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Abstract in English

Improving the hydrolysis of sewage sludge to intensify methane fermentation

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Wastewater treatment is a complex technological process aimed at removing physical, chemical, and biological contaminants from wastewater. Effective implementation of this process enables the safe discharge of treated wastewater into water bodies or the ground,

in accordance with applicable environmental requirements. During treatment, in addition to treated wastewater, sewage sludge is produced, which, due to its composition and volume, requires appropriate and rational management. The processes involved in their treatment, including dewatering, sanitization, transport, and disposal, generate significant operating costs for wastewater treatment plants.

Stabilized sewage sludge can be used for various purposes, such as fertilizing plants not intended for human or animal consumption, the reclamation of degraded land, composting, or landfilling. Biological, chemical, and thermal methods are used for its stabilization,

with biological methods- particularly anaerobic digestion- being the most commonly employed. It should be emphasized, however, that the effectiveness of anaerobic fermentation does not always guarantee that the sludge parameters will meet the requirements specified in the Announcement of the Minister of Climate and Environment dated November 18, 2022. Consequently, pretreatment of sewage sludge prior to the fermentation process is increasingly being employed. This treatment intensifies the course of hydrolysis and the entire fermentation process, contributing to increased stabilization efficiency, improved quality and quantity of the resulting digestate, and also affects the yield and composition of the biogas produced. The effectiveness of sewage sludge stabilization is significantly influenced by its composition, particularly the content of poorly biodegradable organic substances.

This group includes, among others, cellulose, a polysaccharide that is the primary structural component of plant cell walls and one of the most common polymers in the environment. Cellulose is also a significant component of the organic matter entering sewer systems, mainly due to the use of hygiene products by humans. Its presence in wastewater and sewage sludge directly affects the course of technological processes in treatment plants and the efficiency of sludge management. Given the need for effective sewage sludge management and the reduction of operating costs, intensive research is being conducted on methods that enable both the intensification of stabilization processes and the economic optimization of these activities.

The following doctoral dissertation presents methods for the effective treatment of sewage sludge without additional environmental burden from chemicals, through the use of cellulolytic microorganisms present in sewage sludge. The doctoral dissertation comprises five publications- one review and four research papers, describing the impact of autochthonous

isolated during the study, resulted in an 11% increase in biogas production compared to untreated sewage sludge. Additionally, a 6% increase in cellulose degradation was observed compared to the control sample. Fungi are considered the most effective of all cellulolytic microorganisms. Consequently, the research described in this publication served as the starting point for further studies, which were expanded upon in **Publications 3, 4, and 5**.

Further research planned as part of the doctoral dissertation involved the use of indigenous cellulolytic bacteria, isolated from sewage sludge, to increase the rate of sewage sludge hydrolysis. However, during the research, it was not possible to isolate pure bacterial cultures. It was also impossible to store the isolated microorganisms, as the tested methods led to the loss of the cellulolytic properties of the isolated bacteria. Freezing in glycerin, freeze-drying, and slant inoculation were tested. In each of the tested cases, the isolated bacteria lost their cellulolytic properties. These results pointed the research toward mixed cultures with cellulolytic potential, i.e., bacteria isolated from sewage sludge that were capable of growing and multiplying in a synthetic medium where the sole carbon source was carboxymethylcellulose. A mixed culture of indigenous bacteria with cellulolytic potential was characterized in terms of enzymatic activity using the Filter Paper Assay (FPA) and carboxymethyl cellulose (CMC), as well as their ability to degrade pure cellulose and cellulose present in sewage sludge.

Comparative studies of long-term enzyme contact with sludge are presented in **Publication 3**, which also examined the effect of enzymatic treatment of sewage sludge on the degree of cellulose degradation and methane potential.

Bound enzymes were isolated from a mixed culture of indigenous bacteria with cellulolytic potential. Enzyme isolation was performed using thermomechanical methods that could be easily implemented in a wastewater treatment plant. To preserve and enhance their usability, the isolated enzymes were immobilized on an inorganic carrier- activated carbon. **Publication 3** describes the use of enzymes produced by indigenous cellulolytic bacteria. As in the case of fungal activity, the enzymatic activity of a mixed culture of cellulolytic bacteria and the degradation efficiency of pure cellulose were determined.

The methane potential of sludge inoculated with isolated enzymes was also determined, both in immobilized and non-immobilized forms. The performance of the isolated enzymes was compared with that of the commercial cellulolytic enzyme mixture Cellic CTec2 (Novozymes, Denmark).

