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Effect of Fenton's reagent on the intensification of the hydrolysis phase of methane fermentation of excess sludge and microbiological indicators

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ABSTRACT

Sludge oxidation with Fenton's reagent leads to the production of hydroxyl radicals, which are a strong oxidizing agent and thus reduce the time of final degradation of organic pollutants that are difficult to decompose. The aim of the study was to demonstrate the effect of in-depth oxidation with Fenton's reagent on the course of hydrolysis and the microbiological indicators of excess sludge subjected to methane fermentation compared to conventional fermentation. In the case of oxidation of excess sludge with Fenton's reagent, the iron ion dose of 0.08 g·Fe^{2+}/g total solids (TS) was considered the most favorable process condition, with a Fe^{2+}:H_2O_2 ratio of 1:5. A 28% degree of sludge disintegration, a 7-fold increase in the value of soluble chemical oxygen demand, and a 3-fold increase in the concentration of volatile fatty acids were observed compared to the initial values. The use of higher doses of Fe^{2+} ions, that is, 0.1 and 0.12 g·Fe^{2+}/g TS, and a proportion of Fe^{2+}:H_2O_2 greater than 1:5 did not increase the process efficiency. The disintegration of excess sludge with Fenton's reagent using a dose of 0.08 g of Fe^{2+} ions and hydrogen peroxide at a ratio of 1:5 resulted in the reduction of the number of microorganisms in individual groups. The highest decrease was recorded in the group of mesophilic microorganisms, from the initial value of 70 × 10^4 colony-forming units (CFU)/cm^3 before the process to 30 × 10^4 CFU/cm^3 after disintegration.

Keywords: Fenton's reagent; Excess sludge; Hydrolysis; Methane fermentation; Microbial indicator

1. Introduction

The currently observed intensive production of sewage sludge poses a challenge to conventional sludge treatment technologies. The upward trend in sludge generation is caused by rapid demographic and industrial development. As a by-product of wastewater treatment, sludge contains a variety of contaminants, has a high organic content, and is characterized by high water content. Different chemical compositions and properties of sewage sludge impose the need to apply different technological solutions during its disposal and processing. Sludge with a high content of decomposable organic matter is subjected to stabilization. These processes are multi-step in nature in terms of both physico-chemical and biological transformations [1].

Wastewater treatment in biological reactors leads to the production of excess sludge, which is transferred in the technological line of wastewater treatment to separate closed digesters. This sludge is formed with the growth of microorganisms when dissolved and colloidal contaminants are removed from wastewater. Depending on the treatment methods used, excess sludge contains about 97% water and between 30% and 50% mineral matter. Furthermore, it is characterized by a significant amount of facultative bacteria, which affects their low susceptibility to decomposition under anaerobic conditions. To accelerate the degradation of macromolecular compounds before the process of anaerobic

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stabilization, excess sludge is subjected to initial disintegration during which the walls and cell membranes of microorganisms are destroyed and organic compounds are released from their interior [2–5]. According to Kocwa-Haluk and Woźniakiewicz [6], there are more than 300 types of microorganisms in excess sludge and they account for 5%–25% of the organic matter, with the remaining part being dead organic matter. The low reduction of organic compounds in excess sludge and the low biogas production are due to the ability of heterotrophic bacteria to survive in the anaerobic environment. Therefore, a prerequisite for increasing the efficiency of anaerobic stabilization is the destruction of relatively anaerobic bacteria [7,8].

Disintegration is a method that offers an increase in the efficiency of biochemical degradation of sludge under anaerobic conditions. The idea of disintegration is to release organic substances from the cells of microorganisms living in excess sludge. Disintegration leads to the fragmentation of sludge flocs, the destruction of microbial cell walls, and the release of intracellular substances into the supernatant liquid. Organic matter provides a substrate for heterotrophic microorganisms that utilize it in the methane fermentation process. Methane fermentation is the key method of sludge disposal in large wastewater treatment plants, that is, with a capacity of over 15,000 m^3/d.

Intensive research is currently being conducted on disintegration based on both stand-alone and hybrid methods. Indicators such as soluble chemical oxygen demand (SCOD) and concentration of volatile fatty acids (VFAs) are important criteria for evaluating sludge degradation. As the values of these indices increase, an increase in the susceptibility of excess sludge to biochemical decomposition under anaerobic conditions is observed. An increase in the concentration of organic matter in the dissolved form in disintegrated sludge occurs due to the initiation of processes of a lysis nature [9–14]. According to Müller et al. [15], the semi-rigid structure of the cell envelope is a barrier against osmotic lysis. Furthermore, according to Chen et al. [16] the hydraulic retention time (HRT) is one of the key parameters in the methane fermentation of sludge and determines the reactor efficiency, expressed through the production of biogas and the reduction of volatile solids. A high HRT value of about 20–30 d is necessary to obtain a 30%–50% degree of fermentation of excess sludge. Therefore, it is necessary to support the process of methane fermentation through the process of cell lysis leading to increased bioavailability of polymeric substances (EPS) [17–19].

The high rate of degradation of organic substances, as well as the versatility and high efficiency, make the advanced oxidation method with the use of Fenton's reagent a promising technology applicable in wastewater and sludge treatment [20,21].

According to Michalska et al. [22], it is possible to use the classic Fenton process in the treatment of highly loaded industrial wastewater, disposal of landfill leachate, degradation of pesticides and herbicides, degradation of plant materials, and pre-treatment of sludge. During oxidation, Fenton's reagent destroys the cells of the excess sludge microorganisms, which leads to the release of intercellular material [23].

The Fenton process is one of the advanced oxidation methods, based on the use of hydrogen peroxide and the reaction catalyst in the form of iron ions. The essence of the process is the production of free hydroxyl radicals and obtaining a high degree of oxidation with a potential of 2.8 V. In the presence of Fe^{2+} or Fe^{3+} ions in the water environment, the decomposition of hydrogen peroxide into water and oxygen is observed [24–26]. Hydrogen peroxide is a genotoxic agent, while the decomposition of hydrogen peroxide causes the release of hydroxyl radicals ($^{\bullet}OH$). During the Fenton process, a faster release of radicals is obtained in an acidic environment with a pH ranging from 3 to 5. Free hydroxyl radicals are characterized by strong redox potential and cause DNA chains of microorganisms to break in the sludge. Free radicals also cause permeabilization of the cell membrane, DNA damage, altered membrane fluidity, and induction of apoptosis [27,28].

