# The Effectiveness of Microbial Filter in Recirculating Aquaculture System

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## ABSTRACT

*Aquaculture production accounts for nearly 50% of fish produced for consumption, estimates suggest a five-fold increase in production is required to meet the protein needs of the community. However, increasing production will also increase the negative impact on the environment. Recirculating Aquaculture Systems (RAS) is a fishery production system that reprocesses water used to meet water quality requirements for aquaculture activities. The diversity of RAS-filtering microbial communities in freshwater and marine systems is based on studies using 16S rRNA and functional gene-specific probes or 16S rRNA gene libraries rather than culture-based techniques. Aquaculture systems rely on nitrification to convert toxic ammonia to nitrite and then nitrate, to prevent the accumulation of potentially toxic or harmful forms of nitrogen (non-ionized ammonia, nitrite). Nitrification is accomplished using biofiltration. Several phylotypes of 16S rRNA genes associated with heterotroph denitrification, including Pseudomonas sp., Aqua spirillum sp., and a wide variety of A-Proteobacteria, were found in the anaerobic nitrate-stimulated enrichment derived from the marine RAS nitrate community, possibly contributing to the nitrate-reducing activity. The results obtained demonstrated that nitrifying microorganisms in RAS are different from those used traditionally to model the nitrifying capacity of RAS. Some researchers have hypothesized that each RAS biofilter should have a different composition of the microbial community formed by the operational controls and components implemented in the RAS.*

**Keyword :** *Aquaculture, Denitrification, Nitrification, RAS, Recirculating*

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## 1. Introduction

The development of aquaculture technology can reduce community dependence on capture fisheries. According to [1], aquaculture production accounts for nearly 50% of fish produced for consumption, estimates suggest a five-fold increase in production is required to meet the protein needs of the community. However, increasing production will also increase the negative impact on the environment. Aquaculture recirculation systems or commonly referred to as RAS have been developed to address the waste and storage capacity limits of aquaculture facilities [2].

Recirculating Aquaculture Systems (RAS) is a fishery production system that reprocesses water us ed to meet water quality requirements for aquaculture activities [3]. Recirculating Aquaculture Systems (RAS) were developed to address pollution concerns and limitations of the conventional terrestrial storage capacity of aquaculture facilities [4][2]. The components of the RAS are like those used in wastewater treatment, including the removal of solids and the removal of nitrogenous waste from excess animal manure and undigested feed. Advances in RAS technology and the advantages of overflow systems have led to increased use of RAS, especially among countries that place a high value on minimizing environmental impact [5] and in urban areas where space is limited [6].

RAS bio-filters are designs that reduce or eliminate harmful metabolic waste products that have been designed for specific fish species although generic systems have been developed [7]. In general, a bio-filter is a compartment that includes a fixed medium for microbial attachment and growth, i.e., through a bio-film, or allows microbial growth to be delayed [8]. The biofilter will integrate aerobic and anaerobic microbial processes to remove waste products - nitrogen in the form of ammonia excreted by fish, and carbon and nitrogen accumulated from uneaten feed and manure [9].

The diversity of RAS-filtering microbial communities in freshwater and marine systems is based on studies using 16S rRNA and functional gene-specific probes or 16S rRNA gene libraries rather than culture-based techniques. The biofilter bacteria include members of Actinobacteria, Bacterioidetes/Chlorobi, Firmicutes, Nitrospirae, Planctomycetales, and Proteobacteria. Formation of bio-films in marine RAS and found g-Proteobacteria to be the most abundant group followed by An-Proteobacteria [10].

### 1.1 Nitrification

In the aquatic environment, nitrogen is a major concern in its various forms [11]. The role of nitrifying bacteria in mineralization processes is well known in aquaculture circles [12][13]. nitrogen is a major concern in its various forms Nitrification is accomplished using biofiltration in which nitrifying bacteria normally coexist with heterotrophic microorganisms that metabolize biologically degradable organic compounds [16]. Nitrification proceeds at dissolved oxygen (DO) levels as low as 2.0 mg/l and can even be interrupted by a diurnal anaerobic cycle while still removing large amounts of ammonia [17], as some are bactericidal [18]. The role of nitrifying bacteria in the mineralization process is well known among aquaculture circles, but the underlying bacteriology remains mysterious [12][13].

Biofiltration and recommendations were made for future recirculating aquaculture research [13][19]. Currently biological filters are commonly used to remove ammonia in aquaculture systems [14][15]. Aquaculture systems rely on nitrification to convert toxic ammonia to nitrite and then nitrate, to prevent the accumulation of potentially toxic or harmful forms of nitrogen (non-ionized ammonia, nitrite) [20].

### 1.2 Denitrification

Under anaerobic conditions (in specialized compartments or bio-film layers deep within), the combination of high nitrate/nitrite levels and organic carbon sources – derived from uneaten feed and fish waste or provided as a supplement promotes heterotrophic determination in freshwater RAS and the sea [21]. Several phylotypes of 16S rRNA genes associated with heterotroph denitrification, including *Pseudomonas* sp., *Aquaspirillum* sp., and a wide variety of A-Proteobacteria, were found in the anaerobic nitrate-stimulated enrichment derived from the marine RAS nitrate community, possibly contributing to the nitrate-reducing activity [22].

