1 Introduction

1.1 Introduction

The most growing challenge in world is to provide food security and nutrition to 6.4 billion people in the use of natural resources with sustainable development in economic and environmental terms in context of climate change, economic and financial uncertainty, with growing competition for natural resources (UNWFP, 2005).

From prehistoric times, oceans, lakes and rivers have been a major source of aquatic food that contributes to the nutritional, economic and social well-being. However the natural resources are limitedly renewable and need to manage properly for sustainable development. The development of advance fishing gear cause dramatic lose in aquatic food, because of illegal, unreported and unregulated fishing and other abusive fishing techniques (FAO, 2011b).

Fish contain all the essential amino acid, and outstanding source of essential fatty acid. It is also rich source of vitamins (D, A and B) and minerals (including calcium, iodine, zinc, iron and selenium). Fish contains hydroxyapatite that can heal bone injury rapidly, unsaturated fatty acids (EPA, DHA and HUFA) is highly protective against cardiovascular disease, foetal and infant development of the brain and nervous system. So its valuable nutrients can helps in unbalanced diet that can countering obesity. It is a cheap and easily available major animal protein sources for the developing world (FAO, 2014).

Microorganisms are outnumbering than all other organisms in both biomass and diversity. They are a major part of global ecosystem services and natural capital in the world. Their diversity plays an important role to maintain biosphere, and provides large amounts of resources. Microbes are the primary producer, without them there will be no any living creatures on the earth (Zhou et al., 2009). Microbes are very much significant in energy and nutrient transfer in any ecosystem. The biogeochemical cycle can‘t be complete without microbe‘s activities. The metabolism of microbes is very complicate and proceeds in many extreme condition (Carlson et al., 2007).

1.2 World aquaculture

Aquaculture is a rapidly growing food producing sector that grown 8.9% per year since 1970, where 1.2% in capture fisheries and 2.8% in terrestrial farmed meat production. The annual amount of fish used as direct aquaculture feed in the Asia-Pacific region in 2004 was in the range of 2.47 to 3.88 million tons (FAO, 2016).

In 2014, the global aquaculture production is 101.1 million tonnes in live weight. The farmed aquatic plants were 27.3 million tonnes ($5.6 billion) and fish harvested from aquaculture was 73.8 million tonnes ($165.8 billion). The aquaculture production consisting of 49.8 million tonnes (44.1%) of finfish ($99.2 billion), 16.1 million tonnes of molluscs ($19 billion), 6.9 million tonnes of crustaceans ($36.2 billion), and 7.3 million tonnes of other aquatic animals including frogs ($3.7 billion) (FAO, 2016).

In 2014, global total capture fishery production was 93.4 million tonnes. Capture production in marine waters was 81.5 million tonnes (Northwest Pacific has 21.4 million tonnes 27% , Western Central Pacific has 12.4 million tonnes 15%, the Southeast Pacific has 8.9 million tonnes 11%, and the Northeast Atlantic has 8.4 million tonnes 10%), and in inland waters were about 11.9 million tonnes in 2014 (FAO, 2016).

It was estimated that 56.6 million people were engaged in the primary sector of capture fisheries and aquaculture in 2014, among them 36% were engaged full time, 23% were part time, and the remaining were either occasional fishers or of unspecified status. Moreover, fish provided more than 3.1 billion people with almost 20% of their average per capita intake of animal protein (FAO, 2016)

1.3 Nepali Aquaculture: Its growth and development

In Nepal, aquaculture has not a long history. In past, the certain group/cast people were involved in capture fisheries and completely depend on river, pond and lakes. The course of aquaculture was remarkably changed after commencement of Aquaculture Development Programme which was supported by ADB, UNDP and FAO in 1981-82. Since then the aquaculture was start to grow in Nepal. Although 185 species of indigenous fish have been reported in Nepal, fish culture was restricted to 7 carp species mostly. These include indigenous major carps: Rohu (*Labeo rohita*), Mrigal (*Cirrhinus mrigala*), Catla (*Catla catla*) and exotic carps: Silver carp (*Hypophthalmicthys molitrix*), Big head carp (*Aristichthys*

nobilis), Grass carp (*Ctenopharyngodon idella*) and Common carp (*Cyprinus carpio*). Besides that Nile tilapia (*Oreocromis niloticus*), Rainbow trout (*Onchorhynchus mykiss*), Catfish (*Clarias gariepinus*) are also culture. Asala (*Scizothorax* sp), Katle (*Neolissochilus hexagonolepis*), Mahseer (*Tor tor*), Silver barb (*Puntius gonionotus*) and Giant freshwater prawn (*Macrobrachium rosenberghii*) are under experimental study (SAARC, 2012).

Table I: The fishery production in Nepal

S. No.	Particular	Total area (ha.)	Fish Production (mt.)	Productivity
1	Fish production from aquaculture practices			
1.1	Pond fish culture	8,600	37,427	4.35
1.2	Other area (Ghols)	2,900	5,220	1.8
1.3	Paddycum fish culture	100	45	0.45
1.4	Cage fish culture	70,000	350	
1.5	Enclosure fish culture	100	140	1.4
1.6	Trout fish culture	12,000	192	
			43,374	
2	Fish production from capture fisheries			
2.1	River	395,000	7,110	0.02
2.2	Lakes	5,000	850	0.17
2.3	Reservoirs	1,500	385	0.26
2.4	Marginal/ swamps/ ghols	11,100	5,990	0.17
2.5	Low land irrigated paddy field	398,000	7,165	0.02
			64,874	
Seed production (_000)				
3	Fish seed production			148,501
3.1	Public sector			31,982
	a. Hatchling			132,296
	b. Fry			15,462
	c. Fingerling			8,260
3.2	Private sector			
	a. Fry			116,519

(FRD, 2015)

1.4 Rainbow Trout culture in Nepal

The first salmonid fish was introduce in Nepal was made on 1969 by importing Atlantic salmon (*Salmo salar*) and brown trout (*S. trutta*) from Kashmir, India, and rainbow trout (*Onchorynchus mykiss*) from the United Kingdom, and sockeye salmon (*O. nerka*) from Japan in 1979-80, which were not successful (Gurung and Basnet, 2003). After Recognizing the significance of trout in cold water fishery in Nepal, 50,000 eyed-eggs of *Oncorhynchus mykiss* were again imported form Miyazaki, Japan in 1988 and then intensive studies on rainbow trout farming was started. In 2006/07, the JICA Soft type follow up project provides further studies on seed production, capacity building, demonstration of trout production processes and technology dissemination (Wagle et al., 2013).

Nepal has high potential in trout culture as abundant cold-water resources in the hill and mountain region (54 districts). Nowadays the institutes for trout farming technologies development, scaled up in 16 hill districts, 85 private trout farms are in operation. Farming of rainbow trout in flowing through system that use raceways in common practice. Most of trout farm adopted series type raceways system (Wagle et al., 2013).

It is a potential cash crop in mountain region niche. The Government of Nepal include Trout Culture in One Village One Product in commodity of Nuwakot, Sindhupalchok and Rasuwa districts that for in-situ participatory research and subsidy in trout seed management (hatchery strengthening), capacity building (specialized and resource person training, observation tours, interactions), technology dissemination (publication, audio-visual production, training, demonstration) (Wagle et al., 2013).

The introduction of trout have not invasion into indigenous cold water fishery (particularly *Schizothorax* sp.). Previously it was considered that trout predation may cause serious impact on indigenous cold water fishery. But in Nepali cold water Asala were not affected by the presence of trout in natural waters. Trout and Asala can co-exist in same environment. The trout are not prolific breeder and need a specific habitat to spawn in the natural environment and even if trout are stocked and succeeded to reproduce naturally in cold waters, native species have not impacts of trout (Gurung and Basnet, 2003).

1.5 Microbes in aquaculture

Microorganisms play important role in aquaculture, particularly in productivity, nutrient recycling, and feed for the cultured species. Microorganism contributes in maintain water quality, disease control and aquaculture effluent management, that help in minimize environmental impacts of the aquaculture (Moriarty, 1997). The phytoplankton is chief source of oxygen in aquaculture. Extensive, semi-intensive or some intensive aquaculture systems, mostly depend on microbial food. The planktons are directly consumable food resource and detritus bacteria indirectly contribute via decompose organic matter and release inorganic nutrients that stimulate primary production (Moriarty, 1985).

1.6 Aquaculture Effluents

The rapid growth in aquaculture was achieved by development of intensive aquaculture with a high-stocking density fish pond. But in such practice has a limited self-purification that arise the environmental impacts. The major problems are disease transmission, nutrient-loaded effluent, prophylactic use of chemicals including antibiotics, massive water use (FAO, 2016).

The major sources of pollutant in aquaculture are chemical that used for maintaining water quality, drugs for disease control (Mugg et al., 2007) aqua feeds comprise a solid particulate fraction including uneaten and undigested feed, and a dissolved fraction comprising metabolic products, principally ammonia, urea and phosphate (Hargreaves, 1998).

Basically, the discharge of aquaculture effluent increase in suspended solids as well as dissolved nutrient. The suspended organic matter increase biological oxygen demand due to degradation of organic matter via bacteria. The rapid consumption of oxygen cause anoxic condition. The anoxic condition is detrimental to any aquatic organism. The dissolved nutrients are mostly nitrogen and phosphorus. These nutrients affect benthic fauna, plankton and bacterial communities growth diversity and abundance (Steicke et al., 2011). The effects of effluent mostly depend upon temperature of water that influence in metabolism of aquatic fauna (Ariel and Jutta, 2014). Anoxic condition cause breakdown of calcium-carbonate-phosphate [$Ca_3(HCO_3)_3PO_4$] at sediment in bottom and recycle the phosphate into water that also cause rise in phosphate level in water which initiate eutrophication (Avnimelech, 1983).

The uses of antibiotics to cure pathogen also affect the non-targeted bacterial that play significant roles in aquatic environments (Kawasaki et al., 2016).

Aquaculture has some serious concerns are the following:

(a) Destruction of wetlands and other sensitive aquatic habitat by aquaculture projects;

(b) Conversion of agricultural land to ponds;

(c) Water pollution resulting from pond effluents;

(d) Excessive use of drugs, antibiotics, and other chemicals for aquatic animal disease control;

(e) Inefficient utilization of fish meal and other natural resources for fish and shrimp production;

(f) Salinization of land and water by effluents, seepage, and sediment from brackish water ponds;

(g) Excessive use of ground water and other freshwater supplies for filling ponds;

(h) Spread of aquatic animal diseases from culture of organisms to native populations;

(i) Negative effects on biodiversity caused by escape of non-native species introduced for aquaculture, destruction of birds and other predators, and entrainment of aquatic organisms in pumps; and

(j) Conflicts with other resource users and disruption of nearby communities. Fertilizers and feeds are applied to ponds to promote shrimp and fish production, and normally, no more than 25% to 30% of the nitrogen and phosphorus applied to ponds in fertilizers and feeds is recovered in fish or shrimp at harvest (Boyd, 2003).

1.7 Concept of microbes in Nitrogen management

The aquatic animals are ammonotelic and due to toxic nature of ammonia, it gets more attention than other aquaculture effluents. About 20 to 30% of the nitrogen in feed was assimilated by fish, remaining were release to the culture environment as waste (Hargreaves, 2013). The major elimination of ammonia from culture system was via phytoplankton uptake during photosynthesis, autotrophic nitrification and heterotrophic bacterial assimilation (Ebeling *et al*., 2006). The loss of ammonia through volatilization and nitrification are minor (Hargreaves, 1998).

The algal cell consumes 0.7g N/m^2/day of ammonia to produce oxygen of 10.7g/m^2 of oxygen with 4g C/m^2/day of carbohydrate. The nitrification and denitrification are closely coupled in aquatic sediments and limited by oxygen. The heterotrophic bacterial uptake of nitrogen depends upon C: N and oxygen. The bacteria are fed on organic carbonaceous substrates along with nitrogen, to produce the protein that for cell growth and multiplication (Avnimelech, 2003).

1.8 Ammonia Nitrogen removal techniques

The ammonia can be managed in several way but economic mean most be considered on the basis of species cultured. The idea about the general water quality of the pond is essential before any modification or manipulation in aquaculture systems.

The microorganism treatment for removal of ammonia nitrogen was via outside cultural unit (Earthen treatment ponds or bio filtration) or within culture unit (Bio-flocs, periphyton) (Crab et al., 2007).

1.7.1 Stop feeding or reduce feeding rate

The water quality can be significantly improve only by reducing daily feeding i.e.>50kg/ha/day in species that can feed natural food (Yeo et al., 2004). The reduction of nitrogen contain i.e. protein in feed can reduce ammonia excretion (Hargreaves and Tucker, 2004).

1.7.2 Add ion exchange materials

Zeolites are naturally occurring, synthetic minerals, crystalline hydrated alumino-silicates. Zeolites are used for cation exchange, molecular sieving, and catalysis processes. Zeolites are also use to remove ammonia nitrogen in aquaculture (Zhou, 2015) that assumes to enhance growth of rainbow trout (Danabas and Altun, 2011).

Ion-exchange resin method, based on separation of ammonia from water through an ion-exchange resin, which can regenerate by electrochemical ammonia oxidation instantaneously through chemical desorption (Gendel and Lahav, 2013).

1.7.3 Nitrification

In aquatic sediment, nitrifying microbes remove the ammonia from aquatic media via nitrification and denitrification that release nitrogen molecule. Here, inorganic carbon and oxygen are used to form a new microbial cell (Veuger et al., 2013). The autotrophic nitrifying and sulphur bacteria consume oxygen and carbon dioxide while oxidising ammonium, nitrite and sulphide respectively (Moriarty, 1997). The nitrification and denitrification are strongly coupled process and limited by availability of oxygen. The efficiency of N removal via this mechanism is quite small (Hargreaves, 1998). The growth of nitrifying bacteria is relatively slower than heterotrophic and in race way system they can be washout from culture system (Ebeling et al., 2006).

1.7.4 Add a source of organic carbon

The principle based on heterotrophic growth of bacteria that consume the ammonia along with the carbohydrate and oxygen. The heterotrophic bacteria require C: N of 15 to grow and consume the ammonia. In Addition, of organic matter to meet the ratio to promotes the immobilization of the dissolved ammonia in water. The process can occur in anywhere and oxygen is major limiting factor (Hargreaves, 2006).

1.9 Status of removal of ammonia via bacteria in aquaculture

When the ratio of carbon and nitrogen in aquaculture is 15-20, heterotrophic bacterial oxidised organic carbon for energy source and ammonia as raw material to form protein, thus nitrogen is converting into bacterial biomass (Crab et al., 2012).

The aggregate biomass of algae, bacteria, protozoa and organic debris are organised to form floc also called biofloc. Species such as shrimp and tilapia that can tolerate poor water quality and are filter feeder that consume biofloc and digest microbial protein. The dry weight protein content of biofloc is estimates between 30 and 45%. Fat content is estimates between 1 and 5% (Hargreaves, 2013).

The first biofloc was practice in shrimp culture by Ifremer-COP (French research institute for exploitation of the sea, oceanic centre of pacific) in the early 1970‘s. In late 1980‘s and early 1990‘s by Israel and the USA use same model in tilapia culture. In 1988, at the Sopomer

farm in Tahiti and Belize Aquaculture farm the first commercial application of biofloc technique in shrimp culture (Day, 2015).

