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A New Approach to Assess the Effect of Various Humic Compounds on the Metabolic Activity of Cells Participating in Methanogenesis

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Abstract: The possible use of the concentration of intracellular adenosine triphosphate (ATP) as a parameter enabling quick and adequate evaluation of the metabolic activity of methanogenic cells was demonstrated in the work. This approach was used to analyze the effect of introducing potassium humate and fulvic acids (1–10 g/L) into media with four different methanogenic consortia producing biogas. The ATP concentration was analyzed by the bioluminescent luciferin–luciferase method at the beginning and end of the process. During the entire process, the biogas composition, biogas efficiency, and the kinetics of methanogenesis in the presence of humic compounds were determined. The increase in the concentration of potassium humate led to a decrease in the overall energy status of the cells and reduced methanogenesis efficiency. However, fulvic acids introduced into the media stimulated methanogenesis in half of the tested consortia, which was accompanied by an increase in ATP concentration in cell samples. So, a positive correlation between the metabolic activity of cells in biogas formation and the concentration of ATP was observed. ATP concentration control appears to be an attractive tool for finding compounds that suppress methanogenesis in landfills.

Keywords: ATP; biogas; methanogenesis; anaerobic consortia; potassium humate; fulvic acids

1. Introduction

Landfills of solid household waste pose a serious long-term risk to the environment and public health. This is due to the active production of landfill gas, which mainly contains CH_4 and CO_2 with a low addition of H_2S, H_2, and NH_3 [1]. Uncontrolled emission of landfill gas can cause fires and explosions, since the biochemical processes occurring inside the landfill lead to an increase in the temperature and thus to the spontaneous combustion of waste [2,3]. For this reason, in many developed countries, special procedures are used to minimize landfill gas emission. In particular, landfill gas is sometimes captured for its further use in power plants to generate electrical and heat energy. However, such methods are difficult for technological implementation [4]. Therefore, methods based on the oxidation of methane by coating landfills with a layer of compost containing methanotrophic bacteria are used [5,6]. However, such systems rapidly decompose, and as a result, methane synthesis increases again [6]. In this regard, the search for new solutions to reduce the intensity of landfill gas production and the proportion of CH_4 in its composition is highly relevant [7]. Obviously, under real conditions, it is impossible to completely suppress the process of methane formation; therefore, it is possible to solve the problem by decreasing the rate of gas emission and changing the composition of the emitted gas by reducing the methane content therein.

CH_4 is formed as a result of the metabolic activity of methanogenic microbial consortia that are formed spontaneously under natural conditions and consist of hydrolytic and acetogenic bacteria

as well as methanogenic archaea [8]. Various organic substances (long-chain fatty acids, aromatics, xenobiotics, ammonia, heavy metal salts, etc.) can affect the methanogenic activity of such complex consortia [9,10]. The inhibition of the methanogenic activity of anaerobic consortia by various humic compounds (HCs), which are complex organic compounds containing various functional (carboxyl, phenolic, hydroxyl, etc.) groups, has been actively studied recently [11]. The following results concerning the influence of HCs on the functioning of anaerobic sludge have been reported: 75% inhibition of methane accumulation can be provided by the addition of 1 g/L HC to a medium with hydrogenotrophic methane-forming bacteria present in anaerobic sludge, with the exception of *Methanospirillum hungatei*, for which the inhibitory concentration of HC is 5 g/L [12]. It was found that the methanogenic activity of the acetoclastic methanogen *Methanosarcina barkeri* is inhibited by a concentration of 1 g/L HC, but the activity of *Methanosaeta concilii* decreases markedly only at a concentration of 3 g/L HC. Granular anaerobic sludge was found to be less susceptible to the inhibitory effect of HCs than pure cultures of methane-forming bacteria. An effect of sodium humate on the methanogenic activity of granular anaerobic sludge was shown.

