Nitrogen transformations in aquaponic systems: A review

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ABSTRACT

In recent years, aquaponic systems have gained significant popularity as soilless agriculture systems for organic fruits and vegetables production with concomitant remediation of aquaculture effluent. Aquaponics is a potential sustainable food production system that integrates aquaculture with hydroponics in which nitrogen-rich effluent from the fish production is utilized for plant growth. Because nitrogen is one of the most important inputs in an aquaponic system, it is critical to investigate the nitrogen transformations in the system for enhanced recovery of resources. Since studies on nitrogen transformations and nitrogen utilization efficiency (NUE) in aquaponic systems have been very limited, this review critically examines the important fates of nitrogen from input to outputs (e.g., ammonia nitrogen generation, nitrification, nitrate assimilation and nitrogen loss) to improve NUE in aquaponic systems. Various factors affecting the nitrogen transformations are also discussed. Furthermore, an example of nitrogen imbalance between nitrate uptake and nitrate generation rates in an aquaponic system was demonstrated. This review aims to advance our current understanding of nitrogen transformations and outlines future research needs in aquaponic systems, a sustainable model for efficient water and nutrient managements, and food production.

Keywords: Aquaponic system, aquaculture, hydroponic system, nitrogen transformation, resource recovery

1. Introduction

The growing food insecurity, uncontrollable rising of food prices, water scarcity and poverty, especially in developing countries, coupled with concerns of climate pattern, have resulted significant global challenge (FAO, 2011; IPCC, 2013; Mukuve and Fenner, 2015). To overcome these issues, agriculture-based sustainable farming was suggested to produce food by conserving water and recycling nutrients, and converting wastes (water) into high-value resources. Aquaponics is a soilless agriculture system that synergistically combines aquaculture and hydroponics (Love et al., 2015). Some of the merits of aquaponic systems (APS) are recovering nutrients, minimizing water demand and increasing profitability by simultaneously producing two cash crops.

Nitrogen is an essential element for all living organisms because it is the component of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), amino acids, protein and other cell components (Pratt and Cornely, 2014). The major source of nitrogen input in aquaponic systems is fish feed, which is excreted by the fish in the form of ammonia nitrogen (90%) (Timmons et al., 2002). Thus, ammonia nitrogen excreted by fish provides nitrogen source for plant growth. As the aquaculture effluent flows into the hydroponic component, nutrients are transformed by biological nitrification and denitrification, and assimilation by plants in the form of nitrate (NO_3^-) and ammonium (NH_4^+) (Graber and Junge, 2009; Zou et al., 2016). Aquaponics combines the advantages of both aquaculture and hydroponics, while eliminating their respective disadvantages (e.g., cost of treatment of aquaculture effluent and cost of nutrient solutions in hydroponics) when operating individually. It also reduces the operating costs when considering each system individually (Love et al., 2015). Aquaponics has great potential to become a

sustainable technology for nitrogen-rich waste remediation with simultaneous year-round production of high quality fish and vegetables while conserving the water.

Although a wide variety of system designs, plant and aquatic species have been examined over the past decades, an in-depth study, especially with respect to the mechanisms and dynamics pertaining to nitrogen transformations in aquaponic systems is still lacking (Hargreaves, 1998; Rakocy, 2007). In order to promote the development of aquaponic systems and to minimize the environmental impacts associated with its widespread application, it is vital to identify the key mechanisms of nitrogen transformations in soilless aquaponic systems.

This paper critically examines the pathways of nitrogen transformations for improving nitrogen utilization efficiency (NUE) in aquaponic systems by evaluating the important processes of bioconversion of nitrogen into products and the processes of possible nitrogen loss. Various factors affecting nitrogen transformations are discussed. An example of nitrogen imbalance between nitrate uptake and nitrate generation rates in an aquaponic system was demonstrated. This review aims at advancing our understanding of microbial nitrogen transformations in aquaponic system and to provide information on how this data can be employed in designing and operating an efficient aquaponic system.

2. Types of aquaponic systems

A typical aquaponic system consists of a fish tank (aquaculture), a biofilter (for nitrification) and a grow bed (hydroponics) (Love et al., 2015). There are three types of most commonly used aquaponic systems (Fig. 1), classified based on types of grow bed, namely nutrient film technique (NFT), floating-raft (deep water culture) and media-filled (flood and

drain) (Engle, 2015). NFT type provides high oxygen to the plant roots that facilitates high yield of vegetables. However, NFT is only suitable for small vegetable species because the grow bed cannot support high quantity of roots due to potential blockage of recirculating flow (Chérif et al., 1997; Engle, 2015). Thus, efficient solid removal is critical for NFT to prevent the clogging in the grow bed channel. Floating-raft type is most commonly adopted in an aquaponic system because it allows the plant roots to freely absorb the nutrients in the water without clogging the water channel (Engle, 2015; Liang and Chien, 2013; Timmons et al., 2002). NFT and floating-raft aquaponic systems require a biofilter for nitrification and a sedimentation tank for solid removal (Engle, 2015; Nelson, 2008). Media-filled type is the simplest aquaponic system that does not require separate biofilters because it contains media (e.g., pumice stones or clay beads) in the grow bed for nitrification (Zou et al., 2016). A siphon is used to fill and drain the water in order to supply oxygen by direct contact between plant roots and the air (Bernstein, 2011). However, clogging and insufficient oxygen levels in the grow bed are commonly observed during a long-term operation of aquaponic systems.

Fig. 1. Aquaponic systems based on types of grow bed.

