



# Nutrient removal by floating aquatic macrophytes cultured in anaerobically digested flushed dairy manure wastewater

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## Abstract

The potential of three floating aquatic macrophytes to improve the water quality of anaerobically digested flushed dairy manure wastewater (ADFDMW) was evaluated. In undiluted ADFDMW (total chemical oxygen demand 2010 mg/l), growth of water hyacinth (*Eichhornia crassipes*) was inhibited and both pennywort (*Hydrocotyle umbellata*) and water lettuce (*Pistia stratiotes*) failed to grow. In a 1:1 dilution of ADFDMW, all three plants grew successfully. However, growth of pennywort and water lettuce was limited while water hyacinth growth was robust. High salinity appears to be the principal reason for inhibition, as well as possibly uncharacterized soluble compounds.

In terms of reductions in nutrients, chemical oxygen demand (COD), solids and salinity, water hyacinth performed better than water lettuce and pennywort in diluted ADFDMW. Reduction in nutrients and COD followed first-order kinetics, with water hyacinth exhibiting the highest rates. For water hyacinth, total Kjeldahl nitrogen was reduced by 91.7%, ammonium by 99.6%, total phosphorus by 98.5%, and soluble reactive phosphorus by 96.5% in 31-day batch growth. A polyculture of the three plant species in 1:1 diluted ADFDMW exhibited the next best performance. The high biomass yield of the diluted water hyacinth culture corresponded with high EC and Na<sup>+</sup> reductions, suggesting that EC measurement might be a simple tool to monitor performance of water hyacinth growth and nutrient reduction under high plant growth rate conditions.

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**Keywords:** Floating aquatic macrophytes; Flushed dairy manure; Nutrient reduction; Water hyacinth (*Eichhornia crassipes*); Water lettuce (*Pistia stratiotes*); Pennywort (*Hydrocotyle umbellata*); Salinity; Anaerobic digestion; Water quality

## 1. Introduction

Excess nutrients from agricultural operations can result in significant impairment of surface and ground-water quality. These pollutants often enter surface

waters from diffuse or non-point sources associated with surface runoff and from point sources typically associated with concentrated farming activities such as the production of livestock (Knight et al., 2000). Management practices that target excess nutrients from animal manure include oxidation ponds, facultative lagoons, constructed wetlands, storage ponds and land spreading, and composting (NRCS, 1999).

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The high productivity and nutrient removal capability of aquatic plants have created substantial interest in their use for wastewater treatment and resource recovery. The application of aquatic plants for treatment of animal wastes has mainly involved constructed wetlands. Currently, livestock producers in at least 26 states across the USA use constructed wetlands to treat animal production wastewater (Hunt and Poach, 2001). Constructed wetlands have an appeal as a farm waste management practice because they are a low cost, simple technology, and ideally require little maintenance or management after construction (Hammer, 1992).

Floating aquatic macrophyte-based treatment systems (FAMTS) also have potential for removing and recovering nutrients in wastewaters from animal-based agricultural operations. In addition to the advantages cited by Hammer (1992) for wetlands, FAMTS have the following positive attributes: (1) high productivity of several large-leaf floating plants; (2) high nutritive value of floating plants relative to many emergent species; and (3) ease of stocking and harvesting (Boyd, 1974). An efficient harvesting system is a necessary requirement for large-scale FAMTS. In the context of engineered aquatic treatment systems, long rectangular channels with aspect ratios (length:width) of 4–15:1 are usually recommended (Tchobanoglous, 1987). Such a design allows harvesting by lifting the plants from the edge of the channels by hand, crane or mechanical conveyors. Much of the work on harvesting, processing and drying of aquatic plants has been carried out by Bagnall (1979). Harvested biomass of floating macrophytes can potentially be used for composting, soil amendments, anaerobic digestion with methane production, and processing for animal feed. Further, harvested aquatic biomass could be mixed with separated manure solids to increase the amount of nutrients available for exporting off the farm.

Among the floating aquatic plants, water hyacinth (*Eichhornia crassipes*), a nuisance weed, has been extensively researched at laboratory and pilot scale and has been evaluated on a large scale for nutrient removal from wastewaters (Reddy and Smith, 1987). However, most of the studies have focused on the treatment of municipal wastewater. Relatively few studies have been reported on the use of floating plants in animal manure-based wastewater treatment. They include the

application of water hyacinth (*Eichhornia crassipes*) and duckweed (*Lemna* spp.) in the treatment of pig and dairy manure-based wastewater (Whitehead et al., 1987; de Casabianca-Chassany et al., 1992; Polprasert et al., 1992; DeBusk et al., 1995; Costa et al., 2000). DeBusk et al. (1995) found that water hyacinth failed to thrive on undiluted flushed dairy manure from a primary lagoon, but obtained satisfactory growth rates on 1:1 diluted primary lagoon effluent. None of the reported studies have employed FAMTS on anaerobically digested flushed dairy manure wastewaters (ADFDMW).

The objective of the current study was to compare the potential of three floating aquatic macrophytes, along with a polyculture of the three, in reducing the nutrient, salinity and organic content of effluent from an anaerobic digester receiving flushed manure from a dairy farm. Since anaerobic digestion reduces the organic content and increases the availability of manure nutrients (Wilkie and Mulbry, 2002), the combination of anaerobic digestion and FAMTS for flushed dairy manure treatment may provide an effective integrated waste management system.

