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A Comparative Assessment of Hydroponically Grown Cereal Crops for the Purification of Aquaculture Wastewater and the Production of Fish Feed

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Abstract: Hydroponically grown wheat, barley and oats were examined for their ability to remove nutrients from aquaculture wastewater. Wheat, barley and oats seeds were germinated in water in a hydroponics system. The seedlings then received wastewater from an aquaculture system stocked with Arctic charr. During the experiment, the crops grew rapidly and fairly uniformly and showed no signs of mineral deficiency although fungal growth was evident. The average crop heights and yields at harvest were 19.0, 25.5 and 25.2 cm and 64, 59 and 42 t ha^{-1} for wheat, barley and oats, respectively. The hydroponically grown wheat, barley and oats were able to significantly reduce the pollution load of the aquaculture wastewater. The TS, COD, NH_4^+-N, NO_2^--N, NO_3^--N and PO_4^{3-}-P reductions ranged from 53.3 to 57.7%, from 55.7 to 78.7%, from 76.0 to 80.0% from 85.1 to 92.9%, from 62.1 to 79.3% and from 74.1 to 93.0%, respectively. The compartments containing barley produced the highest quality effluent, which was suitable for reuse in aquaculture operations. The average TS, COD, NH_4^+-N, NO_2^--N, NO_3^--N and PO_4^{3-}-P concentrations and pH of the final effluent from the compartments containing barley were 442, 64, 0.50, 0.02, 5.89 and 0.61 mg L^{-1} and 6.65, respectively. The nutritive value of the three wastewater grown crops was assessed to determine the suitability of using the plants as a component in fish feed. The three terrestrial crops meet the energy, fat, Ca, Mg, P, Na, S and Mn dietary requirements of aquatic animals, exceed the carbohydrate, crude fiber, Cl, K, Cu, Fe, Se and Zn requirements of fish and shellfish and do not contain sufficient amounts of protein to meet the dietary requirements of fish and shellfish. The crops will require supplementation with a high protein source that contains low concentrations of carbohydrates, crude fiber, Cl, K, Cu, Fe, Se and Zn. Common protein sources that could be used for supplementation included fishmeal, bone meal and blood meal.

Key words: Aquaculture, wastewater, hydroponics, wheat, barley, oats, water quality, fish feed, nutrition

INTRODUCTION

Aquaculture has become the fastest growing food production sector in the world due to: (a) significant increases in the demand for fish and seafood, (b) the industry's continuity of supply, consistency of quality and control of production, (c) scientific advances in nutrition, disease control, rearing techniques and genetics and (d) the decline in wild fisheries[1]. In 2002, the industry produced 51.4 million tonnes of finfish, shellfish and aquatic plants valued at US $60.0 billion[2]. The rapid growth of the aquaculture industry has been accompanied by an increase in environmental impacts. The production process generates substantial amounts of polluted effluent, which contains particulate and dissolved organic matter and nutrients. Aquaculture effluents exert adverse environmental impacts when discharged to receiving waters as the organic matter loading reduces dissolved oxygen levels and contributes to the buildup of bottom sediments and high nutrient loading stimulates excessive phytoplankton production[3-5].

Many of the technologies employed for the treatment of municipal wastewaters have also been utilized for the remediation of aquaculture effluents with varying degrees of success[6-11]. These technologies include: screening, sedimentation and centrifugation, coagulation, activated carbon and ion exchange and biofiltration. Because of the large flow rates and low nutrient concentrations associated with aquaculture operations, these pollution control measures tend to be quite costly and also have the disadvantages of sludge production, high energy demand and frequent maintenance requirements[4, 12-13].

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In addition to the generation of large amounts of waste, the use of fishmeal and fish oil as prime constituents of feed is another non-sustainable practice in aquaculture[3, 14-15]. Aquaculture feeds are amongst the most expensive animal feeds and typically account for half of the total cost of aquaculture production, with protein being the most expensive component[14, 16-17].

Hydroponics, the cultivation of plants in nutrient enriched water with or without the support of a medium such as sand or gravel, has been integrated with aquaculture systems to produce a valuable by-product, recover nutrients and improve water quality[18-20]. Hydroponics is typically integrated with intensive, recirculating aquaculture facilities because the low water exchange and high feeding rates associated with these systems lead to an accumulation of dissolved nutrients in the wastewater. In these integrated systems, nutrient rich effluent from the aquaculture facility provides moisture and nutrients for the production of plants[21].

The primary aim of this study was to perform a comparative assessment of the feasibility of using three cereal crops (wheat, barley and oats) to purify the wastewater from an aquaculture operation and their suitability as fish feed. The specific objectives were to evaluate: (a) the plant growth and yield, (b) the effectiveness of these plants in reducing the pollution load of the aquaculture wastewater as measured by a reduction in TS, COD, NH_4^+-N, NO_2^--N, NO_3^--N, PO_4^{3-}-P and pH and (c) the suitability of recycling the treated wastewater for fish culture, (d) the nutritive value of these plants as measured by energy, carbohydrates, crude protein, crude fat, crude fiber, Ca, Cl, Mg, P, K, Na, S, B, Cu, Fe, Mn, Mo, Se and Zn contents and (e) the suitability of these plants as a component in fish feed.

EXPERIMENTAL APPARATUS

The hydroponic system (Fig. 1) consisted of a frame, growth troughs and aeration, lighting, cooling, irrigation, supernatant collection and control units.

The frame (Fig. 2) was constructed of angle iron with a width of 244 cm, a depth of 41 cm and a height of 283 cm. The back and the top were covered with 0.6 cm thick plywood sheets. The frame consisted of three shelves (76 cm apart). Each shelf was divided vertically into two cells by dividers made of 1.2 cm thick plywood sheets. The frame supported the growth troughs and all other systems.

The plant growth unit consisted of six troughs. Each trough was made of galvanized steel and was divided into three compartments. Each compartment held a tray that acted as the plant support medium and consisted of a wire-mesh base (16 openings cm^{-2}) with 5 cm high metal sides. The dimensions of each trough and plant supporting tray are shown in Fig. 2. The trays were positioned in the troughs so that the plant roots were in contact with the liquid waste. The placement of trays was maintained by means of supports welded into the corners of each compartment 5 cm below the top edge of the trough.

