Biotechnological Exploitation of Lignocellulosic Wastes for Biomethane Production and Algae Cultivation in the Digestate

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***Abstract*—The use of renewable energy sources and applying appropriate conditions for realization of anaerobic digestion of agricultural waste is carried out for obtaining higher yields of biomethane as an energy carrier. The influence of substrate type, quantity and pretreatment, together with temperature regime on biomethane production was estimated. Both substrates generated higher biogas yields at the higher temperature regime and after pretreatment. The organic loading of 35 g/L was found to be most appropriate. The obtained liquid phase of anaerobic digestate was utilized as medium to maintain and enhance green microalgal growth. The ability of microalgae to photosynthetically fix carbon dioxide producing various biologically active substances, their short growth cycle and easy accummulation of biomass was involved. Due to their ability to colonize different environments these microorganisms represent promising sources for new products and applications. Good growth and development was observed for the microalga *Scenedesmus acutus* in digestate after adsorption with active carbon, using the macro and micronutrients present. This approach may lead to reducing costs and environmental impacts. Accumulated algal biomass (5 g/L) was afterwards introduced back in the reactor, realizing twice increased quantity of biogas on the second day of the anaerobic digestion process, generating biomethane.**

***Index Terms*—biomethane, anaerobic digestion, lignocellulosic wastes, digestate, microalgae**

I. Introduction

Today's civilization faces the rising energy and environmental problems associated with the depletion of fossil energy sources, and energy needs are steadily increasing due to the accelerating economic growth. This calls for new solutions to preserve the ecological and economic stability of our planet. In this regard, the renewable energy sources, due to their wide availability, should be utilized through innovative biotechnological approaches. In recent years, one of the global problems of mankind has been linked to the search for new alternative sources of energy [1].

On the other hand, organic waste from agriculture, households, etc. are one of the major pollutants of the environment. These wastes can be used to obtain many products, including energy. The potential of biomethane production is not well seized yet in the context of biogas to be considered as an important topic related to several economical activities producing a lot of organic wastes [2]. The biological methods of degradation of such waste for biofuel production are an alternative that finds broader and broader application. Biogas is a renewable, as well as a clean source of energy. Gas generated through biodigestion is non-polluting as the concern for the environment is a major reason. Biogas is an environmentally friendly fuel and the expansion of biogas production systems will be a contribution to conversion from fossil to renewable energy systems [3]. As crop residues are raw materials that will always be available, it makes it a highly sustainable option. The use of organic waste to generate energy is also encouraged by the reduction of carbon dioxide emissions from fossil fuels, the need for safe energy supply and revitalization of rural areas [4]. The anaerobic biotechnological processes for biomethane production need no aeration, which reduces the cost of waste treatment compared to aerobic ones. Lignocellulosic waste includes plant biomass - straw, maize, sunflower and tobacco stems, vine rods, wood and releases from wood processing, etc. Bulgaria has about 6.10^6 tons available of straw per year. Half of it remains unused and burnt or rotten in the field. By involving it as a source for bioenergy through anaerobic biodegradation, thousands of tons of fuel oil

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can be saved. In agricultural areas, biogas technology could be used for reducing organic matter load, through transformation of organic carbon into biogas [5]. Anaerobic digestion is a process of organic matter mineralization by microorganisms into biogas (mainly methane and carbon dioxide) and digestate (with liquid and solid phases) in the absence of oxygen [6]. The process is carried out by heterogeneous microbial populations involving multiple biological and substrate interactions. The products obtained are of energetic and ecological significance. The investigations on the quality of the digestate obtained from the anaerobic biodegradation of the above mentioned substrates, have shown that it is a secondary biomass rich in macro- and microelements that can be necessary constituents of a nutrient medium. Different alternatives of digestate valorisation, apart from land applications are possible as the use of the digestate liquid for replacing freshwater and nutrients in algae cultivation or the use of solid digestate for energy production. The interest in microalgae biomass has increased in both fundamental and applied research fields. Algae biotechnology is constantly evolving into a global industry, using its biochemical diversity for a wide range of applications [7]. The digestate, corresponding to the anaerobically non-degraded fraction has been mostly used at farms for improving soils. However, its increasing production induces problems related to greenhouse gas emissions during storage and high nitrogen content that constrains its use to land application only [8]. A suitable alternative is the cultivation of microalgae, which will utilize the nutrients such as nitrogen and phosphorus as they can become valuable biomass, which in turn can find different applications - biochemicals, bionutrients, biofertilizers and biofuels. Microalgae biomass is known to have great potential as a source of new bioactive compounds with industrial and health applications in human, animal and aquatic life [9]. Both organic and inorganic carbon sources from biogas production can significantly improve the growth and increase algal biomass. After clarification of the digestate with a suitable adsorbent, microalgae could extract and use them for growth and development. This alternative approach may, in some cases, replace the direct application of digestate to soil [10].