Based on the conducted studies, a 27% increase in cellulose degradation was observed in inoculum and substrate samples (fermenting sludge supplemented with fresh sludge) treated

cellulolytic microorganisms and the enzymes they produce on the efficiency of cellulose degradation and methane production. The research was preceded by a detailed literature review (**Publication 1**), which analyzed methods for enhancing methanogenic fermentation with a view to improving sludge hydrolysis and methane production itself. The review presented the impact of chemical, physical, and biological treatment on sewage sludge hydrolysis and the effectiveness of each method in terms of the determination of hydrolysis products such as volatile fatty acids (VFA), chemical oxygen demand (COD), and methane potential. Furthermore, inhibitors of the process and substances that significantly affect the efficiency of methanogenic fermentation are described. **Publication 1** highlighted, in particular, the limited number of studies related to optimizing the use of biological sewage sludge treatment methods as means to intensify methanogenic fermentation and the hydrolysis stage. A theoretical review of the topic allowed for the identification of poorly studied methods of preliminary sewage sludge treatment, referred to as biological, and in particular those involving autochthonous microorganisms and the enzymes they produce. The literature review also enabled the planning of research aimed at filling this research gap, taking into account the needs and capabilities of the industry.

Publication 2 describes the isolation of cellulolytic fungi from sewage sludge. Fungi capable of growing under aerobic and anaerobic conditions were isolated, with the aim of utilizing them at various stages of sewage sludge management. Twelve strains of cellulolytic fungi were isolated. Seven of them were capable of growing under aerobic and anaerobic conditions.

Five strains were identified using Sanger sequencing of the ITS1 region. All of the isolated fungi belonged to the genus *Aspergillus*. It was determined that the isolated fungi were capable of degrading pure cellulose. Based on enzymatic activity and the efficiency of pure cellulose degradation, one strain, designated G3 (*Aspergillus terreus* S011), was selected for cellulose degradation tests in sewage sludge and for the methane potential test.

The vast majority of studies described in the literature utilize pretreatment conducted prior to the methane fermentation chamber. In the studies described in **Publication 2**, biological treatment was applied as a form of support for the process already underway during the Automatic Methane Potential Test (AMPTS). The tested sewage sludge was inoculated with mycelium of the isolated fungus G3 (*Aspergillus terreus* S011). The study utilized the microorganism itself rather than the enzymes it produces, with the aim of intensifying the treatment and simplifying the sludge pretreatment method, in view of its potential industrial application. The introduction of an autochthonous cellulolytic fungus of the genus *Aspergillus*,

with isolated non-immobilized enzymes, and a 66% increase in the case of fermented sludge not fed with a fresh batch of excess and primary sludge. Additionally, methane production was intensified by 21.7% in sludges inoculated with isolated non-immobilized enzymes and by 18.9% in sludges inoculated with isolated immobilized enzymes. For commercial enzymes, an increase in methane production of 42.5% and 27% was observed for non-immobilized and immobilized enzymes, respectively.

Publication 4 presents the short-term effects of enzymatic treatment using isolated and commercial enzymes, both non-immobilized and immobilized on an inorganic carrier, namely activated carbon. During the test described in **Publication 4**, the effect of dosing isolated and commercial enzymes on the concentration of volatile fatty acids, chemical oxygen demand (COD), and extracellular polymeric substances (EPS) was evaluated. The test allowed for the observation of changes in the concentrations of the measured parameters during the first hours following the inoculation of the sludge with enzymes.

In sewage sludge samples inoculated with isolated non-immobilized and immobilized enzymes, an increase in VFA production of 25% and 128%, respectively, was observed. An increase in COD concentration of 272% and 73% was also observed. In the case of inoculation of sludge with commercial non-immobilized and immobilized enzymes, an increase in VFA concentration of 1856% and 175% was observed. The total COD concentration in samples inoculated with commercial non-immobilized and immobilized enzymes increased by 1,792% and 670%, respectively.

Publication 5 presents the effect of inoculating sewage sludge with isolated and commercial immobilized and non-immobilized enzymes on the physicochemical parameters of sewage sludge during a 15-day test determining the methane potential of the tested sludges. Additionally, the effect of enzymatic treatment of sewage sludge on the microbial community residing in the sludge was also verified. Based on the conducted studies, it was determined that the selected type of treatment and the applied enzyme concentration do not significantly affect the composition and microbial community of the sewage sludge. A comparison of the physicochemical results described in **Publication 4** and **Publication 5** revealed that the use of isolated and commercial enzymes, both immobilized and non-immobilized, allows for the same sludge parameters and methane production rates to be achieved in a shorter time than in the case of untreated sludge. **Publication 5** further identified correlations between physicochemical parameters and the abundance of the most common bacterial species, indicating strong relationships between the presence of specific microorganisms and the concentration of the parameters studied. At the same time, it was demonstrated that enzyme immobilization was

more significant than the type of enzyme used. In situations where a negative correlation was observed with non-immobilized enzymes, the use of their immobilized counterparts resulted in a positive correlation between the abundance of a given species and the measured parameter.