

CHAPTER TWO
II. REVIEW OF LITERATURE

2.1. World production of food fish:

Capture fisheries and aquaculture supplied the world with about 148 million tonnes of fish in 2010 (with a total value of US\$217.5 billion), of which about 128 million tonnes were utilized as food for people. Preliminary data for 2011 indicate increased production of 154 million tonnes, of which 131 million tonnes were destined as food (Table 1 and Figure 1, all data presented are subject to rounding). With sustained growth in fish production and improved distribution channels, world fish food supply has grown dramatically in the last five decades, with an average growth rate of 3.2 percent per year in the period 1961–2009, outpacing the increase of 1.7 percent per year in the world's population. World per capita food fish supply increased from an average of 9.9 kg (live weight equivalent) in the 1960s to 18.4 kg in 2009, and preliminary estimates for 2010 point to a further increase in fish consumption to 18.6 kg¹ (Table 1 and Figure 2). Of the 126 million tonnes available for human consumption in 2009, fish consumption was lowest in Africa (9.1 million tonnes, with 9.1 kg per capita), while Asia accounted for two-thirds of total consumption, with 85.4 million tonnes (20.7 kg per capita), of which 42.8 million tonnes was consumed outside China (15.4 kg per capita). The corresponding per capita fish consumption figures for Oceania, North America, Europe, Latin America and, the Caribbean were 24.6 kg, 24.1 kg, 22.0 kg, and 9.9 kg, respectively. Although annual per capita consumption of fishery products has grown steadily in developing regions (from 5.2 kg in 1961 to 17.0 kg in 2009) and in low-income food-deficit countries (LIFDCs, from 4.9 kg in 1961 to 10.1 kg in 2009), it is still considerably lower than in more developed regions although the gap is narrowing. A sizeable share of fish consumed in developed countries consists of imports, and, owing to steady demand and decline in domestic fishery production (down 10 percent in the period 2000–2010), their dependence on imports, in particular from developing countries, is projected to grow in coming years (FAO, 2012).

2.2. Aquaculture:

Aquaculture supplied the world with about 63.6 million tonnes in 2011 (Table1).

2.3. Fish production in Egypt:

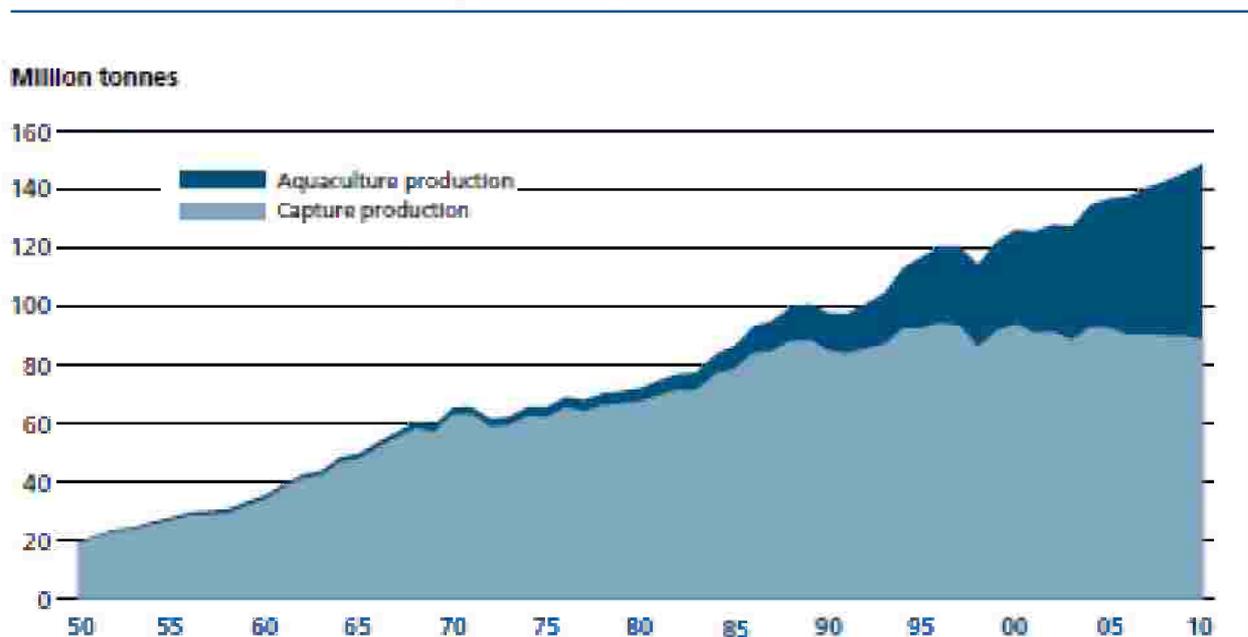
Total fish production (capture and aquaculture) of Egypt was 1,371,975 tonnes in 2012 (Table 2). Aquaculture production was 1,017,738 with (74.18%) of the total fish production of Egypt. Mullet supplied the total Egyptian production with about 163176 metric tonnes of fish in

2012 (with a total value of EGP 3153926 million), aquaculture participated with 129651 metric tonnes (79.45%) (GAFRD, 2012).

Table(1). World Fisheries (Capture and Aquaculture) Production* and Utilization (FAO, 2012).

	2006	2007	2008	2009	2010	2011
Capture	(Million tonnes)					
Inland	9.8	10.0	10.2	10.4	11.2	11.5
Marine	80.2	80.4	79.5	79.2	77.4	78.9
Total Capture	90.0	90.4	89.7	89.6	88.6	90.4
Aquaculture						
Inland	31.3	33.4	36.0	38.1	41.7	44.3
Marine	16.0	16.6	16.9	17.6	18.1	19.3
Total Aquaculture	47.3	49.9	52.9	55.7	59.9	63.6
TOTAL WORLD FISHERIES	137.3	140.2	142.6	145.3	148.5	154
Utilization						
Human consumption	114.3	117.3	119.7	123.6	128.3	130.8
Non-food uses	23.0	23.0	22.9	21.8	20.2	23.2
population(<i>billions</i>)	6.6	6.7	6.7	6.8	6.9	7.0
Per capita food fish supply(<i>kg</i>)	17.4	17.6	17.8	18.1	18.6	18.8

Notes: Excluding aquatic plants. Totals may not match due to rounding. Data for 2011 are provisional estimates.



Figure(1). World Fisheries (Capture and Aquaculture) Production. (FAO, 2012).

Table (2). Fish production of Egypt (tonnes), GAFRD 2012.

Resources		Production (tonnes)	Percent of total
Marine Fisheries	Mediterranean Sea	69332	5.05
	Red Sea	44866	3.27
	Sub-total	114168	8.32
Northern Lakes	Manzala	62272	4.54
	Burulous	52076	3.80
	Idko	6576	0.48
	Maryout	7427	0.54
	Sub-total	128351	9.36
Coastal Lagoons	Bardaweel	3844	0.28
	Port Fouad	95	0.01
	Sub-total	3939	0.29
Inland Lakes	Qaroun	4410	0.32
	Rayan land3	3451	0.25
	Nasser	26290	1.92
	Bitter and Temsah	2894	0.21
	Toshky	2301	0.17
	New valley	1780	0.13
	Sub-total	41126	3.00
River Nile and canals	Nile system	66623	4.86
	Sub-total	66623	4.86
Fish culture	Government	6509	0.69
	Private	720412	52.51
	Cages	249358	18.18
	Rice fields	34537	2.52
	Semi intensive culture	1451	0.11
	Intensive culture	2444	0.18
	Sub-total	1017738	74.18
Grand total		1371975	100

