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Recent Used Techniques and Promised Solutions for Biofiltration Treatment of Fish Wastewater

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Abstract

Overpopulation and lack of resources have become serious problems in recent years. Therefore, there is an urgent need to find renewable sources of food to confront this problem. Aquaculture is an inexpensive and important source of protein, as aquaculture has increased significantly, especially during the past two decades. The wastewater produced from those farms includes many harmful elements such as ammonia (NH_3), organic matter (OM), phosphorous(P) and dissolved organic carbon (DOC). So, it has become necessary to find an appropriate method to treat the wastewater produced from those farms. There are many ways to treat wastewater from fish farms, including physical, chemical or biological methods. Biological treatment is one of the best methods used in wastewater treatment from aquaculture, as it is environmentally friendly and less expensive than other methods. The biofiltration process depends on the use of microorganisms that convert ammonium (NH_4^+) to nitrite (NO_2^-) and then to nitrate (NO_3^-), and this is done using Recirculating Aquaculture Systems (RASs). The main component of the biofilter is the filter media, which can be compost, wood chips, beads, or other organic or inorganic materials. There are many types of microorganisms used to treat wastewater like Microalgae, bacteria, fungi and yeast. The choice of the type of microorganism used depends on the chemical nature of the pollutants.

Keywords: wastewater, ammonia (NH_3), Biological treatment, ammonium (NH_4^+), nitrate (NO_3^-), Recirculating Aquaculture Systems (RASs), filter media.

1. Introduction

Overpopulation, climate changes and limited resources are among the most important problems facing the world at present. Therefore, the need arose to provide adequate food for the growing population, as aquaculture represents an important source of protein food and thus contributes to solving the global food problem. Aquaculture has also been recognized by international experts as one of the most effective techniques for obtaining protein because it uses grains less than animal protein [1, 2]. Aquaculture is a process in which fish are produced in the same quantity that they are produced under natural conditions, whether in fresh or salt water [3]. The Food and Agriculture Organization (FAO) has determined that fish represents about 17% of the protein consumed [4]. There are more than 3.1 million people around the world who depend on fish as their main source of protein. There is a prediction that the percentage of capture fisheries will halve in 2030 compared to 60% of production in 2011. It is expected that aquaculture will increase by 30 million tons during the same interval. If aquaculture is improved, we will be able to solve the food problem of more than a billion people around the world by 2050 [5].

In Egypt, different wastewater is treated for two important purposes. The first is to provide a non-conventional source of water. Since Egypt suffers from a large water deficit, this water that has been treated can be reused again to achieve the principle of Zero Liquid Discharge (ZLD). The second goal is to preserve the current sources of water from pollution if this polluted water is drained directly into the various sewers and water channels without treatment [6].

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Recent years especially the past two decades have seen a large intensification of the industry of aquaculture. Presently aquaculture production accounts for close to 50% of the consumption of fish worldwide. This intrinsic development in the aquaculture section attributes to the increase in fish demand, declining fisheries and improved technologies [7]. Aquaculture can aid in enface the problem of excessive demand for seafood and minimize the influence on fish bonds. Nonetheless, intensive fish farm productions add huge amounts of fish to face nutritional demands and consequently produce large quantities of waste [8]. So, the aquaculture industry now is considered to make a reverse and passive effect on the surrounding environment. Most governments have established strict regulations that deal with the discharge of aquaculture wastes that haven't been treated to the environment [7].

The effluent of the aquaculture includes nutrients, different organic and inorganic compounds such as NH_4^+, P, DOC and OM. The high levels of nutrients lead to environmental damage to the receiving water bodies [9]. Several regulations have been set up to control the quality of the discharge derived from aquaculture to the environment. Where the output discharge rules have been set by the Environmental Protection Agencies (EPA) which determines the amount of water produced from each farm separately. Whereas Increased residues in fish farm water lead to fish diseases, increase NH_3 in water, reduce oxygen (O_2) concentration and filter blockage. Negative damage from aquaculture should be minimized. Using chemicals to treat wastes may result in health risks, also there are many restrictions on their use. there is a need for fish farms that meet the demand for fish production without harming the environment or human health. Therefore, there has been a trend toward discovering a treatment method that is environmentally friendly and doesn't cause any damage and is also affordable [7].

Because of the risks of using antibiotics in general and in fish farms in particular, the most effective and healthiest way to treat aquaculture wastes is bioremediation. Biological treatment is defined as the method by which microorganisms can analyze pollutants before being released into the environment. These microorganisms are known as Bioremediating Agents or Bioremediators. As they can fully remove the wastes produced by the fish-farm facilities [7].

Biofiltration is an authoritative technology due to it being a cost-effective and environmentally friendly technology compared with other physico-chemical technologies, so it is used to control low and moderate concentrations of wastewater that contain odors and volatile organic compounds (VOCs) [10]. Biological treatment has many applications, like cleaning polluted areas such as soil, water and air. Where pollutants are converted to inorganic compounds such as carbon dioxide (CO_2), water and methane [11]. Bioremediation can be divided into two parts: biosorption and bioaccumulation. Biosorption is a rapid and reversible process. Many factors affect the biosorption of minerals like pH, biomass concentration, particle size and temperature. Biological absorption is independent of cell metabolism and can therefore occur in living or dead biomass. Bioaccumulation involves processes inside and outside the cell [12].

Fish secretions and particulate diffusion are major sources of NH_3. It can be disposed of by nitrification by setting a dynamic filter for sand and gravel. NH_3 is oxidized by different genera of bacteria such as Nitrosomonas, Nitrosovibrio, Nitrosococcus, Nitrolobus and Nitrospira. NO_2^- can also be oxidized by Nitrobacter, Nitrococcusand Nitrospira. Nitrification can be performed as follows [13].

$$NH_4^+ + 11/2\ O_2 \longrightarrow NO_2^- + 2H^+ + H_2O \quad (1)$$

$$NO_2^- + 11/2\ O_2 \longrightarrow NO_3^- \quad (2)$$

Where pollutants can be disposed of by microorganisms through various processes such as absorption, adsorption, and biodegradation. In aquaculture, bacteria are one of the best microorganisms that can analyze their contaminants. It can be used as the main means of bioremediation of fish farm contaminants by developing its natural metabolic activities [7]. Wastewater treatment can sometimes include optional basins, anaerobic and aerobic treatment including bacteria and protozoa. Fungi are also important in wastewater treatment as they withstand ambient conditions such as low pH. Dispersed bacteria and OM can be absorbed at the beginning of the treatment process by the presence of rotifer [14]. Microorganisms used in biological treatment may already be present in the contaminated area or isolated from elsewhere and brought to the contaminated area. Wastewater must inoculate with microbes that could degrade the contaminants in it. These microbes are either naturally present or synthesized in the laboratory to analyze these contaminants [11]. Algae, bacteria, fungi and yeast are microorganisms used in biological treatment as shown in Figure 1, from 2004 to 2014 according to the ISI Web of Science. They considered bacteria and fungi the most common microorganisms [12].

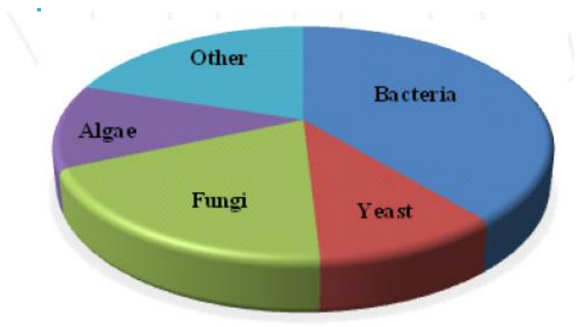


Fig. 1. Types of microorganisms that are used in biological treatment [12].

The cost of biological treatment depends on the nature of the pollutants, the aquatic environment and the ecological environment of the pollutants. Bacteria are used as energy sources or nutrients. There are several ways to stimulate the biological treatment process including the addition of nutrients, O_2, substrates or the introduction of microorganisms with the required capacities [15].

