Organic matter decomposition in simulated aquaculture ponds

Beatriz Torres Beristain

Organic matter decomposition in simulated aquaculture ponds

Beatriz Torres Beristain

Proefschrift

Ter verkrijging van de graad van doctor
Op gezag van de rector magnificus
van Wageningen Universiteit,
Prof. Dr. Ir. L. Speelman,
In het openbaar te verdedigen
Op dinsdag 15 April 2005
des namiddags te half twee in de Aula

A Domingo, Yolanda y Alejandro

Table of contents

General introduction

Global aquaculture production doubled during the last decade, making it the fastest growing animal food production sector in the world (FAO, 2002). Ponds are the most commonly used aquaculture production system accounting for about 40% of the world production. A vast majority of the farmed freshwater fishes and nearly all farmed crustaceans are cultured in ponds. For simplicity, in this document, we will refer to all organisms cultured in ponds as 'fish'. In intensive ponds, organic matter will accumulate during the culture cycle due to the administration of high levels of external inputs (mainly fertilizers and feeds). Aquaculture ponds are complex ecosystems, which will only produce the targeted fish production if nutrient cycling and waste decomposition are properly managed. This is not easy to accomplish.

Aquaculture ponds among aquatic systems

Aquaculture ponds share physical, (gas balance, sedimentation-resuspension, water circulation, dilution), chemical (pH, organic matter decomposition) and ecological (food web structure, prey-predator relationship) characteristics with other aquatic ecosystems. Some key physico-chemical parameters and the amount of fish produced in organically loaded water bodies are shown in Table 1.

Table 1. Comparison of physico-chemical parameters and fish production of aquatic systems.

	Eutrophic shallow lake[2]	Extensive Aquaculture ponds[2]	Intensive outdoor ponds	Oxidation Pond
Chla µg L^{-1}	47 ± 25	68.35 ± 7.42	257 ± 18	1929 ±1675
TSS (mg L^{-1})	13 ± 8	--	139 ±18	164 ±122
PO_4-P (mg L^{-1})	-	1.48 ± 0.67	-	8.7 ± 4.2
NH_3-N (mg L^{-1})	-	0.70 ± 0.08	0.99 ±0.11	6.9 ±4.8
NO_3-N (mg L-1)	-	4.20 ± 0.34	0.64 ±0.07	1.3 ± 2.0
COD (mg L^{-1})	8.2 ± 2	-	-	467 ±255
Secchi (cm)	88 ±43	37.58 ± 3.23	-	-
Trophic state index (TSI)[3]	68	72	85	105
Fish production (kg ha $^{-1)}$	133	5000	102204 (±58163)	325
Reference	(ILEC, 1986)	(Azim *et al.*, 2001)	(Milstein, 1990)	(Wrigley *et al.*, 1988)

[1]Total fish production per year 8,745 (lake area= 22,000 ha and calculated per 4 months production)
[2]Average pond values of Rohu culture with no substrate
[3]TSI based on chlorophyll *a* = 9.81 ln(CHL-a) + 30.6 (Iwashita *et al.*, 2004)

The trophic state index (TSI) developed by Carlson (1977) classifies a water body according to the organic load using chlorophyll-a concentration, total phosphorous concentration, or

Secchi disk transparency. The TSI values given in Table 1 were calculated based on chlorophyll *a* concentration (Carlson, 1977; Iwashita *et al*., 2004). The shallow lake was classified as eutrophic (TSI: 60-69) while the other systems (aquaculture and oxidation ponds) as hyper-eutrophic ponds (TSI: 70-100) (Iwashita *et al*., 2004). Eutrophic and hyper-eutrophic systems are very variable environments sharing characteristics like dense algae blooms dominated by toxic or inedible algal species, shifts between anoxia and oxygen supersaturation, high nutrients concentration, thermocline development and light limitation (Sommaruga and Robarts, 1997). In shallow eutrophic and hyper-eutrophic lakes dominated by phytoplankton, a turbid state prevails contrary to the clear water state, dominated by macrophytes, that only exists in oligotrophic lakes (Scheffer *et al*., 1993). Aquaculture ponds are deliberately fertilized with organic manures, chemical fertilizers or agricultural wastes. Overall fish yield increases as primary productivity increases, but the nutrient and energy transfer efficiency declines at the highest eutrophication levels due to the stressful and variable environment generated at high eutrophication levels (Vadeboncoeur, 2002). In Table 1, the intensive aquaculture outdoor ponds produced a very high fish yield in comparison with the other three systems. This high production was a result of the management practices applied: daily artificial feed input (30% protein content), water exchange (1.7 volumes per day), removal of sediments and artificial aeration (paddle-wheel and airlifts) (Milstein, 1990). The tremendous high fish yield obtained in this intensive system was linked to discharge of high amounts of nutrients and high energy consumption. Valuable information about nutrient cycling and food web structure from natural systems can help to diminish the energy and nutrients use in aquaculture ponds. The knowledge generated through aquaculture systems about water quality, fish biomass production, and fish health linked to management practices (e.g. aeration, water exchange, organic load, chemical and biological products addition), may will contribute to eutrophication and waste management in natural systems.

Fish feed a costly input
Because of the continuing demand of seafood, aquaculture production intensifies. This intensification demands the utilization of artificial feed to supplement the natural food or to fulfill totally the nutritional requirements of the cultured species. An intensive system is typified by high fish densities, high feeding rates, and by high levels of aeration and mixing. A usual fish biomass can be 10 kg m^{-2} with a daily feed input in the order of 200g m^{-2} (2% body weight) (Avnimelech *et al.,* 1995). Fish feed is an expensive input, both from an economic and ecological point of view. Economically because the cost of fish feed is one of

the major expenses (above 50%) during the growing cycle (El Sayed, 1999). Furthermore the deterioration of the aquatic environment demands the use of aerators and water exchange that also imply additional costs. Ecologically because fish feed have high protein content, mainly from fishmeal, and therefore aquaculture is blamed to increase the pressure on wild pelagic species which are used to make fishmeal. For example to produce 1 kg of carnivorous specie the protein required is around 2 to 5 kg of protein from fish meal (Naylor *et al*., 2000). High stocking densities imply more feed input, more metabolites produced by the fish, more feed spills and thus more organic matter decomposition. In addition the nutrients that are not assimilated by the cultured species are accumulated in the fish pond or washed out to the environment. The nitrogen and carbon retained by fish is on average 29% and 13% respectively (Avnimelech and Ritvo, 2003).

Organic matter decomposition and bacteria biomass production
Depending on the biochemical processes, mediated by microorganisms, carbon and nitrogen can be integrated into the trophic food web, mineralized or buried as inert components. In fishponds the organic matter is decomposed by microorganisms using aerobic or anaerobic pathways depending on the conditions that favor the one or the other. Aerobic and anaerobic conditions coexist in aquaculture ponds, e.g. at the sediment water interfaces or in biofilms. A large accumulation of organic matter in pond soil increases the oxygen demand and favours the development of anaerobic patches. When the organic matter accumulation is very high the small anaerobic patches may enlarge in size. Under aerobic decomposition, 50% of the organic matter metabolized is converted into bacterial cells (Henze *et al*., 2002), and therefore with the high load of the intensive aquaculture systems, the bacteria biomass is expected to be high. The nutrient ratio of the substrate also influences the organic matter decomposition rate and the bacterial biomass production. Carbon and nitrogen are incorporated into the bacterial biomass tissue at a fixed rate. The C/N ratio of bacterial cells is around 5 (Rittmann and McCarty, 2001). If we consider the extra carbon needed for respiration, the optimal C/N ratio may increase up to 10 under aerobic conditions. If the C/N ratio is very high, like in natural systems, then N will become very soon limiting (Berard *et al.*, 1995). Due to the use of nitrogenous fertilizers and protein rich feeds the typical C/N ratios found in ponds are low. In recirculating aquaculture systems, where feed rich in protein (40-50%) are commonly used, the C/N ratio can even be as low as 2 and bacteria biomass formation is then dependent on carbon supplementation (Schneider *et al*., 2004). Typical C/N ratios of some aquatic environments are shown in Table 2. Because microbial assemblages are the basis of the

heterotrophic food web and the link with higher trophic levels (Schroeder, 1978) its of great interest to exploit the bacteria biomass as a direct food source for culture species and in this way to increase the overall energy transfer efficiency.

Table 2. C/N ratio of different aquatic systems

Systems	C/N ratio	Reference
Marine systems	17-40 (range 6.99-27.63)	Danovaro *et al*., 1999
Lakes	12.5 (range 6-30)	Cimbleris and Kalff, 1998
Fish pond earthen tilapia	9.5 (range 7.10 -10.55)	Jimenez-Montealegre *et al*., 2002
African Catfish recirculation system	around 2.3	Schneider *et al.,* 2004

Autotrophic and heterotrophic food webs

In terms of organic matter and oxygen balance there is a strong co-relation between primary producers (autotrophs), and decomposers (heterotrophs) (Fig 1) (Naeem *et al.*, 2000). Algae fix inorganic carbon (CO_2) into algal biomass and release large quantities of soluble organic matter that are readily utilized by heterotrophic bacteria. Bacteria break down organic matter releasing mineralised nutrients, carbon dioxide and growth factors (e.g. vitamins) (Hargreaves, 1998; Naeem *et al.*, 2000). In addition algae provide oxygen through photosynthesis while bacteria consume oxygen during organic matter decomposition. Both autotrophic and heterotrophic food webs play an important role as food sources to the cultured animals. Every management action affects these two food webs.
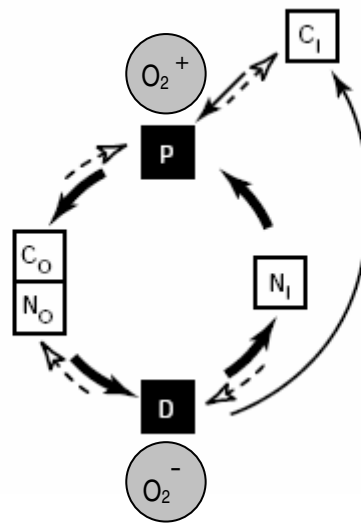
Figure 1. The fundamental producer-decomposer co-dependency. Filled boxes represent biomass and open boxes represent material pools. The biomass compartments shown are producer (**P**) and decomposer (**D**). The material pools are organic carbon (Co), inorganic carbon (Ci), organic nutrients (No) and inorganic nutrients (NI). Solid arrows indicate major flows, whereas dashed open arrows indicate minor flows. Grey circles show the oxygen consumed by **D** (O_2^-) and produced by **P** (O_2^+). Modified from Naeem *et al.*, 2000

Pond Management

Management practices influencing the load and decomposition rates in ponds include water exchange, sediment removal, aeration, fallowing period between crop cycles and liming. The daily amount of organic matter sedimentating during each pond culture cycle increases steadily over time in response to the increased daily feeding load (Jimenez-Montealegre, 2001). Sedimentation and resuspension processes strongly affect the rate of organic matter decomposition. In aquaculture ponds, both the fish biomass and the individual fish size influence the sedimentation and resuspension processes (Jimenez-Montealegre *et al*. 2002). The practical consequence of fish driven resuspension is that anoxic sediments are brought in contact with the more oxygen rich water column. This action integrates both, pelagic and benthic food web systems (Vadeboncoeur, 2002). Intensity increment in aquaculture systems demands management practices that allow augmenting the fish biomass, improving the overall nutrient assimilation efficiency, while enhancing the decomposition and mineralization of organic matter.

Problem definition and thesis objective

Excessive nutrient loading of aquaculture ponds can disrupt the natural ecological processes occurring in aquatic systems. High primary productivity and external organic matter inputs (e.g. fish feed, manures, fertilizers) feed the detrital food web. Dysfunction or disconnection of this detrital food web to higher trophic levels decreases not only the organic matter and energy transfer efficiency, but also the decomposition of the remaining organic matter. The decomposition pathways of this accumulated organic matter determine the water quality and therefore the survival and growth of the cultured species. In aquaculture ponds, management practices, including feeding, influence the microbial organic matter decomposition. In this context, it is important to gain better insight how the parameters: oxygen availability, C/N ratio and resuspension, influence the organic matter decomposition under intensive rearing conditions in ponds. Therefore, the main objective of the present thesis was to study the effect of these parameters on decomposition of fish feed under intensive rearing conditions.

Thesis outline

A review about the microbial ecology and role in aquaculture ponds was elaborated. Aquatic systems vary in nutrient richness, which is sometime classified as trophic level, the latter based on the relation between carbon production and Chl-a levels. A strong relation between algae and bacteria on one hand and organic matter on the other hand exists. The complexity of the system became apparent when specific species-species relationships were involved. The organic substrates added to intensive aquaculture can stimulate or inhibit fish production depending on their nature and quantity. Bacterial biomass formation is an important factor especially as a link between dissolved matter and higher trophic levels. Water quality parameters are strongly affected by the microbial decomposition processes. Currently probiotics are intensively used in aquaculture ponds. However, there is still scarce experimental evidence in relation of its mechanism of action. The improvement of microbial performance in aquaculture ponds is possible by changing the C/N ratio with carbohydrate addition, thereby recycling protein through accumulation of nitrogen in the bacteria biomass **(Chapter 2).**

In the next three chapters experiments were designed to test the decomposition of fish feed under different conditions in *lab-scale* microcosm systems simulating aquaculture ponds. Several studies of organic matter decomposition tried to understand the organic matter

decomposition processes in batch systems where there is only an initial and final measurement. One of the particularities of this work is the fed-batch system. With this approach we tried to simulate the daily feed input with the addition of fresh organic matter as actually occurs also in aquaculture systems.

The aim of chapter three was to evaluate the decomposition of 23 and 49% protein diets under aerobic and anaerobic conditions and to monitor changes in water quality. A microbial community from tilapia recirculation systems was used as inoculum. This fully controlled experiment followed the decomposition of a heavily loaded organic system which simulates the intensive fish pond conditions. The complex oxygen availability conditions occurring in the water, sediment and water-sediment interface in the pond were reduced as fully aerobic or anaerobic treatments in order to simplify the system and understand these extreme situations. Decomposition products were quantified and decomposition rates of carbon and nitrogen were calculated (**Chapter three).**

Chapter four describes development of bacterial biomass during decomposition of fish feed in a simulated intensive aquaculture system, testing two fish feed with different protein content under aerobic and anaerobic conditions. Bacterial biomass forms an important link between the various trophic levels in a pond ecosystem. The oxic status of the medium determines the fate of organic carbon e.g. how much it escapes or remains in the system and in which form. The process of bacteria biomass formation is important to understand carbon transformations after entering the system as fish feed. Information regarding the amount of carbon that is mineralized as CO_2 and the amount that is remaining in the system as bacteria biomass or other decomposition products will improve our understanding of detrital food webs (**Chapter four**).

To investigate the effect of oxic-anoxic gradient on organic matter the decomposition of a 49% protein fish feed was analyzed in ten marine *lab-scale* systems with microbial communities inoculated from the biofilter of a turbot fish farm recirculation system. The effect of oxygen gradient on fish feed decomposition was addressed simulating the effect of shifts for short and longer periods between aerobic and anaerobic conditions. The long term (6 to 18 hrs) decomposition of organic matter under aerobic or anaerobic conditions was simulated with switches between oxygen and nitrogen gasses introduced into the systems. The influence of short changes between aerobic and anaerobic conditions was simulated using

different resuspension levels of settled organic matter. Two additional fully aerobic and anaerobic treatments were also run to have all ranges of oxygen (**Chapter five)**.

To conclude a general discussion about the decomposition of the organic matter in aquaculture systems was elaborated. This discussion included the results obtained during the simulated intensive aquaculture pond system reported in the previous chapters. The limitations of the experimental setup were pointed out as well as some suggestions concerning to further research (**Chapter six).**

References

Avnimelech, Y., Mozes, N., Diab, S., Kochba, M., 1995. Rates of organic carbon and nitrogen degradation in intensive fish ponds. Aquaculture 134, 3-4.

Avnimelech, Y., Ritvo, G., 2003. Shrimp and fish pond soils: processes and management. Aquaculture 220, 549-567.

Azim, M.E. 2001. The potential of periphyton-based aquaculture production systems. Fish Culture and Fisheries. PhD Thesis, Wageningen, The Netherlands, 219 pp.

Berard, A., Volat, B., Montuelle, B., 1995. Bacterial activity and its trophic role in a eutrophic pond. Archive für Hydrobiologie 134, 499-513.

Carlson, R.E., 1977. A trophic state index for lakes. Limnology and Oceanography 22, 361-369.

Cimbleris, A.C.P., Kalff, J., 1998. Planktonic bacterial respiration as a function of C:N:P ratios across temperate lakes. Hydrobiologia 384, 1-3.

Danovaro, R., Marrale, D., Croce, N.D , Parodi, P., Fabiano, M., 1999. Biochemical composition of sedimentary organic matter and bacterial distribution in the Aegean Sea: trophic state and pelagic-benthic coupling. Journal of Sea Research 42, 117-129.

El Sayed, A.F.M., 1999. Alternative dietary protein sources for farmed tilapia, *Oreochromis spp*. Aquaculture 179, 1-4.

FAO (Food and Agriculture Organization of the United Nations), 2002. The State of World Fisheries and Aquaculture 2002. , Food and Agriculture Organization of the United Nations, Rome 150 pp.

Hargreaves, J.A., 1998. Nitrogen biogeochemistry of aquaculture ponds. Aquaculture 166, 3-4.

Henze, M., Harremoes, P., Jansen, J., Arvin, E., 2002. Wastewater treatment, biological and chemical processes. Third edition. Springer Verlag, Berlin, Germany. 430 pp.

ILEC (International Lake Environment Committee), 1986. World Lakes Database.

http://www.ilec.or.jp/database/database.html

Iwashita, K., Kudoh, K., Fujii, H., Nishikawa, H., 2004. Satellite analysis for water flow of Lake Inbanuma. Advances in Space Research 33, 284-289.

Jimenez-Montealegre, R., 2001. Nitrogen transformation and fluxes in fish ponds: a modelling approach. Fish Culture and Fisheries. Wageningen, Wageningen, 185 pp.

Jimenez-Montealegre, R., Verdegem, M., Zamora, J.E., Verte, J., 2002. Organic matter sedimentation and resuspension in tilapia (Oreochromis niloticus) ponds during a production cycle. Aquacultural Engineering 26, 1-12.

Milstein, A., 1990. Water quality in an intensive outdoor commercial fish culture system with mechanically stirred ponds. Israeli Journal Aquaculture Research-BAMIDGEH 42, 99-109.

Naeem, S., Hahn, D.R., Schuurman, G., 2000. Producer-decomposer co-dependency influences biodiversity effects. Nature 403, 762-764.

Naylor, R.L., Goldburg, R.J. , Primavera, J., Kautsky, N., Beveridge, M.C.M., Clay, J., Folke, C., Lubchenco, J., Mooney, H., Troell, M., 2000. Effect of aquaculture on world fish supplies. Nature 405, 1017-1024.

Rittmann, B.E., McCarty, P.L., 2001. Environmental Biotechnology: Principles and Applications. New York , McGraw-Hill Book Co 754 pp.

Scheffer, M., Hosper, S.H., Meijer, M.-L., Moss, B., Jeppesen, E., 1993. Alternative equilibria in shallow lakes. Trends in Ecology & Evolution 8, 275-279.

Schneider, O., Sereti, V., Eding, E.H., Verreth, J.A.J., 2004. Yields and nutrient balances of bacterial production on carbon supplemented fish waste. In: S. Adams and J.A. Olafsen (Eds.) Biotechnologies for Quality, Barcelona, Spain, 729-730.

Schroeder, G.L., 1978. Autotrophic and heterotrophic production of micro-organism in intensely-manured fish ponds, and related fish yields. Aquaculture 14, 303-325.

Sommaruga, R., Robarts, R.D., 1997. The significance of autotrophic and heterotrophic picoplankton in hypertrophic ecosystems. FEMS Microbiology Ecology 24, 187-200.

Vadeboncoeur, I., 2002. Putting the lake back together: reintegrating benthic pathways into lake food web models. BioScience 52, 44.

Wrigley, T.J., Toerien, D.F, Gaigher, I.G., 1988. Fish production in small oxidation ponds. Water Research 22, 1279-1285.

Microbial ecology and role in aquaculture ponds

Beatriz Torres-Beristain[a], Marc Verdegem[a] and Yoram Avnimelech[b]

[a]Fish Culture and Fisheries Group, Department of Animal Sciences, Wageningen Institute of Animal Sciences, Wageningen University and Research Centre, P.O.B. 338, 6700 AH, Wageningen, The Netherlands

[b]Department of Civil & environmental, Technion. Israel Institute of Technology, Haifa 32000, Israel

Abstract

Aquaculture production systems are complex eco-systems containing high biomass of the target animal and ample nutrients and feed residues to support a complex array of organisms, algae, bacteria and protozoa as well as planktonic and benthic invertebrates. Densities of bacteria in ponds are on average $1.86*10^7$ cells ml^{-1}. The bacterial organic carbon is in the range of 3-20 g C m^{-3}, as compared to an average daily primary productivity of 3-7 g C m^{-2}. Estimates are that bacterio-plankton production exceeded phytoplankton production even in extensive systems. The role of the heterotrophic microbial population rise when ponds are fed. The addition of 1 kg of formulated feed would yield about 125 g bacterial biomass. A heterotrophy ratio (HR) was defined as: HR = Daily external C addition / Daily autotrophic C assimilation.

Ponds having about 1750 kg fish ha^{-1},or fed daily by a ration of about 35 kg C ha^{-1}, have a HR of 1. When HR > 1, pond processes are mostly affected by bacteria.

 The cycling of organic matter in the pond is influenced by sedimentation and resuspension processes. Resuspension brings organic matter and nutrients back into the water oxygen rich water column where organic matter decomposition occurs much more efficient, yielding less toxic components than in the sediment.

Controlling bacterial processes offers an interesting management tool for aquaculture. Addition of specific micro-organisms to grow out ponds is complicated and there are as yet no scientific proofs as to its success. Yet, the benefit of introducing external micro-organisms to small closed systems or short tern production units (hatcheries, nurseries) was proven. The control of microbial processes by adjusting the environmental conditions was shown to be a very potent means. Active suspension ponds (ASP) are ponds where high bacterial biomass is generated by limiting water exchange, raising organic substrates and microbial biomass. Adjusting C/N ratio in these ponds enables a reliable control of water quality and leads to an effective recycling of feed protein, up to roughly doubling protein utilization by fish.

In light of the important role of bacteria in pond systems, there is a need and justification to further study the nature of this population, its dynamics and interaction with the other components of the pond eco-system.

Introduction

Aquaculture systems are always complex ecosystems containing the cultured animals (fish, shrimp or other), algae, zooplankton, bacteria and other organisms. For simplicity, in this work the term fish covers all cultured species. Traditionally, the role of algae in driving the autotrophic food web was emphasized, giving little attention to the role of bacteria and the decomposition of organic matter.

Hepher and Schroeder initiated the concept of heterotrophic food web in their pioneering works (Schroeder, 1978; Schroeder and Hepher, 1979). In these works, they studied the food web originating from organic matter applied to ponds. The pathways were initiated through the breakdown of the organic matter by bacteria, serving as the basis of the heterotrophic food web on which zooplankton, benthic organisms and fish feed. Avnimelech and co-workers (Avnimelech and Mokady, 1988; Avnimelech *et al.*, 1992ab) suggested managing the heterotrophic food web by the development of active suspension ponds, intensive ponds with zero or limited water exchange, accumulating high amounts of organic substrates. Inorganic nitrogen accumulation is controlled through the addition of carbonaceous substrates, raising the C/N ratio and leading to the immobilization of nitrogen through the production of microbial proteins (Avnimelech *et al*, 1994). Fish harvest the bacteria, as bacterial flocs and utilize this protein source (Avnimelech *et al.*, 1989; Milstein *et al.*, 2001a; McIntosh *et al.*, 2000).

A pioneering work was done at Solar aquafarms in California demonstrating a closed tilapia culture system (Fitzimmons, 2002), as presently practiced in a number of commercial farms. A similar system was adapted for shrimp culture, first as an experimental system in Waddell Mariculture Research Center in South Carolina (Hopkins *et al.*, 1993) and then as a commercial system in Belize Aquaculture (McIntosh *et al.*, 2000; Burford *et al.*, 2003a) and other farms. The term heterotrophic system, as opposed to autotrophic system, was used and became a popular term in aquaculture.

Another use of bacteria in pond management is the application of "probiotics" in ponds, hatcheries and nurseries. Many probiotics products are found in the market and in commercial use. Probiotics are biological additives, mostly microbial inoculates, claimed to improve or stabilize water quality, to reduce disease threats and to enhance the health status of cultured animals.

A review on the role of microorganisms in ponds by Moriarty was published in 1997 (Moriarty, 1997), dealing mostly with the methodological matters. A book on microbial processes in aquaculture (Maeda, 1999), dealt mostly with probiotics. More recently the World Aquaculture

Society, in cooperation with The Oceanic Institute held a workshop dealing with Microbial approaches to aquatic nutrition, proceedings of which were published in 2002 (Lee and O'Bryen, 2002). In light of new information and growing interest in the use of bacteria in aquaculture it seems timely to review the relevant information in this field both scientifically as well as to discuss the practical options and conclusions.

1. Heterotrophic biomass in algae driven ponds

Aquaculture ponds are typified by the presence of high levels of algae, higher than most natural water bodies. Chlorophyll a (Chla) concentrations and primary productivity in water bodies of different trophic levels and in a sample of aquaculture ponds are given in Table 1. Chla concentrations and primary production values in ponds are in the range expected for eutrophic or hyper-eutrophic water bodies. The average Chla concentrations and primary productivity in the examined ponds were 103 ($\pm$68) mg/m^3 and 3.2 ($\pm$1.8) g C/m^2*day, respectively. The variation is high, especially since extensive non-fertilized ponds as well as intensively fed and fertilized ponds were included in the sampled data set. Other data compilations yielded similar results: Tucker and Martin (1991) estimated that the typical catfish ponds in the USA contain more than 100 mg m^{-3} Chla (200-400 mg m^{-3} during the summer). Boyd (1991) estimated Chla levels in fertile fish or shrimp ponds to be 60-150 mg m^{-3}.

Del Giorgio and Peters (1993) found a positive correlation between primary production (PP, g C m^{-2} d^{-1}) and Chla concentration (in mg m^{-3}), using a dataset from 118 freshwater lakes worldwide:

$$(1) \qquad PP = 10.3 \ Chla^{1.19} \ (r^2 = 0.75)$$

Chlorophyll-a values in freshwater lakes varied between 0.3 and 120 mg m^{-3}. However, they stated that for Chla levels above 150-200 mg m^{-3} the primary production rise is milder. Therefore, the above mentioned relation may be wrong in hyper-trophic lakes and aquaculture ponds, where Chla levels are often above the 120 mg m^{-3} limit (Sommaruga, 1995; Teichert-Coddington and Green, 1993.

Table 1. Selected values for chlorophyll a concentrations and primary production in fish ponds and lakes.

System	Chlorophyll a, (mg m^{-3})	Carbon production (g C m-^{2}day^{-1})	Reference
Non Fertilized ponds, Carp, Israel	103.4	3.3	Hepher, 1962
Fertilized ponds, Carp, Israel	212.3	6.6	Hepher, 1962
Intensive shrimp pond	88.8	4.7	Burford, 1997
Ponds effluents	43.4	3.87	Burford *et al.*, 2003b
1Poly-culture, no fertilizer	12.5	0.9	Yusoff and McNabb, 1989
Poly-culture, P added	46.7	1.7	Yusoff and McNabb, 1989
Poly-culture, P and N added	109.2	2.6	Yusoff and McNabb, 1989
Tilapia pond, beginning of cycle	109	1.2	Jimenez-Montealegre, 2001
Tilapia pond, end of cycle (ca 60 days)	205	3.8	Jimenez-Montealegre, 2001
Average	103	3.2	

Range for lakes

Oligotrophic lakes	0.3-3	0.05 – 0.3	Wetzel, 1975
Mesotrophic lakes	2 – 15	0.25 – 1.0	Wetzel, 1975
Eutrophic lakes	10-500	> 1.0	Wetzel, 1975

According to Chang *et al.*, (2003), the ratio of assimilated carbon (mg C m^{-2} d^{-1}) to Chla concentration is in the range of 10 to 100. One reason for the wide range of the assimilated carbon:Chla ratio may be the fact that different algal groups contain different Chla percentages (Kalff and Knoechel, 1978). A ratio of 35 mg C assimilated to Chla concentration as reported by Cloern *et al.*, (1995) can be regarded as a medium value. The average ratio found for the ponds listed in table 1 is 39.8 $\pm$24.7 mg Chl-a per 1 g C m^{-2} d^{-1} , in a very good agreement with that reported by Cloern, yet the variability is high, as expected. However, the rather good fit, obtained for ponds of different climatic conditions, management and fish biomass hints that the overall algal community and functioning in ponds is similar.

Bacterial development and density is associated with primary production, which in many environments is the main source of organic matter. Bacteria utilize algal exudates from live cells and lysis products from dead algae, whereas algae use the inorganic nutrients released by the bacteria. The number of bacterial cells was found to increases linearly with the Chla concentration (Gasol and Duarte, 2000).

Bird and Kalff (1984) constructed a regression model to predict bacterial abundance as a function of Chla concentrations, based on data from fresh water and marine water bodies. The range of Chla concentrations for which this equation was calculated is from 0.5 to 120 mg m^{-3}. The correlation between the acridine orange direct count (AODC, number ml^{-1}) and Chla (mg m^{-3}) was:

(2)   log AODC= 5.867 + 0.776 log Chl *a*

Sommaruga and Robarts (1997) found that the bacterial abundance increases with the trophic state of a water body. However, after reviewing several hypertrophic systems they concluded that the bacterial production and biomass responded to the Chla concentration less than predicted with eq. (2). Therefore, care must be taken to use equation (2) when the Chla concentration is above 120 mg m^{-3}.

Microbial biomass indexes were calculated for the ponds described in Table 1, using equation (2) and were plotted against the Chla concentration (Figure 1).
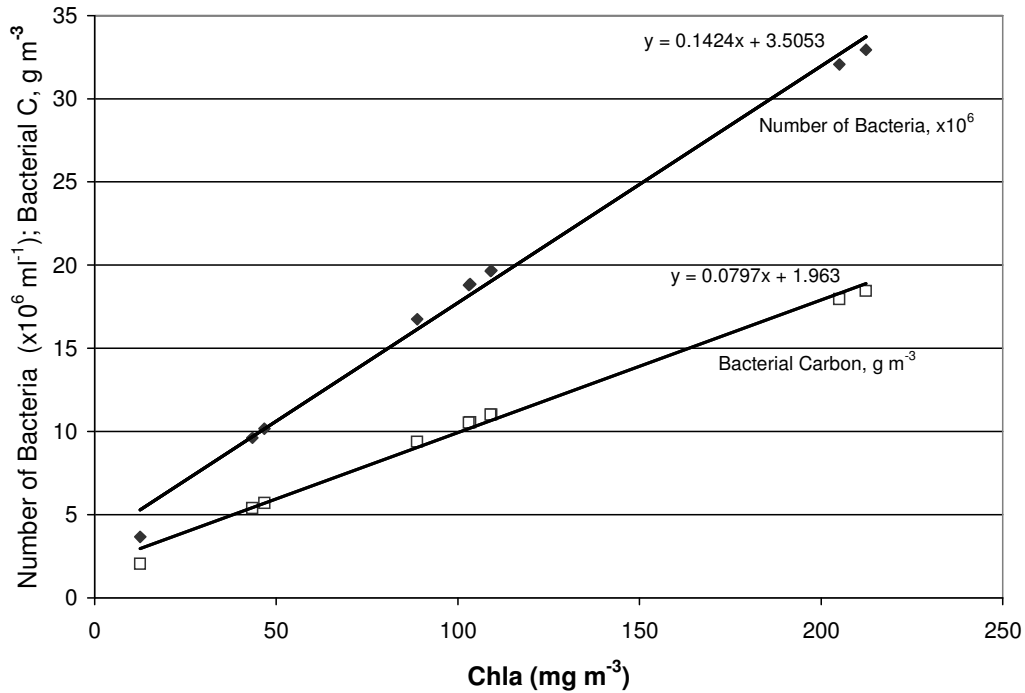
Figure. 1. Bacterial carbon content and bacteria abundance cells ml^{-1} from aquaculture ponds (Table 1.) calculated in base of chlorophyll-a measurements using Bird and Kallf (1984) equation.

The microbial volume was calculated, using an average specific volume of 0.7 μm^3, an average of values used for bacteria in inland water as given by Wetzel, (2001, Table 17-1). Bacterial carbon content was calculated using the factor $5.6 \ 10^{-13}$ g C μ m^{-3} bacterial volume (Wetzel, 2001, Table 17.1). Bacterial carbon levels as a function of Chla concentration are also given in Figure 1. It is seen that bacterial population varies within the range of Chla commonly found in ponds, and is in the range of 1.1×10^6 to 4.74×10^7, with an average of 1.86×10^7 cells ml^{-1}. The bacterial organic carbon is in the range of 3-20 g C m^{-3}, as compared to an average daily primary productivity of 3 to 7 g C m^{-2} (Wetzel, 1975).

Coveney and Wetzel (1995) discussed the coupling of bacterioplankton to phytoplankton in an oligotrophic lake (Lake Lawrence, MI). Estimates of bacterioplankton production exceeded that of phytoplankton by factors of 1.33 – 3.35. Bacterial respiration for a 2 years study was found to be 68-171 g C m^{-2} yr^{-1} as compared with net phytoplankton production of 51 g C m^{-2} yr^{-1}, indicating that "the overall metabolism of the pelagic zone in Lawrence Lake is heterotrophic". The coupling of bacteria to algae activities was assumed to be partially due to dissolved organic carbon (DOC) liberated by algae. However, they concluded that both algae and bacteria are limited by regulating factors such as very low phosphorus supply. The gap between the bacterial

respiration as related to phytoplankton C fixation is possible due to external supply of organic matter, mostly with the incoming water. Aquaculture ponds are much richer in added organic matter, mostly introduced with the feed, and thus higher bacterial productivity is expected like in eutrophic systems. In a hypertrophic shallow reservoirs, the bacterioplankton production exceed the phytoplankton production by 5 to 25 times (Balogh and Vörös, 1997).

2. Algae-bacteria interaction

Bacteria and algae are ubiquitous and abundant microorganisms in aquaculture ponds that coexist at different parts of the pond: as plankton in the water column, in benthic environments, on the sediment or attached at the surface in a periphyton biofilm. The first obvious dependency between algae and bacteria is related to biomass and oxygen availability. Algae produce organic matter and oxygen, and bacteria mineralize organic matter and consume oxygen. The relations between the phytoplankton community and the heterotrophic organisms such as bacteria that depend on it are still not well understood and quantified (Hansson *et al.*, 1998). Algal diversity is inversely correlated with the eutrophic level of a system, in oligotrophic conditions the microbial population has lower activity but greater taxonomic diversity (Cole, 1982). In eutrophic systems like aquaculture ponds, cyanobacteria are more predominant than algae. Cyanobacteria blooms are more predictable as several studies showed a correlation between environmental factors and cyanobacteria blooms (Scheffer, 1998).

Nutrients are added to promote primary production. This supports the culture species as well as increasing oxygen production. However, dense algal growth will increase turbidity, reduce light penetration through the water column and subsequently limit further photosynthesis. When Chla exceed 100-300 μg Chla l^{-1}, algal turbidity shades the water column and gross primary productivity does not further increase (Hargreaves and Tucker, 2003).

Table 2. Algae blooms related with aquaculture animals mobility or mortality

Algae bloom	Group	Toxin	Cultured animal	Note	Reference
Schizothrix calcicola strain UTEX B-1936	Cyanobacterium		*Litopenaeus vannamei*	Damage to the gastrointestinal linen. May jeopardize the culture by slowing shrimp growth rate and biomass yield	(Perez-Linares *et al.*, 2003)
Trichodesmium erythraeum	cyanobacterium	saxitoxin	pearl oysters (*Pinctada maxima*)	Blooms of T. erythraeum were observed at the time of the pearl oyster mortality. However not conclusive relation of the toxin with the mortalities	(Negri *et al*, 2004.)
Chaetoceros wighami	Flagellate		Salmon	Acute mortalities and sublethal effects on salmon. Fish were inappetant and lethargic in all four farms, with some fish showing respiratory distress	(Treasurer *et al.*, 2003)
Microcystis aeruginosa	cyanobacterium	Microcystin-LR, a hepatotoxin	Catfish *Ictalurus punctatus* (Rafinesque)	Fish exposed to pond water containing this toxic bloom were killed within 24 h. Necropsy of fish revealed congested liver and spleen tissues	(Zimba *et al.*, 2001)
Anabaena circinalis	cyanobacterium	paralytic shellfish poisoning (PSP)	*Alathyria condola*	Bioaccumulation high levels of toxin in the viscera	(Negri and Jones, 1995)
Karlodinium micrum	dinoflagellate	hemolytic, ichthyotoxic, and cytotoxic substance	hybrid striped bass	fish kill events associated with blooms	(Deeds *et al.*, 2002)

19

Furthermore, such over-enrichment combined with specific environmental factors can cause an uncontrolled phytoplankton bloom of unwanted species that in some cases can be toxic for cultured animals. Phytoplankton blooms may also produce substances that give off-flavors to the cultured animal. In such a case, harvest will be delayed and profitability reduced (Hargreaves and Tucker, 2003). Some examples of cultured species affected by algal blooms are shown in Table 2.

Once the algal bloom crashes, it will stimulate bacterial growth, consuming oxygen and often creating anoxic conditions, putting fish survival at risk (Alonso-Rodriguez and Paez-Osuna, 2003). There is a strong correlation between algae massive die-offs and subsequent bacterial development. Coveney *et al*. (1977) showed that peaks in phytoplankton abundance are followed with peaks in bacterial abundance. Bacteria respond to the biomass availability of senescent algae but also to the exudates of living algae. The latter is supported by the observation that the bacterial community starts to increase right from the beginning of an algal bloom (Cole, 1982). An example of algal bloom-bacterial cycles in natural systems can be found in Kuwait Bay. This Bay is enriched by aquaculture and sewage effluents. During August and September 2001, a bloom of the dinoflagelate *Ceratium furca* caused massive mortality in Sea bream net pens (100–1000 dead fish day^{-1}). After that, an outbreak of the bacterium *Streptococcus agalatiae* killed over >2500 metric tons of wild mullet. As the mass fish mortality progressed, various harmful algal blooms (HAB) were observed including *Gymnodinium catenatum, Gyrodinium impudicum*, and *Pyrodinium* var. *compressum* (Glibert *et al*., 2002).

Bacteria-algae interactions are still poorly understood (Simon *et al*., 2002). These interactions include competition, mutualism, inhibition, stimulation and coexistence. Primary producers, which are nutrient limited (mainly N and P), can only use dissolved inorganic nutrients while bacteria, can use both dissolved and particulate nutrients. Therefore, bacteria can compete with algae for dissolved nutrients (Aota and Nakajima, 2000). The algae are generally ineffective in competing for available organic substrates at substrate concentrations maintained by active bacterial heterotrophic activity (Wetzel, 2001). When there is a strong carbon limitation (low C:N and C:P ratios) bacteria tend to be out-competed. However, under N or P limitation (high C:N and C:P ratio) algae will be less competitive. At intermediate C:N and C:P ratios algae and bacteria will be both active (Thingstad and Pengerud, 1985).

