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Agricultural waste utilization for biomethane and algae-based fertilizer production for circular economy

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Abstract

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After obtaining of biogas during the anaerobic digestion processes, the liquid fraction can be applied directly as fertilizer (if it meets the relevant sanitary and hygienic norms), or the rich in mineral composition digestate to be used as a medium for development of microalgae. The algal biomass thus obtained could be applied as fertilizer, and the unused part of it could be re-utilized as raw material in other methanation processes, alone or in combination with other raw materials or wastes. Microalgae can easily colonize different environments. These microorganisms represent promising sources for new products and applications. The obtained liquid phase of anaerobic digestate was utilized as medium for enhanced growth of green microalgae. The ability of microalgae to photosynthetically fix carbon dioxide with production of valuable substances, their short growth cycle and easy biomass accummulation was used. In this study, good growth and development was observed for the microalga *Scenedesmus obliquus* in digestate after a clarification process, based on the macro and micronutrients present therein. This approach may lead to reducing the production costs along with environmental impacts.

Keywords: anaerobic digestion; digestate; green microalgae; biofertilizer

Introduction

Lignocellulosic agricultural materials are quite common and abundantly found wastes. Corn stalks in particular, account for a huge share of the annual production of lignocellulosic waste materials. Their utilization in anaerobic digestion processes for biomethane production is of interest as a way to cope with one of the great ecological problems nowadays that is the replacement of fossil fuels and waste management (Gil, 2021). On the other hand, its complex structure makes its microbiological biodegradation and assimilation challenging (Shi et al., 2021). Anaerobic biodegradation of organic wastes leads to production of biogas. Biogas produced is a renewable and clean source of energy (Ganev & Beschkov, 2022). Gas generated through biodigestion is non-polluting as the concern for the environment comes as a major reason (Kasap et al., 2012). Anaerobic digestion (AD) is an environmentally benign and economically viable process for treating organic wastes with the purpose of generation of value added products (Paul & Dutta, 2018). The great quantities of digestate produced after anaerobic digestion of lignocellulosic wastes cause problems related to transport costs, gas emissions and sludge accumulation, while unutilized nutrients remain available. Alternative paths for valorization to reduce its environmental impact and improve the economic profitability of anaerobic installations are sought. One approach, is involving this waste by-product as a fertilizer or use its potential as a source of nutrients, for growing microalgae (Lee et al., 2021). Algae are important natural source of high-value compounds with wide applications as food additives, in pharmacy and even in biofuels production as source of lipids for biodiesel plants. As photosynthetic organisms they need of light which became one of important factor for productivity control when they are subjected to artificial growth (Popova & Boyadjiev, 2008). Algae can reduce organic components of digestate at the expense of increasing biomass production (Tawfik et al., 2022). This biomass, additionally, could be immobilized and in this form applied for soil improvement. Biofertilization is a sustainable agricultural practice that applies biofertilizers to increase the soil nutrient content, leading to higher productivity, being at the same time eco-friendly with no pollution. It leads to clean products for clean food and health benefits (Abdel-Raouf et al., 2016**).**

Materials and Methods

Digestate source and processing

The digestate obtained from two anaerobic digestion processes of previously milled corn stalks was used. Anaerobic digestion processes were performed on a laboratory scale at 35°C and 55°C, as previously described (Hubenov et al., 2020).

Aliquotes of both mesophilic and thermophilic digestates was sieved for particles removal and used for further processing. A two step decolorisation includes precipitation of fine particles and microbial cells for 24 h. After their removal using centrifugation at 4500 rpm for 10 min and subsequent introducing of active carbon for another period of 24 h. After active carbon separation from the clarified liquid it was used as a cultivation medium without any need of dilution with fresh water (Hubenov et al., 2022).

Microalgal cultures and immobilisation

Monoalgal, non-axenic cultures of green *Scenedesmus obliquus* (Chlorophyta) from the culture collection of the Institute of Botany (Trebon, Czech Republic) were used. An initial algal culture density of 0.5 $mg.ml^{-1}$ dry weight (DW) participated in the experiments. Cultivation was carried out at 25°C and continuous illumination (132 µmol photons $m^{-2}.s^{-1}$). Carbon source was provided by bubbling sterile 2% CO_2 (v/v). Standard culture medium of Zachleder & Setlik (1982) was used in the control cultivation.