It was found that the presence of sodium humate in concentrations up to 2 g/L does not significantly affect the accumulation of CH_4 and even has a certain stimulating effect on the methanogenic activity of anaerobic sludge, which on average is increased by 19% as a result of the buffering and regulating ability of sodium humate [13]. In another study of several methanogenic consortia, partial inhibition of methanogenesis was observed only at an HC concentration of 10 g/L and complete inhibition was shown at 20 g/L HC [14]. It should be noted that delayed hydrolysis of cellulose, which was used as the main substrate, was observed when the HC concentration was increased to 2 g/L, and the entire process of methanogenesis was slightly delayed. This was explained by the inactivation of hydrolytic enzymes functioning at the first stage of methanogenesis [15]. Consortia with higher hydrolytic activity were less susceptible to inhibition of HCs [14]. Thus, research is being actively conducted on the use of HCs and their derivatives for controlling the intensity of methanogenesis. However, it was found that there are many factors influencing the complex process of biogas accumulation. So, a simple and adequate method for quick monitoring of the process conducted under changing conditions should be used to evaluate the efficiency of methanogenesis.

In the known studies, the effect of various factors, including the introduced concentration of HCs on the metabolic activity of anaerobic sludge, was evaluated by measuring the amount of methane released or the hydrolytic activity of anaerobic cells. The use of these parameters to monitor methanogenesis is informative but time and energy consuming under laboratory conditions, and analyzing the parameters in situ is not possible at all. It has been suggested that a much more efficient technique for such monitoring can be developed based on the determination of the concentration of intracellular adenosine triphosphate (ATP) in sludge samples.

The ATP concentration can be quickly and easily determined using bioluminescence with great accuracy, even with an ultralow content of the substance in the sample (as low as $10^{-14} \div 10^{-12}$ M), using luciferin–luciferase reagents [16,17]. Portable luminometers make measuring the ATP concentration possible both in the laboratory and in situ, providing a method for quickly analyzing the presence of cells in test samples and their energy status. The latter is determined by the content of intracellular ATP, since ATP is used for intracellular energy storage by all living cells [18]. It plays a central role in any type of metabolism and is the most important energy supplier in many biochemical reactions. At the same time, the intensity of all metabolic processes, including the accumulation of any extracellular metabolites in a cell, is reflected in its energy status. It has been previously proved that measuring the ATP level is an efficient tool for assessing the metabolic potential of cells in the processes of cryoimmobilization, xenobiotic destruction, and antibiotic synthesis, including monitoring the cell viability of anaerobic biogas-producing consortia [19–22]. The time required for preparing a sample for analyzing its ATP concentration is several minutes, and the measurement itself takes no more than 1 min. All this contributes to the attractiveness of ATP monitoring as an informative method with high detection sensitivity for the rapid assessment of the state of cells producing biogas. Moreover, due to

its ease of use, the ATP monitoring method could be readily implemented in landfills, where the use of methanogenesis inhibitors is highly relevant.

The purpose of this work was to evaluate the activity of various natural methanogenic consortia using the bioluminescent luciferin–luciferase method of ATP determination and to compare this method with the traditionally used approach based on studying the characteristics of methanogenesis: the process efficiency and the methane content in the accumulated biogas. In particular, the new technique was applied to assess the effect of different concentrations of potassium humate (PH) and fulvic acids (FA) on methanogenesis.

It was important to estimate the correlation between the ATP measurements and the metabolic activity of cells in order to assess the perspectives of the ATP-based approach to monitoring the methanogenesis efficiency.

2. Materials and Methods

2.1. Characteristics of the Consortia and HCs

FA of high-moor peat (Tver region) and PH from brown coal (HUMINTECH GmbH, Grevenbroich, Germany), obtained [23] using the standard method recommended by the International Humic Substances Society (IHSS), were used in this work. These HCs were added to a nutrient medium used for methanogenesis at different concentrations (up to 10 g dry weight/L).

Four anaerobic consortia were used: I—natural anaerobic consortium isolated from the Kashira landfill (Kashira, Russia) (cultivation temperature 37 °C); II—natural anaerobic consortium taken from an inactive landfill (Pokrov, Russia, thermophile, 55 °C); III—anaerobic consortium obtained from a digester which treated cattle waste (Dmitrov, Russia, 37 °C); IV—anaerobic consortium obtained from the wastewater of a food plant (Kashira, Russia, 37 °C). The main characteristics of the consortia are presented in Table 1. The methanogenic consortia were stored at 35 °C in a thermostat with the addition of 1 g chemical oxygen demand (COD)/L (milk whey) every week until their use.

Table 1. Characteristics of methanogenic consortia used in this work.

