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Biomass and oil production by *Chlorella vulgaris* and four other microalgae — Effects of salinity and other factors

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ABSTRACT

Five nominally freshwater microalgae (*Chlorella vulgaris*, *Choricystis minor*, *Neochloris* sp., *Pseudococcomyxa simplex*, *Scenedesmus* sp.) with a known ability to produce high-levels of lipids for possible use as fuel oils were evaluated for their ability to thrive and produce lipids in seawater and brackish water. Only *C. vulgaris* was found to thrive and produce lipids in full strength seawater. Seawater tolerant strains of *C. vulgaris* are unusual. Lipid productivity in nutrient sufficient seawater exceeded 37 mg L^{-1} d^{-1} and was nearly 2-fold greater than in freshwater. Although other microalgae such as *C. minor* had higher lipid productivities (e.g. 45 mg L^{-1} d^{-1}), they did not thrive in seawater. The lipid content of the *C. vulgaris* biomass was nearly 16% by dry weight. The calorific value of the seawater-grown *C. vulgaris* biomass exceeded 25 kJ g^{-1}. Compared to continuously illuminated cultures, a 12/12 h light-dark cycle reduced lipid productivity of *C. vulgaris* by ~30%, but did not affect the lipid content of the biomass. Biomass yield on phosphate was nearly 27% higher in seawater compared to in freshwater. While *C. vulgaris* has been extensively studied in freshwater, it has not been examined to any detail in full strength seawater. Studies in seawater are essential for any future large scale production of algal oils for biofuels: seawater is available cheaply and in large amounts whereas there is a global shortage of freshwater.

1. Introduction

Microalgae are attracting much attention as potential sources of fuel oil and essential medicinal oils. Microalgae can be grown photosynthetically using water, carbon dioxide, mineral salts and freely available sunlight. Microalgae are generally more efficient oil producers than commercial oil crops such as oil palm and soybean (Chisti, 2007). Growing microalgae does not require arable land. Concentrated carbon dioxide, the main carbon source for photoautotrophic culture of microalgae, is available from coal-fired electricity generating facilities, for example. Water is a necessary resource for algal culture. Depending on species, microalgae can be grown in freshwater, including domestic wastewater, brackish water and seawater. This work assesses the ability of five nominally freshwater microalgae to produce biomass and lipids in saline media, particularly in full-strength seawater. The microalgae used, had all been previously identified as potentially good producers of lipids but only in freshwater media. Biomass and lipid productivities in freshwater, brackish water and seawater are reported. One species (*Chlorella vulgaris*) that was found to be highly productive in seawater-based media was studied in more detail for biomass and oil production in full-strength seawater.

Although wastewater can be used to grow algae such as *Chlorella* (Chiu et al., 2015), its supply is insufficient for producing any significant amount of algal oil for fuel (Chisti, 2013). Furthermore, the average lipid productivity of microalgae in effluent of municipal wastewater treatment plants has been reported to be less than 10 mg L^{-1} d^{-1} (Chiu et al., 2015). When higher productivities are observed (Chiu et al., 2015), they are invariably a consequence of heterotrophic growth on dissolved organic carbon present in municipal wastewaters. Therefore, wastewater is not a substitute for seawater for large-scale production of algal fuel oils. Existing studies of microalgae using freshwater media are of little relevance to any future large-scale production of algal oils for fuels (Chisti, 2013). This is because freshwater is in short supply globally and its use for producing microalgae will undoubtedly compete with its existing uses in production of food and fodder.

Chlorella biomass is a human food supplement and has recognized benefits as a nutritional additive to animal feeds (Chiu et al., 2015; Griffiths et al., 2014; Kotrbáček et al., 2015). *Chlorella* grown in freshwater media is used as an aquaculture feed, but is too expensive for use as bulk animal feed (Kotrbáček et al., 2015). Extensive work on freshwater production of *C. vulgaris* biomass and oils has been reported (Griffiths et al., 2014; Liang et al., 2009; Liu and Chen,

