

Application of a Single-Tank Sequencing Batch Reactor for Long-Term Zebrafish Care

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Abstract

Sequencing batch reactors are efficient wastewater treatment systems, however, they are not adapted to treat aquaria. We aimed to compare the efficacies of a modified single-tank sequencing batch reactor (SSBR) and a regular overflow filtration system. Zebrafish were maintained in an acrylic aquarium connected to an SSBR or in a tank connected to a regular overflow filtration system for 27 weeks. Water change or aeration using air stone was not performed. The water quality in both groups was within the normal range until 26 weeks, after which the nitrite and nitrate levels increased. The average temperature was, $29.46 \pm 0.71^\circ\text{C}$ and $29.78 \pm 0.70^\circ\text{C}$; pH, 8.22 ± 0.14 and 8.20 ± 0.12 ; ammonia levels, 0.1 ± 0.001 and 0.01 ± 0.005 ; nitrite level, 0.04 ± 0.06 and 0.05 ± 0.09 mg/l; and ammonia removal rate, 0.12 and 0.12 mg/l/h, in the control and SSBR groups, respectively. The average dissolved oxygen concentration was 5.81 ± 0.48 and 6.18 ± 0.50 mg/l; and the average nitrate level was 5.59 ± 6.47 and 10.17 ± 15.31 mg/l, in the control and SSBR groups, respectively. Beneficial microorganisms such as bacteria, diatoms, nematodes and ciliated protozoa were found in the biological reactors. The SSBR provides a long-lasting, healthy aquarium environment with optimal aeration and a diverse microbial community, while enabling the culture of a small number of fish. Zebrafish are common experimental animals in clinical research, especially in veterinary and medical science. This modified system could be beneficial for small-scale animal laboratories with limited budgets.

Keywords: recirculation aquaculture system, sequencing batch reactor system, bioreactor, ornamental fish, zebrafish care

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Introduction

Zebrafish are a useful animal model; however, researchers unfamiliar with aquatic animals encounter challenges in raising and caring for them. Commercially available zebrafish rearing equipment is expensive, complex and requires a large space for fish tank racks. Smaller and cost-effective holding systems, such as the flow-through system (Hohn and Petrie-Hanson, 2007) and SideRack system (Burg *et al.*, 2014), require space for a standing rack and the assembly of a complex filtration reactor. In cases such as embryonic toxicity tests, a small number of zebrafish broodstock is sufficient due to the large number of eggs produced per female (Braunbeck and Lammer, 2006). Therefore, it is adequate to rear a small number of zebrafish in a regular aquarium equipped with a simple filtration system. There are three main categories of water filtration systems: mechanical, chemical and biological. Mechanical filtration removes solid waste from a recirculating water system. Biological filtration uses microorganisms to detoxify water by processing nitrogenous waste. Chemical filtration uses a chemical reaction to bind and remove contaminants. All three steps are critical, interrelated and usually proceed in a sequence: mechanical, biological and, finally, chemical filtration (Steicke *et al.*, 2009). The accumulation of food debris, microflora and organic matter causes sludge to clog the filter every three to four weeks; this reduces the efficiency of the filtration system. Therefore, changing the water and cleaning the filtration system frequently is essential but can be time-consuming.

The sequencing batch reactor (SBR) is a well-known wastewater treatment system. It uses oxygen to activate facultative aerobes that digest organic waste. It is suitable for low volumes of wastewater, is easily operable and small compared to other aerobic water treatment systems, such as completely mixed, oxidation ditch and stabilisation activated sludge (Jin *et al.*, 2014). The SBR is used to treat different types of effluents, such as municipal, domestic and hypersaline waters, under different conditions (Mace and Mata-Alvarez, 2002). The system consists of two tanks: one for mechanical filtration and the other for aeration; however, a third tank can be added for sedimentation (Jagaba *et al.*, 2021). The SBR system includes a continuous wastewater filtration cycle (Kargi and Uygur, 2003). Each cycle consists of five steps: (1) adding wastewater to the reactor; (2) aerating the wastewater; (3) allowing the sludge to settle; (4) discharging the clear supernatant; and (5) leaving the system idle before the next cycle. SBR systems are used in some aquaponic farms that produce fish and vegetables simultaneously at a semi-commercial level, however, they are rarely applied to large-scale commercial aquaculture due to construction and fuel costs (Goddek *et al.*, 2019; Palm *et al.*, 2018).

SBR has never been used for zebrafish rearing systems. The fish rearing water is considerably less contaminated than sewage. Therefore, we applied the SBR in a low-cost model for zebrafish care to extend time intervals between water changes and filtration system cleaning.

The aim of this study was to compare the efficacy of the SSBR with that of a regular overflow filtration system in zebrafish rearing.

