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Effect of flow rate on water quality parameters and plant growth of water spinach *(Ipomoea aquatica)* in an aquaponic recirculating system

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ABSTRACT

Recirculating aquaculture–hydroponic systems were designed to provide an artificial, controlled environment that optimizes the growth of fish (or other aquatic species) and soil-less plants, complete control of water quality, the production schedule and the fish product, while conserving water resources. Nutrients removal such as inorganic nitrogen and phosphate is essential for aquaculture wastewater treatment to protect receiving waters from eutrophication as well as for potential reuse of the treated water. In this study, a prototype of an aquaponic system was built at the Freshwater Hatchery Unit on the University Malaysia Terengganu campus. The system consists of a fish culture tank, hydroponic trough, sump, sand filter and water holding tank. Hydroponic troughs were planted with water spinach (*Ipomoea aquatica*) that been used to treat wastewater from an aquaculture system stocked with African catfish. The unplanted hydroponic trough was concurrently run as a control unit. The effect of five different water flow rates was tested in order to relate nutrients removal, water quality with plant growth. The results showed that the aquaponic recirculating system removed 5-day biochemical oxygen demand (47–65%), total suspended solids (67–83%), total ammonia nitrogen (64–78%), and nitrite-nitrogen (68–89%), and demonstrated positive correlated with flow rates. Total phosphorus and nitrate-nitrogen removal rates varied from 43% to 53% and 42% to 65%, respectively, and were negatively correlated with flow rates. It was found that all flow rates were efficient in nutrient removal and in maintaining the water quality parameters within the acceptable and safe limits for growth and survival of fish.

Keywords: Flow rate; Water quality parameters; Water spinach; Aquaponic; Recirculating systems

1. Introduction

Aquaculture, the controlled cultivation and harvest of aquatic plants or animals, places great demands on water resources. The industry typically requires range from 200–600 m^3 of water for every kilogram of fish produced [1]. In Malaysia, African catfish are cultured in open or semi-open systems in ponds, tanks or raceways. Open aquaculture systems imply that effluents are discharged to the environment with enhanced nutrient and solid

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concentrations. Depending on the species and culture technique, up to 85% of phosphorus, 80–88% of carbon and 52–95% of nitrogen input into a fish culture system may be lost to the environment through feed wastage, fish excretion, fecal production and respiration [2]. Remediation of aquaculture effluents is important because, in many areas, water is a limited resource and depending on the receiving water body, the total mass loading of nutrients from effluents can contribute to significant environmental degradation [3].

Several technologies are available for wastewater treatment such as activated sludge process, aerobic lagoon, and constructed wetland. During the past three decades, constructed wetlands have been used to treat acid mine drainage, industrial wastewater, agricultural runoff, and effluent from livestock operation. Previous studies [4–7] have demonstrated that constructed wetlands efficiently remove the major pollutants from catfish, shrimp and rainbow trout pond effluents, including suspended solids, organic matter, nitrogen, and phosphorus under flow rates ranged between 0.18 to 53.9 m^3/d. Lin et al. [6] reported that constructed wetland effectively removed total suspended solids (55–66%), 5-day biochemical oxygen demand (37–44%), total ammonia (64–66%) and nitrite (83–94%) from the recirculating water under various flow rates (42.7–53.9 m^3/d). However, the high construction cost, large land requirement and the potential risk of soil clogging that provokes a general failure of the system have limited its application in an intensive wastewater treatment system [8].

Hydroponic plants have been widely used in wastewater treatment systems because they efficiently absorb dissolved compounds in wastewater as nutrients for plant growth [9–11]. Aquaponics, the integration of hydroponics with aquaculture systems, has been used to produce a valuable by-product, recover nutrients and improve water quality. In these integrated systems, nutrients, which are excreted directly by the fish or generated by the microbial breakdown of organic wastes, are absorbed by plants cultured hydroponically. Fish feed provides most of the nutrients required for plant growth. As the aquaculture effluent flows through the hydroponic component of the recirculating system, fish waste metabolites are removed by nitrification and direct uptake by the plants, thereby treating the water, which flows back to the fish-rearing component for reuse. Thus, a harmful by-product of fish production becomes a beneficial input for plant production.

Aquaponics has several advantages over other recirculating aquaculture systems. The hydroponic component serves as a biofilter, and therefore a separate biofilter is not needed as in other recirculating systems. Integrating fish culture with plants has been tested in hydroponic systems where effluent was used as nutritive solution. These systems were designed for lettuce, tomatoes and other crops [12–15]. The technology associated with aquaponics is complex. It requires the ability to manage simultaneously the production and marketing of two different agricultural products. Modern aquaponic systems can be highly successful, but they require intensive management and they have special considerations. However, information on using aquaponics for treating recirculating aquaculture water under a various flow rate has not yet been extensively studied.

