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Biological Approaches in Management of Nitrogenous Compounds in Aquaculture Systems

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ABSTRACT

Aquaculture is the fastest growing food-producing sector accounting for almost 43% of the world's food fish. There is however a need to increase aquaculture production in the next two decades in order to satisfy the minimum protein requirement for human nutrition. There are many constraints that limit the maximum production in aquaculture systems such as water quality and adequate live feeds. With the development of modern aquaculture farming, extensive culture has given way to intensive culture systems. In intensive systems, cultured organisms are fed protein-rich formulated feeds. Uneaten feed along with metabolic wastes and other organic matters decompose resulting in an increase of toxic nitrogenous compounds causing deterioration of water quality which is toxic to cultured organisms. The discharge of a large amount of nutrient-rich wastes from these aquaculture systems, the majority of which are nitrogenous compounds, promotes eutrophication in water bodies. In general, an increase of nitrogenous compounds has adverse effects on the environment and on aquaculture production. The aim of this paper is to highlight some of the trends in biological management of nitrogenous substances in aquaculture systems.

Keywords: aquaculture, ammonia, nitrogen, management

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INTRODUCTION

Aquaculture is the farming of aquatic organisms in natural, controlled marine or freshwater environments. It is one of the fastest growing industries and provides 43% of all the fish consumed by humans (FAO 2007). The Asia-Pacific region accounts for more than 89% of the world aquaculture production in quantity. In order to maintain the current per capita consumption of aquatic food, an additional 40 million tonnes will be required by the year 2030 (FAO 2006).

Aquaculture is an important economic activity contributing to the world protein supply. One of the possible benefits suggested with increase in aquaculture production is to alleviate pressure on wild fisheries (Naylor *et al.* 2000; Pauly *et al.* 2002; Goldburg and Naylor 2005). To meet the growing demand for fish and seafood throughout the world, traditional farming systems have given way to intensive aquaculture. In addition, factors such as limitations in water quality and quantity, and cost of land are driving the aquaculture industry toward more intensive practices. However, large inputs of resources in intensive aquaculture systems are not normally efficiently utilized resulting in release of wastes to the environment (Folke and Kautsky 1989).

Nutrition of fish in natural ecosystems or in extensive ponds is dependent mostly on the natural food web. However, in intensive aquaculture systems, formulated protein-rich feed is commonly used for culturing aquatic organisms. Intensive shrimp pond culture produces high organic wastes consisting of solid matter viz. uneaten feed, feces, phytoplankton and dissolved metabolites for instance ammonia, urea and carbon dioxide. Wickins (1985) showed that an average of 11% of a mixture of wet and dry pelleted feed remained un-eaten by *Penaeus monodon.* In addition, fish excrete ammonia as their principal nitrogenous waste which is a major constraint to successful aquaculture practices. Together with the excess feed accumulated in the bottom of

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ponds and tanks, these wastes decompose resulting in an increase of toxic nitrogenous compounds. The accumulation of a high concentration of total ammonia nitrogen leads to a decrease in aquaculture production. To maintain good water quality and to overcome the toxic effects of nitrogenous compounds, frequent water exchange is the most common practice in aquaculture farming. However, frequent water exchange to overcome poor water quality problems is laborious, expensive (Thompson *et al.* 2002) and may increase the risk of introducing disease-causing agents. Moreover, the discharge of nutrient-rich water and sediments from aquaculture systems has contributed to negative environmental impacts (Jones *et al.* 2001) causing eutrophication in rivers and coastal waters which leads to conditions unsuitable for aquatic organisms. As a result, aquaculture waste, if not properly managed, could cause a negative impact on the aquatic environment.

To maintain a healthier environment in aquaculture systems, good water quality is essential for growth and survival of cultured species. There are several strategies to control water quality in aquaculture systems such as the use of chemicals like ozone, chlorine, oxidizing agents, chelating agents and physical methods like filtration and aeration which have both beneficial and negative impacts (Dupree 1981). These strategies have led to the development of bioremediation which is environmentally friendly and has shown to boost productivity in aquaculture. Bioremediation involves degradation of hazardous waste to environmentally safe levels by selected microorganisms, bivalves and algae (Rao and Sudha 1996), and thus reduces toxic compounds produced by the cultured animals. In addition, bioremediation makes use of natural processes to transform contaminants to forms that are harmless to the organisms (Beck *et al.* 1997) and promotes a sustainable environment for aquaculture.

A number of techniques have been developed and evaluated with varying degree of success to control nitrogenous compounds in aquaculture systems. This study highlights some of the different biological approaches used for effective management of nitrogenous substances in aquaculture wastewater.

RECYCLING OF NITROGENOUS WASTES

Nutrient cycling is an essential process in the aquatic ecosystem to return the organic wastes into useful forms that can be used by autotrophs. Nitrogen, sulphur, carbon and phosphorus cycles are the major nutrient cycling processes occurring in the aquatic ecosystem and bacteria play a key role in the formation of inorganic compounds. In aquaculture systems, nitrogenous compounds are constantly produced, converted and consumed. The nitrogen cycle occurs in four steps, nitrogen fixation, ammonification, nitrification and denitrification carried out by different aquatic microorganisms. During the nitrogen fixation process prokaryotic microorganisms fix free nitrogen to form ammonia through nitrogenase activity and these include bacteria and cyanobacteria which are referred to as diazotrophs. The role of *Azotobacter*, *Clostridium*, *Desulfovibrio* and photosynthetic nitrogen-fixing bacteria in nitrogen fixation in marine ecosystems has been well documented (Sisler and ZoBell 1951; Pshenin 1963; Truper and Genovese 1968; Wynn-Williams and Rhodes 1974a, 1974b; Herbert 1975; Lakshmanaperumalsamy *et al.* 1975). Under natural conditions, ammonia or nitrates are present in low concentrations in the aquatic ecosystem and production of organic matter by autotrophs depends greatly on the availability of both. Autotrophs utilize ammonia and nitrate as a source of nitrogen for synthesis of protein (Rheinheimer 1974) which is in turn consumed by herbivores such as fish and other aquatic animals. Protein-mineralising bacteria such as *Pseudomonas*, *Bacillus* and *Vibrio* act on these aquatic animals after death, breaking them down to amino acids and peptides. Ammonifying bacteria further break down the amino acids liberating NH_3 in the process. Liberated NH_3 serves as a source of nitrogen for phytoplankton (Koike *et al.* 1986) and aquatic macrophytes.

