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Use of a filtering process to remove solid waste and antibiotic resistance genes from effluent of a flow-through fish farm

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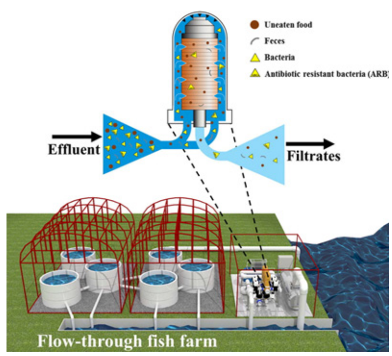
HIGHLIGHTS

- A filtering process removed solids and ARGs from effluent of a flow-through fish farm.
- Reduction in targeted ARGs was achieved by removing particles via filtration.
- Most of the reduction in ARGs resulted from reduction in *tet*.
- Composition of bacterial communities was not significantly altered via filtering.

GRAPHICAL ABSTRACT



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ABSTRACT

The objective of this study was to investigate reduction in antibiotic resistance genes (ARGs) via targeting solid waste in effluent from a flow-through aquaculture in South Korea. The level of suspended solids in the filtrates was approximately 12.5 ± 2.3 mg/L, corresponding to a removal efficiency of 68.8 ± 5.7% irrespective of variations in the size of the filter pores. The total number of particles in the effluent was reduced to the lowest numbers of particles using a filter pore size of 25 μm, corresponding to a removal efficiency of 40.3%. Among the 23 ARGs conferring resistance to tetracyclines, beta-lactam antibiotics, sulfonamides, quinolones, macrolides, florfenicol and multidrug, tetracycline resistance genes were the most prevalent with a relative abundance of 67.5%. Of eleven tetracycline resistance genes (*tetA*, *tetB*, *tetD*, *tetE*, *tetG*, *tetH*, *tetM*, *tetQ*, *tetX*, *tetZ*, *tetB/P*) analyzed, the relative abundance of *tetG* was the highest in the effluent. The removal efficiency of the total number of particles showed similar patterns to the removal efficiency of ARGs depending on the size of the filter pores. Levels of ARGs in the filtrates were reduced to approximately 60.5% of those of the ARGs in the effluents. With a filter pore size of 25 μm, a maximum removal efficiency of 66.0% was achieved. In particular, the relative abundance of detected tetracycline resistance genes decreased only after passing through the filters, perhaps reflecting the presence of high quantities of tetracycline resistance genes in particles from the fish farm. Using Illumina sequencing based on a 16S rRNA gene, the dominant phyla were found to be *Bacteroidetes*, *Proteobacteria*, *Planctomycetes* and *Verrucomicrobia* in the effluent. Although the overall composition of the bacterial communities was not significantly changed via filtering tests, only the relative abundance of *Bacteroidetes* and *Proteobacteria* was changed. These results demonstrate that a filtering process in aquaculture facilities can be used to reduce solid waste as well as ARGs from aquaculture farms.

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1. Introduction

Declines in wild fish stock by exploitation of popular marine species, combined with growing human demand for protein derived from aquatic life, has led to expansion of aquaculture (Dolan et al., 2013). Among several current aquacultural methods, flow-through aquaculture is an inland operation in which water moves through the aquaculture structure to maintain the desired level of water quality (Mirzoyan et al., 2010). Many commercially important flatfish (*Paralichthys olivaceus*) have been successfully domesticated using flow-through systems along the coast of Jeollanam Province, where one third of total aquaculture in South Korea is located.

These flow-through systems require large amounts of water in order to remain clean and clear via dilution effects. However, many effluents are discharged into aquatic environments having received little or no treatment system (Mirzoyan et al., 2010). Typical flow-through systems are that low concentrations of pollutants such as solids, nitrogen, organic compounds and phosphorus in high flows cannot be handled effectively. Although concentrations of pollutants in effluents are low, significant amounts of pollutants are discharged from flow-through systems as a result of high flow rates. Particularly, solid waste from flow-through systems presents a central issue for water quality in areas neighboring aquaculture systems. Considering an average flow rate of 43,200 tons/day and concentrations of solid waste averaging 19.4 mg/L from the aquaculture targeted in our study, approximately 0.9 tons of solid waste is discharged to areas neighboring aquaculture systems every day. Unavoidable turbulence in the system leads to rapid fragmentation of solids at high solid loads, negatively affecting the physicochemical and biological marine ecosystem (Schumann et al., 2017).

Solids originating in flow-through systems are comprised of uneaten feed, feces, fish excretion and bio-floc mixed with various chemicals and pathogens (Pfeiffer et al., 2008; Schumann et al., 2017). Antibiotics such as tetracycline and amoxicillin have been indiscriminately used to control aquatic disease (Chen et al., 2017). As a result, feces and uneaten feed contain antimicrobials, retaining their antimicrobial activity in the water and sediment of aquaculture (Buschmann et al., 2012). Continuous exposure to these antimicrobials in aquaculture can promote elevated antibiotic resistance (Zhao et al., 2017). Indeed, diverse antibiotic resistance genes (ARGs) have been detected in solids in the water and sediment of aquaculture (Di Cesare et al., 2013; Harnisz et al., 2015; Zhao et al., 2017). Studies have reported that solid waste from aquaculture can act as a potential pollutant containing antibiotics and ARGs (Luo et al., 2010; Xiong et al., 2015). ARGs may be disseminated via plasmid horizontal transfer serving as the major mechanism (Luo et al., 2014). Even after bacteria die, free DNA containing ARGs can be transmitted to other bacteria in a marine environment (Zhu, 2006). Selection of antimicrobial resistant bacteria in a marine environment can threaten human health by transfer of ARGs from marine microbes to fish pathogens (Burridge et al., 2010; Buschmann et al., 2012).

