Effluent Water Characterization of Intensive Tilapia Culture Units and its Application in an Integrated Lettuce Aquaponic Production Facility

by

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Abstract

In an intensive aquaculture project, effluent water characterization from three systems were evaluated (biofloc, polygyser and opposing flows). There were no differences in the mineral additions to the water per unit of feed while comparing the polygyser and opposing flow systems, both of which are bead bio-filter based systems. When both were compared to the biofloc system which lacks a bio-filter, the latter provided approximately 50 to 300% more mineral content in the water which varied based on the component discussed. This study suggests that the biofloc system provides a higher mineral content (and hence a higher nutrient value) for integrated applications such as a hydroponic grow bed. Characterizing the mineral content and feed nutrition value of effluent sludge indicates that the polygyser and opposing flow systems would be better than biofloc systems for organic fertilization purposes in an on land application due to a higher mineral value in the sludge since it had a longer time to mineralize. The proximate analysis trials showed that all three effluent sludge sources were a potentially excellent source of feed; they were not different and ranged around 17 to 24% crude protein among other variables tested.

Lettuce trials showed that using settled fish water or unsettled fish water produced the same results and growth parameters. Lettuce grown in a commercial hydroponic solution produced better quality lettuce unless the fish effluent water being exchanged on a daily rate had nitrate, soluble phosphorus, potassium and calcium at 27 mg/L, 21 mg/L, 33 mg/L and 21 mg/L, respectively. It is only at these levels that the quality and growth of the lettuce produced by the fish effluent water was comparable to that of the commercially produced hydroponic lettuce.

Tissue analysis of lettuce grown under three different treatments (fish effluent with solids, fish effluent without solids and commercial hydroponic solution) showed no differences, indicating that mineral uptake in lettuce was limited by the lowest mineral component found in the nutrient solution.

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LITERATURE REVIEW

Aquaculture

Aquaculture is defined as the production of living organisms in water which encompasses both plants and animals in fresh, marine or brackish water (Jasper, 1992). The products from aquaculture are usually targeted for food and fiber, but as the industry has developed products suited for the ornamental, pharmaceutical and medical industries have resulted. Different systems of aquaculture are segregated based on their levels of intensity ranging from extensive systems where human input is minimal, followed by semi-intensive and then intensive systems where man has the upper hand in controlling both water quality and availability of food (Brown, et al., 1986).

Within the aquaculture industry, production units can be divided into open and closed (or water reuse) systems. Open systems take water in from one point and discharge it at another; hence they use the water once. Closed systems involve reusing the water and only adding new water to the system when water is lost. Closed culture units often have a series of filtration and processing components that help maintain water quality (Berghage, et al., 1999). Aquaculture is poised to provide more than one third of the world demand of fish, shellfish and other marine organisms, but concerns over the negative environmental impact of aquaculture have been brewing. The issues range from mangrove destruction in the case of shrimp production to sea bed pollution in case of large scale off shore aquaculture (Wurts, 2000).

One of the key issues of concern is the effluents produced in such systems. Each type of system differs in effluent quality based on production capacity, and effluents are a major concern

because they often are discharged in canals, creeks, rivers or seas. Currently, the industry is developing guidelines and technological innovations to tackle environmental issues by implementing practices to lessen pollution loads and to make use of aquaculture waste in an economical and beneficial way (EPA, 1980).

Tank Culture

Tank culture is one of the intensive ways of producing fish. The quantity of fish per unit volume in tank culture is many times greater than in pond culture. Tank culture systems are efficient because of their lower requirements of land as well as water resources (Rakocy and Mcginty, 1989). The higher productivity level in tank culture is achieved by aerating the water, providing complete feeds, exchanging the water partially or continuously, as well as removing wastes and amending certain water parameters when needed (Schonbeck et al., 1991). A distinction should be made between tank culture systems that discard the water after it goes through the culture units (flow through systems) and those that filter and recycle water (re-circulating systems) (Rakocy, 2002).

Tank culture of tilapia or other species offers several advantages compared to cage or pond culture. Disrupting breeding at high stocking densities allows both sexes to reach marketable size in a shorter period of time due to lack of energy expenditure on reproductive development. The fish stocking density and the environmental parameters in re-circulating units can be managed to a high degree to achieve maximum production levels (Shaw and Cantliffe, 2003). Tank culture has also made it possible for tilapia and other fish species to be grown beyond their normal geographical range by maintaining them in environmentally controlled structures keeping in mind that all tank culture systems are not in greenhouses. The filtration systems that accompany re-circulation systems are complex and expensive and backup systems

for aeration are a must to prevent massive dissolved oxygen depletion and massive fish mortality.

Tank Culture Considerations

Stocking densities in tilapia (*Oreochromis niloticus*) tanks are initially very high. However, as the fish grow, fish numbers are reduced by transferring some to other tanks to reduce crowding and to ensure adequate water quality to support the rest of the fish in the tanks. Stocking densities range from 100 to 1,000 fish / m^3. To assure the popular market size of around 250 grams, a stocking rate of 200 tilapia / m^3 is recommended (Seawright et al., 1998). The most environmentally suitable conditions need to be provided to achieve ideal growth rates, with variables of greatest importance including dissolved oxygen (DO), un-ionized ammonia-nitrogen, nitrite-nitrogen, carbon dioxide, alkalinity, and solid waste. Solid wastes result from using pelleted feed, where the portion of the feed that is not assimilated is excreted as feces producing a biological demand on the system (Losordo et al., 1998). These solids can be settleable, suspended, floatable and dissolved. The nature and the chemical constituents of these wastes are related to the feed formulation as well as to the fish species and the environmental conditions being used.

Of the many components of feed, nitrogen (N) content is of major importance. When N is not completely assimilated by the fish it can cause eutrophication when discharged in large amounts into natural waters. Nitrogen levels in feed are reflected in crude protein (CP) concentration (%N X 6.25 = % Crude Protein). Crude protein concentration for fish feed varies with species. For example, crude protein ranged from 26 to 30 % CP for tilapia (Hargreaves and Tucker, 2004; Pompa and Masser, 1999), 38 to 50% CP for hybrid striped bass (Hobson et al., 1981) and 34 to 38% CP for rainbow trout just to name a few. Usually around 20 to 30% of the

N contained in feed is recovered in fish flesh at harvest, with the remainder potentially entering the culture system as ammonia nitrogen (NH_4) (Graber and Junge, 2008). Ammonia is considered a pollutant and it should be removed with a biological filter. Another important consideration in tank culture is the need to add atmospheric oxygen to the water and release the excess carbon dioxide from the respiratory processes. This can be achieved by many methods such as air diffusers, surface agitators and other instruments that tend to be highly efficient. Pure oxygen injection is an option that is becoming popular in closed systems because it can increase DO concentrations above that which is possible with traditional aeration (Schuenhoff et al., 2002).

Treatment of Aquaculture Effluents

One of the main products of any aquaculture system is the effluent, which tends to be high in nutrient and organic matter concentration. Discharge of nutrient rich effluents into natural water bodies can cause eutrophication or excessive plant growth and especially blooms of phytoplankton and other algae (Boyd and Tucker, 1998). Eutrophication can lead to a drop in DO levels at night and very high levels of DO during the day. When nutrients become limited, massive die offs of phytoplankton may occur. Another concern is potential development of some blue-green algae species which are toxic and can have an adverse effect on the ecosystem in which aquaculture effluents are discharged. Many countries have established water quality regulations to protect against eutrophication. These regulations often are based on the effluent limitations into certain bodies of water relating to their use. The procedure followed in the United States is called the Clean Water Act (CWA). The CWA requires all rivers and tributaries in each state to be assigned a use classification, and each use category has specific water quality

standards. Streams cannot be downgraded to a lower class with the intention of the CWA to cause degraded streams to improve in water quality over time (Boyd, 2000).

Several basic water treatment methods exist: mechanical, gravitational, chemical and biological (Timmons et al., 2002). Mechanical filters are used for the separation of liquids and solids (removing particulate matter from water). Common types of mechanical filters are stationary screens, rotary screens, and pressurized or non-pressurized sand filters. Gravitational treatment uses the force of gravity to extract particles from a fluid, and sedimentation is the main method by which this is accomplished. In sedimentation, water is held under quiescent conditions in a tank or basin long enough for particulate matter having greater density than that of the liquid to settle down. Sometimes, coagulants are used to flocculate particles and increase the settling velocity (Timmons et al., 2006). Chemical filtration (CF) of water is accomplished in adsorption units which rely on the accumulation of substances at a surface or liquid-solid interface. The CF process is driven by the lyophobic forces between solutes and the affinity forces produced by the adsorbing phase. Carbon filters are one of the most common forms of chemical filters, along with foam fractionation and ion exchange filtration (Timmons and Losordo, 1994).

Biological filtration (BF) is one of the most important methods by which water treatment takes place in many aquaculture units. The BF process is defined as the bacteriological conversion of organic nitrogenous compounds into nitrate. The steps of BF processes are the same as those found in the familiar nitrogen cycle. The primary process of BF is to change ammonia to nitrite and nitrite to nitrate (Timmons and Losordo, 1994). These processes tend to come in succession as soon as the substrate of one reaction becomes available and hence a lag between them tends to occur. To provide a medium on which BF reactions occur, a substrate is

provided in a properly sized filter to accommodate all the ammonia produced in a system. The size of the filter relates to the amount of feed being used as well as the N content of the feed which is proportional to the crude protein (CP). The most common forms of bio-filters are trickle filters and rotating biological contactors (RBC). The plastic media used in RBC technology have specific surface areas of up to 200 m^2 / m^3 and can achieve a nitrification rate of approximately 76 g total ammonia nitrogen (TAN) / m^3 (Wheaton et al., 1994).

Trickle filters which are configured as a non flooded system tend to have large air spaces and can achieve a nitrification rate of approximately 90 g TAN / m^3 (Malone et al., 1993). A subset of BF uses plant filters to remove inorganic nutrients such as nitrates, nitrites, ammonia and phosphates. Plants used can range from small algae, which is a process heavily employed in the sewage disposal systems to larger aquatic plants. Some of these plants have potential as animal feed and can be easily harvested and separated from the water. A very important consideration in the design of such living filters is the nutrient uptake rate of plants which determines the necessary filter retention time as well as other important design parameters (Rennert, 1994). In a study by Hand et al., (1973) working with *Lemna minor* (duckweed), there was a linear relationship between the total Kjeldahl N and retention time in days in the system they used, where after 2 days about 21% of the N was removed, while at 10 days about 86% of the N was gone. Duckweed application also reduced the amount of phosphorus by around 11% in 2 days. Hand et al., (1974) showed that it is possible to remove up 95% of the effluent N using a semi-continuous marine algae culture system which was salt water fed.

The Case for Sustainable Aquaculture

With the ever increasing demand for seafood, fish and other water based products and limited ability to increase the capture of fisheries products from natural waters; aquaculture has

become extremely important in world food supply. Aquaculture is projected to grow by another 50 million metric tons in the next twenty five years which suggests that many issues regarding the sustainability of this practice are to be exposed and many challenges need to be addressed (Aubin, 2006). For a food system to be sustainable its use by the current generations must not diminish the food resources for future generations (Bardach, 2008). Sustainability has a social, environmental and economic aspect which should all be aligned to seek the best benefit out of the system. Fish and all other aquatic products provide around 19% of all protein consumed and contribute directly to food security as nutritious protein for human consumption worldwide (Bardach, 2008). Aquatic products have been increasingly used as a source of food supplies around the world, but with the rise of the "eco-friendly" and the "green revolution" in the last decade, concerns about how sustainable aquaculture really is has become an issue. One of the major issues stated by Bloom (1995) is that fish population densities used in aquaculture is a major factor in producing pollution. Intensive aquaculture culture systems can produce up to 50 tons of biomass or more per hectare, and hence water exchange and excessive effluent discharge have become a major issue in making aquaculture a questionable industry from an environmental standpoint. Consumers increasingly demand a more responsible form of seafood production, which has lead to the introduction of sustainable certification programs and eco-friendly labeling. The eco-friendly movement has lead to many innovative solutions to decrease the negative effects of the industry, such as the development of alternative protein sources for aquaculture diets to replace the massive amounts of fish meal used and these now include soybeans and other plants' meals (Naylor et al., 2000).

Another innovation is low-pollution diets where by manipulating the diets and feeding strategies in a production facility, the efficiency of the system is increased and waste outputs are

reduced. Chemical advances such as introducing somatotropin in feed has been shown to improve feed conversion efficiency, reduce ammonia excretion and improve N retention in the fish (Pedini, 2000). In this same line of developing solutions to make the industry more sustainable, many have suggested that nutrient recycling, which is the conversion of N back to protein or other harvestable products, can be a practical and efficient manner to control and treat effluent associated with aquaculture. Integration of aquaculture with other animal and plant based systems has become a viable option to make use of the extra nutrients available from a production facility to reduce nutrient discharge. Ultimately, turning aquaculture into a sustainable industry will enable an ability to adapt to the increasing product demands leading to an advantage over traditional production practices (Wurts, 2000).

Horticulture and Hydroponics

Horticulture is the industry of producing plants and plant cultivation products ranging from the processes of collecting seeds, preparing land or artificial soils, sowing seeds, tending to plants during growth, and finally harvesting, post harvest processing and distribution (Jensen, 1997). The production of horticulture products includes many methods such as field production, greenhouse production, outdoor container-plant production and other systems. Olericulture is the sub-study of horticulture involved in production of vegetables for mainly human consumption. According to the economic research survey of the United States department of agriculture (ERS/USDA), the value of the nursery and greenhouse products industry in Alabama was $291 million in 2007.

Hydroponics is a specialized method for horticulture production involving the growth of plants without soil. Hydroponics has developed over time by determining what substances work best as substrates along with which nutrient solution work best (Resh, 2004). In many ways,

hydroponics is a science in its infancy, used in commercial production only in the last 50 years. Hydroponics is becoming increasingly popular as demand on traditional soil-based horticulture increases. Clear advantages of soil-less hydroponic culture systems include ability to sterilize the root-zone substrates with chemicals, solarization, or steam; to completely control nutrient formulations of the hydroponic solution; spacing of plants limited only by light availability and not nutrient availability; weeds and soil-borne diseases are eliminated; and water stress does not occur (Alleman, 1985). Studies done by Wolverton (1987) showed that on a per acre equivalent basis wheat production was 143% more in hydroponic production as when compared to normal soil culture, while potatoes, cabbage, tomatoes and lettuce where 87%, 138%, 54% and 233%, respectively, more in hydroponic production than from normal field conditions. Under greenhouse conditions where the only difference was soil versus soil-less culture, the increased production of tomatoes grown hydroponically is usually 20 to 25 percent (Resh, 2004). However when compared to conventional outdoor culture where soils cannot be sterilized or adequately fertilized we observe a yield increase in hydroponics by 4-10 times over conventional soil-grown conditions.

Integrated Aquaculture Systems

Historically, family farms were integrated. The family produced food, fiber, fuel and other essentials on the same plot of land. Waste water from one activity was an input for another, e.g, animal manure was used as fertilization for plant crops. Integrated systems allowed farmers to make higher use of diversified resources and decreased depletion of certain system components such as fertilizer. In recent history, farmers shifted to more monoculture type production systems, where feed lots, up to hundreds of acres in size, are dedicated to one type of animal, and where many acres are planted with one crop over and over again. Although these

systems are more efficient in the short run relative to the revenue generated, they present a high risk long-term issue (Edwards, 1993). Hence, considering integrated systems with the aquaculture industry might provide valuable resources to reduce production costs, enhance productivity and help eliminate some of the negative consequences such as water pollution via discharge of effluents.

Integration of aquaculture with other industries has been attempted in many situations, and this type of integration can be defined on the basis of diversifying an agriculture production system towards linking subsystems to each other, leading to a synergy where ‘‘an output from one sub-system in an integrated farming system which may otherwise have been wasted becomes an input to another sub-system resulting in a greater efficiency of output of desired products from the land/water area under the farmer’s control’’ (Edwards, 1993). Linking aquaculture and other human activities not only revolves around agriculture, e.g., stocking fish in irrigation dams or canals, it also includes the roles of sewage treatment and nutrient recovery such as in hydroponic-fish systems. Waste heat energy from power plants and other sources can be used in fish culture systems to increase sub-optimal water temperature (Lightfoot et al., 1993; Lightfoot and Pullin, 1994, 1995; Pullin and Prein, 1995; Pullin, 1998).

Aquaculture can be linked to an agriculture system in at least two basic ways. A pond can be fertilized with farm lagoon water such as animal manures with wastes converted to fish flesh. The other way is to use the inorganic nutrients in effluents from aquaculture grow-out units to fertilize crop plants. Aquaculture is actively and physically integrated into another running enterprise through modifying the design and the operation of the first (Castro el al., 2006). The most common entry point for integrated systems for small stakeholders is through the use of on-farm nutrient sources to fertilize fish ponds since inorganic commercial fertilizers and feeds tend

to be expensive. These fertilization sources include plant waste, grasses and manure allowing natural fish food to develop in the form of phytoplankton, zooplankton and other benthic organisms (Colman and Edwards, 1987; Kwei Lin et al., 1997; Knud-Hansen, 1998).

Examples of animal-fish systems include livestock-fish systems where slaughter house waste products such as skin, inner organs and scraps of meat are used as feed for carnivorous fish species. The most successful of these systems are integrated with a large feedlot that provides large amounts of manure ensuring high fish production rates (Pullin and Shehadeh, 1980; Little and Muir, 1987). Another source of nutrients from an integrated livestock-fish system is urine from feed lots. Urine is nitrogen rich and provides enough nutrients in the water to trigger primary productivity; the problem usually is the inability to maintain a regular supply (Knud-Hansen, 1998). Another example of integration of fish and animal industries is the fish-poultry industry. Poultry manure and poultry litter are both of relatively high nutrient content and often available in large quantity. Sometimes, poultry houses in Thailand, Indonesia and the Philippines are constructed above ponds and the wastes fall directly into the water. Poultry litter or manure that is stored and transported to ponds later on tends to have lower N content due to the loss through volatilization, drying, and storage hence decreasing fertilization efficiency (Palada et al., 1999).

Plant Integration and Aquaponics

One of the most appealing forms of integrated aquaculture systems is integrated plant-fish systems which tend to be the most convenient for application due to the plant's requirement of nutrient rich water in the form of fertigation to allow for proper growth. The major waste of the aquaculture grow out unit, nutrient rich effluent, allows excellent synergy between the two systems when in balance with plant fertilization needs. Aquaponics is the combination of re-

circulating aquaculture and hydroponics, where the nutrient rich water that results from raising fish provides a source of nutrients for growing plants (Neori et al., 2004). Aquaponic systems tend to be governed and controlled by a series of components such as bacteria required to convert nutrients from their produced form to one usable by plants and a set of sedimentation tanks and solid removal systems to make the water suitable. In turn, this closed-loop system increases water quality for fish production by removing otherwise harmful nutrients through assimilation into plant biomass (Rakocy, 2002).

Aquaponics is an ideal solution for fish farmers who want to treat nutrient rich water and diversify their farm by utilizing fish waste for plant production. In some cases integration of aquaculture and plant production may be sought after through environmental stewardship or through necessity due to water conservation concerns. Advantages of integration include but are not limited to: eliminating the cost and expertise involved in mixing traditional hydroponic nutrients, a source of inexpensive 'organic' or 'natural' fertilizer source, and integration reduces water consumption when compared to conventional aquaculture and plant production practices. Many conventional pesticides cannot be used in aquaponic or integrated systems due to toxicity concerns in regards to fish production. These toxicity concerns have lead to the use of organic certified pesticides, beneficial insects and in some cases insect barriers. The reduction in pesticide use coupled with the natural fertilizer source has potential to increase the value of plant products through organic certification and labeling. Another advantage to integrated systems is the use of carbon dioxide naturally vented from respiration of the fish tanks. There are some challenges and limitations of aquaponics systems such as: compromising the best pH to fit the fish and the plants, having a reliable power source for the oxygen demands of the system, large initial capital investment, and an avenue for marketing and shipping products.

In integrated fish-plant systems, plants are considered to be ideal secondary crops because they have the ability to grow very rapidly in response to high levels of dissolved nutrients that are generated by decomposition and breakdown of uneaten feed and feces (Rakocy et al., 1992). This is especially relevant since nutrient levels in a re-circulating system tend to concentrate and can become far greater than the concentrations found in the commercial hydroponic nutrient solutions. Work by Rakocy et al., (1992) showed that the rate of salt buildup in re-circulating aquaponics systems may be a problem when total dissolved solid concentrations start exceeding 2,100 mg / L. Work done by Losordo (1998) showed an optimum ratio can be achieved when plant production is maximized and salt build up is low, hence the optimum number of leaf lettuce plants (summer Bibb) to fish (tilapia) was seen to be 1.9:1 when using a water exchange rate of 1 percent per day and feeding a 36% protein diet, yielding up to 2.4 grams of feed per plant in the cultivation bed.

