

Use of Barley for the Purification of Aquaculture Wastewater in a Hydroponics System

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Abstract: Barley was examined for its ability to remove nutrients from aquaculture wastewater. The effects of seed sterilization using ethanol and bleach and seed density on germination and plant growth were investigated. Surface sterilization of barley seeds had a negative impact on germination. Increasing the ethanol concentration and/or the bleach concentration reduced the germination percentage. Barley seeds were first germinated in water in the 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 or disease. The average crop height at harvest was 25.5 cm and the yield varied from 25 to 59 t ha⁻¹, depending on the seed density. The hydroponically grown barley was able to significantly reduce the pollution load of the aquaculture wastewater. The TS, COD, NH₄⁺-N, NO₂⁻-N, NO₃⁻-N, and PO₄³⁻-P reductions ranged from 52.7 to 60.5%, from 72.9 to 83.1%, from 76.0 to 76.0%, from 97.6 to 99.2%, from 76.9 to 81.6% and from 87.1 to 95.1%, respectively. However, the effluent produced from the hydroponics system had slightly higher levels of TS (420-485 mg L⁻¹) than the 480 mg L⁻¹ recommended for aquatic animals. A sedimentation/filtration unit should be added to the hydroponics system.

Key words: Aquaculture, wastewater, hydroponics, barley, sterilization, forage, pollution

INTRODUCTION

Aquaculture, the controlled cultivation and harvest of aquatic plants or animals, places great demands on water resources. The industry typically requires anywhere from 200-600 m³ of water for every kilogram of fish produced^[1]. Although some aquaculture systems (raceways and pond culture) are much more water consumptive than others (recirculating systems), the industry generally requires more water per unit area or per unit of product than most other plant or animal production systems^[2]. Consequently, aquaculture operations produce large quantities of effluent containing particulate and dissolved organic matter and nutrients. 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 waste, fish excretion, fecal production and respiration^[3,4].

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^[5,6]. McIntosh and

Fitzsimmons^[7] reported mean effluent concentrations from an inland, low-salinity shrimp farm in terms of TN, NH₃-N, NO₂-N, NO₃-N, TP, reactive phosphorus, alkalinity, COD, BOD, TSS and VSS of 9, 0.17, 0.261, 9.8, 0.74, 0.33, 96, 23, 6.40, 46.8, 24.1 mg L⁻¹, respectively. Tovar *et al.*^[8] reported that for each tonne of gilthead seabream (*Sparus aurata*) produced, 9104.57 kg of TSS, 843.20 kg of POM, 235.40 kg of BOD, 36.41 kg of NH₄-N, 4.95 kg of NO₂-N, 6.37 kg of NO₃-N and 2.57 kg of PO₄-P were released to the environment.

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^[9-13]. 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^[14].

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The primary aim of this study was to evaluate the feasibility of using barley plants to purify the wastewater from an aquaculture operation. The specific objectives were to evaluate: (a) the effectiveness of surface sterilization on barley seed germination percentages, (b) the effect of seed quantity on plant height and yield, (c) the effectiveness of barley plants in reducing the pollution load of the aquaculture wastewater as measured by TS, COD, $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$, $\text{NO}_3^-\text{-N}$, $\text{PO}_4^{3-}\text{-P}$ and pH and (d) the suitability of recycling the treated wastewater for fish culture.

EXPERIMENTAL APPARATUS

The hydroponics system (Fig. 1) consisted of a frame, growth troughs and aeration, lighting, 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 PVC 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