3. Total ammonia nitrogen (TAN) generation

Feed provides nutrients for cell growth and energy for assimilation (fish muscle growth), free energy for metabolism and heat losses (Lekang, 2013). The production rate of TAN can be approximated as given below (Ebeling et al., 2006; Timmons et al., 2002):

$$P_{\text{TAN}} = F \times \text{PC} \times 0.092 \quad (1)$$

Where P_{TAN} is the production rate of TAN (kg/day); F is feeding rate (kg/day); PC is protein content (in fraction); and 0.092 is the fraction of excreted TAN per protein input. Adult fish normally consume 1 to 3% of their body weight in feed, while juvenile fish consume around 7% of their body weight (Bernstein, 2011). In addition, feed conversion ratio (FCR) shows the ratio of the total weight of fish feed to total wet weight gained by fish at the end of the culture period (Endut et al., 2010). FCR value depends on fish species and feed (Hu et al., 2014).

Aquaponic systems are generally operated using freshwater because the growth of plants and roots is stressed by osmotic pressure and is inhibited by high salinity (Duan et al., 2013). Ammonia excretion pathways in fresh water fish are different from those in marine fish, because of different mechanisms of osmoregulation. Fresh water fish (hypertonic) urinate to maintain low blood concentration of solutes while marine fish (hypotonic) balance the high blood concentration by drinking seawater and reducing the urine excretion (Evans, 2003; Weihrauch et al., 2009). Thus, fresh water fish provides a higher NH_4^+ generation rate than the fish in marine environments when feeding with the same rate (Ip and Chew, 2010). In fresh water, fish release ammonia nitrogen via urine through transamination and deamination process of feed protein in the liver. Excess L-amino acids derived from the protein are firstly transformed to glutamate by the catalytic reaction with alpha-ketoglutarate. Glutamate dehydrogenase enzyme then deaminates the glutamate to NH_4^+ . This biochemical pathway regenerates alpha-ketoglutarate enzyme as the product which is reused in the same cycle. The produced NH_4^+ and free ammonia molecules (NH_3) appear in blood stream and partially accumulate in white muscle depending on electrochemical gradients between blood stream and the amount of white muscle (Wilkie, 1997). Since it is very difficult for the NH_4^+ to permeate across the phospholipid of biological membranes from the blood stream, passive NH_3 diffusion drives the excretion of ammonia

nitrogen to the bulk water through branchial epithelium of fish (Ip and Chew, 2010; Wilkie, 2002). High partial pressure of NH_3 in blood drives the NH_3 to permeates through gill epithelium via aqueous pores (aquaporins) and paracellular routes, diffuses to gill water (unstirred boundary layer) and bulk water, respectively. Fig. 2 (left box) shows the pathways of TAN generation by fish. (At the gill water layer, the passing NH_3 will be protonated via the dissociation of dissolved CO_2 which is permeable within the gill (Ip and Chew, 2010). CO_2 can also dissociate inside the gill cytosol by hydration reaction with carbonic anhydrase enzyme (CA). The released ammonia is then trapped with H^+ which is generated from the original dissolved CO_2 and the other H^+ ion. The latter is actively excreted to apical side by the mediation of H^+ -ATPase (Wilkie, 2002, 1997). Thus, the partial pressure of NH_3 in the blood and outside the gill is controlled by the gradients of NH_3 concentration and ambient pH, which equilibrates the fraction of NH_4^+ and NH_3 . The summation of NH_4^+ and NH_3 is known as total ammonia nitrogen (TAN). Fig. 2 illustrates an aquaponics system, and the nitrogen cycle in different components.

Fig. 2. Schematic diagram of an aquaponic system and nitrogen transformations pathways in different components.

The other pathway of TAN generation is the hydrolysis of organic N such as fish urine and feces. Urea hydrolysis occurs in biofilms of aquaponic biofilters. The hydrolysis rate can be approximated by first-order kinetics because urea concentrations in aquaponic systems are significantly lower than the half-saturation coefficients (e.g., 33.1 g/L for *Escherichia coli* MJK2) of hydrolytic bacteria from Michaelis-Menten equation (Connolly et al., 2015). Fish excrete high amount of feces, and the microorganisms synthesize new cells in the presence of organic carbon (van Rijn, 2013). Total solids generations of about 230, 585, 448 and 224

kg/metric ton fish produced were reported in RAS for tilapia (*Oreochromis* sp.), Nile tilapia (*O. niloticus*), Gilthead seabream (*Sparus aurata*) and Atlantic salmon (*Salmo salar*), respectively (van Rijn, 2013). Nitrogen content in fish feces varies from 10 to 40% depending on nitrogen content of fish feed and fish species (Lupatsch and Kissil, 1998; Schneider et al., 2004; van Rijn, 2013). The solids can be periodically removed by draining the sedimentation tank or biofilters.

