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Effluent treatment in an aquaponics-based closed aquaculture system with single-stage nitrification–denitrification using a down-flow hanging sponge reactor

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ABSTRACT

A laboratory-scale demonstration of the aquaculture effluent treatment was conducted in a system that combined a down-flow hanging sponge (DHS) reactor and a hydroponic cultivation bed (HCB). *Cyprinus carpio* was used as a model aquaculture fish and *Cupsicum frutescens* was used as a model hydroponic plant. The aquarium water was circulated through the HCB and DHS using a submerged pump. The experiment was divided into six phases in which the sodium acetate solution was supplied using different feeding patterns. The sodium acetate solution acted as a carbon source and not only eliminated nitrogen through denitrification but also increased the alkalinity through biological degradation of the acetate. Denitrification was observed to eliminate 7.7% of the total nitrogen at the inlet out of which 53.5% was converted by *C. frutescens* to form a fruit. The microbial community in the sludge that was retained in the DHS contained both nitrifying and denitrifying bacteria. *Nitrososhaera* was the dominant ammonia-oxidizing bacterium, whereas *Nitrospira* was the dominant nitrite-oxidizing bacterium. Further, *Opitutus* acted as the dominant denitrifying bacterium. No major bacterial pathogen was detected in the DHS–HCB system. The study confirmed that the DHS system provided single-stage nitrification–denitrification and that the overall DHS–HCB system provided a low-cost and high-performance aquaculture effluent treatment system that is capable of being used for safe food production.

1. Introduction

Aquaculture is becoming an increasingly important area of food production, accounting for 45.3% of the aquatic animals that were produced for human consumption in 2015 as compared with 25.7% in 2000 (Food and Agriculture Organization of the United Nations (FAO), 2017). However, its effluent contains toxic pollutants and discharges, such as ammonia and nitrite, which are major causes of eutrophication (Jegatheesan et al., 2007). Additionally, fish bred in aquaculture farms may pose a health risk if they are raised in an environment that is polluted by harmful substances such as heavy metals (Ju et al., 2017).

Recirculating aquaculture systems (RAS) can prevent both environmental pollution and food contamination; further, it also requires less amount of water replacement (Blidariu and Grozea, 2011). The nitrification–denitrification process is extensively used for nitrogen elimination from RAS (Eding et al., 2006). However, an alkaline agent is required during the nitrification stage, whereas a carbon source is required during the denitrification stage (Lam et al., 2015; Lahav et al., 2009). This makes nitrogen elimination to be more expensive in RAS than that in marine fisheries.

Nitrate, which is a less toxic nitrogen compound oxidized from ammonia via nitrite, is an essential nutrient for plant growth. This has caused the development of a system that combines RAS and hydroponics, which is known as aquaponics. Using the otherwise-polluting aquaculture runoff as a plant nutrient helps the aquaponics to allow simultaneous production of both fish and vegetables (Blidariu and Grozea, 2011). While this simultaneous production improves the economics of RAS, supplementary plant nutrients and a light source for performing photosynthesis are still required (Liang and Chien, 2013; Hu et al., 2015). To ensure a high fish-survival rate, a high nitrification rate must also be maintained.

The down-flow hanging sponge (DHS) reactor is a type of trickling-filter reactor in which the sponge acts as the filtering medium. Such reactors offer highly efficient ammonia elimination and are further used

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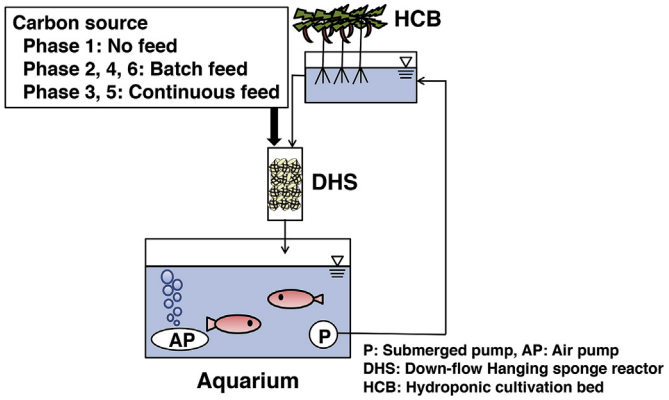

Fig. 1. Schematic of the closed aquaculture system.

for the post-treatment of industrial wastewater, sewage, and aquaculture effluent in anaerobic treatment systems (Kubota et al., 2014; Adlin et al., 2017; Watari et al., 2017). The sponge medium combines an aerobic zone at the surface with an interior anoxic zone. The DHS reactor, therefore, offers single-stage nitrification–denitrification.

In the current study, the aquaculture effluent treatment was investigated using a laboratory-scale aquaponics-based closed aquaculture system that was equipped with a DHS reactor. The performance of the proposed system and the mechanism of nitrogen elimination were investigated. In addition, the microbial communities in the system were characterized.