An aeration unit was installed in each compartment to provide oxygen to the immersed roots of the growing plants. The main air supply was connected to a manifold (PVC pipe of 2.54 cm outside diameter) on each shelf using PVC tubing of 0.635 cm outside diameter. The air flow from the main supply to the manifold on each shelf was controlled by a pressure regulator (Model 129121-510, Aro, Brayn, OH). Six aeration units were connected to the manifold on each shelf using tygon tubing of 0.635 cm outside diameter. Each aerator consisted of a main tube with three perforated stainless steel laterals coming off it at right angles to the main. Each lateral was approximately 30 cm long whereas the main was 26.5 cm long.

The lighting unit was designed to provide approximately 360 hectolux of illumination per trough. This was achieved by a mixture of fluorescent and incandescent lamps. Six 34 W cool white fluorescent lamps (122 cm in length) and two 60 W Plant Gro N Show bulbs were fastened above each trough.

A cooling unit was designed to continuously remove the heat produced by the lamps to avoid heating of the wastewater on the upper and middle shelves. For each of these two shelves, a 5 cm diameter PVC pipe, having 6 mm diameter holes spaced 6 cm apart and facing out, was placed under the backside of the troughs. Two metal blocks supported the front side of the trough. This provided a 5 cm space between the trough and the lighting unit of the shelf below it. A 5 cm diameter PVC pipe acting as a manifold was attached vertically to the left side of the frame, through which air was blown by means of a motor driven fan (Model AK4L143A type 821, Franklin Electric Company, Bluffton, IN).

The wastewater application unit consisted of: (a) a wastewater storage tank, for storing the wastewater, (b) a pump, to transfer the wastewater from the storage tank to the growth troughs, (c) six valves, to control the amount of wastewater fed to each cell and (d) an irrigation system, for applying the wastewater onto the plant supporting trays in the growth troughs. The wastewater storage tank was constructed of plastic and had a capacity of approximately 100 L. A mixing shaft, with a 40 cm diameter impeller, was installed through the center of the cover of the tank to agitate the wastewater in the tank. Four 2.5 cm baffles were installed vertically along the inside wall of the tank to promote complete mixing. A 1 hp motor (Model NSI-10RS3, Bodine Electric Company, Chicago, IL) with speed reducer was mounted on the tank cover to drive