Consortium	Dry Matter (g/L)	Ash (%)	Biomass VSS (g/L)	* Activity (mg COD/g VSS/day)		Initial Concentration of ATP, $\times 10^{-12}$ mol/mL
				Acidogenic	Methanogenic	
I	64.3 ± 3.1	42.4 ± 0.9	35.3 ± 1.1	2210 ± 30	315 ± 5	15.7 ± 0.6
II	57.1 ± 2.3	37.8 ± 1.6	35.4 ± 1.2	1550 ± 30	86 ± 1	26.4 ± 0.8
III	56.6 ± 1.6	38.5 ± 1.5	34.8 ± 1.1	2180 ± 30	195 ± 10	4.4 ± 0.3
IV	58.2 ± 2.2	42.7 ± 1.7	33.0 ± 1.1	1970 ± 30	157 ± 1	15.1 ± 0.6

* COD—chemical oxygen demand, VSS—volatile suspended solids.

The concentration of the substrate was estimated via the standard method of COD determination [24]. Potassium dichromate was used as an oxidizing agent and glucose was used as a control oxidizable substrate. The concentration of the reduction product $Cr_2O_7^{2-}$ was detected spectrophotometrically at 600 nm on an Agilent UV-853 spectrophotometer (Agilent Technologies, Waldbronn, Germany).

The dry weight of anaerobic consortia was determined via a standard gravimetrical method by drying a sample at 105 °C to a constant weight.

Volatile suspended solids (VSS) of anaerobic consortia was determined after the combustion of a dried sample in a muffle furnace at 600 °C for 5 h, and the ash content (%) of the latter was calculated.

Determination of both the acidic and methanogenic activity of anaerobic consortia was carried out using nutrient media based on glucose and acetate as a substrate, respectively, according to a previously reported method [25]. Measurements were carried out until the content of hydrogen, methane, and carbon dioxide in the gas phase ceased to change in time. The specific acidogenic activity of sludge (mgCOD/gVSS/d) was calculated by the slope of the linear part of the curve in the graph

(glucose concentration vs. time). The specific methanogenic activity of the sludge (mg COD/gVSS/d) was calculated from the slope of the linear part of the curve in the graph (methane concentration vs. time).

2.2. Methanogenic Tests

To study the effect of HCs on microorganisms, methanogenic consortia were cultivated in 100 mL anaerobic serum bottles used as bioreactors (Sigma-Aldrich, St. Louis, MO, USA) with a nutrient medium prepared on the basis of 0.1 M phosphate buffer (pH 7.2) and containing 1 g COD/L of milk whey as the carbon source. The bottles were filled with helium for 0.5 h to remove the air.

The inoculum-to-substrate ratio (both expressed in gVS/L) was 3.5 ± 0.1. The total operational volume (medium + inoculum) was 50 mL. Medium (consortium + milk whey without addition of HCs) was used as a control. The process was conducted without mixing at 37–55 °C for two weeks.

The efficiency of methanogenesis (E) was calculated as follows:

$$E = (Q/Qmax) \times 100\ (\%) \quad (1)$$

where Q (mL) is the volume of biogas produced in a reactor with a test sample (sum of all gases), and Qmax (mL) is the theoretical maximum volume of biogas (Equation (2)).

$$Qmax = (C_I V_{lph}) \times 0.35 \times 1000\ (mL) \quad (2)$$

where V_{lph} is the volume of the liquid phase in the reactor (L), C_I is the initial concentration of organic substances in a sample (g COD/L), and 0.35 is the volume of methane produced from 1 g COD at 0 °C.

$$Qm = \{(C/100 \times P_{tot} T_0 V_{gph})/(T_1 P_0)\} \times 1000 \quad (3)$$

where C is the dry methane content in the gas phase (%); V_{gph} is the volume of gas phase in the reactor (L); T_0 is the temperature under normal conditions, 0 °C; T_1 is the operating temperature in the reactor; P_0 is the pressure under normal conditions, 1 atm; and P_{tot} is the total pressure in the reactor (atm).

2.3. Analytical Methods

The cell metabolic activity was determined by measuring the intracellular ATP concentration in samples of anaerobic consortia using the bioluminescent luciferin–luciferase method and the Microluminometer 3560 (New Horizons Diagnostics Co, Baltimore, MD, USA) [17,21,26]. For this purpose, samples of fermentation medium after vigorous mixing were collected at the beginning and end of the process (0.1 mL), transferred to dimethyl sulfoxide (0.9 mL), and allowed to stand at 25 °C for 2 h to extract intracellular ATP. Then, the sample was diluted with distilled water (1:10), 50 µL of the resulting solution was added to the bioluminometer cuvette containing 50 µL of the luciferin–luciferase reagent (Lumtek, Moscow, Russia), and the luminescence intensity was measured. The ATP concentration was determined using the calibration curve plotted for standard ATP solutions ($10^{-13} \div 10^{-7}$ M).

