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Research Article

APPLICATION OF RESPIROMETRIC TESTS FOR ASSESSMENT OF METHANOGENIC BACTERIA ACTIVITY IN WASTEWATER SLUDGE PROCESSING

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ABSTRACT

Production of a methane-rich gas ('biogas') is contemporary popular sludges processing technology which allows to generate thermal and/or electric energy. Formal requirements issued by the European Union to promote so called renewable energy resources made these process more attractive leading to its application in WWTPs which were designed based on different sludge handling processes. Authors (as active design engineers) noted that dimensioning sludge digestion chamber is usually based on SRT assessment without any emphasis on sludge characteristics. Bio-mass characteristics and the estimation of its activity with respect to methane production are of great importance, from both scientific and practical points of view, as anaerobic digestion appears to be one of crucial processes in municipal wastewater handling and disposal. The authors propose respirometric tests to estimate a biomass potential to produce 'a biogas' and several years' laboratory and full scale experience proved its usefulness and reliability both as a measurement and a design tool applicable in sludge handling. Dimensioning method proposed by authors, allows to construct and optimize operation of digestion chambers based on a methanogenic activity.

Keywords: Sewage sludge, methanogenic activity, sludge digestion, renewable energy, energy recovery.

INTRODUCTION

Role of anaerobic digestion in contemporary sludge processing

Wastewater treatment processes, especially based on so called 'activated' sludge concept, are based on a general principle that an atmospheric air (rarely pure oxygen) is introduced to a mixture of municipal (or in some cases, industrial) wastewater, which creates proper conditions for bacteria growth, using a organic matter (as well as other nutrients) present in human excreta and other wastes carried with wastewater. This growth produces concentration of bacteria suspended in treated wastewater called 'activated sludge', which is composed mainly of saprotrophic bacteria but also has an important protozoan flora such as: amoebae, *Spirotrichs*, *Peritrichs* including *Vorticellids* and a range of other filter feeding species. In activated sludge plants (also multiphase biological reactors) once the wastewater has received proper treatment, excess mixed liquor is discharged into settling tanks and the treated supernatant is discharged to receiving waters or undergoes further treatment before discharge. Part of the settled material, the 'sludge', is returned to the head of the aeration system to re-seed the new wastewater inflowing the biological, while the excess sludge which eventually accumulates beyond what is returned is called Waste Activated Sludge (WAS) and must be removed from the treatment process to keep the ratio of biomass to food supplied in balance. Application of anaerobic process to WAS process was initially focused on stabilization of wastewater sludge with the use

of methanogenic bacteria. Anaerobic digestion has been recognized the proper technology for WWTP of a capacity over 10 000–20 000 m^3/day average flow. Later on, it was found that a by-product of anaerobic stabilization of wastewater sludge is a methane-rich gas (usually called a 'biogas'), which is combustible and can be easily converted into heat. Latest attempts towards sustainability of municipalities led WWTPs operators to re-think a role of anaerobic processes in overall energy balance of these facilities. Methane can be converted to thermal and electrical energy was found promising renewable source of energy. Another advantage was that carbon dioxide emission due to digestion of methane for energy production is of 22% lower than summarized CO_2 produced during aerobic stabilization and CO_2 production, related to electrical energy supply for aeration purposes.

Problems arose with first attempts towards increase of methane production efficiency, due to complex character of methanogenesis phenomena. Digestion in fact is a biochemical decomposition of organic matter from sludge by bacteria Anaerobic degradation of organic material has been described as a multi-step conversion of many, parallel, biochemical reactions. Many groups of anaerobic bacteria are involved in this process. Four main stages of this degradation can be distinguished:

I hydrolysis of complex organic materials to soluble products (hydrolytic fermentative bacteria),
II acidogenesis – generation of intermediary products, such as short-chain fatty acids, (hydrogen producing and acetogenic organisms),
III acetogenesis – acetate production (hydrogen-producing, hydrogen-consuming acetogenic organisms),
IV methanogenesis – methane production (methane-forming bacteria).