The aquatic plants selected for this study included water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), and pennywort (*Hydrocotyle umbellata*). Previous studies have shown that, among the floating macrophytes, the selected plants were more productive than small-leaf plants such as salvinia (*Salvinia rotundifolia*), azolla (*Azolla caroliniana*) and duckweed (*Lemna minor*) (Reddy et al., 1983). However, water hyacinth and water lettuce are sensitive to temperature (Clough et al., 1987; Aoi and Hayashi, 1996). Freezing temperatures for a sustained period of more than 24 h can result in plant death. To overcome this problem, cold tolerant plants can be used in polycultures along with these plants. Pennywort was found to be an effective counterpart to water hyacinth for the winter season (Clough et al., 1987).

Wastewater treatment in aquatic macrophyte systems occurs by several mechanisms including: solids settling, plant uptake of contaminants, biotransformation, and physico-chemical reactions. This paper compares the biomass yield, nutrient removal, organic matter reduction, and salinity reduction within the culture media for the selected floating aquatic macrophytes in batch growth on diluted and undiluted ADFDMW.

## 2. Methods

### 2.1. Batch studies

Batch studies under greenhouse conditions were initiated in June 2002. The experiment consisted of two sets of five rectangular plastic containers (0.5 m length  $\times$  0.36 m width  $\times$  0.4 m height) with a working depth of 0.3 m each, a surface area of 0.18 m<sup>2</sup> and a capacity of 50 L of wastewater. One set contained undiluted ADFDMW and the other set had the wastewater diluted with tap water in a 1:1 ratio. The flushed dairy manure underwent mechanical solids separation and settling prior to being pumped into a 400 m<sup>3</sup> fixed-film anaerobic digester operating at ambient temperature and a 2-day hydraulic retention time (Wilkie, 2003). The characteristics of the ADFDMW are presented in Table 1.

Both diluted and undiluted treatments consisted of five sub-treatments without replication. These included: monocultures of water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), and pennywort (*Hydrocotyle umbellata*), a polyculture of the three plants, and a container with no aquatic plants, referred to as “control/algal” culture, since the growth of algae was apparent in both containers that lacked

aquatic plants. The plants were collected from Lake Alice, which is located on the University of Florida campus (Gainesville, FL). To allow for some adaptation, the plants were grown in media of 1:4 diluted anaerobic digester effluent for 1 month prior to the start of the experiment.

The experiment was conducted over a 1-month period, during which observations were made of changes in appearance and growth of the plants. The temperatures of the cultures were recorded on the days of sampling. The minimum and maximum daily air temperatures and total daily radiation for the period of the study were downloaded from the Florida Automated Weather Network (FAWN, 2002). Losses in culture volume due to evapotranspiration were countered by addition of deionized water to the original level every other day, by which time the level had generally decreased by about 1 cm. Water sampling was performed on the second day following the volume adjustment such that deionized water additions would minimally impact measurements.

The containers were initially stocked with plants such that half of the surface area was covered. In order to obtain the initial biomass dry weight, two sets of plants of similar fresh weight were taken for each sub-treatment at the beginning of the experiment. One set was used for the experiment, while the other was dried for 72 h at 70 °C and this measured weight was used to estimate the initial operational density. The final biomass dry weight per square meter was calculated using the dried weight of the harvested biomass.

Table 1  
Characteristics of anaerobically digested flushed dairy manure wastewater

Parameter	Units	Mean value	S.D.
TKN	mg/l	257	$\pm 16$
NH <sub>4</sub> -N	mg/l	136	$\pm 8$
NO <sub>3</sub> -N	mg/l	0	nd
TP	mg/l	34	$\pm 2$
SRP	mg/l	10	$\pm 1$
TCOD	mg/l	2010	$\pm 52$
SCOD	mg/l	942	$\pm 12$
Acetic acid	mg/l	166	$\pm 24.9$
TS	mg/l	3530	$\pm 35$
VS	mg/l	2260	$\pm 10$
SS	mg/l	1020	$\pm 11$
VSS	mg/l	905	$\pm 8$
pH	pH units	7.89	nd
EC	$\mu$ S/cm	2510	$\pm 10$
ORP	mV	-320	nd
DO	mg/l	0	nd
Alkalinity	mg CaCO <sub>3</sub> /l	1260	$\pm 96$
Na <sup>+</sup>	mg/l	59.1	$\pm 6.1$

S.D., standard deviation of triplicate subsamples; nd, no data.

### 2.2. Sampling and analysis

The treatments were evaluated by measuring the physical and chemical parameters in grab samples, which were consistently taken during mid-morning. Samples were obtained by dipping a 100 ml graduated cylinder at three places across the surface of the container and combining them. Care was taken to minimize disturbance of the plants. The wastewater in each container was sampled 8 times over a 31-day period, on days 0, 3, 7, 11, 15, 20, 24, and 31. The parameters measured included temperature, electrical conductivity (EC), dissolved oxygen (DO), oxidation–reduction potential (ORP), pH, alkalinity, total solids (TS), suspended solids (SS), volatile solids (VS), volatile suspended solids (VSS), total chemical oxygen demand

(TCOD), soluble chemical oxygen demand (SCOD), total Kjeldahl nitrogen (TKN), ammonium ( $\text{NH}_4\text{-N}$ ), nitrate ( $\text{NO}_3\text{-N}$ ), total phosphorus (TP), soluble reactive phosphorus (SRP) and sodium ( $\text{Na}^+$ ) ion.

