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EFFECT OF ZINC AND COPPER ON ANAEROBIC STABILIZATION OF SEWAGE SLUDGE

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Abstract

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The most frequently found metals in municipal sewage sludge are copper and zinc. The aim of this study was to the evaluate effect of these metals on anaerobic microorganisms during sewage sludge stabilization. Anaerobic fermentation tests were carried out in 24 batch fermenters with hydraulic retention time 21 days at mesophilic temperature conditions 38 °C ± 0.2 °C. Five different concentrations of metal ionts (100-1000 $mg \cdot l^{-1}$) were tested. Cumulative biogas and methane production were used as the comparative parameters of inhibition. Hypothesis, which predicted presence of inhibitory effect of zinc and copper on anaerobic microorganisms, mainly on methanogenic *Archaea*, was confirmed. The lowest concentration of zinc and copper which cause significant inhibition of biogas production was 400 mg $Zn^{2+} \cdot l^{-1}$ and 1000 mg $Cu^{2+} \cdot l^{-1}$, which cause reduction of 10.3 ± 2.0 % and 82.8 ± 1.1 %, respectively. The lowest concentration of zinc and copper which lead to significant inhibition of methane production is 400 mg $Zn^{2+} \cdot l^{-1}$ and 600 mg $Cu^{2+} \cdot l^{-1}$, which caused to reduction of 16.1 ± 3.2 % and 17.4 ± 2.2 %, respectively. The reduction in methane production is higher than in biogas production.

Keywords: biogas production, methane, toxic metals, heavy metals, inhibition, wastewater treatment plant, municipal sewage sludge

INTRODUCTION

Anaerobic sewage sludge treatment is a complex microbiological process involving various types of anaerobic and facultative bacteria. This way of sludge treatment involves degradation and the stabilization of organic matter, with reduction in odour, pathogens and the mass of solid organic material that requires further processing. This is accomplished by the biological conversion of organic matter to methane (CH_4) and carbon dioxide (CO_2) (Parkin and Owen, 1986; Kelleher *et al.*, 2002).

Toxic materials causing anaerobic reactor upset and failure are often present in substantial concentrations in wastewater and sludge (Chen *et al.*, 2008). Heavy metals were confirmed in significant concentrations in municipal wastewater and sewage sludge (Swanwick *et al.*, 1969; Chen *et al.*, 2008). Heavy metals are often present in industrial and municipal wastewaters and sewage sludges in significant concentrations, the most frequently found are copper (Cu), zinc (Zn), lead (Pb), mercury (Hg), chromium (Cr), cadmium (Cd), iron (Fe), nickel (Ni), cobalt (Co) and molybdenum (Mo) (Altaş, 2009). The primary source of heavy metals in urban wastewater is industry, which represents up to 50 % of the total heavy metal content in sewage sludge. Domestic sources are mainly associated with leaching from plumbing materials (Cu and Pb), gutters and roofs (Cu and Zn) and galvanised materials, the use of detergents and washing powders containing Cd, Cu and Zn, and the use of body care products containing Zn (Appels *et al.*, 2008).

Many essential metals (e.g. Cu, Zn, Se) are required for the activation or functioning of many enzymes and coenzymes in anaerobic fermentation (Chen *et al.*, 2014). However, the excessive amounts of heavy metals can lead to the inhibition of anaerobic microorganisms (Li and Fang, 2007 in Chen *et al.*, 2014). The *Archaea* are commonly considered to be the most sensitive to toxicity of all microorganisms in the overall consortium for anaerobic conversion of organic matter to methane. However, acclimation to toxicity and the reversibility of toxicity are frequently observed. Inhibition is usually indicated by a decrease of the steady-state rate of methane production and accumulation of volatile fatty acids (Kroeker *et al.*, 1979).