The Fenton process involves two parallel processes, coagulation and oxidation, affecting changes in the concentration of organic matter, especially in the supernatant. The hydroxyl radicals $^{\bullet}OH$ are highly reactive but slowly mobile forms. Therefore, the organic matter present in the supernatant is first subjected to the advanced oxidation process [29,30].

The Fenton reaction produces the hydroxyl radicals in acidic solution by iron catalyzed decomposition of H_2O_2 according to the reaction [31]:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^- \quad (1)$$

The hydroxyl radicals formed according to reaction (1) react with the target organics. The $^{\bullet}OH$ radical is the main reactant in a process capable of decomposing a wide range of organic substances by oxidation [31].

According to Eq. (2), Fe^{3+} ions can again be reduced to Fe^{2+} (2). Almost all chemical compounds can react with hydroxyl radicals, which is directly determined by their high oxidation potential [32].

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + {}^{\bullet}HO_2 + H^+ \quad (2)$$

The speed and extent of the Fenton reaction depend on the iron concentration, the dose of hydrogen peroxide, and the pH of the solution [31].

As a result of chemically disintegrated wastewater treatment with Fenton's reagent and then methane fermentation, Zieliński et al. [21] observed a significant impact of the tested disintegration method on increasing the total biogas production.

Subjecting excess sludge to disintegration leads to an increase in methane fermentation efficiency and improves its susceptibility to dewatering [33,34]. Excess sludge, characterized by a flocculent structure and composed of polymer-agglomerated microbial cells, is resistant to biodegradation. To increase the susceptibility to biochemical decomposition under anaerobic conditions, excess sludge should be subjected to the modification that allows for breaking down the macromolecular structure of the sludge flocs and releasing the organic matter from the microbial cells.

Therefore, the aim of the study was to demonstrate the effect of in-depth oxidation with Fenton's reagent on the course of hydrolysis and the microbiological indicators of excess sludge subjected to methane fermentation compared to conventional fermentation.

2. Experimental part

2.1. Substrate

Excess sludge from a wastewater treatment plant with a daily wastewater flow of approximately 50,000 m^3 was used as the substrate. The tested excess sludge was collected from the sludge delivery pipeline to the mechanical thickener. The digested sludge was collected from a pipeline transporting sludge from separate closed digesters to separate open digesters. Sampling was done randomly and once, and the samples were subjected to technological analysis and testing on the day of sampling. Triplicates of selected physico-chemical determinations were performed and standard deviations were evaluated. Table 1 presents a general characterization of excess sludge.

Excess sludge and digested sludge were tested for microbiological composition. Quantitative analysis was performed for mesophilic, psychrophilic, *Salmonella* spp., and *Escherichia coli* microorganisms. Characterization of selected groups of microorganisms in excess sludge is shown in Table 2.

2.2. Physical and chemical analysis of excess sludge

In the case of disintegration of excess sludge with Fenton's reagent, analyses of selected indicators such as SCOD and VFA were performed. These indicators express the degree of liquefaction of organic substances contained in the sludge. Based on the results obtained, the most favorable conditions for the disintegration of excess sludge were determined.

Table 1
Selected physical and chemical parameters of sewage sludge

Symbols	Excess sludge	Digested sludge
pH	6.85	7.50
TS, g/dm^3	12.3	25.11
VSS, g/dm^3	8.24	14.22
Acidity, mval/dm^3	0.8	6.4
Alkalinity, mval/dm^3	4.4	68.75
VFAs, CH_3COOH/dm^3	51	854
SCOD, mg·O_2/dm^3	164	1,872
TP, mg·P/dm^3	8.04	12.36
Ammonium nitrogen, mg·N–NH^{+4}/dm^3	45	567
Total Kjeldahl nitrogen, mg/dm^3	95	754

TS – total solids; VSS – volatile suspended solids; VFAs – volatile fatty acids; SCOD – soluble chemical oxygen demand; TP – total phosphorus.

The course of sludge methane fermentation was controlled based on physical and chemical analyses and microbiological indicators. For selected physico-chemical determinations, the sludge after methane fermentation was stirred to homogenize and the temperature was reduced to 25°C. The pH of the sludge was evaluated using Elmetron pH-meter, type CP-411, according to the PN-EN 12176 standard [35]. The content of water, dry mass, and mineral and organic compounds in the sludge was measured according to the PN-75/C-04616/01 standard [36]. In the centrifuged supernatant liquid, total alkalinity and acidity were determined according to PN-91/C-04540/05 [37], volatile fatty acids – according to PN-75/C-04616/04 [38] and ammonium nitrogen – according to PN-73/C-04576/02 [39]. For the determination of total phosphorus, total Kjeldahl nitrogen, and SCOD, samples of the supernatant liquid were subjected to a wet digestion process using strongly oxidizing mineral acids. SCOD and total phosphorus values were measured using a HACH DR/4000 Spectrophotometer (HACH LANGE). SCOD values were recorded at a wavelength of 620 nm, while for total phosphorus, absorbance was measured at a wavelength of 690 nm, and the concentration of phosphate contained in the sample was read from the calibration curve.

Soluble chemical oxygen demand (SCOD) was determined by the dichromate method using tests for HACH 2I00N IS Spectrophotometer according to ISO 7027 [40]. Determination of total Kjeldahl nitrogen was performed by the titration method in accordance with PN-EN 13342 standard [41].

The degree of digestion of the tested sludge was calculated according to the PN-75/C-04616/07 [42] and the following formula:

$$S_1 = 100 \cdot \left[1 - \frac{\left(a_1\left(100 - a\right)\right)}{\left(a\left(100 - a_1\right)\right)} \right] \quad (1)$$

where S_1 is the degree of sludge digestion, %; a_1 is the content of organic matter in digested sludge, %; a is the content of organic matter in the test sludge, %.

