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Research Article

Assessing Peracetic Acid Application Methodology and Impacts on Fluidized Sand Biofilter Performance

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Nitrifying biofilters oxidize harmful ammonia excreted by fish into less toxic nitrate within recirculating aquaculture systems (RAS). Biofilter performance and resulting RAS water quality largely depend on a robust microbiome that effectively converts nitrogenous wastes; however, occasional use of water disinfectants may also be necessary to reduce or eliminate specific fish pathogens. Disinfectants and sanitizers such as peracetic acid (PAA) work by disrupting microbial activity and could unintentionally alter the microbially-driven nitrification biofiltration process if allowed to circulate within an RAS. Furthermore, the target concentration and application method of PAA may influence the level of biofilter disruption. For this study, 12 replicated experimental-scale fluidized sand biofilters were dosed with PAA to achieve target concentrations ranging from 1.0-–2.5 mg/L, a typical low-dose treatment range to reduce or eliminate opportunistic pathogens. Two application methods were compared, including (i) a single pulse of PAA added every other day for five days, and (ii) smaller doses of PAA added every five minutes over four hours. The PAA decay was monitored and predosing and postdosing water quality parameters were assessed. Regardless of the target concentration or application method, PAA addition within the tested range did not cause significant disruption to the biofilters' nitrification processes. This research demonstrates that PAA may be a viable water sanitizer for the RAS industry, although further research to refine safe application protocols is necessary.

1. Short Communication

There is a significant interest by the aquaculture industry in utilizing peracetic acid (PAA; CH_3CO_3H) as a water disinfectant to reduce or eliminate potential opportunistic pathogens (e.g., *Flavobacterium* spp). The efficacy of PAA in killing a range of bacteria, fungi, yeasts, and viruses has been demonstrated [1, 2], and its rapid degradation into nontoxic byproducts of acetic acid, hydrogen peroxide, and water largely eliminates pollution and discharge issues. Previous research on PAA application in aquaculture settings has primarily focused on disease prevention through PAA's bactericidal activity [3, 4]. Several toxicity studies indicate that low-dose PAA treatments are tolerated by commercially raised rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*) at various life stages [5–10].

Intensive fish production technologies, such as recirculating aquaculture systems (RAS), commonly used to raise these species, rely on microbial-based treatment processes, including nitrifying biofilters. Bacteria established on biofilter media are integral to converting toxic fish metabolites (i.e., ammonia) into less harmful nitrate. Because PAA is a broad-spectrum antimicrobial product, nitrification could be impaired if biofilters are exposed to sufficient concentrations of PAA due to the destruction of nitrifying bacteria. Disruption of nitrification, even if temporary, can result in

significant adverse outcomes for fish, as ammonia and nitrite can be highly toxic when above certain concentrations. The current literature on the effects of PAA treatments on nitrification is limited [11–13]. Additionally, the application method of PAA and target concentration may also influence water quality conditions. This study sought to add to existing knowledge by evaluating the impacts of low-dose PAA concentrations on the nitrification efficiency of fluidized sand biofilters by comparing two PAA application methods: (i) a single pulse of PAA added every other day for five days, and (ii) smaller doses of PAA added every five minutes over four hours.

Twelve replicated pilot-scale fluidized sand biofilters (122 cm tall × 15 cm interior diameter; MAHI International, Indianapolis, IN, USA) were each coupled via polyvinyl chloride piping with a polyethylene sump (56.8 L), creating independent biofiltration units (63 L total volume; Figure 1). Each biofilter received 990 g quartz silica sand (#1 Dry, 30 mesh; RJ Glass, Duncansville, PA, USA), which equated to a postflushing static sand bed depth of approximately 23 cm. Biofilters were each seeded with 9.75 mL of nitrifying bacteria (FritzZyme® TurboStart®; Fritz Aquatics, Mesquite, TX, USA) and fed daily 1.65 g of ammonium chloride (AniMed; Winchester, KY, USA) until the nitrification process was fully established, which was accomplished in approximately one week. Nitrification was verified by an increase in nitrate-nitrogen concentration (data not shown). Thereafter, water that had been pretreated by a microscreen drum filter within a semicommercial-scale RAS for rainbow trout (*Oncorhynchus mykiss*) was provided as inlet water to the biofilters. Fish culture water averaged 16 ± 0.05°C with an alkalinity of 262 ± 2 mg/L as $CaCO_3$ and pH of 7.5 ± 0.0, with a range of 0.06–0.32 nitrite-nitrogen (NO_2-N) and 0.04–0.40 total ammonia nitrogen (TAN). No additional ammonium chloride was added after the seeding application. Each biofiltration unit was designed with dual effluent pipes, and RAS effluent received a single pass through the sand bed before discharge; however, during system seeding and experimental treatment phases, water was recirculated back through the sump, simulating the closed RAS design (~8.4 min HRT). Throughout the experiment, fluidized sand bed expansion (50–60%) was maintained by regulating biofilter inflow via a submersible sump pump (7.5–7.6 L/min) (Danner supreme magnetic drive, 500 gph; Islandia, NY, USA). Thre biofilter function was confirmed before PAA Application 1 and 2 through TAN and NO_2-N removal efficiencies (88 ± 1% and 55 ± 3%, respectively). The removal efficiency (RE) was calculated by subtracting the effluent concentration from the influent and dividing it by the influent.