Dissolved oxygen, EC, pH, alkalinity, ORP, TS, VS, SS, VSS, TCOD and SCOD were measured according to standard methods (APHA, 1998). EPA methods 350.1, 351.2, 353.2, 365.1, 365.4, and 200.7 (USEPA, 1983) were used for the analyses of  $\text{NH}_4\text{-N}$ , TKN,  $\text{NO}_3\text{-N}$ , SRP, TP, and  $\text{Na}^+$ , respectively. Organic acids were determined by high-performance liquid chromatography (Hewlett-Packard 1090 series II chromatograph equipped with refractive index and UV<sub>210</sub> detectors) with a Bio-Rad Aminex HPX-87H ion exclusion column. The treatments were analyzed for both nonvolatile organic acids (oxalic acid, oxalacetic acid, fumaric acid, pyruvic acid, malonic acid, methyl malonic acid, succinic acid, and lactic acid) and volatile organic acids (formic acid, acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, valeric acid, isocaproic acid, and caproic acid).

### 2.3. Statistical analysis

First-order kinetic equations were used to describe TKN,  $\text{NH}_4\text{-N}$ , TP, SRP, TCOD, SCOD and EC reduction in the plant cultures and control/algal systems. First-order rate constants were calculated from linear regressions using the following integrated rate equation:

$$\ln \frac{C_0}{C_t} = kt \quad (1)$$

where  $C_0$  (mg/l) is the initial concentration of a parameter in plant culture,  $C_t$  (mg/l) is concentration at time  $t$  (days), and  $k$  is the first-order rate constant (per day).

In the case of EC, the parameter was scaled to a background level of 150 mS/cm prior to the rate parameter estimate. The parameter data and sampling times were entered into an MS Excel spreadsheet and the linearized form of the data was calculated in accordance with equation (1). The LINEST function without an intercept was used to estimate the rate ( $k$ ) using simple linear regression and to obtain the standard error and degrees of freedom for this estimate. A Student's  $t$ -test was performed on the rate constants to ascertain the validity of the first-order kinetic model.

The performances of the control/algal systems were compared to those of the plant cultures by performing a Student's  $t$ -test to determine if the differences between the  $k$  values were statistically significant using the method of independent samples and unequal variances (Steel and Torrie, 1980). The same test was conducted to compare the performance of water hyacinth with the other plant cultures in the diluted treatments.

## 3. Results

### 3.1. Experimental conditions

The average wastewater temperature during sampling for the undiluted and diluted control/algal cultures and the diluted pennywort culture was 28 °C, while the other plant cultures had an average temperature of 27 °C. The lower temperature in the plant cultures, except for pennywort, was due to the plant cover, which shaded the wastewater. The average minimum and maximum ambient temperatures during the study period were 20.4 and 30.3 °C, respectively, and the temperature fluctuations ranged from 16.4 to 35.6 °C. The total daily radiation ranged between 6.51 and 27.28 MJ/m<sup>2</sup> (FAWN, 2002).

### 3.2. Visual observations

The leaf margins of water lettuce and pennywort plants in the undiluted treatments started crisping and browning by the second day of the experiment and became necrotic before finally wilting by the end of the first week. These signs first appeared on the older leaves and progressed to the younger ones. Since water lettuce and pennywort did not survive in the undiluted treatments, these systems along with the undiluted polyculture were not monitored for the rest of the study.

The plants established rapidly in the 1:1 dilution, with the surface area of the containers being completely covered by the second week of the experiment, except for the pennywort monoculture. Compared to water hyacinth and water lettuce which can float as individual plants prior to establishing a mat, pennywort has a long creeping stem that prevents individual plants from floating freely until a dense mat of intertwined stems is formed, which explains the late start

Table 2

Biomass yield of floating aquatic macrophytes after 31-day batch growth in anaerobically digested flushed dairy manure wastewater

Treatment	Dilution	Initial biomass (g dry wt. m <sup>-2</sup> )	Final biomass (g dry wt. m <sup>-2</sup> )	Biomass yield (g dry wt. m <sup>-2</sup> )	Productivity (g dry wt. m <sup>-2</sup> per day)	Average growth rate (per day)
Water hyacinth	0	283	1050	767	24.7	0.087
Water hyacinth	1:1	292	1900	1608	51.9	0.178
Pennywort	1:1	160	240	80	2.6	0.016
Water lettuce	1:1	90	120	30	1.0	0.011
Polyculture	1:1	190	880	690	22.3	0.117

in its growth. In addition, the presence of the algae *Chlorella* was detected in the pennywort culture. In the polyculture, the water hyacinth provided support for the creeping stem of pennywort and helped the plant to establish more rapidly. This was visible from the harvested biomass, where it was difficult to separate the intertwined water hyacinth and pennywort plants. The vertical growth of water hyacinth and pennywort shaded the water lettuce in the polyculture and consequently resulted in reduced growth of the latter plant. In the diluted plant cultures, the wastewater color changed from dark green to light rust-brown over the 31-day period.

Algae established in the diluted control treatment by the end of the second week and by the end of the third week in the undiluted control. The color of the wastewater also changed from dark green to brown due basically to the settling of particulate matter, before turning pale green as a result of algal growth. Algae in the undiluted control were identified as single-cell green algae, while the diluted control contained *Sphaerocystis*.

### 3.3. Biomass production

The initial and final biomass operational densities (g dry wt./m<sup>2</sup>), biomass yields, productivities and average growth rates for the plant cultures are listed in Table 2. While the diluted and undiluted water hyacinth cultures started with similar plant densities, the biomass yield of water hyacinth in the diluted culture was double the yield for the undiluted culture indicating that growth was inhibited in the undiluted ADFDMW. Although the initial biomass densities for pennywort and water lettuce were substantially lower than that for water hyacinth, it is apparent that their low biomass yields were partly due to their lower average growth rates. The polyculture exhibited a biomass yield and average growth rate at levels intermediate between these extremes.