The aim of this study was to estimate the influence of the substrate type (wheat straw and corn stalks), quantity and pretreatment, as well as temperature regime on biomethane production, followed by evaluation of the capability of different algal strains to exploit waste products (nutrients) resulting from the anaerobic digestion of agricultural waste.

II. Materials and Methods

All experiments were started using culture fluid from an anaerobic laboratory bioreactor with stable biomethane production. Immediately before each experiment, nutrient media were prepared for mesophiles and thermophiles and introduced in the bioreactors. Two laboratory bioreactors with working volumes of 14 dm^3 at 55°C and 10 dm^3 at 37°C and speed of stirrer 100 rpm worked at semi-continuous mode for 30 days. The temperature was maintained with an electronic controller to be (37 ± 0.5 ºC) and (55 ± 0.5 ºC). During the experiments, the pH was maintained in the normal range (6.5 to 8.5). The two laboratory bioreactors operate under anaerobic conditions, realized by nitrogen purging.

Analytical methods included measurement of total solids - determined by dehydration at 105°C and volatile solids - by burning at 575°C. Released gas volume was estimated using a graduated cylinder in a gas holder working on water displacement method. The measurements of the gases released were performed with a "Dräger" (Germany) type specimen X-am 7000.

Residual cellulose was determined during the biodegradation process spectrophotometrically with an anthrone reagent according to the method of Updegraff (1969) [11].

The profile of Volatile Fatty Acids (VFA) formed during the anaerobic process was also determined. The VFA concentrations were measured with a Focus GC (Thermo Scientific) gas chromatograph equipped with a Split/Splitless column, TG-WAXMS (length - 30 m, ID - 0.25 mm, film - 0.25 μm) and FID.

Wheat straw and corn stalks were physically pretreated using ultrasound sonication in a Lab750 for pipe (SinapTec, France) apparatus at a solid/solvent (distilled water) ratio 1:30 (wt/vol) for 1 h. After pretreatment the liquid phase was discharged and the solid fraction was dried in Escalibur® Food dehydrator at 70 °C.

Chemical pretreatment was carried out with 28% NH_4OH, polyethylene glycol-4000 (3%) and water in a ratio of 1:0.5:20: NH_4OH: H_2O. The mixture underwent heating at 90 °C for 5h in a water bath. This was followed by rinsing till neutral pH.

NaOH based substrate pretreatment was carried out with 4% NaOH in terms of weight (w/w). The straw or corn stalks were soaked in the solution and incubated for 24 hours at 55 °C, then filtered and washed thoroughly with distilled water to neutral pH. Drying at 80 °C for 8-12 hours was also performed. After homogenization the required amount was added to the bioreactor.

Definite quantity (100mL) of the digestate was centrifuged at 15000 rpm and the supernatant was subjected to decolorization. Adsorption was performed with introducing active carbon - from 2 to 32g in 100ml of liquid digestate after the end of the process of biomethane production. Active carbon (Fluka) was used for clarification of the obtained digestate to become suitable for cultivation of algae. After 24 hours at room temperature, another centrifugation was carried out and the supernatant was used as a cultivation medium.

Algal culture of *Scenedesmus acutus* was obtained from the collection of IPPG – BAS. All extensive culture experiments were conducted at day light in special vessels (20 ml) at room temperature. pH was 7.0. The growth of the algal culture was estimated following the increase in its weight. For this purpose, 10 ml of the algal suspension were centrifuged at 6000 g for 20 min

(Rotofix 32A, Hettich). The supernatant was removed and the cells were dried at 105°C for 16 h.