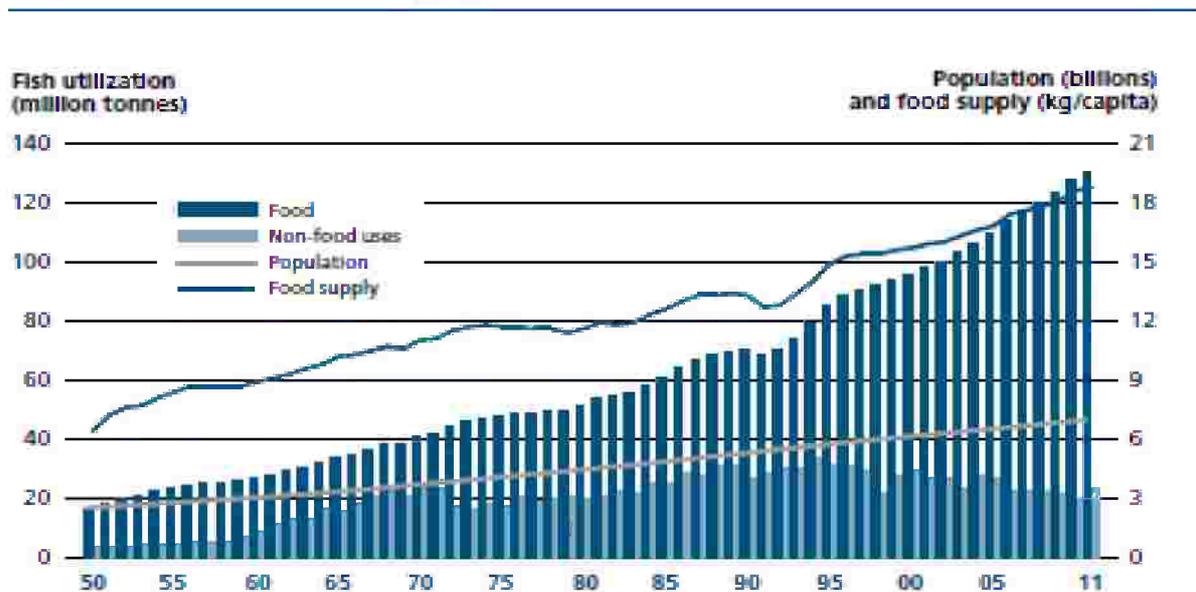


Figure (2). World Fish Utilization and Supply, Excluding China. (FAO, 2012).

2.4. Mullet species in Egypt:

Two decades ago tilapia and mullet were the main species reared in extensive earthen ponds. Presently, 14 different species of finfish and two species of crustacean are cultured in Egypt. Ten are native and six are introduced species. The native species are: Nile tilapia, *O. niloticus*, blue tilapia, *O. aureus*, North African catfish, *Clarias gariepinus*, flathead grey mullet, *M. cephalus*, thinlip mullet, *Mugil ramada*, bluespot mullet, *Valamugil seheli*, European seabass, *Dicentrarchus labrax*, gilthead seabream, *Sparus auratus*, meager, *Argyrosomus regius* and penaeid shrimp. The introduced species are: common carp, *C. carpio*, grass carp, *C. idellus*, silver carp, *H. molitrix*, bighead carp, *Aristichthys nobilis*, black carp, *Mylopharyngodon piceus* and the giant river freshwater prawn, *Macrobrachium rosenbergii*. The main cultured species are tilapias - mainly *O. niloticus* (about 35% of total aquaculture production), carps - mostly *C. carpio* (35%), mullets - mainly *M. ramada* and *M. cephalus* (23%), African catfish - *C. gariepinus* (3%), gilthead sea bream, *S. auratus*, (2%), and sea bass, *D. labrax*, (2%). Tilapia, carps, and mullet dominate the other cultured species and tolerate water salinity of up to 4 ppm, with mullet making up the bulk of stocked fish at higher salinities (Sadek *et al.*, 2006).

Out of the 17 genera and 80 species belonging to the family Mugilidae Nelson, (1984) and Saleh, (2008) stated that only three species belonging to the family Mugilidae are of aquaculture importance due to their higher growth rates and market acceptance. The flathead

gray mullet, *M. cephalus*, thinlip mullet, *L. ramada* and the bluespot mullet, *V. seheli*, are the most commonly cultured species of mullet in Egypt.

2.4.1. Flathead grey mullet

Scientific classification

Subphylum:	<i>Vertebrata</i>
Superclass:	<i>Gnathostomata</i>
Class:	<i>Actinopterygii</i>
Subclass:	<i>Neopterygii</i>
Division:	<i>Teleostei</i>
Subdivision:	<i>Euteleostei</i>
Superorder:	<i>Acanthopterygii</i>
Order:	<i>Mugiliformes</i>
Family:	<i>Mugilidae</i>
Genus:	<i>Mugil</i>
Species:	<i>Mugil cephalus</i>

The flathead grey mullet, *M. cephalus*, (Figure3) is a very important aquaculture species in the Mediterranean, Southeast Asia, Taiwan Province of China, Japan, and Hawaii (Saleh, 2006). It was the first species of mugilidae used for aquaculture in Egypt. This species has been used for traditional aquaculture and culture - based fisheries since the late 1920s and is still of major importance today also in other Mediterranean countries and Taiwan Province of China (Faouzi, 1936; Saleh and Salem, 2005 and Basurco and Lovatelli, 2003).

The species can reach a length of up to 120 cm externally. Males are difficult to distinguish from females, except for the more slender shape of males when sexually ripening (Virgona, 1995). Their color is olive - green dorsally, with sides that are silvery shading to white ventrally. They have thin lips and the pectoral fins are short, not reaching the first dorsal fin. The grey mullets found in coastal waters of the tropical and subtropical zones of all seas are catadromous, frequently found in estuaries and freshwater environments (Figure 3). Adults form large near the surface over sandy or muddy bottoms and dense vegetation and migrate offshore to spawn in large aggregations (Eschmeyer *et al.*, 1983). The larvae move inshore to extremely shallow water, which provides protection from predators as well as a rich feeding ground. After reaching 5 cm in length, the young mullets move into slightly deeper waters (Saleh, 2006). The

species is mainly diurnal and feeds on zooplankton, benthic organisms, and detritus. Adult fish tend to feed mainly on algae while inhabiting freshwater. Reproduction takes place in the sea from July to October. Females spawn 5 to 7 million eggs provided with a notable vitellus.



Figure (3): Biological features for flathead grey.

2.4.2. Global distribution of the flathead grey mullet

Cosmopolitan in coastal waters of the tropical, subtropical, and temperate zones of all seas. Eastern Pacific: California, USA to Chile (Eschmeyer *et al.*,1983). Western Pacific: Japan to Australia (Harrison and Senou, 1997). Western Indian Ocean: from India to South Africa (Smith and Smith, 1986). Western Atlantic: Nova Scotia, Canada to Brazil (Robins and Ray, 1986); Cape Cod to southern Gulf of Mexico (Smith, 1997); absent in the Bahamas and most of West Indies and Caribbean (Robins and Ray 1986 and Humann, 1994). Eastern Atlantic: Bay of Biscay to South Africa, including the Mediterranean Sea and Black Sea (Thomson, 1990).

2.5. Mullet and aquaculture Impacts:

2.5.1. Flathead grey mullet and culture systems

Flathead grey mullet has been farmed for centuries in extensive and semi-intensive ponds in many countries, Subsistence farming in ponds and enclosures has been traditional in the Mediterranean region, South East Asia, Taiwan Province of China, Japan, and Hawaii. Traditional vallicoltura methods employed for raising mullet are now advanced, especially in Italy. In the Russian Federation, mullet aquaculture has been practised in the Black Sea and Caspian Sea regions since 1930. This species was first introduced to be cultured with carp in Israel in 1957. In the Philippines, mullet has been raised with milkfish since 1953. The intensive

culture of mullet in Hong Kong was successful in fertilized ponds with the traditional practice of carp polyculture since 1940. It has been reported that mullet have been farmed in India since ancient times; for example, it has been extensively cultured in Bengal, Madras and Kerala since 1947. However, this is not reported its statistical return to FAO; production is presumably 'hidden' within the category 'Osteichthyes'. Flathead grey mullet is also cultured in Korea and is considered as an important foodfish in the southwest region. In Taiwan Province of China, nearly 40 per cent of the total commercial production (fisheries and aquaculture) of *Mugil cephalus* has been pond reared since the 1960s, being cultured with carp in ponds. In the US, mullet has been cultured as bait fish since the 1940s. Small-scale trials of mullet culture have been carried out in Saudi Arabia and other Gulf States. Also, flathead grey mullet is a very important aquaculture species in Egypt, where its farming has been traditional in the hosha system in the delta region for centuries. Since the early 1960s, flathead grey mullet has also been cultured in semi-intensive ponds with tilapia and carps in Egypt (Oren, 1981 & Harrison, and Senou 1999)

