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QUANTITY AND QUALITY OF BIOGAS PRODUCED FROM THE POULTRY SLUDGE OPTIMIZED BY FILAMENTOUS FUNGI

IŁOŚĆ I JAKOŚĆ BIOGAZU OTRZYMANEGO Z OSADU PODROBIOWEGO OPTYMALIZOWANEGO PRZEZ GRZYBY STRZEPKOWE

Abstract: One of the methods for recovery and utilization of waste products from the poultry industry is to subject them to the methane fermentation process in the biogas plant. These are waste with a high content of fatty compounds and proteins, including keratin. Their specificity is characterized by rapid possibility of spoilage, rancidity and problems of further management. These wastes are characterized by varying degrees of complexity, thus their use as a raw material for the biogas fermenter should be preceded by a pre-treatment. An example of waste generated in poultry processing is biological sludge. Optimizing this material with highly enzymatic fungi could accelerate the degradation of the organic matter contained and, as a result, increase the energy efficiency of this type of waste. Quantitative and qualitative parameters of biogas produced from biological sludge processed by isolated filamentous fungi with high metabolic potential were determined. Laboratory tests were based on the modified methodology included in the standards DIN 38414- S8 and VDI 4630. Based on the results obtained, it was found that the pre-optimization of biological sludge by fungal strains with different metabolic potential, influences on the yield of biogas production, including methane. There was an increase in the biogas yield from the biological sludge processed by the mixed fungal consortium (by 20 %) and the strain marked as F1 (by 14 %) as compared to the non-inoculated material, which was also reflected in the amount of methane produced in the case of the mixed fungal consortium (by 28 %) and the strain marked as F1 (by 12 %).

Keywords: filamentous fungi, biological sludge, poultry waste, pre-treatment, biogas

Introduction

The use of biomass energy, especially waste, becomes increasingly popular. Therefore, more and more investments are being made related to the possibility of producing biogas from plant biomass and much other agricultural waste [1, 2]. One of the most important parameters influencing the efficiency of the biogas process is the type and quality of the raw materials used - the batch [3, 4]. Hence, components containing nutrients with high energy potential are highly desirable. These requirements are met by waste products from the poultry industry, mainly due to the content of fats and proteins [5-7]. Their use in

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methane fermentation in agricultural biogas plants allows them to use the energy contained, thus eliminating their negative environmental impact. However, it is extremely important to prepare the substrate as a batch for fermentation. Various pre-treatment methods (chemical, physical, mechanical) of the input material can significantly improve its energy efficiency during the methane fermentation process [8-10]. The biological method with single microorganisms, mixtures of strains or enzymes is very promising [11-14].

The aim of this work was to demonstrate that the centrifuged biological sludge obtained from the poultry industry, optimized by highly-active fungal enzymes, influences on the quantity and quality of biogas obtained.

Materials and methods

The laboratory experiment was carried out on a biological sludge after centrifugation from a treatment plant located in one of the poultry processing plants in Western Poland. Chemical analysis of the tested material was carried out for the content of organic carbon as well as total nitrogen and total sulfur. For this purpose, a CNS elementary analyzer from Coestech was used.

