19th International Conference on Environmental Science and Technology

Kos, Greece, 3 to 6 September 2025

Microalgae cultivation in ammonia rich digestate subjected to different pretreatment stages

Palikrousis T.[1], Manolis Ch.[1], Kalamaras S.[1], Samaras P.[1,*]

[1]Department of Food Science and Technology, International Hellenic University, Alexandrian IHU campus at Sindos, 57400 Thessaloniki, Greece

*corresponding author: Samaras P.
e-mail: samaras@ihu.gr

Abstract Anaerobic digestion has been beneficial for the energy valorization of a broad variety of residues. However, the effluent from the digesters, the digestate, usually has high ammonia nitrogen, requiring additional measures for its management. Photosynthetic microalgae assimilate nitrogen, and can be used for the treatment of digestate, while grown biomass can be valorized in the formation of added value products. The aim of this work was the examination of the efficiency of *Chlorella sorokiniana* for the treatment of various digestate samples subjected to subsequent treatment stages. Results showed that *Chlorella* can efficiently be used in digestate, although high ammonia content may inhibit growth. Cells composition depends on operation conditions as well as on substrate properties.

Keywords: microalgae; digestate; nitrogen recovery

1. Introduction

Anaerobic digestion (AD) is an increasingly adopted technology for the stabilization of organic waste and renewable energy generation via biogas. However, one of its major by-products—digestate—often contains high levels of ammonium nitrogen (NH_4^+-N), particularly when nitrogen-rich substrates such as livestock manure especially from poultry farms, sewage sludge, or food waste are used. Elevated ammonia concentrations, higher than 1,000 mg/L NH_4^+-N can limit land application of digestates due to phytotoxicity and environmental concerns, including nitrate leaching, ammonia volatilization, and greenhouse gas emissions (Yang et al., 2022). Therefore, considerable efforts are taken in finding sustainable solutions for digestate treatment and valorization of contained nutrients.

In recent years, microalgae-based technologies have emerged as promising alternatives for nutrient recovery from waste streams. Microalgae can assimilate nitrogen and phosphorus from aqueous media, using sunlight and CO_2 to grow and produce high-value biomass. This biomass can be further converted into biofuels, fertilizers, animal feed, or bioproducts, thereby closing nutrient cycles and aligning with circular economy strategies (Abdelfattah et al., 2023). Several studies have demonstrated the feasibility of using microalgae like *Chlorella vulgaris* or *Scenedesmus obliquus* to treat anaerobic digestate. These microalgae not only remove nutrients but also produce biomass that can be valorized into biofuels, animal feed, or bioplastics, contributing to circular economy principles (Liu et al., 2017). Furthermore, microalgal systems offer the added benefit of CO_2 fixation, making them suitable for integration with biogas upgrading units (Rawat et al., 2011).

Nevertheless, digestates with high ammonia content pose certain challenges for microalgal cultivation, such as ammonia toxicity, high turbidity, and dark coloration that limit light penetration. Nevertheless, the selection of appropriate ammonia-tolerant strains (e.g., *Chlorella vulgaris*, *Scenedesmus obliquus, Coelastrum sp.*) can significantly improve system resilience and nutrient removal efficiency (Scarponi et al., 2021, Psachoulia et al., 2022). Recent studies have shown nitrogen removal efficiencies exceeding 80% in diluted digestate conditions under optimized parameters, with simultaneous production of algal biomass rich in proteins and lipids (Psachoulia et al., 2024). Moreover, coupling microalgae cultivation with CO_2 from biogas upgrading systems enhances carbon capture while providing a low-cost carbon source for photosynthesis.

Despite its potential, microalgae-based treatment is not yet widely commercialized, mainly due to seasonal variability, and especially the need for process optimization; species growth rate and biomass composition are greatly affected by the operation conditions and certainly the particular species used in the corresponding process. The aim of this work was the examination of the growth of specific microalgae species, *Chlorella Sorokiniana*, in samples of digestates that have been subjected to different pretreatment steps, and the evaluation of the composition of the cells as a function of cultivation time, nutrients substrate and operation conditions.

2. Materials and Methods

Digestate samples employed in this study were collected from the various stages of a digestate treatment plant,

operating in an 1 MW_{el} biogas plant fed by various waste streams including local animal husbandries, food industries and food waste. About 20 L of each sample were collected in a plastic tank and transferred to the laboratory for analysis and use as microalgae substrate.

Microalgae cultivation conducted in 250 mL Erlenmeyer flasks. Approximately 150–155 mL of digestate samples were inoculated with 15–20 mL of *Chlorella Sorokiniana* preculture, resulting in cultures of total initial volume of 170 mL and initial Optical Density (OD680nm) of approximately 0.35. Atmospheric air passed through 0.2 μm filters was sparged into each flask while in certain samples 2.5 mL/min of CO_2 were supplied for enhancing biomass growth. Cool white LED strips (6000 K, 7.2 W/m) were employed to uniformly illuminate the cultures with 1200 lux light intensity. A daily photoperiod of 16 h lighting followed by 8 h darkness was applied. Species were cultivated in around 17-day batches.