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Nomenclature

A	Illuminated surface area of a culture bottle (m^2)
A_{nnn}	Spectrophotometric absorbance at nnn nm
C	Biomass concentrations at time t ($g\,L^{-1}$)
C_L	Total lipid concentration in the chloroform extract ($mg\,mL^{-1}$)
C_0	Initial concentration of biomass ($g\,L^{-1}$)
DCW	Dry cell weight ($g\,L^{-1}$)
d	Diameter of a culture bottle (m)
I	Incident irradiance ($\mu mol\,m^{-2}\,s^{-1}$)
k	First-order rate constant for biomass loss in the dark (h^{-1})
N_f	Final concentration of nitrate ($mg\,L^{-1}$)
N_i	Initial concentration of nitrate ($mg\,L^{-1}$)
PAR	Photosynthetically active radiation
P_f	Final concentration of phosphate ($mg\,L^{-1}$)
P_i	Initial concentration of phosphate ($mg\,L^{-1}$)
Q_L	Final lipid productivity ($g\,L^{-1}\,d^{-1}$)
Q_X	Final biomass productivity ($g\,L^{-1}\,d^{-1}$)
q_N	Specific nitrate consumption rate ($mg\,g^{-1}\,d^{-1}$)
q_P	Specific phosphate consumption rate ($mg\,g^{-1}\,d^{-1}$)
t	Duration of the batch culture (d)
t_f	Final time (d)
t_i	Initial time (d)
V	Working volume of a culture bottle (L)
V_c	Volume of the chloroform extract (mL)
X_e	Quantity of the dry biomass extracted (mg)
X_f	Final concentration of biomass ($g\,L^{-1}$)
X_i	Initial concentration of biomass ($g\,L^{-1}$)
$Y_{X/I}$	Biomass yield on incident light ($g\,\mu mol^{-1}$)
$Y_{L/I}$	Lipid yield on incident light ($g\,\mu mol^{-1}$)
$Y_{X/N}$	Yield coefficient of biomass on nitrate ($g\,mg^{-1}$)
$Y_{X/P}$	Yield coefficient of biomass on phosphate ($g\,mg^{-1}$)
y	Weight fraction of lipids in the biomass

Greek letters

μ	Specific growth rate (d^{-1})

2016; Liu and Hu, 2013; Lohman et al., 2015; Ördög et al., 2016; Potvin et al., 2011; Rajanren et al., 2015; Ras et al., 2011; Scragg et al., 2002; Singhasuwan et al., 2015; Sirisansaneeyakul et al., 2011; Wong et al., 2016), but studies in seawater-based media are rare. In nitrogen-limited freshwater batch cultures, the lipid productivity of *C. vulgaris* has ranged from 3 to 173 $mg\,L^{-1}\,d^{-1}$ with an average being ~47 $mg\,L^{-1}\,d^{-1}$ (Griffiths et al., 2014). In nutrient sufficient media, lipid productivity of many microalgae is generally lower than in nutrient-limited culture (Ördög et al., 2016; Scragg et al., 2002).

Apparently, many strains of *C. vulgaris* do not withstand full strength seawater (Alyabyev et al., 2007; Matos et al., 2015) and this explains a lack of studies in seawater for this microalga. Electrical conductivity of freshwater-based culture media is generally less than 1000 $\mu S\,cm^{-1}$. In contrast, a seawater based medium will have a minimum conductivity at 25 °C of around 54,000 $\mu S\,cm^{-1}$. In one study, increasing conductivity of the culture medium from around 1000 $\mu S\,cm^{-1}$ (i.e. a freshwater-based medium) to only around 2800 $\mu S\,cm^{-1}$ reduced biomass productivity of *C. vulgaris* from around 0.1 $g\,L^{-1}\,d^{-1}$ to less than 0.02 $g\,L^{-1}\,d^{-1}$ (Matos et al., 2015).

A desalination concentrate with a maximum total dissolved salts concentration of 2.3 $g\,L^{-1}$ mixed with a freshwater-based medium (Bold's basal medium) to the level of 25% by volume, has been used to culture *C. vulgaris* (Matos et al., 2015), but the dissolved salts concentration in the final medium was only about 0.6 $g\,L^{-1}$ higher than in a typical freshwater-based medium. In contrast, seawater contains around 40 $g\,L^{-1}$ of dissolved salts, or nearly 67-fold greater than the saline medium used by Matos et al. (2015).