In this study, hydroponic troughs planted with water spinach, *Ipomoea aquatica*, were integrated with an indoor recirculating aquaculture tank with a limited water exchange to regulate the water quality for intensive culture of African catfish (*Clarias gariepinus*). The main objectives of the study are to: (1) investigate the performance of the water spinach in treating aquaculture wastewater under medium water flow rates; and (2) examine the effect of flow rate on water quality and plant growth in the integration system.

2. Materials and methods

2.1. Experimental aquaponic recirculating system

The aquaponic recirculating system was constructed in a greenhouse (Fig. 1) somewhere near the Freshwater Hatchery Unit on the campus of University Malaysia Terengganu. The system consists of three culture tanks, four hydroponic troughs (growing bed), two sand filtration tanks for solid removals, one sump system for denitrification unit, one water holding tank and reservoir (pre-aeration). Pipelines made of polyvinyl chloride were installed to connect the culture tank and hydroponic trough to recirculate the water.

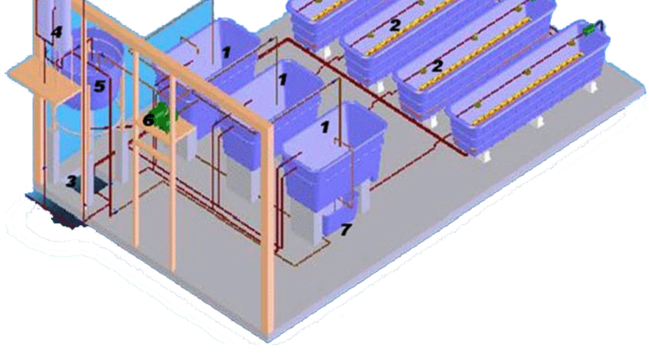

Fig. 1. Layout of prototype of aquaponic recirculating system. 1 fish tank, 2 growing bed, 3 sump, 4 sand filter, 5 water storage tank, 6 air blower, 7 pre-aeration tank.

Three culture tanks arranged in series were used in the rearing of African catfish (*Clarius gariepinus*). Tube diffusers, connected to an air blower were installed in the culture tank to supply oxygen for fish culture. Water level in each culture tank was kept at 0.85 m deep to maintain the water volume at 1000 L. Water lost through evaporation, transpiration and sludge removal is replenished with water in the pre-aeration tank.

In this study the square tank with the conical pattern around the ends of the tank was used in which the perforated sheet will be placed between the tank and conical part to create areas of no turbulence, allowing for more rapid settling of particulate waste (Fig. 2). This will allow most of the settleable solids to be concentrated and removed from the tank while most of the water flows out toward the end, into the hydroponic trough by gravity. The water flow rate was controlled by a valve.

Three of the hydroponic troughs were planted with water spinach (*Ipomoea aquatica*) and the other was not planted to study the effect of using plant on water quality parameters. Hydroponic troughs were filled with gravel the size of 0.5–40 mm and porosity of 0.60. Aquaculture wastewater from the fish tank flowed by gravity into the hydroponic trough and the sump, which is the lowest point in the system. The water was then pumped vertically to the sand filtration tanks for particulate removal. After exiting the sand filter the water went directly to storage tank and was flowed by gravity back to the fish tank.

2.2. *Hydraulic condition and culture*

Five experiments were carried out at different flow rates into recirculating aquaculture system (RAS), each operating for 3–4 weeks in order to relate to nutrients removal, water quality and growth of the plant. African catfish with the same stocking density about 25 kg/m^3 were introduced into the culture tank and acclimatized for 1 week after setting the desired tested water flow rate. The feeding rate was adjusted according to the intake rate at around 2–5% of bodyweight/day. The fish were fed with a commercial floating pellet manually twice a day at around 9 am to 5 pm each day. These commercial diets contained around 32% protein and 10% moisture. The food size was adjusted to compensate for changes in fish size. No water discharge or displacement except for replacing water lost through evaporation, transpiration, and sludge removal. Ten percent of fish were taken from the culture tank to measure their length and body weight to estimate the growth rate of the fish. Table 1 illustrates the hydraulic condition for this study.

