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Using CO_2 to enhance carbon capture and biomass applications of freshwater macroalgae

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Abstract

A major limiting factor in the development of algae as a feedstock for the bioenergy industry is the consistent production and supply of biomass. This study is the first to access the suitability of the freshwater macroalgal genus *Oedogonium* to supply biomass for bioenergy applications. Specifically, we quantified the effect of CO_2 supplementation on the rate of biomass production, carbon capture, and feedstock quality of *Oedogonium* when cultured in large-scale outdoor tanks. *Oedogonium* cultures maintained at a pH of 7.5 through the addition of CO_2 resulted in biomass productivities of 8.33 (±0.51) g DW m^{-2} day^{-1}, which was 2.5 times higher than controls which had an average productivity of 3.37 (±0.75) g DW m^{-2} day^{-1}. Under these productivities, *Oedogonium* had a carbon content of 41–45% and a higher heating value of 18.5 MJ kg^{-1}, making it an ideal biomass energy feedstock. The rate of carbon fixation was 1380 g C m^{-2} yr^{-1} and 1073.1 g C m^{-2} yr^{-1} for cultures maintained at a pH of 7.5 and 8.5, and 481 g C m^{-2} yr^{-1} for cultures not supplemented with CO_2. This study highlights the potential of integrating the large-scale culture of freshwater macroalgae with existing carbon waste streams, for example coal-fired power stations, both as a tool for carbon sequestration and as an enhanced and sustainable source of bioenergy.

Keywords: algal biomass, bioenergy, carbon dioxide, carbon sequestration, feedstock quality, freshwater, macroalgae, *Oedogonium*

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Introduction

Australia has very large reserves of coal and natural gas that provide a low cost and consistent source of base load electricity generation. Over 80% of Australia's electricity is produced through the combustion of coal. This combustion produces ca. 190–200 Mt CO_2 –e annually and accounts for ca. 35% of Australia's total CO_2 emissions (Department of Climate Change & Energy Efficiency, 2012). These emissions now incur a cost (carbon tax) and there is a global responsibility to reduce emissions of greenhouse gases where possible (Lo & Spash, 2012). However, options to reduce these emissions are limited to either decommissioning existing low cost 'dirty' power stations to build relatively more costly 'clean' renewable energy systems (solar, wind) or to develop and implement carbon capture and storage (CCS) techniques to sequester carbon before it is released to the atmosphere (Sims *et al.*, 2003; Schrag, 2007; Wall, 2007). These CCS techniques are in their infancy, but can be broadly divided into abiotic and biotic approaches (Lal, 2008). Abiotic approaches involve capturing carbon directly from exhaust gas and injecting it in deep reservoirs where it will theoretically be contained for thousands of years (Lackner, 2003; Haszeldine, 2009; Pires *et al.*, 2011). Biotic approaches revolve around plant photosynthesis, where carbon is converted to glucose and incorporated into the structural framework of the plant biomass (Nilsson & Schopfhauser, 1995; Ho *et al.*, 2011). This captured carbon can then be recycled as feedstock biomass for bioenergy or converted into biochar for long-term carbon storage (Lehmann, 2007; Lal, 2008; Mathews, 2008; Bird *et al.*, 2011). A limitation of using terrestrial plants to capture carbon is that they can only access CO_2 after it has been released to the atmosphere. In contrast, for aquatic plants, waste CO_2 can be dissolved directly into the culture water enabling waste carbon to be directly accessed and converted into biomass (Benemann & Tillett, 1993).

Macroalgae are large multicellular algae and their cultivation is a promising mechanism to service large-scale biomass applications (Kraan, 2010; Jung *et al.*, 2013). Macroalgae have high productivities and can be cultured using high nutrient wastewater on non-arable land, thereby avoiding the fuel vs. food debate associated with terrestrial bioenergy crops (Kraan, 2010; Nigam & Singh, 2011; Acién Fernández *et al.*, 2012). Macroalgal cultiva-