Microscopy observations were made on Light Microscope (Carl Zeiss Jena).

Elemental analysis of the obtained digestate was performed with EuroEA 3000 automatic analyzer.

All experiments were conducted at least in triplicate. The data presents average values.

III. Results and Discussion

The main factors influencing the anaerobic biodegradation of lignocellulosic organic wastes are the composition and concentration of the substrate, the need of pretreatment and the organic loading of the reactor. Due to its specific composition, the use of lignocellulosic waste requires optimization and detection of its methanogenic potential. During the experiments, the pH was maintained in the normal range from 6.5 to 8.5. In the resulting biogas, CH_4 content of 37% to 72% and CO_2 of 15% to 39% were observed. The influence of temperature regime was also evaluated for both substates used, initially at constant loading of 5g/L (Fig. 1). Conveying the process at higher temperature lead to higher biogas yield for both substrates tested-wheat straw and corn stalks, probably due to the fact that the substrate becomes more susceptible to enzyme attack at higher temperatures because of loosening of its complex structure.

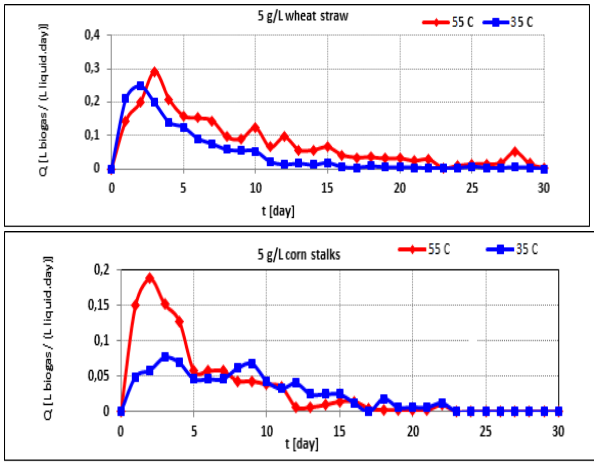

Figure 1. Biogas yield at 37°C and 55°C with substrates –corn stalks and wheat straw.

Passos *et al.* (2013) detected an increment of the methane yield from 4% to 62% applying temperature pretreatment at 55 to 95°C, respectively [12]. New experiments with gradual increase in substrate concentration showed that when loading reached 35g/L of wheat straw, highest biogas quantity was realized (Fig. 2). Measuring the biomethane percent in the biogas showed the highest yield with the same loading but with the other substrate corn stalks – 69%. Further increased loading (45 g/L) did not lead to better yield, probably due to substrate inhibition.

For comparison of data, the biodegradation degree was calculated according to the following formula: biodegradation degree: $BD = \frac{ODM_i - ODM_o}{ODM_i}.100$, BD–biodegradation degree, ODMi–input organic dry matter, ODM–output organic dry matter. Fig. 3 presents the calculated biodegradation degree in relation to cellulose biodegradation in a process with a substrate corn stalks 35g/L and 55 °C. The degree of biodegradation was calculated to be 68 % (Fig. 3). The differences in the obtained experimental values were statistically analyzed by oneway ANOVA at significance level of $P<0.05$, using GraphPAD InStat Software (San Diego, CA, USA).

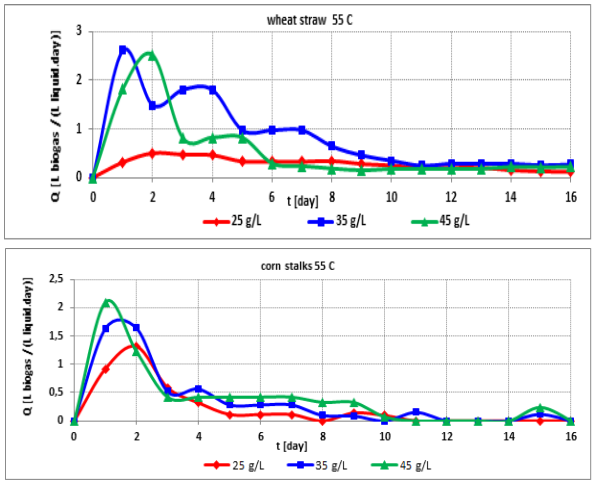

Figure 2. Influence of substrate loading on biogas production.