Materials and Methods

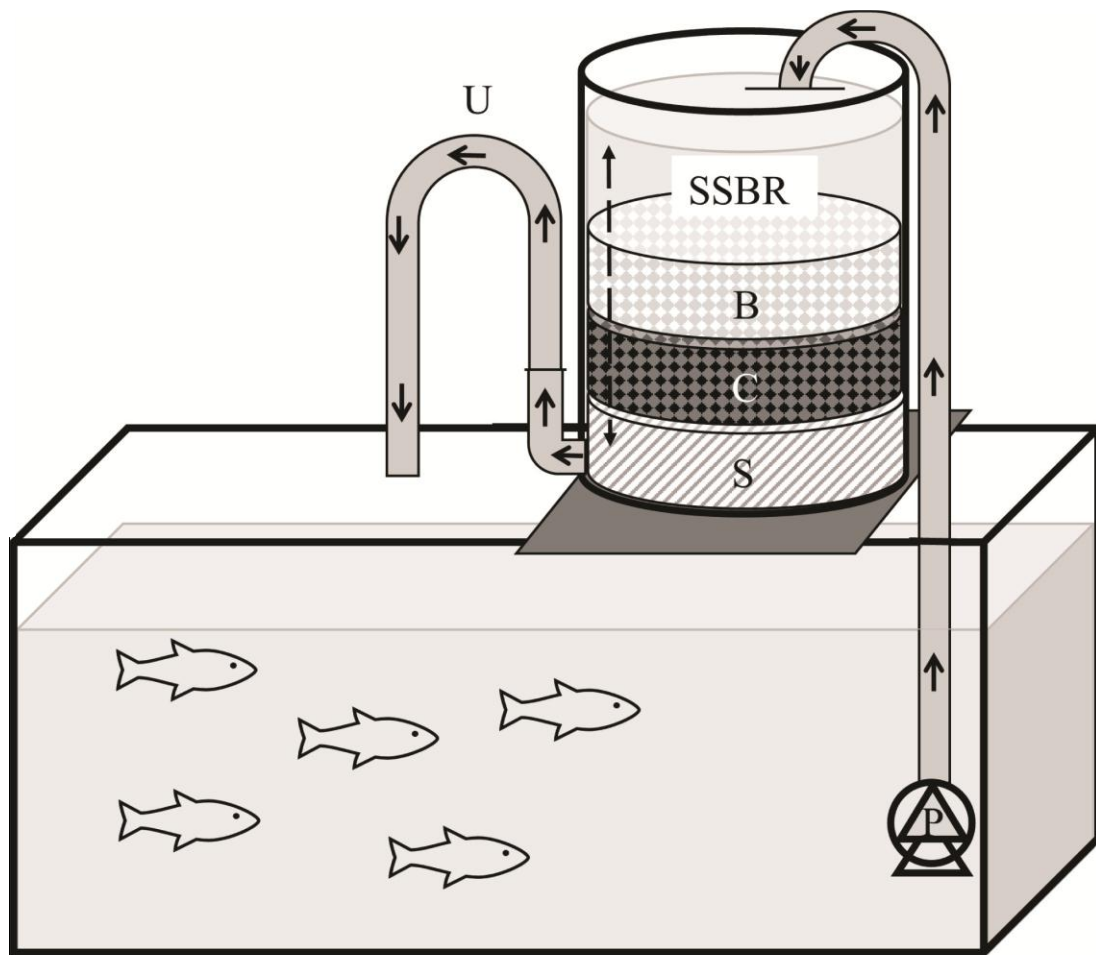
Animal rearing: The experiment was conducted in two phases using 120 adult long-fin zebrafish (*Danio rerio*) of both sexes; the average weight of each fish was 0.5 ± 0.03 g. The fish were obtained from a local ornamental fish farm in Nakornpatom Province, Thailand. Forty individuals were acclimatized in a 200-L glass aquarium for 1 week in municipal tap water flowing through an activated carbon pack in a stainless-steel filtration system (TMD Co., Ltd., Bangkok, Thailand). Aeration was achieved using a sandstone connected to an air pump; the average dissolved oxygen level was 6.42 ± 0.15 mg/l. A connected water heater maintained the water temperature at 29°C. The fish were fed frozen tubifex worms (Ocean free; Qian Hu Co., Ltd., Bangkok, Thailand) and dry feed (Tetramin flakes; Tetra, Melle, Germany), twice a day, which were completely consumed within 5 mins (Lawrence, 2007). During acclimatization, 20 L of water was replaced daily.

The study was conducted at the Veterinary Medical Aquatic Animal Research Center, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand. The descriptions of the zebrafish used in this study adhere to the experiments on fish reporting guidelines (Brattelid and Smith, 2000). All animal husbandry and experimental conditions were approved by the Chulalongkorn University Animal Care and Use Committee (Protocol number: 1931089).

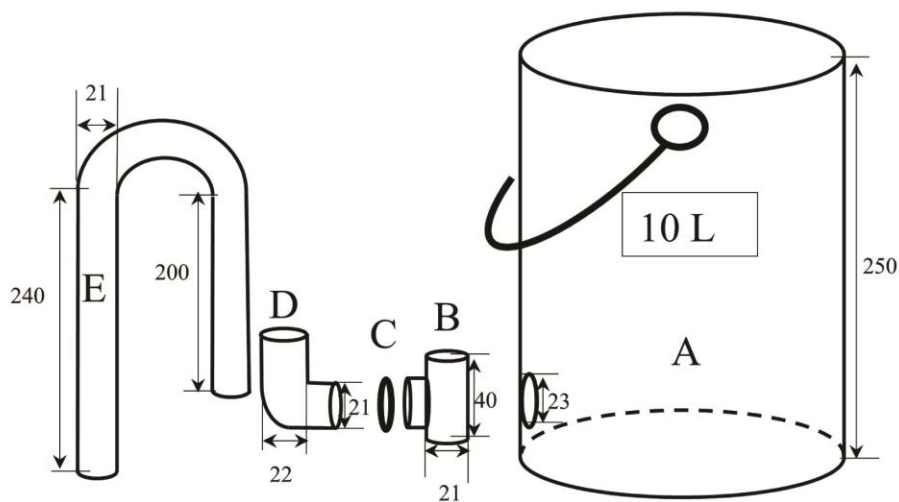
Experiment 1: SSBR and Overflow Filtration Reactor

(OFR) Function Test: The SSBR unit comprised of a plastic bucket containing 100 g of polyester sponge (AW103; Qian Hu Co., Ltd.) at the bottom, a net bag containing 500 g of activated carbon pellets (FM131; Qian Hu Co., Ltd.) in the centre, and a net bag containing 100 g of porous cellulose beads (Endonite Light, Plesca Co., Ltd., Tokyo, Japan) at the top. An 18 W submersible pump (Venus aqua; Kaixing Electrical Appliance Co., Ltd., Zhongshan, China) was connected to a water inlet pipe, and a 10-L polypropylene water container was connected to an inverted U-shaped siphon tube water outlet (Fig. 1). The pump was operated continuously and there were three steps in each cycle: (1) water was pumped into the reactor until it reached a level equal to the bend of the inverted U-shaped siphon tube, (2) water was drained from the reactor through the siphon tube using differential air pressure back into the fish tank and then (3) the water in the reactor was emptied, and air entered the siphon tube to stop drainage. The total filtration rate was 40 cycles/h at approximately 320 L/h.

The OFR unit was set up similarly to the SSBR but without connecting to the inverted U-shaped siphon tube (Fig. 2). The submersible pump was continuously operated to pump water from the zebrafish tank into the reactor. The water continuously flowed through the filtration material back to the fish tank.