There are many types of bacteria used in this process such as Acromobacter, Alcaligenes, Arthrobacter, Bacillus, Cinetobacter, Corneybacterium, Flavobacterium, Micrococcus, Mycobacterium, Nocardia, Pseudomonas, Vibrio, Rhodococcus and Sphingomonas [15]. The minerals used in the biological treatment are shown in Figure 2 Cr, Cu, Cd, and Pb make up about 70% of the use [12].

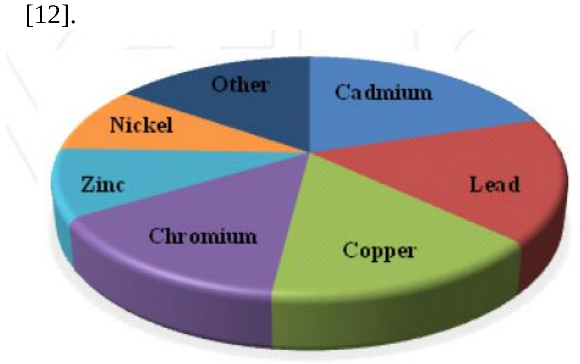


Fig. 2. Metals used in biological treatment [12].

Water produced from fish farms is recycled using Recirculating Aquaculture Systems (RASs). It leads to saving water and can also be treated and used again as well as using waste in the production of organic fertilizer [16]. RAS reduces the amount of water used compared to other conventional systems. To maintain water quality, solids, CO_2 and NH_3 must be removed, and pH, temperature and dissolved oxygen level (DO) must be maintained at appropriate levels as shown in figure 3 [17].

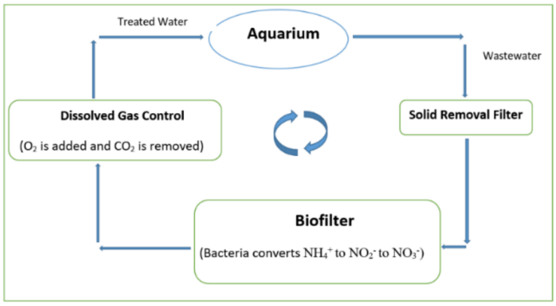


Fig. 3. Recirculating Aquaculture System (RAS) (amended and redrawn [17]).

2. Aquaculture in Egypt

Fish farming is an important and inexpensive source of protein, it also allows for the regeneration of natural fisheries. It accounts for about 50% of the total fish production in the world, and it is considered one of the most growing production sectors. The number of fish species farmed around the world is 424, which provides a source of protein for the poor. Aquaculture has grown with an annual growth rate of 8.8%. The contribution of the African continent to aquaculture is still negligible, at 2.7 percent, while that percentage is increasing significantly, especially in Egypt, Nigeria and Uganda. The region's production in 1995 reached 110,200 tons, while in 2018 the production reached 2,196,000 tons, which increased by 20 times. While Egypt is one of the most productive countries for aquaculture in Africa, followed by Nigeria, Uganda, Ghana, Tunisia, Kenya, Zambia, Madagascar, Malawi and South Africa. Where tilapia and african catfish are among the most important species farmed in Egypt. Aquaculture is considered one of the promising industries in these two countries due to the high demand for fish and the institutional commitment. Despite this success, there are some limitations, such as insufficient fingerlings, high cost of feed, and no reliance on technology [5, 18].

Fish farming began in Egypt about 2,500 B.C. and now it provides more than 79% of Egypt's fish needs. As most of the production comes from small and medium farms [3]. Modern management methods have been adopted to increase the production of aquaculture in Egypt. Whereas, over the past 200 years, there has been a great development in aquaculture in Egypt because of the shift from the traditional, extensive systems of fish farming to intensive fish farming, and the technology of producing aquatic feed has also appeared [5, 19]. Despite the success of aquaculture in Egypt, and the presence of lakes and the Nile, which has large areas of fisheries, this resource is not being exploited sufficiently. The price of fish in Egypt has increased from 0.86 $/kg in 2013 to around 1.53 $/kg in 2017 despite an increase in fish production from 1.45 million tons in 2013 to 1.82 million tons in 2017.

Egypt is ranked seventh in the production of fish farms in the world and first at the level of the continent of Africa. Its production accounts for 73.8% of the total aquaculture in Africa in terms of volume and 64.2% in value [19, 20].

Nile tilapia is one of the most important types of fish farmed in Egypt and accounts for 65.15% of the total production [18]. Aquaculture employs more than 6.2 million employees in Africa, thus it helps reduce the unemployment rate, and it also leads to economic development and contributes to food security. Aquaculture faces some obstacles such as the lack of capital, lack of resources and the need for wise management of the entire sector [5].

The fastest growing sector of food production in the world is Aquaculture, as it has huge potential to face animal protein needs. Egypt's annual production of fish exceeds 1.5 million tons annually, at a value of $2 billion. Fish farms contribute around 77% of the total fish production in Egypt and employ around 580,000 employees. Aquaculture production should also be increased to bridge the gap between supply and demand for fish. Aquaculture in Egypt is based on a wide variety of fish and shellfish. Tilapia is one of the largest of those species in terms of production quantity, as it represents around 67% of the total cultivated species [3]. Egypt ranks second after China in the cultivation of tilapia, with a value of around 900,000 USD, and its production is steadily increasing. Tilapia, carp and mullet are among the most cultured species in Egypt, where they make up 95%. In smaller quantities, shrimp, seabream, sea bass and meager are cultured as shown in figure 4 [21].

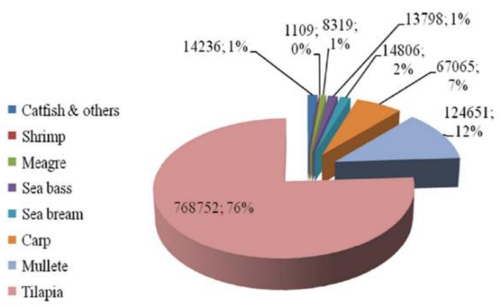


Fig. 4. Fish Farms Production [21].

The most cultured species of tilapia are the red tilapia hybrid, blue tilapia (O. aureus) and nile tilapia (Oreochromis niloticus). Although the production of farmed tilapia in Egypt ranks second in the world, after China, it cannot be exported to those markets due to Egypt's inability to meet the food safety standards for the European Union and the American market. Hence, all this massive production is consumed locally. Carp production contributes around 17% of the total production and ranks second after tilapia. Grass carp (Ctenopharyngodon Idella), silver carp (Hypophthalmichthys molitrix) and Common carp (Cyprinus carpio) are the most common cultured species. While the mullet represents about 10.5% of the total aquaculture in Egypt. The most common types are thick-lip gray mullet (Chelonlabrosus), black keeled mullet (Liza carinata), leaping mullet (Liza saliens), flat-head gray mullet (Mugil cephalus – L. 1758), blue spot mullet (Valamugilseheli), thin-lipped mullet (Chelonlabrosus) and golden gray mullet (Liza aurata). As for marine fish farming in Egypt, they are European sea bass and gilthead seabream and they represent 3% of total aquaculture production [5].

Although aquaculture in Egypt uses different systems, most of the aquaculture is carried out in semi-intensive ponds. While extensive and intensive aquaculture is constantly growing, the extensive system relies on the use of ground ponds while the intensive system uses concrete tanks. A permit to establish aquaculture farms is obtained from the Ministry of Agriculture, with the knowledge that the use of the Nile water in aquaculture is prohibited by the current laws [5]. Egypt possesses large areas of fish wealth, the Nile River and the lakes, all these resources are not properly exploited. Despite the increase in Egypt's fish production, it is still not meeting the needs of consumers [20]. The growth of aquaculture has resulted in the provision of more than one million tons of fish per week, however, it does not provide adequate training for fish farmers. In 2012 attention to this problem began by starting training in Best Management Practices (BMP) for farmers, tilapia, which is the most common species farmed in Egypt. Since the eighties of the last century, aquaculture production in Egypt has increased dramatically, as large areas of land have been allocated for the benefit of aquaculture on the edge of the Nile Delta Lake, Manzala, Burullus and Edku [22].