The aim of this study is to observe effect of essential metals such as zinc and copper on the anaerobic stabilization of sewage sludge and eventually specify the inhibitory effect of these metals on the anaerobic stabilization of sewage sludge and biogas production. Hypothesis predicts the presence of the inhibitory effect of zinc and copper on anaerobic microorganisms, mainly on methanogenic *Archaea*.

MATERIALS AND METHODS

Sludge samples were collected directly from the anaerobic sewage sludge stabilization tank located at the wastewater treatment plant (WWTP) in Brno, Czech Republic, Population Equivalent (PE) 513,000. The anaerobic stabilization tank processes the mixture of primary and secondary sludge (50 : 50 v/v) at mesophilic condition (38 °C) with hydraulic retention time 18–20 days. After the collection, the sewage sludge sample was transported to the laboratory as soon as possible. This sewage sludge sample used for the batch anaerobic fermentation tests was specified by the analysing of total solids (TS), volatile solids (VS), pH, redox potential, conductivity and the contents of zinc and copper.

To determine the sewage sludge total solids (TS) and volatile solids (VS) content, a muffle furnace LMH 11/12 (LAC Ltd., Czech Republic) was used. Fresh sample was dried at 105 °C ± 5 °C to determine total solids (TS) content according to Czech Standard Method CSN EN 15934. Volatile solids (VS) content was determined by the incineration of the sample at 550 °C ± 5 °C according to CSN EN 15169. Sewage sludge pH, redox potential and conductivity were analysed by using pH/Cond meter 3320 (WTW GmbH, Germany) in accordance with CSN EN 12176, CSN 757367 and CSN EN 27888 standards. The contents of zinc and copper in dried sample were determined by handheld spectrometer and metal analyser DELTA PROFESSIONAL (BAS Rudice, Czech Republic).

Biogas production and quality was measured using batch anaerobic fermenters at the temperature 38 °C ± 0.2 °C according to German Standard VDI 4630. Two anaerobic fermentation experiments were carried out. For both experiments, 3 systems, that each consists of 8 batch fermenters of volume 5 dm^3, were used. All 24 batch fermenters were filled up with 3.0 dm^3 of sludge sample. For both experiments, fresh sewage sludge was used. Glycerine (8 ml) was added to each fermenter as a carbon source for microbial growth. In both experiments, 4 batch fermenters were used as blank, with addition 50 ml of distilled water. Into remaining fermenters 5 different amounts of zinc chloride ($ZnCl_2$) and copper chloride ($CuCl_2$) stock solution in concentration 60 grams of metal per litter were added in experiment No. 1 and experiment No. 2, respectively. Distilled water was added in order to achieve same volume (3.058 dm^3) in all batch fermenters, Tab. I. Each tested concentration of zinc and copper was added into 4 fermenters.

Hydraulic retention time for both batch fermentation experiments was 21 days. Over this defined period, the biogas produced was collected in wet gas meters and was measured daily. Methane (CH_4) and carbon dioxide (CO_2) content was measured during both experiments by gas analyzer COMBIMASS® GA–s (BINDER GmbH, Germany). Biogas production was converted to standard conditions ($T_0 = 273$ K, $p_0 = 101\,325$ Pa). The volume of biogas and methane produced by the sample was converted to biogas yield (BY) and methane yield (MY), by expressing them as cubic meter per kilogram of substrate volatile solids (VS).

All measurements were done in triplicate. All measured values are expressed as arithmetic mean ± standard deviation. Significant differences in biogas (methane) production among control and different zinc and copper concentrations were determined by the analysis of variance (ANOVA) at $P < 0.05$ using the Tukey test. STATISTICA 12 software was used.

I: *Experiments setup*

Concentration of metal [mg • l^{-1}]	Volume of metal chloride solution [ml]	Volume of distilled water [ml]
Blank	0.0	50.0
100	5.0	45.0
200	10.0	40.0
400	20.0	30.0
600	30.0	20.0
1000	50.0	0.0

RESULTS

The sewage sludge samples used for the batch anaerobic fermentation tests were specified by the analysing of total solids (TS), volatile solids (VS), pH, redox potential, conductivity (see Tab. II) and the content of zinc and copper (see Tab. III).