Potentiometric measurements were conducted to control the pH of the prepared media and the samples analyzed in the experiments. A Corning Pinnacle 530 pH meter (Corning Incorporated, Corning, NY, USA) was used. During the experiment, the pH of the medium was monitored in all the samples at the beginning and end of the process.

The content of H_2, CH_4, and CO_2 in the gas phase was controlled using a Crystallux-4000M gas chromatograph (RPC "Meta-chrom", Yoshkar-Ola, Russia) as published previously [27]. The data are presented as means of at least three independent experiments ± standard deviation (±SD). Statistical analysis was performed using SigmaPlot (ver. 12.5, Systat Software Inc., San Jose, CA, USA).

3. Results

3.1. Influence of HCs on the ATP Level of Cells in the Natural Anaerobic Consortia Catalyzing Methanogenesis

The specific concentration of intracellular ATP in the fermentation medium was monitored before and after methanogenesis in the presence of HCs (Table 2). When varying the concentration and type of the natural HCs (PH or FA) introduced into the medium, it was found that the presence of PH in contact with methanogenic consortia led to a decrease in the overall energy status of the cells. The inhibitory effect of PH on cell metabolism, affecting the concentration of ATP, increased with the increase of the PH concentration (in the range studied). Consortia III and IV were found to be the most sensitive ones to the presence of PH in the culture broth.

Table 2. Influence of humic compounds (HCs) on the intracellular adenosine triphosphate (ATP) concentration ($\times 10^{-12}$ mol/mL) in cell samples before and after anaerobic cultivation of methanogenic consortia in media with different concentrations of potassium humate (PH) and fulvic acids (FA).

HC, g/L	Consortium			
	I	II	III	IV
0 (control)	14.5 ± 0.5	6.6 ± 0.3	6.3 ± 0.3	10.2 ± 0.4
PH				
1	12.7 ± 0.4	5.2 ± 0.2	0.7 ± 0.1	1.5 ± 0.2
5	4.5 ± 0.3	1.7 ± 0.2	0.3 ± 0.1	0.9 ± 0.1
10	0.5 ± 0.1	0.9 ± 0.1	0.2 ± 0.1	0.3 ± 0.1
FA				
1	20.1 ± 0.6	5.9 ± 0.3	25.2 ± 0.6	2.3 ± 0.2
5	24.9 ± 0.7	3.5 ± 0.2	17.6 ± 0.5	1.5 ± 0.2
10	32.9 ± 0.9	2.1 ± 0.2	15.4 ± 0.4	0.6 ± 0.1

Unlike PH, which only affected the cells negatively, the effect of FA on the energy status of the cells in methanogenic consortia was ambiguous. So, the concentration of intracellular ATP in the cells of anaerobic consortia I and III was increased by a factor of 1.3–5.7 compared with the initial values of this parameter. The maximum increase in ATP in consortium I was observed with an increase in FA concentration in the medium to 10 g/L, whereas for consortium III, the maximum energy level in the cells was noted at 1 g/L. At the same time, the introduction of FA into the medium with consortia II and IV had an inhibitory effect on the metabolic activity of these cells, and with an increase in the concentration of FA, the inhibitory effect in these biocatalytic systems only intensified.

3.2. Analysis of the Biogas Production Efficiency and CH_4 Content in Biogas Accumulated by Methanogenic Consortia in the Presence of Different Concentrations of HCs

When studying the efficiency of biogas accumulation under the action of natural anaerobic consortia, it was shown that FA, introduced into the nutrient medium in concentrations from 1 to 10 g/L, stimulated biogas accumulation in the case of consortia I and III (Figure 1a,e) or weakly affected methanogenesis in the case of consortia II and IV (Figure 1c,g). With the introduction of PH in concentrations of 1–10 g/L, the efficiency of methanogenesis under the action of all four anaerobic consortia was lower with this indicator for samples without HCs (Figure 1).

The CH_4 content in biogas observed in our experiments when various HCs were introduced into the tested media is shown in Figure 1b,d,f,h.