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tion is typically synonymous with seaweeds, with over 16 million tones cultivated annually (Jung *et al.*, 2013). In contrast, apart from a few isolated studies which have utilized freshwater macroalgae for the bioremediation of high nutrient effluents from animal agriculture or human sewage (Mulbry & Wilkie, 2001; Wilkie & Mulbry, 2002; Mulbry *et al.*, 2008, 2010), they have been largely overlooked as a large-scale source of sustainable biomass (Gao & McKinley, 1994; Kraan, 2010; Rowbotham *et al.*, 2012). This is surprising considering the co-location of major CO_2 emitters with inland coal mines, where freshwater is available within the industrial ecology framework of coal-fired electricity generation. Notably, industrial flue gas typically contains between 12 and 15% CO_2 with the remaining 85% being composed of nitrous and sulfur oxides which can be toxic (reviewed Van den Hende *et al.*, 2012). However, flue gas composition is dependent on the energy source combusted and experimental studies on the effects of flue gas are recommended for each flue source (Van den Hende *et al.*, 2012). Only one experimental study has examined the benefits of using flue gas as a carbon source for macroalgal cultivation with the marine red alga *Gracilaria conferta* being successfully cultured with flue gas at high productivities for 13 months (Israel *et al.*, 2005). As such the coupling of freshwater macroalgal production with the use of CO_2 presents an ideal model for intensive biomass production with a reduction in CO_2 emissions.

Inadequate carbon levels are often the primary limiting factor of macroalgal biomass production. Under intensive culture, the combination of high photosynthetic activity, low water exchange (Menéndez *et al.*, 2001; Mata *et al.*, 2007) and the very slow rate of CO_2 diffusion from the atmosphere (Denny, 1990) can rapidly deplete the available carbon (Israel *et al.*, 1999; Mata *et al.*, 2007). In solution, inorganic carbon forms part of the carbonate buffer system and is available as one of three species – carbon dioxide (CO_2), bicarbonate (HCO_3^-) or carbonate (CO_3^{2-}) – with the relative proportion of each being dependent upon the pH, and to a lesser extent salinity and temperature (Lobban & Harrison, 1997). At a pH of 8 in freshwater the concentration of CO_2 is effectively zero (<1.5%), with ca. 95% of the dissolved inorganic carbon (DIC) in the form of HCO_3^-, while at a pH of 10 HCO_3^- is reduced to 26% of the DIC with the unusable CO_3^{2-} form accounting for the remainder. All algae examined to date are unable to utilize CO_3^{2-} as a carbon source, most species of marine green algae can use HCO_3^-; however, it must first be converted into CO_2 through a carbon concentrating mechanism (CCM), which can then diffuse directly into the chloroplasts (Choo *et al.*, 2002).

Despite the broad understanding of carbon preferences, and the effect of carbon availability on the growth of marine macroalgae, the carbon utilization of freshwater macroalgae has yet to be quantified. Determining carbon usage is therefore a critical first step in assessing the potential of freshwater macroalgae as a biomass feedstock and carbon sink. The second critical step is to quantify the effects of the form and concentration of DIC on biomass production, as both are inextricably linked to the use of CO_2 as a source of carbon. The focus of this study is a freshwater green macroalga, *Oedogonium crispum* that has recently been identified as a robust and competitively dominant species in small scale cultures (Lawton *et al.*, 2013). We now determine the carbon utilization of *Oedogonium,* and secondly quantify the trade-offs in increasing the supply of DIC through CO_2 addition on the biomass production, carbon capture and feedstock quality of *Oedogonium* in large-scale tank culture.

Materials and methods

Study species

Oedogonium is a genus of unbranched filamentous green algae made up of small cylindrical cells. This genus has a worldwide distribution and is a common component of natural ecosystems where it grows either attached to the substrate or as free floating mats. It is a competitively dominant species that overgrows other freshwater macroalgae under high nutrient environmental conditions (McCracken *et al.*, 1974; Simons, 1994). The original biomass used in this experiment was collected from an irrigation channel in the Brandon sugar cane growing region (Latitude: 19.55°S; Longitude: 146.35°E). Stock cultures of *Oedogonium* were maintained at the Marine & Aquaculture Research Facilities Unit, at James Cook University, Townsville (Latitude: 19.33°S; Longitude 146.76°E). The study species is identified as *O. crispum* (hereafter referred to as *Oedogonium*) using morphological characteristics and taxonomic keys (Entwisle *et al.*, 2007).