Metabolic requirements of each specific bacteria or phytoplankton species make them worse or better competitors under specific determined circumstances. For example phytoplankton competes with chemoautotrophic nitrifying bacteria for ammonia. When concentrations are low, phytoplankton are more effective competitors for ammonia than nitrifying bacteria and the reverse occurs when concentrations are high (Hargreaves, 1998).

Bacteria and algae may specifically inhibit each other performances. Bacterial growth can be suppressed by cultures of planktonic or bentic microalgae while algae can be inhibited by algicidal bacterial products. Besides that, the same bacteria can supply a growth factor for one species while inhibiting another species (Fukami *et al.*, 1997). Following this relationship, Hold *et al.*, (2001) studied the diversity of bacteria in both toxic and non-toxic dinoflagellate cultures. Their study suggested a species-specific association between some bacteria and algal species. This association is a potentially important regulatory factor for population dynamics of both organisms, and may also be involved in the induction and control of toxin production. Carrasquero-Verde (1999) showed that *Heterosigma carterae* (flagellate) was toxic to salmonids only when heterotrophic bacteria were present in the culture, suggesting that the toxicity can be induced through bacteria interactions.

A very strong relationship between bacteria and algae can be found in periphyton structures and its development. Periphyton communities are comprised by bacteria, fungi, protozoa, phytoplankton, zooplankton, benthic organism and some invertebrates and their larvae (Azim, 2001). Any substrate under water will support a periphyton community. The substrate will first be coated by organic substances, further colonized by bacteria and finally by algae and invertebrates, all of them embedded in a mucopolysaccharide matrix where organic detritus is trapped (van Dam *et al*., 2002). The diversity of bacteria and algae found in the periphyton layer varies depending on pH, alkalinity, temperature and light intensity. Azim *et al*. (2001) found that the algal periphyton community contained 50 different genera. Moreover the community structure and species mix influence the flux of nutrients and energy. These relations are responsible for the heterogeneity inside the biofilm, for example, microenvironments with oxic and anoxic zones (van Dam *et al.*, 2002). Aquaculture ponds are a highly eutrophic system where relations between primary producers and decomposers are of vital importance. A healthy aquaculture pond will have a balance between bacteria and algae biomass and species composition. The importance of the algae-bacteria interactions in a fish pond depends on the rate of organic matter loading, environmental factors and pond management practices.

3. Effects of added organic substrates.

Microbial growth depends on the availability of organic substrates. Quantity and quality of the added substrates are relevant. Readily biodegradable substrates, in contrast with stable compounds, are effective in promoting bacterial growth. Most inputs into the pond are readily degradable. Feed pellets contain starch and proteins, having a first order decomposition rate (K) of about 0.8 day^{-1} (van Keulen and Seligman, 1987). The mineralization rates of feed pellets tested in laboratory microcosms, was found to be 0.26 day^{-1} under aerobic conditions, as compared to about 0.06 day^{-1} under anaerobic conditions (Torres-Beristain, submited). Avnimelech *et al*. (1995) found that degradation of organic matter in mixed – aerated tanks and in commercial active suspension ponds followed a first order kinetics with a rate of 0.14-0.16 day^{-1}, which is in good agreement with the above mentioned laboratory data. A first order decomposition constant of dead algae cells and algal cells exudates have a first order decomposition rate of about 0.1 day^{-1} (Westrich and Berner, 1984; Wetzel, 1975). On the other hand, refractive organic matter in sediments degrade at a rate of 0.4 yr^{-1} (Avnimelech *et al*., 1995), and humic compounds from terrestrial origin entering the pond with run-off water degrade much slower (K=8.3 $x10^{-5}$ day^{-1} (van Keulen and Seligman, 1987). These components, although conventionally considered as organic matter, will not support large bacterial communities.

Fish biomass in conventional ponds is in the range of 1 to 40 ton ha^{-1} (0.1 to 4 kg m^{-2}), and reaches 100 kg m^{-2} in super intensive systems. Daily feed addition is roughly 2-3% of fish biomass, i.e. 0.002 – 120 g m^{-2} in conventional ponds and 2000-3000 g m^{-2} in super intensive systems. The daily organic carbon input is half of the above-mentioned feed inputs since feed contains on the average 50% organic carbon. A primary production of about 3.5 g C m^{-2} d^{-1}, as the average value for ponds reported here is equivalent to the daily carbon addition through the feed to a pond with a fish biomass of about 0.175 kg m^{-2}, or 1750 kg ha^{-1}. Feed carbon additions to ponds with a higher fish biomass will supersede the carbon assimilatory capacity of the algae. As mentioned above, there are no totally autotrophic, algal controlled, systems, and there are no totally heterotrophic systems. There is always a mix between the two. A useful concept can be the heterotrophic ratio (HR) that is defined as:

HR = Daily external C addition / Autotrophic carbon assimilation rate

On average, in ponds with a fish biomass of 1750 kg ha^{-1}, HR is 1. When HR > 1 pond processes are mostly affected by bacteria.

The addition of large amounts of labile organic matter to the pond raises the bacterial biomass and bacterial activity. Microbial metabolism of organic substrates leads to (a) the oxidation of the substrate to produce CO_2 and energy and (b) the bio-synthesis of microbial cell materials:

  (3)   **Organic C** $\rightarrow$ **CO_2 + Energy + C** (assimilated in microbial cells).

The percentage of the assimilated carbon with respect to the metabolized organic carbon is defined as the microbial conversion efficiency (E) and is in the range of 40-60% (Gaudy & Gaudy, 1980). Moreover, carbohydrate digestibility by fish is 40-60% (Verdegem *et al*., 2000) so it can be assumed that about 50% of the organic carbon input through the feed ends up in the pond as un-utilized feed or feces. In consequence, one kg of feed generates about 125 g bacterial biomass (1000 g feed x 50% carbon x 50% released x 50% microbial efficiency).

The rate of feed conversion into microbial biomass depends on the conditions in the pond. Under aerobic conditions, the degradation rate of feed materials is about 10-80% per day (K=0.1-0.8 day^{-1}), thus the conversion takes place within hours or up to a few days. However, if the added organic substrate settles down to the anoxic pond bottom, the degradation rate is slower and the bacterial conversion efficiency will be much lower. Therefore, maintaining the organic matter under aerobic conditions is important in order to get better recycling of feed residues through the pond's food web.

Microbial growth rate (dx/dt) is related to the concentration of organic substrate (S) through the Monod equation (Monod, 1949):

  (4) dx/dt = μ_{max} x (S)/(K_x + S),

where μ_{max} is the maximal growth rate (determined by the system environmental variables) and K_x is a constant depended on the substrate quality and the specific microbial community. When K_X is higher than S (i.e. substrate concentration is the limiting factor), as usually the case, equation (4) is reduced to a first order kinetic:

  (5) dx/dt = (μ_{max}/K_x) x S,

i.e., microbial growth rate becomes a direct linear function of substrate availability.

The degradation of the organic substrate (dS/dt) is considered to follow first order kinetics (-K x S), where K is a degradation constant. Organic substrates are added daily to the pond as feed or organic fertilizers and through primary production. Assuming that the daily addition of organic matter (B) is constant over time then the equation becomes:

  (6)   **dS/dt = B - K * S**

Which after integration becomes:

  (7) S = (B - e^{-Kt} *(B - KS_0))/K

Equation (7) describes the evolution in time of the substrate concentration S, with S_0 as the initial concentration. However, when time is long, the exponent e^{-Kt} approaches zero, and equation (7) becomes:

(8) S = B/K

i.e., the substrate concentration approaches a steady state, where daily addition is equal to daily degradation and the concentration remains constant over time (Avnimelech *et al*. 1995).

Computed substrate concentrations as a function of time, for several rate constants are presented in Figure 2. It can be seen, that the time to achieve a steady state decreases with the increase of the degradation rate constant. For a rate constant of about 0.15 day^{-1}, as typical to fish ponds (Avnimelech *et al*., 1995), steady state is approached within a period of a few weeks. The model described above is an approximated model, not taking in account cloudy days, algal die-offs, fish diseases or changes in feeding rate over time. Nevertheless, the model leads to interesting conclusions. One is that the organic substrate pool and the resulting microbial community are roughly constant over time, once the steady state is achieved. The steady state is controlled by 2 factors, the rate of feed addition and the organic carbon decomposition rate. Avnimelech *et al*. (1995) reported that the degradation rate constants for organic carbon are similar in intensive ponds and experimental tanks. The average rate constants found in experimental tanks was 0.16 day^{-1}, as compared with 0.145 ± 0.068 day^{-1} found for 113 sampling events in commercial ponds. Roughly Similar rates (0.26 day^{-1}) were obtained in laboratory oxic-mixed microcosms in Wageningen (Torres-Beristain, submited). These findings suggest that the overall features of microbial communities in ponds are similar. Thus, we can expect as a first approximation, to find similar organic matter concentrations and similar bacterial biomasses in aerated-mixed ponds with similar feed addition, worldwide.
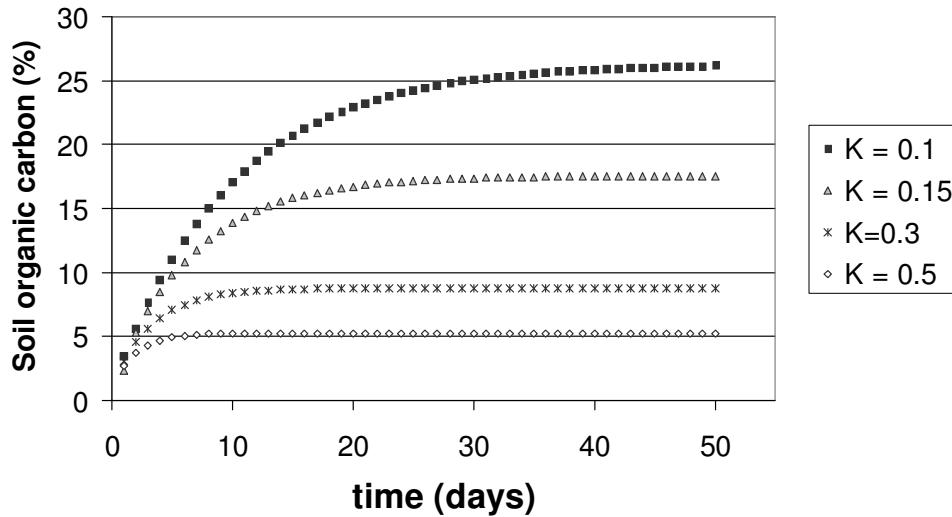
Figure 2. Organic carbon accumulation as a function of time base on the equation (7)

4. Sediments and other hotspots.

In the preceding chapters, ponds were treated as uniform mixed systems. This is not true for most ponds. Typically, ponds have regions with low concentrations of organic matter and others with very high concentrations, since organic sediments tend to accumulate in the deeper parts of the pond. In addition, wind direction and other factors affect spatial distribution of algae, suspended matter and oxygen. Unless thoroughly mixed, ponds are not uniform systems. The equations discussed in the preceding chapters do not take the heterogeneity in account and thus over-simplify the pond environment.

A dominant process creating different zones in the pond is sedimentation. Organic rich particles are constantly settling down and accumulating at the pond bottom. Concentrations of organic matter at the pond bottom are in the order of a few percents (10^{-2}), as compared to 10^{-5} in the water body (Avnimelech & Ritvo, 2003). Bacterial density in the sediments is in the range of 10^7-10^9 g^{-1} dry soil (Ram *et al*, 1982; Burford *et al*, 1998) as compared to 10^6 ml^{-1} in the water column (Moriarty, 1986). The high concentration of organic substrates and the low light penetration lead to low algal activity, result in a high heterotrophic ratio in the pond bottom. The process of resuspension (Avnimelech *et al.*, 1999; Jimenez-Montealegre, 2001) brings organic matter and nutrients back into the water phase. In addition to having a high load of organic matter, the sediment is typified by an oxi-stratification, with a very thin aerobic layer overlying a thick anaerobic layer (Meijer and Avnimelech, 1999; Revsbech *et al.*, 1983). The oxidized layer is a few mm thick, and can be as thin as a fraction of a mm in undisturbed clay bottom

soils. A very steep decline in oxygen availability occurs at the water – sediment interface, concurring with a transition in microbial community. The transition zone from aerobic to anaerobic conditions is an area where aerobic products such as nitrates are reduced and soluble anaerobic products such as H_2S, Fe^{2+} or organic acids are effectively oxidized (Revsbech *et al*. 1983). Transfer of matter between the reduced sediment, the thin oxidized layer and the overlying water is mainly controlled by diffusion, the rate of which is rather low (Meijer & Avnimelech, 1999). However, fish searching for food stir the sediment and increase the rate of transfer (Avnimelech *et al*., 1999; Ritvo *et al*, 2004). The resuspension process raises organic residues to the overlying aerobic water and thus facilitates its degradation. Jimenez-Montealegre (2001) found in a 77-day experiment with tilapia a high correlation between fish weight/biomass and total solids resuspension rate. On average the resuspension rate of total solids was 105.6 gr m^{-2} day^{-1} with a fish density of 1.5 tilapia m^{-2} and an average fish weight of 144 g. Stocking a bottom feeder like common carp, even at low densities, leads to an increased resuspension in poly-culture ponds (Ritvo *et al*., 2004) and raise the production capacity of ponds (Milstein *et al*., 2001a). The sediment water interface is a site of intensive fish activity and the detailed effects of the prevailing and changing conditions in this layer are yet to be resolved.

Besides the sediment, one may find in ponds other hotspots with high organic matter concentration and high heterotrophic ratio, such as sites where algae and debris accumulate on aquatic plants or artificial substrates. The addition of a submerged substrate area equal to the pond surface area enhanced fish production by 70 – 160 % in fertilized non-fed ponds (Azim, 2001). The enriched microbial communities growing on these substrates are efficiently harvested by fish (Azim *et al*., 2004). Thus, even in ponds with a low heterotrophic index, one finds zones where microbial communities dominate.

5. Microbial activity and water quality.

The maintenance of water quality in ponds is tightly affected by algal and/or microbial processes. Oxygen level, accumulation of inorganic nitrogen, pH, alkalinity and CO_2 levels as well as accumulation of organic residues are all tied intimately to the activity of microorganisms.

In fish ponds, the dissolved oxygen concentration in the water column needs to be maintained above 4-5 mg l^{-1} (Boyd, 1990). Oxygen is consumed in ponds during aerobic bacteria break down of organic matter. In intensive and semi intensive systems more oxygen is consumed than provided through photosynthesis and surface re-aeration, hence aeration is needed. Providing aeration, although energy consuming, is usually feasible, and farmers are able to maintain the oxygen concentration in the water column above critical levels. More difficult to control is the oxygen level at the water-sediment interphase, where a large diversity of bacterial groups co-exist along the oxic-anoxic gradient (Brune *et al*., 2000).

Organic carbon is mineralized by both aerobic an anaerobic respiratory mechanisms. Anaerobic carbon mineralization includes denitrification, sulphate reduction and methanogenesis. Organic matter decomposition has an optimal pH of 7.5-8.5. The pond pH is adjusted through liming. Liming also increases alkalinity, improves carbon availability and may enhance phosphorus solubility (Boyd, 1995).

Nitrification is an oxic process and is performed in two steps: oxidation of ammonia to nitrite and oxidation of nitrite to nitrate. Different groups perform each step: ammonia-oxidizing bacteria (*Nitrosomonas, Nitrosococcus, Nitrosospira, Nitrosolobus, Nitrosovibrio, etc.*) and nitrite oxidizing bacteria (*Nitrobacter, Nitrococcus, Nitrospira, Nitrospina, etc.*) (Hagopian and Riley, 1998). Only 5 to 10% (25 to 70 mg N m^{-2} d^{-1}) of the total nitrogen input is oxidized through nitrification in earthen catfish ponds (Hargreaves, 1998; Gross *et al*., 2000) Several factors can limit nitrification in aquaculture pond. First, nitrifying bacteria are aerobic bacteria that have the tendency to attach to biofilms. The sediment is the main site where decomposition of organic matter takes place and ammonium is released. Yet, due to oxygen limitation, nitrification at that site is limited. Second, because heterotropic bacteria have a very fast growth rate in environments with high concentration of labile organic matter, heterotrophic bacteria will out-compete successfully the nitrifying bacteria that have a significantly slower growth rate (Strauss and Lamberty, 2000, Golz *et al*., 1999).

Water exchange is a normal practice in semi-intensive and intensive aquaculture ponds. The principal reason to apply water exchange is to flush out excess phytoplankton and other organic

matter, to eliminate metabolic toxic products from fish, mainly ammonia, and to increase the dissolved oxygen concentration (Hopkins, 1993). The dilution of the system has also an impact on the microbial population and processes. For instance, nitrifying bacteria, which are slow growers need several days develop, and are easily washed out from the ponds at high exchange rates (Avnimelech *et al*., 1992a; Hargreaves, 1998), preventing the consolidation of a nitrifying bacteria population. An effective bacterial decomposition of organic matter in the sediment and water column will reduce the necessity of water exchange (Milstein *et al*, 2001b). The advantage of reducing water exchange rates, even up to the level of zero-exchange systems (Burford *et al*., 2003a), is the synergistic effects between algae and bacteria, leading to more stable water quality and improved feed conversion (Pruder, 2001).

The beneficial use of probiotics in aquaculture is an issue intensively dealt with recently. Different definitions of probiotics are used. Some consider probiotics as microbial feed supplements that improve the health of the animals (e.g. Gatesoupe, 1999), while others (e.g. Moriarty, 1998) expand this definition to microbial amendments that improves health, growth or growing conditions in the pond. Other terms used are bio-control or bio-remediation.

Probiotic products may act within or on the animal, usually through the principle of competitive exclusion and immunostimulant effects (Gullian *et al*., 2004). These activities depend on the bacterial interaction with and within the target animal and much less on the pond ecology. A number of cases where added probiotics increased production (Garg *et al*, 1998; Moriaty, 1998) or helped in preventing disease (Prabhu *et al*, 1999) were reported. However, as stated by Irianto and Austin (2002), the mode of action was rarely investigated. The different mechanisms of bacterial interactions with cultured animals were critically reviewed by Verschuere *et al*., (2000). The effects and efficiency of probiotics agents in specifically act against disease inducing organisms is beyond the scope of the present review.

It is tempting to add "good" bacteria to the pond, bacteria that will develop in the water or within the fish and will maintain desired conditions. Maeda (1999) described methods to select bacteria that can raise shrimp survival. Other studies described laboratory selection of bacteria that potentially raise growth of fish. However, the potential benefit of adding external bacteria to a grow-out pond is not trivial. In order to be effective, the added inoculum has to survive and out-compete the existing community during the entire duration of the production cycle. The added bacteria should be more effective than the native ones in maintaining water or sediment quality or in counteracting diseases. The efficiency of probiotics depends to a large extent on the pond ecology. Microorganisms that have a competitive advantage within a system will develop in that system. However, addition of less competitive organisms will not lead to a lasting change

in the community structure. This is different during the time period when a system progresses towards a steady state. For instance, addition of nitrifying bacteria during the first days after stocking an intensive pond may shorten the nitrification lag period (e.g. Bower and Turner, 1981), a time lag occurring due to the slow development of nitrifying bacteria. Similarly, inoculation of biofilters may shorten the adaptation period needed before a full capacity may be achieved (e.g. Verschuere *et al*., 2000). The possibility that "pioneer organism" added as an external inoculum will survive (Verschuere *et al*., 2000) along a pond growing season is rather slim, both due to the fact that a huge indigenous inoculum is present in the pond bottom and water column and due to the long duration of the growing season, covering many microbial generations.

Successful application of probiotics has been reported for small, highly controlled systems with short production cycles, such as larval cultures, nurseries and aquaria systems (Nogami and Maeda, 1992; Garriques and Arevalo, 1995; Ringo and Birkbeck, 1999; Alabi *et al*, 1999). When the added bacterial culture is not competitive, the application needs to be repeated every few days (Moriarty 1998).

Less conclusive results are available for grow-out ponds. Unfortunately, the high volume of commercial activity in promoting the use of probiotics is supported by very few objective, statistically tested reports on the effect of probiotics on pond water and pond soils quality. Duvall *et al*., (2001) tested the effects of 4 microbial products on sediment and water quality. None of the tested products had any significant effect on sediment bacteria or algal growth rates. Sonnenholzner and Boyd (2000) did not found effects on bacteria activity or organic matter decomposition, using three different probiotics products. When commercial probiotics were evaluated in mariculture systems, no significant differences between treatments where observed in nitrogen dynamics and fish growth (Thoman, 2001). Queiroz and Boyd (1998) studied addition of a commercial bacterial inoculum on three catfish ponds. No significant or consistent effects on water and sediment quality were found. Yet, survival and net fish production were significantly higher in the treated ponds, but the underlying working mechanism of the inoculum could not be explained. McIntosh *et al*. (2000) tested the effects of a commercial product on shrimp production in tanks without water exchange. No effect was found regarding water and sludge quality, shrimp survival and growth. The authors concluded that the natural microbial flora in the shrimp tanks was sufficient to maintain proper water quality. More objective and reliable research is needed to evaluate the effect of probiotics on production, survival and water quality in grow-out ponds, and to explain the underlying mechanisms.

6. Microbial controlled ponds.

A basic characteristic of intensive ponds is the inorganic nitrogen enrichment. Only about 30% of feed nitrogen input is recovered by fish harvest (Avnimelech & Ritvo, 2003). The rest is released to the water. Unlike organic carbon of which a large fraction is released as CO_2 most of the nitrogen remains in the pond. This leads to the accumulation of inorganic nitrogen, often up to toxic levels of ammonia and nitrite.

Inorganic nitrogen in conventional ponds is controlled through the activity of algae. Algae photosynthesize CO_2 and water to produce sugars. To subsequently synthesize protein, algae have to take up inorganic nitrogen, preferably ammonium, from the water. The average C/N ratio in algae is about 7 (Redfield *et al.*, 1963) thus, for every 7 photosynthesized carbon atoms, one nitrogen atom is removed from the water and immobilized in the algal biomass. The N-removal capacity is linked to the primary productivity, which is about 3.5 g C m^{-2} day^{-1}, and thus constraint to about 0.5 g N m^{-2} day^{-1}. However, this mechanism fails on cloudy days or following algal crashes.

Intensive ponds are enriched with inorganic nitrogen, lowering the C/N ratio in the pond. Adding organic substrates with high carbon content raise the pond C/N ratio again. The carbon rich organic substrate is metabolized by bacteria that immobilize inorganic nitrogen to synthesize microbial proteins:

(**9**) $(CH_2O)_n + O_2 + NH_4 \rightarrow$ Microbial protein + CO_2
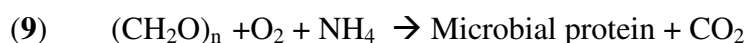
This process is basic and common to heterotrophic microorganisms that are naturally present in any pond. Adding the carbon rich substrate encourages microbial metabolism and growth, immobilizes inorganic nitrogen and serves as a means to control water quality (Avnimelech, 1999). Many carbon sources can be used. The criteria to select carbonaceous substrates should be its bio-availability, ability to be dispersed in the water and its cost. A readily bio-degradable substrate is preferable in very intensive systems. The substrate should be soluble or given in fine powdered form, so as to slow its sedimentation rate and to keep it suspended in the water as much as possible. Finally, one should select substrates that are not costly. Carbonaceous substrates such as molasses, cassava meal, wheat or other flour have been successfully used. It is possible to add carbonaceous substrates as an emergency measure in cases of an increase in inorganic nitrogen levels, e.g. after a period of cloudy days. An addition of 20-25 g carbonaceous substrate is needed to immobilize 1 g of inorganic nitrogen. A detailed discussion of the quantitative effects of C/N ratios is given by Avnimelech (1999).

In addition to water quality control, this process leads to the buildup of microbial proteins that can contribute to fish protein nutrition. During the last years evidence on the utilization of bacteria as a source of feed by fish and shrimp was rather intensively studied and published. It was found that tilapia common carp and shrimp can harvest bacteria and potentially utilize it as a source of protein (Beveridge *et al*., 1989, 1991; Avnimelech *et al*., 1989). To demonstrate it, Avnimelech *et al*. (1988) cultured fish in tanks fed mostly with cellulose powder and ammonium salt. Though tilapia cannot directly utilize cellulose, it fed on the bacteria that degraded the cellulose. The assimilation of the cellulose was demonstrated also through the use of ^{13}C enrichment (Avnimelech *et al*., 1989). Using tracer bacteria to measure bacteria ingestion Mattena *et al*. (1995) confirmed that tilapia and common carp can ingest bacteria in suspension. It was assumed that tilapia, as a filter feeder harvests bacterial flocs and digests the microbial protein. Shrimp are not typical filter feeders, yet it was found in a pioneering work conducted in the Oceanic Institute (Moss *et al*., 1992) that they grow faster in "green water" as compared to well water, apparently demonstrating that they do harvest suspended particles. Thompson, *et al* (2002) shows that the shrimp *Farfantepenaeus paulensis* feeds intensively on biofilms. Burford *et al*. (2004), evaluated protein uptake by shrimp growing in microbial dominated ponds using ^{15}N concluding that shrimp do utilize protein by harvesting bacterial flocs and that 18 to 29 % of the nitrogen uptake by the shrimp was from microbial flocs. These basic data led to the development of ponds where both feeding and water quality control are based upon the manipulation of microbial biomass.

Carbonaceous substrates can be added not just as an emergency means to reduce nitrogen accumulation but can be supplied as a part of the feed, adjusted in a way as to prevent ammonium release and to recycle most of the inorganic nitrogen not ingested by the fish, into microbial protein. It was shown, both theoretically and empirically (Avnimelech *et al*., 1994; McIntosh *et al*., 1999, 2001) that this goal is reached when feed protein level is reduced to about 20%. Normally, protein recovery in harvested fish or shrimp is about 25% of protein added with the feed (Avnimelech & Ritvo, 2003). Using low protein feed in microbial controlled ponds, protein utilization is almost doubled. Avnimelech *et al*., (1994) obtained 45% protein utilization with tilapia and reduced feed cost by 40% compared to control ponds. McIntosh found practically identical utilization in microbial dominated shrimp ponds in Belize aquaculture (McIntosh *et al*, 1999).

The creation of flocs, their composition and nutritive value is an essential feature of active suspension ponds. These flocs or aggregates are common in aquatic systems and are made of senescent organic matter, particulate and dissolved, algae, bacteria and protozoa sustained in an

extracellular polymeric substance (EPS) (Laspidou and Rittmann, 2002). Zimmermann-Timm (2002) reviewed the characteristics of aggregates in rivers. Such aggregates are abundant components of running water, yet they also exist in marine environment as marine snow (Boeckelmann *et al*, 2000). These aggregates are important components of the natural food web, because most predators of higher trophic levels are only able to eat small-sized organisms if they are attached or part of the aggregates. Aggregation usually depends upon the presence of EPS serving as a binding agent. An important factor in aggregate formation is the probability of collision between particles. Thus, the more bacteria and algae are present, the more aggregation one can expect. Turbulence and the presence of clay particles were reported as important factor in aggregation (Argaman and Kaufman, 1970; Avnimelech *et al*., 1992b).

The nutritional quality of bacteria aggregates largely depends on the quality of the trapped organic matter (particulate and dissolved) and the EPS which mainly consists of polysaccharides and proteins (Nielsen Per *et al*., 1997; Bura *et al*, 1998). Tacon *et al*., (2002) evaluated the feeding value of the microbial flocs. He found that they contain the essential amino acids in the right proportion. In addition, it was shown that the flocs contain vitamins and trace metals, making it possible to reduce the inclusion of these expensive additives in commercial feeds and by this to lower feed cost by about 25%.

The use of microbial assemblages as an essential link in the food web is very efficient way of transferring energy and feed elements. Bacteria utilize about 50% of metabolized feed to synthesis cells, as compared to about 10% utilization by higher organisms (Pauly and Christensen, 1995). This means that only 10% of the feed eaten by a higher organism is available to the next level in the food chain (fish or other) as compared to 50% in the case of direct microbes utilization.

The recycling of protein has important economical advantages as well as very important environmental benefits. First, by doubling protein utilization, nitrogen release to the environment is reduced. In addition, aquaculture is blamed of over-harvesting marine animal proteins as a feed source for cultivated species (Naylor *et al*., 2000). It was shown in the studies mentioned above, that by recycling proteins, the need for imported proteins can be cut 50%.

Conclusions

Aquaculture production systems are complex eco-systems containing high biomass of the target animal and ample nutrients and feed residues to support a complex array of organisms, algae, bacteria and protozoa as well as planktonic and benthic invertebrates. The role of algae as primary producers and as a part of the natural water quality control is widely accepted. Yet, algae are always accompanied by other organisms, a fact that is often neglected. Looking into the subject index of any aquaculture text book or proceedings, the term "algae" appears many times. Bacteria are mentioned at a much lower frequency.

It was shown here, that conventional ponds contain $10^6 - 10^7$ bacteria ml^{-1}. The bacterial organic carbon is in the range of 3-20 g C m^{-3}, and bacterial production is usually higher than algal production. Bacterial biomass in non-fed ponds is proportional to algae biomass, as bacteria feed upon soluble organics excreted from the algae cell and upon dead and senescing algae. Adding feed to the pond further increase microbial biomass. One kg feed generates about 125 g bacterial biomass.

Algae and bacteria necessarily coexist in the pond system, the algae as primary producers and bacteria mostly as decomposers, recycling nutrients in the pond. Algal biomass is limited by light intensity within the water while the heterotrophic bacterial biomass is limited by the supply of organic substrate. A heterotrophy ratio (HR) was proposed, defined as:

HR = Daily external C addition / Daily autotrophic C assimilation.

Ponds having about 1750 kg fish ha^{-1},or fed daily by a ration of about 35kg C ha^{-1}, have a HR of 1. When HR > 1, pond processes are mostly affected by bacteria. Moreover, even in ponds with low HR, there are zones, such as the sediment or surfaces accumulating debris, where heterotrophic activity dominates. Microbial activity affects water quality and cannot be neglected.

Microbial biomass and activity can be controlled to a much higher degree than our control on algae biomass, speciation and activity. Limiting water exchange leads to a higher accumulation of organic residues and thus raises microbial biomass. Bacterial metabolism is affected by the redox of the pond. Rate of organic matter degradation is about an order of magnitude higher under aerobic conditions as compared to anaerobic ones. Moreover, the decomposition products are different for aerobic vs. anaerobic conditions.

Controlling bacterial processes offers an interesting management tool for aquaculture. Addition of specific micro-organisms to grow out ponds is complicated and there are as yet no scientific proofs as to its success. Yet, the benefit of introducing external micro-organisms to small closed systems or short tern production units (hatcheries, nurseries) was proven. The control of microbial processes by adjusting the environmental conditions was shown to be a very potent means. Active suspension ponds (ASP) are ponds where high bacterial biomass is generated by limiting water exchange, raising organic substrates and microbial biomass. Adjusting C/N ratio in these ponds enables a reliable control of water quality and leads to an effective recycling of feed protein, up to roughly doubling protein utilization by fish.

In light of the important role of bacteria in pond systems, there is a need and justification to further study the nature of this population, its dynamics and interaction with the other components of the pond eco-system

Acknowledgements

References

Alabi, A.O., Cob, Z.C., Jones, D.A., Latchford, J.W., 1999. Influence of algal exudates and bacteria on growth and survival of white shrimp larvae fed entirely on microencapsulated diets. Aquaculture International 7, 137-158.

Alonso-Rodriguez, R., Paez-Osuna, F., 2003. Nutrients, phytoplankton and harmful algal blooms in shrimp ponds: a review with special reference to the situation in the Gulf of California. Aquaculture 219, 317-336.

Aota, Y., Nakajima, H., 2000. Mathematical analysis on coexistence conditions of phytoplankton and bacteria systems with nutrient recycling. Ecological Modelling 135, 17-31.

Argaman, Y.A., Kaufman, W.J., 1970. Turbulence and flocculation. ASCE, Journal of the Sanitary Engineering Division 92, 223-241.

Avnimelech, Y., Mokady, S., 1988. Protein biosynthesis in circulated fishponds. In: Pullin, R.S.V., Bhukaswan, T., Tonguthai, K. and Maclean, J.L., (eds). The Second International Symposium on tilapia in Aquaculture ICLARM., Manila, Philippines, ICLARM Conference Proceedings 15, pp 301-309.

Avnimelech, Y., Mokady, S., Schroeder, G.L., 1989. Circulated ponds as efficient bioreactors for single cell protein production. Israeli Journal of Aquaculture Research-BAMIDGEH 41, 58-66.

Avnimelech, Y., Diab, S., Kochba, M., 1992a. Control of nitrogen in intensive aerated and mixed ponds. Israeli Journal of Aquaculture Research-BAMIDGEH 44, 145-XX.

Avnimelech, Y., Mozes, N., Weber, B., 1992b. Effects of aeration and mixing on nitrogen and organic matter transformations in simulated fish ponds. Aquacultural Engineering 11, 157-169.

Avnimelech, Y., Kochva, M., Diab, S., 1994. Development of controlled intensive aquaculture systems with a limited water exchange and adjusted carbon to nitrogen ratio. Israeli Journal of Aquaculture Research-BAMIDGEH 46, 119-131.

Avnimelech, Y., Mozes, N., Diab, S., Kochba, M., 1995. Rates of organic carbon and nitrogen degradation in intensive fish ponds. Aquaculture 134, 211-216.

Avnimelech, Y., Kochva, M., Hargreaves, J.A., 1999. Sedimentation and resuspension in earthen fish Ponds. Journal of World Aquaculture Society 30, 401-409.

Avnimelech, Y., 1999. Carbon/nitrogen ratio as a control element in aquaculture systems. Aquaculture 176, 227-235.

Avnimelech, Y., Ritvo, G., 2003. Shrimp and fish pond soils: processes and management. Aquaculture 220, 549-567.

Azim, M.E. 2001. The potential of periphyton-based aquaculture production systems. Fish culture and Fisheries. PhD Thesis, Wageningen, The Netherlands, 219 pp.

Azim, M.E., Wahab, M.A., Biswas, P.K., Asaeda, T., Fujino, T., Verdegem, M.C. J., 2004. The effect of periphyton substrate density on production in freshwater polyculture ponds. Aquaculture 232, 441-453.

Balogh, K., Vörös, L., 1997. High bacterial production in hypertrophic shallow reservoirs rich in humic substances. Hydrobiologia 342-343, 63-70.

Beveridge, M.C. M., Begum, M., Frerichs, G.N., Millar, S., 1989. The ingestion of bacteria in suspension by tilapia Oreochromis niloticus. Aquaculture 81, 373-378.

Beveridge, M.C.M., Sikdar, P. K., Frerichs, G. N., Millar, S., 1991. The ingestion of bacteria in suspension by the common carp Cyprinus carpio L. Journal of Fish Biology 39, 825-831.

Bird, D.F., Kalff, J., 1984. Empirical relationships between bacterial abundance and chlorophyll concentration in fresh and marine waters. Canadian Journal of Fisheries and Aquatic Sciences 41, 1015-1023.

Boeckelmann, U., Manz, W., Neu Thomas, R., Szewzyk, U., 2000. Characterization of the microbial community of lotic organic aggregates ('river snow') in the Elbe River of Germany by cultivation and molecular methods. FEMS Microbiology Ecology. 33, 157-170.

Boyd, C.E., 1990. Water quality in ponds, Auburn University. 482 pp.

Boyd, C.E., 1991. Empirical modeling of phytoplankton growth and oxygen production in aquaculture ponds. In: D.E. Brune and J.R. Tomasso (Eds), Aquaculture and water quality. World Aquaculture Society, Baton rouge, LA, USA pp 363-395.

Boyd, C.E., 1995. Chemistry and efficacy of amendments used to treat water and soil quality imbalance in shrimp ponds. shrimp farming, Aquaculture'95, Baton Rouge, Louisiana, World Aquaculture Society, 1995.

Bower, C.E., Turner, D.T., 1981. Accelerated nitrification in new seawater culture systems: Effectiveness of commercial additives and feed media from established systems. Aquaculture 24, 1-9.

Brune, A., Frenzel, P., Cypionka, H., 2000. Life at the oxic–anoxic interface: microbial activities and adaptations. FEMS Microbiology Reviews 24, 691-710.

Bura, R., Cheung, M., Liao, B., Finlayson, J., Lee, B.C., Droppo, I.G., Leppard, G.G., Liss, S.N., 1998. Composition of extracellular polymeric substances in the activated sludge floc matrix. Water Science and Technology 37, 325-333.

Burford, M., 1997. Phytoplankton dynamics in shrimp ponds. Aquaculture Research 28, 351-360.

Burford, M.A., Peterson, E.L., Baiano, J. C. F., Preston, N. P., 1998. Bacteria in shrimp pond sediments: their role in mineralizing nutrients and some suggested sampling strategies. Aquaculture Research 29, 843-849.

Burford, M.A., Thompson, P.J., McIntosh, R.P., Bauman, R.H., Pearson, D.C., 2003a. Nutrient and microbial dynamics in high-intensity, zero-exchange shrimp ponds in Belize. Aquaculture 219, 393-411.

Burford, M.A., Costanzo, S.D., Dennison, W.C., Jackson, C.J., Jones, A.B., McKinnon, A.D., Preston, N.P., Trott, L.A., 2003b. A synthesis of dominant ecological processes in intensive shrimp ponds and adjacent coastal environments in NE Australia. Marine Pollution Bulletin 46, 1456-1469.

Burford, M.A., Thompson, P.J., McIntosh, R.P., Bauman, R.H., Pearson, D.C., 2004. The contribution of flocculated material to shrimp (Litopenaeus vannamei) nutrition in a high-intensity, zero-exchange system. Aquaculture 232, 525-537.

Carrasquero-Verde, J.R., 1999. Role of associated bacteria in Heterosigma carterae toxicity to salmonids. Aquatic Toxicology 45, 19-34.

Chang, J., Shiah, F.-K., Gong, G.-C., Chiang, K.P., 2003. Cross-shelf variation in carbon-to-chlorophyll a ratios in the East China Sea, summer 1998. Deep Sea Research Part II: Topical Studies in Oceanography 50, 1237-1247.

Cloern, J.E., Grenz, C., Vidergar-Lucas, L., 1995. An empirical model of the phytoplankton chlorophyll : carbon ratio the conversion factor between productivity and growth rate. Limnology anOceanography 40, 1313–1321.

Cole, J.J., 1982. Interactions between bacteria and algae in aquatic ecosystems. Annual review of ecology and systematics 13, 291-314.

Coveney, M.F., Cronberg, G., Enell, M., Larsson, K., Olofsson, L., 1977. Phytoplankton, zooplankton and bacteria standing crop and production relationships in an eutrophic lake. Oikos 29, 5–21

Coveney, M.F., Wetzel, R.G., 1995. Biomass, production, and specific growth rate of bacterioplankton and coupling to phytoplankton in an ologotrophic lake. Limnology and Oceanography 40, 1187-1200.