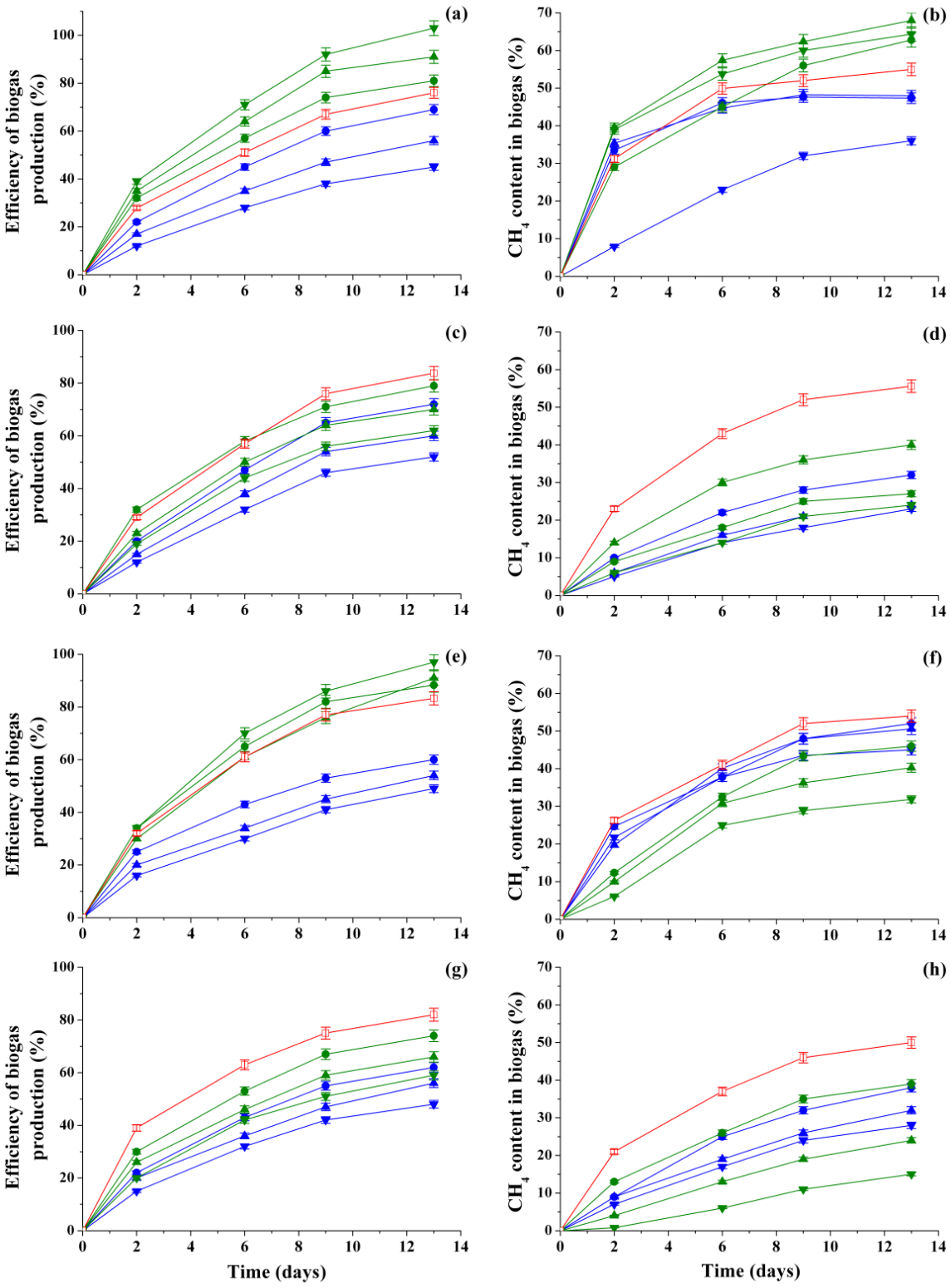

Figure 1. Efficiency of biogas production (**a**,**c**,**e**,**g**) and CH_4 content in biogas (**b**,**d**,**f**,**h**) during anaerobic cultivation of natural anaerobic consortia (I-a, II-b, III-c, IV-d) in media containing 1 g COD/L milk whey and different concentrations (g/L) of PH (blue lines) and FA (green lines): 1-●, 5-▲, and 10-▼, where the red line corresponds to the control sample (without HC in the medium). The theoretical maximal conversion of substrate to biogas was assumed as 100% efficiency of biogas production.