pH drift assay

A pH drift assay was conducted to determine the ability of *Oedogonium* to utilize different species of carbon (CO_2, HCO_3^-) during photosynthesis. Basal culture medium was prepared using dechlorinated tap water enriched with Guillards f/2 growth media (0.1 g l^{-1}) and CO_2 was bubbled through this culture water to reduce the initial pH to 6.7. Biomass for this trial was harvested from an outdoor (2500 l) culture of *Oedogonium* and excess water was removed using a centrifuge (246 × g) before adding 0.01 g of algal biomass to each of 102, 120 ml graduated culture vessels. These culture vessels

were overfilled with freshly prepared growth media (pH 6.7) to remove any air pockets, which could enable CO_2 to diffuse into the growth media and influence rates of pH change. Culture vessels were randomly positioned inside a culture chamber (Sanyo model MLR-351) with constant irradiance (230 μmol photons m^{-2} s^{-1}) and were repositioned every 30 min to prevent edge effects or the formation of a boundary layer around algal filaments. After 30 min in culture, six culture vessels were destructively sampled and their pH measured using a WP81 handheld pH probe. This procedure was repeated every 30 min for the first hour and then every 15 min for the following 2 h before hourly sampling for the next 7 h until the pH reached a stable level for at least three consequent measurements. These stable measurements represent the pH compensation point where the DIC uptake by the algae equals the CO_2 released by respiration and/or photorespiration into the medium.

Carbon supply and biomass productivity

Culture system. Experiments were conducted in large outdoor tanks measuring 10 m long and 3 m wide with a flat base and were filled to a depth of 0.5 m giving a total volume of 15 000 l to reflect the scale of industrial applications of *Oedogonium* culture. *Oedogonium* biomass was maintained in suspension with an aeration frame placed around the entire edge of the tank, with three additional lines being evenly spaced between the two outside lines to give an aeration manifold of 56 linear meters with 1 mm holes spaced every 15–20 cm. A diffusion hose ran parallel to the three central aeration lines to supply CO_2 (food grade 99.9%) to the tanks. The pH of each culture tank was continuously monitored using a probe-pH controller with the controller activating a solenoid valve that added CO_2 to the culture when the preset pH was exceeded. To prevent a rapid drop in pH when CO_2 supply was activated, a Dwyer 70 ml flow meter was used to control the rate of CO_2 addition. This fine scale control of CO_2 delivery enabled daytime pH values to be maintained within 0.2 pH units of the predetermined pH treatments (7.5 and 8.5). CO_2 supply was turned off overnight and pH in treatment tanks converged to 8.1, corresponding with respiration and CO_2 off-gasing. Notably, this effect was reversed as soon as photosynthesis and CO_2 delivery resumed the following morning (Fig. 1). pH values in our control treatment also declined overnight although pH values in this treatment rarely dropped below 9 at any time (Fig. 1). This experiment was conducted during the Austral winter and water temperatures ranged between a night time minimum of 12.9 °C and daytime maximum of 23.2 °C over the 4 week growth period with a mean daily temperature of each tank ranging between 18.4 °C (±2.2) and 19.1 °C (±2.24). Daily (6:00–18:00 hours) Photosynthetically Active Radiation averaged 39.9 (±7.5) mol m^{-2}, with daily peaks ranging between 622 and 2117 mol m^{-2}.

Experimental design. To determine whether increasing the total DIC, or increasing the relative proportions of CO_2 vs. HCO_3^-, is the primary mechanism for increasing biomass production of *Oedogonium* an upper limit was placed on the pH of each of two 15000 l culture tanks, while a third tank acted as a control where the pH responded to the natural fluctuations associated with photosynthesis and carbon fixation. The two pH levels, 8.5 and 7.5 were used as these values correspond to pH levels which have elevated proportions of either HCO_3^- or CO_2, respectively (Table 1). These pH values were maintained during daytime by bubbling CO_2 between 06:30 and 17:30 hours at a flow rate of 1.5 l min^{-1} and 2.5 l min^{-1} for the 8.5 and 7.5 pH treatments, respectively. By limiting the upper pH level the proportions of CO_2, HCO_3^- and CO_3^{2-} available in the culture water are regulated. The concentration of DIC and each carbon species available in the culture water was calculated weekly using the software CO_2 sys (Lewis *et al*., 1998) based on the total alkalinity, pH, and temperature of the water for each treatment. The daily average pH of 9.7 was used to calculate carbon availability for the control treatment. Total alkalinity was calculated using potentiometric titration by the Australian Centre for Tropical Freshwater Research at James Cook University.