2.5.2. Aquaculture Impacts

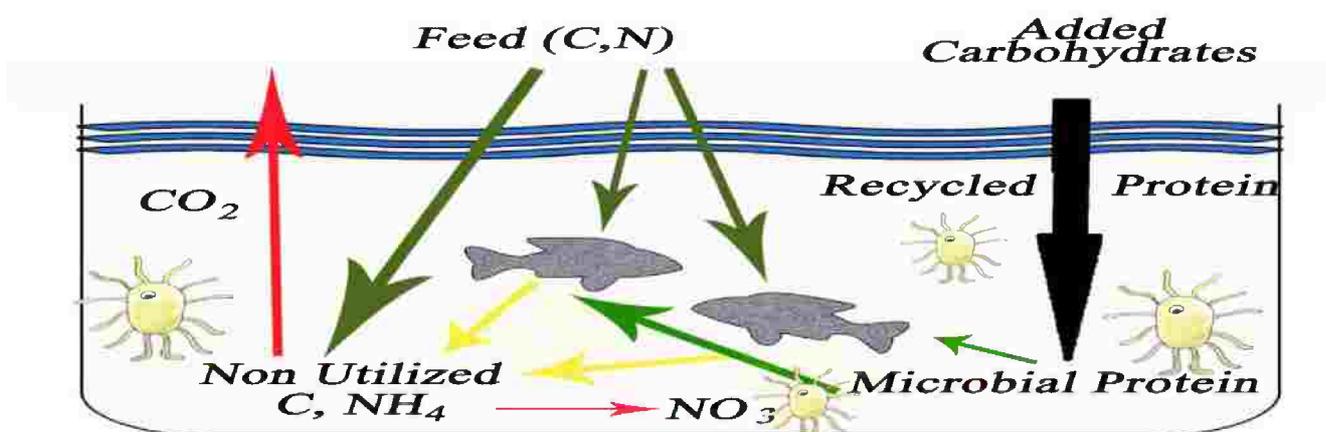
Aquaculture is a critical industry for supporting the world's demand of seafood protein and will play an even more important role as the global population continues to increase (Jackson, 2007). The current worldwide growth rate of the aquaculture business (8.9-9.1% per year since the 1970s) is needed in order to cope with the problem of shortage in protein food supplies, which is particularly situated in the developing countries (Gutierrez-Wing and Malone, 2006 Matos *et al*, 2006).

Along with, this aquaculture produces large quantities of wastes that contain solids (e.g. feces and uneaten feed) and nutrients (e.g. nitrogen and phosphorus) which can be detrimental to the environment, if managed improperly. These solids and nutrients originate from uneaten feed, feces, and animal urea or ammonia (Maillard *et al*. 2005 and Sharrer *et al*. 2007). If released directly to the environment, these solids and nutrients are pollutants resulting in environmental issues such as eutrophication (Wetzel 2001) or could be directly toxic to aquatic fauna (Timmons *et al*. 2002 and Boardman *et al*. 2004). The accumulation of these metabolites has been reported to negatively affect the performance of fish. For long, the most common method for dealing with these pollutants has been using continuous replacement of the pond water with external fresh water (Gutierrez-Wing and Malone, 2006). However, the water volume needed for even small to medium aquaculture systems can reach up to several hundreds of cubic meters per day but this

approach came to deadlock for several reasons. First of all in the case of fresh water system, water became scarce or expensive to an extent of limiting aquaculture development. Secondly, the releases of untreated pond water to the environment either river or marine system, became prohibited by the environmental authorities in most countries. In Egypt that suffers from the water shortage, Egyptian laws and policies regarding using fresh water limit a wider application of a combination of aquaculture and agriculture, commonly called integrated fish farming, according to the Law 124/1983, which severely restricts the use of freshwater for aquaculture.

A second approach is established for the removal of the major part of the pollutants in the water as is performed in recirculating aquaculture systems (RAS) with different kinds of biologically based water treatment systems (Gutierrez-Wing and Malone, 2006). The amount of water that needs to be replaced on a daily basis is generally reduced to about '10% of the total water volume (Twarowska *et al* 1997). However, this technique is costly in terms of capital investment. (Gutierrez-Wing and Malone, 2006). RAS ponds do not recycle feed; any feed that is not immediately consumed by the fish and all unutilized feed excretions are removed and disposed, at a high cost (Timmons *et al*. 2002).

Application of bio-flocs technology (BFT) offers a solution for both problems waste water and shortage in protein. It depends on lower investment and maintenance costs and incorporating the potential to recycle feed (Timmons and Ebling, 2007). Biofloc combines the removal of nutrients from the water with encouraging the growth of microbial biomass (heterotrophic bacteria) which consume ammonia for growth that lead to decrease pond water exchange. On the other hand, bacteria could be used by the culture species as additional food source.



(Fig. 4). Scheme of Biofloc Technology pond (Avnimelech 2012).

2.6. Definition and component of bio-floc:

Biofloc system defined as aquaculture without water exchange under high stocking density with strong aeration and biota (predominantly, -aerobic and heterotrophic), formed by microbial flocs or bio-flocs is known as ZEAH (Zero Exchange Aerobic Heterotrophic) (Avnimelech, 2007 and De Schryver *et al*, 2008) or more recently as bio-flocs technology (BFT). De Schryver *et al.* (2008) defined the biofloc technique as a co-culture of heterotrophic bacteria and algae which are grown in flocs under controlled conditions within the culture pond. The system is based on the knowledge of conventional domestic wastewater treatment systems and is applied in aquaculture environments; where microbial biomass is grown on fish excreta resulting in a removal of these unwanted components from the water.

Burford *et al.* (2003) reported that forming bio-flocs contains bacteria, other micro-organisms, protozoa, and zoo plankton. Microbial flocs consist of a heterogeneous mixture of micro-organisms (floc-formers and filamentous bacteria), colloidal particles, organic polymers, cations and dead cells and can reach more than 1000 μ m in size (Jorand *et al.* 1995). Typical flocs are irregular in shape, have a broad distribution of particle sizes, are fine, easily compressible, highly porous (up to more than 99% porosity), and are permeable to fluids (Chu and Lee, 2004).

Beside natural composition of biofloc, chemical compositions were discussed where, biofloc severed from tilapia culture tanks was reported for 38% protein, 3% lipid, 6% fiber, 12% ash, and 19 KJ g^{-1} energy (on dry matter basis). Moreover, it was found that biofloc under these conditions contained 27-28% polyunsaturated, 28-29% monounsaturated, 30-35% saturated fatty acids, and 10-12% unknown peaks (Azim and Little, 2008).

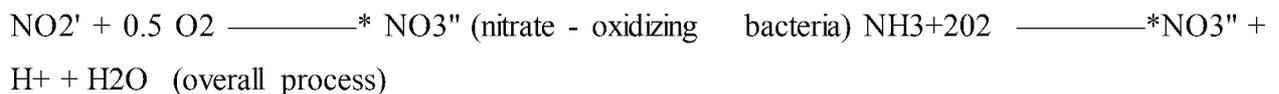
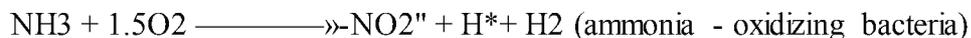
2.7. Mode of action of bio-floc technique.

Microbial growth needs aerobic condition beside optimal C/ N ratio to breakdown 40-60% of metabolized organic matter to produce new bacteria. Such heterotrophic production is an important component of fed and fertilized ponds stocked with particulate feeding fish but is limited by the need for constant aerobic conditions.

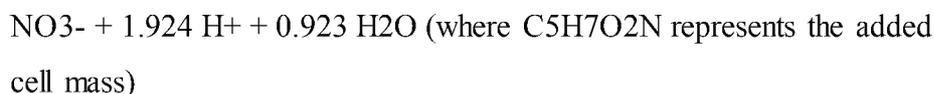
When carbon and nitrogen are well balanced according to either the use of lower protein diet or supplying additional carbon sources, e.g. glucose, sucrose, and starch to the pond, the

inorganic nitrogen components (ammonia, nitrite, and nitrate) in pond will be converted into bacterial biomass (Avnimelech, 1999 ;Crab *et al.* 2007 and Hargreaves, 2006). As such, nutrients from excretion and remnant feed are recycled into bacterial biomass and formed bioflocs (Avnimelech, 2006). These result in water quality control and in situ feed production.

