Algal Research 24 (2017) 40–63

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

Assessment of potential zooplankton control treatments for wastewater treatment High Rate Algal Ponds

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ARTICLE INFO

Article history:
Received 9 December 2016
Received in revised form 21 February 2017
Accepted 16 March 2017
Available online 23 March 2017

ABSTRACT

Cladocerans and rotifers rapidly consume beneficial microalgae and reduce the performance of High Rate Algal Ponds (HRAPs) for wastewater treatment and algal production. Potential zooplankton control treatments for HRAPs have been proposed and tested at a laboratory scale including CO_2 asphyxiation, biological control using competitor species, filtration, and mechanical disruption using hydrodynamic shear stress. This paper aims to validate these treatments using outdoor mesocosms with physicochemical and operational conditions similar to those of full scale HRAPs. A continuous CO_2 concentration of ~100 mg/L maintained low pond water zooplankton densities, while a continuous concentration of ~180 mg/L killed all microcrustaceans and rotifers present. As biocontrol agents, the cladoceran *Moina tenuicornis* at ~2000 individuals/L reduced average rotifer densities by 90% while the ostracod *Heterocypris incongruens* at ~1000 individuals/L removed all rotifers. Mechanical filtration using 300 μm and 500 μm filters eradicated *M. tenuicornis* after one and four filtration periods, respectively. Mechanical hydrodynamic stress killed up to 100% of microcrustaceans, and ~50% of larger rotifers. Furthermore, phototaxis-induced migration promoted higher densities of *M. tenuicornis* in the upper layer of the water column in an 8 m^3 HRAP during periods of low solar radiation, suggesting that mechanical treatments should be performed at night and to the upper layer of the pond water. Overall, CO_2 asphyxiation appeared to be the most reliable, versatile, and effective zooplankton control treatment.

1. Introduction

1.1. Zooplankton control in wastewater treatment HRAPs

High Rate Algal Ponds (HRAPs) with artificial CO_2 addition are simple reactors to reclaim nutrients and energy from wastewater (WW) as algal biomass, and provide higher productivity and nutrient removal compared to traditional pond systems [1,2]. However, being open systems with near neutral pH and high food concentration, HRAPs are particularly susceptible to contamination with zooplankton species that can establish and survive at high densities in the wastewater environment. When high densities of the zooplankton species consume the dominant microalgal species, they can reduce the microalgal biomass and negatively affect HRAP performance [3], reducing both productivity and nutrient removal [4].

HRAPs for microalgae cultivation have been used for over 70 years, although zooplankton contamination still limits their extensive use worldwide. Zooplankton control is widely recognized as necessary for efficient and consistent WW nutrient removal, algal productivity, and HRAP stability [5–9]. However, to date, the availability of treatments options for zooplankton control in hectare scale WW HRAPs is scarce.

Montemezzani et al. proposed potential options to control zooplankton in HRAPs [10]. Treatments included mechanical treatments such as filtration, hydrodynamic cavitation, shear stress and bead mills; chemical treatments such as CO_2 asphyxiation, promotion of the lethal un-ionized ammonia toxicity, use of biocides, and the chitinase inhibitor chitosan; and biocontrol treatments including competitor and predatory organisms such as the cladoceran *Moina tenuicornis*, the ostracod *Heterocypris incongruens*, and species of the carnivorous rotifer *Asplanchna*. However, these treatments were based on the literature review of existing technologies to control zooplankton in laboratory cultures, experimental ponds, ballast waters, and aquaculture systems, and were never assessed in microalgae cultures with conditions typical of WW HRAPs.

Options such as filtration, un-ionized ammonia toxicity, and use of biocides [11–13], have been used in pilot scale HRAPs with only

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http://dx.doi.org/10.1016/j.algal.2017.03.013
2211-9264/

moderate success. Some treatments have been also used to control zooplankton in smaller systems, or different pond system than HRAPs. For example acute application of CO_2 was used to inactivate zooplankton in experimental enclosures [14], and in 1.5 m^3 microalgae cultures [15]. The use of chemical substances to control zooplankton can also be effective, and have been previously reported [8,16–21]. However, chemicals are not usually applicable in WW HRAP systems because the beneficial zooplankton established in the maturation ponds could be killed by the residual toxic substances in the water that flows from the treated HRAPs.

Moreover, there are treatment strategies that are not practical due to the high cost required to treat large volumes of water and the detrimental effect that treatments have on microalgae. For example, moderate heating [22] kills zooplankton species [22–24], but also a large portion of the microalgae, and the energy cost required to increase the temperature of large amounts of water (3000–5000 m^3) of hectare scale HRAPs makes this treatment costly.

Zooplankton treatments should control zooplankton to low densities rather than completely eradicate them [4]. In particular, the eradication of larger zooplankton species such as cladocerans may reduce competition for shared food resources and mechanical interference, which naturally limit densities of less desirable smaller species, such as rotifers, that generally are more difficult to control [4,25]. Furthermore, moderate densities of filter feeding zooplankton species such as the rotifer *Brachionus* spp. and cladoceran *Moina* spp. can be beneficial by altering algal morphology to forms that enhance biomass harvestability [4]. Montemezzani et al. assessed potential treatments to control zooplankton density to desired levels at a laboratory scale using microalgae cultures collected from pilot HRAPs [25]. Acute injection of CO_2 into the water resulted in more rapid asphyxiation of cladocerans than rotifers, showing potential for use to selectively control particular types of zooplankton. Moreover, the high CO_2 concentrations in water associated with the zooplankton treatment are expected to increase microalgal growth [26] and productivity, by providing additional carbon for the photosynthetic activity. Biocontrol using *M. tenuicornis* was effective in reducing smaller species of rotifers whereas biocontrol using *H. incongruens* severely reduced the densities of all rotifer species, particularly in high mixing conditions to ensure they were brought into contact. Hydrodynamic shear stress was more effective in killing cladocerans (by disrupting their large, brittle exoskeleton) than smaller soft-bodied rotifers, showing the potential to select for larger zooplankton species. However, these treatments needed to be assessed in microalgae cultures with physicochemical (nutrient concentration, pH, temperature, light radiation) and operational (hydraulic retention time (HRT), mixing, CO_2 addition) conditions typical of WW HRAPs. This study is aimed to validate the treatments previously tested at laboratory scale [25], using outdoor mesocosms with physicochemical conditions typical of WW HRAPs.