The first stage of the research was a screening resulting in the isolation of 150 microbial strains with particularly high metabolic activity from the environment of the poultry industry. These were, among others, feathers (hen, duck, turkey and goose), slime from the basin of liquid waste, biological sludge, as well as compost. The microorganisms were grown on media containing protein, fat and starch. Biological sludge was optimized, which was carried out using microorganisms with high degradation activity towards protein and lipid compounds. Microorganisms with protein degradation abilities were determined on the agar medium with 10 % defatted milk and microorganisms with fat degradation activity on Tributyrin Agar medium (with the following composition per liter of distilled water: 5.0 g peptone, 3.0 g yeast extract, 10.0 g tributyrin, 15.0 g agar). The isolated microorganisms, taken into account their enzymatic activity index (the ratio of hydrolysis zone diameter to the colony diameter), 4 fungal isolates were selected (Table 1). The strains were analyzed based on macro- and microscopic features of their morphological structures [15-18]. We used the following symbols: A1 - strain of *Cladosporium* sp., C1 - strain similar to *Verticillium* sp., F1 - strain of *Cladosporium* sp., J1 - strain of *Cladosporium* sp. Selected fungal cultures were amplified on Potato Dextrose Agar (PDA) medium at 30 °C/48 h, and then the final inoculum was prepared according to the scheme (Fig. 1), which was at the level of 10^6 - 10^8 CFU·cm⁻³ (CFU - colony forming units). Densities of fungal strains were determined by serial dilutions technique using Rose Bengal Agar (RBA) medium. A mixture of all analyzed fungi was created it was named MIX. A 250 g sample was prepared and incubated in a Grant OLS 200 incubator for 10 days at 24-25 °C (Fig. 2).

Table 1

Enzymatic activity and density of fungal strains used

Symbol strain	Activity index (IA) [-]		Density [CFU·cm ⁻³]
	lipolytic	proteolytic	
A1	4.0	1.3	2.2·10 ⁸
C1	4.3	1.3	2.3·10 ⁷
F1	2.0	1.2	1.1·10 ⁶
J1	2.7	2.3	3.7·10 ⁸

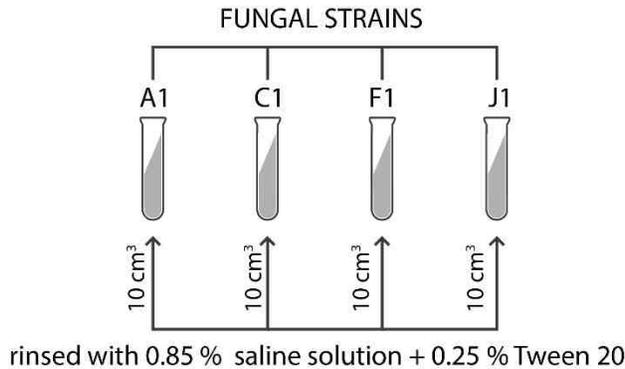


Fig. 1. Diagram of preparation of fungal inoculum (source: own study)

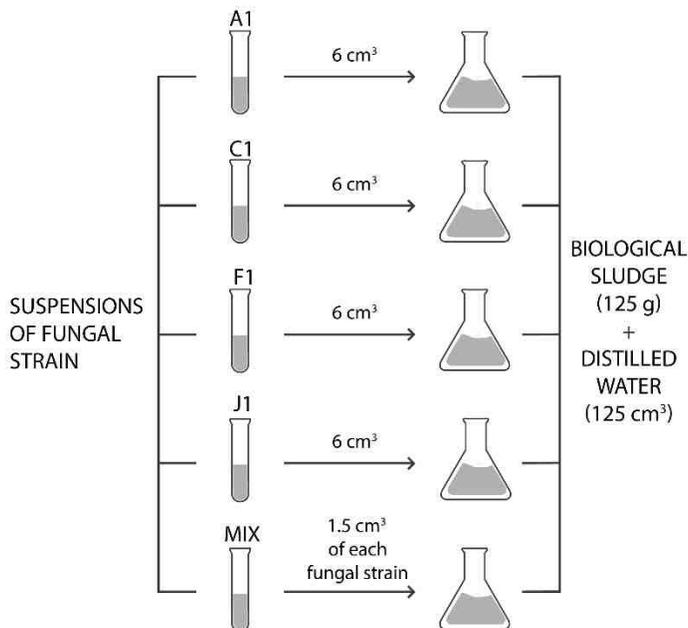


Fig. 2. Preparation of the batch material prior to its introduction into the biogas fermenter (source: own study)