To reap the benefits of an integrated system, several trials have been conducted to show what this new synergy can provide. In Alberta, Canada an integrated tilapia crop system was developed by Savidov et al., (2007) to evaluate the commercial feasibility of an integrated system in the temperate climate of the northern region of North America. In their system, yields of tomatoes and mini-cucumbers reached 20.7 kg / plant / year and 33.4 kg / plant / year, respectively, which exceeded the average yields produced in commercial hydroponic systems being employed in the same geographic area that achieved 16.8 kg / plant / year and 28.1 kg / plant / year of tomatoes and mini-cucumbers respectively. In the above mentioned system, tilapia was grown out at 24.8^{o}C on a 24-week cycle. The feed conversion ratio (FCR) was 1.3 and the fish were raised to 400 gram size. In the same experiment, early in the cycle the low feed input to

the fish tanks resulted in low nutrient concentrations in the water (Savidov et al., 2007). Hence, nitrogen, phosphorus, potassium and iron deficiencies were commonly seen in the crops.

Another set of trials (Takeda et al., 1993), studied the growth rates of strawberries grown in aquaculture effluents with respect to the flow rate and established that a specific flow rate was required, in this case, 100 ml/min, to maintain plant growth and yield when compared to regular hydroponic production systems. Trails done by Takeda et al. (1993) were based on an integrated trout production system where 340 gram trout were stocked at a density that produced a constant total nitrate-nitrogen rate of 11 ppm, total phosphorus rate of 0.3 ppm and a total potassium rate of 4.7 ppm. They revaluated the growth of the strawberries at flow rates of 33, 67 and 100 ml / min, and found that 100 ml / min was required to achieve the best crop.

In another experiment at the University of the Virgin Islands, an integrated aquaponic system producing both tilapia and basil was assessed to determine if aquaponics was better from a batch or staggered system approach (Rakocy et al., 2004). These researchers used a system capable of producing 4.37 metric tons of tilapia per year in either a batch or staggered method. When connected to a 500 m^2 hydroponic bed production site, the yield of basil was 2.0 and 1.8 kg / m^2 using batch and staggered production of tilapia respectively, compared to 0.6 kg / m^2 in regular field cropping. Signs of nutrient deficiencies where much more common in the batch system than that of the staggered one due to the different levels of feeding provided in each adopted production scheme (staggered or batch). When fish were fed to satiation in the batch system, it provided 81.4 g / day / m^2 of basil production while it provided 99.6 g / day / m^2 for the staggered production (Watten and Busch, 1984).

Nutrient Release, Evaluation and Accumulation

The amount of nutrients released in the effluent water from an aquaculture system is of central importance in identifying the best use for this nutrient enriched water. The main usable components of fish effluent are calcium, magnesium, potassium, ammonia, nitrate, phosphate, sulfate, iron, manganese, zinc, copper, boron, and molybdenum (Al-Hafedh et al., 2008). Identifying concentrations of these nutrients present in effluent water as a function of the feed input, fish stocking density and fish species becomes a very useful tool in assessing the productivity of the water output of any aquaculture system. Through identifying what comes out of an aquaculture system and by knowing what the system integrated to the aquaculture unit requires, a good estimate about total productivity of the whole system can be quantified.

The extent of the environmental impact of an aquaculture system is dependent on the feeding techniques, the feed composition, and the water body receiving the effluent (Ackefors and Enell, 1994). The most critical of these matters is the feed composition and how it relates to its digestibility and feed conversion by fish because that will determine the level of the discharge (Aubin, 2006). When compared to many other industries, aquaculture has a low impact on the environment. Nevertheless, the industry should strive to reduce its potential for causing eutrophication by decreasing the amounts of phosphorous (P) and N in the feed which now stands at around 1% and 7% respectively. The industry has worked on enhancing feed conversion ratio (FCR) values to levels where the amount of feed used is more efficient.

Aquaponic effluents contain many plant nutrients, but the levels of N and P in the effluent are of greatest concern, due to their high economic value. Nitrogen excretion is the result of catabolism of amino acids resulting in the production of ammonium, carbon dioxide, bicarbonate, and a small amount of urea. Fish feeds usually contain 0.5 to 0.8% phosphorus

(Bergheim and Sivertsen, 1981). Phosphorus discharged in aquaculture effluents is often a limiting nutrient in natural water bodies, and can cause eutrophication (excessive productivity of phytoplankton blooms) in receiving waters.

When assessing the nutrient output and budgets of fish tanks in which tilapia are stocked, stocking densities must be considered. A study done in Saudi Arabia with 12 indoor fiberglass tanks stocked with hybrid tilapia (*Oreochromis niloticus* x *O. aureus*), different stocking densities were reared for four weeks, during which N and P budgets were estimated (Siddiqui and Al-Harbi, 1999). Three fish densities were used: 1kg, 5kg and 15 kg / m, with tilapia fed to satiation twice daily with a 34% protein feed. Results showed that to produce one kilogram of fish mass, 87.1 to 95.6g N and 12.6 to 13.8g P were released into the water as waste. Only 21.4% of the N and 18.8% of the P provided to the system in feed was incorporated into the fish flesh (Siddiqui and Al-Harbi, 1999). Another trial done by Dontke and Clanton (1999), showed that each 1kg of tilapia fish flesh produced using a 32% protein feed released 64.5 to 78.2g of N and 7.5 to 14.4g of P.

In an aquaculture system, the nutrient load increases in proportion to the feeding rate. Doughty and McPhail, (1995) showed that 1kg (dry weight) of 36% protein feed used in a system stocked at 22 kg tilapia/m^3 released 26.7 g of nitrate nitrogen, 1.7 g of phosphate phosphorus, 2.6 g of sulfate sulfur, 57.8 g of potassium, 14.2 g of calcium and 2.0 g of magnesium. In the same system, when the aquaculture effluent was re-circulated through a 10 m^2 bed of leafy green soil-less production, the nutrient accumulation was reduced to 22.0 g of nitrate nitrogen, 0.9 g of phosphate phosphorus, 2.1 g of sulfate sulfur, 55.4 g of potassium, 12.3 g of calcium and 1.8 g of magnesium (Doughty and McPhail, 1995). It is worth mentioning here that the accumulation of nutrients is one of the critical management considerations when using fish

culture effluents as a fertilizer for vegetable or other crop plants. A proper balance between the secondary vegetative system and the primary aquaculture unit needs to be reached to ensure the efficiency of both.

A preliminary evaluation of organic waste produced by an aquaculture system to act as a source of inorganic nutrients for hydroponics was attempted by Rakocy et al. (2007). Both an aquaponic and green water tank culture system were continuously aerated to facilitate mineralization of fish effluent material and then used to grow crops and compared with a commercial hydroponic fertilizer. As mineralization of the organic matter occurred the concentrations of the electrical conductivity (EC), total dissolved solids (TDS), Sodium (Na), chloride (Cl), magnesium (Mg), potassium (K), nitrate nitrogen (NO_3-N), sulfate sulfur (SO_4-S), phosphate phosphorus (PO_4-P) and molybdenum (Mo) gradually increased while concentrations of NH_4-N and iron (Fe) decreased and those of manganese (Mn), zinc (Zn) and copper (Cu) fluctuated. The degree of mineralization ranged between 20 to 80% for Mg, Mn, Cu, B and Mo. The NO_3-N concentration increased from 2.3 to 313.0 mg / L while the Fe concentration decreased by 98% due to the uptake of bacteria and other micro organisms. The study concluded that while the commercially developed hydroponic fertilizer by Resh (2004) is ideal for production, the nutrients extracted from aquaculture effluent would reduce the synthetic nutrient requirement for plant fertilization. Although the nutrients from the fish system effluents are significantly lower than commercial grade nutrient solutions, supplementation with additional nutrients to achieve better results and lower overall production costs is possible (Rakocy et al., 2007).

Nutrient Budget and Dynamics in Fish Effluent-Plant Systems

Nutrients interactions with each other in aquaponics are dynamic with changing factors of feed input, fish species, water exchange rates and others. Thus, nutrient dynamics affect nutrient budgets and production of fish and plants. Many farmers tend to flush out commercial hydroponics systems completely on a weekly basis and replace it with a new nutrient solution, but this practice may not be cost effective. In addition, many tend to measure levels of individual ions and nutrients and amend the nutrient solution accordingly, a practice that may not be needed (Nash and Brown, 1980). In an integrated aquaponics system, monitoring the nutrient levels might seem to be the most plausible method to determine nutrient requirements needed to reach optimal production. However, this might not be the best option, because plants quickly remove some nutrients while other minerals, elements and compounds accumulate in the water. Farmers usually use the electrical conductivity (EC) value of the water as a measure of the nutrient strength of the solution.

Plant nutrients are categorized into three main groups, the first being the ones such as N, P, K and Mn that are actively absorbed by the roots and hence their levels decline from a solution in a short time. The second group of elements are more slowly removed such as Zn and Mo. The third group are such as Na elements taken in passively and tend to accumulate in the system (Wolverton, 1987). Hence, in an integrated system where fish effluent is providing a combination of nutrients, some nutrients tend to be taken up more readily than others, creating an imbalance in the solution. Elemental levels that are provided by the fish water do not decrease proportionally with time and some tend to accumulate to toxic levels while others become deficient. Bugbee (2004) showed that analysis of hydroponic or aquaponic water media is unnecessary, often inaccurate and often times hard to interpret. Bugbees's work suggests that

plant tissue analysis is a more effective method to determine the deficiencies in the plant nutrient solution.

A study conducted at Texas A&M University monitored changes in the concentration of dissolved nutrients over time at different Nile tilapia *(Oreochromis niloticus)* stocking densities for comparison with a commercial hydroponic solution in which romaine lettuce *(Lactuca sativa)* was grown. Nutrient concentrations and proportionality ratios between the nutrients quickly changed because nutrient uptake from fish effluent by the plants was not proportional (Seawright et al., 1998). The study suggested a disparity between what is being provided in the system by the fish and what is being used by the plants. Moreover, the rates and ratios of nutrient generation from fish culture and for plant uptake change over time and also change with plant species. This experiment observed that concentrations of K, Mg, Mn, P, Na and Zn accumulated at a faster rate as the amount of fish to plants increased. It also was shown that manipulating dietary content may theoretically affect nutrient release into fish effluent. Nitrogen and Ca levels could be stabilized at required concentrations without amendment if feed contained 41.6% crude protein (CP) and de-nitrification was controlled in the system (Clarkson and Lane, 1991). Nair et al, (1985) also found that nutrients do not accumulate at equal rates in an integrated fish-plant system if standard diets are being used and direct deficiencies of P, SO_4, K and Fe are observed.

One of the most important nutrients in plant production is N, and in most cases, integrated systems are being developed based primarily on the potential value of transforming N in effluent to vegetable biomass and protein. Nitrogen transfer from aquaculture to agriculture in integrated systems has been difficult to quantify, and understanding N utilization by crops has been poorly documented. Much work has been done in effort to understand N in integrated systems with varying results. Azevedo, et al. (1999) used labeled nitrogen ^{15}N to determine N

transfer from a fish system and its utilization by plants in an integrated system. Different treatments were amended at rates of 0%, 25%, 50% and 100% N. The results showed that the system efficiently recovered the N from the fish feed, but indicated that the effluent alone does not supply sufficient N for lettuce production. The trials showed that 39.1% of the inorganic N was recovered from fish effluent when it was supplemented in a 1:1 ratio with N nutrient stock. The lowest N recovery of 15.7% was for fish effluence without N amendment. The reason for this is that plants can only utilize inorganic N and much of the nitrogen in straight fish culture effluent is in organic form. In the trials, only 22.3% of the total N was in usable inorganic form. Although the inorganic N was almost completely removed from the effluent, it was not enough to provide a large yield of plant biomass.

In an attempt to model N transformation in intensively aerated fish ponds used in integrated aquaculture-hydroponics, Kochba et al. (1994) found that inputs of N included feed and influent water, and outputs were uptake by fish, removal of sediment and discharge in effluent. In this trial, internal transformations between inorganic and organic N forms was computed. Ammonia is mineralized through excretion by the fish or microbial degradation of organic N. The reverse process of immobilization of N takes place when algae or other microorganisms absorb ammonia and use it in protein synthesis. While developing this model, a long list of factors were taken into consideration to include pond area, water volume, feed added, N content of feed, mineralization rates, drainage levels, water inputs and outputs as well as C/N ratio in algae and their levels. After going through this process they suggested that a quantitative model to predict both the organic and inorganic N levels in the water is needed and that many factors tend to affect it. The common conclusion was reached that the water exchange rate was the largest and most decisive factor that affected the N levels in the effluent. The results were

further confirmed (Boesch et al., 2001) when adjusting between different exchange rates in the water tanks of their re-circulating systems between 10% and 25%, a difference in N output varied between 17 to 24%. Hence both researchers (Kochba et al., 1994; Boesch et al., 2001) suggested that determining the proper water exchange rate at the needed biomass and feeding rate is essential to maintain a proper N output in the effluent discharge to best fit the plant culture as well as stay suitable for the fish system.

Gross et al. (2000) assessed the N balance of a channel catfish pond at Auburn University. The input of feed (4.85% N) accounted for 87.9% of the N found in the pond. There were four main N loss pathways that included: fish harvest, 31.5%; de-nitrification, 17.4%; NH_3 volatilization, 12.5%; and bottom accumulation, 22.6%. It was suggested that feed is the largest N input in the ponds. If growers adopt improved feeding practices such as more frequent feedings per day and use good quality feed the N recovered in fish will be increased and the amount of ammonia released in the effluent will be reduced. It is worth mentioning in integrated aquaculture-hydroponic systems, only the inorganic N can be used directly by plants. Aeration and water circulation enhanced nitrification and oxidation of organic N into usable inorganic N.

Effluent Nutrient Recycling and Uptake Via Horticulture Crops

To be able to see the potential application of integrated systems between the aquaculture and horticulture industries the efficiency of nutrient recycling in relation to the levels of nutrients being released into the water body must be assessed. Studies have been conducted with various types of fish species and crop species as well as under different management conditions ranging from varying stocking densities to varying feed to plant ratios. Graber and Junge (2008) in Zurich worked on measuring nutrient recovery from fish effluent after they planted three different crops: eggplant, tomatoes and cucumbers. The study showed that over a period of 105

days, the tomato crop was the most efficient nutrient removing plant with nutrient uptake rates similar to those of tomatoes grown in commercial hydroponic systems. The tomatoes removed 0.52, 0.11 and 0.8 g / m^2 / day of N, P and K for the aquaponic system as compared to removal of 0.43, 0.07 and 0.4 g / m^2 / day of N, P and K from the commercial hydroponic solution. The authors concluded that nutrient recycling aquaponics could be used by rural communities and many wastewater treatment facilities to provide income. Another similar experiment showed that under the same conditions for both cultures, only the water sources differed, nutrient recycling by aquaponics ranged from 9 to 69% for N and 5 to 27% for P. In hydroponics systems these values were 25 to 36% for N and 9 to 48% for P (Colman and Edwards, 1987). Both these studies showed that although the water from the aquaponic systems contained approximately three times less N and around ten times less P, tomatoes reached almost identical yields in both systems. Potassium was one of the most important nutrients in the fish effluent, but it was approximately 45 times less than what is usually provided in synthetic formulation. Although low K concentrations did not affect tomato yield, it affected the tomato quality greatly as reflected in 185% more dry matter yield in hydroponic production than in aquaponic production (Graber and Junge, 2008).

In parts of the world where water resources are scarce, integrated aquaculture-hydroponic systems become a viable and attractive option that recycles nutrients, makes double use of water, and produces two or more products. This comes at the heart of water conservation and responsible agricultural practices. A study was conducted in Saudi Arabia where an indoor aquaponic system was operated to maximize water reuse and allow for year round intensive food production. After a period of 13 months, 268 kg of Nile tilapia was harvested per cubic meter and total water consumption was 320 liters per kg of fish produced. Lettuce production was 42

heads of lettuce per square meter of the integrated growing beds (Al-Hafedh et al., 2008). In the same study 1.4% of the total water was added daily to compensate for evaporation and transpiration losses. Normal industry water consumption levels for Nile tilapia production in ponds is 667 liters of water per kg of fish produced (Boyd, 2004).

The results of many trials integrating water effluent treatments using lettuce and other horticulture product production reached the conclusion that K was always a limiting factor which is highly deficient in fish water and in some cases P was low as well (Clarkson and Lane, 1991). Water conservation is a major benefit of integrated systems in arid and semi-arid regions. Sustainability advocates see integrated systems as an ideal track to save water, dispose of aquaculture effluent and provide "free" fertilizer. In a trial conducted by Brazil's Universidade Federal Rural do Semi-Árido (UFERSA) Institute that studies rural development in semi arid regions, in an experiment where plots were fertilized with cow, chicken and commercial compost manures and then amended with either fresh well water or fish effluent water, the treatments using fish effluent showed higher fruit number and increased mean weight of the products (Castro et al., 2006). Treatments amended with fish water increased fruit numbers from 7 to 32% and fish water provided certain limiting factors that were not sufficiently provided by the organic manure sources. Possible factors were higher K and P, as well as higher ammonia, nitrite and nitrate nitrogen levels when compared to well water (Castro et al., 2006).

The studies discussed in this section generally conclude that fish effluent can be used to irrigate horticulture crops in both hydroponic and field systems to provide better yields and conserve water. Fish effluent can compliment, and in some cases, substitute for organic fertilizers in horticulture production while achieving similar results (Chaves et al., 1999 a).

One of the major issues in the field of integrated fish-plant systems is the resulting sludge and the solids in the system. The main issue is whether or not the sludge or remaining solids should be removed before waste water usage in these integrated systems. In the majority of hydroponic integrated systems, solid removal comes as an integral part of the design, to prevent these solids with a high oxygen demand from binding to the roots and suffocating the plants (Rakocy, 2002). These matters will be discussed further later, because when discussing integration of aquaculture with normal soil based horticulture production, sludge removal is not a big consideration. Palada et al. (1999) at the University of the Virgin Islands studied yields of bell pepper grown in concentrated sludge from settling tanks (FS), filtered fish water (FW), non filtered fish water (NW), granular inorganic fertilizer (IF) and dry composted cow manure (CM). The most significant indicator was the total marketable yields which for FS, FW, NW, IF and CM were 6.62, 2.82, 5.8, 8.98 and 4.68 tons/ha, respectively. The results clearly show that fish effluent in integrated bell pepper production is not as good as inorganic commercial fertilizers which were attributed to lower levels of K, N and P in fish effluent. But, when the fish effluent is concentrated by collecting the heavy sludge and applying it to fields, the yield produced reached 66.3% of the commercial level production, a significant gain considering that no direct cost is attributed to this fertilization application.

A similar study of the uptake rates of different N forms by plants suggested much of the N in fish effluent is in organic nitrogen form and unavailable to plants (Moritsugu et al., 1995). Crops tend to take up N in some forms at faster rates than other forms. Crops like rice, barley and lettuce take up ammonia N at faster rates than nitrate N, while another group of crops including Chinese cabbage, spinach and radish take up nitrate N at considerably higher rates than ammonia (Moritsugu et al., 1995). This might explain wide variations of treatment results that

were obtained by Palada et al. (1999). There might have been different nitrification rates in the tanks causing different proportions of ammonia to nitrate nitrogen. Differences in ratios of ammonia to nitrate N could have caused the observed yield difference even though the total N concentration were similar.

In conclusion, integrated plant production systems can be an effective way of treating fish effluent, but the quantity and quality of the crops produced are not always up to commercial levels. This apparently results from low concentrations of N, P, and especially K in comparison to plant requirements. Nevertheless, use on crops may be a very efficient way to remove nutrients after which this effluent can be recycled in the system or applied on field crop production. Crops can have an efficiency comparable to constructed wetlands used to treat municipal wastes. Aquaponics systems have been shown to remove 250 to 630 g of N / m^2 and 45 to 75 g of P / m^2 of which at 144 to 423 g of N / m^2 and 33 to 44 g of P / m^2 are recovered in harvestable plant products (Graber and Junge, 2008).

Nutrient Requirements For Hydroponically Grown Crops

Hydroponic crop production ranges from simple herbs to vegetables and fruits with the concentration of nutrients required in hydroponic solution varying with plant species. Generally plant matter contains 80-95% water; the other 5 to 20% dry matter composed mainly of carbon, hydrogen, oxygen and other elements (Resh, 2004). Most researchers state that plants need thirteen nutrients in large (macro) or small (micro) amounts as follows: macronutrients - N, K, Ca, Mg, P and S; micronutrients - Cl, B, Fe, Mn, Zn, Cu and Mo. A hydroponic system must provide a nutrient solution that makes available these nutrients in a balanced form to prevent deficiency disorders. The actual recipe of nutrients to be provided tends to vary based on the crop, the climate and the season. Temperature is very important, and optimum temperatures tend

to induce higher nutrient demands (Resh, 2004). The most well known nutrient formulations were developed by Hoagland (1919) and have been adjusted and modified over time. Nutrient solutions typically have concentrations in the following ranges: Ca (150-250 ppm); Mg (50-100 ppm); Na (0-20 ppm); K (120-400 ppm); $N\text{-}NO_3$ (100-200 ppm); $N\text{-}NH_4$ (0-50 ppm); $P\text{-}PO_4$ (50-300 ppm); $S\text{-}SO_4$ (30-200 ppm); Cl (0-30 ppm); Fe (0-5 ppm); Mn (0.1-1.5 ppm); Cu 0.01-0.1 ppm); Zn (0.01-0.6 ppm); B 0.1-0.6 ppm); Mo (0.001-2.5 ppm) (Resh, 1989). These nutrient levels are also adjusted within the growing season of the same crop to suit the growth stage, and environmental conditions such as light availability, temperature and humidity.