The curves of cumulative biogas production during both fermentation experiments are shown in Fig. 1. Specific biogas productions after 21 days are shown in Tab. IV and V. The lowest concentration of zinc which causes significant inhibition of biogas production is 400 mg $Zn^{2+} \bullet l^{-1}$. This concentration of zinc leads to the reduction of 10.3 ± 2.0% in the biogas yield. The addition of zinc in the concentration 600 and 1000 mg $Zn^{2+} \bullet l^{-1}$ leads to the reductions of 47.8 ± 1.5%, 70.9 ± 1.0% in biogas production, respectively. The lowest concentration of copper which causes the significant inhibition of biogas production is 1000 mg $Cu^{2+} \bullet l^{-1}$. This concentration of copper causes the reduction of 82.8 ± 1.1% in the biogas yield. There is no significant inhibition of final biogas yield caused by tested metals in concentrations till 200 mg $Zn^{2+} \bullet l^{-1}$ and 600 mg $Cu^{2+} \bullet l^{-1}$. However, the curves of biogas production are affected after the addition of tested metals in all concentrations, which means that anaerobic microorganisms need longer adaptation period after the addition of metals in higher concentration.

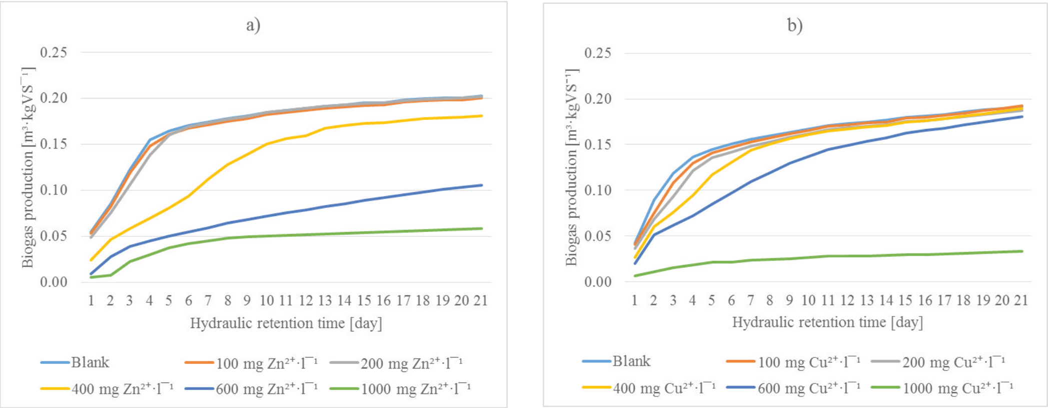
The content of methane in biogas generated during both fermentation experiments is shown in Fig. 2. The curves of cumulative methane production during hydraulic retention time are shown in Fig. 3. Specific methane yields at the end of both experiments are shown in Tab. IV and V. The lowest concentration of zinc which leads to the significant inhibition of methane production is 400 mg $Zn^{2+} \bullet l^{-1}$. This concentration leads to the reduction of 16.1 ± 3.2% in the methane yield. The reductions of 63.4 ± 2.3%, 91.4 ± 4.3% in methane production can be observed after the addition of zinc in concentration 600 and 1000 mg $Zn^{2+} \bullet l^{-1}$, respectively. The lowest concentration of copper which leads to the significant inhibition of methane production is 600 mg $Cu^{2+} \bullet l^{-1}$. This concentration leads to the reduction of 17.4 ± 2.2% in the methane yield. The reduction of 96.8 ± 2.2% in methane production can be observed after the addition of copper in concentration 1000 mg $Cu^{2+} \bullet l^{-1}$. There is no significant inhibition of final methane yield caused by tested metals in concentrations till

