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Free ammonia offers algal crop protection from predators in dairy wastewater and ammonium-rich media

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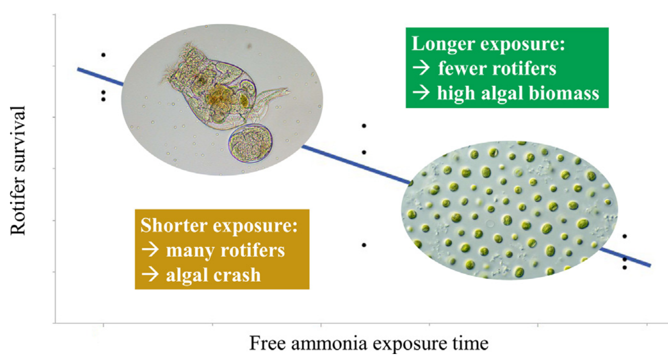
HIGHLIGHTS

- Free NH_3 concentrations that inhibit rotifers do not affect a model algal species.
- Exposure time to free NH_3 was optimized for algal production and pest control.
- 6- and 12-h of free NH_3 exposure inhibited rotifers with no effect on algae.
- This approach may provide a sustainable, low-cost algal crop protection strategy.

GRAPHICAL ABSTRACT



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ABSTRACT

Cost-effective methods for protecting crops from grazing organisms like rotifers are needed to reduce the risk of pond crashes in mass algal cultures. We present a novel strategy to optimize the exposure time to free ammonia, via control of media pH, in both defined media and dairy anaerobic digester effluent to suppress rotifers and maintain algal productivity. We tested five different free ammonia exposure times (0, 1, 2, 6, and 12 h) and found a significant nonlinear effect of exposure time ($p < 0.0001$) but not pH ($p > 0.9$) on rotifer survival. In both media types, 6–12 h of elevated free ammonia significantly reduced *Brachionus plicatilis* rotifer survival with no negative effects on *Nannochloropsis oculata*, while shorter exposure times were insufficient to inhibit rotifers, leading to severe algal culture crashes. These results suggest that algal crops can be protected from rotifers, without productivity loss, by elevating free ammonia for 6 or more hours.

1. Introduction

In order to meet ambitious targets for quickly reducing greenhouse gas emissions, a diverse portfolio of low-carbon energy sources will be needed. Advanced biofuels and bio-products from microalgae have considerable potential to help meet these goals. Algae are much more productive than terrestrial biofuel crops, leading to greater yields of biofuel per unit area (Chisti, 2008, 2007; Jones and Mayfield, 2012). Moreover, algae can be grown on marginal land using brackish water or wastewater, which reduces the food vs. fuel issues associated with first generation biofuel crops (Chisti, 2008, 2007; Jones and Mayfield, 2012). Mass algal cultivation could even promote reforestation and carbon capture by displacing conventional animal feed crops (Walsh et al., 2015).

Despite the many benefits of harnessing algae biomass for a variety of bio-products, there are still barriers that must be over-

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come to enhance the viability of such systems. Competition with inexpensive petroleum fuels makes algal biofuel less viable and incentivizes industry to pursue higher-value but lower-volume products like nutraceuticals. Therefore, mass commercialization of algae will require minimization of production costs. Additionally, there are many grazing pests (e.g. rotifers, ciliates and cladocerans) and pathogens (e.g. bacteria, viruses, and fungi) that can cause mass algal cultures to crash, reducing annual productivity (Day, 2013; Shurin et al., 2013). For example, grazers like rotifers exert particularly strong top-down pressure on algae; *Brachionus plicatilis* can consume up to 3000 cells per hour (Montagnes et al., 2001) which causes cultures to crash if left untreated (Van Ginkel et al., 2015). Development of cost-effective methods for protecting algal crops against pests and pathogens is therefore vital for the successful scale up of the algae biomass industry.

One prevalent strategy for eliminating pests from algal cultures is to use chemical controls that selectively eliminate the pest while minimizing harm to the algae. This approach has been effective for inhibiting rotifer growth using free ammonia, copper, rotenone, hypochlorite and quinine (Lincoln et al., 1983; Pradeep et al., 2015; Schlüter and Groeneweg, 1985; Van Ginkel et al., 2015; Xu et al., 2015) and also for inhibiting chytrid fungi using hydrogen peroxide (Carney and Lane, 2014). All of these inputs are effective at inhibiting pests without severely affecting algae. Ammonia, however, could be particularly efficient as addition of ammonium could play a double role as fertilizer for algae and as a broad-spectrum inhibitory agent for grazing pests like rotifers and cladocerans (Arthur et al., 1987; Gersich and Hopkins, 1986; Lincoln et al., 1983; Schlüter and Groeneweg, 1985). Specifically, free ammonia (NH_3) reduces *Brachionus* rotifer reproduction at concentrations as low as 2.4 mg L^{-1} (de Araujo et al., 2000) and causes complete mortality at concentrations over 5 mg L^{-1} free NH_3-N (Schlüter and Groeneweg, 1985). Algae are generally much less sensitive to free ammonia, although this varies by strain. *Scenedesmus obliquus* can tolerate up to 28 mg L^{-1} free NH_3-N (Abeliovich and Azov, 1976) and *Chlorella pyrenoidosa* can tolerate up to 20 mg L^{-1} free NH_3-N (Tan et al., 2015) without inhibition of photosynthesis or growth rate. Similarly, *Nannochloropsis oculata* and *Chlorella sorokiniana* were not inhibited by the highest level tested (16 mg L^{-1} free NH_3-N), although other species were inhibited (Gutierrez et al., 2016). Importantly, the lipid content of *N. oculata* was not significantly altered with exposure to high free ammonia levels, indicating that ammonia will not affect the species' biomass composition relevant to biofuels production (Gutierrez et al., 2016).

