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Research paper

Use of freshwater macroalgae *Spirogyra* sp. for the treatment of municipal wastewaters and biomass production for biofuel applications

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Freshwater macroalgae has competitive advantages compared to microalgae and marine macroalgae, such as lower separation and drying cost requirements and an abundance of available freshwater media. Municipal wastewater containing large quantities of nutrients (particularly nitrogen and phosphorus) is a valuable and underutilized resource. In this study, the cultivation of the naturally isolated filamentous freshwater macroalgae *Spirogyra* sp. was investigated in three different types of municipal wastewater including primary (PW), secondary (SW) and centrate (CW) wastewaters. Two different types of reactors including closed column photobioreactors and open rectangular aquarium reactors were operated under no and low air flow rates of less than (18 ± 2) $cm^{-3} \cdot min^{-1}$, respectively. The SW, PW diluted with water to a 20 % volume fraction and CW diluted with water to a 2 % volume fraction appeared to promote ash-free biomass productivities of (2.17-6.68) $g \cdot m^{-2} \cdot d^{-1}$ and specific growth rates of (16.4–29.7) $\% \cdot d^{-1}$. Nitrogen and phosphorus removal efficiencies ranged from (50.6–90.6) % and (60.4–99.1) %, respectively. Based on ultimate analysis, the biomass produced a higher heating value of (12.4–17.1) $MJ \cdot kg^{-1}$, and also showed relatively consistent protein ((16.7–19.5) % of the dry mass fraction), carbohydrate ((41.5–55.0) %) and lipid ((2.8–10.0) %) contents. These results indicate the feasibility of using *Spirogyra* sp. to recover nutrients from multiple municipal wastewater sources with the simultaneous production of biomass that contains value-added biochemical components for bioenergy and biofuel applications.

1. Introduction

With growing global socio-economic expansion, an urgent need exists for the development of efficient and sustainable methods to address both wastewater pollution from municipal and industrial activities, as well as greenhouse gas emissions [1]. Compared to physio-chemical processes, biological processes such as activated sludge treatment are widely used for municipal wastewater treatment [2]. Most conventional biological wastewater treatments consist of three stages: primary treatment to remove solids, secondary treatment for the microbial degradation of organic substances and nutrients, and tertiary or facultative treatment as a final polishing stage for nutrient removal and/or disinfection prior to the release of effluent into the environment. During this process, carbon and nitrogen present in wastewater are converted into carbon dioxide (CO_2) and nitrogen gas, and phosphate is removed as a sludge, which requires additional disposal. Recently, new sustainable treatment options have emerged targeting the recovery and beneficial use of the nutrients and carbon present in wastewater as an alternative to conventional high-energy aerated biological treatment methods [3]. One such method is the cultivation of algae in wastewater, where algal-based treatment technologies can allow for efficient nutrient and energy recoveries while absorbing CO_2 and producing an oxygenated and treated effluent. Furthermore, algal-based wastewater treatments eliminate the need for multiple anaerobic/anoxic/aerobic tanks and chemical additions common in more conventional operations. Algae exhibit high areal productivities, rapid growth rates and their cultivation in wastewater does not compete with land-based food crops [4]. The resulting high-value algal biomass can be converted to a broad range of third generation biofuels such as biodiesel, bioethanol and biomethane [5].

To date, much of the research into algal bioremediation of wastewater and the production of biofuels has focused on

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microalgae [6–8]. Comparatively, macroalgae also exhibit high areal productivities and their ability to form dense floating mats on the water surface may offer significant reductions in cost and energy during the biomass harvesting and dewatering steps compared to suspended microalgae [9]. In addition, macroalgal biomass has shown potential for the production of a variety of liquid and solid biofuels, using similar conversion pathways as with microalgae [10–12]. Current macroalgae-to-liquid fuel conversion pathways include the biochemical conversion of carbohydrates to bioethanol [13] and biobutanol [14], lipid extraction and esterification of fatty acids for biodiesel production [15], and thermochemical conversions such as hydrothermal liquefaction (HTL) to produce a liquid biocrude [12]. Comparatively, HTL provides an attractive advantage as it utilizes the whole organic fraction of the biomass, converting proteins and carbohydrates, in part, and the entire lipid fraction to oil [16–18]. HTL can also process wet biomass, which eliminates the need for dewatering and drying, thereby, reducing costs [16,19].

To date, the majority of current research conducted on the production of alternative third generation biofuels from macroalgae has focused on marine seaweeds, with a much smaller focus on freshwater filamentous macroalgae [20,21]. Although marine macroalgae could represent an important bio-feedstock resource, their need for a saline growing environment limits their potential use in the bioremediation of most municipal and industrial wastewater streams, which are primarily freshwater based. Therefore, freshwater macroalgae may have strong potentials for bioremediation applications and biofuel production as they are able to utilize these freshwater waste streams for growth.