2.3. Microbiological analysis of excess sludge

Microbiological analysis was performed for un-modified and disintegrated sludge and the sludge subjected to

Table 2
Characteristics of selected groups of microorganisms in excess sludge

Microbiological determinations	Excess sludge	Digested sludge
Mesophilic microorganisms, CFU/cm^3	$30 \times 10^4 – 49 \times 10^6$	$10 \times 10^4 – 72 \times 10^6$
Psychrophilic microorganisms, CFU/cm^3	$80 \times 10^4 – 61 \times 10^6$	$10 \times 10^4 – 80 \times 10^6$
Escherichia coli, coliform titer	$10^{-3} – 10^{-5}$	$10^{-3} – 10^{-6}$
Salmonella spp.	Not isolated	Not isolated

methane fermentation in the next stage of the study. The number of mesophilic microorganisms, psychrophilic microorganisms, *Salmonella* spp. bacteria, and *Escherichia coli* type bacteria (which is a pathogenic species) was evaluated. A solid medium (nutrient agar) was used to culture mesophilic and psychrophilic microorganisms. *Escherichia coli* was cultured on Eijkman's liquid medium, and, to confirm the presence of microorganisms, the culture was performed on Endo solid medium. Before testing, several dilutions from 10^{-8} to 10^{-1} were prepared from the collected sludge sample. During the analysis of the presence of psychrophilic and mesophilic microorganisms, a sample of 0.1 mL was taken from each dilution and transferred into Petri dishes with a solid medium. Incubation of mesophilic microorganisms was performed for 24 h at 37°C. Psychrophilic microorganisms were incubated at 20°C for 72 h. After a specific period of time, the count of the bacterial colonies was estimated. The determination of the *Escherichia coli* titer was carried out using the tube fermentation method. A 1 mL sample from each dilution was inoculated onto Eijkman's liquid medium. The microorganisms were incubated at 20°C for 48 h. A change in color from purple to yellow indicated a positive test result, confirming the presence of microorganisms in the sample. To confirm the result, the transiently stained samples were transferred onto Petri dishes with Endo solid medium. Incubation was conducted for 24 h at 37°C. The appearance of red colonies with a characteristic metallic sheen was an indicator of a positive test result. *Salmonella-Shigella* (SS) agar was used to detect *Salmonella* spp. Test sludge samples were diluted with saline and then 0.1 cm^3 was inoculated onto the solid medium. The incubation was conducted for 48 h at a constant temperature of 37°C [43,44].

2.4. Conditions for disintegration with Fenton's reagent

The first stage of the study involved disintegration of excess sludge with Fenton's reagent, and the obtained results were the basis for determining the most favorable modification conditions, for which the highest value of soluble chemical oxygen demand (SCOD) and the increase in the concentration of VFAs were obtained.

The disintegration using Fenton's reagent was performed in glass vessels with an active volume of 0.25 L. The tested excess sludge was acidified with a 2-molar H_2SO_4 solution to a pH value of 3 before the oxidation process. A weighed amount of $FeSO_4 \cdot 7H_2O$ in the solid state was added to the acidified sludge and a specific dose of a 30% solution of H_2O_2, called perhydrol, was dosed. Reagent doses were determined relative to the dry mass content of the sludge. The disintegration was carried out for iron ion doses ranging from 0.02 to 0.14 g·Fe^{2+}/g total solids (TS). Hydrogen peroxide was measured in ratios of 1:1–1:10 relative to the weight of iron ions. The prepared excess sludge samples treated with an appropriate dose of iron ions and hydrogen peroxide were stirred with a magnetic stirrer at a constant stirring speed of 200 rpm. The reaction time for sludge oxidation was 60 min. After this time, the samples were alkalized with a 4-molar solution to a pH value 7 optimal for the methane fermentation of sewage sludge.

2.5. Methods for evaluation of the effectiveness of disintegration method

The effectiveness of the selected disintegration methods was evaluated by determining the increase in organic matter liquefaction expressed by the increase in SCOD value and VFAs concentration. Microscopic observation of the structure of the modified sludge was also an important tool for evaluating the effects of the disintegration methods used. Microscopic observations of the structure of unprocessed and modified excess sludge were performed with an Olympus BX41 microscope with instrumentation for taking photographs. Microphotographs were taken using a 500x magnification, and the assessment of changes in the structure of excess sludge was made using a visual method taking into account selected morphological features of the sludge.

2.6. Conditions for the anaerobic stabilization process

The examination concerned the course of methane fermentation conducted conventionally and supported by a high-efficiency oxidation method.

Methane fermentation of excess sludge was carried out under static conditions in specially designed methane fermentation systems that were models of digesters with an active volume of 0.5 L. The sludge was stabilized at a constant temperature of 37°C, typical of the processes conducted under mesophilic conditions. During the methane fermentation, excess sludge was mixed with digested sludge at a volume ratio of 10 to 1 to initiate the process. During anaerobic stabilization conducted in model digesters, selected physico-chemical determinations of sludge and microbiological indicators were performed on consecutive days of the process.

Anaerobic stabilization was performed for:

- raw excess sludge,
- excess sludge disintegrated using Fenton's reagent with an iron ion mass of 0.08 g·Fe^{2+}/g TS and a ratio of Fe^{2+}:H_2O_2 of 1:5.

3. Results and discussion

3.1. Sludge disintegration using Fenton's reagent

The disintegration of excess sludge was conducted using a strong oxidizing agent (Fenton's reagent). The effect of Fenton's reagent on the disintegration efficiency of the excess sludge was determined by the increase in SCOD and VFA concentration. According to Şahinkaya et al. [45], the disintegration of the excess sludge subjected to oxidation in the Fenton process proceeded in two stages through both processes: fast and successive slow disintegration stages. The rapid disintegration step of the sludge can be described according to a zero-order kinetic model. The SCOD values obtained from the oxidation of excess sludge are shown in Fig. 1.

Modification of the excess sludge with Fenton's reagent resulted in an increase in soluble organic

matter concentration determined based on SCOD values. The increase in the index occurred with the increase of applied Fe^{2+} ion doses and H_2O_2 dose. A similar trend was observed by Winterbourn et al. [46], who stated that the Fe dosage enhanced the sludge disintegration by the Fenton reaction as well as the H_2O_2 dosage. Depending on the dose of hydrogen peroxide added to the excess sludge, a different pattern of oxidation of organic substances contained in the sludge was observed when the tested doses of iron ions were applied. In the case of the iron ion doses tested, for the Fe^{2+} dose of 0.02 Fe^{2+}·g/g TS, the smallest increase in SCOD values was obtained compared to other doses. On the other hand, oxidation with iron ion doses of 0.08, 0.10, and 0.12 g·Fe^{2+}/g TS showed an intensive increase in SCOD, already for using the smallest of the applied dose of hydrogen peroxide, with the ratio of Fe^{2+}:H_2O_2 of 1:1. Analysis of the effect of Fenton's reagent on the disintegration process reveals a significant increase in the degree of disintegration in the range of iron ion doses of 0.02–0.08 g·Fe^{2+}/g TS. Using an iron ion dose of 0.1 g·Fe^{2+}/g TS and 0.12 g·Fe^{2+}/g TS yielded no significant increase in the SCOD value compared to the iron ion dose of 0.08 g·Fe^{2+}/g TS. According to the literature data [44] the high ratio of iron to oxidized substrates may have contributed to the formation of so-called hydroxyl free radical scavengers and thus reduced process efficiency. It was found that for each iron ion dose used in the study, there was a limiting value of the ratio of iron ions to hydrogen peroxide above which there was no significant increase in SCOD values. The changes in SCOD values recorded during oxidation were used to evaluate the most favorable Fe^{2+}:H_2O_2 ratio for individual Fe^{2+} doses by determining the increment in SCOD of disintegrated sludge compared to un-modified sludge (Table 3).

