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Control of zooplankton populations in a wastewater treatment High Rate Algal Pond using overnight CO_2 asphyxiation

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A B S T R A C T

High Rate Algal Ponds (HRAPs) with addition of CO_2 are open pond wastewater treatment systems that recover nutrients as microalgal biomass. Such ponds are vulnerable to contamination by opportunistic zooplankton species able to survive the wastewater HRAP environment. The high food availability and a near neutral pH can promote the rapid development of high densities of zooplankton that can reduce treatment performance by consuming microalgae. Zooplankton control using night time CO_2 asphyxiation was selected from promising zooplankton control methods previously screened at laboratory and mesocosm scales, and used to control zooplankton densities in an 8 m^3 HRAP over 14 months. Increasingly higher flow rates (1 to 6 L/min) of pure CO_2 were tested by using 13 control treatment events. CO_2 was injected during night time, and treatment events were repeated for a number of consecutive nights sufficient to control zooplankton density to $\leq 10\%$ of that before treatment. Treatments with higher CO_2 flow rates promoted more rapid reductions of zooplankton density (12 nights to 1), and were associated with higher maximum CO_2 concentrations (100 to 420 mg/L), and lower pH ($\sim$6 to $\sim$5). Compared to the control HRAP, CO_2 treatment decreased the average population densities of some zooplankton species over the experimental period: *Moina tenuicornis* (41.3%), *Paracyclops fimbriatus* (43.9%), *Filinia longiseta* (59.8%), but was associated with higher average population densities of others: *Heterocypris incongruens* (174.4%), *Asplanchna sieboldi* (177.8%), *Cephalodella catellina* (200.0%), and *Brachionus calyciflorus* (234.9%). However, the population densities of the rotifers *B. calyciflorus* and *C. catellina* were always reduced following CO_2 treatments with flow rates $\geq$ 2 L/min. The cladoceran *Daphnia thomsoni* and the rotifer *Brachionus urceolaris* established only in the control HRAP. Zooplankton control by CO_2 asphyxiation improved the overall performance of the treated WW HRAP compared to the control in several ways, including increasing algal biomass (VSS) (150.8%), productivity (151.4%), chlorophyll-*a* concentration (161.8%), particle size (MCSA) (115.8%), and average settleability efficiency (189.2%). Overnight CO_2 asphyxiation showed the potential to control zooplankton and to promote better WW HRAPs performance.

1. Introduction

High Rate Algal Ponds (HRAPs) are 200–500 mm deep closed-loop, paddlewheel-mixed ponds of up to a few hectares in size [1], used to provide economical and efficient near tertiary-level wastewater (WW) treatment [2,3] as well as reclaim water, nutrients and energy from organic wastes. Algal biomass can be recovered in harvest ponds by gravity settling of mainly colonial microalgae associated with bacterial flocs, and can be used for biofuel production, fertilizer and animal feed [4,5]. Before being discharged into the environment, the algal harvest pond effluent may be further treated in a series of maturation ponds where zooplankton graze on the remaining microalgae still suspended in the water. HRAPs operated with CO_2 addition for pH control and to provide additional carbon for microalgal growth have a pH between 7 and 8, and offer an ideal environment for contamination and development of high densities of zooplankton [6]. Moreover, HRAPs have a high concentration of food (mainly bacteria and microalgae), and lack higher predators such as fish that can consume zooplankton, which further contribute to the establishment of zooplankton species that can survive WW conditions. Once established, zooplankton that can ingest the dominant microalgae, often rapidly consume the microalgal biomass [7,8], and reduce the productivity and the nutrient removal capacity of HRAPs [6].

The necessity to control zooplankton densities in WW HRAPs is

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widely recognized [9,10,11,12,13,14], and required for both consistent WW nutrient removal and microalgal productivity [15]. Zooplankton control methods should not reduce microalgal growth because algal biomass is essential for the WW nutrient removal, and should not disrupt the structure of colonial algae and algae-bacterial flocs because large particles are essential for a good settleability of the suspended biomass [16]. In particular, the effect of the zooplankton control methods should be limited to the HRAPs, and not reduce the zooplankton density in maturation ponds into which they flow, where they provide an important function in further polishing the HRAP effluent. Potential options for zooplankton control such as filtration [17,18,19], centrifugation [9], heating [20], cavitation [21,22], UV radiation [23], increased concentration of CO_2 [15], deoxygenation [24], un-ionized ammonia toxicity [25,26,27], biocides [28,29,30,31], chitinase inhibitors [32], altering hydraulic retention time (HRT) [33], and biocontrol using competing or carnivorous zooplankton [34,35], have been previously proposed. However, only a few of these zooplankton control methods (e.g., filtration, un-ionized ammonia toxicity, and use of biocides) have been used for zooplankton control in HRAPs [33,27,36]. Zooplankton control methods should control zooplankton populations to low levels, maintaining them as part of a stable community rather than totally eradicating them [6]. This is because moderate populations of zooplankton are expected to reduce the potential establishment of different zooplankton species that are less easy to control. For example, biotic resistance (the ability of a native community to keep out newly arriving species) from existing zooplankton has been shown to play an important role in reducing the establishment rates of new zooplankton arriving at ponds, and promoting healthy populations of desired species may reduce the establishment rates of less desirable zooplankton [37]. Moderate densities of certain zooplankton species may also be beneficial because they can release chemicals and metabolites that can induce the formation of microalgae colonies and cells with spines [38]. This can reduce the capacity for grazers to ingest the larger food particles relative to single celled algae without spines [39,40,41], and increase the biomass settleability [33,42,43,44]. Zooplankton eradication should be avoided also because the high costs required to remove or kill all individuals is likely to be pointless when HRAPs are contiguous with other ponds (e.g., maturation ponds), and cross contamination occurs continuously. However, when contaminant zooplankton can rapidly consume dominant microalgal species, the control methods should rapidly (within 1–2 days) reduce the density of zooplankton to prevent severe reductions of HRAP biomass.