II: *Sewage sludge samples characteristics*

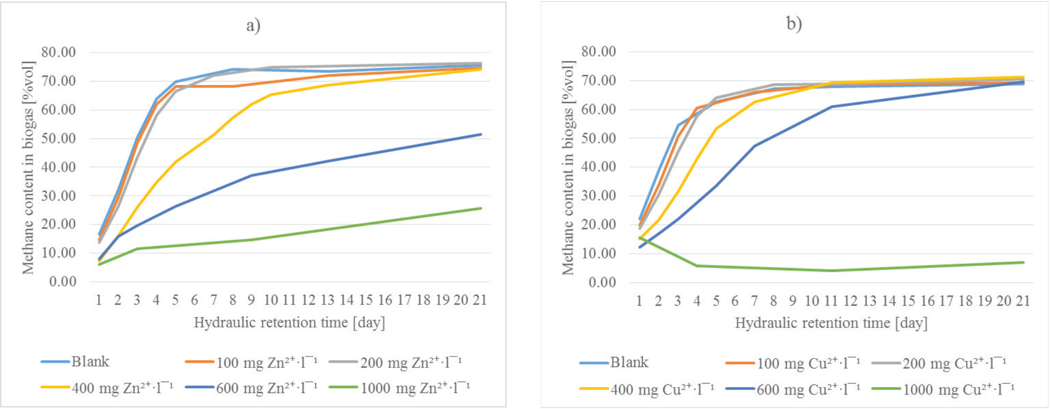
Sample	pH [–]	Redox potential [mV]	Conductivity [mS • cm^{-1}]	Dry matter [%]	Organic dry matter [%]
Sewage sludge No. 1	7.42 ± 0.01	−30.40 ± 0.65	6.99 ± 0.02	3.18 ± 0.01	51.15 ± 0.10
Sewage sludge No. 2	7.52 ± 0.02	−21.50 ± 0.50	7.12 ± 0.02	3.23 ± 0.01	57.41 ± 0.07

III: *Metals content in sewage sludge samples*

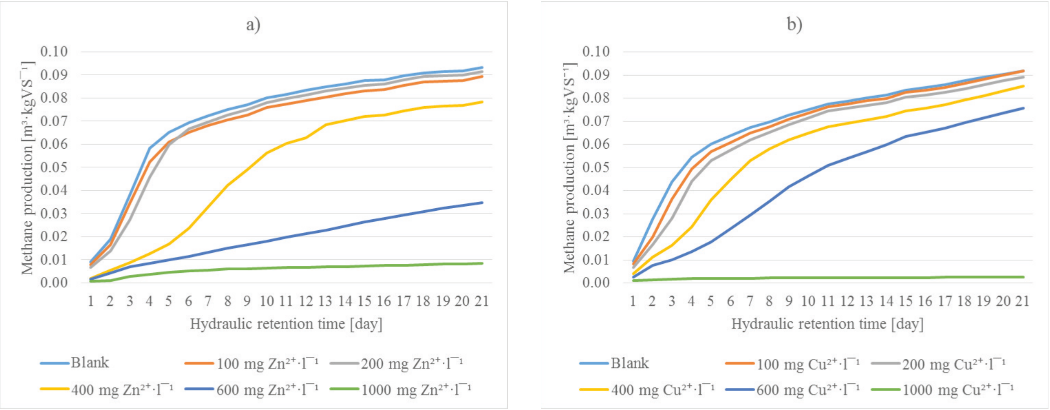
Sample	Content of Zn		Content of Cu	
	[mg • l^{-1}]	[mg • kgTS^{-1}]	[mg • l^{-1}]	[mg • kgTS^{-1}]
Sewage sludge No. 1	37.46 ± 2.35	1178.00 ± 74.00	8.33 ± 2.45	262.00 ± 77.00
Sewage sludge No. 2	37.46 ± 2.35	1382.00 ± 79.00	7.30 ± 2.40	226.00 ± 74.00



