

## Article

# Improvement of Biogas Yield by Pre-Treating Poultry Waste with Bacterial Strains

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**Abstract:** Poultry waste is increasingly used as a substrate for the methane fermentation process in a biogas plant. However, not all waste materials processed in the meat industry meet the criteria for optimal process management and cost-effective methane efficiency. An example may be centrifuged biological sludge, etc. Treatment of such material used as a substrate by introducing new metabolically and enzymatically active strains of bacteria could be beneficial for the fermentation process in a biogas plant and provide increased energy efficiency. The aim of the study was to compare the amount and quality of biogas obtained from biological sludge from the processing of poultry vaccinated with metabolically diversified bacterial inoculum after initial incubation of the batch before the actual process in a biofermenter. Laboratory tests were carried out in accordance with the guidelines contained in the DIN 38 414-S8 and VDI 4630 standards. Based on the obtained results, it was found that the optimized biological sludge can be used as a substrate in the methane fermentation process in a biogas plant. The material processed by the combination of bacterial strains marked with the symbols A/C, E/G, and F/H showed a significantly increased efficiency of biogas, including methane, compared to the non-grafted material. This is a good predictor for industrial applications, process feasibility, economic viability, and environmental sustainability that should be compiled based on the results obtained from this study.



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**Keywords:** bacterial strains; bacterial enzymatic activity; biological sludge; poultry waste; pre-treatment; biogas production

## 1. Introduction

Poultry production in Poland and in the world is growing on a larger scale, which is associated with a large demand for this type of meat. In the first quarter of 2021 (according to the Central Statistical Office), a total of 697 thousand jobs were produced in poultry industry plants employing 50 or more employees. Moreover, tons of poultry meat have been produced—8% more than last year. According to KOWR analysts, poultry production in 2021 may increase by 6–7% compared to 2020. In previous years, in 2018, according to estimates by the United States Department of Agriculture (USDA), global chicken production increased by 2%, to 95, 5 million tons. Higher production was recorded on the markets of the world's leading producers of poultry meat, i.e., in the United States (an increase of 2.2%), the European Union (by 2.4%) and China (by 0.9%). According to the USDA forecast, in 2019 and the following years, the global production of chickens will reach a record level of 98.4 million tons, 3% higher than in 2018. The dynamically growing production in China (an increase of 8%) will have the greatest impact on the

increase in world production) and further development of production at the remaining largest global producers, i.e., the USA, Brazil, and the EU [1]. In 2019, the determinants of poultry production growth in Poland are export demand and internal consumption. The European Union is a net exporter of poultry products. In 2018, 1.8 million tons of poultry products (in carcass equivalent) were sold outside the EU customs territory, which is 6.5% more than in 2017. The increase in the volume of EU exports was mainly due to increased sales to Ukraine, the Philippines, and Ghana [2].

Due to the fast growth rate of the poultry industry, a significant impact on the elements of the natural environment is observed through the production of huge amounts of waste and post-slaughter pollution [3]. A number of negative processes take place at landfills for poultry processing, such as rancidity, spoilage, and uncontrolled decomposition of organic compounds and fermentation processes [3,4]. One of the methods of processing poultry waste may also be fermentation in biogas plants. This waste is rich in fats and proteins, hence, it has a high potential as a substrate for energy production in biogas plants [4,5]. A biogas plant is a complete plant for converting biomass into biogas. The process that produces biogas is called methane fermentation. Bacteria living in anaerobic conditions are responsible for its course.

There are four stages in the methane fermentation process. The first stage is hydrolysis, during which organic compounds are broken down into simple organic compounds under the action of water and enzymes. The second stage is the organic acid formation phase (acidogenesis), in which the hydrolysis products are further broken down into fatty acids such as acetic, propionic, and butyric. The next phase is the formation of acetic acid (acetateogenesis). In this phase, higher fatty acids are further broken down into acetic acid. During the decomposition of organic matter into fatty acids, and then into acetic acid, they release, among others, carbon dioxide and hydrogen. From these compounds, methane bacteria synthesize methane in the last stage of biogas production, called methanogenesis. In the digestion chamber of a biogas plant, all these steps run in parallel. The microorganisms that are responsible for the various stages of fermentation live side by side. Connected by food dependencies, they live in a relative balance. The presence of all groups of microorganisms and their correct proportions determine the proper course of fermentation, and, therefore, also the quality of the obtained biogas. The imbalance between microorganisms changes the composition of the biogas, even to the complete elimination of methane. In the transformation of methane fermentation, the speed at which the intermediate products of one phase are formed is proportional to their distribution in the next.

The most favorable course of the total biomass decomposition is obtained at the same rates of changes that take place in acid and methanogenic fermentation. However, not all processed waste materials have optimal substrate quality. Various modifications of the enrichment method and alternative parameters are still sought, which can improve the quality of the waste substrate being a carrier of renewable energy sources and improve the efficiency of the biogas production process and increase its efficiency. The basic parameters of the fermentation process include various factors, such as the optimal growth temperature for mesophilic bacteria within the range of 25–45 °C and thermophilic bacteria above 55 °C and this significantly facilitates mass transport [3,6], the pH of the process, the C/N ratio—the optimal value corresponds to a ratio in the range 10:1–25:1, some literature references indicate 100:3 as the maximum value of C:N. If the maximum value is exceeded, nitrogen will be used by the methanogenic fermentation bacteria and this will reduce the amount of biogas produced. If the quotient falls below the lowest value, nitrogen will be released in the form of ammonia and will increase the pH of the environment. This, in turn, will disturb the nitrogen balance and have a toxic effect on methanogenic bacteria. Ammonia (NH<sub>3</sub>) is more than 20-fold more toxic than the ammonium ion. The COD:N ratio is considered optimal when it ranges from 400:7 to 1000:7, the ratio N:P:S = 7:1:1, and C:N:P:S = 600:15:5:1. In addition to the above-described base substances bacterial growth, sodium, iron, magnesium, calcium, soluble forms of potassium, and trace elements (copper,

zinc, selenium, nickel, molybdenum, manganese) are also necessary for their metabolism. However, the abundance of agricultural waste in the appropriate amounts of the mentioned components does not require the adjustment of the input composition [3]. In addition, other process components such as water abundance, inhibitors, hydraulic retention time, mixing, nutrients rich in nitrogen, carbon compounds, phosphorus, sulfur, and other elements are among the basic nutrients for maintaining adequate viability of microorganisms [6–59].

