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Phytoremediation of Anaerobic Digester Effluent for Water Purification and Production of Animal Feed

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Abstract: The application of phytoremediation for purification of an anaerobically treated dairy manure and production of forage crops was investigated. Four crops (two cereals and two grasses) were examined for their ability to grow hydroponically and to remove pollutants (nutrients) from dairy wastewater. The preliminary experiments showed that timothygrass and orchardgrass did not perform well as aquatic plants. Only 24 and 29% of the seeds germinated after 19-21 days giving a crop yield of 21 and 19 t ha^{-1} for timotygrass and orchardgrass, respectively. Wheat and barley grow very well as aquatic plants with a seed germination of 83 and 73 (in 7 days) and a crop yield of 106 and 86 t ha^{-1} for wheat and barley, respectively. The effect of light duration, seeding rate, wastewater application rate and fungicidal treatment on the wheat crop yield and pollution potential reduction were studied. The results indicated that with this system, a wheat forage crop could be produced in 21 days from germination to harvest. A treatment combination of wastewater application rate of 900 mL day^{-1}, a seeding rate of 400 g and a light duration of 12 hrs gave the best results for crop yield (3.81 kg of wheat $tray^{-1}$). Based on thirteen harvests per year, a total possible yield of 3300 t ha^{-1} per year can be achieved with the system. This is more than 102 times grater than the yield obtainable from a filed grown conventional forage of 245 t ha^{-1} per year. Wheat had a superior nutritional value (higher digestible energy, higher carbohydrates, fat, protein and mineral contents and less crude fiber) compared to the other field forage crops. It also contained higher macro and micro nutrients (Sodium, Magnesium, Manganese, Iron, Copper, Boron, Selenium, Iodine and Cobalt) than field forage crops. Removal efficiencies of 72.4, 88.6 and 60.8 % can be achieved for the total solids, Chemical Oxygen Demand (COD) and ammonium nitrogen, respectively. A nitrate nitrogen concentration of 7.1 mg L^{-1} was also found in the effluent from the hydroponic system. This is below the Canadian Environmental and Health Guidelines of 10 mg L^{-1}.

Key words: Phytoremediation, digester effluent, dairy waste, seeding rate, COD, nitrogen, solids

INTRODUCTION

Pollution of ground and surface water by manure produced from dairy farms causes widespread environmental and health concerns. Manure contains residues of feed and bedding in addition to the urine and faces from animals, which exert an adverse environmental impact when the manure finds its way to surface and ground waters. The organic matter loading into surface water contributes to building up of bottom sediments and reduces dissolved oxygen levels that results in fish kills. Nutrient loading into surface water causes eutrophication that impairs the quality of water by stimulating excessive algae production, which changes the color and taste of water[1].

Anaerobic digestion of animal manure with energy recovery through methane productions is an effective mean of solving the manure pollution problem, while producing gas which could be used for space and water heating of farm houses and animal shelters, on farm grain drying and as a fuel for heating greenhouses during the could winter[2-4]. However, the pollution potential of the digester effluent, as measured by the concentrations of total solids and chemical oxygen demand (COD), is very high requiring further treatment of the digester effluent before final disposal.

Phytoremediation is a low tech, low cost emerging clean up technology for wastewaters. It is defined as the engineered use of green plants to remove, or render harmless, various environmental contaminates such as

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inorganic and organic compounds. This definition includes all plant influenced biological, chemical and physical processes that aid in the uptake and degradation of contaminates by plants and microbes attached to them[5]. Application of phytoremediation in the treatment of various wastewater has been reported by several researchers[6-10]. Therefore, development of an aquatic plant system for nutrient recovery from the anaerobic digester effluent and/or diluted animal manure may result in purification of these wastewaters while producing another value added product that can be used as animal feed. Combining anaerobic digestion with phytoremediation may serve the double purpose of reducing the pollution potential and producing energy and forage crops, thereby helping to preserve surface water and improve the economics of farming by reducing the demand for energy, commercial fertilizer and animal feed.

The possibility of using agricultural plants for purification of anaerobically treated dairy manure and their suitability as animal feed were investigated. The specific objectives were to: (a) investigate the effect of seeding rate, wastewater application rate, light duration and fungicidal treatment of seeds on the growth of of two young cereal plants (wheat and barley) and two young forage plants (timothygrass and orchardgrass) hydroponically, (b) to evaluate the suitability of these plants as animal feed as measured by their germination percentage, height, yield and nutritional values and (c) to assess the ability of hydroponically produced crops to reduce the pollution potential of the wastewater as measured by the total solids chemical oxygen demand and nitrogen compounds.

MATERIALS AND METHODS

Germination System/ A germination system (Fig. 1) was constructed from wood, galvanized steel and glass materials. The system has a width of 44 cm, a depth of 42 cm and a height of 114 cm it contains ten removable metal trays on which the seeds are germinated. Four fluorescent tubes located at the back of the unite were used to provide light when required for germination. A temperature and humidity sensor placed inside the system gave a digital readout on a meter mounted on the system. A thermostat-controlled heater was used to provide supplemental heat when required. A fan located on the back wall provided cooling when the lights were on.

Hydroponic System. The hydroponic system (Fig. 2) consisted of a frame, growth troughs and aeration, lighting, cooling, waste application, supernatant collection and control systems. The frame (Fig. 3) was constructed of angle iron with a width of 244 cm, a depth of 41 cm and a height of 283 cm. The sides, back and the top were covered with 0.6cm 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. This provided a better control of the light and feeding.

The hydroponic system has six growth 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 was made with a wire-mesh base (16 openings cm^{-2}) and 5 cm high metal sides. The trays were positioned in the troughs so that the plant roots were in contact with the wastewater. This was done by means of supports welded into the corners of each compartment 5 cm below the top edge of the trough. An outlet from each compartment allowed the supernatant from that compartment to be collected separately.

An aeration unit was installed in each compartment to provide oxygen to the immersed roots of the growing crops. 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 a manifold on each shelf using plastic tubing of 0.75 cm outside diameter. Small screw valves mounted on each manifold were used to control the airflow to each aeration unit, which consisted of a main tube (of 0.6 cm outside diameter) 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 system was designed to provide approximately 430 hectolux (hlx) of illumination (19280 lumen per trough). A mixture of fluorescent and incandescent lamps achieved this. Six 40 W cool white fluorescent lamps (122 cm in length) and two 100 W incandescent bulbs were fastened above each trough. The lights were controlled by an electronic circuit, which was set to provide the required number of hours of light per trough per day.