The quantity and quality of water allocated to aquaculture in Egypt are limited due to the limited water resources in Egypt. Though aquaculture is one of the most important industry sectors in Egypt, underground water and canal water are used, and the use of the Nile water is not allowed. As the use of water containing pollutants leads to a decrease in fish production as well as a decrease in its quality, which consequently leads to a negative impact on the user [21]. One of the important factors affecting aquaculture is water quality. As fish production in the aquaculture system depends on the integration between the feeding systems and the aquatic systems. Whereas, the feeding system is dependent on complementary feeding, while aquaculture depends on the adequate use of water of physical and chemical quality [23]. Environmental degradation, pollution, damage to water resources and poor infrastructure are among the most important defects of traditional aquaculture, which lead to an imbalance in the environment [1].

Aquaculture has developed from primary ponds to intensive aquaculture, and consequently, the sources of pollution such as feed, antibiotics and hormones have increased in the fish farm waters. The properties of the microbial biochemical reaction can be used to improve water quality [24]. There is an urgent need to increase water resources due to the increase in water use by more than double the rate of population increase. Hence the need to recycle wastewater and use it again in various fields. Recycling wastewater and treating it also leads to preserving the environment from pollution as it works to form natural fertilizers [25].

3. Aquaculture Wastewater

Aquaculture is considered one of the causes that led to the deterioration of the environment due to the wastewater generated from fish farms, which negatively affects the water system [26]. It is one of the most important defects in the development of fish farms. This is because the wastewater contains OM, P and nitrogen (N). Consequently, removing these pollutants in the wastewater produced from fish farms has become a necessity to protect the surrounding environment from pollution. This is done by following simple and promising technological methods for treating fish farm water [27]. Around 1–3 kg dry weight feed (assuming a food conversion ratio of about 1–3) is needed to produce 1 kg live weight fish. Around 36% of the feed is excreted as a form of organic waste. One of the most important pollutants in the wastewater produced from fish farms is the waste containing N, the most important of which is NH_3. Although both NH_3 and NH_4^+ may be toxic to fish, unionized ammonia (NH_3) is more toxic form to the fact that it is uncharged and lipid soluble. It has great damage to the environment when it is discharged without the required treatment [9].

There are many compounds from fish farm water that are either soluble (P and N) or in the form of solids. These solids can carry about 30 % - 84 % of the total P present in the water and about 7 % - 32 % of the total N present in the water. The rest is moved out of the farm in the dissolved part because it is too large to be separated using the particle separation technology used in the treatment of fish farm wastewater [16]. Nitrogenous compounds (NC) (NH_3, NO_2^- and nitrate (NO_3^-)), P and DOC cause environmental damage at high levels. NH_3 is the output of fish breathing and decomposition of excess OM. The balance between NH_4^+ and NH_3 depends on pH and temperature. Total ammonium nitrogen (TAN) is the sum of the two forms [9]. Chemoautotrophic bacteria (Nitrosomonas and Nitrobacter) oxidize NH_4^+ to NO_2^- and NO_3^- ions. Though, bacteria, aquatic plants and algae digest these ions as a source of N [28]. In aquaculture plants wastewater, N and P are found in small quantities, while suspended solids (SS) water is present in large quantities (2000–3000 mg/L) [15].

For example, there are large quantities of waste from fish farms discharged into the water without treatment, for example, there are 14250 tons/year NH_3, 10688 tons/year NO_3^- and 404 tons/year P being discharged into the Mediterranean Sea from Kufr El Sheikh, which leads to huge negative effects. On the environment and living organisms [23]. Ammonia-N is toxic to fish cultured at concentrations higher than 1.5 mg N/L. 01.025 mg N/L is considered the acceptable level of NH_3 in fishponds. Though, the toxicity threshold is based on the species, size, refractory organics, surface-active compounds, fine solids, metals and NO_3^- [9]. While the concentration of solids in contaminated fish farm water is about 5 – 50 mg/L and this concentration may vary depending on the management of the aquaculture system [16].

As all these harmful substances lead to the death of the fish, it has become necessary to use a wastewater treatment system to provide a suitable environment for the fish. Where a physical filter is used to remove SS and a biological filter is used to remove NH_3 and N. To get rid of SS, sedimentation ponds can be used in the pretreatment of wastewater from fish farms due to their low cost and less water waste. In addition to using the solid liquid separator to get rid of large SS only, it is also used to reduce pressure on the filter, increasing filter efficiency and thus improving water quality. Sedimentation ponds are one of the simplest methods of removing large particles and fish waste from fish farm wastewater, but it is less efficient than a solid liquid separator. Centrifugal force is used by the solid liquid separator to get rid of sized particles to avoid hindering the work of post-treatment units which includes helical rings and spiral separators. These units are removed with particles with a diameter of 80 mm or greater, which represents 80% of the total particles present in the wastewater of the fish farms [1].

Fish are the main source of NH_3 in fish farms, whether it is produced from food residues or excretions. The rate of NH_3 excretion is related to the feed and the protein level in the feed. NH_3 is the final product of protein metabolism, which remains in the form of non-ionized ammonia (NH_3) and ionized ammonia (NH_4^+). NH_3 one of the most important parameters of water efficiency is due to its toxicity to fish. However, NH_4^+ is toxic only in high concentrations [26]. The organic and inorganic materials are degraded by microorganisms that break down these proteins into NH_3. NH_3 is one of the most toxic substances to fish and has negative effects on the surrounding environment, unlike NH_4^+.

Ammonium oxidation results in NO_3^- and NO_2^-, which are two nutrients in aquaculture [29]. The dynamic process in which N is transferred from one form to another is rapidly represented by the different forms of N in the water. The life cycle of N is shown in figure 5 [17, 30].

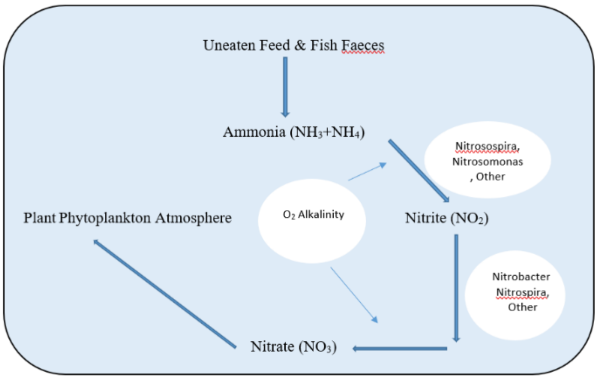


Fig. 5. The nitrogen cycle in the aquatic system (amended and redrawn after [17]).

Nitrogen Compounds (NC) in the aquatic ecosystem revolve in an endless circle, involving many stages. Firstly, the accumulation of NH_3 from fish waste, uneaten food, dead microorganisms and other causes. NH_3 is converted to NO_3^- using nitrifying bacteria, which can also convert CO_2 into carbohydrates. These bacteria utilize energy from the conversion of NH_3 to NO_3^- and from converting CO_2 into organic carbon (OC). NH_3 is removed either by autotrophic or heterotrophic nitrification. The main autotrophic bacteria genera in the nitrification process are Nitrosomonas, Nitrosovibrio, Nitrolobus, Nitrococcus, Nitrosococcus, Nitrospira and Nitrobacter. Heterotrophic bacteria and some fungi such as Aspergillus flavus may also be used. Secondly, the conversion of NO_2^- to NO_3^- by nitrite oxidoreductase enzyme. Thirdly, denitrification is in which anaerobic bacteria are used to minimize accumulated NO_3^- to a less harmful level of nitrogen gas, which is released from the system. NO_3^- resulting from the nitrification process can be absorbed by microorganisms and thus the cycle ends before denitrification [7, 15]. As shown in figure 6, they explained that NH_3 is nitrified N to NO_3^- with NO_2^- as an intermediate. In an aerobic environment, facultative heterotrophic bacteria more efficiently use O_2 for the oxidation of OM. In anoxic denitrification, these bacteria decrease NO_3^- and NO_2^- to nitrogen gas by electron capture and energy from biodegradable OM [31].