The biofoc forming technique is also used in catfish (*Clarias gariepinus*) culture, where the addition of molasses enhance the water quality by forming biofloc and become source of natural feed that improve growth (Hapsari, 2016). In common carp (*Cyprinous carpio*) culture, the addition of molasses result higher growth and survival rate of than no treatment (Sartika, 2015).

In a study of fish fingerlings of tilapia reared under biofoc with limited water-exchange condition own less ectoparasites in gills and ectoderms mucous as compare to conventional water exchange system (Mauricio et al., 2013).

1.10 Objective

(a) General objective

To examine the sucrose treatment which intensifies microbe‘s activities that help to achieve sustainable trout culture

(b) Specific objectives

i. To improve the efficiency of feed.

ii. To explore the role of microbes in
water quality variation,
maintain potential beneficial level of ammonia in water

1.11 Rational of the study

The carnivorous trout culture requires the large amount of (45%) protein in their diet. The protein metabolisms cause the larger amount of ammonia in excreta. The ammonia has toxic effect of aquatic animal. So, the addition of soluble carbohydrate in culture medium enhance the heterotrophic microbial activities, that minimize the ammonia contain of culture medium. Also the low level of ammonia in trout culture causes the higher growth without increase in feed, because of non-essential protein formation mechanism. The non-essential amino acids are used as energy source for fish. Such process can be mile stone for sustainable trout culture as it can aid in minimize water exchange, lower amount of protein contain feed formulation, minimize water pollution, biosecurity and environmental impacts.

2. Literature review

2.1 Sustainable aquaculture

Historically, natural fishery has been views as a limitless resource. Today, fish constitutes the primary source of animal protein for over a billion peoples in world. The average fish consumption was increased from 9.9kg in the 1960s to 14.4kg in the 1990s and 19.7kg in 2013. The preliminary estimation show the consumption in 2014/15 grows to beyond 20kg. In 2013, the global population fish consumption was about 17% of the animal protein and 6.7% of overall protein consumed (FAO, 2016). The rapid expansion and intensification of aquaculture must consider the use of basic natural resource of water and land without damaging the environment along with economic and social sustainability (Crab et al., 2012).

Although aquaculture sector contribution surpassed wild caught fishery, total industrial compound aqua feed production was 7.6 million tons in 1995 and 29.3 million tons in 2008, such increment cause over fishing of non-commercial fish species (FAO, 2011b). The fish stocks of 31.4% were estimated as biologically unsustainable i.e. overfished (FAO, 2016). 4-5kg of wet fish will yield 1kg of fishmeal and 100g of fish oil (FAO, 2011b). The chemical use to improve water quality and antibiotic to treat disease with massive water exchange can harm wildlife (Mugg et al., 2007). The feed formulation with improper composition of fish requirement cause nutrient-loaded effluent can lead to eutrophication (Hasan, 2001).

In order to sustain the natural resource, conservation and reliable utilization that ensures attainment to continuous satisfaction of human needs for present and future generations. The major issues are increasing the feed efficiency; minimize water exchange, shifting of fishmeal with crop based ingredients (World Bank, 2007). The major targets to achieve sustainable aquaculture are 10% improved feed efficiency in input use, significant intensification, shifting energy supply, shifting species mix (higher share of freshwater species, lower share of marine species), and replacement of fishmeal and fish oil with crop based ingredients (Waite et al., 2014).

Salmonid fish are carnivorous fish that fed with high concentrations of fish and plant-based protein. Even plants as a source of protein have limitations, e. g. soybean meal have many anti-nutritional factors among others; protease inhibitors, phytic acid and saponins (Cho, 2001).

2.2 Nutrient in aquaculture

Nutrients that are ingested but not assimilate in animal‘s gut wall are releases as faeces, which are not usually available to primary producers. Heterotrophic Microbe decomposes and remineralises nutrient subsequently become available to primary producers. Excreted nutrient is most available for primary producers (algae and vascular plants) and heterotrophic microbes (bacteria and fungi). N and P of excrete are actually important for primary producers and heterotrophic microbes. The N: P in excreta depends upon N: P in feed and animal body composition (Vanni, 2002).

Commercial fish feeds typically contain excess P as all P in the feed is not available (digestible and absorbable) to the fish. Many common feed ingredients such as fish meal and soybean meal contain non-absorbable forms of P. Dietary P consume by fish, but not digest and absorb, will appear in the waste as faecal P (Flimlin et al., 2003). The P adsorb by sediment is an anion exchange process that decline 90% of P into sediment. The ortho-phosphate is only available P for plankton, measured 0.001-0.05ppm (Boyd, 1971). The phosphorus in pond is in low concentration as it hold equilibrium with calcium i.e. calcium-carbonate-phosphate [$Ca_3(HCO_3)_3PO_4$] that sediment in bottom (Avnimelech, 1983).

The aquaculture enriched with both nitrogen and phosphorus grows the highest concentration of phytoplankton. P: N requires of 1:6, show that phosphate is critical but not limiting as nitrogen (Gregory, 2007).

2.3 Dissolved oxygen in aquaculture

Dissolved oxygen is primary water quality factor that limits aquatic production. Good water quality is the key factor for the success of aquaculture that ensures the survival, growth rate and reproduction of the cultured species (Hargreaves, 2006). In most of ponds, the oxygen content of the water is governed by the activities of algae and bacteria. The algae produce oxygen by means of photosynthesis. Consumption of oxygen is predominantly by bacteria through the activity of the heterotrophic decomposition that alter the water quality factors, i.e. DO, pH, content of ammonia, alkalinity, etc. (Moriarty, 1997).

The lack of oxygen in aquaculture is detrimental to cultured species. The primary symptoms of anoxic (0% oxygen) or hypoxia (>30% oxygen) are stop feeding and unusually passive. Due to lack of oxygen in blood fish budge out the gill for more water exchange that

consumes further energy. The depletion of oxygen cause weakness fish and become more susceptible to disease. During hypoxia or anoxic the medication is more detrimental and must improve the oxygen level before medication. Dissolved oxygen levels can affect respiration of fish, as well as ammonia and nitrite toxicity. When the oxygen level is near saturation or even near super saturation, it increases growth, reduces feed conversion ratio and increases in volumes of fish production. When oxygen levels fall below 60% in the water, trout begins to lose appetite. Tissue hypoxia declines in swimming activities and reduces resistance to disease (Avkhimovich, 2013).

2.4 Trout culture effluent

The salmonid fish culture is potential source of aquaculture effluent. The species are carnivorous and need higher amount of protein in diet (Avkhimovich, 2013). The major source of energy is protein and lipid metabolism. The protein metabolism cause higher ammonia excretion and higher amount of oxygen is consumed (Cho, 2001).

In Nepal, the rainbow trout farmed in flowing through system that uses series raceways, the dissolved oxygen contain in water was decrease. However, the NO_2 and NO_3, NH_4 and PO_4 become rise in water. Such decline in water quality was reflected in fish mortality as higher mortality was observed in 15^{th} race way (FRD, 2014).

The physic-chemical parameter of water such as temperature, pH, conductivity, total hardness, and bicarbonate alkalinity change between inflow and outflow were within satisfactory standard limits for trout culture. However, ammonia nitrogen and phosphorus get rise in water (Pulatsül and Çamdeviren, 1999; Pulatsu et al., 2004).

2.5 Ammonia in aquaculture

The ammonia–nitrogen is the limiting factor for aquaculture production after dissolved oxygen (Ebeling, 2006). The ammonia produce in water via metabolism of protein, also enter from degradation of nitrogen-containing organic matter, fertilizer runoff, and industrial sources. In water, ammonia occurs in two forms: ionized ammonia, NH_4^+, and un-ionized ammonia, NH_3^0. The relative proportion of each form is dependent on pH, temperature and salinity (Hanna et al., 2013). The un-ionized ammonia is more detrimental because it is lipid soluble and is uncharged which makes it easy to move across the biological membranes

(Körner *et al*., 2001). The un-ionized ammonia diffuses back into fish via the gills and form NH_4^+ in blood. The ammonia toxicity increases in mucus secretion at gills cause chloride hyperplasia, tengiectasis on lamella and hyperemia on epithelial. The rainbow trout gills lamellae get deformed when expose to 0.1mg/l for 2 hours (Hanna et al., 2013).

The accumulation of ammonia causes reduction in glycogen stores, NADH and adenylates in fish tissues (McKenzie et al., 2003). The reduction metabolic energy cause hyperventilation began to sideways spiral swimming and to death (Hanna et al., 2013). Such interruption in energy production causes glycogen vacuolation in liver, physiologically hydropic degeneration, cloudy swelling, vacuolization and focal necrosis (Hanna et al., 2013).

The sub lethal exposure of ammonia causes glomerulonephritis and hyperemia in kidney (Hanna et al., 2013). NH_4^+ can replace K^+ in Na^+-K^+ - ATPase (NKA) and in Na^+/K^+ /$2Cl^-$ co-transport (Nabila et al., 2013).

The lethal concentration of ammonia in brain break glutamine starves the nerve cells of ATP, causing paralysis and eventually death (Robert et al., 2014).

Certain metabolic pathways are in fish through which ammonia detoxification could occur. Glutamate dehydrogenase fixes ammonia onto alpha-ketoglutarate to form glutamate, and glutamine synthetase enzyme convert glutamate into glutamine. Also the amino groups can be transferred from glutamate by transamination (Wood, 2004). Typically the appetite suppressing effects is been observed in rainbow trout that because of the acute hyperammonemia-associated reduction in food intake will protect fish from further toxicity (Van et al., 2005). The toxic effects of ammonia will observed once protective mechanisms, has been overcome. Such ability is not seen in tropical fishes (McKenzie et al., 2003). Such effect also observe in embryo of trout as there is no effect on total amino acid in embryonic tissue but yolk contain amino acid were lower than non-exposed. This prevents ammonia accumulation in embryonic tissue, by conversion of ammonia to urea in embryonic tissues (Shelby et al., 2001).

The protein requirement for trout is 25% in feed and more than that requirement is for energy source as the trout prefers protein. The addition of non-essential amino acid (such as alanine, glutamine, and glycine) in feed can increase the growth over the fish meal based diet (Cowey and Cho, 1993).

2.6 Microbes and its application for sustainable aquaculture

The largest fluxes of organic matter in the most of ecosystem, bacteria are responsible for a major aerobic respiration, all of the anaerobic respiration, and a large amount of the remineralization. In allochthonous organic input in ecosystem, the secondary production of bacteria can be co-equal or even larger than that of the primary production of phytoplankton. The oligotrophic aquatic ecosystems can be subsidized by terrestrially produced organic matters that maintain required net heterotrophic (Cole, 1999). The growth and productivity of microorganisms are limits by nutrient but not relate to consumer (Pace and Funke, 1991).

Nitrogen released to the culture system as waste can be incorporated into bacterial cells. Consumption of this microbial protein by culture species contributes to growth. That benefit is reflected in improved feed conversion ratio (Hargreaves, 2013). This fact can deliver a highly quality, safe, attractive and economically affordable intensive aquaculture system. The organic matter and dissolved oxygen requirement for minimum or no exchange of water can be artificially added. The diversity in microbial community improves better waste utilization and reduces pathogenic strain dominance (Avnimelech, 1999). The heterotrophic bacteria also eliminate H_2S, CH_4 and organic acids by redox reaction and convert into nutritionally useful organic matter through biogeochemical cyclic processes (Moriarty, 1985).

In aquaculture, the microbial can either be antagonistic to pathogen (bio control and probiotics), food or improves the water quality (Panigrahi and Mohapatra, 2010). For the disease management the microbial approach is innovative in contrast to antibiotic, antifungal and probiotic application. Microbes acts internally as well as externally against pathogen. Internally the synthesis of PHA can be beneficial as antibacterial and nutritive. PHA granules are synthesized under conditions of physiological and nutrient stress (Parlane, 2012). When these polymers are degraded in the gut, enzymatic hydrolysis could significantly accelerate its digestibility. The enzyme hydrolysis is carried out by extracellular depolymerizes activities which, acting as a preventive or curative protector against Vibrio sp. infections and stimulate growth of fish larvae (Mauricio et al., 2013).

The protein content of biofloc varies from 25 to 50%, mostly 30 and 45%. Fat content varies from 0.5 to 15%, with most estimates between 1 and 5% (Hargreaves, 2013).

Fin condition, gill histology, proximate composition, blood hematocrit and plasma cortisol levels of fish were not significant differ with normal culture (Azim and Little, 2008).

2.7 Heterotrophic microbes in aquaculture

The bacteria that utilize organic matter as energy source are heterotrophic bacteria. The bacteria act upon the complicate organic molecule and broke into simple molecule releasing the micronutrient contain. Heterotrophic bacteria feed on any form of organic material, such as solid fish waste, uneaten fish food, and even dead bacteria. Heterotrophic bacteria need high amount of DO. The naturally occurring fungi are also involved in decomposition of organic matter (Moriarty and Pullin, 1987). Thus heterotrophic microbes regulate most of ecosystem via intermediate or final product of metabolism. The active metabolism regulates biogeochemical cycling. It significantly contributes in microbial food chain (Pace and Funke, 1991).

The heterotrophic microbe has advantage over phytoplankton as sunlight is not the limitation. The continuous aeration can achieve less fluctuation in water quality and has a better stability over time (Hargreaves, 2006). Bacterial decomposition seems to be more important than fungal decomposition in aquatic systems (Moriarty, 1987).

Ammonia control through the heterotrophic pathway is often more stable and reliable than algal uptake or nitrification (Hargreaves, 2013), as bacterial nitrogen up take rapidly 10 times increased than nitrification (Crab et al., 2007). Heterotrophic microbes enhance water quality by maintaining the C: N of 12 to 15:1 that favours the heterotrophic pathway for ammonia control (Hargreaves, 2013).

In laboratory condition, sediment suspension modified with ammonium about 10mg/l, add twenty times higher amount of glucose, results almost all the ammonium disappeared after a period of about 2 hours, with no linked to production of NO_2 or NO_3((Avnimelech, 1999). This promote nitrogen uptake by bacterial growth decreases the ammonium concentration more rapidly than nitrification (Crab et al., 2012). The microbial biomass production is 40 times greater than nitrification (Ebeling et al., 2006).

Intensive heterotrophic bacteria growth in aquaculture systems can retain 7% feed N and 6% feed P. The use of activated sludge process to treat water in a recirculation system that produced sludge mix with grains for later re-use as fish feed for carps (Maurício et al., 2013). Despite of the biofloc culture system is successfully introduce in Shrimp, Tilapia, Cat fish and Carps fishery, in cold water fishery (trout culture) the technique has not introduce yet. Because of the suitable species for biofloc culture species that are adapted in high solid

content and mostly tolerate of poor water quality (Hargreaves, 2013). But in opposite the trout are very much sensitive to water quality (FAO, 2011a). Thus cultural system should be modified that make suitable for both species (trout and Heterotrophs) in cultural practice is made.

