378.1	7.1	0.00245	0.01282	99.7
	100		4,431	255.9	874.2	6.9	0.00164	0.22938	99.7
	500		2,781	212.2	1,697.2	6.4	0.10750	0.17018	99.9
Metox 30	Control		7,234	438.4	216.0	7.1	–	–	–
	10		6,873	432.2	218.0	7.1	0.00155	0.00467	99.7
	50		6,076	368.2	296.8	7.0	0.01168	0.01168	99.8
	150		4,870	250.6	312.0	7.0	0.00701	0.07012	99.8
ChlorfenvinphosEnolofos	Control		4,174	364.1	55.0	7.4	–	–	–
	200		4,444	296.8	147.0	7.1	4.5290	10.6810	92.4
	500		1,676	111.7	600.0	7.1	17.4526	98.8970	76.7
	1,000		1,715	112.1	648.5	7.0	84.1221	213.084	70.3
	Control		3,433	349.0	nm	nm	–	–	–
	100		2,307	192.2	nm	nm	0.49290	1.09710	96.4
Fenitrothion	Control		10,619	565.4	77.1	7.3	–	–	–
	500		9,944	517.2	111.4	7.2	0.34762	15.8490	96.7
	3000		7,604	348.6	964.6	6.9	15.7784	62.3850	97.4
	5,000		3,061	224.5	1,876.8	6.6	22.3809	213.208	95.3
Owadofos	Control		7,234	438.4	113.0	nm	–	–	–
	300		5,183	412.8	976.0	nm	0.15100	0.81100	99.4
	500		2,096	115.9	2,420.0	nm	0.34050	0.84420	99.5
	3,000		777	30.5	2,571.0	nm	13.6230	98.7420	92.5
Malathion	Control		9,040	672.4	85.7	7.3	–	–	–
	500		8,750	711.4	141.4	7.0	0.39600	1.72500	99.6
	5,000		7,526	326.5	968.5	6.6	134.615	397.500	89.4
	10,000		7,272	220.4	1,307.0	6.5	200.000	400.000	94.0
Sadofos	Control		3,433	317.9	72.6	7.2	–	–	–
	100		3,242	348.6	108.0	7.1	0.15270	0.17590	98.7
	500		1,596	188.6	288.0	7.0	0.69260	0.19440	99.4
	1,000		741	61.7	756.0	6.9	3.10500	17.4210	92.1

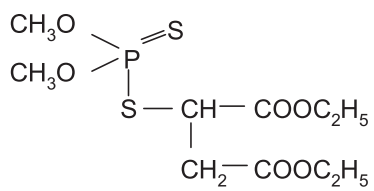
Note: nm—no measure. Toxic doses were bolded.

Fenitrothion is classified as toxicity class II. LD_{50} for rats (*per os*) is 503 mg/kg [34].

The concentration range used for the tests was from 1 to 10,000 g/m^3 (5×10^{-5} do 0.6 g as/g d.o.m).

Owadofos 50 contains 50% chemically pure fenitrothion; the other components are xylene and emulsifiers. The concentration levels used for the tests were from 300 to 5,000 g/m^3, which conformed with 0.01do 0.16 g as/g d.o.m.

Malathion is a derivative of thionothiolophosphoric acid with the molecular formula of $C_{10}H_{19}O_6PS_2$ and the structural formula (4):



(4)

The insecticide is classified as class III toxicity. LD_{50} for rats (*per os*) is 2,100 mg/kg [34]. The effect of malathion on the sludge methane digestion, as well as its persistence and thereby its susceptibility to decomposition in anaerobic conditions was tested applying concentration levels of 0.5–2,000 g/m^3, which conformed with 3.2×10^{-5}–1.39 g as/g d.o.m. The technical preparation of malathion, commercially named as Sadofos liquid 30, contains 30% chemically pure malathion, the other components are a dissolvent and emulsifiers. It belongs to the third class of toxicity due to its acute toxicity to mammals. LD_{50} for rats (*per os*) is 2,800 mg/kg [34]. The effect of Sadofos on the progress of methane digestion as well as its susceptibility to decomposition in anaerobic conditions was analyzed using concentrations of 100–20,000 g/m^3 (2.7×10^{-3}–0.54 g as/g d.o.m).

