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Waste management in recirculating aquaculture system through bacteria dissimilation and plant assimilation

Zipporah Moraa Gichana[1] · David Liti[2] · Herwig Waidbacher[1] · Werner Zollitsch[1] · Silke Drexler[1] · Joseph Waikibia[3]

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Abstract
Wastewater management and disposal in aquaculture is becoming increasingly important due to stringent water regulations regarding waste discharges into natural water systems. Recirculation aquaculture is one of the technologies designed to reduce waste discharge through the nitrification process. However, nitrification results in nitrate accumulation which is normally reduced by dilution through water exchange. Water exchange is only possible with sufficient water. Although nitrification is a conventional process, it has limitations because the autotrophic bacteria require long start-up and multiplication periods. The nitrifiers require high levels of oxygen with relatively higher aeration costs. Moreover, the bacteria are sensitive to rapid changes in pH, temperature, and flow rate. Denitrification can be a solution to the limitations of nitrification since denitrifiers are most abundant in the natural environment and have higher growth rates than nitrifiers. In addition, the process reduces energy costs since there is no need for aeration, water consumption is also reduced drastically since water exchange is minimized. Organic loading can be reduced when fish waste is utilized as a carbon source. An alternative process to manage aquaculture wastes is through anaerobic ammonium oxidation (anammox), where ammonia and nitrite are converted into nitrogen gas. Anammox can efficiently reduce ammonia and nitrites from culture water, but it has not received wide application in aquaculture. Aquaculture wastewater contains nutrients which are essential for plant growth. The plants maintain good water quality by absorbing the dissolved nutrients. Denitrification, anammox, and nutrient uptake by plants are feasible strategies to reduce wastes from aquaculture effluents.

Keywords Anammox · Aquaponics · Denitrification · Nitrification · Recirculating aquaculture

✉ Zipporah Moraa Gichana
zippmoraa@yahoo.co.uk

[1] Institute of Hydrobiology and Aquatic Ecosystem Management (IHG), University of Natural Resources and Life Sciences, Gregor-Mendel-Straße 33/DG, 1180 Vienna, Austria

[2] Department of Biological Sciences, University of Eldoret, P.O. Box 1125-30100, Eldoret, Kenya

[3] Department of Botany, Jomo Kenyatta University of Agriculture and Technology, P.O. Box 62000-00200, Nairobi, Kenya

Introduction

Aquaculture wastes emanate mainly from feed, which is not consumed or feed which is consumed and excreted in the form of ammonia through gills or from feces (Meriac et al. 2014). According to Cripps and Bergheim (2000), all materials or nutrients which are not retained as fish biomass and are not removed during harvesting can be regarded as waste. In fish, only 1/3 of feed nutrients are digested, absorbed, and utilized in the metabolic processes and retained in biomass while the rest is excreted as non-fecal or fecal losses into the environment (Schram et al. 2014; Meriac et al. 2014). According to Meriac et al. (2014)) and Chen et al. (1997), fecal loss is one of the main sources of solid waste in aquaculture and consists of undigested feed nutrients. The fecal waste includes dissolved components such as phosphorus and nitrogen-based nutrients as well as suspended solids (Losordo and Westers 1994). Non-fecal losses are generally metabolites of feed nutrients absorbed by fish but not retained as biomass. The non-fecal loss is excreted mainly as nitrogen in the form of ammonia and urea (Meriac et al. 2014). Most of these nutrients from aquaculture waste are discharged into the surrounding waters (Turcios and Papenbrock 2014). Neto and Ostrensky (2015) estimated that for every tonne of tilapia produced, 1040.63 kg of organic matter is produced and discharged into the environment out of this organic loading, a load of 44.95 kg of nitrogen, and 14.26 kg of phosphorus. The discharged wastes can cause oxygen depletion and eutrophication in the receiving environment (Iwama 1991). It is therefore important to effectively manage the release of aquaculture wastes to prevent eutrophication of the receiving environment and ensure long-term sustainability of aquaculture and the integrity of the recipient environment (Bureau and Hua 2010).

The general recommendation to reduce wastes especially ammonia in aquaculture is to improve protein digestibility and utilization (Cho and Bureau 2001). Although fish feed containing fish meal as the major protein source is highly digestible, increasing prices of fish meal have forced feed producers to use plant ingredients as substitutes for fish meal (Bureau and Hua 2010). However, plant ingredients have lower digestibility and increases solid waste production in aquaculture (Bureau and Hua 2010). Wastewater management in aquaculture is generally done through exchange of water. However, water exchange is only possible if there is adequate water supply (Meriac et al. 2014). Besides, the exchange of water is untenable due to increased discharge of untreated effluents on the receiving environment (Iwama 1991). Recirculation aquaculture systems (RAS) have been necessitated due to stringent environmental requirements regarding discharge of wastes to the environment, limited water supply, and inadequate space for development of aquaculture as well as recovery of nutrients from culture system through aquaponics. RAS can reduce water consumption by 90–99% compared with other aquaculture systems (Timmons and Ebeling 2007). RAS systems are usually under a greenhouse and therefore fish can be grown independent of seasonal effects (Badiola et al. 2012). RAS is popular in production of fish and fingerlings, though its contribution to the total aquaculture production is insignificant compared with other systems (Martins et al. 2010).

The basic RAS design consists of three main components; the fish rearing unit, the solids separation unit and bio-filtration unit (Bovendeur et al. 1987). In addition, the system is designed to replace 5–10% of the total water volume per day to prevent nitrate and soluble organic matter accumulation (Freitag et al. 2015). The bio-filtration units in RAS provides an environment for attachment and growth of bacteria which improve water quality through the nitrification process (Blancheton 2000). The system is designed to control the amount of nitrogenous wastes using different types of nitrification biofilters. The conventional biofilters are integral part of the RAS where chemoautotrophic bacteria oxidize ammonia to nitrate in a two-step process known as nitrification (Wheaton et al. 1991). Three types of bacteria are involved in this process, the *Nitrosomonas* which convert ammonia to nitrite and the *Nitrobacter* and *Nitrospira* which convert nitrite to nitrate (Ryan et al. 2017).

Though RAS offers suitable culture conditions for fish, one challenge in the system is that it leads to accumulation of nitrates, which at high levels lowers the immunity of fish (Hrubec et al. 1996; Lekang 2008). Nitrate concentrations as high as 1000 mg L^{-1} in RASs with high feed input and low water renewal rates have been reported (Freitag et al. 2015). The nitrate levels may accumulate with time to the extent of limiting fish production (Davidson et al. 2009, 2011; Martins et al. 2009; Freitag et al. 2015). In addition, increased feeding rate can result in accumulation of soluble organic matter, heavy metals, and phosphorus (Davidson et al. 2009).

The other challenge in RAS is the insufficient removal of fine solids by the current techniques of solid removal such as mechanical filters (Martins et al. 2010). Furthermore, the fine solids together with organic matter in aquaculture wastewater support growth of heterotrophic bacteria which compete with nitrifiers for nutrients and space (Malone and Pfeiffer 2006). The competition between the nitrifiers and heterotrophic bacteria can influence the rate of ammonia oxidation by suppressing the nutrient availability of the later and subsequently affecting the efficiency of the functioning of the biofilter (Michaud et al. 2006). The heterotrophic bacteria also produce significant quantities of bacterial biomass which may clog the biofilter and reduce nitrification capacity (Ebeling et al. 2006).

The application of the denitrification process removes both nitrogen and phosphorus wastes in recirculating aquaculture. The process of denitrification as means to control both nitrogen and phosphorus was proposed as an elegant way to reduce accumulation of nutrients in the culture water (Shnel et al. 2002). Phosphorus is removed from culture water during oxic or anoxic conditions but released under anaerobic conditions. During the latter conditions, acetate or other low molecular weight organic compounds are converted to polyhydroxyalkanoates (PHA), poly-P and glycogen are degraded, and phosphate is released. Under oxic and anoxic conditions, PHA is converted to glycogen, phosphate is taken up and polyphosphate is synthesized by bacteria. Under oxic and anoxic conditions, growth and phosphate uptake is regulated by the energy released from the breakdown of PHA (van Rijn et al. 2006). Under either oxic or anoxic conditions, denitrifying bacteria store phosphorus when it is more than their metabolic requirement. Denitrification reduces energy costs because of minimal aeration, water consumption is also reduced drastically since water exchange is reduced. Organic carbon can be reduced in the culture system since endogenous carbon from fish waste can be utilized as a carbon source for denitrification (van Rijn et al. 2006).

However, denitrification relies on expensive exogenous organic carbon such as methanol because endogenous carbon such as fish waste requires degradation of complex organic matter for example carbohydrates and proteins via hydrolysis to soluble organics. The soluble organics are then fermented into volatile fatty acids (VFAs) that are utilized for denitrification (van Rijn et al. 2006; Mirzoyan et al. 2010). Hydrolysis is slower than denitrification, therefore, this process is a limiting step in the use of fish waste as a carbon source for heterotrophic denitrification (Klas et al. 2006a, b). Besides, fluctuations in water quantity and quality make it difficult to control the exact amount of soluble carbon that must be added to support bacterial growth. In addition, organic residues from the soluble carbon such as methanol may leach into the culture water and contaminate it (Singer et al. 2008). Anaerobic ammonium oxidation (ANAMMOX) was established as an alternative to reduce the cost of organic carbon. In this process, ammonia and nitrite are transformed into nitrogen gas (Strous et al. 1999). Besides, some nitrate generated during the nitrification and the anammox process can be assimilated by plants in aquaponic systems (Zou et al. 2016). Therefore, these processes improve nutrient recycling and water quality in recirculating aquaculture and minimize waste discharge to the environment (Martins et al. 2010). In aquaponic systems, plants play a significant role in maintaining water quality by removing dissolved nutrients, which they use for growth. This significantly reduces nutrient loads in the discharges and improves the quality of wastewater (Goddek et al. 2016). The purpose of this review is to discuss waste production and its management in RASs.