The composition of substrates used as fermentation batch consisted of an equal weight ratio (1:1) of material optimized by the appropriate fungal strain and swine liquid manure. The swine manure came from a swine farm located in the county of Drawsko in the West Pomeranian Voivodeship. The bottles with analyzed material were placed in a water bath at 37 ± 1 °C. Methane fermentation was carried out using modified German standards DIN 38 414 - S8 [19] and VDI 4630 [20], in triplicate for each sample. Measurement of the amount of biogas produced was performed on the basis of the eudiometric burette, while its qualitative components, i.e. methane, carbon dioxide, oxygen, hydrogen sulfide and

ammonia, using the GA 2000 Plus biogas analyzer. In order to convert the volume of biogas into normal conditions, it was necessary to measure ambient temperature, relative humidity and atmospheric pressure. Measurements of individual parameters were performed every 24 hours for 46 days. The results obtained were normalized according to the aforementioned standards. The triple pH value, dry matter content [21], and organic dry matter [22] were also measured in the case of material analyzed, swine liquid manure and the final product, i.e. post-fermentation mass.

Statistical analysis was performed using the Statistica 12 software. The significance of differences of the mean yields of biogas and methane between the tested samples was determined by the non-parametric Kruskal-Wallis ANOVA test.

Results and discussion

Laboratory-scale tests have shown that the yields of biogas and methane from biological sludge processed by different variants of fungal strains were varied. The fungal strains presented high proteolytic activity ranged from 1.2 to 2.3 IA and the lipolytic activity from 2.0 to 4.3 IA (Table 1). Biological sludge was characterized by the following chemical substances contents: organic carbon - 38.05 % d.m. (dry matter), total nitrogen - 6.57 % d.m., total sulfur - 0.787 % d.m. Weiland [23] argues that pre-treatment of the substrate and the addition of microelements may contribute to the enhanced potential of biogas production. The highest yield of biogas for the 46-day methane fermentation was characterized by material processed by the mixture of fungal strains (MIX) and fungal strain F1 (375.1 $\text{Nl}\cdot\text{kg}^{-1}$ o.d.m. (normalized litres of gas per kilogram of organic dry matter and 355.4 $\text{Nl}\cdot\text{kg}^{-1}$ o.d.m.) (Fig. 3). Lower biogas yield was obtained in materials processed by the strain marked as J1 and A1 (192.2 $\text{Nl}\cdot\text{kg}^{-1}$ o.d.m. and 151.6 $\text{Nl}\cdot\text{kg}^{-1}$ o.d.m.). The lowest biogas yield was characterized by the biological sludge processed by the fungal strain C1 (73.8 $\text{Nl}\cdot\text{kg}^{-1}$ o.d.m.). The yield of biogas from untreated control material was 311.8 $\text{Nl}\cdot\text{kg}^{-1}$ o.d.m.

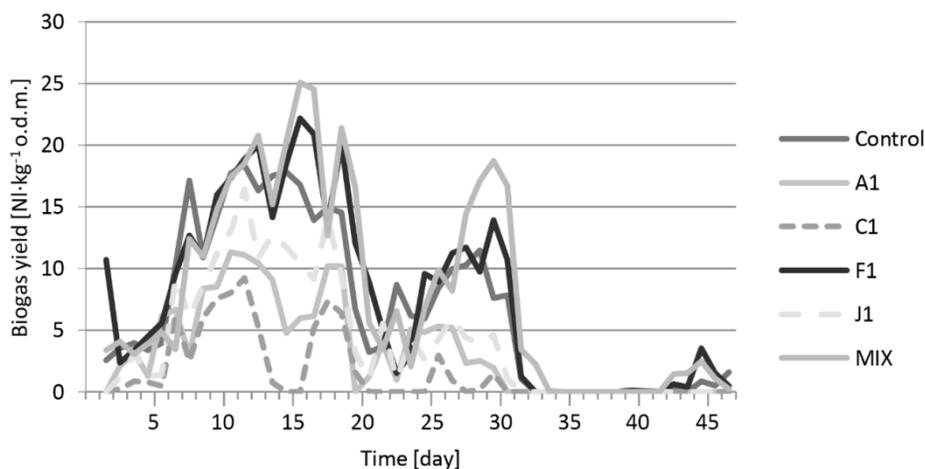


Fig. 3. Daily amount of biogas produced during methane fermentation in tested samples