Methanogenic bacteria play a key role in conversion process by keeping the hydrogen partial pressure low – a condition necessary for the growth of acetogenic bacteria. The balance activity of the mixed population of bacteria is needed to secure stability of anaerobic digestion. However, this stability can be easily disturbed by different factors causing a rapid increase of volatile fatty acids concentration following the decrease methane production. Failure of digesters and anaerobic reactors has been affected by a number of compounds that may be present in sewage. One group of these are heavy metals [6]. The basic pre-requisite of proper operation of this process is a balance between substrates and quantity of bacterial microorganisms. As particulate matter cannot transfer through the microorganisms' cell it is required that organic and inorganic substrates are in soluble form. Conversion of an organic matter into methane and carbon dioxide leads to decrease of degradable organic matter content in sludge down to 50–55% The process is performed on so called technical level when approx. 50% of total organic matter is being decomposed [1]. Quantity of digestion gas obtained from organic matter may vary depending on the proportions of main sludge constituents: carbohydrates, proteins and fats. Usually it is estimated that 1std m^3 of a biogas can be obtained from 1 kg of decomposed organic matter. Average composition of digestion gas is the following: CH_4 – 65–70%, CO_2 – 25–30%, and N_2, H_2, H_2S < 1%, however it may vary from case to case.

Process characterization

Typical characterization of a biomass being treated anaerobically is determination of a Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS) concentrations. Basing on these parameters some rough estimation of process performance can be done, and traditional methods of sludge digestion chambers dimensioning are using these data. However, there is no way to differentiate between active biomass (volatile compound in bacteria cells) and substrates for the reaction (short-chain fatty acids). That is why various methods are developed focusing on the characterization which describes the exact ability of a biomass to convert substrates into methane under anaerobic conditions. This is not only a 'curiosity driven' experiment but rather a search for a tool applicable in designing and operating sludge processing facilities.

Methods applied for process estimation

Three groups of measurements technology have been applied by now, including [7, 13]:

I. Assessment of microorganisms' quality and quantity
   a. Assessment on most probable number of microorganisms,
   b. Immunological tests,
   c. Microscopic observation.

II. Analyses of chemical composition of microorganisms
   a. Tests on presence of a F_{420} co-enzyme,
   b. Assessment of etheric bounds (C-O-C) in cells,
   c. Hydrogenaze activity tests.

III. Biochemical activity tests
   a. Determination of quantity and dynamics of gas production,
   b. Determination of quantity and specific velocity of methane production based on substrates and VSS;
   c. determination of decomposition of substrates (short-chain fatty acids)

All methods based on determination of methanogenic bacteria species were found impracticable due to long time of these microorganisms' growth. Problems associated with incubation of pure anaerobic bacteria were described by numerous authors [11, 16, 17, 22]. Time (duration) of isolation of pure anaerobic cultures extends to months and even years and are rarely presented in papers. That is why some authors tended to use F_{420} co-enzyme as a parameter [9, 10, 14], however due to high adverse impact of ambient conditions on method's accuracy this procedure has not been widely applied. Biochemical methods appear to be most promising ones. The IIIa method is not precise enough to determine the activity of microorganisms due to complex of gas content. Therefore, remaining IIIb – based on calculation of methane quantity and IIIc as a rate of substrate conversion to CH_4 and CO_2 appear to be the most prospectus methods [9, 19, 20, 23]. The authors of the present paper proved that respirometric batch tests, as described below, are reliable enough.