3. Materials and Methods

3.1 Materials

3.1.1 Experimental setup apparatus

a) Bucket (20 lit, 15 lit)
b) Water supplying
c) pipes with stopper
d) Siphoning Pipe
e) Pellet fish feed
f) Scoop net

3.1.2 Analytic apparatus

a) Conical flask (100 ml, 200 ml)
b) Test tube (30 ml)
c) Pipettes (0.5 ml, 1 ml, 2 ml, 5 ml, 25 ml)
d) Beakers (50 ml, 100 ml, 250 ml, 500 ml, 1000 ml)
e) BOD bottles (300 ml)
f) Aluminum foil
g) Measuring Cylinders (25 ml, 50 ml, 100 ml, 500 ml, 1000 ml)
h) Volumetric Flask (100 ml, 1000 ml)
i) Burette
j) Glass tubes (50 ml)
k) Borosilicate glass
l) Erlenmeyer flasks

3.1.3 Instrumental apparatus

a) Vernier Meter (6391A-LABQUEST2)
b) Weighing balance
c) Distillation plant (8532275)
d) Spectrometer (SSI UV-2101)
e) Refrigerator (Samsung-RR1915CCASA/IG)
f) Altimeter-Barometer (BKT 381/B381)
g) Incubator (ULM-500)
h) Electronic balance (H2K-FA110)
i) Magnetic stirrer (MLH)
j) Thermometer

3.1.4 Chemicals for water quality test

1. Manganese sulphate ($MnSO_4.4H_2O$)
2. Potassium iodide (KI)
3. Sodium azide (NaN_3)
4. Sodium hydroxide (NaOH)
5. Sulfuric acid ($H_2SO_{4)}$)
6. Starch
7. sodium thiosulphate ($Na_2S_2O_3.5H_2O$)
8. potassium iodate (KIO_3)
9. Sodium salicylate ($HO.C_6H_5.COONa$)
10. tri sodium citrate ($Na_3C_6H_5O_7.2H_2O$)

11. Sodium hypochlorite (NaOCl)
12. Ammonium chloride (NH_4Cl)
13. Sodium nitroprusside ($Na_2(Fe(CN)_5NO)_2H_2O$)
14. EDTA (Ethylene-diamine-tetraacetic-acid)
15. Bromcresol green
16. Ascorbic acid
17. Ammonium molybdate ($(NH_4)_6Mo_7O_{24}\cdot 4H_2O$)
18. Potassium antimonyl tartrate ($(K(SbO)C_4H_4O_6.^1/_2H_2O$)
19. Phosphate (KH_2PO_4)
20. Cupric sulphate ($CuSO_4.5H_2O$)
21. Zinc sulphate ($ZnSO_4.7H_2O$)
22. Hydrazine solution ($HS.(NH_2)H_2SO_4$)
23. Sulphaniamide ($C_6H_4SO_2(NH_2)_2$)
24. Sodium carbonate (Na_2CO_3)
25. Potassium nitrate (KNO_3)
26. Magnesium sulphate ($MgSO_4.7H_2O$)
27. Eriochrome Black T
28. N-(1-naphthyl)-ethylenediamine dihydrochloride ($C_{10}H_7HNCH_2CH_2NH_2.2HCl$)

3.2 Experimental setup

The research was conducted in FRD, Godawari, Lalitpur, Nepal (Latitude $27^0$36‘9.65‘‘N, Longitude $85^0$23‘27.54‘‘E, 1574.90 m). A five cm diameter circular pipe was use as main pipeline to supply the water. The six circular pipes of one cm diameters were used to divide the water from main pipe to each individual bucket. The rectangular steel frame with net was used to cover whole experimental setup and also to hold the pipeline at uniform height to ensure the equal amount of water supply at instantaneous period. Each bucket was also covered with wooden frame with PVC net that prevent the entrance of outer material and also to escape the fish. The buckets were perforated at height of 18 cm for outlet of water. The five holes of 3mm diameter were made at five sides of each bucket. Each buckets were supplied with water of 43±2.5ml/sec.

The completely randomized block with treatment of sucrose and non treatment was carried to find out the effectiveness of treatment of sucrose in water to minimize the TAN in water in trout culture. Also the effect of TAN on growth of fish. Triplicates were maintained for each treatments.

Photograph I: Experimental setup maintained in the outdoor rearing facilities in FRD

3.3 Fingerling maintenance and assessment

Fry of Rainbow Trout (*Oncorhynchous mykiss)* was supplied from Trisuli Fishery Research Centre to FRD. The fry were initially feed with boiled hen egg albumin for month to acclimatize in the environment of Godawari. The bucket was disinfected with saltwater over night. Twenty-five numbers of fingerlings having an individual weight of 0.8±0.05 g were taken and transfer to bucket where it was again left for 4 days to acclimatize. The sucrose treatment was done after proper acclimatized that shown by proper feeding intensity. The feed composition was shown in table II. In addition the fingerlings were feed with buffalo liver once a day. The bucket was siphoned once or twice as required per day and complete cleaned in every 3-4 days as required.

3.4 Feed formulation

The shrimp was purchase from market and washed to remove the sand contain in it. The washed shrimp was then taken to heater drier to dry up completely. The soya bean was roasted in furnace. Then the whole ingredient (given in Table II) was mixed together in uniform manner and put into grinder, from where the desire sized pellet feed was prepared.

Table II: The ingredient and amount of ingredient for feed formulation

Ingredients	Amount (g/Protein contain)
Big shrimp powder	45 (27.67)
Soya bean powder	35 (15.26)
Wheat flour	20 (2.54)
Total	100 (45.47)
Extra addition	
Vitamin B $_{complex}$	2 g
Mineral	2 g
Vitamin 'C'	0.2 g

(FRD, 2015)

3.5 Calculation of quantity of carbohydrate required

According to Ebeling et al., (2006) the required amount of C: N=15 for heterotrophic bacterial metabolism. Initially the sucrose was added g by multiplying 15 with TAN concentration. But in race way it was hard to maintain that ratio, thus it was maintained by continuous addition of sucrose through fine pipe with diameter of five mm provided with stopper. In bottle, 24 g of sucrose was dissolved in 2.5 lit of water, and 1 ml/40sec of sucrose solution was dropped in each buckets.

Here, Sucrose solution concentration= 24 mg/ml,

Water loss/sec=43 ml

Sucrose loss/sec=0.6 mg

Photograph II: Sucrose solution supplied to each bucket through pipeline

3.6 Water quality test

3.6.1 Physical parameter

3.6.1.1 Temperature

The temperature was measured with the electronic thermometer. The half part of metallic tip of thermometer was dipped into water and continues stirred up until value change was stopped.

3.6.1.2 Electric conductivity

The sample water was taken in beaker and the electrode of vernier meter was dipped into water. The height of dipped was marked in electrode and stirred up until value change was stopped.

3.6.1.3 Turbidity

The sample water of 10 ml was taken in glass container of turbidity meter of vernier meter. The glass container was kept into turbidity meter and covered. The value was measured in vernier meter.

3.6.2 Chemical parameter

3.6.2.1 pH

The pH value was measured using the pH meter.

3.6.2.2 Dissolved Oxygen- Winkler's azide modified method (APHA, 1999)

Reagents preparation

a. Manganous sulphate solution:

Manganous sulphate of 48g was dissolved in 100ml distilled water, and then filter up the solution. The container was wrapped with aluminium foil.

b. Alkali-iodide-azide reagent:

Sodium hydroxide of 50g and potassium iodide of 15g were dissolved in 100ml distilled water. Then Sodium azide of 10g was dissolved in 40ml distilled water and mixed.

c. Sulphuric acid, H_2SO_4, conc.:

Conc. Sulphuric of 1ml was equivalent to about 3 ml alkali-iodide-azide reagent used.

d. Starch:

Laboratory-grade soluble Starch of 2g was dissolved in 100ml of hot distilled water.

e. Standard sodium thiosulphate titrant:

Sodium thiosulphate of 6.205g was dissolved in distilled water. Sodium hydroxide of 0.4g was added and diluted to 1lit. The solution was standardized with iodate solution.

f. Standard potassium iodates solution, 0.0021M:

Potassium iodate of 812.4mg was dissolved in distilled water and dilute to 1lit.

The Winkler's azide modified method iodometric test, based on the treatment of divalent manganese solution to dissolved oxygen, followed by addition of strong alkali in a glass-stoppered bottle. Dissolved Oxygen oxidizes bivalent maganous to trivalent state to equivalent quantity of dissolved oxygen. The maganous again reduce to bivalent state in the presence of iodide ions in an acidic medium. The amount of iodine liberated was equivalent to dissolved oxygen. The amount of iodine was determined by titrated with sodium thiosulphate. The titration end point was determined using a starch indicator.

$$MnSO_4 + 2KOH \longrightarrow Mn(OH)_2 + K_2SO_4$$

$$2Mn(OH)_2 + O_2 \longrightarrow 2MnO(OH)_2$$

$$2MnO(OH)_2 + 3H_2SO_4 + 2KI \longrightarrow 2MnSO_4 + K_2SO_4 + 3H_2O + I_2$$

$$I_2 + Na_2S_2O_3 \longrightarrow NaI + Na_2SO_4$$

The azide effectively removes interference caused by nitrite, which is the most common interference in biologically treated effluents and incubated BOD samples.

Collection of Samples

The water sample was collected in 300ml narrow-mouth BOD with glass-stoppered. The water sample was collected from the surface of water with very gently flow of water in BOD with avoiding bubble formation. Record sample temperature to nearest degree Celsius or more precisely.

Fixation of Dissolved oxygen

In collected sample the 1ml of Manganous sulphate solution was added and immediately again 1ml of alkali-iodide-azide reagent was added.

The sample was allowed to precipitate. Then 1ml conc. Sulphuric acid was added and the bottle was shook for complete dissolve the precipitated. Then a few drop of starch solution was added and titrated with standard Sodium thiosulphate from pale yellow straw colour to clear solution.

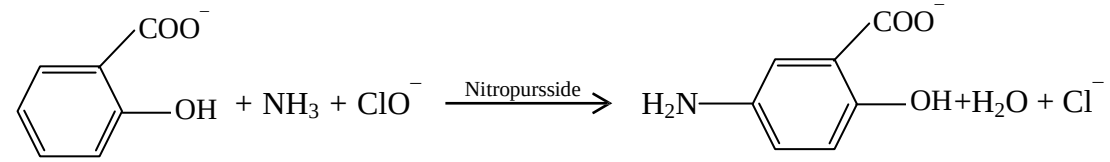
Calculation

For titration of 200 ml sample, 1ml of 0.025*M* Sodium thiosulphate = 1mg DO/lit.

$$\text{Dissolved oxygen (mg lit)} \quad \frac{\text{Volume of thiosulphate} \quad \text{Normality of thiosulphate} \quad 1000}{\text{Volume of sample}}$$

3.6.2.3 Total Ammonium Nitrogen- Salicylate-hypochlorite method (Bower and Holm Hansen, 1980)

The ammonia reacts with sodium-salicylate and hypochlorite in presence of sodium-nitroprusside, to form 5-amino, salicylate. The reaction pH is 9.8 and sodium citrate is used to prevent metal hydroxide precipitation.



Salicylate Hypochlorite 5-aminosalicylate

Reagents Preparation

a. Sodium salicylate solution:

Sodium salicylate of 440g with Sodium Nitroprusside of 0.2g was dissolved in 1lit of water. The chemical was highly photo sensitive and kept in dark bottle at 5^0C and stable for 3 month.

b. Alkali-iodide-citrate solution:

Sodium hydroxide of 18.5g and tri-sodium citrate of 100g was dissolved in 1 lit. Stable for indefinitely period.

c. Sodium hypochlorite solution:

Sodium hypochlorite (4%) of 5ml was diluted in 80ml of distill water. It is highly unstable compound and rapid degrade when exposure to light and room temperature.

d. Alkaline-hypochlorite solution:

The alkaline citrate solution and hypochlorite were mixed in 9:1 ratio. The solution was stable for 1hour only.

e. Stock Ammonium chloride solution (1g/lit.NH_3-N):

Ammonium chloride was dried at 110^0C for 1hour and then ammonium chloride of 3.819g was dissolved in 1lit.

f. Standard Ammonium chloride solution

The stock solution was diluted of 1ml into 1000 ml of distill water. The standard solution is prepared as follow:

Table III: The proportion of stock solution and distill water for standard ammonium solution preparation

NH_3-N (mg/lit.)	Diluted solution	Distill water
0	0	25
0.01	0.25	24.75
0.025	0.625	24.375
0.05	1.25	23.75
0.1	2.5	22.5
0.2	5	20
0.5	12.5	12.5
1	25	0

The 25ml of sample water was transferred in a 50-ml Erlenmeyer flask, followed by 3ml sodium salicylate-nitroprusside solution was added and well shook, then again 5ml alkaline-hypochlorite solution was added and immediately transferred the solution in dark for color development at room temperature (22-27°C). At least after 1hour but before 3hour the absorbance of solution was measured at 640nm in spectrophotometer.

For correction of curve, at least two other standards were prepared by diluting stock ammonia solution into the sample concentration range. The process was done once a week for validation of curve.

Calculation

A standard graph was plotted between Concentration of ammonia-N and absorbance and resolved the regression equation. The value of absorbance is resolve in equation to determine the value of ammonia-N contain of sample.

3.6.2.4 Alkalinity- Titrimetric Method (APHA, 1999)

Alkalinity of water is its acid-neutralizing capacity. Alkalinity of many surface waters is primarily a function of carbonate, bicarbonate, and hydroxide content. The measured values also include borates, phosphates, silicates, or other bases if these are present.

Alkalinity entirely due to carbonate or bicarbonate content, the pH at the equivalence point of the titration is determined by the concentration of carbon dioxide at that stage using Phenolphthalein indicator of pH 8.3 also called Phenolphthalein alkalinity. For complete alkalinity Bromcresol green or a mixed bromocresol green-methyl red indicator is used for pH 4.5.

Reagents preparation

a. Sodium carbonate solution, approximately 0.05N:

Primary standard Sodium carbonate of 5g was dried at 250°C for 4hours and cool in a desiccator. A Sodium carbonate of 2.65g was dissolved in a 1lit volumetric flask. The solution was stable for 1week.

b. Standard sulphuric acid:

Conc. Sulphuric acid (6 N) of 20ml was acid diluted to 1000ml with distilled water.

c. Bromocresol green indicator solution, pH 4.5 indicator:

Bromocresol green of 100mg was dissolved in 100ml distilled water.

The sample was titrated with standard Sulphuric acid using bromocresol green indicator. The end point was determined by changed in colour from blue to pale yellow straw. Bromocresol green indicator determines the end-point at pH 4.5.

Standardization of acid:

Standardize Sodium carbonate solution was titrated with Sulphuric acid. The end point was determined potentiometrically to pH of about 5.

Calculation

$$\text{Alkalinity } (CaCO_3 \text{ mg lit.}) \quad \frac{\text{Amount of acid} \quad \text{Normality of acid} \quad 50{,}000}{\text{Amount of sample}}$$

3.6.2.5 Hardness, EDTA Titrimetric Method (APHA, 1999)

Ethylene-diamine-tetraacetic acid and its sodium salts (EDTA) form a chelated soluble complex when added to certain metal cations (Calcium and Magnesium). Eriochrome Black T in aqueous solution containing calcium and magnesium ions at a pH of 10.0 ± 0.1, the solution became wine red. When EDTA was titrated, the calcium and magnesium will be complexes, and solution turns from wine red to blue, marking the end point of the titration. Magnesium salt of EDTA was added to the buffer; which automatically introduces sufficient magnesium to indicate the end point and obviates the need for a blank correction.

