



Peracetic acid degradation and effects on nitrification in recirculating aquaculture systems

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ABSTRACT

Peracetic acid (PAA) is a powerful disinfectant with a wide spectrum of antimicrobial activity. PAA and hydrogen peroxide (HP) degrade easily to oxygen and water and have potential to replace formalin in aquaculture applications to control fish pathogens, for example the ectoparasite, *Ichthyophthirius multifiliis*. We studied water phase PAA and HP decay in three aquaculture situations, i) batch experiments with two types of system waters, ii) PAA decay at different fish densities, and iii) degradation of PAA in submerged biofilters of recirculating aquaculture systems (RAS). Furthermore, effect of PAA on the nitrification activity and the composition of the nitrifying population were investigated.

PAA and HP decay showed first order kinetics. High dosage PAA/HP in water with low COD inhibited HP removal, which was not observed in water having a higher COD content. PAA decay was significantly related to fish stocking density, with half life constants for PAA of 4.6 and 1.7 h at 12 and 63 kg m⁻³, respectively. PAA application to RAS biofilter showed rapid exponentially decay with half life constants of less than 1 h, three to five times faster than the water phase decay rates.

Biofilter surface specific PAA removal rates ranged from 4.6 to 13.9 mg PAA m⁻²h⁻¹ and was positively correlated to the nominal dosage. Low PAA additions (1.0 mg L⁻¹) caused only minor impaired nitrification, in contrast to PAA application of 2.0 and 3.0 mg L⁻¹, where nitrite levels were significantly increased over a prolonged period, albeit without fish mortality. The dominant ammonium oxidizer was *Nitrosomonas oligotropha* and the dominant nitrite oxidizer was *Nitrospira*. Based on the present findings and other recent results from field and in vitro studies, application perspectives of PAA are discussed.

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1. Introduction

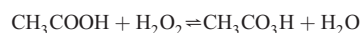
There is a pertinent use of chemical additives and antibiotics related to aquaculture in order to improve water quality and treat fish diseases, respectively (Buchmann and Bresciani, 1997; Jørgensen et al., 2009). The chemical sanitizers are generally used to control fish pathogens in the systems and are applied to the water phase (Noble and Summerfelt, 1996; Boyd and Massaut, 1999; Rintamaki-Kinnunen et al., 2005a). White spot disease, caused by the ectoparasite *Ichthyophthirius multifiliis* (Matthews, 2005) is one of the most common parasitic outbreaks in recirculating aquaculture systems (RAS) and cause significant loss if not treated timely and correctly. To control outbreaks of *I. multifiliis*, formaldehyde is most commonly used and it is in many ways an ideal chemical to add to RAS, as its treatment efficiency is very high, without harming the fish nor the biofilter at the concentrations used for treatment (Pedersen et al., 2007). However, recent concern on potential environmental effects of

excess formaldehyde discharge, as well as worker safety issues, has lead to an intention of phasing out the use of formaldehyde. Therefore, there is an urgent need to find and implement suitable therapeutic candidates to replace formaldehyde. In order to facilitate a safe and fully sustainable implementation of a new chemical, an array of new additional information about the chemicals is required.

Use of UV in combination with ozone have proven to be a feasible solution to control pathogens (Summerfelt and Hochheimer, 1997; Summerfelt et al., 2009), but to date this approach is not commercially applied in full-scale open Danish RAS systems. Hydrogen peroxide is an obvious candidate due to the minor environmental footprint, and proven efficiency towards *I. multifiliis* infections (Heinecke and Buchmann, 2009). However, the rapid degradation and potential impact on biofilter performance in RAS cause caution in the use of HP (Schwartz et al., 2000; Møller et al., in press). Sodium chloride has also proven to be effective towards *I. multifiliis* infections, when used at concentrations of 10–15 kg m⁻³. It too has obvious limitations however, when very large water volumes are to be treated, both in terms of economy and handling, as in terms of the resulting discharge to a water course. At present, alternatives to formaldehyde are thus needed in controlling white spot disease.

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Peracetic acid (PAA) is a strong disinfectant with a wide spectrum of antimicrobial activity (Baldry, 1983; Kitis, 2004). The oxidation potential of PAA is larger than that of chlorine, hypochlorite and hydrogen peroxide and hence, due to a very high treatment efficacy, the use of PAA as a disinfectant for municipal wastewater effluents has recently received considerable attention (Antonelli et al., 2006; Falsanisi et al., 2006; Rossi et al., 2007; Santoro et al., 2007). PAA is available in commercial solutions in an acidic quaternary equilibrium mixture of hydrogen peroxide (HP), acetic acid (AA) and water in the equilibrium:



The equilibrium stability and decomposition of PAA is pH-dependent (Yuan et al., 1997), and the commercial products are often stabilized by acidification.

The biocidal active form is undissociated at pH less than 8.2 (Wagner et al., 2002). PAA has far more antimicrobial effect than HP (Block, 1991), but the combination of PAA and HP has been found to be synergistic (Alasri et al., 1992). Various commercial compounds exist (Peroxyacetic acid; proxitane, Divosan[®], Wofasteril; Peraqua and PerAqua Plus[®]; Promox; Parasan[™]; Incimaxx Aquatic) with different combinations of PAA and HP concentrations. Commercial compounds have a PAA content ranging from 3 to 40% and an HP content from 14 to 35%. In the present study we used PerAqua Plus[®] (PA+) containing 12% PAA and 30% HP according to the manufacturer (Brenntag, DK).