A cooling system 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 placed under each trough provided a 5 cm space between the trough and the lighting system of the shelf below it. A 5 cm diameter PVC pipe was attached vertically to the left side of the frame and acted as a manifold through which air was blown by means of a motor driven fan (Model AK4L143A type 821, Franklin Electric, Bluffton, IN).

The waste application system consisted of a control system, a feeding tank and a pump. A 100 L feeding tank was used for storing the effluent from the anaerobic digester. A mixing shaft, with a 40 cm diameter impeller, was installed through the center of the cover of the feeding tank to agitate the wastewater. 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, Chicago, IL) was mounted on the tank cover to drive the mixing shaft and impeller.

A variable speeds pump (Model 110-23E, TAT Pumps, Logan, OH) with a capacity of 138 cm^3/revolution was used to transfer the wastewater from the feeding tank to the applicators through a manifold (PVC pipe of 2.5 cm outside diameter) connecting a system of feeding tubes to six wastewater applicators through six solenoid valves. The wastewater was pumped from the feeding tank to the wastewater applicators through the solenoid valves that controlled the amount of wastewater fed to each cell. 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 attached to the applicator 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 (three tray length). When a guide on an applicator hit a micro switch located at each end of the shelf, the motor stopped, waited a few seconds and then reversed and the applicator traveled in the opposite direction. This process continued for the designated feeding time, which was controlled by computer.

The supernatant from each tray was collected in a separate container (2.7 l each) located at the bottom of the system. The outlets were connected to plastic tubes of 1 cm outside diameter, which passed through a solenoid valve. Prior to feeding, the outlet tubes were closed using solenoid valves so that the fresh wastewater was retained in the compartments to allow time for the plants to assimilate the fresh nutrients being added.

A computer was used to operate and control the various components of the hydroponic system. A basic computer program allowed the user to configure the operating frequency and duration of the lighting, wastewater application and supernatant collection systems. The computer was connected to a data coordinator which had 24 digital output ports and 24 digital input ports. The digital output ports were connected to electronic circuits which were responsible for the lighting, cooling, wastewater application and supernatant collection systems.

Selected Crops. The potential of growing filed crops hydroponically for wastewater purification and production of forage crops was investigated. Four filed crops were selected for this study: two cereals (wheat and barley) and two grasses (orchardgrass and timothygrass).

Wheat: Wheat is an annual plant with flat blades and terminal spike. And its height may vary from 60 to 150 cm. The plant passes through a vegetative phase followed by a reproductive or fruiting period. Vegetative development stages include germination, root growth, tillering, joining, culm elongation and heading[11]. When the wheat grain is exposed to moisture, water is absorbed and the grain swells. The embryo begins to push out both the coleorhize and the coleoptile. The coleoptile turns upward to form the stem while the coleorhiza turns downward to from the primary root. Wheat will germinate between 4^oC and 37 ^oC, with 20-25 ^oC being the optimum. The minimum moisture content for germination is 35-40% of the grain dry weight and germination is more rapid as moisture increases above this level. Light is not of great importance in controlling wheat germination[12]. Young wheat plants consist mainly of crown, roots and basal leaves.

Barley: Barley is an annual plant which can grow up to 170 cm in height. The lateral spread of barley roots is usually 15-30 cm while they can penetrate up to 195 cm deep. The growing barley plant passes through a vegetative phase followed by a fruiting phase similar to those of the wheat plant[11]. The minimum temperature required for barley to germinate is 3-5 ^oC, with 30 ^oC being the maximum. On germination, the coleorhize elongates and breaks through the pericarp. Soon afterwards, the seminal roots break through the end of the coleorhizae. The temperature largely determines the length of time barley takes to flower and mature after the floral structure has been laid down. Barley is a long day cereal and flowers earlier when nights are short and days are long.