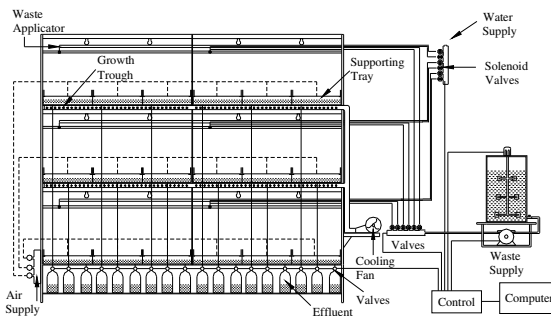


Fig. 1: The hydroponics system

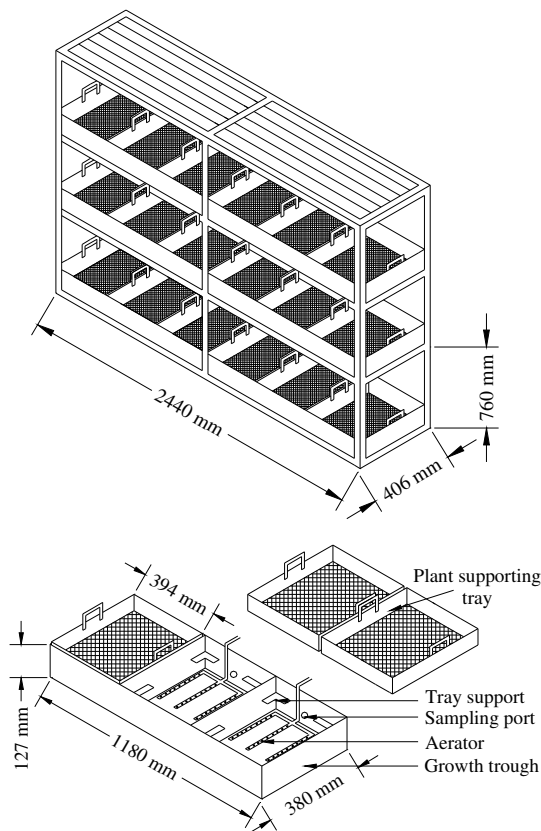


Fig. 2: The frame, growth trough and plant support tray

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 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 $138 \text{ cm}^3 \text{ rev}^{-1}$ was used to transfer the wastewater from the storage tank to the irrigation system. The pump was connected to the irrigation system using PVC 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 PVC 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 nonvolatile timing. A separate program (BASIC Stamp Windows Editor version 2.2.6, Parallax, Inc., Rocklin, CA) was used to set the real time clock.

MATERIALS AND METHODS

Experimental Materials: The barley 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*). The chemical analyses for the aquaculture wastewater are presented in Table 1.

Seed Sterilization: Germination experiments were conducted to determine the effects of surface sterilization on the germination of barley seeds. In this study, germination refers to emergence of the radicle (root) through the seed coat. Surface sterilizations were performed to limit problems associated with fungal infections observed in previous studies. Eight chemical treatments were investigated: 50% ethanol, 70% ethanol, 10% commercial bleach, 20% commercial bleach and combinations of 50% ethanol followed by 10% commercial bleach, 50% ethanol followed by 20% commercial bleach, 70% ethanol followed by 10% commercial bleach and 70% ethanol followed by 20% commercial bleach. Each treatment consisted of 5 replicates.

Surface sterilization was performed by soaking 100

Table 1: Chemical analysis of aquaculture wastewater

Parameter	Concentration
Total solids (mg L ⁻¹)	827 ± 28
Suspended solids (mg L ⁻¹)	104 ± 13
Total chemical oxygen demand (mg L ⁻¹)	157.97 ± 9.32
Soluble chemical oxygen demand (mg L ⁻¹)	102.34 ± 8.56
Ammonium-Nitrogen (mg L ⁻¹)	2.08 ± 0.5
Nitrite-Nitrogen (mg L ⁻¹)	1.27 ± 0.09
Nitrate-Nitrogen (mg L ⁻¹)	21.64 ± 0.60
Total phosphorus (mg L ⁻¹)	6.30
Orthophosphate (mg L ⁻¹)	4.49 ± 0.18
Potassium (mg L ⁻¹)	74.67 ± 0.32
Calcium (mg L ⁻¹)	59.90 ± 0.95
Sodium (mg L ⁻¹)	114.67 ± 0.58
Sulfur (mg L ⁻¹)	6.97 ± 0.12
Chloride (mg L ⁻¹)	86.67 ± 0.58
Magnesium (mg L ⁻¹)	5.06 ± 0.07
Manganese (mg L ⁻¹)	0.2
Iron (mg L ⁻¹)	0.03 ± 0.01
Copper (mg L ⁻¹)	0.06
Zinc (mg L ⁻¹)	0.2
pH	7.00 ± 0.13

seeds in either ethanol or bleach for 1 or 20 min, respectively and then rinsed with distilled-deionized water. Seeds subject to a combination of chemical treatments were initially washed in ethanol for 1 minute and rinsed with distilled-deionized water then washed in bleach for 20 min and rinsed with distilled-deionized water. A control group of seeds was soaked in distilled-deionized water for 20 min. After sterilization, seeds were placed in five 9-cm Petri dishes on filter papers, each was moistened with 3 mL of distilled-deionized water. Each Petri dish contained 20 seeds and was sealed with a strip of parafilm to prevent evaporation. Seeds were germinated in the dark for 7 days at room temperature (22°C). The number of seeds which had germinated was recorded on days 1, 3, 5 and 7. At the completion of the test, the germination percentage for each crop was calculated as the average of five replicates.