Deeds, J.R., Terlizzi, D.E., Adolf, J.E., Stoecker, D.K., Place, A.R., 2002. Toxic activity from cultures of Karlodinium micrum (Gyrodinium galatheanum) (Dinophyceae)--a dinoflagellate associated with fish mortalities in an estuarine aquaculture facility. Harmful Algae 1, 169-189.

Del Giorgio, P.A., Peters, R.H., 1993. Balance between phytoplankton production and plankton respiration in lakes. Canadian Journal of Fisheries and Aquatic Science 50, 282-289.

Duvall, R.J., Anderson, L. W.J., Goldman, C.R., 2001. Pond enclosure evaluation of microbial products and chemical algicides used in lake management. Journal of Aquatic Plant Management 39, 99-106.

Fitzsimmons 2002. Tilapia aquaculture in recirculating systems. Aquacultural Engineering. Society News. 6, 2-4

Fukami, K., Nishijima, T., Ishida, Y., 1997. Stimulative and inhibitory effects of bacteria on the growth of microalgae. Hydrobiologia 358, 185-191.

Garg, S.K., Bhatnagar, A., Narula, N., 1998. Application of Azotobacter enhances pond productivity and fish biomass in still water ponds. Aquaculture International 6, 19-231.

Garriques, D., Arevalo, F.D., 1995. An evaluation of the production and use of a live bacterial isolate to manipulate the microbial flora in the commercial production of Penaeus vannamei postlarvae in Ecuador. In: C.L. Browdy and J.S. Hopkins, Editors, Swimming through Troubled Water. Proceedings of the Special Session on Shrimp Farming, Aquaculture '95, World Aquaculture Society, Baton Rouge, USA (1995), pp. 53–59.

Gasol, J.M., Duarte, C.M., 2000. Comparative analyses in aquatic microbial ecology: how far do they go? FEMS Microbiology Ecology 31, 99-106.

Gatesoupe, F.J., 1999. The use of probiotics in aquaculture. Aquaculture 180, 147-165.

Gaudy, A. F. Jr., Gaudy, E. T., 1980. Microbiology for Environmental Scientists and Engineers. New York, McGraw-Hill Publ. 127 pp.

Glibert, P.M., Landsberg, J.H., Evans, J.J., Al-Sarawi, M.A., Faraj, M., Al-Jarallah, M.A., Haywood, A., Ibrahem, S., Klesius, P., Powell, C., Shoemaker, C., 2002. A fish kill of massive proportion in Kuwait Bay, Arabian Gulf,2001: the roles of bacterial disease, harmful algae, and eutrophication. Harmful Algae 1, 215–231.

Golz, W.J., Rusch, K.A., Malone, R.F. 1999. Modeling the major limitations on nitrification in floating-bead filters. Aquacultural Engineering 20, 43-61.

Gullian, M., Thompson, F., Rodriguez J., 2004. Selection of probiotic bacteria and study of their immunostimulatory effect in Penaeus vannamei. Aquaculture 233, 1-14.

Gross, A., Boyd, C.E , Wood, C.W., 2000. Nitrogen transformations and balance in channel catfish ponds. Aquacultural Engineering 24, 1-14.

Hagopian, D.S., Riley, J.G., 1998. A closer look at the bacteria of nitrification. Aquacultural Engineering 18, 223-244.

Hansson, L.A., Bronmark, C., Nystrom, P., Greenberg, L., Lundberg, P., Nilsson, P.A., Persson, A., Pettersson, L.B., Romare, P., Tranvik, L.J., 1998. Consumption patterns, complexity and enrichment in aquatic food chains. Proceedings of the Royal Society of London Series B Biological Sciences. May 26, pp. 901-906.

Hargreaves, J.A., 1998. Nitrogen biogeochemistry of aquaculture ponds. Aquaculture 166, 181-212.

Hargreaves, J.A., Tucker, C. S., 2003. Defining loading limits of static ponds for catfish aquaculture. Aquacultural Engineering 28, 47-63.

Hepher, 1962

Hold, G., Smith, E., Rappe, M., Maas, E., Moore, E., Stroempl, C., Stephen, J., Prosser, J., Birkbeck, T., Gallacher, S., 2001. Characterisation of bacterial communities associated with toxic and non-

toxic dinoflagellates: Alexandrium spp. and Scrippsiella trochoidea. Microbial Ecology 37, 161-173.

Hopkins, J. S., Hamilton, R. D., II, Sandifer, P. A., Browdy, C. L., Stokes, A. D., 1993. Effect of water exchange rate on production, water quality, effluent characteristics and nitrogen budgets of intensive shrimp ponds. Journal of World Aquaculture Society 24, 304-320.

Irianto, A., Austin, B., 2002. Probiotics in Aquaculture. Journal of Fish Diseases 25, 633-642.

Jimenez-Montealegre, R., 2001. Nitrogen transformation and fluxes in fish ponds: a modeling approach. Fish Culture and Fisheries. PhD Thesis, Wageningen, The Netherlands, 185 pp

Kalff, J., Knoechel, R., 1978. Phytoplankton and their Dynamics in Oligotrophic and Eutrophic Lakes. Annual Review of Ecology and Systematics 9, 475-495.

Laspidou, C., Rittmann, B., 2002. A unified theory for extracellular polymeric substances, soluble microbial products, and active and inert biomass. Water Research 36, 2711-2720.

Lee, C.S., O'Bryen P. 2002. Microbial approaches to aquatic nutrition within environmentally sound aquaculture production systems. World Aquaculture Society, Baton Rouge, LA, USA, 187 pp.

Maeda, M. 1999. Microbial processes in aquaculture. Society for the Biological Creation and Enhancement of the Aquatic Environment. BIOCREATE Press. London, 102 pp.

Matena, J., Simek, K., Fernando, C.H., 1995. Ingestion of suspended bacteria by fish: A modified approach. Journal of Fish Biology 47, 334-336.

McIntosh, R.P., Drennan, D.P., Bowen, B.M., 1999. Belize Aquaculture: Development of an intensive sustainable, environmentally friendly shrimp farm in Belize. In: 5th Simposio Centroamericano de Acuacultura, San Pedro Sula, Honduras, March 3 – 5th 1999, pp. 85-99.

McIntosh, D., Samocha, T.M., Jones, E.R., Lawrence, A.L., McKee, D.A., Horowitz, S., Horowitz, A., 2000. The effect of a commercial bacterial supplement on the high-density culturing of Litopenaeus vannamei with a low-protein diet in an outdoor tank system and no water exchange. Aquacultural Engineering 21, 215-227.

McIntosh P.R. 2001. Changing paradigms in shrimp farming: V. establishment of heterotrophic bacterial communities. Global aquaculture Advocate 4, 53-58.

Meijer, L. E., Avnimelech, Y., 1999. On the use of micro-electrodes in fish pond sediments. Aquacultural Engineering 21, 71-83.

Milstein, A., Avnimelech, Y., Zoran, M., Joseph, D., 2001a. Growth performance of hybrid bass and hybrid tilapia in conventional and active suspension intensive ponds. Israeli Journal of Aquaculture Research-BAMIDGEH 53, 147-157.

Milstein, A., Zoran, M., Kochba, M., Avnimelech, Y., 2001b. Effect of different management practices on water quality of intensive tilapia culture systems in Israel. Aquaculture International 9, 133-152.

Monod J. 1949. The growth of bacterial cultures. Annual Review of Microbiology 3, 371-394.

Moriarty, D.J.W., 1986. Bacterial Productivity in ponds used for Culture of Penaeid Prawns. Microbial Ecology 12, 259-269.

Moriarty, D.J.W., 1997. The role of microorganisms in aquaculture ponds. Aquaculture 151, 333-349.

Moriarty, D.J.W., 1998. Control of luminous Vibrio species in penaeid aquaculture ponds. Aquaculture 164, 351-358.

Moss, S.M., Pruder, G.D., Leber, K.M., Wyban, J.A., 1992. The relative enhancement of Penaeus vannamei growth by selected fractions of shrimp pond water. Aquaculture 101, 229-239.

Naylor, R.L., Goldburg, R.J., Primavera, J.H., Kautsky, N., Beveridge, M.C.M., Clay, J., Folke, C., Lubchenco, J., Mooney, H., Troell, M., 2000. Effect of aquaculture on world fish supplies. Nature 405, 1017-1024.

Negri, A.P., Jones, G.J., 1995. Bioaccumulation of paralytic shellfish poisoning (PSP) toxins from the cyanobacterium Anabaena circinalis by the freshwater mussel Alathyria condola. Toxicon 33, 667-678.

Negri, A.P., Bunter, O., Jones, B., Llewellyn, L., 2004. Effects of the bloom-forming alga Trichodesmium erythraeum on the pearl oyster Pinctada maxima. Aquaculture 232, 91-102.

Nielsen Per, H., Jahn, A., Palmgren, R., 1997. Conceptual model for production and composition of exopolymers in biofilms. Water Science and Technology 36, 11-19.

Nogami, K., Maeda, M., 1992. Bacteria as biocontrol agents for rearing larvae of the crab Portunus trituberculatus. Canadian Journal of Fisheries and Aquatic Sciences. 49, 2373-2376.

Pauly, D., Christensen, V., 1995. Primary production required to sustain global fisheries. Nature 374 255-257.

Perez-Linares, J., Cadena, M., Rangel, C., Unzueta-Bustamante, M.-L., Ochoa, J.-L., 2003. Effect of Schizothrix calcicola on white shrimp Litopenaeus vannamei (Penaeus vannamei) postlarvae. Aquaculture 218, 55-65.

Prabhu, N.M., Nazar, A.R., Rajagopal, S., Khan, S.A., 1999. Use of probiotics in water quality management during shrimp culture. Journal of Aquaculture in the Tropics. 14, 227-236.

Pruder, G.D. 2001. Biosecure Zero-Exchange System: Maturation and Growout of Marine Animals. In: Biotechnology-Aquaculture Interface: The Site of Maximum Impact Workshop. ARS-The Oceanic Institute Workshop, National Conservation and Training Center, U.S. Fish and Wildlife Service, Shepherdstown, West Virginia, March 5-7, 2001.

Queiroz, J.F., Boyd, C.E., 1998. Effects of a bacterial inoculum in channel catfish ponds. Journal of World Aquaculture Society 29, 67-73.

Ram, N., Zur, O., Avnimelech, Y., 1982. Microbial changes occurring at the sediment-water interface in an intensively stocked and fed fish pond. Aquaculture 27, 63-72.

Redfield, A.C., Ketchum, B.H., Richards, F.A., 1963. The influence of organisms on the composition of sea water. In M.N. Hill (Ed.), The Sea, Vol. II. Willey –Interscience N.Y. pp 26-77.

Revsbech, N. P., Jorgensen, B.B., Balckburn, T.H., Cohen, Y. 1983. Micro-electrodes studies of the photosynthesis and O2, H2S and pH profiles of a microbial mat. Limnology and Oceanography 28,1062-1074.

Ringo, E., Birkbeck, T.H., 1999. Intestinal microflora of fish larvae and fry. Aquaculture Research 30, 73-93.

Ritvo, G., Kochba, M., Avnimelech Y. 2004. The effects of common carp bioturbation on fishpond bottom soil. Aquaculture 242, 345-356

Scheffer, M., 1998. Ecology of shallow lakes, Chapman & Hall 357 pp.

Schroeder, G.L., 1978. Autotrophic and heterotrophic production of micro-organisms in intensely-manured fish ponds, and related fish yields. Aquaculture 14, 303-325.

Schroeder, G., Hepher, B., 1979. Use of agricultural and urban wastes in fish culture,. In T.V.R. Pillay and W.A. Dill (Editors), Advances in Aquaculture. Fishing News Books Ltd., Farnham, Surrey, England. pp. 487–489

Simon, N., Biegala, I., Smith, E., Vaulot, D., 2002. Kinetics of attachment of potentially toxic bacteria to Alexandrium tamarense. Aquatic Microbial Ecology 28, 249-256.

Sommaruga, R., 1995. Microbial and classical food webs: A visit to a hypertrophic lake. FEMS Microbiology Ecology 17, 257-270.

Sommaruga, R., Robarts, R.D., 1997. The significance of autotrophic and heterotrophic picoplankton in hypertrophic ecosystems. FEMS Microbiology Ecology 24, 187-200.

Sonnenholzner, S., Boyd, C.E., 2000. Managing the accumulation of organic matter deposited on the bottom of shrimp ponds--do chemical and biological probiotics really work. Journal of World Aquaculture Society 31, 24-28.

Strauss, E.A., Lamberti, G.A., 2000. Regulation of nitrification in aquatic sediments by organic carbon. Limnology and Oceanography 45, 1854-1859.

Tacon, A.G. J., Cody, J.J., Conquest, L.D., Divakaran, S., Forster, I.P., Decamp, O.E., 2002. Effect of culture system on the nutrition and growth performance of Pacific white shrimp Litopenaeus vannamei (Boone) fed different diets. Aquaculture Nutrition 8, 121-137.

Teichert-Coddington, D., Green, B., 1993. Comparison of two techniques for determining community respiration in tropical fish ponds. Aquaculture 114, 41-50.

Thingstad,T.F., Pengerud, B., 1985. Fate and effect of allochthonous organic material in aquatic microbial ecosystems. An analysis based on chemostat theory. Mar. Ecol. Prog. Ser., 21, 47–62.

Thoman, E.S., Ingall, E.D., Davis, D.A., Arnold, C.R., 2001. A nitrogen budget for a closed, recirculating mariculture system. Aquacultural Engineering 24, 195-211.

Thompson, F.L., Abreu, P.C., Wasielesky, W., 2002. Importance of biofilm for water quality and nourishment in intensive shrimp culture. Aquaculture 203, 263-278.

Treasurer, J.W., Hannah, F., Cox, D., 2003. Impact of a phytoplankton bloom on mortalities and feeding response of farmed Atlantic salmon, Salmo salar, in west Scotland. Aquaculture 218, pp. 103-113.

Tucker, S.C., Martin, F.J., 1991. Environment – related off-flavors in fish. In: Brune, D.E. and Tomasso, J.R (Eds), Aquaculture and water quality. World Aquaculture Soc., Baton rouge, LA, USA pp 133-179.

van Dam, A., Beveridge, M.C.M., Azim, M.E., Verdegem, M., 2002. The potential of fish production based on periphyton. Reviews in Fish Biology and Fisheries 12, 1-31.

van Keulen, H., Seligman, N.G., 1987. Simulation of water use, nitrogen nutrition and growth of spring wheat, Pudoc, Wageningen, 310 pp.

Verdegem, M.C.J., van Dam, A.,Cabarcas-Nuñez, A.A., Oprea., L., 2000. Bio-energetic modeling of growth and waste production of nile tilapia (Oreochromis niloticus L.) in recirculation systems.In: K. Fitzsimmons and J. Carvalho (Eds.) Proceedings of the 5th International Symposium on Tilapia in Aquaculture, 3-7 September Rio de Janeiro, Brazil, pp.368-381.

Verschuere, L., Rombaut, G., Sorgeloos, P., Verstraete, W., 2000. Probiotic bacteria as biological control agents in aquaculture. Microbiology and Molecular Biology Reviews 64, 655-671.

Westrich, J.T., Berner, R.A., 1984. The role of sedimentary organic matter in bacterial sulfate reduction: the G model tested. Limnology and Oceanography 29, 236–249.

Wetzel, R.G., 1975. Limnology. , W.B. Saunder, Philadelphia and London. 743 pp.

Wetzel, R.G., 2001. Limnology. Lake and river ecosystems, Academic press. 1006 pp.

Yusoff, F.M., McNabb, C.D., 1989. Effects of nutrient availability on primary productivity and fish production in fertilized tropical ponds. Aquaculture 78, 303-319.

Zimba, P.V., Khoo, L., Gaunt, P.S., Brittain, S., Carmichael, W.W., 2001. Confirmation of catfish, Ictalurus punctatus (Rafinesque), mortality from Microcystis toxins. Journal of Fish Disease 24, 41-47.

Zimmermann-Timm, H., 2002. Characteristics, dynamics and importance of aggregates in rivers. International Review of Hydrobiology 87, 197-240.

Effect of C/N ratio and oxic conditions on organic matter decomposition in lab-scale intensive fresh water systems

Torres-Beristain[a], B., Pilarcyzk[b], M., Verdegem[a], M.C.J.

and Verreth[a], J.A.J.

[a] Fish Culture and Fisheries Group, Wageningen Institute of Animal Sciences, P.O Box 338, 6700 AH Wageningen, Wageningen University, The Netherlands.
[b] Polish Academy of Sciences, Institute of Ichthybiology and Aquaculture, 43-520 Chybie, Poland.

Abstract

The accumulation of organic matter is a common problem in aquaculture ponds. The main source of organic matter in intensive fish ponds is the formulated feed. Uneaten feed and feed derived metabolites accumulate in the sediment where they are decomposed under aerobic or anaerobic conditions. In the present study lab-scale systems were used to study the microbial decomposition of formulated feeds. To avoid interference with other organism, zooplankton and phytoplankton were excluded from the system. The aim of this study was to evaluate the decomposition of 23 and 49% protein diets under aerobic and anaerobic conditions and to monitor changes in water quality. The decomposition of the fish feeds was registered in terms of CO_2 production and COD changes in the microcosms. Decomposition of organic nitrogen was followed based on Kjeldhal nitrogen, NH_4^+, NO_3^- and NO_2^- measurements. For both tested diets the amount of carbon mineralized was influenced by the aerobic/anaerobic conditions. The remaining carbon in the anaerobic treatments was 3.2 times higher than the amount remaining in the aerobic treatments. The decomposition rate constants for carbon under aerobic and anaerobic conditions were 0.26 and 0.06 per day, respectively. For nitrogen the ammonification rate constants under aerobic and anaerobic conditions were 0.03 and 0.02 per day, respectively. The C/N ratio of the organic matter decreased over time in all treatments and this decrease was faster and sharper in aerobic than in anaerobic environments. Even though ammonia was available very limited nitrification was observed, most likely due to substrate inhibition by high ammonia levels.

1.   Introduction

In intensive and semi-intensive pond culture, formulated feed constitutes the main nutrient input (Hargreaves, 1998) and are the most expensive production cost (Sevilleja, 1985). Cultured animals retain on average 30-50% of the feed, the rest contributing directly or indirectly to the nutrient load of water and sediment (Naylor *et al*., 2000). The accumulated organic matter will decompose mainly in the sediment because aquaculture ponds are shallow and the residence time of the particles in the water is short (Connolly and Coffin, 1995). Aerobic decomposition will occur as long as oxygen remains available, after that, anaerobic conditions will take over. The oxygen only penetrates the top 0.01 – 1 mm layer of the sediment, below that anoxic conditions prevail (Meijer and Avnimelech, 1999). Both aerobic

and anaerobic decomposition pathways coexist in aquaculture ponds, however pond managers normally try to stimulate aerobic decomposition and to minimize the development of anoxic conditions.

Healthy water quality conditions are crucial for successful culture. Poor water quality causes stress, reduced growth, and increases the susceptibility to disease and mortality (Conte, 2004). In intensive ponds the principal factor affecting water quality is the amount of feed supplied and the subsequent release of metabolites and decomposition products (Milstein, 1990). Management practices like aeration, water exchange or sediment removal are aimed at diminishing the impact of these metabolites and decomposition products on water quality. A major concern for water quality management is the accumulation of NH_4^+ and NO_2^- as a result of gill excretion and the decomposition of nitrogen-rich wastes (Avnimelech $et\ al.,$ 1994). The dietary protein is the principal source of these inorganic nitrogen species. The amount of crude protein in formulated feeds is usually higher that 20% and is normally 30-45% for carnivorous species (Tacon $et\ al.$, 1995).

Finding a right balance between accumulation and decomposition of organic matter in fish ponds while maintaining favorable oxygen levels for culture is a common problem in aquaculture pond management. Previous studies concentrated on the budget, fluxes or transformations of nutrients in fish ponds (Kochba $et\ al.$, 1994, Jimenez-Montealegre, 2001; Jackson $et\ al.$, 2003). Studying the decomposition of fish feeds in ponds is difficult because it is not possible to distinguish between the supplied feeds and natural produced organic matter in ponds. Because in intensively managed ponds formulated feeds are the principal nutrient input, it is interesting to get insight in the decomposition of feeds under aerobic and anaerobic conditions, as well as on the effect of dietary protein level on the decomposition process. Therefore, in the present study, the decomposition of organic matter was monitored in lab-scale microcosm that simulated the daily feed addition to intensively managed ponds.

2. Materials and Methods

The decomposition of fish feed was monitored during 49 days in 2-l microcosms. Daily feed loading rates were similar to application rates used in intensive fish ponds, assuming a biomass of 2 kg m^{-3} that is fed at 2% body weight. The treatments used in this experiment are given in Table 1. A 23 and 49 % protein diet were each tested under aerobic and anaerobic conditions, resulting in 4 treatments that were carried out in duplo. Each 2-l microcosm unit was filled with filtrated fresh water (0,2 µm mesh filter) and inoculated with bacteria taking from the biofilter of a recirculation system stocked with tilapia for several years. To create aerobic and anaerobic conditions, pure O_2 or pure N_2 gas was led into the flasks at 2 ml minute^{-1} and a pressure below 2 atmospheres. The daily feeding rate was 40 mg l^{-1}·d^{-1} from the start till day 20 and due to the high amount of ammonia produced the feed rate was reduce 50% from day 20 to 34. From day 35 till 49 the initial feeding rate of 40 mg l^{-1}·d^{-1} was resumed.

Table 1. Description of treatments, considering the supply of a 23 or 49% protein diet, and bubbling O_2 of N_2 gas through the microcosms.

Treatment	Symbol	Oxygen Circumstances	Diet treatment (C/N ratio)
aerobic-low protein	O_2LP	Aerobic	23% protein (12.8)
aerobic-high protein	O_2HP	Aerobic	49% protein (6.3)
anaerobic-low protein	N_2LP	Anaerobic	23% protein (12.8)
anaerobic-high protein	N_2HP	Anaerobic	49% protein (6.3)

2.1 Experimental Unit

The components of each microcosm unit are presented in Fig 1. The unit consisted of a high-pressure gas container (O_2 or N_2) connected to a mass control meter (Brooks Instruments, The Nederlands) and to a 2-l glass flask used as bioreactor. All the connections (3 mm$_{OD}$) and connectors were air-tight and made from stainless steel. The reactor was placed in a uniformly mixed 22±0.1 ^{o}C water bath. A magnetic stirrer in each bioreactor kept the solids in full suspension. The gas outlet from the unit was connected with 3-mm$_{ID}$ elastic Tygon® pipe to a 250 ml test tube filled with 100 ml 1.0 M NaOH to trap CO_2. For the anaerobic microcosms an additional 250 ml test tube to trap H_2S in a 5.5% zinc acetate solution was incorporated

before the CO_2 trap. When needed, a sample could be taken from the gas outlet to check for methane formation by using gas chromatography.
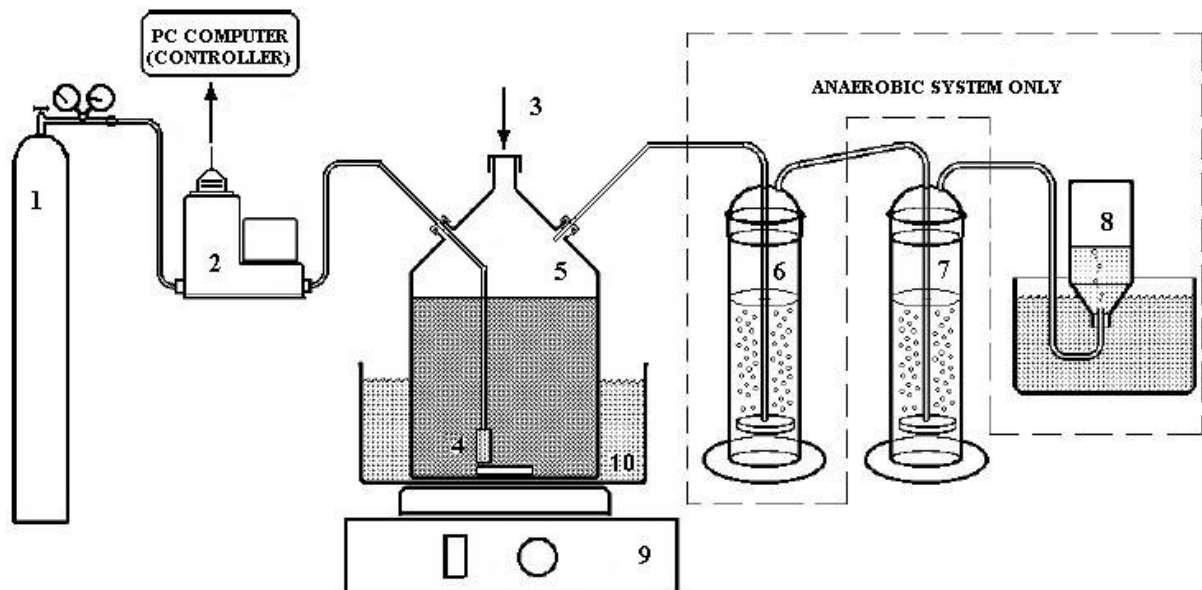

Figure 1. Experimental unit scheme: 1 - pressure gas (oxygen/nitrogen) container; 2- mass flow control meter ; 3 - sampling port; 4 - air stone; 5 - flask with microcosm; 6 - H_2S trap; 7 - CO_2 trap; 8 - gas sampling bottle; 9 - magnetic stirrer; 10 - thermoregulated water bath. No H_2S trap was incorporated in the aerobic units.

2.2 Water quality parameters

Water quality parameters which can be used as indicators for organic matter decomposition including pH, redox, inorganic N species, CO_2 and COD were measured. The pH measurements were daily taken using a WTW pH-meter model pH325 (WTW, Germany). Redox potential (ORP) was taken using a SenTix ORP electrode and pH325 pH-meter (WTW, Germany). Analysis of 10 ml of sample for NH_4^+, NO_3^- , NO_2^- , CO_2 and Lowry protein were done using SKALAR auto analyzer procedures (Skalar, San plus system). Ammonia nitrogen was determined by the modified Berthelot reaction with indophenol-blue development. The color intensity was measured at 660 nm. Nitrite was diazotized with sulfanilamide coupled with α-naphtylenediamine dihydrochloride, after which the absorbance was measured at 540 nm. Nitrate determination was based on a reduction of nitrate to nitrite and measured using the same method as for nitrite (APHA, 1998). CO_2 measurements were done using a bicarbonate determination using phenolphthalein as a pH indicator, and measuring the color decrease at 550 nm. Soluble proteins were measured using the Lowry procedure. The water sample was hydrolyzed with an alkaline copper solution, the resulting

hydrolase reacts with folin-cooiocalture phenol reagent producing a blue color that was measured at 660 nm (Lowry *et al.*, 1951). Total nitrogen was determined using the macro Kjeldahl method (Tecator, Germany) (APHA, 1998). Chemical Oxygen Demand (COD) was determined by color development through oxidation of the samplewith sulfuric acid and potassium-dichromate at 150 ^{o}C according to 5220 method (APHA, 1998). H_2S gas was trapped in a 5.5% zinc acetate and 1% sodium acetate solution. The concentration of sulfides was measured using the methylen blue method (APHA, 1998). The outlet gas of the anaerobic treatment was trapped in 200 ml glass bottles that were kept sealed until the methane concentration was measured with gas chromatography. The chromatograph (Carlo Erba Instruments) had a flame ionisation detector (FID) and used a 6 m × 3.18 mm (inner diameter) glass column, packed with Porapak Q (50/80 mesh). The temperature of the detector and injector was 150°C. The carrier gas used was N_2 (flow 20 ml min^{-1}). The increase in turbidity was monitored by measuring the optical density (OD) with a spectrophotometer at 600 nm according to Begot *et al.* (1996). Each sampling day the water that was removed from the microcosm for chemical analysis was replaced with fresh water. A dilution factor based on volume of water refreshment was calculated for each sampling day.

2.3 Statistical analysis

Differences among the four treatments were tested using split-plot ANOVA with treatments as the main factor and time as the sub-factor for redox, ammonia, nitrite, Lowry protein, total ammonia nitrogen (TAN) and COD (Gomez and Gomez, 1984). Significant differences between treatment means were based on Tukey test. A separate split-plot ANOVA was carried out with gas type and protein level as main factors and time as sub-factor, to determine the effect of gas (aerobic or anaerobic conditions) and dietary protein on the same parameters. A 5% probability level was applied for all the tests. All the analyses were done using statistical package SAS 6.12 (SAS Institute, Cary, NC 27513, USA).

Table 2. Physico-chemical parameters ANOVA and mean multicomparisons (Tukey test).

Variable	TREATMENTS				Standard error	Significance Level[1]			
	O_2LP	O_2HP	N_2LP	N_2HP		Oxic-anoxic	Diet	Oxic-anoxic *Diet	time
Redox	221 [a]	203 [a]	-313 [b]	-365 [b]	7.5	***	**	ns	***
NH_4-N (mg l^{-1})	12.2 [b]	31.4[a]	13.1 [b]	31.4 [a]	0.75	ns	***	ns	***
NO_3-N (mg l^{-1})	0.98[b]	2.21[a]	-	-	0.05	***	***	***	***
Protein (Lowry) (mg l^{-1})	31 [b]	48 [ab]	69 [ab]	117 [a]	15	***	***	**	***
Kjeldhal nitrogen (mg l^{-1})	192 [b]	396 [a]	192 [b]	359 [a]	12	ns	***	ns	***
Chemical Oxygen Demand (COD) (mg l^{-1})	257 [b]	274 [b]	786 [a]	782 [a]	18	***	ns	ns	***
CO_2 production (mg l^{-1})	978 [a]	850 [a]	321 [b]	295 [b]	59	***	ns	ns	***

[1] Significance level ns = not significant, **= $P \leq 0.01$, ***= $P \leq 0.001$. Means in the same row with one letter in common are not significantly different at $P \leq 0.05$; a>b>c.

3. Results

The results of the split-plot analysis on the physico-chemical parameters redox, NH_4^+-N, NO_3^--N, dissolved protein, Kjeldhal nitrogen and chemical oxygen demand, are shown in Table 2. At the beginning of the experiment the zinc-acetate trap was placed after the NaOH trap and did not capture H_2S. Therefore, the traps were switched and H_2S detection started on day 28 until the end of the experiment, reaching cumulative values as high as 6 mg l^{-1}. The diet type did not influence the H_2S results significantly. Methane was detected in the anaerobic treatments reaching a production of around 30 µg l^{-1} d^{-1} at the end of the experiment.

3.1 Fish feed proximate composition

The proximate composition of the low (23%) and high protein diet (49%) are shown in Table 3. Using carbon percentages of 72 for fat, 53 for protein, and 43 for carbohydrates (Henze *et al.*, 2002), the total carbon content in each diet was calculated. The carbon input of the two diets was very similar with 408 mg C g feed^{-1} in the 23% protein diet and 418 mg C g feed^{-1} in the 49% protein diet.

Table 3. Proximate composition of the experimental diets

Fish feed	DM g kg^{-1}	Fat[1] g kg^{-1}	Protein[1] g kg^{-1}	Ash[1] g kg^{-1}	Carbohydrates[2] g kg^{-1}	carbon content[3] (mg C g feed^{-1})
23 % protein content	905.9	41.7	232.7	40.4	591.0	407.7
49 % protein content	894.4	104.7	485.2	105.5	199.1	418.1

[1] based on dry weight
[2] Calculated as difference between dry matter (100%) and the sum of fat, protein and ash.
[3] Calculated according to Henze *et al* (2002), as explained in the text.

3.2 Optical density and pH

The increase in optical density over time by treatment is given in Figure 2, and was very similar between replicate treatments, as shown by the small standard errors.
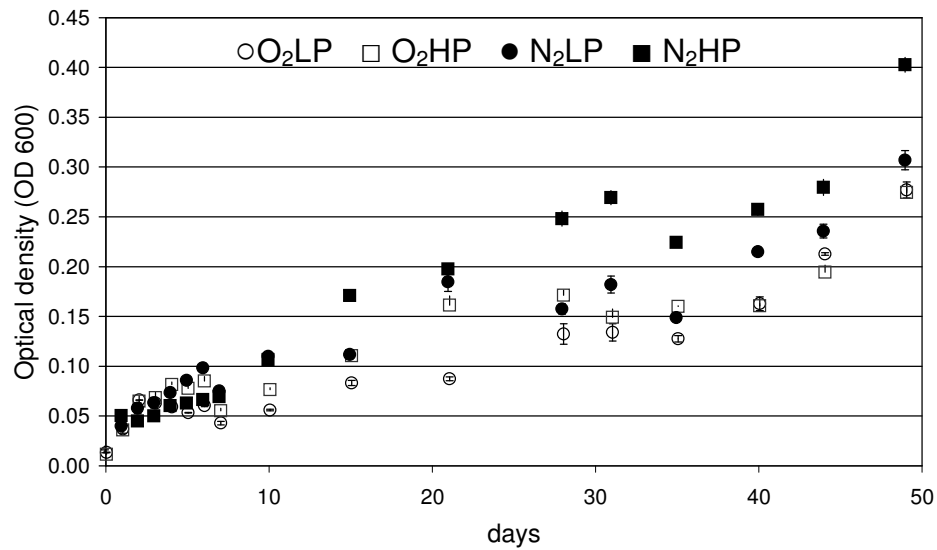
Figure 2.Optical density development (bars indicate standard error).

Daily pH measurements are given in Figure 3. The pH decreased in the aerobic treatments from 8 to 7 during the first 10 days. From then onwards until the end of the experiment the values remained relatively stable, varying between 7 and 7.5. The pH in the anaerobic treatments was influenced by diet type. The pH value in treatment N_2HP decreased from 7 on day 10 to 6 on day 21, and from then onwards remained relatively stable between pH 6.4-6.8 until the end of the experiment. In treatment N_2LP the pH dropped more reaching a level of 5 on days 13 and 26. Between day 22 and 49 the pH fluctuated around 6.
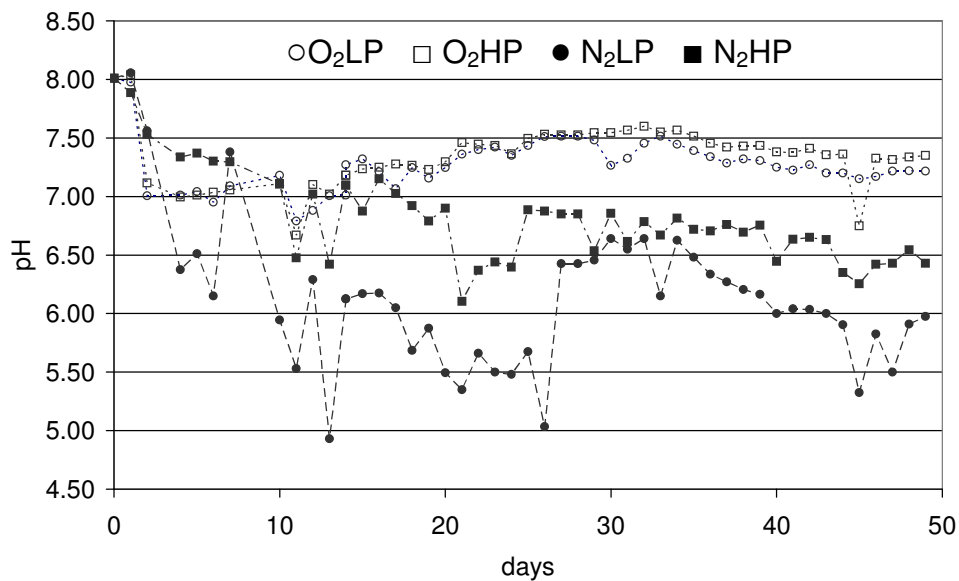
Figure 3. The average pH per treatment. No standard errors are given.

3.3 Redox

The average redox potential in the various treatments is plotted in Figure 4. Constant redox potential values were reached during the experiment. After the 5th day the anaerobic treatments reached complete anaerobic conditions with an average redox potential of -399±78.7 mV. In the aerobic microcosms the mean redox potential was 213 ± 40.0 mV. The redox potential was highly different between the aerobic and anaerobic treatments (P < 0.001). In the high protein diets the redox potential was lower than in the low protein diets (P < 0.01).

3.4 CO_2 production

Significantly more (P < 0.01) CO_2 was produced in the aerobic microcosms than in the anaerobic ones (Table 2). On average, 2366 mg CO_2 was produced in the aerobic microcosms compared to 872 mg CO_2 in the anaerobic microcosms, equaling a 2.7 times higher CO_2 production under aerobic conditions (Fig 5).
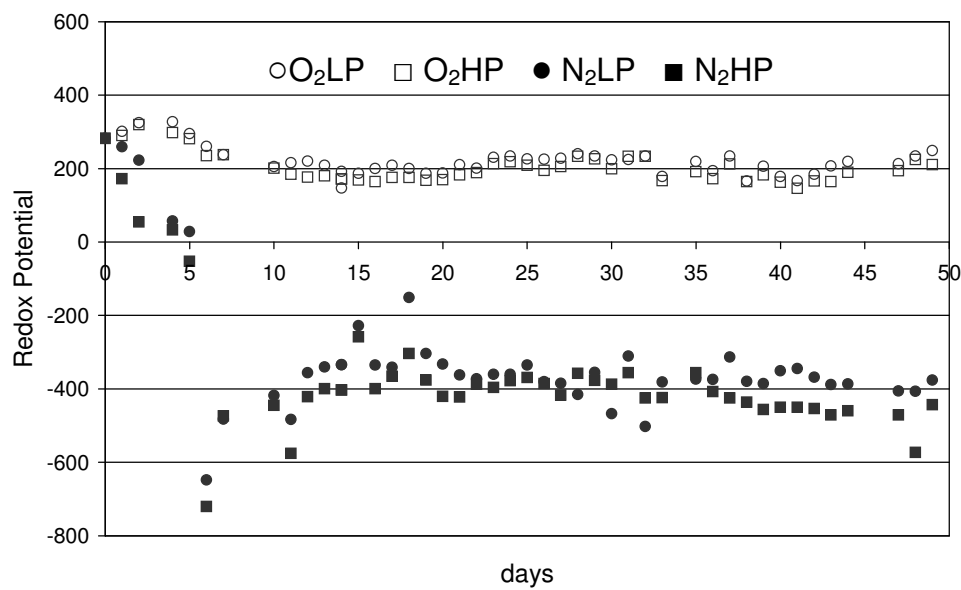

Figure 4. The average redox potential per treatment. No standard errors are given. For statistical differences between treatments see Table 2.
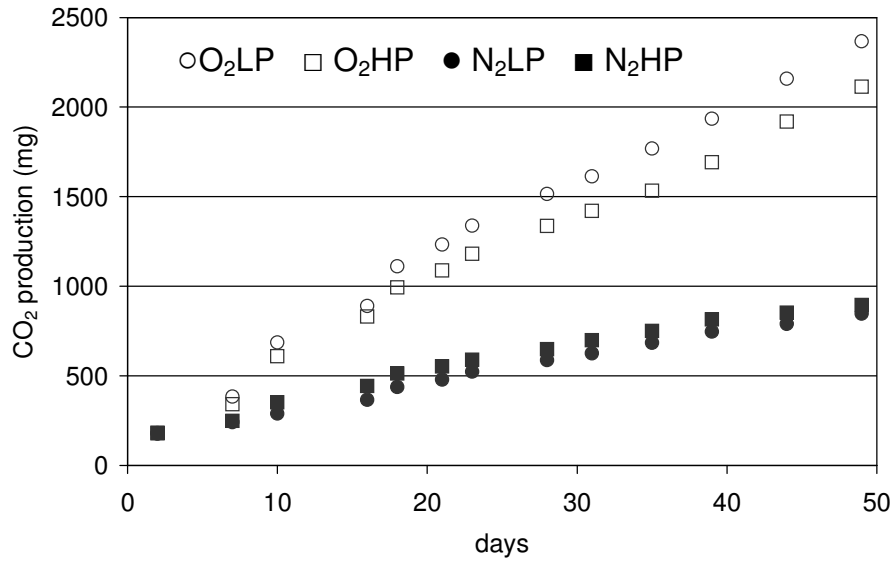
Figure 5. The cumulative CO_2 production by treatment. No standard errors are given. For statistical differences between treatments see Table 2.