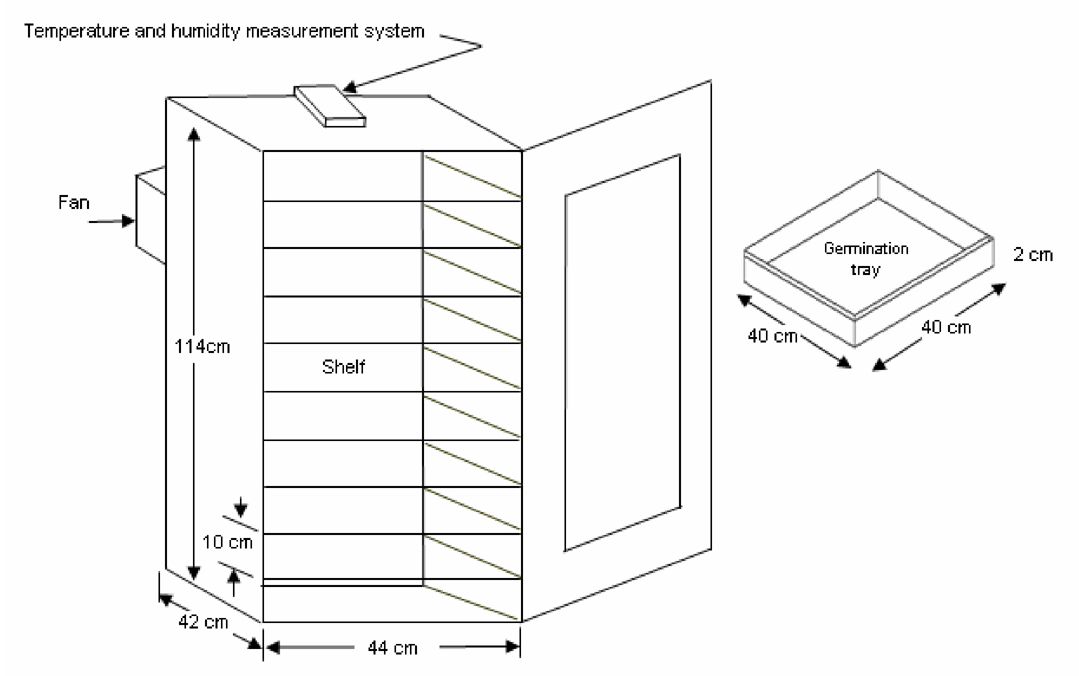

Fig. 1: The germination system

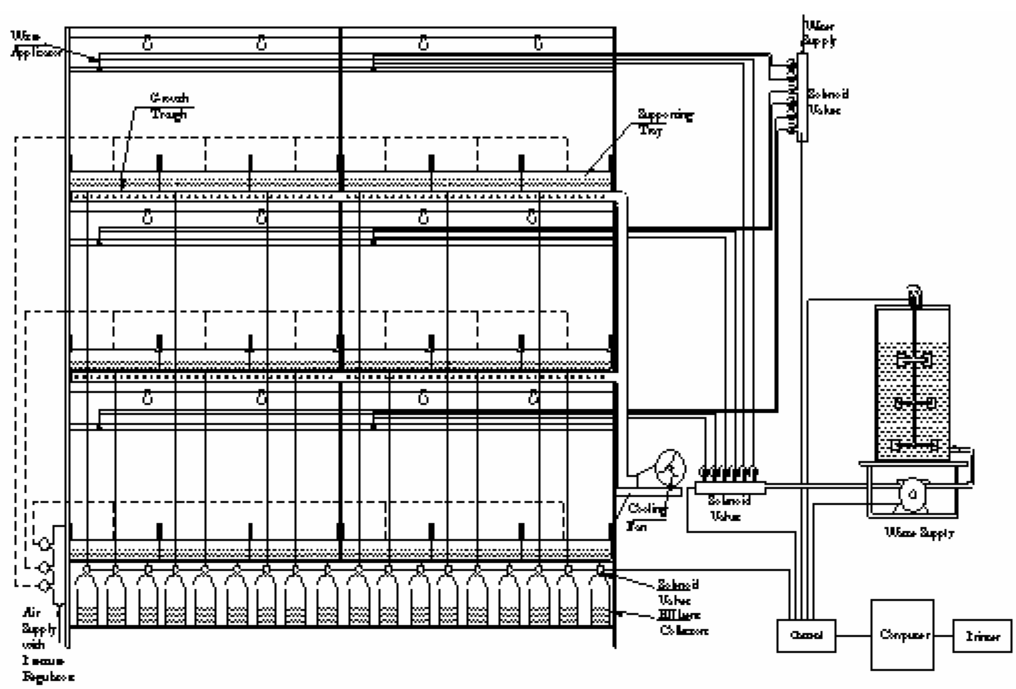

Fig. 2: Hydroponic system

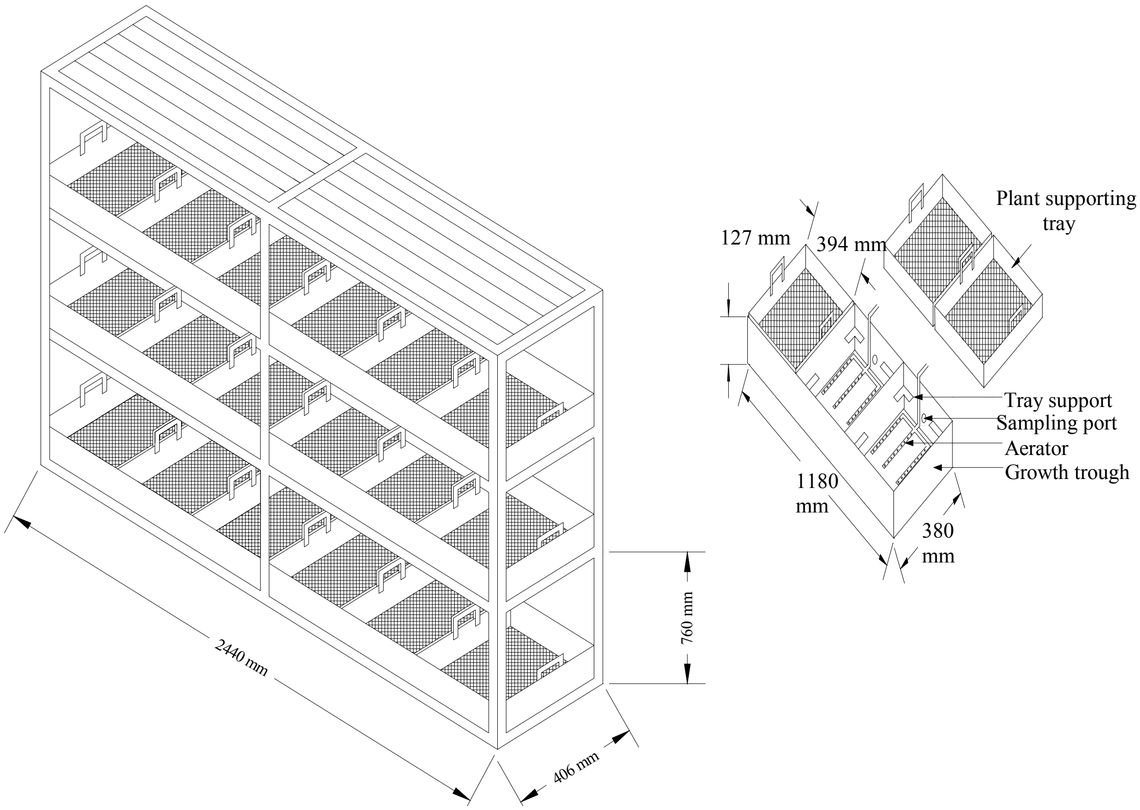

Fig. 3: The frame, plant support and the aeration system

Orchardgrass: Orchardgrass is a perennial, drought tolerant and cool season grass that grows in clump. It requires light for germination and a temperature between 20 and 30 °C[12]. After germination, the scutellum remains within the testa and acts as a secretory and absorbing organ supplying the developing embryo with food material from the endosperm. As the coleoptile elongates, the plumule grows, finally splitting the coleoptile. Vegetative growth occurs first and then stems elongation. The inflorescence of orchardgrass differentiates at the base of the plant and moves upward by elongation of the internods. The flowering culms grow up to 100-130 cm in height. The optimum day temperature for growth is 21 °C and temperatures above 28 °C greatly reduce growth[13]. Orchardgrass is shade tolerant and third of incident light can be intercepted for as long as three years without any detrimental effect on yield. It can also stand high light intensities.

Timothygrass: Timothygrass is widely adapted and easy to establish and maintain. It is perennial bunch grass characterized by a dense cylindric spikelike inflorescence and erect culms. It can grow to 80-110 cm in height and has flat elongated leaves and produces many tillers when plants are isolated[13]. It requires light for germination and a temperature of 20-30 °C. Optimum temperatures for growing timothygrass under controlled environmental conditions have been found to be 18-22 °C. Germination and growth of timothygrass follow the same pattern and stages as Orchardgrass. Individual shoots of timothygrass are biennial growing vegetatively in the first year and flowering in the second. It is a high yielding grass and one of the most palatable hay species.