Maintaining the kinetic equilibrium in individual phases of fermentation is an important element for its proper course. Methanogenic bacteria are responsible for the closure transformation process and they must be provided with favorable environmental conditions. Disruption of any of the first three phases may reduce the activity of methanogenic bacteria, resulting in a decrease in the amount of biogas produced.

Other sources of obtaining biogas from animal and plant residues include:

- The bioaugmentation processes which can increase biomethane production [59].
- The cellulose-containing co-substrate from the dried leaves which after preliminary preparation can intensify biogas evolution and reduce the lag phase of the production of methane in the anaerobic digestion of poultry litter [60].
- The complete utilization of kitchen waste and its conversion to biogas can be achieved with a comprehensive treatment technology consisting of pre-treatment, wet solution, oil-water separation, and anaerobic digestion. The lubricant is converted into an ester by acid catalytic pre-esterification and alkaline catalytic transesterification followed by vacuum distillation to produce biodiesel and biological desulphurization to effectively clean the biogas [61].
- Production of biogas and methane using Buswell's equation using a mixture of maize silage and sweet sorghum versus sweet sorghum alone and their combination in central Ukraine in 2013–2016. Field data were obtained from mixed cultivation of Dovista sweet sorghum and Monica 350 hybrids [62].
- In biogas production, the effect of anaerobic simultaneous digestion of chicken feather (CF) and horse dung (HD) was also investigated [63].
- The use of a mixture of chicken manure with sawdust with ordinary barley straw in combination with *Pleurotus ostreatus* fungi gave the highest amount of biogas in all mass proportions [64].
- The massive amount of poultry (PM) and buffalo (BD) manure is generated by the growing poultry and buffalo industries in Pakistan, causing serious environmental problems such as air, water, and soil pollution. The research aims to use PM and BD to generate biogas in order to meet the energy needs of people and to save the environment that has been degraded as a result of inappropriate disposal of selected waste. The anaerobic co-fermentation technique was used to treat poultry manure and buffalo manure [65].
- Anaerobic co-digestion of KW and PM could be a sustainable way of producing clean and renewable energy in the form of biogas while minimizing environmental impact. In this study, the anaerobic co-digestion of KW with PM was studied to assess the rate of cumulative biogas (CBG) production and methane percentage in four digester setups (D1, D2, D3, and D4) operated in batch mode [66].

The aim of the research was to determine the quantity and quality of biogas produced from centrifuge sludge in the poultry industry after their optimization by consortia of bacteria with high metabolic potential, which include acid-producing, acetate, and methanogenic bacteria.

## 2. Materials and Methods

### 2.1. Methodology Used

The study used biological sludge after centrifugation from the biological sewage treatment facility located in the poultry processing plant in Western Poland. The method used for centrifuging the biological sludge in our case was the most optimal for further analysis. The manuscript by using new unique strains marked with the symbols A/C,

E/G, and F/H showed a significantly increased efficiency of the biogas method, including methane, compared to the non-grafted material is a new look at the methodology, making it more innovative.

## 2.2. Other Alternative Methodologies Used

In addition to the presented methodology, new innovative applications are also used in technologies such as: autoclaving with sorting (RotoSTERIL technology—which can also be treated as pre-treatment of mixed waste), pressure extrusion of mixed waste, depolymerization, methane fermentation, and hydromechanical separation. Some of these technologies are a complete recovery process (i.e., from the waste substrate to the final product), others are only a certain preliminary stage, although they may positively affect the full technological process.

In the case of raw municipal waste, there are two main concepts of its management in the fermentation process: fermentation of unsorted municipal waste, represented, e.g., by heaps (a) energy, GICON, and Strabag technology (Poland, Parzniew) or the fermentation of the squeezed FOOK fraction postulated by the VM PRESS company, or the autoclaved and segregated (Bioelectra Group SA) biodegradable waste fraction; fermentation of municipal waste sorted at the source—postulated in Directive (b) 2008/98/EC, due to the possibility of easier disposal of digestate; often thermophilic, combined with the technology of biogas enrichment and liquefaction of biomethane for transport purposes—a method particularly popular in Sweden.

## 2.3. Analyzed Material

In the analyzed material, the organic carbon content, as well as total nitrogen and sulfur, were determined using Coestech's CNS elementary analyzer. Biological sludge is characterized by the following content of organic carbon –38.05% DM (dry matter), total nitrogen –6.57% DM, and total sulfur –0.787% DM.