Reagents for Hardness

a. Buffer solution:

Ammonium chloride of 16.9g was dissolved in 143ml conc. Ammonium hydroxide. Disodium salt of EDTA of 1.179g and magnesium sulphate of 780mg was dissolved in 50ml distill water and mixed in ammonium chloride solution.

b. Standard EDTA

EDTA of 3.723g was dissolved in 1 lit distill water.

The 50ml of sample was taken and 1 to 2ml buffer solution was added. A small pinch of dry-powder Eriochrome Black T indicator was added. Then it was titrated with standard EDTA slowly, with continuous stirring, until the last reddish tinge disappeared.

The titrated standard ETDA required for sample must be less than 15ml otherwise dilute sample and EDTA titrant must completed within 5 min, measured from time of buffer addition.

Calculation

$$\text{Hardness } (CaCO_3 \text{ mg lit.}) \quad \frac{\text{Volume of E TA} \quad 1{,}000}{\text{Volume of sample}}$$

3.6.2.6 Phosphate, Ascorbic Acid Method (APHA, 1999)

Ammonium molybdate and potassium antimonyl tartrate reacted in acid medium with orthophosphate to form a heteropoly acid-phosphomolybdic acid that was reduced to intensely colored molybdenum blue by ascorbic acid.

Reagents for Phosphate

a. Sulphuric acid (5N):

Conc. Sulphuric acid of 70ml was diluted to 500ml with distilled water.

b. Potassium antimonyl tartrate solution:

Potassium antimonyl tartrate of 1.3715g was dissolved in 500ml distilled water. Bottle was Stored in a glass-stopper.

c. Ammonium molybdate solution:

Ammonium molybdate of 20g was dissolved in 500ml distilled water. The solution was stored in a glass-stopper bottle.

d. Ascorbic acid, 0.1M:

Ascorbic acid of 1.76g was dissolved in 100 ml distilled water. The solution was stable for about 1 week at 4°C.

e. Combined reagent:

All the reagents were mixed at 50: 5: 15: 30 of 5*N* H_2SO_4, potassium antimonyl tartrate solution, ammonium molybdate solution, and ascorbic acid solution respectively. If turbidity was formed in the combined reagent, shook and let stand for a few minutes until turbidity disappeared before proceeding. The combined reagent was stable for 4hours.

f. Stock phosphate solution (1g/lit PO_4^{3-}-P):

Phosphate was dried at 110^0C for 4 hours and anhydrous Phosphate of 816.54mg was dissolved 1000ml in distilled water.

g. Standard phosphate solution:

Standard phosphate solution was formed from stock phosphate solution; 1ml was diluted to 1000ml with distilled water. The standard solution was prepared as follow:

Table IV: The proportion of stock solution and distill water for standard phosphate solution

PO_4^{3-}-P (mg/lit.)	Stock solution	Distill water
0	0	20
0.0047	0.5	19.5
0.009	1	19
0.019	2	18
0.028	3	17
0.047	5	15
0.074	8	12
0.112	12	8
0.139	15	5

Initially, the sample water was treated with phenolphthalein, if color developed; the Sulfuric acid solution was added drop wise until color disappeared. Then 20ml of sample water was pipetted into 30ml test tube and 4ml of combine reagent was added and mixed thoroughly. At least, after 10 min but no more than 30 min, the absorbance of each sample was measured at 880nm.

Calculation

The standard graph was plotted between PO_4^{3-}-P and absorbance and resolved the regression equation. The value of absorbance is resolve in equation to determine the value of PO_4^{3-}-P contain of sample.

3.6.2.7 Nitrite and nitrate, Colorimetric method (APHA, 1999)

Nitrate was reduced to Nitrite with hydrazine sulphate. The Nitrite (originally present) plus reduced Nitrate was determined by diazotization with sulphanilamide and coupling with *N*-

(1-naphthyl)-ethylenediamine dihydrochloride to form a highly colour azo dye that was measured colorimetrically.

Reagents for Nitrite and Nitrate

a. Zinc-copper catalyst solution:

Cupric sulphate of 0.0354g and Zinc sulphate of 0.9g was dissolved in distill water and fill up to 1lit.

b. Sodium hydroxide solution (1N):

Sodium hydroxide of 4g was dissolved into 100ml distill water.

c. Hydrazine solution:

Hydrazine sulphate of 1.71g was dissolved in 1lit distill water. It is highly toxic chemical.

d. Sulphanilamide solution:

Sulphanilamide of 1g was dissolved in 100ml of 1:10 diluted Hydrochloric acid (i.e. 1ml HCl + 9ml distill water)

e. N-(1-naphthyl)-ethylenediamine dihydrochloride

N-(1-naphthyl)-ethylenediamine-dihydrochloride of 0.1g was dissolved in 100ml of distill water.

f. Stock Potassium nitrate solution (NO_3^- 1g/lit.):

Potassium nitrate ($KN0_3$) was dried at 105-110^{0}C for 4hour. Potassium nitrate of 1.629g was dissolve in 1lit of distill water.

g. Standard Potassium nitrate solution:

The stock solution was diluted of 1ml into 1000ml of distill water. The standard solution was prepared as follow:

Table V: The proportion of stock solution and distill water for standard Nitrate solution

$N0_3^-$ (mg/lit.)	Stock solution	Distill water
0	0	20
0.01	0.2	19.8
0.03	0.6	19.4
0.05	1	19
0.08	1.6	18.4
0.1	2	18
0.3	6	14
0.5	10	10
1	20	0

Collected water samples were preserved at 4^0C with adding conc. H_2SO_4 <2 pH. The 20ml of sample water was pipetted into a 50ml of test tube. Then 3ml of Zinc-cupper catalyst, Sodium hydroxide, and Hydrazine sulphate were added one by one and mixed well. Then the mixed solution was kept in water bath at 30-40^0C for 15-10 min. During this the solution was continuously stirred for equal distribute of heat around tubes. Again 11 ml of Sulphanilamide was added and shaken. After 5 min 3ml of *N*-(1-naphthyl)-ethylenediamine-dihydrochloride was added and shaken again. Then absorbance was measured at 540nm with a 5cm light path.

Calculation

The standard graph was plotted between concentration of nitrite and absorbance and resolved the regression equation. The value of absorbance is resolve in equation to determine the value of nitrate and nitrite contain of sample.

3.7 Growth analysis

1. Mean weight gain (g) = Mean final weight – Mean initial weight
2. Net yield (g/m^2) = Total biomass at harvest – total biomass at stocking
3. Specific growth rate(S G d-1) $\frac{(\text{ og final weight} - \text{log of initial weight}) \quad 100}{\text{earing period in day}}$

4. Average daily weight gain $\frac{\text{(final mean weight – initial mean weight)}}{\text{earing period in day}}$

3.8 Heterotrophic bacterial metabolism

Reactions for electron acceptors: G (KJ/mol)

1. $\mathbf{1/2}H_2O \rightarrow 1\ 4O_2 + H^+ + e^-$ 18.675

Reaction for electron donors:

2. $1/24C_6H_{12}O_6 + 1/4H_2O \rightarrow 1\ 4CO_2 + H^+ + e^-$ − 10.0
3. $1/8NH_4^+ + 3/8H_2O \rightarrow 1\ 8NO_3^- + 5/4H^+ + e^-$ 8.245
4. $1/10CH_3COCOO^- + 2/5H_2O \rightarrow 1\ 5CO_2 + 1/10HCO^{-3} + H^+\ e$ − 8.545

When an electron donor is used for both energy and cell synthesis the split between the electron flows is given by:

$f_e+f_s=1$

f_s fraction of electron donor used for synthesis

f_e fraction of electron donor used for energy

Using the estimated values for f_e and f_s, the half reactions can then be combined to describe biological processes according to the following relationship:

$R = R_d - f_sR_c - f_eR_a$

Where:

R overall balanced reaction

R_d half-reaction for the electron donor

R_c half-reaction for the synthesis of microbial biomass

R_a half-reaction for the electron acceptor

$$f_s \quad \frac{1}{1 \quad A}$$

Also,

$$A = \frac{\frac{G_p}{n} + G_{pc}}{- .G_r}$$ (McCarty, 1975)

G_r = Rxn (2) − Rxn (1) = −10.0 − (18.675) = −28.675

G_p = Rxn (2) − Rxn (3) = −10.0 − (−8.545) = −1.455 (m = −1)

$G_n = 0$

$$A = \frac{1.455 \text{ X } 0.6 - 7.5}{0.6\ (-28.675)} \ 0.385$$

$f_s = 0.7$ and $f_a = 0.3$

$R = -\ _d - 0.70 * R_s - 0.30 * R_a$

The following equations are developed.

R_d : $0.0417\ C_6H_{12}O_6 + 0.250\ H_2O \rightarrow 0.250\ CO_2 + H^+ + e^-$

$-f_s * R_c$: $0.03[0.2\ CO_2 + 0.05\ HCO^{-3} + 0.05\ NH_4^+ + H^+ + e^- \rightarrow 0.05\ C_5H_7O_2N + 0.45\ H_2O]$

$-f_e * R_a$: $0.7[0.25\ O_2 + H^+ + e^- \rightarrow 0.5\ H_2O]$

Stoichiometric yields will be the following equation:

$NH_4^+ + 1.18C_6H_{12}O_6 + HCO_3^- + 2.06O_2 \rightarrow C_5H_7O_2N + 6.06H_2O + 3.07CO_2$

In this process for each g of ammonia– nitrogen into microbial biomass consume 4.71g of dissolved oxygen and 3.57g of alkalinity and 15.17g carbohydrates. Also 8.07g of microbial biomass (4.29g organic carbon) and 9.65g of CO_2 (2.63g inorganic carbon) are produced (Ebeling et al., 2006).

The heterotrophs metabolism was calculated as before and after with control design method. Heterotrophic consumption of,

DO	=	DO in control – DO in treatment
Ammonia	=	Ammonia of control – Ammonia of treatment
Alkalinity	=	Alkalinity of control – Alkalinity of treatment

The effect of treatment of sucrose was calculated as Before-and-after with control design:

Mean weight gain = Weight gain in treatment – Weight gain in control

Net yield = Net weight gain in treatment – Net weight gain in control

ADG = ADG in Treatment – ADG in control

3.9 Statistical analysis

All the values are expressed in mean ± standard deviation. The effect of treatment of sucrose was calculated as Before-and-after with control design. The acceptable range of water parameter was analysed with Inferences about Central Values with statistical value. The variation of water parameter with in replica is analysed with ANOVA: single factor, variation of water parameter with treatment is analysed with ANOVA: two-factor with replica (Ott and Longnecker, 2010).

4. Results

4.1 Physical Parameters

4.1.1 Temperature

The variation in temperature lies within acceptable range throughout the experiment. The temperature of water was 16.5±0.91^{0}C (P<0.05). The temperature of water lied in between 15.4 - 20.5^{0}C.

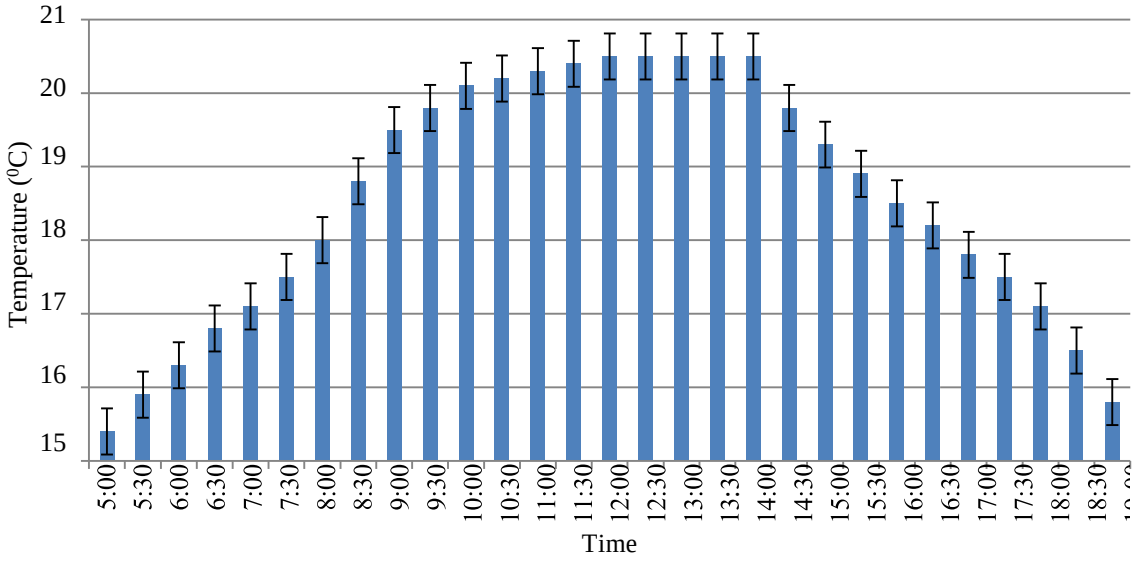


Fig. I: The temperature variation in day

4.1.2 Electric conductivity (EC) and Turbidity

The EC of source was 377, treatment was 375.33±1.5 and control was 356.67±8.02. The turbidity of source was 48.9, treatment was 33.4±6.08 and control was 28.6±1.49.

4.2 Chemical parameters

4.2.1 pH

The pH of water in source was 7.7±0.15 (7.86-7.43), treatment was 8.17±0.03 (8.31-8.05) and control was 8.16±0.02 (8.29-8.05). The variation of the source with treatment and control was significant (P<0.05), but variation between control and treatment was insignificant (P>0.05).

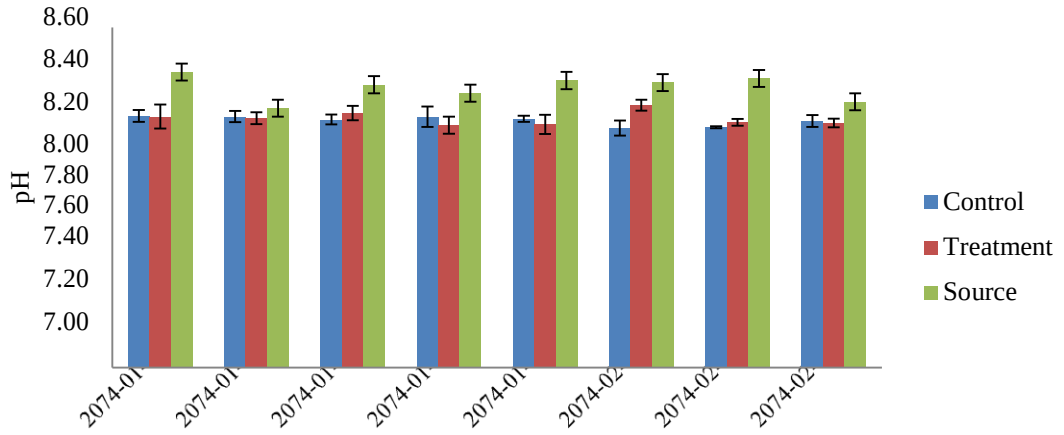


Fig. II: The pH variation in water due to sucrose treatment and without sucrose treatment with source of water.

4.2.2 Dissolved Oxygen

The dissolved oxygen contain in water of sucrose non-treatment was 5.48±0.1mg/l ($P<0.05$), and sucrose treatment was 5.39±0.1mg/l at 16.5^0C. The DO contain in water was not significantly ($P>0.05$) differ between control and treatment.

