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Nitrogen and carbon balance in a novel near-zero water exchange saline recirculating aquaculture system

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ABSTRACT

In response to increasing demand for aquaculture products and strict new regulations on organic matter and nitrogen discharge, inland closed recirculating aquaculture systems (RASs) are being developed as a viable eco-sustainable alternative to traditional aquaculture (e.g. ponds, raceways and cages) because of their minimal environmental impact and controlled operation. Fish feed is virtually the only source of carbon and nitrogen to the system. It is estimated that 20 to 30% of the feed nitrogen and 50% of the feed carbon are assimilated or utilized by the fish, while the rest is released to the water. Understanding the fate and utilization of these elements can help optimize RAS efficiency and economics. The fate of carbon and nitrogen was studied by mass balance in a novel near-zero discharge (<1% water exchange of system's volume per day) saline research scale RAS. The system included a fish tank attached to three treatment loops: (a) a solid filter followed by an aerated nitrification fixed-film reactor, (b) a single-stage anoxic denitrification activated sludge bioreactor which utilizes fish sludge as a carbon source, and (c) an anaerobic bio-digester (upflow anaerobic sludge blanket [UASB]) for treatment of excess denitrification biomass for the production of biogas. About 50% of the introduced carbon (from feed) was removed by fish assimilation and respiration, and another 10% by aerobic biodegradation in the nitrification bioreactor. In the denitrification reactor, 10% carbon was removed and 25% carbon was introduced into the UASB reactor, of which 12.5% was converted to methane, 7.5% to CO_2 and the rest (5%) remained as nondegradable carbon in the UASB. Using the UASB can save up to 12% of the system's energy demands, both directly as energy (methane) input and indirectly by reducing the system's oxygen demand. Of the feed nitrogen, 29% was assimilated by the fish and bacteria in the nitrification reactor and 40–50% was removed in the denitrification reactor, of which 10–20% was removed by anammox. Lastly, ~20% of the nitrogen was removed in the UASB reactor, likely by precipitation. It was demonstrated that the system was operating at high stocking density, with almost complete nitrogen and carbon removal and energy recovery.

Statement of relevance: The fate of carbon and nitrogen was studied by mass balance in a novel near-zero discharge (<1%) saline RAS. A novel approach which may significantly reduce pollution, save water and energy and improve intensive aquaculture operations was demonstrated. It was postulated that the system operated at high efficiency, with almost complete nitrogen and carbon removal and energy recovery.

1. Introduction

Aquaculture is among the fastest growing animal food-producing sectors, accounting for almost half of the total food fish supply (FAO, 2014). If not adequately addressed, current aquaculture practices can have negative environmental impacts as a result of eutrophication of water bodies, landscape modification, and changes in biodiversity (Tovar et al., 2000). For this reason, regulations have been introduced related to water use (quantity and quality) and waste discharge (Jokumesen and Svendsen, 2010). These and other concerns (e.g. disease control and weather effects) have motivated the industry to explore land-based recirculating aquaculture systems (RASs) as an alternative to the traditional open ponds and cage culture systems (Avenue and Kong, 1995; Timmons and Ebeling, 2007).

In RASs, water from the fish tank is circulated through bioreactors that treat it for return and reuse in the growth tank. This concept provides enhanced control over water quality, fish performance, biosecurity and energy use (Ebeling, 2000; Timmons and Ebeling, 2007; Tal et al., 2009). In the RAS, fish feed is virtually the only source of carbon and nitrogen solids, which are the major sources of pollution. It is estimated that by weight, the amount of solids produced in a RAS accounts for about 30 to

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60% of the applied fish feed (Chen et al., 1994). The solid waste is composed mainly of fish excretions and a small percentage of uneaten feed. Its volatile (organic) fraction ranges from 50 to 92% and it typically contains a low total solid content of 1.5–3% in the effluent (Mirzoyan et al., 2008). RAS solids and sludge is typically removed by sedimentation or physical filtration (Chen et al., 1994; Timmons and Ebeling, 2007). While freshwater RAS sludge can be used as fertilizer, saline RAS sludge usages are very limited (if at all), due to its high salinity (Sharrer et al., 2007). Today, sludge disposal from most RASs is done off site (Piedrahita, 2003), but it requires high volumes of water and is a potential source of pollution.

Fish feed contains 25–65% protein (Lovell, 1988), corresponding to 4.1–10.7% organic nitrogen. Only about 20–30% of the nitrogen from the applied feed is retained by the fish (Heinsbroek and Kamstra, 1990; Gross et al., 2000; Islam, 2005; Sandu and Hallerman, 2013), while the rest is excreted into the water. Therefore, it is estimated that about 75% of the fish protein nitrogen is released into the water, with a significant portion composed of total ammonia nitrogen (TAN) (Ebeling et al., 2006). Ammonia is toxic to many fish, even at low concentrations (a few milligrams per liter) (Randall and Tsui, 2002; Timmons and Ebeling, 2007). The most common TAN treatment in RASs is aerobic nitrification (Guerdat et al., 2010; van Rijn, 1996), which results in the accumulation of less toxic nitrate in the system. The nitrate concentration is usually kept at high and non-toxic levels by water exchange (20–40% system volume per day) (Hu et al., 2012). This practice might pollute the environment if released untreated. In advanced RAS an anoxic denitrification unit is introduced (Martins et al., 2010; van Rijn, 2013). Denitrification refers to the biological reduction of nitrate to di-nitrogen gas (N_2). In this process, approximately 1 mol of alkalinity is released to the water for each mole of reduced nitrate (van Rijn et al., 2006). Denitrification is mostly performed by anoxic heterotrophic bacteria and requires an organic carbon source. The latter can be provided by the addition of organic substrates, such as molasses, methanol or acetic acid (van Rijn et al., 2006; Hamlin et al., 2008), or by adding fish sludge (van Rijn and Rivera, 1990; Klas et al., 2006a). During the denitrification process, denitrifying microbial biomass is produced.

