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Extended Abstract in English

Improving the hydrolysis of sewage sludge to intensify methane fermentation

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Discipline: Environmental Engineering, Mining and Energy

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1. Scope of the research

The scope of the research included the isolation, characterization, and identification of cellulolytic microorganisms present in sewage sludge. The sewage sludge used for the isolation of microorganisms was treated using previously isolated microorganisms to evaluate this type of biological hydrolysis method as a means of intensifying the decomposition of organic matter, with particular emphasis on cellulose, and intensifying methanogenic fermentation.

2. Research Objective

The objective of the study was to verify the potential of using indigenous cellulolytic bacteria and fungi to intensify biogas production through the biological hydrolysis of organic matter in the studied sludge, particularly cellulose.

The study aimed to evaluate the use of microorganisms already present in the sewage sludge from the treatment plant as a low-cost and environmentally friendly means of supporting the hydrolysis of hard-to-degrade organic substances and the resulting intensification of biomethane production.

3. Research Hypothesis

The proposed research hypothesis posits that increasing the number of indigenous microorganisms in sewage sludge will have a positive effect on the decomposition of organic matter and biogas production. Microorganisms present in a given environment have the ability to function and multiply through the effective utilization of available matter as a food source. An excessive amount of cellulose in the dry matter of fermented sludge indicates inefficient utilization of this polymer by microorganisms.

Therefore, introducing microorganisms previously isolated from this environment into the sludge may positively influence more efficient cellulose degradation, reducing the amount of dry organic matter in digested sludge, increasing the amount of methane produced by improving the decomposition of complex organic substances and reduce the amount of digested sewage sludge overall.

4. Description of the Research Subject

The research subject consisted of sewage sludge collected from the Central Wastewater Treatment Plant in Gliwice. The study utilized mixed sludge destined for the fermentation chamber and sludge that had already undergone fermentation in the Separate Fermentation Chamber (SFC). Cellulolytic microorganisms were isolated from sludge collected from the SFC as the environment in which these microorganisms should occur and be capable of effectively degrading cellulose under specified conditions. The efficiency of cellulose degradation by the isolated microorganisms and the enzymes they produce was verified using sewage sludge entering the SFC.