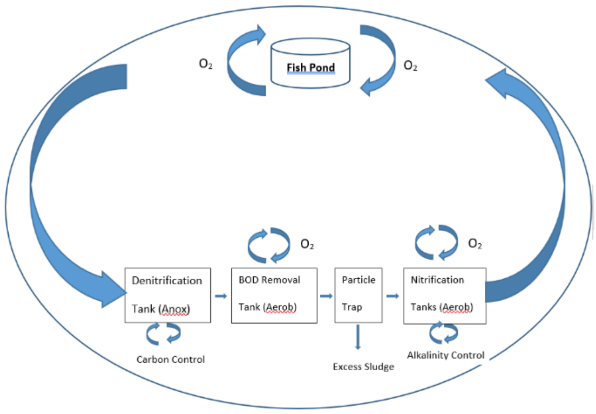


Fig. 6. The main functions in the RAS (amended and redrawn after [31]).

The fish farming system is used to remove NC due to the high toxicity of NH_3 and NO_2^-. However, urea ($CO(NH_2)_2$) can be removed in the aquaculture system at 0.014 $gN/m^2/d$ using a moving bed biofilter (MBB) [32]. There are several methods for NO_3^- removal, like adsorption, electrodialysis, chemical denitrification, ion exchange, membrane separation and biological denitrification [33]. NO_3^- can be converted to N using denitrification filters as it provides a suitable environment for the growth of anaerobic bacteria such as Pseudomonas, Bacillus and Alcaligenes, as shown in Figure 7 [13].

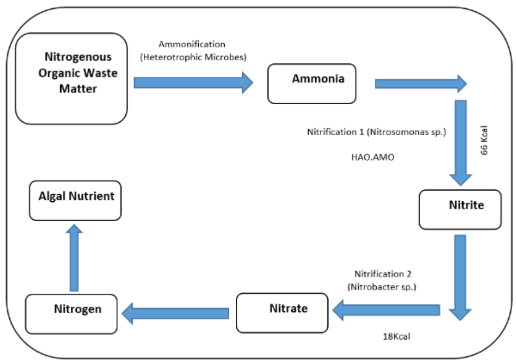


Fig. 7. Nitrification Process(amended and redrawn after [13]).

NH_3 is a proton acceptor and can be highly mixed in water. Factors affecting the conversion of NH_3 to NH_4^+ include temperature, pH and salinity. As NH_3 is un-ionized, it is more toxic to fish than NH_4^+. The total of both NH_3 and NH_4^+can be measured through TAN and none of them can be measured individually. Free NH_3 is produced in warm conditions and is very toxic to fish [34]. Significant NH_3 degradation is observed when a mixture of Nitrosomonas and Nitrobacter is used. Maximum degradation of NH_3 is measured on the 22^{nd} day of the biological treatment at 135 mg/L. An economical solution for aquaculture wastewater treatment was anaerobic treatment followed by aerobic treatment using Bacillus sp. Bacillus cereus and Aeromonas veronii are efficiently used to minimize the biochemical oxygen demand (BOD) in aquaculture output by more than 50 % [11]. NH_3 and NO_2^- should be kept below 0.1 mg/L. Nitrates should be maintained at a level below 50 mg/L. Nitrogen removal may be calculated according to [35]: -

$$N\ removal = \frac{B^t - B^0 * tissue\ N}{\left(\frac{B^t + B^0}{2}\right) * t} * \frac{DW}{FW} * 10^3 \qquad (1)$$

Where:

N removal: (gm N/gm DW day)
B_t: Biomass during the last day (t) (gm)
B_0: Biomass during the first day (gm)
DW: Dry weight (gm)
FW: Fresh weight (gm)

Fish farms produce from 42 to 66 kg of N per 1 ton of fish. As the concentration of NH_3 exceeds a certain limit, the fish will go into a coma and ultimately die. Therefore, the appropriate means must be used to control the concentration of NH_3 and NO_3^- to protect the environment and fish [26]. Excessive use of food and fish excreta leads to damage to the surrounding environment, including N pollution by 30 - 40% [36].

4. Wastewater Treatment Methods

TAN, total organic carbon (TOC) and BOD result from protein rich wastes from fishponds. Physical, chemical and biological processes are the methods that are used to treat water produced from fish farms. Physical units use physical forces to remove pollutants. Sedimentation or mechanical filtration such as screen filtration or expendable granular media filtration are accomplished for solid particles removal. Chemical units are used with biological processes and physical treatment units to treat wastewater from fish farms. The disadvantage of most chemical processes is that the chemicals stay for a large part in the water. Disinfection by ozonation is the major chemical process used in aquaculture. Disinfection by ultraviolet (UV) may be utilized due to the absence of toxic by-products. Nitrification is done by 2 general types of fixed film filters: emerged (rotating biological contactors (RBCs), trickling filters (TFs)) and submerged (fluidized bed biofilters (FBBs), bead filters (BFs)) [9].

Various conventional approaches are adopted for the removal of the wastes in aquaculture systems, however, they face huge problems like membrane fouling, high cost and the generation of toxic by-products. Electrochemical technology is used to face such matters. The advantages of electrochemical treatment contain small equipment sizes, ambient operating conditions, high efficiency, rapid start-up and minimal sludge generation. The bioelectrochemical reactors (BERs) are the best system, that can produce energy from wastewater (using microbial fuel cells) or a valuable product like hydrogen (using microbial electrolysis cells). BERs could remove contaminants at high efficiencies (≈99%) and therefore they have the least impact on the environment [28].

Despite the diversity of wastewater treatment methods among physical, chemical and biological methods, each of them has its drawbacks, which may be either high cost or ineffective in removing pollutants. However, the biological method has proven more efficient than the other methods. Whereas in the anaerobic biological treatment method, an unpleasant odor and turbidity are generated, and the pollutant removal efficiency is low. On the contrary, the aerobic biological treatment methods proved to be more efficient than the anaerobic biological treatment methods in terms of temperature, pH and organic load imbalances. In addition, the aerobic biological treatment needs a large surface area to ensure good ventilation [37]. Table 1 shows a comparison between anaerobic and aerobic systems [14].

Table. 1. Comparison between anaerobic and aerobic systems

Parameters	Aerobic	Anaerobic
Energy requirement	High	Moderate
Temperature sensitivity	Low	High
Effluent quality	High	Medium
Odor	Low potential to produce	High potential to produce
Nutrient requirement	High	Low
Organic Removal Efficiency	High	High

The biological treatment is mainly based on microorganisms, which break down the pollutants and keep the system stable. Also, biological treatment has many advantages compared to other treatment methods in terms of use and maintenance costs as well as the impact on the environment [14]. Mechanical and biological treatment of RASs allow water to be recycled and minimized water discharge. RASs improve water quality and decrease water discharge, but typically they aren’t zero-waste designs. To ensure that the biofilter operates efficiently, solids should firstly be removed [8].

The use of the RASs began in the 1960s, as the systems used at that time were fixed aquaculture systems that use gravel as a filter medium, as well as multi-stage aquaculture systems. The development of RBCs increased in the 1970s, as did pretreatment before biological treatment. In the 1980s, the main pattern of fish farming appeared, with the emergence of a unified pattern of industrial agriculture in Europe, which had several advantages such as easy management and preservation of the environment. Biological treatment and biofilms were used in fish farm water recycling systems in the 1990s to purify water, increase O_2 levels, and control temperature. The establishment of a system to recycle the aquaculture system is through environmental science, information and biology, and the use of biological or physical filters to remove residues, fodder residues, NO_2^- and NH_3 from the water, add O_2, remove CO_2, then adjust the temperature and return the water to the reservoir, which is done several times and subsequently increase the efficiency of the aquaculture process [1].

