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Effects of Heavy Metal Ions (Fe^{3+}, Cu^{2+}, Zn^{2+} and Cr^{3+}) on the Productivity of Biogas and Biomethane Production

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Abstract

It was shown that the concentration of heavy metal ions Fe^{3+}, Cu^{2+}, Cr^{3+} and Zn^{2+}, which are contained in the fermentation medium, affects the process of biogas production. The aim of this paper was to establish the concentration of ions, which makes it possible to increase the yield of biogas and the methane content in it. The total yield of biogas per unit of dry organic matter, methane and the kinetics of changes in the yield of biogas during fermentation of cattle manure in the presence of different concentrations of metal ions in the anaerobic environment. It was established that the content of Fe^{3+}, Cu^{2+} is up to 80 mg/dm^3, Cr^{3+} and Zn^{2+} is up to 50 mg/dm^3 in the medium lead to increased methane production by anaerobic association of microorganisms due to the effect on the activity of enzyme systems in comparison with the sample without the addition of metal ions. It was found that the rational concentrations to increase the methane yield in the biogas obtained by fermentation of cattle manure are: Fe^{3+}—20 - 40 mg/dm^3, Cu^{2+}—40 - 60 mg/dm^3, Cr^{3+}—10 mg/dm^3. The increase in the concentration of metal ions above rational values leads to a decrease in the methane content in biogas. It was shown that zinc ions have a positive effect on methane production, but reduce the total biogas yield and, accordingly, the degree of conversion of organic raw materials. Therefore, the rational concentration of Zn^{2+} in the fermentation medium is 10 mg/dm^3.

Keywords

Heavy Metal, Iron, Zinc, Copper, Chromium, Biogas, Biomethane

1. Introduction

The effect of heavy metal ions (Me^+) on the process of anaerobic fermentation is associated with the uncontrolled content of metal-containing compounds in the raw material entering the fermentation (for example, organic municipal waste [1]), which can lead to inhibition of anaerobic association of microorganisms by the following mechanisms:

- Metal ions can interact with electron donor (hydroxyl, carboxyl, amino and sulfhydryl) groups of organic compounds, changing their structure and influencing their biological action [2];
- Metal ions can irreversibly replace divalent cations of the active centers of enzyme systems and other components of cells, which causes growth inhibition and death of microorganisms [3];
- Oxidizing metals—have a detrimental effect on microorganisms due to irreversible oxidation of enzymes and structural components of cells [4];
- Metal ions can act as antimetabolites, inactivating their catabolism or accelerating it [5].

In an anaerobic process, with the participation of a complex anaerobic association of microorganisms, metal ions can be involved in many physicochemical processes, including precipitation in the form of sulfides (except Cr^{3+}), carbonates and hydroxides; sorption on solids of the anaerobic system; formation of complexes in solution with intermediate metabolites, substrates and products formed during fermentation; binding to the cell wall of bacteria, etc. [6].

The role of iron in the vital activity of the anaerobic association of microorganisms is manifested in biological action in the composition of enzymes: electron transfer chains, hydrogenases, CO-dehydrogenase, methane monooxygenase, NO-reductase, superoxide dismutase, nitrite and nitrate reductase, etc. [7].

The most accepted mechanism of bioavailability of iron metal is related to corrosion: H_2, which is formed, can be used to reduce CO_2, which is an intermediate product in the stages of anaerobic fermentation, to methane by hydrogenotrophic methanogens [8].

During the accumulation of iron in the environment, microorganisms encapsulate the metal, which can damage the cell structure.

Iron (III) ions affect the granulation of anaerobic sludge due to the fact that they act as centers of granule formation, increase the diameter of the granules and improve its granularity [9].

Inhibition of the activity of microorganisms by iron (II) oxide can be caused by its high concentrations and as a subsequent pH value, which provides conditions that are incompatible with the development of bacteria [10].

