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Thermal pretreatment and bioaugmentation improve methane yield of microalgal mix produced in thermophilic anaerobic digestate

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ABSTRACT

Liquid fraction produced in anaerobic digestion (AD) of biodegradable waste can be treated on-site with microalgae, which can be recycled back as substrate to the biogas plant. For this research, a pilot high rate algal pond (HRAP) was set with connections to a full scale biogas plant that enabled the use of waste heat and CO_2 from a combined heat and power gen-set (CHP). The microalgal mix produced in the thermophilic anaerobic digestate supernatant was tested as a substrate for biogas production in the thermophilic AD (i.e. untreated, bioaugmented with anaerobic bacteria *Clostridium thermocellum*, and thermally pretreated, respectively). The methane potential of the untreated microalgal mix was low (157.5 ± 18.7 mL CH_4/g VS). However, after the thermal pretreatment of the microalgae, methane production increased by 62%, while in the bioaugmentation with *C. thermocellum* under thermophilic conditions (T = 55 °C) it was elevated by 12%. The outcome of our pilot trial suggests that microalgae produced in the thermophilic biogas digestate represent a prospective alternative AD feedstock. At the same time, microalgae reduce the digestate nitrogen and COD to the level sufficient for the outflow to meet the quality required by the sewage system (ammonia-nitrogen max 200 mg/L, nitrite max 10 mg/L).
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1. Introduction

In the European Union, huge quantities of liquid anaerobic digestate are produced as side product in anaerobic digestion of biodegradable waste into biogas. Due to a high nutrients value (NPK), application of digestate as fertilizer in agriculture is a generally widespread practice [1] [2], however, its use in urban and water sensitive areas is limited. Some common issues connected to digestate management are emissions of ammonia and methane, contamination with pathogens, over-fertilization, and consequently eutrophication as nutrients leach into groundwater or inland water [3]. For logistical and agro-technical reasons, the whole digestate is often separated into liquid and solid fraction. The latter of the two represents a non-problematic fertilizer, while the liquid fraction, i.e. digestate supernatant, hereinafter referred as digestate, on the other hand, carries a significant portion of nutrients and causes high transport costs to fields, as well as high costs in case of treatment at wastewater treatment plants. Although the digestate is produced continuously all year round, it cannot be applied on agricultural land immediately after its production due to the limitations of crop growth stage, soil type, and its stabilization level. In addition, it needs to be stored for several months [2], [3], [4], thus alternative solutions are tested for continuous digestate management. Recent research is focused towards the development of new environmentally safe and feasible solutions for digestate treatment [5].

Algal treatment of digestate is one of the appropriate alternative technologies that can be applied under favorable geographical conditions. Introducing algal technology at a biogas plant site could represent a potential economic benefit for the plant due to the

Abbreviations: AD, anaerobic digestion; BMP, biochemical methane potential; CHP, combined heat and power cogeneration unit; COD, chemical oxygen demand; CSTR, continuous flow stirred-tank reactor; HRAP, high rate algal pond; HRT, hydraulic retention time; NPK, nitrogen, phosphorous, potassium; OLR, organic loading rate; PAR, photosynthetic active radiation; SCFA, short chain fatty acids (volatile fatty acids); TS, total solids; VS, volatile solids.

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reduction of the logistics and waste water treatment costs. In addition, microalgal biomass, the by-product of this treatment, as a nitrogen rich substrate, contributes to the optimization of the C/N ratio in the substrate for anaerobic digestion, as well as provides higher biogas yields. Also, by maintaining a proper N:P balance in the digestate, no costs for mineral nutrient sources for algae cultivation occur [6]. Overall, biogas production can be coupled to algae production with several benefits and with the possibility of using waste heat and CO_2 (flue gases) in algal pond [7]. In this biorefinery concept, microalgae use mineral nutrients from the biogas digestate, CO_2, and organic matter, while at the same time producing O_2 and the valuable biomass. This results in a reduction in nutrients, CO_2 sequestration, and oxygenation of the biogas digestate. Moreover, microalgae contribute to the removal of heavy metals [8], organic pollutants (e.g. hormone disruptors) [9] and pathogens [10]. The produced microalgal biomass can be used for anaerobic digestion [11] or other products, for example biofuels [12] or bioplastics [13].

A closed loop for nutrient recycling can be established, when anaerobic digestate is used as a nutrient source for microalgae followed by the use of the microalgal biomass for biogas production. The microalgal biomass, i.e. microscopic algae 5–50 μm, including cyanobacteria, is a prospective substrate for biogas production [14] and when anaerobic digestate is used as a nutrients source for microalgae production, the loop for recycling of nutrients is closed [15–17].

Previous research on the biomethane potential of a microalgal biomass has shown certain obstacles in the anaerobic digestion of microalgae. These obstacles are mostly due to the complex and rigid cell walls of the microalgae, which first need to be disrupted, so that the intracellular organic matter becomes available for anaerobic microorganisms in AD. Another important obstacle for AD is the low C/N ratio of microalgae that might cause ammonia inhibition that results in accumulation of short chain fatty acids (SCFA) and process failure [18–20].