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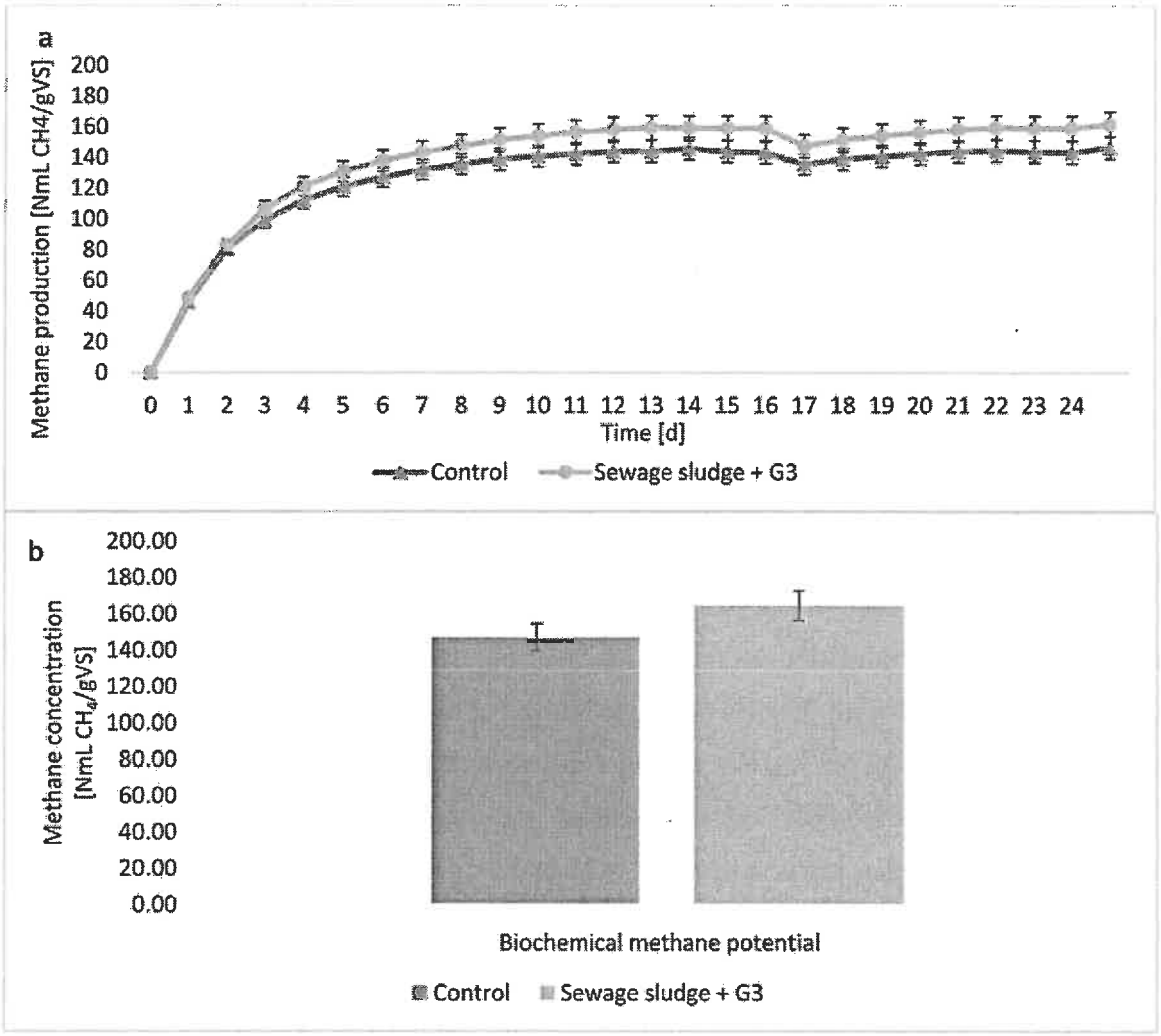

Fig. 1 Comparison of biomethane production for sludge inoculated with the G3 strain of *Aspergillus terreus* and untreated sludge