Cu^{2+} is a high-potential oxidant (Eo) (Cu^{2+}/Cu^0 = +338 mV), which is able to replace divalent metals in the active centers of enzymes [11]. Microorganisms can reduce Cu (II) ions to Cu (I) and Cu (0) (for redox systems formed by Cu (II), Cu (I) are characterized by lower values of standard potentials—+160 mV Cu^{2+}/Cu^+, and + 518 mV for Cu^+/Cu^0) [12].

Microorganisms tightly regulate the concentration of cytoplasmic Cu^{2+} to minimize its toxic effects, while providing a sufficient amount of proteins containing copper ion (Cu-proteins). In turn, Cu proteins in bacteria are periplasmic or extracellular rather than cytoplasmic [11]. If the Cu^{2+} content becomes too high for the vital activity of microorganisms, the inclusion of cell defense mechanisms are activated: Cue (Cu efflux) system [13], CsoR (copper sensitive operon) system [14], oxidation of Cu (I) to obtain less toxic ion Cu (II) [12].

The process of methanogenesis at high concentrations of copper ions is influenced by factors such as the presence in the environment of chelating agents (inhibitory effect is observed at higher concentrations), density of microorganisms (with increasing concentration of microorganisms—the maximum allowable salt content increases), pH (at neutral value or slight leaching—permissible concentration of copper ions increases), the ratio of iron and copper ions (the higher the content of iron ions, the higher the value of the inhibitory concentration of copper ions).

Zn (II) is part of enzymes that affect phosphate, carbohydrate, protein metabolism, RNA and ribosome synthesis and regulation of redox potential of cells (hydrogenase, dehydrogenase formate, superoxide dismutase, etc.), it is a stabilizer of membrane components, determining their reactivity [7] [15] [16]. The minimum concentration of zinc ions that is required for different types of microorganisms varies widely.

Inhibitory content of zinc ions in the environment—3 - 400 mg/dm^3, toxic—250 - 600 mg/dm^3 depending on the species of microorganisms and living conditions [17].

Cr (III) is an important trace element required for glucose, lipid and amino acid metabolism, play a regulatory role in the processes of replication and transcription in microorganisms [18] [19] [20], but in high concentrations causes a negative effect on cellular structures. Cr (VI) is 1000 times more toxic and mutagenic than Cr (III) due to the oxidative capacity of compounds [21]. When chromium (III) methionine is added to the medium at a concentration of 0.5 - 1.5 mmol/dm^3, activation of anabolic processes, cellulose and amylolytic activity, cell growth of microorganisms is observed, as a result of which their mass increases [22]. Chromium (III) chloride in concentrations up to 1.0 mmol/dm^3 increases cell biomass growth. Increasing the concentration of chromium (III) salts to 2.5 mmol/dm^3 leads to inhibition of hydrolytic processes and cell growth.

Under anaerobic conditions, Cr (VI) can serve as the ultimate acceptor of electrons in the respiratory chain for a wide range of electron donors, including carbohydrates, proteins, fats, hydrogen, NAD(F)N [23], both cytoplasmic and membrane enzymes are involved in the reduction of Cr (VI) under anaerobic conditions [24].

For different types of anaerobic microorganisms and their associations, there is a range of heavy metal ions in the environment, which does not adversely affect biogas production: for Fe^{3+}—4 - 150 mg/dm^3, for Cr^{3+}—28 - 50 mg/dm^3; for Cu^{2+}—0.3 - 5 mg/dm^3, for Zn^{2+}—5 - 20 mg/dm^3.

The goal of the research is to establish the dependence of the yield of biogas and methane on the concentration of Me^+ ions in the anaerobic environment.

2. Materials and Methods

2.1. Microorganism Association

As an association of microorganisms for the research used a prepared suspension (inoculum) from fresh manure of one cow, which fed on field grass. Before preparing the suspension, the main parameters of manure were determined (**Table 1**): dry matter (DM) (GOST 26713-85 1986 [25]), dry organic matter (DOM) (GOST 26712-94 1986 [26]), ash content (A) (GOST 26714-85 1986 [27]), pH were determined using a stationary laboratory ionometer MI-150 (RF). To obtain a suspension, 1 kg of manure was placed in a 5 liter plastic bottle and added 3.6 dm^3 of distilled water. The mixture was manually stirred and the pH was determined (pH range should be 6.5 - 7.5). The bottle was placed in a thermostat TC-80M (without lighting, temperature 37˚C ± 1˚C) for 60 days, periodically draining the biogas formed in the bottle and stirring the medium with oscillating movements. After 60 days, the inoculum was selected to determine DM, DOM, A and pH (**Table 1**).