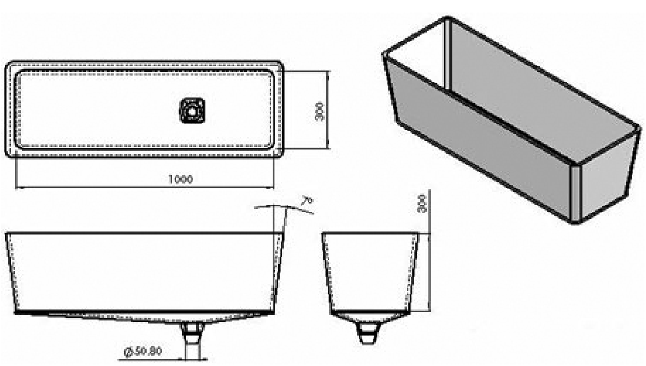

Fig. 2. Schematic diagram of culture tank

Table 1
Hydraulic conditions for operating the aquaponic recirculating system

Q^a (L/min)	Q (m^3 d^{-1})	HLR[b] (m d^{-1})	HRT[c] for overall system (h)
0.8	4.6	0.64	4.5
1.6	9.2	1.28	2.3
2.4	13.8	1.92	1.5
3.2	18.4	2.56	1.1
4.0	23.0	3.20	0.9

[a]Flow rate of the aquaponic recirculating system.
[b]Hydraulic loading rate, which is flow rate (Q) divided by total surface area of the trough.
[c]Hydraulic retention time, which can be computed as (surface area × water depth × porosity of gravel trough/flow rate).

2.3. *Plant growth*

Approximately 15–20 g of water spinach seed was required to completely cover the surface of one trough. The seedlings were placed into the holes evenly spaced 5–8 cm apart and planted with two seeds per hole. The experiment was conducted in triplicate and one trough was utilized as controls and contained gravel only. During the germination period (days 2–4), seed germination and seedling height were observed and recorded daily. A sprinkler was used to irrigate the plant in the trough. Effluent samples were collected from each trough once a week for chemical analyses. During the growth period (days 5–28), the height, leaf length and leaf width of 20 plants in each trough were measured and recorded daily. The plants were harvested at height ranging from 45 to 50 cm. Each growing trough was cleaned and the biomass of plants was measured and recorded.

2.4. *Sampling and analysis*

Water samples were taken once a week from each culture tank, influent and effluent of the hydroponic trough, sump, water holding tank and inflow of culture tank. The samples were analyzed for 5-day biochemical oxygen demand (BOD_5), total suspended solid (TSS), total ammonium nitrogen (TAN), nitrite nitrogen (NO_2^--N), nitrate nitrogen (NO_3^--N) and total phosphorus (TP). Dissolved oxygen, pH, and temperature were also monitored. Weekly sampling was carried out between 8.30 am and 9.30 in each sampling date and refrigerated at 4°C in labeled polythene bottles for chemical analysis. The BOD_5 and TSS analyses were performed according to the Standard Method (APHA 1998). The TAN, NO_2^--N, NO_3^--N and TP measurements were performed using Hach DR4000 spectrophotometer according to a salicylate, diazotization, cadmium reduction and ascorbic acid methods, respectively. The DO and pH of the sample were measured using a DO meter YSI 55A and pH cyber scan waterproof respectively.

2.5. Statistical analysis

The Statistical Package for the Social Sciences (SPSS), Version 16, and Microsoft Excel were used to calculate the mean, standard deviation, and one-way ANOVA. Differences of mean were evaluated for significance by the range tests of Tukey HSD (p = 0.05) for homogeneous variances (Levene test) and by the range test of Dunnett T3 (p = 0.05) for inhomogeneous variances, respectively [7].

3. Results

3.1. Plant growth

After 3 days, the radicles of water spinach (*Ipomoea aquatica)* had broken through the seed coat and were visible on 70–80% of the seeds. During the germination period, the plant seedlings in all growing troughs grew rapidly and fairly uniform and appeared healthy with green color. At the end of the germination period the plants were approximately 4.0 cm in height. The plants continued to grow rapidly and fairly uniformly and showed no signs of mineral deficiency or disease during the growth period (days 5–28). At the end of the growth period, the plants reached the market size at an average height of 45–50 cm. Fig. 3 demonstrates the effect of flow rate on the growth of water spinach in terms of plant height.

The effects of water flow rate on plant growth were tested using a one-way ANOVA and differences of mean were evaluated for significance by the range tests of Tukey HSD (p = 0.05) for homogeneous variances (Levene test) using SPSS version 16.0. It appears that flow rate has a significant influence on the plant growth (Table 2).