Plant Growth: Approximately 250 g of seed was required to completely cover the surface of one tray. Therefore, three different quantities of seeds were tested (200, 250 and 300 g) to determine the effect of seed density on plant growth and nutrient uptake. Two hundred grams of seed was tested to determine if reducing the seed density had a detrimental effect on nutrient uptake and the filtering capacity of the root mat. Three hundred grams of seed was tested to determine if increasing the seed density would adversely affect crop growth. The wastewater application rate was fixed at 690 mL/compartments/day and was calculated based on the phosphorus requirement of barley 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 hrs. Therefore, the lighting system was programmed to provide a daily photoperiod of 14 hrs.

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). Each group of seeds was surface sterilized by soaking the seeds in 10% bleach for 20 min 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 aeration system was turned on and pressure regulators were adjusted to 0.340 atm. Two compartments were utilized as controls and contained wastewater only. The experiment was conducted in duplicate.

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 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.

During the growth period (days 8-21), the crop height in each tray was measured and recorded. The lighting, cooling and wastewater application systems were activated. 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 activated. 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 hrs. The biomass of each tray was measured and recorded.

Analyses: All effluent samples were analyzed for the following parameters: total solids (TS), total chemical oxygen demand (COD), ammonium-nitrogen (NH₄⁺-N), nitrite-nitrogen (NO₂⁻-N), nitrate-nitrogen (NO₃⁻-N), phosphate-phosphorus (PO₄³⁻-P) and pH. The TS, COD, NO₂⁻-N and PO₄³⁻-P analyses were performed according to procedures described in Standard Methods for the Examination of Water and

Wastewater^[15]. The NH_4^+ -N measurements were performed using the Kjeltac 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^[16]. The pH of the wastewater was measured using a pH meter (Model 805MP, Fisher Scientific, Montreal, QC).

RESULTS AND DISCUSSION

Seed Sterilization: Table 2 shows the average germination percentages for barley seeds following the various surface sterilization treatments. The effects of surface sterilization on seed germination percentage at day 7 were tested using a two-sample t-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). The results are presented in Table 3. The germination percentage of bleach (10%) treated barley seeds was not significantly different ($p = 0.100$) from that of the control. However, all other surface sterilization treatments adversely impacted barley seed germination ($p = 0.000$).

MacKenzie^[17] reported a germination percentage of 73% for barley seeds placed on moist paper towels in a germination chamber and incubated at 20°C for 7 days. Ramakrishna *et al.*^[18] evaluated the effectiveness of ethanol and sodium hypochlorite (NaOCl) in killing internal and surface contaminating microorganisms in barley seeds as well as on seed germination. In the experiment, 100 seeds were immersed in 95% ethanol for 40-50 seconds and then in 12.5, 25 or 50% NaOCl solutions for 5, 15 or 30 min. The researchers reported germination percentages of 60-80% for untreated seeds and noticed a decrease in seed germination associated with the increase in NaOCl contact time. Seed germination was not significantly affected by surface sterilization at a contact time of 5 min but was reduced by 61-68% when the contact time was increased to 30 min.

Plant Growth: Within 48 hrs of beginning the experiment, the seeds in all trays began to absorb water and swell. After 2 days, the radicles (part of the plant embryo that develops into a root) had broken through the seed coats and were visible on 55-60% of the seeds. By day 3, the plumules (primary bud of a germinating seed) and root mats had started to develop. During the germination period, the barley seedlings in all compartments grew rapidly and fairly uniformly and appeared healthy with green color. At the end of the germination period (day 8) the plants were

approximately 14.0 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 8-21). At the end of the growth period, the plants reached an average height of 25.5 cm (Fig. 3). The seed quantity (200, 250 and 300 g tray⁻¹) did not have an effect on crop height.

Clarkson and Lane^[10] 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 good plant growth with a height reaching 20-22 cm in 6-10 days. Kamal and Ghaly^[19] evaluated the potential of using hydroponically grown barley for reducing the nutrient content of wastewater from a recirculating aquaculture system stocked with tilapia. The researchers reported a crop height of 25-26 cm after 21 days and found that seed quantity (200, 250 and 300g) did not have any effect on crop height. MacKenzie^[17] reported a crop height of 33.0 cm when barley was hydroponically grown on an anaerobically digested dairy manure for a 21 day period.

Crop Yield: Figure 4 shows the above ground biomass and root mats of barley at the end of the growth period. The average crop yields at harvest ranged from 25 to 59 t ha⁻¹ depending on the seed quantity. The effects of seed quantity on crop yield at harvest (day 21) 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). The crop yield was significantly influenced ($p = 0.027$) by the seed quantity (Table 4).

