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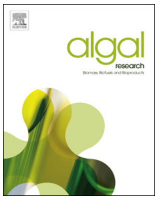

Potential of five different isolated colonial algal species for wastewater treatment and biomass energy production

Abbas Mehrabadi [a,*], Mohammed M. Farid [a], Rupert Craggs [b]

[a] Chemical and Materials Engineering Department, University of Auckland, New Zealand
[b] National Institute of Water and Atmospheric Research Ltd. (NIWA), PO Box 11-115, Hamilton 3200, New Zealand

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ABSTRACT

Wastewater treatment high rate algal pond (WWT HRAP) performance and the potential for low-cost biofuel production from its biomass are affected by the composition of the algal community. While several unicellular algal species have been evaluated extensively for their wastewater treatment and biofuel production potential, there has been little focus on the colonial species that typically predominate in high rate algal ponds and which have the benefit of being easily harvested by cost-effective, simple gravity settling. This study investigates the wastewater treatment performance of five wastewater colonial algal species that are common in high rate algal ponds: *Mucidosphaerium pulchellum*, *Micractinium pusillum*, *Coleastrum* sp., *Desmodesmus* sp. and *Pediastrum boryanum* and their potential value for biofuel production in terms of their biochemical composition and biomass energy yield. The algae were isolated from pilot-scale WWT HRAPs and used in batch monoculture experiments with pre-frozen and pre-filtered primary settled sewage that were conducted over 10 days under simulated New Zealand summer and winter conditions. Under summer conditions, all species showed similar efficient nutrient removal (>95% of NH_4^+-N and >85% of PO_4^{3-}-P within 4 days) whereas, under winter conditions, only the *Mucidosphaerium pulchellum* and *Micractinium pusillum* cultures showed efficient nutrient removal by the end of the experiment. Algal biomass yield, lipid and energy content varied with species and were higher in summer than winter (respectively 991–1700 mg/L, 14.4–48.2 wt% and 19.1–25 kJ/g in summer, and 158.7–584 mg/l, 15.1–31.4 wt% and 17.7–21.1 kJ/g in winter). The *Mucidosphaerium pulchellum* and *Micractinium pusillum* cultures had the highest biomass yield, lipid and energy content under both summer and winter conditions. However the settleability of *Micractinium pusillum* is much better than *Mucidosphaerium pulchellum* suggesting that of the colonial algal species tested, *Micractinium pusillum* has the greatest potential for both wastewater treatment and low-cost energy production in WWT HRAP.

1. Introduction

The techno-economic feasibility of biofuel production from algal biomass has been questioned for several years and it has been highlighted that even the use of conventional raceway ponds for biofuel production alone is not currently financially viable [1–6]. One opportunity to lower algal-based biofuel production costs is where the algal biomass is produced as an essentially free by-product of tertiary-level wastewater treatment in High Rate Algal Ponds (HRAP) [7,8]. Wastewater treatment HRAPs are a component of enhanced wastewater treatment pond systems which have received much attention worldwide as an upgrade option for traditional wastewater treatment ponds, due to their higher nutrient removal rates and the ability to recover resources in the form of algal biomass [9,10].

To effectively combine low-cost tertiary-level wastewater treatment and low-cost algal production in WWT HRAPs for biofuel, the WWT HRAP must be mainly dominated by algal species that have both high treatment and energy production potential. The main characteristics of beneficial algal species for wastewater treatment are: 1) high nutrient removal capacity at typical wastewater nutrient loads, 2) ability to grow under seasonally variable environmental conditions, and 3) easy harvest by simple gravity settling [11,12]. The important characteristics of algal species for low-cost biomass energy yield in WWT HRAP without impacting their wastewater treatment function include: 1) high year-round productivity, 2) high energy content (resulting from beneficial biomass chemical composition), and 3) high settleability to achieve the highest settleable algal biomass yields [8].

Over recent years, many studies have been conducted to investigate beneficial algal species for biofuel production using wastewater (municipal/industrial/animal manure) as a nutrient source [13–18]. The

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[*] Corresponding author.
E-mail addresses: ameh820@aucklanduni.ac.nz, mehrabadi_abbas@yahoo.com (A. Mehrabadi), m.farid@auckland.ac.nz (M.M. Farid), Rupert.Craggs@niwa.co.nz (R. Craggs).

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majority of these studies have found high biofuel production and wastewater treatment potential of the tested algae. However, only a limited number of algal species including *Chlorella* sp., *Scenedesmus* sp., *Ankistrodesmus* sp., and *Monoraphidium* sp. have been assessed [13–18] which are typically motile or poorly-settleable unicellular algae that require chemical flocculation / energy consumption for efficient removal. Moreover, most studies have been carried out under simulated moderate conditions without taking into account natural seasonal variation in performance. In addition nutrient and operational stress conditions (such as cultivation under N starvation or high light intensity) have been suggested to improve algal biomass quality for biofuel production while they may negatively affect wastewater treatment performance [13,14,17,18,19,20].