3.5 Chemical Oxygen Demand (COD)

The 23 and 49 % protein diets that were given daily had a COD of 1236 mg and 1306 mg O_2 per g of feed, respectively. The COD concentration in the microcosms was not influenced by diet type ($P > 0.05$) (Table 2). After 49 days 3 times more organic matter remained in the anaerobic microcosms compared to the aerobic ones ($P < 0.001$) (Figure 6). The difference in COD concentration between aerobic and anaerobic media increased gradually during the experiment. After 49 days, on average 22% of the COD applied through the feed remained in the aerobic microcosms compared to 66% in the anaerobic ones ($P < 0.001$). Using linear regression, the C accumulation was calculated as well as the decomposition rate constant (Table 4).
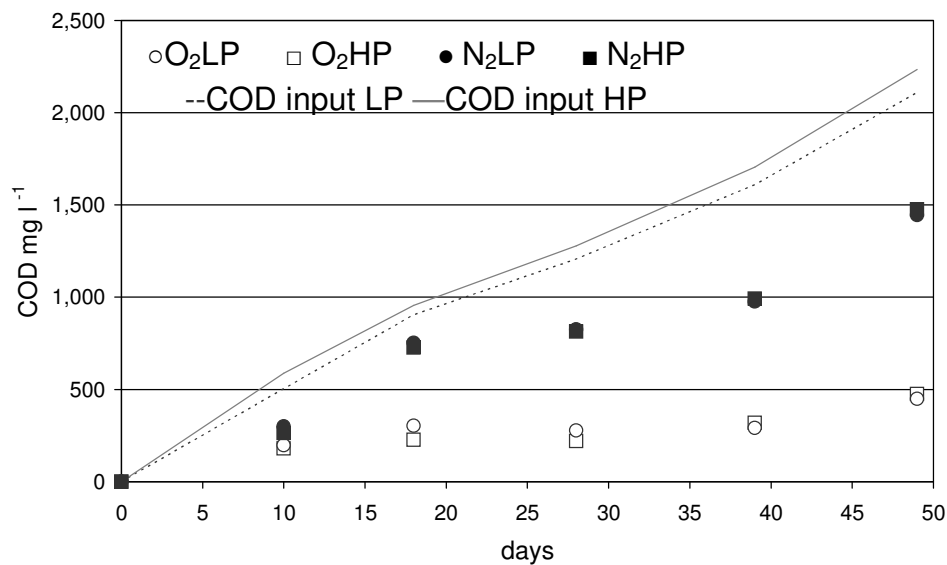
Figure 6. The amount of organic matter, expressed as COD, remaining in the system, by treatment. No standard errors are shown. For statistical differences between treatments see Table 2.

Table 4. Accumulation rates of carbon (from day 28 till 49) and ammonia nitrogen (from day 35 till 49) and the associated decomposition rate constants.

Treatment	Carbon accumulation rate [mg C l^{-1} d^{-1}]	K_C [1/ d^{-1}]	NH$_4^+$ accumulation rate [mg NH$_4^+$-N l^{-1} d^{-1}]	K_N [1/ d^{-1}]
O$_2$LP	3.7	0.25	0.46	0.04
O$_2$HP	2.3	0.27	1.10	0.03
N$_2$LP	8.5	0.07	0.81	0.02
N$_2$HP	9.1	0.06	1.07	0.03

3.6 Nitrogen cycle

The ammonia concentration increases in all treatments during the experiment. With the 49% protein diet treatments, the ammonia production was higher than with the 23% protein diet treatments. The ammonia concentration in the microcosms was not different between the aerobic or anaerobic treatments ($P < 0.001$) (Table 2). The ammonia production rates varied in time depending on the feeding rate (Figure 7). Three lines describing the data at the different feedings rates were fitted and the slopes were compared. From the start of the

experiment until day 20 the feeding rate input was 40 mg l^{-1} d^{-1} and the rate of ammonia production was 0.78 mg l^{-1} d^{-1} for the low protein and 2.05 mg l^{-1} d^{-1} for the high protein diet. From day 20 to 34 the feeding rate was reduced to 20 mg l^{-1} d^{-1} and the ammonia production also decreased to 0.23 mg l^{-1} for the low protein and 0.41 mg l^{-1} for the high protein diet. When the feeding was resumed to 40 mg l^{-1} from day 35 onwards, the ammonia production rate increased again to 0.63 mg l^{-1} d^{-1} for the low protein and 1.08 mg l^{-1} d^{-1} for the high protein diet (Figure 7). The ammonia concentration at the end of the experiment was calculated using linear regression assuming that the feeding rate stayed constant at 40 mg l^{-1} d^{-1}. Under this assumption about 1.4 to 1.6 times more ammonia would have been produced. For the low protein diet treatment 36.8 mg l^{-1} was estimated but only 26.1 mg l^{-1} were really measured. For the high protein diet the calculated level was 96.4 mg l^{-1} compared to the measured 59.9 mg l^{-1}.

During the experiment NO_2^- could not be detected. The nitrogen accumulation as ammonia and the nitrogen decomposition rate constants are shown in Table 4. The K_N was in the range from 0.02 to 0.04 and showed no influence from the protein content of the diet neither from the oxic status of the treatment (Table 4). Nitrification is an aerobic reaction and therefore only occurs in the aerobic treatments. The initial NO_3^- concentrations dropped and the values in the high protein treatments ranged from 1.3 to 3.0 mg l^{-1} (Figure 7). The nitrate levels fluctuated around 0.5 mg l-1 in treatments fed with low protein diets.

In all treatments total Kjeldhal nitrogen (TKN) was positively related to the feed input level ($P < 0.001$). No differences were found between the aerobic and anaerobic treatments ($P > 0.05$) (Table 2). Therefore, the aerobic and anaerobic TKN data were grouped and plotted as high and low protein treatments. Two times more TKN was produced by the high protein diet than by the low protein diet (Figure 8). In the same figure also the dissolved proteins (Lowry-protein) were plotted. More dissolved proteins were present in the high protein treatments ($P < 0.001$) and under anaerobic conditions ($P < 0.001$).
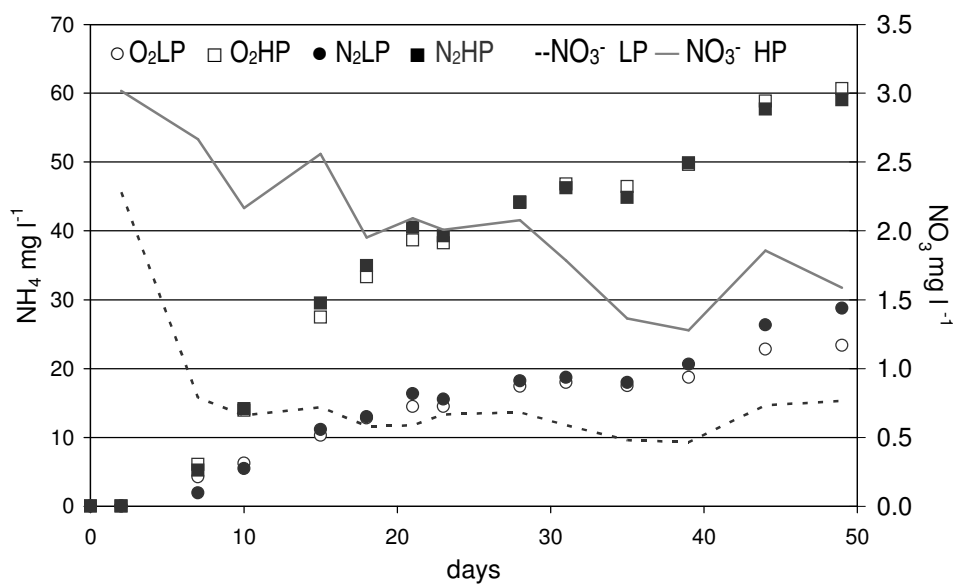
Figure. 7. The ammonia and nitrate (secondary Y axis) concentrations by treatment during the experiment. The nitrate levels were pooled by high or low dietary protein level. No standard errors are shown. For statistical differences between treatments see Table 2.
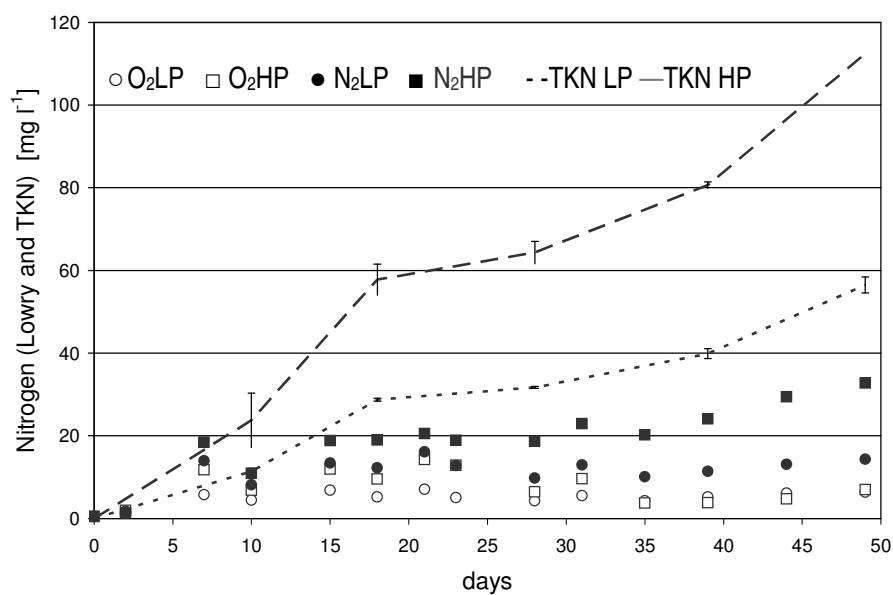

Figure 8. Total Kjeldhal nitrogen for low (TKN-LP) and high protein diet treatments (TKN-HP) and dissolved protein (Lowry method) for all the treatments.

3.7 C/N ratio

The C/N ratio of the low protein and high protein diet added daily to the microcosms was 12.8 and 6.3, respectively (Table 5). The C/N ratio decreased during the experimental period in all treatments. In the aerobic treatments, the C/N ratio decreased to 3.7 and to 1.7 for the 23% and 49% protein diet, respectively. For anaerobic treatments, the C/N ratio decreased to 10.0 and to 5.7 for the low and high protein feed, respectively. The C/N ratio decrease was faster and sharper in aerobic environments than in anaerobic environments (Table 5).

Table 5. Change of C/N ratio during the experiment

Treatment	Initial	Molar C/N ratio		
		Day10	Day 28	Day 49
O_2LP	12.8	6.8	3.0	3.7
O_2HP	6.3	2.8	1.8	1.7
N_2LP	12.8	10.9	11.3	10.9
N_2HP	6.3	6.0	5.7	5.7

4.   Discussion

The influence of aerobic and anaerobic environments on organic matter mineralization

The model of organic matter decomposition is described by a first order kinetics equation (Reddy *et al*., 1986). Intensive aquaculture ponds are fed daily with artificial fish feed and therefore it is necessary to add a term explaining the simultaneous addition and degradation of organic matter. Avnimelech *et al*., (1995) included the daily fish feed addition and developed a model described by equation (1) where C is the organic carbon component, t is the time, K_C is the decomposition rate constant and B is the daily input of component C. The mineralization rate constant for nitrogen (K_N) can be calculated substituting C by N as the organic nitrogen component.

$$dC / dt = B - KcS \qquad (1)$$

In the present study, the organic carbon mineralization rate constant K_C was dependent of the oxic status of the systems. The K_C was significantly higher in aerobic treatments ($K_C= 0.26$) than in anaerobic treatments ($K_C=0.06$) (Table 4). The average K_C calculated by Avnimelech *et al* (1995) for aerated tanks was 0.15. The organic nitrogen mineralization rate constant K_N was evaluated using the ammonia concentration values. The K_N calculated by Avnimelech *et*

al. (1995) was on average 0.087, i.e., higher that the ones calculated in the present study (K_N= 0.02-0.04). The closeness of decomposition rate constants of pure feed and measured in ponds (Avnimelech *et al*., 1995) suggests that feeds drive the decomposition processes in ponds.

Measuring changes in optical density over time is a rough and common method to quantify bacterial growth. In all treatments the turbidity of the medium increased over time with increasing fish feed inputs (Figure 2). The optical density was slightly higher in anaerobic systems than in aerobic systems. The measured changes in optical density can be due to an increase (1) in bacterial cell counts, (2) in fermentation products or (3) a combination of (1) and (2). Due to the interference of fermentation products under anaerobic conditions, it is difficult to compare bacterial growth between aerobic and anaerobic systems based on optical density. Nevertheless, direct measurement of bacterial biomass revealed that more bacteria were present under aerobic conditions than under anaerobic conditions (Torres-Beristain *et al*., submitted). In addition, higher amounts of dissolved proteins, measured as Lowry protein, were also found under anaerobic conditions (Figure 8, Table 2). A higher dietary protein level also causes more dissolved proteins to be present in the microcosms (Figure 8, Table 2). This confirms than under anaerobic conditions a larger fraction of the nutrients end up in the associated nutrient rich microbial matrix, the main contributor of extracellular substances (Schroeder, 1987).

Across the experimental period pH decreased in the aerobic treatments between 0.75 to 1 units, while in the anaerobic treatments pH decreased between 0.5 to 3 pH units (Figure 3). The pH decline under aerobic conditions was due to the increase of dissolved CO_2 (Milstein, 1993). Although the dissolved CO_2 was less in anaerobic systems, the pH decline in these systems was higher most probably due to the accumulation of volatile fatty acids (Vidal *et al*., 2000).

Under aerobic conditions, half of the decomposed organic carbon was incorporated in bacteria biomass and the other half was mineralized to CO_2 (Henze *et al*., 2002). Anaerobic decomposition is a multi-step process and less bacteria biomass and CO_2 is produced because some carbons are incorporated in intermediate fermentation products (Henze *et al*., 2002). Higher amounts of CO_2 were produced under aerobic conditions than under anaerobic conditions (Figure 5). The organic carbon accumulation rates were higher under anaerobic than aerobic conditions (Table 4). In the anaerobic system, 3.2 times more carbon was

56

accumulated than in the aerobic treatments (Figure 6). These values are based on total COD and include all organic matter present including undecomposed organic matter, bacteria biomass, organic fatty acids and all other intermediate decomposition products. More organic fatty acids and intermediate decomposition products were present under anaerobic conditions.

The influence of diet C/N ratio on organic matter mineralization

As a result of the decomposition process, a certain fraction of the carbon input was leaving the system as CO_2 (Figure 5). Higher amounts of CO_2 were produced under aerobic than under anaerobic conditions. The dietary protein level had no effect on CO_2 production (Table 2). Whereas quite some carbon was leaving the system as CO_2, nitrogen mainly accumulated because no denitrification could occur in these pure aerobic or anaerobic systems. When for the same C/N ratio, the aerobic and anaerobic decomposition is compared, it is clear that the C/N ratio decreases faster and steeper in the aerobic environment, probably because of the higher carbon removed from the system (Table 5). Other studies had also observed a decrease of the C/N ratio during culture which in part can be explained by a higher volatilization of C than N (Kochba *et al*., 1994; Mohanty *et al.,* 1994; Burford *et al*., 2003).

Based on a bacterial C/N ratio of 5 (Rittmann and McCarty, 2001), and considering a 50% of efficiency in carbon assimilation (Gaudy & Gaudy, 1980) the optimal substrate C/N ratio can be estimated around 10. A C/N ratio 10 is also recommended for bioremediation applications (Alexander, 1999). Mohanty *et al*. (1994) demonstrated that the rate of nitrogen mineralization was very fast at a C/N ratio of 5-10, moderately fast at C/N ratio of 10-20 and slow at C/N ratio of 20-40. Berard et al., (1995) suggested that at a C/N ratio above 10 the organic matter will be mainly assimilated into bacterial biomass while at a C/N ratio below 10 a large carbon fraction will be lost as CO_2. In the present study, decomposition rates slowed down towards the end which is presumably related to the decrease of C/N below optimal levels. A similar pattern was observed when the reduction of C/N ratio from around 8 to 2 was related to the lack of bacterial growth in intensively managed closed systems (Burford *et al*., 2003). Liu and Han (2004) carried out several nutrient addition treatments to improve microbial activity. They added carbon (glucose) to increase the C/N ratio and they found an enhancement of the ammonia uptake by heterotrophic bacteria suggesting 5.4 as an optimal C/N ratio. Also very high C/N ratios were associated with a slow down in microbial activity. Mohanty *et al*., (1994) reported these critical C/N ratios to be between 20 and 25.

Nitrification

The amount of ammonia present in the microcosms was determined by the amount of dietary protein administrated, and no differences were found between aerobic or anaerobic treatments (Table 2). The daily N addition from a feeding rate of 40 mg l^{-1} d^{-1} of a 23% or 49% protein diet represented 1.5 mg N l^{-1} d^{-1} and 3.2 mg l^{-1} d^{-1}, respectively. Ammonification (ammonia liberation) occurred at a similar rate under aerobic and anaerobic conditions, while K_N was similar for all treatments (Table 4). Similar ammonification rates were also found in aerobic and anaerobic treatments after amino acid addition (Torres-Beristain *et al*., submitted). Jacobsen and Jörgensen (1975) found higher nitrogen release under anaerobic conditions than in aerobic, but this difference was probably due to the immediate conversion of ammonia to nitrate in the aerobic environments, compromising the ammonification measurements.

Nitrification is an aerobic process and therefore only occurs in oxygen rich environments. In the present study only a small fraction of ammonia was transformed to NO_3^- in spite of the high ammonia concentrations. Higher values of NO_3^- were found for the 49% protein diet than for the 23 % protein diet (Figure 7). The amount of NO_3^- was expected to increase in time because denitrification was not able to occur in these complete aerobic and anaerobic systems. However the amount of NO_3^- did not increase and the nitrate concentration stayed more or less constant or even decreased. A possible explanation of the lack of nitrification might be a competition with heterotrophic bacteria for NH_4^+ or substrate inhibition mechanism produced by high organic matter loads or ammonia concentrations.

The inhibition of nitrification in aquatic environments by organic carbon has been reported previously (Hanaki *et al*., 1990; Strauss and Lamberti 2000, 2002). Strauss and Lamberti (2000) added glucose as a carbon source and they observed a decreased nitrification when carbon level increased. These authors stated that at a high C/N ratio typical for nitrogen limited environments, the heterotrophic bacteria are more successful to capture the available NH_4^+ since they are more abundant and grow faster than the chemo-autotrophic nitrifying bacteria. In the present study NH_4^+ was not a limiting factor because the systems were heavily loaded with fish feed and the high bacteria activity produced high concentrations of dissolved CO_2 and NH_4^+. Hanaki *et al* (1990) showed that the addition of organic matter provokes heterotrophic growth while inhibiting nitrification. Also in bacterial biofilm the organic load correlates positively with heterotrophic growth. However nitrification was suppressed

probably due to space competition between heterotrophic and nitrifying bacteria (Wijeyekoon, *et al*., 2004). Another option is that the nitrification process was possibly inhibited by substrate inhibition by NH_4^+ or CO_2 or both. Indeed, nitrifying bacteria use CO_2 as a carbon source and ammonia as a nitrogen source (Straus and Lamberty, 2000), and both CO_2 and NH_4^+ were present in very high concentrations.

TAN uptake by algae is another way to remove the NH_4^+-N from aquaculture ponds. If the average daily primary production assuming a 1 m pond depth is 3.5g C m^{-3} day^{-1} is multiplied by the C/N ratio of the algal cells (7 ; Redfield *et al*., 1963), this results in 0.49 mg N l^{-1} day^{-1} theoretical ammonia uptake by phytoplankton. This primary production would not be enough to remove the amount of ammonia produced at the end of the experiment by the two diets. In average the systems fed with low protein diets produced 0.64 mg N l^{-1} day^{-1} and the systems fed with high protein diet produced in average 1.09 mg N l^{-1} day^{-1} exceeding by two folds its capacity.

5.   Conclusions

Aquaculture ponds are highly eutrophic aquatic systems with fish feed as principal input. In this study, we demonstrated that under aerobic decomposition less carbon remained in the system because more carbon was lost as CO_2. The C/N ratio input decreased in all the treatments as a result of bacteria activity. The protein breakdown produced very high ammonia concentrations for aquaculture systems which was twice as high in the 49% protein diet treatments than in the 23% protein diet treatments. Very limited nitrification was observed, only in the beginning of the experiment, and substrate inhibition is suggested as a possible cause. Further research should focus intermediate aerobic-anaerobic environments to get a more realistic simulation of natural conditions.

References

Alexander, M., 1999. Biodegradation and bioremediation, second edition. Academic Press, San Diego, USA. 453 pp.

American Public Health Association (APHA), 1998. Standard methods for the examination of water and wastewater, 20th Edition. American Public Health Association, New York, USA.

Avnimelech, Y., Kochva, M., Diab, S. 1994. Development of controlled intensive aquaculture systems with a limited water exchange and adjusted carbon to nitrogen ratio. Israeli Journal of Aquaculture 46, 119-131.

Avnimelech, Y., Mozes, N., Diab, S., Kochba, M., 1995. Rates of organic carbon and nitrogen degradation in intensive fish ponds. Aquaculture 134, 211-216.

Begot, C., Desnier, I., Daudin, J. D., Labadie, J. C., Lebert, A., 1996. Recommendations for calculating growth parameters by optical density measurements. Journal of Microbiological Methods 25, 225-232.

Berard, A., Volat, B., Montuelle, B., 1995. Bacterial activity and its trophic role in a eutrophic pond. Archive für Hydrobiologie 134, 499-513.

Burford, M.A., Thompson, P.J., McIntosh, R.P., Bauman R.H., Pearson, D.C., 2003. Nutrient and microbial dynamics in high-intensity, zero-exchange shrimp ponds in Belize. Aquaculture 219, 393-411.

Connolly, J.P., Coffin, R.B., 1995. Model of carbon cycling in planktonic food webs. Journal of Environmental Engineering 121, 682-690.

Conte, F.S., 2004. Stress and the welfare of cultured fish. Applied Animal Behaviour Science 86, 205-223.

Gaudy, A.F.Jr., Gaudy, E.T., 1980. Microbiology for Environmental Scientists and Engineers. , McGraw-Hill Companies, New York, USA. 736 pp.

Gomez, K.A., Gomez, A.A., 1984. Statistical Procedures for Agricultural Research. 2nd edition. John Wiley and Sons, New York, USA, 704 pp.

Hanaki, K., Wantawin, C., Ohgaki, S., 1990. Effects of the activity of heterotrophs on nitrification in a suspended-growth reactor. Water Research 24, 289-296.

Hargreaves, J.A., 1998. Nitrogen biogeochemistry of aquaculture ponds. Aquaculture 166, 181-212.

Henze, M., Harremoes, P., Jansen, J., Arvin, E., 2002. Wastewater treatment, biological and chemical processes. Third edition. Springer Verlag, Berlin, Germany. 430 pp.

Jacobsen, O.S., Jörgensen, S.E., 1975. A submodel for nitrogen release from sediments. Ecological Modelling 1, 147-151.

Jackson, C., Preston, N., Thompson, P.J., Burford, M., 2003. Nitrogen budget and effluent nitrogen components at an intensive shrimp farm. Aquaculture 218, 397-411.

Jimenez-Montealegre, R., 2001. Nitrogen transformation and fluxes in fish ponds: a modeling approach. Fish Culture and Fisheries. PhD Thesis, Wageningen, The Netherlands, 185 pp.

Kochba, M., Diab, S., Avnimelech, Y., 1994. Modeling of nitrogen transformation in intensively aerated fish ponds. Aquaculture 120, 95-104.

Liu, F., Han, W., 2004. Reuse strategy of wastewater in prawn nursery by microbial remediation. Aquaculture 230, 281-296.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurements with the folin-phenol reagent. Journal of Biological Chemistry 193, 265-275.

Meijer, L.E., Avnimelech, Y., 1999. On the use of micro-electrodes in fish pond sediments. Aquacultural Engineering 21, 71-83.

Milstein, A., 1990. Water quality in an intensive outdoor commercial fish culture system with mechanically stirred ponds. Israeli Journal of Aquaculture-BAMIDGEH 42, 99-109.

Milstein, A., 1993. Water quality and freshwater fish culture intensification: the Israeli example. Aquaculture and Fisheries Management 24, 715-724.

Mohanty, A.N., Chatterjee, D.K., Saha, P.K.,. Pani, K.C., 1994. Effect of varying C/N ratios on the mineralization of organic nitrogen in fish pond soils. Journal of Aquaculture in the Tropics 9, 9-14.

Naylor, R.L., Goldburg, R.J., Primavera, J., Kautsky, H..N., Beveridge, M.C.M., Clay, J., Folke, C., Lubchenco, J., Mooney, H., Troell, M., 2000. Effect of aquaculture on world fish supplies. Nature 405, 1017-1024.

Reddy, K.R., Feijtel, T.C., Patrick, W.H., 1986. Effect of soil redox conditions on microbial oxidation of organic matter. In: Y. Chen and Y. Avnimelech (Editors), the role of organic matter in modem agriculture. Martinus Nijhoff Publ., Dordrecht-Boston-Lancaster, UK. pp. 117-156.

Redfield, A.C., Ketchum, B.H., Richards, F.A., 1963. The influence of organisms on the composition of sea water. In: Hill, M.N., (Ed.), The Sea, Vol. II. Willey –Interscience N.Y., USA. pp 26-77.

Rittmann, B.E., McCarty, P.L., 2001. Environmental Biotechnology: Principles and Applications. McGraw-Hill, Boston, USA. 754 pp.

Sevilleja, R.C., 1985. Tilapia production in freshwater fishponds of Central Luzon, Philippines, pp. 115-126. In Smith, I.R.,E.B. Torres and E.O. Tan (eds.) Philippine tilapia economics. ICLARM Conference Proceedings 12, 261 p. Philippine Council for Agriculture and Resources Research and Development, Los Banos, Laguna and International Center for Living Aquatic Resource Management, Manila, Philippines.

Schroeder, G.L., 1987. Carbon pathways in aquatic detrital systems. Pages 217-236 in: D.J.W. Moriarty and R. S. V. Pullin (eds.), Detritus and microbial ecology in aquaculture. ICLARM conference proceedings 13, Manila, Philippines. 420 pp.

Strauss, E.A., Lamberti, G.A., 2000. Regulation of nitrification in aquatic sediments by organic carbon. Limnology and Oceanography 45, 1854-1859.

Strauss, E.A., Lamberti, G.A., 2002. Effect of dissolved organic carbon quality on microbial decomposition and nitrification rates in stream sediments. Freshwater Biology 47, 65-74.

Tacon, A.G.J., Phillips, M.J., Barg, U.C., 1995. Aquaculture feeds and the environment: The Asian experience. Water Science and Technology 31, 41-59.

Vidal, G., Carvalho, A., Mendez, R. Lema, J.M. 2000. Influence of the content in fats and proteins on the anaerobic biodegradability of dairy wastewaters. Bioresource Technology 74, 231-239

Wijeyekoon, S., Mino, T., Satoh, H., Matsuo, T., 2004. Effects of substrate loading rate on biofilm structure. Water Research 38, 2479-2488

Bacterial biomass production in lab-scale intensive fish pond

Beatriz Torres-Beristain, Marc Verdegem, Johan Verreth

Fish Culture and Fisheries Group, Wageningen Institute of Animal Sciences, P.O Box 338, 6700 AH Wageningen, Wageningen University, The Netherlands

Abstract

Fish feed is the most expensive input added to intensive aquaculture ponds. Sixty to 80% of the feed ends up increasing the organic matter load of the system. Microbial processes play an important role in the decomposition of organic matter in ponds, influencing water quality. Success in pond production mainly depends on the farmers´ skill to strike the right balance between production and decomposition. Factors influencing bacterial decomposition of organic matter in ponds are still poorly understood. This study describes the contribution of bacteria to the decomposition of fish feed in a simulated intensive aquaculture system, testing two fish feed with different protein content under aerobic and anaerobic conditions. Bacterial abundance, bacteria biomass, bacteria respiration and bacterial efficiency were different ($P < 0.05$) under aerobic and anaerobic conditions. The bacterial abundance at the end of the experiment was 3.4×10^9 cells ml^{-1} in the aerobic treatments and 1.9×10^9 cells ml^{-1} in the anaerobic treatments. The amount of carbon fixed by bacteria was estimated based on the bacterial biomass. Considering a 1m deep fish pond, in aerobic systems the amount of carbon fixed by bacteria was 19 g m^{-2} day^{-1} compared to 8 g m^{-2} day^{-1} in anaerobic systems. The dietary protein level and the linked C/N ratio did not influence the feed decomposition rate ($P > 0.05$).

1. Introduction

In intensive aquaculture ponds, allochthonous sources of organic matter, including artificial feed, inorganic fertilizer, manure and crop residues, can exceed the autochthonous primary productivity. These inputs are expensive with feed being the most high-priced input applied to aquaculture ponds, representing over 50% of the operational costs (El Sayed, 1999). Large fractions of these inputs are not directly used by the culture organisms and accumulate on the bottom. For instance, Jimenez-Montealegre *et al*. (2002) estimated that 35% of the feed in low density tilapia ponds remained uneaten in the sediment. Simulating the N-cycle in semi-intensive tilapia ponds, Jimenez-Montealegre (2001) assumed that 90% of the uneaten feed, 70% of the feces and 20% of phytoplankton biomass precipitates daily to the pond bottom. The organic matter sedimentation in fish ponds steadily increases during the culture cycle, mainly because the daily feed portion increases in concordance to the growing fish biomass.

The C/N ratio of the substrate influences the organic matter mineralization by bacteria. In natural systems in the Netherlands the sediment C/N ratio varied between 10 and 49, with a

mean around 20, depending on the organic matter origin (Lahr *et al*., 2003). In intensive aquaculture ponds the most important input is fish feed. The percentage of protein in aquaculture feeds can range from 20% for omnivorous species up to 55% for carnivorous species. Such nitrogen-rich inputs cause the C/N ratio to drop to 9.5 and even 6 (Jimenez-Montealegre, 2001). Heterotrophic aerobic bacteria have a C/N ratio of around 5 (Rittmann and McCarty, 2001), but also release CO_2 as metabolic waste. A good C/N ratio allowing for complete decomposition of organic matter under aerobic conditions is around 10 (Alexander, 1999). Higher or lower C/N ratios will result in incomplete mineralization of the organic matter.

Normally, aerobic conditions predominate in the water column and anaerobic conditions prevail just below the sediment surface (Meijer and Avnimelech, 1999). The microbial density in the sediment is 100-1000 times higher than in the water column (Avnimelech *et al*., 1999). In locations in the pond where the accumulation of organic matter in the sediment is large, the overlaying water is stripped from oxygen and anaerobic patches develop. Therefore, in aquaculture ponds aerobic and anaerobic decomposition occur side by side. The relative importance of aerobic and anaerobic processes in ponds depends on the organic loading rate and management practices like aeration, resuspension and sludge removal (Avnimelech and Ritvo, 2003). Under aerobic conditions about half of the metabolized carbon is respired as CO_2 and the other half is assimilated into bacterial cell biomass (Henze *et al*., 2002). Under anaerobic conditions a big part of the organic matter is converted into extracellular low molecular weight compounds. In consequence, a large fraction of the carbon input will be retained in the pond in the form of energy rich organic compounds (Schroeder, 1987).

Bacteria utilize particulate and dissolved organic matter from the water and sediments. Through bacterial decomposition nutrients which were enclosed in the organic matter are either remineralized, making them available for algae, or stored into bacteria biomass making them accessible to higher trophic levels. Thus, bacteria may play an important role in nutrient cycling, particularly in the hypertrophic aquaculture ponds. Bacteria biomass can be an important nutrient source for the cultured animals. Studies showed that bacteria can be consumed directly by tilapia or carp (Beveridge *et al*., 1989, 1991) and by shrimp (Thompson, *et al* 2002). Unfortunately, to date quantitative information on the dynamics of bacterial production in relation to organic matter decomposition in ponds is scarce. The goal of the present study was to monitor the decomposition of fish feed in lab-scale fresh water pond

systems. Special attention was given to the estimation of bacterial biomass, bacterial respiration and bacteria activity as a result of fish feed decomposition.

2. Materials and Methods

2.1 Experimental Design

Intensive fish pond conditions with high organic loading were simulated in 2-l microcosm glass flasks. The flask were filled with filtered (0.2µm) fresh water and inoculated with a bacteria community collected from the biofilter of a recirculation system which was stocked with tilapia for years. The 2x2 factorial experiment was carried out with diet type (23 % and 49 % protein) and oxic status (aerobic and anaerobic) as factors (Table 1). The mixture was stirred constantly and incubated at 22 ^{o}C in a water bath. Fish feed was added daily to the flask in an attempt to mimic feeding practices. The daily feeding rate was 40 mg l^{-1} until day 20. Due to the high amount of ammonia produced the amount of feed was decreased to 20 mg l^{-1}·day^{-1} between day 20 and 34. From day 35 until the end of the experiment the feeding rate was resumed to the original rate of 40 mg l^{-1}·day^{-1}. Samples for bacteria counts were taken on day 2, 16, 31 and 49. The CO_2 production was measured every three to five days. To evaluate bacteria activity an arginine test was carried out at the end of the experiment (Alef and Kleiner, 1986). The gas outlet of the microcosms was analyzed using gas chromatography for methane detection. A flame ionisation detector (FID) was used (Carlo Erba Instruments) and a glass column (6 m × 3.18 mm inner diameter), packed with Porapak Q (50/80 mesh). The temperature of the detector and injector was 150°C

Table 1. Experimental Design

Treatment	Symbol	Oxic status	Diet (C/N ratio)
aerobic-low protein	O_2LP	Aerobic	23% protein (12.8)
aerobic-high protein	O_2HP	Aerobic	49% protein (6.3)
anaerobic-low protein	N_2LP	Anaerobic	23% protein (12.8)
anaerobic-high protein	N_2HP	Anaerobic	49% protein (6.3)

2.2. Bacteria Fixation

To harvest bacterial cells 10 ml sample of the microcosm water was centrifuged at 35,000 rpm for 10 min. The cells formed a pellet on the bottom of the tube and the supernatant was

discarded. The pellet containing the bacteria cells was resuspended in 1.5 ml of 1x Phosphate Buffered Saline (PBS), transferred to an Eppendorf tube and very well mixed, centrifuged again at 35,000 rpm for 10 min and the supernatant was discarded. Bacteria fixation was carried out by incubating the pellet over night with 300 µl of 1x PBS, and 900 µl of 4% paraformaldehyde solution at 4°C. The sample was centrifuged at 35,000 rpm for 10 min and the supernatant was discarded. Two times the pellet was resuspended and washed with 1x PBS centrifuged and the supernatant was discarded. The pellet was finally resuspended in 2 ml final volume PBS /ethanol (1:1) and stored at -20 °C. The 4% paraformaldehyde solution was prepared using 33 ml of H_2O_{dest} heated to 60-65 °C, and 2g of paraformaldehyde was added while stirring the solution. To dissolve completely the paraformaldehyde, 10 N NaOH was added drop–wise until the solution was clear. 16.5 ml of 3x PBS buffer was added, and the pH was adjusted between 7.2-7.4 at 20°C. The solution was filtrated through 0.45 µm filter, stored at 4°C and used within 24 h.

2.3. Bacteria counting and bacterial production calculations

The method used for cell enumeration and cell dimensions measurement was direct cell counting with the fluorescent dye 4′,6-diamidino-2-phenylindole (DAPI) method (Porter and Feig, 1980). Subsamples of each sample collected per flask were ultrasonicated at 40 KHz during 9 minutes to break the cell clumps without destroying them. This was done in a water bath filled with ice to avoid warming up the sample. Bacteria subsamples were put into a 5-mm diameter well of a teflon coated slide (5-20 µl), the amount added depended on the cell concentration. The slides were dried for about 10 min at 46°C. A photograph of each view was taken using a CCD (Charge-Coupled Devices) camera and analyzed afterwards using image analysis software (analySIS®). The staining was performed by dropping into each well 2 µl of Vectashield mounting medium with 4′,6-diamidino-2-phenylindole (DAPI) (Vector Laboratories, Inc). The slide was covered with a glass cover slip. Each subsample was analyzed separately. Detection of cells was carried out with a fluorescence microscope using a total magnification of 1000X. Twenty views of each subsample were analyzed. Bacteria numbers, area, length and width were measured on each photograph. From these measurements the volume of bacterial cells can be calculated and converted to biomass applying an estimate of the carbon content of bacterial cells (Moriarty, 1987). In literature, different methods and conversion factors are used to calculate bacteria biomass. In this study bacterial biomass was calculated, using a factor of 220 fg C μm^{-3} to convert volume into carbon content (Bratbak and Dundas, 1984).

The biovolume was calculated using the formula (Bratbak, 1993):

$$V_{cell} = \frac{\pi}{4} * W^2_{cell} * (L_{cell} - \frac{W_{cell}}{3}) \tag{1}$$

where V_{cell} = cell volume(μm^3); W_{cell} =Width cell (µm) and L_{cell} = length cell (µm)

This formula is for cocci and for straight rod bacteria with hemispherical ends (Bratbak, 1993). A rough measurement of bacteria abundance is through the assessment of the turbidity of the medium measuring optical density (OD) with a spectrophotometer at 600 nm (Begot *et al.*, 1996).

2.4. Bacterial respiration and bacterial growth efficiency (BGE)

Bacterial respiration is the oxidation of organic carbon and its transformation to CO_2. The flasks from all the treatments were permanent connected with 3 mm inner diameter elastic Tygon® with air tight connections to a 250 ml test tube with NaOH (100 ml, 1 M) to trap the CO_2. Every 3-4 days subsamples of NaOH solution were frozen and the NaOH solution from the test tube was changed. The analyses were done using SKALAR analyzer procedure for bicarbonates, using phenolphthalein as a pH indicator, and measuring the decrease in color at 550 nm.