Oedogonium was stocked at 7.5 kg (0.5 g l^{-1}) and maintained in a tumble culture in each of the three 15000 l tanks. Algal biomass was initially acclimated to each of the three pH treatments for 3 weeks prior to the biomass production experiment. To quantify productivity, algal biomass was harvested weekly, centrifuged using a domestic washing machine (246 × g) to remove excess water, weighed and restocked at 0.5 g l^{-1}. Each week, a fresh weight to dry weight ratio was determined by drying a sample of freshly spun algae from each treatment overnight at 60 °C. Algal productivity was calculated using the equation: $P = \{[(B_f - B_i)/\mathrm{FW} : \mathrm{DW}]/A\}/t$, where B_f and B_I are the final and initial algal biomass, FW:DW is the fresh to dry weight ratio, A is the area of our culture tanks, and t is the number of days in culture. The fresh to dry weight ratio ranged between 3.9 and 5.1.

Because large-scale cultures were run for multiple weeks, we used an unreplicated randomized block design to partition variation associated with differences between weeks by rotating experimental treatments among each of the three tanks weekly. The same algal biomass was maintained in the same pH treatment each

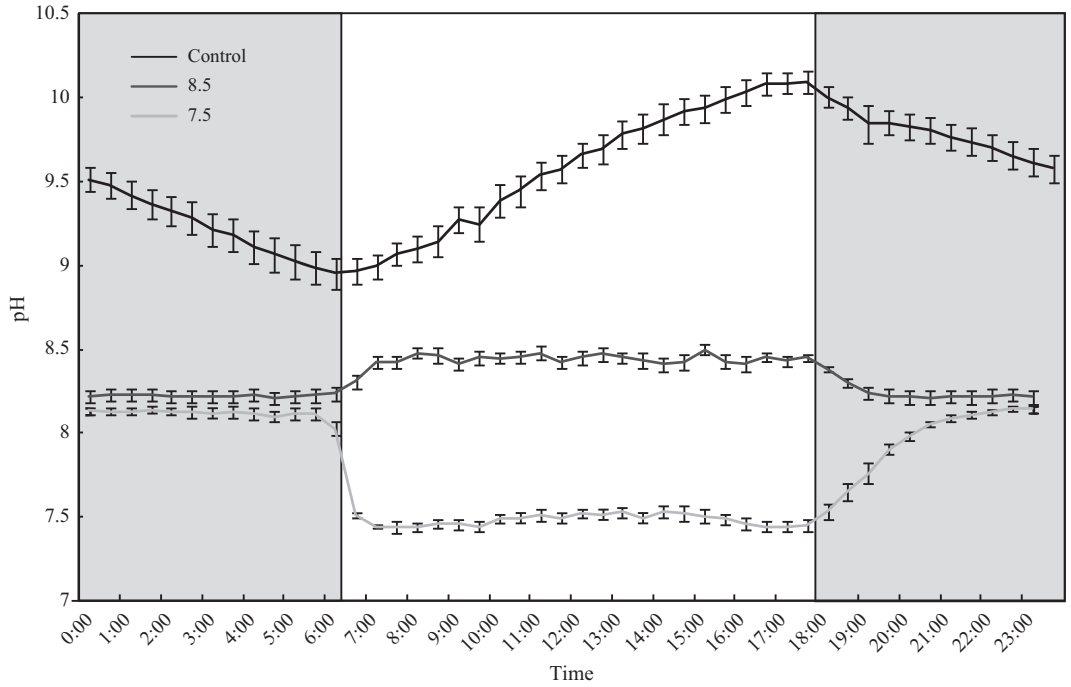

Fig. 1 Mean daily pH fluctuations in *Oedogonium* cultures over 28 days. pH was controlled in two treatments (pH 7.5 and 8.5) through the addition of CO_2 gas between 06:30 and 17:30 hours. Our control treatment had no CO_2 added. Shaded sections represent day-night cycles.

Table 1 Proportion of the total dissolved inorganic carbon that is accounted by each of the three carbon species, CO_2, HCO_3^- and CO_3^{2-}, at each of the three pH treatments used in the biomass production experiment and the pH compensation point for *Oedogonium*

pH	CO_2 (%)	HCO_3^- (%)	CO_3^{2-} (%)
7.5	4.26	94.86	0.88
8.5	0.41	91.17	8.42
Control (average pH 9.7)	<0.01	25.51	74.49
10.7 (compensation point)	0	6.86	93.14

week. A mixed model ANOVA was used to assess the effects of CO_2 addition on biomass production yields with time (week) as the blocking factor. Residual plots were used to ensure ANOVA assumptions were met. To improve normality, biomass production yields were $\log_{10}$ transformed. Tukey's HSD post hoc test was then used to identify where differences among group means occurred.