METHODS

Description of a test stand

Tests were performed using a respirometry method. The test stand was located at the Cracow University of Technology Research Lab [1, 2, 3, 4, 5]. The AER-208 (Challenge Systems, USA) respirometer consisted of two basic parts: apparatus to measure gas volume and interface that transmits the obtained values to a computer for data registration. Sludge samples were placed in small air tight bottles (reaction chambers) of 0.5 litre volume, covered with rubber plugs and, additionally, secured with metal cups. Gas volume measurements were conducted in 8 separate compartments made of transparent plastic and mounted on a base. Photocells were installed in the base of each segment to monitor the number of gas bubbles. Primary sludge was transported from the 120 000 p.e. wastewater treatment plant. The test stand is presented in photograph 1, details of measuring device are shown in photo 2. The test stand allowed to adjust temperature within 0 to 50 °C range.

Photo 1. Test stand - general view

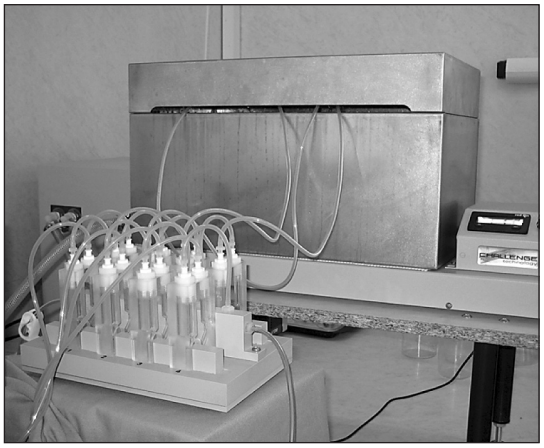

Photo 2. Test stand - details of measuring device

Experimental protocols

Temperature: the incubation temperature was 35 °C;
pH: with regard to the sensitivity of methanogenic bacteria to pH;
Experimental system: A mechanically stirred system was applied to ensure a full mixing of samples;
Sample feeding: VFA substrate, mineral nutrients and yeast extract;
Substrate and sludge concentration: The samples were fed with volatile fatty acids (VFA) mixture. It was assumed that a substrate concentration in samples should range from 2 to 5 g/L and the concentration of sludge from 3 to 5 g VSS/L extract [1, 15].

The methanogenic activity of sludge was evaluated based on the measurements of the amount of methane produced during the fermentation process in the reaction vessels filled with anaerobic sludge, VFA substrates, mineral nutrients and yeast ex-tract. The sludge methanogenic activity was determined by the amount of fatty acids, as g/L COD, which was consumed during the fermentation process and converted into methane (mL CH_4). The COD was referred to 1 gram of sludge VSS (volatile suspended solid) per 1 day. Based on the amount of gas produced during the batch methanogenic test, cumulative gas production curves were plotted. Using those curves, maximum gas production (mL CH_4) was calculated. From the maximum slope of the curves, the gas production rate R (mL CH_4/h), which determined the amount of gas produced during 1 hour, was obtained. The methanogenic activity (ACT) in g COD_{CH4}/g VSS·d, was calculated as follows [12, 15]:

$$ACT = (R \cdot 24) / (CF \cdot V \cdot VSS) \quad (1)$$

where: R – gas production rate, mLCH /h;
CF – conversion factor, mLCH_4/g COD;
V – effective liquid volume of reactor vessels, L;
VSS – sludge sample concentration, g VSS/L.

ESTIMATION OF INHIBITORY EFFECT OF HEAVY METALS ON METHANOGENIC ACTIVITY

Procedure applied

Respirometric tests can be used for estimation whether, and to what extent, heavy metals influence gas production. For these specific tests, Cr (III) chromium was tested (pollution typical for obsolete small scale tannery industry, being important ecological problem in Southern Poland), however the same procedure can be used for other inhibitory factors [1]. Short-chain fatty acid (SCFA) substrates were added to eliminate the impact of excessively low food to microorganism (F:M) ratio [21]. Results were presented as methanogenic activity vale, which expresses the ability of sludge to convert methane to substrate under unfavourable conditions.