The amount of organic carbon metabolized (A) by bacteria was calculated as the sum of bacterial biomass and bacteria respiration for the aerobic system. In the anaerobic system the metabolized carbon is the sum of bacterial biomass, bacteria respiration and organic acids. Bacterial growth efficiency (BGE) is the percentage of assimilated carbon with respect to the total metabolized organic carbon (A). BGE is the percentage of metabolized organic carbon from the fish feed (A), which is converted into bacterial biomass

$$BGE = \frac{BacBiomass}{A} \times 100 \tag{2}$$

2.5. Bacterial Activity- Arginine-ammonification

Bacteria abundance does not give any information about the metabolic status or production of new cells in the microbial community. In order to have an indicator of bacterial activity an ammonification test was done. Most of the heterotrophic bacteria liberate NH_4^+ when they use nitrogen rich compounds (aminoacids) as a C source (Alef and Kleiner, 1986). Bacteria

activity was measured at the end of the experiment on day 49 using a modification of the Alef and Kleiner (1986) method. Six samples (8 ml) of each flask were put in 10 ml tubes. 2 ml of distilled water was added to 2 of these subsamples. The tubes were closed and frozen immediately. The other four sub samples were added with 2 ml 0.2% arginine solution, closed, and incubated in a water bath at 30 ^{o}C. After 3h 2 subsamples were taken out, frozen and the rest of the tubes were removed after 6h of incubation, and frozen to stop all the processes. All the subsamples were analyzed for ammonia nitrogen based on color development from the modified Berthelot reaction; the color intensity was measured at 660 nm using SKALAR auto analyzer procedure (Skalar, San plus system).

2.6 Statistical analysis

Differences among treatments were tested using split-plot ANOVA with treatments as the main factor and time as the sub-factor for measurements from bacterial abundance, bacteria biomass, bacterial respiration, metabolized carbon (A) and bacterial growth efficiency (BGE) (Gomez and Gomez, 1984). Significant differences between treatment means were based on Tukey test. A separate split-plot ANOVA was carried out with gas type and protein level as main factors and time as sub-factor, to determine the effect of gas (aerobic or anaerobic conditions) and dietary protein level on bacteria abundance, bacteria biomass, bacteria respiration, metabolized carbon and bacterial growth efficiency. A 5% probability level was applied for all the tests. All the analyses were done using statistical package SAS 6.12 (SAS Institute, Cary, NC 27513, USA).

3. Results

3.1 Proximate analysis

The proximate analysis of the low protein (23%) and high protein diet (49 %) is shown in Table 2. The percentages of carbon were 72 for fat, 53 for protein, and 43 carbohydrates (Henze *et al*., 2002). These values were used to calculate the amount of carbon content in each diet. The amount of carbon input from the two diets was very similar with 408 mg C kg feed^{-1} in the 23% protein diet and 418 mg C kg feed^{-1} with the 49% protein diet.

Table 2. Proximate composition of the experimental diets

Fish feed	Analytical				Calculated	
	DM g kg^{-1}	Fat[*] g kg^{-1}	Protein[*] g kg^{-1}	Ash[*] g kg^{-1}	Carbohydrates g kg^{-1}	Carbon content (mg C kg feed^{-1})
23 % protein content	905.9	41.9	232.7	40.4	591.0	407.6
49 % protein content	894.4	104.7	485.2	105.5	199.0	418.1

[*] based on dry weight

3.2 Bacterial abundance and bacteria biomass

Bacterial growth was monitored by optical density (OD) with a spectrophotometer at 600 nm (Figure 1). For all the treatments optical density increased in time about 5-7 fold. Although anaerobic treatments yielded generally a higher OD, differences between treatments were not significant.
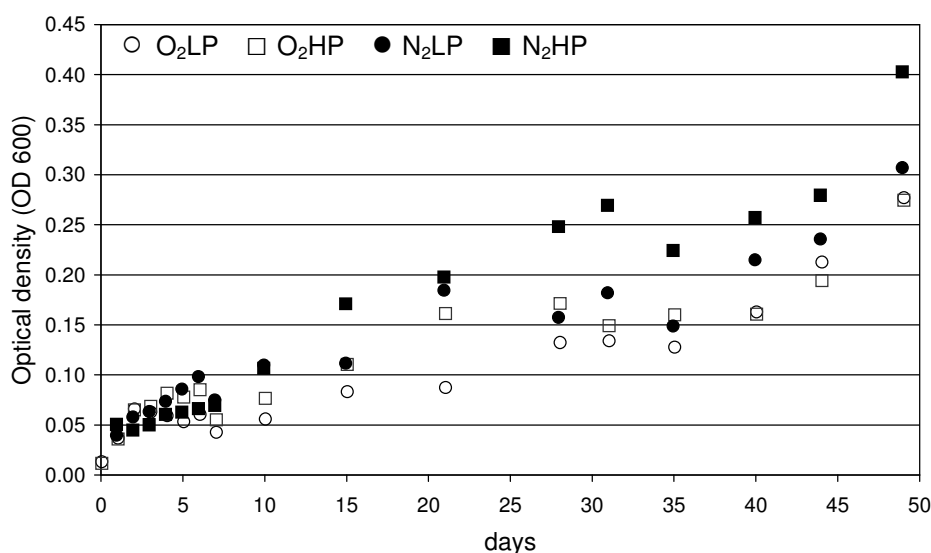
Figure 1.Evolution of bacteria growth measured as optical density

In all the treatments bacteria counts increased during the experimental period (Figure 2). Higher bacteria counts were observed for aerobic treatments than for anaerobic treatments, however because of the high internal variance no statistical differences were found (P>0.01). The mean bacterial count in the aerobic environment was 1.2×10^{9} ml^{-1} and in the anaerobic environment was 6.3×10^{8} ml^{-1}.
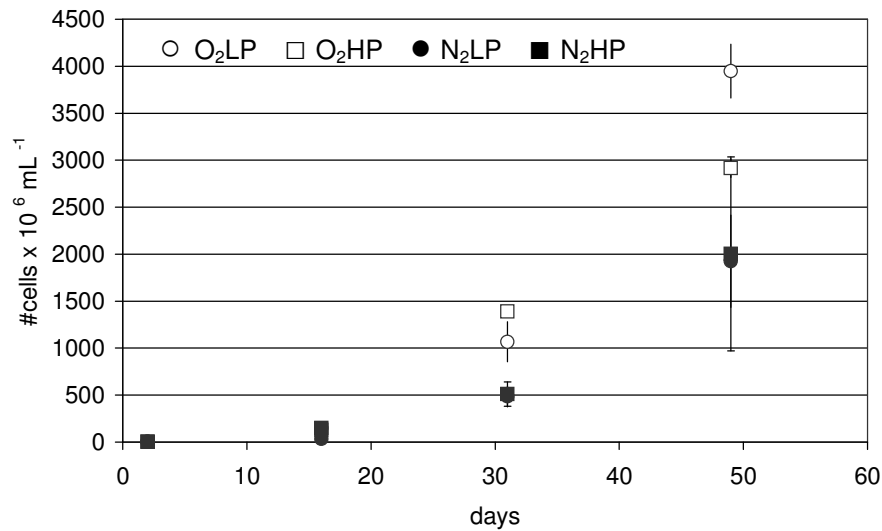
Figure 2. Bacterial abundance (#cells $x10^6$ ml^{-1}) in simulated intensive aquaculture pond. (bars indicate standard error).

To calculate the carbon content, bacteria volume was converted to carbon weight. Table 3 shows the total amount of carbon content in bacterial cells at the end of the experiment. The amount of carbon accumulated in bacterial cells increased significantly during the experimental period ($P<0.001$) (Figure 3). Bacteria biomass in aerobic treatments (328 mg C) was significant different ($P<0.01$) from that in anaerobic treatments (141 mg C).

Table 3. Bacterial biomass present in the microcosms at the end of the experiment.

	TREATMENTS			
	O₂LP	O₂HP	N₂LP	N₂HP
Bacterial Biomass[1] (mg C)	903	979	345	437

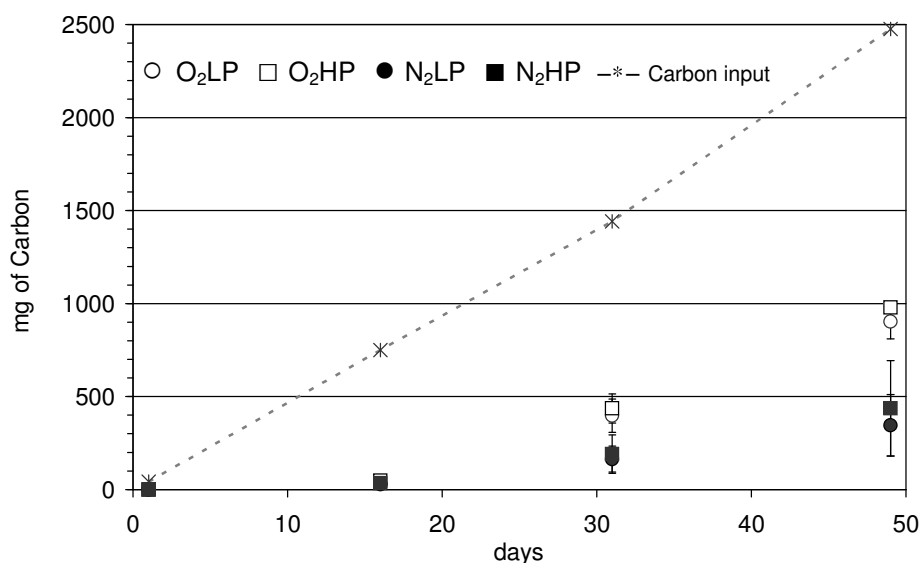[1]Values are means with two replicates

Figure 3. Bacteria production per treatment from bacteria volume calculations.

Bacteria biomass, bacteria respiration, metabolized carbon and bacterial growth efficiency are shown in Table 4. All the parameters changed over time (P<0.001). There was a significant effect of the oxic condition on bacterial respiration, bacterial biomass, and bacteria growth efficiency (P>0.05). The amount of metabolized carbon was not significantly affected by any treatment, neither by the aerobic/anaerobic treatments nor by the diet treatments.
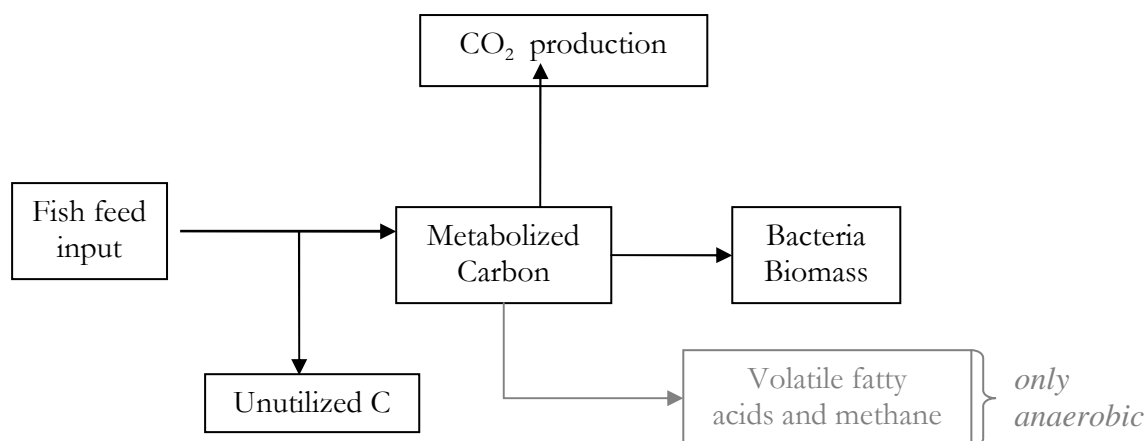
Figure 4. Schematic representation of carbon fate in the simulated aquaculture ponds for aerobic and anaerobic decomposition of fish feed

The fate of carbon under aerobic and anaerobic conditions during decomposition of fish feed is shown in Figure 4. The percentages corresponding to both environments at the end of the experiment are shown in Table 5. The aerobic bacterial biomass present was more that 2-fold higher than the bacterial biomass under anaerobic conditions. The CO_2 production under aerobic conditions was 2 times the production of CO_2 in the anaerobic system. The part corresponding to the volatile fatty acid (VFA) production was set as 33 %, based on van Rijn *et al*. (1995) who measured the VFA production for anaerobic fish feed decomposition.

Table 4. Bacteria respiration, bacteria biomass, metabolized carbon, bacterial growth efficiency and bacteria abundance ANOVA and mean multicomparisons (Tukey test).

Variable	TREATMENTS				Standard Error	Significance Level[1]			
	O_2LP	O_2HP	N_2LP	N_2HP		Oxic-anoxic	Diet	Oxic-anoxic *Diet	time
Bacteria Respiration (mg of carbon)	380 [a]	338 [a]	125 [b]	110 [b]	16	***	ns	ns	***
Bacteria Biomass (mg of carbon)	312 [a]	344 [a]	126 [b]	156 [b]	38	**	ns	ns	***
Metabolized carbon (mg of carbon)	598 [a]	597 [a]	605 [a]	623 [a]	51	ns	ns	ns	***
Bacteria growth efficiency (%)	44 [a]	39 [a]	11 [b]	10 [b]	2	***	ns	ns	***
Bacteria abundance (cells ml^{-1})	9.1×10^{8} [a]	8.7×10^{8} [a]	2.8×10^{8} [a]	3.6×10^{8} [a]	2.2×10^{8}	ns	ns	ns	***

[1] Significance level ns = not significant, *= $P \leq 0.05$, **= $P \leq 0.01$, ***= $P \leq 0.001$. Means in the same row with equal letters are not significantly different at $P \leq 0.05$; a>b>c.

In aerobic systems the fraction corresponding to unutilized feed refers to the amount of carbon that was not quantified as bacteria biomass neither as CO_2 respiration. This fraction was estimated on average as 37% of the total carbon input. In anaerobic systems unutilized feed refers to the amount that was not quantified as bacterial biomass, respired carbon or the percentage corresponded to volatile acids. The average level found was 44% of the total carbon input. Please note that these values differ from the values given in Table 5, where the relative percentages of the 3 pools present in the microcosms on days 2, 16, 31 and 49 are given.

Table 5. Organic carbon percentages allocated in the different pools during the experimental period in aerobic and anaerobic conditions.

	Day	Remaining Carbon[1]	CO_2	Bacteria biomass
AEROBIC	2	41	58	0.30
	16	63	31	5
	31	37	29	34
	49	30	25	46
ANAEROBIC[2]	2	45	57	0.31
	16	81	15	5
	31	73	13	14
	49	70	10	19

[1] Under anaerobic conditions 33% of the remaining carbon is assumed to be carbon that was metabolized by acido forming bacteria and remains as volatile fatty acids (van Rijn *et al*., 1995)

[2] Methane production was measured in one of the repetitions of anaerobic low protein treatment, representing 1.3% of the metabolized carbon at day 49.

3.3 Bacteria cell volume frequencies

The bacterial volume ranged between 0.05 and 1.6 μm^3, with the highest volume frequencies being found in the range of 0.2 to 0.3 μm^3 (Figure 5). When volumes bigger than 1.7 μm^3 were found, they were considered to be bacteria flocks and discarded. The total number of cells counted was 7,054 for aerobic and 3,769 for anaerobic conditions.
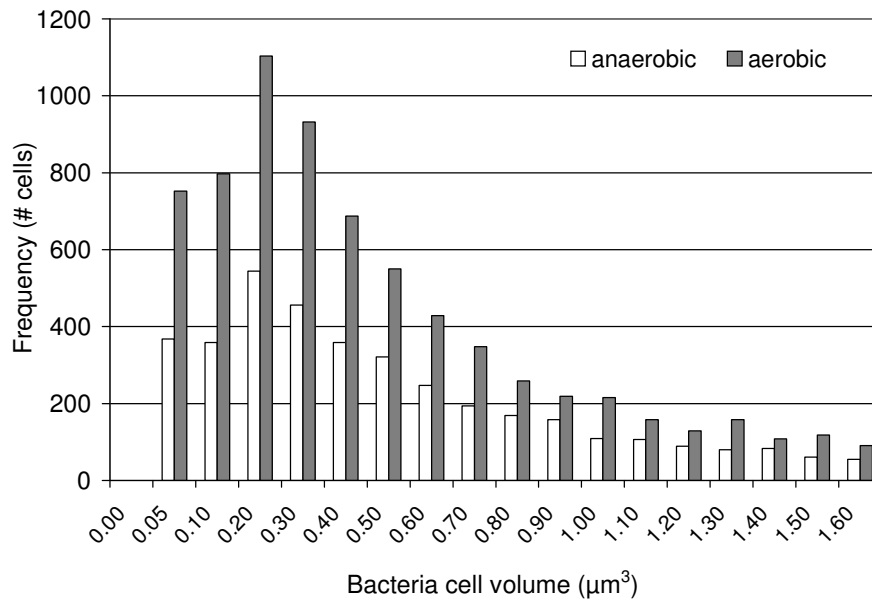
Figure 5. Frequency distribution of bacterial cell volume in simulated aquaculture systems.

3.4 Bacterial Activity: arginine ammonification

Most heterotrophic bacteria liberate NH_4^+ when they use a nitrogen rich compound (e.g. an amino acid such as arginine) as carbon source. Figure 6 shows the ammonia concentration in the subsample from the different treatments after incubation at 30 ^{o}C after 6 hours as explained in material and methods. Mean ammonia production was different ($P < 0.05$) between the low and high protein feeds (48 mg l^{-1} vs 86 mg l^{-1}). Aerobic treatments (77 mg l^{-1}) were not different from the anaerobic ones (87 mg l^{-1}) ($P>0.05$). When ammonia production is plotted against time (Figure 7) the mean NH_4^+ production after 6 hours was 9.37 mg l^{-1}h^{-1} for aerobic systems and 8.4 mg l^{-1}h^{-1} for anaerobic systems.
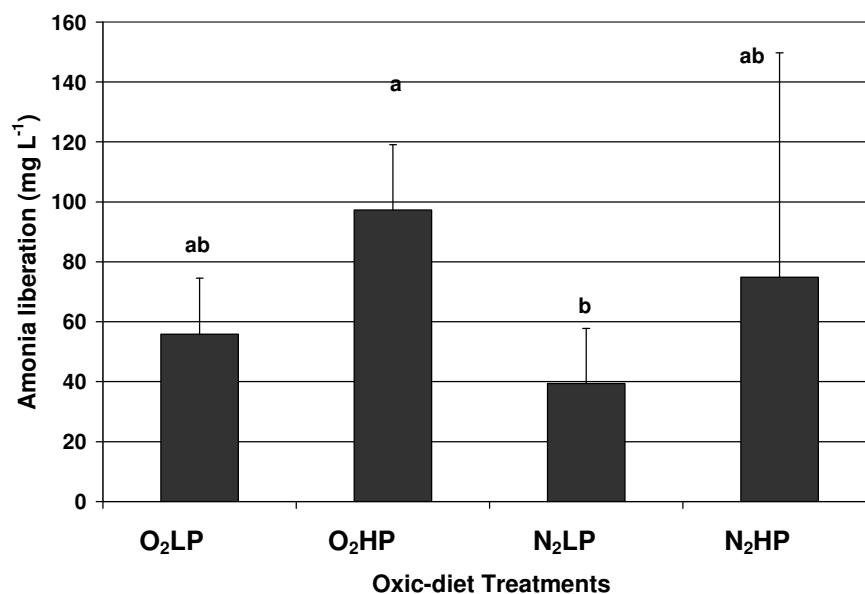
Figure 6. Ammonia concentration during arginine ammonification after 6 hrs (30°C). Error bars represent the standard deviation.
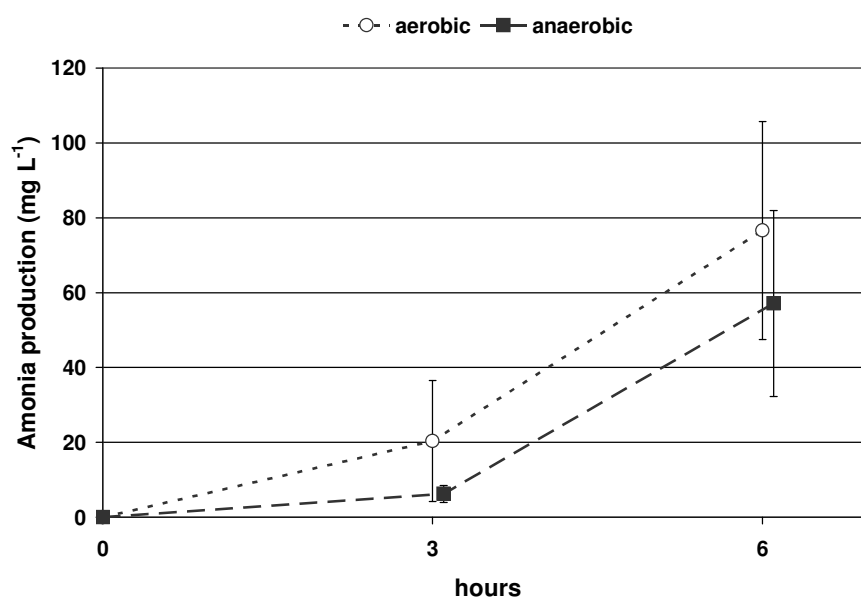

Figure 7. Ammonia production during arginine ammonification test. Error bars represent the standard deviation

4. Discussion

The importance of bacteria in the cycling of nutrients and water quality in aquaculture ponds was already described in previous studies (Moriarty, 1997). In the present study a quantitative estimation of bacterial biomass lab-scale freshwater pond systems was done testing the influence of feed C/N ratio and oxic conditions. The central question was how much organic matter added to the pond was converted into bacterial biomass.

Bacterial abundance and bacteria biomass

In intensive aquaculture ponds there is an increase of organic matter input during the rearing cycle, especially due to the artificial feed. Bacterial density in ponds increases with nutrient load. In the present experiments this can be observed with the increase in optical density in all treatments. Bacterial abundance refers to the number of bacteria per volume in water (#cell ml^{-1}) or number of bacteria per dry weight in sediments (# cells g^{-1}). In the present study, the microcosms were constantly stirred, hence all the particles were always kept in suspension. The bacteria abundance was measured per unit volume (#cell ml^{-1}) and at the end of the experiment the number of bacteria were 3.4×10^9 cells ml^{-1} in aerobic treatments and 1.9×10^9 cells ml^{-1} in anaerobic treatments. These values are considerably higher than those reported in other intensive pond systems, where values of bacterial abundance range between 8.3×10^6 ml^{-1} and 2.6×10^7 ml^{-1} (Moriarty, 1986). In intensive systems without water exchanges, the mean bacterial abundance ranged from 3.3 to 5.4×10^7 ml^{-1} (Burford *et al.*, 2003). Anaerobic optical density measurements were slightly higher than aerobic. This does not correspond with the bacteria counts because higher bacterial counts where found in aerobic treatments. The optical density in the anaerobic conditions can also be affected by intermediate decomposition products and the extracellular nutrients in addition to the microbial cells themselves.

The total amount of feed added at the end of the experiment was on average 2.39 g l^{-1} (2390 g m^{-3}), and the amount of carbon added was 1.24 g l^{-1} (1,238 g m^{-3}). In the present study the carbon stored in the bacterial biomass pool is calculated from biovolume using the factor 220 fg C μm^{-3} (Bratbak, 1993). Biovolume was calculated by measuring width and length of the bacteria using image analysis software (analySIS®). These calculations are more reliable than estimates based on a constant cell:carbon factor which does not take into account the bacteria cell dimensions. The amount of carbon allocated in the bacterial biomass in aerobic systems, was on average at the end of the experiment, 941g m^{-3} for aerobic systems and 391 g m^{-3} for

anaerobic systems. Dividing this carbon production by the experimental days and considering a 1 m depth pond, a secondary production of 19 g C $m^{-2}day^{-1}$ and 8 g C m^{-2} day^{-1} was calculated for the aerobic and anaerobic systems respectively. Comparing these amounts of carbon stored in the bacterial biomass with the amounts of carbon fixed by primary production (ranging between 4 and 8 g m^{-2} day^{-1}; Jimenez-Montealegre *et al*., 2001), it becomes evident that the organic carbon originating from fish feed and stored as bacterial biomass can be higher than the carbon originating from primary production. Primary production is surface limited, while bacteria production is volume limited.

Bacteria Biovolume

In sewer systems mean cell volumes vary between 0.13 μm^3 and 0.37 μm^3 (Jahn and Nielsen, 1998). In the present study, the frequency graph shows a "normal distribution" of bacterial volume (Figure 5). This distribution was also observed in waste water samples, with cell volumes varying between 0.25 to 0.35 μm^3. In our lab-scale aquaculture pond, also rich in nutrients, the most frequent bacterial volume was also found between 0.2 to 0.3 μm^3 for both aerobic and anaerobic treatments. In a study on different aquatic environments, Bernard *et al*. (2000), found that the medium-sized cells ($\pm$ 0.3 μm^3) develop faster than larger or smaller cells. This could explain the relatively higher abundance of bacteria among 0.2 to 0.3 μm^3 volume compared to other sizes.

Bacterial respiration and bacterial growth efficiency

In the present study the microbial community was the only CO_2 producer. Bacteria under aerobic conditions utilize about 50% of metabolized feed to synthesize cells (Gaudy and Gaudy, 1980). Bacterial growth efficiency (BGE) is the percentage of the total metabolized carbon which is converted into bacterial biomass. Different values of BGE have been reported in aquatic environments for planktonic bacteria. This range from 5% to 60 % (del Giorgio and Cole, 1998). To calculate the BGE obtained in the present study a proper estimation of the metabolized carbon is necessary. In the aerobic systems, it was calculated as the sum of bacterial carbon and respired carbon (CO_2). In the anaerobic systems, beside bacterial carbon and CO_2, also volatile fatty acids (VFA) and methane should be taken into account. However methane was observed in only one repetition (H_2LP) and was not higher than 1% of the total metabolized carbon. Therefore it was decided not to consider methane in the balance. For VFA the situation is different. Van Rijn (1995) estimated that VFA constituted 33% of the total metabolized carbon in anaerobic digestion of fish feed with 25% protein. In the present

study, we did not measured VFA, but used the value of van Rijn (1995) (33%) to calculate the total metabolized carbon in the anaerobic systems. Using this approach, we found a BGE of 42% under aerobic conditions and 11% under anaerobic conditions. The typical values of 40-60% for BGE reported in literature (Gaudy and Gaudy, 1980) were in agreement with the values found in the present system for aerobic conditions.

Bacteria biomass and extracellular substances

Very often the term bacterial biomass refers to the combination of cell biomass, extracellular polymers and all substances imbedded in the matrix (Jahn and Nielsen, 1998). The carbon resulting from bacterial metabolization is stored into three pools. Part of the carbon is used by bacteria to build own body mass, part is lost as CO_2 due to respiration and the last part represents extracellular substances. The amount of carbon allocated in extracellular substances can be very important, therefore when referring to bacterial biomass, the amount of these substances should be considered. The ratio cell/extracellular substances is quite variable and depends on environmental factors (Nielsen Per and Palmgren 1997). In the present study, extracellular substances were not included in the bacterial production data but were included in the pool of "unutilized carbon" (Figure 4). This pool refers to the amount of carbon that was not taken into account as bacterial carbon, CO_2 or volatile fatty acid. As a consequence, our values of bacteria biomass may be underestimated since the carbon allocated as extracellular substances was not considered. The percentage at the end of the experiment of "unutilized carbon" was 37 % in aerobic systems and 42 % in anaerobic systems. Some studies show that the amount of carbon allocated in cellular biomass is a minor part of the total. For example in the biofilm of a sewer system cellular biomass constituted, on average, 15% of the organic matter of a microbial matrix (Jahn and Nielsen, 1998). In anaerobic conditions a large part of the substrate carbon is transformed into extracellular low molecular weight compounds which are accumulated in the system. When these compounds are associated with extracellular polymers, they may become available to larger detritus feeders (Schroeder, 1987).

Nutrients cycling

In ponds, sedimentation of suspended particles results in accumulation of organic matter on the bottom, where the available oxygen will be quickly depleted. Schroeder (1987) calculated 3 g of O_2 m^{-2} day^{-1} as the maximal amount of oxygen taken up by pond sediments. Under anaerobic conditions, less CO_2 is produced/liberated and more carbon remains in the system.

However the amount of carbon stored in bacterial cells is higher under aerobic conditions (Figure 2).

The two tested diets had very different protein contents (23 %, e.g., C/N ratio of 12.8 and 49%, e.g., a C/N ratio of 6.3) but this did not influence bacterial production. In contrast to this, the oxic conditions of the decomposition process did affect the bacterial production significantly. Even though the two diets had different protein content, the amount of carbon that was in both feeds was practically the same: for the 23% protein content diet, 407.6 mg C kg^{-1} feed, and for 49% protein content diet, 418.1 mg C kg^{-1} feed. Consequently the amount of carbon that was metabolized for both diets was practically the same. For the 23% protein content diet this was 801.7 mg C and for 49% protein content diet this was 813.7 mg C.

Besides being decomposers, bacteria are the base of the food chain and the link to higher trophic levels. In aquatic systems, where water acts as a support and transport medium, the transfer of bacterial production to higher trophic levels is very effective (Anderson, 1987). In aquaculture systems the food chain can be shortened by directly exploiting algal-fish or detritus-fish combinations (Anderson, 1987). With such combinations, microbial assemblages, including extracellular substances, can be consumed directly by the cultured animals.

Bacteria activity: arginine ammonification

Simple amino acids are quickly used by bacteria. Arginine ammonification is a general technique to evaluate the total activity of the microbial population of soils under aerobic and anaerobic conditions (Alef and Kleiner, 1986; Alef and Kleiner, 1987). Most, if not all, heterotrophic bacteria are able to ammonify arginine (Alef and Kleiner, 1986). In the present study arginine ammonification was used to evaluate heterotrophic bacterial activity in water samples in both aerobic and anaerobic environments. The results show that the microbial populations were very active and responded to the amino acid addition in both the aerobic and anaerobic environments. The initial ammonia concentration values were higher for the high protein diet than for the low protein diet and higher values of ammonia production were obtained in the aerobic environment; however these differences were not significant.

5. Conclusions

This study analyzed the relation between fish feed decomposition and microbial biomass in aquatic systems. The same amount of carbon passed through the microbial population in all the treatments. The oxic status largely determined the fate of the organic carbon. Higher amounts of carbon were allocated as bacteria biomass in aerobic treatments. The influence of the C/N ratio of the feed was not statistically relevant. The total amount of metabolized carbon was neither affected by the C/N ratio (12.8 and 6.3) nor by the oxic status in the present incubation experiments. The amount of carbon remaining in the system was determined by the oxic status. More carbon accumulation occurred under anaerobic conditions because less CO_2 was lost than under aerobic conditions. In intensive aquaculture systems like the ones simulated here, production of bacterial biomass can reach very high levels compared to natural primary production. Future research should concentrate on the detailed characterization of the carbon fractions with focus on volatile fatty acids and the role of extracellular substances in the nutrition of culture animals.

References

Alef, K., Kleiner, D., 1986. Arginine ammonification, a simple method to estimate microbial activity potentials in soils. Biology and Fertility of Soils. 18, 233-235.

Alef, K., Kleiner, D., 1987. Estimation of anaerobic microbial activities in soils by arginine ammonification and glucose-dependent CO_2-production. Soil Biology and Biochemistry 19, 683-686.

Alexander, M. 1999. Biodegradation and Bioremediation. Academic Press, San Diego USA. 453 pp.

Anderson, J.M., 1987. Production and decomposition in aquatic ecosystems and implications for aquaculture. Pages 123-147. In: Detritus and microbial ecology in aquaculture. D. J. W. Moriarty and R. S. V. Pullin, ICLARM 420 pp.

Avnimelech, Y., Kochva, M., Hargreaves, J.A., 1999. Sedimentation and Resuspension in Earthen Fish Ponds. Journal of World Aquaculture Society. 30, 401-409.

Avnimelech, Y., Ritvo, G., 2003. Shrimp and fish pond soils: processes and management. Aquaculture 220, 549-567.

Begot, C., Desnier, I., Daudin, J. D., Labadie, J. C., Lebert, A., 1996. Recommendations for calculating growth parameters by optical density measurements. Journal of Microbiological Methods 25, 225-232.

Bernard, L., Courties, C., Servais, P., Troussellier, M., Petit, M., Lebaron, P., 2000. Relationships among Bacterial Cell Size, Productivity, and Genetic Diversity in Aquatic Environments using Cell Sorting and Flow Cytometry. Microbial Ecology. 40, 148-158.

Beveridge, M.C.M., Begum, M., Frerichs, G.N., Millar, S., 1989. The ingestion of bacteria in suspension by tilapia Oreochromis niloticus. Aquaculture 81, 373-378.

Beveridge, M.C.M., Sikdar, P.K., Frerichs, G.N., Millar, S., 1991. The ingestion of bacteria in suspension by the common carp *Cyprinus carpio L.* Journal of Fish Biology 39, 825-831.

Burford, M.A., Thompson, P.J., McIntosh, R.P., Bauman, R.H. and Pearson, D.C., 2003. Nutrient and microbial dynamics in high-intensity, zero-exchange shrimp ponds in Belize. Aquaculture 219, 1-4.

Bratbak, G., Dundas, I., 1984. Bacterial dry matter content and biomass estimations. Applied and Environmental Microbiology. 48, 755-757.

Bratbak, G., 1993. Microscopic methods for measuring bacterial biovolume:epifluorescence microscopy, scaning electron microsopy, and transmission electron microscopy. Pages 309-317 in: Handbook of Methods in Aquatic Microbial Ecology. P. F. Kemp, B. F. Sherr, E. B. Sherr and J. Cole J. London, Lewis Publishers: pp 777

del Giorgio, P. A. and Cole, J. J., 1998. Bacterial growth efficiency in natural aquatic systems. Annual Review of Ecology and Systematics 29, 503-541.

El Sayed, A.F.M., 1999. Alternative dietary protein sources for farmed tilapia, *Oreochromis spp.* Aquaculture 179, 149-168.

Gaudy, A.F.J. and Gaudy, E.T., 1980. Microbiology for Environmental Scientists and Engineers. New York, McGraw-Hill Companies, New York, USA. 736 pp.

Gomez, K.A., Gomez, A.A., 1984. Statistical Procedures for Agricultural Research. 2nd edition. John Wiley and Sons, New York. USA, pp 704.

Henze, M., Harremoes, P., Jansen, J., Arvin, E., 2002. Wastewater treatment, biological and chemical processes. Third edition. Springer Verlag, Berlin, Germany. 430 pp.

Jahn, A. and Nielsen Per, H., 1998. Cell biomass and exopolymer composition in sewer biofilms. Water Science and Technology 37, 17-24.

Jimenez-Montealegre, R. 2001. Nitrogen transformation and fluxes in fish ponds: a modeling approach. Fish Culture and Fisheries. PhD Thesis, Wageningen, The Netherlands, 185 pp.

Jimenez-Montealegre, R., Verdegem, M.C.J., van Dam, A., Verreth, J.A.J., 2002. Conceptualization and validation of a dynamic model for the simulation of nitrogen transformations and fluxes in fish ponds. Ecological Modelling 147, 123-152.

Lahr, J., Maas-Diepeveen, J.L., Stuijfzand, S.C., Leonards, P.E.G., Druke, J.M., Lucker, S., Espeldoorn, A., Kerkum, L.C. M., van Stee, L.L.P., Hendriks, A.J., 2003. Responses in sediment bioassays used in the Netherlands: can observed toxicity be explained by routinely monitored priority pollutants?. Water Research 37, 1691-1710.

Meijer, L. E., Avnimelech, Y., 1999. On the use of micro-electrodes in fish pond sediments. Aquacultural Engineering 21, 71-83.

Moriarty, D.J.W., 1986. Bacterial Productivity in ponds used for Culture of *Penaeid* Prawns. Microbial Ecology. 259-269.

Moriarty, D.J.W., 1987. Methodology for determining biomass and productivity of microorganisms in detrital food webs. Detritus and microbial ecology in aquaculture. D. J. W. Moriarty and R. S. V. Pullin, ICLARM 420 pp.

Moriarty, D.J.W., 1997. The role of microorganisms in aquaculture ponds. Aquaculture 151, 1-4.

Nielsen Per, H., Jahn, A., Palmgren, R., 1997. Conceptual model for production and composition of exopolymers in biofilms. Water Science and Technology. 36, 11-19.

Porter, K. G., Feig, Y. S., 1980. The use of DAPI 4'6-diamidino-2-phenylindole for identifying and counting aquatic microflora. Limnology and Oceanography. 25, 943-948.

Rittmann, B.E. and McCarty, P.L., 2001. Environmental Biotechnology: Principles and Applications. New York, McGraw-Hill Book Co 754 pp.

Schroeder, G.L. 1987. Carbon pathways in aquatic detrital systems. Detritus and microbial ecology in aquaculture. R. S. V. D.J.W. Moriarty and Pullin, Detritus and microbial ecology in aquaculture 420 pp.

Thompson, F.L., Abreu, P.C., Wasielesky, W., 2002. Importance of biofilm for water quality and nourishment in intensive shrimp culture. Aquaculture 203, pp. 263-278.

van Rijn, J., Fonarev, N., Berkowitz, B., 1995. Anaerobic treatment of intensive fish culture effluents: digestion of fish feed and release of volatile fatty acids. Aquaculture 133, 9-20.

Effect of mechanical resuspension and aerobic-anaerobic shifts in fish feed decomposition in simulated fish ponds

Beatriz Torres-Beristain[a] Marc Verdegem[a], Eva Kerepeczki[b]

and Johan Verreth[a]

[a]Fish Culture and Fisheries Group, Wageningen Institute of Animal Sciences, P.O Box 338, 6700 AH Wageningen, Wageningen University, The Netherlands.
[b]Research Institute for Fisheries, Aquaculture and Irrigation P.O.Box 47 H-5540 Szarvas Hungary

Abstract

The microbial decomposition of organic matter in aquaculture ponds is affected by the oxic-anoxic conditions gradient at the soil-water interface and by resuspension. To investigate these interactions, the decomposition of a 49% protein fish feed was analyzed in ten marine lab-scale systems with different occurrences of resuspension events or time periods of serial exposure to aerobic and anaerobic conditions. To test pure aerobic and anaerobic conditions a constantly mixed aerobic microcosm was compared with a constantly mixed anaerobic microcosm. The resuspension events lasted 15 minutes and were applied 1) two times a day (Rs1), 2) one time per day (Rs2), 3) once every two days (Rs3) and 4) once every four days (Rs4). Four additional treatments tested alternate shifts between aerobic and anaerobic conditions by bubbling O_2 or N_2 through the experimental microcosms for different time periods per day: 18 h O_2-6 h N_2, 6 h O_2-18 h N_2, 12 h O_2-12 h N_2, two times 6 h O_2-6 N_2. All treatments were executed in duplicate. The fish feed added daily to aquaculture ponds provides fresh organic matter that is easy to degrade under both aerobic and anaerobic conditions. The degree of coupling between oxic and anoxic conditions in the system has a strong effect on the products accumulating and escaping from the culture system. Pure oxic or anoxic conditions proved to be less favorable than mixed aerobic-anaerobic systems with respect to the metabolites accumulating in the system. Short 15-minutes resuspension events and a continuous alteration of oxic and anoxic conditions at 12-hour time intervals proved to be the best options to minimize the accumulation of organic matter in culture systems. The correct coupling of aerobic-anaerobic conditions in space and time is the key to maintain a good balance between decomposition and assimilation of carbon and nitrogen components in aquaculture production systems.