The statistical analysis revealed significant differences in mean biogas yields between analyzed samples (Fig. 4). The use of a mixed fungal consortium (MIX) with differentiated enzymatic activity has proven to be an effective way to convert the biomass contained in biological sludge. Such processing of the test material resulted in a 20 % increase in biogas yield as compared to the control material. Divya et al. [24] reported that the effects on methane fermentation stability and biogas production are exerted by chemical and physical properties of the raw material used. Parameters of the reaction are also important, i.e. temperature, pH, hydraulic retention time (HRT), C/N ratio, volatile fatty acids, etc. [25-27]. However, too fast hydrolysis of raw material applied for methane fermentation could limit the development of methanogenic bacteria. This situation could have occurred in the case of biological sludge due to enzymes secreted by fungal strains A1, C1 and J1, which in turn reduced the production of biogas in these samples. Zhang et al. [28] claim that poultry waste is a batch substrate containing significant nitrogen amounts, and thus it may contribute to the accumulation of ammonia in the fermentor and thereby to inhibit the methane fermentation process.

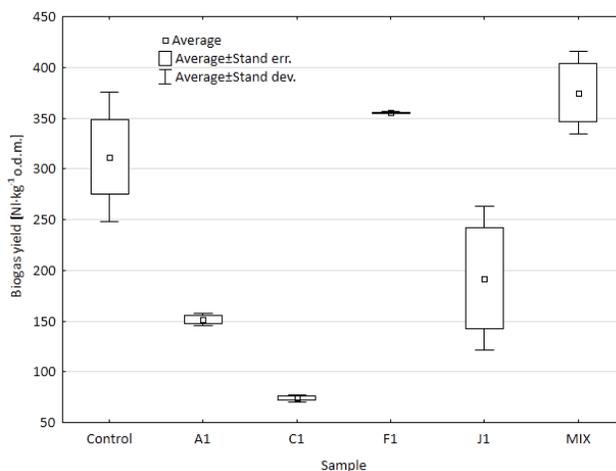


Fig. 4. Average values of biogas production in test samples

For the analyzed samples, the relationship between biogas yield and methane yield was found. The highest methane yield was found in the biological sludge treated by MIX of fungal strains and F1 fungal strain (214.72 NI CH₄·kg⁻¹ o.d.m. and 187.45 NI CH₄·kg⁻¹ o.d.m.). Lower methane yields were obtained in materials optimized by J1 and A1 strains (101.42 NI CH₄·kg⁻¹ o.d.m. and 77.98 NI CH₄·kg⁻¹ o.d.m.). The lowest methane yield was recorded in the case of biological sludge processed by the fungal strain C1 (34.87 NI CH₄·kg⁻¹ o.d.m.) (Fig. 5). The biological sludge not inoculated by fungal strains was characterized by the following methane yield: 167.59 NI CH₄·kg⁻¹ o.d.m.