In a study conducted by NASA's biomedical station in the Kennedy Space Center to evaluate the possibility of hydroponic crop production during long trips in space, Wheeler and Sager (1995) studied the nutrient, acid and water budgets of some hydroponically grown crops that included wheat, potato, lettuce and soybean. A nutrient solution containing 70 ppm N, 10 ppm P, 56 ppm K, 12 ppm Ca, 10 ppm Mg, 10 ppm S, 0.1 ppm Fe, 0.1 ppm Mn, 0.01 ppm Zn, 0.01 Cu, 0.1 B and 0.001 Mn was used (Wheeler and Sager, 1999). However, depending upon species and conditions of the growing season, the quantity of nutrients removed from the nutrient solution differed greatly (Wheeler and Sager, 1999; and Adler et al., 1996). This suggests that a particular nutrient solution may be more suitable for one crop than another.

Lettuce Nutrient Requirements and Specifics of Nitrogen Demand

One of the most successful crops in hydroponic production has been lettuce. With a short growth cycle, low nutrient requirement and fast growth, lettuce is one of several very attractive crops for farmers employing expensive technology and seeking fast economical returns. The most commonly produced variety of lettuce is *Lactuca sativa L*. which is produced in most temperate and tropical climates. This plant plays a major role in providing vitamins, minerals and

substantial fiber into the human diet (Ryder, 1998). The typical macro nutrient composition of lettuce as a percentage of dry weight follows: 3.4 to 4.5% N, 0.4 to 0.6% P, 4.5 to 6% K, 1 to 1.7% Ca, 0.14 to 0.27% Mg, and 0.25 to 0.5% S with micro nutrient composition in part per million at 100 to 200 Fe, 40 to 150 Mn, 25 to 40 Zn, 5 to 15 Cu, 30 to 50 B, and 0.1 to 1 Mo (Morgan, 2007). Lettuce hydroponic nutrient formulations tend to vary slightly from other crops in their ratio of nitrogen (N) to potassium (K) because vegetative plants (such as lettuce) require more nitrogen than fruiting plants (such as tomatoes). Fruiting plants will require higher K rates for enhanced fruit growth and quality (Morgan, 2007). Lettuce needs less N during shorter days than during longer, brighter and warmer days (Copper, 1979).

While nutrient solutions tend to have similar compositions, it is essential to tailor their recipes to the water source so that added elements do not cause toxicities (Izzeldin et al., 1980). Usually, other considerations for a hydroponic system tend to vary based on the system being employed where factors involving flow rates and dissolved oxygen become an important consideration. Nutrient pH range should ideally be set at 5.5 to 6.1 with an electric conductivity (EC) of 1.2 to 2.2 mS / cm (Resh, 2004). A study by Hand et al., (1985) indicated that EC levels were higher than 1.2 to 2.2 mS / cm caused tip burn, but a recent study by the same group showed that increasing the solution conductivity from 0.5 to 2.0 mS / cm resulted in increased lettuce total dry weight, especially in romaine varieties of lettuce.

In a study from the Institute of Agronomy in Perugia, Italy in a hydroponic system supplied with 50, 100 and 200 ppm nitrogen, relative growth rate on a dry weight basis was not strictly related to nitrogen levels, while on a fresh weight basis the relationship between these two parameters was linear. In these trials, the yield of the lettuce *(Lactuca sativa L.)* was 58, 73 and 145 kg / ha on fresh weight basis. The lack of this linearity on dry weight basis was

attributed to a lower potassium to nitrogen ratio in the solutions of higher nitrogen concentration as opposed to those with a lower nitrogen level which tends to directly affect the amount of dry weight (Tei et al., 2000). These results were mirrored in another trial in Costa Rica by Latra et al., (2008) where the levels of nitrogen in a floating hydroponic system were varied by a factor of 2 fold in 3 treatments. The amount of foliar nitrate increased proportionally on the fresh weight basis but the relationship was not observed on dry weight basis. In both experiments, the only variable was nitrogen content; hence, the effect of potassium was amplified. To explore what would happen with a proportional increase of all nutrients, a trial was conducted with three treatments of N-P-K (in ppm) as follows: treatment 1: 50-10-25, treatment 2: 100-20-50, and treatment 3: 200-40-100. In these trials, the increase in yield compared to treatment 1 was 23.7% for treatment 2 and 47.2% for treatment 3, and indicated that N is not always the single most decisive factor in lettuce yields (Chaves et al., 1999 b).

The effect of nitrogen 'form' on lettuce growth is also of major importance. An experiment was designed to provide the same concentration of total nitrogen in the solution, in the forms of KNO_3, NH_4-NO_3, $(NH_4)_2SO_4$ and CO-(NH_3). The shoot (plant section without the roots) fresh weight yield per plant was 283, 260, 215 and 247 grams for the treatments where nitrogen was sourced from nitrate, ammonium nitrate, ammonium sulphate and urea respectively. Approximately the same results were obtained when the trials were repeated the next season (Abd-Elmoniem et al., 1996). The nitrate fed plants had larger root systems than ammonia fed plants which were characterized by small and very dark green leaves. These results were explained by Clarkson and Lane, (1997) in a review of Abd-Elmoniem's article, by saying that ammonium has to be incorporated into organic compounds in the roots whereas nitrate is mobile in the xylem and can also be stored in vacuoles found in the roots and shoots; and in order for

nitrate to be used it needs to be reduced to ammonia at the site. Nitrate can travel more easily to the target cells where it is in demand resulting in slightly higher growth rates when used to fertilize lettuce.

Aquaponic Lettuce Production in Fish Culture Effluent

As mentioned before, lettuce is a favorite crop for hydroponic producers because of its low nitrogen and potassium requirement as compared to other crops (Nelson, 2008). When lettuce is grown in aquaponic systems, it tends to mature in 40 to 45 days. The most common system used for growing lettuce is the nutrient film technique (NFT) but other methods such as the raft and A-frame system can also be used. Ideally, the pH for lettuce should be at 5.8 to 6.2, but since this is relatively low for fish culture, a good compromise is a pH of 7, but can even be grown at pH levels as high as 7.5 (Nelson, 2008). In a study conducted in the Scottish Agricultural College, lettuce's growth response to fish culture effluent was assessed for a 35 day cycle with growth characteristics and water quality parameters tested. The lettuce system was capable of reducing nitrate and orthophosphate levels in the water by 20 and 3 gram / m^3, respectively. The mean head weight achieved by the lettuce was 156 g and nutrient deficiency symptoms were not noted despite nutrient concentrations being significantly below recommended commercial nutrient solutions due to nutrient being supplied constantly at this level (Beltrao et al., 2000).

In another experiment, growth of lettuce in fish effluent was compared to a commercial nutrient solution (Rosenthal et al., 1993). Fresh weights of lettuce did not vary significantly between treatments, but when dried, the commercial hydroponic lettuce dry weight exceeded the aquaponics lettuce by of 27.1%. The difference was attributed to potassium deficiency and lower levels of other major elements, but typical nutrient deficiency symptoms were not observed. In

another set of trials, lettuce was stocked at 30 heads / m^2 and concentration of nitrate and orthophosphate in the nutrient solution declined by 17.4 and 4.1 g / m^3 / day, respectively. However, in the trial, no differences in fresh weight were observed, which agrees with other studies where hydroponic lettuce was grown on a wide range of nutrient concentrations without deficiencies or phyto-toxicity revealed (Copper, 1979; Resh, 1989). A review on aquaponic systems suggest that fish effluent might be suitable to fertigate hydroponically grown lettuce, but minor supplementation of some nutrients would be essential. An advantage to integrated lettuce systems is a reduction in the nutrient levels in the fish water hence reducing the requirements for water exchange and enhancing profitability of the system (Clarkson and Lane, 1991).

Sludge and Methane Production Potential

The breakdown process enabling production of smaller and simpler molecules from larger organic particles is the basic biochemical transformations in an aquaponic system. Gas production from the decomposition of animal and vegetable remains was noticed as early as 1682 by scientist Robery Boyle (Stafford et al., 2000). Methane producing bacteria are known as methanogens which comprise all those bacteria that produce gas methane. These types of bacteria have been isolated from anaerobic digesters and include groups such as lactobacillus, methanobacterium, methanosarcina as well as methanobacillus (Stafford et al., 2000).

Methanogens are considered to be the most strictly anaerobic microorganisms known and tend to occur naturally in the rumen of cows, marshes, brackish water and sewage digesters (Hobson et al., 1981). The sewage digester is the chamber into which the organic material for methane production is added with the bacterial seed. These digesters can be batch, continuous, high rate or other types based on the setup (Hobson et al., 1981). In order to make anaerobic digestion a viable process, the digestibility and chemical constituents of the waste must be

determined (Stafford et al., 2000). Animal waste for methane production should be in a slurry to facilitate handling (Varel et al., 1977). Usually the potential gas production is related to the loading rate into a digester which is set at 0.7 to 5 kg / m^3 / day, and exceeding this rate may cause toxic buildup of ammonia due to the high nitrogen content of farm manures (Stafford et al., 2000). Wastes of different animals vary in chemical composition and the solid content of each source should be evaluated separately before deciding which wastes to use. Fish culture effluent and sludge may be collected in an aquaculture facility and can be compared to human sewage sludge which is defined as the primary sewage which settles after raw sewage enters a treatment facility and settles in a settling tank. Treating this primary sludge does not only provide methane rich gas by mesophilic treatment but also helps to reduce the sludge volume by oxidation in the system (Stafford et al., 2000).

Little work has assessed potential methane production of sludge from aquaculture facilities, but in many instances, researchers have assessed sludge production rates. Rafiee and Saad (2004) evaluated the sludge production capability at different stages of red tilapia grown in a re-circulating aquaculture systems. That study showed that a large amount of manganese, zinc and magnesium, an intermediate amount of iron, copper, calcium and phosphorus and a small amount of nitrogen and potassium added in feed can be recovered in the sludge. The rest of the feed nutrient input is either captured in fish flesh or enters the water in soluble forms (Rafiee and Saad, 2004). Another study by Summerfelt and Penne (2007), evaluated septic tank treatment of effluent from a small-scale, commercial, re-circulating aquaculture system. The re-circulating aquaculture systems (RAS) had a total volume of 249.9 m^3 with a mean standing crop of 25.6 Kg of fish / m^3 of mixed species including largemouth bass, hybrid striped bass, and rainbow trout. Inflow to the septic tank contained 25.3% total N and 55.1% of total P added into the system as

fish feed. The outflow from this septic tank reduced total N, total P, biological oxygen demand (BOD), settleable solids and dissolved solids by 59.5%, 35.3%, 5.1%, 92.6% and 9.6% respectively. The solids collected in the septic tank were transferred to a methane digester. The septic tank contents were pumped out every month and dewatered to 14.9% moisture via air drying. The residue contained 3.6% of the total N and 2.6% of the total P added in the feed. The septic tank "pump-out" material was considered to be marketable as a soil amendment for greenhouse use (Summerfelt and Penne, 2007).

Growth of Aquatic Plants

Another potential use of fish effluents and sludge was examined by a team in Thailand where they tried to recover nutrients that was collected from the bottom of earthen ponds during fish culture (Yi et al., 2002). The growth of lotus (*Nelumbo nucifera)* and tilapia in co-culture, and the growth of tilapia alone and lotus alone, were evaluated. Annual nutrient losses from mud in a 1-ha pond stocked at a density of two tilapia per cubic meter was about 2.4 tons of N, and 1 ton of P. Nutrient removal in lotus biomass was 300 kg N and 43 kg P (Yi et al., 2002), which was considered a novel way of effectively removing nutrients from old ponds by planting the pond beds with aquatic plants that root in an anaerobic medium and withdraw nutrients. Lotus yields whether in co-culture with tilapia or alone, did not show any differences in yields. Tilapia had significantly higher yield when cultured alone, this is without lotus nutrient removal, and resulted in lower primary productivity in the pond (Yi et al., 2002; Nagler et al., 2003).

MATERIALS AND METHODS

Nutrient Water and Effluent Sludge Characterization

Overview and Objectives - The objective of these studies is to assess the discharge of three intensive aquaculture production systems (Bio-floc, Polygyser and Opposing flow) to evaluate the nutrient value of the effluent and sludge, leading to a set of relations that will allow estimation of the nutrient load in the effluent from each system based on how much feed was added. The three systems are different, and they have water of different quality. Hence, the objective is to assess the discharge of the systems under the practices applied.

Bio-floc technology, also named BFT, is a new technique in aquaculture production where a dense and active aerobic microbial community in the water column is manipulated to control water quality by immobilizing ammonia in microbial protein to allow recycling of feed residues into a form that can be eaten again by the fish and hence raise feed efficiency (Avnimelech et al., 1989, 1992; Crab et al., 2007). In this situation a high C:N ratio leads to immobilization of ammonia in the microbial biomass and limits accumulation of TAN in the water. In tilapia culture, the microbial floc which develops can be harvested by fish and digested which can replace a significant fraction of the protein in the feed (Avnimelech, 2007; Burford and Kiebzak, 2003; McIntosh, 2000).

Polygyser systems were developed to allow water reconditioning in re-circulating aquaculture systems. Polygyser filters provide a variety of functions allowing water to be reused for longer periods of time and help prevent the accumulation of harmful levels of suspended solids and dissolved organic and toxic nitrogenous compounds (Downing et al., 1964). Drop

bead filters are classified as expandable granular biofilters (EGB) and function as a physical filter first to remove solids (clarify), while at the same time, they allow growth of desirable bacteria on the beads allowing the removal of dissolved nitrogenous wastes via a bio-filtration process (Sharma and Alhert, 1977). The beads in an EGB allow it to be cleaned while providing a very high surface area for the bacteria to attach to it. The water generally enters the filter through a distribution manifold and passes upward through the beds. Physical and biological purification occurs before the water flows out (Shieh and Motta, 1979). By backwashing an EGB filter, the sludge can be removed.

The third system to be assessed is an opposing flow system which utilizes air to circulate water to the fish culture tank from the clarifier. As the air rises through the water column it provides oxygen and creates an opposing circulating flow which enables solids to be gently drawn into the clarifier from outflow ports located at the bottom of the system and from each side of the tank. In the clarifier, the biofilter, made of shredded plastic material allows biofiltration to take place. Water is returned from both sides of the tank via small spouts forcing fish to align themselves against the current. The fish always face the sides and hence aligning themselves along the length of the tank. This constant swimming of the fish allows them to metabolize the high protein feed into flesh which will provide a higher yield when compared to fish grown in circular tanks (Ritvo et al., 2004). One blower in this system provides oxygen in the biofilter via air diffusers, the second feeds the culture tank air lines, and the third operates the air lift system that is used to transport water in the system.

Procedure - This experiment was conducted at two sites where the three systems to be assessed were located.

The bio-floc system is located the E.W. Shell Fisheries Center, North Auburn, Auburn, AL (32°36′14″N - 85°29′30″W) where fish were stocked in a 27.4 m x 3.8 m x 1.2 m tank with a volume of 125 m^3, constructed of plywood with steel I-beam and metal cable reinforcements, and lined with a 12 mm polyethylene liner. Aeration was provided from 1.5 hp air-blowers (Sweetwater®, AES Inc., Apopka). The polygyser system at Wilson's Farm in West Alabama has a dimension of 29.3 m^2 (3.2 m x 9.2 m) and an average depth of 1.2 m with a filter volume of 3.5 m^3, also in rectangular configuration and located in an insulated metallic barn. The opposing flow system located on the same farm in West Alabama (33°13'71"N - 87°31'46"W), has a dimension of 41.5 m^2 (3.9 m X 10.5 m) and an average depth of 1.2 m with a filter volume of 4.7 m^3, also in rectangular shape within the same insulated barn. The number of tanks evaluated for each system was also different, with one biofloc system with the above dimensions, and five of each of the polygyser and the opposing flow systems.

Water and sludge sampling was conducted over a period of 8 weeks for each trial session. The first session extended from April 5, 2010 until May 24, 2010 during which water temperature fluctuated between 23^{o}C and 31^{o}C. The second session extended from August 2, 2010 until September 20, 2010 during which water temperature fluctuated between 24^{o}C and 29^{o}C. Water samples were collected weekly from each of the systems at a 40 cm depth using a water sampling cup. The sludge was collected while the filters of the polygyser and the opposing flows were being backwashed. Sludge of the biofloc system was collected by connecting a settling basin to the tank for the purpose of collecting solids. Water and sludge samples were refrigerated and transported to the laboratories at Auburn University (Auburn, Alabama USA) where analysis was done promptly using the Kjeltec 8400 auto sampler systems.

Nitrite was determined by the diazotition method; nitrate was determined by the phenol-disulfide acid method; total ammonia nitrogen was determined by the phenate method; soluble reactive phosphorus was determined on filtrates by the ascorbic acid method. Potassium was determined by flame spectrometry; hardness was determined by titration with EDTA; alkalinity was estimated by acidimetry, and total suspended solids were determined using a multifiltration and weighing method. While pH and electric conductivity (EC) were measured by a portable meter fitted with parameter specific probes. All of these methods are described in the standard methods manual (Eaton et al., 1978).

Sludge samples were analyzed in the Biosystems Engineering Lab at Auburn University (Auburn, Alabama USA). Total organic N was determined by combustion using an Elementar Vario-Vax; nitrate and ammonia were determined by colorimetry using KCl extract and a Lachat Autoanalyzer; moisture content was determined by drying at 105^{o}C for 16 hr. Calcium, total K and mineral matter were processed by digesting using a MARS Express microwave and using nitric acid. Total P determination was determined with a Thermo 650 Induced Coupled Plasma Spectroscopy (Thermo Scientific). The electrical conductivity (EC) and pH were measured by a portable meter fitted with parameter specific probes (YSI 556 Multiparameter System). All of these methods are described in the Recommended Methods of Manure Analysis (EPA, 1996). When water was being exchanged, the rate was determined and recorded to facilitate accurate evaluation of nutrient output. Weekly feeding rates and fish biomass was recorded during the trial.

Lettuce Production Potential Trial

Overview and Objectives – The purpose of this part of the study was to evaluate the possibility of using the effluent water from the fish tanks to grow lettuce. Lettuce was selected due to its short

production cycle allowing several trials per season. In addition, lettuce is a high value crop with relatively low nutrient requirements and also has well and easily identifiable deficiency and toxicity symptoms.

The goal of this project was to develop a relationship showing the nutrient level required in effluent from the aquaculture system to produce marketable lettuce. Many publications reflect the need to separate suspended solids from water before using it for lettuce culture, thus raw effluent and effluent free of suspended solids and soils was tested against a control of a commercial hydroponic solution.

Procedure - This experiment involved growing lettuce of the variety *Lactuca sativa L.* 'Charles' under three treatments each with twelve replicates in a randomized block design. Each replicate was composed of a 6-liter water bucket fitted with an air line to provide aeration. A styrofoam square measuring 0.04 m^2 was placed over the bucket, into which the lettuce plant was placed. The lettuce was sown in oasis blocks 2 weeks before it was transplanted into the hydroponic buckets. After transplantation, the crops were grown for 28 days to reach their market size (42 days from the time of sowing). Each bucket was filled with one of the following solution treatments: unsettled effluent from the bio-floc fish culture tanks, settled effluent water from the bio-floc fish tanks and conventional fertilizer treatment. The first treatment (treatment A) used water directly from the biofloc system in the north Auburn Fisheries station without any settling. The second treatment (treatment B) was the same water that had passed through a settling basin to remove the majority of the solids. The third treatment (treatment C) was set at 150 mg/L N-NO_3, 50 mg/L TAN, 240 mg/L dissolved Ca, 125 mg/L potassium, and 35 mg/L reactive soluble phosphorus.

Each trial involved 36 buckets, 12 each for treatments A, B and C. The 12 buckets of treatment C were topped off with reservoir water every day to ensure constant volume, while treatments A and B were exchanged on a daily basis with water from the respective sources. Dissolved oxygen concentration was measured at the end of each trial and weekly water samples were randomly taken from three buckets of each treatment and analyzed for several variables including nitrate, nitrite, potassium, soluble phosphorus, alkalinity, hardness, and calcium. Nitrite was determined by the calorimetric diazotizing reagent method; nitrate was determined by the phenol-disulfide acid method; total ammonia N was determined by phenate method; soluble reactive P was determined by application of the ascorbic acid method to filtered samples. Potassium was determined by flame spectrometry; hardness was determined titration with EDTA; alkalinity was estimated by acidimetry and total suspended solids were determined using a multi filtration and weighing method. Electric conductivity (EC) and pH were measured by a portable meter fitted with parameter specific probes.