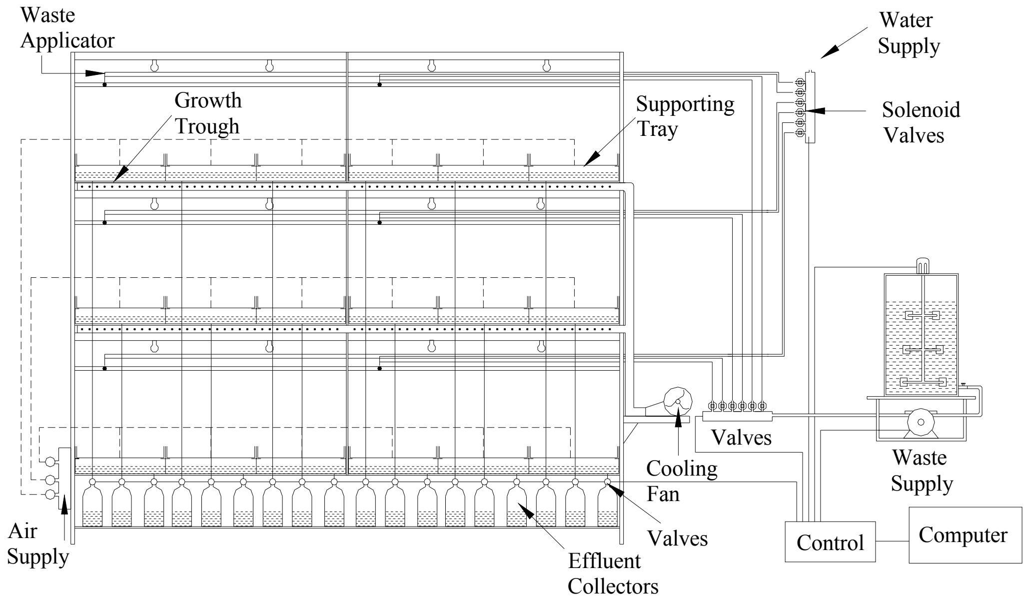

Fig. 1: The hydroponics system

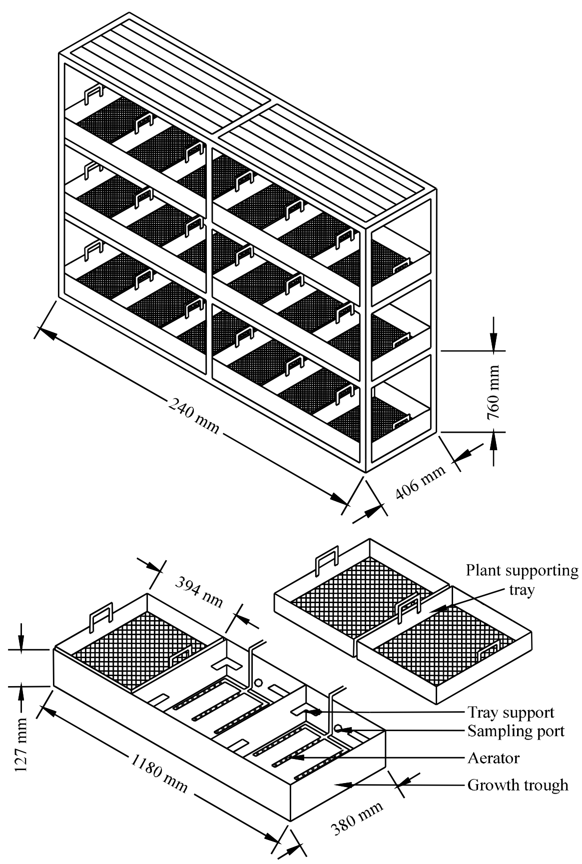

Fig. 2: The frame, growth trough and plant support tray

the mixing shaft and impeller. The wastewater storage tank was connected to the pump using tygon tubing of 3.175 cm outside diameter. A variable speed pump (Model 110-23E, TAT Pumps Inc., Logan, OH) with a capacity of 138 cm^3 revG^1 was used to transfer the wastewater from the storage tank to the irrigation system. The pump was connected to the irrigation system using tygon tubing of 1.905 cm outside diameter. Six valves were used to control the amount of wastewater fed to each growth trough. The timing and duration of opening/closing of the valves were controlled by an electronic circuit. Each wastewater applicator was fabricated from stainless steel pipe with holes punched along the lower edge to allow the wastewater to flow out. The wastewater entered the applicator at the center of the top edge. To overcome the problem of clogging, a water line with six solenoid valves was attached to the applicator and was used to flush out the applicator after feeding periods. The wastewater application system was fully automated and consisted of a motor driven pulley arrangement on each shelf to which the applicator tubes were attached. The motors (Sigma Model 20-3424SG-24007, Faber Industrial Technologies, Clifton, NJ) ran at 6 rpm and were controlled by an electronic circuit. The system was set up so that each applicator traveled 122 cm (3 tray lengths). When a guide on an applicator hit a micro-switch located at each end of the shelf, the motor stopped. After a 3 second delay, the applicator traveled in the opposite direction. This process continued for the designated feeding time which was controlled by computer. Each compartment contained a sampling port located 2.0 cm from the bottom of the trough. Each sampling port was connected to a 2.7 L glass bottle using tygon tubing of 1.27 cm outside diameter and a valve.

A microcontroller (BASIC Stamp 2P24, Parallax, Inc., Rocklin, CA) was used to run the various components of the hydroponics system including the lighting, cooling, irrigation and supernatant collection units. Addressable latches were used to effectively increase the microcontroller's 24 input/output pins to the required number. The microcontroller was programmed using BASIC computer software (BASIC Stamp Windows Editor version 2.2.6, Parallax, Inc., Rocklin, CA). A real time clock (Dallas Semiconductor X1226, Maxim Integrated Products, Inc., Sunnyvale, CA) and a 1-Farad supercapacitor provided nonvolitile timing. A separate program (BASIC Stamp Windows Editor version 2.2.6, Parallax, Inc., Rocklin, CA) was used to set the real time clock.

Table 1: Chemical analysis of aquaculture wastewater

Parameter	Value
Total solids (mg LG^1)	826.67±28.87
Suspended solids (mg LG^1)	103.33±13.63
Total chemical oxygen demand (mg LG^1)	157.97±9.32
Soluble chemical oxygen demand (mg LG^1)	102.34±8.56
Ammonium-Nitrogen (mg LG^1)	2.08±0.50
Nitrite-Nitrogen (mg LG^1)	1.27±0.09
Nitrate-Nitrogen (mg LG^1)	21.64±0.60
Total phosphorus (mg LG^1)	6.30±
Orthophosphate (mg LG^1)	4.49±0.18
Potassium (mg LG^1)	74.67±0.32
Calcium (mg LG^1)	59.90±0.95
Sodium (mg LG^1)	114.67±0.58
Sulfur (mg LG^1)	6.97i±0.12
Chloride (mg LG^1)	86.67±0.58
Magnesium (mg LG^{-1})	5.06±0.07
Manganese (mg LG^1)	0.20±
Iron (mg LG^1)	0.03±0.01
Copper (mg LG^1)	0.06±
Zinc (mg LG^1)	0.20±
pH	7.00±0.13

MATERIALS AND METHODS

Experimental materials: The wheat, barley and oats seeds were purchased from Walker's Livestock, Dartmouth, Nova Scotia. The wastewater used in the study was obtained from an intensive, recirculating aquaculture facility stocked with Arctic charr (*Salvelinus alpinus*) located in Truro, Nova Scotia. The chemical analyses for the aquaculture wastewater are presented in Table 1.

Experimental procedure: A seed quantity of 300 g tray^{-1} provided good cover of the plant support tray and was selected for this study. The wastewater application rate was fixed at 690 L compartmentG^1 dayG^1 and was calculated based on the phosphorus requirements of wheat, barley and oats and on the phosphorus concentration in the aquaculture wastewater. The day length at a latitude of 45°N during the crop growing season (May 1st to Sept 31st) is approximately 14 h. Therefore, the lighting system was programmed to provide a daily photoperiod of 14 hours.

On day 1, the plant support trays were labeled and weighted using an analytical balance (Model PM30, Mettler Instrument Corporation, Hightstown, NJ). The required amounts of seed were also weighed using an analytical balance (Model PM4600, Mettler Instrument Corporation, Hightstown, NJ). Surface sterilization of seeds was performed to limit problems associated with fungal infections observed in previous studies. Each group of seeds was surface sterilized as recommended by Snow and Ghaly[22] by soaking the seeds in 10% bleach for 20 minutes and then rinsing with distilled-deionized water. The seeds were then placed on the trays in the growth troughs. With the valves controlling the sampling ports in the closed position, each growth trough was filled with tap water to a level such that the seeds were in contact with the water, but not submerged. The experiment was conducted in duplicate. The aeration system was turned on and pressure regulators were adjusted to 0.340 atm. Two compartments were utilized as controls and contained wastewater only.

During the germination period (days 2-7), seed germination and seedling height were observed and recorded daily. Tap water was added to each compartment as required to compensate for water losses due to evaporation. Effluent samples were collected from each compartment on day 8 before the addition of wastewater and refrigerated at 4°C in labeled bottles until needed for chemical analyses.

The lighting, cooling and wastewater application systems were activated on day 8. During the growth period (days 8-21), the crop height in each tray was measured and recorded. Effluent samples were collected from each compartment on a daily basis before the addition of wastewater and refrigerated at 4°C in labeled bottles until needed for chemical analyses. The lighting, cooling and wastewater application units were deactivated and the experiment was terminated on day 21. Each tray was removed from its compartment and allowed to dry at room temperature (22°C) for 24 hours. The biomass of each tray was measured and recorded. Crop samples were collected from each tray for nutritional analyses.