The activated suspension technique (AST) recently referred to as biofloc technology (BFT) is based on the use of constant aeration to allow aerobic decomposition and maintain high levels of bacterial floc in suspension (Avnimelech *et al.* 1986). Under such conditions, dense microorganisms develop, functioning both as a bioreactor controlling water quality (Avnimelech *et al.* 1989, 1994) and as a protein food source for the fish. Three ammonia-nitrogen conversion pathways naturally present in aquaculture systems are known as photoautotrophic (by algae), autotrophic (bacterial conversion of ammonia- nitrogen to nitrate- nitrogen), and heterotrophic (bacterial conversion of ammonia-nitrogen directly to microbial biomass) (Ebeling *et al.* 2006). The following chemical equations describe the different stages to get rid of nitrogen along with the formation of microbial biomass. Autotrophic nitrification is a two-step process where ammonia is biologically oxidized into nitrite (nitration) and then to nitrate (nitration) with oxygen as terminal electron acceptor (Rettmann and Me Carty,2001):

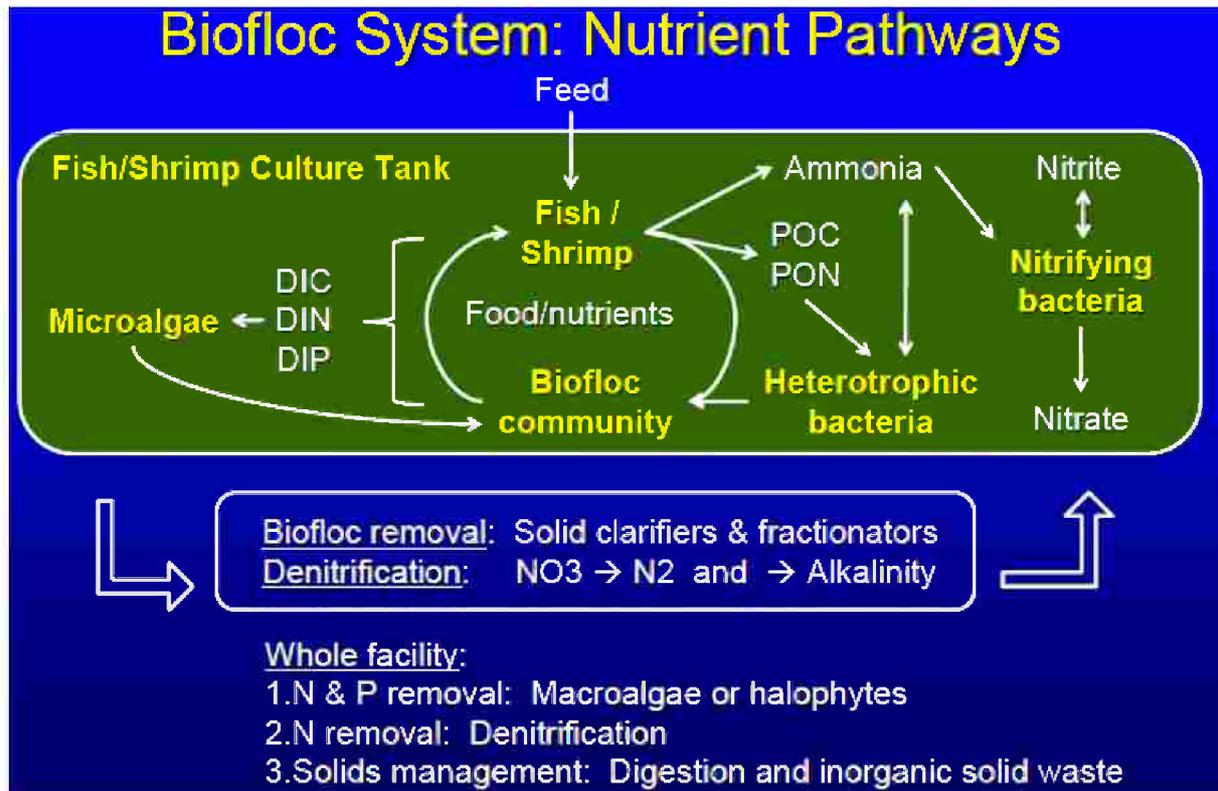


The stoichiometry of the nitrification process (Ebling *et al.*, 2006) is (reactants and products of an oxidation of 1 mole NH₄-N, quintets given in molar units)



The same stoichiometry given in grams is:





(Fig. 5). Bio-floc System Ammonia Nitrite and Nitrate pathways (Emerenciano, *et. al* 2012).

2.8. Factors influencing floc formation and structure:

2.8.1.. Mixing intensity

The mixing intensity imposed by a chosen aeration device at a certain power input will determine the steady-state floc size; this is the equilibrium between the rate of aggregation and the rate of breakage, and the floc size distribution (Chaignon *et al.*, 2002; Spicer and Pratsinis, 1996). In aquaculture, energy dissipation in general is in the range of 0.1–10 W m^{-3} (Boyd, 1998), however, in highly intensive systems, more realistic values can reach up to 100 W m^{-3} . At higher mixing intensities and thus higher shear rates, the average floc size decreases due to increased floc breakage. The relationship between floc size and mixing intensity has been represented by Parker *et al.* (1972) with the power law relationship $d = CG^{-x}$, where d is the maximum stable floc size, G is the average velocity gradient, C is the floc strength component and x is the stable floc size component. For BFT, the steady-state floc size is an important feature as it has already been shown that the quality of food for different aquaculture species is also dependent on the food size (Garatun-Tjeldsto *et al.*, 2006; Knights, 1983).

2.8.2. Dissolved oxygen (DO)

The dissolved oxygen (DO) level is not only essential for the metabolic activity of cells within aerobic flocs but it is also thought to influence floc structure. Martins *et al.* (2003) showed that at low DO values (0.5-2.0 mg IT1) a sludge volume index (SVI) of on average 250 ml g⁻¹, compared with the higher DO values (2.0-5.0 rag IT1) where the SVI was 100ml g⁻¹. This can be ascribed to the presence of a higher amount of filamentous bacteria compared to the zoogloal bacteria at DO levels of less than or equal to 1.1 mg L⁻¹. Bioflocs with a higher floc volume index (FVI) are produced at lower DO levels in the bio-flocs ponds.

2.8.3. Temperature

The temperature is one of the major importance of the microbial metabolism, (Wilen *et al.* 2000) found that de flocculation of the flocs occurred at lower temperature (4 °C) compared to higher temperatures (18-20 °C). This might be due to a decrease of the microbial activity within. (Krishna and Van Loosdrecht, 1999) observed that higher temperatures (30-35 °C) resulted in bulking of the sludge volume index (SVI >500 mL g⁻¹) due to the excessive production of extracellular polysaccharides, so it was expected that an intermediate water temperature of 20-25 °C would be best to obtain stable flocs with an intermediate floc volume index of about 200 mL g⁻¹ (De Scbryver *et al.* 2008)'.

2.8.4. Organic carbon source and loading rate

Under biofloc system, organic carbon can be supplied either as additional organic carbon source (e.g. glucose, acetate, glycerol.) or by changing the feed composition thus increasing its organic carbon content (Avnimelech, 1999). It can be added in small amounts and thus almost continuous mode or be added in larger doses but at regular time intervals (e.g. 1 day⁻¹) (Martins *et al.*, 2003). Goldman *et al.* (1987) found C/N ratios N10:1 was optimal for optimizing biofloc production while minimizing ammonia regeneration. Also Fontenot *et al.*, (2007) reported that the C/ N ratio of 10:1 gave the best results in terms of maximum inorganic nitrogen removal from waste water.