### 3.4. Nutrient reduction

The percent reductions of nutrients for both undiluted and diluted treatments are shown in Table 3 and

Table 3

Percent reduction of parameters in anaerobically digested flushed dairy manure wastewater after 31-day batch growth of floating aquatic macrophytes

Parameter	Control/algal undiluted	Water hyacinth undiluted	Control/algal 1:1 dilution	Water hyacinth 1:1 dilution	Pennywort 1:1 dilution	Water lettuce 1:1 dilution	Polyculture 1:1 dilution
TKN	84.0	84.4	82.1	91.7	87.5	87.6	88.5
NH <sub>4</sub> -N	99.9	99.6	99.8	99.6	99.0	99.2	94.9
TP	89.9	82.0	86.5	98.5	71.3	64.2	92.6
SRP	83.7	60.6	84.7	96.5	60.9	48.5	84.5
TCOD	65.2	74.0	65.8	80.5	72.2	79.6	73.4
SCOD	73.7	59.0	53.3	67.6	58.9	61.0	59.5
SS	72.6	80.6	56.7	92.0	74.3	80.6	81.7
VSS	72.4	90.7	59.3	96.1	85.5	88.6	89.2
EC	45.8	52.2	43.2	89.2	29.7	37.1	50.8
Na <sup>+</sup>	22.4	31.3	32.2	93.5	39.5	35.5	42.4
Alkalinity	20.6	16.7	38.5	61.5	7.7	38.5	38.5

Table 4

Initial and final levels of parameters in anaerobically digested flushed dairy manure wastewater after 31-day batch growth of floating aquatic macrophytes

Parameter	Control/algal undiluted		Water hyacinth undiluted		Control/algal 1:1 dilution		Water hyacinth 1:1 dilution		Pennywort 1:1 dilution		Water lettuce 1:1 dilution		Polyculture 1:1 dilution	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
TKN (mg/l)	257	41.2	227	35.3	164	29.2	164	13.6	160	19.9	162	20.1	173	19.9
NH <sub>4</sub> -N (mg/l)	136	0.12	130	0.43	61	0.10	69	0.29	69	0.66	69	0.56	69	3.53
NO <sub>3</sub> -N (mg/l)	0	0.00	0	0.20	0	0.00	0	0.00	0	0.20	0	0.00	0	0.84
TP (mg/l)	33.9	3.41	32.0	5.74	19.7	2.66	16.5	0.24	17.7	5.08	16.6	5.95	18.1	1.30
SRP (mg/l)	9.97	1.63	11.48	4.52	7.95	1.22	9.61	0.34	9.01	3.52	9.92	5.11	8.08	1.25
TCOD (mg/l)	2007	698	1860	483	1023	350	1103	215	935	260	985	201	1030	275
SCOD (mg/l)	943	248	783	321	409	191	354	115	398	164	395	154	413	167
SS (mg/l)	1018	279	800	155	497	215	487	39	432	111	463	90	518	95
VSS (mg/l)	905	250	723	67	435	177	440	17	385	56	405	46	465	50
pH	7.89	8.89	7.81	8.00	7.91	8.50	7.81	8.32	7.80	8.00	7.90	7.72	7.81	8.02
EC (μS/cm)	2510	1360	2510	1200	1450	824	1450	157	1450	1020	1450	912	1450	713
Na <sup>+</sup> (mg/l)	60.6	47	52.4	36	30.4	20.6	30.8	1.99	33.2	21.4	34.4	20.8	34.7	20
ORP (mV)	-320	92	-320	71	-250	70	-285	113	-260	102	-280	145	-250	90
DO (mg/l)	0	2.3	0	1.3	0	2.5	0	1.8	0	3.6	0	2.4	0	2.3
Alkalinity (mg CaCO <sub>3</sub> /l)	1259	1000	1200	1000	650	400	650	250	650	600	650	400	650	400