2.2. Anaerobic Fermentation with Metal Ions

To study the effect of metal ions on the process of anaerobic fermentation used laboratory methanetanks volume 1.0 dm^3, occupancy rate 70%, with wet-type gasholder. The concentration of DOM in the methane tank was 8%. For this purpose, 300 grams of manure, 200 ml of homogenized inoculum and 200 ml of distilled water, the required concentration of metal salt were placed in each methanetank (**Table 2**). The mixture was stirred, pH was measured (if necessary, adjusted with 0.1 M NaOH or HCl to pH 7). Hay-fed cow manure was used to study the effect of iron and copper ions, and grass-fed cow manure was used to study the effect of chromium and zinc ions. The hermetically sealed methanetank

Table 1. Parameters of raw materials and inoculums.

Parameter	Cattle manure		Inoculum
	Food—grass	Food—hay	
CP, %	16.49 ± 0.82	17.04 ± 0.85	1.54 ± 0.08
COP, %	13.66 ± 0.68	13.87 ± 0.69	1.00 ± 0.05
A, %	17.17 ± 0.85	18.58 ± 0.92	14.71 ± 0.74
pH	7.2 ± 0.36	7.1 ± 0.35	7.7 ± 0.39

Table 2. Concentration of metal ions in the anaerobic system.

Metal ion source	Experimental concentration, mg/dm^3
Fe^{3+}: $FeCl_3 \cdot 6H_2O$	20, 40, 60, 80,
Cu^{2+}: $Cu(NO_3)_2 \cdot 6H_2O$	10, 20, 40, 60, 80
Zn^{2+}: $ZnSO_4 \cdot 7H_2O$	10, 20, 50
Cr^{3+}: $Cr_2(SO_4)_3 \cdot 6H_2O$	10, 20, 35, 50

was placed in a thermostat at a temperature of 37˚C ± 1˚C. The medium was stirred daily. Biogas yields were recorded during the explication period of the experiment: 22 days for medium containing iron and copper ions, 13 days for medium containing zinc and chromium ions.

2.3. Methane Measurements in Biogas

The qualitative composition of biogas was measured at maximum yield: on the 7th day in the study of the influence of copper ions, on 5—iron (III), on 10—zinc ions and on 8—chromium (III). Biogas analyzer SAZQ (China) was used to measure methane in biogas.

3. Results

3.1. The Effect of Iron Ions

The effect of different concentrations of iron ions on the fermentation process of cattle manure is shown in **Figure 1**. As a control point for the experiment used the sample without metals**.**

3.2. The Effect of Copper Ions

The effect of different concentrations of copper ions on the fermentation process is shown in **Figure 2**.

3.3. The Effect of Zinc Ions

The effect of different concentrations of Zn^{2+} on the fermentation process is shown in **Figure 3**.

3.4. The Effect of Cr^{3+}

The effect of different concentrations of chromium (III) on the fermentation process is shown in **Figure 4**.

4. Discussion

As shown in **Figure 1(a)**, Fe^{3+} has a positive effect on the activity of the anaerobic association of microorganisms in the anaerobic fermentation of cattle manure. Compared to the sample without the addition of iron salts, the samples under study have a higher biogas yield. In samples containing 40 and 60 mg/dm^3 Fe^{3+}, biogas formation began on the day of fermentation. At a content of 20 mg/dm^3 Fe^{3+}, biogas formation occurred starting from 2 days. At a content of 80 mg/dm^3 Fe^{3+}, the yield of biogas slows down.