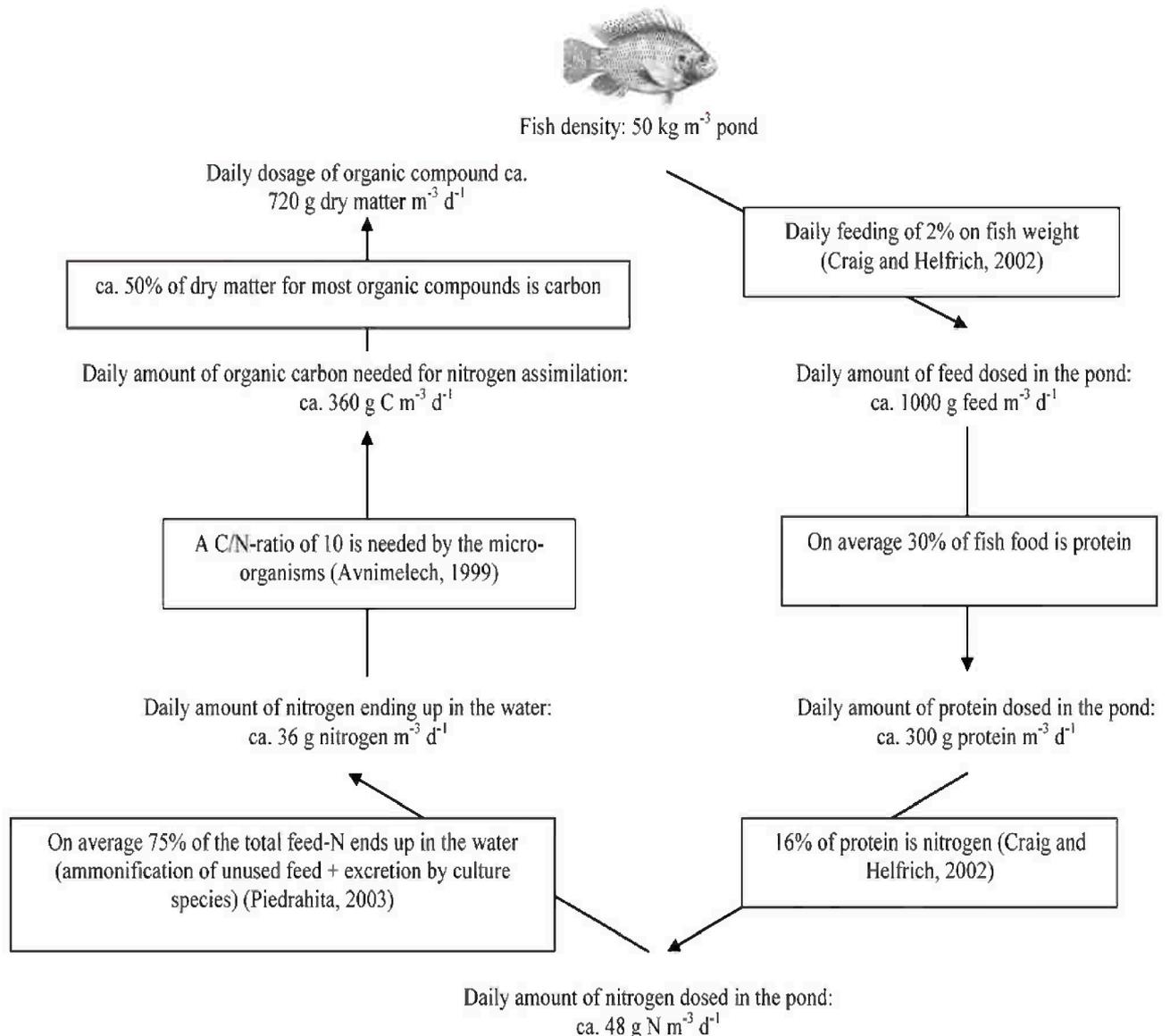


Fig. (6). Schematic calculation of the daily amount of organic carbon needed by bio-flocs to remove the nitrogen excreted in an intensive aquaculture pond of 50 kg fish m⁻³. Schryver, *et. al* (2008).

2.8.5. pH

The stability of the bio-flocs is determined by pH value (Mikkelsen *et al* 1996). Azim and Little (2008) reported that the biofloc systems lose buffering capacity when pH low so, it was necessary to be corrected through additions of NaHCO₃ to be in the average (6.7, range 5.0-8.5). Kuhn *et al.* (2010) reported that the average pH (8.02, range 6.75- 9.28) to shrimp under biofloc condition, while in case additional stressors like handling are absent it seems that tilapia are able to acclimate to pH 4.0 without negative impacts on physiology (VanGinneken *et al.*, 1997). Upper range levels also exist like a pH value of ca. 10 for the Klamath Large-scale and

Shortnose sucker (Portz *et al.*, 2006). In general, next to the fact that it is not an easy parameter to control, possible changes in pH are limited to the optimal range for the cultured animals to avoid mortality and disfunctioning

2.8.6. Carbon /Nitrogen ratio:

Balanced carbon / nitrogen are needed for optimal microbial growth under the biofloc system. Goldman *et al.* (1987) found C/N ratios 10:1 were optimal for optimizing bio-floc production while minimizing ammonia regeneration. High C/N ratio in feed (>N15) leads to the immobilization of ammonium total ammonium nitrogen (TAN) in the microbial biomass and limits the accumulation of TAN in the water (Avnimelech, 1999). Asaduzzaman *et al.* (2008) reported that increasing C/N ratios from 10 to 20 can significantly increase the yield of shrimp (*M. rosenbergii*).

2.9. The advantages of Biofloc system:

Bio-floc technology plays important role in aquaculture as it contribute to the solution of many problems such as, high feed cost and water quality, ect...., the following illustrate these roles.

2.9.1. Bio-floc technique-and water quality:

The most important reasons that lead to poor water quality that fish use proteins for energy production to a large extent, unlike terrestrial animals that use mostly carbohydrates and lipids (Hepher, 1988). Fish protein requirement, therefore, is about two to three times higher than that of mammals. Ammonium is one of the end products of protein metabolism (Walsh and Wright, 1995). All these reasons contribute to the high nitrogen residues in aquaculture water.

In water, NH₃ (ammonia) and NH₄⁺ (ammonium) are in equilibrium depending on the pH and the temperature (Timmons *et al.* 2002). The sum of the two forms is called total ammonium nitrogen (TAN). Although both NH₃ and NH₄⁺ may be toxic to fish, unionized ammonia is more toxic than attributable ammonia due to the fact that it is uncharged and lipid soluble and consequently traverses biological membranes more readily than the charged and hydrated NH₄⁺ ions (Korner *et al.* 2001). Ammonia-N is toxic to commercially cultured fish at concentrations above 1.5 mg N/l. In most cases, the acceptable level of unionized ammonia in aquaculture systems is only 0.025 mg N/l (Neori *et al.* 2004 and Chen *et al.* 2006). However, the

toxicity threshold strongly depends on the species, size, fine solids, refractory organics, surface-active compounds, metals, and nitrate (Colt, 2006). Microbial process is the key step in waste water treatment, to degrade or remove this waste. Adding carbohydrates enhances the water quality in the pond (Burford *et al.* 2003, 2004; Hari *et al.* 2004 and Avnimelech, 2005). The addition of carbohydrate to intensively well-mixed production systems will reduce the TAN concentration through immobilization by bacterial biomass (Avnimelech *et al.* 1999). which cause a significant reduction in NO₂ concentration in the water column, which can be attributed to low availability of TAN as substrate for nitrification and hence the production of NO₂ (Avnimelech, 1999 and Hari *et al.* 2004). The nitrification ultimately leads to the formation of NO₃, so one would also expect to find differences in nitrate concentration as a result of carbohydrates addition (Hari *et al.* 2004).

With balancing carbon and nitrogen, a solution of ammonium in addition to organic nitrogenous waste will be existed, by converting into bacterial biomass (autotrophic and heterotrophic) (Avnimelech, 1999). Highly oxygen supplementation promoted nitrogen uptake by bacterial growth and decreased the ammonium concentration more rapidly than nitrification by autotrophic bacteria (anaerobic). Immobilization of ammonium by heterotrophic bacteria (aerobic) occurs much more rapidly because the growth rate and microbial biomass yield per unit substrate of heterotrophs are a factor 10 higher than that of nitrified bacteria (autotrophic) (Hargreaves, 2006).

