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# Influence of carbohydrate addition on nitrogen transformations and greenhouse gas emissions of intensive aquaculture system

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## HIGHLIGHTS

- Addition of soluble starch to intensive aquaculture system enhanced heterotrophic bacterial growth and denitrification.
- Soluble starch addition minimized the nitrous oxide emissions from intensive aquaculture system by 83.4%.
- Soluble starch addition had significant adverse effects in controlling GHG emissions from aquaculture systems.

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## ABSTRACT

Aquaculture is one of the fastest-growing segments of the food economy in modern times. It is also being considered as an important source of greenhouse gas (GHG) emissions. To date, limited studies have been conducted on GHG emissions from aquaculture system. In this study, daily addition of fish feed and soluble starch at a carbon-to-nitrogen (C/N) ratio of 16:1 (w/w) was used to examine the effects of carbohydrate addition on nitrogen transformations and GHG emissions in a zero-water exchange intensive aquaculture system. The addition of soluble starch stimulated heterotrophic bacterial growth and denitrification, which led to lower total ammonia nitrogen, nitrite and nitrate concentrations in aqueous phase. About 76.2% of the nitrogen output was emitted in the form of gaseous nitrogen (i.e., $N_2$ and $N_2O$) in the treatment tank (i.e., aquaculture tank with soluble starch addition), while gaseous nitrogen accounted for 33.3% of the nitrogen output in the control tank (i.e., aquaculture tank without soluble starch addition). Although soluble starch addition reduced daily $N_2O$ emissions by 83.4%, it resulted in an increase of daily carbon dioxide ($CO_2$) emissions by 91.1%. Overall, starch addition did not contribute to controlling the GHG emissions from the aquaculture system.



## 1. Introduction

Global food fish supply has increased dramatically in the last five decades, with an average growth rate of 3.2% per year in the period of 1961–2009, to meet the rising world demands for protein. In 2010, capture fisheries and aquaculture supplied about 148 million metric tons of fish globally (FAO, 2012). Because world fishery productions have leveled off since the 1970s, aquaculture plays a crucial role in meeting the increasing global demands for fish.

Since the expansion of aquaculture is restricted by land and water requirements, intensive aquaculture, which aims at raising fish at mass densities as high as 30 kg/m$^3$ in closed or semi-closed systems with sufficient oxygen, fresh water and feed, has been increasingly popular to overcome space and resource limitations (Delong et al., 2009). However, the development of intensive aquaculture has created serious environmental problems.

The most important issue during the management of intensive aquaculture is avoiding the accumulation of toxic inorganic nitrogen species (especially, $NH_3$ and $NO_2^-$) in the aqueous phase (Durborow et al., 1997; Hargreaves and Tucker, 2004). In intensive aquaculture systems, fish are fed a high protein diet with protein levels varying from 25 to 55% (Pillay and Kutty, 2005). Protein is digested by fish, producing mainly ammonia, and is excreted to the surrounding aqueous phase. In the aqueous phase, ammonia exists in two forms: un-ionized ammonia ($NH_3$) and ionized ammonium ($NH_4^+$). The sum of $NH_4^+$ and $NH_3$ is usually referred to as total ammonia nitrogen (TAN). Ammonia can further be oxidized to $NO_2^-$ and $NO_3^-$ by nitrifying bacteria present in the aquaculture system. Inorganic nitrogen species, especially $NH_3$ and $NO_2^-$, are toxic to fish. High concentrations of $NH_3$ and $NO_2^-$ can stimulate the release of corticosteroid hormones into the venous circulation, which may inhibit

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fish growth and cause a variety of physiological dysfunctions (Tomasso, 1994).

One of the most common approaches to prevent excess nitrogen buildup is through water exchange. This approach, however, has several environmental issues, e.g., requires a perpetual supply of fresh water and the generation of nitrogen-rich effluent, among others (Avnimelech, 2009). Another approach is to enhance nitrification to facilitate the conversion of ammonia and $NO_2^-$ into relatively non-toxic $NO_3^-$ by using nitrifying biofilters. Although nitrifying biofilters have been successfully employed in aquaculture systems and are effective in ammonia and $NO_2^-$ removal, they are costly and most importantly, do not provide an opportunity for nutrients recycling (Avnimelech, 2006; Crab et al., 2007). This is particularly significant because protein in fish feed is the most expensive component of aquaculture operational costs especially when taking into consideration the fact that only about 25% of the nitrogen consumed by fish is converted to fish mass (Hargreaves, 1998).

An additional strategy, namely carbohydrate addition, has been demonstrated to be effective in achieving low or zero-water exchange intensive aquaculture systems (Gao et al., 2012). By adding carbohydrate to aquaculture systems and regulating the carbon-to-nitrogen (C/N) ratios, the growth of heterotrophic bacteria can be stimulated, which results in the removal of inorganic nitrogen through assimilation. As bacterial biomass increase, they tend to form noticeable aggregates (i.e., bioflocs), which serve as a potential source of food for fish. Thus, the utilization of protein in the fish feed is enhanced due to nutrients recycling by heterotrophic bacteria. Recently, this approach has become increasingly popular for closed-water shrimp and tilapia cultivation (Asaduzzaman et al., 2010; Widanarn et al., 2012).