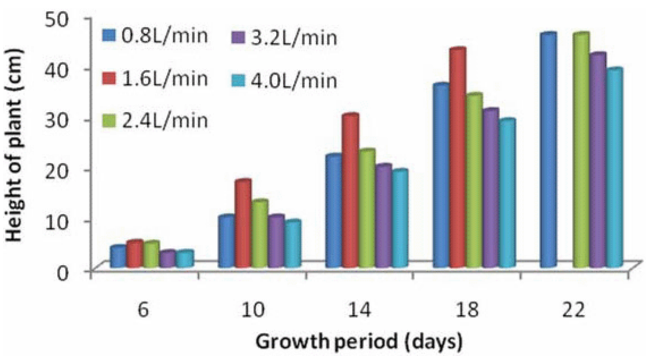

Fig. 3. Plant growth rate in aquaponic recirculating system.

Table 2
Average plant growth at various flow rates

Flow rate (L/min)	Plant growth (cm/d)	Tukey HSD[a] subsets (σ = 0.05)
0.8	1.70	1
1.6	2.11	2
2.4	1.75	2
3.2	1.59	2

[a]Treatment with different numbers is significantly different at p = 0.05 level.

3.2. Fish production

Overall production performance of African catfish in all water flow rates is shown in Table 3. The observed growth rate was different between small and big fish. The mean daily growth rate for flow rate at 0.8 L/min and 3.2 L/min is 0.86 and 0.81, respectively. The relatively low value was probably due to the smaller fish size and number of fish stocked. Feed conversion ratio (FCR) values were in the range of 1.23–1.39. The FCR recorded are not far above the ideal value of 1.0 for the culture of African catfish in a recirculating system and the FCR value 0.85 reported in the culture of African catfish by Eding and Kamstra [17]. However, the recorded FCR are better than the range 1.26–3.34 reported in conventional culture of African catfish in concrete tanks as reported by Faturoti [18]. In our study, the same feed was used and the ration was fixed similarly in all culture tanks. Stocking at flow rate of 1.6 L/min gave the best production performance (Table 3).

3.3. Water quality

Continuous flow operation of the aquaponic system was initiated with a low water flow rate of 0.8 L/min. Plants grew actively in the hydroponic trough. The percentage removal of water quality variables and physicochemical parameters at various flow rates are shown in Table 4 and Table 5 respectively. It was found

Table 3
Production performance for African catfish cultured at different flow rate (mean ± standard deviation)

Parameters	Flow rate, L/min				
	0.8	1.6	2.4	3.2	4.0
Duration of culture (days)	35	35	35	35	35
Initial stocking density (kg/m^3)	25.4 ± 0.4	25.3 ± 0.3	25.3 ± 0.3	25.4 ± 0.5	25.4 ± 0.4
Number of fish/m^3	702.0 ± 2.0	361.0 ± 3.0	204.0 ± 5.0	711.0 ± 4.0	380.0 ± 6.0
Feed rate (3% of body weight) kg/d	0.76 ± 0.01	0.76 ± 0.01	0.76 ± 0.01	0.76 ± 0.01	0.76 ± 0.01
Initial weight (g/fish)	34.3 ± 2.5	66.7 ± 2.5	118.0 ± 1.7	34.0 ± 2.0	63.33 ± 2.1
Final weight (g/fish)	64.3 ± 2.5	126.7 ± 3.5	216.3 ± 3.5	62.3 ± 2.1	114 ± 1.7
Final stocking density (kg/m^3)	45.2 ± 1.9	45.7 ± 1.6	44.1 ± 1.6	44.3 ± 1.6	43.3 ± 1.0
Specific growth rate[a]	1.80 ± 0.10	1.83 ± 0.05	1.73 ± 0.01	1.73 ± 0.26	1.68 ± 0.13
Daily growth rate[b] (g/fish/d)	0.86 ± 0.03	1.71 ± 0.03	2.81 ± 0.04	0.81 ± 0.12	1.45 ± 0.10
Feed conversion ratio (FCR)[c]	1.27 ± 0.02	1.23 ± 0.01	1.33 ± 0.04	1.34 ± 0.21	1.39 ± 0.08
Tukey HSD[d] subsets (σ = 0.05)	1	1	1	1	1

[a]Specific growth rate calculated as SGR = ln final weight (g) − ln initial weight (g) × 100 × $days^{-1}$.
[b]Daily growth rate calculated as DGR = weight gained by fish (g)/culture days.
[c]Feed conversion ratio calculated as FCR = total weight of dry feed given/total wet weight gain.
[d]Treatment with the same number is not significant at the $p = 0.05$ level.