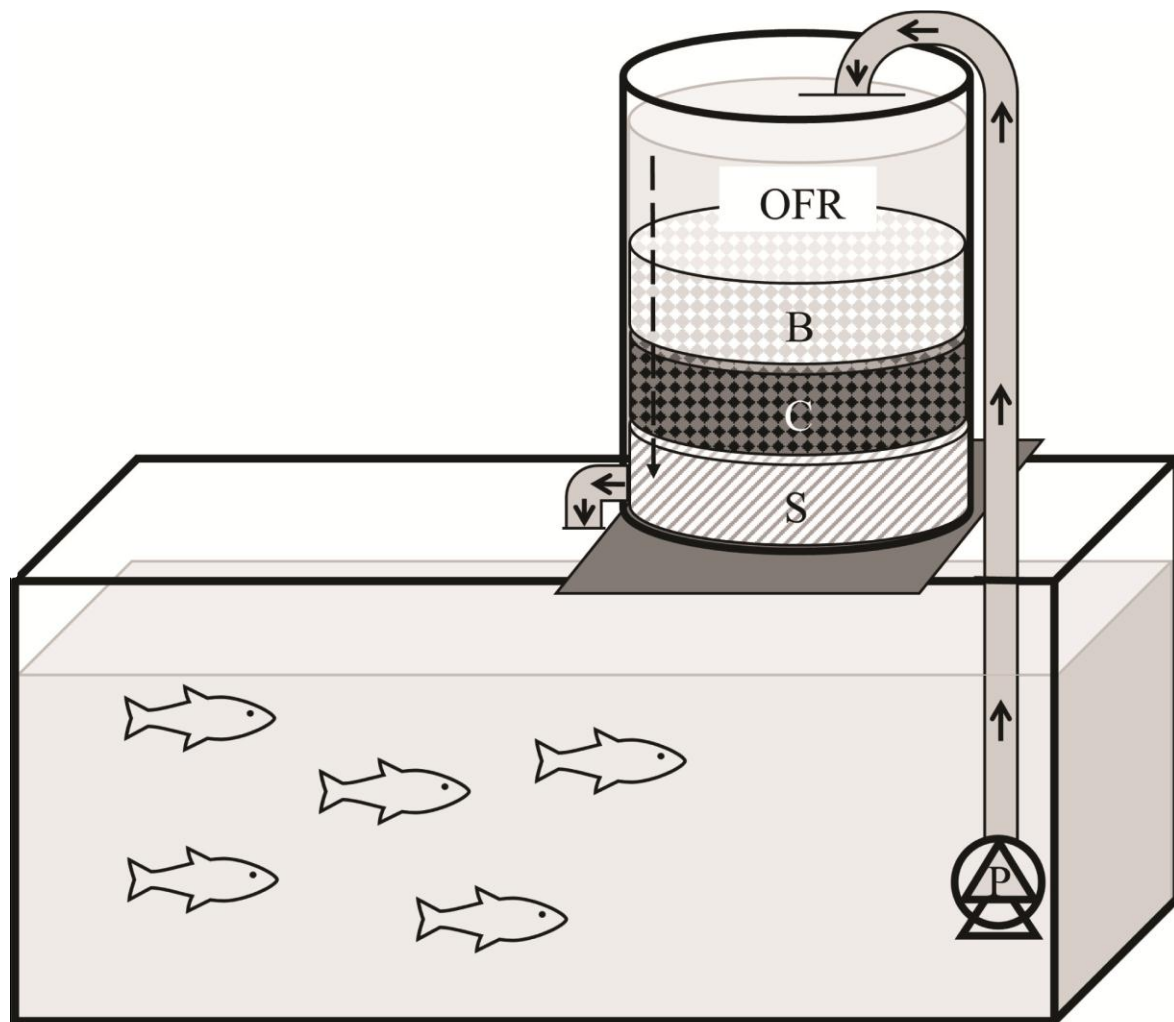


1.1

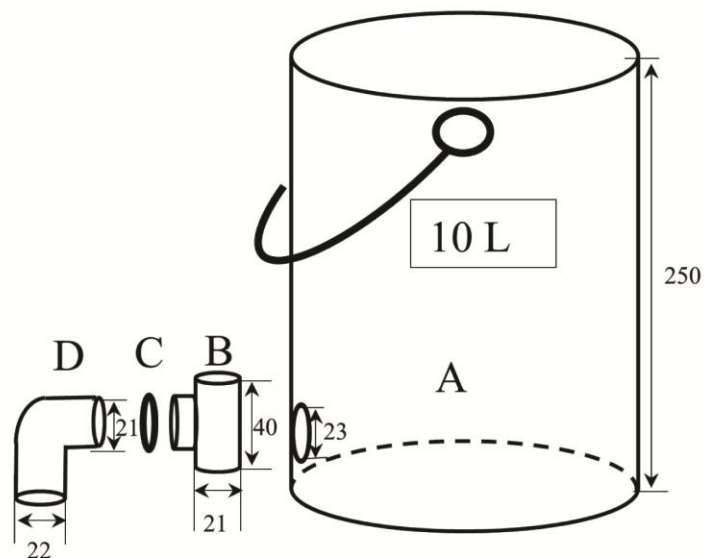


1.2

Figure 1 Illustration of the single-tank sequencing batch reactor (SSBR) (1.1), containing a polyester sponge (S), activated carbon pellets (C) and porous cellulose beads (B). The submersible pump (P) continuously draws water from the aquarium through the water filtration inlet. When the water fills up to the upper part of the U-shaped siphon tube (U), the water is drawn into the tube by differential back air pressure and flows through the water filtration outlet until the water level in the tank is lower than the that in the filtration outlet. Subsequently, the water accumulates in the tank again. The water filtration cycle in the tank is continuous over the rearing period. For construction (1.2), a water container can be made by drilling a hole in the side of a bucket (A). A T-shaped polyvinyl chloride (PVC) joint (B) is inserted inside the water container and the joint is covered with a rubber O-ring (C) to prevent water leakage. An L-shaped PVC joint (D) is connected to the T-shaped PVC joint outside the water container. The U-shaped PVC pipe (E) is connected to the L-shaped PVC joint on one side while the other side is positioned over the aquarium for water drainage. In this experiment, an SSBR unit included a water bucket, accessory parts and filter material (cost = approximately 20 USD, excluding the aquarium and electric water pump, which cost approximately 15 USD). (Measurement unit = mm).



2.1



2.2

Figure 2 Illustration of the overflow filtration reactor (OFR). The submersible pump (P) continuously draws water from the aquarium through a water filtration inlet into the reactor and the water from the reactor slowly flows through the outlet back to the aquarium, continuously, over the rearing period. The construction of OFR is similar to that of the single-tank sequencing batch reactor (SSBR) except that the T-shaped PVC joint (B) and O-ring (C) are connected to a vertically flipped L-shaped polyvinyl chloride (PVC) joint (D). (Measurement unit = mm).