Biomass processing and bioenergy potential. After each weekly harvest, 100 g of *Oedogonium* biomass from each treatment was frozen at −80 °C before being freeze dried for analysis of CHONS (ultimate analysis) (OEA Laboratories UK). To account for residual moisture in the dried biomass a 1.5 g subsample was heated at 110 °C in a moisture balance until constant dry weight was reached. The ash content of this biomass was then quantified in triplicate through the combustion of 500 mg samples at 550 °C in a muffle furnace until constant weight was reached. To quantify the suitability of *Oedogonium* biomass as a potential biofuel the higher heating value (HHV) was calculated for the biomass cultured at each of the three pH treatments. The HHV is based on the elemental composition of the biomass and is a measure of the amount of energy stored within. The HHV was calculated using the following equation

$$\mathrm{HHV}(MJ/Kg) = 0.3491 * C + 1.1783 * H + 0.1005 * S - 0.1034 * O - 0.0151 * N - 0.0211 * A$$

where C, H, S, O, N, and A are the carbon, hydrogen, sulfur, oxygen, nitrogen, and ash mass as percent dry weight, following Channiwala & Parikh (2002).

Carbon accounting. The amount of CO_2 that was incorporated into the *Oedogonium* biomass relative to that which was off-gased and lost to the atmosphere was estimated by combining the flow rate, adjusted for the mass flow of CO_2 gas, with the total time that the solenoid in each tank was activated. CO_2 was supplied to the culture tanks between the hours of 06:30 and 17:30 hours each day. The total amount of carbon

incorporated into growth was estimated using the proportion of the biomass made up of carbon multiplied by the total amount of biomass produced in each of the three pH treatments. The amount of carbon fixed in our control treatments was subtracted from each of the 7.5 and 8.5 pH treatment cultures to account for the amount of carbon that is fixed without CO_2 addition.

Results

pH drift assay

Photosynthesis by *Oedogonium* increased the pH of culture water from an initial pH of 6.7 to a final pH compensation value of 10.71 (Fig. S1). Carbon fixation occurred rapidly at pH values below 9.5. *Oedogonium* raised the pH through photosynthesis from 6.7 to 9.5 within 2.75 h; however, it took a further 5 h to raise the pH to the compensation point of 10.7. The most rapid pH increase occurred between pH 7.5 and 8.5, which took 15 min. *Oedogonium* is able to effectively utilize both CO_2 and HCO_3^- as a carbon source although carbon fixation is impaired as the pH increases above 10.5 and the proportion of DIC that is CO_3^{2-} approaches 100% (Table 1).

Carbon availability and biomass production

The addition of CO_2 significantly increased yields of *Oedogonium* (ANOVA, $F_{2,6} = 10.91$, $P = 0.039$). *Oedogonium* biomass cultured at a pH of 7.5 and 8.5 had a productivity 2.47 and 1.85 times higher than biomass cultured without CO_2 addition, although only the 7.5 treatment was significantly different to the control (Tukey HSD, $P < 0.05$)(Fig. 2). *Oedogonium* cultured at a pH of 7.5 provided a consistent yield that ranged between 7.68 and 9.84 g DW m^{-2} day^{-1}. In comparison, the productivity at a pH of 8.5 varied considerably between weeks, ranging between 4.25 and 8.84 g DW m^{-2} day^{-1}. Similarly, the productivity in the control treatment was also variable between weeks and generally grew slowly, ranging between 1.9 and 5.2 g DW m^{-2} day^{-1}. Controlling the pH of culture tanks increased the total carbon available for photosynthesis (CO_2 and HCO_3^-) by 5.9 and 7.5 times in the 8.5 and 7.5 pH treatments relative to the control (Fig. 2). Similarly, the amount of free CO_2 available for photosynthesis increased with decreasing pH. The ratio of HCO_3^- to CO_2 decreased from 7039 : 1 in our control to 222 : 1 in our 8.5 pH treatment, and to 22 : 1 in the 7.5 pH treatment (Table 1).