After cultivation microalgae were harvested and immobilized with Ca alginate, then added to the soil for growing peppers *Capsicum annuum* L. For immobilization of microalgae 150 ml of microalgal suspension, containing $5x10^6$ cells/ml was centrifuged at 4000 rpm for 10 min. The cells were suspended and mixed with 80 ml of 2% alginate solution and slowly mixed with a stirrer for 15 min. The alginate beads were obtained by dropping the alginate–algae mixture from a syringe into 2% $CaCl_2$ solution. The beads formed were left for 1 h in the solution to harden. These beads were washed off several times with sterile distilled water until their pH reached around 7.

Analysis

Chemical oxygen demand (COD) and nitrogen and phosphorus uptake from digestate during algae cultivation was determined using DR 3900 Spectrophotometer (Hach Lange, GmbH, Germany) by respective test kit LCK 314 for COD, LCK 350 for PO_4-P, LCK 338 for total nitrogen (TN), LCK 302 for NH_4-N (Hach Lange, GmbH, Germany).

Growth of *Scenedesmus obliquus* was estimated by measurement of the dry weight (DW) of algal cultures. The algal suspension was centrifuged (Rotofix 32A, Hettich), then supernatant was removed and cells were dried at 105°C for 16 h. Dry biomass concentration (mg. ml^{-1}) was calculated according to Makarevičienė et al. (2012).

The specific growth rate [µ] was calculated using the following formula: $\mu = \ln(mt_2/mt_1)/(t_2 – t_1)$, where mt are the dry weights at different days ($t_1 = 0$ and $t_2 = 7$) (Levasseur et al., 1993).

Light microscopy images were obtained, using a Levenhuk D870T 8M (Levenhuk LTD, Tampa, FL, USA).

Testing of immobilized algal biomass as plant fertilizer

The calcium alginate/algae biomass beads were tested as plant fertilizer using peppers "Nocera Rosso" (*Capsicum annuum* L.). Lactuca (*Lactuca sativa* L.) plants were used for illustration of raw digestate applicability as fertilizer. As indicators abundance of growth, stem height and leaf area were measured and compared.

Results and Discussion

A renewable source and in the same time agricultural waste – corn stalks was involved in anaerobic digestion at appropriate conditions to obtain biogas and biomethane as an energy carrier. The digestate obtained after an anaerobic digestion process was used as a medium for cultivation of algae after adsorption with activated carbon for clarification. Photosynthetic microalgae used the nutrient residues from anaerobic digestion of agricultural waste and with their short growth cycle and easy accummulation of biomass could prove their economic perspectives- their low-cost cultivation has a great potential for many applications.

Analyses were carried out to estimate the possibility of using the digestate as a cultivation medium. Digestate analysis results before and after cultivation are given in Table 1.

Table 1 Elemental analysis of the digestates before and after cultivation

Parameters, mg.l^{-1}	Mesophilic digestate		Thermophilic digestate	
	Before cultivation	After cultivation	Before cultivation	After cultivation
PO_4-P	3.98	0.18	3.77	0.02
TN	280	81	278	198
NH_4-N	186.4	3.4	246.0	88.6
COD	668	1582	591	1298

Chemical oxygen demand (COD) was measured, showing indirectly the presence of organic carbon. At the beginning it was 668 mg.l^{-1} and in the end of the process - 1582 mg.l^{-1} for the mesophilic digestate and 591 mg.l^{-1} at the beginning and 1298 mg.l^{-1} at the end for the thermophilic digestate. The results show that this parameter increased at the end of the process probably due to some extracellular products accumulated. Phosphorus and nitrogen compounds are consumed, and in the case of nitrogen this is mainly due to the consumption of amine nitrogen.

The anaerobic digestion carried out at 55°C (realized predominantly by thermophilic microorganisms) has some advantages for the methanization process itself and for the sanitation characteristics of the resulted digestate (Hubenov et al., 2021). It helps to eliminate possible pathogenic microorganisms and permits direct application of obtained digestate as a fertilizer. In our study thermophilic digestate was applied to meadow soil for growing Lactuca. Its introduction lead to more intensive growth compared to normal soil used (Figure 1).

Another direction is production of microalgal biomass, which for sure is safer applied as a biofertilizer. Algae proved to grow in the waste effluent as a medium (Figure 2), even growth in the examined digestates was more intensive compared to the control variant (Figure 3).