As shown in **Figure 1(b)**, the highest yield of biogas 4300 dm^3 for the entire fermentation period was obtained at a content of 20 mg/dm^3 Fe^{3+}, which is 76.79 ± 3.84 dm^3/g DOM (**Figure 1(c)**), and 35% more compared to biogas yield in the control sample. When the concentration of iron ions increases to 60 mg/dm^3, the yield of biogas and methane content in it decreases. At the same time with increasing content of iron ions decreases the methane content in biogas (**Figure 1(b)**). At such concentrations of iron ions, the yield of biogas exceeds the yield of the control sample by 19.3% and reaches the values of 67.86 ± 3.39 dm^3/g DOM (**Figure 1(c)**). Based on the fact that gas formation decreases after 9 days of fermentation,

Figure 1. (a) Daily yield of biogas (V) at different concentrations of Fe^{3+}; (b) Total yield of biogas (V) and methane (V) for 22 days of fermentation at different concentrations of Fe^{3+}; (c) Total biogas yield for 22 days of fermentation per 1 gram of DOM.

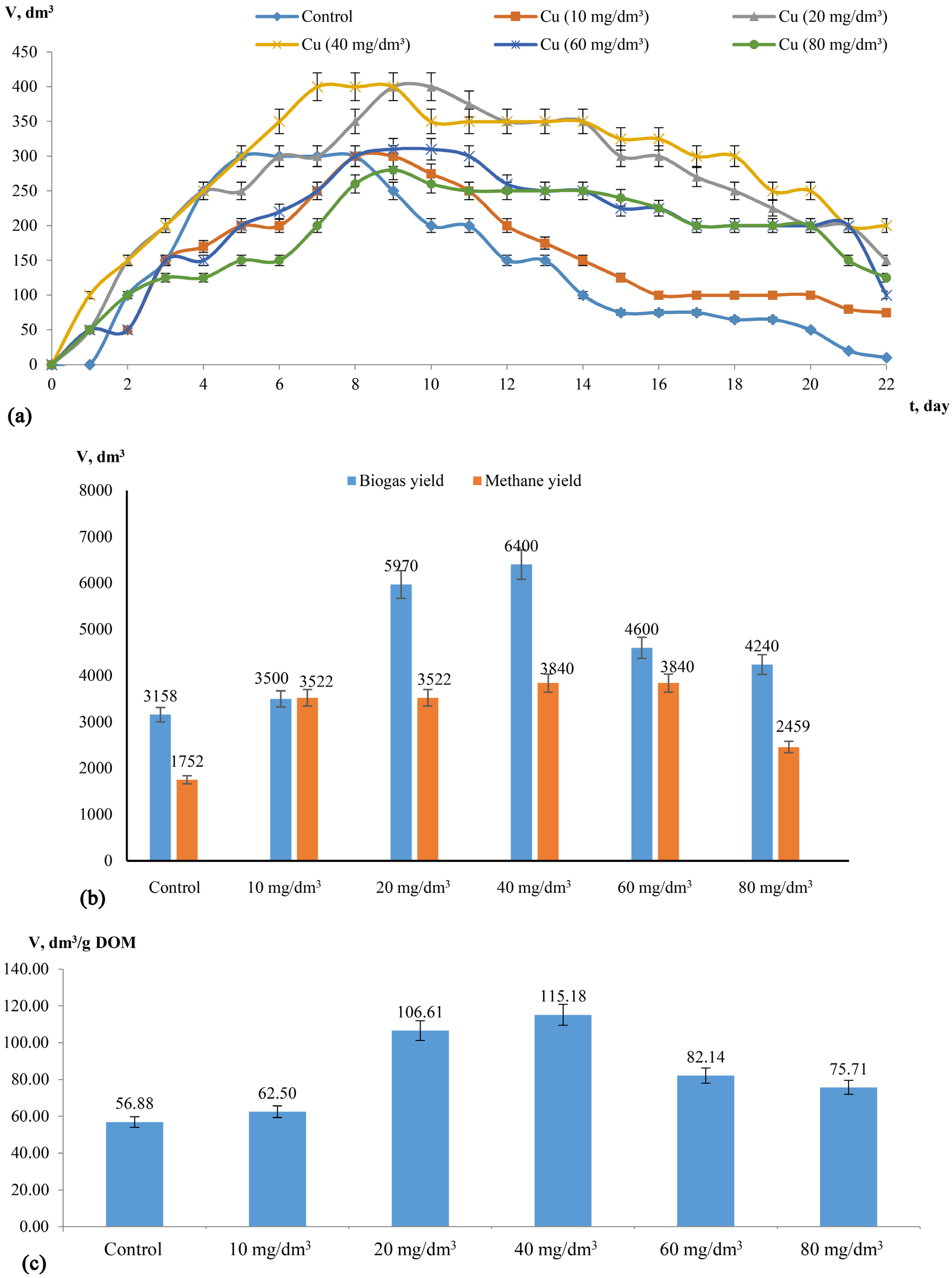

Figure 2. (a) Daily yield of biogas (V) at different concentrations of Cu^{2+}; (b) Total yield of biogas (V) and methane (V) for 22 days of fermentation at different concentrations of Cu^{2+}; (c) Total biogas yield for 22 days of fermentation per 1 gram of DOM.