The measurements of the amount of methane produced during the fermentation process were conducted from reaction vessels filled with:

- anaerobic biomass, SCFA substrates, mineral nutrients – as a control sample and
- anaerobic biomass, SCFA substrates, mineral nutrients, various dosage of Cr(III) – as treatment samples
- the investigations included three test series with the following doses of Cr(III): 200 mg/L, 500 mg/L, 1500 mg/L, 2000 mg/L, 3000 mg/L.

The 500 mL reaction vessels filled with anaerobic biomass, desired amount of substrate, mineral nutrients, yeast extract in control sample and toxicant added in treatment sample, were closed and sealed then placed in water bath at 35 °C. The samples were mixed with a magnetic stirrer during the entire test period. At the beginning of the test, all vessels were saturated with nitrogen. The methane produced during the anaerobic process was transported by pipes through a respirometer system and was recorded by the computer system. A chromatographic analysis of gas quality was carried out for each sample separately (gas chromatograph; column Porapak QS, 80–100 Mesch, 4m, HWD-430; hydrogen). A control analysis of samples for COD, VFA, VSS and pH were performed as well.

Based on the amount of gas produced during the batch methanogenic test and chromatography analyses of methane content, cumulative methane production curves were plotted (Figure 2). Using those curves, the maximum methane production was determined.

RESULTS AND DISCUSSION

Inhibitory effect

During the research significant differences in gas production amount were observed [2]. Basing on the results of methane production obtained from 3 series carried out with Cr(III) concentration from 200 to 3000 mg/L (1 mg/L equals to 1 ppm), cumulative gas production curves were plotted. The curves were drawn for control samples and samples with toxicant in the batch tests (Figure 1). According to gas chromatographic analyses the amount of methane produced from samples during the test period was quite high: 80% and 70–74% for control and toxic samples respectively.

It can be stated that doses 200 and 500 mg Cr(III)/L caused decrease of methane production

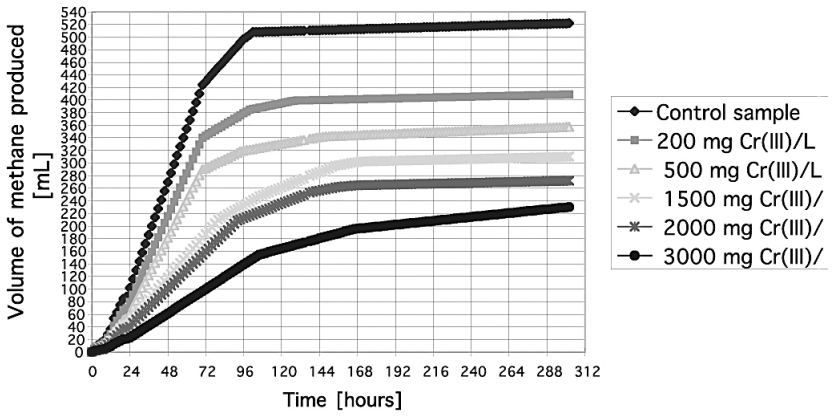

Fig. 1. Total methane amount (expressed in volumetric units) produced during a 12-day test period

of approx. 20%, as compared to the reference sample. Increase of inhibiting compound dose up to Cr(III) 1500 and 2000 mg Cr(III)/L led to approx. 45% decrease of methane production. The concentration of Cr(III) equal to 3000 mg/L caused an approx. 47% decrease of methane production.

However, methane is produced until entire organic matter is converted its reaction rate tends to decrease as the concentration of substrates drops being consumed by microorganisms. After certain period of time the amount of methane produced is relatively small, and from energetic point of view energy required for facility operation becomes higher than the amount of energy recoverable from biogas. That is why for technical purposes, like digestion tanks design and/or operation optimisation solid retention time (SRT) in digestion tanks, estimation of length of period of maximum production rate is a crucial point. It is important to note that decrease of methanogenesis rate is a sign of stabilisation, showing that large part of organic matter has been decomposed into stable by-products. It means that the end of maximum gas production is the end of stabilisation. Length of period of maximum methane production can be estimated basing on summarisation curves plotted for each of Cr(III) doses.

