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Partitioning of wastewater treatment high rate algal pond biomass and particulate carbon

Karl.A. Safi *, Jason.B.K. Park, Rupert.J. Craggs

National Institute of Water and Atmospheric Research, P.O. Box 11-115, Hamilton, New Zealand

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ABSTRACT

The partitioning of algae, bacteria, grazers and detritus in two wastewater treatment high rate algal ponds (HRAPs) was investigated in relation to particulate carbon (PC) over a year. Algae dominated the pond biomass accounting for ~61% of the PC. Changes in algal biomass correlated with changes in PC with both varying seasonally and having spring or summer maxima. The algal biomass itself was dominated by larger cells or colonies in the 20–200 μm size fraction with *Pediastrum* sp. prominent. Bacterial biomass, in contrast, only accounted for ~13.5% of the PC and varied less seasonally. Grazer biomass was lowest at ~4% of the PC on average and was dominated by either zooplankton or microzooplankton. Grazer biomass however, varied the most and reached ~14% of the PC during a spring zooplankton bloom that markedly reduced algal biomass. The remaining ~21.5% of the PC was made up of dead algal, detrital matter, and mucilage that tended to aggregated into bio-floccs. The PC of an efficiently operating HRAP is shown to be driven by high algal biomass with low bacterial and grazer biomass. If this balance is lost grazers may grow to levels that enable them to reduce algal biomass and productivity compounding pond instability.

1. Introduction

High rate algal ponds (HRAPs) are a promising technology for wastewater treatment as they provide improved treatment over conventional wastewater stabilisation ponds and have potential biofuel production applications [1–3]. HRAPs are shallow (~0.3 m deep) raceway ponds with gentle mixing (mean horizontal water velocity ~ 0.15–0.3 m/s) typically provided by a paddlewheel [4,5]. These ponds are specifically designed to promote algal growth and assimilation of soluble carbon and nutrients from wastewater. The oxygen produced during photosynthesis also promotes the breakdown of organic compounds by heterotrophic bacteria [5]. Therefore, HRAPs have similarities to well mixed super-eutrophic natural waterbodies as both favor microalgae production.

Different environmental (light and temperature), operational (mixing, DO, pH, CO_2 and nutrients) and biological (zooplankton grazers and algal pathogens) conditions influence the standing crop of HRAP biomass and overall productivity. The fixed or particulate carbon (PC) in HRAPs is contained within the various organic components including algae, bacteria, protozoans (ciliates and flagellates), zooplankton (rotifers and cladocerans), and other organic matter (e.g. dead algal cells, detrital matter, and mucilage). The component composition of the biomass, in turn, significantly influences productivity, settleability, and PC. Recent pilot-scale studies have focused on optimizing HRAP design and operation to achieve maximum algal production, algal biomass dominance and optimal harvest composition [3,4,6,7]. Under optimal conditions HRAP biomass will be dominated by typical eutrophic water algal species growing as large settleable colonies (colony diameter of 50–200 μm), that form large bio-floccs (diameter: >500 μm) associated with bacteria and other organic matter [8]. The dominance of settleable colonial algae is desirable in HRAP's as it improves biomass harvest efficiency. In contrast ponds that are operating inefficiently may have low algal biomass; be dominated by small non-flocculating single celled algae; or have high bacterial or grazer biomass [4].

In natural waterbodies algae populations are controlled by grazers which are predated on by fish and other higher level predators, transferring algal biomass/carbon through the food web to higher trophic levels. HRAP algal biomass and production are maximized when grazer populations and grazing pressure are low. However, the lack of fish and other predators in HRAPs, means that they are susceptible to grazing by "blooms" of herbivorous zooplankton (e.g. rotifers and cladocerans) or protozoans (e.g. amoebae, ciliates and flagellates) particularly when small algae dominate. While some common zooplankton species (e.g. rotifers) have a preference to graze on small algae, ~8 μm [9], larger zooplankton (e.g. *Moina* sp.) can graze even the large colonial algae in HRAPs [10]. For example, a previous pilot-scale HRAP study [3] reported that algal biomass concentration was substantially reduced from ~250 to <20 mg/L within 4 days when a population of *Moina* sp. increased above ~500 individuals/L. Large *Pediastrum* sp. colonies (~20–200 μm) dominated the algal biomass at this time indicating that grazing pressure by *Moina* sp. was effective even on large colonial algae. Since large zooplankton grazers have much slower growth rates than algae