Treating excess sludge with Fenton's reagent by using the range of iron ion doses of 0.02–0.06 g·Fe^{2+}/g TS yielded three-, four- and fivefold increases in SCOD values using the Fe^{2+}:H_2O_2 ratio of 1:3. The highest (sevenfold) increase in SCOD was obtained by oxidizing the excess sludge with a dose of iron ions of 0.08 g·Fe^{2+}/g TS while maintaining the Fe^{2+}:H_2O_2 ratio of 1:5. Application of higher doses of Fe^{2+} ions such as 0.1 and 0.12 g·Fe^{2+}/g TS, and the Fe^{2+}:H_2O_2 ratio higher than 1:5 did not increase process efficiency. Therefore, analysis of the relationship of SCODnon-ox./SCODox. found that the use of Fe^{2+} ion dose above 0.08 g/g TS does not increase the efficiency of oxidation of organic substances contained in excess sludge. The effect of Fenton's reagent on the disintegration of the excess sludge was also determined based on the increase in VFA concentration. The changes in VFA concentration recorded during the oxidation process are shown in Fig. 2.

It was found based on the results that the use of Fenton's reagent had a positive effect on the generation of VFAs during the oxidation of excess sludge. Increasing the proportion of iron ions and the dose of hydrogen peroxide during the process contributed to an increase in the concentration of VFAs.

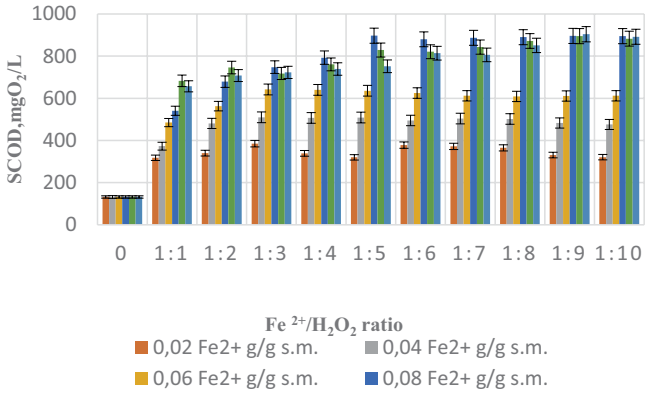

Fig. 1. Effect of Fenton's reagent on SCOD values depending on the ratio of chemical reagents used.

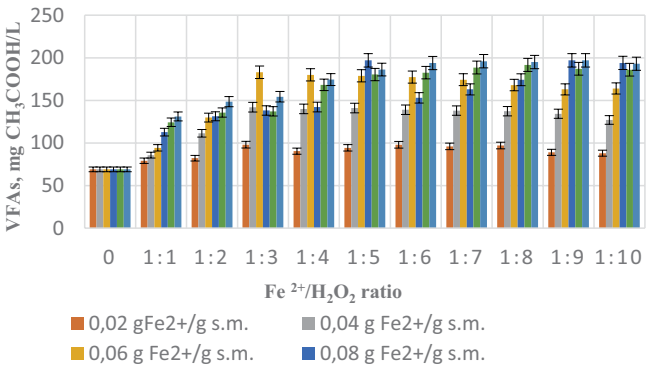

Fig. 2. Effect of Fenton's reagent on VFA concentration depending on the ratio of chemical reagents.

Table 3
Most favorable Fe^{2+}:H_2O_2 ratios for Fe^{2+} ion doses based on the increment in SCOD for sludge modified with Fenton's reagent

		Most favorable ratio of Fe^{2+}:H_2O_2	SCOD of non-modified sludge, mg·O_2/L	SCOD of oxidized sludge, mg·O_2/L	Relationship $SCOD_{non\text{-}ox.}/SCOD_{ox.}$
Dose of Fe^{2+}·g/g TS	0.02	1:3	132	385	1/3
	0.04	1:3	132	510	1/4
	0.06	1:3	132	642	1/5
	0.08	1:5	132	897	1/7
	0.10	1:9	132	895	1/7
	0.12	1:9	132	904	1/7

A similar trend was reported by Winterbourn et al. [46], who observed that oxidation with Fenton's reagent led to an increase in VFA concentration of lignocellulosic biomass with low biodegradability.

For the iron doses tested, the lowest increase in VFA concentration was obtained with an iron ion dose of 0.02 g·Fe^{2+}/g TS and the Fe^{2+}:H_2O_2 ratio of 1:1. Oxidation of sewage sludge at a higher range of doses of chemical reagents increased process efficiency. Disintegration using an iron ion dose of 0.08 g·Fe^{2+}/g TS while maintaining the Fe^{2+}:H_2O_2 ratio of 1:5 showed the highest (about threefold) increase in the concentration of VFAs. The use of higher doses of Fe^{2+} ions such as 0.10 and 0.12 g·Fe^{2+}/g TS and the Fe^{2+}:H_2O_2 ratio above 1:5 resulted in no increase in process efficiency for an iron ion dose of 0.08 g·Fe^{2+}/g TS. A similar trend was observed for SCOD of excess sludge modified by the method studied in the above-mentioned range of iron ion doses and the Fe^{2+}:H_2O_2 ratio.