Among the 120 zebrafish, 80 were randomly divided into four groups, placed in 20-L acrylic aquaria and designated as groups 1–4 (the average weight of fish in groups 1, 2, 3, and 4 was 0.54 ± 0.03 , 0.47 ± 0.03 , 0.50 ± 0.03 , and 0.52 ± 0.03 g, respectively). Each aquarium contained 20 zebrafish in 15 L of activated carbon-filtered water. Groups 1 and 2 were housed in tanks connected to the OFR, whereas 3 and 4 were placed in tanks connected to the SSBR. Each tank had a filtration unit. Preincubation of the filter material was not performed and sandstones were not included. The fish were fed daily with frozen tubifex worms and dry feed *ad libitum*. Fresh carbon-filtered water was added daily to compensate for evaporative loss, for maintaining the water volume at 15 L. The fish were reared for 27 weeks. Weekly checks (Wednesday at 9–11 am) were performed for the following parameters: water temperature and pH (HI98103 checker; HANNA, Woonsocket, RI, USA); dissolved oxygen (DO) (oxygen meter, YSI550A; YSI Inc., Yellow Springs, OH, USA); total ammonia nitrogen ($\text{NH}_4\text{-N}$), nitrite ($\text{NO}_2\text{-N}$), and nitrate ($\text{NO}_3\text{-N}$) levels (DR/890 portable colorimeter; Hach, Loveland, CO, USA); and alkalinity (Alkalinity test kit; Model AL-DT; Hach). Porous cellulose beads were sampled after 24 weeks of rearing and were observed using scanning electron microscopy (SEM) (JSM IT500HR; JEOL Ltd., Tokyo, Japan). Zebrafish were collected and weighed at the end of Experiment 1 and the filtration tank was subsequently used for Experiment 2.

Experiment 2: Efficiency of Nitrogen Waste Removal:

The zebrafish in groups 1 to 4 were moved out of the tanks and the water was replaced with new carbon filtered-water after the completion of Experiment 1 at 27 weeks. The remaining 40 zebrafish were divided into two groups of 20 zebrafish each; the groups were designated as groups 5 and 6. The filtration reactor was in operation, and 0.5 mol ammonium chloride (NH_4Cl) was added to the water to measure the ammonia removal rate. Groups 5 and 6 were housed in separate 20-L tanks that contained 15 L of carbon-filtered water; the tanks were aerated with sandstone without any filtration system. Frozen tubifex worms and dry feed were provided every day. The water in all groups was evaluated at 0, 1, 2, 3, 4, 5, 6, 24, 28, 48, 72, 96 and 120 h post-loading (hpl) of NH_4Cl , for ammonia, nitrite, and nitrate levels.

Statistical Analysis: Water parameters were analysed using the One-Way ANOVA and Post Hoc multiple comparisons with Bonferroni test, using SPSS Statistics Version 22.0 (SPSS Inc., Chicago, IL, USA). $p < 0.05$ were considered significant. Survival and growth rates were analysed using descriptive statistics and the specific growth rates were calculated from the total weight gain of the fish.

Results and discussion

Maintaining healthy fish in a recirculating water system requires a high-quality biological filter to break down the toxic nitrogen waste produced in the tank. The establishment of colonies of beneficial microorganisms in the filter will enable breaking down

the waste products into less harmful compounds. The inorganic nitrogen compounds, such as ammonia, nitrite and nitrate play an important role in fish health in aquarium systems. The nitrogen compounds can be converted from one form to another via nitrification and denitrification by the action of microorganisms. Rearing zebrafish in a tank without aeration, filtration or periodic water changes affects water quality; it is detrimental to fish health due to the gradual increase in the nitrogen compound concentrations (Lawrence and Mason, 2012). The ammonia level is dependent on several factors such as fish density, feed type and amount, fish waste and microbial cycle in the reactor. In a simulation of zebrafish transport in plastic bags at 25 fish/l in a closed container, the ammonia levels at pH 6.92 considerably increased to 23.50–27.40 mg/l at 72 h (Dhanasiri *et al.*, 2011). Ammonia is highly toxic to most fish and a concentration of 25.81 mg/l is the critical point for liver and kidney cell damage in zebrafish. However, in terms of fish health, even lower concentrations can have a sub-lethal effect (Al Zaidan, 2017). Exposure of fish to sub-lethal ammonia concentrations affects the physiological, biochemical, histological and behavioural responses (Wicks *et al.*, 2002). The accumulation of nitrite, another toxic form of nitrogen waste, in unhealthy aquaria at a concentration >2 mg/l affects fish by converting hemoglobin to methemoglobin, causing anoxia (Lewis and Morris, 1986). However, under aerobic conditions, nitrifying bacteria in the bioreactor help convert nitrite in the aquarium to nitrate, which is less harmful to fish. Zebrafish can flourish in water containing up to 50 mg/l nitrates (Harper and Lawrence, 2010; CCAC, 2020). However, nitrate removal by periodical water change or denitrification treatment is necessary to prevent accumulation of nitrate at higher concentrations. In this study, the functionality of SSBR and OFR were compared based on their ability to reduce ammonia, nitrite and nitrate and on the other water parameters in the system (Fig. 3 and Fig. 4). The ammonia levels were not elevated in either of the SSBR and OFR groups; the levels were less than 0.20 mg/l. The nitrite level was lower than 0.2 mg/l until week 18; however, it was slightly increased in the OFR2 (0.32 ± 0.07 mg/l) and SSBR2 groups (0.63 ± 0.12 mg/l) after week 26. The nitrate concentration in OFR1 increased slightly at week 24 (9.40 ± 8.33 mg/l) and reached the highest level (36 ± 8.33 mg/l) after week 26. The nitrate concentration in OFR2 increased slightly at week 25 (9.40 ± 3.97 mg/l) and reached the highest level (19.20 ± 3.97 mg/l) after week 26. At week 24, the nitrate concentration increased to 31.3 ± 12.67 and 54.9 ± 17.60 mg/l, in SSBR1 and SSBR2, respectively. However, the mean concentration of ammonia, nitrite and nitrate between the two groups was not significantly different ($p > 0.05$). In Experiment 2, the aquarium contained 20 zebrafish in 15 L of water without the bioreactor (groups 5 and 6); the ammonia level increased to 4.30 ± 1.6 mg/l within 5 days, while there was no increase in the levels of nitrite and nitrate (Fig. 5). Therefore, nitrification and denitrification did not occur in the absence of the reactor, as indicated by the high ammonia levels, without a concomitant increase in the nitrite or nitrate levels. The low levels of nitrogen waste in the SSBR and OFR link tanks could

be attributed to the high-performance of the filtration systems, which convert ammonia and nitrite to nitrate. The filtration systems were efficient until 26 weeks. After which, the depletion of the carbon source hindered denitrification, resulting in increased levels of nitrate, especially in the SSBR group, which had a higher level of DO.