Methane production under inhibitory conditions was expressed in relation to a reference (control) sample which was inhibitor free. Table 1 summarizes results of tests on an inhibitory effect.

It is visible that volume of methane decreases as the inhibitory dose rises, on the other hand a length of maximum productivity period extends with doses. Based on the amount of the gas produced during the batch methanogenic test, cumulative gas production curves were plotted). Using those curves, the maximum gas production (mL CH_4) was calculated. From the maximum slope of the curves, gas production rate R (mL CH_4/h), which determined the amount of gas produced during 1 hour, was obtained.

Exact dimensioning of digestion chambers

Determination of proper retention time in digestion chambers is of crucial importance in design optimization of these facilities [3]. It is especially difficult when there is no 'real sludge'. In this specific case, the sludge was obtained in small portion as continuous operation of the plant was still based on a pattern: digestion of primary

Table 1. Period of maximum methane production at various Cr(III) doses

Cr(III)dosage [mg/L]	Time of max CH_4 production [hours]
control	approx. 96
200	120–144
500	144–168
1500	approx. 168
2000	to be repeated
3000	exceeds test period

and simultaneous aerobic stabilization of a WAS. The estimation procedure of these parameters for predicted mixture of a primary sludge and WAS has been proposed on the basis of cumulative curves of gas production for digestion without an inoculation. Basing on the plotted curve VSS content vs. time the net length of the start-up ('yield') phase was estimated, similarly a length of effective gas production phase ('decay') was estimated. The procedure for mixed sludge (1:1 proportion) is shown in Figure 2. The maximum theoretic effective gas production period was estimated as an interval between intersection point of tangent to a VSS content curve with a time-axis and a final point of effective gas production period, which led to general SRT equal to 23 days adopted for design purposes.

The procedure for this proportion was illustrated in Figure 1. Duration of a start-up period was estimated equal to 27 days but it must be recognized that these tests were completed without an inoculation. The effective digestion period has been determined as 23.5 days. If the digestion time was extended over this value, it did not result in better stabilization of sludge. This value was adopted for a real-term design. Lab tests proved that common digestion of primary and WAS sludge is technically feasible but requires some capital improvements of the plant. Basing on our tests, it was decided by the plant owner that the designed capacity of the biogas generation system will be 2000 St dm^3/day with possible extension of gas production to maximum value of approx. 28000 St dm^3/day. Results of tests were approved, then adopted as a process calculations by a designer [3].

Application of respirometric tests on assessment of disintegration of sludge

Recent application of respirometric tests was in the field of sludge disintegration. This technology was invented to improve overall methane-rich gas production by partial disintegration of microorganism cells. Before respirometric tests were applied, typical assessment of efficiency of disintegration was done by steady state measurements of COD solubilisation, change of sludge floc' size or change in protein concentration [1, 10, 18]. The results from these measurement technology cannot be simply incorporated into design procedures of wastewater treatment plants. The data necessary for a process engineer usually include a real change (increase) in a biogas yield and an effective digestion time within the chamber (SRT in digestion chamber). The methanogenic activity procedure was invented as an accurate tool for pre-diction of a net gas production and estimation of its dynamic [8,9,15]. Typical scope of tests were as follows:

- determination of sonication parameters based on initial calculations of a sludge disintegration degree for the WAS;
- fermentation tests performed at a respirometric test stand for the WAS, after its disintegration at various parameters;
- methanogenic activity tests – batch tests of the WAS.