While poorly-settleable unicellular species have been cultivated on wastewater under controlled conditions for biofuel production, research on algal-based wastewater treatment ponds has shown that WWT HRAPs are often populated by colonial species which have similar wastewater treatment performance to unicellular species but can be cost-effectively harvested using gravity settling [11,21]. Park et al. [22] conducted an experiment in outdoor pilot-scale WWT HRAPs populated by >80% *Pediastrum boryanum* (a readily settleable colonial species) and showed that >80% NH_4^+-N and 50–75% PO_4^{3-}-P were removed year-round. They found that not only high nutrient removal was achievable by *Pediastrum boryanum* dominance but also high harvest efficiency was achieved, which would improve the economic viability of WWT HRAP for combined wastewater treatment and low-cost energy production. Our previous study has also shown >70% year-round nutrient removal in outdoor pilot-scale WWT HRAPs dominated by colonial species such as *Micractinium* sp., *Mucidosphaerium pulchellum*, *Coleastrum* sp., *Desmodesmus* sp., and *Pediastrum* sp. [23]. In a comparative lab-scale experiment, Sutherland et al. [9] found that under continuous mixing the nutrient removal capacity of colonial species (*Mucidosphaerium pulchellum* and *Pediastrum boryanum*) and poorly settleable unicellular species (*Chlorella* sp.) were similar. However, compared with the two other species, *Pediastrum boryanum* was highly settleable so that >55% of culture biomass settled at 10 min. While a number of colonial microalgal species have been assessed for wastewater treatment potential [9,11,22,24,25] there has been little focus on algal biomass quality (i.e. biochemical composition and biomass energy content) for biofuel production. It has been found that colonial species can be maintained in WWT HRAP by recycling a small (<20%) portion of the harvested biomass back to the pond [11,22,24]. This could provide an opportunity to maintain the most beneficial species for both wastewater treatment and production of algal biomass for biofuel in WWT HRAP. Therefore, the aim of this study was to investigate the performance of typical WWT HRAP colonial algal species for efficient wastewater treatment (in terms of nutrient removal) as well as biomass energy yield (in terms of growth rate, biochemical composition and energy content) for further use as biofuel feedstock.

2. Materials and methods

Laboratory-scale batch experiments were conducted to determine the wastewater treatment and energy production potential of five common colonial algal species isolated from pilot-scale WWT HRAPs at the Ruakura Research Centre, Hamilton, New Zealand (37°47′S, 175°19′E). Performance was compared under New Zealand summer and winter simulated conditions (as used previously by Park et al. [25]) using pre-frozen pre-filtered primary settled sewage.

2.1. Microalgal species isolation

Colonial algal species which were predominantly present in the pilot-scale WWT HRAP at the Ruakura Research Centre (see Mehrabadi et al. [23] for more details) were isolated, identified and grown in pure culture under both New Zealand summer and winter simulated conditions.

The isolation procedure involved a combination of serial dilution and selection of a single healthy colony of each species using a microscope (Leica DM 2500). The single colonies were placed into autoclaved flasks with 100 mL sterile growth medium (Bold 3N growth medium modified by replacing $NaNO_3$ with $(NH_4)_2SO_4$).

Five species were isolated including *Mucidosphaerium pulchellum*, *Micractinium pusillum*, *Coleastrum* sp., *Desmodesmus* sp., *Pediastrum boryanum* (Fig. 1). *Mucidosphaerium pulchellum* (HC Wood) C. Bock, Proschold & Krienitz is a colonial species with 4–64 spherical cells connected by mucilaginous stalks and forms colonies with a diameter of up to 80 μm [26]. *Micractinium pusillum* has small spherical cells (diameter: 3–7 μm) which develop cell wall spines (length: 20–35 μm) and grow as colonies (diameter: up to 150 μm) in the presence of zooplankton grazers (e.g. *Brachionus calyciflorus*) [27]. *Coelastrum sphaericum* Nägeli grow as hollow spherical colonies of closely-packed cells (8–128 cells per colony) with a diameter of up to 100 μm [28]. *Desmodesmus abundans* (Kirchner) E. Hegewald has flat ellipsoidal cells (length: 10–15 μm, width: 5–8 μm) that grow as either unicells or 4-celled colonies and develop cell wall spines in presence of zooplankton grazers (e.g. *Daphnia*) [29]. *Pediastrum boryanum* is a star-shaped flat colonial species typically with 8 to 32 and sometimes 64 cells. The number of cells is fixed from when the juvenile colony emerges from the parent cell and the colony size increases as the cells grow (colony diameter: 4–80 μm). A silica skeleton with horn-like projections on the outer cells makes *Pediastrum* colonies denser than the other colonial species [9,25].

2.2. Culture conditions

The isolated species were grown under both New Zealand summer and winter simulated conditions in growth chambers as used by Park et al. [25] (6150CP-6400CP, Contherm Scientific Ltd.) to grow sufficient biomass for use as inoculum in the experiment. Day/night temperature, light/dark cycle and light intensity for simulated summer conditions were: 25/19 °C, 14/10 h, and 250 μmol photon/m^2/s, and for simulated winter conditions were: 13/9 °C, 10/14 h, and 120 μmol photon/m^2/s.

The purity of cultures was regularly monitored and just prior to the experiment, the cultures were subsampled and grown up over 5 days (summer) and 10 days (winter) to provide and exponential phase inoculum.

2.3. Wastewater collection, storage and characterization

A 100 L volume of primary settled sewage was collected from the Ruakura sewer for use as the growth medium in the experiment. To ensure consistency of the growth medium the wastewater was initially placed in a freezer at −18 °C for 72 h and then stored in a cold room maintained at 4 °C. Physicochemical parameters of the fresh wastewater including COD, BOD_5, total nitrogen, total phosphorous and total Kjeldahl nitrogen (TKN) were measured using standard methods for wastewater [30].

2.4. Experimental set-up

A 100 mL volume of pure algal culture (that had been diluted with deionized water to have a biomass concentration of 12 mg/L) was pipetted into an autoclaved Erlenmeyer flask with 900 mL of pre-frozen filtered primary settled sewage. To avoid interference between wastewater solids and the algal culture biomass, pre-frozen wastewater was filtered through a 0.7 μm glass fibre filter (LabServ, LBS0GFF.047) before use. Triplicate flasks for each algal species were placed in each of the summer and winter simulated condition growth chambers (6150CP-6400CP, Contherm Scientific Ltd.). The algal cultures were bubbled with pre-filtered 1% CO_2-air mixture (0.2 L/min) to avoid