There were four trials in 2010. The first trial seed were sown on 27 March and transplanted on 9 April into the hydroponic buckets. Plants were grown from the week of April 10 until harvest 4 weeks later on 3 May. The second trial seed were sown on 10 April and transplanted into the hydroponic buckets on 23 April. Plants were grown from 26 April until harvest 4 weeks later on 17 May. The third trial seed were sown on 19 July and transplanted into the hydroponic buckets on 2 August. Plants were grown from 2 August until harvest 4 weeks later on 30 August. The fourth trial seed were sown on 2 August and transplanted into the hydroponic buckets on 22 August. Plants were grown from 22 August until harvest 4 weeks later on 20 September.

One week after planting, plants were assessed for growth weekly where several indices were collected. For each plant, two widths and a height measurement was recorded and a SPAD-502 (Minolta, Spectrum Industries Inc.) (a measure of chlorophyll level in the foliage) reading was taken. Four week after planting shoot and root fresh and dry weight were recorded. Samples were dried in a forced air oven drier at 45^{o}C for 14 days and dry weight was recorded. Blocks used for tissue analysis were pulverized and sent to a commercial laboratory (Brookside Laboratories, New Knoxville, OH) to obtain tissue composition data for each treatment.

Data collected was analyzed using SAS statistical software - ANOVA global linear method and the Student-Newman-Keuls multi-parametric comparison of mean separation test.

RESULTS AND DISCUSSION

Discharge Water Characterization

Dissolved nutrient and sludge analysis from the three systems were completed over a 16 week period split into two parts during which the ambient air temperature fluctuated within the range of 13.2^{o}C to 33.8^{o}C. During this time, to accommodate the production cycle used in the facilities, sampling protocols were adjusted to fit the daily management tasks performed on each system. The data collected were used to develop a model for each system which took into consideration inflowing nutrients from the water sources at each site and thus allowed estimation of the nutrient load of each variable component per kilogram of feed added into the system per liter of water exchanged. In addition, a scenario providing a feeding rate at a fixed water exchange rate or an exchange rate at a fixed feeding rate was recommended to achieve the necessary nutrient load in the water for lettuce production based on their nutritional requirement.

Tabulated below (Tables 1-3) are the results of trials done on the three systems, each table illustrates how the tested variables increased or decreased relative to kilograms of feed added per liter of water exchanged through the system daily.

Table 1: Nutrient components resulting in the exchanged water / Kg of feed added / L / day in the evaluated polygyser system.

Variable [Z]	**Average ± Standard Deviation** [Y]	**Range**
Nitrite (X 10^{-6})	3.58 ± 2.97	0.4 - 10.22
Nitrate (X 10^{-6})	46.96 ± 30.12	17.95 - 90.19
Total ammonia nitrogen (X 10^{-6})	47.4 ± 101.65	0.47 - 329.96
Soluble reactive phosphorus (X 10^{-6})	25.81 ± 38.41	1.4 - 101.31
Potassium (X 10^{-6})	13 ± 7.22	6.89 - 30.73
Total Hardness (X 10^{-6})	201.72 ± 154.71	43.09 - 549.37
Total Alkalinity *Reduction* (X 10^{-6})	647.65 ± 904.92	2,902.24 - 0.79
Ca (X 10^{-6})	244.66 ± 270.56	41.02 - 690.60
EC (X 10^{-6} µmhos/cm)[X]	147.12 ± 125.64	27.31 - 393.38
Total suspended solids (X 10^{-6})	11.19 ± 13.59	0.46 - 37.1

[Z] Nutrient components of the polygyser system given in mg/L added into the systems' exchanged water per Kg of feed per liter of water exchanged, over a period of 16 weeks. All nutrient measurements are in mg/L unless noted.

[Y] SAS statistical software - Student-Newman-Keuls multi-parametric comparison of mean separation test, ($P \leq 0.05$).

[X] Conversion factor of unit of electric conductivity: 1 µ.mhos = 10^{-3} m.mhos = 10^{-6} mhos

Table 2: Nutrient components resulting in the exchanged water / Kg of feed added / L / day in the evaluated opposing flow system.

Variable [Z]	**Average ± Standard Deviation** [Y]	**Range**
Nitrite (X 10^{-6})	1.56 ± 2.52	0.03 - 11.45
Nitrate (X 10^{-6})	48.26 ± 50.48	5.21 - 195.46
Total ammonia nitrogen (X 10^{-6})	55.58 ± 122.70	0.97 - 570.27
Soluble reactive phosphorus (X 10^{-6})	8.98 ±18.98	6.36 - 78.46
Potassium (X 10^{-6})	11.89 ± 12.03	1.46 - 47.81
Total Hardness (X 10^{-6})	353.01 ± 530.02	19.7 - 1715.21
Total Alkalinity *Reduction* (X 10^{-6})	189.73 ± 288.99	963.12 - 395.47
Ca (X 10^{-6})	529.77 ± 728.08	33.91 - 3006.49
EC (X 10^{-6} µmhos/cm)	195.75 ± 123.03	28.07 - 495.51
Total suspended solids (X 10^{-6})	13.45 ± 34.93	0.08 - 185.32

[Z] Nutrient components of the opposing flow system given in mg/L added into the systems' exchanged water per Kg of feed per liter of water exchanged, over a period of 16 weeks. All nutrient measurements are in mg/L unless noted.

[Y] SAS statistical software - Student-Newman-Keuls multi-parametric comparison of mean separation test, ($P \leq 0.05$).

Table 3: Nutrient components resulting in the exchanged water / Kg of feed added / L / day in the evaluated bio-floc system.

Variable [Z]	**Average ± Standard Deviation** [Y]	**Range**
Nitrite (X 10^{-6} mg/L)	0.3 ± 0.19	0.18 - 0.8
Nitrate (X 10^{-6} mg/L)	80.63 ± 29.56	42.28 - 138.52
Total ammonia nitrogen (X 10^{-6} mg/L)	4.71 ± 1.87	2.62 - 9.21
Soluble reactive phosphorus (X 10^{-6} mg/L)	64.08 ± 23.55	36.4 - 113.76
Potassium (X 10^{-6} mg/L)	86.6 ± 32.34	50.32 - 156.49
Total Hardness (X 10^{-6} mg/L)	429.67 ± 137.37	267.7 - 720.42
Total Alkalinity *Reduction* (X 10^{-6} mg/L)	13.59 ± 15.8	12.55 - 37.2
Ca (X 10^{-6} mg/L)	72.58 ± 15.33	52.89 - 103.16
EC (X 10^{-6} µmhos/cm)	3681.68 ± 1027.75	2583.05 - 5625.51
Total suspended solids (X 10^{-6} mg/L)	828.42 ± 265.82	483 - 1357.68

[Z] Nutrient components of the bio-floc system given in mg/L added into the systems' exchanged water per Kg of feed per liter of water exchanged, over a period of 16 weeks. All nutrient measurements are in mg/L unless noted.

[Y] SAS statistical software - Student-Newman-Keuls multi-parametric comparison of mean separation test, ($P \leq 0.05$).

Comparing all three systems different variables tend to be made available in the water at different rates due to the nature of the biological filtration systems or for the lack of bio-filtration in the biofloc system.

Fig. 1: Nitrite evaluation of effluent water in each system.

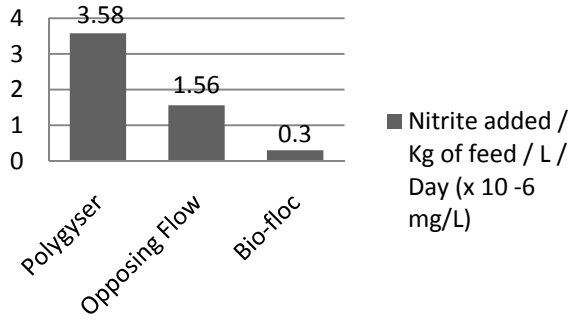


Fig. 2: Nitrate evaluation of effluent water in each system.

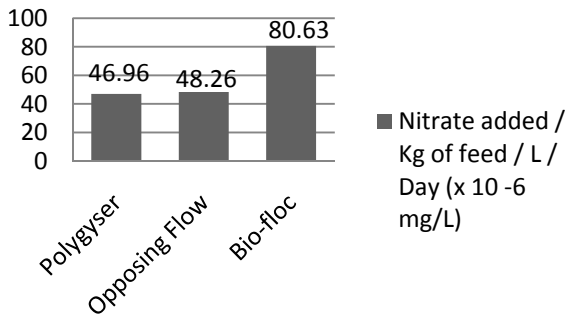


Fig. 3: Total ammonia nitrogen evaluation of effluent water in each system.

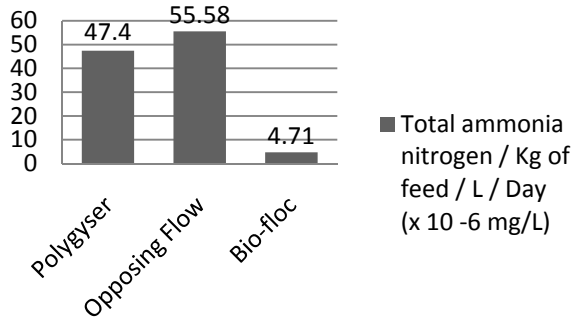


Fig. 4: Soluble reactive phosphorus evaluation of effluent water in each system.

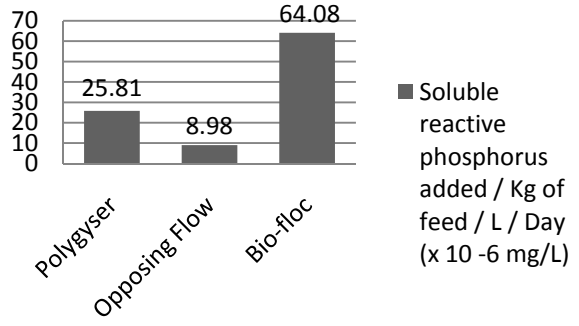


Fig. 5: Total alkalinity evaluation of effluent water in each system.

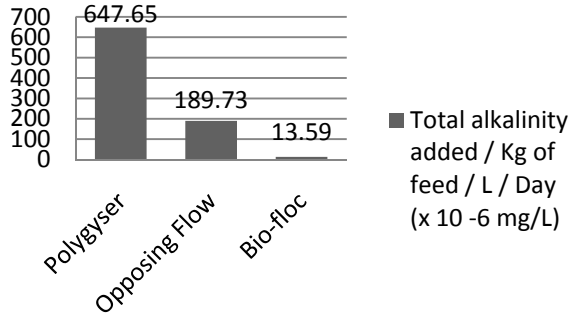


Fig. 6: Potassium evaluation of effluent water in each system.

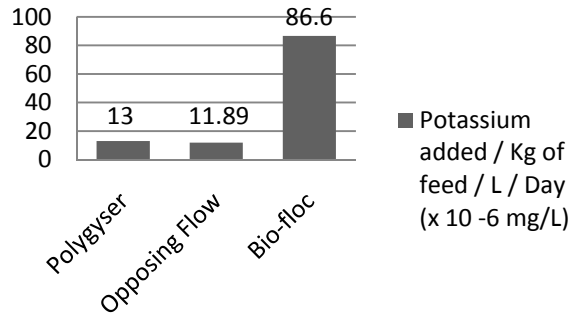


Fig. 7: Total hardness evaluation of effluent water in each system.

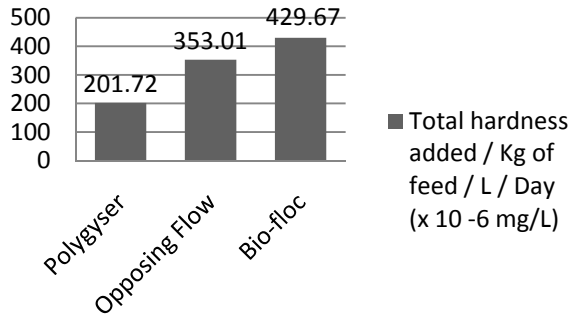
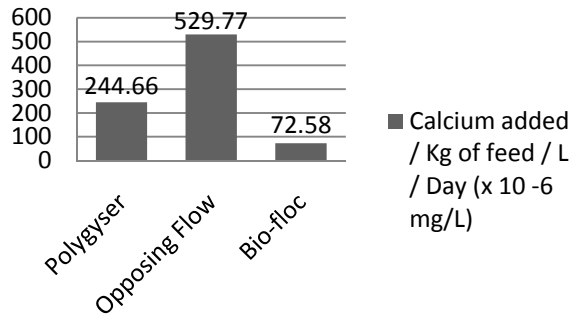
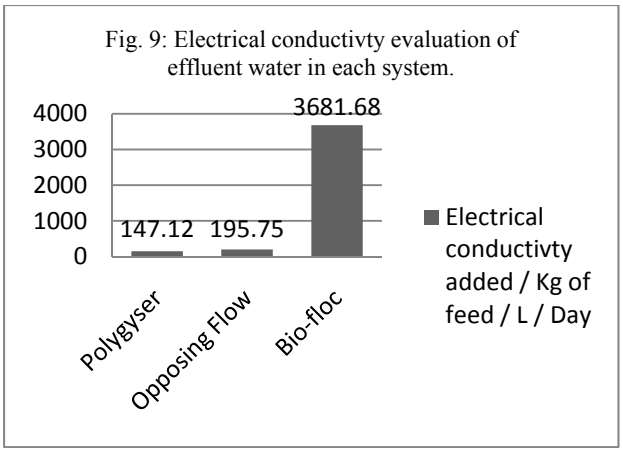


Fig. 8: Ca evaluation of effluent water in each system.





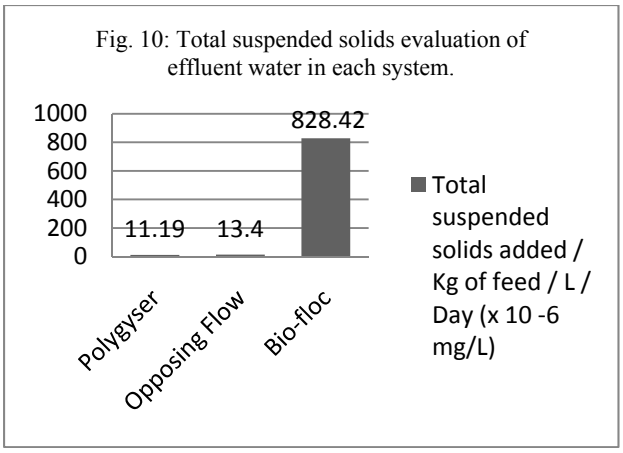


Fig. 1 to 10 graphically compares the different average additions (per Kg of feed per liter of water exchanged) of parameters evaluated during the experimental trials of the three evaluated aquaculture systems.

There were no differences between the addition or the reduction of each of the ten variables between the polygyser (PG) and opposing flow (OF) systems (Fig. 1-10). When both of these systems were compared to the biofloc system, the differences were significant. For nitrite, the polygyser and the opposing flow systems showed higher additions into the water averaging $3.58 \pm 2.97 \text{ X } 10^{-6}$ mg/L/kg of feed and $1.56 \pm 2.52 \text{ X } 10^{-6}$ mg/L/kg of feed, respectively, compared to $0.3 \pm 0.19 \text{ X } 10^{-6}$ mg/L/kg of feed of nitrite added to the biofloc system (Fig. 1). The same results were seen for total ammonia nitrogen (TAN) where the polygyser and the opposing flow system averaged $47.4 \pm 101.65 \text{ X } 10^{-6}$ mg/L/kg of feed and $55.58 \pm 122.70 \text{ X } 10^{-6}$ mg/L/kg of feed respectively while the biofloc stood at $4.71 \pm 1.87 \text{ X } 10^{-6}$ mg/L/kg of feed (Fig. 3). These results mimic experimental data produced by Avnimelech (2006) were he compared a biofloc system to a bead-type, bio-filter. He suggested the reason for these results is that the bacterial flock, which is abundant in the hyper-aerated, biofloc system, are very efficient at reducing both ammonia and nitrite levels by changing them to nitrate through nitrification (Avnimelech, 2006).

For nitrate, the polygyser, opposing flow and biofloc systems averaged 46.96 ± 30.12 X 10^{-6} mg/L/kg of feed, 48.26 ± 50.48 X 10^{-6} mg/L/kg of feed and 80.63 ± 29.56 X 10^{-6} mg/L/kg of feed, respectively (Fig. 2). The biofloc system showed approximately 41% higher nitrate levels in the water when compared to the other systems. For K, the polygyser, opposing flow and biofloc systems averaged 13.0 ± 7.22 X 10^{-6} mg/L/kg of feed, 11.89 ± 12.03 X 10^{-6} mg/L/kg of feed, and 86.6 ± 32.34 X 10^{-6} mg/L/kg of feed, respectively (Fig. 5). The polygyser and the opposing flow systems showed similar results, while the biofloc system released about seven fold more K per unit of feed than did the other two systems. For soluble reactive P, the polygyser, opposing flow and biofloc systems averaged 25.81 ± 38.41 X 10^{-6} mg/L/kg of feed, 8.98 ±18.98 X 10^{-6} mg/L/kg of feed, and 64.08 ± 23.55 X 10^{-6} mg/L/kg of feed, respectively (Fig. 4). The higher rates of nitrate, K and soluble reactive P in the biofloc system are attributed to the higher level of suspended solids and lack of flow through any mechanical filtration apparatus. Increasing retention time of the solids increases the amount of nutrients released through decomposition and bacterial conversion. In systems with an independent bio filter that is properly backwashed, solids and the floccules that collect in the filter are flushed out along with a high proportion of the dissolved components contained in the filter. These results are similar to a study done by Naegel (1977) in which nitrate discharge was two to three times higher per unit of feed in biofloc systems than in bead-type, bio-filters, while soluble reactive P and K were 5 to 10 fold higher.

Total suspended solids and electrical conductivity were much higher in the biofloc system when compared to the other two systems due to the increase in TSS and water retention time and due to lack of removal of the suspended solids and the significant production of bacterial flocks in the water column (McMurty et al., 1997). Total suspended solids additions to

the water in the polygyser, opposing flow and the biofloc systems averaged at 11.19 ± 13.59 X 10^{-6} mg/L/kg of feed, 13.45 ± 34.93 X 10^{-6} mg/L/kg of feed, and 828.42 ± 265.82 X 10^{-6} mg/L/kg of feed, respectively (Fig. 10). The electrical conductivity averaged 147.12 ± 125.64 X 10^{-6} µmhos/cm, 195.75 ± 123.03 X 10^{-6} µmhos/cm, and 3681.68 ± 1027.75 X 10^{-6} µmhos/cm, respectively (Fig. 9). Work done by Rakocy showed that levels of added total suspended solids may exceed 2,409.71 X 10^{-6} mg/L/kg of feed due to the bacterial culture developing in the system (Rakocy, 2002).

The final three parameters tested during the trial periods showed mixed results. Starting with alkalinity reduction, the polygyser, opposing flow and biofloc systems each had a reduction of 647.65 ± 904.92 X 10^{-6} mg/L/kg of feed, 189.73 ± 288.99 X 10^{-6} mg/L/kg of feed, and 13.59 ± 15.8 X 10^{-6} mg/L/kg of feed, respectively (Fig. 7). For hardness, in the polygyser, opposing flow and biofloc systems averaged 201.72 ± 154.71 X 10^{-6} mg/L/kg of feed, 353.01 ± 530.02 X 10^{-6} mg/L/kg of feed, and 429.67 ± 137.37 X 10^{-6} mg/L/kg of feed, respectively (Fig. 6). Average for calcium input were 244.66 ± 270.56 X 10^{-6} mg/L/kg of feed, 529.77 ± 728.08 X 10^{-6} mg/L/kg of feed, and 72.58 ± 15.33 X 10^{-6} mg/L/kg of feed, respectively for the three systems (Fig. 8).

A general review of comparable systems was done by Kuhn et al., (2007) concluded that the best system to be integrated into a hydroponic bed is a biofloc unit or a system lacking a bio-filter system due to the expected higher nutrient level when compared to bio-filter based systems. The main reason being that the higher amount of suspended organic matter in the water column tends to break down over time, providing more nutrients into the water as opposed to being removed by a filter and made unavailable for conversion. They also suggested that the importance of solids removal is to prevent anaerobic conditions around the roots of plants in zones where dissolved oxygen levels are too low to support healthy plant growth. Their findings

suggested that for N-NO_3, K, and SRP bio-floc systems exceeded the other systems by a range of 36.7 to 184.9% in terms of nutrient availability per unit of feed fed to fish. To use the developed ratios to estimate the levels of nutrients in the water, the factors needed to be adjusted in form to fit being multiplied by the exchange rate and the feed rate efficiently (Appendix B).