2.9.2. Biofloc as fish feed:

On the other hand. Bioflocs can be consumed by the cultivated aquatic organism and hereby act as a source of feed, however, some researches showed that carbohydrate addition in extensive shrimp ponds improved the nitrogen retention efficiency and had a positive effect on production (Hari *et al.* 2004). In the same context, (Hari *et al.* 2006) concluded that carbohydrates addition in combination with reduction of the dietary protein level improved the sustainability of shrimp farming through increased nitrogen retention in harvested shrimp biomass. Reduced demand for feed protein reduced concentrations of potentially toxic TAN and NO₂ in the system, and reduced water based- nitrogen discharge to the environment. The same conclusion was reported by (Arnold *et al.* 2009; Ballester *et al.* 2010; Hari *et al.* 2004; Kuhn *et al.* 2008 and Megahed, 2010).

2.9.2.1. Bio-floc effects on fish performance and feed utilization:

Avnimelech (2007) reported that Fish in (BFT) pond grew better than fish in treatment without (BFT) pond. Azim and little (2008) concluded that Individual tilapia fish weight at harvest was 9-10% higher in the BFT treatments than in treatments without Biofloc. BFT treatments contributed to 44-46% greater individual weight gain and net fish production than those in the control confirming the utilization of bio-floc by fish as food.

As for shrimp, Hari *et al.* (2006) showed that SGR and individual shrimp weight gain were higher ($P < 0.05$) in carbohydrate- added treatments compared to either fish fed on high or low protein diets (25 or 40) without carbohydrate supplementation. Same was observed by (Kuhn *et al.* 2009) reported that microbial floe diets enhanced its growth by an average of 65.1% over mean growth of control diets (without biofloc). More specifically, shrimp fed on microbial flocs on average grew, 49.2 and 84.8% respectively faster than controls (absence biofloc). Xu *et al.* (2012) reported that the growth of shrimp fed dietary protein 30% or 35% under BFT were significantly better than control (without BFT), and growth did not differ significantly among treatments fed on dietary protein level 25%, 30% and 35% with BFT. On the other hand, some authors concerned with to what extent biofloc affects feed utilization. Hari *et al.* (2006) reported that the best FCR and PER values noticed for shrimp fed on dietary protein level 25% and 40% with added carbohydrate (1.1) compared with the same treatment without added carbohydrate. Same observations were recorded by (Xu *et al.* 2012 and Xu and Pan, 2012).

2.9.2.2. Bio-floc and Blood parameters of fish:

Few studies suggested that biofloc may affect some of blood parameters, where (Azim and little, 2008) reported that biofloc system did not affect hematocrit blood level compared with reticulating system. On the other hand, blood cortisol concentrations also did not vary significantly between the treatment and control tanks. However, after 1 h stress, the concentrations increased by 8 times in the treatment having 35% dietary protein level under (BFT) and by five times in the control having 35% dietary protein level under (RAS). This difference might be due to an increased initial load of, and challenge from, bacteria entering the fish at the tagging site in the biofloc treatment.

2.9.2.3. Bio-floc and chemical composition of fish:

Azim and little, (2008) reported that there are no significant differences in chemical composition of Nile tilapia (*Oreochromis niloticus*) fish between the tanks under biofloc and

control. Also (Xu and Pan, 2012) reported that no significant differences were observed ($P>0.05$) in chemical composition of the shrimp whole body between bioflocs treatments and the control in the moisture and protein content but the crude lipid and ash content tended to increase in bioflocs treatments.

2.9.3. The effect of biofloc on pathogenic diseases and survival rate:

Not only the nutritional value of the bio-flocs is important but also some internal compounds may be beneficial to the aquaculture species such as short chain fatty acids as bio-control agents against pathogenic diseases which are of particular interest.

The capability of accumulating and storage compound poly-p-hydroxybutyrate (PHB). PHB accumulating bacteria have been shown before to protect different aquaculture animals from bacterial infections (De Schryver *et al.* 2010; Defoirdt *et al.* 2007; Dinh *et al.* 2010 and Halet *et al.* 2007). PHB levels in bioflocs alternate between 0.5 and 18% of the dry matter, (Crab, 2010a; De Schryver and Verstraete, 2009). Bioflocs contain a sufficient PHB level to protect cultured animals from infection by pathogenic bacteria (Halet *et al.*, 2007). Interestingly, it was found that bioflocs grown on glycerol were able to protect gnotobiotic brine shrimp (*Artemia franciscana*) against pathogenic *Vibrio harveyi*, and that the beneficial effect was likely due to interference with the pathogen's quorum sensing system (Crab *et al.* 2010a). Kuhn *et al.* (2009) showed that shrimp are healthiest and grow best in aquaculture systems that have high levels of algae, bacteria and other natural biota. Since several research articles have been published on the benefits of using *Bacillus* to improve shrimp growth performance, survival, immunity, and disease resistance in aquaculture (Verschuere *et al.* 2000), biofloc reactors were inoculated with a probiotic bacillus mixture in an attempt to produce probiotic bioflocs (Decamp *et al.* 2008; Tseng *et al.* 2009 and Verschuere *et al.* 2000). Based on that, (Crab, 2010a) showed that the water of shrimp tanks fed bioflocs inoculated with *Bacillus* had on average 5 times lower *Vibrio* load when compared to the shrimp tanks fed on artificial feed. He concluded that bioflocs technology has protected brine shrimp (*Artemia franciscana*) larvae from vibriosis.

This role of biofloc in disease resistance has a positive effect on the survival rate where, Lezama-Cervantes and Paniagua-Michel, (2010) reported that survival of challenged nauplii increased 3-fold after the addition of live bioflocs. Hari *et al.* (2006) showed that shrimp survival ranged between 77% and 83% under biofloc condition while, final survival rates of shrimp reached (93% to 100%) under biofloc conditions as reported by (Kuhn *et al.* 2009). The same conclusion was suggested by (Xu and Pan, 2012). Xu *et al.* (2012), reported that Mean survival

rates were above 85% under biofloc condition, with no significant differences ($P>0.05$) among all experimental shrimp groups. Azim and Little, (2008) showed that survival rate of Nile tilapia (*Oreochromis niloticus*) fish was 100% in treatments (24%, 35% dietary protein with biofloc) and control tanks.

2.10. The disadvantages of biofloc system:

Despite the many advantages of biofloc, there are some problems in this system as poor FCR and level of production. This may be due to several reasons; increased turbidity because biofloc reduces the visibility and hence artificial feed. Under biofloc conditions, it is important to maintain 500 mg L^{-1} of total suspended solid (TSS); the TSS may reach up to 1000 mg L^{-1} ; meanwhile, water quality parameters were not stable: high fluctuation of pH and alkalinity, high concentrations of inorganic nitrogen species might have chronic effects on fish health (Little *et al.* 2008). Therefore it is important to fine tune of biofloc conditions to improve fish production.

2.11. Bio-floc and cultured species:

A potential alternative, referred as BFT, could utilize heterotrophic bacteria to convert nitrogen in aquaculture into bacteria biomass (De Schryver and Verstraete, 2009), which could potentially be used to feed fish, thereby increasing the efficiency of nitrogen. BFT, i.e. a co-culture of aquaculture species and heterotrophic bacterial biomass within the same solution (called an in situ BFT-aquaculture system) has already been exploited in pond aquaculture systems for tilapia (Azim and Little, 2008; Crab *et al.*, 2009), shrimp (Asaduzzaman *et al.*, 2008; Burford *et al.*, 2004), shrimp and tilapia together (Asaduzzaman *et al.*, 2009), and catfish and tilapia together (Brune *et al.*, 2003). However, the in situ BFT aquaculture system does not appear to be appropriate for species that do not graze on biofloc directly such as carnivorous fish. As a result, further development of mixed systems has been advocated in which culture units are portioned with flocs to develop a hybrid technology of BFT and RAS (Avnimelech and Teubal, 2006; Azim and Little, 2008; De Schryver and Verstraete, 2009).

