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Winter-time CO_2 addition in high rate algal mesocosms for enhanced microalgal performance

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ABSTRACT

Carbon limitation in domestic wastewater high rate algal ponds is thought to constrain microalgal photophysiology and productivity and CO_2 augmentation is often used to overcome this limitation in summer. However, the implications of carbon limitation during winter are poorly understood. This paper investigates the effects of 0.5%, 2%, 5% and 10% CO_2 addition on the winter-time performance of wastewater microalgae in high rate algal mesocosms. Performance was measured in terms of light absorption, photosynthetic efficiency, biomass production and nutrient removal rates, along with community composition. Varying percentage CO_2 addition and associated change in culture pH resulted in 3 distinct microalgal communities. Light absorption by the microalgae increased by up to 144% with CO_2 addition, while a reduction in the package effect meant that there was less internal self-shading thereby increasing the efficiency of light absorption. Carbon augmentation increased the maximum rate of photosynthesis by up to 172%, which led to increased microalgal biovolume by up to 181% and an increase in total organic biomass for all treatments except 10% CO_2. While 10% CO_2 improved light absorption and photosynthesis this did not translate to enhanced microalgal productivity. Increased microalgal productivity with CO_2 addition did not result in increased dissolved nutrient (nitrogen and phosphorus) removal. This experiment demonstrated that winter-time carbon augmentation up to 5% CO_2 improved microalgal light absorption and utilisation, which ultimately increased microalgal biomass and is likely to enhance total annual microalgal areal productivity in HRAPs.

1. Introduction

High rate algal ponds (HRAP) form part of an advanced pond system first developed in the 1950s for the treatment of wastewater and nutrient recovery in the form of microalgal biomass for use as fertiliser, feed or as a feedstock for biofuel production. The central concept behind HRAP wastewater treatment is that microalgal photosynthesis provides the necessary oxygen that drives aerobic bacterial degradation of organic compounds, which, in turn, provides the CO_2 required for photosynthesis (Oswald 1988). The design of HRAPs allows for microalgae to grow profusely thus enhancing nutrient removal via its assimilation into their biomass. This results in combined secondary and partial tertiary wastewater treatment within the HRAP (Rawat et al. 2011). HRAPs have been shown to provide improved and more consistent wastewater treatment than traditional oxidation ponds, as well as higher microalgal productivity for a wide range of wastewaters, including municipal, industrial and agricultural, at a range of scales, including mesocosm, pilot scale and full-scale (Garcia et al., 2006, de Godos et al. 2009, Cai et al. 2013, Craggs et al. 2014). However, despite the already high microalgal biomass yields from wastewater HRAPs improved microalgal productivity is still regarded as a priority in order to make resource recovery from these ponds economically feasible (Rawat et al. 2011).

Carbon is an essential nutrient for microalgal production, comprising approximately 50% of its organic biomass, and growth can become limited when the demand for carbon exceeds supply. Heterotrophic metabolism of organic matter, resulting in the generation of CO_2, supplies an estimated 25–50% of the dissolved inorganic carbon (DIC) required by microalgae in these pond systems (Borowitzka 1998). However, microalgal productivity in HRAPs is still regarded as being carbon limited due to the low

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carbon / nitrogen ratio of wastewater (typically 3:1) compared to microalgal biomass (typically 6:1) (Benemann 2003). Carbon limitation in the HRAPs is indicated by elevated daytime pH, with summer-time pH values reaching in excess of pH 11 (Sutherland et al. 2013). High pH affects the inorganic carbon species, photosynthetic capacity and nutrient removal rates by the microalgae and inhibits aerobic bacteria growth (Azov and Goldman 1982, Park and Craggs 2011, Sutherland et al. 2015a). Improvements in the summer-time performance of wastewater HRAPs, in terms of both nutrient removal and microalgal biomass production, have been achieved through the addition of carbon (Azov et al. 1982, Benemann 2003, Park and Craggs, 2010 and 2011). Carbon augmentation has typically been achieved by CO_2 gas being bubbled directly into the culture to a set point of pH~ 8, although a more recent study investigated the effects of CO_2 addition along a range of pH set points from pH 6.5–8 (Park and Craggs 2010, Sutherland et al. 2015a).

While there have been a number of studies investigating the role of carbon augmentation during the summer-time, we are unaware of any study on the benefits of winter-time carbon augmentation. In an 18 month study of four full-scale wastewater HRAPs Craggs et al. (2012) recorded a winter-time average HRAP culture pH > 9, suggesting potential carbon limitation in winter. In this paper, we investigate the hypothesis that increasing CO_2 supply in wastewater HRAPs during winter further enhances microalgal photo-physiology, nutrient removal and biomass production.