Anaerobic digestion (AD) has long been used to stabilize sludge and reduce its volume, as well as to produce biogas (Marchaim, 1992; Ward et al., 2008; Mirzoyan et al., 2010). During the digestion process, the sludge undergoes considerable changes in its physical, chemical and biological properties (Appels et al., 2008; Chandra et al., 2012). Typical AD is divided into four stages occurring simultaneously in the reactor: hydrolysis, acidogenesis, acetogenesis and methanogenesis; the ultimate product is biogas, which is mainly composed of methane (CH_4) and carbon dioxide (CO_2), with small levels of hydrogen sulfide (H_2S) and ammonia (NH_3) (Appels et al., 2008).

AD of aquaculture sludge followed by biogas production is a fairly new concept with only limited information in the literature; even less is known about saline aquaculture sludge from RASs (Tal et al., 2009; Mirzoyan et al., 2012). The use of an upflow anaerobic sludge blanket (UASB) reactor for anaerobic conversion of saline aquaculture sludge to biogas production has been suggested (Mirzoyan et al., 2008). In the UASB reactor, sludge flows upward through a "microbial blanket" where it is degraded by anaerobic microorganisms and biogas is produced. Mixing in the reactor results from natural movement of the microbial flocs that are attached to biogas bubbles. When the biogas is released from the floc, it settles back down and the gas is collected. The main advantages of the UASB reactor are the low operational costs and simplicity of operation while providing high solids-removal efficiency for wastes with low (1–4%) total solid content (Marchaim, 1992). Experiments with the UASB have demonstrated its potential to reduce saline aquaculture sludge mass followed by biogas production in an experimental marine RAS (Tal et al., 2009). Although some reports have indicated that the efficiency of marine solid-waste reduction to biogas is not optimal according due to low C:N ratio (Mirzoyan, 2009), Quinn et al. (2016) demonstrated efficiencies of 90% with the development of appropriate methanogenic consortia of microorganisms.

The goals of this study were to reduce water exchange in an intensive RAS to close to zero (<1% of system's volume per day) by anaerobic biological treatment of solid waste composed primarily of carbon and aerobic/anaerobic treatment of nitrogen waste products. It was hypothesized that understanding and optimizing the carbon and nitrogen cycles would support development of an intense, sustainable RAS that would maintain high water quality sufficient for maximum fish performance, and generate of biogas to offset the external energy input, resulting in a smaller environmental footprint.

2. Materials and methods

2.1. Experimental system

A research scale pilot RAS for the culture of 250 kg/m^3 catfish (*Clarias gariepinus*) was designed as shown in Fig. 1. The system consisted of a 1 m^3 fish tank (Dolav, Israel) attached to three water-treatment loops. The first treatment loop included an upflow solid filter (220 L) composed of packed plastic beads (Aridal Bioballs, Israel; porosity of 80%, surface area of 860 m^2/m^3; Sklarz et al., 2010) and a nitrification unit consisting of two aerated moving bed reactors (60% media; target DO concentration > 5 mg/L) configured in series (390 L and 470 L). Water from the fish tank was recirculated through the first loop at a rate of 3 m^3/h with a 0.57-kW magnetic pump (Pan-World, Japan) that was controlled with a frequency controller (EDS800, ENC). Fish sludge from the solid filter was backwashed to an 800-L buffer tank by a 0.05-kW aquarium pump (Rio 14, Hyper Flow) every few hours for further treatment in the second loop.

The second treatment loop was designed to remove nitrate by denitrification and was composed of a single-stage anoxic activated sludge bioreactor with configuration based on Klas et al. (2006b). The reactor was composed of a 760-L continuously stirred tank reactor (CSTR) and a 290-L settler. Water from the first loop was continuously pumped to the CSTR at 300 L/h by a 0.03-kW aquarium pump. The CSTR was equipped with a 0.37-kW mechanical overhead mixer with frequency controller (EDS800, ENC). A blade (0.09-kW) turning at a rate of 2 rev/min was used to prevent sludge accumulation on the settler edges (Kora, Israel). Biomass from the settler was recirculated to the activated tank at a rate of 100 L/h by 0.18-kW peristaltic pump (Boxer 4500, Admor) with a frequency controller. The backwash of the fish sludge was pumped from the buffer tank into the denitrification reactor at rate of 60 L/h using a 0.05-kW peristaltic pump (Boxer 4500, Uno).

Excess microbial biomass resulting from microbial growth in the denitrification reactor was treated in the third treatment loop which was composed of a UASB reactor. Briefly, the reactor consisted of a 700-L cylindrical column ($H = 2.5$ m; $R = 0.3$ m); an inverted funnel at the top of the cylinder allowed for efficient solid–liquid–gas separation and biogas collection (Mirzoyan and Gross, 2013). Denitrification biomass (30 L) from the settler bottom was pumped daily at 60 L/h (upflow velocity = 0.21 m^2/h) with a 0.05-kW peristaltic pump (Boxer 4500, Uno). The overflow (30 L) from the UASB was discharged and accounted for a daily water exchange of 0.8% of the system's volume per day.

2.2. Experimental design

Initially, saline groundwater with electrical conductivity (EC) of 3.4 mS/cm was transferred to the RAS. Catfish (*C. gariepinus*; $n = 90$) were introduced and the system was operated for over 3 months to allow for acclimation of all of its biological reactors. The fish tank was then emptied and refilled with fresh saline groundwater (Table S1.) and 347*C. gariepinus* catfish with an average weight of 310 ± 11 g were introduced. Fish were fed daily for 147 days at a rate of 1–2% of