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Impact of ozonation and residual ozone-produced oxidants on the nitrification performance of moving-bed biofilters from marine recirculating aquaculture systems

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ABSTRACT

In marine recirculating aquaculture systems (RAS) ozone is often used in combination with biofiltration for the improvement of process water quality. Especially for disinfection purposes ozone residuals are required, that lead to a fast formation of secondary oxidants in seawater, summed up as ozone-produced oxidants (OPO). We studied the impact of OPO on nitrifying biofilter bacteria in a series of laboratory batch experiments by exposing (i) cell suspensions of the ammonia-oxidizing bacteria (AOB) *Nitrosomonas marina* strain 22 and the nitrite-oxidizing bacteria (NOB) *Nitrospira* strain Ecomares 2.1, (ii) a pure culture of the NOB *Nitrospira* strain immobilized on biocarriers, as well as (iii) a heterogeneous biofilm culture settled on biocarriers from a marine RAS for 1 h to different OPO concentrations up to 0.6 mg/l chlorine equivalent. Subsequent activity tests detected a negative linear correlation between OPO concentration and nitrifying activity of suspended pure cultures. Immobilization on biocarriers increased the tolerance of AOB and NOB dramatically, suggesting the biofilm matrix to be highly protective against OPO. Furthermore, we investigated the chronic effect of moderate ozonation at OPO concentrations of 0, 0.05, 0.10 and 0.15 mg/l chlorine equivalent on biofilter performance in a 21 d exposure experiment using 12 experimental RAS, stocked with tilapia (*Oreochromis niloticus*). Chronic exposure experiments could not reveal any harmful impact on biofilter performance for OPO concentrations up to 0.15 mg/l, even at continuous exposure. Surprisingly, nitrifying activity was enhanced at all OPO concentrations compared to the control without ozonation, suggesting moderate ozonation to promote biological nitrification. It can be concluded that rather health, welfare and performance of most cultivated fish species are the limiting factors for ozone dosage than nitrification performance of biofilters. The results may further have practical implications in relation to design and operational strategy of water treatment processes in RAS and might thus contribute to the optimization of an effective and safe treatment combination of biofiltration and ozonation.

1. Introduction

Over the last decades, fish and shrimp production in land based recirculating aquaculture systems (RAS) has become increasingly important in the aquaculture industry.

Increasing costs for make-up water, waste-water discharge, temperature control and separation of waste streams are driving RAS production more and more towards intensification of water re-use. The increase in applied feed load per water volume results in more concentrated waste levels in the system making an effective water treatment and waste management to essential key factors in modern RAS technology more than ever (Blancheton et al., 2007; Martins et al., 2010).

Water treatment in RAS consists in general of mechanical (e.g. filtration, sedimentation, foam fractionation) and biological (e.g. nitrification, denitrification) treatment processes in order

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to remove particulate matter as well as dissolved organic and inorganic substances, respectively. In recirculating aquaculture systems (RAS) biological water treatment is commonly used for the reduction of dissolved organic matter by heterotrophic microbial processes as well as for the removal of toxic ammonia through biological nitrification, involving a two-step oxidation of ammonia via nitrite to nitrate by aerobic chemoautotrophic bacteria (Eding et al., 2006). Being the main excretory N-metabolite of fish, ammonia is highly toxic to most aquatic species (e.g. Schram et al., 2010; Thurston et al., 1981) and needs to be eliminated from the water before accumulating to harmful concentrations. Hence, the removal of toxic ammonia via biofilter nitrification exhibits special priority in water treatment of RAS. In the biological nitrification process, ammonia is first oxidized to nitrite by ammonia-oxidizing bacteria (AOB), such as *Nitrosomonas* spp. or *Nitrosospira* spp., followed by the subsequent conversion of nitrite to the much less toxic nitrate by nitrite-oxidizing bacteria (NOB), such as *Nitrospira* spp. or *Nitrobacter* spp. For aquacultural systems nitrification was recently reported to be mainly associated with *Nitrosomonas* spp. and *Nitrospira* spp. in marine, brackish and fresh water (Foesel et al., 2007; Keuter et al., 2011; Kruse et al., 2013; Schreier et al., 2010; Sugita et al., 2005; Tal et al., 2003).

Although nitrification might partly be attributed to planktonic bacteria, the predominant part of nitrifying bacteria is attached to surfaces implicating surface biofilms to be the main source of biological nitrification. Various types of biofilters such as trickling-, fixed bed-, moving bed- or fluidized bed-filters are widely applied in RAS to immobilize bacteria on high surface media (Timmons et al., 2002).

Developed in the late 1980s and early 1990s the moving bed biofilm reactor (MBBR) has been widely applied for water treatment in both, wastewater and aquaculture industry (Rusten et al., 2006) and recently established as a popular and reliable biofilter type in RAS aquaculture. MBBRs are distinguished by combining several advantages of alternative biofilter types such as a volume-effective relation between active surface area and reactor volume, a stable and low-maintenance operation, no need for periodic backwashing and no susceptibility for clogging (Rusten et al., 2006).