The cellulose beads in the SSBR and OFR systems provided a substratum and functioned as the carbon (starch) source, for the growth of microorganisms (Deng *et al.*, 2018). After 24 weeks, almost all the cellulose beads were digested and gelatinized. Therefore, the activity of the denitrifying microorganisms were inhibited owing to the lack of carbon source. In such cases, methanol is supplemented to the bioreactors as a carbon source for enhancing the denitrification process. (Pungrasmi *et al.*, 2013).

Aerobic microorganisms perform nitrification and anerobic microorganisms perform denitrification. The DO concentration must be >1 mg/l for nitrification by aerobic microorganisms and <0.5 mg/l for denitrification to support anaerobic microorganisms (Irvine and Busch, 1979). The optimal temperature range for nitrification and denitrification is 25 to 35°C and both processes cease at lower temperatures (Barakat *et al.*, 2016). Several bacteria reduce nitrate to gaseous nitrogenous compounds in the presence of O_2 (Takaya *et al.*, 2003). In the SSBR and OFR systems, the continuous flow of water maintained a DO concentration that prevented anerobic conditions. Therefore, aerobic denitrification occurred in the SSBR and OFR systems, in the presence of a high DO concentration and optimal temperature (29–30 °C) and pH (8.2–8.3) ranges (Fig. 4). The average pH and temperature in the OFR1, OFR2, SSBR1, and SSBR2 systems were not significantly different ($p>0.05$). The average DO concentration in the OFR1 and OFR2-linked tanks was 5.79 ± 0.38 and 5.84 ± 0.57 mg/l, whereas that in the SSBR1 and SSBR2-linked tanks was 6.18 ± 0.47 and 6.17 ± 0.53 mg/l, respectively. There were significant differences between the DO in OFR1 and SSBR1 ($p=0.02$), and that in the OFR1 and SSBR2 ($p=0.03$) systems. We aimed to develop a simple system for fish culture by minimizing the instruments used and reducing the number of water changes. Despite the lack of sandstone and air pump, the DO concentration was maintained at high levels, because of the mechanical aeration in the SSBR. In the SSBR, the inverted U-shaped siphon tube initiates an intermittent water flow in the reactor. However, in the OFR, the water slowly overflowed to the aquarium with very little mechanical aeration. Therefore, mechanical aeration is higher in the SSBR resulting in a higher DO in the SSBR compared to that that in the OFR.

In a conventional single-tank reactor, the oxygen level is sufficient for the conversion of ammonia and nitrite to nitrate through nitrifying microbial activity, however, it is not suitable for denitrifying microorganisms. This results in the accumulation of nitrates in the water during long-term operation. Nitrifying microorganisms use bicarbonate as the carbon source for their growth. When bicarbonate is consumed, the alkalinity is reduced. The reduction of alkalinity impacts the buffering capacity and lowers

the pH of the water. During denitrification the alkalinity increases (Pungrasmi *et al.*, 2013).

When nitrification and denitrification are well balanced, the denitrifying microorganisms will convert nitrates to nitrogen gas and hydroxyl ions. This increases the alkalinity in the system. However, when the oxygen levels are high, the denitrifying microorganisms use oxygen as an electron acceptor instead of nitrate; nitrate is not converted to nitrogen gas. However, under certain conditions, aerobic denitrification occurs, especially, when a suitable substrate is provided.

The alkalinity in both SSBR and OFR fluctuated between 98 ± 34.6 and 236 ± 39.3 mg/l (Fig. 4) without the addition of sodium bicarbonate, indicating the possibility of aerobic denitrification. The partial water changes at regular intervals or the addition of bicarbonate increases the alkalinity and prevents the decrease in pH. However, in the aquariums equipped with the OFR or SSBR, the pH did not fluctuate sharply, even with the lack of water changes and bicarbonate addition.

The efficiency of nitrogen waste removal was evaluated by adding ammoniumchloride into the aquarium connected to the SSBR or OFR without fish in groups 1 to 4. The ammonia level was 14.40 ± 0.2 mg/l at the initial loading time (Fig. 5). The level gradually decreased with the increase in nitrite and nitrate levels due to nitrification and denitrification; the ammonia levels decreased to 0 mg/l within 120 hpl. The ammonia removal rate in the two bioreactors were approximately 0.12 mg/l/h. However, we could not prove that nitrogen cycling in the SSBR was better suited for reducing nitrogenous waste than that in the OFR. This was because we used a relatively small number of zebrafish, resulting in a lower amount of fish waste. This low level of nitrogenous waste was insufficient to highlight the difference between the filtration systems. In this study, no mortality occurred during the 27 weeks and the zebrafish reared in aquaria connected to either type of reactors survived well with a normal specific growth rate of 2.15–2.62%/d. Substantial water movement tends to flush anions for nitrification and denitrification and the intermittent wetting and drying tends to increase nitrification and denitrification by enhancing water movement (Barakat *et al.*, 2016). The water in the OFR tank overflows slowly, however, that in the SSBR moves up and down, causing the filtration material to periodically become wet and dry several times a day. This pattern of wetting and drying is similar to that in the tidal flats in coastal ecosystems, where DO and organic matter supply change daily (Miththapala, 2013). This phenomenon supports the aerobic and anaerobic microbial remineralisation in the sediment, which is confirmed by the high microbial cell numbers in the sediments (Schutte *et al.*, 2019). In nature, tidal flats, which allow microbial access to organic matter from water and oxygen from air, occur only twice in 24 h. In contrast, the microbes in the SSBR filter had access to organic matter and air for a total of 960 cycles within 24 h. This could have influenced the diversity of microbial growth in the reactor, owing to a higher rate of nitrification and denitrification, as reported previously (Hayatsu *et al.*, 2008). Different colors of

porous cellulose beads were collected from the SSBR, in contrast to the beads from OFR, which only have a light brown color as shown in (Fig. 6). Denitrifying fungi, nitrifying archaea, anammox bacteria, aerobic denitrifying bacteria and heterotrophic nitrifying microorganisms play key roles in nitrogen cycling. Aerobic bacterial colonies of three colours, namely, black, white and yellowish-brown, were observed on activated sludge granules in SBR. The yellowish-brown granules were the most common type consisting of coccoid- and bacilli-shaped bacteria, whereas black and white granules were colonised by filamentous bacteria of different diameters (Yun *et al.*, 2019). The porous cellulose beads from the OFR were more gelatinized and softened than those in the SSBR; therefore, making them unsuitable for microbial colonization compared to the porous cellulose beads from the SSBR found covered with bacilliform and filamentous bacteria, diatoms, ciliated protozoa and nematodes under SEM analysis (Fig. 6). The intermittent water flow of the SSBR contributed to the diversity of these microorganisms and built a growing environment for aerobic dominant bacteria, enhancing the nitrification activity in the SSBR. However, the microorganisms in the bioreactor were not classified; therefore, we could not determine the effects of microorganisms on the SSBR.