Analyses: All effluent samples were analyzed for total solids (TS), total chemical oxygen demand (COD), ammonium-nitrogen (NH_4^+-N), nitrite-nitrogen (NO_2^--N), nitrate-nitrogen (NO_3^--N), phosphate-phosphorus (PO_4^{3-}-P) and pH. The TS, COD, NO_2^--N and PO_4^{3-}-P analyses were performed according to procedures described in Standard Methods for the Examination of Water and Wastewater[23]. The NH_4^+-N measurements were performed using the Kjeltec Auto Analyzer (Model 1030, Tecator, Höganäs, Sweden) according to the Kjeldahl method. The NO_3^--N analysis was performed according to the phenoldisulfonic acid technique described in Methods of Soil Analysis[24]. The pH of the wastewater was measured using a pH meter (Model 805MP, Fisher Scientific, Montreal, QC). Plant tissue analyses (energy, carbohydrates, crude protein, crude fat and crude fiber) were performed at Maxxam Analytics Inc., Mississauga, Ontario according to procedures described in Official Methods of Analysis of AOAC International[25]. The elemental composition (Ca, Cl, Mg, P, K, Na, S, B, Cu, Fe, Mn, Mo, Se and Zn) of the wastewater and plant tissue was determined in the Minerals Engineering Center, Dalhousie University using flame atomic adsorption spectroscopy.

RESULTS AND DISCUSSION

Crop growth and yield: Within 24 h of commencing the experiment, the seeds in all trays began to absorb water and swell. After 2 days, the radicle (part of the plant embryo that develops into a root) and plumule (primary bud of a germinating seed) had broken through the seed coat and were visible on the majority of seeds. During the germination period, the crops in all trays grew rapidly and fairly uniformly and appeared healthy with green color. By the end of the germination period (day 8), the wheat, barley and oats seedlings were approximately 11.0, 14.0 and 11.5 cm in height, respectively. During the growth period, the crops continued to grow rapidly and showed no signs of mineral deficiency, however fungal growth was evident in all trays. At the end of the growth period (day 21), wheat, barley and oats were approximately 19.0, 25.5 and 25.2 cm in height, respectively. The increase in plant height over time is shown in Fig. 3.

Clarkson and Lane[26] evaluated the feasibility of using hydroponically grown barley to reduce the mineral content of wastewater from an aquarium stocked with common carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*) and reported

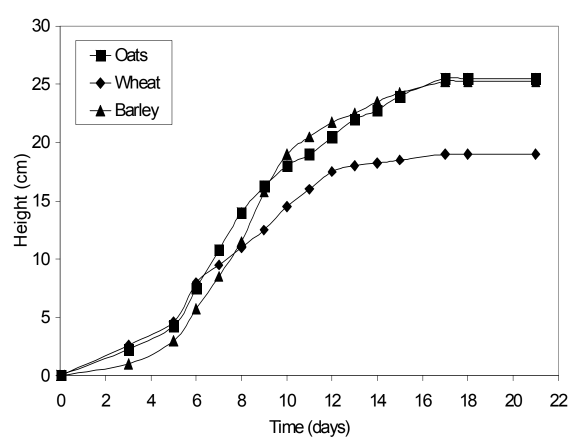

Fig. 3: Average crop height

above ground biomass

root mat

(a) wheat

above ground biomass

root mat

(b) barley

above ground biomass

root mat

(c) oats

Fig. 4: The above ground biomass and root mats of wheat, barley and oats at the end of the growth period

good plant growth with a height reaching 22 cm after 10 days. Kamal and Ghaly[27] evaluated the potential of using hydroponically grown barley and oats for reducing the nutrient content of wastewater from a recirculating aquaculture system stocked with tilapia and reported crop heights of 25 and 26 cm after 21 days. Mackowiak *et al*.[28] used a thin film nutrient delivery system to grow wheat and reported a crop height of 51 cm after 72 days. MacKenzie[29] reported a crop height of 33 cm when barley was hydroponically grown on an anaerobically digested dairy manure for a 21 day period.

Figure 4 shows the above ground biomass and root mats of wheat, barley and oats at the end of the growth period. The average wheat, barley and oats yields at harvest were 945, 883 and 636 g tray^{-1} (64, 59 and 42 t ha^{-1}), respectively. This is within the range reported in the literature for plants grown on aquaculture wastewater.

Bouzoun[30] reported fresh weight yields ranging from 2.949 to 6.609 kg when reed canarygrass was hydroponically grown on domestic wastewater. Pettersen[31] examined the ability of hydroponically grown barley to reduce the nutrient salt content of aquaculture wastewater and reported yields ranging from 1 to 65 t ha^{-1} depending on light intensities and materials used for root support. MacKenzie[29]evaluated the use of hydroponically grown barley to reduce the nutrient content of an anaerobically digested dairy manure and reported a crop yield of 81 t ha^{-1} at a seed quantity of 250 g tray^{-1}. In this study, the growth rates are lower than those reported by other investigators because aquaculture effluents are characteristically high in volume, but low in nutrient content compared to municipal and agricultural wastewaters which are relatively low in volume and high in nutrient content[13].

Effluent quality: During germination, seeds rapidly absorb water from the surrounding environment. The swelling that results from the rapid influx of water leads to rupture of the seed coat and leakage of internal substances from the seed. This rapid leakage of cellular and vacuolar constituents is referred to as seed exudation[32]. Seed exudates generally consist of carbohydrates, amino acids, organic acids, inorganic ions and other miscellaneous compounds all of which alter the quality of the surrounding growth medium[33-34]. Table 2 shows the effluent TS, COD, NH_4^+-N, NO_2^--N, NO_3^--N and PO_4^{3-}-P concentrations at days 8 and 21 of the experiment and the removal efficiencies for each water quality parameter. The effects of crop type on the reductions of these parameters were tested using a one-way analysis of variance (ANOVA) and a Duncan's multiple range test with differences considered significant at the $p \leq 0.05$ level (95% confidence interval) using SPSS (SPSS 14.0.1, SPSS Inc., Chicago, IL).