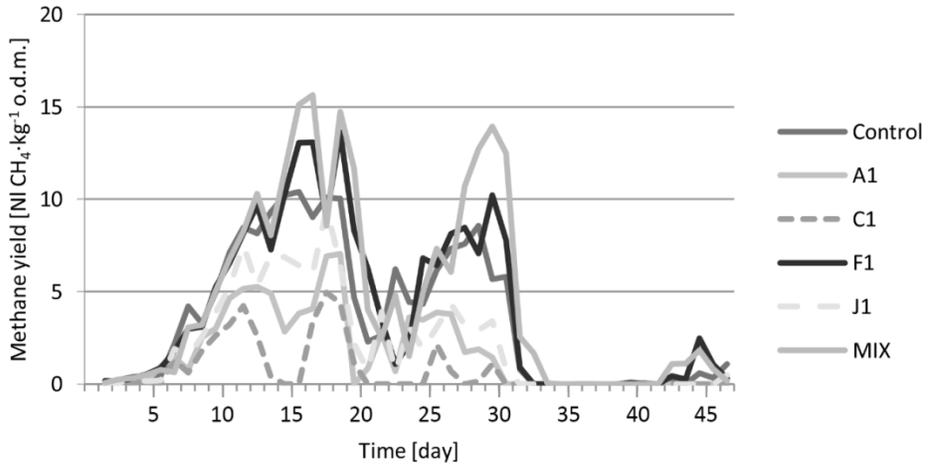


Fig. 5. Daily amount of methane produced in the analyzed materials

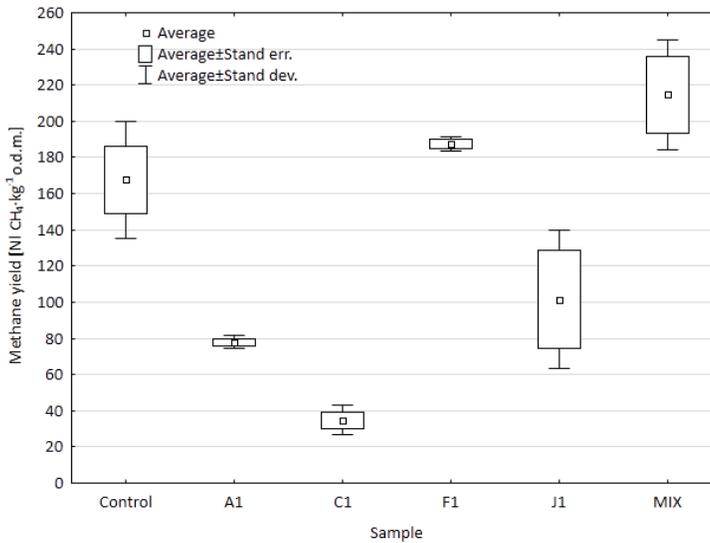


Fig. 6. Average values of methane production in tested samples

The statistical analysis revealed significant differences in average values of methane yields between the analyzed variants (Fig. 6). Increasing the methane yield in MIX combinations (by 28 %) and F1 (by 12 %) as compared to the control material may indicate a favorable effect of pretreatment of the material under study by the above-mentioned fungal strains variants. Ali and Sun [29] demonstrated that biological pretreatment using *Aspergillus terreus* and *Trichoderma viride* fungi as well as chemical pretreatment can remarkably accelerate degradation of lignocellulosic substrates used for methane fermentation and thus favorably affect the amount of biogas and methane obtained.

The same conclusions were obtained with the example of the fungus of *Ceriporiopsis subvermispota* genus by Amirta et al. [30] and Zhao et al. [31]. Valladão et al. [32] have found a beneficial enzymatic effect (hydrolysis + microorganisms) on the treatment of wastewater from poultry slaughterhouses on methane production.

