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Enhancing microalgae cultivation in anaerobic digestate through nitrification

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HIGHLIGHTS

- Microalgae growth was inhibited at NH_4^+-N concentrations above 100 mg/L.
- Microalgae growth in digestate was suppressed by high NH_4^+-N and competing microbes.
- Nitrification based pre-treatment was effective in alleviating NH_4^+-N toxicity.
- Excellent nitrogen and phosphorus recovery from nitrified digestate by microalgae.
- Integrated nitrification and algae cultivation possible using membrane bioreactors.

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ABSTRACT

High ammonium concentration is considered a major challenge in cultivating autotrophic microalgae in anaerobic digestate. In this research, the feasibility of applying nitrification as pretreatment to alleviate ammonium toxicity on microalgae was investigated. Batch experiments conducted in synthetic medium showed that microalgae growth was inhibited at NH_4^+-N > 100 mg/L, but NO_3^--N was benign at concentrations as high as 350 mg/L. Microalgae growth in 2–50% digestate (v/v) was also affected adversely by invading heterotrophic microorganisms. Digestate pre-treatment using activated sludge mitigated these challenges by converting NH_4^+-N to NO_3^--N, and reducing organics content in the digestate. Microalgae exhibited excellent growth and nutrients removal in nitrified digestate (5–30% mixed with municipal wastewater) in batch mode. For example, COD, NH_4^+-N, NO_3^--N and PO_4^{3-}-P removal in 10% digestate using two-stage bacterial-microalgal process were 87%, 100%, 30% and 77%, respectively. In continuous mode, using a microalgae-based membrane photobioreactor (MPBR) operating downstream to membrane bioreactor (MBR), 91% COD, 97% NH_4^+-N and > 99% PO_4^{3-}-P could be continuously removed from 10% digestate. Although NH_4^+-N removal in the process was mainly through nitrification, total nitrogen removal was > 75% at steady state. The effects of lower NH_4^+-N toxicity in the MPBR was also manifested in terms of high microalgae biomass accumulation of about 5 g/L. These results indicate that nitrification can be a promising pretreatment for anaerobic digestate for use in microalgae cultivation.

1. Introduction

Anaerobic digestion (AD) is a waste-to-energy technology employing a consortia of anaerobic microorganisms for treatment of municipal or industrial solid waste [1]. AD results in continuous production of methane-rich biogas, and intermittent release of effluent digestate comprising of undigested solids, organic and inorganic compounds, metal salts and microorganisms [2]. Typically, anaerobic digestate is characterized by extremely high levels of nitrogenous compounds, especially NH_4^+-N, which may reach concentrations above 3 g/L [3]. High nutrients levels in digestate has emerged as a major challenge, as AD expands its footprint in urban waste management.

Due to the high content of nitrogenous compounds, digestate has been widely applied as agricultural fertilizer [4]. However, with the growing distance between digesters and agriculture farms, transportation of liquid digestate is no longer a practical option, as the high costs and fuel demand may negate the energetic benefits associated with AD. Agricultural application of digestate may also be constrained by the

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large land requirements for disposing the digestate on a regular basis, irrespective of seasonal variations in fertilizer demand [3]. Furthermore, the presence of pathogens and heavy metals may affect the acceptability of the digestate among the farmers [5]. Therefore, there is need for a better treatment route for safe and sustainable disposal or reuse of digestate in the environment.

Recently, microalgae have been applied in nutrients recovery from AD digestate [2,6,7]. The use of microalgae in digestate treatment has several advantages: high growth and nutrient uptake rates, large fertilizer demand, carbon capture and biomass generation. Besides, high growth rates of microalgae can substantially reduce land area for digestate application. Additionally, there is a possibility of sourcing CO_2 from biogas as carbon source for microalgae cultivation, which can facilitate simultaneous biogas upgradation and digestate treatment [8]. However, microalgae cultivation in digestate requires alleviation of ammonia toxicity, which may be detrimental for the microorganisms at high concentrations [9]. Moreover, the presence of readily biodegradable organic compounds makes digestate susceptible to bacterial invasion, and the resulting turbidity may reduce photosynthetic efficiency, nutrients removal and biomass productivity [10]. Additional limitations may arise from a relatively low phosphorus to nitrogen (P/N) ratio in the digestate, which may not fulfil the stoichiometric P demand of microalgae.

In order to make digestate suitable for microalgae cultivation, the preferred strategy is to dilute the digestate with synthetic culture medium, secondary/tertiary wastewater or seawater [3,11]. This approach has been successful in mitigating NH_4^+-N inhibition, lowering the turbidity, and enhancing P/N ratio. Protection of microalgae from NH_4^+-N toxicity can also be achieved by changing the oxidation state of nitrogen to a more amenable form, such as nitrate [12]. Nitrification can not only reduce nitrogen toxicity, it may also reduce the concentrations of organic compounds and improve P/N ratio. Thus, a two-stage process based on digestate nitrification and subsequent microalgae application can be suitable for nutrients recovery from digestate, although it would involve separation of anaerobic bacteria from microalgae. However, these processes can be simplified by using membrane bioreactors (MBR), such that an MBR with aerobic heterotrophic microorganisms is operated in conjugation with a membrane photobioreactor (MPBR) with autotrophic conditions [13]. Membrane filtration would prevent mixing of the microorganisms, while allowing operation at lower HRTs with high biomass retention.