The efficacy of zooplankton control methods such as CO_2 asphyxiation, hydrodynamic shear stress, filtration, and biocontrol using competing cladocerans and ostracods were previously assessed in laboratory experiments using batch microalgae and zooplankton cultures [45]. Control methods were then validated under typical WW HRAP physical and chemical (nutrient concentration, pH, temperature, light radiation), and operational (HRT, mixing, CO_2 addition) conditions using outdoor mesocosms operated as semi-continuous cultures [46]. Asphyxiation using CO_2 was the most versatile, selective, and effective zooplankton control method. Other researchers have used CO_2 addition to kill zooplankton in experimental enclosures in the form of dry ice [47], to reduce the zooplankton density in 1.5 m^3 microalgae cultures bubbling pure CO_2 [48], and in a high CO_2 gas mixture (2% O_2; 12% CO_2; 84% N_2) it was used to kill copepods and crustaceans in 1.5 L experimental enclosures [49]. However, to date, successful use of CO_2 to control zooplankton in large HRAPs has not been demonstrated.

Here we compare the performance and zooplankton community dynamics of paired 8 m^3 HRAPs, where one HRAP was treated with night time injection of CO_2 to control zooplankton density, and the other HRAP was untreated as a control, over a period of 14 months. The zooplankton community, the biotic interactions between grazers and microalgae, and the performance of the HRAPs in terms of biomass productivity and settleability were monitored. Prior to this experiment, the two HRAPs had been monitored in terms of zooplankton dynamics and WW treatment performance for a period of 14 months to assess their similarity in performance and zooplankton dynamics when both were zooplankton control methods were not in place [6]. A protocol for zooplankton management in WW HRAPs is proposed based on all our experimental work.

2. Material and methods

2.1. Operation of the paired HRAPs

The two identical WW HRAPs (West and East) were located at the Ruakura Research Centre, Hamilton, New Zealand (37°46′29.5″S - 175°18′45.4″E). Each HRAP consisted of a single-loop raceway with a central baffle, lined with black high-density polyethylene (HDPE) plastic, with semi-circular ends, a depth of 300 mm, a volume of 8 m^3, and a surface area of 32 m^2. Each pond was circulated at an average surface velocity of 0.15 m/s using a 1 m wide, steel paddlewheel with 8 blades. The HRAPs received 1 m^3/d of settled domestic WW collected from the main WW pump station at the Ruakura Research Centre, which was added at hourly intervals. In winter, when microalgal growth is reduced, 1 m^3 of settled WW was added to each HRAP daily to give a HRT of 8 days. In spring/autumn and summer the HRT was reduced to 5 days by dilution of the influent with de-chlorinated tap water to simulate recirculation of treated effluent from which the algae had been harvested [50]. CO_2 was automatically added to both HRAPs to control the pH to a maximum of 8. The CO_2 was stored in CO_2 gas cylinders (BOC Gas Ltd., New Zealand), equipped with gas regulators and flow meters (0–12 L/min range). The pond water pH was measured every 5 s with a pH probe (Sensorex mod. S265C/CD) and when the pH exceeded 8, CO_2 was bubbled into the ponds (2 L/min) using gas diffusers placed on the bottom of the HRAP downstream of the paddlewheel, until the pH was reduced to 7.8. The pH probes were calibrated monthly with pH standard solutions. The effluent flowed by gravity from a drainage outflow pipe located on the bottom of the HRAPs into 250 L settling tanks where the biomass suspended in the culture was settled and removed from the tank bottom daily using a peristaltic pump (Masterflex, Cole-Parmer, HV-07523-60, Chicago, USA). The supernatant flowed from the settling tank into a cascade of four maturation ponds where the resident zooplankton community consumed the remaining microalgae. At the beginning of the monitoring period, the two HRAPs were emptied, carefully cleaned to remove all zooplankton diapausing eggs in the sediment, and inoculated with the same assemblage of naturally occurring algae that had established prior to cleaning.

2.2. Sampling protocol and environmental, physical and chemical analyses

The suspended zooplankton and microalgae were sampled in front of the paddlewheel weekly at 09:00 am, using a 2.5 L bucket dipped into the water down to 50 mm from the HRAP bottom, and with the open end facing the paddlewheel. Diapausing eggs, copepods, and ostracods were collected from the HRAP bottom using 100 mL plastic cylindrical beakers with open tops (ø 60 mm), which were held in position by laboratory stands and clamps. The beakers accumulated settled material over 1 week, were placed in three low mixing (< 0.1 m/s water velocity) areas with high sedimentation [51,6], and were carefully capped with screw lids before being removed from the water. Daily solar radiation, evaporation and rainfall were downloaded from the NIWA National Climate Database (http://cliflo.niwa.co.nz/). The pH and temperature of the HRAPs were continually measured using a Datasonde 4a (Hydrolab, HACH Environment, CO, USA), and data were logged at 15 min intervals using a data logger (CR10X, Campbell Scientific Inc., UT, USA).