1. Introduction

Marine ponds, including shrimp ponds, are more and more intensively used and heavily fed with formulated protein diets. Major environmental impacts of aquaculture have been associated with high-input high-output intensive systems (FAO, 2002). Therefore in intensive aquaculture pond the balance between organic matter accumulated, decomposed or discharged is of great importance.

Intensive aquaculture ponds are shallow systems where the organic matter settles on the bottom. The amount of sedimented organic matter increases during the growing cycle due to primary production and feed input increase (Jimenez-Montealegre, 2001; Jimenez-Montealegre *et al.*, 2002). The oxidation of the accumulated organic sludge consumes the oxygen available at the substrate and when the oxygen is finished the settled organic matter will decompose anaerobically. The proportion of the organic matter decomposed under aerobic or anaerobic conditions as well as the coexistence or alternation of oxic and anoxic periods depends on the management practices applied. Factors like feeding regimes, aeration, resuspension and sludge removal alter the oxic status of the systems in time and space (Milstein *et al.*, 2001; Avnimelech and Ritvo, 2003). The accumulation of organic matter and its decomposition under aerobic or anaerobic is strongly affected by the sediment handling. For instance, in experimental intensive shrimp ponds without water exchange Hopkins *et al.*, (1994) tested the effect of sediment removal (each week), resuspension (every day) and undisturb sediment on water quality, nitrogen balance and shrimp production. Removed sludge represented 67% of the nitrogen budget while 22% and 15% of the nitrogen was left in the sediment for the resuspended and undisturbed sediment treatments respectively. Almost no shrimp survival was registered in the undisturbed sediment treatments while the survival of the shrimp was 33% and 54% in removed and resuspended sediment treatments respectively.

To decompose organic matter waste water treatment plants rely on aerobic or anaerobic processes. Sequential aerobic/anaerobic or coupled anaerobic-aerobic processes are used to reduce the amount of sludge, including bacterial biomass, and to consume the dissolved organic components generated (Low and Chase, 1999). Aquaculture waste water is heavily loaded with organic matter and high total nitrogen concentration. In the case of aquaculture recirculating systems the inorganic nitrogen is removed with an aerobic-anaerobic decomposition sequence in physically separated reactors: the ammonia nitrogen is nitrified in an aerobic trickling filter and the resulting nitrate is subsequently denitrified to nitrogen gas in an anaerobic fluidized bed reactor (van Rijn, 1990; Arbiv *et al.*, 1995).

In aquatic ecosystems both aerobic and anaerobic processes coexist naturally. In ponds, continuously complex interactions occur between oxic and anoxic processes. The flux of organic matter through the pond is largely driven by decomposition processes that in turn are influenced by two opposing processes affecting the aerobic-anaerobic situation. During daylight hours a labile thermocline develops making mixing of the water column more

difficult. This is in part counteracted by fish or current driven resuspension of organic matter that brings anoxic sediments back in contact with the oxygen rich water column. The transition zones between aerobic and anaerobic layers are characterized by high bacterial activity (Fry, 1987). Nitrifying bacteria develop in the narrow border-area between the aerobic and anaerobic zones that are rich in nitrate and organic carbon (Riise and Roos, 1997). In a simulated pond environment where an oxygenated water layer was constantly in contact with the sediment, nitrate did not accumulate and nitrogen was removed form the system by a sequence of nitrification-denitrification processes (Avnimelech *et al.*, 1992). Nitrogen loss through denitrification is enhanced by mixing between anoxic sediments and the aerobic water column (Hargreaves, 1998; Gross *et al.*, 2000).

In summary, in aquatic ecosystems the balance between aerobic or anaerobic decomposition processes influences the amount and the quality of detritus that accumulates. Coupling between aerobic and anaerobic conditions can lead to a reduction in the accumulation of carbon and nitrogen species in the system. The goal of this research was to investigate the effect of two modes of coupling between oxic and anoxic conditions on organic matter decomposition and accumulation of nutrients in ponds. The first mode investigated were 15-min resuspension events, the second mode investigated were serial 6, 12 and/or 18 hour shifts between oxic and anoxic conditions.

2. Material and methods

2.1 Experimental Design

Twenty 2-l glass flasks were filled with filtered (0.22 μ) marine water and inoculated with bacteria taken from the biofilter of a marine turbot recirculation system. High quality fish feed (49% protein) was added at a rate of 20 mg l^{-1} d^{-1}. Aerobic and anaerobic conditions were created with a constant oxygen supply (O_2-MIX) and with constant nitrogen flow (N_2-MIX), respectively. To test the effect of resuspension events on feed decomposition, oxygen was introduced into the air above the water surface and the systems stayed stagnant until resuspension was applied. Four different combinations of 15-minutes resuspension events were created: 1) two times a day, applying 12 hour time intervals between resuspension events (Rs12), 2) once every 24-hours (Rs24), 3) once every 48-hours (Rs48) and 4) once every 96-hours (Rs96). During periods of no resuspension anaerobic conditions developed at the sediment-water interphase while the water column above stayed oxygenated. Four other treatments simulated serial aerobic-anaerobic shifts by alternating O_2 and N_2 gas inflow into

the water of the flasks. In these treatments the gas-inlet-pipe was connected to an air stone positioned in the middle of the microcosm in which the water column was permanently mixed by a magnetic stirrer. The N_2–O_2 combinations applied each 24-hour period were: 1) 18 hr O_2: 6 hr N_2 (18O_2), 2) 6 hr O2: 18 hr N_2 (6O_2), 3) 12 hr O_2: 12 hr N_2 (12O_2), 4) two times 6 hr O_2: 6 hr N_2 (6+6O_2). The gas outlet of each experimental unit was connected with 3-mm$_{ID}$ elastic Tygon® pipe to a 250 ml test tube filled with 100 ml 1.0 M NaOH to trap CO_2 . Figure 1 shows the general scheme of the experimental unit. All the treatments were done in duplicate. Table 1 describes and visualizes the ten treatments tested.
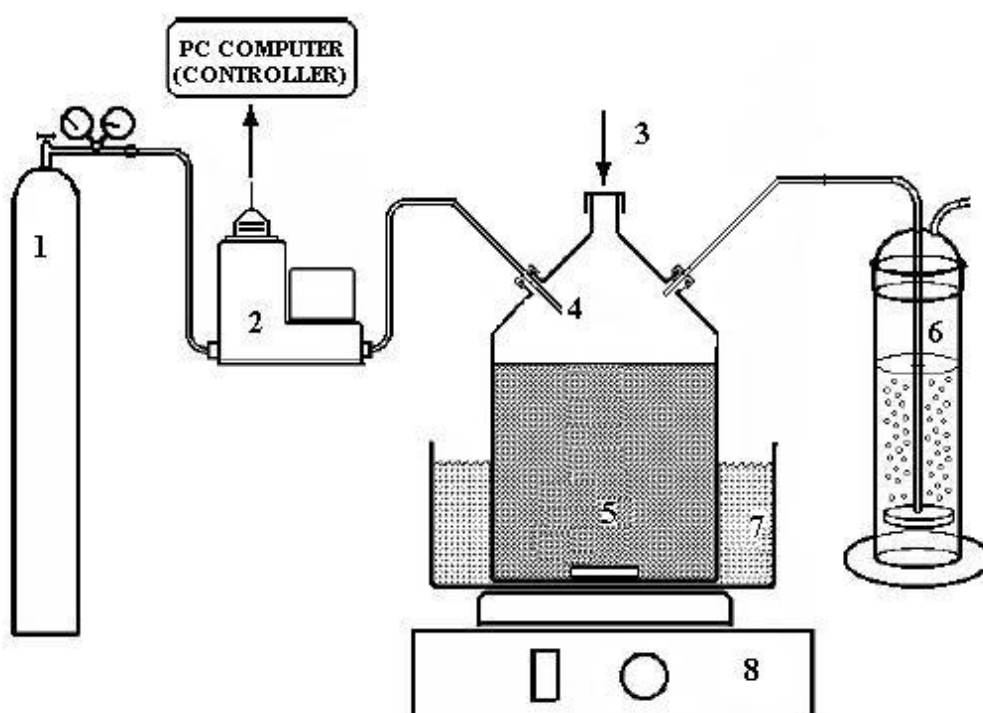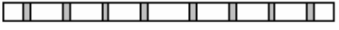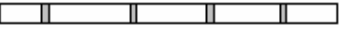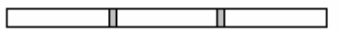

Fig 1. Experimental unit scheme: 1-pressure gas (oxygen/nitrogen) container; 2- mass control meter, 3-sampling port; 4-gas inlet; 5-flask with microcosm; 6-CO_2 trap; 7-thermoregulated water bath 8-magnetic stirrer.

Table 1. Experimental treatments

	Treatment	Description	Treatment symbol
1	Mixed Aerobic	Complete mix 24 hrs a day aerobic	O_2-Mix
2	Mixed Anaerobic	Complete mix 24 hrs a day anaerobic	N_2-Mix
3	Resuspension two times a day	O_2 at surface level, anoxic bottom	Rs12
4	Resuspension 1 times a day	O_2 at surface level, anoxic bottom	Rs24
5	Resuspension every two days	O_2 at surface level, anoxic bottom	Rs48
6	Resuspension every 4 days	O_2 at surface level, anoxic bottom	Rs96
7	¾ day O_2 - ¼ day N_2	Mainly aerobic with 6hrs anaerobic	$18O_2$
8	¾ day N_2 - ¼ day O_2	Mainly anaerobic with 6hr anaerobic	$6O_2$
9	½ day O_2 - ½ day N_2	Aerobic-anaerobic shifts every 12 hrs	$12O_2$
10	Two times:¼ day O_2 -¼ day N_2	Aerobic-anaerobic shifts every 6 hrs	$6+6O_2$

2.2 Water quality parameters

Water samples were taken regularly for water quality analysis. The water removed was replaced with the original marine filtered water to maintain a constant 2-l volume in the microcosms. Each sampling the dilution factor was recalculated to correct the experimental values. The increase in turbidity was monitored by measuring the optical density (OD) at 600 nm. The pH measurements were taken using a WTW pH-meter model pH325 (WTW, Germany). Redox Potential (ORP) was taken using a SenTix ORP electrode and pH325 pH-meter (WTW, Germany). Analysis of 10 ml of sample for NH_4^+, NO_3^- and NO_2^- were done using SKALAR auto analyzer procedures (Skalar, San plus system). Ammonia nitrogen determination was based on the modified Berthelot reaction with indophenol-blue development, measuring the color intensity at 660 nm. Nitrite was diazotized with sulfanilamide while coupling with α-naphtylenediamine dihydrochloride. The reaction produces a reddish purple color whose absorbance was measured at 540 nm. Nitrate determination was based on reduction of nitrate to nitrite and measured using the same method as for nitrite (APHA, 1998). CO_2 measurements were done using phenolphthalein as pH indicator and measuring the color absorbance at 550 nm. Soluble proteins were measurd using the Lowry procedure. The water sample was hydrolyzed with an alkaline copper solution, the resulting hydrolase reacts with folin-cooiocalture phenol reagent producing a

blue color that was measured at 660 nm (Lowry *et al.*, 1951). Total nitrogen was determined using the macro Kjeldahl method (Tecator, Germany) (APHA, 1998). Chemical Oxygen Demand (COD) was determined by color development through the sample oxidation with sulfuric acid and potassium-dichromate at 150 ^{o}C. The samples were diluted 4 times to reduce the interference with chloride (APHA, 1998). Dissolved proteins were measured with the Lowry method but analysis results were complete out of range because some components in the marine water interfered with the Lowry reaction. Therefore, the results from the Lowry analysis are not reported.

The continually mixed aerobic and anaerobic treatments served as controls. In the figures given, the treatments with 15-minutes resuspension events or with 6, 12 or 18 serial aerobic-anaerobic shifts were clustered, showing the cluster means. This was done to keep the figures simple and easy to read. However, statistical differences between treatments are also presented in tables (see section 2.3.). Total nitrogen (TN) was calculated as the sum of Kjeldhal nitrogen, which includes the ammonia and organic nitrogen, with nitrite and nitrate. Total inorganic nitrogen (TIN) was calculated as the sum of ammonia, nitrate and nitrite nitrogen and total organic nitrogen (TON) was calculated as the difference between TN and TIN. Nitrogen loss was calculated as the difference between nitrogen input and TN.

2.3 Statistical analysis

Differences between controls and treatments within each cluster group were tested using split-plot ANOVA with treatments as the main factor and time as the sub-factor (Gomez and Gomez, 1984). The parameters analyzed were redox potential, ammonia, nitrite, total ammonia nitrogen (TAN), TIN, TON, nitrogen loss (N-loss), CO2 production and COD. Significant differences between treatment means were analyzed using Tukey test. A 5% probability level was applied for all the tests. The pH data were not analyzed by ANOVA because of non-linearity. All the analyses were done using statistical package SAS 6.12 (SAS Institute, Cary, NC 27513, USA).

3. Results

A 49% protein feed was daily added to all the treatments. The cumulative feed and the protein input are given in Figure 2. The carbon content per gram of fish diet was 418 mg. By day 28 each flask had received 1.16 g fish feed dry weight input including 493 mg of carbon and 520 mg of protein.
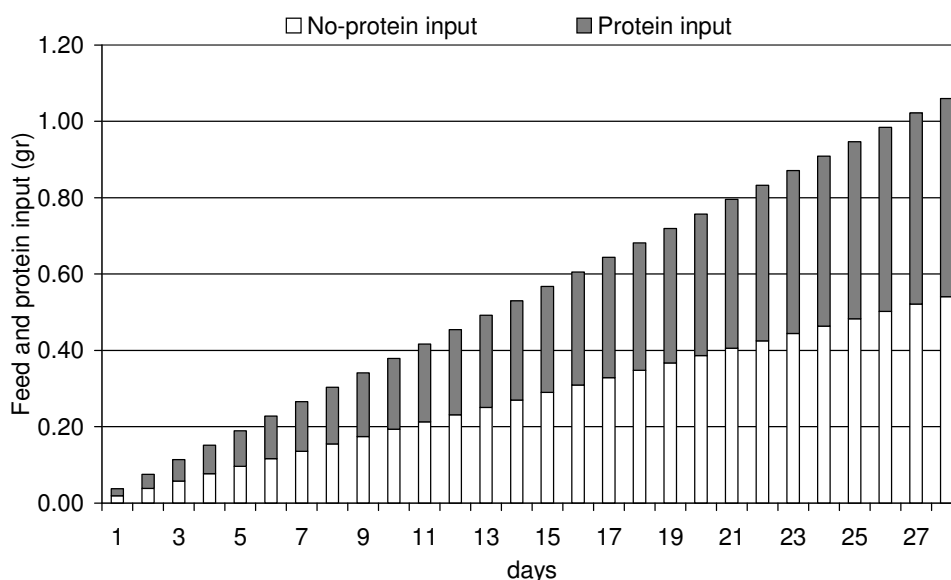

Figure 2. Cumulative feed input during the experimental period on dry weight basis, showing the protein fraction applied.

The statistical differences between treatments of clustered data (resuspension and aerobic-anaerobic shifts) and control treatments for CO_2, NH_4^+, NO_3^- Kjeldhal nitrogen, N-loss, TIN and TON are shown in Table 2.

The results of the redox potential are shown in Figure 3. The mixed aerobic treatment (O_2-Mix) as well as the surface layer of the stagnant treatments (Res-surface) showed a positive redox potential in a range of 100 to 200 mV. The mixed anaerobic treatment (N_2-Mix) and the bottom of the stagnant treatments (Res-bottom) rapidly decreased reaching -431 and -343 mV, respectively, from day 8 onwards. No redox potential measurements were taken for aerobic-anaerobic shift treatments because the time needed for the redox electrode to stabilize was very long, and the time shifts were very different between treatments.
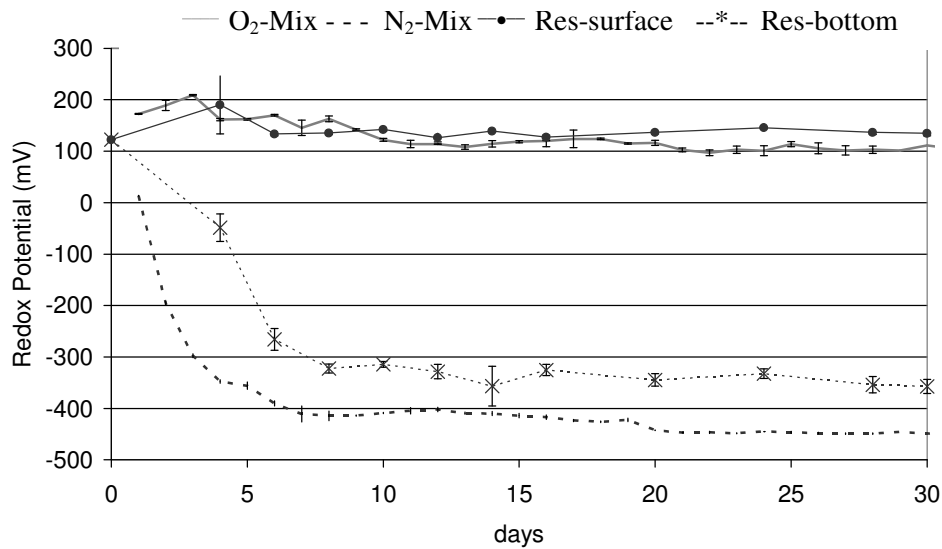
Figure 3. Redox potential values during the experiment for mixed-aerobic and mixed-anaerobic treatments and for surface and bottom measurements in resuspension treatments during stagnant time intervals. Bars represent the standard deviation of clustered data.

All treatments showed an increase in optical density over time. The aerobic-anaerobic shift treatments showed the steadiest increase, with less internal variations (Figure 4). The optical density values for the resuspension treatments had a high variability on the standard error bars.
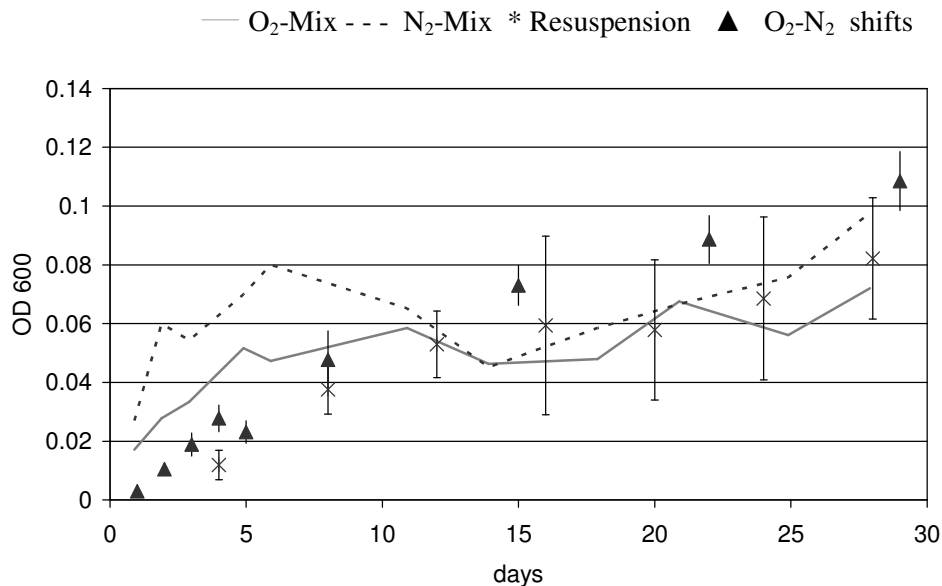
Figure 4. The optical density (600 nm) in the microcosms during the experiment. Bars represent the standard deviation of clustered data.

Table 2. Treatment means for physicochemical parameters. Means in the same row under the heading 'resuspension' or under the heading 'aerobic-anaerobic shifts' with no superscript letter in common are statistically different (P≤ 0.05); a>b>c.

|	RESUSPENSION							AEROBIC-ANAEROBIC SHIFTS						
	O_2-Mix	N_2-Mix	Rs1	Rs2	Rs3	Rs4	Standard error of the mean	O_2-Mix	N_2-Mix	$18O_2$	$6O_2$	$12O_2$	$6+6O_2$	Standard error of the mean
CO_2	511.82 [a]	85.70 [b]	492.14 [a]	262.54 [ab]	426.80 [ab]	404.89 [ab]	64.4	511.82 [a]	84.70 [b]	552.41 [a]	501.64 [a]	344.80 [a]	488.89 [a]	43.72
NH_4^+	7.16 [b]	11.54 [a]	1.14 [c]	1.17 [c]	1.34 [c]	1.81 [c]	0.34	7.16 [a]	11.54 [b]	1.18 [c]	2.80 [c]	1.10 [c]	3.12 [c]	0.43
NO_3^-	4.24 [a]	0.24 [c]	2.51 [b]	2.58 [b]	3.01 [b]	2.83 [b]	0.093	4.24 [b]	0.24 [c]	13.51 [a]	11.78 [a]	0.68 [cb]	13.55 [a]	0.69
TKN	15.69 [b]	21.38 [a]	7.03 [c]	7.85 [c]	9.76 [c]	6.99 [c]	0.58	15.69 [a]	21.38 [a]	3.55 [c]	7.94 [bc]	5.52 [bc]	9.53 [b]	1.04
TIN	12.35 [a]	12.87 [a]	3.85 [b]	4.03 [b]	4.58 [b]	5.074 [b]	0.44	12.35 [b]	12.87 [b]	16.27 [ab]	16.7 [ab]	2.48 [c]	18.72 [a]	0.81
TON	9.36 [a]	10.42 [a]	6.84 [ab]	7.89 [ab]	8.43 [ab]	5.62 [b]	0.82	9.36 [a]	10.42 [a]	2.1 [c]	4.86 [abc]	4.21 [bc]	6.1 [abc]	1.034
Nloss[1]	5.87 [b]	0 [b]	34.67 [a]	31.33 [a]	27.44 [a]	35.19 [a]	1.53	5.87 [cb]	0 [c]	12.22 [b]	10.66 [b]	34.62 [a]	7.69 [cb]	1.49

[1] At the end of the experiment

The pH values in the mixed-aerobic and mixed-anaerobic treatments were systematically higher than in the other treatments with average values of 7.3 (±0.14) and 7.6 (±0.27), respectively. The average pH in the aerobic-anaerobic shifts treatments and in the resuspension treatments dropped compared to the mixed-aerobic or mixed-anaerobic treatments. The average value for the resuspension clustered treatments was 6.8 (±0.38) and for the aerobic-anaerobic shift treatments was 6.6 (±0.5).
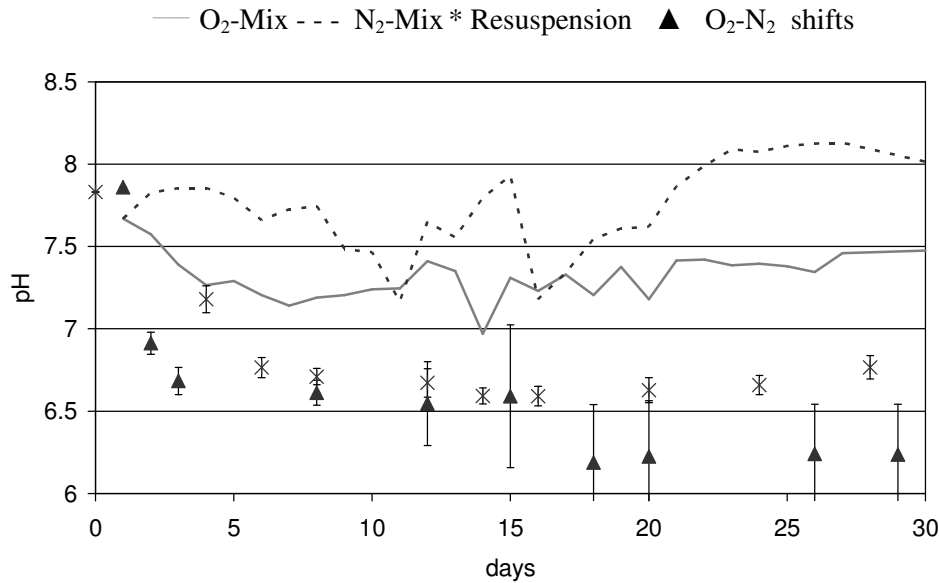
Figure 5. pH measurements during the experiment. Bars represent the standard deviation of clustered data.

CO_2 production differed strongly between the mixed-aerobic and mixed-anaerobic treatments (Figure 6). The CO_2 production in all other treatments was slightly lower than the CO_2 production in the mixed-aerobic treatment, be it not significantly different ($P > 0.05$, Table 2). The CO_2 production in the mixed-anaerobic treatment was lower than in the other treatments ($P < 0.05$, Table 2).
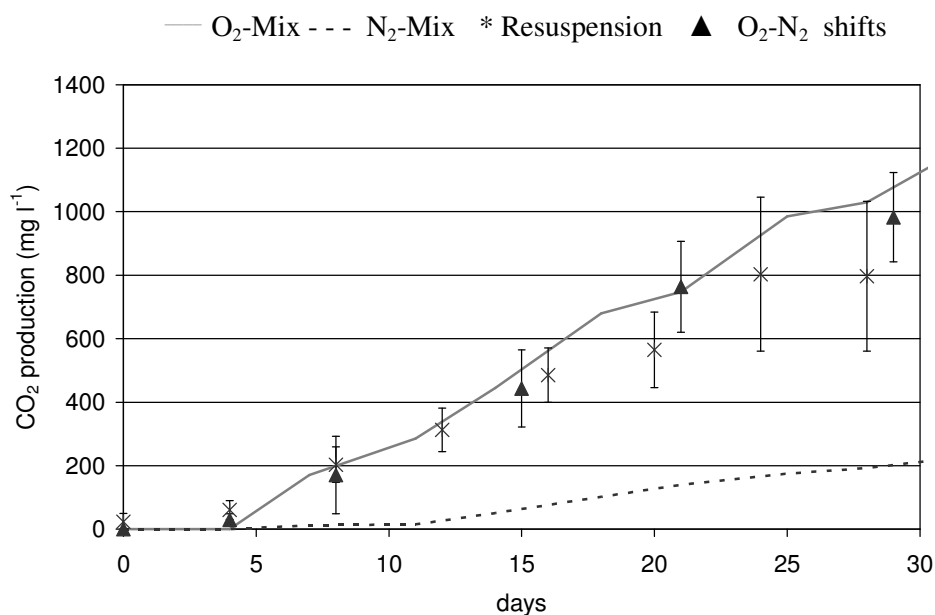
Figure 6. The cumulative CO_2 production in O_2-Mix, N_2-Mix, Resuspension and O_2-N_2 shift treatments. Bars represent the standard deviation of clustered data.

Figure 7 reviews the carbon, expressed as COD, remaining in the microcosms. COD increased over time in all treatments. The mixed-anaerobic treatment had the highest amount of carbon remaining in the system. The variation among the clustered data was considerable especially for the resuspension treatments. No differences within resuspension and aerobic-anaerobic shifts clusters were found (P > 0.05, Table 2).

Total Kjeldhal nitrogen (TKN) measurements include organic nitrogen and the ammonia produced as a result of protein ammonification (Figure 8). In mixed-aerobic and mixed-anaerobic treatments there was accumulation of TKN whereas TKN did not increase in the other treatments, indicating protein was broken down and/or ammonium was transformed. In Figure 8, the means of the clustered aerobic-anaerobic shift treatments and resuspension treatments are given.
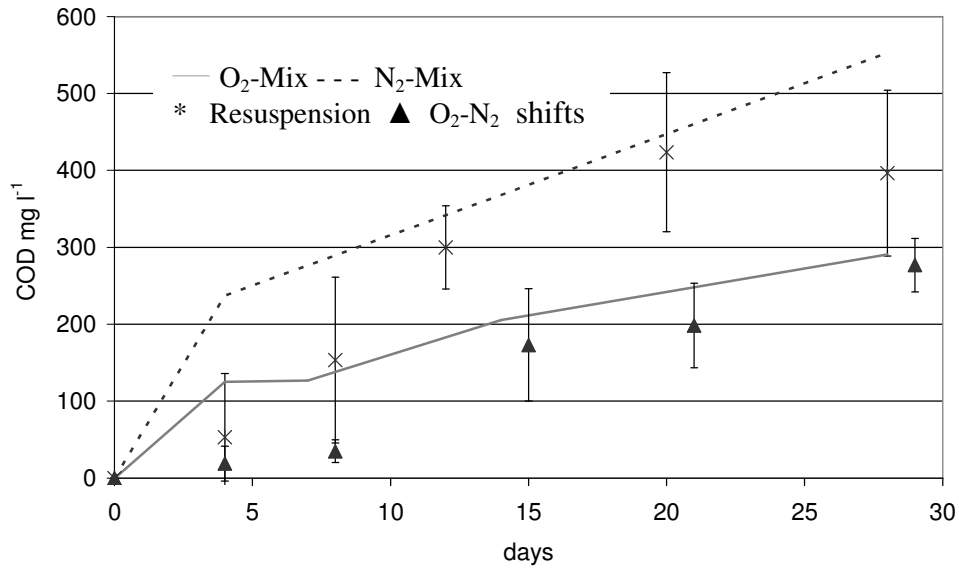
Figure 7. Changes in COD in the microcosms during the experiment for the O_2-Mix, N_2-Mix, Resuspension and O_2-N_2 shifts treatments. Bars represent the standard deviation of clustered data.
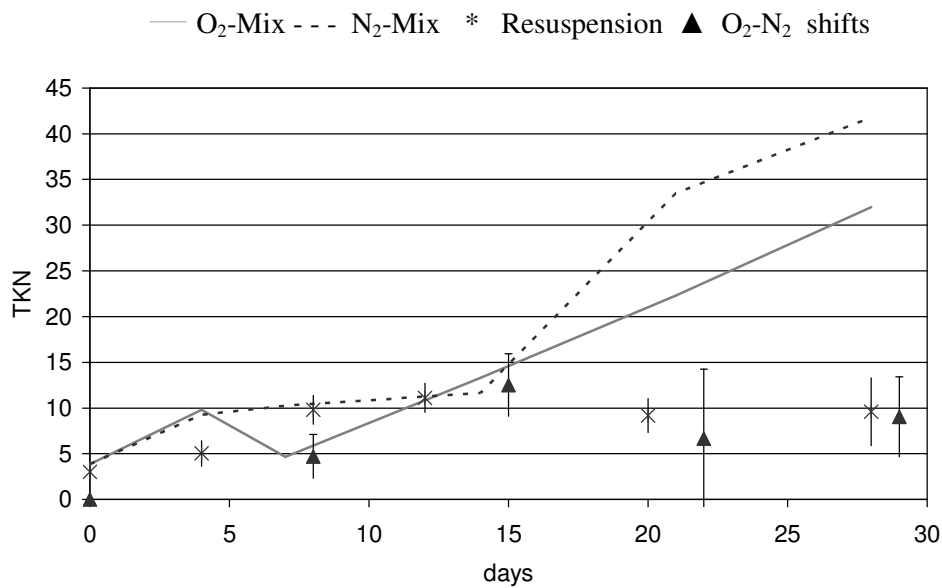
Figure 8. Changes in Total Kjeldhal nitrogen (TKN) in the microcosms during the experiment for the O_2-Mix, N_2-Mix, Resuspension and O_2-N_2 shifts treatments. Bars represent the standard deviation of clustered data.

Higher ammonia concentrations where recorded for the mixed-anaerobic treatment followed by the mixed-aerobic treatment. In the resuspension treatments and the aerobic-anaerobic shift treatments ammonia concentrations increased slowly until day 10 and from then onwards diminished to concentrations ranging in the resuspension treatments between 0.07 and 0.5 mg l^{-1} and between 1.7 and 3.0 mg l^{-1} in the aerobic-anaerobic shift treatments (Figure 9).
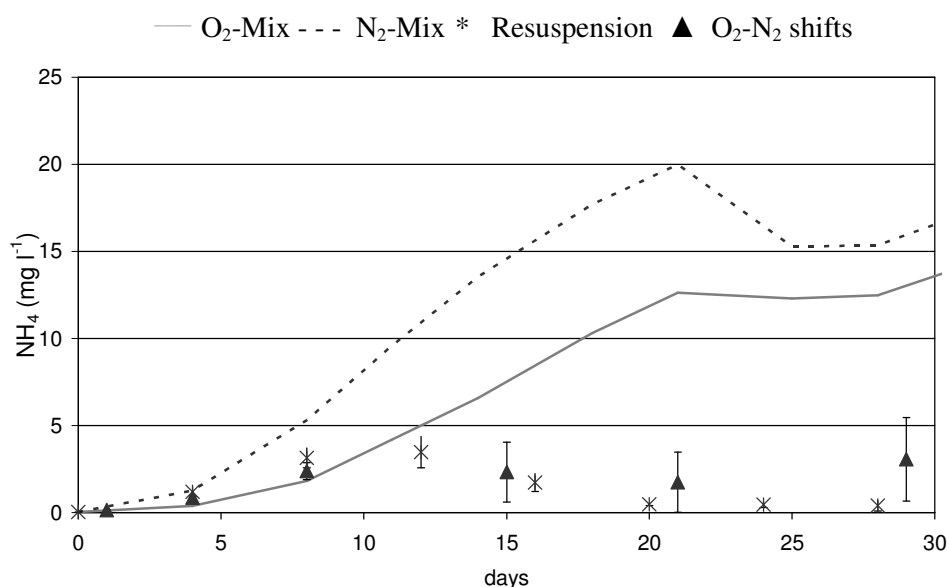
Figure 9. Changes in ammonia concentrations (NH_4^+) in the microcosms during the experiment for the O_2-Mix, N_2-Mix, Resuspension and O_2-N_2 shifts treatments. Bars represent the standard deviation of clustered data.

In the resuspension treatments the concentrations of nitrate increased steadily but slowly. In the mixed-aerobic treatment the average concentration remained around 5 mg l^{-1} from day 10 onwards till the end of the experiment. No nitrate concentrations were recorded in the mixed-anaerobic treatment (Figure 10). The highest nitrate concentrations were found in the aerobic-anaerobic shift treatments at the end of the experiment. Large standard deviations for the clustered aerobic-anaerobic shift treatments are related to the fact that the NO_3^- concentration in treatment $12O_2$ was lower than in treatments $6O_2$, $18O_2$ and $6+6O_2$ ($P < 0.05$, Table 2).
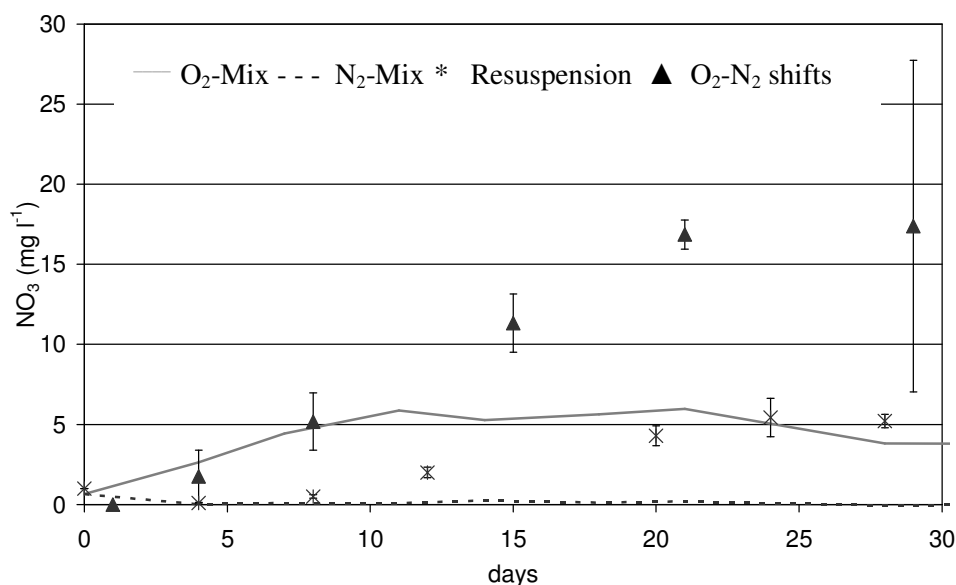
Figure 10. Changes in nitrate concentrations (NO_3^-) in the microcosms during the experiment for the O_2-Mix, N_2-Mix, Resuspension and O_2-N_2 shifts treatments. Bars represent the standard deviation of clustered data.

To follow the fate of the nitrogen in the different treatments a nitrogen balance was calculated including TON, TIN and TN-loss (Figure 11). In the mixed-aerobic and mixed-anaerobic treatments the remaining nitrogen was more or less equally divided between organic (TON) and inorganic (TIN) species and the N-loss was negligible. In the aerobic-anaerobic shift treatments most of the nitrogen was retained in the form of inorganic (TIN) species demonstrating a high degree of mineralization. More than 70% of the nitrogen inputs in the resuspension treatments disappeared from the system, suggesting a high degree of denitrification. Interestingly, a similar 76% nitrogen loss occurred in the $12O_2$ treatment, while not in the other aerobic-anaerobic shift treatments.
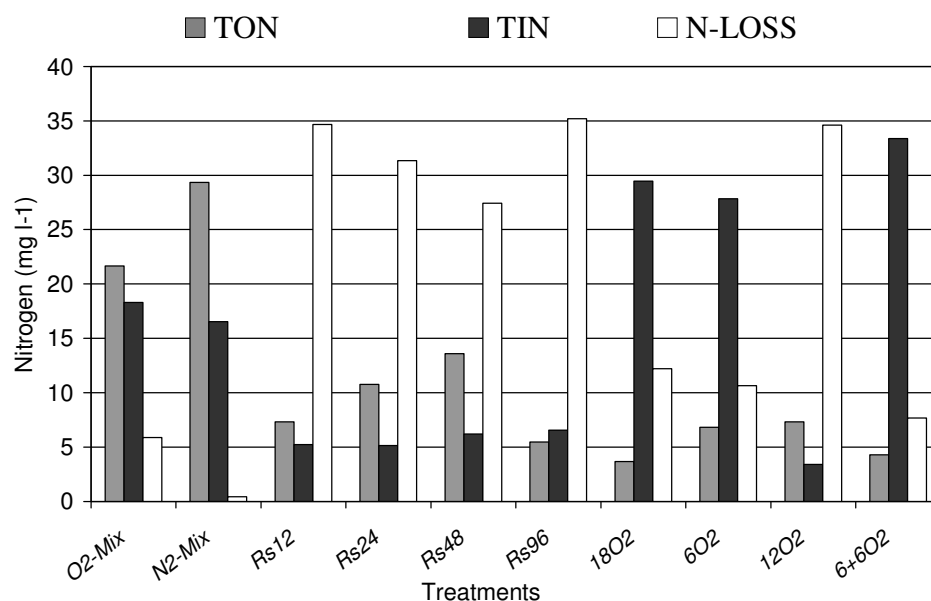
Figure 11. Nitrogen balances of all the treatments at the end of the experiment. TON (total organic nitrogen), TIN (Total inorganic nitrogen) and N-LOSS (nitrogen loss)

4. Discussion

It is generally accepted that organic matter decomposition occurs faster under aerobic than under anaerobic conditions (Reddy and Patrick, 1975). However this view was challenged by Kristensen *et al.,* (1995) who concluded that the organic matter decomposition was not obviously faster under aerobic than anaerobic conditions. The decomposition rate depends primarily on the mixture and type of the organic components (e.g. proteins, carbohydrates, lipids) present and the decomposition stage the mixture is in.