2.3. *Measuring equipment and analytical scope*

The methane digestion of wastewater sludge was carried out in a laboratory using a periodic method. The fermentation chambers were bioreactors with a volume of 3 L, placed in a 12-stand water thermostat. The bioreactors were connected to scaled gas burettes filled with a saturated brine solution, which played the role of the digester gas (measuring instruments). The digestion process was carried out at a temperature of 35 ± 2℃. The progress of the process was observed according to the binding norms and standards in Poland, this meant checking the volume of the digester gas emitted, the temperature, and the pressure every day.

The following items were determined in the residues before the digestion process: sludge dry substance, organic dry matter, pH, and volatile acids. When testing the sludge after the digestion process, as well as the above, the following items were also analyzed: The pesticide residues in the sludge and in the sludge liquid applying the chromatographic quantitative analysis. The tests were performed by means of two chromatographers, with the use of the TID detector upon preparation of appropriate extracts (pure *n*-hexane of the Merck company was used for extraction). The calculations of the results of analyses were performed on the basis of the retention data from the analyses of samples and specimens. The gas chromatography was used in order to test the composition of the digester gas, with the use of the Chrom five apparatus. The sums of daily gas accumulations and gas efficiency included in tables and on charts are referred to the normal conditions, that is, $p = 1{,}013$ hPa and $T = 273$ K. The course of the biogas production curves on the charts was determined by means of the Rosenbrock's statistical estimation method and the quasi–Newton method using the STATISTICA 10 programme.

3. Results

The inhibiting effect of chemically pure active substances, as well as their related commercial products, on the process of methane digestion leads to changes in the basic digestion process parameters, such as: A decrease in the production and yield of biogas and an increase in the concentration of volatile fatty acids when compared to the control test. In Table 1, changes in the sludge digestion parameters depending on the different chosen dose of insecticides added are presented (toxic doses are in bold).

Table 1 shows not only the typical parameters of the process control (biogas production, VFA, pH), but also the concentrations of the insecticide residual after the digestion process, which made it possible to determine the efficiency of disposing of this compound under the conditions of methane digestion. An example of the process of changes in the digester gas production in the tests on sludge containing metoxychlor—a representative of chlorinated hydrocarbons is shown in Fig. 1. As the concentrations of this compound increases, the gas production decreased when compared to the control test. The inhibiting effect of metoxychlor on the process of methane digestion was observed in the test corresponding with a concentration of 100 g/m^3. The gas production had dropped by 34%, and the average yield by 31% when compared to the control test. As well as the inhibition of the digestion process, there was an increase in the concentration of volatile acids of up to

Table 2
Composition of the digester gas—tests containing chosen insecticides [4,8]