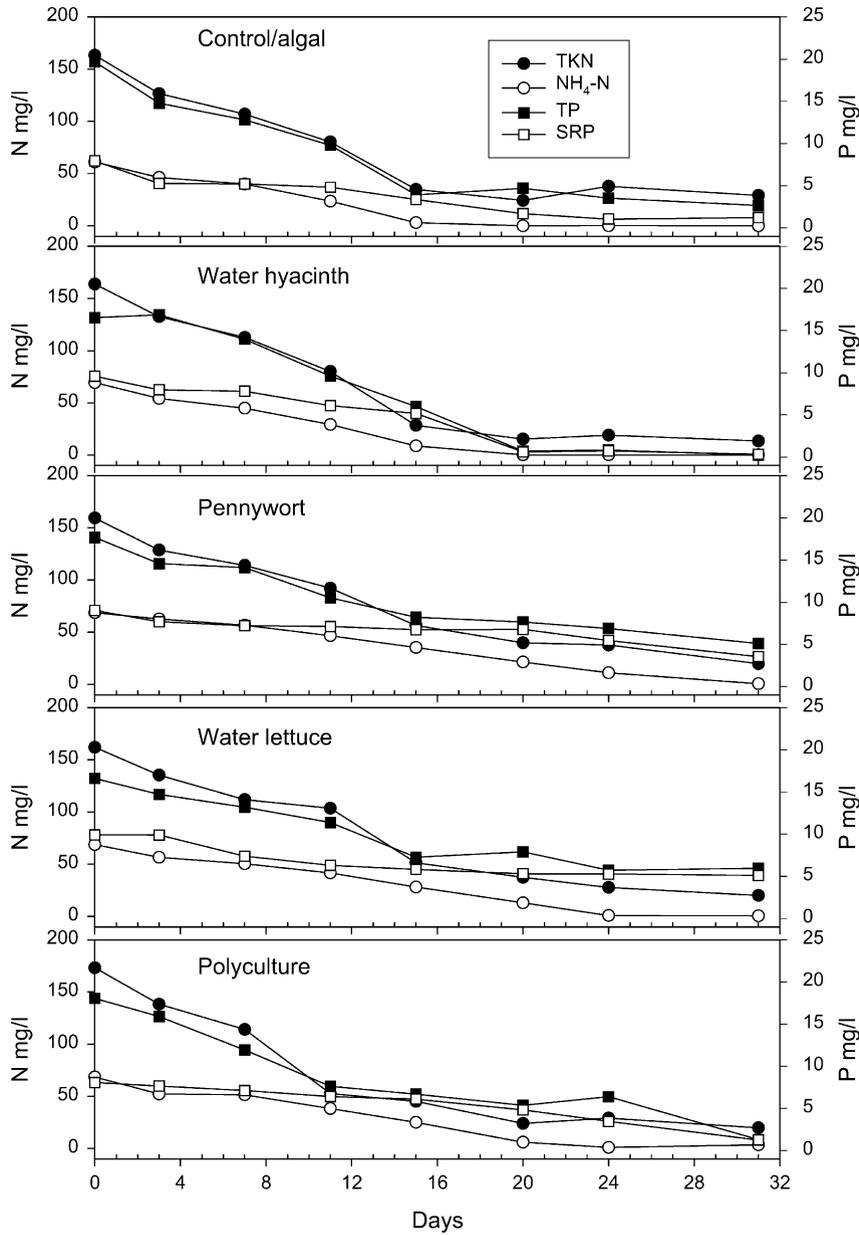


Fig. 1. Levels of TKN, NH<sub>4</sub>-N, TP and SRP in 1:1 dilution of anaerobically digested flushed dairy manure wastewater during 31-day batch growth of floating aquatic macrophytes.

the initial and final levels of TKN, NH<sub>4</sub>-N, NO<sub>3</sub>-N, TP and SRP are given in Table 4. The course of nutrient reductions is shown in Fig. 1 for the diluted treatments. The highest level of TKN reduction (Table 3) occurred in the diluted culture of water

hyacinth. While the control/algal culture exhibited a similar TKN reduction compared to water hyacinth in the undiluted treatments, in the diluted treatments all the plant cultures yielded a higher TKN reduction compared to the algal control. Ammonium levels

reached low values in all treatments by the end of the study, with 99.9 and 99.8%  $\text{NH}_4\text{-N}$  reduction in the undiluted and diluted controls, respectively, within 31 days. Among the plant cultures, both water hyacinth systems had the greatest reduction of  $\text{NH}_4\text{-N}$ , at 99.6% for the same period (Table 3).

None of the treatments had any initial  $\text{NO}_3\text{-N}$ , but some nitrification was detected during the experiment in all treatments except for the diluted water hyacinth culture and the two control/algal cultures, where none was detected (data not shown). At the end of the experiment,  $\text{NO}_3\text{-N}$  was detected only in the undiluted water hyacinth culture and the diluted cultures of pennywort and the polyculture (Table 4). The highest level of  $\text{NO}_3\text{-N}$  detected was 9.6 mg/l on day 24 for the undiluted water hyacinth culture (data not shown). Water lettuce exhibited the second highest  $\text{NO}_3\text{-N}$  level of 8 mg/l, also on day 24, but this decreased to undetectable levels by the end of the experiment.

The highest levels for both TP and SRP reductions occurred for the water hyacinth culture grown in the diluted ADFDMW, with TP reduction of 98.5% and SRP reduction of 96.5% (Table 3). In the undiluted treatments, the control/algal culture exhibited higher TP and SRP reductions compared to water hyacinth. In the diluted plant cultures, only water hyacinth and the polyculture gave a higher TP reduction compared to the control/algal culture and only water hyacinth exhibited a higher SRP reduction than the control/algal culture. The SRP in the diluted water hyacinth culture was reduced by 90% within 20 days, whereas SRP was reduced by only 70% in the control/algal culture during the same period (Fig. 1).

### 3.5. Suspended solids and COD reduction

The initial and final levels of TCOD, SCOD, SS, and VSS for both undiluted and diluted treatments are given in Table 4 and the percent reductions of these parameters are listed in Table 3. The course of TCOD and SS, along with pH, is shown in Fig. 2 for the diluted treatments. In general, TCOD changes over time followed the same trend as changes in SS. Around 50% of the decrease in these parameters occurred within the first week of the experiment. This reduction was mainly brought about by sedimentation in the control/algal systems and additionally by filtration through the root systems in the plant cultures.

Algal growth in the control/algal systems led to an increase of SS in the wastewater, as shown in Fig. 2 (days 20–24). In both the diluted and undiluted treatments, plant cultures exhibited higher levels of TCOD and SS reductions compared to the corresponding control/algal cultures (Table 3).

Initially, the undiluted ADFDMW averaged 942 mg/l of SCOD (Table 1) and was composed of 166 mg/l acetic acid (equivalent to a COD of 177 mg/l or 18.8% of SCOD) and trace amounts of propionic acid, with none of the other organic acids present at detectable levels (<1 mg/l detection limit). The remaining fraction of SCOD was presumably comprised of phenolic residues from tannin derivatives inherent in the wastewater. After day 3, none of the acids, including acetic acid, were detected in either diluted or undiluted treatments. The undiluted control/algal culture exhibited higher SCOD reduction compared to water hyacinth. In the diluted treatments, SCOD reduction in the plant cultures was higher than in the control (Table 3).