2.11.1. Bio-floc and Shrimp culture

Bioflocs technology has been applied and developed in high intensive farming systems of several shrimp species, such as *Penaeus monodon*, *Litopenaeus vannamei*, and *Macrobrachium rosenbergii* farming (Burford *et al.*, 2003; Crab *et al.*, 2010b; Hari *et al.*, 2006). Biofloc technology is based upon the production of shrimp with zero or minimal water exchange

resulting in the accumulation of organic substrates and subsequent development of dense microbial population, mostly aggregated in bioflocs (Avnimelech 2012). Several authors have cited that the high volume of water needed (20–64m³) to produce 1 kg of shrimp under traditional production practices (Hopkins *et al.* 1993; Timmons and Losordo 1994; Moss *et al.* 2001). In contrast, much less water is used by biofloc-rich systems. For example, Otoshi *et al.* (2009) showed that 163 L of water was needed to produce 1 kg of the Pacific white shrimp, *Litopenaeus vannamei*, in a super-intensive production system. A similarly low level of water usage (169 L/1 kg shrimp produced) was documented by Krummenauer *et al.* (2011) in 35-m³ raceways. Furthermore, Samocha *et al.* (2010), in an experimental zero-exchange super-intensive system, reported the use of only 98 L of water to produce 1 kg of food shrimp.

By adding a carbon source to the culture medium in limited-discharge systems (i.e., by changing the C/ N ratio), it is possible to obtain a significant enhancement of bacterial growth and fixation of toxic nitrogen metabolite species (Chamberlain *et al.* 2001; Ebeling *et al.* 2006; Hari *et al.* 2006; Avnimelech, 2009; Crab *et al.* 2010a). In addition to the improvement in water quality, the increase in bacterial biomass, which provides supplemental feed, is known to be associated with improved shrimp survival and growth and to reduce the releases of nutrient-rich water into receiving streams (Timmons *et al.* 2002; Wasielesky *et al.* 2006; De Schryver *et al.* 2008; Avnimelech 2009; Krummenauer *et al.* 2011).

The bioflocs technology (BFT) for shrimp production has been proposed as a sustainable practice capable of reducing environmental impacts and preventing pathogen introduction. The microbial community associated with BFT not only detoxifies nutrients, but also can improve feed utilization and animal growth. Biofloc system contains abundant number of bacteria of which cell wall consists of various components such as bacterial lipopolysaccharide, peptidoglycan and β -1, 3-glucans, and is known as stimulating nonspecific immune activity of shrimp as Pacific white shrimp, *Litopenaeus vannamei*, is one of the most important farmed species in the world. However, farming activities of this species have been largely affected by diseases, mostly viral diseases such as the white spot syndrome virus (WSSV). Producers and researchers are constantly looking for methods to reduce massive shrimp losses due to disease outbreaks. Growing shrimp using biofloc technology (BFT) was proposed as a tool to reduce water exchange and minimize the introduction of viral pathogen through incoming water. In addition, observations on the effects of BFT on reducing viral disease outbreaks were reported (Avnimelech 2012).

The biofloc technology system (BFT) is characterized by rich microbial communities that form flocs in the water column. Growing shrimp under these conditions has been found to have several advantages compared to conventional aquaculture practices. These systems are characterized by high yields, small footprints, and reduced environmental impacts (Browdy *et al.* 2001; Neal *et al.* 2010). The use of limited water exchange minimizes the introduction of pathogens with the incoming water. Moreover, increasing awareness of biosecurity reduces crop losses from disease outbreaks. Furthermore, because the assimilation of nitrogen compounds is enhanced under these growing conditions, the same water can be used for several production cycles with no negative impact on yields. Another advantage stemming from this technology is the ability to construct and operate these facilities inland, far from high-priced coastal areas (Ray *et al.* 2009; Vinatea *et al.* 2010; Samocha *et al.* 2011).

2.11.2. Bio-floc and Tilapia fish

The tilapias are very heterogeneous in the food items they consume. The feeding habits of tilapia have been examined under various conditions including polyculture, supplementary feeding and intensive pond enrichment (Grover *et al.* 1989). Tilapias are generally considered herbivorous despite the role of animal matter in the diet of adult tilapia is considered enigmatic. Fry and early juveniles are known to feed on zooplankton (Bowen, 1982). Hephner and Pruginine, (1981) reviewed that (*O. niloticus*) feeds principally on phytoplankton of which diatoms are important items. (*O. niloticus*) fry also feed on macrophytic detritus, rotifers, and other zooplankton, insect and water mites. Dempster *et al.* (1995) and Azim *et al.* (2003) reviewed that tilapias being capable of both filter feeding and detritivory are ideal candidates for such system (BFT). Also Dempster *et al.* (1995) showed that filter feeding on only micro algae could not provide full energy demands for the fish (tilapias) emphasizing the importance of other larger size food particles in natural systems such as sediment water interface detritus, surface concentrated scums of cyanobacteria and periphyton. Beveridge *et al.*, (1989); Beveridge and Baird, (2000) reported that most of the tilapias are known to utilize in situ produced food particles including suspended bacteria.

Due to the nature of the food for tilapia fish that are commensurate with biofloc technology noted for performance improvement, Azim and Little (2008) concluded that the nutritional quality of biofloc was appropriate at least for herbivorous and omnivorous fish species including tilapias. Crabe *et al.*, (2009) concluded that the use of bio-floc technology proved to be effective in overwintering of tilapia by maintaining appropriate water temperature,

good water quality and high fish survival in low/no water exchange, greenhouse ponds. Avnimelech *et al* (1994) reported that tilapia in biofloc ponds is fed a ratio 20% less than conventional one and fish yields in biofloc ponds were high. Avnimelech and Kochba (2009) concluded that the daily net uptake of microbial protein by tilapia from a biofloc suspension of about 200 mg l⁻¹ amounted to 242mg N kg⁻¹ equivalent to the daily uptake of 1.56 g protein, about 25% of the normal protein ration given to tilapia.

2.11.3. Bio-floc and Channel catfish culture.

Channel catfish are omnivorous, which means they eat a wide variety of food items depending on what is available in their environment. Recent research demonstrated that outdoor biofloc systems can be used to produce high yields of channel catfish (*Ictalurus punctatus*) (Green, 2010). However, studies have not yet been performed to determine the development and composition of phytoplankton communities and related off-flavor problems in these biofloc production systems. In addition, most channel catfish production in the southeastern United States of America is conducted in earthen, embankment-type ponds. In these earthen ponds, phytoplankton will assimilate and reduce NH₃-N concentrations in the pond waters (Hargreaves, 2006). Cyanobacteria (blue-green algae) usually dominate the phytoplankton communities in catfish ponds because of their ability to regulate cell buoyancy by collapse and reformation of intracellular gas vesicles in the poorly mixed and often stratified water column; this physiological mechanism allows cyanobacteria to outcompete other types of phytoplankton for sunlight (Paerl and Tucker, 1995). Certain species of cyanobacteria are undesirable due to their production of odorous compounds that can accumulate in the flesh of fish and subsequently result in an “off-flavor” and unmarketable product.

Recently, Bartholomew and Kevin (2013) carried out an experiment to evaluate the effect of biofloc system on net yield and water quality for channel catfish using a completely randomized experimental design. The stocker of catfish was (217.0 g/fish, 30.5 cm/fish) stocked into nine continuously aerated tanks (18.6 m², 15.5 m³) at 1.4, 2.1, or 2.8 kg/m³. Fish were fed daily to apparent satiation on a 32% protein floating extruded feed. Dissolved oxygen and temperature were measured continuously using sensors connected to a data logger. Water quality variables (pH, TAN, NO₂N, NO₃N, total alkalinity, settle able solids, total suspended solids, total volatile solids, and chlorophyll *a*) were measured on a weekly basis. Also, water samples were periodically analyzed for the off-flavor compounds, 2-methylisoborneol (MIB) and geosmin.

Survival was high, did not differ among treatments, and averaged 97.2%. At the end, they stated that the increase in the mean net yield of channel catfish in relation to initial biomass was curvilinear. The mean net yield ranged from 3.8 - 5.5 kg/m³. The mean individual weight of catfish at harvest decreased linearly as stocking biomass increased. Low, variable concentrations of MIB and geosmin were present in biofloc tank water during most of the study. No significant difference among treatments was detected for MIB or geosmin concentrations in BFT tank water. Concentrations averaged 3 ng/L MIB and 2 ng/L geosmin.