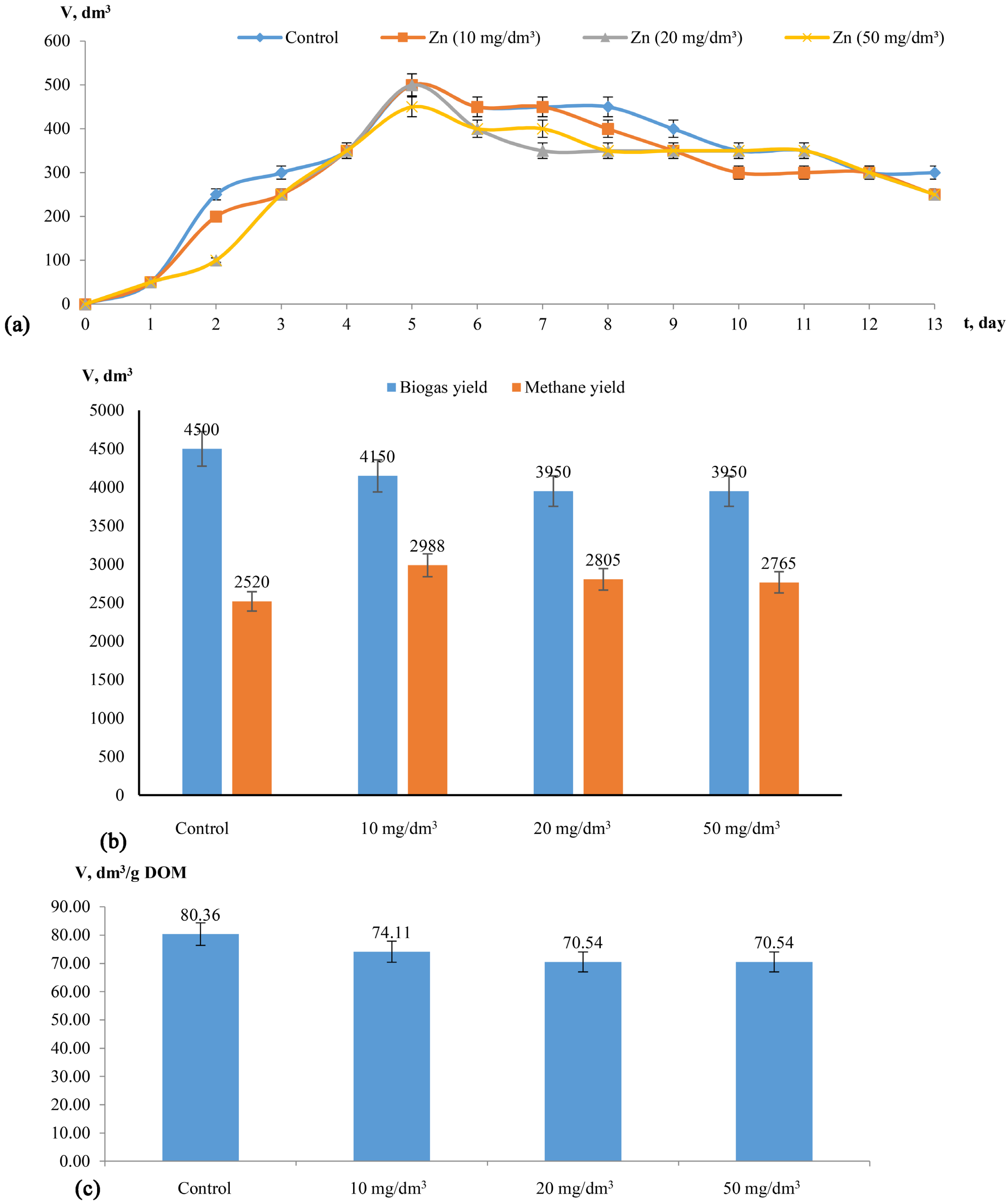

Figure 3. (a) Daily yield of biogas (V) at different concentrations of Zn^{2+}; (b) Total yield of biogas (V) and methane (V) for 22 days of fermentation at different concentrations of Zn^{2+}, (c) Total biogas yield for 13 days of fermentation per 1 gram of DOM.