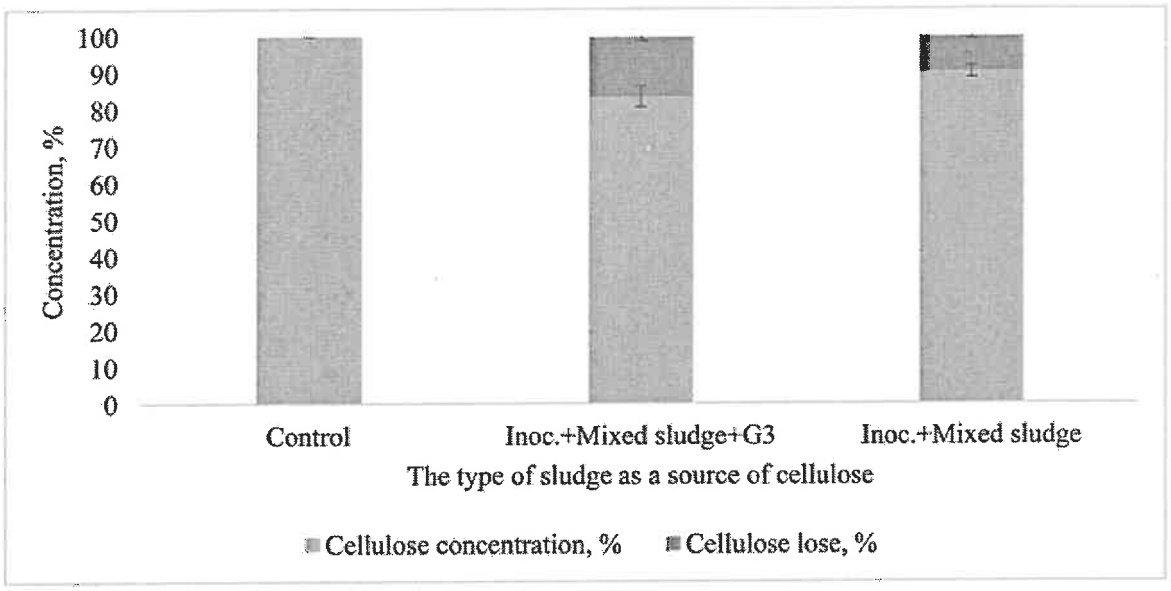

Fig. 2 Percentage reduction in cellulose content following inoculation of sewage sludge with the fungus G3-*Aspergillus terreus* S011.

The efficiency of cellulose degradation in the sewage sludge was 6.7%.

Isolating pure strains of cellulolytic bacteria was significantly more difficult than isolating cellulolytic fungi. Initially, the isolation process was conducted using a selective medium in which sodium carboxymethylcellulose was the sole carbon source. Congo Red dye served as an indicator of cellulose degradation on the medium. Each attempt to isolate a pure strain, from inoculation to growth, took about a month, or no growth was observed. Passaging the bacteria onto a new solid medium led to the loss of the ability to degrade cellulose in the form of sodium carboxymethylcellulose on the solid medium. Publication 4 describes a renewed attempt to isolate autochthonous cellulolytic bacteria. This was carried out using a liquid medium, in which the source of cellulose was again sodium carboxymethylcellulose. Repeated passage in the liquid medium allowed for the isolation of a mixed culture of cellulolytic bacteria. However, it was not possible to isolate a single culture on a solid medium due to the observed loss of cellulolytic properties. The mixed bacterial culture, growing in a liquid medium where sodium carboxymethylcellulose was the sole carbon source, was identified using Next-Generation Sequencing (NGS). Based on the results obtained, it was observed that the mixture contained bacteria characterized as cellulolytic, as well as many others capable of degrading other organic substances. The composition of the mixed bacterial culture is shown in Figure 3.

Sequences that accounted for less than 1% of the community were grouped under the category "Others."

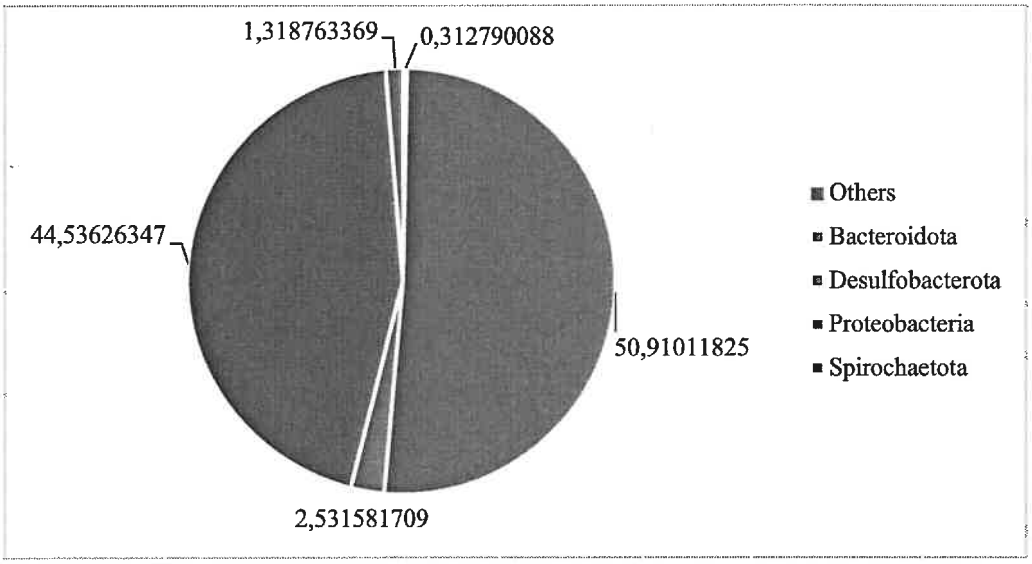

Fig. 3 Sequences that accounted for less than 1% of the community were grouped into the "Others" category.