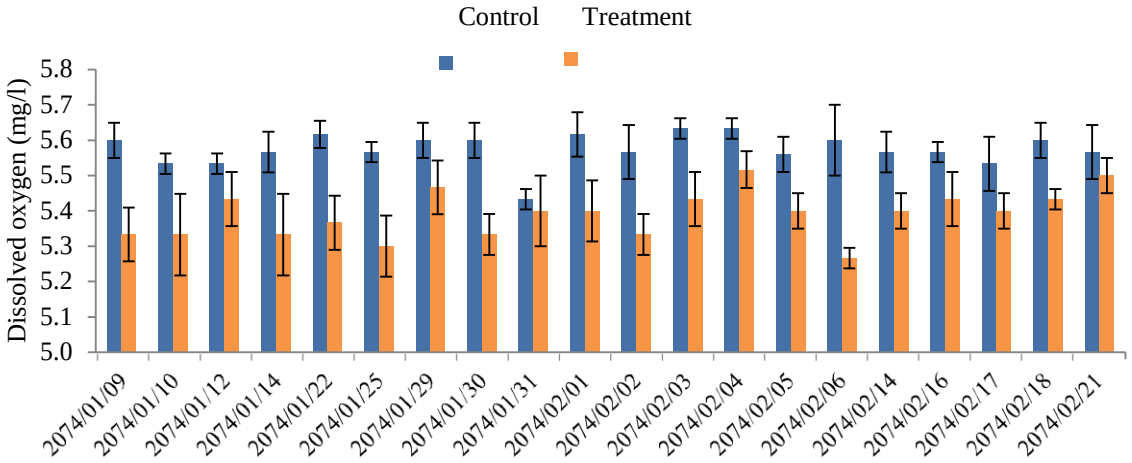


Fig. III: The variation of dissolved oxygen contain of water due to sucrose treatment and without sucrose treatment with 43±2.5ml/sec of water exchange.

Even though the dissolved oxygen varies sharply in the experiment period, the dissolved oxygen was negatively correlated to temperature i.e. 0.97 and mostly depend up on the temperature of water.

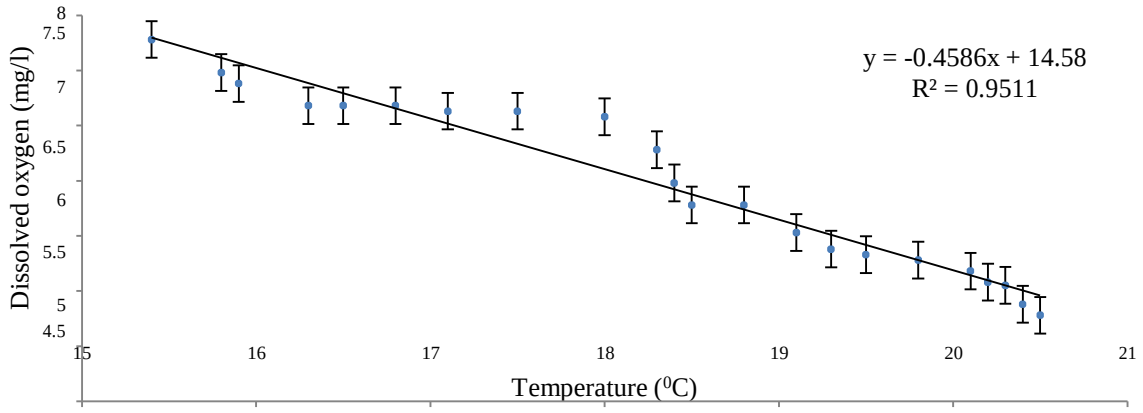


Fig. IV: The correlation between Dissolved oxygen and temperature

4.2.3 Total Ammonia Nitrogen (TAN)

The total ammonia nitrogen contain in water of source was 0.4091±0.0772mg/l, in water without sucrose treatment was 0.4487±0.0424mg/l, and in sucrose treatment was 0.2578±0.0741mg/l. The variation in TAN in control and source was insignificant (P>0.05) and treatment has significant (P<0.05) variation with source and control.

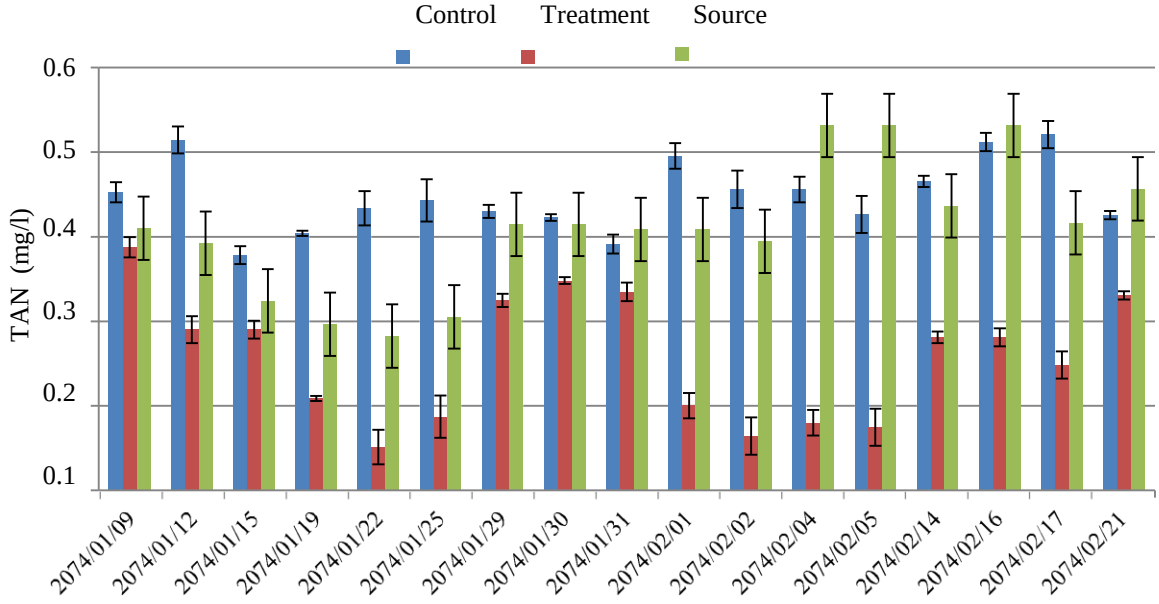


Fig. V: The variation of TAN contain of water due to sucrose treatment and without sucrose treatment with 43±2.5ml/sec of water exchange.

4.2.4 Alkalinity

The alkalinity of water was 161.17±7.62mg/l of $CaCO_3$ ($P<0.05$), the alkalinity of water source was 165.34±9.86mg/l of $CaCO_3$ (180-135), control was 158.97±5.31mg/l of $CaCO_3$ (180-150) and the treatment was 159.2±4.79mg/l of $CaCO_3$ (180-142.5).

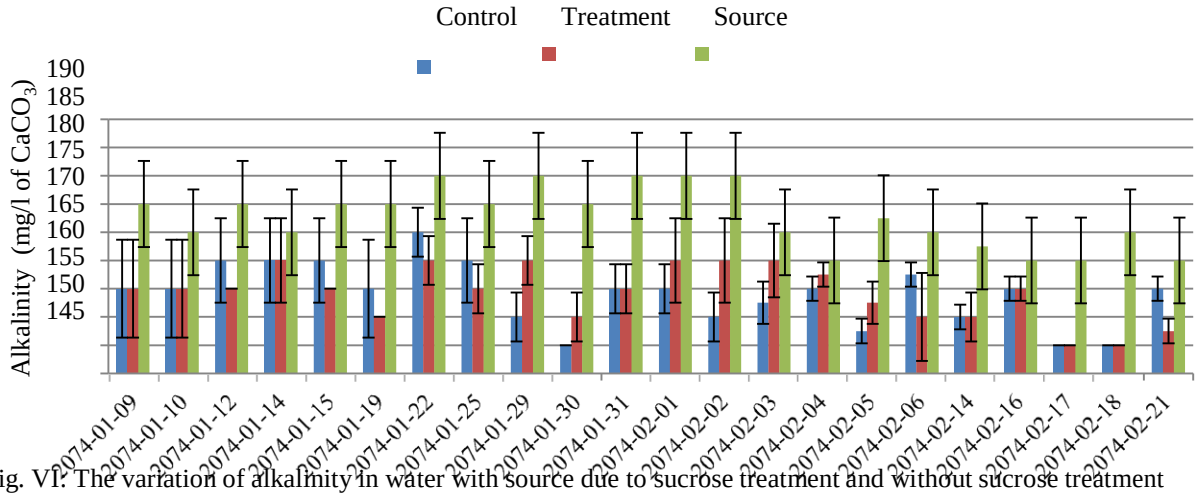


Fig. VI: The variation of alkalinity in water with source due to sucrose treatment and without sucrose treatment with 43±2.5ml/sec of water exchange.

4.2.6 Hardness

The hardness of source water was 74.125±2.85mg/l of $CaCO_3$ (79-70), control water was 62.71±1.95mg/l of $CaCO_3$ (65-59) and treatment was 66.43±2.73mg/l of $CaCO_3$ (73-59). The variation of the source with treatment and control was significant ($P<0.05$), but variation between control and treatment significant ($P<0.05$).

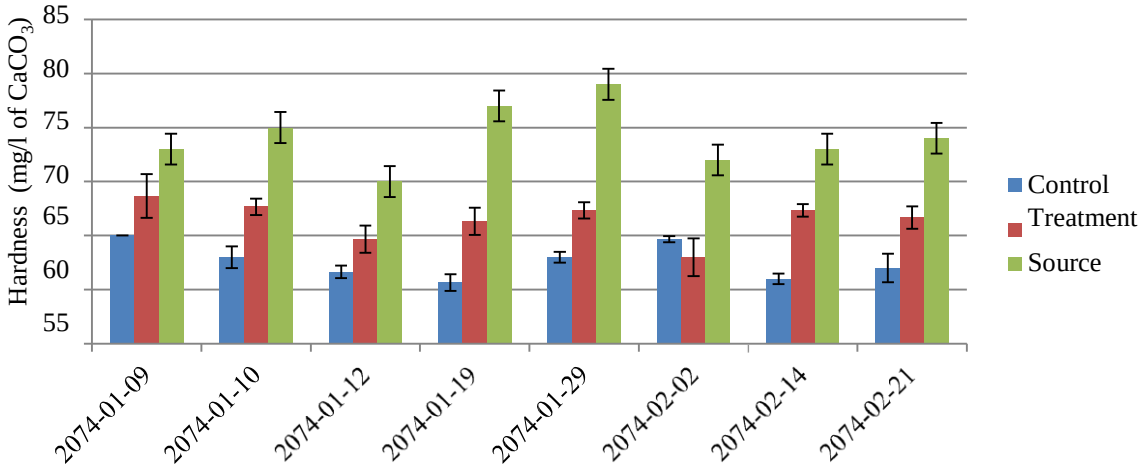


Fig. VII: The variation in hardness of water due to sucrose treatment and without sucrose treatment with source.

4.2.6 Phosphate

The phosphate contain of source was 13.8±2.2µg/l (20-12.3), treatment was 12.3±1.1µg/l (13-10.7) and control was17.2±1.2µg/l (19.3-15.4). The variation of phosphate contain of water between source and treatment was insignificant (P<0.01) and variation with control was significant (P>0.01).

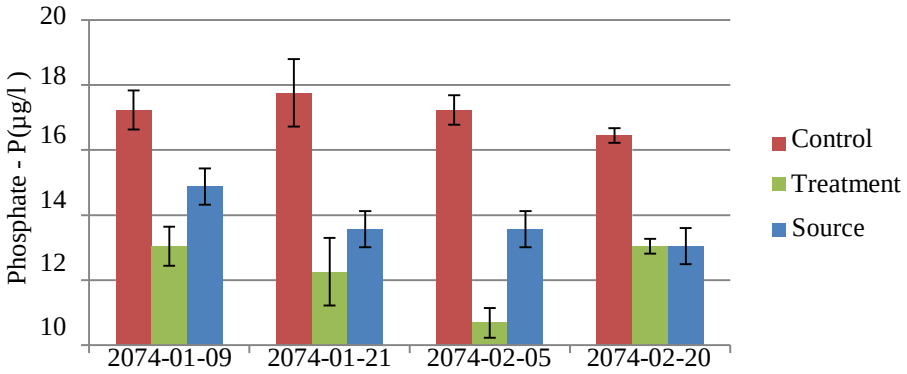


Fig. VIII: The variation in phosphate contain of sucrose treatment and non-treatment of water with source water

4.2.7 Nitrite and nitrate

The nitrate and nitrite contain of source water was 108±8µg/l (120.2-102.4), treatment was 92.5±6µg/l (0.1032-0.0852) and control was 102.2±15µg/l (135.1-86.2). The variation of nitrite and nitrate in between source, treatment and control was insignificant (P>0.01).

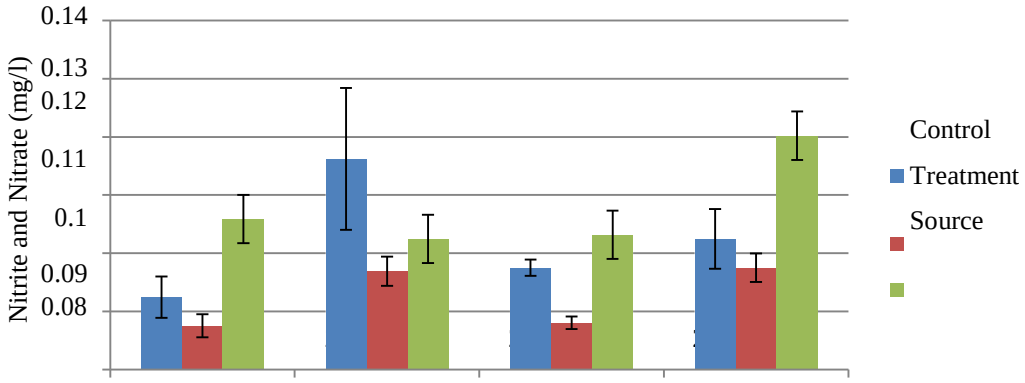


Fig. IX: The variation in Nitrite and nitrate contain of water in sucrose treatment and non-treatment with source water

4.3 Growth

4.3.1 Mean weight gain

The initial wt. of fish rearing was 0.8±0.05g in both control and treatment. The average mean weight after 15 days was significant varied (P<0.01) in control 1.27±0.027g and in treatment 1.42±0.020g. Similarly after 30 days, the average mean weight in control was 1.78±0.041g and in treatment was 2.05±0.047g. Again after 45 days, the average mean weight in control was 2.31±0.05g and in treatment was 2.69±0.07g. The variations are also significant (P<0.01) in 30 days as well as 45 days.