### 3.6. Changes in EC, ORP, DO, pH, alkalinity and $\text{Na}^+$

Fig. 3 depicts the evolution of EC, ORP and DO in the diluted treatments and the course of pH is shown in Fig. 2. The initial and final levels for these parameters, as well as alkalinity and  $\text{Na}^+$ , for both the undiluted and diluted treatments are listed in Table 4. The percent reduction of EC,  $\text{Na}^+$  and alkalinity is shown in Table 3. The initial EC of the undiluted treatments was 2510  $\mu\text{S/cm}$ , while initial EC of the 1:1 dilutions was 1450  $\mu\text{S/cm}$ . Since EC provides a measure of the dissolved salt content of a solution, the uptake of ions from solution would consequently have a bearing on conductivity readings. Reduction of EC due to algal activity was similar in both controls. Among the aquatic plants, the greatest reduction in conductivity was achieved with water hyacinth, where EC was reduced at the end of the study by 52.2 and 89.2% in the undiluted and diluted cultures, respectively (Table 3).

The undiluted wastewater had an ORP of  $-320$  mV, while the initial ORP recorded for the diluted treatments ranged from  $-285$  to  $-250$  mV (Table 4). These values reflect the anaerobic state of the digested dairy wastewater. As dissolved oxygen was detected in the

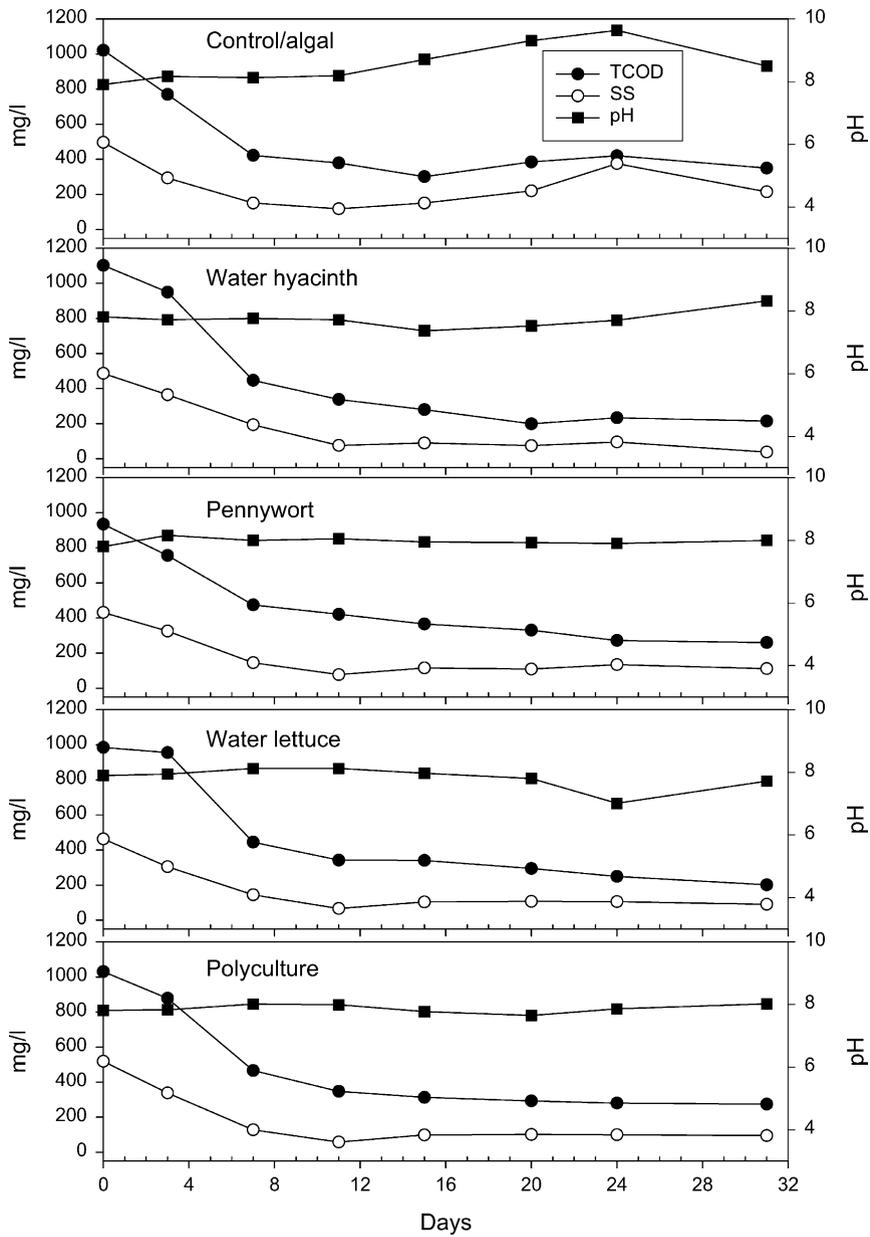


Fig. 2. Levels of TCOD, SS, and pH in 1:1 dilution of anaerobically digested flushed dairy manure wastewater during 31-day batch growth of floating aquatic macrophytes.

various treatments, the ORP shifted from negative to positive (Fig. 3).