Anaerobic decomposition occurs basically in two steps. In step one particulate organic matter (POM) is transformed into dissolved organic matter (DOM). In step two the DOM is further mineralized leading to the release of for instance CO_2 and NH_4^+ (Kristensen *et al.*, 1995). To understand the results of anaerobic decomposition this two steps process need to be taken into account. The fate of carbon or nitrogen in aquatic systems is differently influenced by aerobic or anaerobic conditions.

Redox potential

Abrupt shifts in the aerobic-anaerobic state of aquatic system are accompanied by dramatic changes in the redox potential and inhibit or kill specific species groups of bacteria while benefiting other groups. This leads to a modification of composition of the microbial community (Kristensen *et al*., 1995). The redox potential is not necessarily uniform throughout an aquatic ecosystem. In the resuspension treatments the redox potential was always positive at the surface of the microcosms while it was constantly negative at the bottom (Figure 3). The frequency of resuspension events as well as the level of organic loading influences the position of the aerobic-anaerobic interface (Brune *et al*., 2000). No significant differences were found between the 4 resuspension treatments for surface and bottom redox potential values (P > 0.05) presented in Figure 3. The redox potential values observed at the bottom in all resuspension treatments were on average 100 mV higher than the redox potential in the mixed-anaerobic treatment (Figure 3). This might be explained by the presence of NO_3^- in the resuspension treatments which was absent in the mixed-anaerobic treatment, acting as an alternative electron acceptor and diminishing the decrease in redox potential (Reddy and Patrick, 1975).

CO_2 production

The breakdown of organic carbon in aerobic system was straight forward and could be monitored on the basis of CO_2 production measurements. As is shown in Figure 6 the amount of carbon mineralized to CO_2 was around 5 times higher in the mixed-aerobic treatment than in the mixed-anaerobic treatment. In fresh water systems, only 2.5 times more CO_2 was produced in a mixed-aerobic treatment than in a mixed-anaerobic treatment (Torres *et al*., submited). In water logged soils, Reddy and Patrick (1975) also found twice the amount of CO_2 produced under aerobic than under anaerobic conditions. In the present experiment, the CO_2 production in all the aerobic-anaerobic shift treatments was similar to the mixed-aerobic treatment but much higher than in the mixed-anaerobic treatment (Figure 6). The N-loss registered in the resuspension treatments (Figure 11) indicates that denitrifying bacteria were quite active. The CO_2 production levels in the aerobic-anaerobic shift treatments were equally high (Figure 6), but the N-loss in these treatments was much smaller (Figure 11). The results show that coupling of aerobic and anaerobic processes through small resuspension events or through serial shifts in the aerobic-anaerobic state of aquatic systems lead to a similar CO_2 production as in a completely mixed-aerobic system. However, resuspension events have a

different effect on the fate of nitrogen in the system than serial shifts in the aerobic-anaerobic state of the system.

COD accumulation and CO$_2$ production

The mixed-anaerobic treatment had the higher organic carbon accumulation, measured as COD, than the mixed-aerobic treatment (Figure 7). The same pattern was observed in mixed simulated aquaculture ponds (Avnimelech *et al*., 1992). In the mixed-anaerobic treatment, as well as in the resuspension and aerobic-anaerobic shift treatments, a large fraction of the accumulated carbon is dissolved organic carbon produced through fermentation processes (Blackburn, 1987).   The COD accumulation in the resuspension treatments was slightly higher than in the aerobic-anaerobic shift treatments, but the results were highly variable (Figure 7). Combining the data from carbon which is either loss (CO$_2$) or remaining in the system (COD), the carbon mineralization seems to occur very effective in treatments where aerobic and anaerobic conditions were alternated, especially in those treatments where longer (6 h or more) aerobic conditions occurred. In resuspension treatments the carbon mineralization was apparently less effective because relatively more COD remained in those systems. It is hypothesized that in the latter systems, the particulate organic matter (POM) remained most of the time at the bottom where anaerobic conditions prevailed and the POM was converted into dissolved organic carbon (DOC) (Schroeder, 1987). This DOC diffused slowly to above oxygenated water column, except during resuspension events, which seem to be sufficient to trigger still a considerable CO$_2$ production.

Nitrogen transformations

Nitrogen is constantly transformed in aquatic environments. TKN includes total ammonia nitrogen and the organic nitrogen from bacteria, dissolved organic matter and undecomposed fish feed. The highest TKN values were observed in the mixed-aerobic and mixed-anaerobic treatments (Figure 8), with the build-up of TKN occurring at the same speed in the mixed-aerobic and mixed-anaerobic treatments. Reddy and Patrick (1975) also found that protein is broken down at the same rate under aerobic and anaerobic conditions. Nitrification did not occur in the mixed-anaerobic treatment because of the lack of oxygen. In the mixed-aerobic treatment nitrification started from the beginning of the experiment and NO$_3^-$ reached its maximum around day 10. Subsequently, the nitrification was inhibited and NH$_4^+$-N accumulated in the system (Figure 9 and 10). This inhibition of the nitrification process in the mixed-aerobic treatment was also found in a previous experiment (Torres *et al*., submitted).

Possible explanations are substrate inhibition or heterotrophic competition for carbon source (Hanaki *et al.*, 1990; Strauss and Lamberty, 2000). In the resuspension and the aerobic-anaerobic shifts treatments nitrification occurred causing ammonia concentrations to stay low (Figure 9). Even though the ammonia levels for the resuspension treatments and the aerobic-anaerobic shift treatments were similar, suggesting that similar amounts of nitrate were produced, the nitrate concentrations where higher in the aerobic-anaerobic shift treatments than in the resuspension treatments. This suggests that NO_3^- was consumed more efficiently and that the denitrification rate was higher in the resuspension treatments.

Denitrification is an anaerobic process where NO_3^- is used as a terminal electron acceptor and N_2 gas is produced (Reddy and Patrick, 1975). Therefore the denitrification process demands the physically coexistence or consecutive existence of aerobic and anaerobic conditions. Alternation of aerobic-anaerobic conditions proved to be an effective way to promote denitrification (Avnimelech and Raveh, 1974; Reddy and Patrick, 1975 & 1976). In addition soluble organic compounds, mainly volatile fatty acids, generated during anaerobic periods, fueled the denitrification process acting as a carbon source for nitrifying bacteria (van Rijn *et al.*, 1995; Aboutboul *et al.*, 1995) and therefore increasing the CO_2 production. In the present experiment in the resuspension treatments the well oxygenated water column assured the NO_3^- supply to the anoxic bottom. The average N-loss was 70% ($\pm$ 1.5) in the resuspension treatments and was not influenced by the different resuspension regimes (P > 0.05) (Figure 11). While the consecutive aerobic-anaerobic shift treatments the N loss was on average 20 % ($\pm$ 5.0) with exception of the $12O_2$ treatment which had a pattern similar to the Rs12, and had a N-loss of 73%. It was striking that only the Rs12 treatment showed a decomposition pattern similar to the resuspension treatments. Apparently a 12 hour shift is necessary for both nitrifiers and denitrifiers to develop sufficiently. These bacteria are known to be slow growers (Avnimelech *et al.*, 1992).

Conclusions

Ammonification was carried out at the same rate under aerobic or anaerobic conditions (49% of the input) and the decomposition of the original fish feed input was accomplished at similar rates under aerobic and anaerobic conditions. However, the accumulation of carbon as dissolved organic carbon was higher under anaerobic conditions. In aerobic systems nitrifiers can recycle the generated NH_4^+ and produce NO_3^-. Inorganic nitrogen, mainly ammonia which is very toxic for fishes accumulated in the mixed-aerobic and mixed-anaerobic treatments. NO_3^- accumulated in the aerobic-anaerobic shift treatments (with treatment $12O_2$ as an exception), which is less toxic to aquatic life. The resuspension treatments had the lower accumulation of inorganic nitrogen species like NH_4^+ or NO_3^- and a higher N-loss. The fish feed added daily to aquaculture ponds provides fresh organic matter that is easy to degrade under both aerobic and anaerobic conditions. The degree of coupling between oxic and anoxic conditions in the system has a strong effect on the products accumulating and escaping from the culture system. Pure oxic or anoxic conditions proved to be less favorable than mixed aerobic-anaerobic systems with respect to the metabolites accumulating in the system. Short 15-minutes resuspension events and a continuous alteration of oxic and anoxic conditions at 12-hour time intervals proved to be the best options to minimize the accumulation of organic matter in culture systems. The correct coupling of aerobic-anaerobic conditions in space and time is the key to maintain a good balance between decomposition and assimilation of carbon and nitrogen components in aquaculture production systems.

References

Aboutboul, Y., Arviv, R., van Rijn, J., 1995. Anaerobic treatment of intensive fish culture effluents: volatile fatty acid mediated denitrification. Aquaculture 133, 21-32.

American Public Health Association (APHA), 1998. Standard methods for the examination of water and wastewater, 20th Edition. American Public Health Association, New York, USA.

Avnimelech, Y., Raveh, A., 1974. The control of nitrate accumulation in soils by induced denitrification. Water Research 8, 553-555.

Avnimelech, Y., Mozes, N., Weber, B., 1992. Effects of aeration and mixing on nitrogen and organic matter transformations in simulated fish ponds. Aquacultural Engineering. 11, 157-169.

Avnimelech, Y., Ritvo, G., 2003. Shrimp and fish pond soils: processes and management. Aquaculture 220, 549-567.

Arbiv, R., van Rijn, J., 1995. Performance of a treatment system for inorganic nitrogen removal in intensive aquaculture systems. Aquacultural Engineering 14, 189-203.

Blackburn, T.H., 1987. Role and impact of anaerobic microbial processes in aquatic systems. Pages 32-53 in: D. J. W. Moriarty and R.S.V. Pullin (Eds) Detritus and microbial ecology in aquaculture. Manila, Philippines, ICLARM pp. 420

Brune, A., Frenzel, P., Cypionka, H., 2000. Life at the oxic–anoxic interface: microbial activities and adaptations. FEMS Microbiology Reviews 24, 691-710.

FAO, 2002. The state of the world fisheries and aquaculture 2002. Food and agriculture organization of the united nations. FAO. Rome pp 150.

Fry, J.C., 1987. Functional roles of the major groups of bacteria associated with detritus. Pages 83-122 in: D. J. W. Moriarty and Pullin, R. S. V. (Eds) Detritus and microbial ecology in aquaculture Manila, Philippines, ICLARM pp. 420.

Gomez, K.A., Gomez, A.A., 1984. Statistical Procedures for Agricultural Research. 2nd edition. John Wiley and Sons, New York, USA, pp 704.

Gross, A., Boyd, C.E., Wood, C.W., 2000. Nitrogen transformations and balance in channel catfish ponds. Aquacultural Engineering 24, 1-14.

Hanaki, K., Wantawin, C., Ohgaki, S., 1990. Effects of the activity of heterotrophs on nitrification in a suspended-growth reactor. Water Research 24, 289-296.

Hargreaves, J.A. 1998. Nitrogen biogeochemistry of aquaculture ponds. Aquaculture 166, 181-212.

Hopkins, J.S., Sandifer, P.A., Browdy, C.L., 1994. Sludge management in intensive pond culture of shrimp: Effect of management regime on water quality, sludge characteristics, nitrogen extinction, and shrimp production. Aquacultural Engineering 13, 11-30.

Jimenez-Montealegre, R., 2001. Nitrogen transformation and fluxes in fish ponds: a modeling approach. Fish Culture and Fisheries. PhD Thesis, Wageningen, The Netherlands, 185 pp.

Jimenez-Montealegre, R., Verdegem, M.C.J., van Dam, A., Verreth, J. A. J., 2002. Conceptualization and validation of a dynamic model for the simulation of nitrogen transformations and fluxes in fish ponds. Ecological Modelling 147, 123-152.

Kristensen, E., Ahmed, S.I., Devol, A.H., 1995. Aerobic and anaerobic decomposition of organic matter in marine sediment: Which is fastest? Limnology and Oceanography 40, 1430-1437.

Low, E.W., Chase, H.A., 1999. Reducing production of excess biomass during wastewater treatment. Water Research 33, 1119-1132.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurements with the Folin-phenol reagent. Journal of Biological Chemistry 193, 265-275

Milstein, A., Zoran, M., Kochba, M., Avnimelech, Y., 2001. Effect of different management practices on water quality of intensive tilapia culture systems in Israel. Aquaculture International 9, 133-152.

Reddy, K.R., Patrick, J.W.H., 1975. Effect of alternate aerobic and anaerobic conditions on redox potential, organic matter decomposition and nitrogen loss in a flooded soil. Soil Biology and Biochemistry 7, 87-94.

Reddy, K.R., Patrick, J.W.H., 1976. Effect of frequent changes in aerobic and anaerobic conditions on redox potential and nitrogen loss in a flooded soil. Soil Biology and Biochemistry 8, 491-495.

Riise, J.C., Roos, N., 1997. Benthic metabolism and the effects of bioturbation in a fertilised polyculture fish pond in Northeast Thailand. Aquaculture 150, 45-62.

Schroeder, G.L., 1987. Carbon pathways in aquatic detrital systems pages 217-236 in: D.J.W. Moriarty and R.S.V. Pullin (Eds) Detritus and microbial ecology in aquaculture Manila, Philippines, ICLARM. pp 420.

Strauss, E.A., Lamberti, G.A., 2002. Effect of dissolved organic carbon quality on microbial decomposition and nitrification rates in stream sediments. Freshwater Biology 47, 65-74.

van Rijn, J., Fonarev, N., Berkowitz, B., 1995. Anaerobic treatment of intensive fish culture effluents: digestion of fish feed and release of volatile fatty acids. Aquaculture 133, 9-20.

General discussion

GENERAL DISCUSSION

The main objective of this thesis was to investigate the decomposition of organic matter under intensive fish pond conditions and to evaluate the effect of C/N ratio, resuspension events and oxygen availability on decomposition. The role of the microorganism in aquaculture ponds was reviewed, focusing on the decomposition of organic matter and its influence on pond dynamics. Decomposition of organic matter was studied in lab-scale 2-l microcosms with daily feed addition simulating feeding levels applied in intensively managed ponds. No phytoplankton developed in the microcosms and no fishes were stocked.

In aquaculture ponds, external nutrient inputs (e.g. fertilizer, manures, crop residues, commercial feeds) are applied to increase fish production. This organic matter load is not only assimilated by the fish, but also influences the water quality and ecological processes in the pond. In part, pond management aims to optimize nutrient efficiency and to minimize nutrient discharge to the environment (Tacon *et al.*, 1995; Tacon and Forster, 2003). Towards the end of a culture cycle in intensively fed ponds, the fish feed input rapidly becomes the main factor affecting water quality (Milstein, 1990). A common practice to avoid oxygen problems and dilute the toxic compounds is to exchange big volumes of water per day (Hopkins *et al.*, 1993). In the last years, a "green water" approach was developed to reduce the water use in intensive ponds. This approach consists of the integration and balance of the bacterial and algal sub-systems, stimulating at the same time the pond productivity and controlling water quality (Moss *et al.*, 1992; Moss 2001).

In some of these studies the water exchange was indeed reduced (Kochba *et al.*, 1994; Funge Smith and Briggs, 1998; McIntosh, *et al.*, 2001) often to the point of zero exchange (Hopkins *et al.*, 1995; McIntosh, 2001; Tacon *et al.*, 2002; Thakur and Lin 2003; Burford *et al.*, 2003). However the remaining bottleneck is to optimize and control the decomposition and production processes and to link them to higher trophic levels. Large amounts of organic matter, are added to intensive systems, mainly in the form of fish feed. The high nutrient concentration of the system stimulates the production of both the detrital and algal food webs. However, the microbial biodiversity (algae and bacteria) tend to decrease in these eutrophic systems (Cole, 1982). In addition, the relationship between both food webs becomes more fragile. Some undesirable species like cyanobacteria (blue-green algae) can become dominant. These algae are not readily used as food and they are relatively poor water oxygenators (Paerl

and Tucker, 1995). When large quantities of organic matter are decomposed by bacteria available oxygen gets depleted and products potentially toxic to aquatic life can be produced under anaerobic conditions. Furthermore, outbreaks of pathogenic bacteria and viruses are often linked to poor environmental conditions in intensive production systems (Kautsky *et al*., 2000). In intensive ponds, the organic load easily reaches the pollution threshold. Under such circumstances the success of the culture will depend to a great extend on how skillfully the decomposition of organic matter is managed by the farmer.

Organic matter decomposition: aerobic vs anaerobic

In aquaculture ponds aerobic and anaerobic conditions co-exist. The main fraction of the unconsumed organic matter will accumulate in the sediment. This organic matter is decomposed first using oxygen as electron acceptor and when oxygen is finished, a sequence of other electron acceptors will oxidize the organic matter (e.g. NO_3^-, MnO_2, $FeOOH$, SO_4^{-2}, and CO_2) (Reddy *et al*., 1986). To determine the effect of aerobic and anaerobic conditions on the organic matter decomposition and the metabolites formed, experiments simulating intensive aquaculture ponds were carried out (Chapter 3). The aerobic or anaerobic conditions influenced the amount of organic carbon that was mineralized (CO_2), assimilated (bacteria) or partially decomposed (organic acids) in the system. Under aerobic conditions half of the decomposed organic matter was mineralized as CO_2 and the other half was used to build bacteria biomass (Henze *et al*., 2002). In our experiments, the aerobic treatments produced higher amounts of bacterial biomass and CO_2 than the anaerobic treatments. Under anaerobic conditions several consecutives steps are involved in organic matter decomposition, including anaerobic acid production and anaerobic methane production (Henze *et al*., 2002). In our experiment methane was measured in only one of the duplicate anaerobic treatments and in very small amounts. The organic carbon remaining in our system, (including organic acids, bacteria biomass and undecomposed organic carbon), was 3.2 times higher under anaerobic than under aerobic conditions. This remaining carbon is assumed to consist of low molecular compounds associated with the microbial extracellular matrix (Schroeder, 1987; van Rijn *et al*., 1995).

The model of organic matter decomposition is described by a first order kinetics equation in which the daily addition and degradation of fish feed (Avnimelech *et al*., 1995) are combined. This model is described by equation (1) where *C* is the organic carbon component, *t* is the time, K_C is the decomposition rate constant and *B* is the daily input of component *C*.

$$dC / dt = B - KcS \qquad\qquad (1)$$

The organic carbon mineralization rate constant K_C was dependent on the oxic status of the systems. The K_C was significantly higher in aerobic treatments ($\overline{K_C} = 0.26$) than in anaerobic treatments ($\overline{K_C} = 0.06$). In consequence, more organic carbon remained in the anaerobic treatments than in aerobic treatments (Chapter 3).

In intensive aquaculture ponds, heterotrophic bacteria decompose large amounts of fish feed. Fish feed is a nitrogen rich substrate. When decomposing, it produces high amounts of ammonia. Only a small fraction is incorporated into bacterial biomass. The amount of ammonia produced during decomposition (23% or 49% protein diet) was similar under pure aerobic and anaerobic conditions (Chapter 3). From bacteria activity tests using arginine-addition, similar ammonification rates were obtained under aerobic and anaerobic conditions (Chapter 4). Ammonia can be consumed by nitrifying bacteria under aerobic conditions and transformed into harmless nitrate (Hagopian and Riley 1998). In our pure aerobic mixed treatments nitrification did not occur, even though oxygen, ammonia and carbon sources were present (Chapter 3). This was attributed to the excess of organic matter (feeding rate of 40 mg l^{-1}day^{-1}). In aquatic environments, nitrification inhibition by organic carbon has been previously reported (Hanaki et al., 1990; Zhu and Chen, 2001). Therefore the feeding rate was reduced to the half (20 mg l^{-1}day^{-1}) in the next experiment (Chapter 5), but still no nitrification occurred. However, in all treatments where aerobic-anaerobic conditions were combined nitrification was observed. From the sanitation point of view, in intensively fed ponds with great amounts of feed spills, the maintenance of aerobic conditions is needed to faster mineralize the maximal amount of organic carbon. However from the ecological point of view, the organic carbon accumulation/preservation under anaerobic conditions is not necessarily negative if it can be brought in contact with oxic conditions regularly. For farmers, controlling mixing events between aerobic and anaerobic zones in the pond is the key factor to maintain healthy conditions and to use the valuable nutrients introduced.

The amount of bacteria biomass was quantified under both aerobic and anaerobic conditions (Chapter 4). The bacterial biomass is important because it forms the link between dissolved nutrients and higher trophic levels. In ponds, food webs are shorter than in natural systems

because zooplankton and macrofauna are nearly completely eliminated by intense predation and the dominating pathways are algae-fish or detritus-fish (Anderson, 1987). In all the microcosm treatments, bacterial density increased with the nutrient load. More bacteria biomass was produced under aerobic conditions than under anaerobic. The bacterial abundance at the end of the experiment was 3.4×10^9 cells ml^{-1} in aerobic treatments and 1.9×10^9 cells ml^{-1} in anaerobic treatments. These values are considerably higher than values found in commercial growth out pond systems, most probably due to the controlled lab conditions (constant temperature, permanent stirring, daily feeding) that favoured bacterial development. Under aerobic conditions organic carbon decomposition was very effective. An efficiency of 50% (between 40 to 60%) is normally assumed and half of the carbon is mineralized as CO_2 and the other half is accumulated as bacteria biomass (Gaudy and Gaudy, 1980). In natural systems the bacterial growth efficiencies vary over a broad range (5- 60%), depending on the environmental factors (del Giorgio and Cole 1998). The results found in the present study corroborate with the observations previously reported in literature. Bacterial growth efficiencies under aerobic conditions varied between 8 and 63%. From the sanitation point of view, higher amounts of CO_2 production imply that a large part of the organic matter is decomposed and that organic carbon is mineralized and removed from the system. In pond culture it is of great importance to recycle the unused dietary nutrients and the metabolic wastes by converting them into bacterial biomass. The direct or indirect consumption of this bacterial biomass by the cultured animals will improve the feeding efficiency and cost.

Aerobic –Anaerobic: the role of coupling

Accumulation and decomposition occurs concurrently at the pond bottom, leading to the formation of stratified sediment, each stratum having its own carbon content and dominant type of electron acceptor (Spring *et al.*, 2000). In this stratified environment the microbial communities are also layered. The factors that affect the relative position of the oxic-anoxic boundary also affect the microbial communities (Brune *et al.*, 2000). Bacteria living in the oxic-anoxic interface are bacteria with a great metabolic versatility and the adaptive capacity to survive under a broad range of aerobic-anaerobic conditions. In aquaculture ponds, a dramatic oxygen depletion due to for instance an algal crash, can kill some aerobic bacteria, but several studies showed that facultative aerobic bacteria can adapt quickly to the new anoxic situation, while aerobes like nitrifying bacteria would remain latent (Ram *et al.*, 1981). In the present study the aerobic-anaerobic range was simulated restricting oxic and anoxic

conditions in time and space. In the mixed treatments, long periods (between 6 to 18 h) of O_2 or N_2 gas supply were alternated. In the stagnant systems, the majority of the time the aerobic conditions were restricted to the water column. Sediment material remained at the anaerobic bottom and only when a 15 minutes resuspension event occurred the sediment was mixed with the above oxygenated water column (Chapter 5).

The reduced products and the dissolved organic substances present in the anaerobic areas stimulated the formation of bacteria biomass when mixed with the aerobic layers (Blackburn, 1987). For example the reduced H_2S is re-oxidized and used by sulfur oxidizing bacteria (Zopfi et $al.$, 2001). When aerobic and anaerobic conditions are coupled, reduced substances are oxidized and bacterial production is stimulated. However, when anaerobic conditions dominate, not all the reduced substances will get oxidized (Hargreaves and Tucker 2003). When the oxic and anoxic conditions cannot be coupled, the anoxic zone will develop into a toxic environment for the cultured animals. The coupling and connection of aerobic-anaerobic processes, as well as the amount of organic matter oxidized, determined the water quality of the system. So in intensively fed ponds, farmers should avoid long periods of leaving the sediment undisturbed, and introduce short periods of mixing. The frequency of mixing can be low, but when mixing occurs, it should be thoroughly.

Nitrogen removal is strongly influenced by the coexistence of aerobic and anaerobic circumstances, because nitrification is an aerobic process while denitrification is an anaerobic process (Hargreaves, 1998). Therefore, the magnitude of nitrogen removal depends on the degree of coupling between aerobic and anaerobic conditions. Denitrification uses NO_3^-, which is only produced under aerobic conditions, as terminal electron acceptor. For an effective nitrification, a constant NO_3^- supply is needed. Coupling between oxic and anoxic conditions is possible either through the continuous coexistence of an aerobic and anaerobic layer which are mixed at regular time intervals, or through consecutive changes in oxygen availability over time. These two mechanisms were tested in Chapter 5. The coexistence of aerobic and anaerobic conditions was tested with the creation of an aerobic water column and anaerobic sediment. Resuspension events (15-minutes) disturbed the system with a frequency varying from two times a day to every four days. The consecutive occurrence of aerobic and anaerobic conditions was simulated by alternating the O_2 and N_2 flows through the microcosms. Nitrogen removal was observed in all the treatments where aerobic and anaerobic were coupled in space or time. During anaerobic decomposition a large part of the

organic carbon remained in the microcosms as low molecular weight compounds, most likely volatile fatty acids (van Rijn *et al*., 1995). The coupling of aerobic-anaerobic processes also fuels denitrification by providing this low molecular weight compounds as a carbon source for the heterotrophic denitrifying bacteria (Aboutboul *et al*., 1995). Anaerobic conditions always prevail under the first millimeters of aquatic sediments. In aquaculture ponds, anaerobic conditions in the sediment surface are common due to the high amount of organic matter added. A well oxygenated water column above this sediment is needed to maintain aerobic conditions. The coexistence and coupling of both aerobic and anaerobic processes stimulates optimal organic matter decomposition, a high bacterial biomass production and limits the production of toxic compounds for the aquatic life.

C/N ratio, the quality factor

The organic matter decomposition processes are influenced by the C/N ratio of the substrate. The C/N ratio of commercial fish feeds is low compared to natural organic matter sources (e.g. macrophytes, algae, run-offs) due to its high protein content. In Chapter 3 the decomposition of two fish diets with different protein content and C/N ratio were followed under aerobic and anaerobic conditions. The initial C/N ratios were 12.8 and 6.3 for 23 % and 49 % protein diet, respectively. During the decomposition process, carbon was mineralized to CO_2 and removed from the systems, while nitrogen species, mainly ammonia, accumulated. Therefore, the C/N ratio of the organic matter decreased in all treatments. A decrease in C/N ratio during a decomposition process has been observed in other studies (Kochba *et al*., 1994; Mohanty *et al* 1994; Burford, *et al*., 2003). In our experiments higher amounts of CO_2 were produced under aerobic conditions than under anaerobic conditions. The amount of ammonia produced was entirely dependent of the protein content of the diet. The diet with lower C/N ratio produced the higher amounts of dissolved inorganic nitrogen compounds. Due to the high CO_2 production followed by volatilization under aerobic conditions and the concurrent accumulation of nitrogen compounds, the C/N ratio decreases faster aerobically than anaerobically. The C/N ratio range where the decomposition of organic matter is optimal is around 10 (Alexander, 1999). No effect of C/N ratio (diet treatments) on CO_2 production was observed in our microcosms. Compared to the C/N ratios found in natural (20-40) or in recirculation systems (2-3), the C/N ratios tested in the microcosms (12.8 and 6.3) were probably not divergent enough to find clear differences. In addition, both fish diets provided similar amounts of organic carbon. A high C/N ratio (>30) slows down microbial activity (Mohanty *et al*., 1994), but also a low C/N ratio limits bacteria biomass synthesis (Schneider

et al., 2004). Beside the C/N ratio, an important factor affecting the organic matter decomposition is the organic matter quality. Fish feed mainly consists of easily degradable starches, proteins and fats, making it highly reactive. For example, easily decomposable organic carbon, like the inputs provided by fish feed, stimulated heterotrophic activity and inorganic nitrogen consumption, effectively outcompeting nitrifying bacteria (Strauss and Lamberti, 2000, 2002). In intensive aquaculture ponds the decomposition of fish feed is easy and fast because it consists of labile compounds and the C/N is not too low initially. The problem starts when inorganic nitrogen compounds accumulate causing the C/N ratio to drop and slowing down the decomposition process. The protein content in the diet should be carefully chosen, taking into account not only its nutritional value but also the C/N ratio, and its effect on bacterial production. The latter stimulates fish or shrimp production and increase farming profits (Chapter 2).

Organic load, the quantity factor

In intensive aquaculture ponds one the most important pond management practices to control is the feeding level. The amount of organic matter added to the aquaculture ponds through feed is the main factor affecting water quality (Milstein, 1990). Among water quality parameters ammonia concentration is very problematic and related with mortality and low growth rates in intensive systems (Krom *et al.*, 1985). In the present thesis two feeding levels of intensively managed pond systems were simulated. At the rate of 40 mg l^{-1}day $^{-1}$ two diets with low protein content (23%) and high protein content (49 %) were added (Chapter 3). A second feeding rate of 20 mg l^{-1}day $^{-1}$ was tested for the 49% protein content diet (Chapter 5). Figure 1 shows that the ammonia concentrations increased over time in all treatments. The highest ammonia concentrations were produced by the 49% protein content diet under a 40 mg l^{-1} day^{-1} feeding regime. The high protein diet under a feeding regime of 40 mg l^{-1} day^{-1} produced 2.02 mg NH_4^+-N l^{-1}day^{-1}. This value was more than double the ammonia production of the other two cases: 0.81 mg NH_4^+-N l^{-1} day^{-1} for the high protein diet at 20 mg l^{-1} day $^{-1}$ and 0.76 mg NH_4^+-N l^{-1} day^{-1} for the low protein at 40 mg l^{-1} day $^{-1}$.
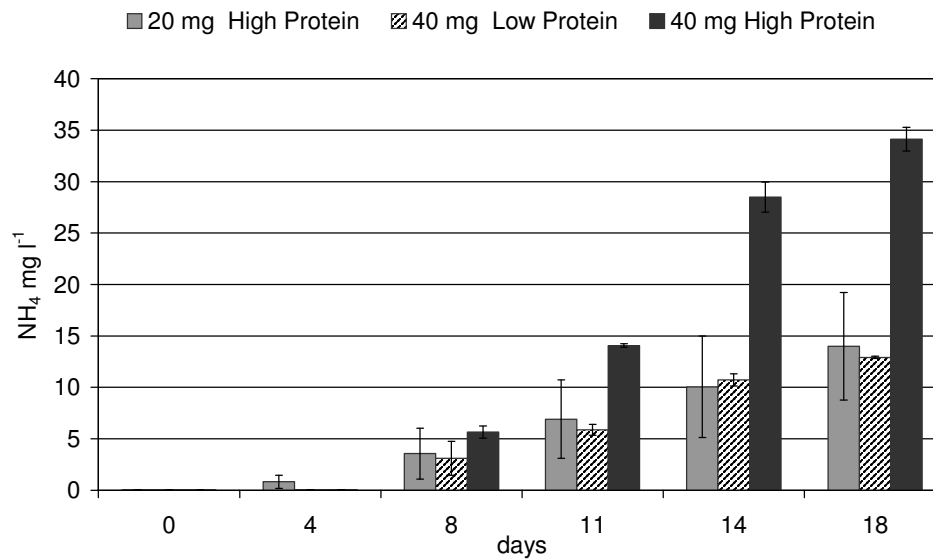
Figure. 1 The ammonia concentrations by feeding level and protein content. The 40 mg l^{-1} day^{-1} feeding level was tested for the high (49%) and low (23%) protein diets and the high protein diet (49%) was tested for 20 mg l^{-1} day^{-1} feeding rate. Bars represent the standard deviation of aerobic and anaerobic treatments.

The amount of organic matter added to the system and the pathway under which it is decomposed determine the amount of carbon converted into CO_2. The more intensive the system, the more organic matter is added to the ponds, and the more CO_2 is produced. The mechanisms controlling carbon cycling (decomposition vs. preservation) through different trophic levels is a hotly debated issue of global consequence (Hedges and Keil 1995; Berner, 1995; Hartnett *et al*., 1998; Sabine *et al*., 2004). Aquatic ecosystems can behave as CO_2 sinks (net autotrophic), when CO_2 fixation by phytoplankton is higher than CO_2 production due to microbial mineralization. They also can behave as CO_2 sources (net heterotrophic) when the organic carbon mineralization exceeds CO_2 fixation. In aquaculture ponds where N and P are added, primary production increases and CO_2 is fixed and converted into biomass. The carbon is stored as phytoplankton and passed on to higher trophic levels. However, massive phytoplankton crashes, typical for intensive aquaculture systems, and external carbon inputs as formulated feed, fuel the detrital web causing CO_2 production to exceed CO_2 fixation. Aquaculture ponds behave as net CO_2 producers. When swamps, typically assumed as carbon sinks, were used for aquaculture production they released 375 t ha^{-1} of carbon (De la Cruz, 1986). We can theoretically calculate the amount of CO_2 produced in a pond for an entire production cycle. The estimated CO_2 production is 52.2 t ha^{-1}. This estimation is based on a fishpond with 10 kg m^{-2} fish biomass and a daily feed input of 200 g m^{-2} (50% carbon

content), during a 120 days growing cycle and aerobic mineralization (50% efficiency) of not retained fish feed (13% carbon retention). In this estimation the amount of carbon introduced into the system by photosynthesis was not considered. These findings suggest that aquaculture ponds potentially have a large influence on the release of green house gasses. Their impact will depend on the organic load and the management practices. In terms of production and pollution, the idea that "more is better" does not apply in aquaculture ponds. The feeding level should be applied with caution and in combination with other management practices which maximize carbon re-utilization.

Experimental set up

The lab-scale microcosm was designed to resemble the organic load conditions of an intensive aquaculture system. With this batch feeding set up, it was possible to follow the decomposition process, and the inputs and outputs. The 2-liters experimental unit allowed optimal control of the oxic and temperature conditions in the medium. The system was easy to sample and measure, and inexpensive to run. However, the fact of being a microcosm system has a limitation in itself. It only simulates partially the real conditions and only some factors are taken in account. In real ponds there is more heterogeneity: microniches, numerous and different organisms and complex interaction of biotic and abiotic factors. It is important to have in mind the advantages and limitations of the experimetal set up. The information generated through the present work can help understand the decomposition processes occuring in aquaculture ponds, especially in relation with its main organic input: fish feed.

Further research suggestions

A more detailed characterization of organic decomposition products is necessary especially under anaerobic conditions. More research is needed on the phytoplankton component to better understand of the microbial loop. Bacteria biomass quantification including extracellular substances would help to evaluate nutrients cycling through the food web. Molecular techniques can be used to describe the microbial diversity of a fish ponds and its modification over the rearing period or by management practices. Further, it would be most interesting to study the effects, mechanisms and efficiency of probiotic products in aquaculture ponds.

Conclusions

Many of the management practices in aquaculture ponds are applied to minimize the effects of organic matter accumulation. The quantity and quality of the added inputs, as well as the decomposition pathways, determine the water quality in the system. A C/N ratio ranging between the tested values (6.3 and 12.8) had a similar influence on the carbon mineralization (< 50 days). A decrease in C/N ratio was observed in all the treatments during the experimental period, this reduction was especially fast under aerobic conditions. The C/N ratio in the diet determined the concentration of nitrogen species in the water. Higher amounts of ammonia were produced by high protein content diets. In the aerobic treatments no nitrification was measured even though the essential components, oxygen and ammonia, were present, most likely was related with the high organic load. Less accumulation of organic carbon occurred and more bacteria biomass was produced under aerobic conditions. Short 15-minutes oxic-anoxic mixing periods every 1-4 days, or oxic conditions for at least 6 h per day, were sufficient to obtain good organic matter decomposition. Nitrification and denitrification were registered for all the systems in which aerobic and anaerobic conditions coexisted in time or space. The coupling of aerobic and anaerobic conditions can effectively decompose organic matter and stimulate the nutrient cycling through the autotrophic and heterotrophic food webs. Nitrification was inhibited in pure aerobic conditions. The increase in culture intensity, (fish stoking densities and feed inputs), should be accompanied by management practices aiming to combine aerobic and anaerobic decomposition processes. The use of 2-l microcosms made it possible to identify the principal factors governing organic matter decomposition in ponds, keeping all other environmental factors uniform, which is impossible in real pond conditions. The results obtained form the 2-l microcosm studies clarified some of the principal factors governing organic matter decomposition in ponds. The next challenge is to apply these findings under farming conditions in ponds.

References

Aboutboul, Y., Arviv, R., van Rijn, J., 1995. Anaerobic treatment of intensive fish culture effluents: volatile fatty acid mediated denitrification. Aquaculture 133, 21-32.

Alexander, M., 1999. Biodegradation and bioremediation, second edition. Academic Press, San Diego, USA. 453 pp.

Anderson, J.M., 1987. Production and decomposition in aquatic ecosystems and implications for aquaculture. Pages in: D.J.W. Moriarty and R.S.V. Pullin (Eds). Detritus and microbial ecology in aquaculture. Manila, Philippines, ICLARM pp. 420

Avnimelech, Y., Mozes, N., Diab, S., Kochba, M., 1995. Rates of organic carbon and nitrogen degradation in intensive fish ponds. Aquaculture 134, 3-4.

Blackburn, T.H., 1987. Role and impact of anaerobic microbial processes in aquatic systems Pages 32-53 in: D. J. W. Moriarty and Pullin, R. S. V. (Eds) Detritus and microbial ecology in aquaculture. Manila, Philippines, ICLARM pp. 420.

Berner, R. A. 1995. Sedimentary organic matter preservation: an assessment and speculative synthesis--a comment. Marine Chemistry 49, 121-122.

Brune, A., Frenzel, P., Cypionka, H., 2000. Life at the oxic–anoxic interface: microbial activities and adaptations. FEMS Microbiology Reviews 24, 691-710.

Burford, M.A., Thompson, P.J., McIntosh, R.P., Bauman, R. H., Pearson, D.C., 2003. Nutrient and microbial dynamics in high-intensity, zero-exchange shrimp ponds in Belize. Aquaculture 219, 393-411.

Cole, J.J., 1982. Interactions between bacteria and algae in aquatic ecosystems. Annual review of ecology and systematics 13, 291-314.