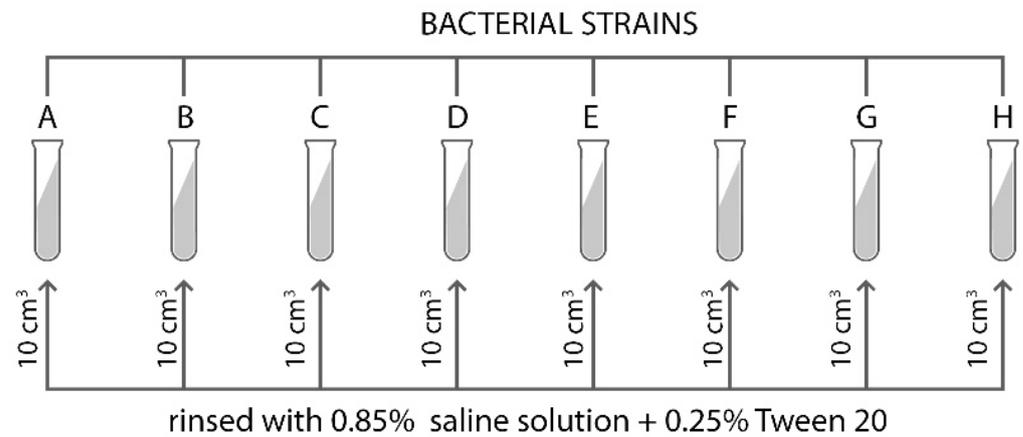
In an earlier stage of the study, 150 microbial strains with high metabolic potential were isolated from poultry waste at different stages of its management, i.e., slime generated in the liquid waste pool, biological sludge after centrifugation, feathers (from chickens, ducks, turkeys, and goose), and proper compost. The research material was subjected to the action of microorganisms characterized by high enzymatic activity towards protein and fat. Microorganisms with a lipolytic capacity were grown on a Kosewska medium [9], while proteolytic according to the recipe by Kędzia and Koniar [10]. After the initial selection, these strains were evaluated including calculation of the activity index. As a result, 8 of several tens of units of outstanding bacterial strains were selected (Table 1). Bacterial cultures were propagated on Tryptone Soya Agar (TSA) medium at 30 °C/48 h. The selected bacteria were identified by amplification of 16S rDNA fragment in accordance with the methodology recommended by MicroSeq.

**Table 1.** Enzymatic activity and density of the bacterial strains used.

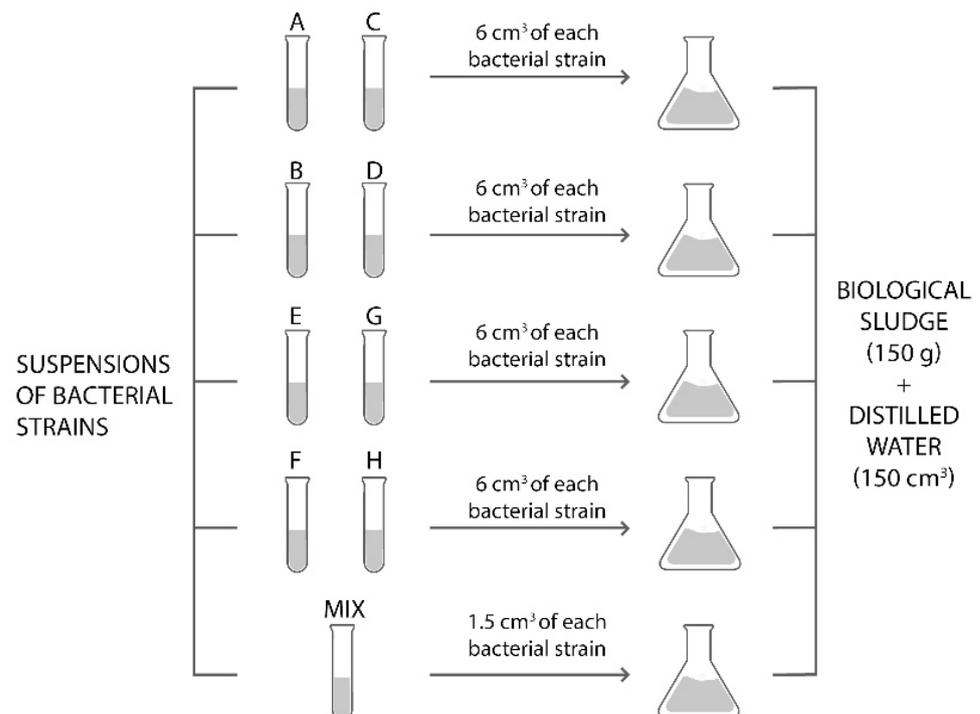
Symbol Strain	Activity Index (IA)		Density [CFU·mL <sup>-1</sup> ]
	Lipolytic	Proteolytic	
A	1.3	3.6	1.7 × 10 <sup>10</sup>
B	1.4	4.2	8.5 × 10 <sup>9</sup>
C	1.5	1.5	2.6 × 10 <sup>10</sup>
D	1.5	1.5	2.5 × 10 <sup>10</sup>
E	1.2	2.6	1 × 10 <sup>10</sup>
F	1.1	1.8	1 × 10 <sup>6</sup>
G	1.2	3.8	3 × 10 <sup>6</sup>
H	1.3	2.4	8.7 × 10 <sup>6</sup>

#### 2.4. Preparation of Sediment Samples

Preparation of sediment samples for optimization was preceded by the preparation of proliferated bacterial cultures inoculum. The material was rinsed with 0.85% saline solution +0.25% Tween 20 in an aliquot of 10 cm<sup>3</sup> (Figure 1). After dilution of activated sediment (150 g) with distilled water (150 cm<sup>3</sup>), suspensions of bacterial strains combinations (A/C, B/D, E/G, F/H, and mixed bacterial consortium—MIX) were introduced into the mixture. Two bacterial strains in an amount of 6 cm<sup>3</sup> each were then added to the flasks with a solution of the biological sludge; in the case of strains mix, 1.5 cm<sup>3</sup> of each bacterial strain (Figure 2). The densities of bacterial suspensions are shown in Table 1. Incubation and optimization were performed on a shaker for 10 days at a temperature of 24–25 °C. Due to relatively high density, samples were additionally mixed by hand using a glass rod twice a day.