2.11.4. Bio-floc and Artemia

A lab-scale experiment was carried out by Hoa. *et al.* (2013), to determine the possible use of bioflocs as a feed for *Artemia*. The bioflocs were developed at four different salinities (35, 60, 80 and 100 ppt) in the plastic tanks using cassava flour as a carbon source. *Artemia* nauplii were stocked communally to sexual maturity (adult stage) in a 10 l glass bottle with 3 replicates and stocking density of 100 ind. l⁻¹ to determine the survival and growth, and .Then, *Artemia* adults were cultured as individual couple in a plastic Falcon tube containing 50 ml medium of 80 ppt salinity with 40 replicates for monitoring the reproductive characteristics and life span. Five feeding treatments consisting of mixed microalgae harvested from the fertilization pond served as a control feed was compared to four bioflocs collected at different salinities. The results showed that seven days post-inoculation, survival of *Artemia* was in the range of 49.4 - 66.1%, of which the group receiving the control feed had significantly higher value than those fed on bio-floc grown at 35 and 100 ppt ($P < 0.05$). A similar pattern was observed for total length. The pre-productive period of all feeding treatments was similar, ranging between 14.3 and 14.7 days. The reproductive performances (reproductive period, total offspring per female, number of brood) of *Artemia* females in the control treatment were not significantly different from the group fed on the biofloc harvested at salinities of 60, 80 and 100 ppt, except the 35 ppt biofloc treatment. The life span of *Artemia* females follows the same response as observed for the reproductive performances. Moreover, the results indicated that total number of offspring encysted in all biofloc treatments was higher than in the control although there was no significant difference among treatments ($P > 0.05$). It can be concluded that biofloc could be a suitable food for *Artemia*.

Also *Artemia nauplii* had been reared in 1 l cones at stocking density of 2 nauplii / l by Toi *et al.* (2013), each culture tube contained 800 ml of filtered artificial seawater (FASW) at salinity 33 mg/l. Three feeding regimes, standard feeding regime (SF) or ad libitum regime, half

of SF (1/2 SF), and one third of SF (1/3 SF), were offered to *Artemia*. Sucrose was daily added to each feeding regime as carbon source to produce C/N ratio 5, 10 and 15 for in 15 days of experimental culture period. The *Artemia* culture cones were placed in a controlling temperature bath at 28.0 ± 1.0 °C, and each culture cone was supported with continuous aeration. Three replications were performed for each treatment. Their results showed that the poorest survival in SF regime was shown by C/N ratio 15 (SF15), while *Artemia* survival in the C/N ratio 5 (SF5) and 10 (SF10) was similar to the NF control. In the 1/2SF regime, a significantly better survival was exhibited by all C/N ratios (1/2SF5, 1/2SF10, and 1/2SF15) as compared to the 1/2SF control ($P < 0.05$). In 1/3SF regime, significantly higher survival was shown by C/N ratio 10 (1/3SF10) and C/N ratio 15 (1/3SF15) as compared to 1/3SF control treatment and C/N ratio 5 (1/3SF5). The C/N ratio manipulation in the SF regime produced no effect on *Artemia* growth ($P > 0.05$). However, there was a trend of improved *Artemia* growth by sucrose addition. In 1/2SF regime, *Artemia* in 1/2SF10 and 1/2SF15 tended to grow faster compared to the 1/2SF control and 1/2SF5, but a significant effect was only detected between 1/2SF15 on the one hand and the 1/2SF and 1/3SF5 on the other ($P < 0.05$). Similar results as shown in SF regime were observed in the 1/3SF regime. There were no significant differences among the treatment although *Artemia* in the 1/3SF5 and 1/3 SF15 tended to grow faster compared to the 1/3control. Among three feeding regimes, the best *Artemia* performance in terms of growth was shown in the SF treatments, whereas two C/N treatments in the 1/2SF regime, 1/2SF10 and 1/2SF15, could equal SF growth ($P > 0.05$). Moreover, *Artemia* treated in the 1/3SF regime with C/N ratio manipulation could not equal the growth in the SF control ($P < 0.05$), but *Artemia* in the 1/3SF5 and 1/3SF10 had similar growth as *Artemia* in the 1/3SF control ($P > 0.05$) Toi, *et. al.* (2013).

2.11.5. Flathead grey mullet and Bio-floc

Flathead grey mullet is a diurnal feeder, consuming mainly zooplankton, dead plant matter, and detritus. Mullet have thick-walled gizzard-like segments in their stomach along with a long gastrointestinal tract that enables them to feed on detritus. They are an ecologically important link in the energy flow within estuarine communities. Feeding by sucking up the top layer of sediments, flathead grey mullet remove detritus and microalgae. They also pick up some sediment which functions to grind food in the gizzard-like portion of the stomach. Mullet also graze on epiphytes and epifauna from sea grasses as well as ingest surface scum containing microalgae at the air-water interface. Larval flathead grey mullet feed primarily on micro

crustaceans. Copepods, mosquito larvae, and plant debris have been found in the stomach contents of larvae fewer than 35 mm in length. The amount of sand and detritus in the stomach contents increases with length, indicating that more food is ingested from the bottom substrate as the fish matures. Also the adult mullet consider euryhaline fish because it has been found in waters ranging from zero salinity to 75 percent, while juveniles can only tolerate such wide salinity ranges after they reach lengths of 4–7 cm (Harrison & Senou, 1999).

Therefore, much of the nutritional research with fish species has focused on minimizing crude protein in the diet for mullet (Albertini-Berhaut, 1974; Alexis, and Papaparaskeva-Papoutsoglou 1986; and El-Dahhar, 2000 b, c; and Luzzana *et al.*, 2005). These researches tended to reduce the cost of feed. In a laboratory study El-Dahhar (2000 b) fed striped mullet (*Mugil cephalus*) larvae of 0.2g initial BW at incremental dietary crude protein levels from 14 to 38% to determine protein requirements. They found that 26% dietary crude protein level is the level needed for maximum growth and feed utilization. Also, Alexis and Papaparaskeva-Papoutsoglou (1986) fed young grey mullet (*M. capito*) of average initial BW 2.5g, five semi-purified diets containing 12–60% protein. They found that 24% protein was required for maximum growth at 23 oC while, Alexis and Papaparaskeva-Papoutsoglou (1986) found that the dietary protein content of 15% resulted in sufficient growth equal to 26, 37 and 50% dietary crude protein levels for grey mullet of 12.5g initial BW. The optimal protein level of the diet can be lowered if the energy level is increased, due to protein sparing action of energy nutrients with mullet and other fish species Lee and Putnam, (1973) with rainbow trout; Machiels and Henken, (1985) with African catfish; Serrano *et al.*, (1992) with red drum; Shiau and Peng, (1993) with Nile tilapia; Jantrarotai *et al.*, (1998) with hybrid *Clarias* catfish and El-Dahhar, (2000b. c) with striped mullet).

Also El-Dahhar (2007) stated that we can reduce the protein level from 26 % CP to 22% CP with increasing the metabolized energy (ME) level up to (250 kcal/100g diet). When he fed striped mullet at three dietary protein levels (18%, 22% and 26%) and three dietary ME levels (200, 225 and 250 kcal/100g diet), he observed that weight gain, survival and feed efficiency improved as dietary energy increased up to 250 kcal/100g diet. Differences in performance among protein levels in this trial were most pronounced in fish fed on 26% protein diets, which resulted in the fastest growth. In relation to the interaction between dietary ME and protein levels, striped mullet larvae exhibited the greatest survival, weight gain and feed utilization when they were maintained at 22% dietary protein with 250 kcal ME /100g diet than the other protein

levels at all ME levels used. These differences in survival and weight gain can be attributed to the increased feed intake and feed efficiency exhibited at 250 kcal ME /100g diets.

All of this leads us to examine the rearing of flathead grey mullet under bio-floc conditions and determine the optimal level of protein with this system and the effect of different salinities on flathead grey mullet fingerlings under BFT system.