MacKenzie^[17] 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. Pettersen^[20] examined the ability of hydroponically grown barley to reduce the nutrient salt content of aquaculture wastewater. The researcher reported barley yields ranging from 1 to 65 t ha⁻¹ depending on light intensities and materials used for root support.

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^[21]. Seed exudates generally consist of

Table 2: Average germination percentages for barley seeds following surface sterilization

Chemical Treatment	Germination (%)			
	day 1	day 3	day 5	day 7
control	9 ± 2	18 ± 6	35 ± 3	60 ± 2
ethanol (50%)	7 ± 6	11 ± 4	22 ± 3	35 ± 3
ethanol (70%)	7 ± 4	10 ± 5	19 ± 1	30 ± 2
bleach (10%)	15 ± 6	27 ± 12	33 ± 12	57 ± 3
bleach (20%)	4 ± 4	17 ± 9	24 ± 11	50 ± 2
ethanol/bleach (50% / 10%)	5 ± 6	17 ± 4	23 ± 2	33 ± 4
ethanol/bleach (50% / 20%)	2 ± 3	15 ± 8	20 ± 3	26 ± 2
ethanol/bleach (70% / 10%)	3 ± 1	11 ± 3	22 ± 3	30 ± 2
ethanol/bleach (70% / 20%)	4 ± 3	11 ± 8	20 ± 5	25 ± 2

These values are the average of 5 replicates.

Table 3: Results of two-sample t-test for barley seeds subject to various surface sterilization treatments at day 7 as compared to the control

Source	DF	\bar{X}	s	SE	T-value	P-value
control		60	2.00	0.89		
ethanol (50%)	8	35	3.00	1.30	15.50	0.00
ethanol (70%)	8	32	1.67	0.75	23.67	0.00
bleach (10%)	8	57	3.00	1.30	1.86	0.10
bleach (20%)	8	50	2.00	0.89	7.91	0.00
ethanol/bleach (50%/10%)	8	33	4.00	1.80	13.50	0.00
ethanol/bleach (50%/20%)	8	26	2.00	0.89	26.88	0.00
ethanol/bleach (70%/10%)	8	30	2.00	0.89	23.72	0.00
ethanol/bleach (70%/20%)	8	25	2.00	0.89	27.67	0.00

Differences are considered significant at the $p \leq 0.05$ level (95% confidence interval).

carbohydrates, amino acids, organic acids, inorganic ions and other miscellaneous compounds all of which alter the quality of the surrounding growth medium^[22, 23].

Vancura^[24] reported the presence of sugars (maltose, galactose, glucose, fructose, xylose and ribose), organic acids (oxalic, malic, glycolic, succinic and fumaric) and amino acids (asparagine, aspartic acid, serine, glycine and alanine) in barley seed exudates. Kovacs^[25] concluded that a number of aliphatic (malic, lactic, citric, glycolic and succinic) and aromatic acids (p-hydroxybenzoic, protocatechuric, vanillic and salicylic) are exuded by germinating barley seeds. In this study, the presence of seed exudates in the wastewater during the germination period was evaluated using total solids, chemical oxygen demand, nitrogen and phosphorus analyses. Table 5 shows the influent and effluent TS, COD, NH₄⁺-N, NO₂⁻-N, NO₃⁻-N and PO₄³⁻-P concentrations at days 8 and 21 of the experiment and the removal efficiencies for each water quality parameter. The effects of seed quantity and the presence of barley 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 total solids (TS) concentration in each compartment increased during the germination period due to the release of dissolved and suspended matter from the developing seeds^[21-23]. The increase in TS concentration was not significantly affected by seed quantity. The aquaculture wastewater contained 87% dissolved solids and 13% suspended solids. Feces, uneaten feed and bacterial biomass are the main sources of total solids (TS) in aquaculture effluent^[26-28]. At the end of the growth period, TS reductions of 27.4% and 52.7, 59.4 and 60.5% were achieved in the controls and in the compartments containing barley (200, 250 and 300 g tray⁻¹), respectively. The TS reductions were not significantly influenced by seed quantity ($p = 0.408$), but were significantly influenced by the presence of barley ($p = 0.000$) as shown in Table 5.

Ghaly *et al.*^[29] evaluated the use of hydroponically grown barley to reduce the TS concentration in wastewater from a recirculating aquaculture system stocked with tilapia. The TS concentration in the aquaculture wastewater ranged from 1990 to 2060 mg L⁻¹. The researchers reported TS reductions of 85.0,

Table 4: Average barley yields at harvest

Seed Quantity (g)	Biomass (kg/tray*)	Crop Yield (t/ha)	Duncan Subsets ($\alpha = 0.05$)
200	0.424	25 ± 1	1
250	0.477	35 ± 4	2
300	0.636	59 ± 3	3

Treatments with different numbers are significantly different at the $p \leq 0.05$ level.