5. Biological Wastewater Treatment (Biofiltration)

The biological wastewater treatment process utilizes microorganisms to remove nutrients and OM from wastewater. This method is the most effective way to treat wastewater compared to physical or chemical methods, as it is environmentally friendly. In biological treatment, a bioreactor is used in a medium on which microorganisms grow, which convert harmful pollutants in wastewater into less harmful substances [25]. The operation theory of biofilter is the wastewater passes over a fine porous medium (diameter of 2 ± 3 mm) wherever the SS is filtrated and the OM is degraded by utilizing the fixed film biomass therefore purification occurs. These eventually lead to the clogging of the biofilter. A clogging cycle commonly continues from 24 to 48h [38]. The bioremediation process depends on the ability of the biomass to bind ions or molecules in the water. Also, bioremediation can be developed and used in the future, as it is low cost and environmentally friendly. In bioremediation, a wide range of algae, bacteria and fungi can be used to break down pollutants [12]. Probiotics is a method of biological control as it is a competitive exclusion process to improve a specific environment by introducing harmful organisms, like parasites or specific pathogens.

Basics of biological treatment: -

- Biodegradation of contaminants from harmful compounds to other less harmful compounds.
- All ambient conditions should be provided to allow microbial growth necessary for faster decomposition of waste [13].

There are a lot of advantages of using biological wastewater treatment processes:

1- Relative low operating and investment costs.

2- The increasing competitiveness of these technologies compared to the more conventional processes, like the adsorption, absorption, incineration or condensation process [39].

The operation of a new biofilter takes up to six weeks, depending on the environment. Initially, the ammonia level is steadily increasing, which the Nitrosomonas bacteria analyze and convert to NO_2^- until the Nitrobacter bacteria convert it into NO_3^-. Adaptation can be accelerated by pollinating the new system with water from an existing system [17].

Ammonium is converted to nitrite:

$$NH_4^+ + 1.5\ O_2 \xrightarrow{\text{Nitrosomonas}} NO_2^- + 2\ H^+ + H_2O \quad (3)$$

Nitrite is converted to nitrate:

$$NO_2^- + 0.5\ O_2 \xrightarrow{\text{Nitrospira/Nitrobacter}} NO_3^- \quad (4)$$

The overall reaction for ammonium conversion to nitrates is:

$$NH_4^+ + 2\ O_2 \longrightarrow NO_3^- + 2\ H^+ + H_2O \quad (5)$$

The main obstacle in the work of the RAS is to remove nitrate–nitrogen (NO_3^--N) from the water which affected the economic and environmental sustainability of these systems. It is necessary to improve practical and cost-effective ways to minimize RAS (NO_3^--N) loads to be preserved or improved productivity with the achievement of water quality aims and good environmental management [40].

The biofilter consists of a closed chamber containing contaminant-degrading microorganisms. The packed media of biofilter must provide a high ability for water uptake, have a long working life and provide a low pressure drop for the water passing through the packing material [41]. Biofiltration depends on supplying the pollutants water with a wet fixed-bed bioreactor (FBR) containing a porous layer which is the basis for supporting biomass growth.

Biofilter media are usually made of wood bark, peat, compost, soil, activated carbon or other organic material, that can degrade the pollutants by the microorganisms [42].

Factors that ensure the success of the biological treatment process are eliminating excess N, reducing the accumulation of hydrogen sulfide (H_2S), minimizing the accumulation of sludge and decreasing the concentration of NH_3. There are several steps by which biological treatment can be improved:

1. Studying the output of fish production units.
2. Identifying the formed bacteria and the possibility of isolation.
3. Conducting experiments for biological treatment of fish farms wastewater using isolated bacteria [15].

The biological treatment process is dependent on the use of bacteria to convert the dissolved waste into biomass. The biological treatment is used to treat wastewater from fish farms and reuse it again for different purposes. Various types of microorganisms can be used in the bioremediation process. Such as the use of algae, bacteria and protozoa to remove organic and inorganic wastes in the biofilm treatment system [26]. The cost of the bioabsorption process is low due to the biomass can be produced from industrial waste, which may be reused more than once. The process of bioaccumulation is very expensive because it requires the presence of living cells that can't be reused. Also, table 2 shows a comparison between the main parameters of the bio-absorption and bioaccumulation process [12].

Table. 2. A comparison between the main parameters of the bio-absorption and bioaccumulation process

Characteristics	Bioaccumulation	Biosorption
pH	A great change in pH has a large influence on living cells	pH solution has a large influence on heavy metals and sorption capacity. The procedure may take place in a great pH range
Cost	Ordinarily High. This process takes place in the presence of the cells of living organisms that must be supported	Ordinarily low. Industrial waste provides biomass. production of biosorbent and Transportation influence cost.
Rate of removal	The rate of removal is slower than in the biosorption due to the long time that intercellular accumulation takes	At a fast rate, most mechanisms occur
Selectivity	Better than in the biosorption	Poor. But this may be increased by modifying biomass transformation
Recovery of metals	Biomass doesn't use for other purposes, even if it is possible	The recovery of heavy metals is possible with an appropriate eluent
Regeneration and reuse	Because of intercellular accumulation, reuse is limited	Biosorbents may be regenerated and reused in many cycles
Energy demand	It is required for cell growth	Usually, low

As the nitrification process is used to remove NH_3 from wastewater using biological oxidation. It takes place in two steps, which are converting NH_3 into NO_2^- and then into NO_3^- and it is carried out by bacteria called nitrifiers. The nitrification process is carried out by two specific types of autotrophic bacteria, Nitrosomonas and Nitrobacter [36].

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H+ + 2H_2O + \text{Nitrosomonas} \quad (6)$$

$$2NO_2^- + O_2 \rightarrow 2NO_3^- + \text{Nitrobacter} \quad (7)$$

One of the most important biological treatment methods for wastewater is the active sludge, as it removes N and P. zoogloea is formed for the biodegradation of pollutants through nitrification and denitrification. High efficiency and simplicity of operation are the main advantages of the biological membrane method. As the microorganisms grow on the biofilm and work on the biodegradation of OM, the active sludge method depends on the fixed effect of the microorganisms. The content of N, H_2S, and turbidity, as well as chemical oxygen demand (COD), can be reduced and the growth of harmful microorganisms is prevented through the Gelatin mucosal biofilm formed on the surface of the filter. A biofilm system for aquaculture wastewater treatment usually contains an ozone generator, a UV sterilizer, a protein separator and a biofilter. The biofilter is the appropriate environment for microbes to grow on through the biofilm. Usually, it is dependent on RBCs, FBBs and submerged biofilters (SBs) upon intensive fish farms [24].

6. Types of Biofilter

There are five essential water treatment steps in RAS and they are solids removal, aeration and degassing, circulation, biological filtration and disinfection. Biological wastewater treatment is desired for water reuse and the capability of a biofilter is limited by its nitrification ability. The various types of biofilters utilized in RAS units have their advantages and disadvantages [43]. RBCs, TFs, BFs and fluidized sand biofilters (FSBs) are utilized to remove N from aquaculture wastewater [9].

RBCs, TFs, FBFs, floating packed-bed reactors (FPBRs) and SBs are considered the traditional fixed membrane biofilters used in fish farm water treatment. The new types of biofilters include the three-phase fluidized filter, hybrid biofilter (HB) and MBB. Dolomite, coral reefs with a diameter of 5 mm or crushed oysters can be used as biofilter media in the SBs, while plastic filter medium is one of the most common types. Capital, operating costs and water quality influence the choice of the biofilter. The quality of the biofilter is judged by its ability to remove NH_3, the absence of NO_2^-, microbial growth on the biofilm, the absence of frequent maintenance and the possibility of constructing it on a small area. Each vital filter has its advantages and disadvantages, and through which the required biological filter is chosen. The wastewater from fish farms has a lower concentration of pollutants compared to domestic wastewater. Several factors affect the nitrification process, including temperature, acidity, OM, salinity, DO and substrate concentration [36, 44].

6.1 Trickling Filters (TFs)

It consists of a fixed packed media through which pretreated wastewater trickled downwards through an aerobic biofilm. The major factor is the filter media. The main characteristics of a filter medium are specific surface area, homogeneous water flow and void ratio which influenced the contact time between biofilm and trickling water and influence pore-clogging. The surface required for biofilm growth and homogeneous water flow concerned with dead zones and channels in the system are indicated from the specific surface area [28]. Figure 8 shows the Typically trickling filter [44].

