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Peracetic acid is a suitable disinfectant for recirculating fish-microalgae integrated multi-trophic aquaculture systems

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ABSTRACT

Integrated multi-trophic aquaculture (IMTA) is a promising direction for the sustainable development of aquaculture. Microalgae have good potential to be integrated with recirculating aquaculture systems because they can use the nitrogen excreted from fish and share the same optimal pH value as in aquaculture. As a byproduct, the microalgae biomass can be used for fish feed or biofuel. However, the recirculating fish-microalgae IMTA system is under constant threat from fish pathogens and phytoplankton-lytic bacteria. Therefore, it is necessary to apply proper disinfectants as prophylaxis or treatment which are effective against these threats, but safe to fish and microalgae. For this purpose, peracetic acid (PAA) is a valid option because it is highly effective against fish pathogens and bacteria at low concentrations and degrades spontaneously to harmless residues. In the present study, we exposed the culture of a marine microalgae *Tetraselmis chuii* once per day for four days to four PAA products with differing hydrogen peroxide (H_2O_2)/PAA proportions at two concentrations (1 and 2 mg L^{-1} PAA). The H_2O_2 solutions at equivalent total peroxide (H_2O_2 + PAA) concentrations were tested in parallel. The results show that the growth and photosynthesis of *T. chuii* were not affected by three of the PAA products (Wofasteril® E400, Wofasteril® E250 and Applichem® 150) and equivalent H_2O_2 solutions at both concentrations. In contrast, Wofasteril® Lspez and an equivalent H_2O_2 solution at both concentrations caused irreversible culture collapse, photosynthesis dysfunction and irreversible cell damage. In conclusion, PAA products with low proportions of H_2O_2 are optimal disinfectants for fish-microalgae IMTA systems.

1. Introduction

The growing world population has an increasing demand for high quality proteins. Based on the estimation of Tilman and Clark (2014), the global average dietary composition of fish and other seafood will increase 82% from 2009 to 2050. Increasing the world's supply of fish relies mainly on increasing aquaculture production, since the catch from the fisheries industry has been declining since the late 20th century (Pauly and Zeller, 2016). As discussed by Naylor et al. (2000), many traditional aquaculture practices cause environmental and/or biological pollution and threaten fisheries by the use of fish oil and/or fish meal; they suggested that only sustainable aquaculture could help increase the supply of fish without harming natural ecosystems or fisheries. One option of sustainable aquaculture is the integrated multi-trophic aquaculture (IMTA) system.

Many IMTA systems have been developed and tested. For instance, an aquaponics system is the combination of aquaculture and hydroponics; it uses the waste from aquaculture as the fertilizer for hydroponics (Graber and Junge, 2009). However, hydroponics needs lower pH than aquaculture, so the water from aquaculture must be acidified and buffered. This results in higher technical and management costs and a limited recycling of nutrients. In contrast to plants, many algae species grow at the same pH

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value as fish. Therefore, algae have better potential to be integrated with aquaculture. Cahill et al. (2010) demonstrated that seaweed (macroalgae) performed better at removing ammonia and nitrite from a recirculating aquaculture systems (RAS) than traditional biofilm filtration (i.e., nitrifying bacteria). Gilles et al. (2014) developed an IMTA system consisting of microalgae, plankton and detritivorous/zooplanktivorous fish to replace the filtration unit of a RAS; they observed that an increase of the microalgae biomass led to a decrease of the total ammonia nitrogen. The studies detailed above demonstrate that algae are suitable to be used in the denitrification process of recirculating IMTA systems; as an added benefit, the algae biomass can also be harvested as a byproduct. The algae biomass, especially the microalgae biomass, can be used to produce biofuel (Lardon et al., 2009) or as a sustainable feed for shellfish, fish larvae and live prey (zooplankton) in aquaculture (Hemaiswarya et al., 2011).

The recirculating fish-microalgae IMTA system has several integrated problems. Fish may become infected by various pathogens. Meanwhile, the microalgae are threatened by phytoplankton-lytic bacteria, which can lead to reduced growth and even collapse of the population (Wang et al., 2013). To control fish pathogens, disinfectants can be regularly used as prophylaxis or treatment; however, the use of disinfectants might harm the microalgae culture. Therefore, it is important to find a disinfectant that can effectively control fish pathogens but not harm microalgae. To our knowledge, research has not been undertaken to find a suitable disinfectant for a recirculating fish-microalgae IMTA system.

Peracetic acid (PAA) has been recognized to be a sustainable disinfectant in aquaculture. It degrades completely within several hours after application (Pedersen et al., 2009) and results in harmless residues (Kitis, 2004). Noteworthy, the effective concentration of PAA is less than 2 mg L^{-1} against various pathogens (Pedersen et al., 2013). In contrast, another sustainable disinfectant, hydrogen peroxide (H_2O_2), needs a much higher concentration (over 20 mg L^{-1}) to achieve successful disinfection (Schmidt et al., 2006). Because toxicity highly depends on the dose, PAA has higher potential to fit in the recirculating fish-microalgae IMTA system. The aim of the present study is to test whether PAA at effective disinfection concentrations (up to 2 mg L^{-1}) is toxic to the microalgae cultivation in controlled lab conditions, and thus to determine whether PAA is a suitable disinfectant for recirculating fish-microalgae IMTA systems.