For this purpose, Fenton's reagent was used, which is a strongly oxidizing mixture of hydrogen peroxide with iron ions. Doses of hydrogen peroxide were added to excess sludge at fixed ratios of 1:1 to 1:10 relative to a mass of Fe^{2+} iron ions. An iron ion dose ranging from 0.02 g·Fe^{2+}/g TS to 0.12 g·Fe^{2+}/g TS was used to determine the most favorable conditions for oxidation of excess sludge. It was found that the use of a strong oxidant in the form of Fenton's reagent leads to the increase in the concentration of organic matter in the supernatant liquid. As the volume of doses of Fe^{2+} ions and hydrogen peroxide solution added to the excess sludge increased, an increase in SCOD values was observed. The SCOD value was found to increase up to a certain threshold value, above which successive doses of oxidant added had no significant effect on increasing the disintegration rate of excess sludge. Oxidation of excess sludge with Fe^{2+} iron ion doses of 0.2, 0.4, and 0.6 g·Fe^{2+}/g at relatively low doses of hydrogen peroxide, that is, the Fe^{2+}:H_2O_2 ratio of 1:3 led to a 3, 4 and 5-fold increase in SCOD, respectively. The highest (7-fold) increase in SCOD of sludge oxidized with Fenton's reagent compared to SCOD of untreated sludge was obtained for sludge oxidized with an iron ion dose of 0.8 g·Fe^{2+}/g TS, while maintaining the Fe^{2+}:H_2O_2 ratio of 1:5. These conditions were found to be the most favorable for conducting the oxidation of excess sludge with the reagent studied. For iron ion doses of 0.10 and 0.12 g·Fe^{2+}/g TS, SCOD values remained comparable to those for the sludge prepared with a dose of 0.08 g·Fe^{2+}/g TS. It was found that the application of iron ion doses above 0.08 g·Fe^{2+}/g TS did not result in a higher than 7-fold increase in SCOD values. During oxidation of excess sludge with Fenton's reagent, in addition to the iron ion dose, SCOD values were found to be affected by the dose of hydrogen peroxide applied. It was observed that as the dose of hydrogen peroxide (H_2O_2) increased, the SCOD of the supernatant liquid increased up to a certain limiting value. It was found that the introduction of successively higher doses of hydrogen peroxide into excess sludge did not improve oxidation efficiency. At high doses of H_2O_2 relative to the oxidized substrate, hydrogen peroxide can become a binding agent for •OH radicals and cause their inactivation. Similar to excess hydrogen peroxide, excess iron ions can act as free radical scavengers which in turn can lead to a decrease in oxidation efficiency.

3.2. Effect of oxidation with Fenton's reagent on the structure of excess sludge

Microscopic examinations were performed to assess the structure of excess sludge not subjected to modification and changes occurring in the structure of sludge modified with Fenton's reagent. The un-modified excess sludge was characterized by a uniform structure, with a uniform dispersion of fine solid-phase particles observed throughout the liquid phase (Fig. 3). The analysis of microscopic preparations of the modified sludge showed a clear change in the sludge structure.

Microscopic analysis of chemically modified excess sludge was performed to demonstrate the disintegrating effect of Fenton's reagent on the structure of excess sludge. As a result of the application of advanced oxidation, significant modification of the microbial cells of the prepared excess sludge was observed. The substantial changes in sludge structure were the result of the oxidative effect of hydrogen peroxide. Furthermore, as reported Lu et al. [47] the disintegration of excess sludge was affected by iron ions acting as a catalyst for the Fenton process. The observations of the structure of excess sludge revealed that as the doses of the Fenton reagent increased, there was a fragmentation of the solid phase particles and gradual liquefaction of the sludge particles. For higher hydrogen peroxide concentrations (Fig. 4d–f), the strongly dispersed sludge particles aggregated to form compact clusters. With the oxidation process, the dispersed sludge particles completely covered the field of view of the preparation. Therefore, an iron ion dose of 0.08 g·Fe^{2+}/g TS and a ratio of Fe^{2+}:H_2O_2 1:5 was found to be sufficient for effective disintegration of excess sludge using the stand-alone chemical method. Fig. 4a–f show the structure of excess sludge disintegrated with Fenton's reagent using different oxidation parameters, that is, iron ion mass and Fe^{2+}:H_2O_2 ratio.

3.3. Anaerobic stabilization of excess sludge

Mesophilic methane fermentation was conducted to assess the susceptibility of unprepared and chemically disintegrated excess sludge to biochemical degradation occurring under anaerobic conditions. The sludge was stabilized for 10 d in laboratory flasks, which were used as models of digestion chambers. Selected physico-chemical parameters and microbiological indicators were analyzed before, during, and after the methane fermentation process. Mixtures of excess sludge and digested sludge were subjected to methane fermentation processes at a volume ratio of 10 to 1.

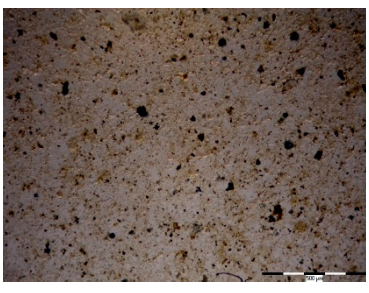

Fig. 3. Structure of un-modified excess sludge.

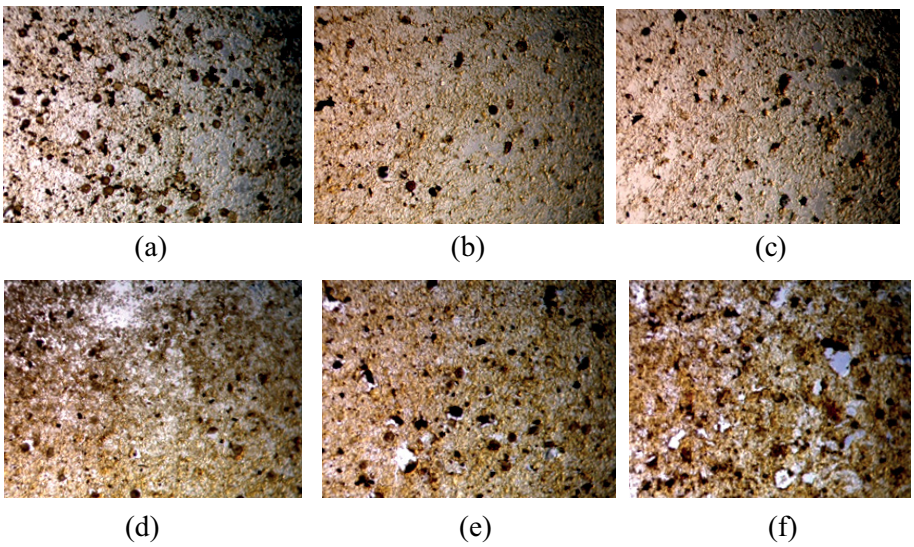

Fig. 4. Structure of excess sludge oxidized with Fenton's reagent using different oxidation parameters, that is, iron ion mass and Fe^{2+}:H_2O_2 ratios: (a) 0.02 g·Fe^{2+}/g TS, Fe^{2+}:H_2O_2 of 1:3, (b) 0.04 g·Fe^{2+}/g TS, Fe^{2+}:H_2O_2 1:3, (c) 0.06 g·Fe^{2+}/g TS, Fe^{2+}:H_2O_2 1:3, (d) 0.08 g·Fe^{2+}/g TS, Fe^{2+}:H_2O_2 1:5, (e) 0.10 g·Fe^{2+}/g TS, Fe^{2+}:H_2O_2 1:9, and (f) 0.12 g·Fe^{2+}/g TS, Fe^{2+}:H_2O_2 1:9.