In aerobic conditions, ammonia is oxidized during the nitrification process by aquatic ammonia-oxidisers such as *Nitrosomonas* and *Nitrococcus* to nitrite. The aquatic nitrite is then oxidized by *Nitrobacter*, *Nitrococcus* and *Nitrospina* to nitrate. Heterotrophs are also able to carry out nitrification but to a lesser extent compared to bacteria. Witzel and Overbeck (1979) reported vigorous nitrate formation by *Arthrobacter* strains isolated from Kleiner Plöner See (Holstein, Germany). In aquaria where aeration is strong, nitrate concentration becomes very high due to oxidation of the high ammonia concentration formed from the metabolic products and excess feeds (pers. obs.).

Denitrification involves reduction of nitrate to free nitrogen. Jetter and Ingraham (1981) listed 73 bacterial genera capable of denitrification. These included common aquatic heterotrophs such as *Alkaligenes*, *Pseudomonas*, *Vibrio* and other less widely distributed genera like *Hyphomicrobium*, *Leptothrix* and *Thiobacillus*. Herbert (1982) showed that *Aeromonas*, *Vibrio*, *Klebsiella*, *Escherichia* and *Clostridium* were very active in NO_3 dissimilation. Most of the nitrogen cycle processes occur simultaneously in aquatic ecosystem.

Anaerobic ammonia oxidation (anammox) and denitrification process

In conventional method, biological nitrogen removal is achieved by nitrification followed by a denitrification process, Recently, the short-cut nitrification–denitrification (SND) and anaerobic ammonium oxidation (anammox) (Mulder 1992) have been invented for nitrogen removal. Another high rate nitrification/denitrification technology known as single reactor system for high activity ammonium removal over nitrite (SHARON) has been developed for the removal of nitrogen components from wastewater (Hellinga *et al.* 1998). Different technologies involving autotrophic nitrogen removal have resulted in the development of single-stage nitrogen removal using anammox and partial nitritation (SNAP) (Furukawa *et al*. 2006), completely autotrophic nitrogen removal over nitrite (CANON) (Sliekers *et al.* 2002), deammonification (Hippen *et al*. 1997) and oxygen-limited autotrophic nitrification–denitrification (OLAND) (Pynaert *et al.* 2004). However, anammox offers a novel, energy saving and cost-effective biological nitrogen removal technique (Kumar and Lin 2010). The anammox reaction which allows ammonia to be oxidized to nitrite under anoxic conditions (Strous *et al.* 1999) is considered to be an important pathway of the nitrogen cycle. The anammox reaction is initiated by the bacterium *Brocadia anammoxidans* in which ammonium oxidation is coupled to nitrite reduction converting ammonium into nitrogen gas without using oxygen and without producing carbon dioxide. It provides an alternative approach to nitrogen removal via denitrification. The ability to oxidize ammonium anaerobically makes *B. anammoxidans* useful for reducing ammonium in recirculating and wastewater systems. In addition, it has the potential of providing significant oxygen and energy savings due the oxidation of only half of the ammonia produced in the system. Furthermore, anammox removes ammonia via autotrophic pathways without the requirement of organic carbon (van Rijn *et al.* 2006) as shown below:

Partial nitrification: $2NH_4^+ + 1{:}5O_2 \rightarrow NH_4^+ + NO_2^- + H_2O + 2H^+$

Anammox: $NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$

Total: $2NH_4^+ + 1{:}5O_2 \rightarrow N_2 + 3H_2O + 2H^+$

Very few studies have been carried out in fish/shrimp culture systems to understand the dynamics of the nitrogen cycle. Barat and Jana (1987) worked on the effect of farm management on the distribution pattern of protein mineralizing and ammonifying bacterial population in fish culture tanks. They reported a higher density of protein mineralizing and ammonifying bacteria in catfish farming system than in carp culture ponds probably due to high protein and ammonia in carnivorous fish farms compared to herbivorous fish ponds. Jana and Roy (1983) studied the activity of microbial populations involved in nitrogen cycle in water and sediments of mono- and polyculture systems of fish farming. Spatial differences in microbial density in these ponds were related to the fish culture practices.

NITROGENOUS WASTE PRODUCTION IN AQUACULTURE – FORMS AND TOXICITY

A number of toxic compounds such as ammonia and nitrite can be found in most polluted aquaculture systems. These compounds can reach toxic levels in culture systems as a result of metabolic activity due to high stocking densities and feeding rates. Ammonia occurs in two forms in water, ionized ammonia (NH_4^+) and unionized ammonia (NH_3) and together these two forms of ammonia are referred to as total ammonia nitrogen (TAN). Ammonia is toxic to fish (Russo and Thurston 1991) and crustaceans (Chin and Chen 1987).