Biochemical composition of Oedogonium. The addition of CO_2 and subsequent pH of culture water had no effects on the biochemical composition of the biomass as quantified by ultimate and proximate analysis (Table 2). Mean carbon content ranged between 42.5 (±1.24 SD) and 43.2% (±1.45) for the three treatments and was relatively stable over the 4 week growth trial. Carbon addition also had no effect on the HHV of *Oedogonium* biomass, which was approximately 18.5 MJ kg^{-1} across all treatments (Table 2).

Carbon accounting. A total of 3.15, 2.4, and 1.19 kg of carbon was sequestered through *Oedogonium* biomass production over the 4 week growth trial in the 7.5, 8.5, and control treatments, respectively. To maintain a pH value of 7.5 and 8.5 a total of 3.75 kg (±0.34) and 1.42 kg (±0.31) of CO_2 was added daily with carbon representing 27.2% of the total gas added. Over the 4 week growth trial *Oedogonium* sequestered carbon at a mean daily rate of 3.75 (±0.23) g m^{-2} day^{-1}, 2.94 (±0.48) g m^{-2} day^{-1} and 1.4 (±0.18) g m^{-2} day^{-1} in the 7.5, 8.5, and control pH treatments, respectively (Fig. 3). This represents an uptake efficiency of 23.8% (±0.03) and 11.23% (±0.01) of the total carbon added to the 8.5 and 7.5 pH treatments. However, this amount is inflated as a proportion of this total carbon sequestered would occur without the addition of CO_2. To account for this, we also estimated the net proportion of added CO_2 that was sequestered by subtracting the amount of carbon fixed in our control treatment from each of our CO_2 addition treatments. The net efficiency of carbon uptake is 7.1% (±0.1) and 11.5% (±0.2) in our 7.5 and 8.5 pH treatments, respectively (Fig. 3). The remaining CO_2 is unaccounted for and is most likely lost to the atmosphere through off-gassing.

Discussion

This study demonstrates, for the first time, that the freshwater genus *Oedogonium*, and freshwater macroalgae more generally, are key candidates for the large-scale culture and supply of feedstock biomass for bioenergy applications. The strong positive response in biomass production using carbon supplementation provides a clear rationale for the integration of macroalgal cultivation within the existing industrial infrastructure of large-scale CO_2 emitting industries. Co-culturing freshwater macroalgae with industrial flue gas could provide a holistic solution for carbon sequestration where a proportion of the carbon emitted can be incorporated into biomass. This carbon can then be recycled by converting the algal biomass into a range of bioenergy products from biogas to liquid and solid biofuels. Alternatively, if long-term carbon storage is the goal then the algal biomass can be converted selectively through pyrolysis to biochar and used as a soil ameliorant (Lehmann, 2007; Sohi, 2012). Biochar has been produced from both

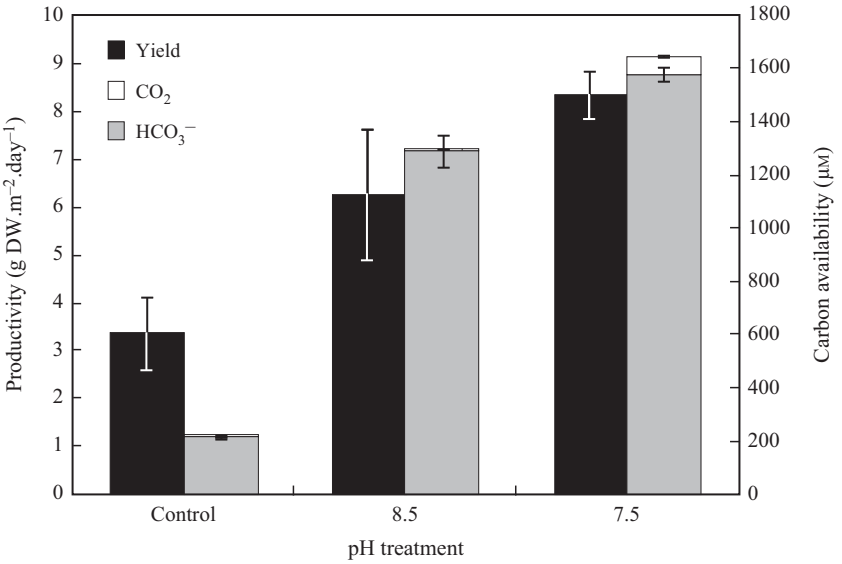

Fig. 2 Biomass production rates of *Oedogonium* and total carbon available (CO_2 and HCO_3^-) for photosynthesis when cultured at three pH treatments (7.5, 8.5, and ambient).