Based on the results described above, the first stage of investigations on methanogenic activity has been performed. It was completed for the following parameters of disintegration:

- Sound intensity: 24·103 W/m^2

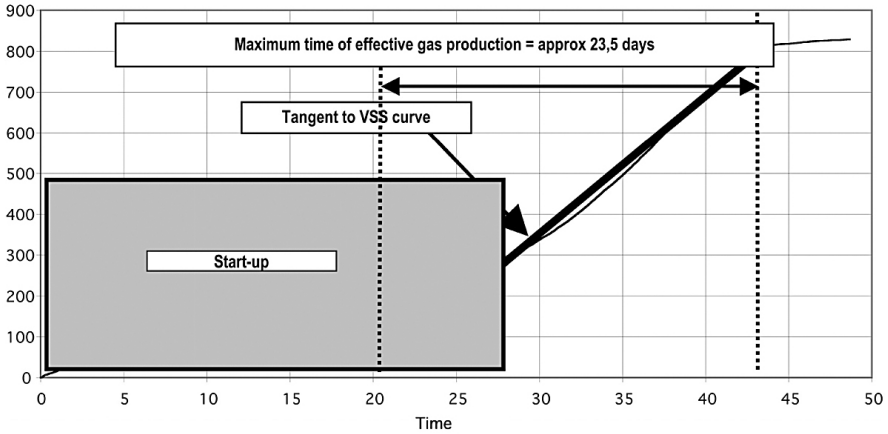

Fig. 2. Determination of a retention time for design purposes. Mixed sludge: primary and WAS (1:1 proportion)

- Sonotrode draught: 4 cm
- Time of disintegration: 3, 5, 7, 9 minutes
- Repeatability of results (two identical samples from each WWTP, digested at the same time)
- Frequency of a biogas measurement: 2 hours
- Duration of tests: 14 days (plus incubation).

Gas production measured during one of the selected series has been presented in Figures 3 and 4 as gas cumulative curves, plotted from the data obtained at a sonication intensity equal to $24 \cdot 10^3$ W/m^2 (ultrasound density $5{,}8 \cdot 10^4$ W/m^3).

They reflected fermentation gas production at a changing sonication time, prior to fermentation. The gas production curve for non-sonicated samples was drawn in each figure to show how the sonication process impacts changes in the gas yield. Figure 3 proves that this time of sonication appears to be too short to decompose cell structure properly. Increase of a sonication time to 5 and then to 7 minutes resulted in higher both gas production and sludge methanogenic activity (ACT) parameter. A further increase of gas production was observed for sonication time of 7 minutes. Figure 4 presents results for both sonication times: 7 and 9 minutes, and confirms that increase of these parameters over 7 minutes did not result in a dynamic increase of gas production. Similar changes in gas production and sludge activity were observed for a lower ultrasound density. Remaining experimental series confirmed the observations presented in Table 2.

Photographs 3 and 4 present microscopic images of the sludge before and after ultrasound disintegration. All images were taken during tests, at the following parameters: intensity $24 \cdot 10^3$ W/m^2; ultrasound density ASW W/m^3; optical magnification 100×.

A non-dyed sample presented in Photo 3 shows a typical image of activated sludge with filamentous bacteria. Following images show changes in a sludge structure after an increase of sonication times (3, 5, 7, 9 minutes). The increase of sonication time makes the microscopic image of sludge more 'fuzzy' and it is hard to observe the structure of sludge flocs and/or presence of filamentous bacteria. Such observations can confirm efficient mechanical decomposition of cells but have no application in a quantitative description of the process.

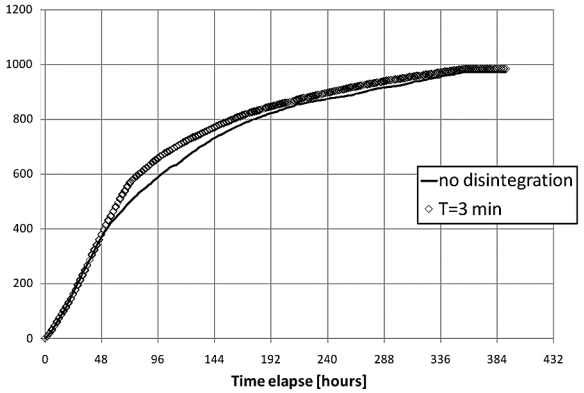

Fig. 3. Gas production cumulative curve (sonication 3 minutes)