*Tray area = 1497.20 cm².

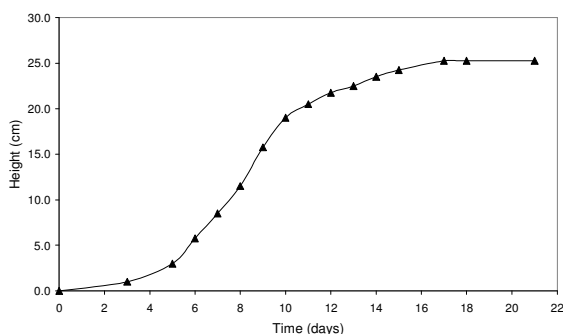


Fig. 3: Average barley height with time



(a) above ground biomass



(b) root mat

Fig. 4: The biomass at the end of the growth period

88.5 and 91.0% after 21 days of plant growth for compartments containing 200, 250 and 300 g tray⁻¹ of barley, respectively. MacKenzie^[17] 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% depending on seed quantity. Bouzoun^[30] utilized a modified hydroponics system called the nutrient film technique for treatment of domestic wastewater. The system consisted of two plywood channels lined with polyethylene, sloped at a 5% grade and planted with reed canarygrass. Over a five month period at a flow rate of 3.2 L min⁻¹, the researcher reported a total suspended solids reduction of 75%.

A number of mechanisms are responsible for the removal of dissolved and suspended solids from the wastewater. One mechanism for the removal of dissolved solids is plant uptake. Sixteen elements essential for plant growth^[31]. Carbon, hydrogen and oxygen are derived from air and water while the remaining nutrients (N, P, K, Ca, Mg, S, Fe, Cl, Mn, B, Zn, Cu and Mo) used by plants are absorbed through the root system from the growth medium^[12]. A second mechanism for the removal of dissolved solids is microbial assimilation. In aquatic, plant-based treatment systems, microbial communities are primarily associated with the surfaces of litter, sediments and submerged plant parts (root zone) or may be dispersed throughout the water column^[32]. Similar to plants, sixteen elements are also essential for microbial growth and maintenance. Dissolved organic carbon is utilized by these microbial communities as an energy source and for cell synthesis. A possible mechanism for the removal of suspended solids is sedimentation, a process by which suspended particles settle from a wastewater under the influence of gravity^[33]. However, sedimentation was not a dominant mechanism for the removal of suspended solids in this study due to aeration. An aeration unit was located at the bottom of each compartment and was designed to force air upward into the wastewater. A second mechanism for the removal of suspended solids is filtration of suspended particles by the root mats of plants^[34, 35].

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^[4]. Excessive sedimentation can abrade or cover respiratory surfaces (gills) of aquatic organisms, offer a suitable habitat for the proliferation