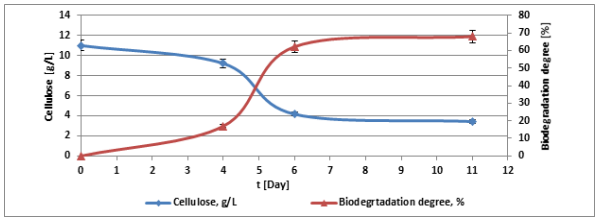

Figure 3. Biodegradation degree in relation to cellulose degradation of corn stalks at 55°C and loading 35g/L.

Experimental work continued towards increasing the volume of gas produced under mesophilic and thermophilic conditions. The low efficiency of hydrolysis is usually the rate-limiting step in the anaerobic digestion of lignocellulosic substrates. Different types of pretreatment have been found to be beneficial for the improvement of the hydrolysis of lignocellulosic substrates and enhancing methane production [13]. Application of this approach was realized for methane yield increase using NaOH, PEG and NH_4OH and ultrasound. Highest increase in the total biogas volume was reached when applying the amphiphilic substance polyethylene glycol to substrate wheat straw (Fig. 4). Pretreatment with ultrasound increased the volume of biogas, when the substrate was corn stalks but less than the increase with the other substrate. The results showed the positive effect of pretreatment, since the substrates used were complex in structure and difficult to digest. They are a mixture of high molecular weight polysaccharides. The main components are cellulose,

hemicellulose and lignin. Cellulose is a structural polymer of glucose residues compounded by β-1, 4 bonds.

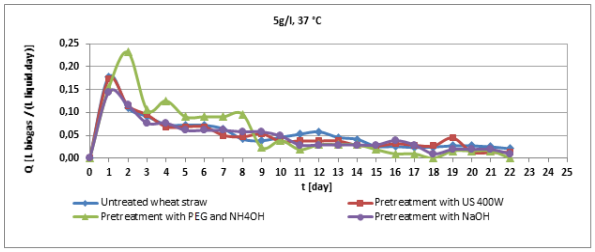

Figure 4. Comparison of the different types of substrate pretreatment on biogas yield.

Alkali pretreatment could efficiently remove lignin in plant tissues, leading to high delignification [14]. Lignin functions in supporting plant structures to avoid microbial permeation.

Another characteristic we determined was the profile of volatile fatty acids formed during the degradation processes at the two temperatures as they are a major metabolic product in the process of anaerobic biodegradation. The concentration of volatile fatty acids was determined in the end of the fermentative process. The highest was the percentage of acetate - 2.45 and 2.6 g/L, followed by that of butyrate- 0.33 and 0.4 g/L and propionate – 0.26 and 0.29 g/L, respectively for the mesophilic and thermophilic conditions. In a typical acidogenic anaerobic digestion operation, the substrate type, different pretreatment techniques, reactor operation, and volatile fatty acids recovery are factors that influence volatile fatty acids content. It is known that they can be obtained from lignocellulosic agro-industrial wastes and various biodegradable organic wastes as key intermediates through dark fermentation processes in addition to their synthesis through chemical routes and after separation to serve as building blocks for several organic compounds [15].

Mixed bacterial consortia are involved in the degradation of lignocellulosic substrates. Light microscopy images of the microbial community in the digestate visualized the presence of spore-forming rod shaped clostridial forms.

From the two methanogenic bioreactors operating at different temperature regimes (37°C and 55°C), anaerobic microbial communities were isolated and cultured on nutrient media for *Ruminiclostridium cellulolyticum* (CM3) 520 DSMZ and Hungateiclostridium 122 DSMZ and Hungateiclostridium 122 DSMZ. These communities were visualized and photo-documented under a light microscope (magnification×1000) and morphologically identified as belonging to the Bacteroidetes and Firmicutes types after Gram staining. Bacterial cells are seen on Fig. 5.