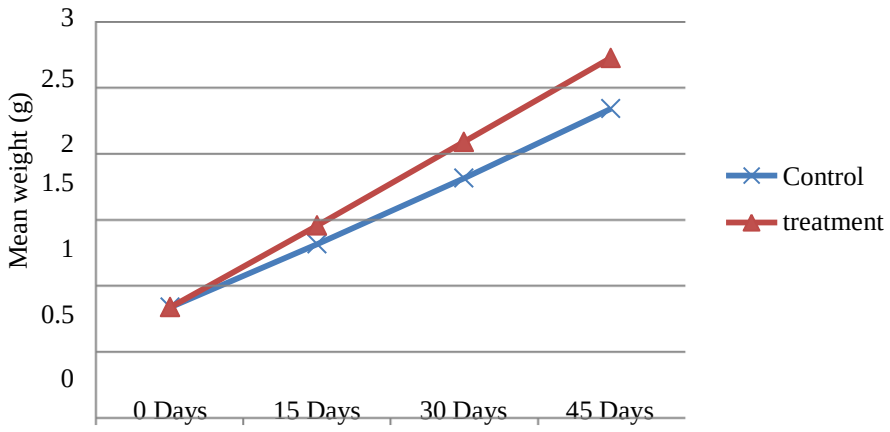


Fig. X: The mean weight gain in sucrose treatment and without sucrose treatment

4.3.2 Net yield

The initial stocking weight of fish was 20±1.25g in all of the experimental set up. The total weight of treatment was 35±1g after 15 days was significantly higher (P<0.01) than that of control, which has was 30.67±1.15g. Also the net yield was significantly higher (P<0.01) after 30 days and 45 days in treatment than control. The net yield of control was 42.67±1.15g after 30 days was and 55.33±2.31g after 45 days. Again in treatment net yield was 50±1.15g after 30 days and 66.33±1.53g after 45 days.

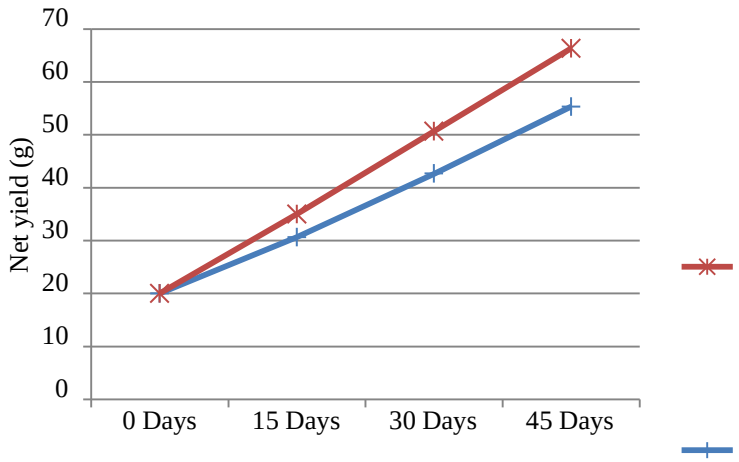


Fig. XI: The net yield of fish in sucrose treatment and without sucrose treatment.

4.3.3 Specific growth rate (SGR)

The SGR was significantly higher in the treatment group than in the controls group. SGR values were virtually identical ($P<0.01$) in replicated groups, and all were significantly lower ($P<0.01$) than in the comparable control group.

Table VI: The specific weight gain rate variation in sucrose treatment and without sucrose treatment.

	15 days	30 days	45 days
Control (g)	1.36±0.061	1.15±0.033	1.02±0.021
Treatment (g)	1.66±0.04	1.36±0.033	1.17±0.028

4.3.4 Average daily weight gain

The average daily weight gain in treatment was 41±0.1mg/day and control was 26±0.1mg/day. The average daily weight gain variation between treatment and control was significant ($P<0.01$).

Table VII: The average daily weight gain in sucrose treatment and without sucrose treatment

	15 days	30 days	45 days
Control (mg/day)	31.87±1.81	20.02±0.84	21.09±1.22
Treatment (mg/day)	41.26±1.34	42.37±2.24	42.34±4

4.4 Heterotrophic bacterial metabolism

The heterotrophic bacterial metabolism consumes the DO of 0.18±0.08mg/l, NH_4^+ of 0.19±0.09mg/l, alkalinity of 3.45±2.073mg/l at 43±2.5ml/sec of water exchange.

Table VIII: Consumption of water contain by heterotrophic metabolism.

Consumables	Stoichiometry	Consumes/ l
DO	4.17mg/ oxygen /mg N	0.18±0.08mg
NH_4^+-N	1mg	0.19±0.09mg
Alkalinity	3.57mg/ Alkalinity/mg N	3.45±2.073mg
pH		0.01
Hardness		7.695mg

5. Discussion

5.1 Water quality parameters

5.1.1 Temperature

The temperature governs all the activities of environment and one of the most important parameter to secure the aquaculture. The water temperature determines the metabolism of aquatic cold blooded animal. In the present experiment, water temperature was 16.5±0.91^{0}C (15.4-20.5^{0}C). The experimental water temperature is conventional with the FAO (2011a) recommended optimal temperature for trout culture i.e. 7-18^{0}C. However, according to NARC (2010) the ideal water temperature for trout culture in Nepal was ranged from 16-18^{0}C. In the current study, the water temperature was mostly depending on sun shine as the day progress the temperature of water rise. However, the temperature remains lowered in rainy and cloudy day i.e. 17-18^{0}C. The experimental water temperature was not higher than 18^{0}C for more than 5hour/day. Also the experiment period was summer season (April-May), the temperature remained at upper limited temperature. The water temperature of rainbow trout culture during summer season in Kakani, Nuwakot, Nepal was found to be 13.4-18.8^{0}C (Bhagat and Barat, 2015), which is similar with our present experiment. Amatya (2000) also found that the trout culture in Modi khola was 7.5-15^{0}C.

5.1.2 Dissolved oxygen

The Dissolved oxygen is the most important parameter for aquaculture. The dissolved oxygen is exclusive source of oxygen for respiration. In this experiment, the dissolved oxygen was 5.48±0.1mg/l at 16.5^{0}C. The dissolved oxygen was related with the water temperature. Similarly, the correlation of water temperature with dissolved oxygen was found to be -0.97.

The dissolved oxygen of the water in this experimental setup lies within the FAO (2011a) recommended dissolved oxygen (>5mg/l) for trout culture. Moreover, FAO recommended dissolve oxygen for incubated egg is 5-6mg/l, which is lower than the NARC (2010). The DO was negatively correlated with pH in our experiment, which is similar with the findings of Moogouei et al. (2010).

According to Carter (2008), the level of dissolved oxygen is required above 8mg/l for swimming fitness of salmonids. The swimming performance of rainbow trout was reduced 30% at dissolved oxygen concentrations of 5.1mg/l and 14^0C. At oxygen levels of 3.8mg/l and a temperature of 22^0C, the swimming fitness was further reduced to 43% (Carter, 2008).
The result was less than the rainbow trout culture in Kakani, Nuwakot, Nepal, in summer (March to May), where the DO was 6.5-8.4mg/l (Bhagat and Barat, 2015) and Kostomuksha (USSR) rainbow trout farm, where dissolved oxygen contains of water was 13.41±1.4mg/l (Avkhimovich, 2013). Nevertheless, higher than Lorestan (Iran) rainbow trout farm, where the dissolved oxygen contains of water was 3.3±0.57mg/l (Moogouei et al., 2010).

5.1.3 Total Ammonia Nitrogen

The source of ammonia in water is excretory product of aquatic animal. The ammonia is second limiting factor for aquaculture production after dissolved oxygen. In this experiment, the TAN of source water was 0.4091±0.0772mg/l, control was 0.4487±0.0424mg/l and treatment was 0.2578±0.08mg/l. The TAN in control was increased due to addition of ammonia via fish excretion. However, in treatment the TAN was decrease than source water showed the reduction of ammonia in treatment via heterotrophic bacterial consumption.
NARC (2010) and Boyd (2013) recommend that the TAN for trout culture is >0.2mg/l. Also Avkhimovich (2013) state the TAN for trout culture is >0.07mg/l. However, the common practices have 0.14-0.21mg/l of TAN in rainbow trout culture in Kakani, Nuwakot, Nepal (Bhagat and Barat, 2015); 2.2±1.62mg/l in Lorestan (Iran) (Moogouei et al., 2010); and 0.2±0.05mg/l in Kostomuksha (USSR) (Avkhimovich, 2013).
As compare to control of our experiment, the TAN of water was increased as FRD (2014) study in the trout farm of Nuwakot, Dhading and Sindhupalchowk, the ammonia-nitrogen was rise from 0.026mg/l at first race way to 0.17mg/l at last race way (15^{th} race way). Similarly in trout farm of Germany, the TAN increase from 0.04±0.03mg/l at inlet to 0.47±0.4mg/l at outlet (Sindilariu et al., 2008), and from 0.038±0.032mg/l to 0.114±0.12mg/l in Karasu stream trout farm (Pulatsu et al., 2004).
In addition, the result of treatments observed in this study is similar to Liu et al (2014), where TAN was lower significantly in maize treatment as carbohydrate source. Similarly at an

optimal C/N ratio between 10 and 15, the nitrogen removal efficiency reached up to 98% (=110 mg N/l reactor/day) bacterial utilization (Schryver and Verstraete, 2009).

5.1.4 Alkalinity, pH and Hardness

The Alkalinity is capacity of water to resist the change in pH and the hardness is amount of Calcium and Magnesium of alkalinity. In water with moderate to high alkaline, there is the relation among alkalinity, pH, hardness and carbon dioxide (Wurts and Durborow 1992). In present experiment, the alkalinity was varies from 165.34±9.86mg/l of $CaCO_3$ of water source to 158.97±5.31mg /l of $CaCO_3$ in control and 159.2±4.79mg /l of $CaCO_3$ in treatment. The pH of water in source was 7.7±0.15 (7.86-7.43), treatment was 8.17±0.03 (8.31-8.05) and control was 8.16±0.02 (8.29-8.05). The hardness was of source water was 74.125±2.85mg/l of $CaCO_3$ (79-70), control water was 62.71±1.95mg/l of $CaCO_3$ (65-59) and treatment was 66.43±2.73mg/l of $CaCO_3$ (73-59).

The alkalinity of water was reduced due to addition of carbon dioxide that release from fish during respiration. The alkalinity of treatment loss more as the bacterial activities also release acid. The pH of water was not remain steady because the alkalinity is varies only slightly than source water, so the pH remain almost same. The water hardness was varies greatly from source water, which shows that the Calcium of water was loss with balancing the acid i.e. carbonic acid. The loss of hardness was almost equivalent with alkalinity of water loss.

According to Hargreaves (2013), the bacterial activities consume alkalinity and release the carbonic acid that causes the depletion in alkalinity of water. However, (Ebeling et al., 2006) suggested that the alkalinity is moderately loss during the bacterial activities. APHA (1999) reported that the water hardness and alkalinity play a major role to restrict changes in water pH. The losses of water hardness contribute to resist is change in pH value. In our experiment, the loss of alkalinity was more in treatment than control but the value was insignificant.

The result was appropriate to FAO (2011a) recommended optimal condition of pH 6-8.5 for trout growth. Likewise the loss in pH was similar to Pulatsu et al. (2004), the pH of the water was not significantly changed and only a minor elevation in pH (0.04). Wagner et al. (1997) conclude the high temperature (19-22^{0}C) alone did not produce stress in trout but along with high pH cause significant mortality. At low temperature, the high pH stress response was

delayed. The mortality occurred at pH levels greater than 9.3 and temperature of 19.9-22.8^{0}C. Carter (2008) determined that juvenile rainbow trout mortality was greatly increased at pH levels of 5.5 and below, and that no eggs survived when exposed to pH levels below 4.5.

5.1.5 Phosphate

Phosphorus is vital to life and is non-substitutable in biological reaction systems. Phosphate is the primary of indicator of fertilization. Excessive of phosphate in water cause eutrophication that detrimental for aquatic life. In present experiment, phosphate contain of source was 13.8±2.2µg/l, treatment was 12.3±1.1µg/l and control was 17.2±1.2µg/l.

Likely in our control the phosphates contain of water was increased significantly than source water. As Kawasaki et al. (2016) reported that the total amount of nitrogen and phosphorus added to each aquaculture pond were estimated to be released as wastewater per pond per year, accounting for 20-30% of nitrogen and phosphorus of feeds. Similarly the FRD (2014) found the phosphates contain of water arise from 0.013mg/l to 0.057mg/l in trout culture. Pulatsu et al. (2004) also reported the phosphorus arises from 0.069±0.034mg/l to 0.117±0.041mg/l in trout culture. Likewise Sindilariu et al. (2008) conclude the phosphate-P arises from 0.02 ±0.02mg/l at inlet to 0.06 ±0.05mg/l at outlets in trout culture.

Pulatsül and Çamdeviren (1999) state the typical value of total phosphorus in aquaculture effluent water was 0.125mg/l. and the water pH play an important role in controlling phosphorus availability in calcareous water bodies. Unlike to that our experimental value was far less than effluent mark.

In common practices the phosphate contain in trout culture in Kakani, Nuwakot, Nepal in summer (March to May) was 0.07-0.18mg/l (Bhagat and Barat, 2015); 0.07±0.01mg/l in Lorestan (Iran) (Moogouei et al., 2010); and <0.05mg/l in Kostomuksha (USSR) (Avkhimovich, 2013).

5.1.6 Nitrite and Nitrate

Nitrite is produce in pond via nitrifying bacteria. The nitrification and denitrification is tight couple reaction that immediately produce nitrate. In experiment, the nitrate and nitrite contain of source water was 108±8µg/l, treatment was 92.5±6µg/l and control was 102.2±15µg/l.

The heterotrophic bacteria prefer also nitrite and nitrate in substitute of ammonia, which was seen in result as the nitrate and nitrite were also decreased in treatment than control. Hosseinzadeh and Nafari (2014) reported that the daily growth of trout was certainly effected by concentration of nitrite.

Pulatsül and Çamdeviren (1999) state the standard value of nitrite-N in aquaculture is 0.83 mg/l, however 0.1 mg/l is limited value. The experimental nitrite and nitrate were below the limited value.

Likewise, in control Fadaeifard et al. (2012) conclude the nitrite and nitrate contain of water in trout culture rise significantly. In the study of the FRD (2014) found that the nitrite and nitrate contain of water rise from 0.39 to 1.99mg/l in study of the trout farm of Nuwakot, Dhading and Sindhupalchowk. Sindilariu et al. (2008) also report the nitrite-N rise from 0.03±0.05 mg/l to 0.08 ±0.06 mg/l and nitrate from 5.28 ±1.23mg/l to 5.09±1.25mg/l in trout farm. Similarly Pulatsu et al. (2004) also report the nitrite-N and nitrate-N concentrations increased from 0.019±0.054mg/l and 0.581±0.33mg/l upstream and 0.108±0.074mg/l and 1.035±0.56mg/l downstream.

The common practice of trout culture in Kakani, Nuwakot, Nepal in summer (March to May) the nitrate contain is 0.04-0.19mg/l (Bhagat and Barat, 2015).

5.2 The effect on growth

The growth of rainbow trout depends upon many physical and chemical parameters. The main aim of our study was to determine the effect of sucrose treatment in trout culture that enhances heterotrophic bacterial activity cause improves water quality that effect in growth as well as environment conservation. The heterotrophic bacterial activity consumes the wastes (nitrogen and phosphorous) of culture medium that harm culture species and environment.

The initial mean weight of fish was 0.8±0.05g. The final mean weight in experiment was 2.69±0.07g in treatment and 2.31±0.05g in control. The initial stock weight was 20±1.25g. The net yield was 66.33±1.53g in treatment and 55.33±2.31g in control.