2. Materials and methods

2.1. Aquaculture effluent treatment system

A schematic of the closed aquaculture system is depicted in Fig. 1. The closed aquaculture system comprised an aquarium (80 L water volume), a DHS (1.5 L), and a hydroponic cultivation bed (HCB, 10 L) (DHS–HCB system). Water was circulated through the aquarium using a submerged pump (NPT-712, Nisso) via the HCB and DHS. The DHS reactor was fabricated from polyvinyl chloride piping and was filled with a 33 mm × 33 mm × 33 mm polyurethane sponge. However, no seed sludge was used. The hydraulic retention time of the DHS–HCB system and flow rate of the water circulation were 8 min and 90 L h^{-1}, respectively. *Cyprinus carpio* (carp) was selected to be the model aquaculture fish species, whereas *Capsicum frutescens* (capsicum) was selected to be the model hydroponic crop. The carp were fed using commercial feed that contained 40% crude protein, 4% crude fat, 2% crude fiber, 11% ash, and 11% moisture. The feeding for the carp was conducted three times at morning, afternoon and evening every day. On days 13 and 105, 100 mmol L^{-1} of dipotassium hydrogenphosphate solution was added to the HCB in order to maintain a balance between the nitrogen, phosphate, and potassium that were supplied to the capsicum.

The operational period was divided into six phases in which different carbon source feeds were used. In phase 1, only the fish feed was supplied to the aquarium. In phases 2, 4, and 6, 100 gCOD L^{-1} of the sodium acetate solution was added in a batch feed to serve as a carbon source for denitrification. During these phases, the sodium acetate solution was added once with a volume of 50–100 mL at every instance where the pH in the aquarium dropped to 6.0. In phase 3, 1 gCOD L^{-1} of the sodium acetate solution was fed continuously to the DHS. In phase 5, this was replaced by acetic acid. During phases 1 and 2, the effect of carbon source addition to nitrogen removal was evaluated. From phases 2 to 4, the optimum feed type of carbon source was considered. By comparing the results in phases 3 and 5, the effect of acidic carbon source utilization on the pH in the aquarium was estimated. The system was installed in a temperature-controlled room, which was maintained at 20 °C. Only the HCB was exposed to sunlight. Further, the aquarium water was not replaced during the course of the experiment.

2.2. Chemical analysis

The nitrate levels and pH were monitored by nitrate ion meter (LAQUAtwin NO^{3-}, Horiba) and pH meter (NPH-690D, Nissan), respectively. The ammonia levels were measured using an ammonia photometer (MI407, Martini), whereas the levels of nitrite were measured using a digital pack test (DPM-NO2, Kyoritsu Chemical-Check Lab). Additionally, the concentrations of chemical oxygen demand (COD) and total nitrogen (TN) were also monitored (DR/2500, Hach). As the statistical methods for experimental data analysis, the descriptive statics method was used in this study.

2.3. Microbial community

On days 72 and 425, analyses of the microbial communities were conducted in the sludges that were retained in the DHS and HCB. DNA extraction was performed using a FastDNA Spin Kit for Soil (MP Biomedicals). A polymerase chain reaction (PCR) amplification of 16S rRNA genes was performed using the universal forward primer Univ515F (5′-GTG CCA GCM GCC GCC GTA A-3′), whereas the universal reverse primer Univ806R (5′-GGA CTA CHV GGG TWT CAT AT-3′) was used for the entire set of bacteria and archaea (Caporaso et al., 2012). PCR products were purified using a QIAquick PCR Purification Kit (Qiagen). Massive parallel 16S rRNA gene sequencing was conducted using a Miseq Reagent Kit v.2 with the Miseq system (Illumina). Sequential data analysis was conducted using the QIIME software package v.1.9.1 (Caporaso et al., 2010). Further, the operational taxonomic units were classified at a sequence identifying level of 97%. The taxonomic classification was performed using the Greengenes database v.13_8. Additionally, related strains of the representative sequences were identified using a web-based BLAST search of the NCBI database.

3. Results and discussion

3.1. Performance of the closed aquaculture system

Fig. 2 depicts the time course of the ammonia concentration, nitrate concentration, and pH in the aquarium. In phase 1, the nitrate accumulated, which caused the pH to drop from 8.10 to 5.62 because nitrification reduced the alkalinity. In phase 2, the sodium acetate solution was added to enhance the denitrification in the DHS and to supply the alkalinity to the aquarium through acetate degradation. The sodium acetate solution was added in a batch feed at every instance where the pH in the aquarium dropped to 6.0. However, while this stabilized the

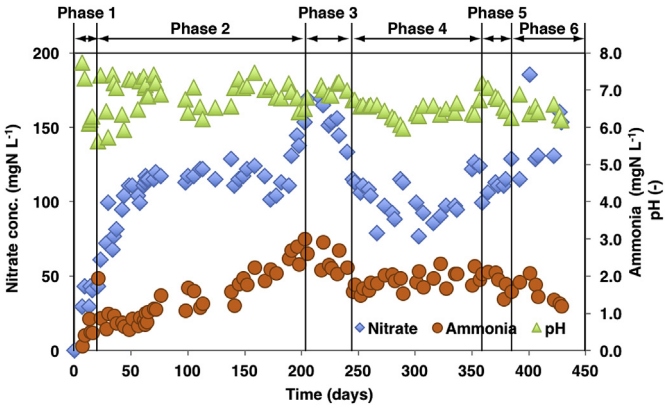

Fig. 2. Time course of the ammonia concentration, nitrate concentration, and pH in the aquarium.