Table 5: Water quality parameters

Parameter	Treatment	Seed quantity (g)	Input ^a (mg L ⁻¹)			Effluent ^b (mg L ⁻¹)	Reduction		Duncan Subsets ($\alpha = 0.05$)
			Wastewater	Released substances	Total		(mg L ⁻¹)	(%)	
TS	Control	-	827 ± 29	-	827 ± 29	600 ± 14	227	27.4	1
	Barley	200	827 ± 29	210 ± 14	1037 ± 32	485 ± 7	552	52.7	2
		250	827 ± 29	210 ± 42	1037 ± 51	420 ± 14	617	59.4	2
		300	827 ± 29	235 ± 14	1062 ± 32	420 ± 99	642	60.5	2
COD	Control	-	158 ± 9	-	158 ± 9	114 ± 5	44	27.6	1
	Barley	200	158 ± 9	118 ± 20	276 ± 22	47 ± 17	229	83.1	2
		250	158 ± 9	136 ± 26	294 ± 28	54 ± 16	240	82.0	2
		300	158 ± 9	172 ± 17	330 ± 19	91 ± 47	239	72.9	2
NH ₄ ⁺ -N	Control	-	2.08 ± 0.50	-	2.08 ± 0.50	0.50 ± 0.71	1.58	76.0	1
	Barley	200	2.08 ± 0.50	0.00	2.08 ± 0.50	< 0.50	1.58	76.0	1
		250	2.08 ± 0.50	0.00	2.08 ± 0.50	< 0.50	1.58	76.0	1
		300	2.08 ± 0.50	0.00	2.08 ± 0.50	< 0.50	1.58	76.0	1
NO ₂ ⁻ -N	Control	-	1.27 ± 0.09	0.00	1.27 ± 0.09	1.16 ± 0.05	0.11	8.7	1
	Barley	200	1.27 ± 0.09	0.00	1.27 ± 0.09	0.03 ± 0.02	1.24	97.6	2
		250	1.27 ± 0.09	0.00	1.27 ± 0.09	0.01 ± 0.00	1.26	99.2	2
		300	1.27 ± 0.09	0.00	1.27 ± 0.09	0.01 ± 0.00	1.26	99.2	2
NO ₃ ⁻ -N	Control	-	21.64 ± 0.60	-	21.64 ± 0.60	7.92 ± 0.44	13.72	63.5	1
	Barley	200	21.64 ± 0.60	6.33 ± 0.46	27.97 ± 0.76	6.46 ± 0.61	21.51	76.9	2
		250	21.64 ± 0.60	6.75 ± 0.00	28.39 ± 0.60	5.91 ± 0.29	22.48	79.2	2
		300	21.64 ± 0.60	7.24 ± 0.08	28.88 ± 0.61	5.31 ± 1.75	23.57	81.6	2
PO ₄ ³⁻ -P	Control	-	4.49 ± 0.18	-	4.49 ± 0.18	2.95 ± 0.25	1.54	34.3	1
	Barley	200	4.49 ± 0.18	2.08 ± 0.01	6.57 ± 0.18	0.85 ± 0.50	5.72	87.1	2
		250	4.49 ± 0.18	3.10 ± 0.48	7.59 ± 0.51	0.41 ± 0.03	7.18	94.6	2
		300	4.49 ± 0.18	7.44 ± 5.62	11.93 ± 5.62	0.59 ± 0.30	11.34	95.1	2

^a day 8, ^b day 21

Treatments with different numbers are significantly different at the $p \leq 0.05$ level

of pathogenic organisms, smother eggs and larvae and bury and smother communities of benthic organisms reducing the biodiversity of the ecosystem^[27, 28]. According to Lawson^[2] and Meade^[36], 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). The average TS concentrations in the final effluents from the hydroponics system were in the range of 420-485 mg L⁻¹.

Chemical oxygen demand: The chemical oxygen demand (COD) concentration in each compartment increased with time during the germination period due to the release of dissolved and suspended organic matter from the developing seeds^[21-23]. The increase in COD concentration was not significantly affected by seed quantity. The total COD concentration in the aquaculture wastewater was 158 ± 9.32 mg L⁻¹. The soluble fraction of the COD was 102 ± 8.56 mg L⁻¹ (approximately 65% of the total COD). Uneaten or

regurgitated food and fecal production are the major sources of organic matter in aquaculture effluents^[4, 37]. At the end of the growth period, COD reductions of 27.6% and 83.1, 82.0 and 72.9% were achieved in the controls and in the compartments containing barley (200, 250 and 300 g tray⁻¹), respectively. The COD reductions were not significantly influenced by seed quantity ($p = 0.495$), but were significantly influenced by the presence of barley ($p = 0.000$) as shown in Table 5.

Ghaly *et al.*^[29] evaluated the ability of hydroponically grown barley for nutrient reduction from aquaculture wastewater and reported COD reductions of 70.2, 79.7 and 85.9% after 21 days of plant growth in compartments containing 200, 250 and 300 g tray⁻¹ of barley, respectively. Gloger *et al.*^[9] 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.*^[38]

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.

One mechanism responsible for the removal of COD from the wastewater is the decomposition of soluble organic carbon by microbial communities^[39, 40]. In aquatic, plant-based treatment systems, submerged plant parts (root zone) are typically covered with an active biofilm. Microbial communities may also be associated with the surfaces of litter and sediments and may be dispersed throughout the water column^[32, 40]. According to Bouzoun *et al.*^[41], plant root density and root surface area are major factors in COD removal. The greater the root surface area per unit volume of tray, the higher the removal of COD because the greater surface area of the finer root system provides more sites for microbial growth. Another mechanism for the reduction in COD is the filtration of suspended particles by plant root mats and absorption of dissolved nutrients by plant roots^[34, 35].

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^[3, 42-44]. Limits for COD concentrations in waters used for the culture of aquatic organisms have not been defined.

Ammonium-nitrogen: The ammonium-nitrogen (NH_4^+ -N) concentrations in compartments containing barley were below detection at the end of the germination period. The aquaculture wastewater contained $2.08 \pm 0.5 \text{ mg L}^{-1} \text{ NH}_4^+$ -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^[45, 46]. Ammonium is also produced through the microbial decomposition of fish feces and uneaten food in a process called ammonification.