The SSBR used in this study was a modified SBR, which combined all the water treatment steps in one chamber; it eliminated the waste-collecting and sedimentation tanks. It augmented the suspended growth system with an attached growth system. The microorganisms in the modified system attach to the filtration material, thereby reducing the cloudiness of the water and allowing the water to recirculate back to the tank without extra sedimentation. This system increases the direct absorption of oxygen from the air for consumption by microorganisms and helps prevent anoxia in the filters.

The SSBR was maintained easily with water supplementation only to compensate for the water loss through evaporation. The experimental setup successfully maintained a healthy environment for adult zebrafish as per the recommendation that a pair of fish can be maintained overnight in 1.5 L or as many as six fishes can be maintained in 2.3 L of water (Matthews *et al.*, 2002). The SSBR model is appropriate for rearing one to two adult zebrafish per litre of water. It only requires a fish tank and a filtration set, which, once assembled, can be maintained for at least 27 weeks. The stable environment minimizes the transmission of potential pathogens and reduces the stress due to water changes, thus supporting the fish immune response and health. However, there are some limitations of the study and future research is required for improving the *in vitro* nitrogen removal and bacterial diversity. In addition, the role of aerobic bacteria in denitrification in the bioreactor should be investigated.

In conclusions, the SSBR method can help maintain smaller numbers of zebrafish in a convenient setup. Compared to alternative filtration methods, such as OFR, SSBR proved suitable for maintaining a healthy aquarium with optimal aeration and a diverse microbial community. Zebrafish are widely used as

experimental animals in clinical research, both in veterinary and medical studies. In this context, the SSBR could benefit small-scale animal facilities with limited budgets. However, the effect of the SSBR on nitrogenous waste management could not be conclusively established in this pilot test owing to the small sample size of zebrafish; this could be an area for further research.

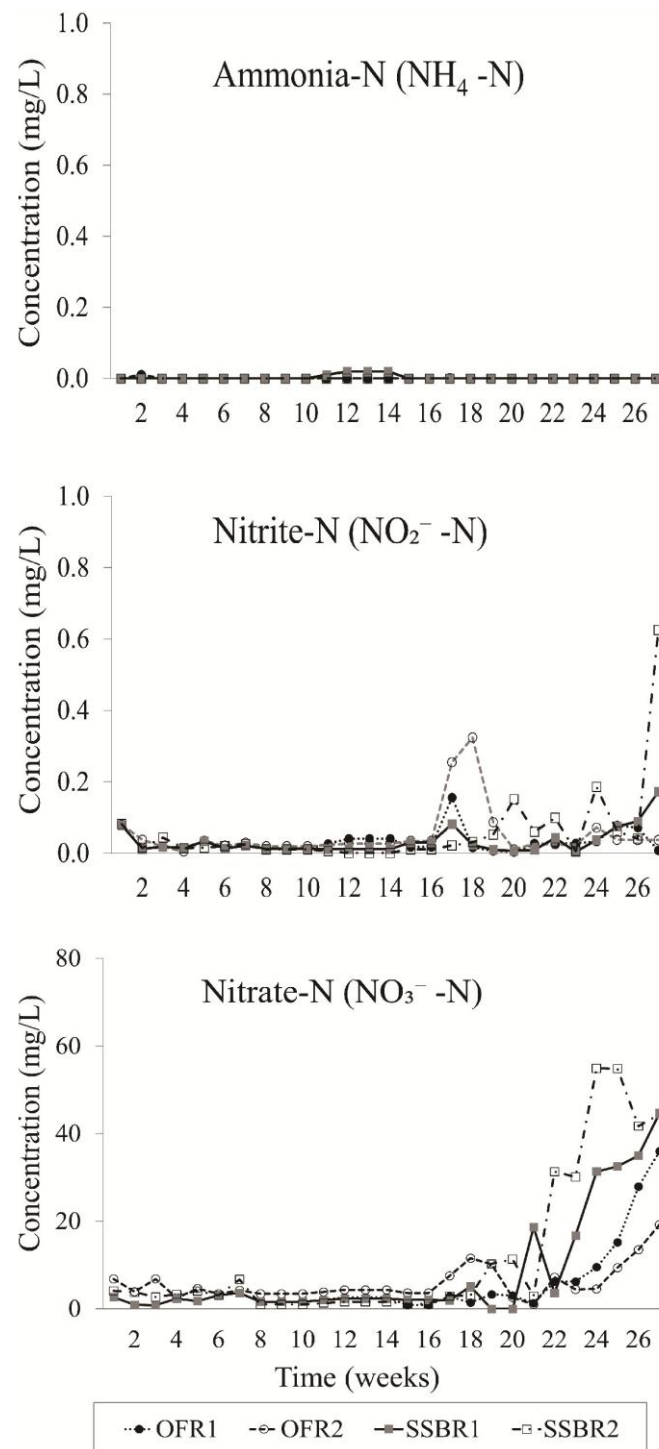


Figure 3 Comparison between the ammonia-N ($\text{NH}_4\text{-N}$), nitrite-N ($\text{NO}_2\text{-N}$), nitrate-N ($\text{NO}_3\text{-N}$) levels in the overflow filtration reactor (OFR) groups (group 1 and group 2) and the single-tank sequencing batch reactor (SSBR) groups (group 3 and group 4). The water parameters were recorded over a 27-week rearing period.