As biological, physical and chemical processes are involved in biofilm nitrification, biofilter performance is significantly affected by several biotic and abiotic parameters, e.g. temperature, pH, alkalinity, salinity, organic matter and substrate content, dissolved oxygen concentration and turbulence level (Chen et al., 2006). Nitrifying bacteria are reported to be particularly sensitive to sudden environmental perturbations and during biofilm formation at biofilter start-up (Rusten et al., 2006).

As chemical disinfection agents are widely used in aquaculture for prophylaxis and therapy these biocides bear a substantial risk of impairing the nitrification performance of biofilters, potentially leading to the accumulation of fish toxic ammonia or nitrite (Pedersen et al., 2009). Along with the temporal change of disease treatment within the past decades several studies investigated the impact of different chemotherapeutics and water born prophylactic disinfectants on biofilter performance in fresh water and marine recirculating aquaculture systems (e.g. Bower and Turner, 1982; Collins et al., 1975, 1976; Levine and Meade, 1976; Schwartz et al., 2000). Recently, Pedersen et al. studied the effects of the current chemical sanitizers peracetic acid (Pedersen et al., 2009, 2013), hydrogen peroxide (Pedersen et al., 2006, 2012; Pedersen and Pedersen, 2012) and formaldehyde (Pedersen et al., 2007, 2010) on nitrification in RAS and their degradation kinetics in biofilters.

In closed RAS ozone, a strong oxidant, is widely used as effective chemical disinfection agent (Colberg and Lingg, 1978; Liltved et al., 2006), which moreover has been proven to efficiently improve water quality and fish performance even at low dosages (Davidson et al., 2011; Summerfelt, 2003; Summerfelt and Hochheimer, 1997). Besides controlling bacteria abundance in the recirculating process water, ozone promotes microflocculation of organic matter, resulting in an improved filtration and skimming of suspended solids (Otte and Rosenthal, 1979; Sander and Rosenthal, 1975). By effectively oxidizing nitrite to nitrate as well as non-biodegradable organics such as colour-causing compounds to biodegradable organics, ozone is further applied to complement and sometimes support biological filtration in water treatment (Schroeder et al., 2011).

Ozone itself decays relatively fast in aquacultural waters, minimizing the risk of toxic ozone residuals impairing cultured organisms and biofilter bacteria in freshwater. However, during ozonation of saline waters highly toxic and persistent by-products are formed, as ozone reacts rapidly with selected halogen ions such as iodide and mainly bromide to different secondary oxidants summed up as ozone-produced oxidants (OPO) (Hoigne et al., 1985). It has been shown that brominated oxidants such as hypobromous acid/hypobromite, bromamines and further bromine species represent the dominant species in ozonated saline waters (Heeb et al., 2014; Crecelius, 1979).

Recent studies investigated the impact of OPO on different marine aquaculture species and found an impairment of health and animal welfare at concentrations as low as $\geq$0.10 mg/l chlorine equivalent (Reiser et al., 2010, 2011; Schroeder et al., 2010). As the dominant OPO are excellent bactericides (Johnson and Overby, 1971), a harmful impact can also be expected on nitrifying biofilter bacteria. In closed RAS the importance of biofilter bacteria is nearly of the same order of magnitude as the cultivated species itself in terms of their huge effect on water quality and consequently on fish health and performance. However, until now not much is known about a potential impact of seawater ozonation and associated residual oxidants on nitrifying bacteria in biofilters.

Thus, the aim of the study was to investigate (i) the sensitivity of relevant nitrifying bacteria towards OPO, (ii) the protective effect of biofilm matrix against OPO, and (iii) the long-term effect of moderate ozonation and associated OPO on nitrification performance of moving-bed biofilters by performing a series of laboratory batch experiments as well as a chronic exposure experiment in 12 separate experimental RAS.

2. Material & methods

2.1. Short-term exposure experiments

In order to characterize the specific tolerances of suspended as well as adherent relevant marine nitrifying bacteria towards OPO, a series of short-term exposure experiments were performed in lab-scale exposing different nitrifying bacteria cultures to initial OPO concentrations of 0; 0.05; 0.1; 0.2; 0.3 and 0.6 mg/l for a period of 1 h at constant conditions.

2.1.1. Experiment 1

To comparatively determine the sensitivity of a relevant marine AOB and NOB towards OPO, cell suspensions of the AOB *Nitrosomonas marina* strain 22 and the NOB *Nitrospira* strain Ecomares 2.1 in pure culture were exposed to ozonated waters of different OPO concentrations. Pure cultures of *Nitrosomonas* and *Nitrospira* strains were cultivated at the Department of Microbiology and Biotechnology, Biocenter Klein Flottbek of the University of Hamburg using mineral salts medium (in 70% seawater, final salinity: 23 ppt) according to Krümmel and Harms (1982) and Spieck and Lipski (2011), respectively. Both exposure experiments for AOB and NOB cultures were conducted consecutively and performed as follows:

In each of five 1 l Duran flasks (Schott) 500 ml of cell suspension was mixed with 500 ml of the appropriate OPO stock solution

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