4. Nitrogen transformations by nitrification

Nitrification is the main process that transforms NH_4^+ to NO_3^- in the presence of oxygen (Hu et al., 2015). TAN is oxidized into nitrite (NO_2^-) by ammonia oxidizing bacteria (AOB) (e.g., *Nitrosomonas*, *Nitrosococcus*, *Nitrospira*, *Nitrosolobus*, *Nitrosovibrio* sp., etc.) and ammonia oxidizing archaea (AOA). The resulting NO_2^- is oxidized to NO_3^- by nitrite oxidizing bacteria (NOB) (e.g., *Nitrobacter*, *Nitrococcus*, *Nitrospira*, *Nitrospina* sp., etc.) (Ebeling et al., 2006; Gerardi, 2002; Panuvatvanich et al., 2009). AOA do not appear to play a role in aquaponic systems although their abundance was reported in similar environment such as soils and oceans (Jung et al., 2014; Xia et al., 2011; Zhang et al., 2012). AOA is responsible for oxidizing ammonia under extremely low NH_4^+ concentrations (about 2 $\mu\text{g N/L}$) due to their physiological diversity, leading to toleration and adaptation to extreme nutrient limitations (Martens-Habbena et al., 2009). The nitrification rate of AOA will not be significantly higher than that of AOB in nitrogen-rich environments, such as aquaponic systems. Thus, nitrification by AOA does not significantly occur in aquaponic systems (Hu et al., 2015; Zou et al., 2016).

In aquaponic systems, TAN needs to be oxidized to NO_3^- because NO_3^- is not toxic to fish even at high concentrations of up to 150-300 mg N/L (Graber and Junge, 2009; Hu et al., 2014). However, TAN and NO_2^- concentrations have to be maintained at low levels (Buzby and

Lin, 2014; Liang and Chien, 2013). For example, TAN and NO_2^- concentrations of 1.6 to 2.9 mg N/L and 0.4 to 1.1 mg N/L, respectively, were observed in a well-operated aquaponic systems using tilapia and basil, while NO_3^- accumulated at relatively high concentrations of up to 54.7 mg N/L (Rakocy et al., 2003). Studies showed that the NO_3^- in aquaponic systems can vary from 10 mg N/L to over 200 mg N/L without stress to tilapia and plants (Lam et al., 2015; Seawright et al., 1998; Sikawa and Yakupitiyage, 2010). Table 1 shows the nitrogen concentrations, nitrogen variations, and trends of nitrate in different types of aquaponic system. NO_3^- accumulations and NO_3^- depletion in recirculating water show the imbalance between plant requirement and ammonia generation. Table 1 shows that NO_3^- accumulation in previous studies of aquaponic systems occurred when NO_3^- generation rate exceeded the amount of NO_3^- that plants could utilize. As a result of the imbalance between NO_3^- generation and NO_3^- utilization, NO_3^- accumulation occurred. This is shown by the trend of NO_3^- concentration in the recirculating water over time. Conversely, NO_3^- concentrations decreased over time when NO_3^- uptake rate by plants was higher than the NO_3^- generation rate. This decreasing trend of NO_3^- indicates NO_3^- depletion in aquaponic systems. The increase in NO_3^- concentration represents the NO_3^- accumulation, and the decrease in NO_3^- concentration represents the NO_3^- depletion (Table 1). Thus, the overall balance of nitrogen in aquaponic systems occurred when the trend of NO_3^- concentration was constant (stable) over time (Table 1). However, studies on optimizing nitrogen in aquaponic systems are still in its infancy.

Table 1. Nitrogen concentrations, nitrogen variations, and trends of nitrate in different types of aquaponic system (APS).

In essence, the fish tank and biofilters in aquaponic systems resemble to RAS in terms of TAN and NO_2^- oxidation, which suggests that the same nitrifying microbial groups exist in both systems (Hu et al., 2015; Kuhn et al., 2010; Prehn et al., 2012). However, gene expressions of the microbial groups were also found on the surface of plant roots in aquaponic systems (Hu et al., 2015; Zou et al., 2016). Using Fluorescence In Situ Hybridization (FISH) and 16S rRNA analysis, the abundance and activity of nitrifiers, such as *Nitrosomonas* sp., *Nitrococcus* sp., and *Nitrospira* sp., were observed in the biofilm of a moving bed biofilm reactor (MBBR) employed in a recirculating aquaculture system (van Kessel et al., 2010). AOB and NOB (*Nitrobacter* sp. and *Nitrospira* sp.) were found in aquaponic systems, particularly on root surface of tomato (*Lycopersicon esculentum*) and Pak choi (*Brassica campestris* L. subsp. *chinensis*) (Hu et al., 2015). AOB and NOB require oxygen as an electron acceptor for their metabolisms. The nitrifiers also utilized NH_4^+ and inorganic carbon (dissolved carbon dioxide and bicarbonate) as nitrogen and carbon source, respectively for cell assimilation. In the biofilters treating ammonia-rich aquaculture effluent, the energy input is utilized in two electron accepting pathways; the synthesis of carbonaceous biomass (6.2%) and the generation of metabolized energy (93.8%) (Ebeling et al., 2006). Thus, nitrogen in the solids generated in aquaponic systems by nitrifiers is not significant compared to NO_3^- generation.

In RAS and aquaponic systems, biofilter is primarily designed for nitrification. Michaelis-Menten equation or Monod's equation can be applied for determining nitrification rate of fixed-film biofilters; however, zero- or first-order kinetics can be assumed to simplify the calculation (Connolly et al., 2015; Hagopian and Riley, 1998; Müller et al., 2007). In large-scale hydroponic biofilter (non-recirculating system) treating eutrophic surface water at long retention time, zero-order reaction was reported for nitrification process of both NO_2^- and NO_3^- (Li et al.,

2009). Ammonia oxidation rates in both fresh and marine aquaculture biofilters can be expressed as zero-order to first-order reactions (Díaz et al., 2012; Endut et al., 2010). Moreover, the kinetics of TAN oxidation depended on the hydraulic loading rate (HLR), carbon-to-nitrogen (C:N) ratio and the media characteristics of grow beds in aquaponic systems (Endut et al., 2010). For example, the first-order constant for TAN oxidation was positively correlated with HLR (Endut et al., 2010), while the zero-order constant for TAN oxidation was negatively correlated with C:N ratio (Michaud et al., 2014). However, the predictability of the models is more accurate when the concentration of dissolved oxygen is sufficient for microbial growth in biofilters. Specific loading rates were also provided to approximately determine the required surface area of biofilters. In engineering design, the removal rate of 0.8 and 1 mg TAN/m²-d at 20 and 25°C, respectively, were suggested (Lekang, 2013). The kinetic constants and orders of nitrification reaction are dependent on many factors. Since the aquaponic systems consist of three major entities: fish, plants, microbial community, more meticulous consideration is needed in adopting the kinetic model.