3.3.1. Conventional methane fermentation of excess sludge

Methane fermentation was conducted for untreated excess sludge. This sludge is characterized by limited susceptibility to biochemical degradation in anaerobic conditions. The sludge digestion rate after 10 d of the process was ca. 16%. The reduction of sludge dry mass was 13%. An increase in dissolved organic matter concentration was found based on the changes in SCOD values during the first three days of methane fermentation. For sludge that was a mixture of excess and digested sludge, the SCOD was 354 mg·O_2/L, whereas a value of 934 mg·O_2/L was recorded on the third day of methane fermentation. On the last day of anaerobic stabilization, the SCOD value was 428 mg·O_2/L. The concentration of VFAs increased gradually until the third day of stabilization. Its value was about six times higher than the initial value determined in the sludge mixture (498 mg·CH_3COOH/L). On the last day of methane fermentation, the concentration of VFAs was 243 mg·CH_3COOH/L. An increase in the total alkalinity of the sludge was observed in the following days of methane fermentation. There was an increase in total acidity values of the sludge observed on 5 consecutive days, from 1.4 mval/L in mixed sludge to 4.8 mval/L on day 5 of fermentation, and a gradual decrease in total acidity on subsequent days of the process. Methane fermentation after 10 d of the process resulted in total acidity of 2.8 mval/L. During the methane fermentation process, an increase in the concentration of biogenic compounds expressed in the value of total nitrogen, ammonium nitrogen, and total phosphorus was observed. Compared to the levels before methane fermentation, the values of total nitrogen, ammonium nitrogen, and total phosphorus increased by about 64%, 77%, and 92%, respectively. Sludge pH values ranged from 6.84 to 7.05, which is appropriate for normal methane fermentation. A higher pH value ranging from 6.96 to 7.30 was recorded in the supernatant liquid. Changes in the values of selected physico-chemical parameters for conventional methane fermentation of excess sludge are presented in Table 4.

During methane fermentation, tests were performed to determine the number of microorganisms present in stabilized excess sludge. Microbiological analysis conducted during the process revealed a decrease in the number of mesophilic and psychrophilic microorganisms, and pathogenic *Escherichia coli* bacteria. The highest microbial inactivation effect was obtained for *Escherichia coli*. The titer of this group of microorganisms increased from 10^{-5} to 10^{-3}. Mesophilic microorganisms were highly resistant to anaerobic environmental conditions. In the case of methane fermentation, there was a slight loss of mesophilic microorganisms from 65–105 CFU/cm^3 before the process to 11–105 CFU/cm^3 on the last day of the process. A similar trend was observed for psychrophilic bacteria, with their number decreasing from 32×10^5 CFU/cm^3 to 10×10^4 CFU/cm^3 during stabilization. The results of the microbiological analysis of the mesophilic methane fermentation of excess sludge are shown in Table 5.

3.3.2. Methane fermentation of excess sludge oxidized with Fenton's reagent

According to the literature [48,49], the hydrolysis phase is considered as limiting methane fermentation. The sludge oxidation with Fenton's reagent intensified the hydrolysis phase of methane fermentation, expressed by an increase in the SCOD and VFA values on subsequent days of the process. As reported in the literature [50], increasing the rate of generation of volatile fatty acids, which are an intermediate product of methane fermentation, and the intensification of biogas production are directly reflected in the reduction of the technologically necessary HRT value.

Methane fermentation was carried out for excess sludge oxidized with Fenton's reagent at a dose of 0.08 g·Fe^{2+}/g TS. It was assumed that the use of a strong oxidizing reagent

Table 4
Changes in physico-chemical parameters of excess sludge in the process of conventional methane fermentation

Index/Unit	Excess sludge	Digested sludge	Sludge mixture	Methane fermentation time, d									
				1	2	3	4	5	6	7	8	9	10
TS, g/L	12.3 ± 0.1	20.41 ± 0.08	14.72 ± 0.21	14.06 ± 0.12	13.96 ± 0.23	13.67 ± 0.13	13.67 ± 0.03	13.28 ± 0.017	13.14 ± 0.014	12.98 ± 0.32	12.53 ± 0.01	12.12 ± 0.72	12.85 ± 0.23
VSS, g/L	8.24 ± 0.07	12.85 ± 0.34	8.79 ± 0.18	8.47 ± 0.13	7.89 ± 0.11	7.85 ± 0.35	7.75 ± 0.3	7.31 ± 0.08	7.19 ± 0.05	7.21 ± 0.2	6.98 ± 0.05	6.37 ± 0.43	7.45 ± 0.01
Acidity, mval/L	0.8 ± 0.06	3.8 ± 0.2	1.4 ± 0.42	3.0 ± 0.35	3.2 ± 0.14	3.8 ± 0.16	4.2 ± 0.4	4.8 ± 0.2	4.0 ± 0.34	4.4 ± 0.5	3.6 ± 0.18	3.0 ± 0.17	2.8 ± 0.04
Alkalinity, mval/L	4.4 ± 0.34	52 ± 3.86	6.8 ± 0.02	7.6 ± 0.1	9.0 ± 1.0	11.6 ± 0.16	12.8 ± 0.4	15.0 ± 0.42	15.2 ± 1.21	16.4 ± 1.29	18.8 ± 3.05	20.4 ± 0.4	21.2 ± 0.72
SCOD, mg·O_2/L	164 ± 7	1281 ± 28	224 ± 21	789 ± 17	916 ± 12	934 ± 24	929 ± 10	917 ± 13	785 ± 13	682 ± 16	591 ± 17	567 ± 20	543 ± 9
VSS, mg·CH_3COOH/L	51 ± 5	531 ± 12	87 ± 24	343 ± 12	462 ± 36	498 ± 26	497 ± 12	480 ± 24	454 ± 17	411 ± 26	360 ± 29	377 ± 12	360 ± 15
Total Kjeldahl nitrogen, mg·N/L	95 ± 0.39	638 ± 0.2	145 ± 0.79	280 ± 0.1	299 ± 10.88	325 ± 0.2	353 ± 1.4	375 ± 0.59	375 ± 1.19	381 ± 0.32	386 ± 9.89	386 ± 0.2	392 ± 0.2
Ammonium nitrogen, mg·N–NH^{+4}/L	45 ± 1.6	540 ± 0.8	78 ± 0.3	246 ± 0.2	302 ± 0.4	316 ± 0.2	322 ± 2.0	322 ± 1.2	324.8 ± 19.8	330 ± 4.0	333 ± 1.6	335 ± 0.2	339 ± 0.2
Total phosphorus, mg·P/L	8.04 ± 0.08	10.27 ± 0.11	2.28 ± 0.13	3.21 ± 0.23	4.20 ± 0.11	6.19 ± 0.15	7.13 ± 0.31	8.60 ± 0.28	9.86 ± 0.08	10.43 ± 0.21	14.78 ± 0.05	17.21 ± 0.06	19.48 ± 0.18
pH of the sludge	6.85	7.21	6.95	6.93	6.87	6.84	6.90	7.01	7.02	6.99	6.94	6.98	7.05
pH of the leachate	7.19	7.79	7.09	7.02	6.99	6.96	7.02	7.09	7.15	7.21	7.30	7.19	7.21