Biofiltration units were treated with a commercially available PAA product consisting of 15% PAA and 10% hydrogen peroxide (H_2O_2) (VigorOx® SP-15; Evonik Active Oxygens LLC, Philadelphia, PA, USA). Stock concentrations of 174,088 and 186,438 mg/L as PAA were verified immediately before PAA Applications 1 and 2 (see below), respectively (Hach LIT2199—PAA and H_2O_2 Titration). Additionally, before administration, the stock concentration was diluted with deionized water (1 : 10 ratio) for safe handling. For both applications, PAA was added directly into each sump, just below the water level and adjacent to the sump's influent pipe; so the PAA addition occurred at the highest level of mixing. During PAA Application 1, biofilters were randomly divided into four groups (i.e., $n = 3$ biofilters per treatment). Each group received single-pulse doses of either: 3.6, 6.3, or 9.1 mg of PAA, corresponding with targeted known therapeutic initial concentrations of 1.0, 1.75, and 2.5 mg/L PAA (or low, mid, and high concentrations), respectively. These therapeutic concentrations have been previously used in experimental RAS for salmon parr [9] with a minimal effect on overall fish health and welfare. For control treatments, deionized water of a similar volume was used. Over a 5-d period, biofilters received three single-pulse doses, with a 48-h rest period between each dose. Approximately ten weeks later, the systems were again randomly assigned into treatment groups. During PAA Application 2, biofilters were continuously pulse-dosed every 5-min over 240-min, receiving either 0.167, 0.205, or 0.246 mg of PAA to produce target concentrations of 1.0, 2.0, and 2.5 mg/L PAA. The higher midrange target for Application 2 was chosen based on the findings during Application 1. Additionally, there was no comparative control during Application 2, as three biofilters were not in commission at the time.

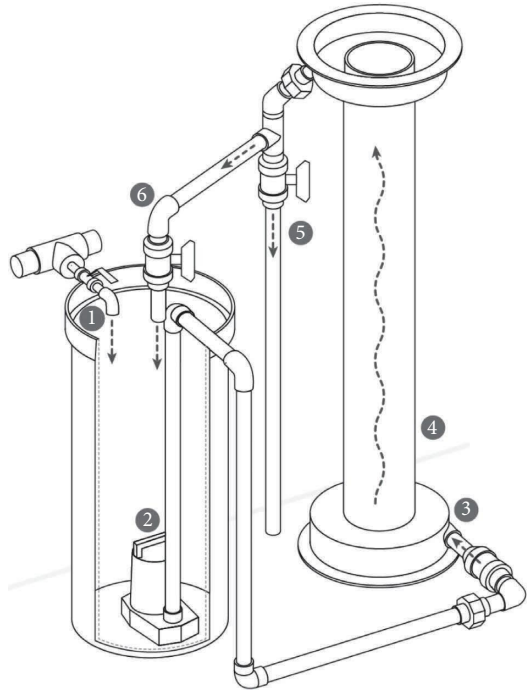

Figure 1: Diagram of fluidized sand biofiltration unit. Sump fed with water from a semicommercial-scale RAS that had been treated by a microscreen drum filter (1), then pumped via submersible pump (2) into biofilter (3). Water upflowed through the fluidized sand bed (4), where it was either discharged (5) or recirculated back into the sump (6) to create a closed loop.