Germination Experiment. Germination tests were carried out using the germination unit to determine the period required for germination and germination percentage for each crop. Since the light does not influence the germination of cereals but is required for the grass seeds, wheat and barley were germinated with no light while orchardgrass and timothygrass seeds were germinated with the light system being turned on. For each crop, four groups of 100 seeds were counted out and placed on moist paper towels on the shelves located in the germination unit and the thermostat was set at 20°C. The seeds were checked on a daily basis to

Table 1: Nutrients requirements[13, 14]

Plant	Amount (kg ha^{-1})			N: P: K
	Nitrogen	Phosphors	Potassium	
Wheat	56	28	17	10:5:3
Barley	56	28	17	10:5:3
Timothygrass	151	39	163	10:2.5:10.8
Orchardgrass	336	49	350	10:1.5:10.5

Table 2: Chemical analysis of the waste water

Parameter	Concentration (mg L^{-1})
Total solids	14090
Suspended solids	2425
Chemical Oxygen Demand	14690
Ammonium Nitrogen	500
Nitrite nitrogen	0
Nitrate nitrogen	0
Phosphorous	106
Potassium	900

ensure that they were constantly kept moist. The germination percentage was calculate on days 3 , 7 and 10. The final number of germinated seeds from each of the four groups of 100 seeds were counted and recorded. The trays were removed from the unit after 7 days for whaeat and barley and after eighteen days for timothygrass and orchardgrass and the final number of germinated seeds from each of the four groups of seeds were counted and recorded. The germination percentage was then calculated for each crop.

Preliminary Experiment. The experiment was carried out to evaluate the performance of two traditional forage crops (timothygrass and orchardgrass) and two grain crops (wheat and barley). Enough seeds of each crop were used in to cover the tray surface evenly. This resulted in 300 grams of wheat, 300 grams of barley, 100 grams of timothygrass and 75 grams of orchardgrass being used. Three trays were used for each crop. Once the seeds were placed on the trays, the troughs were filled with water to a level such that the seeds were in contact with water but not totally immersed. Light was provided to all the seeds as the grass seeds required light for germination and light does not affected the germination of cereal seeds). Water was added as necessary to insure that the seeds were in contact with water.

After germination had taken place the addition of wastewater was started. The four crops received the same wastewater application rate for a period of three weeks. The nitrogen, phosphorus and potassium requirements for the crops are shown in Table 1[13, 14]. These requirements are for the soil plants and were adapted for hydroponic plants. The chemical analysis performed on the wastewater (Table 2) showed that the phosphorus concentration was the growth limiting nutrient. Based on the phosphorous content of the wastewater and phosphorus requirement by the plants, the wastewater application rate was estimated at 150 mL per tray per day (equivalent to 15 mg P/tray per day). The pollution potential reduction of the wastewater was not considered in this experiment. The light duration was maintained at 12 h. The performance of the four crops was observed during the growth period. After three weeks of wastewater addition, the trays were removed from the system, allowed to drain and then weighed. The crop yield of each crop was recorded.

Main Experiment. The primary experiments indicated that wheat showed the most promise for growth in the hydroponic system. Therefore, three quantities of wheat seeds (per try) were tried in this study 300 g (less crowded), 400g (normal covered) and 500g (overcrowded) to determine if increasing the seeding rats will affect the crop germination and growth. It was also decided to see weather increasing the light duration to 24 hrs would significantly improve the crop performance over that of 12 h light duration. Three wastewater application rates of 150, 300 and 450 mL day^{-1} (which provided 15, 30 and 45 mg of phosphorous per day per compartment, respectively) were used in order to evaluate the impact of nutrient loading on plant growth and pollution potential reduction

On day 1, the required quantities of the wheat seeds were weighted and placed on the trays in the growth troughs. The solenoid valves controlling the outlet tubes were turned off and each compartment was filled with water to a level such that the seeds were in contact with the water but not submerged.

During the germination period (days 2-7), water was added to the compartments as required to compensate for loss of water by evaporation. The germination percentage and seedling height was measured in each tray and recorded on days 3, 5 and 7. A sample of the effluent was taken from each compartment on days 8 before the addition of wastewater and stored in a fridge (at 4 ^{o}C) in a labeled bottle for chemical analyses.

During the growth period (days 8-22), the crop height was measured and recorded on days 10, 14, 18 and 22. Wastewater application continued on daily basis. A sample of the effluent was taken from each compartment before wastewater application and stored in the fridge (at 4 ^{o}C) in a labeled bottle for chemical analyses. The solenoid valves were then opened to allow the effluent to exit through the outlet tubes. The experiment was terminated on day 22. Each tray was removed with the crop and allowed to drain. The crop was then weighed and the yield was recorded.

The solids, chemical oxygen demand (COD), ammonium, nitrite and nitrate analyses were performed on the samples taken from the effluent to determined the reduction in pollution potential of the waste materials. The analyses were carried out according to the procedures described in the Standard Methods for Examination of Water and Wastewater[15].

RESULTS AND DISCUSSION

Germination Experiment. The wheat and barely seeds germinated more rapidly than the timothygrass and orchardgrass seeds (Table 3) About 78 and 66 % of the wheat and barley seeds germinated after 3 days while only 11 and 5 % of the timothygrass and orchardgrass seeds germinated in the same period, respectively. By the seventh day, 83 and 73% of the wheat and barely seeds had germinated, respectively. This is in contrast to a germinate rate of 17 and 7% for timothygrass and orchardgrass seeds in the same period, respectively. A maximum of 24 and 29 % seed germination was observed after 18 days for timothygrass and orchardgrass seeds, respectively. Mackenzie[15] found wheat and barely to germinate faster than timothygrass and orchardgrass and report seed germination of 82, 72, 22 and 8 for wheat, barley, timothygrass and orchardgrass, respectively. Yong *et al*.[16] reported 65 to 80% seed germination of barely *(Hordeum vulgare)* after 7 days.

Preliminary Experiment. The height and yield results are presented in Table 4. Timothygrass and orchardgrass did not perform well as a aquatic plants. They required much longer germination periods (10-18 days) than those of wheat and barely (3-7 days) and had very slow rates of growth. They both grow up to 6 cm in height after 3 weeks and produced very low yield (300 and 272 g tray^{-1} for timothygrass and orchardgrass, respectively). On contrast, wheat and barely seeds germinated rapidly (3 days) and their plant growth rates were fairly high (reaching a height of 38 and 33 cm for wheat and barely in 3 weeks, respectively). Welting and loss of color were noticed during the last week of experiments for all crops, except wheat, which remained healthier.