There are many commercial PAA products available and these products are equilibrium-mixtures of PAA, H_2O_2 and water with various H_2O_2/PAA proportions. If these products are applied at the same PAA concentration, the difference in H_2O_2 concentration between the products would result in different acute toxicity values (Liu et al., 2015). Based on our past research, we hypothesize that there will be a difference in toxicity to microalgae with various commercial PAA products and we predict that products with a lower H_2O_2/PAA proportion will be less toxic; consequently, they will be more suitable for prophylaxis/treatment in a fish-microalgae IMTA system.

2. Materials and methods

2.1. Chemicals

We tested four commercial PAA products with various PAA and H_2O_2 concentrations. Three of the products were Wofasteril® PAA formulations (Kesla Pharma Wolfen GmbH, Greppin, Germany): E400 (40% mass/volume [m/v] PAA, 12% m/v H_2O_2), E250 (25% m/v PAA, 30% m/v H_2O_2), and Lspez (3% m/v PAA, 40% m/v H_2O_2); the fourth product was Applichem® 150 (AppliChem GmbH, Darmstadt, Germany; 15% m/v PAA, 24% m/v H_2O_2). All other chemicals used were analytical grade and purchased locally.

2.2. Algae culture

A *Tetraselmis chuii* culture of the strain SAG 8-6 was obtained from the University of Göttingen (Göttingen, Germany) and cultured in 100-mL Erlenmeyer flasks with F/2 medium. The F/2 medium was prepared as described by the Canadian Phycological Culture Center at the University of Waterloo, Canada (https://uwaterloo.ca/canadian-phycological-culture-centre/cultures/culture-media/f2). The F/2 medium was sterilized by passing it through a Sartolab-P20® 0.2 μm micro-filter (Sartorius Lab Products & Services, Göttingen, Germany).

Flasks containing the algae culture were placed on a GFL 3005 shaker (GFL mbH, Burgwedel, Germany) in a KBW 720 incubator (Binder GmbH, Tuttlingen, Germany). The rotation rate of the shaker was 180 × g and the temperature in the incubator was 18 °C. There were two parallel OSRAM L 18W/865 daylight fluorescent lights (OSRAM GmbH, Munich, Germany) 0.5 m above the shaker, providing a light intensity of 2000 to 2700 lx on the surface of the shaking plate; the light cycle was controlled by a timer and set at 16 h light/8 h dark. Before toxicity experiments began, the stock algae culture was acclimated for 2 months under these conditions and the F/2 medium was refreshed once per week.

All work with the algae, including refreshing the medium, translocation and treatments were done under sterilized conditions. All materials that had contact with algae were sterilized by autoclaving or a hot air sterilizer.

2.3. Measuring PAA and H_2O_2 concentrations

The DPD (N, *N*-diethyl-*p*-phenylendiamine sulfate salt) photometric method was used to determine PAA and H_2O_2 concentrations based on a linear relationship between concentration and absorption (Liu et al., 2015).

The absorption-concentration relationship was determined by measuring the 550-nm absorption of solutions containing only H_2O_2 in distilled water at the concentrations of 0.112, 0.224, 0.336, 0.448, 0.56, 0.672, 0.784 and 0.896 mg L^{-1} on a DR3900 spectrophotometer (Hach Lange GmbH, Düsseldorf, Germany). The 550 nm absorption of 0.147 corresponded to 1.0 mg L^{-1} PAA or 0.448 mg L^{-1} H_2O_2, and the 550 nm absorption of 0.294 corresponded to 2.0 mg L^{-1} PAA or 0.896 mg L^{-1} H_2O_2.

The DPD photometric method could not measure the H_2O_2 concentration in the 1 and 2 mg L^{-1} Lspez solutions because the absorption values were too high for the spectrophotometer to read. Therefore, we used an iodometric titration method to determine the H_2O_2 concentrations (Greenspan and MacKellar, 1948). In short, a 5-mL Lspez sample was added to 30 mL of an ice-cold 10% H_2SO_4 solution in an Erlenmeyer flask. Next, 3 drops of 0.025 mol L^{-1} Ferroin solution (Carl Roth, Karlsruhe, Germany) were added to the mixture and titrated with 0.1 mol L^{-1} Ce^{+4} sulfate solution (Bernd Kraft GmbH, Duisburg, Germany) until the color turned from orange to blue. According to the method, 1 mL consumption of the Ce^{+4} sulfate solution corresponded to 1.701 mg H_2O_2.

2.4. Treatment and sampling

A volume of 200 mL of the stock algae culture was transferred to a 3-L Erlenmeyer flask and combined with F/2 medium for a total volume of 1.6–1.7 L. The flask was then placed in the incubator at 18 °C for 72 h to allow the algae to reach exponential growth phase. Next, 30 mL of the algae culture was transferred into 51 100-mL Erlenmeyer flasks. The flasks were immediately divided randomly into 17 groups consisting of a control treatment and 16