Initially, no DO was detected in the cultures as the wastewater originated from an anaerobic digester. Dissolved oxygen was detected in most treatments by the

fourth day of the study. The plant cultures had a lower DO concentration compared to the control/algal systems. Among the 1:1 diluted monocultures, the pennywort culture had a higher concentration, with DO ranging from 1.0 to 3.6 mg/l (days 7–31) compared

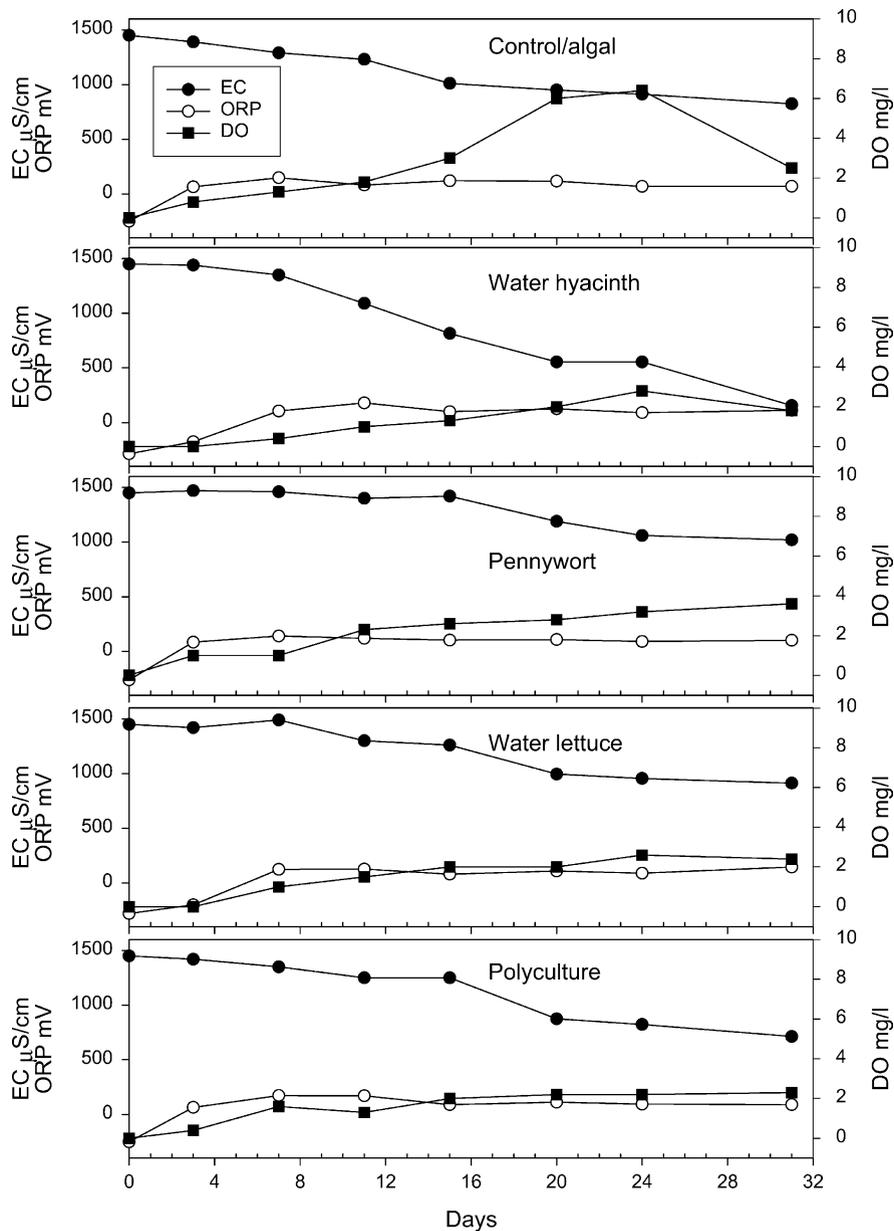


Fig. 3. Levels of EC, ORP and DO in 1:1 dilution of anaerobically digested flushed dairy manure wastewater during 31-day batch growth of floating aquatic macrophytes.

to 0.4–2.8 mg/l in the water hyacinth culture and 1.0–2.6 mg/l in the water lettuce culture (Fig. 3).

The pH level in the control/algal systems increased from 7.91 to 9.64 in the diluted wastewater (Fig. 2) and from 7.89 to 9.12 in the undiluted treatment. The

alkalinity in these cultures decreased by 38.5 and 20.6%, respectively, which most probably resulted from the consumption of carbonate during algal photosynthesis. An average pH of 8.0 was recorded for the aquatic plant cultures. The alkalinity reduction was

Table 5  
First-order rate constants for reduction of nutrients, COD and EC in anaerobically digested dairy manure wastewater during 31-day batch growth of floating aquatic macrophytes<sup>a</sup>