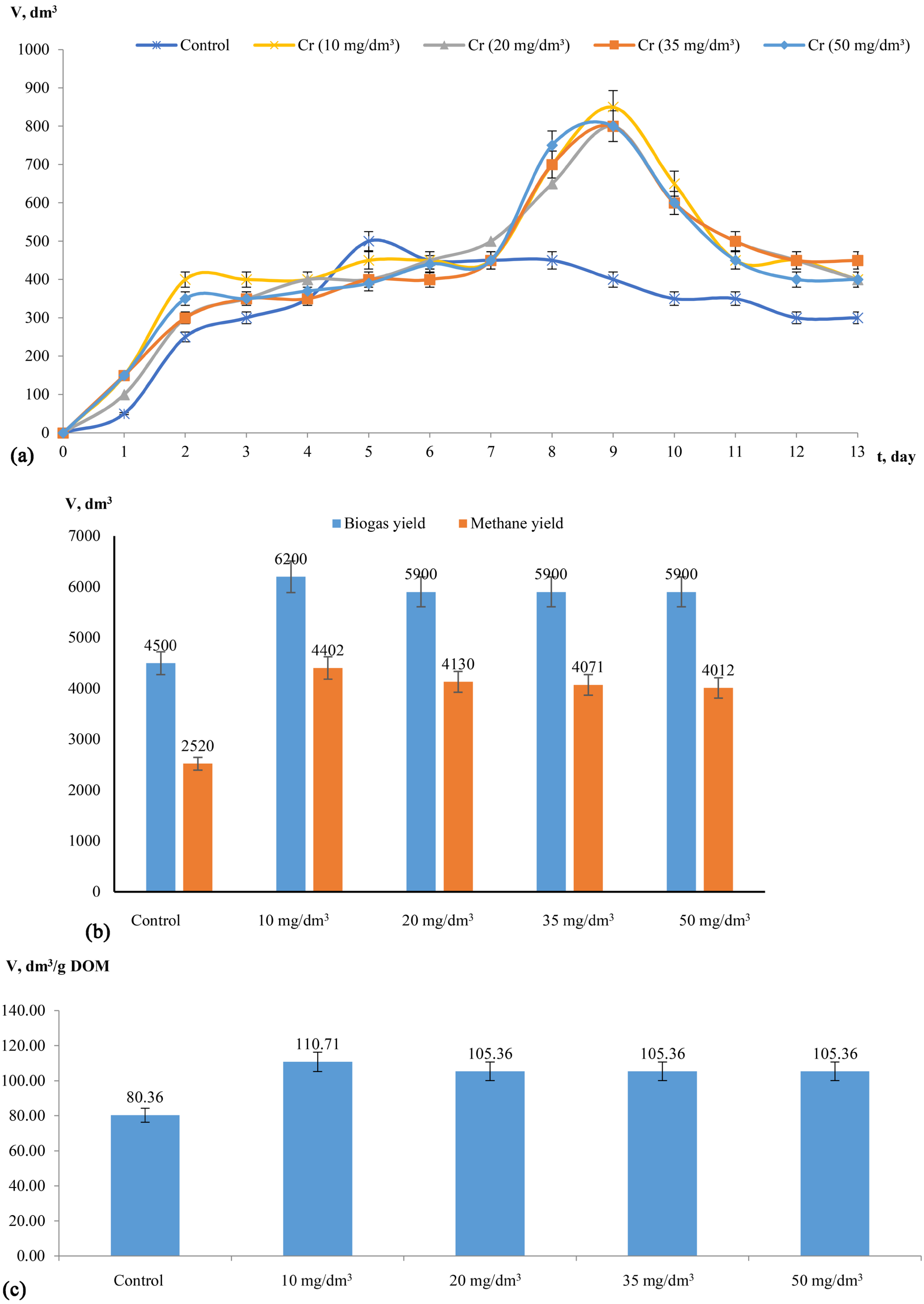

Figure 4. (a) Daily yield of biogas (V) at different concentrations of Cr^{3+}; (b) Total yield of biogas (V) and methane (V) for 22 days of fermentation at different concentrations of Cr^{3+}, (c) Total biogas yield for 13 days of fermentation per 1 gram of DOM.

it can be assumed that Fe^{3+} has a more positive effect on acetogenic and acidogenic microorganisms of the association, due to participation in a number of enzymes: hydrogenase (absorption or release of H_2), monoxide dehydrogenase (CODH)—an enzyme for the formation of acetic acid by anaerobic bacteria [7]. A slight increase in biogas yield after 16 and 20 days for samples containing iron ions of 20 and 40 mg/dm^3, respectively, may be associated with hydrogenotrophic methanogens that use hydrogen and CO_2 to form methane, because the concentration of iron ions decreases in the process of fermentation due to the increase in biomass.

As shown in **Figure 2**, a, the content of 40 mg/dm^3 Cu^{2+} in the anaerobic system has a positive effect on the biogas yield. At the same time, the methane yield is the same for Cu^{2+} 40 and 60 mg/dm^3 concentrations. That is, when the content of copper ions increases to 60 mg/dm^3, the concentration of methane in biogas increases and reaches 83%. While at a content of 40 mg/dm^3 of copper ions, the concentration of methane is 60%, as for other concentrations. Based on the fact that the methane content in biogas increases while reducing its yield, it can be assumed that this concentration of copper ions has a negative effect on microorganisms—hydrogen consumers, which are contained in the association, thereby increasing methane production from CO_2 and hydrogen. This leads to increased methane yield and reduced CO_2 content in biogas.

Copper ions (**Figure 2(b)**) affect methanogenic microorganisms at a concentration of 80 mg/dm^3, the concentration of methane in biogas begins to reduce, also reduces the yield of biogas. The concentration of Cu^{2+} 10 mg/dm^3 slightly improves biogas yield (**Figure 2(b)**), but has a positive effect on methane yield in biogas (**Figure 2(b)**). Instead of the expected inhibitory effect, we obtained a stimulating effect on the yield of biogas and biomethane, which can be explained by the formation of complex copper compounds with environmental components contained in raw materials and high concentrations of microorganisms in the system and sufficient Cu proteins [11].