To reduce the discharge of potential pollutants containing antimicrobials, solid waste should be properly managed using accepted treatment techniques in aquaculture systems (Chen et al., 1993; Merino et al., 2007). Among the most common solid-removal units, mechanical filtration processes are widely used for removal of solid waste in aquaculture systems (Bergheim and Brinker, 2003). Removal efficiencies of filtrating processes for solid waste occur within a wide range of 19–91% (Cripps, 1994; Cripps and Bergheim, 2000). Particle sizes of solid waste found in aquaculture vary widely depending on the species and size of fish and type of fish feed (Ali, 2013). Thus, to maximize removal efficiency of solid waste, the filter pore size in a filtering process should be optimized reflecting conditions in aquaculture systems. If solid waste is effectively eliminated via a filtering process, amounts of ARGs remaining in solids may be minimized in the effluent from aquacultures. In South Korea, there are no environmental regulations mandating effluent treatment facilities in fish farms. Thus, solid waste in effluent from flow-through fish farms in South Korea is discharged without undergoing proper treatment by facilities directly into surrounding aquatic environments. The objective of this study was to use a filtering process to investigate reduction in solid waste and ARGs in effluent from targeted flow-through aquaculture. Filter pore size was determined based on the efficacy of reduction in solids and ARGs in effluent. Also, the type and abundance of ARGs and bacterial communities were explored in the water and sediment of the targeted aquaculture. To the best of our knowledge, this study is the first to investigate variations in ARGs via targeting solid waste from aquaculture systems in South Korea.

2. Materials and methods

2.1. Targeted aquaculture sampling site

Sampling was conducted at a flow-through fish farm located along the coast of Jangheung, Jeollanam Province, South Korea (Fig. 1). The targeted flow-through fish farm has a total of 32 rounded-corner rearing tanks. Each rearing tank has a water volume of 29.2 m^3 (9.4 m^2 × 0.33 m in depth). The farm is fed by water taken directly from the ocean. Water enters an inlet channel which provides water to 32 parallel equal sized tanks. Effluent from the tanks is collected in a channel which discharges directly into the receiving water body. The final effluent is discharged without undergoing proper treatment by facilities. The influent flow for each tank was adjusted to approximately 1000 L/min. The hydraulic residence time (HRT) of each rearing tank was maintained at approximately 30 min by water circulation. In each tank, flatfish (*Paralichthys olivaceus*) are reared at an average density of 13.6 kg/m^3. Approximately 300 kg of feed (about 0.8% of the combined body weight of all the fish in each tank) was provided twice a day. The first feeding of each day was conducted between 06:00 and 07:00; the second, between 16:00 and 17:00. After each feeding, the tanks were cleaned of sediments containing uneaten food, feces and bio-floc. Commercial flatfish feed contains 520 g/kg crude protein, 80 g/kg crude fate, 9 g/kg calcium, 27 g/kg phosphorus, 50 g/kg crude fiber and 150 g/kg crude ash mixed with an antibiotic to prevent aquatic diseases. During this study, 3 kg of oxytetracycline (about 1.0% of total feed weight) was ordinarily added.

Concentrations of solids in the effluent from tanks varied depending on the feeding period and showed a significant increase within a few minutes after feeding (Fig. 2). During the feeding period, the concentration of total suspended solids (TSS) in the effluent increased >2 to 3 times over baseline. In particular, discharged TSS concentrations reached their peak during the cleaning times, registering an approximate 4 times greater increase in TSS concentrations compared to baseline. At this peak, concentrations of total phosphorus (TP) increased by 11%, total nitrogen (TN) by 139%, ammonia by 138% and chemical oxygen demand (COD) by 106% over baseline (data not shown). To maximize removal efficiency of solid waste in filtering tests, effluent samples during cleaning times after feeding were collected at the discharge channel of the flow-through aquaculture. Around 100 L of samples in sterilized plastic bags were stored at 4 °C in cooling boxes and then quickly taken to a laboratory within 3 h. Temperature, dissolved oxygen (DO), pH, salinity and conductivity in the effluent from the aquaculture were analyzed using in-situ measurements (Table S1). Temperature in the effluent was 24.6 °C. The values of pH and DO were measured as 7.99 and 5.3, respectively. The concentration of DO in the influent decreased from 6.3 mg/L to 5.3 mg/L in the effluent, perhaps due to oxygen consumption by fish respiration as well as oxidation by bacteria of organic and nitrogen pollutants in fish farms.

2.2. Filtering tests

Filtering tests were conducted using polyethylene, non-woven fabric filters having four different pore sizes of 100, 50, 25 and 5 μm and