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Evaluation of growth, nutrient utilization and production of bioproducts by a wastewater-isolated microalga

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HIGHLIGHTS

- Wastewater isolate, *Kirchneriella* sp., was grown in laboratory systems up to 60 L.
- Successful growth and rapid nutrient uptake in laboratory media and wastewater.
- N-limitation negatively affected FAME yield, positively affected biodiesel quality.
- Promising bioproduct characteristics: FAME, carbohydrate-rich biomass, and pigments.

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ABSTRACT

Treatment of wastewater while producing microalgal biomass is receiving ever-increasing attention, particularly in the biofuels arena. In this study, a wastewater chlorophyte isolate, *Kirchneriella* sp., was tested for its ability to be mass cultivated, utilize nutrients from defined media and wastewater, and produce bioproducts of commercial interest. Growth studies were carried out in various systems at scales up to 60 L, with *Kirchneriella* sp. showing an excellent amenability to being cultured. Biomass concentrations of greater than 1 g L^{-1} were consistently achieved, nitrogen and phosphorus uptake was rapid, and stable medium-term cultures were maintained. Nitrogen limitation affected biomass yield, fatty acid methyl ester (FAME) yield, and cetane index. In contrast, a low phosphorus condition had no effect. *Kirchneriella* sp. showed an ability to produce several products of commercial value, including carbohydrate-rich biomass, FAME/biodiesel and the pigments β,β-carotene and lutein.

1. Introduction

With a likely increase in the use of fossil fuels ultimately having an impact on global warming (Hill et al., 2006), renewable sources of energy and fuel (e.g. U.S. DOE, 2010) are being increasingly sought. The treatment of wastewater coupled with the production of bioenergy and biofuels via the cultivation of algae is one alternative approach that is receiving considerable attention at present (for example review see Pittman et al., 2011). Several aspects to this approach have both community and commercial appeal, among these are: (1) wastewater is treated to a higher degree (Martínez et al., 2000); (2) arable land is less impacted upon than for other oil crops, e.g. soybean; (3) water and many nutrient inputs may be freely available; (4) operations are generally well co-located with other industry and associated infrastructure; (5) nutrients may be recycled (Yang et al., 2011); (6) biomass or refined protein may be used as agricultural and other energy-generating feedstock (Scott et al., 2010); (7) other valuable bioproducts may be refined, e.g. antioxidants (Foley et al. 2011). Despite these favorable aspects, there remain significant biological, economical and logistical constraints to be overcome if this approach is to be fully realized.

Selection and analysis of algal strains and/or microbial consortia for suitable production of biomass and biofuel-specific feedstock is at the fore of the current biological constraints. Suitable microalgae must be amenable to large-scale culturing (for review of culturing systems see Tredici et al., 2009) and harvesting (Pittman et al., 2011), grow in wastewater containing variable organic and inorganic constituents, have rapid uptake of significant amounts of key nutrients, especially nitrogen (N) and phosphorus (P) (Li et al., 2010), have high productivity of biomass and other bioproduct raw materials (Scott et al., 2010), and can be processed

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economically. In addition, there are also site-specific requirements of the alga to be taken into consideration, such as temperature tolerance, wastewater quality compatibility, and solar irradiance tolerance.

The co-production of materials, energy and chemical products, otherwise referred to as the biorefinery concept (Clark et al., 2009), is also an important consideration if microalgae are to be produced on a commercial scale (Foley et al., 2011). The various fractions into which algal biomass may be partitioned, which is well documented in the literature, include total lipids, carbohydrate, proteins and residual (ash). Examples of bioproducts derived from these fractions include: FAME/biodiesel and pigments from total lipid; biofuels and bioplastics from carbohydrates; and animal feed from protein (U.S. DOE, 2010; Foley et al., 2011). Among the various screening efforts targeting one or more of these bioproducts, Griffiths and Harrison (2009) point out that lipid productivity more so than content is a key characteristic for choosing algal biodiesel candidate species, whilst few examples can be found regarding assessment of algal biodiesel quality parameters such as cetane index value (e.g. Francisco et al., 2010).

Pittman et al. (2011) emphasize the significant benefit of initial laboratory based small-scale and pilot pond-scale experimental analyses for evaluation of wastewater-to-biofuel microalgae. Important parameters for such an evaluation include, but are not limited to, algal growth, biomass concentration and productivity, nutrient uptake and utilization, and production of bioproduct raw materials. In this study, a wastewater-isolated chlorophyte, *Kirchneriella* sp., was tested for its ability to be mass cultivated in systems up to 60 L in capacity, remove nutrients from defined media and wastewater, and produce valuable bioproducts such as biomass, fatty acid methyl esters (FAME), carbohydrates and pigments. Estimations were made of biomass and fatty acid methyl ester (FAME) productivities, with detailed effort focusing on an N- and P-limitation experiment.