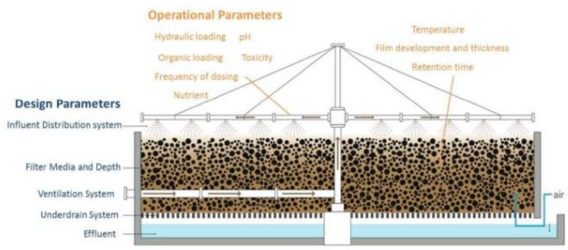


Fig. 8. Typically Trickling Filter [44].

6.2 Rotating Biological Contactors (RBCs)

RBC is dependent on the rotation of a submerged substrate, that is made of high-density polystyrene or polyvinyl chloride, connect to a shaft. Nitrifying bacteria grow on the media and they alternately contact N-rich water and air due to the rotation. CO_2, generated by the bacteria exchanged with O_2 from the air when the RBCs rotate. RBC systems are divided into a group of separate stages. When water flows through the champers, the organic content will decrease. Hence the system developed organic removal. RBCs have low head requirements to move water through the vessel. This provides a low chance of clogging, passive aeration and CO_2 removal. An average TAN removal rate is about 0.42 g/m^{2}day was obtained when RBCs are used in a tilapia RAS. Ammonia removal efficiency is minimized by rising influent DOC levels. No expected relationship between ammonia oxidation performance and the feed loading rate. Ammonia oxidation performance affects by mass and hydraulic loading, organic loading, rotational speed and staging [9]. Figure 9 shows the front and side view of RBC [45].

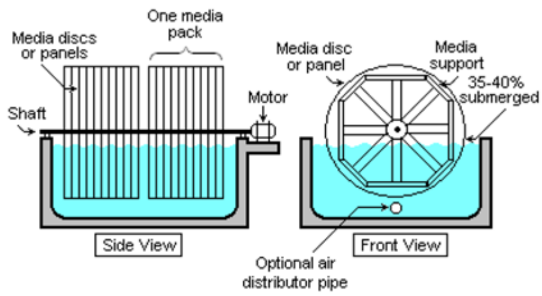


Fig. 9. Rotating Biological Contactor [45].

6.3 Fluidized Sand Biofilters (FSBs)

It is widely used in RAS. Where they should maintain excellent water quality. FSBs have a high specific surface area, 4000–20000 m^2/m^3 and have a moderate cost. But they do not aerate, as do TFs. Thus, extra aeration is required. Also, these filters must be operated within a narrow water flow band to maintain appropriate bed expansion [9]. The FSBs may use for clogging issues solution in TFs, in addition to it removes dissolved wastes from RAS efficiently compared to RBCs, TFs and BFs. The most influential factor in the removal operation is the particle size of the medium. Also, other factors like the media and bed management techniques influence the performance of this system [28].

6.4 Submerged Biofilters (SBs)

It is easy and versatile, simple to maintain and easy to construct. It is used in small fish farms. The source of O_2 in it is water because the filter medium is completely submerged in the water. Its efficiency depends on the filter medium and the hydraulic retention time (HRT). While the dead zone is considered one of the most important problems of the SBs, in this area the amount of O_2 and nutrients needed for bacteria are not available. This problem can be solved by increasing the flow rate of water [46]. An experiment was prepared to consist SBs tanks and the filter medium which consisted of coconut fiber, wastewater and biomembrane. 5 SBs tanks were used to remove NH_3 from wastewater from fish farms. As NH_3 concentrations of 10, 20, 30, 40 and 50 mg/L were used (as R1, R2, R3, R4 and R5, respectively). The filter medium was immersed in water and the NH_3 concentration was measured every 4 hours for a week. The experiment lasted for 156 h, with a depth of 20 and 30 cm of coconut fiber medium. The results showed that the greater the depth of the filter media, the greater the ammonia removal efficiency at the lower concentration due to the increase in the surface area on which the biofilm has grown [26]. Figure 10 shows downflow and upflow submerged biofilter respectively [44].

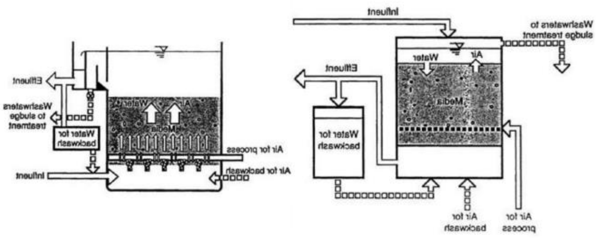


Fig. 10. Downflow and Upflow Submerged Biofilter [44].

6.5 Bead Biofilters (BFs)

It is considered one of the popular and new types at the same time, where the filter medium is plastic beads of polypropylene or polyethylene. Where the wastewater enters in an upward direction and the plastic beads float on it. One of the most important advantages of beads is that the ratio of their large surface area to their volume is sufficient for biofilm to exist, and at the same time small enough to trap SS. While one of the most important problems of the BF is the presence of carbon (C) materials that lead to the growth of heterotrophs bacteria at the expense of nitrobacteria. Also, this filter requires high skill in operation and high energy consumption [46].

7. Biofilter Design Parameters

7.1 Type of Packed Material

The biofilter systems are packed with suitable filter media which able to develop the quality of outputs for recirculation applications. The characteristics of the packed media affect the efficiency of the biotreatment more critically than biofilter flow scheme arrangements. The surface area and structural characteristics of the biofilter packing material are helpful for the improvement of biofilms and the capture of organic suspended matter (OSM) is required to obtain perfect biofilter performance [47]. The filter packing material is coated with a layer of microorganisms such as bacteria and fungi. The microorganisms in the biofilter under these conditions are capable to use the captured OSM as an alternate substrate to support their metabolic activities while the concentration of the initial substrate, such as BOD is low [47, 48]. When a group of microorganisms bind together and stick to the surface, they form what is called a biofilm. The growth rate of chemoautotrophic nitrifying bacteria is slow and therefore biofilm formation is difficult. The cross-adhesion of the biofilm formation of nitrifying bacteria is facilitated by attachment to the biofilter by extracellular polysaccharides. Biofilm is highly effective in removing N in the biofilter, especially when the wastewater is water from fish farms. As the main factor determining the efficiency of the biological filter is microbial activity [36].

The filter packing material affects the general efficiency of the biofilter. Therefore, its choice depends on several considerations such as its surface area and pores. The filter packing material can be compost, wood chips, beads, or other organic or inorganic materials, [47, 48]. Various organic and inorganic media may be used as biofilter media. organic media are low-priced, maintain the water content at the best levels for microorganisms and are appropriate to release inorganic nutrients, while inorganic media show higher contact surface area and durability. Some mixed media for biological wastewater treatment showed more stable nitrification capability, lower acidification, faster startups, lower pressure drop and less compaction than organic media [49]. Coconut is a suitable medium for biofiltration to remove N due to its porosity and large surface area. Where, NH_3 concentrations of 10, 20, 30, 40 and 50 mg/L (in R1, R2, R3, R4 and R5 filters respectively) were used. Coconut filter medium was used at depths of 20 and 30 cm (group A and total B straight). It was observed that the rate of NH_3 removal increased with increasing the concentration of NH_3, as the rate of removal increased from 0.083 mg/L/h in the case of R1 to 0.67 mg/L/h in the case of R5, in case A. In the case of B,ce the rate of elimination increased from 0.27 mg/L/h in the case of R1 to 0.70 mg/L/h in the case of R5. It was also noted that case B is better than case A at the same concentration in terms of efficiency and rate of removal due to the increased depth of the filtration medium, which allows for better growth of the biofilm. Coconut fiber is a good biofilter media for wastewater treatment. Where it was found that the use of coconut fiber instead of gravel as a medium for biofiltration achieved high efficiency in removing BOD_5, COD and ammoniacal nitrogen (NH_3-N) [26].

7.2 The Type and Size of Biofilter

The type and size of biofilter are the most important parameters to both the economic and technical success of a RAS [43]. There are several factors affecting the size of the biological filter, including water salinity, surface area, feed protein, temperature, O_2 concentration in water and fish density [17].