Effluent Sludge Characterization

Sludge was collected from all three systems over the period of the trials and tested for mineral content, nutrient value, and a proximate analysis of feed potential. The mineral concentrations for the sludge from the three systems are depicted in Tables 4-6.

Table 4: Effluent sludge quality of a polygyser system.

Variable [Z]	**Average Percentage ± Standard Deviation** [Y]	**Range**
Total N (%)	10.95 ± 1.59	8.77 - 13.46
Total P (%)	2.54 ± 0.84	1.36 - 4.02
Total K (%)	0.44 ± 0.16	0.22 - 0.74
Nitrate (%)	0.025 ± 0.015	0.007 - 0.051
Ammonia (%)	0.02 ±0.008	0.002 - 0.032
Calcium (%)	1.71 ± 0.31	1.23 - 2.22
EC (µmhos/cm)	11.16 ± 2.25	6.8 - 14.2
pH	7.67 ± 0.52	6.84 - 8.52
Moisture (%)	95.13 ± 1.63	92.43 - 97.41
Mineral Matter (%)	12.07 ± 0.96	10.47-13.94

[Z] Sludge quality evaluation of polygyser system analyzed using recommended methods of manure analysis (EPA, 1996). All nutrient measurements are in % unless noted.

[Y] SAS statistical software - Student-Newman-Keuls multi-parametric comparison of mean separation test, ($P \leq 0.05$).

Table 5: Effluent sludge quality of an opposing flow system.

Variable [Z]	**Average Percentage ± Standard Deviation** [Y]	**Range**
Total N (%)	6.59 ± 1.23	3.34 - 8.37
Total P (%)	2.31 ± 0.49	1.54 - 3.21
Total K (%)	0.64 ± 0.21	0.19 - 1.21
Nitrate (%)	0.025 ± 0.01	0.004 - 0.044
Ammonia (%)	0.015 ± 0.007	0.002 - 0.026
Calcium (%)	5.43 ± 1.31	2.97 - 7.41
EC (µmhos/cm)	15.72 ± 1.92	11.8 - 18.9
pH	7.33 ± 0.63	6.38 - 8.98
Moisture (%)	94.94 ± 1.48	92.89 - 98.61
Mineral Matter (%)	12.15 ± 2.02	8.93 - 16.41

[Z] Sludge quality evaluation of opposing flow system analyzed using recommended methods of manure analysis (EPA, 1996). All nutrient measurements are in % unless noted.

[Y] SAS statistical software - Student-Newman-Keuls multi-parametric comparison of mean separation test, ($P \leq 0.05$).

Table 6: Effluent sludge quality of a bio-floc system.

Variable [Z]	**Average Percentage ± Standard Deviation** [Y]	**Range**
Total N (%)	4.37 ± 1.34	2.68 - 6.44
Total P (%)	1.46 ± 0.18	1.12 - 1.85
Total K (%)	1.04 ± 0.19	0.79 - 1.42
Nitrate (%)	0.01 ± 0.003	0.004 - 0.018
Ammonia (%)	0.076 ± 0.017	0.038 - 0.098
Calcium (%)	2.09 ± 0.41	1.56 - 2.85
EC (µmhos/cm)	13.12 ± 2.12	8.4 - 15.7
pH	7.26 ± 0.65	6.31 - 8.61
Moisture (%)	94.16 ± 2.01	91.25 - 98.33
Mineral Matter (%)	27.82 ± 1.64	24.39 - 30.24

[Z] Sludge quality evaluation of bio-floc system analyzed using recommended methods of manure analysis (EPA, 1996). All nutrient measurements are in % unless noted.

[Y] SAS statistical software - Student-Newman-Keuls multi-parametric comparison of mean separation test, ($P \leq 0.05$).

Fig. 11: Evaluation of total nitrogen in sludge from each system.

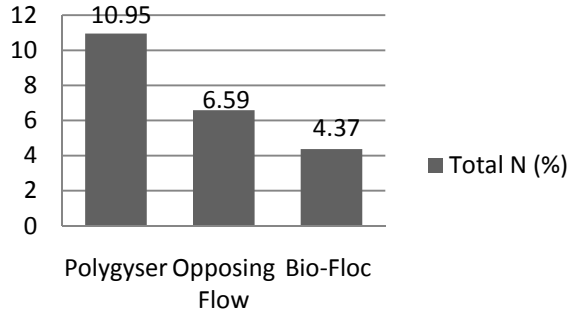


Fig. 12: Evaluation of total phosphorus in sludge from each system.

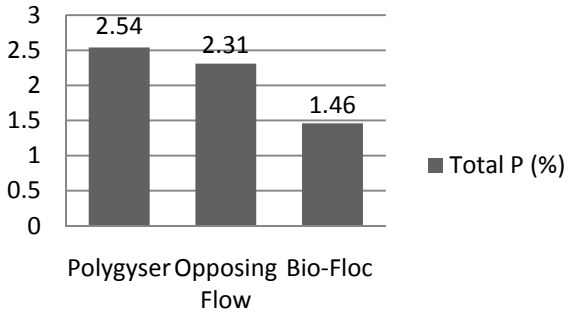


Fig. 13: Evaluation of total potassium in sludge from each system.

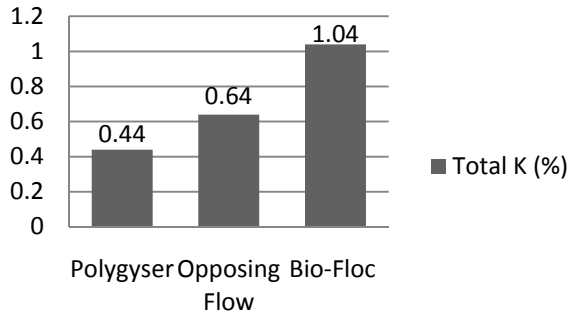


Fig. 14: Evaluation of nitrate in sludge from each system.

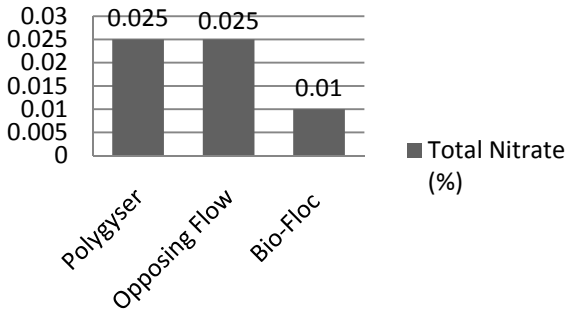


Fig. 15: Evaluation of ammonia in sludge from each system.

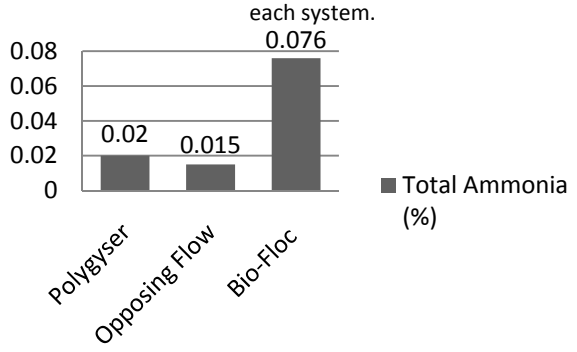
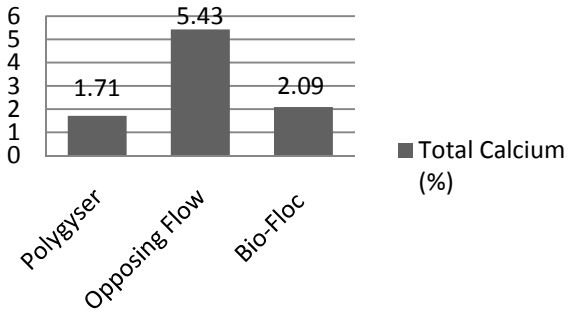


Fig. 16: Evaluation of calcium in sludge from each system.



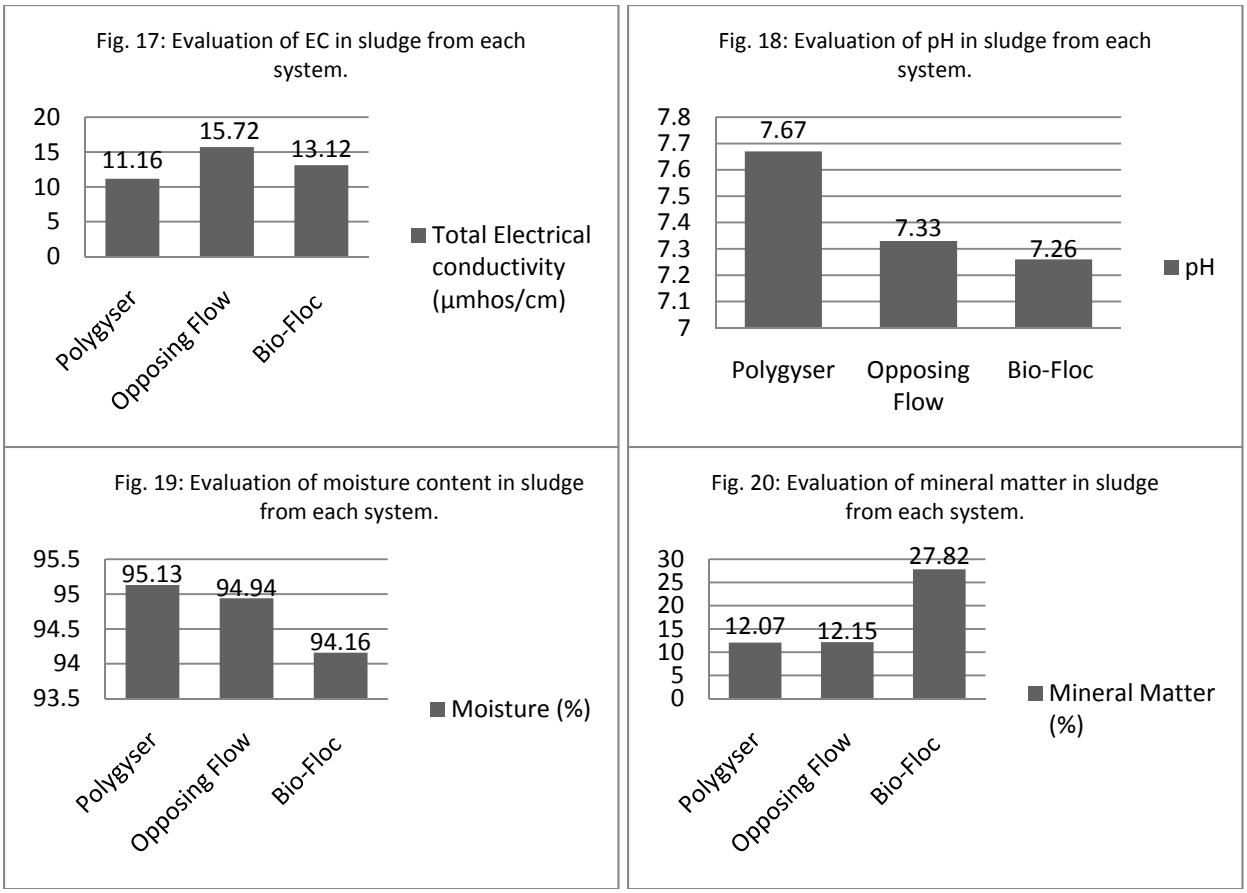


Fig. 11 to 20 graphically compares the different average values of the sludge characteristics evaluated during the experimental trials of the three evaluated aquaculture systems.

The nutrients of interest in assessing aquaculture sludge as an organic fertilizer are N, P and K. For N content the polygyser (PG), opposing flow (OF) and biofloc (BF) systems averaged 10.95%, 6.59% and 4.37%, respectively. The average nitrogen concentration in organic materials and manures used for agricultural purposes is typically between 1 to 13% (Downing et al., 1964); hence the sludge originating from the polygyser system rests at the upper range of that scale while that produced from the biofloc system is at the lower end of the scale. Work done by Rakocy et al., (2007) confirms N level in biofloc systems typically range between 4.9 to 5.3%. P content, polygyser, opposing flow and biofloc systems averaged 2.54%, 2.31% and 1.46%, respectively. Organic P sources typically range between 0.1 to 1%. Swine waste tends to be the

highest at 0.7 to 1.4% (Downing et al., 1964). The biofloc system sludge was significantly lower ($P < 0.05$) in P level than sludge from the other two systems by around 39.7%. An assessment of P levels of sludge from BF systems indicated a level of around 2.2% P (Rakocy, 2002). For K content, sludge from the polygyser, opposing flow and biofloc systems averaged at 0.44%, 0.64% and 1.04%, respectively. On average, K content of animal manure typically ranges between 0.2 and 2% of dry matter, hence, these results are in line with other organic matter sources (Downing et al., 1964). Work done by Rakocy (2002) indicated an average of 0.5% K in the fish sludge from his systems.

Nitrate and ammonia levels were analyzed on the wet samples before drying because they are volatile organic compounds (VOCs) and will be lost from combustion. Nitrate content in sludge from the polygyser, opposing flow and biofloc systems averaged at 0.025%, 0.025% and 0.01% respectively. Ammonia content from the polygyser, opposing flow and biofloc systems averaged at 0.02%, 0.015% and 0.076%, respectively.

Ca content measured in the polygyser, opposing flow and biofloc systems averaged 1.71%, 5.43% and 2.09% respectively. Levels of pH were very consistent and similar for the polygyser, opposing flow and biofloc systems and averaged 7.67, 7.33 and 7.26, respectively. Mineral matter content sampled from polygyser, opposing flow and biofloc systems averaged 12.07%, 12.15% and 27.82%, respectively, suggesting a much higher mineralization rate taking place in the biofloc sludge collection vessel than in the others due to an higher abundance of bacterial floc. Similar results were reported in a previous study with a series of biofloc tanks, where the retention time in the sludge collection tanks affected the mineralization rate to produce mineral contents ranging from 11.3 to 39.6% while the average stood at 23.45 ± 1.67 (Avnimelech, 2007).

Aquaculture sludge in land application trials have demonstrated good value and is an excellent nutrient source for agronomic crops, suggesting that such resources should be valued and not disposed of as waste. In a study at the University of the Virgin Islands, cucumbers were grown with commercial inorganic fertilizers and with de-watered aquaculture sludge. Aquaculture sludge increased production of cucumbers when compared to a commercial, inorganic fertilizer (Osmocote 13-13-13; % N: % P_2O_5 : % K_2O); however, the aquaculture sludge contained a high level of organic carbon ranging between 78 to 85% from which nutrients had to be mineralized by microbes to make them useful to plants (Rakocy et al., 2007). Currently, work is underway to determine methods of de-watering aquaculture sludge. Geo-textile tubes and burlap with the addition of a coagulating polymer are being tested for their efficiency and economic effectiveness (Rakocy et al., 2007).

In brief, results indicate that the aquaculture effluent sludge from different systems produce an organic fertilizer with an analysis of 7.3-2.1-0.7 while other comparable sources of organic manures stand at a grade of 8-5.5-6 for beef cattle and 5.8-5-4 for swine (Nelson, 2008).

Table 7: Proximate analysis of system sludge and other comparable feed ingredients.

	Polygyser sludge	Opposing flow sludge	Biofloc sludge	Alfalfa	Chick-peas	Corn grain	Poultry Manure	Soybean Meal	Dried Whey
Crude protein (%)	16.56	24.56	20.67	18.1	19.1	8.9	24.9	48.7	13.1
Fiber (%)	22.15	13.05	23.9	19.8	4.3	2.3	11.6	26.9	0
NFE (%)	42.98	38.48	35.59	33.95	35.98	56.1	39.6	36.6	77.4
TDN (Mcal/45 Kg)	58.68	54.84	56.18	57.1	77.5	71.2	48.2	50.4	75.2
Calcium (%)	1.68	1.52	2.21	1.38	0.18	0.02	8.1	1.12	1.02
Phosphorus (%)	0.777	0.822	0.947	0.26	0.41	0.26	2.2	0.24	0.81

Proximate analysis data showing the data produced in our trials on dried sludge from the polygyser, opposing flow and bio-floc system, compared to common feed ingredients used in the feed industry such as alfalfa, chickpeas, corn grain, poultry manure, soybean meal and dried whey. Proximate analysis evaluated using standard USDA procedures (1992) by Brookside laboratories (New Knoxville, OH).

Using dried aquaculture sludge as an animal feed or feed amendment is another potential use for this nutrient resource. Proximate analysis done on dried sludge produced from the systems evaluated in our trials reflected good nutritional values for the sludge when compared to other conventional feed ingredients.

In our analysis of the sludge, crude protein (CP) as a concentration averaged 24.56% higher in sludge from the opposing flow system than in sludge from the polygyser and biofloc system whose means were 16.56% and 20.67%, respectively. These levels of CP exceed those generally expected of alfalfa (18.1%), corn grain (8.9%) and dried whey (13.1%) and they are comparable to those of poultry manure (24.9%) (Rennert, 1994). Fiber content was lowest in sludge from the opposing flow system; it averaged 13.05% while the polygyser and biofloc sludge averaged 22.15% and 23.9%, respectively. The N free extract (NFE) of the sludge varieties was not different between systems for the polygyser, opposing flow and biofloc systems; for which values were 42.98%, 38.48% and 35.59%, respectively. By comparison, chickpeas, poultry manure and soybean meals have NFE values of 35.98%, 39.6% and 36.6%, respectively (Schonbeck et al., 1991). Two very important parameters generally evaluated in feeds are total digestible nutrients (TDN) and the net energy of lactation (given in Mcal/45Kg). Analyzing samples for TDN polygyser sludge, opposing flow sludge and biofloc sludge averaged 58.68%, 54.84% and 56.18%, respectively. When these potential feed stuffs are compared to soybean meal, they rank very close: soybean has 50.4% TDN and 51.3 Mcal/45Kg (Rennert, 1994).

To conclude, it is clear that both de-watered sludge and dried sludge from all three of these systems have potential for land application as a fertilizer supplement, or if processed, they can potentially be integrated into feeds for commercial use. Other possible opportunities to use

aquaculture sludge are methane extraction and dry pelletizing to make a fuel. These possibilities should be examined in the future to expand the potential income streams from intensive aquaculture systems and increase their sustainability.

Lettuce Production Potential trial

Integrating tilapia aquaculture units into a hydroponic lettuce growing bed and optimizing the nutrient levels in the water to fit the mineral requirements of the lettuce was the goal of the four, 7-week trials done at E.W. Shell Fisheries North Auburn Station. As mentioned in the methods section, three treatments were run in parallel, one using the fish water with the solids, the second was settled fish water lacking the solids, and the third a control made of commercial hydroponic lettuce fertilizer. After collecting the weekly water chemistry data and lettuce variables, the lettuce heads were harvested and dried for tissue analysis. The different average levels of each mineral component found in the water of each treatment are presented in Tables 1-4. The level of nutrients is a result of the feeding rate that was being used at that time in the tilapia bio-floc system. Details of the effluent characterization were discussed in the previous section.

Table 8: Water quality parameters of an aquaculture trial on *Lactuca sativa L.* 'Charles'.

Variable [Z]	**Treatment A** Average ± SD [Y]	**Treatment B** Average ± SD [Y]	**Treatment C** Average ± SD [Y]
Nitrite (mg/L)	0.12 ± 0.029	0.11 ± 0.032	0.21 ± 0.047
Nitrate (mg/L)	32.06 ± 11.04	30.1 ± 11.81	130.05 ± 17.71
Total ammonia nitrogen (mg/L)	1.68 ± 0.32	1.54 ± 0.26	42.57 ± 4.31
Soluble reactive phosphorus (mg/L)	26.47 ± 8.84	25.32 ± 8.93	30.03 ± 4.34
Potassium (mg/L)	35.28 ± 8.01	34.35 ± 8.97	92.37 ± 5.47
Total Hardness (mg/L)	176.17 ± 5.14	178.75 ± 5.15	404.23 ± 56.73
Total Alkalinity (mg/L)	43.58 ± 4.25	46.25 ± 3.31	39.33 ± 6.81
Ca (mg/L)	24.76 ± 6.05	24.71 ± 6.12	227.11 ± 7.75
Total suspended solids (mg/L)	273.75 ± 25.04	4.44 ± 1.228	0.22 ± 0.06
EC (µmhos/cm)[X]	1172.91 ± 132	1118.58 ± 189.57	759.11 ± 139.27
pH	7.09 ± 0.124	7.14 ± 0.07	6.62 ± 0.21
Temperature (^{o}C)	23.03 ± 0.95	23.09 ± 0.86	23.33 ± 1.47

[Z] Water quality parameters during the first grow out trial of *Lactuca sativa L.* 'Charles' under three treatments A (bio-floc effluence with solids), B (bio-floc effluence without solids) and C (commercial hydroponic solution), from 10th of April 2010 until the 3rd of May 2010. All nutrient measurements are in mg/L unless noted.