For the natural consortium I, the introduction of FA in any of the studied concentrations (1–10 g/L) stimulated the accumulation of CH_4, the content of which by the end of the study in the composition of the biogas was 20–25% higher than that in the control. For all the other consortia (II–IV), the introduction of FA into the culture media led to a decrease in the proportion of CH_4 in the composition of biogas, and in the presence of 10 g/L FA, the methane portion was decreased by a factor of 2–3 compared with the control (without FA). With an increase in FA concentration, the inhibiting effect on methane accumulation in the biogas composition became more pronounced.

The presence of HP in the media used to cultivate methanogenic consortia, regardless of the concentration introduced into the medium (1–10 g/L), invariably led to inhibition of methane accumulation in the composition of biogas produced by consortia I, II, and IV. Consortium III was almost not affected, and the methane content was close to what was obtained in the control sample without the addition of any HC.

The analysis of pH in the study of the kinetics of methanogenesis in media with or without HCs showed that the decrease in this parameter from the initial level by the end of the process was no more than 0.1–0.3 in all the variants of the studied media. Therefore, the effect of pH was not taken into account when interpreting the results.

Figure 2 illustrates the positive correlation between the ATP concentration in the cells participating in methanogenesis and the efficiency of biogas production in the presence of different concentrations of PH and FA in relation to the same parameters observed in the control samples (without additions of HC and FA) at the end of the process. The parameters obtained for control samples were taken as 100%. All the results were calculated based on the data of Table 1 and Figure 1.

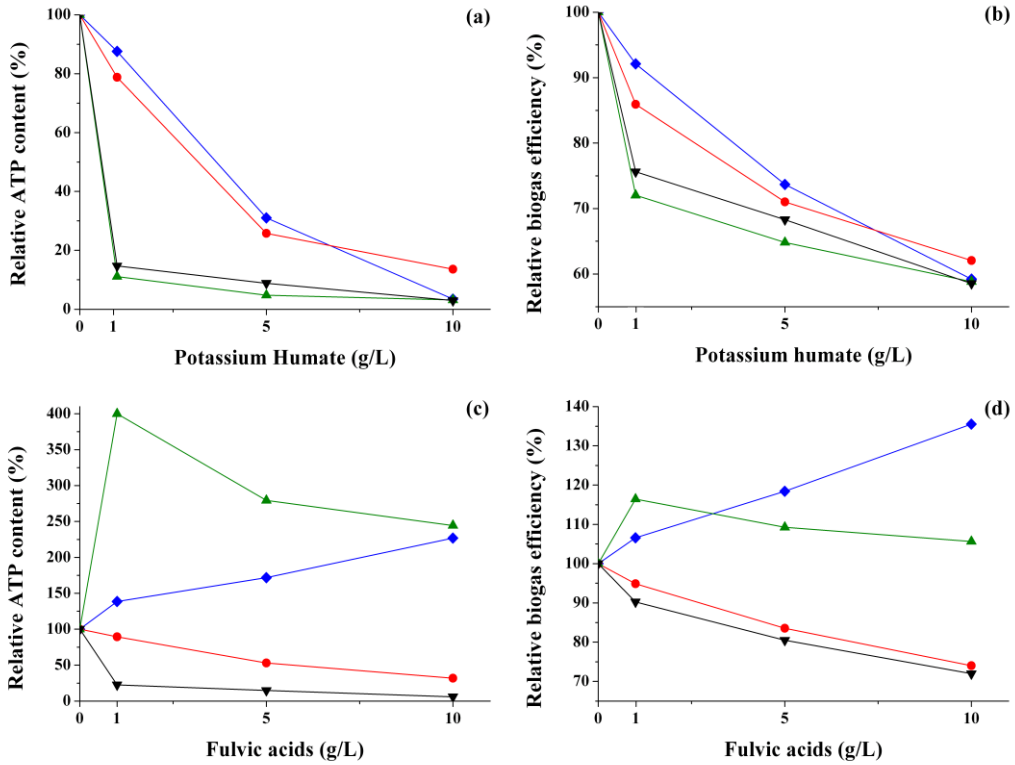

Figure 2. Changes in the level of intracellular ATP of cells of anaerobic consortia (**a**,**c**) and the level of biogas (**b**,**d**) accumulated under the action of anaerobic consortia in the presence of PH (**a**,**b**) and FA (**c**,**d**) in relation to control parameters obtained in the absence of HC at the end of methanogenesis. The parameters observed in control samples (without HC) were taken as 100%. The blue, red, green and black lines correspond to the consortium I, II, III, and IV respectively.