## 2. Microbial Filter in Recirculating Aquaculture System

Nitrifying biofilters are a critical component of most RASs and a critical determinant of operational success. This biofiltration is also cited as the biggest hurdle for RAS start-up and the most difficult component to manage once the RAS is operational [5]. RAS biofiltration acts to remove nitrogenous waste products produced by fish protein catabolism and oxidation processes. Ammonia and nitrite are of major concern to freshwater aquaculturalists, with toxic doses of both species nitrogen depending on pH and the aquatic organism being reared [23][24].

The use of fixed film bioreactors is the most common choice for nitrification and denitrification processes in RAS technology [25]. Management of biological filters begins with the acclimation process, which must be inoculated with suitable nitrifying bacteria. The biomass and metabolism of the bacteria must then be increased to the level required to remove the ammonia produced by the cultured fish [26]. Pond sediments or uncontaminated soil can serve as natural sources of desirable bacteria [27]. Using water or an active medium from an already operating system will speed up this process. Alternatively, stocking a system with a low density of small fish and providing reduced feed levels will provide a limited concentration of ammonia which will form the filter slowly without damaging the fish [28]. Once the filter reaches full acclimation, fish residues can be added and feed levels increased [28]. The most commonly used, and rapid, biofilter pre-acclimation method is through seeding the biofilter with a commercial source of nitrifying bacteria followed by the addition of appropriate concentrations of ammonia and nitrite [29].

According to research by [29], phylogenetic analysis of microbial populations in a recirculating aquaculture system by next generation sequencing showed that there are a large number of bacteria that oxidize ammonia and nitrite in the nitrifying biofilter, while in the nitrifying bioreactor there is a large proportion of bacteria that are heterotrophic and autotrophic. denitrification, as well as those who participate in sulfur cycling. Comparing the stable microbial population established on the biofilter for complete nitrogen removal with the culture composition contained in the commercial inoculum, it can be concluded that the environmental seawater used to fill the RAS serves as an additional source of bacteria, especially in the denitrification reactor. Considering their role in the nitrogen and sulfur cycles, this is a favorable upgrade for commercial inoculums.

## 3. Application Microbial Community on The Biofilm Filter of RAS

Recent studies on freshwater aquaculture biofilters have introduced renewable nitrification processes present in these systems to include archaeal oxidizing ammonia (AOA), various Nitrospira spp., and Nitrotoga [30]. In the research of [31] show results that the biofilter microbial community in freshwater RAS is dynamic, diverse, and more widely distributed by resource availability than is often considered in the design process. The results obtained by [32] and [33], demonstrated that nitrifying microorganisms in RAS are different from those used traditionally to model the nitrifying capacity of RAS. Some researchers have hypothesized that each RAS biofilter should have a different composition of the microbial community formed by the operational controls and components implemented in the RAS [34]. In a study conducted by [31] the most abundant bacteria include *Kribbella, Niabella, Chitinophaga, Byssovorax, Hyphomicrobium*.

As previously stated, in the research of [35] conducted on the brackish water RAS found bacteria or microbes that were different from the microbes used in the fresh water RAS. In this study, the dominant bacteria or microbes were the phylum Bacteriodate, Verrucomicrobia and Acidobacteria. Bacillus, Clostridium, and Trigonala are classified into the phylum Firmicutes, and Bacteroides are classified in the phylum Bacteroidetes. Bacillus secretes proteases that hydrolyze proteins into peptides and amino acids and then release ammonia [36]. In addition, [37] argue that members of the genus Bacteroides also have an important role in the hydrolysis of proteins, lipids, cellulose and also some biopolymers such as polysaccharides.

Meanwhile, the results of research conducted by [38] showed that the two inoculums, namely commercial inoculum for marine RAS or biofilm carriers from RAS, differed in terms of microbial community composition. The microbial or microbial community on the biofilm carrier has the same optimal salinity for growth as standard operating conditions at the RAS facility, and consists of nitrifying bacteria. In contrast, commercial inoculums have a lower optimal salinity for growth, which is in accordance with the manufacturer's recommendation to use inoculums for RAS with freshwater under low salinity conditions, and consist of nitrifying archaea and bacteria. The first nitrification step was carried out by Thaumarchaeota in commercial inoculums and Nitrosomonas in biofilms on carriers, because these microorganisms are known to function to carry out ammonium oxidation [39].

The biofilm carriers of the RAS contain Nitrospira, a genus of bacteria known for their nitrite-oxidizing ability, which most likely performs the second nitrification step. This observation is also supported by previous studies on nitrification in the RAS, where mutualism between Nitosobacter and Nitrospira was a common group of nitrifying bacteria [40]. Slow-growing nitrospirates play an important role in RAS biofiltration by converting toxic nitrite to non-toxic nitrate [41].

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