Sources of wastes in aquaculture

Waste metabolites in aquaculture result from protein metabolism in fish and is subsequently released into the environment in form of ammonia through cationic exchange within the gills and decomposition of feces and feed wastes (Handy and Poxton 1993). The feces and feed wastes can significantly increase the concentration of ammonia nitrogen and biological oxygen demand (BOD) in aquaculture systems. Fish excretes 70–80% of nitrogen as ammonia and 10% as urea (Ebeling et al. 2006). Therefore, ammonia is the main nitrogenous waste produced by fish (Cao et al. 2007). The excreted nitrogen occurs in two forms including ionized (NH_4^+-N) and un-ionized (NH_3-N) (Timmons and Ebeling 2007), and the sum of the two is reported as total ammonia nitrogen (TAN). The unionized form of ammonia is highly toxic to aquatic animals and should be kept in low concentrations preferably in the range of 0.05–0.1 mg L^{-1} in aquaculture systems (Timmons and Ebeling 2007).

The toxicity of ammonia is generally attributed to unionized ammonia because biological membranes are more permeable to NH_3-N than NH_4^+-N (Liew et al. 2013). Nonetheless, high NH_4^+-N concentration in the culture water inhibits diffusion of ammonia from the gills and increase in the blood (Liew et al. 2013). Therefore, ionized ammonia can cause some degree of toxicity, which is significantly less than that of NH_3-N. Additionally, NH_3-N can hinder the nitrification process because it is toxic to both the *Nitrosomonas* and *Nitrobacter* bacteria (Russo 1985). The proportion of the two forms of ammonia in the culture system is mainly affected by pH and temperature (Hargreaves and Tucker 2004). Unionized ammonia dominates in culture water with high pH whereas ammonium dominates in low pH. Generally, when pH is < 8.0, less than 10% ammonia is toxic. However, as pH increases, the proportion of ammonia increases dramatically (Hargreaves and Tucker 2004). The toxicity of unionized ammonia can also increase with temperature increase. For each unit increase in pH, the toxicity of ammonia increases ten times (Durborow et al. 1992). In addition, at any given pH, ammonia is more toxic in warm water than cold water (see Fig. 1) (Hargreaves and Tucker 2004). In pond systems, fluctuations in pH result from photosynthesis, which increases pH whereas respiration reduces pH. Hence, toxic ammonia dominates in the late afternoon and early evening while less toxic ammonium dominates at sunrise to early morning (Hargreaves and Tucker 2004). Toxicity from high levels of ammonia is attributed to increased physiological stress response and results in reduced growth and survival of fish (Tomasso 1994).

Fish feed as a source of pollution in aquaculture

Fish feed is the major source of ammonia in aquaculture systems and the subsequent nutrient loading to the environment (Hargreaves and Tucker 2004). Fish feed increase organic waste

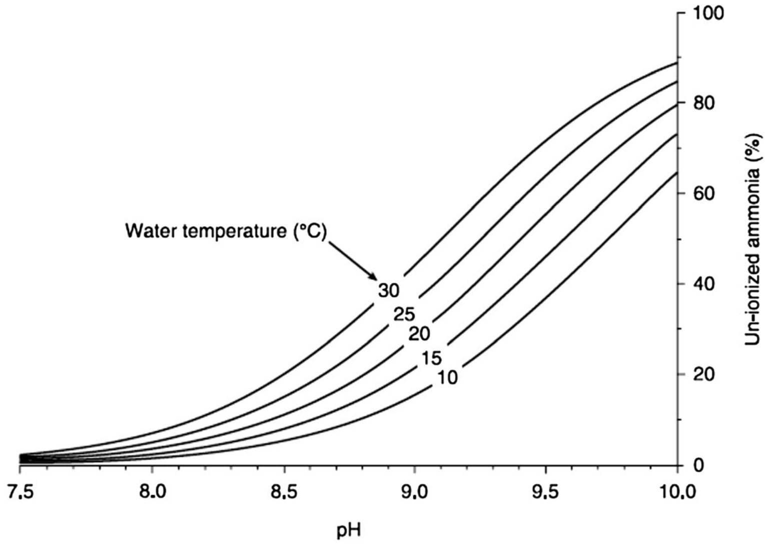

Fig. 1 Effect of pH and temperature on levels of unionized ammonia (from Hargreaves and Tucker 2004)

loads in aquaculture systems, and without proper management, the effect on water quality can be dramatic in receiving water bodies (Tucker and Hargreaves 2009). Some of the feed is not utilized by fish and is either degraded by physical, chemical, or biological processes within the aquaculture systems or discharged to the recipient environment (Boyd and Tucker 2014). Some of the factors that influence feed intake include; quality of feed, feeding strategies, temperature, fish species, fish appetite, water quality conditions, and density of fish. Generally, 80% or more of fish feed is consumed but 10–20% of the consumed feed is excreted as feces (Boyd and Tucker 2014). The uneaten feed decomposes in the culture system and release nutrients. The absorbed nutrients are then converted to microbial biomass and the rest is metabolized and excreted as carbon dioxide and other compounds (Cao et al. 2007; Hargreaves and Tucker 2004). Approximately 60–80% of nitrogen and phosphorus in fish feeds is released into the system as waste, and a higher proportion of carbon in the feed is transformed to carbon dioxide and excreted through respiration (Boyd and Tucker 2014). About 50% of the total nitrogen (N) and total carbon (C) is excreted into the water through the gills, and over 50% of phosphorus is released as particulate phosphorus (White 2013).

Pollution from feed originates from excessive feeding, poor feed quality, and poor digestibility of the feed (Nash 2001). Excess feed results in organic loading which generates additional oxygen demand during bacterial decomposition which subsequently compete with fish for available oxygen. Overfeeding is minimized by providing the correct amount of feed through satiation feeding, regulation of feeding frequency, regulation of feeding duration, and correct timing because cultured fish show marked variations in appetite between and within days (Noble et al. 2007). Poor feed quality and feeding strategy have a major impact on waste production in aquaculture systems (White 2013). Poor quality feed will also lead to poor utilization of feed with more solid wastes and protein discharged to the environment (White 2013). In pond systems, excess nutrients accumulate within the pond or are utilized by algae. However, the nutrients are discharged to the environment during harvest time or water exchange (White 2013). Moreover, poor food conversion ratio (FCR) is reported to cause inconsistent increase in total N and P loading. White (2013) reported an increase of total P and

N loadings by 47 and 36.1%, respectively, for a 25% increase in FCR from 1.6–2.1 for tilapia reared in ponds.

Solid wastes also known as particulate organic matter consist of feces or uneaten food. A build-up of these wastes within culture systems should be prevented as they can cause oxygen depletion and ammonia toxicity when they decompose (Cao et al. 2007). In recirculation aquaculture systems, organic wastes exist in the form of settled solids that accumulate at the bottom of tanks. Fine solids that float within the water column can cause gill irritation and affect the health of fish (Cao et al. 2007. According to Bureau and Hua (2010), waste discharge from aquaculture operations has become a matter of public concern because of its effects on the surrounding aquatic ecosystems. Efforts to manage waste accumulation from aquaculture such as renewal and exchange of water if there is enough water and development of reuse systems where water is reused after purification amongst others has been established. However, it is recommended that reduction of waste in aquaculture should begin with the management of feed quality and feeding strategies since the main source of waste in aquaculture originate from the feed added to the rearing systems.

Mitigation measures for wastes in aquaculture

Feed strategies

One way to reduce nutrient loading in aquaculture systems is by using feeds with nutrient concentrations comparable with the dietary needs of the target fish (Lall and Dumas 2015). Sound feeding management practices are important to ensure that most of the administered feed is consumed by fish (Tucker and Hargreaves 2009). High-quality pellets should be used to reduce fine particles that fish cannot ingest and reduce leaching of nutrients from feed pellets into the culture water (Tangendjaja 2015). Therefore, sound feeding strategies can improve feed utilization and feed conversion ratio and reduce feed wastage. Underfeeding results in decreased growth since most of the feed energy is utilized for maintenance while overfeeding produces more feed waste and reduced utilization of feed (Tangendjaja 2015). Food conversion ratio can be reduced by improving palatability and digestibility of the diet (Lopez 1997). Improved FCR is achieved through careful selection of ingredients with adequate nutrient profiles and high digestibility values. Palatability can reduce feed waste since most of the feed will be consumed by fish. Diet utilization has improved in the last two decades due to the reduction of FCR by more than 50% (Lopez 1997).

Extrusion technology has also been used to improve the performance of feeds. More fish oil in diets increases energy levels and improves protein utilization (Tangendjaja 2015). A large portion of energy comes from fat while more proteins are used for growth because of the protein sparing action (Tangendjaja 2015). Extrusion also enhances utilization of plant proteins and reduces production of dust from feeds (Tangendjaja 2015). Plant protein can also be enhanced using enzymes to allow better utilization of proteins such as soya (Carter et al. 1994). Another method to improve protein utilization and reduce ammonia excretion is to the improve amino acid profile since diets with deficient amino acid results in high ammonia excretion (Alvarado et al. 1994). If a diet is deficient of one single amino acid, utilization of the other excess amino acids is reduced. The fish will, therefore, deaminate the excess amino acids and use them as a source of energy and excrete the amino group as ammonia (Alvarado et al. 1994). But when there is no amino acid deficiency, the excretion of ammonia decreases (Alvarado et al. 1994).

Even though the improvement of FCR reduce waste loads in aquaculture, wastes will nonetheless accumulate (Chatvijitkul et al. 2017). Furthermore, the greatest proportion of dry matter of feed ends up as waste in cultures system even with good feed management strategies and utilization of high-quality feeds (Boyd et al. 2007). This is because the feed conversion efficiency for culture of fish such as tilapia is about 0.59, i.e., 1 kg fish feed results in 0.59 kg of fish resulting in 0.41 kg of waste load. Therefore, feeding results in a large discharge of waste; for instance, tilapia ponds with a daily feeding rate of 200 kg day^{-1} produces a dry matter waste around 150 kg day^{-1} (Chatvijitkul et al. 2017).