De La Cruz, A. A. 1986. Tropical wetlands as a carbon source. Aquatic Botany 25, 109-115.

del Giorgio, P. A. and Cole, J. J., 1998. Bacterial growth efficiency in natural aquatic systems. Annual Review of Ecology and Systematics 29, 503-541.

Funge Smith, S.J., Briggs, M.R.P., 1998. Nutrient budgets in intensive shrimp ponds: implications for sustainability. Aquaculture 164, 1-4.

Gaudy, A.F.J. and Gaudy, E.T., 1980. Microbiology for Environmental Scientists and Engineers. New York, McGraw-Hill Companies, New York, USA. 736 pp.

Hagopian, D.S., Riley, J.G., 1998. A closer look at the bacteria of nitrification. Aquacultural Engineering 18, 223-244.

Hanaki, K., Wantawin, C., Ohgaki, S., 1990. Effects of the activity of heterotrophs on nitrification in a suspended-growth reactor. Water Research 24, 289-296.

Hargreaves, J.A. 1998. Nitrogen biogeochemistry of aquaculture ponds. Aquaculture 166, 181-212.

Hargreaves, J. A. and Tucker, C. S. 2003. Defining loading limits of static ponds for catfish aquaculture. Aquac. Eng. 28, 47-63.

Hartnett, H.E., Keil, R.G, Hedges J.I., Devol, A.H., 1998. Influence of oxygen exposure time on organic carbon preservation in continental margin sediments. Nature 391, 572-575

Hedges, J.I., Keil, R.G., 1995. Sedimentary organic matter preservation: an assessment and speculative synthesis. Marine Chemistry 49, 81-115.

Henze, M., Harremoes, P., Jansen, J., Arvin, E., 2002. Wastewater treatment, biological and chemical processes. Third edition. Springer Verlag, Berlin, Germany. 430 pp.

Hopkins, J. S., Hamilton, R. D., II, Sandifer, P. A., Browdy, C. L., Stokes, A. D. 1993. Effect of water exchange rate on production, water quality, effluent characteristics and nitrogen budgets of intensive shrimp ponds. J. World Aquacult. Soc. 24: 304-320.

Hopkins, J.S., Sandifer, P.A., Browdy, C.L., 1995. Effect of two feed protein levels and feed rate combinations on water quality and production of intensive shrimp ponds operated without water exchange. Journal of World Aquaculture Society 26, 93-97.

Kautsky, N., Ronnback, P., Tedengren, M., Troell, M., 2000. Ecosystem perspectives on management of disease in shrimp pond farming. Aquaculture 191, 145-161.

Kochba, M., Diab, S., Avnimelech, Y., 1994. Modeling of nitrogen transformation in intensively aerated fish ponds. Aquaculture 120, 95-104.

Krom, M. D., Porter, C. and Gordin, H. 1985. Causes of fish mortalities in semi-intensively operated seawater ponds in Eilat, Israel. Aquaculture 49, 159-177.

McIntosh, R.P., 2001, High Rate Bacterial Systems for Culturing Shrimp. Proceedings from the Aquacultural Engineering Society's Second Issues Forum, Ithaca, NY, NRAES-157, pp. 117_/129.

McIntosh, D., Samocha, T.M., Jones, E.R., Lawrence, A.L., Horowitz, S., Horowitz, A., 2001. Effects of two commercially available low-protein diets (21% and 31%) on water and sediment quality, and on the production of Litopenaeus vannamei in an outdoor tank system with limited water discharge. Aquacultural Engineeering 25, 69-82.

Milstein, A., 1990. Water quality in an intensive outdoor commercial fish culture system with mechanically stirred ponds. Israeli Journal Aquaculture Research-BAMIDGEH 42, 99-109.

Mohanty, A.N., Chatterjee, D.K., Saha, P.K.,. Pani, K.C., 1994. Effect of varying C/N ratios on the mineralization of organic nitrogen in fish pond soils. Journal of Aquaculture in the Tropics 9, 9-14.

Moss, S. M., G. D. Pruder, K. M. Leber and J. A. Wyban 1992. The relative enhancement of Penaeus vannamei growth by selected fractions of shrimp pond water. Aquaculture 101, 229-239.

Moss S.M., 2001. Zero-Exchange Systems for Intensive Shrimp Culture: Past Experiences, Biocomplexity Challenges, Future Opportunities. In: Biotechnology-Aquaculture Interface: The Site of Maximum Impact Workshop. ARS-The Oceanic Institute Workshop, National Conservation and Training Center, U.S. Fish and Wildlife Service, Shepherdstown, West Virginia, March 5-7, 2001.

Paerl, H.W., Tucker, C.S., 1995. Ecology of blue-green algae in aquaculture ponds. Journal of World Aquaculture Society 26, 109-131.

Ram, N., Ulitzur, S., Avnimelech, Y., 1981. Microbial and Chemical changes occurring at the mud-wate interface in an experimental fish aquarium. Israeli Journal of Aquaculture Research-BAMIDGEH 33, 71-86.

Reddy, K. R., Feijtel T. C., Patrick, J. W.H., 1986. Effect of soil redox conditions on microbial oxidation of organic matter. The role of organic matter in modern agriculture. Y. Chen, Y. Avnimelech and M. Nijhoff. Dordrecht-Boston-Lancaster, Martinus Nijhoff Publ: 117-156.

Sabine, C.L., Feely, R.A., Gruber, N., Key, R.M., Lee, K., Bullister, J.L., Wanninkhof, R., Wong, C. S., Wallace, D.W.R., Tilbrook, B., Millero, F.J., Peng, T.-H., Kozyr, A., Ono, T., Rios, A.F., 2004. The Oceanic Sink for Anthropogenic CO_2. Science 305, 367-371.

Schneider, O., Sereti, V., Eding, E.H., Verreth, J.A.J., 2004. Yields and nutrient balances of bacterial production on carbon supplemented fish waste. In: S. Adams and J.A. Olafsen (Eds.) Biotechnologies for Quality, Barcelona, Spain, 729-730.

Schroeder, G.L., 1987. Carbon pathways in aquatic detrital systems. Pages 217-236 in: D.J.W. Moriarty and R. S. V. Pullin (eds.), Detritus and microbial ecology in aquaculture. ICLARM conference proceedings 13, Manila, Philippines. 420 pp.

Spring, S., Schulze, R., Overmann, J., Schleifer, K.H., 2000. Identification and characterization of ecologically significant prokaryotes in the sediment of freshwater lakes: molecular and cultivation studies. FEMS Microbiology Reviews 24, 573-590.

Strauss, E.A., Lamberti, G.A., 2000. Regulation of nitrification in aquatic sediments by organic carbon. Limnology and Oceanography 45, 1854-1859.

Strauss, E.A., Lamberti, G.A., 2002. Effect of dissolved organic carbon quality on microbial decomposition and nitrification rates in stream sediments. Freshwater Biology 47, 65-74.

Tacon, A.G.J., Phillips, M.J., Barg, U.C., 1995. Aquaculture feeds and the environment: The Asian experience. Water Science and Technology 31, 41-59.

Tacon, A.G.J., Cody, J.J., Conquest, L.D., Divakaran, S., Forster, I.P., Decamp, O.E., 2002. Effect of culture system on the nutrition and growth performance of Pacific white shrimp

Tacon, A.G.J., Forster, I.P., 2003. Aquafeeds and the environment: policy implications. Aquaculture 226, 181-189.

Thakur, D.P., Lin, C.K., 2003. Water quality and nutrient budget in closed shrimp (Penaeus monodon) culture systems. Aquacultural Engineering 27, 159-176.

van Rijn, J., Fonarev, N., Berkowitz, B., 1995. Anaerobic treatment of intensive fish culture effluents: digestion of fish feed and release of volatile fatty acids. Aquaculture 133, 9-20.

Zhu, S. and Chen, S., 2001. Effects of organic carbon on nitrification rate in fixed film biofilters. Aquacultural Engineering 25, 1-11.

Zopfi, J., Ferdelman, T.G., Jorgensen, B.B., Teske, A., Thamdrup, B., 2001. Influence of water column dynamics on sulfide oxidation and other major biogeochemical processes in the chemocline of Mariager Fjord (Denmark). Marine Chemistry 74, 29-51.

Summary

Resumen

Sammenwatting

SUMMARY

Different kinds of organic and inorganic compounds (e.g. formulated food, manures, fertilizers) are added to aquaculture ponds to increase fish production. However, a large part of these inputs are not utilized by the fish and are decomposed inside the pond. The microbiological decomposition of the organic matter is a critical factor for water quality control and nutrient recycle. Usually, management practices are developed to control the survival and health of the cultured animals and to maintain good water quality. The microbiological processes are affected by these practices but usually unintentionally. A better control of culture conditions and sustainability of aquaculture ponds is possible with an improvement of the microbiological processes. The present thesis is divided in two parts, the first is a literature review of the microbial ecology of aquaculture ponds and the second is the description of a series of experiments in lab-scale aquaculture ponds.

In the first part, the role of the microorganisms in aquaculture ponds is reviewed, focusing on the decomposition of organic matter and its influence on pond dynamics. It was theoretically estimated that the addition of 1 kg of formulated feed would yield approximately 125 g bacterial biomass. This bacterial biomass is potentially a valuable nutrients source for higher trophic levels. Sedimentation and resuspension processes are important factors affecting the decomposition pathways. Both processes are directly related with the feeding rate and the stocking density applied. The rate of organic matter loading, environmental factors and pond management practices influence the functioning of algae-bacteria interactions, which are extremely important in pond processes. Included is a literature that describes commercial probiotic products that claim to solve: nutritious, water quality and pathogens problems in pond aquaculture, were analyzed. Alternative management practices to steer the decomposition process were also presented (**Chapter 2**).

The second part describes all the experiments that were conducted in lab scale microcosm systems, simulating the conditions of an intensive fish-less aquaculture pond with daily feeding rates. In **Chapter 3** the influence of aerobic and anaerobic conditions and the organic C/N ratios on the decomposition process is described. Under aerobic decomposition less organic carbon remained in the systems. The results from this experiment suggest that a C/N ratio ranging between the tested values (6.3 and 12.8) does not have a significant influence on the carbon mineralization in the short term (< 50 days). However, a C/N ratio decrease was

observed in all the treatments during the experimental period; this reduction was especially fast and steep under aerobic conditions. This decrease in C/N ratio of the organic matter might explain why in all treatments the rate of decomposition slowed down at the end of the experiment. The C/N ratio also determined the concentration of inorganic nitrogen compounds in the water. Higher concentrations were found for the richest protein diet treatments. No nitrification was measured even though oxygen and ammonia were present.

Bacterial biomass production was quantified testing two formulated fish feed with different protein content under aerobic and anaerobic conditions **(Chapter 4)**. The oxic status significantly influences the bacterial abundance, bacterial biomass, bacterial respiration and bacterial efficiency. More bacterial biomass was produced under aerobic conditions. The two diets did not influence significantly the bacterial growth. The bacterial abundance at the end of the experiment was 3.4×10^9 cells ml^{-1} in aerobic treatments and 1.9×10^9 cells ml^{-1} in anaerobic treatments. The remaining amount of carbon, fixed in bacterial biomass and expressed on a per area basis, was 19 g m^{-2} day^{-1} for aerobic system and 8 g m^{-2} day^{-1} for anaerobic systems.

In **Chapter 5** the effect of the oxic-anoxic range on fish feed decomposition was investigated. Different ranges, from completely aerobic to completely anaerobic, were tested. To establish intermediate oxic levels the following treatments were used: 1) alternated flows of O_2 or N_2 at different periods and 2) maintaining the coexistence of aerobic and anaerobic layers while applying short resuspension events. Similar amounts of carbon were converted to CO_2 under completely aerobic conditions and under the different ranges of aerobic-anaerobic conditions. Under anaerobic conditions much less carbon was converted into CO_2. This means that actually only limited periods of oxic conditions (or resuspension) are needed to stimulate complete organic matter decomposition. From our results it appears that only 6h per day of aerobic conditions or only once mixing of aerobic and anaerobic layers (i.e. resuspension) per four days are needed to reach the same carbon mineralization as in continuous aerobic conditions. Very limited nitrification was observed in the completely aerobic treatment. Nitrification and denitrification were registered for all the systems when aerobic and anaerobic conditions coexisted in time or space. The highest nitrogen removal (around 70%) was found in the resuspension treatments (and 12 h O_2 flow treatment).

The use of controlled lab scale microcosm simulating intensive aquaculture ponds allowed us to follow the fate of carbon and nitrogen during particular decomposition processes. The results found in the different chapters are discussed in **Chapter 6**. Both the quality and the quantity of the organic matter influenced the decomposition process and its products. The use of high protein diets increased the concentration of nitrogen species affecting the water quality. The aerobic and anaerobic conditions determined the nutrients pathway (mineralized, assimilated or partially decomposed). More bacterial biomass was produced under aerobic conditions than under anaerobic. The coexistence of aerobic and anaerobic conditions stimulated organic matter decomposition; it avoided the accumulation of ammonia while maintaining good water quality conditions.

A better understanding and control of the organic matter decomposition in aquaculture ponds is crucial. The anaerobic decomposition only becomes a problem when it predominates in the sediment, causing the aerobic-anaerobic interface to move up into the water column, and thus remains disconnected from the aerobic decomposition. Management practices that link aerobic and anaerobic processes can stimulate fish production by recycling carbon and nitrogen compounds. The recycling of surplus organic matter through bacterial processes, however, has a limit. Increasing fish pond productivity should come along with practices to stimulate the autotrophic and heterotrophic food webs, without exceeding the capacity of this aquatic system.

.

RESUMEN

Con el fin de incrementar la producción piscícola, es una practica común agregar nutrientes orgánicos e inorgánicos (alimentos formulado, estiércol, fertilizantes) en los estanques de acuicultura. Sin embargo, gran parte de estos nutrientes no llegan a ser utilizados por los peces y son desperdiciados. La descomposición microbiológica de esta materia orgánica excedente es un factor crítico para el control de la calidad del agua y el reciclamiento de nutrientes. Las prácticas de manejo utilizadas en estanques de acuicultura tienen como objetivo mantener una calidad del agua óptima para controlar la salud y supervivencia de las especies cultivadas. Los procesos microbiológicos del estanque de acuicultura son afectados por estas prácticas pero generalmente de una manera empírica. El control de los procesos microbiológicos posibilitaría un mejoramiento de las condiciones de cultivo de los estanques de acuacultura incrementando su sustentabilidad. Esta tesis esta dividida en dos partes. Primero se explora la ecología microbiana en los estanques de acuicultura mediante una revisión de la literatura relacionada, y, posteriormente, se presentan una serie de experimentos a escala de laboratorio que simulan las condiciones de estanques de acuicultura intensivos.

En la primera parte, se revisaron los diferentes roles de las comunidades microbianas existentes en los estanques de acuicultura. Dicha revisión se centró en la descomposición de materia orgánica y su influencia en la dinámica de los estanques. Se estimó teóricamente que la adición de 1 kg de alimento formulado producirá ± 125 g de biomasa bacteriana. Esta biomasa bacteriana es una fuente potencial valiosa de nutrientes para los niveles tróficos superiores. Los procesos de sedimentación y resuspensión son factores importantes que influyen en las rutas de descomposición de la materia orgánica. Ambos procesos, sedimentación y resuspension, están relacionados directamente con la tasa de alimentación y la densidad de cultivo aplicada. Las interacciones alga-bacteria son influenciadas por la materia orgánica adicionada a los estanques, los factores ambientales y las prácticas de manejo. Estas interrelaciones son extremadamente importantes en los procesos ecológicos de los estanques de acuicultura. Los probióticos son productos comerciales (microorganismos o derivados de ellos) que prometen funcionar como aditivos nutritivos, resolver problemas de eutroficación, mejorar la calidad de agua, y combatir microorganismos patógenos. Reportes del uso de probióticos en estanques de acuicultura fueron revisados y analizados. También en este capítulo se presentan varias prácticas de manejo alternativas que promueven los procesos de descomposición de la materia orgánica acumulada (**Capítulo 2**).

En la segunda parte de la tesis se realizaron varios experimentos a escala de laboratorio, simulando las tasas de alimentación diarias de un estanque de acuicultura intensivo. No se incluyeron peces dentro de estos microcosmos. En el **Capítulo 3** se estudió el proceso de descomposición de materia orgánica bajo condiciones aerobias o anaerobias de dos alimentos formulados con diferente contenido de carbono y nitrógeno (cociente de C/N). El carbón orgánico se acumuló a una tasa menor bajo condiciones aerobias que bajo condiciones anaerobias. Los resultados de este experimento sugieren que aunque las dietas estudiadas tenían cocientes diferentes de C/N, (6,3 y 12,8), estas influenciaron la mineralización del carbono de una manera similar, al menos en el corto plazo (<50 días). Se observó que el cociente de C/N de cada dieta influyó de una manera determinante en la concentración de los diferentes compuestos inorgánicos de nitrógeno en el agua. Las más altas concentraciones de amonio fueron encontradas en los tratamientos con mayor contenido proteico. Por otro lado, el cociente de C/N del sustrato remanente en los reactores disminuyó en todos los tratamientos a lo largo del periodo experimental. Esta disminución fue más rápida y abrupta bajo condiciones aerobias que bajo condiciones anaerobias. La tasa de descomposición en todos los tratamientos diminuyó al final del experimento. Esta disminución fue asociada con la reducción disminución de cociente de C/N observada en todos los casos. No se observó nitrificación a pesar de existir las condiciones necesarias (oxígeno y amonio) para este proceso.

Se cuantificó la producción de biomasa bacteriana producida por la descomposición de dos alimentos formulados para pescados con diferente contenido proteico en condiciones aerobias y anaerobias (**Capítulo 4**). La presencia o ausencia de oxígeno afectó significativamente a la abundancia, biomasa, respiración y eficiencia bacteriana. Se produjo mayor biomasa bacteriana bajo condiciones aerobias. La abundancia bacteriana al final del experimento fue de 3.4×10^9 células ml^{-1} en los tratamientos aerobios y 1.9×10^9 células ml^{-1} en los tratamientos anaerobios. El diferente porcentaje de contenido proteico en las dietas no influyó significativamente al crecimiento bacteriano. La cantidad de carbono asimilado como biomasa bacteriana y expresado en términos de superficie fue de 19 g m^{-2} día^{-1} para sistemas aeróbicos y de 8 g m^{-2} día^{-1} para sistemas anaerobios.

En el **Capítulo 5** se investigó la descomposición de alimento para pescado dentro de un rango de condiciones que variaron desde completamente aerobio hasta completamente anaerobio.

Con el fin de establecer los niveles óxicos intermedios entre completamente aerobio y completamente anaerobio, dos tratamientos fueron utilizados: 1) flujos alternados de O_2 o N_2 en diferentes frecuencias 2) coexistencia de sedimento anaerobio y columna de agua aerobia con aplicación de eventos cortos de resuspensión que mezclaban ambos estratos. La mineralización de carbono orgánico y su transformación a CO_2 se dio de manera similar, tanto bajo condiciones completamente aerobias, como en aquellos tratamientos donde hubo alguna introducción de oxígeno. Bajo condiciones anaerobias la producción de CO_2 fue mucho menor. Esto significa que la intervención de periodos cortos de oxígeno estimuló la descomposición de materia orgánica de una manera mas completa. Nuestros resultados muestran que con sólo 6 h por día de flujo de oxígeno o con la aplicación de un periodo de resuspensión mezclando las capas aerobias y anaerobias cada 4 días, fue suficiente para mineralizar el carbono orgánico de manera similar que bajos condiciones aerobias continuas. La nitrificación se vio limitada en los tratamientos donde el flujo de oxígeno se mantuvo constante. En los tratamientos donde coexistieron condiciones aerobias y anaerobias, ya sea en tiempo o en espacio, se registraron tanto nitrificación como desnitrificación. El mayor porcentaje de remoción de nitrógeno (aproximadamente 70%), se observó en todos los tratamientos donde se aplicó resuspensión y en el tratamiento donde se introdujo oxígeno 12 h por día.

El diseño experimental de microcosmos a escala de laboratorio, simulando las condiciones de un estanque de acuicultura intensivo, permitió el seguimiento del proceso de descomposición y la localización de los diferentes compuestos de carbono y nitrógeno. Los resultados reportados en los diferentes capítulos son discutidos en conjunto en el **Capítulo 6**. Tanto la calidad como la cantidad de la materia orgánica influenciaron el proceso de descomposición y sus productos. El uso de alimentos formulados con alto contenido proteico incrementó la producción de compuestos inorgánicos de nitrógeno, afectando la calidad del agua. La disponibilidad de oxígeno determinó la ruta de descomposición y la distribución de los nutrientes (mineralizados, asimilados o parcialmente descompuestos). Más biomasa bacteriana fue producida en los tratamientos aerobios que en los anaerobios. La coexistencia de circunstancias aerobias y anaerobias estimuló la descomposición de materia orgánica de una manera más completa, evitando así la acumulación de amonio y manteniendo buena calidad de agua.

Es necesario un mejor entendimiento y control de los procesos de descomposición de materia orgánica en los estanques de acuicultura para su óptimo funcionamiento. La descomposición anaerobia se convierte en un problema cuando prevalece en el sedimento, la interfase aerobio-anaerobio se mueve a niveles superiores dentro de la columna de agua y permanece desconectada de los procesos de descomposición aerobios. Las prácticas de manejo utilizadas en estanques de acuicultura pueden incrementar la producción de peces mediante el reciclado de compuestos de carbono y nitrógeno. En sistemas acuáticos, el reciclamiento de la materia orgánica acumulada a través de comunidades microbianas tiene un límite. El incremento de la producción de peces debe estar acompañado por prácticas de manejo dirigidas a estimular las cadenas tróficas autotróficas y heterotróficas sin exceder la capacidad ecológica de este sistema acuático.

SAMENWATTING

Verschillende soorten organische en anorganische toevoegingen (o.a. visvoeders of meststoffen) worden gebruikt in kweekvijvers om de productie te verhogen. Een groot deel van deze toevoegingen worden echter niet gebruikt door de vissen maar worden afgebroken in de vijver. De microbiologische afbraak van organische stof is een kritische factor voor het waterkwaliteitsbeheer en in de nutriëntenkringloop. Over het algemeen zijn beheersrichtlijnen ontwikkeld om het overleven en de gezondheid van de gekweekte dieren te garanderen en om een goede waterkwaliteit te behouden. De microbiologische processen worden, meestal onbedoeld, door deze maatregelen ook beïnvloed. Een verbeterd beheer van kweekcondities en verhoogde duurzaamheid in kweekvijvers is mogelijk door een verbetering van de microbiologische processen. Dit proefschrift is onderverdeeld in twee delen; het eerste deel geeft een overzicht van de microbiële ecologie in kweekvijvers en het tweede deel beschrijft een serie experimenten van op labschaal gesimuleerde kweekvijvers.

In het eerste deel, welke een literatuurstudie is, wordt de rol van micro-organismen in kweekvijvers beschreven met een focus op de afbraak van organische materie en de invloed daarvan op de dynamiek van het systeem. Er werd uitgerekend dat er op theoretische basis ongeveer 125 g microbiële biomassa gevormd wordt uit de toevoeging van 1 kg visvoeder. De gevormde biomassa is potentieel een waardevolle bron van nutriënten voor hogere trofische groepen. Sedimentatie en resuspensie zijn belangrijke factoren die de afbraak beïnvloeden. Beide processen zijn direct gerelateerd aan het gebruikte voedingsregime en de populatiedichtheid. De organische belasting, milieufactoren en vijverbeheer beïnvloeden de alge-bacterie interacties, die erg belangrijk zijn in vijverprocessen. De resultaten van een aantal commerciële probiotica producten waarvan beweerd wordt dat ze problemen met nutriënten, waterkwaliteit of pathogenen in kweekvijvers oplossen, werden besproken. Alternatieve beheersmaatregelen die de afbraakprocessen kunnen sturen, werden voorgesteld (hoofdstuk 2).

In het tweede deel worden alle experimenten beschreven die uitgevoerd werden in labschaal microcosm systemen. Hierin werden de condities in een visloze intensieve kweekvijver met een dagelijks voedingsregime gesimuleerd. In hoofdstuk 3 worden de experimenten beschreven waarin de invloed van aërobe en anaërobe condities en de organische C/N ratio's getest werden. Bij aërobe afbraak bleef er minder organisch koolstof achter in het systeem. De

resultaten van deze experimenten geven aan dat de C/N ratio's binnen het geteste gebied (6.3 – 12.8) geen significante invloed hebben op de koolstof mineralisatie op de korte termijn (< 50 dagen). Er werd tijdens de experimentele periode echter wel een vermindering in C/N ratio geobserveerd in alle verschillende behandelingen en deze was het snelst onder aërobe condities. Deze vermindering in C/N ratio van de organische materie zou de waarneming van verminderde afbraaksnelheden aan het eind van het experiment, bij alle behandelingen, kunnen verklaren. De C/N ratio bepaalde ook de concentratie van anorganische stikstof verbindingen in het water. Hogere concentraties werden gevonden in de behandeling met de diëten met de meeste proteïnen. Geen nitrificatie werd gemeten ondanks de aanwezigheid van zuurstof en ammonia.

Bacteriële biomassaproductie werd gemeten door twee visvoeders met verschillende hoeveelheden proteïnen te testen onder aërobe en anaërobe condities (hoofdstuk 4). De oxische status beïnvloedde aanzienlijk de bacteriële aanwezigheid, biomassa, respiratie en efficiëntie. Onder aërobe condities werd meer bacteriële biomassa geproduceerd. De twee diëten hadden geen significante invloed op de bacteriële groei. Bacteriële hoeveelheden aan het eind van het experiment waren 3.4×10^9 cellen ml^{-1} in aërobe behandelingen en 1.9×10^9 cellen ml^{-1} in anaërobe behandelingen. De overgebleven hoeveelheid koolstof, gefixeerd in bacteriële biomassa en uitgedrukt per oppervlakte, was 19 g m^{-2} dag^{-1} voor aërobe systemen en 8 g m^{-2} dag^{-1} voor anaërobe systemen.

In hoofdstuk 5 wordt het onderzoek naar het effect van een oxisch-anoxische gradiënt op de afbraak van visvoeder beschreven. Verschillende gradiënten, van volledig aëroob tot volledig anaëroob, werden getest. Om de tussenliggende oxische niveau's te kunnen testen, werden de volgende behandelingen gebruikt: 1) afwisselende beluchting met O_2 of N_2 gedurende verschillende perioden en 2) behoud van stabiele coëxistentie van aërobe en anaërobe lagen door het gebruik van korte momenten van resuspensie. Vergelijkbare hoeveelheden koolstof werden omgezet naar CO_2 onder volledig aërobe condities en de verschillende gradiënten van gemengd aëroob-anaërobe condities. Onder volledig anaërobe condities werd veel minder koolstof omgezet in CO_2. Dit betekent dat slechts beperkte momenten van oxische condities (of resuspensie) nodig zijn om volledige afbraak van organische materie te stimuleren. Uit onze resultaten valt af te leiden dat slechts 6 uur per dag onder aërobe omstandigheden of eenmaal per vier dagen mengen van aërobe en anaërobe lagen (ofwel resuspensie) nodig is om dezelfde koolstof mineralisatie te bekomen als onder continu aërobe condities. Zeer

weinig nitrificatie werd geobserveerd in de volledig aërobe behandeling. Nitrificatie en denitrificatie werden geregistreerd in alle systemen waarin aërobe en anaërobe condities naast elkaar bestonden in tijd of plaats. De hoogste stikstofverwijdering (ongeveer 70%) werd gevonden in de resuspensiebehandelingen (en in de 12 uur O_2 beluchting behandeling).

Het gebruik van gecontroleerde labschaal microcosm systemen die intensieve kweekvijvers kunnen simuleren, gaf ons de mogelijkheid om koolstof en stikstof onder bepaalde afbraak processen te kunnen volgen. De resultaten uit de verschillende hoofdstukken worden bediscussieerd in hoofdstuk 6. Zowel de kwaliteit als de hoeveelheid van de organische stof beïnvloeden het afbraakproces en de eindproducten ervan. Het gebruik van proteïnerijke diëten verhoogt de concentratie van anorganische stikstof verbindingen, wat de waterkwaliteit beïnvloedt. De aërobe en anaërobe condities bepalen de afbraakroute (mineralisatie, assimilatie of gedeeltelijke afbraak). Meer bacteriële biomassa wordt gevormd onder aërobe condities dan onder anaërobe condities. Het naast elkaar bestaan van aërobe en anaërobe condities stimuleert de afbraak van organische materie en voorkomt de ophoping van ammonia terwijl een goede waterkwaliteit behouden blijft.

Een verbeterd begrip en beheer van de afbraak van organische materie in visvijvers is onmisbaar. De anaërobe afbraak wordt enkel een probleem als het overheerst in het sediment waarbij er geen menging plaats vindt tussen aëroob en anaëroob processen. Beheersmaatregelen die zorgen voor een koppeling tussen aërobe en anaërobe processen, kunnen de visproductie stimuleren door het recycleren van koolstof- en stikstofverbindingen. Het recycleren van een teveel aan organische materie door bacteriële processen heeft een limiet. Verhoogde visvijver productiviteit moet hand in hand gaan met maatregelen om de autotrofe en heterotrofe voedselketens te stimuleren zonder dat de capaciteit van het aquatisch systeem wordt overschreden.

Acknowledgements

It is time to say *thanks.* It is difficult though to include all the people that I had the opportunity to meet during all this time in Holland. First I will like to thank CONACYT-Mexico (Consejo Nacional de Ciencia y Tecnología) for the economical support provided in order to carry out the main part of my PhD. Furthermore to Wageningen University for its support through the sandwich scholarship.

I will like to thank my daily supervisor Marc Verdegem, for his work, patience and coaching during all this long process. You always gave me a hand when I needed. I must thank Johan Verreth, my supervisor and promotor, for his support, especially during the writing phase. Also thank: Omar Calvario in Mexico, who gave me the opportunity to come to Holland to do the PhD; Marten Scheffer, which helped me with his interesting and motivating discussions; Hauke Smit and other people from Microbial Ecology for its time and advices. Thanks to Ronald Booms and Tino Leffering for their work and a lot of help in the lab and to Henk Schipper for his assistance with the fluorescent microscope. For some of the experiments I had the great opportunity to work together with Maciek Pilarczyk and Eva Kerepeczky, Thanks guys! Also I will like to thank all the people that have been in the FCF group during my time here. Greetings to Anne van Dam, Yuming, Pablo, Niel, Ana, Rodrigo, Ekram, Oliver, Ep, Titu, Patricia, Harrison, An, Hanh, Jonnas, Marc Denes, Samad, Catherina, Yasha, Paula Meno and to all the people of FCF. I especially like to give thanks to Viky Serety for her help and advices. Thanks to Elene, Lies, Gerri and Netty for the help with the administration procedures. Thanks to Chris for helping me to deal with the computer stuffs. Also I will like to thanks Gab van Winkel for his counseling. To Yoram Avnimelch for giving me the opportunity to work with him: your advice and aid in the distance were of great value. You were an inspirational source for me!!.

My time here in Holland would have been very different without the company of the friends that I met here. Thanks to Pablo and Bernando for helping me establish, and to nicely camouflage what was waiting for me. I have a lot of nice memories between Wageningen and Gent from Patty, Ramon and Andreas a big kiss for the three of you!. My first findings about the university life in Wageningen was with ISOW, there I found a very enthusiastic and hard worker fellows, thanks for the good memories to Jaime, Jantine, Emma, Marcus and Raquel. A crazy and enjoyable time I had with the "Spanish-Community": Vanessa, Bass, Barbara, Martha, Luis, Ralph, Fanny (the organic girl), Devidin (the pollution guy!), Mike (every body should have a canadian!), and cia! You took care of me with so healthy vegetarian dinners and the not that healthy parties, thanks guys!. Beside parties I also worked, and I had the opportunity to be involved with the NGO-Otherwise and to meet nice people like Kees, Nadja, Annike, Jacco and all the volunteers, thanks to all of you for sharing interesting discussion, work and fun.

A wonderful discovery was the "Greek Dances" and together with it came a bunch of people. I had gorgeous time with you, thanks to Maele, (my shoulder), Cristina, Niko, Andreas, Theo, Erik, Shana, Gerard, Kathrin, Lisa, Geerts, Anike and to all my co-dancers. Especial thanks to my nice friends Athina Amanatidou and Vaso Pali for your great enthusiasm and love. *Filikia*!!

A piece of Mexico was the Mexican community. Thanks for being here. Pablo, Maaike, Eira ,Ivo, Nayely Steffan, Iemke, Arturo, Maricarmen, Theo, Cesar, Rodrigo, Viviana, Juan Carlos, Erika, Michiel, Irineo and Family and the new born generation! Especially Meta was my link between Mexico and Holand!, thanks for all your help and friendship. *Yo Amo a Mexico*!! another great dancing discovery. Even though Leiden was far, it was always worthy to spend some time with you. Thanks to Edoardo Ramos, for his friendship and enthusiasm. To Ariadna, Astrid, Linda, Bernardo, Fatima, Aris, Oscar, Mahmut, Rafa and all the people of the group!! Especially thanks to Meta and Erika to help me keep going even when I didn't want to.

Thanks for my last "master" friends: Itziar, Lucho Gracia, Marcela, Ruben, Santosh, Wenjing, Katlin and Juan, for sharing its time and change my "chip" during our nice dinners! Itziar and Lucho, we were really a family, thanks for your help and frienship during every day of this last year, I will really miss you. Also I will miss my last "family" Ale and Joaquin, thanks guys you brought enjoyment to our house.

Thanks for my Catalan-Family for opening the door of your houses and hearts and making me feel at home. Gracias a la Iaia Maria, Gloria, Mikel, Marta, Bego, Sergi, Rosa Maria, Claudia y Tomas.

One thing that has been permanent and strong in each and every moment of my life has been my family. Un abrazo para todos: mis tías, tíos y primos, thanks to all of you for supporting me in the distance. My Tía Marta and my Abuelita Chela left us before I was able to finish, my thoughts are always with you. Thanks to my parents Domingo y Yolanda for its daily offlines, chats and unconditional love. Ustedes son un ejemplo de vida para mi chapalinos!! Thanks to my brother Alejandro for his love and for a life together. Eres mi héroe chamaco!!

I can not find words to express how much I thank Jordi, my partner of journey, house and love to be in the good, the bad and the really bad moments next to me. Lo terminamos negrito ahora podemos irnos!!!.

Training and Supervision Plan		Graduate School WIAS
Name PhD student	Beatriz Torres Beristain	
Project title	Organic matter decomposition in simulated aquaculture ponds	
Group	Fish Culture and Fisheries	
Daily supervisor(s)	Dr. Marc Verdegem	
Supervisor(s)	Prof. dr. Johan Verreth	
Project term	from 01-09-2000 until 01-03-2005	
Submitted	February 2005 first plan / midterm / **certificate**	

The Basic Package (minimum 2 cp)	year	cp
WIAS Common Course (mandatory)	2001	2.0
Course on philosophy of science and ethics (mandatory)	2001	1.0
Subtotal Basic Package		**3.0**

Scientific Exposure (conferences, seminars and presentations, minimum 5 cp)	year	cp
International conferences *(minimum 2 cp)*		
World Aquaculture 2003, Salvador Bahia, Brasil, May 19-23	2003	1.0
Aquaculture Europe 2003, Trondheim, Norway, 8-12 August	2003	1.0
International Symposium of Microbial Ecology, Cancun, Mexico, August 22-27,	2004	1.0
Aquaculture Europe 2004 Barcelona, Spain, October 20-22	2004	0.6
Seminars and workshops		
WIAS Science Day, Wageningen, The Netherlands	2001,2002, 2004	0.6
Seminar Plus Organic farming:a challenge for animal production systems research	2000	0.3
Seminar Plus on Stable Isotopes	2001	0.3
WIAS Workshop 'Supervising PhD students'	2001	0.1
Stakeholders platforms in Water Management Sept 29- October 1	2004	0.6
Presentations *(minimum 4 original presentations of which at least 1 oral, 0.5 cp each)*		
Oral presentation, World Aquaculture 2003, Salvador Bahia, Brasil	2003	0.5
Poster presentation, Aquaculture Europe 2003, Trondheim, Norway	2003	0.5
Oral presentation, WIAS Science Day, Wageningen, The Netherlands	2004	0.5
Poster presentation, International Symposium of Microbial Ecology, Cancun, Mexico	2004	0.5
Poster presentation, Aquaculture Europe 2004 Barcelona, Spain	2004	0.5
Subtotal International Exposure		**8.0**

In-Depth Studies (minimum 4 cp)	year	cp
Disciplinary and interdisciplinary courses		
WIAS advanced Statistics Course: Experimental Design	2000	0.6
Food Security Course	2001	4.0
Broaden your Horizon	2001	0.8
Stable Isotopes in Studies of Nutrient dynamics	2001	0.6
Estuarine Ecology, Yerseke, Netherlands 10-15 May	2002	1.0
MSc Courses		
Aquaculture Production Systems E45-219	2000	4.0
Practical Course Water Quality H400-202	2001	2.0
Subtotal In-Depth Studies		**13.0**

Professional Skills Support Courses (minimum 2 cp)	year	cp
WIAS Course Techniques for Scientific Writing	2001	0.8
International Fluorescent in situ Hybridization Course	2001	1.0
Safe handling with radioactive materials and sources	2002	1.0
Subtotal Professional Skills Support Courses		**2.8**

Research Skills Training	year	cp
Preparing PhD research proposal	2001	4.0
Subtotal Research Skills Training		**4.0**

Didactic Skills Training (optional)	year	cp
Organisation of seminars and courses		
OTHERWISE (NGO, linked to Wageningen University and Research centre)	2002-2004	2.0
Conferences, and public debates about the interaction of science and society on development		
Membership of boards and committees		
ISOW (International student Organization of Wageningen)	2002	2.0
WAPS (WIAS Associated PhD Students Council)	2004	2.0
Subtotal Management Skills Training		**6.0**
Education and Training Total (minimum 21 cp, maximum 42 cp)		**36.8**

One credit point (cp) equals a study load of approximately 40 hours

Curriculum Vitae

Beatriz Torres Beristain was born on October 16, 1971 in Cordoba Veracruz, Mexico, She was the second child of Domingo Torres Renero and Alma Yolanda Beristain Navarro. She obtained her Bachelor Degree of Science in Biochemical Engineering in 1994 at the Instituto Tecnologico y de Estudios Superiores de Monterrey, (ITESM) Campus Guaymas, Mexico. She obtained a Master of Science in Ecology in 1998 at the Instituto de Ecologia de la Universidad Nacional Autonoma de Mexico (UNAM). She worked for 2 years as a research assistant in several projects on water quality and human health at UNAM. She studied a postgraduate course on Lake Management in 2000 at International Lake Environment Committee (ILEC), in Japan. At different periods of her life she had been working with environmental and development NGOs. In September 2001 she began her PhD at the Fish Culture and Fisheries Group, Wageningen University. After her graduation she is planning to go back to Mexico.

contact e-mail: **torres.beristain@gmail.com**

Cover: prehispanic fish pond modified from Aztec codice

Printed by Ponsen and Looijen BW, Wageningen