Another mechanism of general resistance to copper is the diversity of anaerobic association, as evidenced by the lack of inhibition of *Methanobacterium thermoautotrophicum* in cocultivation with sulfate-reducing bacteria *Desulfotomaculum spp*. at a concentration of Cu^{2+} in a medium of 63.5 mg/dm^3, which causes complete inhibition of *Methanobacterium thermoautotrophicumt* [28]. In this case, part of the hydrogen sulfide reacts with metal ions in the environment with the formation of metal sulfides, so the inhibitory effect on microorganisms is reduced [29]. *Desulfovibrio* strain *R*2 is resistant to 800 mg/dm^3 Cu^{2+} because it contains the plasmid gene for resistance to Cu^{2+} [30]. The content of copper ions in superoxide dismutase (SODM) and hydrogenase in methanogens [31] may also affect the lack of inhibition.

Given the data obtained, it can be taken into account that the concentration range of Cu^{2+} 20… 80 mg/dm^3 increases the biogas yield from 56.88 dm^3/g DOM (control) to 114.29 dm^3/g DOM (**Figure 2(c)**), that can be used in the production of micronutrients for biogas plants.

Figure 3(a) shows that the addition of Zn^{2+} does not stimulate biogas production in the anaerobic association of microorganisms, and even at the lowest concentration (10 mg/dm^3) biogas yield decreases from 80.36 dm^3/g DOM (control sample) to 74.11 dm^3/g DOM, (Figure 3(c)). This confirms the fact that the inhibitory effect of zinc ions on microorganisms at concentrations of 3 mg/dm^3 in the environment [17]. In this case, the methane content in biogas increases from 56% in the control to 72% at a zinc concentration of 10 mg/dm^3 (Figure 3(b)). Concentrations of 20 and 50 mg/dm^3 have the same biogas yield for 13 days of fermentation (Figure 3(b)), but at 20 mg/dm^3 the methane yield is slightly higher than at 50 mg/dm^3 (Figure 3(b)). It is also worth noting that the action of zinc ions increases the content of H_2 in biogas and reaches 7% - 10%, which indicates a positive effect of zinc ions on hydrogen-producing microorganisms.

As shown in Figure 4(a), Cr^{3+} has a stimulating effect on the activity of the association of microorganisms, including methanogens, which is manifested by peak biogas yields for 8 - 10 days for all test samples containing chromium salts. At the same time, the peak value of biogas yield is almost twice the value of the control sample. This confirms the fact that Cr (III) is involved in the metabolism of organic compounds, which can promote the growth of cells of microorganisms, thereby increasing their productivity [22].

Methane production also increases (Figure 4(b)) with the addition of chromium salts: if for the control sample the methane content in biogas was 56%, then for the tested samples—not less than 68%. Figure 4(b) and Figure 4(c) show that the biogas yield at a concentration of 10 mg/dm^3 is 37.8% higher than the control values and 5% higher than the biogas yield in the concentration range of 20 - 50 mg/dm^3. But increasing the concentration of chromium ions, despite the same biogas yield at this concentration range, leads to a decrease in methane content from 70% for 20 mg/dm^3 to 68% for 50 mg/dm^3.

5. Conclusions

It is shown that ions of such metals as Fe^{3+}, Cu^{2+}, Cr^{3+} have a positive effect on the yield of biogas and methane content in it. All experimental concentrations of Fe^{3+}, Cu^{2+}, Cr^{3+} and Zn^{2+} ions lead to increased methane production by the anaerobic association of microorganisms due to the effect on the activity of enzyme systems.

Rational concentrations to increase methane yield in biogas are: Fe^{3+}—20 - 40 mg/dm^3, Cu^{2+}—40 - 60 mg/dm^3, Cr^{3+}—10 mg/dm^3. Zinc ions have a positive effect on methane production, but reduce the overall yield of biogas and, accordingly, the degree of conversion of organic raw materials. Therefore, the rational concentration of Zn^{2+} is 10 mg/dm^3.

Author Contributions

All authors contributed to the concept and design of the study. Experiment planning performed by N. B. Golub, M. V. Kozlovets. Preparation of material, construction of laboratory installation performed by A. V. Shynkarchuk, O. A. Kozlovets. The experiment, data collection was performed by A. V. Shynkarchuk, O. A. Kozlovets. Data analysis was performed by M. V. Kozlovets, N. B. Golub. The first draft of the manuscript was written by A. V. Shynkarchuk, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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