[Y] SAS statistical software - Student-Newman-Keuls multi-parametric comparison of mean separation test, ($P \leq 0.05$).

[X] Conversion factor of unit of electric conductivity: 1 µ.mhos = 10^{-3} m.mhos = 10^{-6} mhos

Table 9: Water quality parameters of an aquaculture trial on *Lactuca sativa L.* 'Charles'.

Variable [Z]	**Treatment A** Average ± SD [Y]	**Treatment B** Average ± SD [Y]	**Treatment C** Average ± SD [Y]
Nitrite (mg/L)	0.102 ± 0.01	0.093 ± 0.01	0.161 ± 0.053
Nitrate (mg/L)	18.72 ± 5.82	17.214 ± 5.11	133.12 ± 13.76
Total ammonia nitrogen (mg/L)	1.35 ± 0.304	1.27 ± 0.37	40.88 ± 4.41
Soluble reactive phosphorus (mg/L)	16.18 ± 4.27	14.78 ± 4.52	29.256 ± 4.38
Potassium (mg/L)	24.07 ± 5.47	23.39 ± 6.59	90.55 ± 25.5
Total Hardness (mg/L)	172.75 ± 4.43	173.33 ± 4.75	401.12 ± 61.46
Total Alkalinity (mg/L)	43.83 ± 5.31	46.25 ± 3.38	39.33 ± 6.44
Ca (mg/L)	20.525 ± 1.19	20.54 ± 1.11	229.87 ± 9.19
Total suspended solids (mg/L)	221.91 ± 64.41	4.25 ± 1.19	0.47 ± 0.129
EC (µmhos/cm)	999.66 ± 135.01	960.58 ± 186.13	747.92 ± 164.41
pH	7.04 ± 0.157	7.05 ± 0.15	6.74 ± 0.29
Temperature (^{o}C)	22.52 ± 0.675	23.08 ± 0.85	23.15 ± 0.96

[Z] Water quality parameters during the second grow out trial of *Lactuca sativa L.* 'Charles' under three treatments A (bio-floc effluence with solids), B (bio-floc effluence without solids) and C (commercial hydroponic solution), from 23rd of April 2010 until the 17th of May 2010. All nutrient measurements are in mg/L unless noted.

[Y] SAS statistical software - Student-Newman-Keuls multi-parametric comparison of mean separation test, ($P \leq 0.05$).

Table 10: Water quality parameters of an aquaculture trial on *Lactuca sativa L.* 'Charles'.

Variable [Z]	**Treatment A** Average ± SD [Y]	**Treatment B** Average ± SD [Y]	**Treatment C** Average ± SD [Y]
Nitrite (mg/L)	0.131 ± 0.08	0.127 ± 0.06	0.19 ± 0.037
Nitrate (mg/L)	26.87 ± 1.09	25.89 ± 0.96	134.11 ± 13.81
Total ammonia nitrogen (mg/L)	1.69 ± 0.27	1.54 ± 0.19	39.47 ± 5.66
Soluble reactive phosphorus (mg/L)	18.53 ± 0.98	17.98 ± 0.72	32.88 ± 4.39
Potassium (mg/L)	122.71 ± 3.72	121.61 ± 2.99	87.77 ± 3.96
Total Hardness (mg/L)	156.02 ± 7.92	152.95 ± 6.43	422.73 ± 54.98
Total Alkalinity (mg/L)	72.39 ± 4.72	68.98 ± 3.65	41.92 ± 6.24
Ca (mg/L)	36.52 ± 5.02	34.55 ± 3.65	244.77 ± 8.24
Total suspended solids (mg/L)	229.33 ± 37.21	3.75 ± 1.92	0.43 ± 0.12
EC (µmhos/cm)	1082.81 ± 89.05	1023.3± 73.56	765.14 ± 127.43
pH	7.11 ± 0.28	7.07 ± 0.19	6.71 ± 0.16
Temperature (^{o}C)	25.89 ± 1.98	26.39 ± 1.73	26.31 ± 2.87

[Z] Water quality parameters during the third grow out trial of *Lactuca sativa L.* 'Charles' under three treatments A (bio-floc effluence with solids), B (bio-floc effluence without solids) and C (commercial hydroponic solution), from 2nd of August 2010 until the 30th of August 2010. All nutrient measurements are in mg/L unless noted.

[Y] SAS statistical software - Student-Newman-Keuls multi-parametric comparison of mean separation test, ($P \leq 0.05$).

Table 11: Water quality parameters of an aquaculture trial on *Lactuca sativa L.* 'Charles'.

Variable [Z]	**Treatment A** Average ± SD [Y]	**Treatment B** Average ± SD [Y]	**Treatment C** Average ± SD [Y]
Nitrite (mg/L)	0.126 ± 0.06	0.109 ± 0.02	0.24 ± 0.024
Nitrate (mg/L)	24.67 ± 0.95	23.65 ± 1.93	136.55 ± 13.02
Total ammonia nitrogen (mg/L)	1.32 ± 0.16	1.35 ± 0.23	49.53 ± 3.84
Soluble reactive phosphorus (mg/L)	19.47 ± 0.78	14.32 ± 0.61	26.43 ± 2.93
Potassium (mg/L)	135.84 ± 4.01	131.54 ± 2.36	87.02 ± 3.39
Total Hardness (mg/L)	154.23 ± 6.94	147.61 ± 6.04	387.25 ± 45.32
Total Alkalinity (mg/L)	68.44 ± 3.91	65.79 ± 3.07	41.23 ± 6.23
Ca (mg/L)	39.71 ± 4.88	36.76 ± 2.97	255.71 ± 6.95
Total suspended solids (mg/L)	294.43 ± 31.65	2.95 ± 1.57	0.38 ± 0.03
EC (µmhos/cm)	1117.54 ± 90.54	954.23± 61.32	775.15 ± 121.87
pH	7.05 ± 0.23	6.98 ± 0.24	6.81 ± 0.32
Temperature (^{o}C)	24.78 ± 1.98	25.15 ± 1.85	25.36 ± 1.69

[Z] Water quality parameters during the fourth grow out trial of *Lactuca sativa L.* 'Charles' under three treatments A (bio-floc effluence with solids), B (bio-floc effluence without solids) and C (commercial hydroponic solution), from 22nd of August 2010 until the 20th of September 2010. All nutrient measurements are in mg/L unless noted.

[Y] SAS statistical software - Student-Newman-Keuls multi-parametric comparison of mean separation test, ($P \leq 0.05$).

The critical minerals in these trials were nitrate, soluble reactive P, K, and Ca because they are the primary limiting nutrients for the lettuce growth. The commercial hydroponic solution (treatment C) was set at specific levels for each of these variables as explained in the methods sections, and utilization of these minerals in the commercial solution took place at the same rate as in each of the trials. Using the control group, nutrient uptake per lettuce plant was measured, each lettuce removed an average of 276 mg of $N\text{-}NO_3$, 78 mg of soluble reactive phosphorus (SRP), 420 mg of K, and 126 mg of Ca from the water. Treatments A and B the fish effluent provided different levels of the minerals in each trial, but within the A and B treatments there were no difference between mineral levels, although treatment A, with the solids, was always slightly higher in each mineral that can be attributed to the breakdown of the suspended solids in the water. In both treatments A and B, in trial 1 (Table 1), $N\text{-}NO_3$ averaged 32 mg/L, SRP averaged 26 mg/L, K averaged 35 mg/L and Ca 24 mg/L. In trial 2 (Table 2), $N\text{-}NO_3$ averaged 18 mg/L, SRP averaged 16 mg/L, K averaged 24 mg/L and Ca 20 mg/L. In trial 3 (Table 3), $N\text{-}NO_3$ averaged 26 mg/L, SRP averaged 19 mg/L, K averaged 122 mg/L and Ca 36 mg/L. In trial 4 (Table 4), $N\text{-}NO_3$ averaged 24 mg/L, SRP averaged 19 mg/L, K averaged 135 mg/L and Ca 39 mg/L. The decrease in mineral content from trial 1 to trial 2 resulted from a fish mortality incident that caused feeding rates to be reduced, which in turn led to a lower nutrient concentration and lettuce productivity. After assessing the results of trials 1 and 2 it was clear based on mineral profiles in effluent water that Ca and K were in short supply. It was also clear from plant coloration, that iron was deficient. In trials 3 and 4, water was supplemented with calcium hydroxide ($Ca\text{-}(OH)_2$) and potassium chloride (KCl) to raise K levels (around 6 fold higher) and approximately double the Ca content. The fish water was also supplemented with iron chelate to maintain a level of 1.5 mg/L iron in the water. It is worth mentioning that using

calcium hydroxide and potassium chloride have a double use of providing carbonates that increase alkalinity and pH as well as chloride which counteract potential high levels of nitrite in the water.

The necessity of measures to improve the composition of the water for hydroponic application was not surprising based on previous work by others (Rakocy, 2002; Rakocy et al., 2007). Rakocy (2002) suggested that Ca and K supplementation be done through base additions and the Fe as a chelate. Significant improvements in lettuce growth performance resulted from the supplementation of Ca, K and Fe during trials 3 and 4.

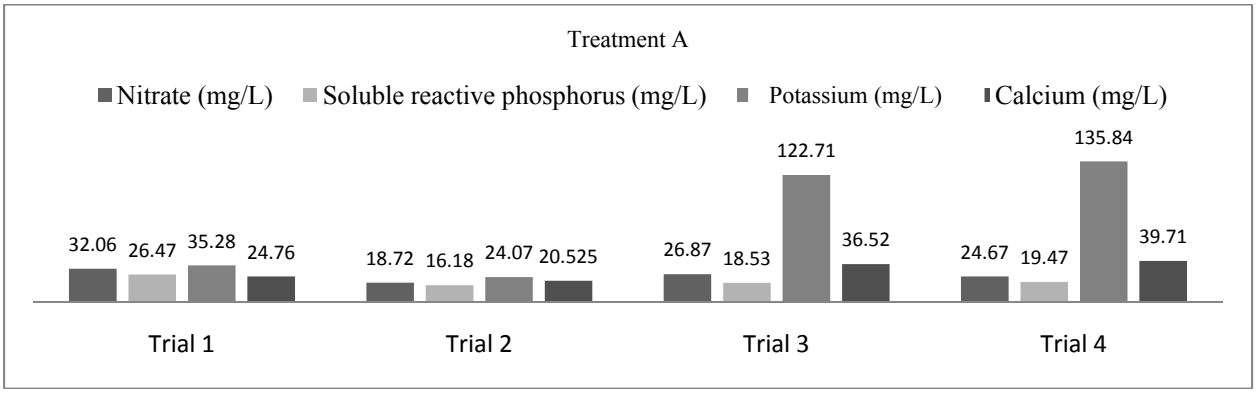


Fig. 21: Average value of critical nutrients (nitrate, SRP, K and Ca) over the period of 4 trials within treatment A (bio-floc effluence with solids).

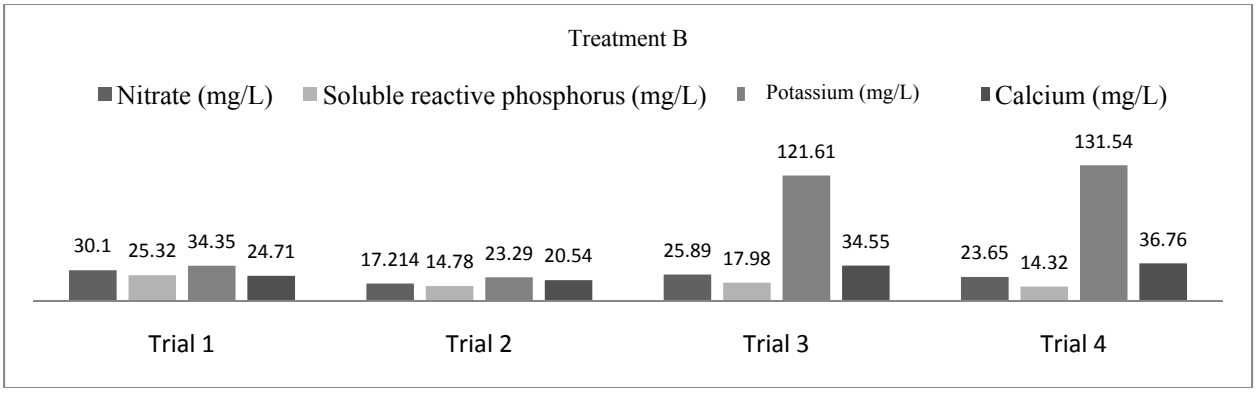


Fig. 22: Average value of critical nutrients (nitrate, SRP, K and Ca) over the period of 4 trials within treatment B (bio-floc effluence without solids).

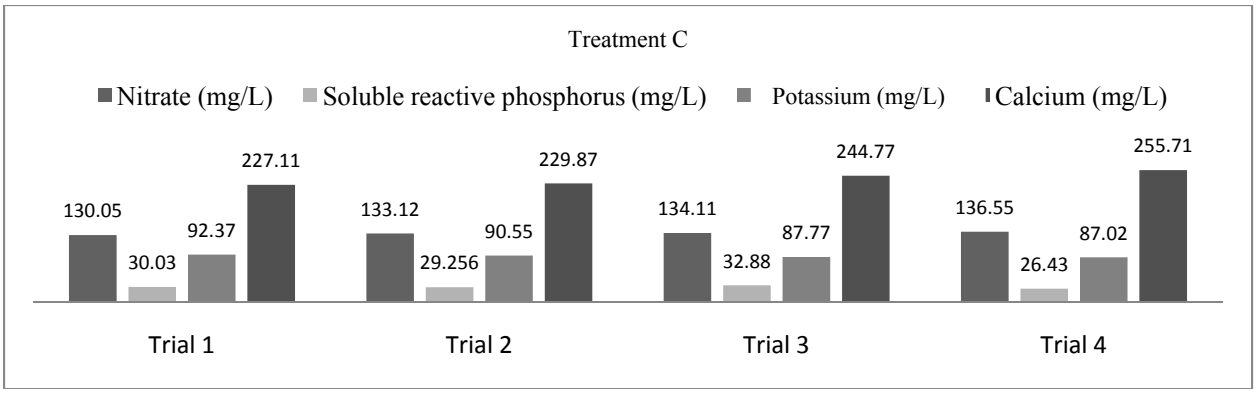


Fig. 23: Average value of critical nutrients (nitrate, SRP, K and Ca) over the period of 4 trials within treatment C (commercial hydroponic solution).

In trial 1, no differences were seen between lettuce produced using the fish water with and without the solids, while differences were seen between them and the commercial hydroponic solution. On fresh weight basis, lettuce produced under treatments A, B and C was 73.6 g, 68.6 g and 139.5 g respectively, while on dry weight values were 4.2 g, 4.5 g and 8.0 g, respectively. The growth index used to assess these crops (length and two widths of lettuce head at harvest divided by 3), averaged 14.2, 14.6 and 18.9 for treatments A, B, and C respectively. The SPAD readings were at 12.9, 11.9 and 25.5 respectively.

In trial 2, no differences were seen between lettuce produced using both the fish water with and without the solids, while differences were seen between them and the commercial hydroponic solution. Fresh weights of lettuce produced in treatments A, B and C were 8.6 g, 5.6 g and 154.4 g, respectively, while dry weights were 0.72 g, 0.58 g and 9.02 g, respectively. The growth index values for treatments A, B and C were 6.9, 5.2 and 18.5, respectively, and the SPAD readings were 22.1, 19.4 and 32.1 respectively.

In trial 3, no differences were seen between lettuce produced using treatments A, B and C. This trial produced results having quality levels and growth parameters similar to the control lettuce grown in a commercial hydroponic solution. The fresh weight of lettuce plants in

treatments A, B and C were 149.2 g, 143.8 g and 152.3 g, respectively, while dry weights were 8.9 g, 9.0 g and 8.99 g, respectively. The growth index averaged 17.9 cm, 17.3 cm and 18.2 cm for treatments A, B and C, respectively, the SPAD readings were 28.0, 29.5 and 29.4, respectively.

Results of trial 4 mimicked those of trial 3. The growth and quality of lettuce produced in the fish effluent and in the commercial hydroponic solution were not different. For the fresh weight basis, the lettuce produced in treatments A, B and C was 155.9 g, 160.2 g and 159.0 g, respectively, while dry weights were 9.4 g, 9.3 g and 9.5 g, respectively. The growth index averaged 19.3, 19.0 and 19.1, for lettuce of treatments A, B and C, respectively, while SPAD readings were 31.2, 31.0 and 31.0, respectively.

The decrease in lettuce performance observed in treatments A and B between trial 1 and trial 2 is attributed to the lower mineral content and nutrient value in the water due to a lower feeding rate. A decrease in nitrate by half, K by one third and P by half was sufficient to decrease the growth rate of the lettuce in fish effluent by a factor of 10 resulting in small, K deficient crops. The total amount of $N\text{-}NO_3$, SRP and K made available for the plant in treatments A and B over the whole 28-day cycle, averaged 18 mg/L for $N\text{-}NO_3$, 16 mg/L for SRP and 24 mg/L for K per day. A total of 3,024 mg (18 mg/L/day X 28 days X 6 liter volume of bucket) of $N\text{-}NO_3$, 2,688 mg of SRP and 4,032 mg of K was provided which exceeds what a lettuce plant requires based on calculations in the previous section. However, Wheeler and Sager (1999) stated that a minimum threshold of minerals was needed daily in the hydroponic solution for the plant to be able to make effective use of it. They explained that if mineral levels are under this critical threshold then the energy expended by the plant to collect these minerals from the water exceeds their value. Critical threshold values for hydroponically produced lettuce for nitrate, soluble

phosphorus, potassium and calcium were 25 mg/L, 20 mg/L, 30 mg/L and 20 mg/L, respectively (Wheeler and Sager, 1999). Ryder also pointed out that under these low nutrient levels, the lettuce crop will extract nutrients at a very slow rates which will reduce production efficiency (Ryder, 1998). This contributes to the explanation of our results because in trial 1, these thresholds were overcome, while in trial 2, we were significantly under the threshold. In trial 1, although the minimum threshold for the critical minerals ($N\text{-}NO_3$, SRP and K) was met and growth did occur, the plants showed deficiencies, predominantly in iron (Fe), K and Ca. Although these minerals were present above minimal threshold levels, they were not sufficient for full commercially successful crop growth; hence based on Leibigs law of the minimum, the rate of other nutrient utilization was limited by these essential nutrient variables and a low value crop resulted.

In trials 3 and 4, lettuce of the desired quality and quantity was produced. In these two trials, lettuce was of equivalent quality to the lettuce produced by the commercial hydroponic nutrient solution. The reason is that during trials 1 and 2 K, Ca and Fe were limiting nutrients in our fish production effluent water acted as the weakest links in the solution mix. Supplementations of the solution with potassium chloride (KCl), calcium hydroxide ($CaOH_2$) and chelated iron, overcame the limitations. Results from these studies confirms with the conclusion of Rakocy (2002) that Ca, K and iron may require supplementation to achieve commercial yields of lettuce production using fish effluent and hydroponic systems.

Evaluating the lettuce quality visually (Fig. 32-35), by head/leaf size and physical quality differences between treatments A and B could not be seen. However the differences were obvious when lettuce in treatments A and B are compared with treatment C. In trials 1 and 2, treatment C outperformed the other treatments with visibly superior green, lush leaves and good

growth rate and whole produced head was marketable. On the other hand, in both trials 1 and 2, treatments A and B produced smaller head/leaf size (more obvious in trial 2), and there was noticeable leaf yellowing, inter-venial chlorosis and leaf edge chlorosis – tell-tale signs of N, Fe and K deficiencies.

Side by side comparison of the lettuce productivity results relative to the water in which they were produced, one can see that since lettuce has a relatively high K requirement, K in the grow-out solution plays a major role in the market quality of the lettuce. Higher K levels in trial 1 than trial 2 (35 mg/L compared to 24 mg/L) were noted, and the quality of the lettuce produced was better for every variable and index measured. In addition, in trials 3 and 4, when K was supplemented to levels around 125 mg/L, the plant quality results were obvious. It was also clear that if adequate nitrate was supplied in the fish effluent to be equal to the amount of N found in the lettuce, rate of growth and lettuce quality did not decline. Ca levels between treatments also varied but were not as critical as K or nitrate.

Table 12: Final growth indices, SPAD and weights of lettuce in trial 1.[Z]

Treatment [v]	Growth Index [u]	SPAD [wu]	Fresh Weight [xu]	Dry Weight [yu]
A	14.21_a	12.92_a	73.61_a	4.19_a
B	14.56_a	11.88_a	68.55_a	4.45_a
C	18.92_b	25.51_b	139.53_b	8.02_b

[Z] Final growth indices (GI) of the first grow out trial of *Lactuca sativa L.* 'Charles' under three treatments: A (bio-floc effluence with solids), B (bio-floc effluence without solids) and C (commercial hydroponic solution), from 10th of April 2010 until the 3rd of May 2010. GI determined by height plus 2 perpendicular widths divided by 3.