1: *Cumulative biogas production during experiment No. 1 (a) and No. 2 (b)*



2: *Methane content in biogas during experiment No. 1 (a) and No. 2 (b)*



3: *Cumulative methane production during experiment No. 1 (a) and No. 2 (b)*

200 mg Zn^{2+}• l^{-1} and 400 mg Cu^{2+}• l^{-1}. However, the curves of methane production are affected after the addition of tested metals in all concentrations, which means that methanogenic *Archaea* need longer adaptation period after the addition of metals in higher concentration.

The Tab. IV shows that the reduction in biogas and methane production after the addition of zinc in the highest concentration(1000 Zn^{2+}• l^{-1}) is 70.9 ± 1.0% and 91.4 ± 4.3%, respectively. The Tab. V shows that the reduction in biogas and methane production after the addition of copper in the highest concentration (1000 Cu^{2+}• l^{-1}) is 82.8 ± 1.1% and 96.8 ± 2.2%, respectively. Which means, that methanogenic *Archaea* are more inhibited by tested metals than the other groups of anaerobic microorganisms.

The lowest concentration of zinc which causes the significant inhibition of biogas and methane production is 400 mg Zn^{2+}• l^{-1}. This concentration of zinc causes the reduction of 10.3 ± 2.0% in the biogas yield and the reduction of 16.1 ± 3.2% in the methane yield (bold in Tab. IV). The lowest concentration of copper which causes the significant inhibition of

IV: *Biogas and methane yield after 21 days – experiment No. 1*

Sample	Specific biogas production [m^3 • kg_{VS}^{-1}]	Relative biogas production [%]	Specific methane production [m^3 • kg_{VS}^{-1}]	Relative methane production [%]
Blank	0.203 ± 0.004a	100.0 ± 2.0	0.093 ± 0.003a	100.0 ± 3.2
100 mg Zn^{2+} • l^{-1}	0.201 ± 0.004a	99.0 ± 2.0	0.089 ± 0.003a	95.7 ± 3.2
200 mg Zn^{2+} • l^{-1}	0.202 ± 0.003a	99.5 ± 1.5	0.092 ± 0.002a	98.9 ± 2.1
400 mg Zn^{2+} • l^{-1}	**0.182 ± 0.004b**	**89.7 ± 2.0**	**0.078 ± 0.003b**	**83.9 ± 3.2**
600 mg Zn^{2+} • l^{-1}	0.106 ± 0.003c	52.2 ± 1.5	0.035 ± 0.003c	37.6 ± 3.2
1000 mg Zn^{2+} • l^{-1}	0.059 ± 0.002d	29.1 ± 1.0	0.008 ± 0.004d	8.6 ± 4.3

V: *Biogas and methane yield after 21 days – experiment No. 2*

Sample	Specific biogas production [m3 • kg_{VS}^{-1}]	Relative biogas production [%]	Specific methane production [m3 • kg_{VS}^{-1}]	Relative methane production [%]
Blank	0.192 ± 0.002a	100.0 ± 1.1	0.092 ± 0.002a	100.0 ± 2.2
100 mg Cu^{2+} • l^{-1}	0.192 ± 0.002a	100.0 ± 1.1	0.092 ± 0.002a	100.0 ± 2.2
200 mg Cu^{2+} • l^{-1}	0.188 ± 0.004a	97.9 ± 2.1	0.089 ± 0.003a	96.7 ± 3.2
400 mg Cu^{2+} • l^{-1}	0.190 ± 0.004a	99.0 ± 2.1	0.085 ± 0.003a	92.4 ± 3.2
600 mg Cu^{2+} • l^{-1}	0.181 ± 0.003a	94.3 ± 1.6	**0.076 ± 0.002b**	**82.6 ± 2.2**
1000 mg Cu^{2+} • l^{-1}	**0.033 ± 0.002b**	**17.2 ± 1.1**	0.003 ± 0.002c	3.2 ± 2.2

biogas and methane production is 1000 mg Cu^{2+}• l^{-1} and 600 mg Cu^{2+} • l^{-1}, respectively. The concentration 400 mg Cu^{2+} • l^{-1} causes the reduction of 1.0 ± 2.1 % in the biogas yield and 7.6 ± 3.2 in the methane yield (bold in Tab. V). Thus, the comparison of Tab. IV and V shows that zinc is more toxic to all groups of anaerobic microorganisms than copper.