Ammonia

Ammonia is primarily present as NH_4^+ and NH_4OH. Undissociated NH_4OH is highly toxic to many organisms especially fish (Trussell 1972). Ammonia in aquaculture system is mainly associated with the type and quantity of organic wastes such as feed, fertilizer, metabolic wastes and decaying matters in the culture system. Feed is one of the primary sources of nitrogen and comprises of proteins from fish meal, bone or soybean. Some farms use trash fish, chicken offal and kitchen wastes as feed. Ammonia is produced when these proteins are metabolized. In addition, fish produces ammonia due to catabolism of amino acids in the liver (Ip *et al.* 2001) and is usually excreted as ammonia through the gills (Wilkie 2002; Evans *et al.* 2005). Crustaceans being ammonotelic excrete ammonia as their chief metabolic/excretory product which is present in aquatic environment as unionized (NH_3) and ionized forms (NH_4^+) (Hanstein 1970). Though ammonia is utilized by phytoplankton and ammonia oxidizing bacteria, it may also escape through other pathways like evaporation and physical agitation. The concentration of ionized ammonia (NH_4^+) which is nontoxic and the unionized ammonia (NH_3) which is toxic is primarily dependent upon temperature and pH of water (Emerson *et al.* 1975; Whitefield 1978). The higher the water temperature and pH, the greater is the concentration of the toxic form. Monitoring of ammonia should be done regularly during the culture cycle since it can be a serious cause of fish mortality and low production. Fish and other cultured organisms in poor quality water are stressful and are vulnerable to diseases (Shishehchian *et al.* 1999).

Toxic effects of ammonia

Ammonia-N is toxic at concentrations above 1.5 mg N/L to commercially cultured fish. However, 0.0125 mg/L is the acceptable level of unionized ammonia nitrogen in aquaculture systems (Colt and Armstrong 1981). Excess ammonia causes many problems. According to Colt and Armstrong (1981), as ammonia level increases in water, excretion rate in fish and shellfish decreases. Low ammonia excretion rate in organisms causes increase in the level of ammonia in blood and tissue. Consequently blood pH rises and causes related adverse metabolic effects on enzyme catalyzed reactions and membrane stability. It also decreases O_2 consumption by tissues, damages gills and reduces the ability of blood to transport oxygen. Chen *et al.* (1991) observed changes in the kidney, spleen, thyroid and blood in shrimp exposed to sublethal concentration of ammonia. All these changes cause stress to the fish resulting in susceptibility of fish to infectious agents. Ammonia toxicity in shrimp farm usually causes reduced growth rate but extreme concentrations may cause mortality (Wickins 1976; Armstrong *et al.* 1978; Jayashankar and Muthu 1983; Chin and Chen 1987; Chen and Chin 1988). Studies done by Fouzi (2008) showed 5.1% prevalence of white spot syndrome virus (WSSV) in seven shrimp species caught in the wild from Malaysia was significantly correlated with total ammonia-N and nitrite.

Nitrite

Nitrite is another nitrogenous compound that is toxic for cultured aquatic organisms. It is an intermediate product of the conversion of ammonia to nitrate by bacterial action and is a natural component of the nitrogen cycle in ecosystem. However, nitrite is a very unstable compound and is readily oxidized to nitrate in the presence of oxygen or reduced to ammonia in anoxic condition. Since the conversion of nitrite to nitrate is accomplished by certain bacteria, environmental conditions that affect bacterial growth and metabolism can impact the rate of conversion and therefore the concentration of nitrite. High concentrations of nitrite can cause problems in intensive culture of commercial fish species and ornamental fishes (Dvorak 2004; Svobodova *et al.* 2005) and in intensive fish and shellfish culture (Chen *et al.* 1991; Boyd 1992; Alcaraz and Espina 1995).

Toxic effects of nitrite

The toxic effects of nitrite vary between species and life stages and are well documented (Lewis and Morris 1986; Jensen 2003). Haywood (1983) showed that acute toxicity can occur at 0.2 mg/L NH_3 in salmonids. Studies by Yusoff *et al.* (1998) showed that *Puntius gonionotus* (Bleeker) fish fry grown at 2 mg/L NO_2-N had significantly lower growth rate and when exposed to 4 mg/L NO_2-N at pH 5 had 100% mortality after 48 h. When absorbed by fish, nitrite reacts with hemoglobin to form methemoglobin, which impairs the oxygen carrying capacity rate causing hypoxia and cyanosis leading to stress and consequent mortality which is known as brown blood syndrome (Colt and Armstrong 1981). This effect is most easily seen in the gills. Nitrite also accumulates in other tissues such as liver, brain and muscle (Margiocco *et al.* 1983). Over longer periods of exposure to nitrite, fish can become anemic.

Nitrate

Nitrate is an important component of nitrogen cycle. It is the final product in the nitrification process and is considered relatively less toxic to fish. For fish culture, 50 mg N/L is generally accepted safe limit for nitrate nitrogen (Gutierrez-Wing and Malone 2006). The acceptable level of nitrate for seawater culture is generally considered to be less than 20 mg/L nitrate–N (Spotte 1979).

Toxic effects of nitrate

The lethal and sub-lethal effects of high concentrations of nitrates in some marine and freshwater organisms are well known. Excess nitrates can slow down growth and retard development, increase susceptibility to diseases, cause low fertility and decrease survival rate. Nitrate concentrations above 30 mg N/L have been associated to marine white spot disease (Burgess 1995). Wickins (1976) reported that the 48-h LC_{50} was 3400 mg/L for nitrate–N in seven species of penaeid shrimps. In the case of *P. monodon* zoea, Muir *et al.* (1991) reported that the survival is affected at a concentration of 0.225 mg/L nitrate–N (1 mg/L nitrate) when exposed for 40 h. In Medaka (*Oryzias latipes*) 100 mg N/L has been shown to be lethal when exposed during adult and growing phases. At a concentration of 75 mg N/L, fertilization and hatching rates are reduced in addition to a decreased growth rate of juveniles. There is retardation in spawning at 50 mg N/L in fish exposed at the juvenile phase (Gutierrez-Wing and Malone 2006).