[V] Treatments: A (fish water with solids); B (fish water without solids); C (hydroponic solution).
[U] Means within columns followed by the same letter are not different (Student-Newman-Keuls multi-parametric comparison of mean separation test, $P \leq 0.05$).
[W] Chlorophyll value using SPAD-502 meter.
[X] Fresh weight of plant at harvest (grams).
[Y] Weight of plant tissue dried at 80°C for 2 weeks (grams).

Table 13: Final growth indices, SPAD and weights of lettuce in trial 2.[Z]

Treatment [v]	Growth Index [u]	SPAD [wu]	Fresh Weight [xu]	Dry Weight [yu]
A	6.85_a	22.07_a	8.56_a	0.72_a
B	5.18_a	19.39_a	5.55_a	0.58_a
C	18.51_b	32.01_b	154.35_b	9.02_b

[Z] Final growth indices (GI) of the first grow out trial of *Lactuca sativa L.* 'Charles' under three treatments: A (bio-floc effluence with solids), B (bio-floc effluence without solids) and C (commercial hydroponic solution), from 23rd of April 2010 until the 17th of May 2010. GI determined by height plus 2 perpendicular widths divided by 3.

[V] Treatments: A (fish water with solids); B (fish water without solids); C (hydroponic solution).
[U] Means within columns followed by the same letter are not different (Student-Newman-Keuls multi-parametric comparison of mean separation test, $P \leq 0.05$).
[W] Chlorophyll value using SPAD-502 meter.
[X] Fresh weight of plant at harvest (grams).
[Y] Weight of plant tissue dried at 80°C for 2 weeks (grams).

Table 7: Final growth indices, SPAD and weights of lettuce in trial 3.[Z]

Treatment [v]	Growth Index [u]	SPAD [wu]	Fresh Weight [xu]	Dry Weight [yu]
A	17.89_a	28.04_a	149.21_a	8.94_a
B	17.32_a	29.48_a	143.75_a	9.03_a
C	18.21_a	29.36_a	152.32_a	8.99_a

[Z] Final growth indices (GI) of the first grow out trial of *Lactuca sativa L.* 'Charles' under three treatments: A (bio-floc effluence with solids), B (bio-floc effluence without solids) and C (commercial hydroponic solution), from 2nd of August 2010 until the 30th of August 2010. GI determined by height plus 2 perpendicular widths divided by 3.

[V] Treatments: A (fish water with solids); B (fish water without solids); C (hydroponic solution).
[U] Means within columns followed by the same letter are not different (Student-Newman-Keuls multi-parametric comparison of mean separation test, $P \leq 0.05$).
[W] Chlorophyll value using SPAD-502 meter.
[X] Fresh weight of plant at harvest (grams).
[Y] Weight of plant tissue dried at 80°C for 2 weeks (grams).

Table 15: Final growth indices, SPAD and weights of lettuce in trial 4.[Z]

Treatment [v]	Growth Index [u]	SPAD [wu]	Fresh Weight [xu]	Dry Weight [yu]
A	19.32_a	31.21_a	155.87_a	9.43_a
B	18.97_a	30.95_a	160.23_a	9.28_a
C	19.13_a	31.02_a	159.04_a	9.53_a

[Z] Final growth indices (GI) of the first grow out trial of *Lactuca sativa L.* 'Charles' under three treatments: A (bio-floc effluence with solids), B (bio-floc effluence without solids) and C (commercial hydroponic solution), from 22nd of August 2010 until the 20th of September 2010. GI determined by height plus 2 perpendicular widths divided by 3.

[V] Treatments: A (fish water with solids); B (fish water without solids); C (hydroponic solution).
[U] Means within columns followed by the same letter are not different (Student-Newman-Keuls multi-parametric comparison of mean separation test, $P \leq 0.05$).
[W] Chlorophyll value using SPAD-502 meter.
[X] Fresh weight of plant at harvest (grams).
[Y] Weight of plant tissue dried at 80°C for 2 weeks (grams).

Table 8: Average lettuce tissue analysis trial 1.[Z]

Treatment[Y]	N (%)[X]	P (%)[X]	K (%)[X]	Ca (%)[X]	Mg (%)[X]
A	3.83_a	0.88_a	6.32_a	2.01_a	0.42_a
B	3.81_a	0.87_a	6.26_a	2.06_a	0.38_a
C	4.11_a	0.66_a	6.23_a	1.76_a	0.57_b

[Z] Tissue analysis of *Lactuca sativa L.* 'Charles' produced during trial 1 under three treatments A (bio-floc effluence with solids), B (bio-floc effluence without solids) and C (commercial hydroponic solution), from 10th of April 2010 until the 3rd of May 2010.

[Y] Treatments: A (fish water with solids); B (fish water without solids); C (hydroponic solution)
[X] Means within columns followed by the same letter are not different (Student-Newman-Keuls multi-parametric comparison of mean separation test, $P \leq 0.05$). Nutrient levels determined by Kjehdahl and inductively coupled plasma (ICP) spectrometry by Brookside laboratories (New Knoxville, OH).

Table 17: Average lettuce tissue analysis trial 2.[Z]

Treatment[Y]	N (%)[x]	P (%)[x]	K (%)[x]	Ca (%)[x]	Mg (%)[x]
A	3.52_a	0.62_a	5.69_a	1.82_a	0.39_a
B	3.16_a	0.55_a	5.28_a	1.66_a	0.38_a
C	3.81_a	0.53_a	4.59_a	1.92_a	0.51_a

[Z] Tissue analysis of *Lactuca sativa L.* 'Charles' produced during trial 1 under three treatments A (bio-floc effluence with solids), B (bio-floc effluence without solids) and C (commercial hydroponic solution), from 23rd of April 2010 until the 17th of May 2010.

[Y] Treatments: A (fish water with solids); B (fish water without solids); C (hydroponic solution)
[X] Means within columns followed by the same letter are not different (Student-Newman-Keuls multi-parametric comparison of mean separation test, $P \leq 0.05$). Nutrient levels determined by Kjehdahl and inductively coupled plasma (ICP) spectrometry by Brookside laboratories (New Knoxville, OH).

Table 18: Average lettuce tissue analysis trial 3.[Z]

Treatment[Y]	N (%)[x]	P (%)[x]	K (%)[x]	Ca (%)[x]	Mg (%)[x]
A	3.87_a	0.57_a	5.33_a	1.74_a	0.53_a
B	4.01_a	0.62_a	5.29_a	1.81_a	0.58_a
C	3.97_a	0.59_a	5.98_a	1.72_a	0.56_a

[Z] Tissue analysis of *Lactuca sativa L.* 'Charles' produced during trial 1 under three treatments A (bio-floc effluence with solids), B (bio-floc effluence without solids) and C (commercial hydroponic solution), from 2nd of August 2010 until the 30th of August 2010.

[Y] Treatments: A (fish water with solids); B (fish water without solids); C (hydroponic solution)
[X] Means within columns followed by the same letter are not different (Student-Newman-Keuls multi-parametric comparison of mean separation test, $P \leq 0.05$). Nutrient levels determined by Kjehdahl and inductively coupled plasma (ICP) spectrometry by Brookside laboratories (New Knoxville, OH).

Table 19: Average lettuce tissue analysis trial 4.[Z]

Treatment[Y]	N (%)[x]	P (%)[x]	K (%)[x]	Ca (%)[x]	Mg (%)[x]
A	4.12_a	0.62_a	6.02_a	1.84_a	0.61_a
B	4.09_a	0.66_a	6.12_a	1.79_a	0.67_a
C	4.21_a	0.64_a	6.08_a	1.78_a	0.61_a

[Z] Tissue analysis of *Lactuca sativa L.* 'Charles' produced during trial 1 under three treatments A (bio-floc effluence with solids), B (bio-floc effluence without solids) and C (commercial hydroponic solution), from 22nd of August 2010 until the 20th of September 2010.

[Y] Treatments: A (fish water with solids); B (fish water without solids); C (hydroponic solution)
[X] Means within columns followed by the same letter are not different (Student-Newman-Keuls multi-parametric comparison of mean separation test, $P \leq 0.05$). Nutrient levels determined by Kjehdahl and inductively coupled plasma (ICP) spectrometry by Brookside laboratories (New Knoxville, OH).

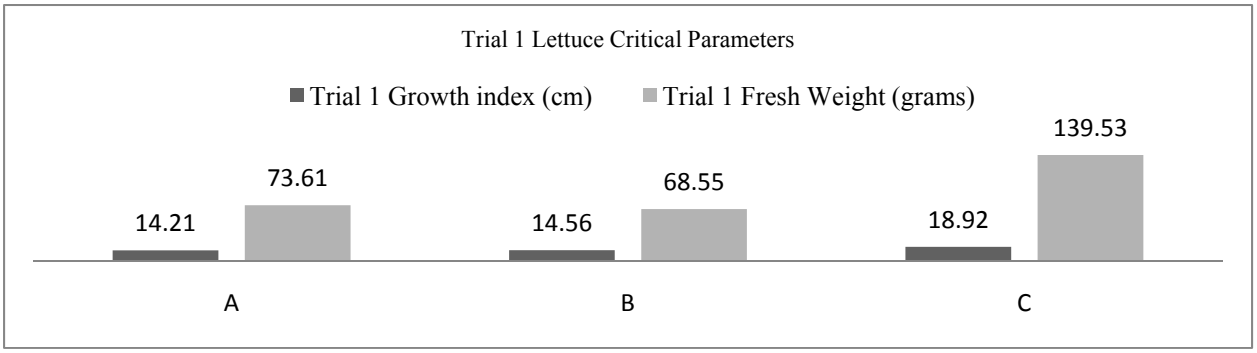


Fig. 24: Graphical representation of average growth indices of the first grow out trial of *Lactuca sativa L.* 'Charles' under three treatments. Treatments: A (bio-floc effluence with solids), B (bio-floc effluence without solids) and C (commercial hydroponic solution), from 10th of April 2010 until the 3rd of May 2010.

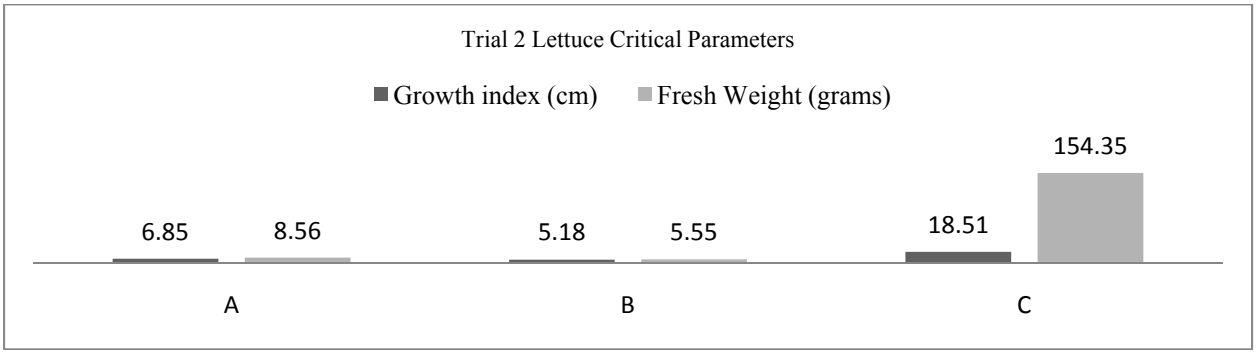


Fig. 25: Graphical representation of average growth indices of the second grow out trial of *Lactuca sativa L.* 'Charles' under three treatments. Treatments: A (bio-floc effluence with solids), B (bio-floc effluence without solids) and C (commercial hydroponic solution), from 23rd of April 2010 until the 17th of May 2010.

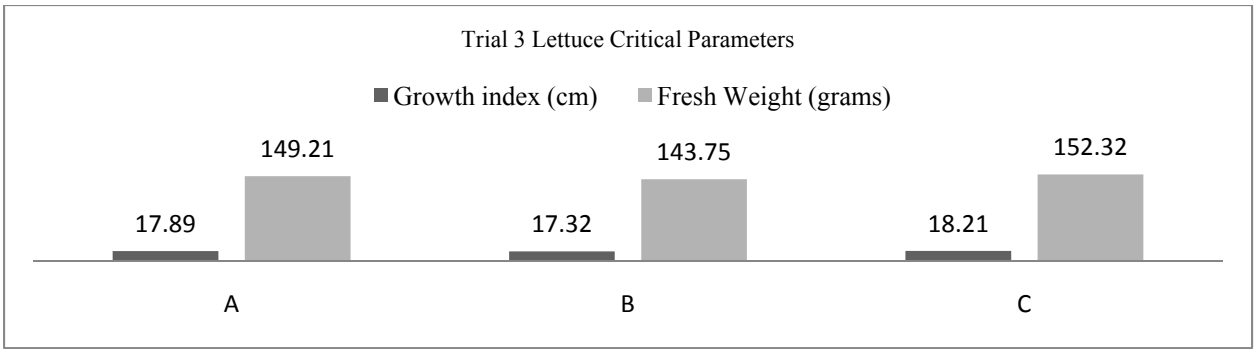


Fig. 26: Graphical representation of average growth indices of the third grow out trial of *Lactuca sativa L.* 'Charles' under three treatments. Treatments: A (bio-floc effluence with solids), B (bio-floc effluence without solids) and C (commercial hydroponic solution), from 2nd of August 2010 until the 30th of August 2010.

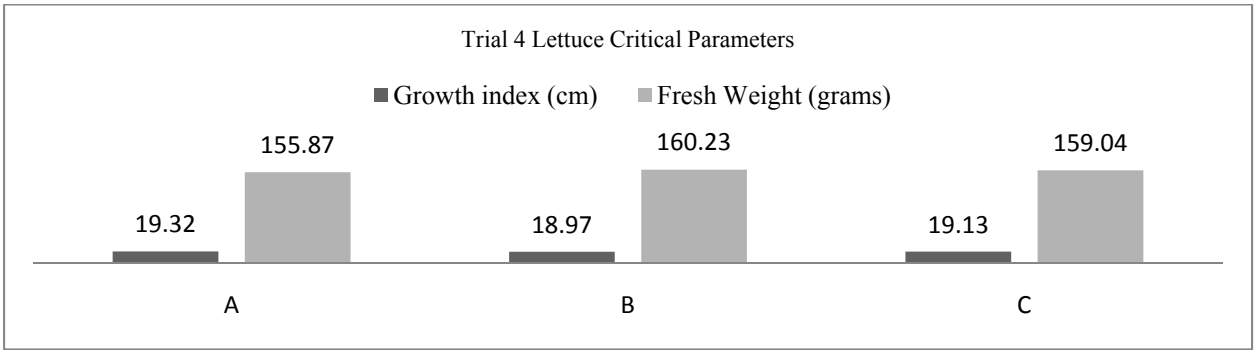


Fig. 27: Graphical representation of average growth indices of the fourth grow out trial of *Lactuca sativa L.* 'Charles' under three treatments. Treatments: A (bio-floc effluence with solids), B (bio-floc effluence without solids) and C (commercial hydroponic solution), from 22nd of August 2010 until the 20th of September 2010.

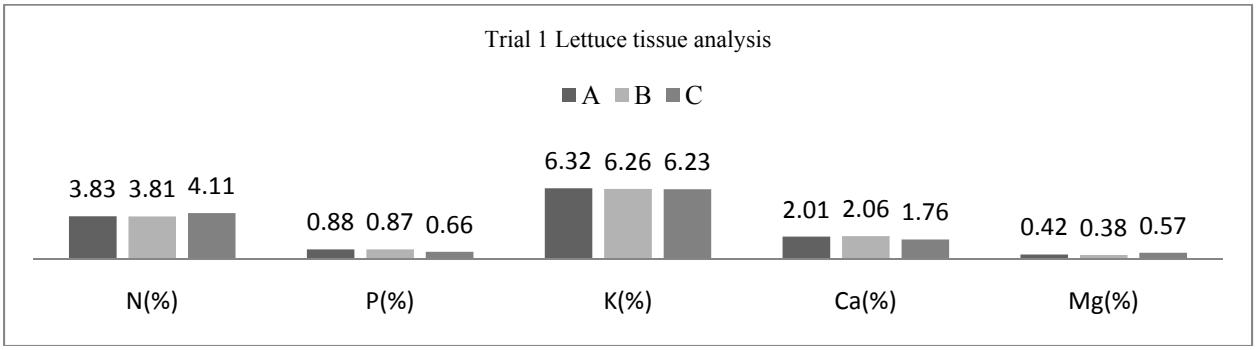


Fig. 28: Graphical representation of average tissue analysis of *Lactuca sativa L.* 'Charles' produced during trial 1.Treatments: A (bio-floc effluence with solids), B (bio-floc effluence without solids) and C (commercial hydroponic solution), from 10th of April 2010 until the 3rd of May 2010.

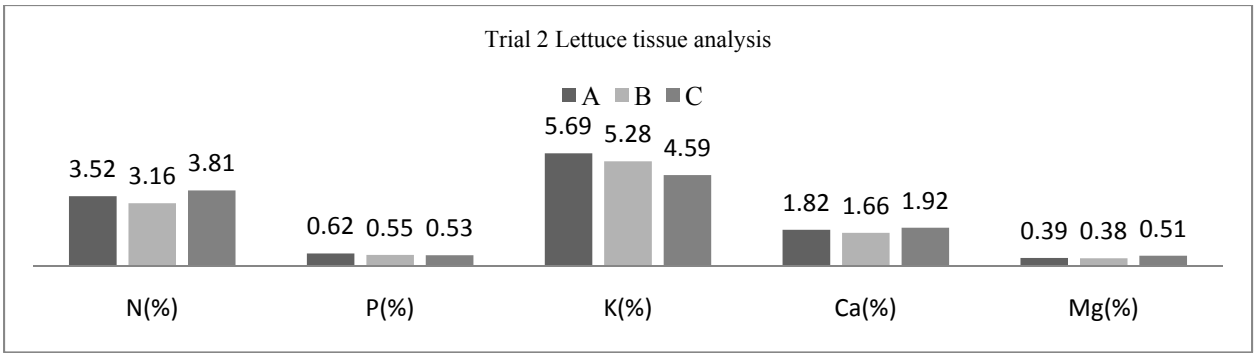


Fig. 29: Graphical representation of average tissue analysis of *Lactuca sativa L.* 'Charles' produced during trial 2.Treatments: A (bio-floc effluence with solids), B (bio-floc effluence without solids) and C (commercial hydroponic solution), run from 23rd of April 2010 until the 17th of May 2010.

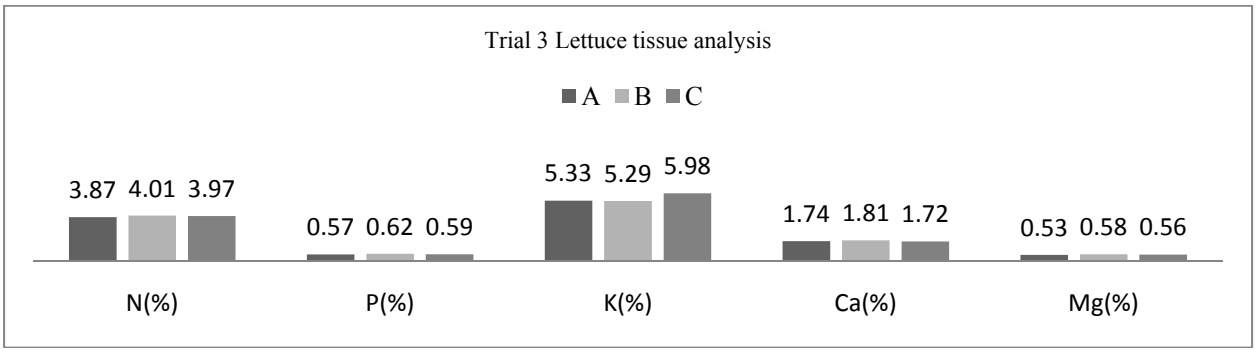


Fig. 30: Graphical representation of average tissue analysis of *Lactuca sativa L.* 'Charles' produced during trial 3.Treatments: A (bio-floc effluence with solids), B (bio-floc effluence without solids) and C (commercial hydroponic solution), run from 2nd of August 2010 until the 30th of August 2010.