DISCUSSION

The results of sludge samples analysis (Tab. II and Tab. III) meet the intervals published by other authors (Alagöz *et al.*, 2015 – 3.83 % TS, 66.16 % VS, Koch *et al.*, 2015 – 2.28–2.37 % TS, 60.20–61.1 % VS, Silvestre *et al.*, 2014 – 3.35 % TS, 69.70 % TS, Zaleckas *et al.*, 2012 – content of metals, Liu *et al.*, 2016 – pH 7.34, 2.58 TS, 55.81 % VS).

If Sarioglu *et al.* (2010) used anaerobic sewage sludge taken from an up-flow anaerobic sludge blanket reactor treating the wastewaters of Pakmaya Yeast Factory, cumulative methane gas production decreased to 55 and 43 %, respectively for 500 and 1000 mg Zn • l^{-1}. Lin and Chen (1999) tested sludges that were obtained from an up-flow anaerobic sludge blanket reactor treating winery wastewater, the concentration at which zinc caused 50 % inhibition of methane production was 690 and 270 mg Zn^{2+} • l^{-1}, respectively at hydraulic retention time 1 and 2 days. This study reaches quite similar results, zinc in concentration 600 mg • l^{-1} causes the inhibition of methane production by 63.4 ± 3.2 %.

Zayed and Winter (2000) observed that the addition of copper chloride ($CuCl_2$) at the concentrations of 10 mg • l^{-1} and 20 mg • l^{-1} led to the inhibition of methane formation of 50 % and 79 %, respectively. Same authors reported the complete inhibition of methanogenesis (but not of acidogenesis) caused by 30–40 mg $CuCl_2$ • l^{-1}. However, the complete inhibition of methanogenesis is caused by 1000 mg Cu^{2+} • l^{-1} in this study. Furthermore, Zayed and Winter (2000) reported the 50 % inhibition of methanogenesis at 40 mg $ZnCl_2$ • l^{-1}, but the lowest concentration of zinc which leads to the significant inhibition of methane production is 400 mg Zn^{2+} • l^{-1} in this study.

It was found by Hickey *et al.* (1989) in Zayed and Winter (2000) that CO_2 production was less inhibited by heavy metals than methanogenesis, indicating a weaker effect of heavy metals on acidogenesis than on methanogenesis. Eckenfelder and Santhanam (1981) in Lin and Chen (1999) also reported that the acidogens responsible for acidogenesis are generally considered to be less sensitive to environmental conditions, e.g. toxicant concentrations, as compared to methanogens. In this study the Tab. IV shows that the reduction in biogas and methane production after the addition of zinc in the highest concentration (1000 Zn^{2+} • l^{-1}) is 70.9 ± 1.0 % and 91.4 ± 4.3 %, respectively. The Tab. V shows that reduction in biogas and methane production after addition of the highest copper concentration (1000 Cu^{2+} • l^{-1}) is 82.8 ± 1.1 % and 96.8 ± 2.2 %, respectively.