Freshwater macroalgae are ubiquitous around the world in natural waterways as well as in engineered wastewater treatment systems. Species such as *Oedogonium*, *Cladophora* and *Spirogyra* have been isolated from municipal and aquaculture wastewaters [10,20] and from naturally occurring water bodies, irrigation channels and wetland areas [3,22–24]. The presence of freshwater macroalgae in wastewater streams, and their competitive dominance over other species, demonstrates their natural capacity to thrive in wastewater and therefore their potential as a bioremediation strategy [25]. Several studies have demonstrated the nutrient removal capabilities of freshwater macroalgae, however, in wastewaters with relatively low nutrient concentrations. For example, Kangas et al. [26] recorded 0.1 $g \cdot m^{-2} \cdot d^{-1}$ for nitrogen and 0.011 $g \cdot m^{-2} \cdot d^{-1}$ for phosphate removal rates using algal turf scrubber (ATS) raceways and agricultural drainage wastewater. Similarly, Cole et al. [27] reported maximum nutrient removal rates of 0.564 $g \cdot m^{-2} \cdot d^{-1}$ for nitrogen and 0.236 $g \cdot m^{-2} \cdot d^{-1}$ for phosphate from aquaculture wastewater using *Oedogonium* sp. Freshwater macroalgal biomass has also shown strong potential as a biofuel feedstock, for both direct combustion and its conversion into a variety of liquid fuels [10,20,21]. Based on its nutrient attenuation capacity and biofuels potential, the application of freshwater macroalgae should be further expanded into the bioremediation of municipal wastewaters with high nutrient concentrations for the production of algal biofuel feedstocks.

The goal of this study was to demonstrate the potential of using a naturally isolated freshwater filamentous macroalgae for the bioremediation of high-level municipal wastewaters and biomass production. Both short-term batch, and long-term semi-continuous growth experiments were conducted using cylindrical photobioreactors (PBRs) and flat plate aquariums, respectively, with a variety of primary, secondary and centrate wastewater media. Nutrient removal efficiencies, treatment efficiencies and removal rates were used as indicators to assess the potential of the macroalgae for wastewater treatment. Biomass productivities, specific growth rates and biochemical composition of the final biomass were employed as indicators for biofuel feedstock potential of the macroalgae.

2. Materials and method

2.1. Macroalgae identification and stock cultures

Freshwater filamentous macroalgae was obtained using a fiber net from the first waste stabilization pond at the Amherstview Water Pollution Control Plant, located in South-Eastern Ontario Canada [28]. The collected macroalgae was washed with distilled water, then the green filamentous species were manually separated and transferred into three flat-plate aquariums ($35\ cm \times 40\ cm \times 50\ cm$) filled with three types of real wastewaters including primary wastewater (PW), secondary wastewater (SW) and a volume fraction of 2% dilute centrate (2-CW), collected from Ravensview WWTP located in Kingston, Ontario, Canada [29]. A relatively pure culture of filamentous macroalgae was then obtained through repeated growth and separations cycles. *Spirogyra* sp. was identified according to the following morphological characteristics: vegetative cells (85–230) μm long and (40–60) μm wide; 1–5 chloroplasts spirally arranged; ellipsoidal zygospores [30,31]. The aquariums were equipped with an Orphek Atlantik Aquarium LED lighting platform, which provided appropriate light spectra for macroalgal growth ranging from (380–440) nm and (650–670) nm with a light intensity of (50–85) $\mu mol \cdot m^{-2} \cdot s^{-1}$. Air pumps (Tetra Whisper, Canada) equipped with membrane filters provided aeration and mixing conditions and the wastewater media were changed every 2 weeks.

2.2. Experimental design

2.2.1. Effect of mixing rate

During the cultivation of stock cultures, a relationship between *Spirogyra* sp. growth and different mixing rates was observed. It was hypothesized that the growth of *Spirogyra* sp. would likely be sensitive to mixing rates and that degradation or cell death would occur with strong mixing. Hence the effects of three different mixing conditions (Air flow rates: 0, (18 ± 2) and (206 ± 14) $cm^{-3} \cdot min^{-1}$ at 296.15 K and 101.3 kPa) on the biomass productivity and nutrient removal efficiency of *Spirogyra* sp. were studied by adjusting the flow rates from air pumps which provided aeration for the PBRs through in-line filters and air diffusers. Experiments were conducted using 2.0 L column-type UTEX photobioreactors (PBRs) in an algal growth chamber (Caron model 6321–1, U.S.). The PBR platforms have been discussed in detail elsewhere [6]. For each treatment, 3 replicate PBRs were employed with 0.8 L of autoclave sterilized (20 min, 120 °C, 103.4 kPa) secondary wastewater (SW) and 0.5 g (fresh weight, FW) of macroalgae (equivalent to 0.625 $g \cdot L^{-1}$). A controlled cultivation environment was maintained in the growth chamber at 23 °C and 2% CO_2 concentration, and with white fluorescent lighting on a continuous lighting regime (mean light intensity of (120 ± 15) $\mu mol \cdot m^{-2} \cdot s^{-1}$). Within the closed growth chamber, CO_2 was fed into the ambient internal environment and each PBR in the chamber was aerated with an internally placed air pump.

Biomass and wastewater samples were collected every 2–3 days during the 13-day experimental period. Biomass was collected and then filtered using vacuum filtration with a 0.45 μm pore size membrane filter (Whatman® membrane filters nylon) for 3 minutes to remove excess surface water. Liquid samples (15 cm^{-3}) were filtered using a 0.22 μm pore size glass fiber filter (Fisher Whatman puradisc-25 mm) to remove microalgal cells and other particles prior to analysis.

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