Insufficient nitrification is a major problem in biofilters, especially during the start-up period. TAN concentration increases drastically when the abundance of AOB is limited. Furthermore, the growth rate and the abundance of AOB are relatively higher than those of NOB during start-up period, leading to the accumulation of NO₂⁻ (Delong and Losordo, 2012). Once the nitrifiers are fully established, the rates of NH₄⁺ and NO₂⁻ oxidation reach steady state. The supplementation of commercially available nitrifying bacteria together with low recirculation rate during the start-up period can also accelerate the nitrification rate (Delong and Losordo, 2012; Kuhn et al., 2010).

5. Nitrogen uptake by plants

Nitrogen uptake is the main pathway of nitrogen recycling into vegetables in aquaponic systems. An efficient aquaponic system should show high yields of plant and fish biomass with low amount of nitrogen loss. The nitrogen transformations between vegetable biomass and gas loss are affected by microbial growth on the surface area of plant roots, carbon source for microbial consumption and contact time, which is affected by HLR (Buhmann and Papenbrock, 2013). Furthermore, plants affect the soil microbial community, functions and interactions among microorganisms, suggesting that plants could also have similar impact on microbial functions and nitrogen transformations in aquaponic systems (Wang et al., 2015).

Nitrogen uptake rate is influenced by many factors such as nutrient concentrations, light intensity, humidity, temperature and ambient carbon dioxide concentration (Tiaz and Zeiger, 2002; Zhang et al., 2008). NO_3^- uptake rate by plant is higher than other nutrient uptake rates, such as Ca^{2+} , Mg^{2+} and K^+ (Seawright et al., 1998). NO_3^- uptake kinetic in plant was studied using stable nitrogen isotope (^{15}N) and the results showed that increase in NH_4^+ concentration resulted in higher uptake rate by the plant and Michaelis-Menten kinetic was the best-fit kinetic model (Inselsbacher et al., 2013; Zhang et al., 2008). However, the preference for NH_4^+ and NO_3^- depends on their concentrations, growing stages and plant's genetic factors (Fink and Feller, 1998); thus making it difficult to model nitrogen uptake by plant. Many other empirical models have been established for growth determination of plant by considering the nitrogen content in plant, which is proportional to the growth rate at a certain season (Fink and Feller, 1998). Nitrogen uptake was also found to increase over the time (age of plant) in a salinized

aquaponic system using shrimp (*Litopenaeus vannamei*) and hydroponic tomato (*Lycopersicon esculentum*) (Mariscal-Lagarda and Páez-Osuna, 2014).

The plants growing in aquaponic systems uptake NO_3^- as the main nitrogen source because NO_3^- concentration in aquaponic systems is higher than NH_4^+ and NO_2^- concentrations (Hu et al., 2015; Rakocy et al., 2003). Inside the root, water and dissolved minerals are transported via xylem for photosynthesis. The movement of water and minerals are translocated by capillary force in the interconnecting organs and the evaporation from plant's leaves, leading to the suction of water and minerals. The evaporation predominantly occurs at stomata (small pores where oxygen and carbon dioxide normally diffuse between leaves and the atmosphere) (Resh, 2013). NO_3^- is assimilated into organic nitrogen by the mediations of many enzymes such as nitrate reductase (NR) and nitrite reductase (NiR), glutamine synthetase (GS), glutamate synthase (glutamine: 2-oxoglutarate aminotransferase, or GOGAT), glutamate dehydrogenase (GDH), aspartate aminotransferase (Asp-AT) and asparagine synthetase (AS), respectively (Tiaz and Zeiger, 2002). Fig. 2 shows the pathways and intermediate compounds of nitrogen assimilation by plants. During translocation and assimilation, inorganic nitrogen influx and efflux also takes place between the roots and the recirculating water. The reason of NO_3^- efflux on its biochemical basis has not been fully discovered (Reddy and Ulaganathan, 2015; Segonzac et al., 2007). However, it has been known that the NO_3^- efflux is mediated by NO_3^- transporters such as NO_3^- transporter 1.5 (NRT1.5) and nitrate excretion transporter 1 (NAXT1) of root plasma membrane (Reddy and Ulaganathan, 2015; Segonzac et al., 2007). The NO_3^- efflux is induced by stressed conditions such as medium acidification, defoliation, mechanical stress, transplant shock and pathogen attacks (Reddy and Ulaganathan, 2015; Segonzac et al., 2007).

This suggests that plants do not fully assimilate nitrogen when nitrate exceeds plant requirement (Evans, 2001; Kalcsits and Guy, 2013).

