



# Effect of CO<sub>2</sub> addition on biomass energy yield in wastewater treatment high rate algal mesocosms



Abbas Mehrabadi<sup>a,\*</sup>, Mohammed M. Farid<sup>a</sup>, Rupert Craggs<sup>b</sup>

<sup>a</sup> Chemical and Materials Engineering Department, University of Auckland, New Zealand

<sup>b</sup> National Institute of Water and Atmospheric Research Ltd. (NIWA), PO Box 11-115, Hamilton 3200, New Zealand

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## ABSTRACT

Carbon limitation in algal-based wastewater treatment ponds typically constrains microalgae growth and consequently biomass energy yield, particularly in summer. This study investigates the effect of CO<sub>2</sub> addition on algal biomass energy yield in wastewater treatment high rate algal mesocosms (WWT HRAM). Two experiments (summer and winter) were conducted using 15 replicate HRAMs under outdoor conditions while the cultures were bubbled continuously with different air:CO<sub>2</sub> mixtures including: air (control mesocosm), 0.5%, 2%, 5% and 10% CO<sub>2</sub>. The effects of CO<sub>2</sub> addition were evaluated by determining the productivity, algal proportion, chemical composition, energy content, and settleability of the biomass. Under summer conditions there was a direct relationship between biomass productivity and CO<sub>2</sub> concentration with the maximum productivity increase (50% higher than control) occurring in the 10% CO<sub>2</sub>-HRAMs. Under winter conditions there was no significant difference in biomass productivity between treatments. In both experiments, the biomass energy content varied slightly (19.3–22.8 kJ·g<sup>-1</sup>) with %CO<sub>2</sub> addition, with a slight trend of increasing at higher %CO<sub>2</sub> level and where the biomass lipid content was higher. CO<sub>2</sub> augmentation led to a change in the HRAM algal composition and consequently changed the biomass settleability. The total biomass energy yield and its gravity harvestable proportion (calculated by multiplying biomass concentration, energy content and harvest efficiency) were highest for the 5% CO<sub>2</sub>-HRAMs in summer and for the 0.5% CO<sub>2</sub>-HRAMs in winter. These results show that CO<sub>2</sub> addition (indicated by maintaining a culture pH of 6–7 in summer and 7–8 in winter) not only improves biomass productivity and energy content but selects for easily harvestable colonial algal species which are less susceptible to zooplankton grazing.

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## 1. Introduction

Microalgal biomass cultivated as a by-product of wastewater treatment in high rate algal ponds (WWT HRAPs) has been highlighted as a promising feedstock to reduce production costs for community-level algal based biofuel production [1–3]. WWT HRAPs offer a niche opportunity by producing harvested algal biomass during near tertiary-level treatment of wastewater [4] without the addition of nutrient fertiliser and using simple gravity sedimentation to harvest and concentrate the biomass to 1–2 wt% solids [4].

The biomass energy yield potential of WWT HRAP is a function of productivity, algal species dominance, chemical composition, and harvestability of the biomass [5,6]. However, all these parameters

become limited by environmental, operational and biological factors. Our previous study of a pilot-scale HRAP showed that the biomass energy yield potential of WWT HRAP was highly dependent on climate conditions (decreasing by >250% from summer to winter) and zooplankton grazing pressure (decreasing by >50% within few days during a summertime zooplankton bloom) [5].

To enhance the biomass energy yield of WWT HRAP different practical strategies can be employed such as: CO<sub>2</sub> addition which can improve biomass productivity, algal and lipid content, and lipid profile; optimizing hydraulic retention time (HRT) which can increase biomass energy content by increasing biomass lipid content; biomass recycling which can increase biomass productivity, energy content and harvestability by promoting the dominance of readily harvestable algae; and zooplankton control which can prevent productivity loss due to grazing and increase the settleability of the biomass (since some of the algal species develop spines as a defence mechanisms in presence of grazers which could further improve the formation of algal-bacterial flocs) [2,6–8]. However, further research is needed to identify the most practical strategy/strategies to improve WWT HRAP performance both in terms

\* Corresponding author.

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

of wastewater treatment and production of energy-rich biomass. This paper will focus on CO<sub>2</sub> addition.

Carbon limitation is one of the main constraints to year-round algal production in full scale WWT HRAP [9,10]. Carbon limitation results in pH elevation and therefore inhibition of the algae and microbial community. This reduces both WWT HRAP treatment efficiency and biomass energy yield [11]. CO<sub>2</sub> to WWT HRAP has several benefits: 1) avoids high pH (>8.5) inhibition and free ammonia toxicity of algal and microbial community, 2) increases the availability of ammonium and dissolved reactive orthophosphate for algal uptake, 3) increases the C/N ratio of the wastewater (typically 3:1) in the pond to overcome carbon limitation for algal assimilation of all wastewater nutrients, 4) enhances the proportion of algae in the pond biomass (typically 2.5–4:1), and 5) improves the lipid content and profile of the algal biomass in terms of lowering polyunsaturated fatty acid content [11–19]. Park and Craggs [15] found that WWT HRAP biomass productivity was improved by 30–50% by CO<sub>2</sub> addition and maintaining the day-time maximum pond water pH below 8 during summer conditions. Sutherland et al. [11] investigated the effects of CO<sub>2</sub> addition on wastewater microalgae performance showing that, in summer, the biomass concentration and the algal biovolume of CO<sub>2</sub> enriched HRAMs (pH 6.5) were enhanced by 22–45% and 100–560%, respectively compared with HRAM without CO<sub>2</sub> addition. They also found similar results under winter conditions where, the maximum photosynthetic rate, biomass concentration and algal biovolume increased by up to 172%, 20% and 181%, respectively in 2% and 5% CO<sub>2</sub> HRAMs [17]. As stated earlier, the culture CO<sub>2</sub> concentration also affects algal lipid content. Sun et al. [20] reported >100% enhancement of the lipid content of batch cultures of *Chlorella sorokiniana* sparged with a 10% CO<sub>2</sub>-air mixture compared to control cultures which were sparged with air.

