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Increasing tetracycline concentrations on the performance and communities of mixed microalgae-bacteria photo-bioreactors

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ABSTRACT

This study investigated the impact of varying concentrations of tetracycline on the performance of mixed microalgae-bacteria photo-bioreactors. Photo-bioreactors were assessed for their ability to remove carbon dioxide (CO_2) from the biogas of anaerobic membrane bioreactor (anMBR), and nutrients from the anaerobic effluent. The varying concentrations of tetracycline had no impact on the removal of CO_2 from biogas. 29% v/v of CO_2 was completely removed to generate > 20% v/v of oxygen (O_2) in all reactors. Removal of nutrients and biomass was not affected at low concentrations of tetracycline (≤ 150 μg/L), but 20 mg/L of tetracycline lowered the biomass generation and removal efficiencies of phosphate. Conversely, high chlorophyll *a* and b content was observed at 20 mg/L of tetracycline. High tetracycline level had no impact on the diversity of 18S rRNA gene-based microalgal communities but adversely affected the 16S rRNA gene-based microbial communities. Specifically, both Proteobacteria and Bacteroidetes phyla decreased in relative abundance but not phylum Chloroplast. Additionally, both nitrogen-fixing (e.g. *Flavobacterium*, unclassified Burkholderiales and unclassified Rhizobiaceae) and denitrifying groups (e.g. *Hydrogenophaga* spp.) were significantly reduced in relative abundance at high tetracycline concentration. Phosphate-accumulating microorganisms, *Acinetobacter* spp. and *Pseudomonas* spp. were similarly reduced upon exposure to high tetracycline concentration. Unclassified Comamonadaceae, however, increased in relative abundance, which correlated with an increase in the abundance of tetracycline resistance genes associated with efflux pump mechanism. Overall, the findings demonstrate that antibiotic concentrations in municipal wastewaters will not significantly affect the removal of nutrients by the mixed microalgae-bacteria photo-bioreactors. However, utilizing such photo-bioreactors as a polishing step for anMBRs that treat wastewaters with high tetracycline concentration may not be effective as evidenced from the lower nutrient removal and occurrence of antibiotic resistance genes.

1. Introduction

The use of anaerobic fermentation for wastewater treatment eliminates the energy costs associated with aerating wastewater while also introducing the potential for recovery of methane generated by anaerobic digestion [1]. By further coupling a membrane separation process to anaerobic digestion, the whole process becomes what is referred to as the anaerobic membrane bioreactor (anMBR). Although anMBR can achieve a good removal of total organic carbon and hence demonstrate great potential for improving efficiency and sustainability of wastewater treatment, they are unable to remove ammonia and phosphate to a level that can meet the discharge or reuse regulations [2–4]. Post-treatment of anMBR effluents is therefore necessary. Additionally, biogas generated from anMBR processes contains approximately 20–40% of carbon dioxide [5], which is a fossil gas that diminishes the calorific content of biogas.

To circumvent the shortcomings associated with anMBR, it had been proposed that the microalgae photo-bioreactors be applied to upgrade biogas by increasing the proportion of methane (CH_4) percentage and to remove nutrients from the anaerobic effluents [6,7]. At the same time, microalgae are considered as one of the most promising feedstock for biofuels and chemicals [8]. However, widespread usage of antibiotics in both domestic and agricultural environments has resulted in higher antibiotic loading rates in wastewater treatment plants (WWTPs) across the world. To exemplify, low concentrations of approximately 1 μg/L of tetracycline is typically detected in domestic wastewaters [9] while hospital wastewater contained over 100 μg/L of tetracycline [10]. A high concentration of tetracycline of up to 23 mg tetracycline

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detected per kg of animal manure was also detected in the livestock wastewater [11].

An earlier study has assessed that anaerobic digestion can robustly treat wastewater with varying concentrations of antibiotics while deriving value-added products and minimizing the dissemination of associated antibiotic-resistance genes [12]. Another study also independently determined that anaerobic microbial communities express biodegradation genes to facilitate the degradation of antibiotics [13]. In particular, by further coupling with membrane separation process, anMBR can remove up to 80% of antibiotics from the influent [14]. However, remnant antibiotics present in the anaerobic effluent can potentially affect downstream technologies such as microalgae photo-bioreactors that are used for polishing the nutrient in the effluent.

Although microalgae photo-bioreactors has been applied to reclaim wastewater contaminated with pharmaceutical compounds [15–19], it is unknown to what extent antibiotics can affect the overall performance of microalgae photo-bioreactors. Earlier studies showed that antibiotics, e.g. chloramphenicol, florfenicol, and thiamphenicol can reduce total chlorophyll content of *Chlorella pyrenoidosa, Isochrysis galbana*, and *Tetraselmis chui* significantly [20], while increase in the concentration of enrofloxcin caused an increase in the total chlorophyll of *Chlamydomonas mexicana* and *Micractinium resseri* [21]. However, most of these studies emphasize on pure microalgae cultures, with only few studies available to report on how stressors like antibiotics would influence the removal of CO_2 and nutrients from anMBR by a mixed microalgae-bacteria photo-bioreactor. Pure cultures of microalgae are difficult to maintain in most wastewater treatment processes as microalgae draws more benefits from their interactions with bacteria. For example, Kim et al. found that the *Rhizobium*, a plant growth promoting bacterium, can enhance the growth of algae through mutualistic interaction found [22]. In the mutualistic relationship of *Emiliania huxleyi* with *Phaeobacter gallaeciensi,* it has been found that bacterium produces antibiotic compounds to protect the host from other bacterial pathogens [23].