The bacteria present in a mixed culture exhibit certain similarities; for example, they utilize carbohydrates for their metabolic processes or efficiently convert fatty acids. In the mixed culture, bacteria abundant in the sludge share similar properties with those from less abundant groups. Since less abundant bacteria possess capabilities similar to those of more abundant bacteria, their presence in the mixture used for enzyme isolation suggests that they can still grow and have access to certain nutrients. Furthermore, most of the identified microorganisms are not strictly cellulolytic but anaerobic and capable of degrading various organic compounds. This indicates that their presence in the mixed culture and the enzymes they produce may support the hydrolysis process by degrading various organic compounds.

These results led to the conclusion that the studied indigenous cellulolytic bacteria are not capable of effectively degrading cellulose on their own, but can do so only in a consortium, supported by other bacteria. Therefore, subsequent steps were carried out using a mixed bacterial culture.

Both the fungi and the mixed bacterial culture were tested for storage stability. The microorganisms were frozen and freeze-dried. It was observed that frozen and freeze-dried fungi could be stored, but the same procedures caused the bacteria to lose their cellulolytic properties. Therefore, enzymes isolated from a mixed culture of indigenous cellulolytic bacteria were used in subsequent studies. The isolated enzymes were tested in comparison with a commercial mixture of cellulolytic enzymes. Immobilization was also performed for the tested enzymes, using activated carbon as a carrier (Publications 3, 4, 5). The studies showed that both the use of isolated cellulolytic fungi and isolated enzymes contributes to increased methane production. It was also confirmed that the isolated enzymes, both immobilized and non-immobilized, retained their effectiveness after one year of storage at 4°C.

Although native cellulolytic fungi are easier to isolate and store, detailed studies were conducted using bacteria and the enzymes they produce. The main reason was the potential for industrial application of this solution, as well as the lower risk to humans associated with working with microscopic fungi, both in the laboratory and on an industrial scale. Consequently, in the sludge subjected to enzymatic treatment using isolated and commercial, immobilized and non-immobilized enzymes, changes in physicochemical parameters such as total nitrogen concentration, ammonium nitrogen, volatile fatty acids, and chemical oxygen demand were also verified. As in the case of studies conducted on fungi, cellulose degradation and methane production were also assessed. Figures 4 and 5 show the course of methane production, while Table 1 presents the percentage of cellulose degradation in the tested sewage sludge during the biogas production test.