The positive correlation between the changes of the investigated parameters (ATP concentration and methanogenesis efficiency) in relation to control samples (Figure 2) was confirmed for all the anaerobic cell types studied. The introduction of PH in the studied concentrations into the medium led to a decrease in the intracellular concentration of ATP and to a decrease in the accumulated biogas amount for all the consortia (Figure 2a,b). At the same time, the introduction of FA into the medium accompanied an increase in the concentration of ATP in the cells and an increase in the methanogenesis efficiency in the case of consortia I and III, and, vice versa, to a decrease in both parameters for consortia II and IV (Figure 2c,d).

4. Discussion

The monitoring of the overall energy status of the cells by determining the concentration of intracellular ATP during the cultivation of natural anaerobic consortia in the presence of FA showed an increase in this parameter for two of the four tested consortia by the end of the process compared with the initial values of the same parameter. On the contrary, the introduction of PH into the medium resulted in a decrease in the concentration of intracellular ATP in all consortia. The difference in the effect of the natural HCs on the microbial consortia studied is probably due to the differences in the composition of these HCs. Recently, researchers have noticed that aromatic and aliphatic groups similar to those found in humic acids are found in the molecules of FA, but the aromatic part of FA molecules is less pronounced [28]. Since aliphatic groups are carriers of hydrophilic properties (as opposed to hydrophobic aromatic groups), FA, with a wider ratio of aliphatic groups to aromatic groups, are different from humic acids and more hydrophilic. When studying the effect of different HCs with various chemical structures, hydrophobicity, and degrees of aromaticity on the methanogenic activity of anaerobic sludge, it was found that FA with less hydrophobicity had no effect on the process of all the stages of methanogenesis. At the same time, HCs with greater hydrophobicity inhibited acetoclastic methanogenic activity and disrupted the metabolic pathway of the conversion of acetate to methane.

In another investigation, in the process of anaerobic cellulose and xylan digestion with crushed anaerobic granular sludge that was realized with the introduction of HCs (Sigma-Aldrich; CAS number 68131-04-4) at concentrations of up to 0.4 g/L, microbiological analysis showed that the concentrations of bacterial cells of the genera *Clostridiales*, *Bacteroidales*, and *Anaerolineales*, as well as hydrogenotrophic methanogens, were significantly reduced in the presence of HCs [29]. It was found that the relative number of bacteria of *Methanobacteriaceae* and *Methanomicrobiales*-WCHA208 was greatly reduced in the presence of HCs, whereas the number of *Methanosaetacea* bacteria did not change.

In still another investigation, an analysis of the specific methanogenic activity of granular anaerobic sludge in wastewater containing sodium humate at a concentration of up to 2 g/L showed an increase of this parameter by 19.2% [13]. These results demonstrated that HCs can enhance the metabolic activity of granular sludge. This is probably due to the retaining effect of HA on the morphology of the sludge particles.

Other researchers, using the example of four anaerobic consortia, showed that HCs at concentrations close to 2 g/L can inhibit the hydrolysis of protein and carbohydrate-rich substrates and reduce the intensity of acetotrophic methanogenesis [14]. However, various inoculums had different HC inhibition levels, possibly due to the differences in bacterial metabolic activity and microbial community composition with certain biochemical properties. The inoculums with higher hydrolytic rates and higher relative abundance of the hydrolytic bacteria of *Actinomycetales* and *Pedosphaerales* were more resistant to HC inhibition.

Thus, the microbial composition of anaerobic consortia, developing in certain places and under certain conditions of formation of methanogenic populations, predetermines their metabolic activity, since the presence in their composition of different types of cells that intensify various stages of methanogenesis can lead to the formation of biogas with an increased CH_4 content in the presence of natural HCs.

It has been established that the use of natural FA in high concentrations does not lead to the suppression of the metabolic activity of methanogenic consortia. At the same time, the introduction of PH into anaerobic sludge in increasing concentrations leads to a decrease in the energy status of all the studied microbial samples of consortia.