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^[33]. Heterotrophic microorganisms responsible for ammonification belong to the genera *Pseudomonas*, *Vibrio*, *Proteus*, *Serratia*, *Bacillus* and *Clostridium*.

At the end of the growth period, NH_4^+ -N reductions of 76.0% were achieved in all compartments. The NH_4^+ -N reductions were not significantly influenced by the seed quantity or the presence of barley. 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.*^[47] evaluated the effectiveness of *Datura innoxia* plants for domestic wastewater purification and reported NH_4^+ -N reductions in the effluent of 93% after 48 hrs of treatment. MacKenzie^[17] 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/day, respectively.

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^[33]. Ammonium (NH_4^+) is one of the major sources of inorganic nitrogen taken up by the roots of higher plants^[47]. It may be assimilated by microorganisms and converted back into organic matter or may be removed from waste streams through the process of nitrification^[39].

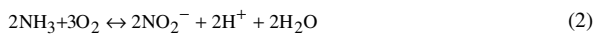
Accumulation of ammonia in water is one of the major causes of functional and structural disorders in aquatic organisms^[48, 49]. Only unionized ammonia is toxic to fish because it can readily diffuse across the gill membranes into the circulation, whereas the ionized form (NH_4^+) cannot^[46, 49]. The average calculated NH_3 -N concentrations in the final effluent from the hydroponics system were in the range of 0.0008-0.0010 mg L^{-1} , which is lower than the recommended ammonia concentration of 0.02 mg L^{-1} for waters used for the culture of aquatic animals^[2, 36].

Nitrite-nitrogen: The nitrite-nitrogen (NO_2^- -N) concentrations were below detection at the end of the germination period in all compartments. The average NO_2^- -N concentration in the aquaculture wastewater was $1.27 \pm 0.09 \text{ mg L}^{-1}$. At the end of the growth period, NO_2^- -N reductions of 8.7% and 97.6, 99.2 and

99.2% were achieved in the controls and in the compartments containing barley (200, 250 and 300 g tray⁻¹), respectively. The NO₂⁻-N reductions were not significantly influenced by seed quantity (p = 0.465), but were significantly influenced by the presence of barley (p = 0.000) as shown in Table 5.

Ghaly *et al.*^[29] examined the use of a hydroponically grown barley for treatment of wastewater from a recirculating aquaculture system stocked with tilapia and reported NO₂⁻-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 (NO₂⁻) and further to nitrate (NO₃⁻) by aerobic bacteria from the genera *Nitrosomonas* and *Nitrobacter*, through a process called nitrification^[50, 51].



Nitrification was facilitated by the continuous aeration of the system compartments during the experiments. Princic *et al.*^[52] reported that the optimum pH range for conversion of NH₄⁺ to nitrite (NO₂⁻) is between 5.8 and 8.5. The pH of the water in all experiments was within this range.

Although NO₂⁻-N is considerably less toxic than NH₃-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^[50, 53]. 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^[2].

The average NO₂⁻-N concentrations in the final effluent from the hydroponics system were in the range of 0.01-0.03 mg L⁻¹. Poxton^[46] recommends a NO₂⁻-N concentration less than 0.02 mg L⁻¹ in water used for the culture of most freshwater fish. The effluents from compartments containing barley at a seed quantity of 250 and 300 g were suitable for reuse in an aquaculture system.

Nitrate-nitrogen: The nitrate-nitrogen (NO₃⁻-N) concentration in each compartment increased with time during the germination period due to the release of dissolved and suspended matter from the developing seeds^[21-23]. The aquaculture wastewater had an average NO₃⁻-N concentration of 21.64 ± 0.60 mg L⁻¹. NO₃⁻-N

accumulates in aquaculture systems as a result of nitrification^[50, 51]. At the end of the growth period, NO₃⁻-N reductions of 63.5% and 76.9, 79.2 and 81.6% were achieved in the controls and in the compartments containing barley (200, 250 and 300 g tray⁻¹), respectively. The NO₃⁻-N reductions were not significantly influenced by seed quantity (p = 0.514), but were significantly influenced by the presence of barley (p = 0.000) as shown in Table 5.

Ghaly *et al.*^[29] investigated the possibility of using hydroponically grown barley for the treatment of aquaculture wastewater and reported NO₃⁻-N reductions in the effluent of 68.8-76.7% after 21 days of plant growth. Clarkson and Lane^[10] 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 four-week period, nitrate-nitrogen concentrations in the effluent were reduced from 33.03 to 3.03 mg L⁻¹ using barley.