* Corresponding author.
E-mail address: karl.safi@niwa.cri.nz (K.A. Safi).

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[11] they can only affect the algal population if given sufficient time to increase their numbers. In contrast, protozoan grazers are capable of growing at similar rates to the smaller (<20 μm) algae on which they graze [12], which may be one reason why smaller algae are less prevalent in HRAPs. Protozoans also graze on bacteria which may assist in keeping bacteria from dominating pond biomass [13].

In addition to grazing HRAPs are also vulnerable to other biological influences such as fungal parasitism and viral infection which can also significantly reduce the algal population within a few days and trigger changes in algal population structure, diversity and succession [14].

A technique to further understand the relationships between organisms and their role in natural water body food-webs is to partition them in terms of their carbon biomass [15–17]. These studies have led to generalizations about the relative importance of different planktonic food web components such as: grazing versus microbial pathways; metazoan versus protozoan grazers; and algae versus bacteria as carbon sources in natural systems. The relative contribution of different organisms to the biomass of wastewater treatment HRAPs and other man-made super-eutrophic systems has not previously been quantified. There is limited information available on the partitioning of food-web components within HRAPs and the relative contribution of each component to PC has not been studied before.

The main purpose of this study was to investigate over one year the changes in the relative contribution of the different biomass components to PC in wastewater treatment HRAPs. Specifically we aimed to; (*i*) identify the size classes of organisms and relative contribution to PC; (*ii*) identify the algal, bacterial, grazer and other components of HRAP biomass and determine their relative contribution to PC; (*iii*) describe any seasonal changes, and; (*iv*) identify the effects of grazing on the pond biological community.

2. Materials and methods

2.1. Experimental pilot-scale high rate algal ponds

The experiment was conducted over one year (from April 2009 to March 2010) using two identical pilot-scale single-loop raceway HRAPs treating domestic wastewater at the Ruakura Research Centre, Hamilton, New Zealand (37°47′S, 175°19′E). Each HRAP had a surface area of 31.8 m^2, a depth of 0.3 m and a total volume of 8 m^3 with semi-circular end walls and with a dividing baffle separating the two raceway channels. The pond water was circulated around each raceway at a mean velocity of ~0.15 m/s by a paddlewheel. More detailed specifications of the HRAPs are described in a previous study [7].

The HRAPs were operated at different hydraulic retention times (HRT) depending on season to account for changes in environmental parameters such as light and temperature and their influence on wastewater treatment and algal growth. Algal production was maintained throughout the year by altering the HRAP HRT from 8 days in winter (June to August 2009) to 4 days in summer (November 2009 to March 2010) by using inflows of 1 and 2 m^3/day respectively. In summer the influent sewage was diluted 1:1 with tap water to reduce nutrient concentration while maintaining the same nutrient load. During the NZ autumn (March–May 2009) and throughout spring (Sept–Nov 2009) the HRT of the HRAPs was maintained at 6 days by using an inflow of 1.3 m^3/day of diluted sewage. CO_2 was added to the HRAPs during the day to maintain the maximum pH below 8.0 to avoid carbon limitation and free ammonia inhibition [6]. Settled biomass was removed daily from an algal harvester (with an HRT of 3–6 h depending on season) and a portion of the gravity harvested algae (*Pediastrum* sp. dominant) was recycled back to one of the HRAP ($HRAP_r$ with recycling) at a recycling ratio of ~10% of daily algal production. The second HRAP was operated as a control without recycling ($HRAP_c$) with all other operational parameters the same as the $HRAP_r$. These operational differences were designed to maintain the dominant algal species, as well as the biomass levels, productivity and settleability of algal populations within the HRAPs. Further details are described in previously published work using these HRAPs [3,4].