During the experiment, it was found that the roots of the plants were dying due to the lack of oxygen in the trough, caused by the heat produced from the light system which raised the water temperature (40 ^{o}C). In additional, a problem was encountered with a white fungal disease, which appeared on the seed during germination and continued to grow until the root mat was covered by fungus. Based on these observations, an aerobic and cooling units were added to the hydroponic system and the seeds were treated with fungicide before the start of the main experiment.

Table 3: Seed germination experiments

Crop	Number of days			
	3	7	10	18
Wheat	78	83		
Barley	68	73		
Timothygrass	11	17	20	24
Orchardgrass	5	7	21	29

The values are the average of 4 replicates

Table 4: Preliminary experiment crop height and yield at harvest

Crop	Height Cm	Yield	
		g tray^{-1}	t ha^{-1}
Wheat	38	1490	106
Barley	33	1206	86
Timothygrass	6	300	21
Orchardgrass	6	275	19

The values are the average of 4 replicates. The tray area = 160 cm^2

Main Experiment. Seed Germination: Within 24 h of placing the wheat seeds onto the germination trays, they started swollen and began to geminate. By day 3, the radical and plumule had broken through the seed coat and were visible on most of the seeds. By day 5, the root mat was starting to develop and the seedlings had grown approximately 1-2 cm in height. At the end of germination period (first 7 days), most of the seedlings were 3-5 cm in height (Table 5) regardless of the seeding rate, light duration and fungicidal pretreatment. A germinated percentage of 89% was achieved in the hydroponic system which is slightly higher than that 83% obtained in the seed germinated experimental.

Renault *et al*.[17] reported that during seed germination plants are particularly sensitive to their chemical environment. Bewley and Black[18] stated that as seeds start to take up water during germination, there is a rapid leakage of solutes, sugars, organic acids, ions, amino acids, proteins and growth enzymes (such as gibberellins and cytokinins) into the medium, all of which affect the quality of the growth medium. However, little is known about the effects of these compounds on the processes of seed germination[19]. Therefore, none of these compounds was measured in this study.

Information on the literature regarding the effect of light on seed germination are contradictory. Evans[12] stated that light dos not effect wheat germination. Mata and Casasola[19] reported that germination was not affected by light but the inundation level had a

Table 5: Crop height (cm) at the end of germination period

Seed Rate (g)	Light Duration (h)	Waste Application Rate (mL day^{-1})					
		300		600		900	
		NF	F	NF	F	NF	F
300	12	4.5	4.0	4.0	3.0	4.0	4.0
	24	4.0	3.5	4.0	3.5	3.0	4.0
400	12	3.0	3.5	3.5	5.0	4.5	5.0
	24	3.0	3.5	4.0	4.0	3.5	4.0
500	12	3.0	3.5	3.0	3.5	5.0	5.0
	24	4.0	3.5	4.0	4.5	5.0	4.5

Values are the average of three measurements NF: No Fungicide added. F: Fungicide added

Table 6: Wheat crop height (cm) and yield (kg) at harvest

Seed Rate (g)	Light Duration (h)	Wastewater Application Rate (9mL day^{-1})													
		300				600				900					
		NF		F		NF		F		NF		F			
		H	Y	H	Y	H	Y	H	Y	H	Y	H	Y		
300	12	38.0	1.79	40.0	2.09	36.0	1.98	35.0	2.01	37.0	2.42	30.0	2.54		
	24	12.0	0.33	36.0	1.58	18.0	1.18	40.0	1.60	40.0	2.60	37.0	2.13		
400	12	39.0	2.45	40.0	2.52	38.0	2.74	31.0	2.62	37.0	3.65	39.0	3.24		
	24	28.0	1.22	35.0	1.28	36.0	2.01	31.0	1.82	40.0	3.02	40.0	3.10		
500	12	40.0	2.71	38.0	2.63	37.0	2.93	35.0	2.94	35.0	3.46	37.0	3.81		
	24	30.0	0.46	31.0	1.74	35.5	2.12	38.0	2.35	35.0	2.97	39.0	2.86		

Values are the average of three measurements NF: No Fungicide added. F: Fungicide added H: height (cm) Y: yield (kg)

Table 7: Comparison of ingredients of hydropomically produced wheat forage to these of commonly used field forages in feeding of cattle in North America[29]

Item	Wheat	Field Forge Crop					
		Alfalfa	Timothygrass	Blue grass	Birds foot	Orchardgrass	Wheat pasture
Dry matter %	22	24	26	36	36	24	21
Carbohydrates (g Kg^{-1})	330	340	360	400	360	340	380
Crude fibre (g Kg^{-1})	65	270	310	270	270	300	180
Crude fat (g Kg^{-1})	93	30	38	39	39	40	40
Total digestible energy (MJ Kg^{-1})	187	157	167	180	180	170	187
Crude protein (g Kg^{-1})	288	190	110	150	150	140	200
Macroelements (g Kg^{-1})							
Phosphorus	9	2.7	2.8	3.0	3.0	3.0	3.6
Potassium	3500	26	19	19.0	26	26	31
Calcium	2.9	13.5	4	3.7	3.3	3.3	3.5
Sodium	3.2						
Chloride	1900	4	5.7	4.2	4.1	4.1	6.7
Microelements (mg Kg^{-1})							
Magnesium	3040						
Manganese	98						
Iron	205						
Copper	6						
Zinc	299	18	28	25	21	21	21
Boron	0.8						
Selenium	0.1						
Iodine	0.5						
Cobalt	0.1	2800	1500	1900	1900	2000	2200
Sulphur	0.2						

Note: According to the author the organic constitutionals can vary by as much as ±5%, the mineral constitutionals can vary by as much as ± 30% and the energy values can vary by up to ±10%

significant effect. Kambizi *et al*.[20] reported that germination under alternating light and dark period was significantly higher than under continuous light or continuous darkness.

The fungicidal pretreatment of seeds did not appear to have any significant effect on the seed germination at all seeding rates and light durations. This is in agreement with Cardwell[21] who reported no significant effect of surface sterilization on the germination of wheat seeds. In addition to fungicides, several pregermination treatments have been reported in the literature. Ramakrishna[22] reported decreases in seed germination with increases in sodium hypochlorite (NaOCl) contact time. Resh[23] reported that maize, rice and chickpea primed with salty water not only germinated faster but also grew vigorously, tolerated drought and pests better and flowered and matured earlier. Merceds *et al*[24] reported that exposure of maize seeds to stationery magnetic fields increased with the rate of germination.