FACTORS AFFECTING THE PRODUCTION OF NITROGENOUS COMPOUNDS

A number of water quality parameters including physical, biological and chemical factors affect the survival, growth and reproduction of aquatic organisms. pH levels in freshwater ponds can fluctuate between 6.6 to 10.2. This is due to carbon dioxide removal because of photosynthesis during daytime causing an increase in pH level, and the release of carbon dioxide during night resulting in decrease of pH level (Boyd 1990). Nitrification is most rapid at pH 7-8 and at temperatures of 25-35°C. According to Chen and Kou (1996), ammonia excretion and total nitrogen excretion in *Macrobrachium rosenbergii* decreased with increased pH level, whereas urea, nitrite and nitrate excretions increased with increased pH level. At high pH levels, excretions of urea, nitrite and nitrate may be related to the detoxification of ammonia. Temperature also affects respiratory metabolism and ammonia excretion of marine invertebrates. Chen and Kou (1996) found that the proportion of ammonia excretion to total nitrogen excreted by prawns was inversely related to temperature, whereas the proportion of urea excretion to total nitrogen excreted increased directly with temperature. Ammonia, urea, organic and total nitrogen excretions of juvenile *M. rosenbergii* increased directly with temperature in the range of 17-32°C. No urea excretion was found at 17°C.

According to a review by Regnault (1987), certain intrinsic and extrinsic factors such as molt cycle, nutritional status and size, temperature, salinity and ambient ammonia affects ammonia-N excretion. Studies have shown the existence of an inverse relationship between ammonia-N excretion and salinity in the shore crab *Carcinus maenas* (Haberfield *et al.* 1975), blue crab *Callinectes sapidus* (Mangum *et al.* 1976), *M. japonicus* (Chen and Chen 1996), tiger shrimp *Penaeus monodon* (Chen *et al.* 1994) and mud crab *Scylla serrata* (Chen and Chia 1996). Their studies showed that ammonia excretion increases when animals are hyperregulating and decreases when they are hyporegulating.

BIOLOGICAL MANAGEMENT OF NITROGENOUS COMPOUNDS IN AQUACULTURE SYSTEMS

Availability of high quality water is one of the most important factors for successful aquaculture. Various measures can be taken to regulate the environmental conditions so that water quality remains within an optimum range for improved growth and survival of stocked culture organisms. Physical, chemical and biological methods are used to treat water in aquaculture systems. Physical methods like sedimentation and filtration are simple, inexpensive and are used as pretreatment of water. Physical methods have limiting effects in removing nitrogenous substances. Chemical methods such as neutralization, coagulation, sterilization and oxidation are expensive and have some toxic effects. On the other hand, biological methods are regarded as the most promising treatment technology and are widely used to minimize toxic nitrogenous compounds in aquaculture systems. A few bacteria such as *Bacillus*, *Pseudomonas*, *Acinetobacter*, *Cellulomonas*, *Rhodopseudomonas*, *Nitrosomonas* and *Nitrobacter* have been recognised as beneficial in converting hazardous organic wastes into environmentally safe compounds (Thomas *et al.* 1992; Rao *et al.* 1997). Even macroorganisms like *Ulva lactuca*, mussels and echinoderms can also be used. A number of biological products including enzyme preparation, plant extracts and yeast extracts are used in aquaculture ponds for improving water quality (Boyd and Gross 1998). **Table 1** shows examples of organisms/systems involved in the biological management of nitrogenous compounds in aquaculture.

Organisms involved in biological management of nitrogenous compounds

1. Bacteria

There is increasing evidence that bacteria play an important role in the regeneration and consumption of dissolved nutrients in the water column. There are several reports on the participation of heterotrophic bacteria in nitrogen removal and have been reported to nitrify many types of nitrogen compounds. Bacteria use nitrogen oxides as a source of oxygen for their respiration in denitrification process. However, denitrification activity is lower than those of autotrophs. Facultative anaerobes have also been reported in denitrification where they use free oxide in the absence of free oxygen. Mevel and Prieur (2000) have reported heterotrophic nitrification by *Bacillus* strains. Studies by Jawahar Abraham *et al.* (2004) showed that a mixture of *Nitrosomonas* sp. and *Bacillus* sp. was the most efficient in removing 96% total ammoniacal nitrogen in microcosm experiments. In an another study, Ghosh *et al.* (2007) found that *Bacillus subtilis* isolated from the intestine of *Cirrhinus mrigala* (Hamilton) when incorporated in the rearing water of livebearing ornamental fishes significantly lowered total ammonia nitrogen concentration.

Table 1 Different organisms/technologies used in biological management of nitrogenous compounds.

Organisms/technologies	Reduction/uptake	References
Bacteria – *Nitrosomonas* and *Bacillus*	96% TAN	Jawahar Abraham *et al.* 2004
Fungus – *Aspergillus niger*	25 mg TAN/L	Hwang *et al.* 2007
Fungus – *Penicillium*	0.72 mg TAN/L	Karim 2008
Macrophyte – *Elodea densa*	0.2 mg NH_4-N/L; 0.4 mg NO_2-N/L	Corpron and Armstrong 1983
Biofilter	3.46 g TAN/m^3/day; 0.77 g NO_2/m^3/day	Al-Hafedh *et al.* 2003
Trickling filter	0.24-0.55 g TAN/m^2/day	Kamstra *et al.* 1998
	0.64 g TAN/m^2/day	Lyssenko and Wheaton 2006
Microbead filter	0.45-0.60 g TAN/m^2/day	Greiner and Timmons 1998
	0.30 g TAN/m^2/day	Timmons *et al.* 2006
Fluidized bed reactor	0.24 g N/m^2/day	Miller and Libey 1985
Ion exchange membrane bioreactor (IEMB)	90% NO_3-N	Matos *et al.* 2009
Seaweed – *Ulva lactuca*	49-56% mean NH_3-N	Cohen and Neori 1991
Seaweed – *Ulva pertusa*	0.45 g N/m^2/day	Wang *et al.* 2007
Periphytic – cyanobacteria	91% TAN; 91% NO_2-N	Khatoon *et al.* 2007
Periphytic – diatoms	62% TAN; 82% NO_2-N	Khatoon *et al.* 2007
Periphyton	0.56 mg TAN/L	Azim *et al.* 2002
AquaMats®	0.22 g ammonia/m^2/day	Verdegem *et al.* 2005
Biofilms	0.42 µg ammonia/L	Ramesh *et al.* 1999
Algal Turf Scrubber™	0.2 mg ammonia/L	Craggs *et al.* 1996
Immobilized nitrifying bacteria	4.2–6.7 mg TAN/L/day	Shan and Obbard 2001