Table 4
Mean values and percentage removal of water quality variables at various flow rates

Parameter	Flow rates (L/min)	Influent (mg/L)	Effluent (mg/L)	Removal (%)	Tukey HSD[a] subsets (σ = 0.05)
BOD_5	0.8	5.5	2.9	47.3	1
	1.6	5.5	2.5	54.5	2
	2.4	5.6	2.5	55.4	2
	3.2	5.7	2.2	61.4	2
	4.0	5.5	1.9	65.5	3
TSS	0.8	73.4	24.2	67.0	1
	1.6	73.2	22.3	69.5	2
	2.4	73.6	20.4	72.3	2
	3.2	73.2	15.4	79.0	3
	4.0	72.7	12.4	82.9	4
TAN	0.8	10.82	3.88	64.1	1
	1.6	10.84	3.43	68.4	1
	2.4	10.81	3.14	71.0	2
	3.2	10.79	2.88	73.3	2
	4.0	10.78	2.34	78.3	3
NO_2-N	0.8	0.58	0.19	67.2	1
	1.6	0.56	0.14	75.0	2
	2.4	0.56	0.11	80.4	2
	3.2	0.57	0.09	84.2	2
	4.0	0.57	0.06	89.5	2
NO_3-N	0.8	18.6	7.0	62.4	4
	1.6	18.8	6.6	64.9	4
	2.4	18.7	7.4	60.4	2
	3.2	18.8	7.8	58.5	3
	4.0	18.9	10.9	42.3	1
TP	0.8	15.8	7.9	50.0	4
	1.6	15.9	7.5	52.8	5
	2.4	15.7	8.2	47.8	1
	3.2	15.8	8.3	47.5	3
	4.0	15.9	9.1	42.8	2

Values given are mean from triplicate data.

Table 5
Physicochemical water parameters of inlet and outlet of the hydroponic trough

Flow rate (L/min)	Sampling location	pH	DO (mg/L)	Temperature (°C)
0.8	Inlet hydroponic trough	7.56 ± 0.14	5.67 ± 0.23	28.74 ± 0.35
	Outlet hydroponic trough	7.48 ± 0.13	4.12 ± 0.15	28.42 ± 0.17
1.6	Inlet hydroponic trough	7.52 ± 0.18	6.19 ± 0.21	28.56 ± 0.32
	Outlet hydroponic trough	7.42 ± 0.21	4.22 ± 0.25	28.34 ± 0.14
2.4	Inlet hydroponic trough	7.47 ± 0.22	6.32 ± 0.32	28.45 ± 0.23
	Outlet hydroponic trough	7.38 ± 0.15	4.42 ± 0.24	27.42 ± 0.24
3.2	Inlet hydroponic trough	7.46 ± 0.12	6.47 ± 0.26	27.75 ± 0.25
	Outlet hydroponic trough	7.34 ± 0.23	4.61 ± 0.21	27.34 ± 0.17
4.0	Inlet hydroponic trough	7.37 ± 0.23	6.65 ± 0.22	27.54 ± 0.35
	Outlet hydroponic trough	7.28 ± 0.09	4.82 ± 0.18	27.32 ± 0.17

Values given are mean ± standard deviation.

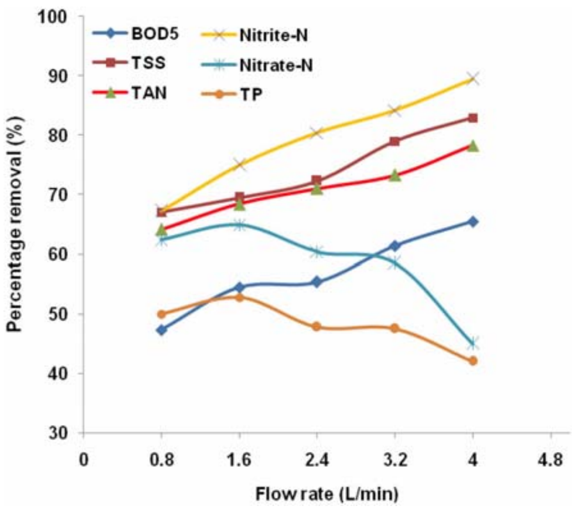

Fig. 4. Percentage removals of water quality parameters at various flow rates in recirculation aquaponic system.

that removal percentage of BOD_5, TSS, TAN and nitrite-N increased with the increasing of flow rates. In contrast to BOD_5, TSS, nitrite-N and TAN, the removal percentage of nitrate-N and TP increased with the increasing of flow rates from 0.8 L/min to 1.6 L/min and decreased with the increasing of flow rates from 1.6 L/min to 4.0 L/min (Fig. 4)

Treatment with the same number is not significant at the p = 0.05 level. The value of pH slightly decreased (p >0.05) from the influent to effluent of hydroponic trough and maintaining at a value of 7.56 to 7.28 during the experiment. Oxygen levels were sufficient resulting from diffused-air aeration in the culture tank. The ideal temperature range for African catfish was shown to be 20–30°C, with an optimum of 27°C for juveniles and 25°C for adults [19]. The water temperature range of 27–29°C during this study lies well within the ideal limits indicated for the optimal growth of African catfish.