Thus, it is much more essential to explore the impact of antibiotics, such as tetracycline on the performance of mixed microalgae-bacteria photo-bioreactor than on pure microalgae photo-bioreactors. An earlier study reported that treating the mixed microalgae photo-bioreactor with high concentrations of tetracycline (up to 30 mg/L) caused reduction in the removal of nutrients and adversely affected the overall dynamic of microalgae community [24]. However, the study only utilized denaturing gradient gel electrophoresis (DGGE) to study the microalgae community and not the bacterial populations. DGGE is a fingerprinting technique that allows the identification of predominant populations but does not allow quantitative measurements of each individual microalgae and bacterial population. Neither does the technique allow one to infer which microalgae or bacterial populations would be perturbed in their nutrient removal process in the presence of antibiotics.

In this study, it is hypothesized that mixed microalgae-bacteria photo-bioreactors are able to remove nutrient and CO_2 from anaerobic effluent that contained varying concentrations of tetracycline. To evaluate this hypothesis, three photo-bioreactors, each with different concentrations of tetracycline, i.e., 1 μg/L, 150 μg/L and 20 mg/L, representative of those found in municipal, hospital and livestock farm wastewaters, respectively [9–11], were carried out along a control reactor (i.e., 0 μg/L tetracycline). The photo-bioreactors performance was evaluated by measuring ammonia, phosphate, CO_2 and O_2 percentage within biogas, as well as chlorophyll concentrations. Both 16S and 18S rRNA gene-based amplicon sequencing were applied to analyze both bacterial and microalgal communities, respectively. This is to provide quantitative values of relative abundance of prokaryotic and eukaryotic populations, and to facilitate the evaluation of which populations play a role in overall reactor performance. Additionally, the occurrence of tetracycline resistance genes was also investigated to determine if mixed microalgae-bacteria photo-bioreactors would contribute to the dissemination of antibiotic resistance genes through the final treated effluent.

2. Materials and methods

2.1. Experimental set-up and sampling

Four media bottles, each containing 450 mL of effluent obtained from a lab-scale anaerobic membrane bioreactor (anMBR) was seeded with mixed microalgae cultures. The seed was originally sampled from a laboratory-scale wastewater bioreactor, and contained predominantly of *Chlorella* spp. but mixed with bacteria as well. Media bottles were individually added with 0 μg/L, 1 μg/L, 150 μg/L and 20 mg/L of tetracycline hydrochloride (TC-HCl), all diluted from 1 g/L stock solution in ultrapure water (Merck Millipore, Billerica, MA, USA). All reactors were vacuumed and flushed with biogas twice prior to carrying out the experiment. Biogas was supplied via gas bag containing 4 L biogas collected from the anMBR to provide carbon dioxide to the microalgae bioreactors at 0.1 mL/min. The biogas was directly introduced to the aqueous media by a tubing connected to the nozzle at the bottom of each reactor. The compositions of biogas in the supply of gas bag were 29% of CO_2, 0.5% of O_2, 45% of CH_4 and 27% of N_2. Effluent from an attached growth anMBR operated as described previously [25] was used as the media for the microalgae reactors. Gas from the headspace was individually recycled within the microalgae reactors at a flow rate of 25 mL/min. Three LED lamps were placed approximately 20 cm from the top of the photo-bioreactors to supply 2428 ± 37 μW/cm^2 of light intensity to the surface of the microalgae cultures in continuous daylight mode. Light intensity was measured over the spectral range of 250 to 1050 nm with ILT950 portable spectroradiometer at a radiometric accuracy of ca. 5 to 10% (International Light Technologies, Peabody, MA, USA). The whole experiment was repeated in triplicate, referred to as run 1, run 2 and run 3 in this study. The initial OD_{680} and pH was 0.007 ± 0.003 and 8.0–8.2, respectively for all reactors among all runs. The initial COD, NH_3-N and PO_4^{3-}-P in the effluent media was < 50 mg/L, 70–130 mg/L and 17 ± 3 mg/L, respectively. Each reactor was sampled for 20 mL of its content daily, and replaced with 20 mL of fresh media with corresponding amount of tetracycline hydrochloride supplemented to maintain the same tested concentration of tetracycline.

2.2. Reactors performance

Ammonia (NH_3-N) and phosphate (PO_4^{3-}-P) were detected by TNT-AmVer (Salicylate)-high range and LCK 348 (Hach, Loveland, Colorado, USA), respectively. Nitrite and nitrate were also determined by Hach kits TNT 839 and TNT 835, respectively, but were found to be present in negligible proportions compared to NH_3-N. Biomass concentration was estimated by weighing the lyophilized mass of the sample divided by the initial sample volume. The biogas components were detected via gas chromatography (SRI 310C, SRI instrument, USA). Chlorophyll pigment was detected as described by [26] with some modifications. Briefly, 2 mL of the microalgae suspension was placed in 2 mL microcentrifuge tube and was centrifuged at 5600*g* for 3 min. The supernatant was discarded and 2 mL of DMSO was added to lyse chlorophyll pigment from biomass pellet. The suspensions were homogenized, incubated in a water bath at 60 °C for 12 min, and then centrifuged at 2000*g* for 5 min. Supernatant was aliquot for OD measurement at 649 and 665 nm wavelengths, with DMSO as the blank control. All measurements were made in duplicate. The pigment contents were calculated as below [26]:

$$\text{Chlorophyll a (Chl a) in mg/L} = 12.47\,(OD_{665}) - 3.62\,(OD_{649})$$

$$\text{Chlorophyll b (Chl b) in mg/L} = 25.06\,(OD_{649}) - 6.5\,(OD_{665})$$

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