Pretreatment and posttreatment water quality was monitored, with samples collected, stored, and processed on-site, following standard methods [14, 15]. Water quality parameters TAN and NO_2-N were sampled weekly, except

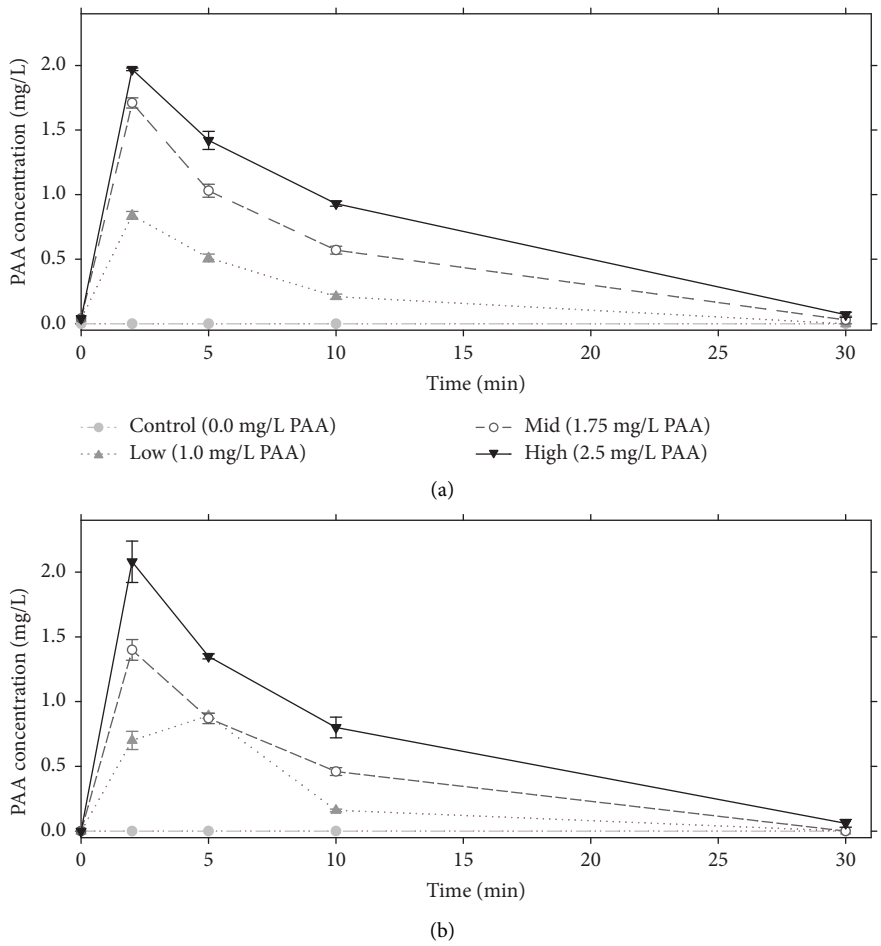

Figure 2: Peracetic acid (mg/L) decay curves after second (a) and third (b) doses of PAA during Application 1 for control, low, mid, and high target concentrations (0.0, 1.0, 1.75, and 2.5 mg/L PAA, respectively).

immediately following PAA applications, when sampling frequency was increased. For background reference, mean (±SE) influent total suspended solids and carbonaceous biochemical oxygen demand were 2.9 ± 0.3 mg/L and 2.0 ± 0.1 mg/L, respectively, throughout the experiment. Dissolved oxygen (Hach LDO101 with HQ40 d, Loveland, CO, USA), measured at the biofilters' outlet, averaged 8.4 ± 0.05 mg/L. The posttreatment water quality was compared statistically using one-way RMANOVA ($\alpha = 0.05$) with Shapiro–Wilk test for normality and Brown–Forsythe equal variance testing (SigmaPlot version 14.0, Systat Software, Inc., CA, USA). Effluent concentrations for NO_2-N were compared statistically, while removal efficiencies for TAN, due to percent format, were arcsine-transformed prior to statistical comparison. In case where normality failed, Friedman RMANOVA on Ranks was run.