A) B)

Fig. 1. *Lactuca* grown in soil (A) and in the same soil supplemented with digestate (B)

Immobilization of the obtained algal biomass

The accumulated biomass of the green microalga *Sc. obliquus* (Figure 4) was immobilized and the obtained preparations were applied to normal meadow soil for growing pep-

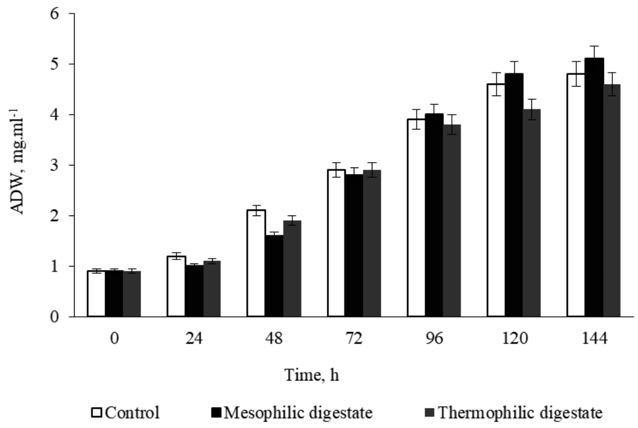

Fig. 2. Growth of *Scenedesmus obliquus* in digestates

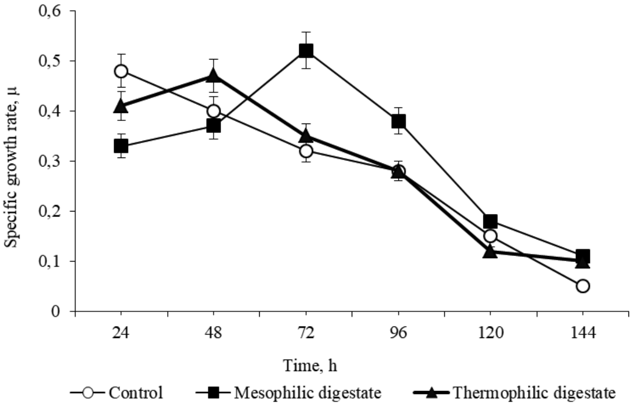

Fig. 3. Specific growth rate for the algal growth in digestate

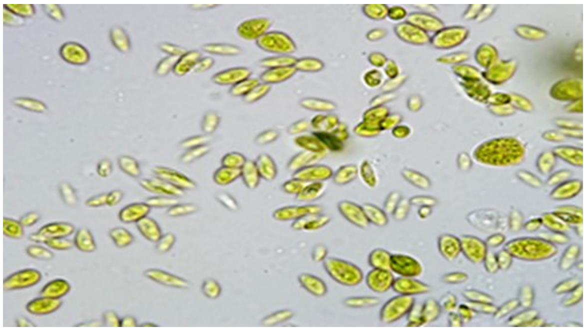

Fig. 4. Light microscopic image of *Scenedesmus obliquus* grown in digestate

Fig. 5. Immobilized algal biomass

per plants (Figure 5). Evident difference was registered for the plants after four weeks, in favor of the growth of pepper *Capsicum annuum* L. in soil supplemented with the immobilized green microalgal suspension (Figure 6). The positive effect was registered after measurements of stem height and leaf surface after four weeks (Table 2). The results showed that the application of the immobilized algal biomass has comparable.

It is shown that algae represent a sustainable and renewable economical source of fertilizer. According to Marks et al. (2019), microalgae favor soil nutrient cycling and promote plant growth by improving nutrient availability. The produced by algae bioactive substances such as exopolysaccharides also act to improve soil structure contribute to the stabilization of soils by the formation of biological soil crusts (Colica et al., 2014), together with the algal cell itself, containing high level – from 30 to 50% sulfated polysaccharides of its dry matter (Domozych et al., 2012). Rachidi et al. (2020) represented microalgae as promising sources of plant biostimulant development, which was revealed in this study. Thanks to the organic