Waste management in recirculating aquaculture systems by use of aerobic biofilters

The most common technique for the removal of ammonia is biological filtration using biofilters. The conventional ammonia removal design is based on solid removal followed by nitrification. Many strategies of solid waste removal have been used including hydro clones, sedimentation, and mechanical filtration (Scott and Allard 1984). Hydro clones can remove 87% of solids (Scott and Allard 1984). Libey (1993) reported that a drum filter with a 40-μm screen mesh size could remove 40% of suspended solids. The RASs are generally designed to use one or more of the numerous biofilter designs including trickling, moving bed, fluidized, floating bead, and submerged filters (van Rijn 1996). The choice of the type of biofilter depends on the culture system and the operator (Colt et al. 2006). Water quality in recirculation aquaculture system is a key limiting factor (Colt 2006). Therefore, to maintain suitable rearing conditions, a suitable biological filter is selected to process the waste produced in the system. Performance characteristics are important for selection of suitable filters for RASs (Todd 2008). But there is little development on the appropriate means to report biofilter performance in aquaculture since the features of biofilter performance have not been standardized (Colt et al. 2006). Although evaluation of biofilter performances in aquaculture is becoming popular, most of the studies have been done on experimental scale with limited application on real production (Todd 2008). The large differences in ammonia and carbon dioxide concentrations between real production and pilot-scale systems confound the understanding of the actual operating characteristics of the biofilters (Todd 2008). There is, therefore, a need for more information on biofilter operations in real production systems.

Ammonia concentration in RASs can be reduced through nitrification process using biological filters. The process is fueled by autotrophic nitrifying bacteria that attach themselves in aerobic biofilters (Wheaton et al. 1991). The biofilters provide an increased surface area for the nitrifying bacteria where total ammonia is oxidized to produce nitrate with nitrite as an intermediate product (Timmons and Ebeling 2007). Autotrophic bacteria belonging to *Nitrosomonas*, *Nitrosococcus*, *Nitrosospira*, *Nitrisolobus*, and *Nitrosovibrio* oxidize ammonia to nitrite, while the *Nitrobacter*, *Nitrococcus*, *Nitrospira*, and *Nitrospina* transform nitrite to nitrate (Haynes 2012; Zehr and Kudela 2011). The basic conversion process that occurs in autotrophic nitrification process is shown in the equations below (EPA 1984).

$$NH_4{}^+ + 1.5O_2 \rightarrow 2H^+ + H_2O + NO_2 \tag{1}$$

$$NO_2{}^- + 1.5O_2 \rightarrow NO_3{}^- \tag{2}$$

For each gram of ammonia oxidized to nitrate in the nitrification process, 4.18 g of dissolved oxygen and 7.04 g alkalinity ($CaCO_3$) are consumed (Timmons et al. 2002).

The process results in the production of 0.19 g of cellular biomass ($C_5H_7O_2N$) and carbon dioxide (Timmons et al. 2002). However, some studies have shown rather different results with 4.57 g oxygen and 7.14 alkalinity being consumed (Timmons et al. 2002). In the first equation, hydrogen ions (H^+) are produced and combine with carbon dioxide to form carbonic acid (H_2CO_3). The formation of the carbonic acid lowers the pH of the water (Todd 2008). During the nitrification process, heterotrophic bacteria consume ammonia/nitrogen when the ratio of organic carbon to ammonia/nitrogen (C/N) is relatively high (Timmons et al. 2002). Heterotrophic bacteria also assimilate organic waste products and convert the waste directly to microbial biomass (Ebeling et al. 2006). Nitrification is measured and quantified by the rate of TAN removal from culture system (Zhu and Chen 1999). The process can effectively reduce TAN thus providing a means to reuse water in a production system (Meade 1985).

The performance of nitrification biofilters depends on the prevailing biotic and abiotic conditions. However, under some set of conditions, the biofiltration process is not fully operational though it is considered an effective solution to remove total ammonia nitrogen and nitrites from RAS (Pedersen et al. 2009). According to Summerfelt (2006), there is no ideal biofilter for all conditions because all biofilters have their advantages and disadvantages. Several studies have reported the performance of different setups of nitrification biofilters. For instance, Suhr and Pedersen (2010) compared the performance of submerged fixed bed biofilters (FBB) and moving bed biofilters (MBB). The study showed that the FBB was more robust to changes in culture conditions and had superior total ammonia nitrogen (TAN) removal (0.46 g m^{-2} day^{-1}) compared with MBB (0.27 g m^{-2} day^{-1}). Nootong and Powtongsook (2012) reported that nitrifying biofilters can mediate the nitrification process almost immediately resulting in TAN and nitrite concentrations below 1.0 mg L^{-1}. However, the performance of biofilters was affected when the operation of solids separation units stopped.

Factors that influence biofilter performance

Total ammonia nitrogen

In lower TAN concentrations (< 3 mg L^{-1}), a linear relationship between the removal of TAN exists in both commercial and laboratory scale systems (Ling and Chen 2005). However, the relationship varies with the size and design of biofilters. Furthermore, other confounding variables such as organic carbon and type of mechanical filtration can confound the relationship.

Organic carbon

Heterotrophic bacteria degrade and metabolize organic carbon derived from fish excreta, dead bacteria, and uneaten feed (Léonard et al. 2002). The heterotrophic and autotrophic bacteria inhabit the biofilter media thus creating a biofilm. The two types of bacteria therefore, compete for nutrients and oxygen resulting in a stratified biofilm structure (Nogueira et al. 2002). Since the heterotrophic bacteria grow faster than autotrophic bacteria (Nogueira et al. 2002), they occupy the outer layer of the stratified biofilm which has high substrate concentration and detachment rates, while the autotrophic bacteria occupy the inner layer of the biofilm (Michaud et al. 2006). Heterotrophs have a growth rate five times greater than autotrophic bacteria (Zhu and Chen 2001). By occupying the outer layer of the biofilter, the heterotrophic bacteria restrict diffusion of nitrogenous substrates and dissolved oxygen to the autotrophic bacteria thus negatively affecting nitrification rate (Chen et al. 2006). Therefore, higher organic carbon decreases the rate of nitrification and result in increased TAN concentrations (Chen et al. 2006). Since increased organic matter in fish culture systems favor heterotrophic bacteria, uneaten feed, feces, and organic carbon inputs should be removed from the system before entering the biofilters. The most appropriate and conventional approach of eliminating heterotrophic bacteria from the system is mechanical filtration (Léonard et al. 2002). Placing the mechanical filter directly before the biofilter results in a maximum removal of particulate organic matter and improvement of nitrification rate.

Alkalinity and pH

Alkalinity is the primary source of inorganic carbon for nitrifying bacteria (USEPA 1984). During nitrification, 7.04 g alkalinity is consumed for every gram of TAN oxidized. For efficient nitrification to occur, carbonate alkalinity greater than 40 mg L^{-1} should be maintained (Biesterfeld et al. 2003). Moreover, alkalinity concentrations of more than 200 mg L^{-1} are required to meet carbon requirement for nitrifying bacteria and buffer for the pH (Chen et al. 2006). Therefore, alkalinity additions using compounds such as sodium carbonate ($NaHCO_3$) and calcium carbonate ($CaCO_3$) are important in RAS with a nitrification unit. However, sodium carbonate is mostly used since it is safer (Chen et al. 2006).

Nitrification process produces hydrogen ions and carbon dioxide that alters the pH in culture water. The optimal pH for the nitrifying bacteria varies widely and varies with the levels of ammonia (Chen et al. 2006). The optimum pH range is between 6.7 and 7.0 for TAN concentration of 0.37 mg L^{-1} and pH 7.5 and 8.0 for 5.0 mg L^{-1} (Groeneweg et al. 1994). To maintain optimum nitrification rates in aquaculture, the pH should be kept within the range of 6.7–7.0 because recommended TAN concentrations for aquaculture operations is less than 3.0 mg L^{-1} (Losordo et al. 2000). A buffer is required to maintain the pH at suitable ranges to increase the nitrification efficiency.

Temperature

The effect of temperature on the removal TAN for some types of biofilters such as fixed film biofilters is more complex compared with suspended growth in a recirculating aquaculture system (Zhu and Chen 2002). In suspended growth systems, higher temperatures enhance the biochemical-driven bacterial processes. In fixed film filters, the effect of temperature on the rate of nitrification is also influenced by substrate diffusion and transport (Fdz-Polanco et al. 1994). In higher-temperature systems, dissolved oxygen becomes a limiting factor for the nitrification process due to the limitation of diffusion process of mass flux into the fixed biofilm (Zhu and Chen 2002). Besides, as temperature increases, the saturation of dissolved oxygen (DO) decreases. In biofilms with both heterotrophic and autotrophic bacteria, competition for DO exists between the inner and outer layers. Increased temperatures increase a layer of heterotrophic bacteria over autotrophic bacteria in systems with high organic carbon. As the layer increases in thickness, diffusion of oxygen is inhibited and mass flux of nutrients is reduced (Zhu and Chen 2002).

Carbon dioxide

Carbon dioxide is a by-product of microbial and macrobiological metabolism. The metabolic activity of cultured fish is proportional to fish feed and is not static in the culture system (Todd 2008). An increase in metabolism results in an increase in oxygen utilization and production of carbon dioxide (CO_2). Biofilters can produce as much as 37% of CO_2 in recirculation systems using fluidized sand filters (Summerfelt and Sharrer 2004). Therefore, there is a need for CO_2 stripping after the biofilters as that is the region where the concentration of CO_2 is highest (Eding et al. 2006). Trickling filters are designed to maximize the removal of CO_2 where carbon dioxide degasses as the water trickles down through the biofilter media (Eding et al. 2006). Carbon dioxide can also be stripped by pumping air through the media (Todd 2008). The effect of carbon dioxide in lowering the pH and acid production in nitrification requires high carbonate inputs to maintain pH and increase the concentration of carbonate.

Though nitrification is considered the conventional approach to reduce ammonia in recirculation aquaculture, nitrate the end product of nitrification may accumulate with time to the extent of limiting fish production (Davidson et al. 2009, 2011; Martins et al. 2009; Freitag et al. 2015). Nitrate concentrations as high as 1000 mg L^{-1} in recirculation aquaculture systems with high feed load and low water renewal rates have been reported (Freitag et al. 2015). In addition, high feeding rate with high protein feed can result in accumulation of soluble organic matter, heavy metals and phosphorus (Davidson et al. 2009). There is also a possibility of nitrite accumulation in systems containing high levels of nitrate (Martins et al. 2010). Besides, environmental regulations require substantial nitrate removal before discharge to the environment in order to reduce eutrophication in natural waters (Suhr et al. 2014). To guarantee better water quality in culture systems, generated wastes should be removed to reduce stress on fish (Wheaton et al. 1994).