Table 5
Microbiological analysis of excess sludge for conventional methane fermentation

		Number of microorganisms, CFU/cm^3		Coliform titer
		Mesophilic microorganisms	Psychrophilic microorganisms	*Escherichia coli* bacteria
Excess sludge		44×10^5	50×10^5	10^{-5}
Digested sludge		14×10^6	80×10^5	10^{-6}
Sludge mixture		65×10^5	32×10^5	10^{-5}
Methane	1	38×10^5	21×10^5	10^{-5}
fermentation	5	24×10^5	18×10^5	10^{-4}
time, d	10	11×10^5	10×10^4	10^{-3}

would lead to the destruction of the cells of excess sludge microorganisms and the release of biological material from their interior, constituting a substrate for heterotrophic living matter in stabilization processes. According to the assumptions, the modification of excess sludge using the proposed method allows for the intensification of methane fermentation and the increase in the efficiency of the production of volatile fatty acids. According to Michalska et al. [22] the volatile fatty acids concentration after chemical pretreatment of biomass was high enough for production of biogas with a high methane content. Combined chemical oxidation and enzymatic hydrolysis increased the efficiency of the methane fermentation process of the studied biomass. After 10-d methane fermentation of excess sludge disintegrated with the highest dose of Fenton's reagent (0.08 g·Fe^{2+}/g TS) and the Fe^{2+}:H_2O_2 ratio of 1:5, sludge digestion rate was ca. 36%. The dry matter reduction rate was 26%. The initial water content of 98.61% increased after 10 d of stabilization to 98.95%. The highest concentration of organic compounds in the dissolved form was recorded on day 3 of the process. The SCOD value was 1,745 mg·O_2/L, more than 2.5 times the initial value, that is, for the sludge mixture. A decrease in the concentration of dissolved organic compounds was observed on subsequent days of methane fermentation. On the 4th day of the process, the highest value of VFAs (909 mg·CH_3COOH/L) was recorded, which translated into about a three times increase of the index compared to the initial value, that is, the sludge mixture. Total alkalinity increased steadily over subsequent days of stabilization. The total acidity of 0.7 mval/L for the sludge mixture peaked at 4.4 mval/L on day 5 of fermentation. During anaerobic stabilization, an increase in total Kjeldahl nitrogen and ammonium nitrogen was observed on subsequent days of the process. There was an increase in the values of these forms of nitrogen and total phosphorus compared to initial values. According to Şahinkaya et al. [45] biodegradability in the fermentation chamber increases because the distortion of sludge flocs, disintegration of bacterial cells, release of extracellular polymer substances, intracellular organic substances and divalent cations into the liquid phase of the sludge, disintegration of the sludge subjected to the oxidation process with Fenton's reagent.

The results of selected physico-chemical parameters of excess sludge disintegrated with Fenton's reagent at a dose of 0.08 g·Fe^{2+}/g TS under methane fermentation are presented in Table 6.

Stabilization of excess sludge disintegrated with Fenton's reagent using 0.08 g dose of Fe^{2+} ions and hydrogen peroxide at a ratio of 1:5 decreased the number of microorganisms in individual groups. The highest decrease was observed in the group of mesophilic microorganisms, from an initial value of 20×10^5 CFU/cm^3 to 54×10^3 CFU/cm^3 on the last day of methane fermentation. The number of psychrophilic microorganisms amounting to 50×10^6 CFU/cm^3 in the sludge mixture on the first 5 d of the process decreased slightly. A significant decrease in the number of microorganisms in this group to 70×10^5 CFU/cm^3 was recorded on the 10th day of stabilization. *Escherichia coli* titer increased slightly from 10^{-3} to 10^{-2} during the fermentation cycle. The quantitative analysis of selected groups of microorganisms is shown in Table 7.

During anaerobic stabilization, a decreasing trend in the number of microorganisms was observed in both un-modified and modified sludge.

It was found that subjecting the sludge to disintegration with Fenton's reagent had a destructive effect on the microbial cells. This led to a decrease in the number of individual microorganisms present in the sludge. In the case of using Fenton's reagent, hydroxyl free radicals with high oxidizing potential play an important role. They effectively act on the cell structure of microorganisms, causing their destruction and directly leading to the reduction of the number of microorganisms in the sludge [51].

To achieve higher rates of sludge treatment, bactericidal agents are used, usually in the form of chemical reagents [52]. The highest effectiveness of Fenton's reagent was observed for *Escherichia coli*. Treating sewage sludge with Fenton's reagent showed that the quantitative changes in this group of microorganisms depend directly on the oxidizing substance introduced into the sludge. The use of an iron ion dose of 0.08 g·Fe^{2+}/g TS and the Fe^{2+}:H_2O_2 ratio of 1:5 led to an increase in process efficiency. There was a significant reduction in the number of *Escherichia coli* microorganisms from 10^{-4} to 10^{-2}.