During PAA Application 1, PAA decay was initially measured at 0, 1, 120, and 240 min after the first pulse-dose (CHEMetrics PAA instrumental test kits; Midland, VA, USA). However, to better capture the curve, second and third doses were sampled 2-min after administration, allowing adequate mixing, and then at 5-min intervals until complete decay (Figure 2). Initial concentrations of 0.84 ± 0.03, 1.71 ± 0.04, and 1.97 ± 0.01 mg/L as PAA were captured for low, mid, and high targets, respectively. Greater than 50% PAA decay occurred within the first 10-min, and most, if not all, PAA had decayed by 30-min. This corresponded to earlier reported decay kinetics of PAA [4].

Generally, biofilters experienced no major disruption to nitrification processes, confirmed through posttreatment water quality sampled 24 hours after each PAA pulse-dose. There were no significant differences between treatments for TAN RE at 1, 3, or 5 days after the initial PAA pulse-dose ($p = 1.000$, 0.859, and 0.958, respectively; Figure 3(a)). However, it should be noted that systems may have been TAN-limited (influent 0.23 ± 0.02 mg/L as TAN and near 100% RE across all biofilters), which may have concealed any underlying deviations. Essentially, low influent TAN and an extended hydraulic retention time resulted in complete TAN removal. No significant differences were noted in NO_2-N effluent concentrations after the initial pulse-dose ($p = 0.080$). However, the NO_2-N level was affected by the high-PAA treatment after the second and third pulse doses ($p = 0.001$, and <0.001, respectively; Figure 3(b)).

PAA Application 2 aimed to gradually increase target concentrations over the treatment period. It should be noted, however, that the PAA decay rate is highly variable based on water temperature and various water quality

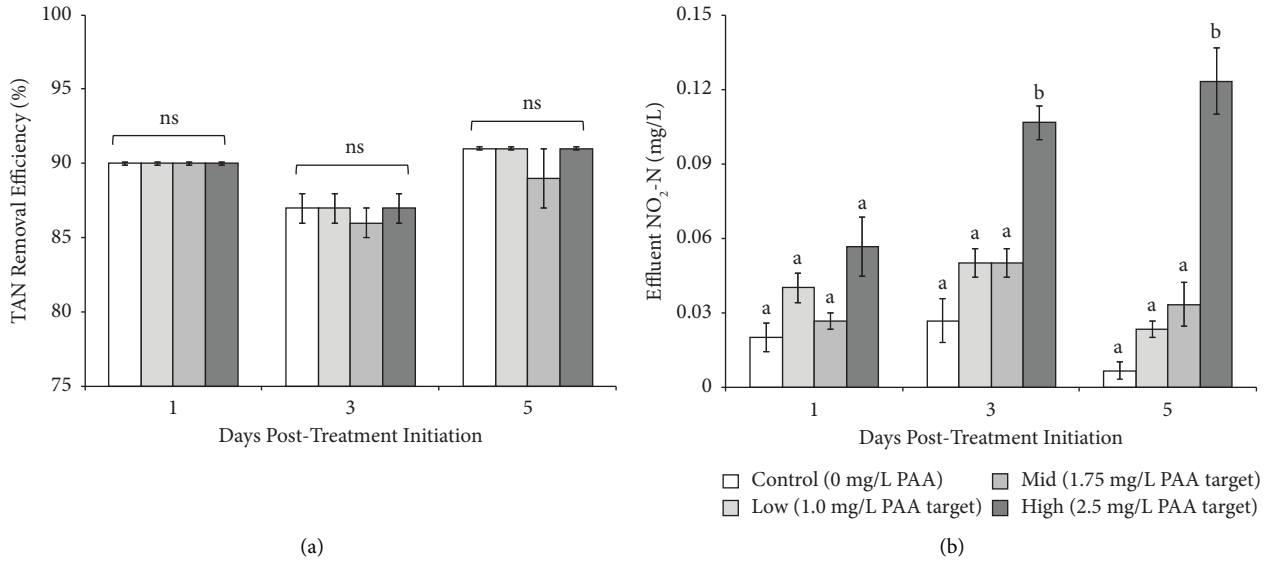

Figure 3: (a) Total ammonia nitrogen (TAN) removal efficiencies (%) and (b) nitrite-nitrogen (NO_2-N) effluent concentrations (mg/L) for 1-, 3-, and 5-days postinitial peracetic acid (PAA) dose during Application 1.