**Figure 1.** Diagram of preparation of bacterial inoculum (source: own study).



**Figure 2.** The scheme of preparation of the research material before introduction into the biogas-producing fermenter (source: own study).

### 2.5. Methodical Execution

After preparing the loads for installation, processed research material was mixed with pig slurry in equal weight proportions, and then introduced into the biogas-producing fermenter, where due to methane fermentation, biogas was generated. The fermentation batch was prepared in three replicates. The test was performed according to the modified method described in German standard DIN 38 414–S8 [11] and VDI 4630 [12]. The experiment was conducted under laboratory installation conditions at a temperature of  $37\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ , characteristic of mesophilic methane fermentation. Measurements of the amount and composition of the produced biogas were carried out obtained every 24 h using the biogas analyzer GA 2000 Plus (Geotech, Keison Products, Essex, UK). In order to normalize the results, the biogas volume value was transformed in accordance with the following equation:

$$V_n = V \times \{(p - e_s) \times 273.15\} / (1013 \times T) \quad (1)$$

where:

$V_n$ —normalized biogas volume [mL]

$V$ —observed biogas volume [mL]

$p$ —atmospheric pressure [hPa]

$e_s$ —pressure of saturated steam [hPa]

$T$ —ambient temperature [ $^{\circ}\text{C}$ ]

The analysis involved measuring the amount of biogas produced, and its quality, with particular attention to the content of methane. The content of oxygen, carbon dioxide, hydrogen sulfide, and ammonia was also tested. In addition, the test material, slurry, and fermented residue obtained after experiments were subject to physicochemical analyses of pH (determined using pH-meter), dry matter [13], and organic dry matter (all machines were from Geotech, Keison Products, Essex, UK [14]) in 3 replicates (Table 2). The laboratory experiment lasted 46 days.

Statistical analysis was performed using Statistica 12 software (Timberlake Consultants Poland, Warsaw, Poland). The significance of differences in mean biogas yield values between the analyzed samples was tested by applying the analysis of variance ANOVA. In order to determine the significance of differences in the average yield of methane between samples, statistical evaluation was performed using the non-parametric test of ANOVA Kruskal–Wallis rank.

### 3. Results and Discussion

Bacteria conducting the methane fermentation process, including Archeons, were able to generate biogas abundant in methane from biological sludge resulting from many characteristic transformations of the substrate.

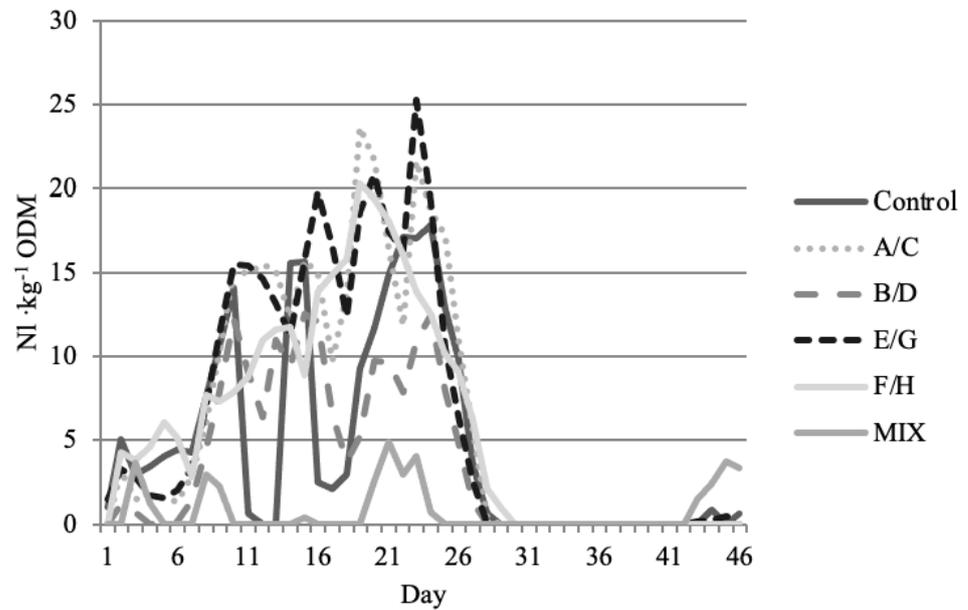
At the stage of hydrolysis and acidogenesis, that is, during “fermentation acidic” bacteria of the genus *Pseudomonas*, *Bacillus*, *Bifidobacterium*, and to a lesser extent *Streptococcus* and *Enterobacterium* have the main share. Acetate bacteria such as *Syntrophomonas* and *Syntrophomonas bacter* are responsible for the conversion of acid fermentation products into acetates and hydrogen, which are the basis for the activity of methanogenic bacteria. Both acidic and acetate bacteria have a long generation time and are very sensitive to environmental changes. Methanogenic bacteria are very diverse in terms of morphological—they are in the form of sticks, spirals, or grains. They specialize in assimilating and processing specific ingredients. Their generation time ranges from 15–18 h [3,6,9]. The rate of their growth depends on the temperature, and with its increase, the rate of their development increases. The optimal conditions for methanogenesis are a temperature of 35–45  $^{\circ}\text{C}$  and a pH of approx. 7. The quality and quantity of biogas are adversely affected by sudden changes in temperature even by 2  $^{\circ}\text{C}$ . That is why it is very important to have designed the methane fermentation system to ensure a constant temperature for individual processes with maximum fluctuations not exceeding 1  $^{\circ}\text{C}/\text{day}$  [28–35]. Maintaining the kinetic