Different approaches of nitrate removal in RAS have been established, the most common method is the exchange of 5–10% of the total water volume with fresh water (Freitag et al. 2015). Nitrate can also be reduced when nitrification is followed by denitrification. Biological denitrification is however, one of the most efficient methods to remove nitrate from wastewater (van Rijn et al. 2006). Most wastewater treatment plants often use heterotrophic denitrification using external electron receptors and organic carbon donors such as methanol and acetate (van Rijn et al. 2006). However, the use of this technology is not largely common in commercial aquaculture because of high investment cost and lack of expertise in the operation of the system (Losordo and Westeman 1994). Furthermore, it is difficult to control the exact amounts of organic carbon compounds to fuel the process due to fluctuations in the quality and quantity of water (Martins et al. 2010). High oxygen concentration in the inflow to the denitrification biofilters can cause excessive consumption of organic carbon and inhibit denitrification (Singer et al. 2008). Besides denitrification, the excess nitrate can be utilized for plant growth (Martins et al. 2010). Nutrient recycling in integrated farms can improve environmental water quality in recirculation systems. Wetlands, algal-controlled systems, and aquaponics can provide alternative and sustainable water treatment strategies (Martins et al. 2010). Plants play a significant role in maintaining water quality by removing dissolved nutrients which they use for their growth subsequently reducing wastewater discharges and water turnover rate (Timmons and Ebeling 2007).

Biological denitrification

Denitrification is an anaerobic microbial process where organic carbon is used to convert nitrate to nitrogen gas through reduction of nitrate and the intermediates nitrite and gaseous nitrous oxide (Pungrasmi et al. 2013).

$$NO_3^- + 2e^- + 2H^+ \rightarrow NO_2^- + H_2O$$

$$NO_2^- + 3e^- + 4H^+ \rightarrow 0.5N_2 + H_2O$$

Denitrification uses nitrate and nitrite as a terminal electron acceptor in the presence of organic carbon source (Gutierrez-Wing et al. 2012). In the absence of oxygen, nitrate becomes the terminal electron acceptor with the removal of a single atom from each nitrate ion to produce nitrite ion. Denitrification process can be heterotrophic or autotrophic, however, most denitrification systems are heterotrophic where organic carbon source fuel the facultative anaerobic bacteria. Heterotrophic denitrifiers utilize costly organic carbon such as methanol, glucose, and acetate as an energy and carbon source whereas autotrophic use inorganic compounds such as carbon dioxide and other compounds such as sulfur, iron, and hydrogen as a carbon source (van Rijn et al. 2006).

Heterotrophic denitrifying bacteria are most abundant in the natural environment and have higher a growth rate than autotrophic bacteria (Gutierrez-Wing et al. 2012). In the natural environment, the process occurs in sediments where anoxic conditions and organic carbon compounds favor the process. Denitrification can remove up to 266 mmol m^{-2} day^{-1} of nitrogen in intertidal flats, marsh sediments, and deep anoxic waters (Dalsgaard et al. 2003). In earthen ponds, nitrogen is removed by algae and plant assimilation, nitrification, and denitrification occur at the pond bottom (van Rijn et al. 2006). Though denitrification is one of the efficient methods to remove nitrate, it is not practically used in aquaculture because of the need for sophisticated equipment, high operating costs and complex operating skills (Pungrasmi et al. 2013). For flow-through systems, which use large quantities of water at low costs, there is little incentive to adopt this technology (Losordo and Westeman 1994).

Heterotrophic denitrification is associated with high denitrification rate, low biomass production and a 40% reduction in electron donor use (Wang et al. 2008). However, in the case of limited organic carbon, the denitrification rate decreases and sometimes produces large quantities of nitrite. Excess carbon relative to nitrogen can expedite the reduction of sulphate to highly toxic sulfide. A carbon to nitrogen ration of 3–6 g COD g^{-1} NO_3-N is required for the denitrification process (van Rijn et al. 2006). Hargreaves (2006) reported that heterotrophic bacteria are the main driving force for TAN removal since they grow ten times faster than the nitrifying bacteria. In their study, the removal of TAN though production of nitrates and nitrites did not occur without a carbon source. A related study reported significantly higher nitrogen removal efficiency for both sequencing batch reactors when a C/N ratio was increased using acetate and glycerol as a carbon source (Verstraete and Schryver 2009). Unlike heterotrophic denitrification, autotrophic denitrification does not require any additional organic carbon sources and produces less sludge thus reducing the cost of treatment process (Christianson et al. 2015). Though autotrophic denitrification is promising, it consumes about 4.57 mg $CaCO_3$ alkalinity and produces 7.54 mg sulfate for each milligram of nitrate reduced (Sahinkaya et al. 2014). Sulfur is also relatively insoluble hence has limited microbial availability at room temperature. Moreover, autotrophs grow at a slower rate resulting in slow denitrification rate

(Christianson et al. 2015). Though autotrophs have a slow growth rate, some studies have reported removal efficiencies of nitrate above 90% (Ramanathan et al. 2014).

During denitrification, nitrate can be reduced to ammonia through dissimilatory nitrate reduction to ammonium (DNRA) (Castine et al. 2012). Dissimilatory nitrate reduction to ammonium competes with denitrification and converts nitrate to ammonium instead of converting nitrate to nitrogen gas (van Rijn et al. 2006). DNRA is regarded as a minor process in the removal of nitrate since only less than 4–10% of nitrate removal is attributed to DNRA (Healy et al. 2012). Castine et al. (2012) demonstrated that carbon can stimulate nitrate ammonifiers and result in competition for nitrate as a substrate. Castine et al. (2012) observed rapid consumption of approximately 90% of the added nitrate by competing pathways such as assimilation or DNRA and only 7.9 ± 2.7% of nitrogen gas was recovered from the added nitrate. Besides, there is a likelihood that some substrates can be digested anaerobically to produce methane instead of denitrification. Other substrates can also be degraded by aerobic biodegradation process in the presence of oxygen to produce carbon dioxide, water and bacterial biomass (Boley and Muller 2005). Figure 2 illustrates the possible nitrogen reduction pathways in recirculation of aquaculture systems.

The rate of denitrification is usually calculated as the difference between the influent nitrate concentrations and effluent divided by hydraulic retention.

Combination of heterotrophic (HD) and autotrophic denitrification (AD) processes makes it possible to overcome the limitations of each of the two processes. Oh et al. (2001) demonstrated that HD and AD can coexist and complete the process of denitrification. Rocca et al. (2006) established heterotrophic-autotrophic denitrification (HAD) plant supported by steel

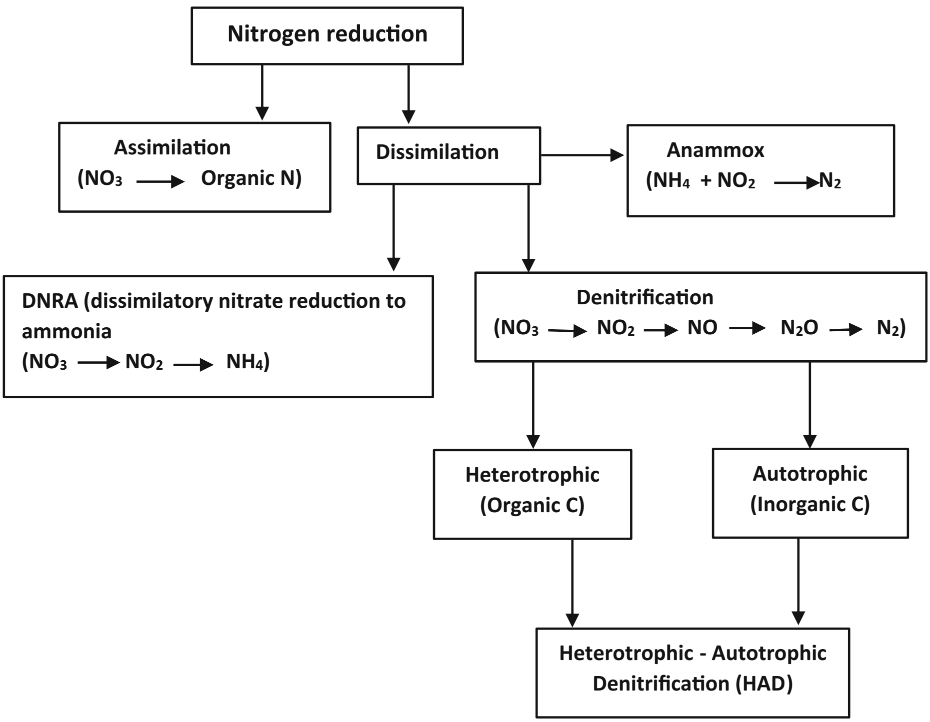

Fig. 2 Possible nitrogen reduction pathways in recirculating aquaculture system

wool and cotton. The study demonstrated high nitrate removal efficiencies. The steel wool was used to reduce dissolved oxygen in water and produced hydrogen which enhanced heterotrophic and autotrophic denitrification. Cotton acted as an organic carbon source for HD and carbon dioxide that was produced by HD, was utilized as an inorganic carbon source by autotrophic denitrifiers. Xu et al. (2015) proved that longer retention times result in the removal of more nitrate (82.6–89%) and sulfate production in HAD. The results from this study indicated that HD and AD can work simultaneously and that HAD can be a feasible process for the concurrent removal of sulfide, nitrate, and chemical oxygen demand (COD). However, the reduction of nitrate to nitrite was much faster than the conversion of nitrites to nitrogen resulting in nitrite accumulation in the HD. Whereas in the AD, the reduction of nitrites to nitrogen was faster than the reduction of nitrate to nitrites. AD can use the surplus nitrite from HD, hence the combination of AD and HD can reduce nitrite accumulation and increase the rate of denitrification (Oh et al. 2001).