4. Discussion

4.1. Plant growth

The hydroponically grown water spinach in a gravel medium, supplied with the nutrient present in aquaculture wastewater, produced apparently normal, healthy looking plants, indicating that there were no major mineral deficiencies or toxicities caused by the wastewater. It was found that their best performance in terms of plant growth was at a water flow rate of 1.6 L/min. The major growth-limiting mineral is usually nitrogen, and highest growth rates and yields are generally seen when nitrogen is supplied as a combination of ammonium and nitrate [20].

4.2. Fish production

The fish production performance at various flow rates is shown in Table 3. The mean daily growth rates range from 0.81 to 2.81 g/fish/day. The volumetric production rate ranges from 43.3 to 45.7 kg/m^3. There is no significant difference (p >0.05) in the feed conversion ratio at various flow rates (Table 3).

4.3. Water quality

The BOD_5 concentration in the system increased with time during the germination period due to the release of dissolved and suspended organic matter from the developing seeds [21]. Uneaten food and fecal production are the major sources of organic matter in aquaculture effluents [22], which was expressed as the BOD_5. At the end of the growth period, BOD_5 reductions of 47–66% and 43–51% were achieved in the system and planted trough respectively. The BOD_5 reductions were significantly influenced by flow rate (p <0.05) as shown in Table 4.

Lin et al. [6] evaluated the use of cattail (*Typha angus-*

tifolia) and common reed (*Phragmites australis*) from an aquaculture system stocked with shrimp and reported BOD_5 removal efficiencies of 37% and 54% for a water flow rate of 42.7 m^3/d and 53.9 m^3/d respectively. One mechanism responsible for the removal of BOD_5 from the wastewater is the decomposition of soluble organic carbon by microbial communities.

In aquatic, plant-based treatment systems, submerged plant parts (root zone) are typically covered with active biofilm. Microbial communities may also be associated with the surfaces of litter and sediments and may be dispersed throughout the water column [23]. According to Bouzoun et al. [24], plant root density and root surface area are major factors in BOD_5 removal. The greater the root surface area per unit volume of tray, the higher the removal of BOD_5 because the greater surface area of the finer root system provides more sites for microbial growth. Another mechanism for the reduction in BOD_5 is the filtration of suspended particles by plant root mats and absorption of dissolved nutrients by plant roots [25]. The oxygen-demanding materials in waters used for the culture of fish and shellfish must be limited for several reasons. Waters rich in organic matter will lead to an increase in oxygen consumption by heterotrophic microorganisms in the water body.

Several authors reported a wide range of total solid concentration in the effluent of rearing tanks at fish aquaculture facilities. Values from 12 to 48 mg/L of this study are in accordance with the published range of 1 to 50 mg/L [26–28]. TSS reduction of 67% to 83% was significantly influenced by the water flow rates (Table 4).

Ghaly and Snow [15] evaluated the use of hydroponically grown barley to reduce the TS concentration in wastewater from a recirculating aquaculture system stocked with Arctic charr (*Salvelinus alpinus*). TS reductions of 27.4% and 52.7%, 59.4% and 60.5% were achieved in the control and in the compartments containing barley (200, 250 and 300 g/tray) respectively. A possible mechanism for the removal of suspended solid is sedimentation, a process by which suspended particles settle from a wastewater under the influence of gravity. However, sedimentation was not a dominant mechanism for the removal of suspended solid in this study due to there is no sedimentation tank used in this system.

At the end of the growth period, a hydroponic trough in this study effectively removed TAN in recirculating water achieving 64–78%. The TAN reduction was also significantly influenced by the water flow rate ($p < 0.05$) (Table 4).

