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Soil is the natural body that provides countless ecosystem services, including space for the growth of plants and other organisms. Thus it is of key importance for growing the human population as a supply of food. Furthermore, the soil is a water filter, a biodiversity hotspot, and an important component of the landscape. Soil fertility is the ability of soil to sustain plant growth and optimize crop yield. Where fertility is not proper, mineral or organic ingredients (fertilizers) may be added to supply the needed plant nutrients. The increase in the price of mineral fertilizers is prompting a search for other sources of ingredients needed to improve soil fertility. Although the application of mineral fertilizers is considered a good agricultural practice, organic fertilizers have the advantage of being cost-effective, improving soil structure and aeration, increasing soil water retention, and stimulating healthy plant development. Organic fertilizers can be derived from various organic wastes and byproducts including a digestate - specific effluent simultaneously produced with biogas during the anaerobic digestion (AD) process. Biogas production as a consequence of AD provides significant benefits. Organic compounds, previously regarded as waste can be channeled through biogas digesters and converted to useful energy carriers. The possibility of obtaining a fuel that can be produced locally, in a decentralized manner is a factor that additionally raises the interest in the development of research focused on AD. At the heart of the AD process, there are cooperations of many groups of microorganisms responsible for, respectively, hydrolysis of polymers, acidic fermentations, acetogenesis, and methanogenesis. In conventional applications, all these microorganisms are kept together in a single reactor system. However, the physical separation of acid-formers (hydrolysis and acidic fermentations) and methane-formers (acetogenesis and methanogenesis) in two separate reactors can enhance AD process, additionally ensuring the obtaining of two valuable sources of energy - hydrogen and methane. Besides the increased stability, two-stage AD provides the combined production of H<sub>2</sub> (stage 1) and CH<sub>4</sub> (stage 2). That is why the development of the two-stage AD process is reported as an innovative support for a single reactor system.

The management of the digestate obtained from the single-stage process is currently attracting the attention of many researchers. However, the disposal of digestate from two-stage systems where we simultaneously produce valuable gases is so far marginalized. In our research group, we have developed a system where we produce biohydrogen-rich gas and acidic effluent in the first bioreactor. The liquid products of molasses fermentation are converted to biogas (rich in biomethane) and digestate in the next step. The central aim of our proposal is an assessment of the fertilizer value of digestate obtained as a result of a two-stage, H2-CH4-yielding AD process. Using innovative techniques of microbiology, chemistry, and agronomy we are planning an evaluation of the input substrate characteristics in the context of digestate fertilizer value. Assessment of the over-time digestate stability. Digestate microbiome analysis in the context of identification of microorganisms potentially beneficial for agronomy. As a model plant for further research, we chose wheat, which is a well-known and food-valuable substrate. In short-time laboratory experiments (pot experiment), we want to determine what is the most optimal doses of digestate and its effect on the status and distribution of elements in the soil and plants. We evaluate selected parameters of wheat growth and soil microbiome emphasizing nitrogen-cycling microbes in fertilized soil. The final stage of the research will be a two-year field experiment, where all the previously mentioned fertilizer properties will be evaluated under natural environmental conditions.

Focusing on the issue of managing the digestate generated in the innovative installation of microbiological biohydrogen and biomethane production from sugar beet molasses, we create a kind of closed circuit in the management of this substrate, increasing the efficiency of the entire process and at the same time providing an environmentally beneficial organic fertilizer.

The results of the project will provide valuable information to microbiologists, soil experts, biotechnologists, and also farmers.