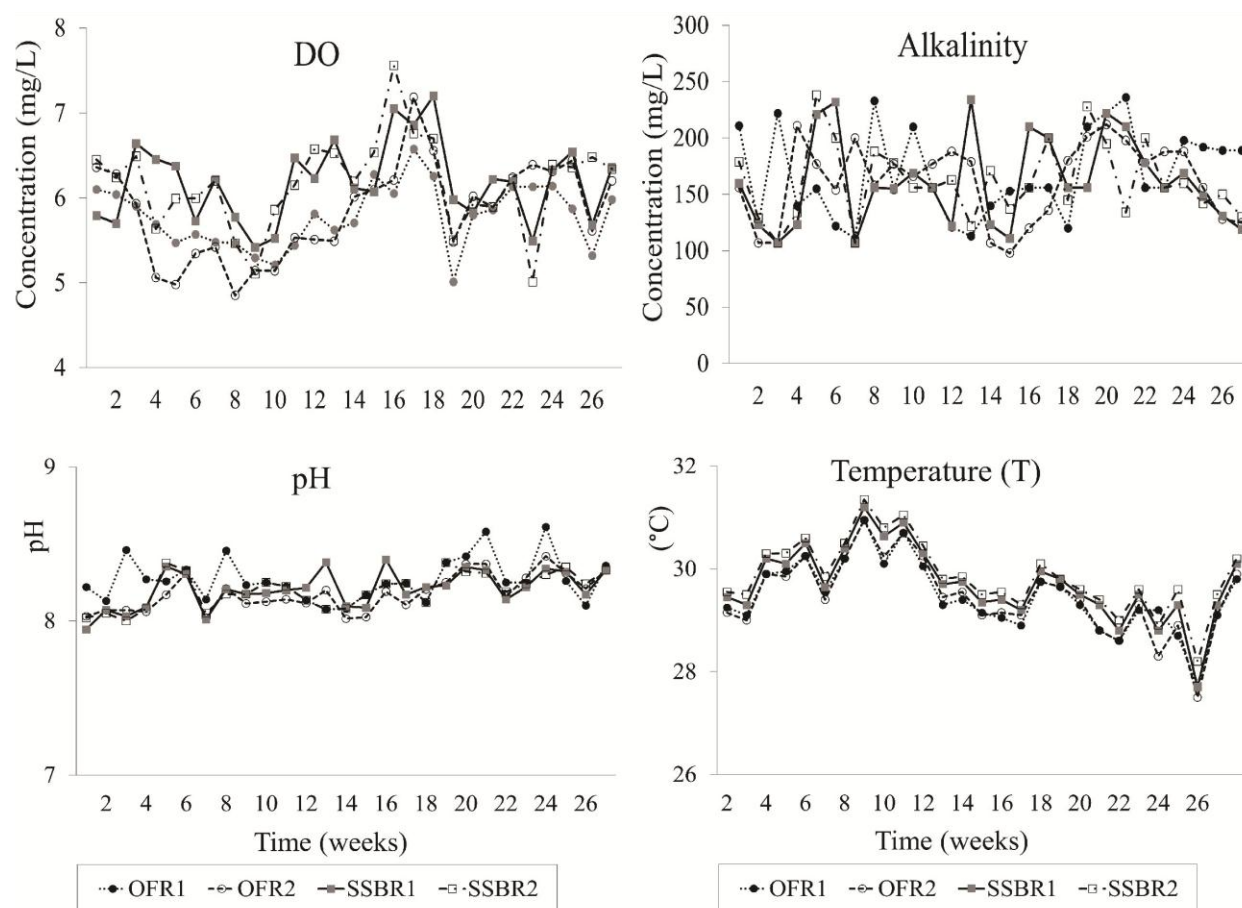


Figure 4 Comparison between the alkalinity, dissolved oxygen (DO), temperature (T), and pH in the overflow filtration reactor (OFR) groups (group 1 and group 2) and the single-tank sequencing batch reactor (SSBR) groups (group 3 and group 4). The water parameters were recorded over a 27-week rearing period.