Table 2 Proximate and ultimate analysis (wt %, on a dry basis, mean of samples, $n = 4$, SD < 1.5) and higher heating value (MJ kg^{-1}), on a dry basis, mean of samples, $n = 4$, SD < 0.7) of *Oedogonium* biomass cultured at three pH treatments over 4 weeks

	Proximate		Ultimate					
pH treatment	Ash	Inherent moisture	C	H	O	N	S	HHV* (MJ kg^{-1})
7.5	11.64	3.20	43.16	6.54	37.02	4.23	0.16	18.66
8.5	12.07	3.02	42.58	6.40	35.57	4.76	0.20	18.42
Control	13.00	3.01	42.46	6.39	34.73	4.93	0.19	18.44

*Calculated from Channiwala & Parikh (2002).

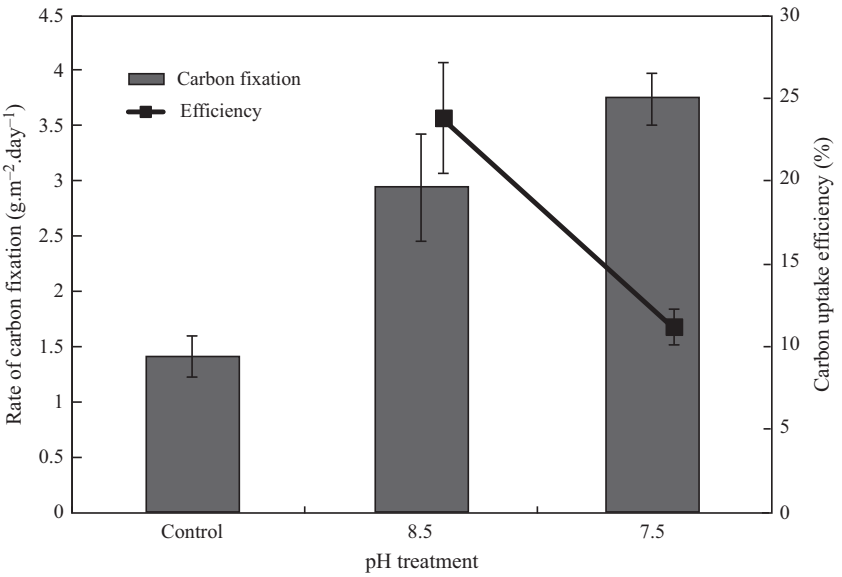

Fig. 3 Rate of carbon fixation (bars) and carbon uptake efficiency (line) by *Oedogonium* cultured at three pH treatments (7.5, 8.5, and ambient).

fresh and saltwater macroalgae using slow pyrolysis, with the resultant biochar retaining >50% of the carbon from the biomass and producing significant order-of-magnitude increases in crop production for low carbon soils (Bird *et al*., 2011, 2012).

The high pH compensation point and ability of *Oedogonium* to utilize HCO_3^- also provide capacity to culture freshwater macroalgae in areas where waste CO_2 is unavailable, such as in high nutrient effluents from agricultural or sewerage wastes. However, despite this ability to maintain photosynthesis at high pH values (>10), the addition of CO_2 to large-scale cultures significantly increased biomass production. Interestingly, cultures maintained at a pH of 7.5 grew at a 33% higher rate than those cultured at pH 8.5. A similar result was observed by de Silva *et al.* (in review) for two seaweed species in which a 26 and 28% increase in biomass production occurred when cultured at pH ca. 7.5 relative to pH ca. 8.5. This increase is proposed to be a result of the increasing fraction of free CO_2 available for photosynthesis at the lower pH culture rather than a response to the overall increase in total DIC. Free CO_2 diffuses directly into the chloroplasts where it is fixed by RuBisCO in photosynthesis (Lobban & Harrison, 1997). In contrast, HCO_3^- must first be converted through a CCM to CO_2 before it can be utilized for growth (Maberly, 1990). The pH 8.5 and 7.5 treatments had a total DIC concentration of 1.30 and 1.65 μM l^{-1}, respectively, however, the ratio of free CO_2 to HCO_3^- increased by a factor of 10 between these treatments. Our results demonstrate that increasing the total DIC will only increase productivity to a point, beyond which an increase in the proportion of free CO_2 is required to further increase rates of biomass production.

