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Impact of hydraulic retention time, backflushing intervals, and C/N ratio on the SID-reactor denitrification performance in marine RAS

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ABSTRACT

In recirculating aquaculture systems (RAS) the high water re-use in combination with insufficient treatment of the process water can lead to the accumulation of nitrate, among other metabolic end products. For the efficient removal of nitrate in a marine RAS, a **S**elf-cleaning **I**nherent gas **D**enitrification Reactor (SID-Reactor) was investigated in this study. Within three consecutive experimental trials the effect of varying hydraulic retention time (HRT), backflushing intervals (BFI), and carbon to nitrogen (C/N) ratios on water quality parameters and denitrification performance (rate and efficiency) were monitored. Different HRTs of 2, 4, and 6 h and additionally BFIs of 10, 30, 60, and 90 min were evaluated. The tested C/N ratios, realized using methanol (MeOH) as a carbon source, were 1.1, 1.5, 1.9, 2.1, 2.3, 2.7, 3.1, and 3.5 (mg MeOH per mg NO_3^--N). The experiments revealed that a HRT of 2 h resulted in the highest denitrification rate (497 g d^{-1} m^3 biocarriers) but a lower denitrification efficiency of 64%. A HRT of 6 h had highest denitrification efficiency of 81% but a lower denitrification rate (253 g d^{-1} m^3 biocarriers). Furthermore, it was evident that backflushing intervals every 10 min resulted in a decreased denitrification efficiency of 29%, while intervals every 90 min increased the maintenance effort. Overall, backflushing intervals every 30 and 60 min showed the best results. A C/N ratio of 2.3 seemed to be sufficient to ensure an optimal denitrification performance, incorporating all single tested water quality parameters. The results of this study allow an easy, efficient and safe application of a SID-Reactor with the purpose of nitrate removal in marine RAS.

1. Introduction

Production of fish in closed recirculating aquaculture systems (RAS) gained increasing interest over the last decades in order to minimize the environmental impact and meet legal frameworks (Badiola et al. 2012; Bregnballe 2015; Dalsgaard et al. 2013; European Commission 2009). Bregnballe (2015) postulated that “super” intensive RAS with a 99.6% degree of recirculation are trendsetting. Thus a high degree of re-use of water is only possible if solid waste treatment and denitrification systems are ensured. Accumulating metabolic end products of fish and bacteria can reach in these systems toxic concentrations for fish, if water consumption is limited and the cultivated species is rather sensitive. Nitrate, as the metabolic end product of nitrification, has a negative impact on health and growth performance of fish at species-specific concentrations (Davidson et al. 2017, 2014; Good et al. 2017; McGurk et al. 2006; Schram et al. 2014, 2012; Scott and Crunkilton 2000; Shimura et al. 2004; Torno et al. 2018; van Bussel et al. 2012).

A denitrification unit included in RAS can reduce and/or eliminate nitrate and its effects on fish and environment. Though uncommon 10 years ago (van Rijn et al. 2006), nowadays an increasing number of denitrification systems is used in RAS, subsequently increasing the research interest in enhanced denitrification practices (Addy et al. 2016). However, conventional denitrification systems are an enormous challenge for RAS staff since maintenance and operation of these systems are demanding. Badiola et al. (2012) reported the major constraints on management and future challenges concerning RAS in a survey, involving RAS based production companies, researchers, system suppliers, and consultants. Main limitations were identified as: poor designs of the systems and poor management due to an absence of skilled people. As Lekang (2013) states, the challenge in RAS is “[…] to bring together both technological and biological knowledge within the aquaculture field”.

Müller-Belecke et al. (2013) introduced the low-maintenance **S**elf-cleaning **I**nherent gas **D**enitrification Reactor (SID-Reactor; patented by

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Müller-Belecke and Spranger 2014), which is also the subject of the current study. They tested the performance of the reactor with different carbon sources (denatured ethanol, methanol, acetic acid, and glycerin) and changing operational modes (low energy demand, low carbon demand, and high performance). It has been demonstrated that the daily routine of operation and maintenance of the reactor was simple and done within a few minutes a day. Hence, the reactor is a promising denitrification unit for RAS. However, to make the SID-Reactor as safe and user-friendly as possible, it is still necessary to evaluate the influence of the most important operating settings to guarantee an optimized and safe denitrification process.