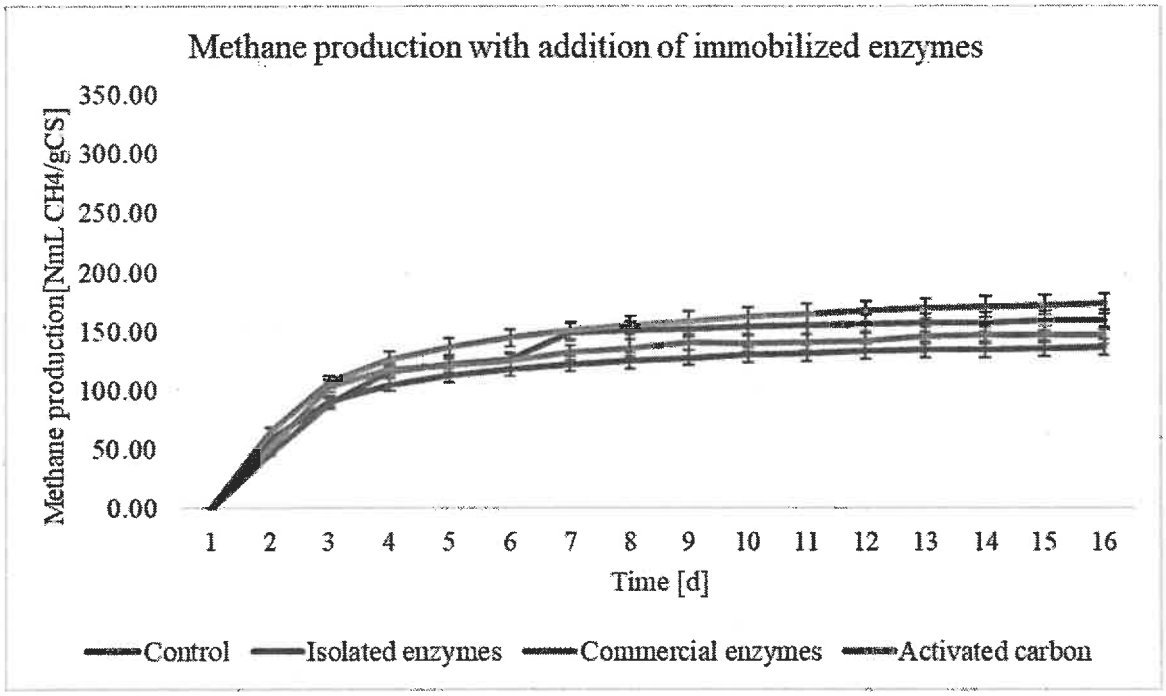

Fig. 4 Effect of biological treatment of sewage sludge using enzymes immobilized on activated carbon.

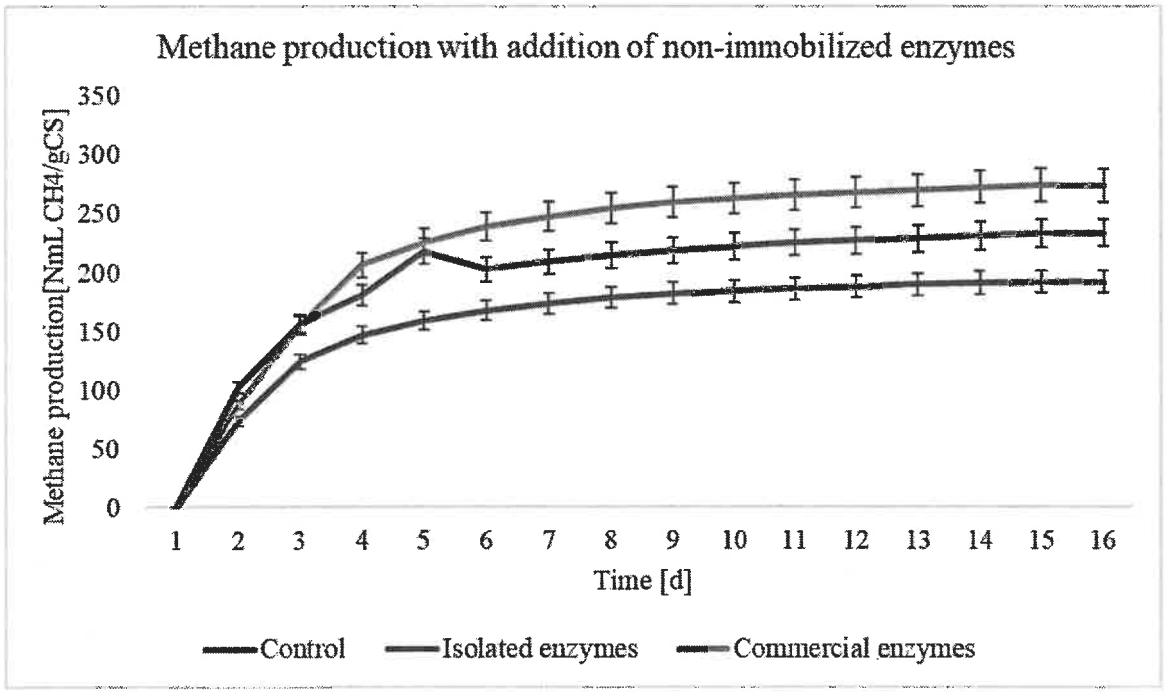

Fig. 5 Effect of biological treatment of sewage sludge using non-immobilized enzymes.