This study is to our knowledge the first to estimate the rate of carbon sequestration and the efficiency of carbon capture by macroalgae cultured in large-scale outdoor systems. The low efficiencies of carbon uptake achieved here are higher than microalgal cultures when flue gas is continuously added, where efficiencies typically range between 4 and 10% (Hu *et al*., 1998; Zhang *et al*., 2001; Acién Fernández *et al*., 2012). Notably, on-demand delivery of flue gas can increase CO_2 uptake efficiencies to 32.5% in open photobioreactors and up to 50% for closed photobioreactors (reviewed by Acién Fernández *et al*., 2012). Moreover, these uptake efficiencies are related to the rate of flue gas injection, with this efficiency declining as the rate of flue gas injection increases (Doucha *et al*., 2005). A similar result occured in this study where an increase in the rate of CO_2 addition caused an increase in both biomass production and the total amount of carbon sequestered per unit area, but a decrease in the efficiency of carbon fixation. This trade-off between productivity and uptake efficiency is likely to be a consistent feature of algal bioremediation strategies, where productivity is maximized by supplying a higher concentration of CO_2, or any other nutrients (or pollutants), but at the expense of uptake efficiency (see also Schuenhoff *et al*., 2006).

While the efficiency of carbon uptake is relatively low, the total amount of carbon converted into biomass is considerable. *Oedogonium* biomass grown without the addition of CO_2 fixed carbon at a rate 481 g C m^2 yr^{-1}, while *Oedogonium* biomass cultured at a pH of 7.5 fixed carbon at a rate of 1.38 kg C m^2 yr^{-1}. This is higher than many alternative bioenergy crops or alternative biological carbon sequestration techniques. The cultivation of the perennial rhizomatous grass *Miscanthus* × *giganteus* over a 16 year period resulted in an annual carbon sequestration rate of between 520 and 720 g C m^2 yr^{-1} (Clifton-Brown *et al*., 2007). Similarly, utilizing low-input natural grasslands for bioenergy enables the sequestration of 440 g C m^2 yr^{-1} (Tilman *et al*., 2006). The potential carbon sequestration of agroforestry is also highly variable and depends to a large extent on the climate and ecological production potential of the system with values ranging from 29 to 1521 g C m^2 yr^{-1} (Dixon, 1995; Albrecht & Kandji, 2003; Ramachandran Nair *et al*., 2009), although this carbon is stored for a considerably longer timeframe relative to bioenergy crops, which are primarily focused on recycling carbon rather than sequestration.

The high comparative total amount of carbon converted into biomass provides a significant advantage for recycling carbon for bioenergy. *Oedogonium* biomass has a carbon content of 41–45% and a higher heating value of 18.5 MJ kg^{-1}, which is considerably higher than the majority of marine seaweeds (22–35% Carbon and 5–17 MJ kg^{-1}) (Lamare & Wing, 2001; Ross *et al*., 2008; Zhou *et al*., 2010; Anastasakis & Ross, 2011; Rowbotham *et al*., 2012) and is comparable to typical values of terrestrial energy crops or woody plants (16–23 MJ kg^{-1}) (Ebeling & Jenkins, 1985; McKendry, 2002; Cantrell *et al*., 2010). Despite comparative calorific values to terrestrial plants there are two major advantages of culturing freshwater macroalgae. Firstly, freshwater macroalgae can be grown on marginal land with non potable water and will not compete directly with valuable farmland that is needed to feed the world's increasing population (Singh *et al*., 2011). Secondly, freshwater macroalgal culture can be integrated into existing industrial infrastructure, whereby water and critical nutrients, such as nitrogen, phosphorous, and carbon, that are needed for biomass production can be recycled from industrial waste streams and converted to biomass, whereas terrestrial crops will require the application of water and fertilizers to maintain high productivities adding additional social and economic costs to

production. The high proportion of carbon within the biomass (42–43%) makes freshwater macroalgae a target feedstock for integration with large-scale point-source carbon emitters and consequently conversion into bioenergy including advanced renewable fuels.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. pH drift assay for *Oedogonium*. Values are the means and standard errors of 6 replicate vessels at each sampling time.