Crop growth and yield: The crop height and yield results at harvest are presented in Table 6. There are conflicting reports in the literature regarding the effect of seeding rate on the performance of plants. Ghaly *et al*.[25] reported that the seeds density did not seem to affected plant height or the growth rate. Horii *et al*.[26] reported that the seeding rate affected the crop growth and yield. In this study, the seeding rate slightly affected the height and crop yield. However, the effect of seeding rate on crop height and yield was influence by the light duration and wastewater application rate.

The plants exposed to 12 hrs light duration grew faster and produced higher yields than those exposed to continuous light. This could be due to the effect of heat resulting from the lighting system. The plants exposed to 12 h light grew to a height between 30 and 40 cm with a yield in the range of 1.79-3.81 kg per tray while the plants exposed to 24 h light grew to a height between 12 and 40 cm with a yield in the range of 0.33-2.97 kg per tray. Cardwell *et al*.[21] stated that light is essential for plant growth and plant growth is generally effected by the intensity and duration of light. Decoteau[27] reported that as the light intensity and/or duration increased photosynthesis rates increased initially up to a limited level (saturation point) and further increases in light intensity did not result in a further increase in the photosynthesis rate. Mackenzi[15] reported that the plants exposed to the 12 h light duration produced higher yield and had a healthy growth than those exposed to the continuous light.

The fungicidal treatment did not appear to have any significant effect on the plant growth and yield at all seeding rates, wastewater application rates and light durations. Mackenzie[15] also reported no significant effect of fungicidal seed treatment on the crop growth. However, other researches reported significant impact of seed pretreatment on plant growth and yield. Harris *et al*.[28]reported that with pretreatment of seeds endophyte infection did not affect vegetative plant growth, but significantly increased crop seed yield. They found that endophyte infected plants (in the control) produced less shoot and dry matter. Horii *et al*.[26]reported that chemical pretreatment of seeds affected seed germination percentage, plant height and weight and flowering.

Increasing wastewater application rate slightly increased the plant height. The plants receiving the 300 mL day^{-1} grew slowly and did not have a healthy appearance. The plants receiving 600 mL day^{-1} appeared healthy during the first seven days of growth period, but started to growth slowly and turned yellow during the final seven days. The plants receiving 900 mL day^{-1} appeared healthy and grew the fastest. The final results indicated that the trays receiving the lowest application rate produced the smallest yields of wheat crop. Mackenzie[15] reported that the trays receiving the lowest wastewater application rate produced the smallest yield due to shortage of essential nutrients.

A treatment combination of a wastewater application rate of 900 mL day^{-1}, a seeding rate of 400 g tray^{-1} and 12 hrs light duration gave the best results for crop yield (3.81 kg of wheat forage per tray). With thirteen harvests per year, a total yield of 3300 tonnes per hectare per year is possible. This is more than 102 times grater than the yield obtainable from filed production of conventional forage of 245 tonnes per hectare per year.

Nutritional Value: The composition of the hydroponically produced wheat forage and several field forage crop are shown in Table 7. Not only the hydroponically produced wheat as a forage crop could be 102 fold of field production of forage crops but it also had a superior nutritional value compared to the other field forage crops. It had higher digestible energy, higher carbohydrates, fat, protein and mineral contents and less crude fiber. It provides some of the macro and micro nutrients (Sodium, Magnesium, Manganese, Iron, Copper, Boron, Selenium, Iodine and Cobalt) that are below the detection limits in the field forage crops. It also had much less sulfur content compared to those found in the forage filed crops.

Effect of seed germination on water quality: The increases in the total solids, COD, ammonium nitrogen concentrations resulting from the germination process are presented In Table 8. The solid and COD concentrations in the wastewater increased with time during the germination period (first 7 days) due to the release of dissolved and suspended solids (sugars,

Table 8: Increases in the solids, COD and NH_4-N concentrations (mg L^{-1}) in the wastewater at the end of germination period

Seed Rate (g)	Light Duration (h)	Wastewater Application Rate (mL day^{-1})																	
		300						600						900					
		NF			F			NF			F			NF			F		
		S	C	N	S	C	N	S	C	N	S	C	N	S	C	N	S	C	N
300	12	160	95	2	60	46	3	205	135	3	93	141	3	195	113	2	70	121	3
	24	400	267	2	280	267	3	300	238	3	240	421	3	260	163	2	260	402	3
400	12	131	101	2	200	184	3	83	123	3	95	137	4	150	201	3	105	307	4
	24	200	224	2	333	332	3	240	324	3	200	301	4	300	291	3	320	526	4
500	12	125	178	3	200	159	3	280	232	3	150	232	3	160	260	3	198	350	3
	24	260	338	3	340	535	3	340	405	3	320	384	3	280	389	3	200	727	3

Values are the average of three measurements S: Solids C: Chemical oxygen demand N: Ammonium nitrogen

Table 9: Total solids concentration mg L^{-1} and solids removal efficiency at harvest

Seed Rate (g)	Light Duration (h)	Wastewater Application Rate (9mL day^{-1})											
		300				600				900			
		Total Solids Concentration (mg L^{-1})		Removal Efficiency (%)		Total Solids Concentration (mg L^{-1})		Removal Efficiency (%)		Total Solids Concentration (mg L^{-1})		Removal Efficiency (%)	
		NF	F	NF	F	NF	F	NF	F	NF	F	NF	F
300	12	2190	2130	84.5	84.9	2890	2510	79.5	82.2	4130	4490	70.7	64.6
	24	1450	1800	89.7	87.2	2700	2925	80.8	79.25	3600	3650	74.6	74.1
400	12	1940	2460	86.2	84.9	3310	4060	76.5	71.4	3425	3890	75.7	72.4
	24	1430	2540	89.9	87.0	2630	3190	81.4	77.4	3570	4480	75.7	68.2
500	12	2090	2490	85.2	82.4	2700	3520	80.9	76.1	4790	4690	66.0	66.8
	24	1970	2440	86.0	84.6	2960	3090	79.0	78.1	4150	3810	70.6	73.0

Values are the average of three measurements The initial COD = 14690 mg L^{-1} F: with fungicide added NF: without fungicide added