While several studies have demonstrated the positive effects of CO<sub>2</sub> addition on wastewater treatment HRAP performance, there is a lack of research on how CO<sub>2</sub> addition influences specific performance parameters (including productivity, chemical composition, energy content and biomass harvestability) of low-cost biomass energy production in WWT HRAP. Hence, this paper investigates the hypothesis that increasing the CO<sub>2</sub> concentration in wastewater treatment HRAP will enhance the total and gravity harvestable biomass energy yield.

## 2. Materials and methods

### 2.1. Experimental set-up

To study the effect of different CO<sub>2</sub> addition concentrations on the biomass energy yield of HRAMs, two outdoor experiments were conducted using fifteen replicate foil-wrapped plastic mesocosms (water depth: 0.3 m; volume: 16 L; surface area: 0.06 m<sup>2</sup>) at the Ruakura Research Centre, Hamilton, New Zealand (37°47'S, 175°19'E). The mesocosms were wrapped to ensure sunlight only entered through the water surface. Experiment 1 was carried out in January 2014 (NZ

summer) over 21 days and Experiment 2 was conducted in July and August 2014 (NZ winter) over 30 days. The HRAMs were inoculated from an adjacent pilot-scale HRAP and they were mixed continuously using magnetic stirrers. During the summer experiment the mesocosms were operated with a 4 day HRT by replacing 4 L of culture with primary settled sewage every morning (at ~9 am). During the winter experiment the mesocosms were operated with an 8 day HRT by daily replacement of 2 L of culture with primary settled sewage. The experiments were conducted without control of zooplankton grazers or dominant algal species.

Supplementary carbon was supplied to the cultures in the form of air:CO<sub>2</sub> mixtures. The CO<sub>2</sub> addition system consisted of four CO<sub>2</sub> gas cylinders, a gas regulator, a gas flow meter (0–12 L·min<sup>-1</sup> range), an air pump and gas diffusers (Fig. 1). The CO<sub>2</sub> gas was blended with air (via the air pump) to provide different CO<sub>2</sub> concentrations including air (control mesocosm), 0.5%, 2%, 5% and 10%. The sparged CO<sub>2</sub> concentration and culture pH were measured regularly during the daytime using a portable gas analyser (Biogas 5000, Geotech) and pH meter (TPS WP-91, TPS Pty. Ltd., Springwood Australia) respectively. The gas blends were continuously bubbled into the cultures at 10 L·min<sup>-1</sup> through a gas diffuser placed on the bottom of the HRAMs. Daily air temperature, solar radiation, evaporation and rainfall data were downloaded from NIWA's National Climate Database (<http://cliflo-niwa.niwa.co.nz/>).

### 2.2. Measurement of nutrient concentration

Concentrations of dissolved nutrients (Ammonium (NH<sub>4</sub><sup>+</sup>-N), nitrate and dissolved reactive orthophosphate (PO<sub>4</sub><sup>3-</sup>-P, DRP)) in the HRAM influent and effluents were analyzed twice a week. Samples were filtered through Whatman GF/F filters and concentrations of ammonium (NH<sub>4</sub><sup>+</sup>-N), nitrate (NO<sub>3</sub><sup>-</sup>-N) and DRP were determined colorimetrically according to standard methods (APHA 2008) using a spectrophotometer (HACH RD2008, Germany).

### 2.3. Algal relative abundance

The relative abundance (%) of algal species present in the HRAM was determined by comparing their biovolume as described previously [5]. Biovolume was calculated by counting the numbers of cells of each algal species and multiplying by the mean cell biovolume for that species, assessed according to the equations of Vadrucci et al. [21].

### 2.4. Measurement of biomass chlorophyll a (chl-a) content

The biomass HRAM effluent chl-a content (as a proxy for the proportion of algae in the biomass) was determined spectrophotometrically using a Shimadzu UV 1601 spectrophotometer as described previously [5]. The chl-a content of biomass filtered from a known volume of HRAM effluent was extracted by boiling in methanol at 65 °C for 5 min and then refrigerating at 4 °C in the dark for 12 h. Chl-a

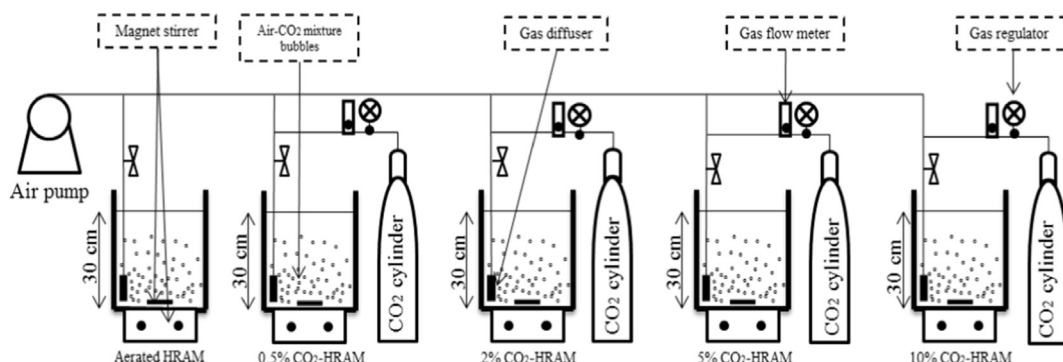


Fig. 1. Schematic diagram of high rate algal mesocosms (HRAMs) supplemented by different air:CO<sub>2</sub> mixtures (triplicates not shown).

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