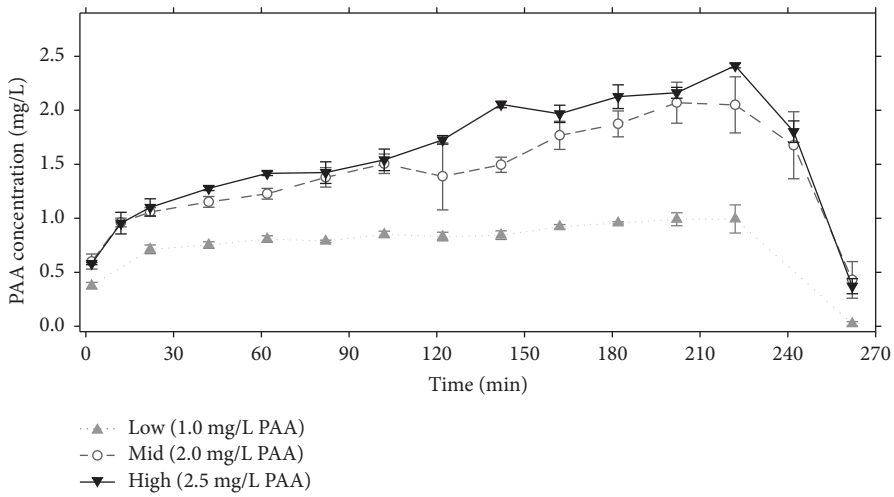

Figure 4: Peracetic acid concentrations (mg/L) for low, mid, and high target concentrations (1.0, 2.0, and 2.5 mg/L as PAA, respectively) during Application 2.

parameters, particularly organics, making it difficult to calculate dosing, even between replicated systems [3, 16–18]. A stable concentration of 0.86 ± 0.02 mg/L PAA for the low-PAA treatment was maintained starting 25-min after PAA administration until dosing was complete, with the target concentration (0.98 ± 0.04 mg/L PAA) maintained for ≥40 min (Figure 4). Both mid- and high-PAA treatments did not plateau in concentration until near the end of the dosing. The mid-PAA treatment demonstrated a higher degree of variability between replicates, but the overall target concentration was maintained for ≥40 min (Figure 4; 2.00 ± 0.10 mg/L PAA). The high-PAA treatment did not reach the target but was maintained at a steady concentration for ≥60 min (Figure 4; 2.08 ± 0.04 mg/L PAA). While the results from Application 2 did not demonstrate static concentrations for the desired time, they clearly expressed the need for continued research on RAS dosing calculation.

Regarding biofilter function, between low-, mid-, or high-PAA treatments at 1- or 3-days postdosing, there were no significant differences in TAN RE ($p = 0.194$ and $p = 0.528$, respectively). Similarly, in both low- and mid-PAA concentrations, effluent NO_2-N concentrations were not significantly different at 3-days' postdosing ($p = 0.297$). Unfortunately, due to slight differences in influent NO_2-N concentration between sampling days, 1-day postdosing results between low- and mid-PAA treatments were not comparable.

In conclusion, this study demonstrated that, regardless of application method, PAA dosed at a range of therapeutic concentrations used to treat opportunistic pathogens (1.0–2.5 mg/L PAA) did not cause significant disruption to fluidized sand biofilter function and associated nitrification. These findings are similar to those of Suurnäkki et al. [12], where the pulsed PAA application impacted short-term

nitrification of fixed bed bioreactors, but no long-term effects were observed. These authors also found that more frequent PAA application decreased overall biofiltration nitrification rates but did not impact TAN removal. Similarly, in the present study, TAN conversion, measured through RE, was nearly 100%. At the highest PAA concentrations, biofilters exhibited slightly increased production of NO_2-N, but this did not impede overall biofiltration function. Without further testing for doses and durations outside of these study conditions, it is recommended that the addition of PAA for pathogen reduction should be carefully administered. It is interesting to highlight that a recent study on Atlantic salmon parr demonstrates that the mode of application has little impact on the physiological consequences of PAA as a loop water disinfectant in RAS [19], hence lending insight into the implications of the chemical data presented in the present study. Further research should investigate gradually higher concentrations, durations, and application methods of PAA to determine the point of impaction for nitrification disruption.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Disclosure

All authors have substantially contributed to this research according to journal standards for authorship qualification. Any trade, firm, or product name used is for descriptive purposes and does not imply endorsement.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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