Table 10: Chemical Oxygen Demand concentration and removal efficiency at harvest

Seed Rate (g)	Light Duration (h)	Wastewater Application Rate (9mL day^{-1})											
		300				600				900			
		COD Concentration (mg L^{-1})		Removal Efficiency (%)		COD Concentration (mg L^{-1})		Removal Efficiency (%)		COD Concentration (mg L^{-1})		Removal Efficiency (%)	
		NF	F	NF	F	NF	F	NF	F	NF	F	NF	F
300	12	1125	1362	92.3	90.7	1600	1253	89.1	91.5	2666	2868	81.9	80.5
	24	1481	1088	89.9	92.6	1603	1250	89.1	91.5	2203	2964	85.0	79.8
400	12	1669	1612	88.6	89.0	2202	2290	85.0	84.4	2073	1673	85.9	88.6
	24	2127	1734	85.5	88.2	1340	1250	90.9	91.5	2061	1481	86.0	89.9
500	12	1771	1980	87.9	86.5	1867	1986	87.3	86.5	1160	2773	92.1	81.1
	24	1797	1804	87.8	87.7	3234	1250	78.0	91.5	3498	3004	76.2	79.6

Values are the average of three measurements. The initial COD = 14690 mg L^{-1} F: with fungicide added NF: without fungicide added

organic acids, ions, amino acids, protein and enzymes) from the seeds as a result of the germination process[25]. Increasing the quantity of seeds increased the amount of solids and other compounds released by the seeds. The values varied from 103 to 340 mg L^{-1} for the solid and from 95 to 405 mg L^{-1} for the COD, depending on the seeding rate and light duration. It seems from the results that the light affected the enzymatic activity, which caused higher solid concentrations under the 24 h light during the germination period.

The ammonium nitrogen in the growth medium increased by 2-4 mg L^{-1} at the end of germination period. Organic nitrogen in the wastewater (proteins and amino acids) was converted into ammonium by the biological process "ammonification". In addition, the solutes released by the seeds may have stimulated the growth of nitrogen fixing organisms, thus increasing the concentration of NH_4 in the growth media as reported by Bewley and Black[18]. The conversion of N_2 to NH_4 has a large metabolic energy requirement and the sugars released from the seeds would serve as a ready source of energy for the

Effluent characteristics: The reduction in pollution potential was measured by the total solids and COD concentration and the presence of nitrogenous compounds.

Total solids: Table 9 shows the concentration of solids and the solid removal efficiency at the harvest time. During the growth period, the solids concentration declined with time due to the development of the crop roots and increased filtration capacity of the root matt. capable of absorbing dissolved solids as plant nutrients. The results showed no effect of the seeding rate on the solid removal efficiency. This could be due to the fact that most of the solids in the effluent (83.0%) were in the soluble form. Mackenzie[15] reported that the solid removal efficiency remained approximately the same for a given waste application rate regardless of the seeding rate. However, other researches reported significant impact of the seeding rate on the solid removal efficiency. Rababah and Ashbolt[31] reported that using more plants in the hydroponic system achieved higher reduction in the nutrient level in the wastewater. Ghaly *et al*.[25] stated that total solids reduction in the effluent was influenced by the seed type and density.

The effluent solid concentration at harvest was in the range of 1940-4690 mg L^{-1} with a removal efficiency of 66.7-86.2 % for the 12 h light duration and in the range of 1430-4480 mg L^{-1} with removal efficiency of 86.2-89.9 % for the 24 h light duration, depending on the seeding rate, wastewater application rate and fungicide application. There are no reports in the literature on the direct effect of light on the solid removal efficiency. The results obtained from this study showed that plants grew much faster and produced higher yield under 12 h light duration and their root system should have filtered higher percentage of the suspended solids and removed higher percentage of the dissolved solids. However, the continuous light increased the temperature of medium which may have increased microbial activities and in turn the solid reduction.

The fungicidal pretreatment did not seem to have any significant effect on the removal efficiency of the total solids. Mackenzie[15] reported that fungicidal application had no apparent effect on the solid removal efficiency under aerobic conditions.

However, the importance of the fungicidal pretreatment of seeds was reported in a study by Ghaly *et al*.[25] who stated that fungicidal pretreatment increased the length of healthy growth period and when the fungus uninfected plants were left to grow for an additional weak, the removal efficiency of the substances released during the germination period increased to 90-95%, depending on the plant type.

The solids removal efficiency was inversely proportional to the wastewater application rate. The removal efficiency was in the rang of 82.0-89.9 % for the 300 mL day^{-1} wastewater application rate, in the range of 71.2-82.2% for the 600 mL day^{-1} wastewater application rate and in the range of 66.0-75.7 % for the 900 mL day^{-1} wastewater application rate. Bouzoun *et al*.[32] reported an inverse relationship between wastewater application rate and solids removal efficiency when using a nutrient film technique for wastewater treatment. Mackenzie[15] reported that lower solids removal efficiencies corresponded with higher initial solid concentrations. Brix.[9] stated that the reduction in suspended solids caused by macrophytes is attributed to filtration of solids by the plant tissue. Ghaly *et al*.[25] stated that not only the plants filtered out all the solids in the wastewater during the growth period, but also removed between 24.2% and 88.0% of the materials released by the seeds during germination.

Chemical oxygen demand: The COD concentration decreased significantly during the growth period (Table 10) because the crop root system was fully developed and the root filtration capacity of suspended solids and absorption of dissolved nutrients increased. Neither the light duration nor the fungicidal pretreatment seem to have any significant effects and the COD removal efficiency. The results also showed no significant effect of the seeding rate on the COD removal efficiency except for slight decrease in the removal efficiency with the increase in the seeding rate at the 300 mL day^{-1} wastewater application rate. Bouzoun *et al*.[32] stated that seeding rates and root density appeared to have been major factors in COD removal, the greater the root density the greater the removal efficiency of COD. Mackenzie[15] reported a positive relationship between the seeding rate and the effluent COD concentration during the germination period but found no effect of the seeding rate on the final removal efficiency.