Total solids: The average total solids (TS) concentration in the aquaculture wastewater was 827±28 mg L^{-1}. Feces, uneaten feed and bacterial biomass are the main sources of TS in aquaculture effluent[35-37]. TS reductions of 27.4, 53.3, 57.7 and 54.0% were achieved at the end of the growth period (day 21) in the controls and the compartments containing wheat, barley and oats, respectively. The TS reductions were significantly influenced by the presence of the crops as compared to the control, but were not significantly influenced by the crop type (Table 2).

Ghaly *et al*.[38] evaluated the use of hydroponically grown barley and oats to reduce the TS concentration in wastewater from a recirculating aquaculture system stocked with tilapia and reported reductions ranging from 85.0 to 91.0% and from 75.5 to 84.5% for compartments containing barley and oats, respectively. MacKenzie[29] evaluated the ability of hydroponically grown wheat to reduce the TS concentration in an anaerobically digested dairy manure and reported reductions ranging from 76.5 to 81.4%. Bouzoun[30] utilized a modified hydroponics system (nutrient film technique) planted with reed canarygrass for the treatment of municipal wastewater over a 5 month period at a flow rate of 3.2 L min^{-1} and reported a TSS reduction of 75%. Rababah and Ashbolt[39] evaluated the feasibility of using hydroponically grown lettuce for nutrient removal from primary treated municipal wastewater and at an irrigation rate of 8 L min^{-1} and reported a 99% reduction in suspended solids over a 7 day period.

Levels of TS in aquaculture wastewaters must be limited for several reasons. Effluents containing high concentrations of suspended solids may form a plume of discolored water in the discharge area reducing light penetration, phytoplankton productivity and feed uptake by visual feeders[40]. Excessive sedimentation can abrade or cover respiratory surfaces (gills) of aquatic organisms, offer a suitable habitat for the proliferation of pathogenic organisms, smother eggs and larvae and bury and smother communities of benthic organisms reducing the biodiversity of the ecosystem[36-37]. According to Lawson[41] and Meade[42], waters used for the culture of aquatic organisms should contain less than 480 mg L^{-1} total solids (80 and

400 mg L^{-1} of total suspended and total dissolved solids, respectively). On the basis of TS, only the compartments containing barley produced effluents suitable for reuse in aquaculture.

Chemical oxygen demand: The aquaculture wastewater had an average Chemical Oxygen Demand (COD) concentration of 158±9.32 mg L^{-1}. Uneaten or regurgitated food and fecal production are the major sources of organic matter in aquaculture effluents[40, 43]. COD reductions of 27.6, 55.7, 78.7 and 70.4% were achieved at the end of the growth period (day 21) in the controls and the compartments containing wheat, barley and oats, respectively. The COD reductions were significantly influenced by the presence of the crops. Barley achieved the highest reductions followed by oats and wheat (Table 2). The difference between the barley and oats was not significant.

Ghaly *et al*.[38] evaluated the ability of hydroponically grown barley and oats for nutrient reduction from aquaculture wastewater and reported COD reductions of 70.2, 79.7 and 85.9% and 64.4, 73.6 and 79.8% after 21 days in compartments containing 200, 250 and 300 g of barley and oats, respectively. MacKenzie[29] examined the feasibility of using wheat in a hydroponics system for the treatment of an anaerobically digested dairy manure and reported COD removal efficiencies ranging from 89.9 to 92.3%, from 89.1 to 89.2% and from 81.9 to 85.0% after 21 days of plant growth at wastewater application rates of 300, 600 and 900 mL compartment^{-1} day^{-1}, respectively. Gloger *et al*.[44] evaluated the contribution of lettuce to wastewater treatment in a recirculating aquaculture system stocked with tilapia (*Oreochromis niloticus*) and reported that the COD removal rate was 54% higher than that of systems containing no plants. Vaillant *et al*.[45] evaluated the use of the nutrient film technique for pollutant removal from domestic wastewater and reported COD removal efficiencies of 90 and 45% in planted and unplanted channels, respectively.

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 column. Oxygen depletion, formation of anaerobic bacterial mats and production of ammonia, hydrogen sulfide and methane gases are problems which may arise when oxygen demand exceeds its supply. These gases are highly toxic to aquatic organisms[46-49]. Limits for COD concentrations in waters used for the culture of aquatic organisms have not been defined.

Ammonium-nitrogen: The aquaculture wastewater contained 2.08±0.5 mg L^{-1} ammonium-nitrogen (NH_4^+-N). In fish and shellfish, ammonia is the major nitrogenous waste product of protein catabolism and it is excreted primarily in un-ionized form (NH_3) through the gills[50-51]. Ammonium is also produced through the microbial decomposition of fish feces and uneaten food in a process called ammonification.

$$\text{Organic} - \text{N} \rightarrow NH_4^+ \qquad (1)$$

Ammonification refers to a series of biological transformations that convert organically bound nitrogen to ammonium-nitrogen under both aerobic and anaerobic conditions. The reactions involved in the decomposition release energy which can then be utilized by the microorganisms for growth and reproduction or to sustain metabolic functions[52]. Heterotrophic microorganisms responsible for ammonification belong to the genera *Pseudomonas*, *Vibrio*, *Proteus*, *Serratia*, *Bacillus* and *Clostridium*. NH_4^+-N reductions of 76.0, 80.0, 76.0 and 76.0% were achieved at the end of the growth period (day 21) in the controls and the compartments containing wheat, barley and oats, respectively. The NH_4^+-N reductions were not significantly influenced by the presence or type of crop (Table 2).

Bouzoun[30] evaluated the feasibility of utilizing hydroponically grown reed carnarygrass 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*.[45] evaluated the effectiveness of *Datura innoxia* plants for domestic wastewater purification and reported NH_4^+-N reductions in the effluent of 93% after 48 hours of treatment. MacKenzie[29] examined the use of a hydroponics system planted with wheat for nutrient removal from an anaerobically digested dairy manure and reported NH_4^+-N reductions ranging from 80.4 to 85.8%, from 64.5 to 72.0% and from 57.4 to 69.8% after 21 days of growth for wastewater applications rates of 300, 600 and 900 mL compartment^{-1} day^{-1}, respectively.