Further our studies continued with testing the obtained digestate after adsorption with activated carbon as a medium for algae cultivation. This pretreatment appeared to depend chiefly on the reduction of the optical density of the digestate, and thus on the better light penetration.

A number of studies indicated the potential of growing the microalge using anaerobic digestion effluent or digestate [16], [17]. Levine *et al.*, reported on inoculation of *Neochloris oleoabundans* in 50, 100 and and 200-fold diluted anaerobic digestion effluent and found that 50-fold dilution lead to the highest growth rates [18]. In our studies the decolorized supernatant was used as a medium for cultivation of green microalgal strains. The pH for all the digestion samples was maintained at 6.8-6.9. Among the major costs encountered for algal cultivation are the costs for nutrients such as CO_2, nitrogen and phosphorous. The digestate was decolorized using a different amount of activated carbon, the best result being achieved with 8 grams of activated carbon per 100 mL of pre-centrifuged digestate (Fig. 6).

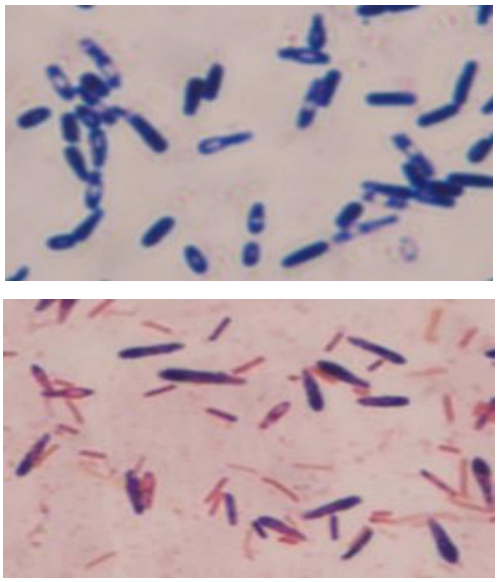

Figure 5. Light microscopy images of the consortia in the digestate.

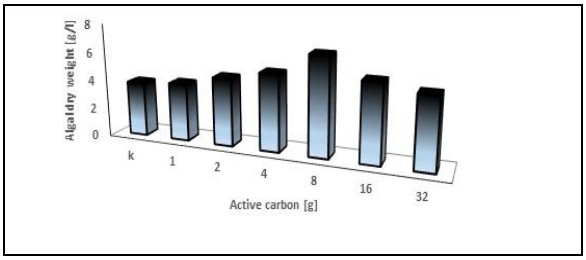

Figure 6. Algal dry weight after growing (7 days) in digestate decolorized with active carbon.

The digestate can ensure the medium for algal growth. Best growth of the tested 7 algal strains at extensive cultivation was obtained for the green microalga *Scenedesmus acutus* (Fig. 7). The Scenedesmus genus counts 7 algal species, typically living in freshwaters as non-motile colonies. Among microalgae, one particularly large group is the green microalgae that belong to the phylum Chlorophyta. *Scenedesmus acutus* –green microalga was tested for growth in the digestate after adsorption. The obtained liquid fraction of the digestate appeared to be an appropriate medium for its cultivation. C/N ratio in the digestate was determined to be in the

optimal range from 15.6:1 to 19.7:1. Diminishing of nitrogen content was established during the growth and development of the green microalga. Results showed satisfactory microalgal growth rate and biomass production. Not diluted and diluted samples were tested for estimation of algal growh. The quantity of algal dry weight measured after 7 days of cultivation in a digestate from a mesophilic bioreactor with pretreated with ultrasound corn stalks as a substrate without dilution was 6.7 g/L. When as a medium digestate of pretreated straw with PEG and NH_4OH was used, the dry weight was 6.5 g/L. For the digestate from a thermophilic bioreactor, dilution (2x) was needed to achieve algal growth. Diminishing nitrogen content proved the growth and development of the algal culture. It was measured to decrease from 0.013g/L to 0.003g/L. Another important feature of microalgae is the ability for phytoremediation to reduce the nutrient content in different waste waters or other effluents due to their ability to assimilate nutrients into the cells. Another approach turned our attention to use the harvested algal biomass by introducing it into the bioreactor and it was digested for biogas production and was analyzed quantitatively. We introduced 5 g of dry microalgal biomass, already accumulated using the digestate as a culture medium into the methanogenic bioreactor to register increased biogas yield with 50% (data not shown).