equilibrium in individual stages of fermentation is an important element for its proper course. Methanogenic bacteria are responsible for the closure process of change and must be provided for the purpose environmental conditions. A malfunction of any of the first three phases can reduce the activity of methanogenic bacteria causing a decrease in the amount of biogas produced. The optimal pH for the process ranges between 4.5–7.5 and it is dependent on the methane fermentation phase. Relationships such as weak acids (carbonic acid, phosphoric acid, volatile organic acids, hydrogen sulfide) and weak bases (ammonium hydroxide) shape the buffering capacity of the system and determine the pH value [7]. Fermentation microorganisms are sensitive to certain substances chemical delivered with the raw materials or being intermediates in the decomposition process. They are called inhibitors, and their toxic effect depends on the form in which they occur, the degree of concentration, and the presence of other harmful substances in the fermentation mass (Table 2). The excess nitrogen becomes an inhibitor of the methanogenesis process, as it slows down the growth of bacteria and interferes with the entire degradation process. It can even destroy entire populations of microorganisms and inhibit fermentation. Above 3 g/L it is toxic to methanogens, and is an inhibitor in the range of 1.5–3 g/L at the appropriate pH. Hence, the C:N ratio should not exceed 100:3. Ammonia formed from excess nitrogen also inhibits fermentation, therefore, a batch with a high content of animal feces should be diluted (animal feces contain a lot of ammonium nitrogen). Another way to lower the ammonia content is to increase its C:N quotient by adding a high carbon component such as straw. At low concentrations (0.05–0.2 g/L), ammonia has a stimulating or neutral effect (0.2–1 g/L), while a value of 3 g/L has a toxic effect on methanogens [1–5]. Another toxic inhibitor is oxygen as it causes the activity of bacteria to be disturbed, and the consequence is an increase in hydrogen concentration, limitation of the development of acetateogens, and disturbance of the entire chain of anaerobic degradation. As a result, fermentation is already inhibited before acetateogenesis and acidification of the environment. Other substances that adversely affect the process of fermentation are heavy metals, such as copper, nickel, and chromium in amounts above 0.1 g/L. Potassium, calcium, and magnesium become toxic in amounts above 2.4 g/L. They also have a toxic effect on pesticides and detergents contained in the mass delivered to the chamber fermentation [1,3,5].

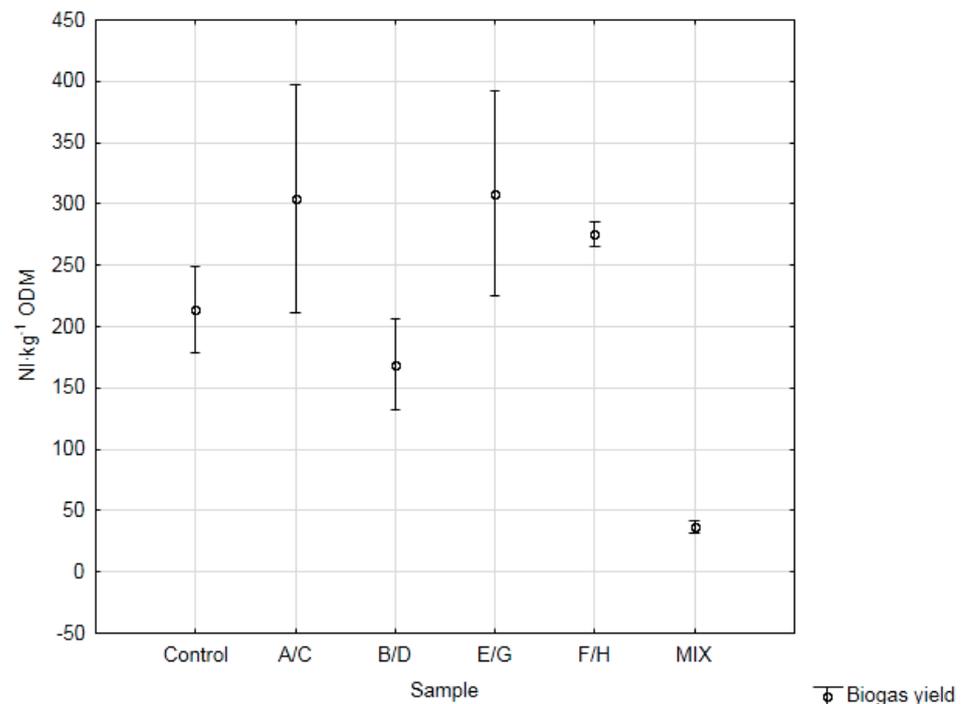
Processing of this material by different combinations of bacterial strains had an impact on the quantity and quality of the obtained biogas. In the control sample, 213.8  $\text{Nl}\cdot\text{kg}^{-1}$  ODM (organic dry matter) was achieved. The greatest amount of biogas (308.4  $\text{Nl}\cdot\text{kg}^{-1}$  ODM) throughout the duration of the methane fermentation process was recorded in material processed by a combination of bacterial strains E/G. Much less potential for biogas production (by 1.4%) had material processed by a combination of strains A/C. Lower biogas yield (by 11%) was found in material being processed through the combination of strains F/H. The lowest amount of biogas was observed in test materials processed by the combination of strains B/D (almost 2-fold lower) and MIX (8-fold lower), as compared to the variant processed by the combination of bacterial strains E/G (Figure 3). Statistical analysis ANOVA showed significant differences in the mean values of biogas yield between samples ( $p = 0.0000$ ) (Figure 4).