The comparison of the results on the efficiency of methanogenesis (Figure 1) and the level of ATP at the end of the process in the cells of natural consortia (Table 2) in the absence or presence of HCs showed that there was a positive correlation between these characteristics. Particularly noteworthy is the fact that the increase in the efficiency of methanogenesis beyond that in the control when FA was introduced into the medium was clearly reflected in the intracellular concentration of ATP. We hypothesize that this phenomenon may be due to the fact that bacterial cells in consortia I and III, where this was observed, could decompose FA and use them as substrates. Such a pathway of HC degradation was noted recently in a study of methanogenic treatment of wastewater entering a refinery under the action of anaerobic sludge in the presence of HCs [30]. The destruction of 1.75 g/L humic acids and 0.34 g/L FA present in wastewater was observed at 16.5% and 27%, respectively.

Additionally, another reason for the observed ATP increase in the case of FA introduction may be due to ATP synthesis catalyzed by ATP synthase, which is dependent on both the Na^+ and H^+ gradient. It was shown recently that it is possible to generate the Na^+ and H^+ gradient during the reduction of Fe (III) in the presence of anthraquinone-2,6-disulfonate (AQDS) by using a *Methanosarcina acetivorans* inverted membrane vesicle [31], and ATP synthesis by ATP synthase was suggested. Recently, conservation of energy to support cell growth solely from extracellular electron transfer was demonstrated for *M. acetivorans* grown with methanol as the carbon source and the extracellular electron acceptor AQDS as the sole electron acceptor [32]. Since anaerobic consortia I and III grew at 37 °C, it must comprise methanogens with cytochromes and the possible generation of the Na^+ and H^+ gradient that can lead to ATP synthesis by ATP synthase.

In this work, the analysis of CH_4 content in the resulting biogas showed that the changes in its concentration did not directly match the variations of the intracellular concentration of ATP, although changes in the composition of biogas were evident when the studied HCs were introduced in different concentrations into the media (Figure 1). A decrease in the portion of methane in biogas was observed due to an increase in the amount of accumulated CO_2. A decrease in the accumulation of CH_4 was also noted in another study on the effect of HCs on methanogenesis with anaerobic sludge when using cellulose or xylan as a substrate [28]. It was shown that an increase in the concentration of HCs to 8 g/L inhibited the efficiency of hydrolysis of the substrates by 40% with a simultaneous decrease in the CH_4 yield. Average daily biogas production and the methane content obtained with the addition of HCs was 2680 ± 10 mL, which was significantly lower than that in the case without HCs (3995 ± 362 mL) [28]. The observed inhibition was explained by the adaptation capacity of the microbial community to the elevated HC concentrations. Additionally, it was found that granular sludge using H_2, formate, or acetate as substrates provided the maximum observed reduction in the total amount of the produced CH_4 (24%) at 5 g/L HC [28]. In our work, the decrease in CH_4 content as a part of biogas was more pronounced for some variants of the anaerobic consortia used (Figure 1).

In summary, it can be stated that, depending on various natural HC types (PH and FA) introduced into the medium in different concentrations, the efficiency of methanogenesis caused by natural anaerobic consortia varied greatly. To suppress methanogenesis using HCs under real conditions, it is necessary to check the effect of each sample. It is obvious that the concentration of intracellular ATP reliably reflects the metabolic activity of the cells of methanogenic consortia and can be used to obtain a prompt response regarding the effect of HCs on the whole process, but it does not directly correlate with the changes in methane content in the accumulated biogas.

5. Conclusions

A comparison of the results obtained by determining the methanogenesis efficiency and the level of ATP in the cells of four natural consortia in the absence or presence of various natural HCs

(PH and FA) showed that there was a positive correlation between these characteristics at the end of the process. An increase in HC concentration in the cultivation medium led to a decrease in the intracellular concentration of ATP in the cells and to a suppression of the formation of biogas as a whole. A decrease in the proportion of CH_4 was noted for three of the four samples of consortia. On the other hand, the presence of FA led to an increase in the ATP concentration, and methanogenesis was stimulated. Thus, ATP measurement can be used for screening among HCs for the most effective candidates allowing methanogenesis intensity to be decreased, and to evaluate the concentration that can significantly suppress biogas formation by certain methanogenic consortia.

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