Based on the laboratory scale study we reached to the conclusion that *Scenedesmus acutus* has the potential to utilize nutrient content of digestate for its mass growth even in the not diluted digestate from a mesophilic process with substrate wheat straw and pretreatment with PEG and NH_4OH, with ultrasound pretreated corn stalks and twice diluted digestate from a thermophilic process.

Thus obtained algal biomass could also be used as a fertilizer, digested or co-digested with new portions of waste agricultural substrates. It is also important for biogas producers to expand the range of substrates. Algae contain polysaccharides, with zero lignin and low cellulose content, which makes them a material more easily consumed and converted to methane by an anaerobic digestion processes [19].

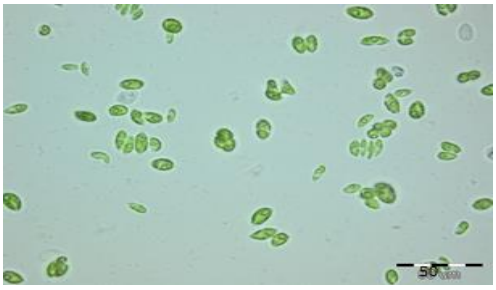

Figure 7. *Scenedesmus acutus* after 7 days growth in not diluted digestate from a mesophilic process.

Introducing the microalgal biomass back into the reactor is the case closes the circle of full utilization. The suggested scheme is a simple and low-cost technology that encourages a circular economy. The strong interest in microalgae anaerobic digestion lies in its ability to mineralize microalgae containing organic nitrogen and phosphorus, resulting in a flux of ammonium and phosphate that can then be used as substrate for growing microalgae or that can be further processed to produce fertilizers. At present, anaerobic digestion outputs can provide nutrients, CO_2 and water to cultivate microalgae, which in turn, are used as substrate for methane and fertilizer generation [20].

IV. Conclusions

Biotechnological exploitation of lignocellulosic wastes is promising for sustainable and environmentally friendly energy production because of the abundant availability of these renewable sources. Improved efficiency of biogas production was registered when both substrates were subjected to pretreatment and temperature increase. We have demonstrated the ability to cultivate microalgae using the digestate obtained after anaerobic processes for biomethane production with nutrient residues from anaerobic digestion of agricultural waste. Most abundant growth and biomass accumulation was registered for *Scenedesmus acutus* grown in non-diluted digestate from a process with substrates that had undergone pretreatment with PEG and NH_4OH and ultrasound at mesophilic conditions.

Conflict of Interest

The authors have declared no conflict of interest.

Author Contributions

Author Contributions: L.K. led the project, supervised all the experiments and prepared the manuscript; H.N. and V.H. conveyed microbiological experiments; I.S., E.Ch. and S. M. followed biodegradation processes accomplishment; J.I. worked on algal strains growth and development.

Acknowledgement

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References

[1] **S**. Alatzas, K. Moustakas, D. Malamis, and S. Vakalis, "Biomass potential from agricultural waste for energetic utilization in Greece," *Energies*, vol. 12, p. 1095, 2019.

[2] S. C. Hernandez and L. D. Jimenez, "The Potential for Biogas Production from Agriculture Wastes in Mexico," in *Biofuels-State of Development*, K. Biernat, Ed., IntechOpen, 2018.

[3] A. Kasap, R. Aktas, and E. Dulger, "Economic and environmental impacts of biogas," *Journal of Agricultural Machinery Science*, vol. 8, pp. 271-277, 2012.

[4] F. Al Zuahiri, D. Pirozzi, A. Ausiello, C. Florio, M. Turco, G. Zuccaro, *et al.*, "Biogas production from solid state anaerobic digestion for municipal solid waste," *Chemical Engineering Transactions*, vol. 43, pp. 2407-2412, 2015.

