



# Variation of biomass energy yield in wastewater treatment high rate algal ponds



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

Wastewater treatment high rate algal ponds (WWT HRAPs) have been recently highlighted as a potential system for low-cost algal energy production since the algal biomass is essentially a free by-product of the wastewater treatment process. This paper investigates the biomass energy yield potential of WWT HRAP (calculated by multiplying biomass productivity and biomass energy content). We address experimentally, for the first time, the influence of algal species dynamics, zooplankton grazing, environmental conditions, biomass chemical composition and biomass algal proportion on overall biomass energy yield. Two parallel identical pilot-scale HRAPs (8 m<sup>3</sup> volume, 0.3 m depth and 31.8 m<sup>2</sup> surface area) were operated for one year and monitored each week for biomass productivity and energy content. Pond effluent nutrient concentration, microalgal relative abundance, biomass chemical composition and chlorophyll-a content were all measured and their effect on biomass productivity and energy content was assessed. The algal species composition and algal proportion in the HRAP effluent varied with season and grazing pressure. The highest biomass lipid content (45wt%) was achieved when effluent ammonia concentration was lowest (<1 mg·L<sup>-1</sup>). Biomass productivity depended on season and zooplankton grazing pressure and biomass energy content increased algal proportion and lipid content of the HRAP biomass. The average biomass energy yield in the HRAPs was 113.3 kJ·m<sup>-2</sup>·day<sup>-1</sup> (based on the average annual biomass energy content of 19.2 kJ·g<sup>-1</sup> and the mean annual HRAP biomass productivity of 5.9 g VSS·m<sup>-2</sup>·day<sup>-1</sup> during the year). Biomass energy yield increased significantly during summer (175 ± 5 kJ·m<sup>-2</sup>·day<sup>-1</sup>) compared to winter (68 ± 18 kJ·m<sup>-2</sup>·day<sup>-1</sup>) since summer environmental conditions were more favorable for biomass growth. Results suggest improving algal proportion and productivity would promote biomass energy yield in WWT HRAP by enhancing biomass energy content and productivity concurrently.

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## 1. Introduction

There is renewed interest worldwide in replacing fossil transportation fuels with algal-based biofuels. Microalgal biomass was first suggested as a feedstock for biofuel production in the 1960s [1]. It can be converted to various kinds of biofuel including: biogas by anaerobic digestion of the whole biomass, bio-oil through thermochemical conversion of the whole biomass, biodiesel by transesterification of the lipid fraction, and bioethanol via fermentation of the carbohydrate fraction [2–4].

Although intensive research has been conducted to try to make algal-based biofuel production an economic reality, there are still many obstacles across the entire process (from cultivation to conversion) [5–10]. The major costs of algae cultivation for biofuel production are: capital cost of algal production system, fertilizer and chemicals, and

pumping of water for cultivation; biomass harvest and dewatering (which have high energy demands as algal species are small (<30 μm) and pond medium is >99% water); and algal biomass biofuel conversion pathways for which there are specific technological limitations [4,6, 11–14]. Even low-cost algal production systems (open raceway ponds) are not yet economical for biofuel production alone and combining algal biofuel production with wastewater treatment is considered to be the most promising option.

Wastewater treatment high rate algal ponds (WWT HRAPs), as part of an advanced treatment pond system, offer a niche opportunity for low-cost algal biomass production since the algal cultivation and harvest costs are included in the wastewater treatment operation. In particular, addition of nutrient fertilizer is not required for microalgal cultivation on human and animal wastewater, and the biomass (algal/bacterial flocs) is relatively easily harvested and may be concentrated to 2 wt% solids by simple gravity sedimentation [3]. Therefore, WWT HRAP could make community-level low-cost algal biofuel production feasible by producing and harvesting biomass as a by-product of the WWT plant.

E-mail addresses: [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).

Efficient wastewater treatment with nutrient recovery and low-cost biofuel production both rely on maximizing algal productivity, which in the context of biofuel production means maximizing energy production. Therefore, any practical strategies that improve the algal yield from wastewater HRAPs should also benefit energy production. The biomass energy yield in WWT HRAP is a function of the biomass productivity and its energy content. Both are affected by the dominant algal species, the proportion of algae in the biomass and chemical composition of the biomass. However, these factors are limited by environmental (light and temperature), operational ( $\text{CO}_2$  concentration, nutrient concentration, cultivation mode, hydraulic retention time, mixing, and algal recycling) and biological conditions (algal species contamination and grazer occurrence) [4]. Therefore, to produce sustainable low-cost energy in the form of biomass in WWT HRAP, a greater understanding of the factors which affect productivity and energy content of biomass is essential.

Several studies have reported the biomass productivity potential of the WWT HRAP [7,15–16] and suggested a number of strategies such as  $\text{CO}_2$  addition, biomass recycling and controlling zooplankton grazers to promote HRAP biomass productivity and culturing under nutrient-limiting conditions that might improve microalgae energy content [5, 11,17–19]. However, these have either been short-term studies in outdoor pilot-scale systems or been carried out under controlled laboratory conditions with little focus on biomass energy yield from WWT HRAP and how it is influenced by different factors. In this paper, the biomass productivity and energy content of two identical WWT HRAPs operated in parallel was measured over a whole year. The variation of biomass energy yield potential of WWT HRAP was related to factors including: biomass chemical composition; algal proportion; algal species dynamics; environmental conditions and zooplankton grazing.