7.3 Clogging

Clogging is one of the most important problems facing biofilters due to excess biomass, especially when the loading rate is high. The clogging may be occurred due to various physico-chemical and biological reasons. This obstruction results in a high pressure drop and a decrease in the removal rate of VOCs. The head loss of biological treatment operation occurs by the accumulation of solids and the growth of biomass. The head loss may be affected by operating factors, including the filtration rate, under normal conditions. For high filtration rates, the clogging increases because the biofilm and the inert particles separated from the medium are distributed deep in the filter [38, 50]. There are several methods for controlling excess biomass in the biofilter, including physical methods that are somewhat effective, such as backwashing with water and stirring, but its disadvantages are its high energy consumption and its negative effect on the filter medium. As for the chemical methods, either using NaOH or NaClO, it can be observed their inefficiency as they negatively affect the microorganisms and the biodegradation process. While introducing protozoa is one of the natural methods that may be used to overcome the previous problems [50]. Periods of interruption due to clogging might extend from a few minutes to several days. When the flow is stopped for intervals up to 12 h, the effect on performance is little. Long and frequent interruptions seriously affect the process [42].

8. Factors Affecting Biofilter Operation

One of the most important factors affecting fish farm production is water quality, as it affects the growth, health and survival rates of fish. Water quality also indicates any physical, chemical or biological properties that may affect fish production [51]. Also, several biotic and abiotic parameters affect biofilter performance, such as pH, temperature, alkalinity, OM, salinity, DO concentration, substrate content and turbulence level, [9, 17, 52]. Also, the lack of nutrients, pollutant toxicity, oxidation, substrate depletion and microbial interaction are the most important factors affecting biological treatment [13]. Nitrifying bacteria are sensitive to surprising environmental troubles and through biofilm forming at biofilter start-up. They are sensitive microorganisms of inhibitors and expose to wide types like high concentrations of NH_3 and nitrous acid, pH out of the optimal range (7.5–8.6) and low DO levels (<1 mg/l). The second step of nitrification ($NO_2 \rightarrow NO_3$) especially, is highly sensitive to even traces of sulfides. Nitrification requires a low C/N ratio because if the C/N ratio is high, the heterotrophic bacteria out-compete nitrifiers for available O_2 and space in the biofilters [9].

There are some parameters for aquaculture water quality for RAS, as shown in table 3. The biotreated aquaculture water is satisfy these parameters for reuse fields. Reuse of aquaculture water could reduce water requirements for aquaculture and reduce the discharge of nitrogenous matter and carbonaceous into receiving water bodies by 90%, thus reducing water contamination problems [47].

Table. 3. General water quality parameters for RAS

water quality parameters	Unit of measure	Water quality guidelines
pH		6 - 9
BOD_5	mg/L	< 5
NH_3	mg/L	0.02 – 0.5
NO_2^-	mg/L	0.2 – 5.0
NO_3^-	mg/L	<1000
Dissolved oxygen (DO)	mg/L	>0.6
Suspended solids (SS)	mg/L	< 10
Carbon dioxide (CO_2)	mg/L	< 20
Alkalinity	mg/L as CaCO3	> 20

But water quality parameters under general conditions in pilot scale RAS are shown in table 4 [17].

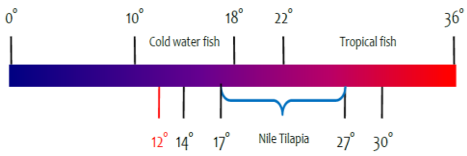
Table. 4. water quality parameters under normal conditions in pilot scale RAS

Parameter	DO mg/L	pH	CO_2 mg/L	Temp °c	TAN mg/L	Salinity ppt	NO_3-N mg/L	NO_2-N mg/L	Density kg/m3
Tilapia	4 - 6	6.5-8.5	≤30-50	20-30	<3.0	≤10-15	100-200	0.05-1.00	85-120
Rainbow trout	6 - 8	6.5-8.0	≤15	2-21	<7.5	0.0-30	<200	<1.00	50-80
European eel	6 - 8	5.0-7.5	10-20	23-28	0.0-5.0	0.0-5	50-100	0-1.50	50-120
Sturgeon	8	7.0-8.0	n.a.	18-25	<3.0	0.0	<25	<0.50	80-100
Arctic charr	9 - 11	6.5-8.5	≤22	5-12	≤1.0	<24-26	<10	<0.50	85-130
Pike perch	6 - 8	6.5-7.5	10-20	22-25	0-10.0	0.0	≤56	0-1.50	15-60

Wastewater is rich in OM resulting from compounds containing N and carbonaceous compounds in addition to the presence of dissolved or suspended solids. The wastewater from fish farms is characterized by its physical and chemical properties, which are represented by the presence of a large amount of COD and organic nitrogen (ON). The main chemical parameters of wastewater are the pH, NH_3^-N, NO_3^--N, BOD_5, COD and phosphorus (P). While, the physical parameters are temperature, turbidity, solids and odor [25]. From a microbiological point of view, the following conditions during the nitrogen removal must be met:
1) Enough O_2, low concentrations of OM and a higher sludge age for the mainly autotrophic nitrification.
2) lack of O2 without the presence of DO, with enough source of organic substrate for heterotrophic denitrification.
N removal in biological wastewater treatment needs to use a combination of controlled nitrification and denitrification [30].

8.1 Dissolved Oxygen (DO)

DO is important for keeping fish alive in fish systems. For good production, it must be maintained at saturation level or at least higher than 5 mg/L as its decrease affects the activity of nitrification bacteria and causes gill problems. O_2 consumption is



associated with the rate of feeding, temperature, the presence of fish wastes and fish size (smaller fish consume more O_2). Warm-water fish such as tilapia adapt better to lower O_2 intake than cold-water fish [17]. Inorganic substances of N and P are used as nutrients. The increase in biomass also helps the growth of microorganisms and increases their number. This ultimately leads to a reduce the amount of DO in water because of the decomposition of microorganisms, which negatively affects organisms that need O_2 [25]. The range of the DO level in the bioremediation process should be between 1.5 to 4 mg/L [15].

8.2 pH

The appropriate pH range for fish farms is between 6.5 and 9. When it increases (water is alkaline), NH_4^+ is converted to NH_3, which is toxic to fish. The pH should be measured periodically (at least once a week) [17]. It is necessary to add an alkalinity raising compound when low pH affects the fish growth and nitrifying performance because of the acidifying effect of nitrification. Hence, an alkalinity control loop is used through the nitrifying reactors, and the addition of an easily biodegradable organic substrate (BOS) into the anoxic tanks for feeds producing a low C/N ratio in the wastes, may also be necessary [31].

8.3 Temperature

Temperature is one of the most important factors affecting the biological treatment process. Ambient temperature determines the temperature of a microorganism as its internal temperature cannot be controlled [25]. Each type of fish has a range of temperatures that is appropriate for its growth. For example, the right range for Oreochromis niloticus (Nile tilapia) is 14 °C to 36 °C and the appropriate growth range is between 27°C to 30°C. The growth phase is between 600-800 gm in 6-8 months [17].

8.4 Total Ammonium Nitrogen (TAN)

The metabolism of fish effluent in TAN ((NH_3) (NH_4^+)) is toxic to fish at concentrations higher than 1.5 mg NH_3-N/L [35]. TAN is affected by temperature and pH e.g., at pH 4.5 nitrification has ceased and TAN concentrations increase [17].