Ghaly and Snow [15] examined the use of barley for nutrient removal from a recirculating aquaculture system stocked with Arctic charr (*Salvelinus alpinus*) and reported 76% of TAN reduction was achieved in all compartments. Bouzoun [29] evaluated the feasibility of utilizing hydroponically grown reed carnary grass to reduce the pollution load of a primary treated municipal wastewater and reported an average NH_4^+-N reduction in the wastewater of 34% over a 5-month period. Vaillant et al. [30] evaluated the effectiveness of *Datura innoxia* plants for domestic wastewater purification and reported NH_4^+-N reductions in the effluent of 93% after 48 h of treatment. Lin et al. [6] examined the use of cattail and common reed in a constructed wetland for nutrient removal from Pacific white shrimp culture and reported TAN reductions ranging from 64–66% for two different hydraulic loading rates.

TAN production with respect to ingested nitrogen was relatively constant regarding the size of fish. In the present study a low water flow rate responsible for low values of dissolved nitrogen wastes due to low fish activity was observed during the lower water flow rate. Dissolved nitrogen excretion increases as swimming activity increases [31].

Several mechanisms exist for the removal of NH_4^+-N from the aquaculture wastewater. Forms of inorganic nitrogen that are associated with particulate matter may be removed from waste streams by sedimentation and filtration/interception by the root mats of plants [32]. Ammonium (NH_4^+) is one of the major sources of inorganic nitrogen taken up by the roots of higher plants [30]. It may be assimilated by microorganisms and converted back into organic matter or may be removed from waste streams through the process of nitrification. Accumulation of ammonia in water is one of the major causes of functional and structural disorders in aquatic organisms [33]. The concentration of TAN in the final effluent were comparatively low in this study (2.3–3.9 mg/L), which is lower than the recommended TAN concentration of 3.0–6.7 mg/L for waters used for the culture of African catfish [17].

The concentration of NO_2^--N in the hydroponic trough effluent was in the range of 0.3–0.49 mg/L. At the end of the growth period, this system effectively removed 67–89% NO_2^--N in recirculating water (Table 4). The NO_2^--N reductions were significantly influenced by the flow rate. Ghaly et al. [34] examined the use of hydroponically grown barley for treatment of wastewater from a recirculating aquaculture system stocked with tilapia and reported NO_2^--N reductions of 98.1% after 21 days of growth. No other reports were available in the literature. In natural waters, ammonium is converted rather rapidly to nitrite (NO_2^-) and further to nitrate (NO_3^-) by aerobic bacteria from the genera *Nitrosomonas* and *Nitrobacter*, through a process called nitrification [35].

Nitrification was facilitated by the continuous aeration of the system compartments during the experiments. Princic et al. [36] reported that the optimum pH range for conversion of NH_4^+ to nitrite (NO_2^-) is between 5.8 and 8.5. The pH of the water in all experiments of this study was within this range. Although NO_2^--N is considerably less toxic than NH_3-N, it may be more important than ammonia toxicity in intensive, recirculating aquaculture systems because it tends to accumulate in the recirculated water as a result of incomplete bacterial oxidation [35,37]. Nitrite toxicity is associated with its ability to diffuse across the gills and into the blood circulation [38]. The average NO_2^--N concentrations in the effluent (Table 4) were in the range 0.06–0.19 mg/L. Eding and Kamstra [17] recommends a NO_2^--N concentration range 0.4–1.5 mg/L in water used for the culture of African catfish.

The aquaculture wastewater had an average NO_3^--N concentration of 18.77 ± 0.22 mg/L. NO_3^--N accumulates in aquaculture systems as a result of nitrification [33]. At the end of the growth period, NO_3^--N reductions in the range 42–65% were achieved in this recirculating system.

Ghaly and Snow [15] investigated the possibility of using hydroponically grown barley for the treatment of aquaculture wastewater and reported NO_3^--N reductions in the effluent of 68.8–76.7% after 21 days of plant growth. Clarkson and Lane [39] evaluated the feasibility of utilizing a nutrient-film technique to reduce the mineral content of wastewater from an aquarium stocked with common carp (*C. carpio*) and rainbow trout (*O. mykiss*). During a 4-week period, nitrate nitrogen concentrations in the effluent were reduced from 33.03 to 3.03 mg/L using barley.

In applications for wetland treatment of aquaculture wastewater and recirculating water, Lin et. al. [4,5] demonstrated efficient nitrate removal (68–99%), whereas Lin et al. [6] and Schultz et al. [7] revealed poor performance in apparent nitrate removal or even an increase of nitrate level from influent to effluent due to the difference of hydraulic loading rate operated in their studies.