From the results obtained, it can be concluded that bacterial strains in combinations A/C, E/G and F/H positively processed biological sludge and significantly increased the biogas yield. Genetic identification of the above strains confirmed the following bacterial species: A—*Micrococcus luteus*, C—*Cellulosimicrobium cellulans*, E—*Brevibacterium luteolum*, F—*Aeromonas veronii*, G—*Bacillus licheniformis*, H—*Bacillus megaterium*. Pre-treatment of biological sludge carried out by these combinations of bacterial strains improved the methane fermentation process, as evidenced by the processes of fermentation. Fermentation of these variants had a much more stable course, which can be attributed to the partial creation of substances contained in the material, by strains forming the above-mentioned inocula. The authors of [15,16] found that biological pre-treatment of the material can accelerate the hydrolysis step during methane fermentation. Similarly, Fraç and Ziemiński [17] concluded that particle size reduction in waste accelerates the hydrolysis step, and thereby increased

the amount of biogas produced. Ojolo et al. [18] observed that mechanical pre-treatment in the form of material grinding has a beneficial effect on biogas production, because of the easier availability of nutrients for bacteria.



**Figure 3.** Daily amount of biogas produced in the methane fermentation of tested materials.



**Figure 4.** Graphical interpretation of variance analysis results for the biogas yield in tested samples.

The significantly lower yield of biogas in the material processed by a combination of bacteria marked as B/D can prove the competition of strains displacing one another. The use of a mixture of bacterial strains significantly reduced the degree of utilization of the analyzed material by methanogenic bacteria. This could be caused by large mineralization of the sample due to bacteria characterized by high and diverse enzymatic activity, as well as a lack of access to suitable substrates for methanogenic bacteria. There is also a

possibility of secondary metabolites accumulation of enzymatic bacteria, which inhibited the development of methanotrophs.

The fermentation of raw biological sludge not subjected to the influence of enzymatically active bacteria had a very unstable course. Intensive biogas production was twice stopped. The phenomenon of irregular biogas production is known and is called diauxic growth [19]. Diauxic growth is the phenomenon whereby a population of microbes, when presented with two carbon sources, exhibits bi-phasic exponential growth intermitted by a lag-phase of minimal growth. Originally, the phenomenon was described by [19] demonstrating diauxic with glucose and lactose in *E. coli*. However, in the case of the sample to be analyzed, these changes can be considered too violent and under unfavorable circumstances, they may be the cause of stopping the biogas production in the fermenter.

The average methane content, regardless of the combination, was at a similar level and amounted to about 60%. The relatively high amount of methane may be due to a high content of protein in the material, which was confirmed by Lalak et al. [20]. Lewicki et al. [21] obtained a very similar methane content of 57.38% in chicken droppings. Performed optimization of biological sludge originating from the poultry waste processing by the addition of bacterial strains with a high metabolic potential resulted in a significant increase in the amount of biogas, which results in an increased yield of methane (Figure 5). The highest methane yield (196 Nl CH<sub>4</sub>·kg<sup>-1</sup> ODM) was recorded in the material processed by a combination of bacterial strains A/C and E/G. Lower methane yields (by 13%) were found in combinations F/H and B/D (by 45%). The lowest gain was observed in a mixed combination of strains, where only 22.19 Nl CH<sub>4</sub>·kg<sup>-1</sup> ODM was produced. In unprocessed material, 130.55 Nl CH<sub>4</sub>·kg<sup>-1</sup> ODM was achieved. Statistical analysis showed significant differences in the average yield of methane in the tested samples (Figure 6).

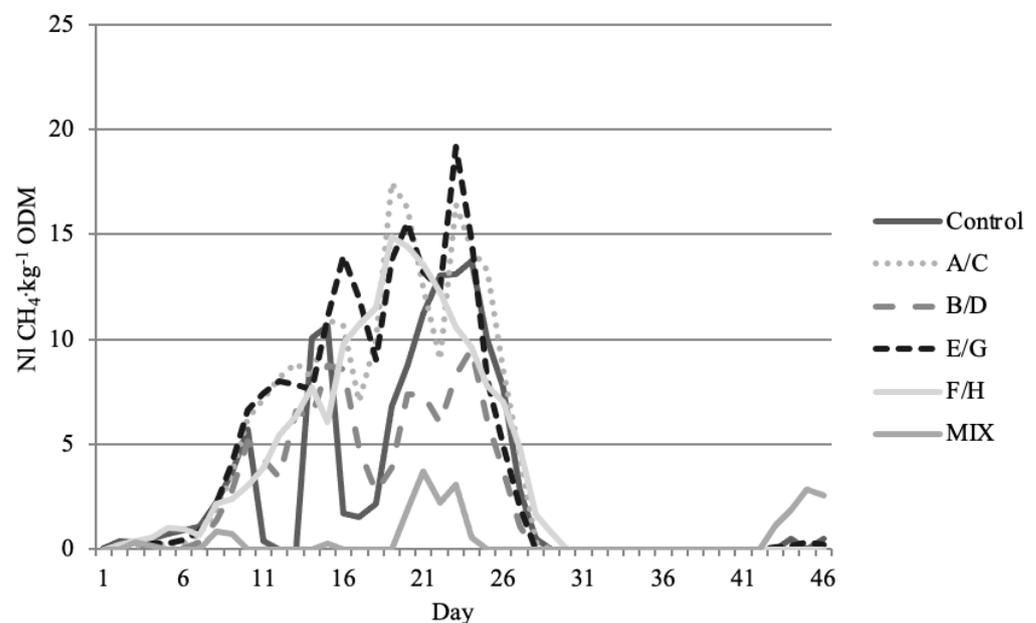
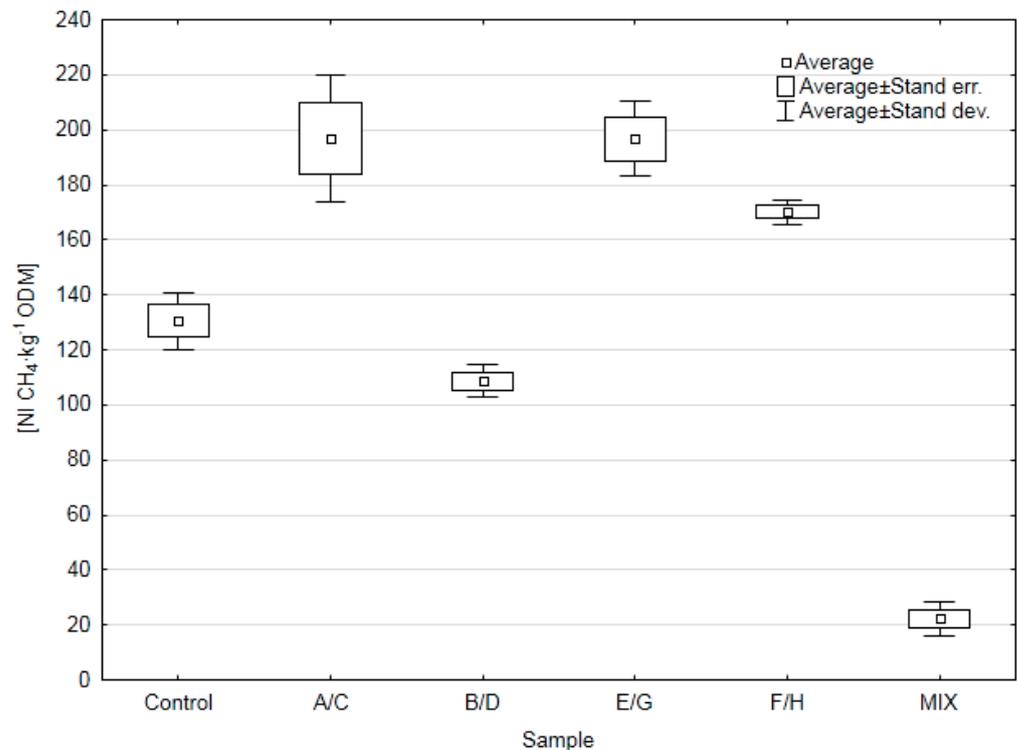