Nitrate reduction using denitrification technology

Denitrification studies have reported significant reduction of nitrate nitrogen from wastewater plants. Manoj and Vasudevan (2012) reported maximum removal rates of 97% in two column reactors with an average of 86 and 80%. However, removal of nitrates was insignificant after 12 weeks due to insufficient carbon source but on addition of carbon, nitrate nitrogen removal became pronounced and reached a maximum of 99% at the 28th week (Manoj and Vasudevan 2012). There was an increase in alkalinity and an increase in pH, an indicator of denitrification (Manoj and Vasudevan 2012). Alkalinity increases with the amount of nitrate nitrogen removed. Approximately 3.6 mg of alkalinity is produced for every milligram of nitrate nitrogen reduced (EPA 1993). A study by Pungrasmi et al. (2013) to determine a suitable carbon source for denitrification showed that natural soil, sand, vermiculite, and pumice stones can successfully reduce nitrate concentration from 100 to 5 mg L^{-1}. A related study reported 0.40–0.47 mg L^{-1} of nitrate removal with palm kernel shell as denitrifying media (Dauda et al. 2014) and Christianson et al. (2015) reported 1.57–1. 82 mg L^{-1} of nitrate removal in autotrophic denitrification.

Several studies have demonstrated the feasibility of denitrification reactors in freshwater and marine recirculation aquaculture systems (Sauthier et al. 1998; Shnel et al. 2002; Sailing et al. 2007; Healy et al. 2012; Pungrasmi et al. 2013; Christianson et al. 2015). Varying denitrification treatment capacities have been demonstrated with or without carbon sources in freshwater systems (Kaiser and Schmitz 1988; Schmitz-Schlang and Moskwa 1992; Knosche 1994). Denitrification using organic carbon compounds released from uneaten fish feed and fish excreta in freshwater recirculation aquaculture systems for tilapia has also been established (Arbiv and van Rijn 1995; van Rijn and Barak 1998; Shnel et al. 2002). Denitrification has also been established in marine systems. Sauthier et al. (1998) reported a denitrification rate of 0.1 kg (NO_3-N) m^{-3} h^{-1} using crushed brick as media and ethanol as carbon source. Table 1 presents denitrification rates using different carbon sources in different reactors.

The various studies above show that varying denitrification rates occur due to the types of carbon sources used. Carbon sources can influence the rate of conversion of nitrate to nitrogen (Shnel et al. 2002). Besides, the configuration of the reactor, hydraulic conditions, nitrate loading rate, temperature and dissolved oxygen can also influence the rate of denitrification (Ghafari et al. 2008).

Table 1 Denitrification rates of different reactors using different carbon sources

Type of reactor	Type of waste water	Substrate	Nitrate removal (g L^{-1} day^{-1})	References
Fluidized	Aquaculture	Sulfur	0.71–0.80	Christianson et al. (2015)
Fixed bed	Aquaculture	Biodegradable polymers	0.168–0.336	Boley et al. (2000)
Sequencing batch reactors	Aquaculture	Acetate	0.11	Verstraete and Schryver (2009)
Moving bed	Aquaculture	Methanol, molasses	0.67–0.68	Hamlin et al. (2008)
Up-flow	Wastewater	Corncobs	0.203	Xu et al. (2009)
Fluidized	Aquaculture	Endogenous	0.402 ± 14	Tsukuda et al. (2015)
Up-flow	Aquaculture	Polyvinyl alcohol	1.4	Park et al. (2001)
PCL-packed reactor	Ground water	Biodegradable polymers PCL	0.19–0.56	Chu and Wang (2013)
Up-flow	Aquaculture	Wood chip	1.365 ± 0.039	Sailing et al. (2007)
Fixed bed	Aquaculture	Sand	0.859	Arbiv and van Rijn (1995)
Pine needle reactor	Ground water	Pine needle	0.002–0.003	Healy et al. (2012)
Up-flow	Aquaculture	BioStyr	0.0081–0.0221	Phillips and Love (1998))
Fluidized bed	Aquaculture	Sand	1.742	Gelfand et al. (2003)
Moving bed	Aquaculture	Plastic media	0.576	Tal and Schreier (2004)
Packed bed	Aquaculture	Brick granules	2.4	Sauthier et al. (1998)
Fluidized	Aquaculture	Sand	1.33	Shnel et al. (2002)

Factors that influence denitrification rate

Denitrification rates and microbial denitrifiers are influenced by the levels of nitrate, oxygen, pH, and temperature (Rivett et al. 2008; Magram 2010), as well as the amount of organic carbon substrate, which is a significant factor in aquaculture denitrification (Singer et al. 2008).

Temperature

Temperature influences the activity of denitrifying bacteria and hydrolysis of endogenous carbon sources (Canziani et al. 1999). Zhou et al. (2011) observed a significant decrease in the efficiency of sulfur-based denitrification from 70 to 50% when temperatures decreased from a range of 20–25 to 5–10 °C in laboratory and pilot scale operations. The most suitable temperature range for denitrifying bacteria is 20–40 °C, below 15 °C the activity of bacteria slows down and almost ceases at temperatures below 5 °C (Wang and Chu 2016). Wang and Chu (2016) observed a 50% decrease in nitrate removal with 5% decrease in temperature. The rate of denitrification proceeds at 5 °C but at a reduced rate while above 20 °C, the rates are constant (Wang and Chu 2016). Cameron and Schipper (2012) reported an increase in nitrate removal rates by 1.7 when temperature increased by 10 °C using softwood as a carbon source to fuel denitrification. The removal rate of nitrate is therefore, a function of temperature and increase with the increase in temperature. Like other physiological processes, denitrification rates increase by a factor of 2 for every 10 °C change in temperature (Warneke et al. 2011).

pH

Denitrification process produces alkalinity with the concomitant increase in pH; approximately 3.57 mg alkalinity is produced per milligram of nitrate reduced (Pungrasmi et al. 2013). In a combined nitrification-denitrification system, the pH of the system is balanced resulting in a stable pH since nitrification consumes alkalinity while denitrification produces alkalinity (Halling-Soerensen and Hjuler 1992). The rate of denitrification is inhibited below a pH of 6.0 and above 8.0, but the optimal rates occur between 7.0 and 7.5 (Cao et al. 2013). Increased pH can suppress the reduction of both nitrate and nitrite in denitrification systems whereas decreased pH completely suppresses denitrification since denitrifiers cannot denitrify under low pH conditions (Cao et al. 2013). Besides inhibiting denitrification, low pH values can result in nitrite toxicity to fish (Cao et al. 2013). The optimum pH values for denitrification are in the range of 6.7–7.5 (Cao et al. 2013) and 7.0–8.0 (Hiscock et al. 1991).

Dissolved oxygen

Denitrification is an anaerobic microbial process that depends on facultative anaerobes to convert nitrate to dinitrogen gas (van Rijn et al. 2006). Therefore, DO of approximately 0.2 mg L^{-1} or less allows denitrification in both freshwater and marine systems (van Rijn et al. 2006). High dissolved oxygen might inhibit the process by suppressing enzymes or by direct competition because oxygen is an efficient electron acceptor compared with nitrate. Nevertheless, Gutierrez-Wing et al. (2012) reported that denitrification can occur in water with DO levels of 4–5 mg L^{-1}. However, the rate of denitrification decreased from 5.5 to 0.5 g NO_3-N L^{-1} day^{-1} when the concentration of DO increased from 0.5 to 4 mgL^{-1}. In a similar study, Xu et al. (2009) reported a 50% decrease in nitrate removal at DO levels higher than 4.0 mg L^{-1} from 85%. The rate of denitrification can still be maintained at more than 3 mg NO_3-N h^{-1} under complete aerobic conditions since anoxic microenvironments can develop in aerobic systems (Hiraishi and Khan 2003). The anoxic microenvironments create a suitable environment for denitrification activity (Hiraishi and Khan 2003). Low dissolved oxygen levels in denitrification making the process more efficient is pointless (Wang and Chu 2016). However, (Klas et al. 2006a, b) maintains that high oxygen limits facultative denitrifiers from utilizing nitrate as the final electron acceptor in their respiratory electron transport chain and reduces the carbon available for denitrifiers through aerobic consumption.

Carbon supply

Organic carbon is one of the most important substrates that influence denitrification process. In conventional wastewater treatment plants, exogenous organic carbon sources such as methanol are often added when there is carbon deficiency, though it is very costly (Arbiv and van Rijn 1995). The use of other carbon sources such as glucose and acetate has been investigated (Lee et al. 2000). The use of these carbon sources results in the production and accumulation of organic acids such as acetic acid that negatively affect bacteria and physiology of fish (Lee et al. 2000). Stief (2001) reported nitrite accumulation when glucose was used as a carbon source to fuel denitrification. Less labile organic compounds lead to significant nitrite accumulation or certain types of carbon can expel true denitrifiers and favor proliferation of facultative organisms which reduce nitrate to nitrite but not further than that (Stief 2001). Several alternative materials have been tested as sources of carbon, for instance, Soares et al. (2000) utilized cotton wool as the only carbon source for reduction of nitrate in well water with high concentrations of nitrate. Singer et al. (2008) also used cotton wool and a degassing technique to remove dissolved oxygen and reported a decrease in nitrate levels and production of nitrite which was later removed in the nitrification biofilter.

Uneaten feed and fish waste which accumulate in recirculation aquaculture systems have also been used as a carbon source, but they have relatively low concentrations of carbon (Hirayama et al. 1988). Several studies have successfully utilized fecal waste as an internal carbon source for denitrification (Gelfand et al. 2003; Shnel et al. 2002; Schuster and Stelz 1998), but the organic matter in feces is in particulate form and is not readily available for microbial use. However, the substances can be converted into volatile fatty acids (VFAs) through hydrolysis and fermentation (Lee III et al. 1995). Hydrolysis is a limiting step in the use of internal organic carbon sources because the process is slow (Klas et al. 2006a, b). Moreover, hydrolysis and fermentation can solubilize total ammonia nitrogen and phosphorus thus the need for additional treatment of the two compounds (Conroy and Couturier 2010). The use of endogenous organic matter in the system as a carbon source can reduce expenses of external carbon sources and solid wastes. Besides, the use of anaerobic ammonium oxidation biofilter can also reduce the need for a carbon source.

Anaerobic ammonium oxidation (anammox)

Anaerobic ammonium oxidation (anammox) is currently a novel, cost-effective, and sustainable autotrophic process of removing nitrogen from wastewater under anoxic conditions using nitrite as the electron acceptor (Strous et al. 1999). The process oxidizes ammonium through nitrite reduction to produce dinitrogen gas. Ammonium oxidation has long been possible only with oxygen to produce nitrite or nitrate. The existence of the anammox process was foreseen by Broda (1977) through thermodynamic calculations and was first reported by Mulder et al. (1995) in a denitrifying fluidized bed reactor. The study revealed that the anammox bacteria in the phylum Planctomycetes were responsible for nitrogen mass imbalances in the fluidized reactor.