The problems regarding the low microalgal C/N ratio can be solved by codigesting the microalgae biomass and substrates with a high C/N ratio. Wirth et al. [21] reported on successful continuous mesophilic AD when the maize silage was added to the algal biomass *Scenedesmus obliquus* (1% contaminated with bacteria, with C/N ratio 8.9) in 1:1 VS ratio to increase the C/N ratio (to 20–30:1) and avoid ammonia or volatile fatty acids inhibition. No significant changes in the Archaeal community were observed by the codigestion of the microalgae or by AD with only *S. obliquus*, however, the predominance of the bacterial order *Clostridiales* was determined in AD and its high cellulase activity led to yet more efficient digestion of the algal biomass. The advantage of the codigestion of the microalgal mix with a carbon rich substrate was again confirmed by the same authors [22] when they added maize silage to the mix of *Chlamydomonas* sp. *Scenedesmus* sp. and mutualistic bacteria.

In addition, the cell wall of many microalgae consists mainly of cellulose which is difficult to degrade [23]. When inspecting the so far performed studies, the microalgal biomass used in research to date differs significantly, therefore results are difficult to compare and methane yields depend on species specifics. The cell wall of *Scenedesmus* sp. e.g. *S. obliquus*, is made of hemicellulose and sporopollenin, i.e. macromolecules which are difficult to degrade and consequently present an obstacle in achieving effective anaerobic degradation [24]. The cell wall of *Monoraphidium* sp. is on the other hand composed of 47% neutral sugars, 16% proteins, 6.1% uronic acids, 0.4% glucosamine, and 31% unknown compounds [25]. In order to achieve higher methane production, microalgal cells need to be subjected to pretreatment. Several techniques for microalgal biomass pretreatment have been tested on lab scale. Thermal pretreatments (in range of 55–170 °C and different pressure) proved to be most effective, taking into account methane yield and net energy utilized for pretreatment, thereby showing the pretreatment of microalgal biomass below 100 °C [20,25], as the most feasible. Other methods used are mechanical pretreatment, biological pretreatments and chemical pretreatments, of which the latter is less promising due to a potential inhibition of AD and higher costs [20].

Pretreatment generally enhances the anaerobic bioavailability and biodegradability of the cell wall compounds. The effect of the chosen pretreatment however depends on the selected species. In case of a less complex cell wall structure, as in e.g. *Monoraphidium* sp., cells were solubilized after thermal pretreatment (95 °C, 10 h), resulting in higher methane production, while in case of species with more recalcitrant cell walls, as in e.g. *Scendesmus* sp. cells were damaged but their methane production was lower than in the less complex ones [20,25].

The increased yield of methane after thermal pretreatment [26–28] and bioaugmentation of anaerobic digestion of the microalgal biomass promises good opportunities for its further large scale application. Bioaugmentation, or the practice of adding specific microorganisms to the AD system to enhance biodegradability, was used in AD to improve methane yield of starch straw [29] or brewery residues [30]. On the other hand, bioaugmentation of the anaerobic digestion with *C. thermocellum*, which is an anaerobic thermophilic bacterium with cellulolytic activity [31], and other anaerobic bacteria, accelerates hydrolysis in AD [29,30,32], which is the rate limiting step in biogas production from complex substrates. Furthermore, addition of *C. thermocellum* resulted in the production of higher concentrations of hydrogen which could promote the development of hydrogenotrophic methanogenesis, resulting in higher methane yield [31]. *C. thermocellum* is an anaerobic Gram positive bacterium capable of producing ethanol directly from cellulose. It also has the ability to undergo a non-growth state (spores and L-forms) under unfavorable conditions [33]. All these properties make *C. thermocellum* a promising candidate for bioaugmentation.

In order to enhance biogas production from the microalgae grown in the anaerobic digestate and recycled back to the biogas digester, the presented research work tests the influence of the microalgae thermal pretreatment and bioaugmentation of AD by *C. thermocellum* under thermophilic conditions.

2. Materials and methods

The microalgal treatment of the anaerobic thermophilic digestate was studied at pilot level at the ALGAEBIOGAS demonstration center in Ljubljana, Slovenia, while the thermophilic anaerobic digestion of the microalgal mix was performed on lab scale.

2.1. Thermophilic anaerobic digestate

The digestate used in the research was produced in a two-stage thermophilic anaerobic digestion at an industrial biogas plant with the capacity of 13,000 t/a. The hydraulic retention time (HRT) was 1–3 days in the hydrolysis tank and additional 14–20 days in digesters with the organic loading rate (OLR) of 4.6 kg VS/m^3/day. The anaerobic digestion took place in three 500 m^3 digesters, the continuous flow stirred-tank reactors (CSTR) with the working volume of 1200 m^3 under thermophilic conditions (with temperature 55.0–55.5 °C). The substrate used for anaerobic digestion was mixed biodegradable waste: food waste, originating from restaurants, canteens, households, markets, sludge from waste water treatment plants from food industry (dairies, slaughterhouses), and leachate water (biodegradable waste from households and

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