Table 2. Plants dimentions after four weeks of growth

	Stem height, cm^2	Leaf area, cm^2
Control	18.9	28.4
Sample	19.2	31.8

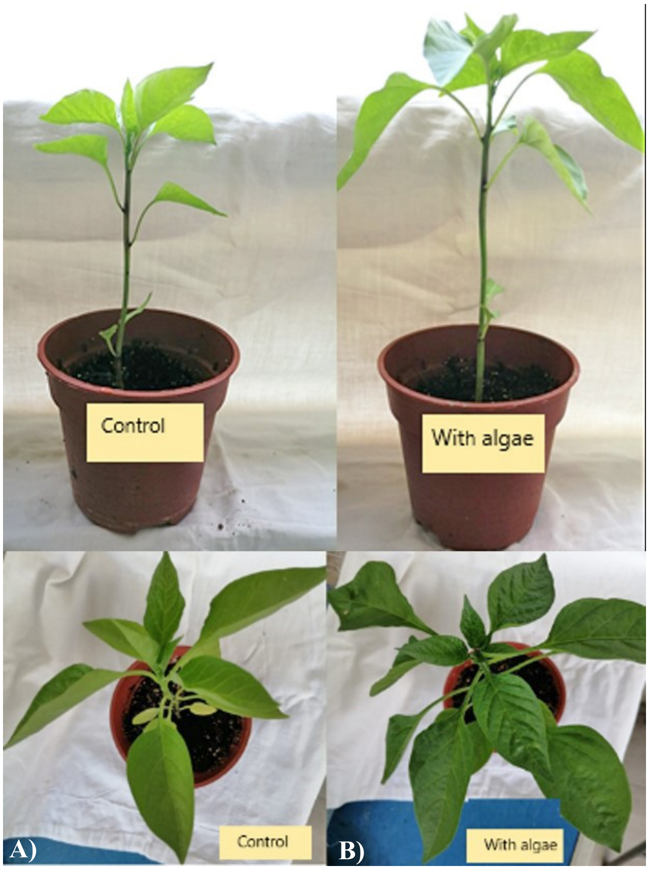

Fig. 6. *Capsicum annuum* L. in soil (A) and in the same soil supplemented with the immobilized green microalgal suspension (B)

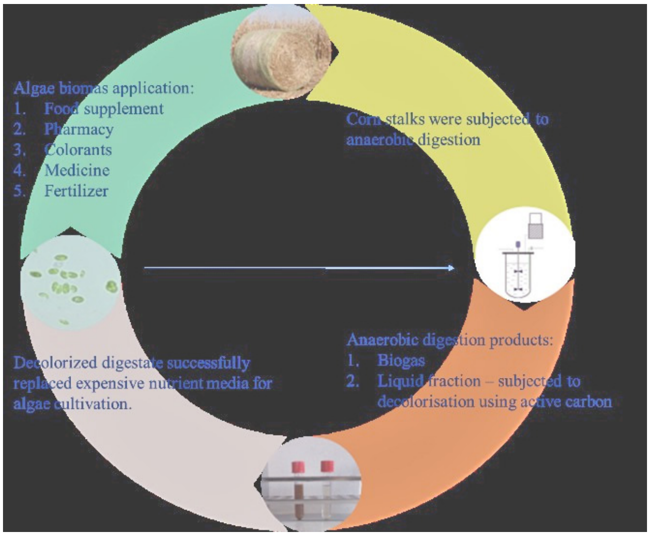

Fig. 7. Concept of circular corn stalks utilization with biomethane and algae-based fertilizer production

nature, algae applied as fertilizers appear to be safer and more environmentally friendly than chemical fertilizers. Using algae-based fertilizers allows keeping arable areas and gardens safer, natural and sustainable.

The Waste Framework Directive of the European Union aims to turn the EU into a recycling society by transformation of linear economy processes into circular economy ones. A concept of corn stalks utilization with obtaining biomethane and algae-based fertilizer or other products as a circular economy approach is presented in Figure 7.

Conclusions

Biotechnological exploitation of lignocellulosic wastes is promising for sustainable and environmentally friendly energy production because of the abundant availability of these renewable sources, which meets the enormous challenge of reducing the volume of wastes available on earth. The green microalga *Scenedesmus obliquus* has the potential to utilize the nutrient content of waste digestate for its mass growth and development. On the other side, algal biomass could be employed as a biofertilizer in a free or immobilized form for stimulating growth of some valuable plants - for food or other purposes. Reducing the cost of microalgal production by using anaerobic digestate obtained as a by-product of bioenergy production processes as a medium for algae cultivation goes along with sparing great quantities of fresh water.

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