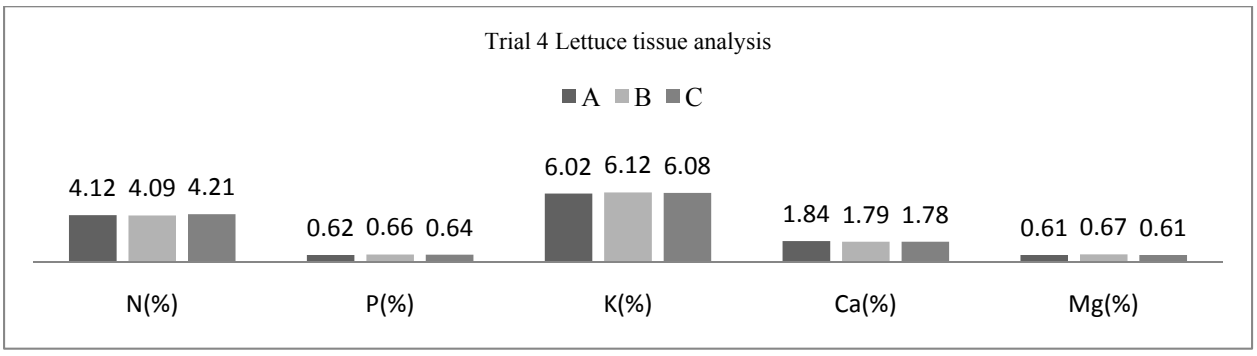


Fig. 31: Graphical representation of average tissue analysis of *Lactuca sativa L.* 'Charles' produced during trial 4. Treatments: A (bio-floc effluence with solids), B (bio-floc effluence without solids) and C (commercial hydroponic solution), run from 22nd of August 2010 until the 20th of September 2010.

Analyzing lettuce tissue from the 4 trials revealed that all variables tested (N, P, K, Ca and Mg), were not different between treatments in each trial or between trials. Literature articles discussing mineral absorption mentioned that minerals are absorbed from the soil or water media

in proportion to their ratios in plant tissue. The only mineral that may show luxury consumption is P (Bugbee, 2004). Between the 4 trials we observed mean N, P, K, Ca and Mg levels of approximately 4%, 0.6%, 6%, 1.8% and 0.5% respectively. The conclusion is that when one mineral is in limited supply, or if a non-tested mineral or micro mineral is limited (iron is an example), the uptake of all other nutrients will decrease to maintain the inherent mineral ratio within the tissue. This can be seen by comparing trials 1 and 2; although, in trial 1, the nitrate level in the fish effluent was approximately 32.06 mg/L, in trial 2 it decreased to 18.7 mg/L. The N content in the tissue of both trials however, remained at 3.5 to 3.8%. Furthermore, comparing these findings to the commercial hydroponic solution with nitrate level around 130.05 mg/L, lettuce with a tissue content of 3.81% N was produced confirming the importance of having a balanced mineral nutrient solution. Any limited nutrient imposes an impact on the use of other minerals present.

Present recommendations will be made for the ideal levels of the critical nutrient variables in the fish effluent to commercially produce marketable lettuce under two conditions: 1) daily water exchange, i.e., fresh supply of water and minerals from the fish system; and 2) a single batch fish effluent for the whole cycle, i.e., lettuce will be grown in the same batch of fish effluent over the entire 4-week growth period. As mentioned earlier, the average lettuce absorption of minerals is 276 mg $N\text{-}NO_3$, 78 mg of SRP, 420 mg of K and 126 mg of Ca provided other essential elements are in sufficient amount. Taking into consideration that hydroponic test beds averaged 6 liters of water volume for each lettuce plant then $N\text{-}NO_3$, SRP, K and Ca concentrations should be started at 46 mg/L, 13 mg/L, 70 mg/L and 21 mg/L respectively, and would be absorbed completely by these lettuce.

However the minimum threshold for each mineral (25 mg/L $N\text{-}NO_3$, 20 mg/L SRP, 30 mg/L K and 20 mg/L Ca) must be added (Wheeler and Sager, 1999) to provide in total 71 mg/L $N\text{-}NO_3$, 33 mg/L SRP, 100 mg/L K and 41 mg/L Ca for each lettuce plant in a 6 liter volume if a lettuce plant is to be produced without water exchange. By this criteria, the minerals found in the solution are sufficient to produce commercial quality lettuce if iron is maintained at 3 mg/L (Rakocy et al., 2007) and other micro minerals are not limiting. If exchanging water is to be used, it is necessary to maintain a minimum mineral level equal to the threshold levels mentioned above and to provide the other minerals needed by the lettuce. Hence, for daily water exchange (over a period of 28 days) the recommended levels of $N\text{-}NO_3$, SRP, K and Ca are 27 mg/L {(46 mg/L ÷ 28) + 25 mg/L}, 21 mg/L, 33 mg/L and 21 mg/L, respectively. Over the cycle of 28 days, the threshold would be maintained and lettuce nutrient requirements would be met. More work needs to determine the nutrient requirements for different stages of the lettuce growth cycle to properly provide it "just-in-time".

Linking these results with those of the effluent water characterization section, an approximate nutrient level in the water for proper lettuce production can be established. The task for aqua-culturists is to adjust feeding rates and water exchange rates using the model created for reaching these levels. The general literature discussing hydroponic vegetable production using aquaculture effluent, states that the following optimal mineral mix is needed in fish effluent in order to produce plant crops efficiently: Ca, 10-82 mg/L; Mg 0.7-12.9 mg/L; K 3-192 mg/L; nitrate 0.4-82 mg/L; soluble P 0.4-15 mg/L (Rakocy, 2002) which mimics the results of this study in which the concentration of critical nutrients fall within the ranges suggested. The exception is soluble reactive P; where we suggest a rate of 21 mg/L as compared to 15 mg/L suggested by Rakocy (2002).

The findings of the present study can be summarized up as follows:

1. Removing solids from the fish production water did not make a difference in the growth rate of lettuce.
2. Potassium, calcium and iron supplementation was critical for commercial quality plants and rate of growth up to the recommended level for each when effluent water held reduced nutrient levels.
3. Establishing a staggered system for both fish production and crop (lettuce in this case) production is critical to ensure a stable rate of nutrient availability from fish feeding to maintain a balanced and predictable nutrient flow and resulting plant output.
4. Ensuring efficient and adequate aeration in the lettuce grow bed is critical for lettuce growth. Since if water is left still, suspended solids in the system will become a problem limiting lettuce plant growth.
5. Adjusting of the pH in the system is important; a pH point appropriate for both plants and fish is necessary. A pH of 7 is ideal; a pH below 6.2 will not be as healthy for fish (in this case tilapia), while a pH of 7.5 or above promotes mineral precipitation.

Additional research is needed for development of a uniform staggered production system and the key guiding principles to advance integrated systems development and construction while always considering local climatological conditions and, most importantly, the chemistry of the original water and target species for culture.

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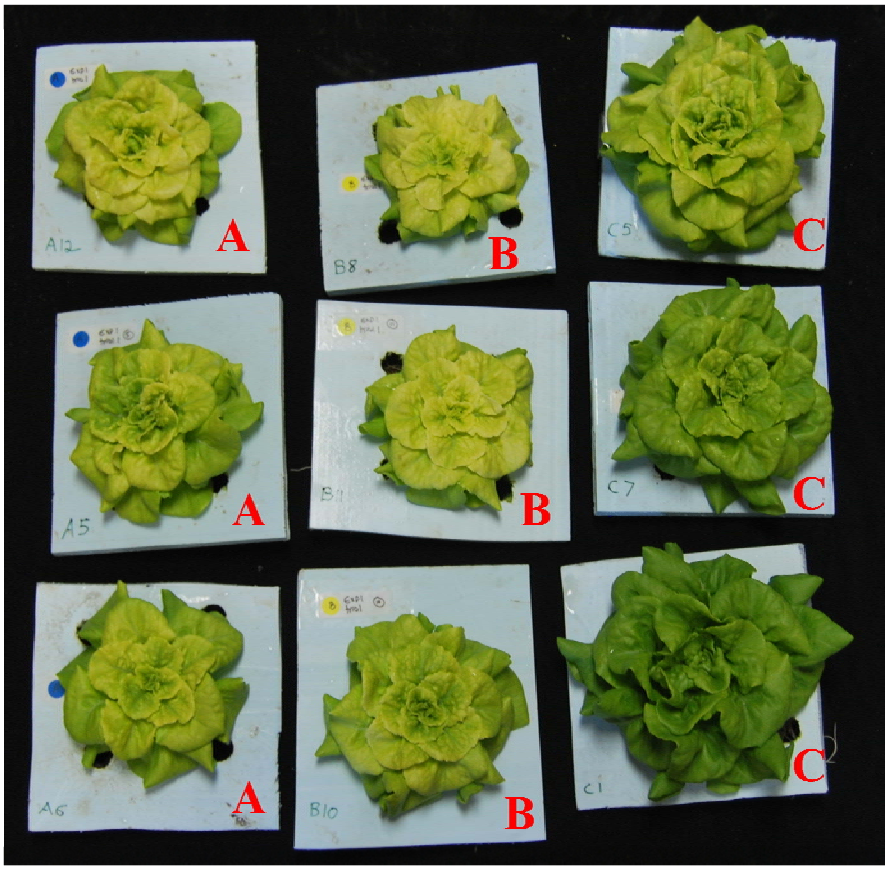
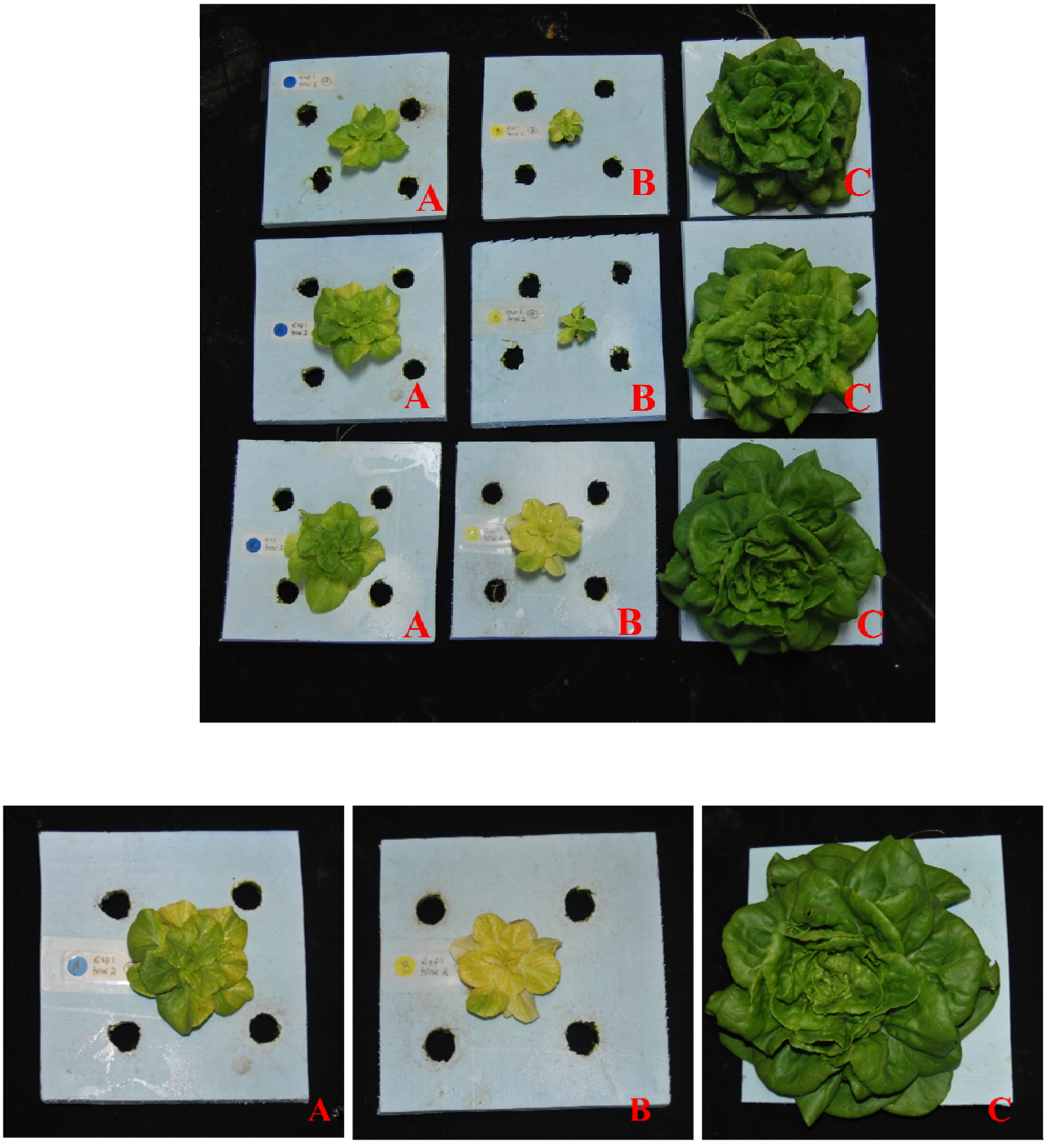
APPENDIX A

Fig. 32: Trial 1 Comparison of *Lactuca sativa L.* 'Charles' under three different treatments.



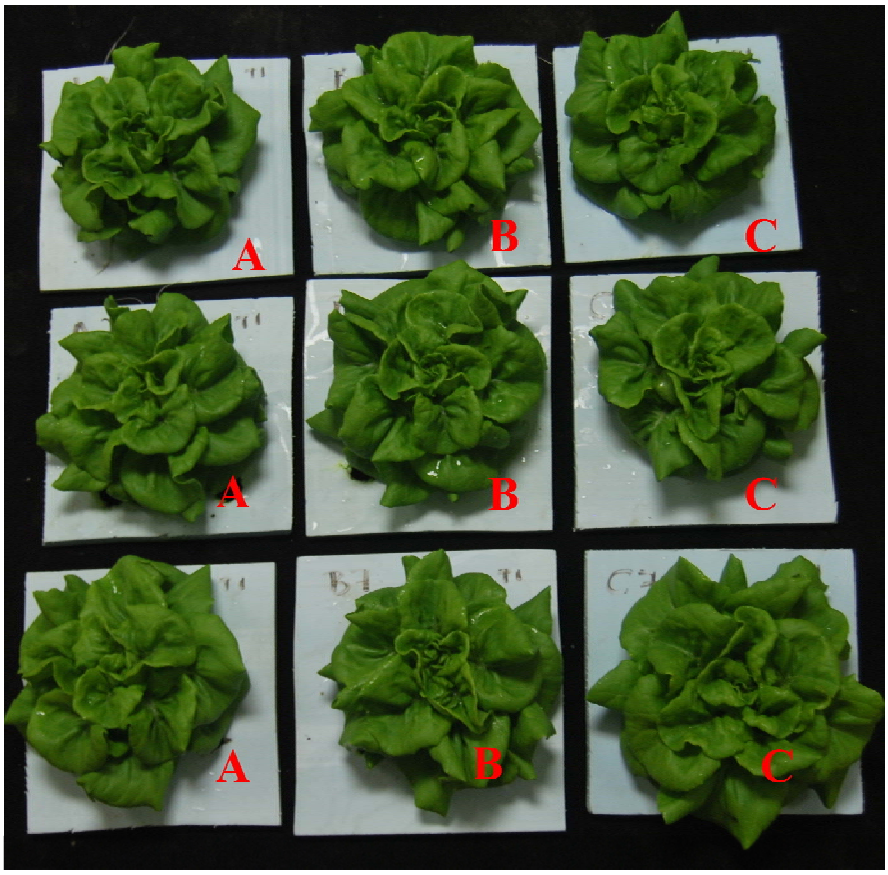
A: fish effluent water with solids; B: fish effluent water without solids; C: commercial hydroponic solution

Fig. 33: Trial 2 Comparison of *Lactuca sativa L.* 'Charles' under three different treatments.



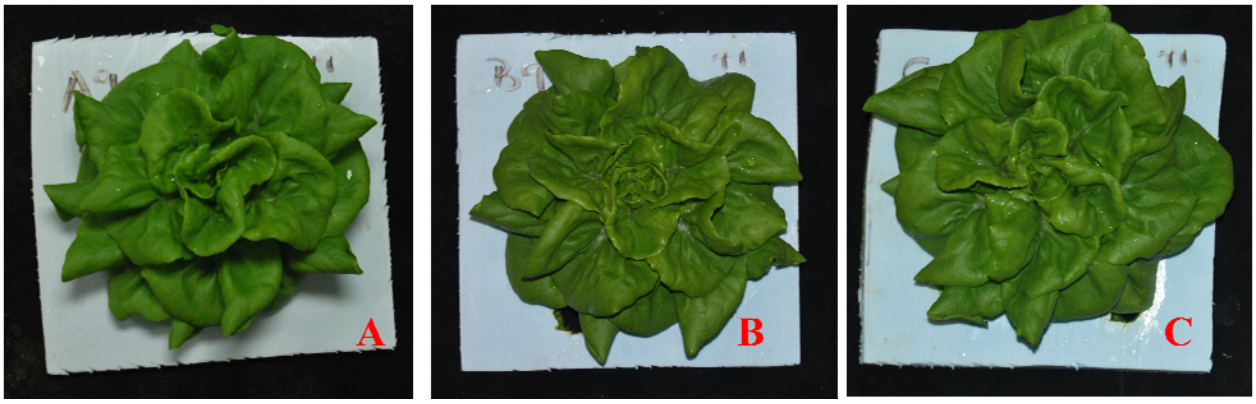
A: fish effluent water with solids; B: fish effluent water without solids; C: commercial hydroponic solution

Fig. 34: Trial 3 Comparison of *Lactuca sativa L.* 'Charles' under three different treatments.



A: fish effluent water with solids; B: fish effluent water without solids; C: commercial hydroponic solution

Fig. 35: Trial 4 Comparison of *Lactuca sativa L.* 'Charles' under three different treatments.



A: fish effluent water with solids; B: fish effluent water without solids; C: commercial hydroponic solution

APPENDIX B

Estimating nutrient output in bio-floc systems:

- Step 1 – Calculate water volume exchanged in liters **(A)** = (System volume in m^3) X (exchange rate (%) / 100) X (1000 L/m^3).
- Step 2 – Calculate system feed added in Kg **(B)** = (Biomass in Kg) X (feed rate (%) / 100).
- Step 3 – Develop the ratio of Kg of feed / Liter of water exchanged **(C)** = (B) / (A).
- Step 4 – Use each of the formulas below to develop an estimate value of the each corresponding nutrient in the water volume exchanged.

Nutrient - Unit	**Corresponding formula to be used**
Nitrite – mg/L	C x 15.31
Nitrate – mg/L	C x 2,822.05
Total ammonia nitrogen – mg/L	C x 141.3
Soluble reactive phosphorus – mg/L	C x 1922.4
Potassium – mg/L	C x 4330.4
Total Hardness – mg/L	C x 21,483.5
Total Alkalinity *Reduction* – mg/L	C x 679.5
Ca – mg/L	C x 3,629.1
EC - µmhos/cm	C x 184,085
TSS – mg/L	C x 20,710.5

- Note: The system is estimated to achieve the values estimated using the formulas when the whole water body has been exchanged.

Estimating nutrient output in polygyser systems:

- Step 1 – Calculate water volume exchanged in liters **(A)** = (System volume in m^3) X (exchange rate (%) / 100) X (1000 L/m^3).
- Step 2 – Calculate system feed added in Kg **(B)** = (Biomass in Kg) X (feed rate (%) / 100).
- Step 3 – Develop the ratio of Kg of feed / Liter of water exchanged **(C)** = (B) / (A).
- Step 4 – Use each of the formulas below to develop an estimate value of the each corresponding nutrient in the water volume exchanged.

Nutrient - Unit	Corresponding formula to be used
Nitrite – mg/L	C x 182.6
Nitrate – mg/L	C x 1,689.1
Total ammonia nitrogen – mg/L	C x 1,422
Soluble reactive phosphorus – mg/L	C x 774.3
Potassium – mg/L	C x 650.71
Total Hardness – mg/L	C x 10,086
Total Alkalinity *Reduction* – mg/L	C x 32,382.5
Ca – mg/L	C x 12,233.33
EC - µmhos/cm	C x 7,356.04
TSS – mg/L	C x 279.75

- Note: The system is estimated to achieve the values estimated using the formulas when the whole water body has been exchanged.

Estimating nutrient output in opposing flows systems:

- Step 1 – Calculate water volume exchanged in liters **(A)** = (System volume in m^3) X (exchange rate (%) / 100) X (1000 L/m^3).
- Step 2 – Calculate system feed added in Kg **(B)** = (Biomass in Kg) X (feed rate (%) / 100).
- Step 3 – Develop the ratio of Kg of feed / Liter of water exchanged **(C)** = (B) / (A).
- Step 4 – Use each of the formulas below to develop an estimate value of the each corresponding nutrient in the water volume exchanged.

Nutrient - Unit	Corresponding formula to be used
Nitrite – mg/L	C x 79.61
Nitrate – mg/L	C x 1,643.6
Total ammonia nitrogen – mg/L	C x 1,674
Soluble reactive phosphorus – mg/L	C x 269.4
Potassium – mg/L	C x 594.55
Total Hardness – mg/L	C x 17,650.5
Total Alkalinity *Reduction* – mg/L	C x 9,486.55
Ca – mg/L	C x 26,489.2
EC - µmhos/cm	C x 9,787.55
TSS – mg/L	C x 336.28

- Note: The system is estimated to achieve the values estimated using the formulas when the whole water body has been exchanged.