In denitrification systems, the adjustment of hydraulic retention time (HRT) shows a high impact on the nitrate removal rate and efficiency. While relatively long HRTs result in high denitrification efficiency (percent of removed NO_3^--N) and low denitrification rates (total amount of removed NO_3^--N per time unit), short HRTs result in the opposite effect (Addy et al. 2016; Lepine et al. 2016; Oh et al. 2001; Timmermans and van Haute 1983; Wang and Chu 2016).

When changing the HRT, generally two opposing scenarios can be expected. (I) At low HRTs a relatively high amount of oxygen (hindering denitrification performance) and nitrate (increasing denitrification performance) enters the reactor via the inlet water. (II) At increased HRTs, a relatively low amount of oxygen (promoting denitrification performance) and nitrate (lowering denitrification performance) enters the reactor via the inlet water. It is necessary to find a balanced setting where as much nitrate as possible can be treated without disturbing denitrification processes by increased oxygen influx.

Another major problem reported for biological filter systems is the clogging through microbial growth and particulate organic matter, thus limiting filter performance (Eding et al. 2006; Lepine et al. 2016; Mara et al. 2003; McMillan et al. 2003; Moretti et al. 1999a; Rakocy et al. 2006; Sastry et al. 1999). Conventional denitrification units based on the moving bed biofilm design or fixed bed design require a backflushing of the biocarriers to prevent clogging and breakdown of the denitrification performance. Backflushing of the denitrification unit is often accompanied by a severe change in environmental conditions for bacteria, causing a temporary breakdown of the denitrification performance.

Safe denitrification in the SID-Reactor relies on the accurate dosage (C/N ratio) of an external carbon source (in this study methanol), which is mandatory to fuel denitrification. An accurate methanol dosage results in a denitrification process able to improve water quality by reducing the nitrate and rising the alkalinity. Underdosing will lower the denitrification rate and stimulate nitrite formation (Hamlin et al. 2008; Sauthier et al. 1998; Timmermans and van Haute 1983; Yang et al. 2012) as well as hydrogen sulfide production (Bregnballe 2015), which are both toxic for aquatic species cultured in RAS. Based on stoichiometric calculations, anticipated 1.9 g of methanol is required to reduce 1.0 g of NO_3^--N (Cheremisinoff 1995). Since the C/N ratio depends on several factors (e.g. type of carbon source and cell synthesis of bacteria) the determination of an accurate dosage is mandatory for safe denitrification processes in specific technical setups.

The aim of this study was to evaluate the effect of (1) different HRTs, (2) varying BFIs, and (3) altered C/N ratios on denitrification performance and water quality parameters in a marine RAS equipped with a SID-Reactor. Three consecutive trials were performed within one semi-industrial scale RAS stocked with European sea bass (*Dicentrarchus labrax*) to evaluate the operational settings and safe use of the SID-Reactor.

2. Materials & methods

2.1. Experimental setup

Recirculation Aquaculture System (RAS).