Time (d)	Gas parameters (%)	Control test	Metoxychlor (100 g/m^3)	Metox (50 g/m^3)	Time (d)	Control test	Owadofos (300 g/m^3)	Fenitrothion (3,000 g/m^3)	Time (d)	Control test	Sadofos (500 g/m^3)	Malathion (5,000 g/m^3)
2	CH_4	68.62	74.16	38.62	2	73.63	47.92	68.23	3	63.74	52.16	69.24
	CO_2	21.43	18.23	41.18		12.54	18.96	18.63		21.12	22.41	16.62
	H_2	1.04	3.08	12.60		5.48	12.41	2.48		12.48	10.08	9.23
	N_2	0.83	1.23	0.73		2.17	15.80	9.41		0.56	0.52	1.54
	H_2S	0.12	0.16	–		0.40	2.60	0.86		0.03	2.10	2.20
	Calorific value (kJ/Nm^3)	27,102	28,834	15,450		26,379	nm	26,802		25,122	21,803	28,043
6	CH_4	72.64	64.18	32.68	5	72.60	42.84	69.54	8	65.26	54.26	73.16
	CO_2	18.93	26.10	42.34		16.92	17.63	16.38		18.26	21.18	17.08
	H_2	0.89	2.19	20.18		3.48	15.61	2.56		9.49	17.82	5.64
	N_2	4.12	0,84	2.16		3.17	10.28	4.29		2.16	3.41	1.16
	H_2S	0.10	0.10	0.42		–	2.23	1.48		0.05	2.12	2.08
	Calorific value (kJ/Nm^3)	27,280	25,493	13,024		26,004	16,727	27,998		22,948	22,199	29,235
16	CH_4	64.23	56.24	Gas amount too small	12	66.81	Gas amount Too small	53.84	12	61.24	48.56	68.26
	CO_2	29.12	33.18			19.49		26.39		24.16	23.10	15.82
	H_2	3.14	9.40			9.53		9.86		8.51	12.91	9.24
	N_2	0.61	2.05			3.16		2.14		1.83	9.23	1.16
	H_2S	0.12	–			–		2.02		0.02	2.38	2.52
	Calorific value (kJ/Nm^3)	25,509	22,391			26,432		20,578		24,658	18,779	27,273

Note: nm—no measure.

Table 3
Effect of the selected insecticides on the process of methane digestion [4,8]

Group	Active substance	Commercial product	Toxic concentration	
			(g/m^3)	g as/g d.o.m
Chlorinated hydrocarbons	Metoxychlor		100.0	7.0×10^{-3}
		Metox 30	50.0	7.7×10^{-4}
Phosphoorganic compounds	Chlorfenvinphos		500.0	4.4×10^{-2}
		Enolofos 50	100.0	4.5×10^{-3}
	Fenitrothion		3,000.0	0.18
		Owadofos 50	500.0	0.016
	Malathion		5,000.0	0.32
		Sadofos 30	500.0	0.013

874.2 g CH_3COOH/ m^3 and the pH was lowered to 6.9 (Table 1). Symptoms of the process inhibition in the case of the tests including malathion (Fig. 2) were present in the test with the concentration of 5,000 g/m^3 of malathion (0.32 g/g d.o.m). Gas production decreased, although only by 17%, yet the gas yield lowered significantly and amounted to 326 L/kg d.o.m., which was only 47% of the yield achieved in the tests carried out in control sample. It was also found that the concentration of volatile acids had increased up to 968.5 g CH_3COOH/m^3, whereas the pH was lowered to 6.6 (Table 1).

The inhibiting effect of insecticides on the process of sludge digestion was also determined on the grounds of changes in the daily digester gas production. Fig. 3 presents an example of changes in this parameter for the tests which included chlorfenvinphos.

The daily production of digester gas decreases after the pesticide has been added. In the tests where from 5.0 to 200 g/m^3 of chlorfenvinphos was used, the average yield decreased by 20% and reached the level of 290 L/kg d.o.m. The first symptoms of the process inhibition were noticed in the sample with 300 g/m^3 (2.8×10^{-2} g as/g d.o.m.) of chlorfenvinphos. This concentration brought about a decrease in gas production by almost 17% when compared to the control test. The concentration of volatile acids increased to 214 g/m^3. It was clearly observed that the digestion process was disturbed in the tests containing from 400 to 1,000 g/m^3 of chlorfenvinphos and, despite the fact that the doses used varied significantly, the symptoms of the process inhibition remained at a similar level. The total gas production was lowered by 50–60%. The concentration of the volatile acids remained at a level of between 518.3 and 648.5 g CH_3COOH/m^3 (Table 1).

Inhibition was also proved by chromatographic analysis of the digester gas composition, in which the percentage content of methane showed a decreasing tendency together with an increase of the pesticide added (Table 2), whereas in the tests where pesticides containing sulfur in a molecule were used, the digester gas showed the presence of hydrogen sulfide in amounts of up to 2.6%.