Since the discovery of Planctomycetes, the bacteria have been found in natural and man-made systems with limited oxygen concentration. Anammox plays a major role in the release of nitrogen to the atmosphere from the ocean (Kartal et al. 2010). Compared with the standard nitrification-denitrification process, anammox reduces the demand for energy by 60%, organic carbon by 100%, sludge production by 80.9% since they produce low biomass resulting in small amounts of sludge production and oxygen demand by 50% because ammonium is nitrified to nitrite rather than nitrates. The process requires inorganic carbon sources such as HCO_3^- and CO_2 for growth and energy for the activity of anammox bacteria (van de Graaf et al. 1996). High concentration of organic matter may affect the activity of anammox bacteria due to the inactivation of enzymes and can result in cell death (Güven et al. 2005). Nevertheless, there have been inconsistent results on the effect of COD to N ratio on the anammox process (Kartal et al. 2010). A study on the short-term effects of acetate and starch demonstrated that COD/N ratios of 2 and 6 had no significant effect on the process (Guillén et al. 2014) while Ni et al. (2012) reported that COD/N ratio above 4 decreased the activity of anammox bacteria. COD/N ratio above 2 may favor the denitrification process and inhibit the anammox process (Chanchoi et al. 2008). Jenni et al. (2014) reported a reduction in abundance of anammox bacteria but increase in *Candidatus brocadiafulgida* with a ratio of 0.8 g COD N^{-1}, and Leal et al. (2016) observed inhibition of the anammox process at COD/N ratio above 3.

Anammox has been applied for treatment of wastewater in different industries producing different wastewater (Lahav et al. 2009). Presently, the process is designed to treat wastewater with high concentrations of ammonia at temperatures between 25 and 40 °C (Lopez et al. 2008). The process has significantly improved the removal of nitrogen in wastewater since its discovery. However, the slow growth of the bacteria limits the scaling up of the process. Its application is also difficult because it is carried out by mixed microbial communities rather than pure cultures (Lopez et al. 2008). Research in recirculation aquaculture systems in both marine and freshwater aquaculture has reported significant anammox bacteria populations (Tal et al. 2003; Klas et al. 2006b; Lahav et al. 2009). Lahav et al. (2009) demonstrated the presence of large quantities of anammox bacteria in the gut and feces of gilthead seabream. The study also reported low nitrate and total ammonium nitrogen concentrations in the anammox reactor throughout the experimental period and high nitrate removal rates at a low organic loading of (solid retention time (SRT) = 12.5 days) to 350 NO_3-N L^{-1} reactor day^{-1} at a SRT of 4 days (Lahav et al. 2009). In municipal water, higher nitrate (90%), COD (85%), and ammonium removal efficiencies (95%) in the range of were reported in anammox reactors (Leal et al. 2016).

Nitrogen reduction through plant assimilation

Nutrient uptake by plants is one of the most documented biological processes for waste removal (Mitsch and Gosselink 2000). Studies in a constructed wetland receiving aquaculture wastewater reported ammonium removal efficiencies of 86 to 96% (Lin et al. 2002). The ability of plants to absorb wastes from wastewater has been successfully established and employed in aquaponic systems. Aquaponics is a closed loop multi-trophic food production system which integrates the RAS and hydroponics (Goddek et al. 2016). The system is designed in such a way that nutrient-rich water from fish tanks flows to the mechanical filter then to the biological filter before being pumped to the hydroponic units which depurate water for plant growth, the water is then channeled back to the fish tanks (Goddek et al. 2015). Aquaponic systems are receiving growing interest as sustainable food production systems due to their capacity to produce fish and plants in an environmentally friendly manner with limited resources (water, land, fertilizer) and nutrient recycling (Vermeulen and Kamstra 2013). The systems reduce the need for mineral fertilizers used in hydroponics because nutrient-rich water from fish tanks can support the growth of plants (Goddek et al. 2016). In recirculating aquaculture, nutrients are recycled through the symbiotic relationship between fish, bacteria and plants. Hence, there is no need for frequent exchange of nitrate-rich water, which is a common practice in recirculating aquaculture systems. Aquaponics represents a promising sustainable food production system for the future since it interlinks aquaculture and hydroponics. The interlinking of the two systems allows some of the shortcoming of aquaculture and hydroponics to be addressed (Goddek et al. 2015).

Nutrient-rich water from aquaculture is efficiently recycled through the biofilter in which residual nutrients are transformed by nitrifying bacteria to nitrites then nitrates (Goddek et al. 2016). As the effluents flow into the hydroponic unit, nutrients are absorbed in the form of non-toxic nitrate and ammonia (Zou et al. 2016) and as a result, improve the quality of water in aquaculture units (Endut et al. 2010). There are three different hydroponic units commonly used in aquaponic systems including; media-filled raised bed, nutrient film technique (NFT) gutter-shaped bed, and deep-water culture (DWC) or floating raft systems. The media-based hydroponic consists of a trough filled with either perlite, pumice, gravel, or expanded clay which supports roots and a microbial substrate. In the media-based unit, water is channeled in an ebb and flow pattern resulting in sequential aeration and nutrition. The NFT system is made up of narrow channels of perforated pipes where roots are partially immersed in a thin layer of water (Goddek et al. 2015), while the floating raft or DWC system consists of floating rafts with perforations where net pots are inserted. The plant pots are filled with media such as pumice and then submerged in water.

The type of hydroponic unit can influence the concentration of nutrients in the aquaponics. For instance, the media in the media-filled hydroponic unit serves as a substrate for nitrifying bacteria, act as a solid filtering and mineralization media (Goddek et al. 2016). This increases the concentration of nutrients available for plant uptake. Nutrient concentration in the aquaponic systems can also be influenced by density and type of fish, protein levels in feed, feeding rate, metabolic conversion, excretion, uneaten feed, and feces and the nutrient requirement of the plants (Endut et al. 2016). These factors can be manipulated to produce nutrients that can sustain plant growth (Licamele 2009). Though nutrient concentrations in aquaponic systems can be balanced by the fish/plant ratio approach. It is complex to determine the exact ratio since fish and plant species have different nutritional requirements that are dependent on the growth stage and external factors such as the system design (Goddek et al. 2016).

Several aquaponic studies have shown the potential of plants to utilize nutrients from aquaculture wastewater and maintain water quality (Table 2). Hu et al. (2015) observed varying nutrient removal rates from different plants. A higher nutrient utilization efficiency (41.3%) was observed in the tomato-based aquaponic system compared with pak choi-based aquaponics (34.4%). The removal of nutrients in the two plants differed due to their different nutrient requirement (Hu et al. 2015). High nutrient efficiency in tomato-based aquaponics was linked to the high biomass of nitrifying bacteria and an extensive root network in tomatoes. Similar studies have shown that plants with extensive root network have a higher removal rate than those with less extensive roots (Endut et al. 2016). The extensive root networks have a sufficient area where nitrifiers develop exopolymeric substances (EPS) that protect the nitrifying bacteria. Studies with herbaceous plants-based aquaponics reported no significant variations in basil (1.29%, 0.58%), peppermint (1.20%, 0.69%), and spearmint (1.53%, 0.69%) in nitrogen and

Table 2 Percent nutrient removal of some plants in aquaponic systems

Type of plant	Fish	Nitrate	Phosphorus	TAN	References
Lettuce	Bester sturgeon	46.97 mg L^{-1}	–	–	Dediu et al. (2012)
Spinach	Rainbow trout	2.9–32 mg L^{-1}	–	–	Petrea et al. (2013)
Water spinach	Catfish	90.04%	–	–	Endut et al. (2016)
Water spinach	Carp and goldfish	40–52%	–	–	Nuwansi et al. (2016)
Water spinach	Marble goby	70%	60%	83%	Lam et al. (2015)
Water spinach	Catfish	79.17–87.1%	75.36–84.94%	78.32–85.48%	Endut et al. (2011)
Mustard green	Catfish	66.67–80.65%	66.79–77.87%	69.0–75.85%	Endut et al. (2011)
Green corn	Tilapia/crayfish	50–95%	50–80%	90%	Gallardo-Collí et al. (2014)
Spinach	Koi carp	57.83–80.01%	39.14–53.18%	–	Hussain et al. (2014)
Tomato	GIFT tilapia	32.29%	12%	64%	Saufie et al. (2015)
Tomato	Cyprinid	Neg>	25%	31%	Klemenčič and Bulc (2015)
Lettuce	Tilapia	13. 58–22.86%	–	83.59–91.50%	Wahyuningsih et al. (2015)
Aubergine	Tilapia	17%	27	–	Graber and Junge (2009)
Tomato	Tilapia	69%	Neg>	–	Graber and Junge (2009)
Lettuce	Murray cod	52.50%	90.90%	–	Lennard and Leonard (2006)
Taro	Tilapia	50%	75%	50%	Hashem et al. (2014)

phosphorus respectively. The plants removed adequate nitrogen wastes reflected by the prominent difference in nitrate and ammonium concentrations between the influents and effluents of the biological filters and production of biomass in plants (Espinosa Moya et al. 2014). Studies on nutrient recovery and uptake in aquaponic systems show varying results. Seawright et al. (1998) reported a significant difference in nitrogen uptake by romaine lettuce when different fish feeding rates were used. Nitrogen uptake was low (8%) with a high feeding rate and high with a low feeding rate (67%), a high quantity of phosphorus (P) was also recorded in solution than in lettuce. Endut et al. (2014) observed a TAN removal efficiencies of 64% in water spinach.