2.2. Sampling, physical and chemical analysis including carbon

Pond water physical properties such as dissolved oxygen (DO), pH and water temperature were recorded at 15 min intervals using a multiprobe DataSonde® (Hydrolab, HACH Environment, USA) coupled with a datalogger (CR10X, Campbell Scientific Inc., UT, USA) (Fig. 1). Daily sunlight radiation, air temperature, and sunshine hours were downloaded from the NIWA climate database for the Ruakura NIWA/AgResearch Station, Hamilton, New Zealand (37°84′70″S, 175°81′90″E), (http://cliflo-niwa.niwa.co.nz/), (Fig. 1).

Samples of HRAP water (1 L) were taken at monthly intervals for one year from April 2009 to March 2010. 100 ml subsamples were taken from the 1 L sample and preserved with Lugol's Iodine solution (1% final concentration) for the analysis of algae, microzooplankton, zooplankton and an estimate of detritus (i.e. large bio-flocs containing dead algal cells, other detrital organic matter and mucilage). Two millilitre subsamples were also taken from the 1 L sample for the analysis of picophytoplankton and bacteria by either flow cytometry or for direct microscopic exanimation. Additional subsamples were taken for duplicate analysis of Chlorophyll-*a* (Chl-*a*) and particulates which were analysed following Standard Methods [18].

2.3. Cell counting, biovolume calculation, and carbon conversion

2.3.1. Phytoplankton

Up to 10 ml of the Lugol preserved subsamples was examined using a Leica inverted microscope (DMI 3000 B) to identify and count >2 μm size algae (Table 1). For enumeration, samples were settled in Utermöhl chambers for >4 h, and then identified and counted with the microscope at 100× to 600× magnification. The dimensions of each taxon were measured (except *Pediastrum* spp.) and the biovolume estimated from approximated geometric shapes (spheres, cones, ellipsoids) following [19,20]. The calculated biovolumes of algae were then used to determine algae cell carbon (μg C/L) using the conversion equations of [21] for different algal groups (Green algae, Diatoms, Dinoflagellates; see Table 1). Biovolumes of the colonial algae *Pediastrum* spp. were determined using a microscopic image analysis technique and details of this method are given in a previous publication [22]. Images were taken with a Leica microscopic camera (DFS 420c) and the dimensions (length and width) of each colony were measured using microscopic image analysis software (Leica Application Suite, LAS version 3.1.0) *Pediastrum* cell carbon was calculated by dividing the measured average colony biovolume by the numbers of cells per colony (8, 16, 32, 64). This was then converted to carbon using the regression equation for chlorophytes (green algae, see Table 1) [21]. Picophytoplankton were examined in duplicate either using Flow Cytometry following the methods [23], or direct counts made using autofluorescence [24]. Cell carbon for eukaryotic picophytoplankton was determined by estimating the average spherical diameter and converting to yield a factor of 820 fg C/cell, while for the cyanobacteria, *Synechococcus* type sp. a factor of 250 fg C/cell was used [12].

2.3.2. Bacteria

Duplicate bacterial samples (~2 ml) were frozen in liquid nitrogen and thawed immediately before counting. Initially counts by flow cytometry underestimated bacterial numbers due to clumping, attachment to mucilaginous cell surfaces and inclusion in aggregated matter even after sonification. To overcome these issues, samples were examined under the microscope after staining with Acridine Orange [29]. Bacteria cell carbon was then calculated using a conversion factor of 108 fg C/cell [30]. This was based on the measured average cell biovolume. As the bacteria are being cultured in a medium that is high in dissolved organic matter they have a larger average cell size than