Microalgae culture growth in each experiment and the respective assimilation of nutrients were monitored by sample collection from cultures every 48 h. Optical density (OD) at 680 nm was measured using a Hach UV-vis spectrophotometer. Determination of biomass concentration, measured as dry cell weight (DCW), was carried out by filtering 5 mL of samples cultures through a pre-weighted glass microfiber filter, dried at 50◦C overnight, and weighted. Microscopic observations of the culture were carried out in an optical microscope while cell numbers were quantified using a Neubauer hemocytometer.

Concentrations of ammonium nitrogen ($N\text{-}NH_4$), nitrate nitrogen ($N\text{-}NO_3$), total nitrogen (TN), soluble phosphorus ($P\text{-}PO_4$) and chemical oxygen demand (COD) were determined in the liquid phase of filtrate samples using standard HACH cuvette tests in a UV–Vis spectrophotometer. In addition, the content of cells in lipids, proteins and hydrocarbons was measured according to the methods described by Psachoulia et al., (2022).

3. Results

Digestate samples subjected to various treatment stages presented different ammonia nitrogen content, depending on the processing level: samples received from the first screening stage had high ammonia content, exceeding 4 g/L depending on the biogas digester feedstock, while lower values were observed in the effluent from a reverse osmosis membrane unit.

Chlorella species were able to grow at all digestate samples, although samples with high nitrogen content seem to inhibit their growth. Therefore, samples dilution up to 10% has to be examined, especially for the sample collected at the exit of the screening stage. On the other hand, the composition of the cells depends on the substrate profile and the corresponding cultivation pattern. Biomass with high protein content was produced from high nitrogen ammonia substrates, while the growth of species with higher lipids content was associated to a diet poor in nitrogen

Therefore, for an efficient valorization of digestate from a biogas unit fed by high nitrogen raw materials, microalgae represent a viable option. Nevertheless, the content of digestate in ammonia nitrogen is crucial for efficient microalgae growth, while operation conditions and feedstock properties affect the development of biomass with specific composition in proteins or lipids.

Acknowledgements

The research project “Strengthening the industrial symbiosis in the value chain of anaerobic digestion” (ALIVE) is implemented in the framework of “(Strategy for Excellence in Universities & Innovation) (ID 16289) - SUB1.1.Clusters of Research and Excellence” under the National Recovery and Resilience Plan “Greece 2.0” funded by the European Union – NextGenerationEU (Implementation Body: ΕΔ ΕΣΠΑ ΥΠΑΙΘΑ (Project Number: ΥΠ3ΤΑ-0560157).

References

Abdelfattah A., Ali S. S., Ramadan H., El-Aswa, E. I., Eltawab R., Ho S. H., Elsamahy T., Li S., El-Sheekh M. M., Schagerl M., Kornaros M. and Sun, J. (2023), Microalgae-based wastewater treatment: Mechanisms, challenges, recent advances, and prospects, Environmental science and ecotechnology, 13, 100205.

Liu X., Ying K., Chen G., Zhou C., Zhang W., Zhang X., Cai Z-H., Holmes T., and Tao Y. (2017), Growth of Chlorella vulgaris and nutrient removal in the wastewater in response to intermittent carbon dioxide, Chemosphere, 186, 977-985.

Psachoulia P., Chatzidoukas C., and Samaras P. (2024), Study of Chlorella sorokiniana Cultivation in an Airlift Tubular Photobioreactor Using Anaerobic Digestate Substrate, Water, 16(3), 485.

Psachoulia P., Schortsianiti S. N., Lortou U., Gkelis S., Chatzidoukas C., and Samaras P. (2022), Assessment of nutrients recovery capacity and biomass growth of four microalgae species in anaerobic digestion effluent, Water, 14(2), 221.

Rawat I., Kumar R. R., Mutanda T., and Bux F. (2011), Dual role of microalgae: Phycoremediation of domestic wastewater and biomass production for sustainable biofuels production, Applied Energy, 88(10), 3411–3424.

Scarponi P., Ghirardini A. V., Bravi M., and Cavinato C. (2021), Evaluation of Chlorella vulgaris and Scenedesmus obliquus growth on pretreated organic solid waste digestate, Waste Management, 119, 235-241.

Yang D., Chen Q., Liu R., Song L., Zhang Y., and Dai X. (2022), Ammonia recovery from anaerobic digestate: State of the art, challenges and prospects, Bioresource Technology, 363, 127957.