6. Environmental concerns and nitrogen loss in aquaponic systems

6.1. Nitrous oxide (N_2O) emission

Aquaponic and aquaculture systems have been considered as the main source of N_2O emission. Aquaponic systems generated nitrous oxide (N_2O) of 1.5-1.9% of nitrogen input (Hu et al., 2015; Zou et al., 2016). N_2O is a potent greenhouse gas with global warming potential of as high as 310 times of CO_2 over 100-year lifespan and has also been reported to destroy the stratospheric ozone layer (Chipperfield, 2009; Hu et al., 2013, 2012). N_2O is generated in both anoxic and aerobic conditions. In aquaponic systems, N_2O emission is induced by several conditions such as insufficient denitrifiers population, NO_2^- inhibition, oxygen inhibition (Hu et al., 2015; Wunderlin et al., 2012), insufficient organic carbon and low pH (Lu and Chandran, 2010; Zou et al., 2016). The difference in the oxidation rate between AOB and NOB also leads to accumulation of nitrification intermediates; thereby increasing the generation of N_2O . N_2O emission from aquaponic systems also occurs during ammonia oxidation via nitrifier denitrification in the grow bed of media-based aquaponic systems (Zou et al., 2016) and in the biofilters of floating-raft and NFT systems (Wrage et al., 2001; Zou et al., 2016). Nitrifier denitrification is the process in which ammonia oxidation and denitrification take place simultaneously in the absence of nitrite oxidoreductase enzyme when oxygen level is low (Kool et al., 2010; Wrage et al., 2001; Zou et al., 2016). Under low DO condition and abundance ammonium in biofilters, nitrifier denitrification coupled with ammonia oxidation can significantly contribute to the generation of NO and N_2O in the presence of AOB such as

Nitrosospira sp. and *Nitrosomonas europia* (Kool et al., 2010; Shaw et al., 2006; Zhu et al., 2013). *Nitrosomonas europaea* was reported as one of the microorganisms producing N₂O through the reduction of NO₂⁻ using NH₂OH as an electron donor under aerobic and anoxic conditions (Beaumont et al., 2004). Such microbes produce more N₂O per cell than the autotrophic nitrifying bacteria (Wrage et al., 2001). The formation of N₂O during ammonia oxidization at low DO condition takes place in aquaponic systems (Hu et al., 2015).

Different strategies have been adopted to reduce N₂O emission in aquaculture. For example, the addition of starch was effective in reducing the daily N₂O emission in intensive aquaculture system (Hu et al., 2014). Aquaponic systems have been recommended to be an alternative method to reduce N₂O emission from aquaculture systems through nitrogen assimilation (Hu et al., 2015). However, studies on N₂O emission from an aquaponic system are still very limited.

6.2. Nitrogen loss via denitrification

Denitrification is a significant pathway in aquaponic systems because it can contribute to nitrogen loss of 25-60% (Hu et al., 2015; Zou et al., 2016). This nitrogen loss reduces NUE and thus the benefit of nitrogen recovery. Denitrification is a main pathway contributing to nitrogen loss due to anoxic condition, particularly in biofilters (Castine et al., 2012; Chen et al., 2012; Lahav et al., 2009; van Rijn et al., 2006). Denitrifiers transform NO₃⁻ to NO₂⁻, nitric oxide (NO), nitrous oxide (N₂O) and finally to nitrogen gas (N₂) under anoxic condition (low dissolved oxygen conditions). Denitrification is carried out by various archaea and facultative heterotrophic bacteria such as *Achromobacter*, *Aerobacter*, *Acinetobacter*, *Bacillus*, *Brevibacterium*, *Flavabacterium*, *Pseudomonas*, *Protus* and *Microoccus* sp. (Gentile et al., 2007;

Hargreaves, 1998; Lu et al., 2014; Michaud et al., 2006). These microbes use NO_3^- as an electron acceptor and utilize dissolved organic carbon in recirculating water as an electron donor. The enzymes involved in each reduction reaction include nitrate reductase, nitrite reductase, nitric oxide reductase and nitrous oxide reductase, respectively (Lu and Chandran, 2010). In aquaponic systems, anoxic condition occurs in biofilters and sedimentation tanks where high concentration of suspended solids is accumulated (Hu et al., 2015).

Carbon limitation affects the activity of denitrifying bacteria, which causes the accumulation of intermediate product, such as NO and N_2O , and also results in NO_3^- reduction to NH_4^+ via the dissimilatory nitrate reduction to ammonia (DNRA) process (Hu et al., 2014; van Rijn et al., 2006). Thus, nitrogen loss via denitrification in aquaponic systems and RAS can be reduced by maintaining sufficient organic carbon and avoiding anoxic zone. Some heterotrophs such as *Alcaligenes faecalis*, *Rhodococcus* sp. CPZ24, *Pseudomonas* sp., *Xanthomonadaceae* sp. and *Sphingomonas* sp. perform heterotrophic nitrification and denitrification simultaneously at low DO condition (< 0.3 mg/L) (Chen et al., 2012; Fitzgerald et al., 2015; Zhao et al., 2012). *Rhodococcus* sp. CPZ24 was reported to transform 85% of ammonia nitrogen to NO_3^- (13%), biomass (24%) and gaseous nitrogen (48%) (Chen et al., 2012). Fig. 3 shows the overall nitrogen transformations with various nitrogen species in aquaponic systems.

Fig. 3. Nitrogen transformations and nitrogen species in aquaponic systems.