The final COD concentrations increased with the increase in the wastewater application rate and as a result the COD removal efficiency decrease. The COD concentrations at the end of the growth period ranged from 1125 to 3498 mg L^{-1} with a removal efficiency in the ranged of 76.2-92.3 %, depending on the wastewater application rate. Mackenzie[15] reported that the reduction in the COD was inversely proportional to

Table 11: Ammonium Nitrogen concentration and removal efficiency at harvest

Seed Rate (g)	Light Duration (h)	Wastewater Application Rate (9mL day^{-1}) 300 NH4 Concentration (mg L^{-1})		300 Removal Efficiency (%)		600 NH4 Concentration (mg L^{-1})		600 Removal Efficiency (%)		900 NH4 Concentration (mg L^{-1})		900 Removal Efficiency (%)	
		NF	F	NF	F	NF	F	NF	F	NF	F	NF	F
300	12	98	93	80.4	81.4	178	180	64.5	64.0	151	202	69.8	59.6
	24	71	116	85.8	76.8	140	135	72.0	73.0	213	205	57.4	59.0
400	12	91	118	81.8	76.4	193	187	61.4	62.6	122	196	75.6	60.8
	24	116	80	76.8	84.0	145	163	71.0	67.4	131	166	73.8	66.8
500	12	96	112	80.8	75.6	125	198	75.0	60.4	229	215	54.2	57.0
	24	140	118	72.0	75.4	162	160	67.6	68.0	207	185	58.6	63.0

Values are the average of three measurements Initial ammonium nitrogen = 500 mg L^{-1} F: with fungicide added NF: without fungicide added

Table 12: Nitrate nitrogen concentration (mg L^{-1}) at the end of germination and growth period

Seed Rate (g)	Light Duration (h)	Wastewater Application Rate (9mL day^{-1}) 300 NF		300 F		600 NF		600 F		900 NF		900 F	
		G	H	G	H	G	H	G	H	G	H	G	H
300	12	1.5	5.5	1.4	5.7	1.4	5.6	1.6	7.0	1.6	10.1	1.5	14.2
	24	1.6	4.1	1.6	4.0	1.6	3.0	1.6	5.0	1.6	6.6	1.6	7.9
400	12	1.6	7.2	1.5	5.6	1.6	8.4	1.6	7.5	1.4	6.7	1.6	7.1
	24	1.4	3.3	1.6	2.6	1.6	4.2	1.5	4.8	1.6	8.0	1.6	9.2
500	12	1.6	7.1	1.6	6.4	1.5	4.4	1.6	7.8	1.5	10.1	1.6	7.6
	24	1.5	3.8	1.5	3.7	1.5	6.1	1.6	5.8	1.4	8.2	1.6	7.8

Values are the average of three measurements Initial nitrate concentration = 0 mg L^{-1} NF: No Fungicide added
F: Fungicide added. G: End of germination period H: At harvest

the wastewater application rate. Gloger *et al*.[33] compared the COD removal rate from fish wastewater by hydroponic tanks that had lettuce plants with that by aerated tanks that had no plants and reported 54% higher COD removal rate for lettuce tanks compared with tanks with no plants. Wilkie and Mulbry[34] studied the recovery of dairy manure nutrients by benthic freshwater algae and achieved a COD reduction of 95%.

Ammonium nitrogen: Table 11 shows the ammonium nitrogen concentration and the removal efficiency at the harvest time. The plants received the lowest wastewater application rate were able to remove a greatest percentage of the ammonium nitrogen (72.0-85.8%) compared to those that received the highest wastewater application rate (57.0-75.6 %). Bouzoun *et al*.[32] stated that the removal efficiency of the total nitrogen increased when the flow rate of wastewater applied in the hydroponic system was decreased. Mackenzie[15] reported that the plants receiving lower wastewater application rates were able to remove a greater percentage of the ammonium nitrogen than those receiving higher application rates. Schaafsma *et al*.[35] reported that a constructed wetland system built to treat wastewater from a dairy farm removed a significant amount of ammonium (56%). There were no ovious trends for the effect of the seeding rate, light duration and fungicide application on the ammonium nitrogen removal efficiency.

Nitrite and nitrate nitrogen: Because the system was aerated continuously, some of the ammonium present in the wastewater was converting to nitrite and nitrate by the nitrification process as follows [19].

$NH_4^+ + 1.5O_2 \xrightarrow{nitrosamines} NO_2^- + H_2O + 2H^+$ (1)

$NO_2^- + 0.5O_2 \xrightarrow{nitrobacter} NO_3^-$ (2)

The nitrite nitrogen concentration in the wastewater during the germination and growth periods was zero while the nitrate nitrogen concentration ranged from 1.4 to 1.6 mg L^{-1} by the end of germination period and from 3.0-14.2 mg/L by the end of the experiment (Table 12). These results showed that the conversion rate of nitrite to nitrate by the *nitrobacter* bacteria was much faster than the conversion rate of ammonium to nitrite by *nitrosamines* bacteria.

Schaafsma *et al*.[35] found a significant increase in nitrate concentration (82%) during the treatment of dairy wastewater in a constructed wetland. Ghaly *et al*.[25] reported that ammonium was oxidized into nitrite and then into nitrate in a hydroponic system treating aquaculture wastewater during the plant growth period of cereal crops and the concentrations were dependent on the quantity and the type of seeds.

CONCLUSION

The results showed that timothygrass and orchardgrass did not perform well as aquatic plants. Only 24 and 29% of the seeds germinated after 19-21 days giving a crop yield of 21 and 19 t ha^{-1} for timotygrass and orchardgrass, respectively. Wheat and barley grow very well as aquatic plants with a seed germination of 83 and 73 (in 7 days) and a crop yield of 106 and 86 t ha^{-1} for wheat and barley, respectively.

A treatment combination of wastewater application rate of 900 mL day^{-1}, a seeding rate of 400 g and a light duration of 12 hrs gave the best results for wheat crop yield (3.81 kg of wheat $tray^{-1}$). Based on thirteen harvests per year, a total possible yield of 3300 t ha^{-1} per year can be achieved with the system. This is more than 102 times grater than the yield obtainable from a filed grown conventional forage of 245 t ha^{-1} per year.

Wheat had a superior nutritional value (higher digestible energy, higher carbohydrates, fat, protein and mineral contents and less crude fiber) compared to the other field forage crops. It also contained higher macro and micro nutrients (Sodium, Magnesium, Manganese, Iron, Copper, Boron, Selenium, Iodine and Cobalt) than field forage crops.

Removal efficiencies of 72.4, 88.6 and 60.8 % can be achieved for the total solids, Chemical Oxygen Demand (COD) and ammonium nitrogen, respectively. A nitrate nitrogen concentration of 7.1 mg L^{-1} was also found in the effluent from the hydroponic system. This is below the Canadian Environmental and Health Guidelines of 10 mg L^{-1}.

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