Accumulation of ammonia in water is one of the major causes of functional and structural disorders in aquatic organisms[53-54]. Only unionized ammonia is

Table 2: Water quality parameters

Parameter	Crop	Influent[a] (mg LG^1) Released substances	Influent[a] (mg LG^1) Total	Effluent[b] (mg L$^{-1)}$	Reduction (mg LG^1)	Reduction (%)	Duncan subsets (α = 0.05)
TS	Control	-	827±29	600±14	227	27.4	1
	Wheat	204±74	1031±79	481±35	550	53.3	2
	Barley	218±46	1045±54	442±38	603	57.7	2
	Oats	163±54	990±61	455±31	535	54.0	2
COD	Control	-	158±9	114±5	44	27.6	1
	Wheat	164±33	332±34	147±41	185	55.7	2
	Barley	142±36	300±37	64±24	236	78.7	3
	Oats	109±40	267±41	79±26	188	70.4	3
NH_4^+-N	Control	-	2.08±0.50	0.50±0.71	1.58	76.0	1
	Wheat	0.42±0.35	2.50±0.61	< 0.50	2.00	80.0	1
	Barley	0.00	2.08±0.50	< 0.50	1.58	76.0	1
	Oats	0.00	2.08±0.50	< 0.50	1.58	76.0	1
NO_2^--N	Control	0.00	1.27±0.09	1.16±0.05	0.11	8.7	1
	Wheat	0.00	1.27±0.09	0.09±0.04	1.18	92.9	2, 3
	Barley	0.00	1.27±0.09	0.02±0.01	1.25	98.4	3
	Oats	0.00	1.27±0.09	0.19±0.17	1.08	85.1	2
NO_3^--N	Control	-	21.64±0.60	7.92±0.44	13.72	63.5	1
	Wheat	6.95±0.91	28.59±1.09	10.42±0.99	18.17	63.6	1
	Barley	6.77±0.47	28.41±0.76	5.89±0.58	22.52	79.3	2
	Oats	6.77±0.38	28.41±0.71	10.76±2.13	17.65	62.1	1
PO_4^{3-}-P	Control	-	4.49±0.18	2.95±0.25	1.54	34.3	1
	Wheat	0.66±0.25	5.15±0.31	0.74±0.26	4.41	85.6	3
	Barley	4.21±0.74	8.70±0.76	0.61±0.22	8.09	93.0	3
	Oats	0.65±0.30	5.14±0.35	1.33±0.32	3.81	74.1	2

[a] day 8, [b] day 21, Treatments with different numbers are significantly different at the $p \leq 0.05$ level

Influent TS = 827±29 mg LG^1
Influent COD = 158±9 mg LG^1
Influent NH_4^+-N = 2.08±0.50 mg LG^1
Influent NO_2^--N = 1.27±0.09 mg LG^1
Influent NO_3^--N = 21.64±0.60 mg LG^1
Influent PO_4^{3-}-P = 4.49±0.18 mg LG^1

toxic to fish because it can readily diffuse across the gill membranes into the circulation, whereas the ionized form (NH_4^+) cannot[51, 54]. The NH_3-N concentrations in the final effluents were 0.003, 0.001, 0.009 and 0.002 mg LG^1 in the controls and in the compartments containing wheat, barley and oats, respectively. Lawson[41] and Meade[42] recommend that ammonia concentrations do not exceed 0.02 mg LG^1 in water used for culture of aquatic animals. Waters suitable for reuse in aquaculture were produced.

Nitrite-nitrogen: The aquaculture wastewater had an average nitrite-nitrogen (NO_2G-N) concentration of 1.27±0.09 mg LG^1. In natural waters, ammonium is converted rather rapidly to nitrite (NO_2G) and further to nitrate (NO_3G) by aerobic bacteria from the genera *Nitrosomonas* and *Nitrobacter*, through a process called nitrification[55-56].

$$2NH_3 + 3O_2 \div 2NO_2G + 2H + 2H_2O \qquad (2)$$

$$2NO_2G + O_2 \div 2NO_3G \qquad (3)$$

Nitrification was facilitated by the continuous aeration of the system compartments during the experiments. Princic *et al*.[57] reported that the optimum pH range for conversion of NH_4^+ to nitrite (NO_2G) is between 5.8 and 8.5. The pH of the water in all experiments was within this range. NO_2^--N reductions of 8.7, 92.9, 98.4 and 85.1% were achieved at the end of the growth period (day 21) in the controls and the compartments containing wheat, barley and oats, respectively. The NO_2G-N reductions were significantly influenced by the presence of the crops. Barley achieved the highest NO_2^--N reductions followed by wheat and oats (Table 2).

Ghaly *et al*.[38] examined the use of a hydroponics system for treatment of wastewater from a recirculating aquaculture system stocked with tilapia. The hydroponics troughs were planted with barley and oats and received wastewater at application rates of 525 and 412 mL compartment^{-1} day^{-1}, respectively. The experiment was conducted for 21 days at which time the researchers reported NO_2^--N removal efficiencies of 98.1 and 96.7% for compartments containing barley and oats, respectively.

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[55,58]. Nitrite toxicity is associated with its ability to diffuse across the gills and into the blood circulation. When nitrite is absorbed by aquatic animals, the iron (or copper) in haemoglobin (haemocyanin) is oxidized from the ferrous (or cuprous) to the ferric (or cupric) state. The resulting product is called methaemoglobin (methaemocyanin) and it is unable to bind and transport oxygen[41]. The average NO_2^-N concentrations in the final effluents from the hydroponics system were 1.16 and 0.09, 0.02 and 0.19 mg L^{-1} in the controls and the compartments containing wheat, barley and oats, respectively. Poxton[51] recommends a NO_2^--N concentration less than 0.02 mg L^{-1} in water used for the culture of most freshwater fish. The compartments containing barley produced effluents that just meet water quality guidelines.

Nitrate-nitrogen: The aquaculture wastewater had an average nitrate-nitrogen (NO_3^--N) concentration of 21.64±0.60 mg L^{-1}. NO_3^--N accumulates in aquaculture systems as a result of nitrification[55,56]. NO_3^--N reductions of 63.5, 63.3, 79.3 and 62.1% were achieved in the controls and the compartments containing wheat, barley and oats, respectively. The NO_3^-N reductions were significantly influenced by the presence of barley (Table 2).