2. Fungi

Compared to bacteria and microalgae, very few studies have been done on fungi with regards to ammonia removal in aquaculture. This may be due to the fact that bacteria are easy to culture and are able to metabolize/mineralize contaminants better when compared to fungi. Experiments done by Hwang *et al.* (2007) showed that the use of *Aspergillus niger* NBG5 removed 25 mg/L total ammonia nitrogen from artificial wastewater within 35 h in a continuously stirred tank reactor. In another experiment by Karim (2008), use of *Penicillium* spp. in combination with *Bacillus* sp. was found to significantly reduce total ammonia nitrogen concentration in *P. monodon* shrimp postlarvae culture. In addition, the postlarvae showed better survival and higher stress tolerance in the treatment tanks compared to the control.

3. Algae

Algae have been a subject of research and development for a few decades in the treatment of municipal wastewater. Use of algae in treating wastewater is less expensive, ecologically safe, efficiently removes nutrients and is comparable to other conventional technologies (de la Noüe *et al.* 1992). According to Chopin *et al.* (2001) and Neori *et al.* (2004), marine microalgae can effectively absorb nutrients from aquaculture effluent. Studies by Gordin *et al.* (1981) and Krom and Neori (1989) have shown that the use of phytoplankton reduce excess dissolved inorganic nutrients from fish effluents. Algae also disinfect the effluent by converting light to heat, which increase the water temperature leading to a decrease of enteric bacteria (Pharhad 1970 as cited in Day *et al.* 1999). However, the phytoplanktons are subject to uncontrollable blooms that are likely to cause changes in water quality, particularly in terms of ammonia and dissolved oxygen (Krom *et al.* 1985).

In mariculture systems, treating of effluents with macroalgae started in the mid 1970s (Haines 1975; Ryther *et al.* 1975; Roels *et al.* 1976; Langton *et al.* 1977; Harlin *et al.* 1979). Macroalgae (seaweeds) can be adapted to cage, tank or pond-based systems due to their adaptability to grow on a variety of substrata and culture (Buschmann *et al.* 1996; Nagler *et al.* 2003; Matos *et al.* 2006). Seaweed that has been used as biofilters include red algae of the genus *Gracilaria* (Gracilariae, Rhodophyta), *G. parvispora* (Nelson *et al.* 2001), *G. edulis* (Gmelin) (Kaladharan *et al.* 1996; Jones *et al.* 2001), *G. tikvahiae* (McLachlan) (Kinne *et al.* 2001), *G. chilensis* (Buschmann *et al.* 2001) and *G. lemaneiformis* (Zhou *et al.* 2006). In addition, *Porphyra* and *Ulva* species have also been used as biofilters (Msuya *et al.* 2006; Blouin *et al.* 2007). De Boer and Ryther (1977) and Fralick (1979) used seaweeds as biofilters to remove dissolved nitrogen from fish pond effluents. Harlin *et al.* (1979) used *Gracilaria* sp. to remove the ammonium produced by the fish *Fundulus heteroclitus*. Similar results were reported by Haglund and Pedersen (1993) in a system using *Gracilaria tenuistipitata*. The use of *Ulva lactuca* in an integrated system of gilthead seabream (*Sparus aurata*) was reported by Vandermeulen and Gordin (1990). They found that *U. lactuca* efficiently removed 85% of total ammonia from fish-pond effluent and found that it was a viable cost-effective way of removing nutrients. Jiménez del Río *et al.* (1994, 1996) also reported that *Ulva* spp. had a higher nitrogen removal capacity than *Gracilaria* spp. In addition, *Ulva* spp. had a higher resistance to epiphytes. Lobban and Harrison (1997) reported that nutrients can be concentrated by macroalgae by a factor of up to 10^5 over seawater levels. In Chile, the integration of *Gracilaria* into salmon aquaculture reduced the release of nitrogen (N) by 56% (Kautsky *et al.* 1996; Troell *et al.* 1997). *Porphyra* is an efficient bioremediator agent due to its morphology (Neori *et al.* 2004). The thin blade of the gametophyte is composed of 1 or 2 cell layers, with all cells involved in nutrient absorption. *Porphyra* has high surface area-to-volume ratio and the coupling between ambient nutrient levels and internal pool is tight, thus enabling a rapid response to environmental nutrient availability (Neori *et al.* 2004). The rate of mass removal of nitrogen equals to the growth rate multiplied by tissue nutrient concentration. Hence, growth rate and tissue nutrient concentration are important for evaluation of the bioremediatory potential. In fact, *Porphyra* is one of the most effective nutrient sequesters among the seaweeds. In addition, the high productivity due to efficient nutrient accumulation in this seaweed makes polyculture systems valuable for the abatement of coastal nutrient loading by finfish aquaculture, while also providing a potentially valuable product upon harvest (Chung *et al.* 2002). Notably, the rate of nutrient uptake by *P. amplissima* can be compared to that of *P. yezoensis*, which is an important species cultivated in Asia. *Porphyra amplissima* can absorb both forms of inorganic nitrogen simultaneously, removing NH^{+4} six times faster than NO^{-3} even when NO^{-3} supply exceeds. *Agardhiella subulata* and *Gracilaria tikvaiae* grew faster with NH^{4+} as nitrogen source (De Boer *et al.* 1978). According to Marinho-Soriano *et al.* (2009), *Gracilaria birdiae* act as a biofilter for efficient removal of nutrients from shrimp pond effluents in addition to production of useful algal biomass. Their study showed that the concentration of NH_4^+ decreased by 34%, NO_3^- by 100% and PO_4^{3-} by 93.5% after a 4-week experiment. In another study by Carmona et al. (2006), *Porphyra amplissima* in an integrated finfish–algal aquaculture system removed 70–100% of N and 35–91% inorganic phosphorus within 3–4 days.