Besides water pollution, the contribution of aquaculture systems to global greenhouse gases (GHG) emissions has aroused great attention in recent years (Williams and Crutzen, 2010; Hu et al., 2012). In aquaculture systems, major GHG emissions include carbon dioxide ($CO_2$), methane ($CH_4$) and nitrous oxide ($N_2O$). $CO_2$ is produced from the decomposition of organic materials and through respiration by fish. High levels of $CO_2$ can be detected in aquaculture systems with high feed loading rates and relatively slow water turnover (Good et al., 2010). $CH_4$ is produced when organic materials are broken down under anaerobic conditions (Adams et al., 2012). In conventional aquaculture systems, however, significant concentrations of $CH_4$ are typically unlikely (Rakocy et al., 2006). $N_2O$ is an important GHG which has a global warming potential (GWP) that is 296 times higher that of $CO_2$ on a 100-year timescale (IPCC, 2007). Hu et al. (2012) estimated that aquaculture could contribute up to 5.72% of global anthropogenic $N_2O$–N emission by 2030 if it continues to increase at the present annual growth rate. However, the mechanisms for $N_2O$ generation in an aquaculture system are highly influenced by environmental conditions and are not well known. Both nitrification and denitrification can contribute to the emission of $N_2O$ from aquaculture systems (Beaulieu et al., 2011; Hu et al., 2013a).

The addition of carbohydrate to aquaculture systems could decrease the abundance of nitrifying bacteria, and thus may minimize nitrification-driven $N_2O$ production (Avnimelech, 1999). Furthermore, the supplementation of external organic carbon could provide electron donors for denitrification and may reduce $N_2O$ generation through denitrification (Hu et al., 2013b). However, carbohydrate addition might increase the concentration of organic materials, which may lead to higher $CO_2$ and $CH_4$ emissions. Nonetheless, there has been a dearth of studies thus far on the emissions of GHGs from zero-water exchange intensive aquaculture system.

The purpose of this study was to investigate the effect of soluble starch addition on GHG emissions from a zero-water exchange tilapia intensive aquaculture system. Nitrogen transformations in aquaculture system with and without soluble starch addition were also examined.

## 2. Materials and methods

### 2.1. System setup

Two aquaculture systems were placed side by side in an air-conditioned room at a temperature around 25 °C and exposed to 24-h lighting. The schematic diagram of aquaculture system is shown in Fig. 1. The system was mainly composed of a plastic tank (KMT85, Tuff Stuff, Terra Bella, CA, USA), with a working volume of 200 L. Air was supplied continuously by an air pump through three diffusers placed at the bottom of the tank, and desired DO concentrations were obtained by controlling the air flow rate. Aeration also provided mixing for the system. In each tank, one biofilter composed of mesh nylon biofilter media bags filled with 1.5 kg of biomedia (Kaldnes @ media, Aquatic Eco-System, Apopka, FL, USA), was placed adjacent to the air diffusers to facilitate the growth of nitrifying bacteria. Semi-transparent acrylic plastic lids were used to minimize water loss by evaporation and to prevent algal growth. Freshwater was occasionally added to the system to compensate water loss through evaporation, when necessary. The aquaculture tank was maintained at pH of around 7.0 by periodic dosing of sodium bicarbonate ($NaHCO_3$).

### 2.2. Experiment design

It generally requires about 4 weeks to establish the required microbial community in a biofilter of an aquaculture system (Avnimelech, 2009). However, since heterotrophic bacteria typically have a maximum growth rate five-fold faster and biomass yields two to three-fold greater than that of nitrifying bacteria, bioflocs could be established rapidly in a matter of days (Grady and Lim, 1980). To overcome the time difference, effluent from a stable operating aquaculture system was used as the initial tank water for the experiment, and the biofilters were inoculated in the stable operating aquaculture system for two weeks prior to the start of the experiment. This facilitated the establishment of nitrifying bacteria in the biofilters, which were used in subsequent experiments.

Each tank was stocked with mixed sex tilapia fish (*Oreochromis niloticus*) with an average weight of 120.5 ± 27.3 g, to obtain an initial stocking density of around 23.5 kg/m$^3$. The fish were obtained from Windward Community College (Honolulu, Hawaii, USA). Fish were grown without water exchange for 8 weeks, and were fed once daily at 10:00 A.M. with 42% protein commercial aquatic feed pellets (Silver Cup Trout Feed, Tooele, UT, USA). The amount of feed per feeding time was determined based on fish response to previous feeding (Casillas-Hernández et al., 2006). Ten minutes after feeding, all the feed pellets remaining above water surface were collected, dried and weighed. The feeding rate was adjusted in the subsequent days so that the leftover (un-consumed) feed 10 min after feeding was no more than 5% of the total feed added. In the treatment tank, soluble starch was added daily along with feed to maintain a C/N ratio (w/w) of 16:1; while the control tank was supplied with fish feed only (Nootong et al., 2011). The amount of daily starch addition was calculated as per the equations proposed by Avnimelech (1999), and about 1.4 g of soluble starch was added for each gram of formulated fish feed. The pre-weighed soluble starch was completely mixed with tank water in a beaker and was then uniformly sprayed over the tank surface water after each feeding.

### 2.3. Analytical method

Water samples from each tank were obtained after feeding every other day and were analyzed immediately for TAN, $NO_2^-$, $NO_3^-$, total phosphate (TP), and chemical oxygen demand (COD) concentrations using HACH reaction kits (Loveland, CO, USA), namely Ammonia TNTplus (TNT 830), Nitrite TNTplus (TNT 839), Nitrate TNTplus (TNT 836), Phosphorus TNTplus (TNT 845), and COD Reagent TNTplus (TNT 822), respectively. Dissolved oxygen (DO) concentrations, temperature,