An unidentified *Chlorella* sp. was reported to grow in full strength seawater (Choi and Lee, 2016). Apparently, the maximum lipid content of the biomass was about 20% by dry weight in a nutrient sufficient medium (Choi and Lee, 2016), but no growth or lipid productivity data were reported. According to Shen et al. (2015), growth of a *C. vulgaris* was not affected by a sea salt concentration of up to 50 $g\,L^{-1}$ and the biomass produced under nutrient deficient conditions contained nearly 40% w/w lipids. In short, there is a general lack of data on seawater culture of *C. vulgaris*. This work aims to address this knowledge gap. In addition, comparative data are provided on four other nominally freshwater microalgae grown in freshwater, brackish water, and seawater media.

2. Materials and methods

2.1. Microalgae

The following five freshwater microalgae (green algae, Chlorophyta) were used: *Chlorella vulgaris*; *Choricystis minor*; *Neochloris* sp.; *Pseudococcomyxa simplex*; and *Scenedesmus* sp. *Scenedesmus* sp. had been isolated by Dr. T. Mazzuca Sobczuk at Massey University, New Zealand. The other algae had been purchased from Landcare Research, Lincoln, New Zealand. The identity of *C. vulgaris* had been confirmed by *rbc*L gene sequencing (Luangpipat, 2013). For the other microalgae, the identity was as determined by the relevant phycological culture collections.

Cultures were maintained on agar slants and petri dishes. BG11 medium (specified later) made with deionized water was used. Freshly inoculated petri dishes were grown at room temperature (22–25 °C) for 10–15 days, under daylight fluorescent light (15–20 $\mu mol\,m^{-2}\,s^{-1}$ at the surface of the dishes) and stored at 4 °C (2–8 $\mu mol\,m^{-2}\,s^{-1}$ light at the surface of the dishes) until needed. Algae were subcultured every 6–8 weeks.

2.2. Growth media

The BG11 medium was used as the basal medium. All media used in maintenance of cultures and preparation of inocula and Duran bottle (borosilicate glass 3.3, LabSerV, Biolab, Auckland, New Zealand) cultures were sterilized by autoclaving (121 °C, 15 min).

The BG11 medium was made by mixing four stock solutions that had been autoclaved (121 °C, 15 min) separately, cooled, and kept at 4 °C until needed. The final medium contained the following per L of deionized water, or other specified water: $CaCl_2{\cdot}2H_2O$, 36 mg; citric acid monohydrate ($C_6H_8O_7$), 6 mg; ferric ammonium citrate ($C_6H_{11}FeNO_7{\cdot}H_2O$), 6 mg; Na_2EDTA ($C_{10}H_{14}N_2Na_2O_8{\cdot}2H_2O$), 1 mg; $MgSO_4{\cdot}7H_2O$, 75 mg; $K_2HPO_4{\cdot}3H_2O$, 40 mg; Na_2CO_3, 0.02 g; H_3BO_3, 2.86 mg; $MnCl_2{\cdot}4H_2O$, 1.81 mg; $ZnSO_4{\cdot}7H_2O$, 222 μg; $NaMoO_4{\cdot}2H_2O$, 390 μg; $CuSO_4{\cdot}5H_2O$, 79 μg; $CoCl_2{\cdot}6H_2O$, 50 μg; and $NaNO_3$, 1.5 g.

For solid media, 15 g agar (Difco™, Agar Noble, France) was added prior to making up the volume to 1 L with deionized water. For solid media only, the following vitamins were added (per L): thiamine-HCl (vitamin B1), 100 mg; biotin (vitamin H), 0.5 mg; and cyanocobalamin (vitamin B12), 0.5 mg. The pH was adjusted to 7.5 using 1 M HCl.

Artificial seawater was prepared by dissolving 40 $g\,L^{-1}$ of sea salt in deionized water or tap water. The sea salt used was either Sigma product no. S9883 (Sigma Chemical Company, St Louis, MO, USA) or natural unrefined Southern Pacific Ocean salt (Pacific Nat-