In this study, overall removal of nitrate in hydroponic troughs declined proportionally with increasing flow rate. It was found that flow rate at 1.6L/min showed highest removal rates of about 65%. This study revealed a poor performance in apparent nitrate removal compare to Lin et al. [4,5] due to the difference of flow rate operated in which lower HLRs were employed in former studies. An increasing flow rate might diminish the contact time for nitrate and denitrifying bacteria, thus decreasing the performance of hydroponic trough for denitrification.

Several mechanisms are responsible for the removal of NO_3^--N from the wastewater. NO_3^--N is the preferred form of inorganic nitrogen taken up by the roots of higher plants [30]. It may also be assimilated by microorganisms in the water column or by biofilms associated with the root mats of plants [40]. Organic-bound nitrogen (proteins, amino acids and urea) was ammonified by microbial processes, which can be either aerobic or anaerobic [41]. Nitrification or oxidation of ammonia (ammonified and excreted ammonia) to nitrate as an oxygen-demanding process occurred in two steps involving microbial species, e.g., *Nitrosomonas* and *Nitrobacter* [42]. The importance of nitrification reported primarily in production of nitrate, which then participates in denitrification reactions, the conversion of nitrate to nitrogen gas.

An increasing water flow rate supported the development of aerobic conditions in the hydroponic trough and hindered denitrification processes. Nevertheless, a low flow rate with lower outflowing oxygen contents promoted denitrification and highest NO_3^--N elimination was observed in lower flow rates (0.8 L/min and 1.6 L/min). Poxton [35] recommended that NO_3^--N concentrations do not exceed 50 mg/L in waters used for the culture of fish and shellfish.

The total phosphorus (TP) concentration in each compartment increased with time during the germination period due to the release of dissolved and suspended matter from the developing seeds [21]. The overall reduction of in TP concentration was significantly affected by flow rate as shown in Table 4. Food residues and fecal matter are the major sources of phosphorus in aquaculture effluent. Phosphorus occurs in aquaculture wastewater primarily as soluble and insoluble phosphates in both organic and inorganic forms [33].

Ghaly et al. [34] examined the use of hydroponically grown barley for removal of PO_4^{3-}-P from aquaculture wastewater and reported PO_4^{3-}-P reductions ranging from 91.8% to 93.6%. Clarkson and Lane [39] evaluated the use of the nutrient film technique for PO_4^{3-}-P removal from aquarium wastewater. During a 4-week period, the PO_4^{3-}-P concentration in the effluent was reduced from 4.4 to 0.3 mg/L using barley.

Several mechanisms exist for the removal of phosphorus from wastewater. Forms of phosphorus that are associated with particulate matter may be removed from wastewater by sedimentation or by filtration/interception by the root mats of plants. In aquatic, plant-based treatment systems, microbial communities responsible for this oxidation process are associated with litter, sediment and the root mats of plants. They may also be suspended throughout the water column. Soluble inorganic phosphate may be removed from waste streams by plant uptake, microbial assimilation, precipitation with cations such as aluminum, calcium, magnesium, iron and manganese and adsorption onto organic matter [42]. Nutrient removal by plant assimilation and uptake is reported as being of minor importance and lying between 10% and 30% of total nitrogen and total phosphorus retention [42,43]. The average TP concentrations in the final effluents from this study were in the range 7.5–9.1 mg/L.

5. Conclusions

Water spinach grew well and showed a positive response to aquaculture wastewater applications in terms of growth and biomass production. No visual signs of mineral deficiency or disease were noticed. The average plant height at harvest was in the range 45–50 cm and the yield range 2.0–2.2 kg/trough. Plant growth was highest at a water flow rate of 1.6 L/min. The plant was able to significantly reduce the pollution load of the aquaculture wastewater stocked with African catfish.

Efficient removal was always achieved in these studies under a wide range of water flow rates because of low pollutant levels of aquaculture wastewater. All flow rates were efficient in nutrient removal and in maintaining the water quality parameters within the acceptable and safe limits for growth and survival of fish. The period of 3–4 weeks for nutrient removal in this study seemed to be shorter than those that occurred in other system cited from the literature. The reason for this might be that a pilot-scale aquaponic system can develop stable removal processes more quickly than other systems. Because the aquaculture wastewater has low nitrogen concentrations, removal of inorganic nitrogen was extremely efficient under various flow rates trials (0.8–4.0 L/min).

The hydroponically grown water spinach was able to significantly reduce the pollution load of the aquaculture wastewater. The BOD_5, TSS, TAN, NO_2^--N, NO_3^--N and TP reductions were in the range of 47–65%, 67–83%, 64–78%, 68–89%, 42–65% and 43–53% respectively. Results from this study showed that both plant growth and production of African catfish were better at a flow rate of 1.6 L/min.

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