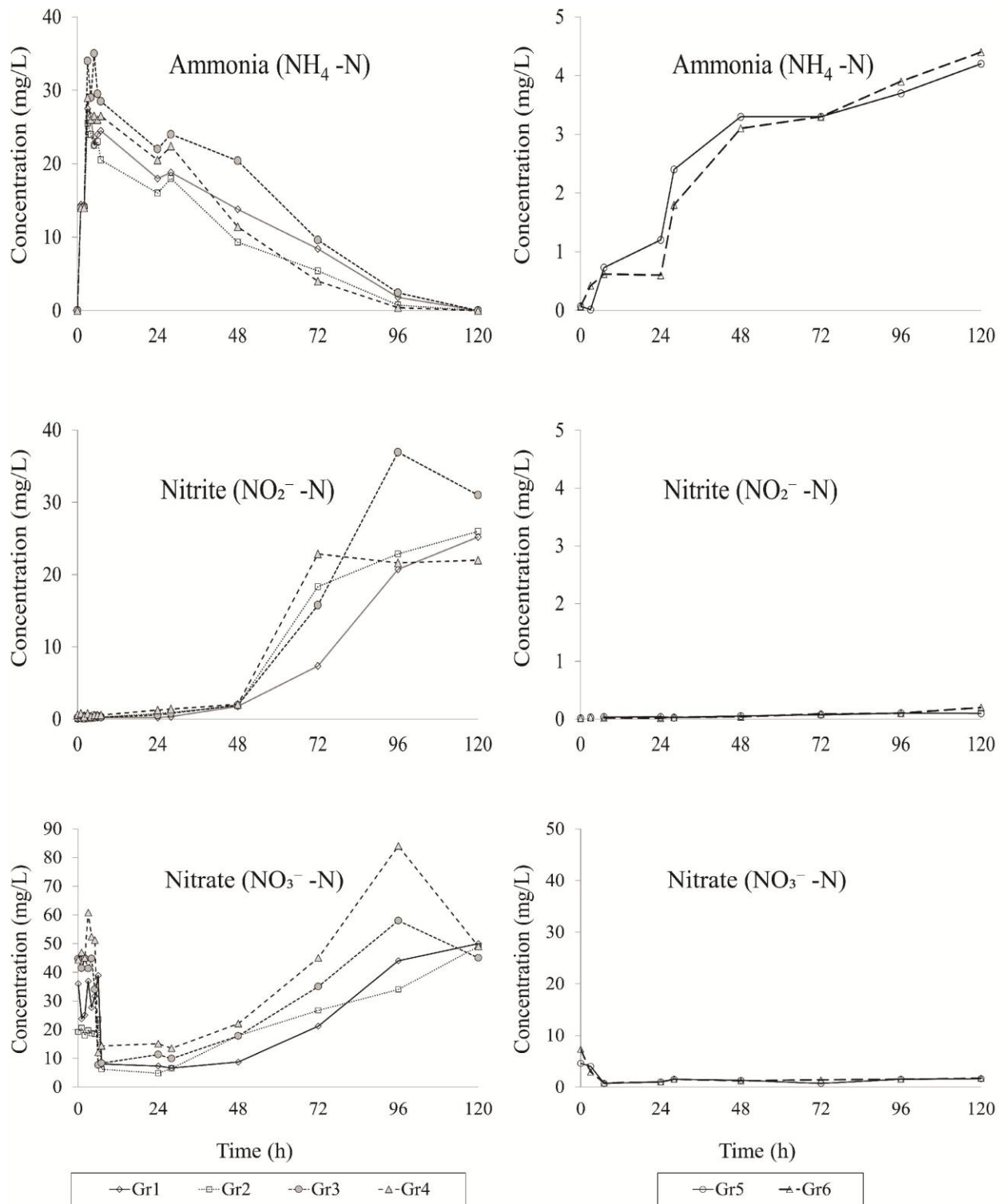


Figure 5 Comparison of ammonia-N ($\text{NH}_4\text{-N}$), nitrite-N ($\text{NO}_2\text{-N}$) and nitrate-N ($\text{NO}_3\text{-N}$) concentrations in Experiment 2 between the overflow filtration reactor (OFR)-linked tanks (group 1 and group 2), the single-tank sequencing batch reactor (SSBR)-linked tanks (group 3 and group 4) and the groups without bioreactor tanks (group 5 and group 6). The water parameters were recorded within 120 h post-load (hpl).

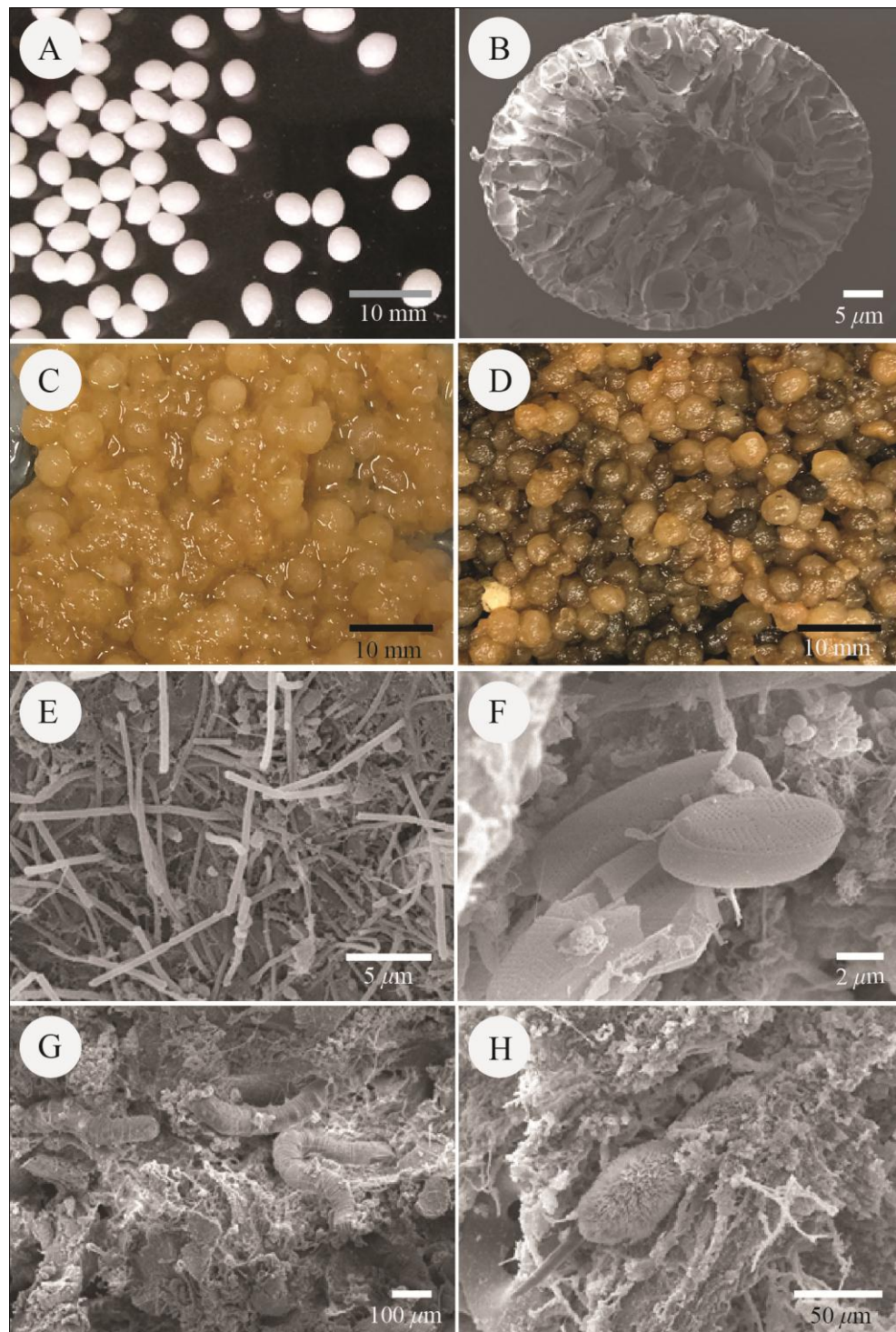


Figure 6 Photograph of white, oval solid porous cellulose beads (A). The beads under scanning electron microscopy (SEM) revealed pores and cavities throughout the surface (B). The beads in the OFR-link tank were light brown, gelatinized and softened (C) while the beads in the SSBR-link tank have a gradient color of light brown to black (D) after 24-week rearing period; the beads pores clogged with microorganisms including bacilliform bacteria, filamentous bacteria (E), diatoms (F), nematodes (G) and ciliated protozoa (H).

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Ethical approval: The study was conducted at the Veterinary Medical Aquatic Animal Research Center, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand. All animal husbandry and experimental conditions were approved by the Chulalongkorn University Animal Care and Use Committee (Protocol number: 1931089).

Data Availability Statement: The dataset generated in the experiment is available within the manuscript or in the supplementary information.

Author Contributions: Aranya Ponpornpisit: Conceptualization, methodology, funding acquisition, data curation, resources, writing - original draft. Malinee Jongjaroenjai: Investigation, project administration. Naowarat Suthamnatpong: Validation, writing - review & editing, Surachet Burut-Archanai: Investigation, visualization.

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