8.5 Volatile Suspended Solids (VSS)

Water quality can be measured by VSS where the biological stability of water protectors is measured at 550±50 °C where the organic part is oxidized and volatilized as gas at 550 ± 50 °C (volatile solids) and the inorganic part (SS) remains. They defined VSS as an indicator of the biomass content in water and may be calculated from [25]:

$$VSS\left(\frac{mg}{L}\right) = \frac{(A-B)}{C} * 1000 \qquad (2)$$

Where:

A Mass of nonfilterable residue on Whatman GF/C filter after evaporation at 550 ◦C (mg)
B Mass of nonfilterable residue on Whatman GF/C filter after ignition at 150 ◦C (mg)
C Volume of the sample used for filtration (mL)

8.6 Biochemical Oxygen Demand (BOD)

BOD can be defined as the amount of O_2 that microorganisms require for the biodegradation of OM. The BOD limit set by the Tamil Nadu Pollution Control Board (TNPCB) is 100 mg/L. The high BOD value increases the concentration of total solids (TS) and SS and the number of microbes [15]. BOD_5 is used to determine the O_2 required for microorganisms in water so that it can complete its mission by oxidizing OM in water to other less harmful substances for 5 days at 20°C [25].

$$BOD_5\left(\frac{mg}{L}\right) = Dilution\ factor\begin{bmatrix}(DO\ of\ the\ diluted\ sample)\\ -ratio\ of\ seed\ in\ diluted\ sample\\ to\ seed\ in\ seed\ control\\ (DO\ of\ the\ seed\ control)\end{bmatrix} = F\,[(DS_0 - DS_5) - f(SC_0 - SC_5)] \qquad (3)$$

$$BOD_5\left(\frac{mg}{L}\right) = Dilution\ factor * [(DO\ of\ the\ diluted\ sample) - (DOofdilutionwater)] = F * [DS_0 - DS_5] - [DW_0 - DW_5] \qquad (4)$$

Where:

DS_0: initial DO in the diluted sample
DS_5: final DO in the diluted sample after 5 days of incubation
DW_0: initial DO in the dilution water
DW_5: final DO in the dilution water after 5 days of incubation
SC_0: initial DO in diluted seed solution (seed control)
SC_5: final DO in diluted seed solution (seed control) after 5 days of incubation
F: dilution factor [total volume after dilution (mL)/volume of undiluted sample (mL)]
f: the ratio of seed in the diluted sample to seed in seed control

[% seed in diluted sample/% seed in diluted seed solution (seed control)]

8.7 Chemical Oxygen Demand (COD)

COD measures the O_2 equivalent in the OM of the sample oxidized by a strong oxidizer. The water sample is heated with potassium dichromate-sulfuric acid solution, after which the remaining chromate is determined. COD is used to determine the degree of contamination [25]. The limit allowed by TNPCB for COD is (350 mg/L) [15].

$$COD\left(\frac{mg}{L}\right) = [Blank\ titration\ (A) - Sample\ Titration\ (B)] * M * 8000 * \frac{Dilution\ factor}{Volume\ of\ sample(mL)} \qquad (5)$$

Where:

A: Volume of FAS titrant used to titrate the blank (mL)
B: Volume of FAS titrant used to titrate the sample (mL)
M: Molarity (mol/L) of FAS titrant; 0.01 M was used
8000: Milliequivalent weight of oxygen × 1000 mL/L

8.8 Total Suspended Solids (TSS)

TSS is one of the most important pollutants present in the wastewater of fish farms, as they lead to an increase in the demand for BOD and thus negatively affect the fish. The fish farm water recycling system relies on removing solid waste quickly. A cylindrical filter can be used to remove larger particles, and a slow sand filter can be used to remove smaller particles. In recent years, microscreen drum filters, parabolic screen filters (PSFs), foam fractionators and swirl separators have been used [1]. TSS is calculated in aquaculture wastewater by the following equation [25]:

$$Formulation\ of\ TSS\ calculation : TSS\left(\frac{mg}{L}\right) = \frac{(A-B)}{C} * 1000 \qquad (6)$$

Where

A: Mass of nonfilterable residue on Whatman GF/C filter after evaporation at 105 ◦C (mg)

B: Mass of the filter paper before sample filtration (mg)

C: Volume of the sample used for filtration (mL)

8.9 Nitrogen (N)

N is an important water element, it is required for the life of living organisms. The source of N in fish farms is either from feed or fish feces as it is produced from the metabolism process. Most of it is in the form of NH_3 with fewer amounts of $CO(NH_2)_2$, which is of less harm than NH_3. And the secretion of $CO(NH_2)_2$ from each type of fish is different from the other type. TAN is preferably maintained at the lowest or below 3 mg/L. While NH_3 is toxic to fish at 1 mg/L, NO_2 is toxic to fish at 5 mg/L and these two elements are 100 times more toxic to fish than NO_3 [17, 32].

8.10 Odors

The wastewater from fish farms is exposed to bacterial rotting, which leads to a foul odor. This is because the pollutants present in the water lead to the depletion of O_2, which leads to the anaerobic decomposition of the OM that breaks down the NC and proteins and releases amines, diamine, hydrogen, methane and NH_3, which leads to poisoning of aquatic organisms and the appearance of an unpleasant odor. Although the unpleasant odors emanating from the wastewater of fish farms are relatively harmless, they have a harmful effect on public life [25].

9. Microorganisms

There are many types of microorganisms used to treat wastewater according to the variety of pollutants present in it. Hence, the choice of the type of microorganism used depends on the chemical nature of the pollutants. The efficiency of the biodegradation process depends on the generation of energy input media and the introduction of O_2 into the hydrocarbons by microorganisms. Contaminants should be converted to harmless substances in biological treatment by microorganisms that may be anaerobic or aerobic. Figure 11 shows Microorganisms that are used in the biological treatment and mechanisms in dead and living biomass. The use of microalgae for biological treatment has increased in recent period. Where microalgae are photosynthetic organisms that grow quickly, they also live-in difficult environments because they can be single or multicellular. It can also remove pollutants such as N, P and C from wastewater during biomass production [12]. The most common bacteria used to oxidize NH_3 is Nitrosomonas species. They also explained that NO_2^- is oxidized by Nitrospira, Nitrococcus and Nitrocystis. While gram-negative bacteria like Psedomonas, Alcaligenes, Paracoccus, and Thiobacillus are used in denitrification processes [11].

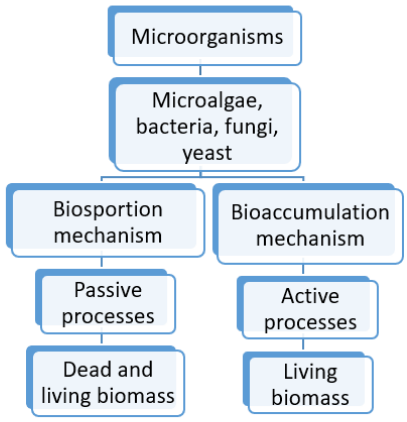


Fig. 11. Microorganisms utilized in the biological treatment and mechanisms of dead and living biomass(amended and redrawn after [12]).

Heterotrophic microorganisms are utilized for reducing dissolved organic contaminants in RAS while biological nitrification is used for the removal of toxic ammonia by two-step oxidation of NH_3 by NO_2^- to NO_3^- by aerobic chemoautotrophic bacteria. They explained that the NH_3 removal by biofilter nitrification offers special priority in RAS. In the biological treatment, NH_3 is first oxidized to NO_2^- by ammonia-oxidizing bacteria (AOB), such as Nitrosomonas spp. or Nitrosospira spp., followed by the next Conversion of NO_2^- to the much less toxic NO_3^- by nitrite-oxidizing bacteria (NOB), such as Nitrospira spp. or Nitrobacterspp. [52].

10. Conclusion

Recent years especially the past two decades have seen a large intensification of the industry of aquaculture. It is considered to have a reverse and passive effect on the surrounding environment. This is because wastewater of aquaculture contains many harmful elements such as NH_3, OM, P and DOC. The high levels of nutrients lead to environmental damage to the receiving water bodies. Biological treatment is one of the best methods used to treat aquaculture wastewater, as it is considered an environmentally friendly method and has a lower cost than other methods. RAS is used in biological treatment, which works to convert NH_4^+ into NO_2^- and then into NO_3^-. There are several types of biofilters such as TFs, RBCs, FSBs and SBs. The design of a biofilter depends on the material of the filter medium, the type and size of the biofilter and the clogging. DO, pH, TAN, BOD, COD, TSS and temperature are considered among the factors affecting the operational process of the biofilter. The most common bacteria used to oxidize NH_3 is Nitrosomonas species. They also explained that NO_2^- is oxidized by Nitrospira, Nitrococcus and Nitrocystis.

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