2. Methods

2.1. High rate algal mesocosm set-up and carbon dioxide augmentation

The experiment was conducted outdoors during winter (July–August 2014) at the Ruakura Research Centre Hamilton, New Zealand (37°47′S, 175°190′E) and run for a period of 28 days. The high rate algal mesocosms (HRAM) comprised of 15 L plastic buckets, with a 300 mm culture depth. The buckets were wrapped in foam to ensure light only entered from the surface of the water. They were placed on individual stirrer plates and mixed continuously with an 8 cm long magnetic stirrer bar. Initial microalgal inoculum was culture from an adjacent pilot-scale HRAP that had been running continuously for a number of years. The cultures were grown on primary settled domestic wastewater and were operated as semi-continuous cultures on an 8 day hydraulic retention time, with a daily exchange of 12.5% of the culture.

Supplementary carbon was supplied to the HRAMs in the form of CO_2 gas / air mixtures. The CO_2 addition system consisted of a CO_2 gas cylinder, gas regulator, air pump, gas flow meter and gas diffusers (0–12 L min^{-1} range). The CO_2 gas was blended with air (via the air pump) to provide CO_2 concentrations of 0.5% (culture pH 8.0 ± 0.1), 2% (culture pH 7.0 ± 0.1), 5% (culture pH 6.4 ± 0.1), 10% (culture pH 6.1 ± 0.1) and a control of air only (culture pH 8.8 ± 0.1), measured on a Biogas 5000, Geotech gas analyser. The gas blends were continuously bubbled into the cultures through a gas diffuser placed on the bottom of the HRAMs.

pH and temperature were measured using a calibrated TPS meter. Organic matter, chlorophyll biomass and dissolved nutrients were measured on days 21, 24 and 28, while species composition, cell counts, light absorption and photosynthesis were measured on day 28.

2.2. Species composition and biovolume determination

Subsamples of HRAM culture for species determination and cell size measurements were settled in a Phyco Tech nanoplankton chamber and viewed on a Leica DMLB microscope at magnifications up to 600×. Microalgae were identified to species level based on the taxonomic descriptions of John et al. (2011). For species counts, 1 mL of HRAM culture was settled in a Sedgwick rafter counting chamber, viewed at 200× magnification and all microalgal present were counted. Cell measurements were made using a calibrated eye graticule and biovolume estimates of the microalgal cells (excluding spines and mucilage) were made in accordance with specific geometric shapes and equations assigned to microalgae genera (Vadrucci et al. 2013).

2.3. Organic matter, chlorophyll-a biomass and nutrient removal

For organic matter a known volume of HRAM culture was filtered through a pre-rinsed, pre-combusted and pre-weighed Whatman GF/F filter, oven dried (105 °C) and then weighed, once cooled, to determine the total suspended solids (TSS) concentration. Filters were then combusted at 450 °C for 4 h, cooled in a desiccator, and re-weighed to determine the ash concentration. The organic matter, also referred to as volatile suspended solids, was estimated as the difference between TSS and ash concentrations. For chlorophyll-*a* (Chl-*a*) a known volume of HRAM culture was filtered onto Whatman GF/F filters and the filters boiled in 100% methanol at 65.5 °C for 5 min then extracted at 4 °C, in the dark, for 12 h. Samples were then centrifuged at 3000 rpm for 10 min and the absorbance of the supernatant read on a Shimadzu UV-2550 spectrophotometer. Chl-*a* concentrations were estimated using the trichromatic equations for methanol (Ritchie 2006). Dissolved nutrient samples were filtered through Whatman GF/F filters and concentrations of ammonium (NH_4–N), nitrate (NO_3–N) and dissolved reactive phosphorus (DRP) were determined colourimetrically according to standard methods (APHA 2008).

2.4. HRAP light attenuation and climate

Light profiles through the cultures were measured using Li-Cor 2π underwater sensors attached to a Li-Cor Li-1000 Quantum logger (Li-Cor Biosciences, Lincoln, Nebraska, USA). The vertical light attenuation coefficient (K_d) was calculated from the regression of log-transformed downwelling irradiance versus depth (Kirk 1994). Depth of the euphotic zone where subsurface light was 1% ($Z_{euphotic}$) was estimated from K_d (Falkowski and Raven 2007). The total light experienced by a cell moving up and down through the water column per day (E_{mix}) was calculated as:

$$E_{mix} = \left(100 \times \left(1 - e^{-K_d\, Z_{mix}}\right)(K_d\, Z_{mix})^{-1}\right) \times \textit{daily surface irradiance}$$

where Z_{mix} is the HRAP depth. Mean E_{mix} based on the 4-day period prior to biomass sampling was determined from total daily surface irradiance. Daily surface irradiance was recorded at an adjacent weather station.

2.5. Microalgal light absorption and package effect

Total particulate light absorption was measured on a Shimadzu UV-2550 spectrophotometer with an integrating sphere, in 1 cm quartz cuvettes following methods detailed by Sutherland et al. (2013). The microalgal absorption coefficient, $a_{ph}(\lambda)$ m^{-1}, was determined as the difference between total particulate absorption and detrital absorption. The specific absorption coefficient per unit of total chlorophyll *a* (TChl-*a* = sum of Chl-*a* and pheophytin-*a*), $a^*_{ph}(\lambda)$ m^2 mg^{-1}, was calculated by dividing $a_{ph}(\lambda)$ by TChl-*a*. $a^*_{ph}(675)$ is a measure of the light absorption by Chl-*a*, while