Additionally, ammonium concentrations are often very high in wastewaters, so a simple manipulation of pH when ammonium levels are high could be used as an integrated pest management strategy. Dairy anaerobic digester effluent (ADE), for example can have over 2000 mg L^{-1} total NH_3-N (Wang et al., 2010). By allowing pH to rise via photosynthesis, to levels that increase the free ammonia fraction, a pond operator could selectively inhibit rotifers at very low or no overall cost, as no additional chemical inputs are needed, and without reduction of algal productivity. Minimizing not only direct costs, but also indirect costs associated with crop protection strategies is vital to sustainable production; environmental and societal costs of agricultural pesticide use have been estimated at \$10 billion yr^{-1} (Pimentel, 2005), which the nascent algae biomass industry would be wise to avoid. In contrast to other recent breakthroughs in algal crop protection (Carney and Lane, 2014; Pradeep et al., 2015; Van Ginkel et al., 2015; Xu et al., 2015), using ammonia already inherent to the wastewater requires neither input costs nor application of exogenous chemical compounds.

Despite the potential for free ammonia dosing to minimize rotifer invasions without harming algal growth, there has been very little research into specific methods for taking advantage of this promising strategy. Tan et al. (2015) suggest that strategic control of free ammonia levels in wastewater could be used simultaneously inhibit pests while minimizing effects on algal biomass. To our knowledge, however, no study has investigated the effects of exposure time of elevated free ammonia levels on rotifers or algae. We therefore sought to provide novel insights into the optimization of this strategy by experimentally manipulating free ammonia exposure time, with the goal of identifying levels with both maximum algal productivity and maximum grazer control. To accomplish this we employed a model saltwater algal species, *Nannochloropsis* oculata, as a representative strain commonly used for large-scale outdoor cultivation of biofuels and bioproducts, as well as the saltwater rotifer *Brachionus plicatilis*, as a representative model grazer capable of rapidly depleting algal cultures. We first subjected each species to a gradient of free ammonia concentrations to verify toxicity levels, and subsequently exposed both species in co-culture to different free ammonia exposure times. Specifically, the objectives of this research were to 1) identify effects of free ammonia concentration on the rotifer *Brachionus plicatilis* in both defined media and dairy wastewater after a 24 h exposure time, 2) identify effects of free ammonia concentration on the saltwater algae species *Nannochloropsis oculata* in both defined media and dairy wastewater after a 24 h exposure time, and 3) identify effects of free ammonia exposure time on longer-term algal productivity in both defined media and dairy wastewater in the presence of *B. plicatilis* rotifers. We hypothesized that increasing free ammonia exposure time would decrease rotifer survival, and that when co-cultured with rotifers, algal production would be highest at an optimum point where rotifers are inhibited but algae are not.

2. Materials and methods

2.1. Media and cultures

Instant Ocean® sea salts (33.6 g/L, Spectrum Brands, Blacksburg, VA, USA) was used to create artificial seawater (hereafter referred to as "ASW") for all media used. F/2 media was prepared by enriching ASW with f/2 nutrients according to a UTEX standard recipe (https://utex.org/products/f_2-medium). From these base media types, we created a defined medium treatment with added ammonium at approximately the same free NH_3-N level as 5% ADE treatment. This solution was composed of f/2 nutrients in ASW and added NH_4Cl (mean 113.9 ± 2.90 mg L^{-1} total NH_3-N across trials), which is hereafter referred to as "f/2 medium." We also created a solution containing ASW with 5% ADE (mean 136.8 ± 6.30 mg L^{-1} total NH_3-N across trials) to assess effects of ammonia in undefined wastewater-based media; this medium is hereafter referred to as "ADE medium." A 5% concentration of ADE was chosen based on previous research indicating that this dilution maximizes growth rates and nutrient removal (Passero et al., 2015). While there were slight differences in initial NH_3-N concentrations between the two media types, they were small when compared to the experimental manipulation of free ammonia, which spanned two orders of magnitude for each experiment. Dairy ADE was collected from Big Sky West Dairy, Gooding, ID, USA, was stored at −20 °C, and was centrifuged prior to use in experiments to reduce potential effects of particulate organic matter. ADE was centrifuged at 10,000 rpm for 10 min for well plate experiments and 20 min for the exposure time experiment. Conductivity of the media was measured before use; 5% ADE medium had a conductivity of 0.2 mS/cm, while the ASW medium had a conductivity of 47 mS/cm. This 4% increase in conductivity upon addition of ADE had no noticeable effects on the species studied.