The result showed the mean weight gain in treatment was significantly higher than in control indicates the impact of TAN in growth of fish. Reinbold and Pescitelli (1990) state the sub-lethal effect of ammonia reduces the fish growth that depends on temperature and dissolved

oxygen. The sensitivity (effect) to ammonia in rainbow trout has been seen to increase as the dissolved oxygen level decreases. Wood (2004) examined the growth of trout under different concentration of TAN, he found that, in control (0μM) 18.4±0.8·g, low TAN (75μM), 23.0±0.7g, and high TAN (225μM), 19.6±0.5g of mean wt. Also he found that higher the temperature the lower the TAN is preferred as, at 15°C, resulted in significant weight gain, as well as increases in condition factor and protein production, at 70μM TAN, the similar effect is found at 6.5°C, at 225μM TAN. Morrows (2009) conclude that higher the TAN significantly reduce tilapia whole body growth, however the low levels of TAN (≤300μM) has no effect on growth.

Maurício et al. (2013) report in juvenile tilapia culture the growth/production between 35% and 24% commercial pellet feed tanks under biofloc are quite similar, but both were higher than clear-water control without biofloc with 35% commercial pellet maintaining good water quality. Avnimelech (1994) found the protein recovery in tilapia culture is arise from 23% to 43% in flocs technique where sorghum is used as supplement carbohydrate and feed pellet with 20% protein contain. Avnimelech (2003) found the addition of wheat flour as carbon substrate on *Tilapia* culture with 30% and 20% protein feed yields, FCR 2.62, 2.17, protein conversion ratios 4.38, 2.42 on 51 days and FCR 2.62, 2.02 Protein conversion ratios 4.35, 2.18 on 30 days.

Hapsari (2016) reported in catfish (*Clarias gariepinus*) culture that the growth of catfish was higher in fermented biofloc than no biofloc and non-fermented biofloc. He found 0.30±0.060g growth in fermented biofloc but 0.10g, and 0.12±0.02g in no biofloc and non-fermented biofloc respectively.

The average daily weight gain per day was significantly higher in treatment than control. The control gain 20.08±1.25mg/day and treatment gain 33.95±2.75mg/day. Wood (2004) also conclude the trout exposed to lower ammonia over the course of 14 months retained up to 10% more protein than that of no ammonia.

The growth of trout in experiment of control was similar to a study of FRD (2015) trout growth feed analysis found that the shrimp based feed has 17.6±1.0mg/day and the blood meal feed has 21±0.6mg/day.

In experiment the SRG is always higher in treatment than control at instantaneous time. The average SRG of control was 1.17±0.038% and treatment was 1.4±0.034%.The SRG value of

experiment was decline with increase in body weight which is similar to Akbulut et al. (2002). The best SRG was obtained by him was at small size is 1.11%.

5.3 Heterotrophic bacterial metabolism

The heterotrophic bacteria oxidised carbohydrate using oxygen for energy source. Thereby it uses ammonia and inorganic carbon (alkalinity) to grow in a medium.

In experiment, the heterotrophic bacterial activities within the water supplied of 43±2.5ml/sec, for 1 mg ammonia reduction required 0.94mg/l of dissolved oxygen, 18.15 mg of alkalinity, and 0.052 of pH and 40.5mg of hardness.

In stoichiometric study of heterotrophic bacterial metabolism, Ebeling et al. (2006) found that for 1mg of ammonia–nitrogen converted to microbial biomass, consume 4.71mg of dissolved oxygen and 3.57mg of alkalinity. The loss of dissolved oxygen was small because of continuous addition of water. The loss of alkalinity was higher than stoichiometric value as bacterial activities add acid in water and also respired carbon dioxide than neutralize the alkalinity.

6. Conclusion

From an experiment, the sucrose treatment as source of carbohydrate in trout culture has no negative impacts on fish. The sucrose treatment brings heterotrophic bacteria to grow along with trout. The heterotrophic bacteria in trout culture had improved the water quality that improves the growth of trout significantly. The heterotrophic bacterial activity causes significant reduction in TAN, phosphate, nitrite and nitrate of trout culture. The consumption of oxygen and alkalinity via heterotrophic bacteria was insignificantly.

The reduction in TAN, phosphate, nitrite and nitrate of outlet from trout culture could contribute to sustainable trout culture. Such reduction contributes increases in growth performance and use of less protein contains feed formulation. The stable water quality causes less susceptible of disease and no need to uses chemical and antibiotic. Moreover the outlet does not harm water shed region aquatic life.

The continuous exchange of water keeping C: N=15 was suitable and has not effect of sucrose treatment in dissolved oxygen. The heterotrophic growth was rapid that are not washout from cultural medium. The alkalinity was stable. The pH is varies but lies with in required limits. Also the hardness of water was loss but not significantly to the standard limits.

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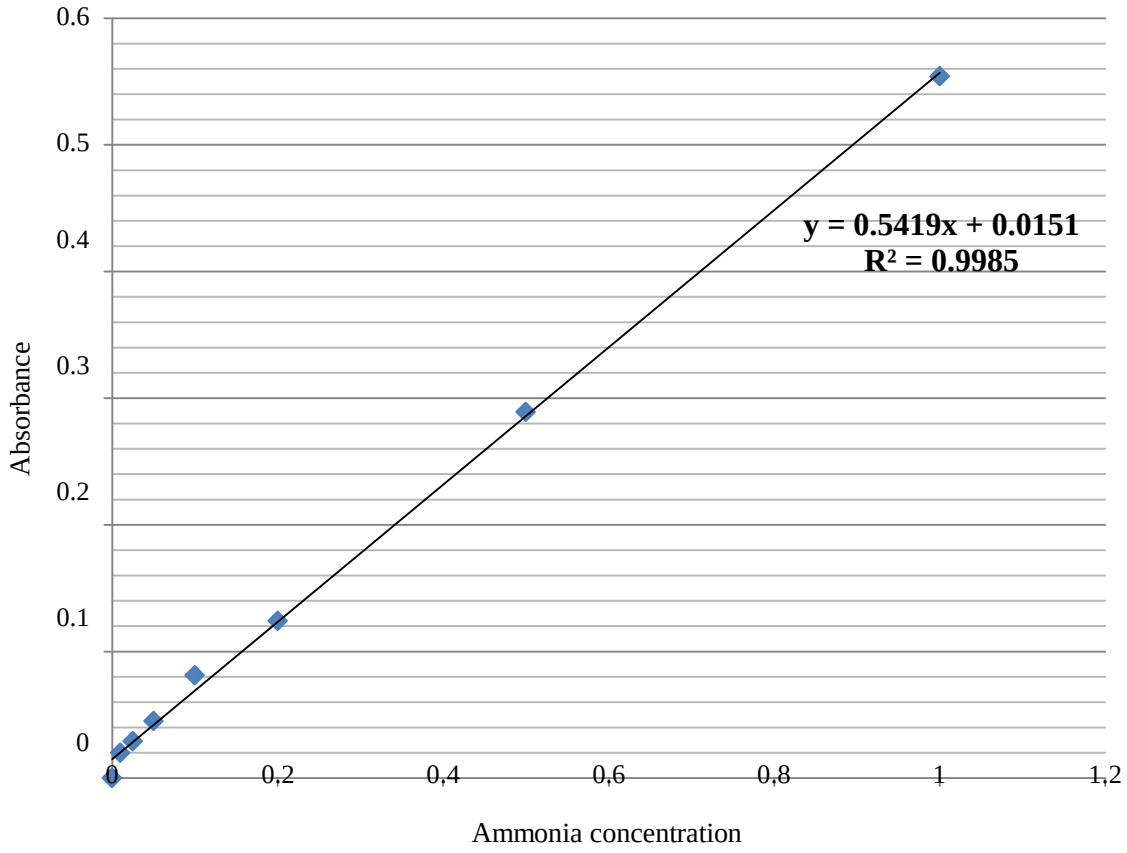
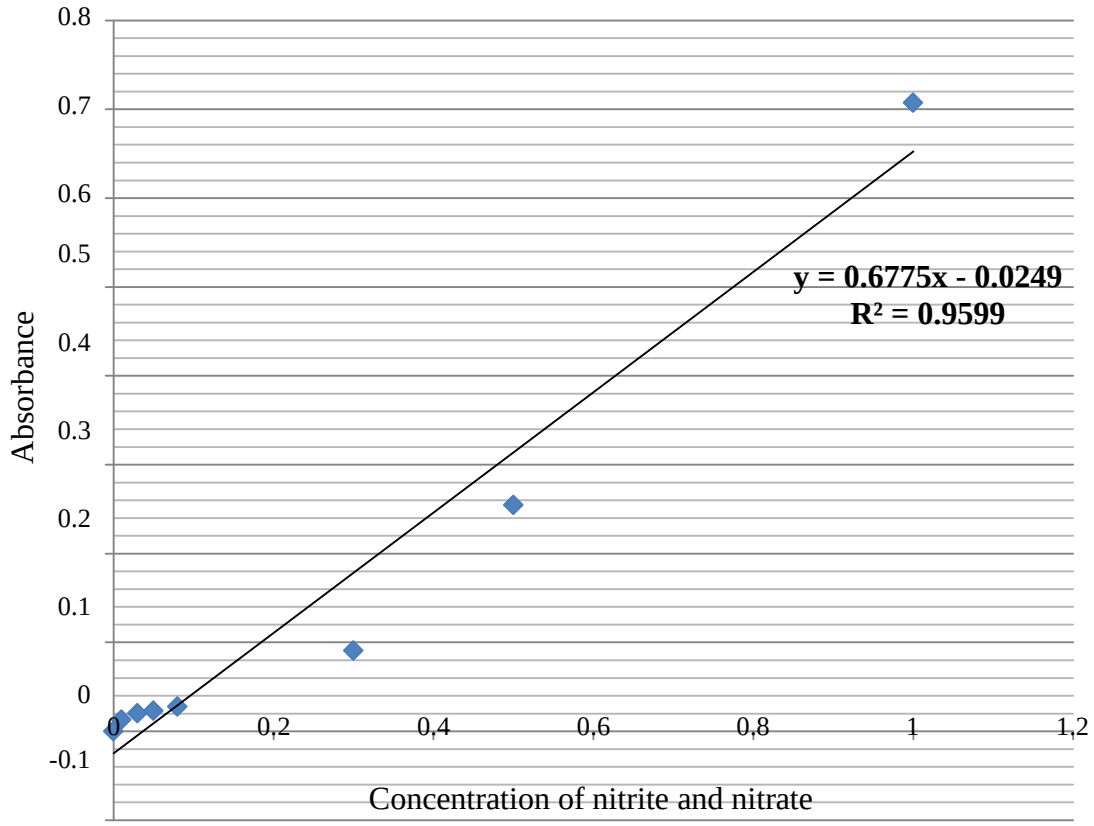
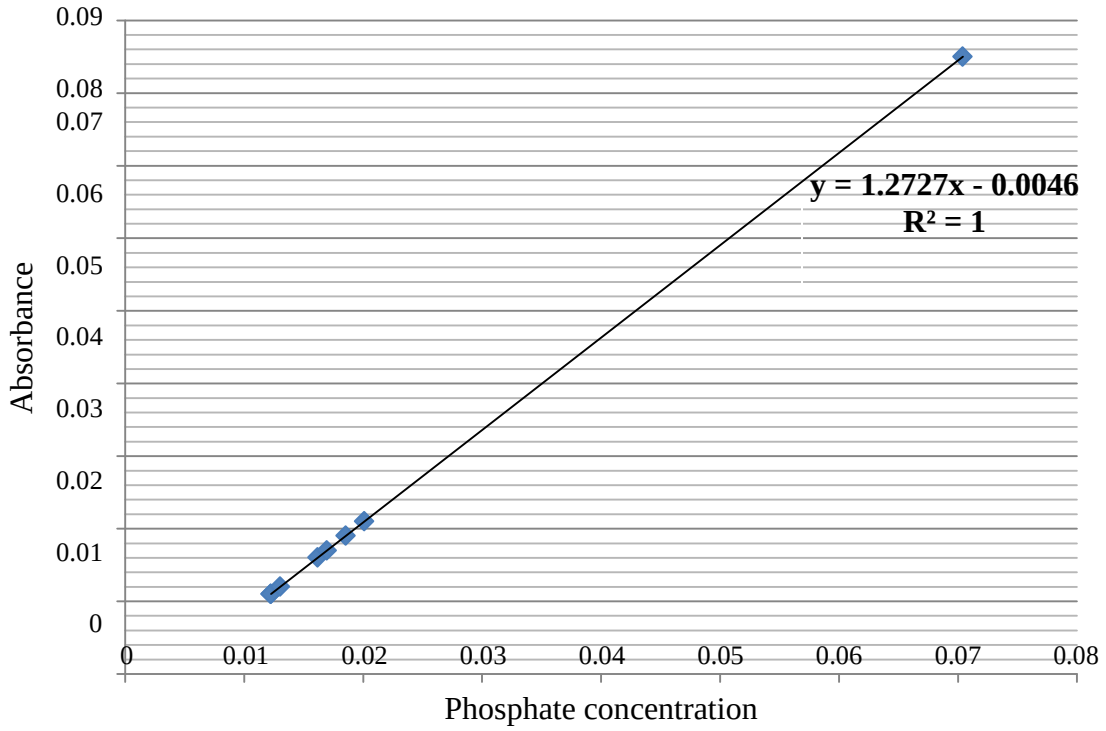
Appendix-I: Standard Ammonia curve



Appendix-II: Standard Nitrite and nitrate curve



Appendix-III: Standard Phosphate curve



Appendix-IV: Temperature analysis for inferences about central values with statistical value

	Temperature (^{0}C)		
	8:00 AM	12:00 PM	4:00 PM
2074-01-09	15.6	20.5	16.3
2074-01-10	15.4	20.3	15.5
2074-01-12	15.5	19.5	15.6
2074-01-14	15.7	19.9	15.2
2074-01-15	15.7	20.3	16.1
2074-01-19	15.6	18.1	16.2
2074-01-22	15.6	19.9	17.1
2074-01-25	15.4	18	15.5
2074-01-29	15.7	17	15.8
2074-01-30	15.5	20.1	16.2
2074-01-31	15.4	20.3	16.4
2074-02-01	15.8	17.6	15.1
2074-02-02	15.4	19.8	16.3
2074-02-03	15.5	19.8	16.5
2074-02-04	15.5	20.2	16.2
2074-02-05	15.7	17.2	15.8
2074-02-06	15.6	18.9	16.2
2074-02-14	15.8	19.5	16.4
2074-02-16	15.8	20.5	16.1
2074-02-17	15.6	20.5	16
2074-02-18	15.5	20.1	16.1
2074-02-21	15.4	20.3	16.1