In this research, microalgae were cultivated in synthetic wastewater and anaerobic digestate with different concentrations of NH_4^+-N and NO_3^--N to study their growth and nutrient assimilation characteristics. In a two-stage sequential bacterial-algal batch process, the digestate was first diluted with municipal wastewater and treated in an activated sludge process. Subsequently, the pretreated and nitrified digestate was used in microalgae cultivation under autotrophic conditions. To investigate the performance of the bacterial-algal process in continuous mode, an MBR with activated sludge was operated in tandem with an MPBR with microalgae. The MBR-MPBR system was operated for carbon removal and nitrification in the MBR, and nutrients recovery and biomass generation in the MPBR.

2. Materials and methods

2.1. Microorganisms, culture conditions, and chemicals

All the chemicals used in this research were of analytical grade and purchased either from Sigma-Aldrich (St. Louis, United States) or Merck (Darmstadt, Germany).

Chlorella vulgaris ATCC 13482 was used throughout this study. The microalgae were cultivated in Bold's Basal Medium (BBM) supplemented with 5% CO_2 enriched air at a rate of 0.2 gas volumes per reactor volume per minute (VVM). The culture flasks were illuminated with 3000 lux light intensity (control experiment) and all the experiments were conducted at a constant temperature of 25 °C. The pH of the culture medium was kept constant at 7–7.5 through HCl or NaOH addition. All media, pipette tips, and Erlenmeyer flasks fitted with cotton plugs were autoclaved before use. Activated cells in late exponential growth phase were used as inoculum for all the experiments.

2.2. Digestate and wastewater

Digestate was collected from a pilot-scale anaerobic digester, operating with food waste, at National University of Singapore. The digestate slurry was autoclaved upon collection, cooled, and centrifuged at 12,000 rpm (Eppendorf 5810R, Germany) for 30 min. The supernatant was collected as the liquid digestate, and it was used to prepare the feed wastewater in all the experiments. The composition of the liquid digestate changed significantly during the period of study due to changes in operating conditions of the digester. The COD, NH_4^+-N, NO_3^--N and PO_4^{3-}-P concentrations in the digestate varied between 5 and 10 g/L, 0.7–1.2 g/L, 90–300 mg/L and 60–190 mg/L, respectively.

Activated sludge was collected from Ulu Pandan wastewater treatment plant of Singapore. Synthetic municipal wastewater was prepared based on the composition of primary filtered wastewater at the same plant with COD, NH_4^+-N, NO_3^--N and PO_4^{3-}-P concentrations of 500 mg/L, 40 mg/L, 2 mg/L and 8 mg/L, respectively. Batch experiments to assess the effects of nitrogen source and concentrations on microalgae were conducted using modified BBM. All the batch studies were conducted in triplicates for reproducibility.

2.3. MBR-MPBR setup and operation

Fig. 1 shows the laboratory scale MBR-MPBR setup. The MBR comprised of a 1 L rectangular tank and a submerged plate-and-frame membrane module with 140 cm^2 filtration area. PVDF microfiltration membranes (Newton & Stokes, Singapore) of 0.1 μm pore size and 0.7 mm thickness were used. Air was pumped continuously into the MBR through a diffuser fitted in the bottom of the tank at the rate of 0.5 L/min. The MPBR comprised of a 3 L cylindrical tank and a plate-and-frame membrane module of 308 cm^2 filtration area. The MPBR was illuminated with fluorescent light tubes of 8000 lux intensity by arranging the tubes parallel to the tank. Humidified 3% CO_2-enriched air was diffused in the MPBR at the rate of 1 L/min. The experiments were conducted at room temperature, which remained stable at 24–26 °C throughout the operating period. Wastewater feed to the MBR was prepared by diluting the digestate 10-folds using synthetic municipal wastewater. Peristaltic pumps (Masterflex L/S Standard Digital Drives, USA) and pump heads (Masterflex L/S Easy Load II, USA) were used to feed the MBR and to withdraw the effluent into a collection bottle. MBR effluent from the collection bottle was used as feed to the MPBR, whereas effluent from the MPBR was continuously withdrawn into the effluent tank. The feed pumps in both MBR and MPBR were controlled using water level sensors. Weighing balances (Sartorius Cubis MSU14202S, Germany) were used to monitor weight changes in the collection bottle, as well as, the effluent tank.

The MBR-MPBR system was operated in three stages. In Stage I (days 1–14), only the MBR was operated to stabilize sludge generation and removal performance. The HRT was set at 1 day. In Stage II (days 15–37), MPBR was operated downstream to the MBR and the operation was conducted until day 37. Since the MBR was operated at an HRT of 1 day, it resulted in 3 days HRT for the MPBR. In Stage III (days 38–70), light intensity in MPBR was doubled by connecting additional fluorescent tubes. Samples were collected from MBR and MPBR regularly to measure cell growth, COD, PO_4^{3-}-P, NO_3^--N, and NH_4^+-N. During stage I, MBR was operated at an SRT of 20 days, but the SRT was decreased to 10 days in Stage II and III. On the other hand, the MPBR was operated under complete biomass retention (SRT > 200 days), and only a small amount of biomass was removed every day for sampling purposes.