PAA can be considered a promising therapeutic for use in aquaculture. PAA has excellent antimicrobial activity and parasitidal effects over a wide temperature range, including temperatures below 10 °C (Block, 1991; Colgan and Gehr, 2001). It is relatively stable at low organic matter content, and it is degraded into harmless, neutral residuals (acetic acid and H₂O₂ and eventually to water), easing the discharge into receiving water bodies. A prerequisite for using PAA in aquaculture though, is that PAA neither poses sublethal effects to the fish treated nor impair the nitrification process in the biofilter at the dosages applied. The nitrification issue has become essential, as intensive aquaculture systems rely on a high degree of water reuse and hence, the need for constant removal of ammonium and nitrite by means of biofiltration. PAA treatment efficacy towards fish pathogens has been evaluated in some aquaculture studies (Rintamaki-Kinnunen et al., 2005a,b; Meinelt et al., 2007a,b; Lahnsteiner and Weismann, 2007), but PAA degradation kinetics and effects on nitrification under aquaculture conditions have, to our knowledge, not been reported. In order to ensure an adequate treatment efficacy, the most important issue is not the amount of PAA applied but the residual levels in the water during the treatment period (Weavers and Wickramanayaka, 1991). This has to be kept in mind as an explanation for the very different treatment efficacies reported from different studies. Therefore, knowledge of the fate and decay rates of PAA and HP under aquaculture conditions is clearly needed.

The aim of this study was to investigate (i) degradation rates of PAA and HP in biofilters and process water from different RAS, (ii) PAA degradation in relation to the fish stocking (biomass), and (iii) effects of PAA and HP on biofilters and stability with regard to accumulation of ammonia or nitrite and activity of the nitrifying communities.

2. Material and methods

2.1. Experimental setup

The experiments were carried out in twelve identical freshwater RAS, each with a total volume of 1700 L (Fig. 1). Each system consisted of a glass fiber rearing tank with approximately 500 L, connected to a swirl separator via a central drain. From the swirl separator, water was lead to a reservoir and pumped (up-flow) to a submerged

biofilter, after which it was passed through a trickling filter and returned to the fish tank. The submerged biofilter was filled with four vertically positioned polyethylene media modules (Bioblok 150 HD[®]; Expo-Net, DK) with a total volume of 0.667 m³ and a total surface area of 100 m². The trickling filter was fitted with the same type of media arranged horizontally (0.167 m³ and a surface area of 25 m²). The pump delivered water with a rate of ca. 100 L min⁻¹, resulting in an elevation velocity in the biofilter of approx. 15 cm min⁻¹. Flow and hydraulics were measured and documented in preliminary experiments using sodium chloride as a tracer while logging electrical conductivity with an EC-probe attached to an HQd40 multimeter (Hach Lange APS, DK).

2.2. Management and operating conditions

All systems were operated at similar conditions for more than three months before the PAA application experiments. Rearing tanks were stocked with rainbow trout (*O. mykiss*) of an average weight of 150 g. Biomass in each tank during the experiment was kept near 12 kg (rearing density ~24 kg m⁻³), by regularly removing excess biomass, with resulting biomass ranging from 10.5 to 13.5 kg per tank. Throughout the experiment, all systems were allocated a fixed amount of 100 g commercial fish feed (DAN-EX 1754) per day during a 6 h period using belt feeders. 40 L of water was manually tapped from the swirl separators and automatically replaced with new tap water, corresponding to a water exchange rate of 2.35% day⁻¹ and a hydraulic retention time of six weeks in the systems. Any uneaten feed pellets were counted and recorded daily by manually emptying the swirl. The relative water renewal rate was 400 L kg⁻¹ feed.

The temperature was kept constant at 17.0 ± 0.3 °C. pH and dissolved oxygen were monitored daily with a Hach Lange HQd40. Oxygen concentrations ranged from 7.2 to 8.5 mg L⁻¹ on a daily basis, and the pH was kept between 7.2 and 7.4 and was adjusted on a daily basis by addition of sodium bicarbonate to compensate for the alkalinity loss due to nitrification. Ammonium and nitrite levels were below 0.2 mg NL⁻¹ and nitrate between 50 and 75 mg NL⁻¹ as monitored on a daily basis. The biomass of all fish in each tank were measured every third week, and surplus biomass was removed to keep the density and the feeding ratio somewhat constant. Fish were visually inspected every day, and mortalities and moribund fish were recorded daily.

2.3. Experimental protocols

The degradation of PAA and HP from the commercial product PA+ (Brenntag, DK) was investigated in three separate experiments: degradation in biofilters from RAS, degradation in the water phase, and degradation in relation to fish densities.

2.3.1. PAA degradation in submerged aquaculture biofilters

This experiment was designed as a single factor experiment with nominal PAA concentration as the fixed factor and PAA degradation rate as the response parameter. Nine RAS were randomly chosen and exposed in triplicate to the different PA+ dosages, corresponding to nominal PAA concentrations of approximately 1, 2 and 3 mg L⁻¹ hereinafter referred to as “low”, “medium” and “high” exposure, respectively. The remaining three RAS served as control systems and were not exposed to PA+. The PA+ addition and degradation experiments were performed in a closed circuit of the RAS (volume ~1170 L) by bypassing the rearing tank (and hence the fish) and the swirl separator during exposure (Fig. 1). Prior to and during these experiments, the fish were not fed. Sufficient dissolved oxygen was maintained in each tank by providing additional aeration with a Resun[®] multichannel airpump and air stones. The trickling filter provided sufficient dissolved oxygen for the biofilter units. Water samples were taken prior to PA+ addition and during frequent, regular intervals afterwards.

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