4. Discussion

The research confirmed that the representatives of insecticides from the group of chlorinated hydrocarbons and phosphoorganic compounds can be toxic to anaerobic biocenosis [4,5,8]. Concentrations having a toxic effect on the methane digestion process are shown in Table 3.

The results of the research allowed for the determination of the toxic doses of insecticides from the group of chlorinated hydrocarbons and phosphoorganic compounds in relation to the microorganisms of the methane digestion (Table 3). Metoxychlor showed the highest toxicity, which at a concentration of 7 mg/g d.o.m. caused 34% inhibition of digester gas production (Table 1, Fig. 1). In the group of phosphoorganic compounds, the highest toxicity toward anaerobic bacteria was presented by chlorfenvinphos, which in only the amount of 4.4×10^{-2} g/g d.o.m, inhibited the production of biogas (Table 3, Fig. 3). The toxicity of this group of compounds with respect to the methane digestion process decreases are presented in the following series [4,8]:

chlorfenvinphos > fenitrothion > malathion

and corresponds with the toxicity series of the whole group of phosphoorganic insecticides:

phosphates > thiophosphates > thiothiolophosphates.

The results of the research carried out for the commercial products were surprising. It was proved that the anaerobic biocenosis shows a greater tolerance to chemically pure compounds than to their commercial products, due to the fact that toxic doses of Metox, Enolofos, Owadofos, Sadofos, were smaller than the doses of chemically pure active substances contained in these preparations (Table 3). This data confirms the hypothesis that the auxiliary substances contained in commercial products such as dissolvents, emulsifiers, and compounds enhancing adherence are selected in such a way so as to increase the effect of the active substance. The studied pesticides can be put into the following series with respect to their toxicity:

chlorinated hydrocarbons > phosphoorganic compounds

and in detail as follows:

metoxychlor > chlorfenvinphos > fenitrothion > malathion

g/g d.o.m. $7 \times 10^{-3} < 4.4 \times 10^{-2} < 0.18 < 0.32$

The commercial products can be put into the following series as far as their relation to anaerobic biocenosis is concerned:

Metox > Enolofos > Owadofos > Sadofos

g as/ g d.o.m. $7.7 \times 10^{-4} < 4.5 \times 10^{-3} < 1.6 \times 10^{-2} < 1.3 \times 10^{-2}$

The results obtained are a new element and supplement the list of compounds having a toxic effect on the digestion process. It was shown in the study in question, that the pesticides from the group of chlorinated hydrocarbons and phosphoorganic compounds are subject to anaerobic biodegradation. The degree of decomposition was: for metoxychlor: 99.3–99.9%, chlorfenvinphos: 38.1–97.6%, fenitrothion: 75.3–99.9% and for malathion: 84.2–99.9% (Table 1) [4,5,8]. This confirms a low persistence of the compounds in anaerobic conditions as well as supplementing the knowledge on the change of pesticides in the environment.

5. Conclusion

(1) Using indirect measuring methods (dosage of toxic substances, changes in the methane digestion parameters, changes in the contents of biogas and residual insecticides after anaerobic digestion) it was found that pesticides (in particular doses), as well as their toxic effect on anaerobic biocenosis, are at the same time biologically decomposed in the analyzed conditions of methane digestion.

(2) The series of insecticide toxicity with respect to anaerobic biocenosis is the following: chloroorganic compounds > phosphoorganic compounds. The compounds studied can be put into a series with respect to their toxicity as follows:

Metoxychlor > chlorfenvinphos > fenitrothion > malathion.

(3) The choice of commercial products from different pesticide groups used for the tests was right, because not only was their toxicity shown, but also, unexpectedly, it was proved that they are more toxic than the chemically pure active substances themselves contained in the preparations.

(4) On the grounds of the research results it is possible to put the analyzed products into the series of lowering toxicity which is as follows: Metox > Enolofos > Owadofos > Sadofos.

(5) The research fills a thus far existing gap in the information concerning the transformations of insecticides in anaerobic conditions, as well as supplementing the list of compounds which have a toxic effect on methane digestion process.

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