## 2. Materials and methods

### 2.1. Environmental variables and HRAP operational parameters

The current study involved operating and sampling two identical pilot-scale WWT HRAPs in parallel (West (WHRAP) and East (EHRAP)) to assess HRAP biomass energy yield potential. The ponds were located at the Ruakura Research Centre, Hamilton, New Zealand ( $37^{\circ}47'S$ ,  $175^{\circ}19'E$ ). Each HRAP was a single-loop raceway with sloped embankments, separated by a central baffle with a depth of 30 cm, surface area of  $31.8 \text{ m}^2$  and total volume of  $8 \text{ m}^3$ . The pond water was circulated with a mean surface velocity of  $0.15 \text{ m} \cdot \text{s}^{-1}$  using a paddlewheel. The HRAPs received  $0.5\text{--}1 \text{ m}^3 \cdot \text{day}^{-1}$  of primary settled domestic wastewater at hourly intervals that was pumped from the Ruakura sewer. The pond hydraulic retention time (HRT) was varied with season from 8 days in winter (Jun.–Aug.) by diluting the influent with tap water (to simulate recycling of treated effluent). During spring (Sep.–Nov.) and autumn (Mar.–May) the HRT was maintained at 6–6.5 days while in summer (Dec.–Feb.) the HRT was maintained at 5 days.

To avoid free ammonia inhibition and carbon limitation, the maximum pH of the HRAPs was maintained below 8 by  $\text{CO}_2$  addition.  $\text{CO}_2$  was automatically injected into the pond water when the pH exceeded 8 and stopped when pH was less than 7.8. The HRAP effluent flowed by gravity from the pond bottom into 250 L settling tanks, from which the settled biomass was harvested daily using a peristaltic pump (Masterflex, Cole-Parmer, HV-07523-60). The HRAPs were run with no control of the dominant algal species or of the zooplankton population. Further details of the HRAP construction and operation were previously described in Park et al. [13] and Park and Craggs [18].

The pH, dissolved oxygen (DO) and temperature of the HRAP water were continually measured using a DataSonde 4a (Hydrolab, HACH Environment, USA). The data were logged at 15 min intervals using a data logger (CR10X, Campbell Scientific Inc., UT, USA) and downloaded weekly. Over the course of study, daily climate data (solar radiation, evaporation and rainfall) were downloaded from NIWA's National Climate Database (<http://cliflo-niwa.niwa.co.nz/>).

### 2.2. Measurement of water quality

Concentrations of water quality variables in the pond influent were periodically analyzed according to standard methods [23]. The pond water ammoniacal-N concentration was sampled weekly as this was the main form of nitrogen in the primary settled sewage [21]. Since the N:P ratio of the domestic wastewater is usually 6:1, which is lower than the N:P ratio of the algal biomass (often 16:1) [7,22], the effect of phosphorus was not considered in this study. Pond influent and water samples were filtered through Whatman GF/F filters (with  $0.7 \mu\text{m}$  pore size) and the concentration of ammonium ( $\text{NH}_4^+\text{-N}$ ) was determined colorimetrically [23] using a spectrophotometer (HACH RD2008, Germany).

### 2.3. Algae assessment and relative abundance

HRAP algal species and their relative abundance were determined weekly during the experimental period using the methodology developed by Park et al. [13]. A well-mixed sub-sample of pond water was settled in an Utermöhl chamber (diameter: 25 mm–volume: 10 mL). Three random pictures were taken using a microscope Leica DM 2500, equipped with a Leica DFC 420 digital camera (Leica Microsystem, Switzerland). The procedure was repeated three times and a total of nine pictures were taken for each HRAP. The numbers of cells of each algal species were counted and multiplied by the mean cell biovolume to obtain their relative abundance. The mean biovolume ( $\mu\text{m}^3$ ) of each algal species was assessed according to Vadrucci et al. [24] equations by measuring the size of 150 cells/colonies using the freeware software “ImageJ” V 1.43u.

### 2.4. Measurement of chlorophyll-a

The biomass chl-a content (which can be used as an indicator of the proportion of algae in the HRAP biomass) was determined spectrophotometrically using the monochromatic equations for methanol of Ritchie [25]. A known volume of pond water was filtered through a 25 mm Whatman GF/F filter (with  $0.7 \mu\text{m}$  pore size), and the filter was placed in a centrifuge tube with 10 mL of pure methanol and placed in a water bath and boiled at  $65 \text{ }^{\circ}\text{C}$  for 5 min. The tubes were cooled and then refrigerated at  $4 \text{ }^{\circ}\text{C}$  in the dark for 12 h for full chlorophyll extraction. The tubes were then centrifuged at 2000 rcf for 15 min and the absorbance of the supernatant was measured using a Shimadzu UV 1601 spectrophotometer.

### 2.5. Measurement of biomass lipid, carbohydrate and protein composition

Samples of HRAP effluent were taken at weekly intervals and the biomass concentrated by centrifugation (2000 rcf, 10 min). The biomass was frozen until the lipid, carbohydrate and protein content was analyzed.

Total lipids were extracted based on a modified procedure adopted from the Bligh and Dyer method [26] and measured gravimetrically. A 20–30 mg sample of the centrifuged frozen biomass was placed in a centrifuge tube with a 20 mL mixture of distilled water, methanol and chloroform (1:2:1). The centrifuge tube was placed horizontally on a shaking table overnight (~6-cm oscillation at ~2 cycles per second). An additional 5 mL of chloroform and 4 mL of distilled water were then added to the tube to give a final ratio of water:methanol:chloroform of 0.9:1:1. The tube was then vortex mixed for 30 s and centrifuged at 3500 rcf for 10 min. Most of lipids are soluble in the chloroform and form a dense layer at the bottom of the centrifuge tube. The remaining cell debris creates the middle layer, while the methanol and water create the top layer. The lipid–chloroform layer was removed using a pipette and then filtered through a GFF filter and transferred into a pre-weighed glass tube. A second and occasionally third re-extraction was conducted by adding another 5 mL of chloroform to the remaining

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