Figure 5. Daily yield of methane in the analyzed variants.

As in the case of research carried out by Sarikaya and Demirer [22], it was concluded that wastes from the slaughter water treatment plant are a good co-substrate in the production of biogas, and its potential and usefulness can be significantly increased by subjecting it to an appropriate treatment. Cavaleiro et al. [23] found that enzymatic pre-treatment of meat industry waste increases methane production. The authors found that even better results can be obtained when using a combination of lipase and protease. On the other hand, Forhad Ibne [24] Al-Imam et al. [25], in their study, also showed that poultry waste has a great potential for biogas production during the methane fermentation process. Ziemiński [15], in their study, also showed that enzymatic treatment proved to be an

effective way of converting the biomass, in this case, lignin-cellulose materials, resulting in an increase in the biogas potential.



**Figure 6.** Average yields of methane in the tested samples.

An increase in the pH value of the resulting fermentation masses was found with respect to the initial substrates used for the process of methane fermentation (Table 2). A similar trend was found by Ali and Sun (2015) in their study.

**Table 2.** Physicochemical parameters of substrates used for the experiment and achieved fermented material.

Sample	pH		Dry Matter [% DM]		Organic Dry Matter [% ODM]	
	Substrate	Digestate	Substrate	Digestate	Substrate	Digestate
Slurry	7.28	8.35	4.42	3.76	71.73	66.84
Control	7.36	8.19	4.59	3.71	52.60	66.73
A/C	7.11	8.08	3.52	3.91	66.48	58.44
B/D	7.7	8.13	3.83	3.54	60.89	59.47
E/G	7.52	8.08	3.83	4.19	67.15	57.28
F/H	7.51	8.24	4.47	3.66	59.86	69.74
MIX	7.66	8.18	4.57	3.51	56.65	72.00

Process of poultry sediments optimization carried out by selected bacteria strains showed lower levels of inhibitors (NH<sub>3</sub>, H<sub>2</sub>S) expressed in ppm as compared to the control. The average content of NH<sub>3</sub> was the following: control—174.20 ppm, A/C—45.68 ppm, B/D—40.51 ppm, E/G—31.52 ppm, F/H—66.30 ppm, MIX—37.44 ppm. The mean content of H<sub>2</sub>S: control—221.50 ppm, A/C—104.62 ppm, B/D—79.11 ppm, E/G—65.45 ppm, F/H—121.10 ppm, MIX—75.39 ppm. This phenomenon may be a result of the hydrolysis, among others, of proteins in the analyzed substrate at the stage of pre-treatment by the inoculum added. As well as increasing the production of volatile fatty acids from activated sludge waste thanks to a novel cation exchange resin support strategy, many parameters such as temperature, C:N ratio, moisture content, or pH are used to assess the stability and quality of the final product [36]. Inoculation has a significant