6.3. Nitrogen loss via assimilation of heterotrophic bacteria

Heterotrophic aerobic bacteria co-exist with nitrifiers and become dominant when organic carbon concentration or C:N ratio increases, leading to the generation of high quantity of

nitrogen loss in anoxic condition, and excess microbial biomass in the form of sludge (Blancheton et al., 2013; Michaud et al., 2014; Zhou et al., 2009). Heterotrophs utilize NH_4^+ , NO_3^- and organic carbon for cell growth so that they will be more dominant when organic carbon is available due to their higher growth rate (~ 5-folds) than the autotrophs (Díaz et al., 2012; Ling and Chen, 2005). Heterotrophs consume about 70% of the internal energy from ammonium generated by the fish for cell synthesis, while 30% of the remaining energy is used for cell metabolisms (Ebeling et al., 2006). This suggests that the cell yield of heterotrophic bacteria is higher than that of autotrophic nitrifying bacteria; contributing to a large amount of microbial biomass production in aquaponic systems. The low energy needed for the synthesis using carbonaceous compounds as electron donor is attributed to the high growth rate of heterotrophs. The high growth rate results in dominance of heterotrophs over autotrophs at high C:N ratio (Ebeling et al., 2006; Michaud et al., 2014). Heterotrophs grow by consuming dissolved organic carbon of decayed biomass and fish excreta as carbon source under aerobic condition. Heterotrophic biomass was reported to account for about 7% of fish feed in aquaculture systems (Hu et al., 2012). This value, however, depends on C:N ratio, nitrogen concentrations, organic matter (chemical oxygen demand (COD)) and operational conditions. In a system where heterotrophs and nitrifiers coexist, the rates of NO_3^- production/TAN consumption were reduced by 24%, 56% and 73% at C:N ratios of 0.5, 1.0 and 1.5, respectively (Ebeling et al., 2006).

6.4. Nitrogen loss via anaerobic ammonium oxidation (Anammox)

Anaerobic ammonium oxidation (anammox) is also likely to exist in aquaponic systems because the water characteristics are similar to those in aquaculture systems, where anammox process has been shown to occur (Lahav et al., 2009; Timmons et al., 2002; van Kessel et al.,

2010; Zou et al., 2016). Anammox bacteria were reported in biofilters treating fresh water aquaculture systems, marine sediments, freshwater and agricultural sediments (Castine et al., 2012; Hu et al., 2011; Magrí et al., 2013; Tal et al., 2006). Anammox process was reported to contribute to nitrogen loss in different ecosystems (Hu et al., 2011). Nitrogen gas can be formed via anammox process under anoxic condition in aquaponic biofilters.

Anammox process can occur simultaneously with nitrification process at low DO conditions (Lahav et al., 2009; Ma et al., 2015; van Kessel et al., 2010). AOB were found at the outer layer of the biofilms in biofilters treating aquaculture effluent, which had the similar characteristics as aquaponic systems (Hu et al., 2015; Zou et al., 2016) and anammox bacteria were detected in the inner layer of the biofilms (the oxygen-depleted zone), suggesting the co-existence of AOB and anammox bacteria (Ma et al., 2015; Magrí et al., 2013; van Kessel et al., 2010).

6.5. Nitrogen loss via ammonia volatilization

Ammonia loss by volatilization is considered to be insignificant in aquaponic systems due to the low concentration of NH_3 . However, aeration and mixing increase the volatilization rate, especially when the pH of recirculating water is above 8.0 (Hargreaves, 1998; Lekang, 2013). Ammonia volatilization rate ($\text{g-N}/(\text{m}^2\text{-d})$) correlates with NH_3 concentration (Zimmo et al., 2004). Since pKa of ammonia is 9.25 at 25°C , NH_3 concentration is relatively low at neutral pH, which is ideal for aquaponic systems (Bernstein, 2011; Nelson, 2008). At high pH, ammonia volatilization rate is affected by TAN concentration, temperature, and aeration rate in the fish tank (Hu et al., 2012).

7. Factors affecting nitrogen transformations in aquaponic systems

7.1. pH

In any aquatic system, pH is a main factor that controls fish metabolism, microbial activities and affects the availability of nitrogen to plants (Kuhn et al., 2010; Tiaz and Zeiger, 2002; Zou et al., 2016). The biological oxidation of NH_4^+ and NO_2^- , and the activity of nitrifiers decreases when the pH is below 6.4 and above 9.0 (Ruiz et al., 2003). However, NUE in a media-based aquaponic system was found at maximum of 50.9% when the pH was maintained at 6.4 and NUE dropped to 47.3% and 44.7% when pH was increased to 7.4 and 8.0, respectively (Zou et al., 2016). pH also governs the solubility of other micro-nutrients such as calcium, phosphorus, potassium, magnesium, etc., which affects the bioavailability of nutrients for plant uptake (Resh, 2013). In addition, N_2O emissions in aquaponic systems at low pH conditions were reported to be higher than that in neutral pH conditions due to the inhibition of functional genes such as genes encoding ammonia monooxygenase, nitrite reductase and nitric oxide reductase (Zou et al., 2016). pH in aqueous phase is buffered by alkalinity. Alkalinity of 100 to 150 mg/L as CaCO_3 is recommended for aquaponic systems (DeLong and Losordo, 2012; Nelson, 2008; Timmons et al., 2002). pH can be periodically adjusted by using potassium hydroxide (KOH) and calcium hydroxide ($\text{Ca}(\text{OH})_2$) (Rakocy et al., 2003). KOH and $\text{Ca}(\text{OH})_2$ also supply the essential nutrients to plants. In addition, alkalinity can be increased with slightly increase of pH by adding weak bases such as calcium bicarbonate ($\text{Ca}(\text{HCO}_3)_2$).