According to Feki et al. [53] advanced oxidation processes (AOPs) are considered an effective method of sludge pre-conditioning, reducing hydraulic retention time, and increasing methane production rates. Increasing the biodegradability of sewage sludge by modification with Fenton's reagent results in the production of hydroxyl radicals ($^{\bullet}OH$) which are capable of decomposing a number of organic substances through oxidation.

Table 6
Changes in physico-chemical parameters of excess sludge disintegrated with Fenton's reagent at a dose of 0.08 g·Fe^{2+}/g TS and the Fe^{2+}:H_2O_2 ratio of 1:5 in methane fermentation

Indicator/Unit	Excess sludge	Disintegrated sludge	Digested sludge	Sludge mixture	Methane fermentation time, d									
					1	2	3	4	5	6	7	8	9	10
TS, g/L	8.77 ± 0.02	13.25 ± 0.19	20.08 ± 0.17	13.91 ± 0.13	13.25 ± 0.01	13.10 ± 0.11	12.73 ± 0.18	12.18 ± 0.15	11.42 ± 0.34	11.29 ± 0.17	11.20 ± 0.04	11.03 ± 0.26	10.46 ± 0.3	10.30 ± 0.26
VSS, g/L	6.38 ± 0.11	6.77 ± 0.21	12.65 ± 0.16	7.38 ± 0.01	6.51 ± 0.04	6.21 ± 0.05	5.77 ± 0.3	5.45 ± 0.08	4.90 ± 0.18	4.84 ± 0.19	4.80 ± 0.05	4.87 ± 0.25	4.28 ± 0.4	4.11 ± 0.11
Acidity, mval/L	3.2 ± 0.28	2.2 ± 0.57	3.4 ± 0.57	0.7 ± 0.71	2.4 ± 0.03	2.6 ± 0.33	2.8 ± 0.85	2.6 ± 0.14	4.4 ± 0.28	2.8 ± 0.2	2.8 ± 0.7	2.6 ± 0.28	3.2 ± 0.8	2.7 ± 0.14
Alkalinity, mval/L	3.6 ± 0.11	0.4 ± 0.14	60.8 ± 0.61	4.8 ± 0.2	10.8 ± 0.5	15.2 ± 0.61	19.8 ± 0.35	26.8 ± 0.81	33.2 ± 0.53	35.6 ± 0.11	37.6 ± 0.6	40.4 ± 0.31	41.6 ± 0.53	48.0 ± 1.15
SCOD, mg·O_2/L	135 ± 1	859 ± 14	2,202 ± 10	1,080 ± 11	1,480 ± 24	1,520 ± 30	1,745 ± 21	1,334 ± 15	1,206 ± 5	1,018 ± 11	799 ± 4	654 ± 1	587 ± 11	529 ± 15
VFAs, mg·CH_3COOH/L	60 ± 15	129 ± 9	711 ± 23	277 ± 17	514 ± 13	669 ± 28	806 ± 9	909 ± 23	514 ± 13	463 ± 17	394 ± 15	380 ± 13	323 ± 5	296 ± 13
Total Kjeldahl nitrogen, mg·N/L	6 ± 0.4	48 ± 0.59	353 ± 3.96	76 ± 1.98	168 ± 3.17	193 ± 2.38	229 ± 0.1	238 ± 0.59	242 ± 0.2	252 ± 0.79	269 ± 1.19	277 ± 0.4	311 ± 0.79	344 ± 0.2
Ammonium nitrogen, mg·$N{\rightarrow}NH^{+4}$/L	2 ± 0.4	17 ± 0.02	269 ± 0.08	45 ± 0.4	118 ± 0.4	168 ± 0.3	214 ± 0.2	235 ± 0.2	238 ± 0.4	246 ± 0.8	252 ± 1.2	257 ± 0.6	276 ± 0.4	325 ± 0.8
Total phosphorus, mg·P/L	1.87 ± 0.08	0.41 ± 0.01	10.35 ± 0.21	0.92 ± 0.03	1.12 ± 0.07	1.47 ± 0.28	1.77 ± 0.04	1.84 ± 0.01	1.98 ± 0.1	2.19 ± 0.08	2.31 ± 0.04	2.44 ± 001	2.63 ± 0.1	4.67 ± 0.14
pH of the sludge	6.83	6.80	7.20	6.97	7.04	7.15	7.28	7.45	7.12	7.39	7.43	7.35	7.27	7.24
pH of the leachate	7.52	6.95	7.81	7.15	7.10	7.23	7.30	7.34	7.27	7.52	7.69	7.69	7.61	7.59

Table 7
Microbiological analysis of excess sludge disintegrated with Fenton's reagent at a dose of 0.08 g·Fe^{2+}/g TS and the Fe^{2+}:H_2O_2 ratio of 1:5 in methane fermentation

		Number of microorganisms, CFU/cm^3		Coliform titer
		Mesophilic microorganisms	Psychrophilic microorganisms	*Escherichia coli* bacteria
Excess sludge		70×10^4	39×10^5	10^{-3}
Disintegrated sludge		30×10^4	26×10^5	10^{-2}
Digested sludge		90×10^5	70×10^6	10^{-4}
Sludge mixture		20×10^5	50×10^6	10^{-3}
Methane	1	21×10^5	38×10^6	10^{-3}
fermentation	5	13×10^4	11×10^6	10^{-3}
time, d	10	54×10^3	70×10^5	10^{-2}

4. Conclusions

Both the literature data and the results of own research indicate that the disintegration method based on the Fenton reaction can be used as a competitive technology compared to conventional technologies used in sewage treatment plants. This method shows intensifies the effect of decomposition of poorly biodegradable organic compounds present in sewage sludge.

As a result of the disintegration by Fenton's reagent, a breakdown of the high-molecular structure of sludge flocs was observed, followed by the release of organic matter from the inside of the cells of microorganisms. Based on the results of the research, the effect of the disintegration method studied on the disintegration degree of modified sludge was found. For the oxidation of excess sludge with Fenton's reagent, an iron ion dose of 0.08 g·Fe^{2+}/g TS and the Fe^{2+}:H_2O_2 ratio of 1:5 were considered the most favorable process conditions. A 7-fold increase in SCOD and a 3-fold increase in VFAs were observed compared to initial values. Disintegration with Fenton's reagent changed the structure of the modified sludge.

It was shown that the use of disintegration prior to the methane fermentation shortens the hydrolysis phase, resulting in an increase in the VFA concentration and SCOD value.

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