4. Macrophytes

The capability of aquatic plants to remove nutrients and their high productivity has created lot of interest for water treatment. Plants remove ammonia and phosphate from water while using sunlight for photosynthesis to produce organic compounds. Floating plants such as water hyacinth (*Eichhornia crassipes*), duckweed (*Lemna* spp.), pennywort (*Hydrocotyle umbellata*), salvinia (*Salvinia rotundifolia*), water lettuce (*Pistia stratiotes*) and azolla (*Azolla* spp.) have been evaluated for nutrient removal from wastewaters (Reddy and Smith 1987). Many other macrophytes such as *Typha latifolia*, *Iris seudacorus*, *Scirpus* sp., and *Glyceria* sp. have also been studied. Studies by Corpron and Armstrong (1983) have shown that the use of *Elodea densa* as a submerged aquatic plant in recirculating *M. rosenbergii* culture system had lower amount of NH_4-N and NO_2-N (0.2 and 0.4 mg/L) compared to the system without the plant (4.0, 5.7 mg/L).

Technologies for biological management of nitrogenous compounds

The use of micro or macroorganisms in aquaculture systems to reduce the hazardous organic wastes to environmentally safe levels can be termed as 'bioremediation'. Micro or microorganism(s) and/or their products when used as water additives to improve water quality, are referred to as bioremediators or bioremediating agents in aquaculture ponds (Moriarty 1998). Bioremediators can comprise of bacteria, fungi, micro and macroalgae and their products like enzymes and metabolites. Bioremediation is a biotechnological tool which has been recently introduced in the aquaculture sector (Moriarty 1996). Examples of organisms/systems involved in the biological management of nitrogenous compounds in aquaculture are given in **Table 1**. Most of the technologies for biological management of nitrogenous compounds encompass the principles of bioremediation which are described below:

1. Biofilms

According to Decho (1990) and Meyer-Reil (1994), biofilms are microbial consortium associated with a matrix of extracellular polymeric substances and are bound to any submersed surfaces. Biofilms are responsible for many biogeochemical cycles especially nitrogen cycling in aquatic ecosystems. In freshwater ponds, biofilms grown on introduced substrates helped to improve water quality, health of cultured organisms and fish production (Shankar *et al.* 1998; Shankar and Mohan 2001). In addition, Thompson *et al.* (2002) found that in intensive shrimp culture ponds, a mature biofilm was able to maintain ammonium at low levels and it was mainly composed of pennate diatoms (*Amphora*, *Campylopyxis*, *Navicula*, *Synedra*, *Hantzschia* and *Cylindrotheca*) and filamentous cyanobacteria. Thus, biofilm has the potential for use in hatchery to improve water quality and larval production.

Periphyton is often referred to as biofilms which comprises of bacteria, fungi, protozoa, planktons and benthic organisms attached to the substratum. Bush *et al.* (1963) were the first to report on 100% removal of nitrogen using attached microalgae from sewage. The different microalgal genera commonly found in such biofilms are *Oscillatoria*, *Navicula*, *Nitzschia*, *Scenedesmus*, *Stigeoclonium* and *Phormidium*. Bender and Phillips (1995) and Zachleder *et al.* (2002) reported that mixed microbial mats consisting of filamentous cyanobacteria (*Oscillatoria* sp.) as the dominant species are efficient in removing nitrogenous compounds and other toxic chemicals from polluted sites. Algal Turf Scrubber^{TM} (ATS) (Adey and Loveland 1998; Craggs *et al.* 1996) uses similar concept to that of microbial mats. The ATS^{TM} is based on naturally occurring bacteria, microalgae and filamentous algae. Laboratory experiments showed that the mats rapidly removed ammonia from 4.1 to 0.2 mg/L as a result of nitrifying bacteria present at the mat/substrate interface (Bender and Phillips 2004).

To avoid the risks of developing harmful organisms as found in natural conditions, biofilms can be developed with selected periphyton species. Experiments by Khatoon *et al.* (2007) using selected cyanobacteria and diatoms coated substrates reported lowest concentrations of total ammonia nitrogen (TAN, 0.03 ± 0.0 mg/L) and nitrite–nitrogen (NO_2–N, 0.01 ± 0.0 mg/L) in *P. monodon* postlarvae rearing system. Two systems have been developed and filed for patent by Yusoff *et al.* (2006) and Shariff *et al.* (2009) in which a consortium of microorganisms grown on substrates have been used for improving water quality by maintaining low levels of total ammonia nitrogen, nitrite nitrogen, soluble reactive phosphorous and pathogenic bacteria. In addition highly nutritive diatoms provide natural food, increase growth and survival of larvae in the *P. monodon* hatchery system. In a freshwater hatchery, Verdegem *et al.* (2005) reported an average removal of ammonia using biofilms grown on bamboo and AquaMats® were 0.69 and 0.22 g/m^2/day, respectively. Ramesh *et al.* (1999) also reported that biofilm improves water quality in freshwater fish ponds by lowering ammonia concentration (0.42 μg/L). According to Azim *et al.* (2002), the average total ammonia concentration in periphyton-based freshwater aquaculture ponds (0.56 mg/L) was significantly lower than the 0.95 mg/L observed in substrate-free ponds.