7.2. Dissolved oxygen (DO)

DO decreases in biofilters, around the root zone of plants in aquaponic systems and aquaculture tanks due to the activities of aerobic microorganisms (e.g., nitrifiers and heterotrophs) and fish (Hagopian and Riley, 1998). Effect of DO has not been well studied in aquaponic systems, but low DO contributes to nitrogen loss via denitrification. Studies using synthetic wastewater (225-450 mg/L of TAN) showed that the activity of AOB reduced when DO level was below 4 mg/L, while the activity of NOB decreased at DO below 2 mg/L (Kim et al., 2005). DO concentration of above 1.7 mg/L was recommended in biofilters to maintain the activity of nitrifiers (Ruiz et al., 2003). However, the recommended DO in fish tanks and the inlet of grow beds is 5 to 6 mg/L to avoid the stress to fish and plants (Bernstein, 2011; Rakocy, 2007). In particular, root rot symptoms may occur when DO is insufficient at high water temperatures (Rakocy, 2007). DO in aqueous phase is also affected by liquid flow pattern and temperature. Aeration systems can be installed at the inlet of grow bed to prevent anoxic zones (Timmons et al., 2002).

7.3. Hydraulic loading rate

Hydraulic loading rate (HLR) is defined as liquid flow rate per unit surface area of grow bed (m/day or m³/m²/day). The optimum contact time of nutrients and microbes present in aqueous phase with plant roots is governed by HLR (Li et al., 2009). The highest NUE of plants in a media-based aquaponic system was reported at the HLR of 1.28 m/day (Endut et al., 2010). Fish biomass yields were not significantly different at different HLRs (Endut et al., 2010). Low HLR leads to oxygen deficiency, which results in denitrification and loss of nitrogen from the aqueous phase. On the other hand, high HLR reduces the contact time between the recirculating

water and the plant roots, and high HLR also washes out the microbes and sediment attached on the plant roots and biofilter media (Endut et al., 2010; Prehn et al., 2012).

7.4. Carbon to nitrogen (C:N) ratios, and ammonia and NO_2^- concentrations

Nitrogen in aquaponic systems can be converted into resources as long as the nitrogen is not transformed into high microbial biomass or nitrogen gas via denitrification. High C:N ratio reduces the abundance of nitrifiers and the nitrification efficiency because the growth rate of nitrifiers is lower than that of heterotrophs (Ebeling et al., 2006; Michaud et al., 2014). Heterotrophs assimilate NH_4^+ and NO_3^- in the presence of organic carbon for cell growth, and turn into sludge, which reduces the nitrogen availability for plant uptake. High concentration of heterotrophs in biofilters drastically lowers the DO and promotes anoxic condition, which causes denitrification and nitrogen loss via N_2O and N_2 . Thus, C:N ratio affects nitrification efficiency, nitrogen emissions, and nitrogen availability for plant uptake.

During the start-up period of aquaponic systems or when feeding rate is increased rapidly, sufficient concentration of nitrifying microorganisms in biofilters is needed to steadily oxidize NH_4^+ and NO_2^- to NO_3^- . If nitrifiers are not present in biofilters, TAN and NO_2^- concentrations increase. High concentrations of TAN and NO_2^- inhibit nitrifiers, and are toxic to fish. Furthermore, NO_2^- concentration of 27 mg N/L reduced the ammonia oxidizing rate by nearly 50% (Nijhof, 1995; Sudarno et al., 2011). NO_2^- is potentially toxic to fish because it has extremely high affinity towards hemoglobin, which reduces the oxygen carrying ability for cell respiration (Hargreaves, 1998). NH_3 and NO_2^- concentrations were suggested to be maintained below 0.06 mg N/L and 8.2 mg N/L, respectively (Popma and Masser, 1999). The toxicity of TAN is dependent on pH as it governs the distribution of NH_3 , which stresses the osmoregulation

of ammonia in the fish (Ip and Chew, 2010). Various factors affecting nitrogen transformations and their relationships with the living species in aquaponic systems are presented in Fig. 4.

Fig. 4. Effects of DO, pH, HLR, C:N ratio, nitrogen concentrations and feed on fish, microorganisms and plants in aquaponic systems.

8. Nitrogen imbalance between uptake rate and utilization rate

NO_3^- is generated in an aquaponic system via nitrification and it is challenging to maintain the balance between NO_3^- generation and its uptake by plants. An imbalance can lead to nitrogen deficiency and large nitrogen loss (as N_2) via denitrification. In this section, nitrogen imbalance is modeled under different scenarios by considering NO_3^- generation and NO_3^- uptake rates by plants at different ages of fish and plants in an ideal aquaponic system. In this modeling, the following assumptions were made: 1) 25 tilapia in a 250-liter fish tank (*Oreochromis sp.*) with initial stocking density of 3.4 kg/m^3 (0.85 kg/tank) and final stocking density of 40 kg/m^3 (10 kg/tank), and 25 plants/grow bed (white cabbage, *Brassica oleracea* var. *capitata*) with the plant density of 6.7 heads/m^2 , 2) C:N ratio of nearly zero due to the high NO_3^- and low COD concentrations, 3) ammonia oxidation efficiency of 100% in the biofilters with no ammonia volatilization during nitrification, and 4) optimal pH, DO and temperature conditions in the aquaponic system.