The estimated central value at 95% confidence

$\bar{y} \pm 1.96 \sqrt{\ } = 17.02 \pm 1.96/\sqrt{\ } = 17.02 \pm 0.24$

$\frac{\bar{y}-}{\sqrt{\ }} \quad \frac{}{\sqrt{\ }}$

Appendix-V: ANOVA analysis of pH

	Control			Treatment			Source
	C1	C2	C3	T1	T2	T3	
2074-01-09	8.12	8.21	8.22	8.11	8.12	8.31	7.89
2074-01-10	8.2	8.12	8.22	8.11	8.21	8.2	7.72
2074-01-12	8.22	8.13	8.15	8.25	8.22	8.12	7.83
2074-01-19	8.29	8.14	8.11	8.14	8.06	8.22	7.79
2074-01-22	8.17	8.20	8.14	8.05	8.15	8.23	7.65
2074-02-02	8.14	8.05	8.19	8.19	8.22	8.29	7.84
2074-02-14	8.12	8.13	8.14	8.19	8.13	8.14	7.86
2074-02-18	8.22	8.15	8.11	8.19	8.15	8.11	7.75

ANOVA: Single factor analysis between control, treatment and source

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between Groups	0.0003	1	0.0003	0.444	**0.515**
Within Groups	0.010	14	0.0007		
Total	0.01	15			

ANOVA: Single factor analysis between control and treatment

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between Groups	0.0841	1	0.0841	38.35526	**2.3E-05**
Within Groups	0.030697	14	0.002193		
Total	0.114797	15			

Appendix-VI: ANOVA analysis of Dissolved oxygen

	Control (mg/l)			Treatment (mg/l)		
	C1	C2	C3	T1	T2	T3
2074-01-09	5.7	5.5	5.6	5.2	5.3	5.5
2074-01-10	5.5	5.6	5.5	5.6	5.2	5.2
2074-01-12	5.5	5.6	5.5	5.3	5.6	5.4
2074-01-14	5.7	5.5	5.5	5.2	5.2	5.6
2074-01-22	5.7	5.55	5.6	5.4	5.2	5.5
2074-01-25	5.6	5.6	5.5	5.2	5.5	5.2
2074-01-29	5.7	5.5	5.6	5.3	5.6	5.5
2074-01-30	5.5	5.7	5.6	5.4	5.4	5.2
2074-01-31	5.4	5.5	5.4	5.6	5.4	5.2
2074-02-01	5.6	5.75	5.5	5.3	5.6	5.3
2074-02-02	5.4	5.7	5.6	5.2	5.4	5.4
2074-02-03	5.6	5.7	5.6	5.3	5.4	5.6
2074-02-04	5.7	5.6	5.6	5.6	5.4	5.55
2074-02-05	5.7	5.6	5.8	5.4	5.3	5.5
2074-02-06	5.6	5.8	5.4	5.3	5.3	5.2
2074-02-14	5.7	5.5	5.5	5.5	5.3	5.4
2074-02-16	5.6	5.5	5.6	5.6	5.4	5.3
2074-02-17	5.4	5.5	5.7	5.4	5.5	5.3
2074-02-18	5.7	5.5	5.6	5.4	5.4	5.5
2074-02-21	5.4	5.7	5.6	5.4	5.6	5.5

ANOVA: Two-factor with replication analysis between control and treatment

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Sample	1.092	1	1.092	65.963	**4.6E-12**
Columns	0.223	19	0.011	0.710	0.797
Interaction	0.183	19	0.009	0.5838	0.907
Within	1.325	80	0.016		
Total	2.824	119			

The estimated central value at 95% confidence

$\bar{y} \pm 1.96 \sqrt{\ } = 5.48 \pm 1.96/\sqrt{\ } = 5.48 \pm 0.18$

Here, the all values of DO are lies within 5.48 ± 0.18.

Appendix-VII: ANOVA analysis of Total Ammonia Nitrogen

	Control (mg/l)			Treatment (mg/l)		
	C1	C2	C3	T1	T2	T3
2074-01-09	0.440	0.437	0.480	0.402	0.382	0.378
2074-01-12	0.549	0.487	0.507	0.322	0.292	0.256
2074-01-15	0.376	0.400	0.358	0.322	0.292	0.256
2074-01-19	0.397	0.409	0.406	0.204	0.231	0.192
2074-01-22	0.387	0.460	0.455	0.139	0.162	0.153
2074-01-25	0.387	0.460	0.483	0.204	0.199	0.159
2074-01-29	0.419	0.430	0.441	0.308	0.336	0.330
2074-01-30	0.432	0.417	0.419	0.342	0.353	0.349
2074-01-31	0.396	0.410	0.367	0.318	0.352	0.335
2074-02-01	0.499	0.464	0.524	0.224	0.209	0.168
2074-02-02	0.407	0.470	0.492	0.159	0.172	0.162
2074-02-03	0.499	0.464	0.524	0.373	0.379	0.396
2074-02-04	0.407	0.470	0.492	0.224	0.116	0.201
2074-02-05	0.437	0.411	0.431	0.201	0.152	0.170
2074-02-14	0.460	0.447	0.489	0.281	0.271	0.291
2074-02-16	0.547	0.506	0.483	0.281	0.271	0.291
2074-02-17	0.528	0.514	0.521	0.262	0.227	0.255
2074-02-21	0.465	0.395	0.416	0.367	0.265	0.360

ANOVA: Two-factor with replication analysis between control and treatment

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	0.939	1	0.939	1195.22	**1.39E-46**	3.97
Columns	0.198	17	0.011	14.8335	3.69E-17	1.76
Interaction	0.201	17	0.011	15.0860	2.36E-17	1.76
Within	0.056	72	0.0007			
Total	1.395	107				

ANOVA: Single factor analysis between source and treatment

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between Groups	0.194	1	0.194	34.03	**1.756E-06**
Within Groups	0.183	32	0.005		
Total	0.377	33			

ANOVA: Single factor analysis between source and control

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between Groups	0.013	1	0.013	3.4271	**0.0733**
Within Groups	0.124	32	0.003		
Total	0.137	33			

Appendix-VIII: ANOVA analysis of Alkalinity

	Control (mg/l)			Treatment (mg/l)		
	C1	C2	C3	T1	T2	T3
2074-01-09	180	150	150	180	150	180
2074-01-10	150	180	150	150	180	150
2074-01-12	150	165	180	180	180	180
2074-01-14	165	180	150	165	180	150
2074-01-15	150	165	180	180	180	180
2074-01-19	150	180	150	150	150	150
2074-01-22	180	165	165	180	165	165
2074-01-25	150	165	180	150	165	165
2074-01-29	165	150	150	180	165	180
2074-01-30	150	150	150	150	150	165
2074-01-31	165	150	165	165	165	150
2074-02-01	150	165	165	180	150	165
2074-02-02	150	165	150	165	150	180
2074-02-03	165	157.5	150	172.5	172.5	150
2074-02-04	157.5	157.5	165	165	157.5	165
2074-02-05	150	157.5	150	150	157.5	165
2074-02-06	165	165	157.5	142.5	150	172.5
2074-02-14	157.5	157.5	150	165	150	150
2074-02-16	157.5	157.5	165	157.5	157.5	165
2074-02-17	150	150	150	150	150	150
2074-02-18	150	150	150	150	150	150
2074-02-21	157.5	157.5	165	157.5	150	150

ANOVA: Two-factor with replication analysis between control and treatment

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Sample	288.07	1	288.07	2.76	**0.10**
Columns	5072.73	21	241.56	2.31	0.00
Interaction	1849.43	21	88.07	0.84	0.66
Within	9187.50	88	104.40		
Total	16397.73	131			

ANOVA: Single factor analysis between source, treatment and control

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between Groups	3853.03	21	183.48	2.31	**0.01**
Within Groups	3487.50	44	79.26		
Total	7340.53	65			

Appendix-IX: ANOVA analysis of Hardness

	Control (mg/l)			Treatment (mg/l)		
	C1	C2	C3	T1	T2	T3
2074-01-09	65	65	65	73	68	65
2074-01-10	63	65	61	68	69	66
2074-01-12	61	63	61	65	67	62
2074-01-19	59	62	61	66	69	64
2074-01-29	63	62	64	69	66	67
2074-02-02	65	65	64	59	65	65
2074-02-14	62	61	60	68	66	68
2074-02-21	65	61	60	69	65	66

ANOVA: Two factor with replica analysis between control and treatment

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Sample	176.33	1	176.33	41.29	**0.00**
Columns	59.58	7	8.51	1.99	0.09
Interaction	63.33	7	9.05	2.12	0.07
Within	136.67	32	4.27		
Total	435.92	47			

ANOVA: Single factor analysis between source and control

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between Groups	529	1	529	99.07	**9.89E-08**
Within Groups	74.75	14	5.33		
Total	603.75	15			

ANOVA: Single factor analysis between source and treatment

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between Groups	235.11	1	235.11	41.157	**1.6E-05**
Within Groups	79.972	14	5.71		
Total	315.08	15			

Appendix-X: ANOVA analysis of Phosphate

	Control (mg/l)			Treatment (mg/l)		
	C1	C2	C3	T1	T2	T3
2074-01-09	0.0123	0.0123	0.0201	0.0185	0.0162	0.0170
2074-01-21	0.0138	0.0123	0.0146	0.0154	0.0185	0.0193
2074-02-05	0.0130	0.0130	0.0146	0.0178	0.0162	0.0178
2074-02-20	0.0138	0.0123	0.0130	0.0162	0.0170	0.0162

ANOVA: Two factor with replica analysis between control and treatment

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Sample	6.96E-05	1	6.9E-05	18.39	**0.0005**
Columns	5.45E-06	3	1.8E-06	0.48	0.7
Interaction	2.68E-06	3	8.9E-07	0.23	0.87
Within	6.05E-05	16	3.7E-06		
Total	0.000138	23			

ANOVA: Single factor analysis between source and control

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between Groups	2.32E-05	1	2.32E-05	51.34	**0.00037**
Within Groups	2.71E-06	6	4.52E-07		
Total	2.59E-05	7			

ANOVA: Single factor analysis between source and treatment

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between Groups	4.54E-06	1	4.53E-06	4.91	**0.068**
Within Groups	5.54E-06	6	9.23E-07		
Total	1.01E-05	7			

Appendix-XI: ANOVA analysis of Nitrite and Nitrate

	Control (mg/l)			Treatment (mg/l)		
	C1	C2	C3	T1	T2	T3
2074-01-09	0.090976	0.086212	0.100151	0.085242	0.092123	0.085242
2074-01-21	0.088682	0.135132	0.12481	0.095564	0.092696	0.102445
2074-02-05	0.097525	0.100359	0.094692	0.087607	0.090441	0.08619
2074-02-20	0.101776	0.092566	0.113111	0.094692	0.103193	0.094692

ANOVA: Two factor with replica analysis between control and treatment

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Sample	0.00055	1	0.00055	5.40	**0.033**
Columns	0.00099	3	0.00033	3.21	0.05
Interaction	0.00026	3	6.88E-05	0.663	0.585
Within	0.00163	16	0.00010		
Total	0.00343	23			

ANOVA: Single factor analysis between source and control

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between Groups	0.0021	3	0.0007	0.881	**0.522**
Within Groups	0.0036	4	0.0008		
Total	0.0057	7			

ANOVA: Single factor analysis between source and treatment

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between Groups	0.0017	3	0.0006	0.9355	**0.5018**
Within Groups	0.0025	4	0.0006		
Total	0.0042	7			

Appendix-XII: ANOVA analysis of Growth

	Control	treatment
0 Days	0.8	0.8
15 Days	1.27	1.41
30 Days	1.78	2.05
45 Days	2.31	2.69

ANOVA: Two factor with replica analysis of mean weight between control and treatment

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Sample	7.82	1	7.82	49.82	3.11E-10
Columns	11.18	22	0.51	3.24	**4.25E-05**
Interaction	3.69	22	0.17	1.07	0.394983
Within	14.44	92	0.16		
Total	37.13	137			

ANOVA: Two factor with replica analysis of net yield between control and treatment

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Sample	28.16667	1	28.16667	1.564815	0.228945
Columns	5578.833	3	1859.611	103.3117	**1.1E-10**
Interaction	14.83333	3	4.944444	0.274691	0.842776
Within	288	16	18		
Total	5909.833	23			

ANOVA: Two factor with replica analysis of SRG between control and treatment

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Sample	0.015287	1	0.015287	157.4343	2.94E-08
Columns	0.208237	2	0.104119	1072.27	**2.97E-14**
Interaction	0.000386	2	0.000193	1.98886	0.179473
Within	0.001165	12	9.71E-05		
Total	0.225076	17			

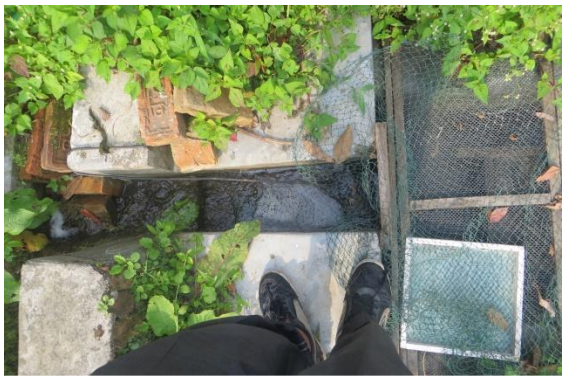
Photographs-III: Experimental setup

Siphoning the unfed material and solid material.

Feed providing to fish

Water collected from different source using pipe lines.

Water dammed to settle dissolved solid of water



Water filtered using net to stop leaves into water reservoir

Water reservoir covered with double net

Photographs-IV: Feed formulation

Prawn drying using heater drier

Dried Prawn

Furnace provided with motor for soya bean roasting

Grinder for feed formulation

Feed for feeding trout sized 0.5-2g

Photographs-VI: Fish weighing

Poring the water and fish from experimental bucket to 5 liter bucket

Collecting fish through hand

Keeping fish into beaker for weighing

Fish weighing

Data collection

Photographs-VII: Analysis of water for DO concentration

Sample collection for DO concentration

DO fixing with treating maganous sulphate

DO fixing with treating alkaline potassium iodide

Dissolving ppt with treating concentration sulphuric acid

Shaking the BOD bottle for proper dissolve ppt

Titration of sample solution with standard sodium thiosulphate solution

Photographs-VIII: Analysis of TAN concentration of water

Transferring 25ml of distill water into Erlenmeyer

Transferring 25ml of sample water into Erlenmeyer flasks

Transferring 25ml of sample water into Erlenmeyer flasks

(a) (b) (c)

Alkaline-hypochlorite solution preparation. (a) adding distill water in beaker. (b) Taking hypochloride using 2ml pipette. (c) Adding Trisodium citrate into hypochloride

(a)

(b)

Reagent adding to sample solution. (a) Adding Sod. salicylate into samples (b) Adding Alkaline-hypochloride solution into samples.

Taking sample into holder

Keeping sample into spectrometer

Taking absorbance reading of sample in spectrometer

Photographs-IX: Analysis of Nitrite and Nitrate concentration of water

Pipette out 25ml of sample water into 50ml tube

Adding zinc - copper catalyst

Adding Sodium hydroxide solution

Adding Hydrazine sulphate solution

Stirring the reagent added sample solution using magnetic stirrer

Weighing Sulphanilamide in electric balance

Diluting the hydrochloric acid

Dissolving the Sulphanilamide in hydrochloric acid

Weighing *N*-(1-naphthyl)-ethylenediamine-dihydrochloride in electronic balance

Dissolving *N*-(1-naphthyl)-ethylenediamine-dihydrochloride in magnetic stirrer

Adding *N*-(1-naphthyl)-ethylenediamine-dihydrochloride in sample solution

Color development in sample solution

Taking sample into tube

Keeping the tube into spectrometer