Bacterial biofilters consist of bacterial biofilms grown on substrates in enclosed system to remove ammonia and harmful nitrogenous compounds. Bacterial biofilters use specific bacterial colonies for conversion of ammonia to nitrate for both freshwater as well as marine systems. Biofilters such as submerged, trickling, biodrums and fluidized beds are commonly used in hatcheries, nurseries, ornamental and commodity fish culturing systems. Microbial populations in biofilters are stable and independent of light conditions compared to algae. Different substrates such as gravel, sand, plastic, polyvinyl chloride (PVC) pipe, polystyrene, wood chips, wheat straw and microbeads have been used in biofilters. Experiment by Al-Hafedh *et al.* (2003) showed that plastic roll as a biofilter medium performed better in removing total ammonia nitrogen compared to PVC pipes and plastic scrub pads. Removal rates of 3.46 g TAN/m^3/day and 0.77 g NO_2-N/m^3/day, as well as TAN and NO_2-N removal efficiencies of 29.37 and 27.3% respectively were achieved using this plastic roll biofilter. Saliling *et al.* (2007) showed that wood chips were found to be better than straw based on rate of mass loss. Liao and Mayo (1974) were the first to report on the use of trickling filters in salmonid hatchery. Miller and Libey (1985) demonstrated that rotating biological contactor had total ammonia nitrogen areal removal rate of 0.19–0.79 g TAN/m^2/day. Studies by Brazil (2006) showed that rotating biological contactor installed in a recirculating aquaculture system rearing tilapia had total ammonia nitrogen areal removal rate of 0.43 ± 0.16 g/m^2/day. In another experiment using commercial scale trickling filter, Kamstra *et al.* (1998) demonstrated the total ammonia nitrogen areal removal rates between 0.24–0.55 g TAN/m^2/day. Lyssenko and Wheaton (2006) reported total ammonia nitrogen areal removal rates of 0.64 g TAN/m^2/day. Experiments done by Greiner and Timmons (1998) using microbead filters observed total ammonia nitrogen areal removal rates of 0.45–0.60 g/m^2/day whereas Timmons *et al.* (2006) using a commercial microbead filter system reported an average total ammonia nitrogen areal removal rate of 0.30 g/m^2/day. Studies done by Miller and Libey (1985) found that the total ammonia nitrogen removal rate was 0.24 g N/m^2/day using fluidized bed reactor. The application of the ion exchange membrane bioreactor (IEMB) concept for removing nitrate from aquaculture tanks or marine aquariums has been investigated by Matos *et al.* (2009). The IEMB system removed nitrate to concentrations below 27 mg/l from initial concentrations of 251 and 380 mg/l. De Schryver and Verstraete (2009) showed that sequencing batch reactors (SBRs) dosed with glycerol or acetate, nitrogen removal efficiency reached up to 98% in lab-scale studies.

Bacterial biofilter technologies are technically effective but their performance at the large production scale has not been studied extensively (Guerdat *et al.* 2010). Most of the evaluation of bacterial biofilters has been done at the laboratory scale (Eding *et al.* 2006). In commercial scale systems, TAN and organic carbon concentrations are different from laboratory scale studies. Therefore, TAN removal rates between laboratory scale studies and actual production conditions are needed. Moreover, its use is not widespread because the system is relatively complex (Shpigel 2005) and may not be cost effective. Besides, nitrifying bacteria are sensitive to high concentrations of ammonia and nitrous acid, low dissolved oxygen levels and pH (Masser *et al.* 1999; Villaverde *et al.* 2000; Ling and Chen 2005). Nitrification by the bacterial biofilm in the biofilter is also affected due to type of substrate, dissolved oxygen concentrations, organic matter, temperature, pH, alkalinity and salinity (Satoh *et al.* 2000; Chen *et al.* 2006). Effective and environment friendly biofilter can be designed taking into consideration their ammonia sequestering capacity, ease of use as well as their effects on the sustainability of the aquaculture system.

2. Macrobiofilters

Shellfish are highly efficient filter feeders and can directly remove particulate matter, reduce turbidity, and remove nitrogen and other nutrients from the water. The use of shellfish in biofiltration systems is also an inexpensive option for the biological removal of toxic nitrogenous compounds from aquaculture effluent water and is one of the best candidates for ecologically sustainable aquaculture. Oysters, mussels, clams acts as natural biofilters and are effective in reducing the small particles from the effluent (Wang 1990; Hopkins *et al.* 1993). According to Shumway *et al.* (2003), 100 kg of nitrogen per year from the environment can be removed by harvesting 10,000 oysters containing 13.6 kg of nitrogen and 1.4 kg of phosphate. The organic particles ingested by the oysters are incorporated into the tissue and captured waste nutrients are converted into secondary cash crops. Experiments conducted by Jones *et al.* (2001) found that total nitrogen and phosphorus concentrations were reduced to 66 and 56%, respectively in a flow through system using Australian native oysters *Saccostrea commercialis*. They also reported that oysters are more effective in a flow-through system compared to filtering effluent in still water (no flow-through). In systems using shellfish as biofilters, flow rate needs to be optimized for optimum particulate filtration by shellfish.

Fed aquaculture, where diet consists of formulated feed and trash fish, discharges heavy nutrient loads into coastal waters, e.g., 35 kg N and 7 kg per ton of cultured fish (Chopin *et al.* 2001). To overcome the problem of high nutrient discharge, farming of fish and seaweed in an integrated system has been developed. Seaweeds are able to remove 90% of the nutrient discharge from fish farms. In 1976, Tenore first reported the successful polyculture of *Ulva* sp. and abalone together. Hughes-Games (1977) and Gordin *et al.* (1981) first described the integrated cultures of marine fish and shellfish with phytoplankton. Since then, several researches have focused on polyculture and integrated mariculture system in which two to three species are usually included in the system. Some researchers integrated macroalgae and fish in their systems (McDonald 1987; Cohen and Neori 1991; Chopin *et al.* 2001; Schuenhoff *et al.* 2003; Neori *et al.* 2004) while others used fish, shrimp and oysters (Wang 1990; Qian *et al.* 1999).