2. Methods

2.1. Algal strain details

Chlorophyte strain CS-933 was isolated from a municipal wastewater treatment lagoon in south-eastern Melbourne, Australia, and deposited in the Australian National Algae Culture Collection (www.csiro.au/ANACC). It should be emphasized that, despite molecular (18S rDNA sequence analysis) and morphological (light microscopy) characterization of strain CS-933 having been undertaken, its taxonomy and that of the Family Selenastraceae remains unresolved (e.g. Krienitz et al., 2011). Therefore, until the family has been fully revised, and despite having morphological affinities with *Monoraphidium* Komárková-Legnerová (M. Fawley, pers. comm.), the strain's original isolation identification, *Kirchneriella* sp. Schmidle, has been retained and is herein referred to as *Kirchneriella* sp.

2.2. Culture systems and methods

A series of experiments were conducted using several culture systems (Table 1). Two base media types were used: MLA (Bolch and Blackburn, 1996) and filtered wastewater (FWW). For all culturing experiments, including unreplicated screening tests and replicated ($n = 3$) experiments "Expt", a 10% inoculum of late logarithmic phase pre-experimental cultures was provided, except for one treatment within Expt 2 which was given a 5% inoculum. General experimental conditions, unless specified otherwise, included incubation at 20 °C, 12:12 h light:dark, 90 ± 10 μmol.photons.$m^{-2}.s^{-1}$, and bubbling with 1% CO_2 in air. Nitrogen (N) was provided in the form of $NaNO_3$ and phosphorus (P) was provided in the form of K_2HPO_4. Initial screening of *Kirchneriella* sp. took place in a static 75 mL Erlenmeyer flask followed by a 500 mL photobioreactor (PBR; described in Rodolfi et al., 2009). Large PBR screening was carried out in a 60 L annular column PBR (described in Chini Zittelli et al., 2006). After an initial batch mode of culturing, stationary phase cultures were then maintained in a semi-batch regime by harvesting 10% of the PBR volume and replacing with fresh MLA medium every 2–3 days for a total of 47 days. A nutrient limitation experiment (Expt 1) was carried out in bubbled 2 L Erlenmeyer flasks containing a final culture volume of 1.5 L. Four initial N and P combinations were tested in triplicate. Apart from static 75 mL flask cultures, which were sampled on day 14 only, all other cultures were aseptically sampled at regular (1–7 day) intervals, with specific samples taken at all major phases of growth. From homogenous 100 mL sub-samples, aliquots were taken for biomass and growth estimation, analysis of nutrients and biochemical constituents, and measurement of photochemical efficiency (Fv/Fm).

Wastewater was collected from the same wastewater treatment plant (WWTP) from which *Kirchneriella* sp. was originally isolated. The WWTP services predominantly residential waste with a small contribution from light industry inputs (see supplementary Table SM1 for heavy metals analysis). In order to test *Kirchneriella* sp. growth on FWW (1.2 μm; Whatman GF/C), two culturing systems were tested. The first consisted of a rack of 5 L-capacity open rectangular trays (polyprolylene; 4 cm optical light path) which were situated under two light boxes and were intended to simulate open wastewater treatment ponds. Each light box contained eight fluorescent tubes on a 20:4 h L:D photoperiod. The three trays contained: (1) FWW only; (2) FWW with 5% *Kirchneriella* sp. inoculum;

Table 1
Details of culturing systems and initial nutrient profiles for all screening and experimental systems.

Experiment[a]	Culturing system	Culture volume (L)	OLP[b] (cm)	Medium	Intial N[c] (mg L^{-1})	Intial P[d] (mg L^{-1})	Intial N:P
Screening	Static flask	0.075	6.0	MLA	28	6.3	4.4
Screening	Bubbled tube PBR	0.5	4.5	MLA	56	6.3	8.9
Screening	Annular column PBR	55	4.5	MLA	56	6.3	8.9
Expt 1 (high N + P)	Bubbled flask	1.5	15.0	MLA	63	6.3	10
Expt 1 (intermediate N)	Bubbled flask	1.5	15.0	MLA	21	6.3	3.3
Expt 1 (low N)	Bubbled flask	1.5	15.0	MLA	7	6.3	1.1
Expt 1 (low P)	Bubbled flask	1.5	15.0	MLA	63	2.1	30
Expt 2 (5% inoculum)	Open tray	5	4.0	FWW	2.9	17.0	0.2
Expt 2 (10% inoculum)	Open tray	5	4.0	FWW	2.9	17.0	0.2
Expt 2 (10% inoculum)	Bubbled bottle	3	13.0	FWW	2.9	17.0	0.2

[a] Expt 1 and Expt 2 were replicated (n = 3). Screening experiments were unreplicated.
[b] OLP = optical light path.
[c] Values shown for Expt 2 are total Kjeldahl nitrogen (TKN).
[d] Values shown for Expt 2 are total phosphorus (TP).