Ghaly *et al*.[38] investigated the possibility of using hydroponically grown barley and oats in the treatment of aquaculture wastewater and reported NO_3^--N reductions of 68.8-76.7% and 68.4-75.1% for barley and oats after 14 days of plant growth, respectively. Clarkson and Lane[26] 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*) over a four-week period and reported that the NO_3^--N concentration in the effluent was reduced from 33.03 to 3.03 mg L^{-1} using barley. Lewis *et al*.[59] evaluated the use of tomato (*Lycopericon esculentum*) hydroponics as a means of preventing the accumulation of NO_3^--N in effluent from a recirculating aquaculture system stocked with channel catfish (*Ictalurus punctatus*) and reported a NO_3^--N concentration in the effluent in the range of 4.99-5.45 mg L^{-1}.

NO_3^--N is not acutely toxic to fish. However, it should not be allowed to accumulate in aquaculture systems because chronic toxicity symptoms and algae and phytoplankton blooms may eventually develop[51,58]. Chronic toxicity symptoms associated with exposure to nitrate include: reduction in the oxygen carrying capacity of the blood, inability of organisms to maintain proper balance of salts, stunted growth and lethargy[60]. The average NO_3^--N concentrations in the final effluents from the hydroponics system were 7.92 and 10.42, 5.89 and 10.76 mg L^{-1} in the controls and the compartments containing wheat, barley and oats, respectively. Poxton[51] recommended that NO_3^--N concentrations do not exceed 50 mg L^{-1} in waters used for the culture of fish and shellfish. Waters suitable for reuse in aquaculture were produced.

Phosphate-phosphorus: The aquaculture wastewater contained 4.49±0.18 mg L^{-1} phosphate-phosphorus (PO_4^{3-}-P). Phosphorus occurs in aquaculture wastewater primarily as soluble and insoluble phosphates in both organic and inorganic forms[52]. The main inorganic form is soluble orthophosphate, which exists in different states ($H_2PO_4^-$, HPO_4^{2-} and PO_4^{3-}) depending on the pH of the medium[61]. PO_4^{3-}-P reductions of 34.3, 85.6, 93.0 and 74.1% were achieved in the controls and the compartments containing wheat, barley and oats, respectively. The PO_4^{3-}-P reductions were significantly influenced by the presence of the crops. Barley achieved the highest PO_4^{3-}-P reductions followed by wheat and oats (Table 2).

Ghaly *et al*.[38] examined the use of a hydroponically grown barley and oats for removal of PO_4^{3-}-P from aquaculture wastewater and reported PO_4^{3-}-P reductions ranging from 91.8 to 93.6% and from 91.4 to 92.3% for compartments containing barley and oats after 21 days, respectively. Clarkson and Lane[26] evaluated the use of the nutrient film technique for PO_4^{3-}-P removal from aquarium wastewater and reported that the PO_4^{3-}-P concentration in the effluent was reduced from 4.4 to 0.3 mg L^{-1} after four weeks using barley. Lewis *et al*.[59] evaluated the feasibility of utilizing hydroponically grown tomatoes (*Lycopericon esculentum*) as a means of preventing the accumulation of PO_4^{3}G-P in effluent from a recirculating aquaculture system stocked with channel catfish (*Ictalurus punctatus*) and reported TP and PO_4^{3}G-P in the effluent of 2.5-27 and 1.13-1.30 mg LG^{1}, respectively. Bouzoun[30] used a nutrient film system for treatment of primary domestic effluent and reported a PO_4^{3}G-P reduction of 10.9% over a 3 month period.

The average PO_4^{3}G-P concentrations in the final effluents from the hydroponics system were 2.95, 0.74, 0.61 and 1.33 mg LG^{1} in the controls and the compartments containing wheat, barley and oats, respectively. Toxicity from high levels of phosphorus has not been reported by aquaculturists[53].

pH: The aquaculture wastewater had an average pH of 7.00±0.13. At the end of the growth period, the average pH of the final effluents was 7.15±0.10, 6.89±0.28, 6.65±0.03 and 7.00±0.12 in the controls and the compartments containing wheat, barley and oats, respectively. In hydroponics systems, fluctuations in the pH of the growth medium are caused by the uptake of cations and anions by the root systems of the developing plants. When cations are taken up more rapidly than anions, the roots will release hydrogen ions into solution and the pH of the medium falls. When anions are taken up more rapidly than cations, the roots release bicarbonate and hydroxyl ions into solution and the pH of the medium rises[62-64].

According to Lawson[41] and Meade[42], the pH of waters used for the culture of fish and shellfish should range from 6.5 to 8.0. When the pH of the growth medium rises above 9.0, it begins to adversely affect most aquatic species and a pH in the range of 11.0-11.5 is lethal to all species of fish[55]. When pH falls within the range of 5.0-6.0, rainbow trout, salmonids and molluscs become rare, the rate of organic matter decomposition declines because the fungi and bacteria responsible for degradation are not acid tolerant and most green algae, diatoms, snails and phytoplankton disappear[51]. Most fish eggs will not hatch when the pH of the surrounding environment reaches 5.0. Changes in water chemistry may also occur as a result of a decrease in pH[55]. Waters suitable for reuse in an aquaculture facility were produced.

Nutritive value: Six major components were considered when analyzing the wastewater grown wheat, barley and oats as potential fish feed: energy, carbohydrates, crude protein, crude fat, macroelements and microelements. Table 3 displays a comparison between the nutritional composition of the wastewater grown barley and the nutritional requirements of aquatic animals.