Several studies have also been done by different researchers in which they combined seaweed culture with land-based fish tanks or open sea fish cages e.g., Troell *et al.* (1999), Neori *et al.* (2000), Buschmann *et al.* (2001), Chopin *et al.* (2001), and Hernández *et al.* (2002). Cohen and Neori (1991) reported mean ammonia-N removal rate of 49–56% when using *Ulva lactuca* to treat marine fishpond effluents. Wang *et al.* (2007) using *Ulva pertusa* as biofilter in the production of juvenile sea cucumber (*Apostichopus japonicus*) demonstrated that *U. pertusa* tank removed 68% of total ammonia nitrogen. The average removal rate of ammonia was 0.459 g N/m^2/day. According to Troell *et al.* (2009) integrated multi-trophic aquaculture (IMTA) system combine fed aquaculture species (e.g. finfish), with inorganic extractive aquaculture species (e.g. seaweeds) and organic extractive species (e.g. suspension- and deposit-feeders). It increase significantly the sustainability of aquaculture, based on a number of potential economic, societal and environmental benefits, including the recycling of waste nutrients from higher trophic-level species into production of lower trophic-level crops of commercial value.

Systems of integrated aquaculture are ideal because the N and P in the animal effluent are necessary requirements for the growth of the seaweeds. Therefore, in integrated systems, the selection of suitable macroalgae, microalgae and shellfish species are important. The species cultured should be able to grow throughout the year and are of economic importance.

3. Immobilization

One of the major problems in bioremediation using algae is the separation or harvesting of the algae biomass from the treated water before discharge. Several methods such as filtration, centrifugation and flocculation have been tried but they are either expensive or not effective. Therefore, immobilization as an alternative method has been investigated. Immobilization involves the fixation of cells into a solid matrix or being retained by a membrane so that their stability is increased. The advantages are faster removal rates of nutrients and cell-free effluent (Hoffmann 1998). Some of the microalgae used for immobilization are *Anabaena*, *Chlorella*, *Chlamydomonas*, *Phormidium*, *Spirulina*, and *Scenedesmus*. Alginate, carrageenan and chitosan are the popular polymers used for immobilization. Some other polymers used for immobilization are collagen, agar, agarose, cellulose, polyurethane, polyvinyl and acrylamide.

In 1985, Chevalier and de la Noüe reported on the use of carrageenan immobilized *Scenedesmus obliquus* for treatment of wastewater and showed a higher uptake rate of NH_4-N. Rai and Mallick (1992) found that using immobilized *Anabaena doliolum* and *Chlorella vulgaris*, the uptake rate for NH_4^+ was 24 and 18% higher, respectively than the free living cells. In another experiment, Vílchez and Vega (1994) used *Chlamydomonas reinhardtii* immobilized in calcium alginate and showed optimum nitrite uptake rate of 14 µmol/mg Chl/h. Studies by Tam and Wong (2000) showed that *Chlorella* immobilized in Ca-alginate and used in a transparent PVC column showed higher nitrate removal rate. Studies have shown nitrogen removal efficiencies of more than 90% over a period of 24 h from wastewater by immobilized algae at different temperatures, pH and light levels (Hoffmann 1998). Poly (allyl amine hydrochloride) (PAA·HCl) polymer hydrogels have also been found to remove efficiently nitrate (NO_3^-), nitrite (NO_2^-) and orthophosphate (PO_4^{3-}) nutrient anions from the aquaculture wastewater. Kioussis *et al.* (2000) reported that the hydrogels are suitable materials in treating aquaculture wastewater effluents and are able to reduce the nutrient anion concentrations to less than 10 mg/l NO_3–N, 0.08 mg/l NO_2–N and 0.3 mg/l PO_4–P.

Apart from algae, bacteria has also been immobilized and used in aquaculture system to reduce ammonia. Shan and Obbard (2001) reported an innovative and economical *in situ* treatment technology using indigenous nitrifying bacteria immobilized onto porous clay pellets for the removal of total ammonia from marine prawn aquaculture water. The total ammonia nitrogen concentrations were maintained at 0.2 mg/L under fed-batch culture conditions. The technology has distinct advantages over conventional water quality control methods that are associated with high capital costs. However, for large scale application the cost of using immobilized microorganisms is expensive. Research is ongoing to find better and inexpensive polymers.

4. Bioflocs

Aquaculture using biofloc technology also offers a solution for a better water quality for the aquaculture industry. In this technology bacterial community is established and maintained in the system. The bacteria accumulate in clumps, also known as flocs. The floc serves as a food source for finfish and shellfish and is also important for nutrient recycling (Serfling 2006). It removes nitrogenous substances from the water with the production of microbial biomass. The development of this material consists mainly of bacteria, algae, fungi and detritus (Burford *et al.* 2004; Holl *et al.* 2006; Serfling 2006) that control levels of ammonia and nitrite. According to Hargreaves (2006), the nitrogen uptake by bacterial growth rapidly decreases the ammonium concentration faster than nitrification.

CONCLUSION

Biological management of nitrogenous compounds in aquaculture using different technologies is important to maintain the water quality which is an essential feature of successful aquaculture. This approach based on the principles of bioremediation is dependent on the environmental conditions and the microorganisms present. Biofilters, biofilms, immobilization, bioflocs technologies and integrated systems are effective in removing nitrogenous compounds in aquaculture systems. Periphyton, bioflocs and integrated systems are sustainable methods and serve the dual purpose of lowering nitrogenous compounds in aquaculture system as well as providing nutrition to cultured organisms. These systems also help in reducing the input of feed which constitute about 50% of production cost. However, additional research in biological management of nitrogenous wastes using different systems is needed especially on the selection of bacteria, microalgae or macroalgae species and study their efficacy on absorbing toxic nitrogenous compounds as well as survival and growth of the target cultured organisms.

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