2. Material and methods

2.1. Experimental set up

Each experiment was conducted with triplicate treatments and controls using outdoor 20 L mesocosms. Mesocosms had a water depth of 300 mm, liquid volume of 16 L, and water surface area of 0.06 m^2. They were foil-wrapped to prevent light penetration through the sides, mixed and aerated with aquarium air stone spargers (100 × 15 mm), using a Hailea ACO 160 W air pump, with maximum flow rate of 145 L/min and 160 W power. The flow rate was ~10 L/min per mesocosm and the air bubbles were sufficiently large to avoid their entrapment under the carapace of cladocerans and resulting flotation of individuals. The mesocosms were located at the Ruakura Research Centre, Hamilton, New Zealand (37°46′29.5″S - 175°18′45.4″E), adjacent to two 8 m^3 pilot-scale WW HRAPs (West and East) which were the source of microalgae and zooplankton used in the experiments. The pilot-scale WW HRAPs were single-loop raceways with semi-circular ends lined with black high-density polyethylene (HDPE) plastic, with a depth of 300 mm, volume of 8 m^3, mixed with an 8 blade steel paddlewheel (1 m wide), average surface velocity of 0.15 m/s, pH controlled between 7 and 8 by addition of CO_2, and a HRT of 8 days achieved by addition of 1 m^3/d of settled domestic WW. The pH of mesocosm cultures was maintained between 7 and 8 by continuous addition of ~0.2% CO_2 v/v in air. A four day HRT was used during January (Austral summer) (CO_2 summer experiment) and an eight day HRT was used during March–April and July–September (Austral autumn and spring) (all remaining experiments), which were achieved with daily (~9 am) replacement of 2 and 4 L of mesocosm culture with primary settled WW.

2.2. Specific treatment conditions

All the treatments tested were chosen based on their minimal negative environmental impact; potential selectivity for particular zooplankton taxonomic groups; cost effectiveness, and lack of negative effects on the beneficial zooplankton communities present in downstream maturation ponds of WW HRAP systems.

We exposed cladoceran and rotifer populations to increasing chronic (1–2 month) CO_2 concentrations and hydrodynamic stress intensities, incubated zooplankton populations with specific densities of *M. tenuicornis* and *H. incongruens*, and used different filter sizes to remove *M. tenuicornis*.

Chronic CO_2 injection was tested instead of the acute injection used in previous laboratory experiments [25] as we expected this alternative treatment strategy to maintain more stable long-term HRAP performance, and reduce the amount of CO_2 required for zooplankton control.

Zooplankton control treatments were assessed in terms of the magnitude of zooplankton reduction, the changes in microalgal biomass concentration, productivity, relative abundance, and settleability. Phototaxis-induced vertical migration of *M. tenuicornis* was demonstrated in the water column of an 8 m^3 HRAP with the aim of only applying mechanical treatments to the surface (zooplankton dense) portion of the pond, to reduce treatment time and costs.

2.2.1. Zooplankton control using CO_2 asphyxiation

Different intensities of zooplankton CO_2 asphyxiation were achieved by continuous (chronic) injection of CO_2/air gas mixes with different percentages of CO_2 (0.5%, 2%, 5%, and 10%) into the mesocosm cultures. The control mesocosms were injected with air, and the experiment was performed twice: initially during summer (21 days, 10/01/2014–30/01/2014), and then during winter (62 days, 15/07/2014–15/09/2014). The starting microalgae and zooplankton cultures were collected on 09/01/2014 (summer) from the East HRAP, and on 15/07/2014 (winter) from the West HRAP. Different ponds were used to have a microalgae consortium composed of colonial species similar to that of WW HRAPs [27]. CO_2 concentration and pH were monitored and adjusted three times per day (09:00 am, 12:30 pm and 04:00 pm) using a gas analyser (Biogas 5000, Geotech), and pH meter (TPS WP-91, TPS Pty. Ltd., Springwood Australia). Ammonia concentration was determined twice per week in the first month, and once per week during the second month (winter experiment) using standard methods [28]. The concentration of CO_2 in 100 mL samples of the mesocosm cultures was assessed by titration of the carbonic acid formed by CO_2 with NaOH standard solutions and phenolphthalein (0.5%) indicator until colour change at pH 8.3 [28,29].

2.2.2. Rotifer control using the cladoceran M. tenuicornis

The inhibitory effect of *M. tenuicornis* on rotifers was assessed by inoculating *M. tenuicornis* into a microalgae culture sourced from East HRAP on 16/03/2015. This was dominated by *Mucidosphaerium* sp. and had a mixed rotifer population (*B. calyciflorus*, *C. catellina*, *F. longiseta*, and bdelloid rotifers). The experiment was conducted over 28 days (16/03/2015–13/04/2015). *M. tenuicornis* were sourced from