The three terrestrial crops meet the energy, fat, Ca, Mg, P, Na, S and Mn dietary requirements of aquatic animals, exceed the carbohydrate, crude fiber, Cl, K, Cu, Fe, Se and Zn requirements of fish and shellfish and do not contain sufficient amounts of protein to meet the dietary requirements of fish and shellfish. There was no evidence in the literature to suggest that the chlorine concentrations observed in the wastewater grown plants would be detrimental to the healthy development of fish and shellfish[65-67]. However, studies have shown that excess dietary K, Cu, Fe, Mn, Se and Zn can cause depressed growth, reduced feed intake, reduced weight gain and nutrient utilization efficiency and reduced body fat and protein deposition in certain species of finfish[68-73]. Since the crops used in the study do not meet the protein requirements of aquatic animals, a protein supplement must be added. Fishmeal is one of the major ingredients in fish feed and is the most common protein source. Other common protein sources include meat and bone meal[74]. Either of these could be used to supplement the crop with protein at the required amount.

Table 3: A comparison between the nutritional composition of wastewater grown terrestrial plants and the nutrient requirements of aquatic organisms

	Terrestrial plants			
Parameter	Wheat	Barley	Oats	Fish Feed[74-82]
Energy (MJ kgG^{1})	16.20	16.00	14.40	12-23
Nutrients (% dm)				
Carbohydrates	63.30	59.80	58.70	10-30
Fiber	29.31	27.17	25.13	1-12
Proteins	21.07	16.13	20.35	32-52
Fats	5.53	4.40	7.25	4-28
Macroelements (%)				
Calcium	0.29±0.04	0.60±0.01	0.56±0.04	0.03-2.90
Chlorine	1.16	1.96	1.25	0.10-0.50
Magnesium	0.23±0.00	0.29±0.00	0.28±0.02	0.04-0.30
Phosphorus	0.75±0.02	0.84±0.02	0.87±0.05	0.45-2.20
Potassium	2.19±0.03	3.80±0.01	2.89±0.06	0.50-1.50
Sodium	0.17±0.03	1.34±0.01	0.77±0.00	0.10-2.30
Sulfur	0.32±0.01	0.32±0.02	0.31±0.02	0.30-1.70
Microelements (mg kg^{-1})				
Boron	8±3	13±2	12±1	
Copper	68±25	117±62	15±0.5	3-10
Iron	1349±73	1679±1255	3182±371	30-170
Manganese	50±3	121±7	77±7	2.4-120
Molybdenum	2±0.45	1±0.74	5±0.96	
Selenium	2.1	1.2	1.6	0.15-0.40
Zinc	741±212	3557±765	1146±52	15-240

These findings are comparable to those reported by other investigators for hydroponically grown crops on nutrient solutions. Sneath and McIntosh[83] evaluated the composition of barley grass and reported that the energy, carbohydrates, crude protein, crude fiber, crude fat, Ca, P, S, K, Na, Mg, Fe, Zn, Mn, Cu and Se ranged from 8.7 to 12 MJ kgG^{1}, 61.3 to 68.85, 11.38 to 24.9, 7.35 to 15.2, 3.18 to 9.27, 0.07 to 0.13, 0.30 to 0.31, 0.16 to 0.22, 0.48 to 0.60, 0.03 to 0.21 and 0.12 to 0.40% and 81 to 168, 21 to 34, 21 to 27, 6 to 11 and 0.9 mg kg^{-1}, respectively. Mackowiak *et al.*[28] evaluated the composition of wheat and reported energy, protein, fat, crude fiber, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn and Mo concentrations ranging from 9.54 to 10.84 MJ kg^{-1}, from 20.5 to 26.5, from 3.4 to 6.9, from 17.5 to 24.8, from 0.74 to 1.14, from 5.21 to 5.24, from 0.60 to 0.69, from 0.22 to 0.23 and from 0.27 to 0.36% and from 147 to 1624, from 58 to 108, from 10 to 13, from 16 to 17 and from 0.77 to 0.89 mg kg^{-1}, respectively. McKeehen *et al.*[84] evaluated the composition of wheat and reported protein, fat, carbohydrates, Na, K, P, Mg, Ca, Mo, Zn, B, Mn, Fe and Cu contents in the ranges of 13.2-23.3, 1.0-1.7 and 58.9-73.9, 0.008-0.0099, 4.11-6.70, 0.11-0.35, 0.09-0.31 and 0.44-0.53% and 3, 8-14, 23-93, 15-58, 79-162 and 5-8 mg kg^{-1}, respectively. Steinberg *et al.*[85] evaluated the composition of wheat and reported that after 22 days of growth the concentration of nutrients in terms of N, P, K, Ca, Mg, S, Fe, B, Mn, Zn, Cu and Mo were 51, 6.2, 27.8, 11.5, 4.5 and 6.3 g kg^{-1} and 176, 80, 123, 37 19 and 18 mg kg^{-1}, respectively.

CONCLUSIONS

During the experiment, the crops grew rapidly and fairly uniformly and showed no signs of mineral deficiency although fungal growth was evident. The average crop heights and yields at harvest were 19.0, 25.5 and 25.2 cm and 64, 59 and 42 t ha^{-1} for wheat, barley and oats, respectively. The hydroponically grown wheat, barley and oats were able to significantly reduce the pollution load of the aquaculture wastewater. The TS, COD, NH_4^+-N, NO_2^--N, NO_3^--N and PO_4^{3-}-P reductions ranged from 53.3 to 57.7%, from 55.7 to 78.7%, from 76.0 to 80.0% from 85.1 to 92.9%, from 62.1 to 79.3% and from 74.1 to 93.0%, respectively. The compartments containing barley produced the highest quality effluent, which was suitable for reuse in aquaculture. The average TS, COD, NH_4^+-N, NO_2^--N, NO_3^--N and PO_4^{3-}-P concentrations and pH of the final effluent from the compartments containing barley were 442, 64, 0.50, 0.02, 5.89 and 0.61 mg L^{-1} and 6.65, respectively. The three terrestrial crops meet the energy, fat, Ca, Mg, P, Na, S and Mn dietary requirements of aquatic animals, exceed the carbohydrate, crude fiber, Cl, K, Cu, Fe, Se and Zn requirements of fish and shellfish and do not contain sufficient amounts of protein to meet the dietary requirements of fish and shellfish. The crops will require supplementation with a high protein source that contains low concentrations of carbohydrates, crude fiber, Cl, K, Cu, Fe, Se and Zn. Common protein sources that could be used for supplementation included fishmeal, bone meal and blood meal.

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