An estimated growth of tilapia stocked in the aquaculture system and the growth curve (gram) can be exponentially expressed as $(2 \times 10^{-5})(\text{day})^{3.0421}$ (Rakocy, 1989). Other assumptions of growing tilapia are that the feeding rate is 1.25% of the fish body weight and the nitrogen content is 16% of the feed protein content (Timmons et al., 2002). TAN generation can be

calculated using eq. (1). However, there is lack of information on vegetable growth, and nitrogen uptake rates in soilless system. In this case, the assumption on nitrogen uptake is based on the growth of white cabbage cultivated in soil (Fink and Feller, 1998). Nitrogen uptake ($N(t)$, mass of nitrogen per unit area) models of white cabbage (*Brassica oleracea* var. *capitata*) were given by eqs. (2) and (3) as a function of time (Fink and Feller, 1998):

$$N(t) = aW(t)n(t) \quad (2)$$

$$n(t) = ge^{-kW(t)} + h \quad (3)$$

Where, $n(t)$ is the nitrogen content of fresh matter (g nitrogen/kg fresh matter as a function of time), $W(t)$ is the fresh weight of plant harvested (mass of fresh matter per unit area), and a , g , h and k are the regression constants. The above equations indicate that the nitrogen uptake exponentially increases over the age and weight of plant at the growth stage and reaches plateau at the maturity stage. The growth ($W(t)$, kg/m²) can be calculated by Richard's function and expressed as $14.3(1 + e^{5.04 - 0.074(\text{day})})^{-1/0.893}$ (Fink and Feller, 1998). Nitrogen uptake (g N/m²) can be calculated using eqs. (2) and (3), respectively. The constants a , g , k and h were given by 0.1, 2.77, 0.11 and 1.77, respectively, and the final fresh weight of the 89 day-harvesting period was 2.13 kg/head (Fink and Feller, 1998). The time increment is 1 ($\Delta=1$ day). Nitrogen uptake rate (g N/day) can be calculated using eq. (4):

$$\text{Nitrogen uptake rate in day } t \text{ (g N/day)} = (N_t - N_{t-1}) / (t - (t-1)) \times A \quad (4)$$

Where, t is the plant's age (day) and A is the grow bed area (3.73 m²).

Considering 2 cycles of the nitrate uptake rate of transplanted vegetables (gN/day) as shown in the Fig. 5 (plant uptake line or solid line) with the nitrate generation rate (gN/day) by

the fish of 120 to 270 days-old (fish line or dash line), the nitrate uptake rate required by the plants was higher than nitrate generation rate by fish in the first cycle (0 to 75 days of plants). Fish stocking density or number of plants can be adjusted to balance between the nitrate generation and nitrate uptake. Fig. 5 shows that the fish stocking density can be increased by 2-folds in the first cycle of vegetables (modified stocking density line or dot line). Moreover, the area of grow bed (number of plants) can be increased by 50% of the original space in the second cycle (modified number of plants line or circle line). With these approaches, the difference between nitrate generation and uptake rate becomes less, leading to the increase of NUE without nitrogen deficiency to the plants. In real aquaponic systems, other pathways occur (e.g., growth of heterotrophs, denitrification, denitrifier-denitrification, etc.). Thus, research on balancing nitrogen with specific fish and plants is necessary to improve NUE and manage nutrients for sustainable aquaponic systems.

Fig. 5. Nitrogen generation rates and nitrogen uptake rates in an aquaponic system (*Oreochromis* sp. and *Brassica oleracea* var. *capitata*).

9. Future research

The possible fates of nitrogen transformations that are important for improving NUE in aquaponic systems and the factors affecting nitrogen transformations have been discussed in this review. However, the quantitative evaluation of nitrogen transformations in various conditions is still limited. Further studies employing nitrogen isotope and natural abundance isotope approach coupled with nitrogen mass balance are needed to elucidate the processes of nitrogen transformations, from nitrogen in fish feed to products. The nitrogen isotope studies will provide information on nitrogen transformation pathways in aquaponic systems, leading to better

understanding of nitrogen cycle that would be useful in developing highly efficient aquaponic system (e.g., high nitrogen recovery efficiency, low nitrogen loss, low GHGs emission). Studies on microbial communities in aquaponic systems as biomarkers, especially using advanced molecular techniques (e.g., 16S rRNA or specific functional genes) are necessary to better understand the biological nitrogen transformations in an aquaponic system. It is also important to conduct life cycle analysis of an aquaponic system focusing on N₂O emission, carbon footprint and water footprint together with techno-economic analysis to elucidate the environmental and economic sustainability of aquaponics technology. Benchmark nutrient budget of nitrogen transformations needs to be comprehensively studied with wide varieties of crops for efficient operation of aquaponic systems. Although it is obvious that aquaponic production conserves nitrogen, only few studies reported that an improved NUE could be economically beneficial in a commercial scale (Engle, 2015).

10. Conclusions

The fundamental understanding of nitrogen transformations could be applied for improving NUE in an aquaponic system. However, quantitative parameters (e.g., generation rates of different forms of nitrogen, and yields of fish and plants) differ from system to system due to the complexity of the biological system. The nitrogen transformations are affected by several factors, which should be systematically controlled in a well-engineered system. This review provides the very first comprehensive evaluation of nitrogen transformations in an aquaponics system.

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Fig. 1. Aquaponic systems based on types of grow bed.

Fig. 2. Schematic diagram of an aquaponic system and nitrogen transformations pathways in different components.

Fig. 3. Nitrogen transformations and nitrogen species in aquaponic systems.

Fig. 4. Effects of DO, pH, HLR, C:N ratio, nitrogen concentrations and feed on fish, microorganisms and plants in aquaponic systems.

Fig. 5. Nitrogen generation rates and nitrogen uptake rates in an aquaponic system (*Oreochromis* sp. and *Brassica oleracea* var. *capitata*).