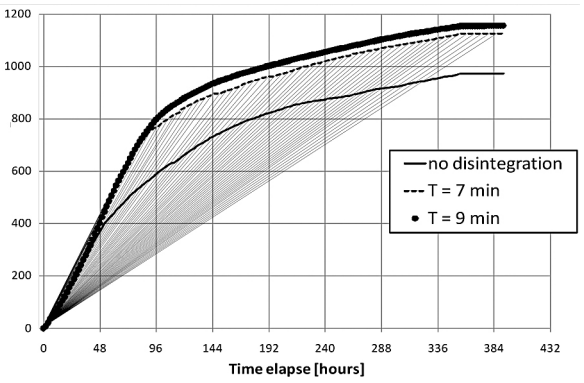

Fig. 4. Gas production cumulative curve (sonication 7 and 9 minutes)

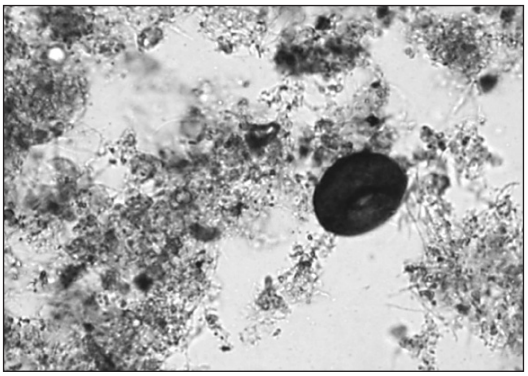

Photo 3. Structure of sludge before sonication

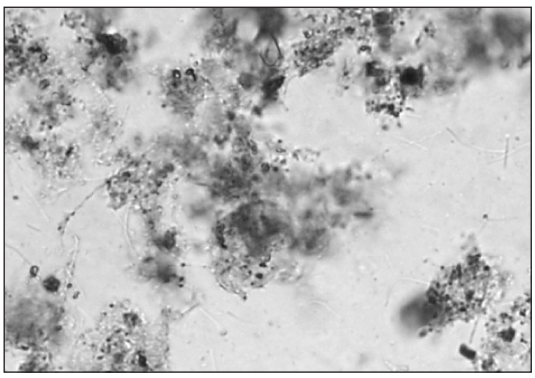

Photo 4. Structure of sludge after 3 minutes sonication

Table 2. Methanogenic activity and disintegration calculations (intensity $24 \cdot 10^3$ W/m^2; ultrasound density $11{,}6 \cdot 10^4$ W/m^3)

Time [min]	0	3	5	7	9
COD reduction [%]	90	92	90	91	91
VSS [g/L]	4.03	4.12	3.99	3.77	3.80
R	4.25	5.6	5.9	6.5	6.8
ACT	0.11	0.16	0.18	0.20	0.21

CONCLUSIONS

1. Common methods of microbial activity assessment are not handy enough in case of methane generating organisms, neither for scientific nor for design purposes, as they do not express a gas production but focus rather on substrates' accessibility.
2. Respirometric tests can be applied in estimating adverse impact of inhibitory factors on a biogas production and its compositions.
3. The method proposed in this paper, based on methanogenic activity, seems to be more flexible. Application of methanogenic activity tests is a valuable tool for a digestion chamber dimensioning, in case the content of sludge is unknown.
4. The proposed design procedures led to exact calculation of the digestion time, which is considered the main parameter for the dimensioning of the sludge processing line at the existing as well as upgraded plant (e.g. in case of changing of general concept of sludge processing).
5. Respirometric tests were successfully applied for estimation of sludge disintegration efficiency. Results obtained with ultrasound disintegration assessment can be easily applied for any other disintegration method.

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