The utilized RAS (Fig. 1, 40 m^3 in total, Kunststoff-Spranger GmbH, Plauen, Germany), was filled with sand-filtered, UV- and ozone-treated North Sea water (practical salinity: 24–30). Light regime was adjusted to 16 h light and 8 h darkness. Water from the ten rearing tanks (each 2.5 m^3) was drained to a drum filter (60 μm mesh size) and further to a pump sump. To ensure oxygen saturation in the rearing tanks, an oxygen cone was included into the RAS. A Moving-Bed-Biofilm-Reactor (MBBR, 4.5 m^3 total volume, 25 m^3 h^{-1} water flow) was filled with 1.5 m^3 of biocarrier (HEL-X®, diameter: 12 mm, surface: 859 m^2 m^{-3}, specific surface area (SSA): 704 m^2 m^{-3}, density: 0.95 g, Christian Stöhr GmbH & Co. Elektro- und Kunststoffwaren KG, Marktrodach, Germany) for aerobe nitrification. Furthermore, the RAS was equipped with a skimmer (Helgoland 500, 11 m^3 h^{-1} water flow, Erwin Sander Elektroapparatebau GmbH, Uetze-Eltze, Germany) supported by ozone (C-Lasky DSI/DTI, 10 g h^{-1} ozone, AirTree Europe GmbH, Baunatal, Germany). Water temperature in RAS was kept constant at 25 °C by a heat exchanger. The pH was kept between 7.3 and 7.5, by adding sodium hydrogen carbonate powder ($NaHCO_3$) to the rearing water. The oxygen saturation of the rearing water was maintained on average $>$ 100% ($>$ 8 mg L^{-1}), guaranteeing a sufficient oxygen supply for the fish as well as the aerobe biofilter systems. Further water parameters (salinity, total ammonia nitrogen (TAN) and NO_2^--N) were monitored and kept in a safe range in accordance to sea bass requirements summarized in Torno et al. (2018). To keep the intended basal nitrate concentration stable at 40 mg L^{-1} throughout the experimental trials, nitrogen in form of a 20% urea and 80% ammonia solution (CH_4N_2O as powder, 25% NH_3^+, Carl Roth GmbH & Co·KG, Karlsruhe, Germany) was added to the system when necessary. The nitrogen was added directly into the MBBR with the help of an automatic dosage pump (RAININ, Dynamax®, Model RP-1, Rainin Instrument, Oakland, CA, USA).

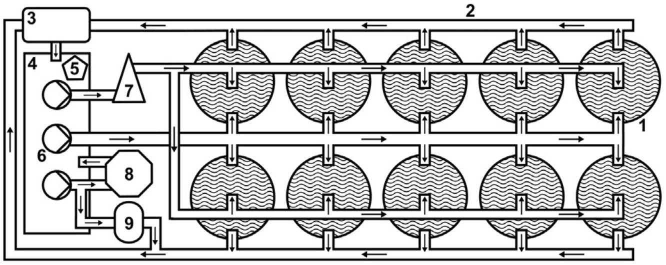

Fig. 1. The 40 m^3 experimental recirculating aquaculture system (RAS). The RAS includes ten 2.5 m^3 rearing tanks (1), an outlet drain (2), a drum filter (3), a pump sump (4), a protein skimmer (5), three circulation pumps (6), an oxygen cone (7), an aerobe MBBR nitrification filter (8), and the anoxic SID-Reactor (9). The water flow is indicated by arrows.

Experimental Animals.

Approximately 1700 European sea bass were obtained from neomar GmbH (Voelklingen, Germany) and acclimatized to the conditions in the experimental RAS in the facilities of the Gesellschaft für Marine Aquakultur (GMA) mbH (Büsum, Germany). Sea bass were distributed in accordance to body size classes (250 to 400 g, 400 to 800 g, and $>$ 800 g) among nine out of ten rearing tanks at an average stocking density of 40 kg per m^3. Fish were fed manually until apparent satiation with a commercial feed (Aller Green, Emsland Aller Aqua GmbH, Gloßen, Germany) twice per day, resulting in a basal nitrogen load into the RAS. Feeding rate was adjusted regularly to 1% of the total biomass per tank.

Denitrification System.

The same SID-Reactor (Kunststoff-Spranger GmbH, Plauen, Germany) was used for the whole experimental duration for the purpose of nitrate removal. The design of the SID-Reactor is based on a combined function principle of a fix bed as well as moving bed biofilm reactor (Müller-Belecke et al. 2013). The reactor (Fig. 2) had a total volume of 0.85 m^3, whereas the water level in the reactor was adjusted to 0.75 m^3. The reactor was filled with 0.45 m^3 floating biocarriers