Inoculating sewage sludge with immobilized and non-immobilized enzymes both commercial and isolated led to an increase in biomethane production. For both immobilized and non-immobilized enzymes, the highest biomethane production was achieved with sludge supplemented with commercial enzymes. The increase in methane potential after 15 days of testing was 18.9% for immobilized isolated enzymes and 27% for immobilized commercial enzymes. When activated carbon alone was used- a method also employed in studies to intensify biogas production- the percentage increase in methane potential was 6.75%. For sludge treated with non-immobilized enzymes, the increase in methane potential was 21.7% for isolated enzymes and 43% for commercial enzymes.

The effect of the presence of isolated and commercial enzymes, both immobilized and non-immobilized, on the composition of the sewage sludge microbial consortium was also evaluated. The analysis showed no significant changes in the composition of the consortium during the study period.

Table 1. Cellulose concentration determined before and after the anoxic methane potential test (AMPTS) for the inoculum (sludge sampled from the fermentation chamber) and a mixture of thickened primary sludge and excess sludge treated with immobilized and non-immobilized enzymes: isolated and commercial. I – inoculum, I0 – at time "0", INE – after AMPTS, SS – mixed sludge, IE – isolated enzymes, CE – commercial enzymes, carb – activated carbon.

Cellulose concentration (% TS)		
Type of the tested sludge	Immobilized enzymes	Non-immobilized enzymes
I0	31.20±3.63	30.52±0.81
INE	28.28±1.84	28.61±0.33
I+SS	29.93±0.69	27.53±1.94
I+SS+IE	28.09±1.92	**22.58±0.75**
I+SS+CE	28.87±0.96	27.98±0.79
I+carb	27.88±1.0	-
I+IE	28.17±3.38	29.64±2.63
I+CE	**11.21±0.37**	28.56±0.88

The main conclusions drawn during and after the completion of the research conducted as part of this study are listed below:

- It is possible to isolate individual cellulolytic strains from sewage sludge; however, their storage- through freezing, freeze-drying, and passage leads to a loss of cellulolytic properties or an increase in growth time.

- Some cellulolytic strains also lose their properties after being separated from other bacteria with which they grew on solid medium. The isolation of autochthonous bacteria in a liquid medium supplemented with carboxymethylcellulose revealed the presence of cellulolytic bacteria as well as other strains that, by their presence in the mixed culture, most likely supported each other's activities.

- Enzymatic treatment of sewage sludge using a mixture of enzymes isolated from a mixed culture of cellulose-degrading bacteria enhances methane and cellulose production and improves sewage sludge hydrolysis, potentially reducing the time required for fermentation.

- The use of microscopic fungi in the form of mycelium as a biological method for treating sewage sludge enhances cellulose degradation and the methane potential of these sludges.

- Enzyme immobilization stabilizes enzyme activity by prolonging the activity of isolated enzymes and reducing the activity of commercial enzymes, thereby protecting the process from excessive acidification caused by increased VFA production.

- The use of enzymatic treatment of sewage sludge with isolated and commercial enzymes, in immobilized and non-immobilized forms at a concentration of 1% of the substrate's dry weight, does not significantly affect the composition of the bacterial community inhabiting the tested sewage sludge.

6. Summary of Original Contributions

The original contributions of this doctoral dissertation include the isolation, characterization, and preparation of the isolated microorganisms for identification. Research verifying the effect of sewage sludge hydrolysis following biological treatment using the fungus *Aspergillus terreus*- G3, as well as immobilized and non-immobilized isolated and commercial enzymes, was also conducted independently. The analysis and interpretation of

all obtained results constituted the author's own contribution. Collaboration with the advisors was a key component of the dissertation work, enabling effective research and planning of subsequent steps without significant delays. Activities carried out in collaboration or as commissioned work included: sequencing of isolated microorganisms and enzyme immobilization.