Several mechanisms are responsible for the removal of NO₃⁻-N from the wastewater. NO₃⁻-N is the preferred form of inorganic nitrogen taken up by the roots of higher plants^[47]. It may also be assimilated by microorganisms in the water column or by biofilms associated with the root mats of plants^[39]. The occurrence of denitrification in the system is an unlikely mechanism for NO₃⁻-N reduction due to the continuous aeration of all compartments during the experiment^[2].

NO₃⁻-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^[46, 53]. 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^[54]. The average NO₃⁻-N concentrations in the final effluents from the hydroponics system were in the range of 5.31-6.46 mg L⁻¹. Poxton^[46] recommended that NO₃⁻-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 phosphate-phosphorus (PO₄³⁻-P) concentration in each compartment increased with time during the germination period due to the release of dissolved and suspended matter from the developing seeds^[21-23]. The increase in PO₄³⁻-P concentration was not significantly affected by seed quantity. The aquaculture wastewater contained 4.49 ±

0.18 mg L⁻¹ PO₄³⁻-P. Food residues and fecal matter are the major sources of phosphorus in aquaculture effluent^[28]. Phosphorus occurs in aquaculture wastewater primarily as soluble and insoluble phosphates in both organic and inorganic forms^[33]. The main inorganic form is soluble orthophosphate, which exists in different states (H₂PO₄⁻, HPO₄²⁻, and PO₄³⁻) depending on the pH of the medium^[39]. At the end of the growth period, PO₄³⁻-P reductions of 34.3% and 87.1, 94.6 and 95.1% were achieved in the controls and in the compartments containing barley (200, 250 and 300 g tray⁻¹), respectively. The PO₄³⁻-P reductions were not significantly influenced by seed quantity (p = 0.274), but were significantly influenced by the presence of barley (p = 0.000) as shown in Table 5.

Ghaly *et al.*^[29] examined the use of a hydroponically grown barley for removal of PO₄³⁻-P from aquaculture wastewater and reported PO₄³⁻-P reductions ranging from 91.8 to 93.6%. Clarkson and Lane^[10] evaluated the use of the nutrient film technique for PO₄³⁻-P removal from aquarium wastewater. During a four week period, the PO₄³⁻-P concentration in the effluent was reduced from 4.4 to 0.3 mg L⁻¹ 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. Soluble and insoluble forms of organic phosphorus are not biologically available until they have been converted into soluble, inorganic forms. Organically bound phosphorus is converted into inorganic phosphates by microbial oxidation. In aquatic, plant-based treatment systems, microbial communities responsible for this oxidation process are associated with litter, sediments and the root mats of plants. They may also be suspended throughout the water column^[39]. 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^[32, 38].

Toxicity from high levels of phosphorus has not been reported by aquaculturists^[48]. The average PO₄³⁻-P concentrations in the final effluents from the hydroponics system were in the range of 0.41-0.85 mg L⁻¹.

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 and 6.68,

6.65 and 6.63 in the controls and the compartments containing barley, respectively. In aquatic macrophyte based treatment 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. Another explanation for the fluctuation in pH is the consumption of carbon dioxide (CO₂) during algal photosynthesis and the production of CO₂ during organic matter decomposition^[34, 55, 56].

According to Lawson^[2] and Meade^[36], 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^[50]. 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^[46]. 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^[50]. Waters suitable for reuse in an aquaculture facility were produced.

CONCLUSIONS

Sterilization of seeds had a negative impact on germination. Increasing ethanol concentration and/or bleach concentration reduced the germination percentage. During the experiment, the crops grew rapidly and fairly uniformly and showed no signs of mineral deficiency or disease. The average crop height at harvest was 25.5 cm and the yield varied from 25 to 59 t ha⁻¹, depending on the seed density. The 300 g of seeds per tray produced the highest crop yield. The hydroponically grown barley was able to significantly reduce the pollution load of the aquaculture wastewater. The TS, COD, NH₄⁺-N, NO₂⁻-N, NO₃⁻-N and PO₄³⁻-P reductions ranged from 52.7 to 60.5%, from 72.9 to 83.1%, from 76.0 to 76.0%, from 97.6 to 99.2%, from 76.9 to 81.6% and from 87.1 to 95.1%, respectively. However, the effluent produced from the hydroponics system had slightly higher levels of TS (420-485 mg L⁻¹) than the 480 mg L⁻¹ recommended for use in

aquaculture operations. A sedimentation/filtration unit should be added to the hydroponics system.

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