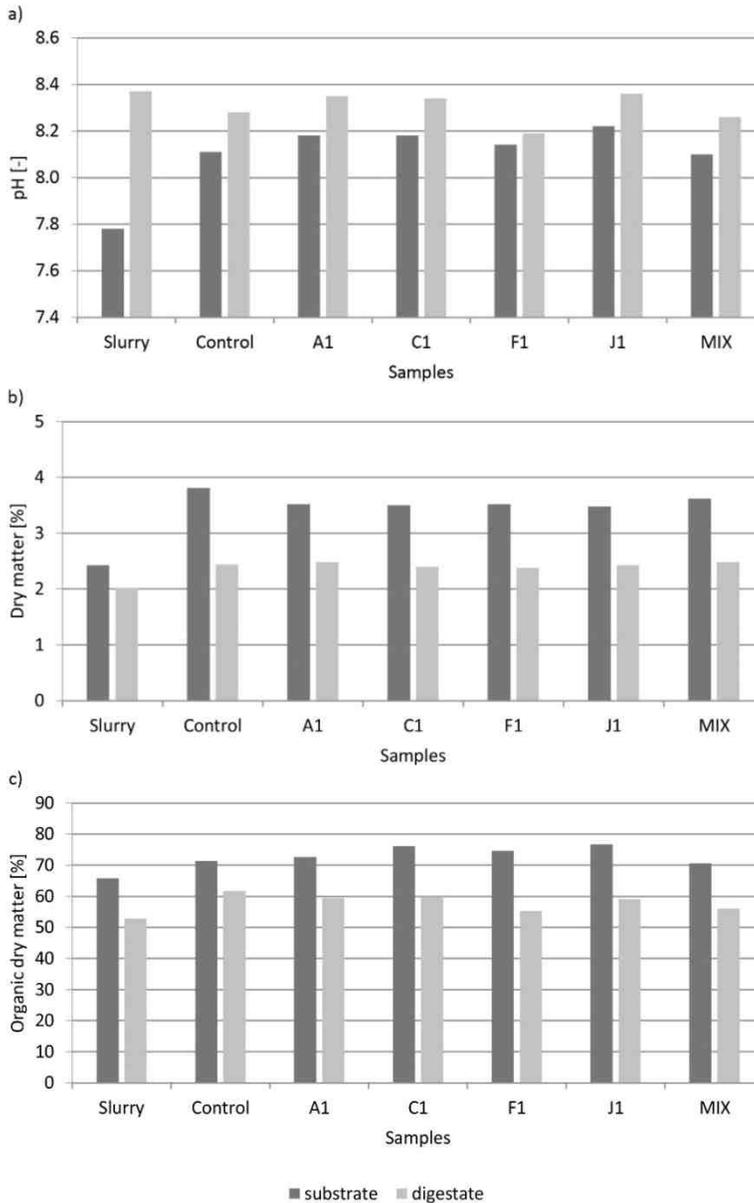


Fig. 7. Basic physicochemical parameters of the tested substrates and the obtained post-fermentation mass: a) pH, b) dry matter, c) organic dry matter

Regardless of the amount of biogas produced, the methane content was 55.39 % on average, as confirmed by other authors [33] on the example of chicken manure.

The experiment was carried out during the methane fermentation process under static conditions, therefore it was not possible to control the fermenter operating parameters, i.e. pH and temperature inside the reactor. Finally, it was observed that the methane fermentation process increased the pH of the post-fermentation mass relative to the batch substrates, as it was in the case in studies by Ali and Sun [29]. In own studies, the dry matter and organic dry matter contents in the resulting post-fermentation pulp were also reduced (Fig. 7).

As a consequence of the methane fermentation process of the biological sludge inoculated with various combinations of fungal strains, the following average concentrations of inhibitors in biogas, i.e. NH_3 and H_2S , were obtained (Table 2).

Table 2

Average content of inhibitors in biogas (NH_3 , H_2S)

Sample	Average content of inhibitors [ppm]	
	NH_3	H_2S
Control	119.32	95.75
A1	47.00	53.16
C1	28.85	35.21
F1	75.61	78.25
J1	21.92	27.78
MIX	121.21	115.14

There was a significant reduction in the content of inhibitors in the biogas in most samples processed by filamentous fungi.

Conclusions

1. Biological sludge subjected to enzymatic pre-optimization using highly active filamentous fungi may be applied as a fermentation batch for biogas-producing plants to achieve larger methane yields.
2. The highest methane yield characterized sludge optimized by mixture of fungal isolates (MIX), in the case of which the methane production was higher by almost 30 % as compared to the control batch.
3. Processing of biological sludge by fungal strains, excluding their mixture (MIX), resulted in a significant decrease in the inhibitors contents (NH_3 and H_2S) in the biogas.

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