The relative toxicity of metals, obtained by using the inhibition of methanogenic activity assay, is Cu > Ni ≈ Zn > Pb according to study by Sarioglu *et al.* (2010), Cu > Zn > Cr > Cd > Ni > Pb according to Lin (1992) in Chen *et al.* (2008) and Cd > Cu > Cr > Zn > Pb > Ni according to Lin (1993) in Chen *et al.* (2008). Nevertheless, based on the comparison of Tab. IV and V is clear that zinc is more toxic to all groups of anaerobic microorganisms than copper in this study carried out with municipal sewage sludge. The results are consistent with the study by Mudhoo and Kumar (2013) presented quite different order of toxic metals: Zn > Cr > Cu > Cd > Ni > Pb.

The operating solids level has significant impact on the heavy metal toxicity in anaerobic fermenters by providing protection from metal inhibitory effect. It has been suggested that inhibition due to heavy metals would be more comparable if metal dosage was expressed as milligram of metal per gram of volatile solids (Hickey *et al.*, 1989 in Chen *et al.*, 2008). Unfortunately, most of the literature only reported the inhibition concentration values in mg • l^{-1}, which makes the comparison of inhibition concentrations more difficult (Chen *et al.*, 2008).

Our results support the hypothesis about the impact of metals addition on methane concentration, thus on methanogenic *Archaea*. The inhibition of hydrogenotrophic methanogenic Archaea, which use hydrogen as substrate, can be proved due to accumulation of hydrogen in biogas during both fermentation experiments. The usual hydrogen concentration in biogas achieves values till 100 ppm in fermenters without the addition of metals. Hydrogen concentration in biogas rises up to 1808 ppm (which is the detection limit of used gas analyser) after the addition of tested metals in concentration 1000 mg • l^{-1}. When we consider fact that more than 50% of biogas is produced from acetate (Garcia *et al*., 2000; Liu and Whitman, 2008; Zinder, 1993) we can conclude, that also the inhibition of acetotrophic methanogenic *Archaea* can be proved.

CONCLUSION

Copper and zinc are the most frequently found metals in municipal sewage sludge. The effect of these metals on the anaerobic stabilization of sewage sludge was studied during 2 fermentation experiments using 24 batch anaerobic fermenters at temperature 38 °C ± 0.2 °C during hydraulic retention time 21 days. Zinc and copper chlorides ($ZnCl_2$, $CuCl_2$) were used as toxic substances in 5 different concentrations of metal ionts (Zn^{2+}, Cu^{2+}); 100, 200, 400, 600 and 1000 mg • l^{-1}. Cumulative biogas and methane production were used as the comparative parameters of tested metals inhibitory effect. Hypothesis, which predicts the presence of zinc and copper inhibitory effect on anaerobic microorganisms, mainly on methanogenic *Archaea*, was confirmed. The lowest concentration of zinc and copper which causes the significant inhibition of biogas production is 400 mg Zn^{2+} • l^{-1} and 1000 mg Cu^{2+} • l^{-1}, which cause the reduction of 10.3 ± 2.0% and 82.8 ± 1.1%, respectively. The lowest concentration of zinc and copper which leads to the significant inhibition of methane production is 400 mg Zn^{2+} • l^{-1} and 600 mg Cu^{2+} • l^{-1}, which leads to the reduction of 16.1 ± 3.2% and 17.4 ± 2.2%, respectively. The reduction in biogas and methane production after the addition of zinc in the highest concentration (1000 mg Zn^{2+} • l^{-1}) is 70.9 ± 1.0% and 91.4 ± 4.3%, respectively. The reduction in biogas and methane production after the addition of copper in the highest concentration (1000 mg Cu^{2+} • l^{-1}) is 82.8 ± 1.1% and 96.8 ± 2.2%, respectively. Which means, that methanogenic *Archaea* are more inhibited by tested metals than the other groups of anaerobic microorganisms. Furthermore, the inhibition of hydrogenotrophic methanogenic *Archaea,* which use hydrogen as substrate, was indicated by the higher concentration of hydrogen in biogas during both fermentation experiments. Hydrogen concentration in biogas rises up to 1808 ppm (which is detection limit of used gas analyzer) after the addition of tested metals in concentration 1000 mg • l^{-1}.

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