impact on the rate of decomposition of organic matter. Numerous microorganisms can transform the main constituents of plant waste such as starch, cellulose, and organic acids into a humus-like product in which nitrogen will be converted from an unstable ammonia form to a stable organic form [37]. Inoculation of municipal waste reduces the molecular weight of proteins and polysaccharides, which accelerates the compost maturation process compared to compost without an inoculum [38]. Research by Ghaffari et al. [39,40] also showed an increase in the activity of the cellulase enzyme and the biodegradation of organic matter. Similar results were obtained by Zeng et al. [41] during the composting of garden waste. The inoculation of *Trametes versicolor* can increase the aromatization of the humic acids contained in waste. Many authors note that inoculation with groups of microorganisms is more effective than inoculation with single species of bacteria [38,41,42]. Wei et al. [38] observed that inoculation with many complementary groups of bacteria (including *Bacillus casei*, *Lactobacillus buchneri*, and *Candida rugopelliculosa*) increases the degree of humification and maturation. Obtained by Barrena et al. [43], the results show that there is no significant difference between the different doses of microorganisms used to vaccinate waste (106, 107, 108 CFU g<sup>-1</sup>), therefore, they considered an optimal dose of 106 CFU g<sup>-1</sup>, which allows reducing the composting time by half, which also significantly reduces the cost of the process. In order to increase the composting efficiency, inoculation should be adjusted to the process conditions, especially the nature of the organic matter contained in the composted material and the temperature [38]. Ohtaki et al. [44] note that the addition of microorganisms, admittedly, does not significantly increase the rate of temperature increase, but increases the time of maintaining high temperature. Sarkar et al. [45] noted that the biodegradation process can be more effective when compost is inoculated with thermophilic bacteria (*Geobacillus* strains). Conceived by Ohtaki et al. [44], studies indicate that native and vaccinated microbial populations evolve continuously, leading to changes in their populations at various stages of the stabilization process. Most microorganisms, with the exception of mesophilic fungi and yeasts, tolerate well or even proliferate in the thermophilic phase. The thermophilic phase does not adversely affect the microbial population, especially when relatively low temperatures, around 50–55 °C, prevail at the beginning of the process. The late appearance of the most intense thermophilic phase, after 2–3 weeks of processing, may give the microflora the ability to better adapt to the conditions present in the pile. At the end of the thermophilic stage, in which easily digestible organic compounds are consumed, bacteria multiply with proteolytic, amyloolytic, and cellulolytic activity [46]. On the other hand, the thermophilic phase in the biostabilization process is very important for killing many unwanted microbes [47]. A positive effect of bioaugmentation in the treatment of metal waste was also noted. Zeng et al. [48] observed that inoculation with White rot fungi can effectively reduce the bioavailability of lead (Pb). The use of microbes cooperating with the aquatic plant *Vallisneria spiralis* L. allowed for the effective removal of chromium (Cr) after 21 days of waste stabilization [49]. Moreover, *Bacillus licheniformis* thermophilic bacteria can efficiently break down proteins and protect against a drop in pH during the process, which can stimulate the proliferation of other thermophilic bacteria. Inoculation of food waste with the lactic acid bacteria *P. acidilactici* accelerated the process by solving the problem of low the pH of the waste. Inoculated mushrooms produced a high concentration of lactic acid, which inhibited the production of acetic acid, which is toxic to many microorganisms. This allowed an increase in the activity of fungi capable of decomposing organic acids, which in turn resulted in an increase in the pH level and acceleration of the process [50]. The biostabilization process is also a source of nitrogen compound emissions. Particular attention is paid to the emission of ammonia, which is one of the main gases produced in this process [51]. The removal of ammonia by microorganisms is becoming the main means of combating these emissions due to many advantages such as low cost and no additional impurities [52]. Inoculation using ammonia-oxidizing bacteria such as *Nitrobacter* and *Thiobacillus* was described by [38]. The results they obtained proved that nitrogen losses can be pronounced when composting material with high nitrogen content. These losses

can be from 74% in relation to the input material [53,54] up to 84.4% from chicken litter [54]. The addition of 1% by volume (10 mL per kg of sample) of a preparation containing ammonification and nitrogen bacteria and *Azotobacter* selected from manure derived from cows, chickens, and pigs increased the initial and final pH value by approx. 0.5 compared to the control sample [55]. The above examples show that the use of techniques significantly improves certain aspects related to increasing the intensity of processes, which could be used in the biostabilization of waste. Unfortunately, the use of dedicated genetically modified organisms is much more beneficial in the case of monocultures. This is due to the lack of competition from other organisms and the ability to adjust the optimal conditions for a given organism. Unfortunately, in the case of biostabilization of municipal waste, the homogeneous environment of bacteria is impossible to apply because municipal waste contains a very diverse microbiocenosis. Under such conditions, genetically modified organisms are not able to compete with heterogeneous microbiocenosis, which will develop more efficiently, using up the available nutrients faster [56–58].

#### 4. Conclusions

Centrifuged biological sludge from the processing of poultry industry waste, after appropriate optimization by active bacterial inoculum, may be a good batch substrate for the biogas-producing facility.

Biological sludge processed by selected bacterial strains marked as A/C, E/G, and F/H with high metabolic potential, was characterized by significantly higher biogas yield, including methane, as compared to the control material.

The process of optimizing the centrifuged poultry sediments preceded by pre-incubation using bacterial inoculum before the treatment can reduce the content of inhibitors ( $\text{NH}_3$  and  $\text{H}_2\text{S}$ ) produced during a proper methane fermentation process. Specification of the parameters at which the fermentation process of methane gas is the most effective in terms of the extraction of methane is of great importance. Providing microorganisms with optimal environmental conditions, it is important that this process proceeds smoothly, as well as to ensure as much as possible maximum biomass decomposition and intensification of the share of methane in biogas. The efficiency of waste biostabilization can be increased not only by optimizing the operational parameters of technological systems. Increasingly, one of the methods of increasing the efficiency of biostabilization processes is the use of the bioaugmentation technique (inoculation). The bioaugmentation technique consists of introducing allochthonous or autochthonous microorganisms into the natural or technical environment in order to intensify metabolic changes. The introduction of the inoculum to the stabilized mass of waste shortens the stabilization time and improves the quality of the stabilizer. Microorganisms introduced into the stabilized mass of waste should accelerate the initial biodegradation, and the resulting intermediate products of transformation should influence the humification.

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