Challenges of aquaponic systems

One of the major challenges in aquaponic systems is the management of nutrient concentrations that can provide plants with optimum concentrations while avoiding any negative impact on the fish, bacteria and the environment (Goddek et al. 2015). There is a need to understand nutrient dynamics to properly manage and balance the concentration of nutrients in aquaponic systems. In these systems, the main nutrient input is uneaten feed, assimilated feed, soluble and solid excreta (Chen et al. 2006). Soluble excreta in the form of ammonia is the most available mineral when converted to the usable form (nitrates) for plants. Whereas uneaten feed and solid feces are unavailable unless they are solubilized from organic material to ionic mineral forms that can be easily assimilated by plants. However, minerals have different solubilization and accumulation rates which influences their concentration in water (Damon et al. 1998). In recirculating aquaculture systems, solid wastes are partially solubilized and filtered out daily resulting in loss of nutrients (Goddek et al. 2015). For instance, 30–65% of phosphorus added to the recirculating aquaculture via fish feed is lost in form of solid excretion from fish and filtered out through mechanical filters (Damon et al. 1998). In addition, organic phosphorus solubilized as orthophosphate can precipitate with calcium to form hydroxyapatite—$Ca_5(PO_4)_3(OH)$ making the orthophosphate unavailable in solution (Krom et al. 2009; Damon et al. 1998). Aquaponic studies report phosphorus concentration range of 1–17 mg L^{-1} (Endut et al. 2010; Al-Hafedh et al. 2008; Lennard and Leonard 2006). However, recommended concentrations in conventional hydroponics are between 40 and 60 mg L^{-1} (Resh 2012; Sikawa and Yakupitiyage 2010). Therefore, phosphorus should be added to the aquaponic systems particularly for fruiting vegetables because it is a macronutrient assimilated by plants for root development and flowering (López-Arredondo et al. 2013).

Most aquaponic systems have been established on a trade-off between the requirements of fish, nitrifying bacteria, and plants. According to Monsees et al. (2017), large fluctuations in pH, temperature, and other water quality parameters experienced in most aquaponic systems might affect fish, bacteria and plants. The fluctuations in water parameters can affect the availability of nutrients in the system. For instance, in conventional hydroponic systems, most nutrients become available at a slightly acidic pH (5.5–6.5) (Graber and Junge 2009). The pH should be acidic to avoid precipitation of Mn^{2+}, Ca^{2+}, Mg^{2+}, Fe^{2+}, and PO_4^{3-} to unavailable insoluble salts when pH is greater than 7. In the hydroponic units, pH levels are controlled by the addition of acids such as nitric acid. Whereas aquaponic systems make compromises in terms of pH, temperature, and nutrient concentrations (Goddek et al. 2015). Compromising water quality parameters such as pH and temperature is not ideal for fish, bacteria and plants because they have different water quality requirements. A pH range of 7.0–9.0 is recommended for fish production and for the nitrifying bacteria to convert ammonia to nitrites then nitrates (Tyson et al. 2001). Whereas, the temperature for most warm water fishes such as tilapia and nitrifying bacteria is 25–30 °C whereas most plants prefer colder water temperature (20–25 °C).

Pests and disease management is another aspect that needs to be addressed to improve the sustainability of aquaponic systems (Goddek et al. 2015; Vermeulen and Kamstra 2013). Hydroponic systems use standard pesticides that cannot be used in an aquaponic system due to the toxicity risk to fish and nitrifying bacteria. Furthermore, fungicides and antibiotics to control and remove fish pathogen in water cannot be used due to the need to maintain nutrient solubilizing organisms and nitrifying biofilm (Nichols and Savidov 2012). Besides, the use of antibiotics in an aquaponic system must be avoided because they are not allowed in plant application (Goddek et al. 2015). Crop practices that decrease the occurrence of pests and diseases have been tried. Decreasing relative humidity around plants, the use of microorganisms with biocontrol activity or extracts of plants such as essential oils have also been applied. However, it is a challenge to select suitable methods for aquaponic systems if the methods are not compatible with living organisms of the systems (Goddek et al. 2015). Hence, innovative and sustainable approaches to control and prevent pests and diseases while minimizing the impacts on fish and desired microorganisms are needed. Currently, aquaponic research is focusing on systems that can maintain optimal conditions for fish as well as the nitrifying bacteria through physical separation of recirculating aquaculture and hydroponic units (decoupled systems). The decoupled systems maintain optimal conditions for each system with periodic water exchange between them (Monsees et al. 2017), sludge can also be subjected to remineralization (Goddek et al. 2016) and organic matter can produce biogas energy (Yogev et al. 2016).

Decoupled aquaponic systems

Several studies have shown that aquaponic systems can minimize consumption of water and resources such as land and fertilizers (Suhl et al. 2016; Goddek et al. 2015). However, most aquaponic systems are designed to provide sub-optimal conditions for both fish and plants in a single water loop process resulting in concerns regarding the sustainability of these systems (Goddek et al. 2016). The single loop systems are also designed to produce plants with lower nutrient requirements such as lettuce. Nutrients are relatively low in these systems because they are consistently removed by plants in the hydroponic unit. Thus, a higher stocking density of fish and high amounts of feed is used to increase the nitrogen content resulting in high feed conversion ratio of 1.7 and 1.8 (Kloas et al. 2015). Moreover, most established systems are small and mainly used for educational purposes (Villarroel et al. 2016), while commercial systems are few and still young (Goddek et al. 2015). To improve the sustainability of these systems, researchers are trying to restructure the traditional aquaponic unit (coupled aquaponic) in the direction of an independent system where each unit (RAS, hydroponics) is controlled separately. Sludge remineralization has also been introduced to recover nutrients from the system (Kloas et al. 2015; Goddek et al. 2016). The remineralization units are integrated as distinct functional units that include separate water cycles that are independently controlled. The new inventions are known as the decoupled aquaponic system (DAPS), this can improve the self-sufficiency of the whole system (Goddek et al. 2016).

One of the first decoupled aquaponic systems was conceptualized in Germany by Kloas et al. (2015), the system is composed of independent recirculating aquaculture system for fish and a hydroponic unit for plants. The two systems were connected by a one-way valve to allow nutrient-rich water to flow into a hydroponic reservoir where the nutrient-rich water can be optimized as fertilizer for the plants. Water loss due to evapotranspiration of plants was replaced via the one-way valve from the RAS. The decoupled system enhanced nutrient flows and the conditions of fish and tomatoes were achieved (Kloas et al. 2015). Recently, Goddek (2017) presented a decoupled system with more than 2 units, the system comprised a third functional unit for the remineralization of sludge derived from RAS. The additional unit improved the control of nutrient flows, increased the production efficiency and minimized further emissions of wastes. Studies have also shown that decoupled systems can improve growth of both fish and plants (Delaide et al. 2018; Goddek et al. 2016). An increased plant growth of 39% was reported in decoupled systems compared with a pure hydroponic system when the hydroponic component was supplemented with additional fertilizer Delaide et al. 2018). Yogev et al. (2016) suggested that the organic matter or sludge can produce biogas energy.

Advantages of decoupled aquaponic systems

Established decoupled aquaponic systems at Wageningen University and Research (WUR) and at the Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB), Germany were able to overcome the limitations of the conventional one loop aquaponic system (Goddek 2017; Monsees et al. 2017). The studies showed that the multi-loop systems can;

- Lower water requirements and fertilizer requirement compared with conventional agriculture
- Increase plant growth by 40% compared with conventional hydroponic systems. Both RAS water and anaerobic digestates contain plant promoting rhizobacteria (PGPR) that could promote plant growth
- Suitable for sensitive fish such as trout thus increasing food diversity
- Create a more resilient economic system, if one subsystem fails, the other can still be going on
- Lower pest and disease rates because the incidences can be managed in the independent units without interfering with the conditions of either fish or plants
- Micronutrients such as zinc that is not available in fish feed can be added in the hydroponic units without negatively affecting the fish.
- Increase nutrient flows in the plant compartment and minimizes effluent discharge through anaerobic nutrient remineralization technology while producing methane for electricity and heat generation.

Although decoupled aquaponics has improved the sustainability of conventional aquaponic systems, they have limitations such as consistently higher nutrient concentration in the RAS unit (without additional fertilizer inputs) due to unidirectional flow design in these systems (Goddek and Keesman 2018). Therefore, a significant amount of nutrients is added to the hydroponic component through mineralization to meet optimal growth conditions for plants. Desalination technology can improve the quality of RAS water to meet fish species requirement and increase nutrient concentration in the hydroponic unit (Goddek and Keesman 2018). According to Subramani and Jacangelo (2015), desalination has the potential to separate dissolved minerals and other minerals from water. The technology can, therefore, provide the desired nutrient concentrations for the hydroponic unit and provide freshwater to the RAS unit. Goddek and Keesman (2018) demonstrated that nutrient concentration can be improved in the hydroponic unit and 40% of water flow to the desalination unit can be mineralized and be reused within the RAS (Fig. 3). Desalination technology can, therefore, be used in

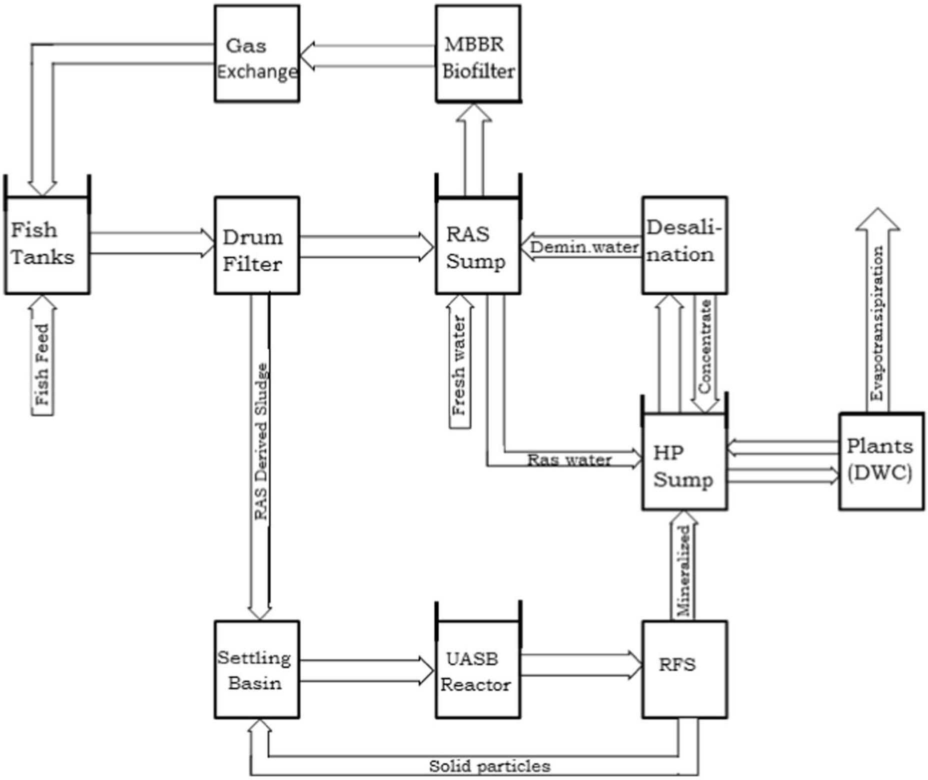

Fig. 3 Schematic representation of a water flow diagram in a multi-loop aquaponic system with desalination unit, proposed by Goddek and Keesman (2018)

optimizing growth of fish and plant conditions by improving nutrient balances in multi-loop aquaponic systems (Goddek and Keesman 2018).