[5] M. Fuchsz, N. Kohlheb, "Comparison of the environmental effects of manure and cropbased agricultural biogas plants using life cycle analysis," *J. Clean. Prod*., vol. 86, pp. 60-66, 2015.

[6] L. Sun, P. B. Pope, V. G. Eijsink, and A. Schnürer, "Characterization of microbial community structure during continuous anaerobic digestion of straw and cow manure," *Microb. Biotechnol.*, vol. 8, no. 5, pp. 815-827, 2015.

[7] T. C. A. Vega, D. K. Y. Lim, M. Timmins, F. Vernen, Y. Li, and P. M. Schenk, "Microalgal biofactories: A promising approach towards sustainable omega 3 fatty acid production," *Microb. Cell Fact*., vol. 11, pp. 96-106, 2012.
[8] F. Monlau, C. Sambusiti, E. Ficara, A. Aboulkas, A. Barakata, and H. Carrère, "New opportunities for agricultural digestate valorization: Current situation and perspectives," *Energy and* Environmental *Science*, vol. 8, no. 9, pp. 2600-2621, 2015.
[9] A. G. Ishaq, H. M. Matias-Peralta, and H. Basri, "Bioactive compounds from green microalga–scenedesmus and its potential applications: A brief review," *Pertanika J. Trop. Agric. Sci*., vol. 39, no. 1, pp. 1-16, 2016.
[10] K. Przygocka, C. Witold, and G. Witold, "Biogas digestate-benefits and risks for soil fertility and crop quality—An evaluation of grain maize response," *Open Chemistry*, vol. 16, no. 1, pp. 258-271, 2018.
[11] D. M. Updegraff, "Semimicro determination of cellulose in biological materials," *Analytical Biochemistry*, vol. 32, pp. 420-424, 1969.
[12] F. Passos, J. Garcia, and I. Ferrer, "Biogas production from microalgae grown in wastewater: Effect of microwave pretreatment," *Appl. Energy*., vol. 108, pp. 168-175, 2013.
[13] Q. Zheng, T. T. Zhou, Y. B. Wang, X. H. Cao, S. Q. Wu, M. L. Zhao, *et al.*, "Pretreatment of wheat straw leads to structural changes and improved enzymatic hydrolysis," *Sci. Rep*., vol. 8, p. 1321, 2018.
[14] A. T. W. M. Hendriks and G. Zeeman, "Pretreatments to enhance the digestibility of lignocellulosic biomass," *Bioresource Technology*, vol. 100, pp. 10-18, 2009.
[15] R. R. Singhania, A. K. Patel, G. Christophe, P. Fontanille, and C. Larroche, "Biological upgrading of volatile fatty acids, key intermediates for the valorization of biowaste through dark anaerobic fermentation," *Bioresour. Technol*., vol. 145, pp. 166-174, 2013.
[16] M. Franchino, E. Comino, F. Bona, and V. A. Riggio, "Growth of three microalgae strains and nutrient removal from an agro-zootechnical digestate," *Chemosphere*, vol. 92, pp. 738-744, 2013.
[17] Y. Yang, J. Xu, D. Vail, and P. Weathers, "Ettlia oleoabundans growth and oil production on agricultural anaerobic waste effluents," *Bioresour Technol*., vol. 102, pp. 5076-5082, 2011
[18] R. B. Levine, M. S. Costanza-Robinson, and G. A. Spatafora, "Neochloris oleoabundans grown on anaerobically digested dairy manure for concomitant nutrient removal and biodiesel feedstock production," *Biomass Bioenerg.*, vol. 35, pp. 40-49, 2009.
[19] A. Vergara-Fernández, G. Vargas, N. Alarcón, and A. Velasco, "Evaluation of marine algae as a source of biogas in a two-stage anaerobic reactor system," *Biomass and Bioenergy*, vol. 32, pp. 338-344, 2008.
[20] E. Uggetti, B. Sialve, E. Trably, and J. P. Steyer, "Integrating microalgae production with anaerobic digestion: A biorefinery approach," *Biofuels Bioproducts and Biorefining*, vol. 8, no. 4, 2014.

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