Sludge mineralization

It is estimated that over 60% of feed input in aquaculture systems ends up as solid waste consisting of feces and uneaten feed (Masser et al. 1992). Therefore, management and removal of these solids are considered the most critical process in managing aquaculture systems (Patterson and Watts 2003) and is one of the major obstacles to the continued development of aquaculture (Piedrahita et al. 1996). Recirculating aquaculture systems are designed with mechanical filters that removes solid wastes and discharges them from the system as sludge (Chen et al. 1997). According to Neto and Ostrensky (2015), sludge contains a lot of nutrients which can be recycled and reintroduced into the aquaponic system via sludge mineralization. Remineralization can be a promising way of increasing nutrient content in aquaponics and reduce both water usage and nutrient emissions. Recent studies show that a high proportion of nutrients can be recovered by digesting sludge in aerobic, anaerobic, and sequential aerobic-anaerobic reactors (Mirzoyan 2009; van Rijn 2013; Monsees et al. 2017; Delaide et al. 2018). Mineralization can be done by manually feeding fresh sludge into reactors as suggested by Monsees et al. (2017) or inoculating the reactors with already digested sludge (Delaide et al.

2018). Aerobic digestion is the biological oxidation of organic matter where bacteria in activated sludge consume organic matter with oxygen and converts them into carbon dioxide (Novak et al. 2003). Whereas, anaerobic digestion is the biological degradation of organic wastes by facultative and obligate anaerobic bacteria (Novak et al. 2003). Anaerobic digestion is normally used for stabilization and reduction of wastewater sludge because it generates 3 to 20 times less sludge and only 5% is converted into biomass. Delaide et al. (2018) quantified tilapia sludge digestion performance in aerobic and anaerobic reactors as shown in Fig. 4.

The results showed better mineralization performance for most essential nutrients except nitrogen (58.75%) and potassium (40.46%) in aerobic reactors. However, Monsees et al. (2017) observed an increase in nitrogen (30%) and potassium (31%) within 14 days. Phosphorus, calcium, and magnesium were 54.25, 62.95, and 57.49% in aerobic reactors and 28.4, 8.41, and 35.77% in anaerobic reactors. Boron, copper, zinc, manganese, and sodium ranged between 13.18 and 62.98% in aerobic reactors and 2.5 and 32.09% in anaerobic reactors (Delaide et al. 2018). Monsees et al. (2017) reported an improvement in nutrient recovery from aquaculture sludge. The study reported a 3.2-fold increase in soluble reactive phosphorus due to a decrease in pH in the aerobic reactor. Nitrate concentration reduced by 16% in the aerated reactor and 97% in the unaerated reactor due to denitrification. Low pH supported leaching of phosphorus and potassium with minor losses in nitrates in aerated reactors (Monsees et al. 2017). pH can be decreased through respiration, nitrification, as well as the addition of glucose which lowers pH after fermentation. Besides, refilling the reactor with new sludge water or concentrated sludge or a prolonged retention time can speed up the pH decrease. Monsees et al. (2017) demonstrated that a continuous reduction in pH increased phosphorus leaching and that the addition of acids increased leaching of different nutrients. The studies indicate potential to recover nutrients such as phosphorus, potassium, and micronutrients that are

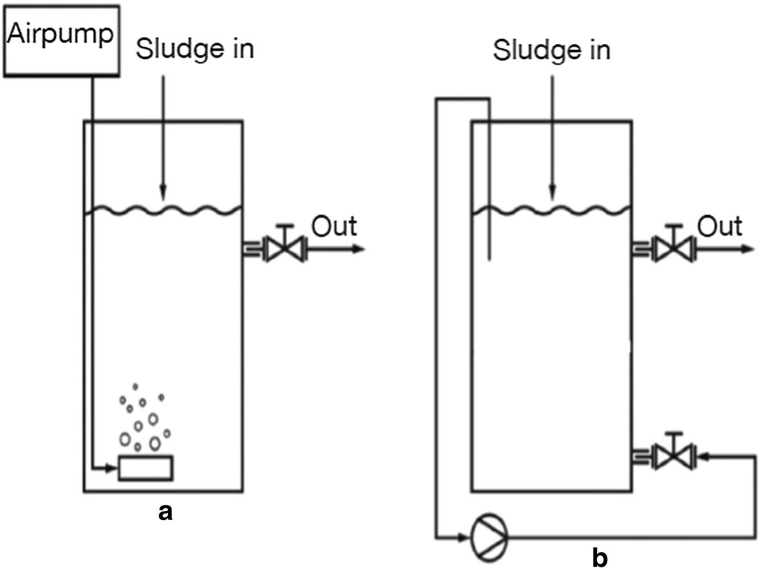

Fig. 4 **a** Aerobic digester (constantly aerated and mixed) and **b** anaerobic digester (constant up-flow velocity of 0.9 m h^{-1} to ensure slow mixing of sludge. A small pump was used to constantly recirculate the top water of the digestor into the bottom inlet). The reactors were 70 cm high with an operating volume of 45 L and a diameter of 30 cm (after Delaide et al. 2018)

limiting in aquaponics and require supplementation. The aerobic reactors were efficient in recovering nutrients from aquaculture sludge compared with anaerobic reactors but can be improved by the addition of acids, bacterial suspension, and carbon sources (Monsees et al. 2017; Delaide et al. 2018).

Remineralization can recycle all the nutrients that enter the system; however, aerobic mineralization is recommended to treat sludge, particularly for effective recovery of phosphates (Monsees et al. 2017). Moreover, aerobic mineralization increases potassium, an important mineral required in high quantities for optimal growth of plants (Hochmuth 2001). Though anaerobic mineralization represents a promising approach to recycle phosphates, it counteracts current efforts to increase and improve the general availability of nutrients in the hydroponic solution due to the removal of nitrates through anaerobic denitrification (Monsees et al. 2017).

Besides improving nutrient concentration in aquaponic systems, sludge can be used to produce biogas. During anaerobic digestion, sludge undergoes substantial changes in its physical, chemical, and biological properties (Novak et al. 2003). Anaerobic digestion produces biogas composed of methane and carbon dioxide with low levels of hydrogen sulfide and ammonia (van Rijn 1996; Novak et al. 2003). The biological degradation of organic matter to methane is a complex process which depends on interactions between different groups of microbes. There are three main stages of anaerobic digestion namely hydrolysis, acetogenesis, and methanogenesis (Sowers 2000). Hydrolysis is the conversion of higher polymers into soluble monomers such as alcohols, fatty acids, and amino acids. These compounds pass through the second stage of digestion (acetogenesis) where carbon dioxide and hydrogen (low molecular weight organic acids) are generated. In the final stage of digestion (methanogenesis), methanogenic bacteria convert acetic acid, formate, and hydrogen to methane (Mirzoyan et al. 2010). The methane produced can be a source of thermal and electric energy (Van Lier et al. 2008).

Conclusions

There are different approaches that can be used to control and manage wastes in recirculating aquaculture including feeding strategies, nitrification, denitrification, HAD, anaerobic ammonium oxidation, and aquaponics. Several studies have shown that these approaches have the potential to maintain the quality of water in recirculating systems. However, selection of a suitable method to manage waste depends on available resources, convenience, cost, as well as its effectiveness to maintain water quality and sustainability. Feeding strategies increase the digestibility of fish feed which improves feed utilization as a result reduces the amount of wastes produced. Nitrification is a conventional approach to reduce ammonia toxicity. However, the process depends on nitrifying bacteria with long start-up and multiplication periods. Besides, the bacteria are sensitive to rapid changes in pH, temperature, filter flow rate, and dissolved oxygen. The rapid changes in water parameters can affect the efficiency of the biofilters. Therefore, there is a need to provide a favorable environment for the growth of autotrophic bacteria and optimum conditions for nitrification to take place. However, it is challenging to minimize organic matter in the influent of nitrifying biofilter because it is difficult to remove fine solids using existing solid removal techniques. Denitrification can be a solution to the above-mentioned challenges because denitrifiers have a higher growth rate than nitrifiers. However, denitrification is not common in aquaculture due to little incentive to adopt the technology as well as the high cost of external carbon source to fuel the process. The combination of HD and AD processes makes it possible to overcome the respective limitations of the two processes. The combination of the two processes can limit the production of nitrites and increase denitrification rate. Anammox is a cost-effective and sustainable process to remove nitrogen from wastewater and can be an alternative to denitrification. However, anammox has not received wide application in aquaculture. Aquaponics is a more sustainable approach to reduce wastes in aquaculture. However, a big share of nutrients in most classical aquaponic systems is still unexploited and often directly discharged to the environment from the mechanical filters. The current multiloop aquaponic systems seem to be an innovative and a more sustainable solution. These systems can help in overcoming the limitations of coupled systems. In these systems, pests and diseases can be managed in independent units, micronutrients that lack in fish feeds can be added without affecting the fish. Besides, sludge from recirculating aquaculture can increase nutrient production through remineralization and can produce biogas. Furthermore, desalination technology can increase nutrient concentration in the hydroponic unit and improve water quality in fish tanks. During remineralization, nutrients can be made available for the plants under low pH conditions. Whereas high pH conditions favor the growth of methanogenic bacteria which produces methane, a source of energy. Therefore, a sequential reactor has the potential to tackle this issue. Hence need for further research in pilot-scale implementations.

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