Treatment	$k_{TKN}$ (per day)	$k_{NH_4-N}$ (per day)	$k_{TP}$ (per day)	$k_{SRP}$ (per day)	$k_{TCOD}$ (per day)	$k_{SCOD}$ (per day)	$k_{EC}$ (per day)
Undiluted <sup>b</sup>							
Control/algal	0.029 ( $\pm 0.001$ )	0.094 ( $\pm 0.009$ )	0.036 ( $\pm 0.003$ )	0.025 ( $\pm 0.002$ )	0.019 ( $\pm 0.002$ )	0.020 ( $\pm 0.001$ )	0.010 ( $\pm 0.001$ )
Water hyacinth	0.026 ( $\pm 0.001$ )	0.047 ( $\pm 0.009$ )	0.026 ( $\pm 0.002$ )	0.013 ( $\pm 0.001$ )	0.024 ( $\pm 0.002$ )	0.013 ( $\pm 0.002$ )	0.010 <sub>nsc</sub> ( $\pm 0.001$ )
1:1 dilution <sup>c</sup>							
Control/algal	0.030 ( $\pm 0.003$ )	0.104 ( $\pm 0.015$ )	0.031 ( $\pm 0.002$ )	0.030 ( $\pm 0.002$ )	0.020 ( $\pm 0.003$ )	0.014 ( $\pm 0.002$ )	0.010 ( $\pm 0.000$ )
Water hyacinth	0.040 ( $\pm 0.003$ )	0.086 ( $\pm 0.009$ )	0.053 ( $\pm 0.006$ )	0.044 ( $\pm 0.005$ )	0.030 ( $\pm 0.003$ )	0.015 ( $\pm 0.008$ )	0.042 ( $\pm 0.010$ )
Pennywort	0.028 <sub>nsc</sub> ( $\pm 0.001$ )	0.042 ( $\pm 0.007$ )	0.020 ( $\pm 0.001$ )	0.008 ( $\pm 0.001$ )	0.022 <sub>nsc</sub> ( $\pm 0.002$ )	0.011 ( $\pm 0.002$ )	0.005 ( $\pm 0.001$ )
Water lettuce	0.030 <sub>nsc</sub> ( $\pm 0.001$ )	0.057 ( $\pm 0.008$ )	0.017 ( $\pm 0.001$ )	0.012 ( $\pm 0.001$ )	0.026 ( $\pm 0.002$ )	0.013 <sub>nsc</sub> ( $\pm 0.001$ )	0.007 ( $\pm 0.001$ )
Polyculture	0.035 ( $\pm 0.002$ )	0.049 ( $\pm 0.006$ )	0.029 <sub>nsc</sub> ( $\pm 0.003$ )	0.018 ( $\pm 0.003$ )	0.025 ( $\pm 0.003$ )	0.014 <sub>nsc</sub> ( $\pm 0.001$ )	0.011 <sub>nsc</sub> ( $\pm 0.001$ )

<sup>a</sup> Values in parentheses are standard errors of rate constants.

<sup>b</sup> A Student's *t*-test was performed to compare *k* values of control/algal with those of water hyacinth. All *k* value differences were highly significant ( $\alpha = 0.01$ ), except for those bearing the subscript: nsc (not significantly different compared to control).

<sup>c</sup> A Student's *t*-test was performed to compare *k* values of control/algal with those of plant cultures and *k* values of water hyacinth with those of the other plant cultures. All *k* value differences were significant ( $\alpha = 0.05$ ), except for those bearing the subscript: nsc (not significantly different compared to control).

highest (61.5%) in the diluted water hyacinth culture (Table 3).

The initial Na<sup>+</sup> levels in the undiluted control/algal and water hyacinth cultures were 60.6 and 52.4 mg/l, respectively, and were only slightly reduced after 31 days (Table 4). In the diluted water hyacinth culture, the Na<sup>+</sup> levels were reduced to less than 2 mg/l compared to much smaller reductions in all other plant cultures and the control (Table 4).

### 3.7. Comparison of reduction rate constants

Total Kjeldahl nitrogen, NH<sub>4</sub>-N, TP, SRP, TCOD, SCOD, and EC followed a first-order decay model and all the rate constants for the regression fits were significant ( $\alpha = 0.05$ ) using a Student's *t*-test (Table 5). Differences between the first-order rate constants for the control/algal culture and the water hyacinth culture in the undiluted wastewater were all highly significant ( $\alpha = 0.01$ ), except for EC. Except for TCOD and EC, the rate constants for the control/algal culture were all greater than the corresponding rate constants for the water hyacinth culture (Table 5).

In contrast, for the diluted wastewater, the water hyacinth culture exhibited significantly ( $\alpha = 0.05$ ) greater first-order rate constants than the control/algal culture for all parameters except NH<sub>4</sub>-N reduction (Table 5). The water hyacinth culture also exhibited the highest reduction rates for all parameters in the 1:1 diluted experiment compared to all other plant cultures.

## 4. Discussion

### 4.1. Visual observations

The water hyacinth plants that survived in the undiluted culture had some of the older leaves becoming necrotic around the margin during the initial days of the study. Plant growth was reduced compared to the diluted water hyacinth culture (Table 2) and the leaves exhibited chlorotic symptoms, being light to yellow green compared to dark green in the 1:1 dilution. The roots of the harvested plants also showed marked differences, with those in the undiluted wastewater being shorter and brown in color compared to longer blackish roots in the diluted treatment.

The leaves of water lettuce and pennywort suffered necrosis in the undiluted cultures when the leaf margins started crisping and browning by the second day. These symptoms are similar to those described by Haller et al. (1974) in a study conducted on the effect of salinity on growth of aquatic macrophytes. Haller et al. (1974) reported toxic effects in water lettuce and water hyacinth when these plants were exposed to diluted seawater with salt concentrations of 1660 and 2500 mg/kg, respectively. Since a common measure of salinity is EC, an EC level was estimated for these figures using a conversion factor of  $1000 \text{ mg/kg} = 1616 \mu\text{S/cm}$ , calculated from values given by Haller et al. (1974). The calculations give a conductivity of  $2683 \mu\text{S/cm}$  as a toxic level for water lettuce and  $4040 \mu\text{S/cm}$  for water hyacinth. These values could explain the survival of water hyacinth versus the toxicity effect on water lettuce in the undiluted culture, which had an initial conductivity of  $2510 \mu\text{S/cm}$ . For terrestrial plants, NRCS (1999) gives soil conductivity values of  $2000 \mu\text{S/cm}$  or less as being low in salts and suitable for all crops, while values above  $4000 \mu\text{S/cm}$  affect plant growth for all but the more tolerant crops. For the aquatic plants used in this study, the lake water from which the plants originated had an average conductivity of  $466 \mu\text{S/cm}$ .

Since the EC of the undiluted wastewater ( $2510 \mu\text{S/cm}$ ) was much less than the estimated toxic value of  $4040 \mu\text{S/cm}$ , the reduced water hyacinth growth in the undiluted culture could be attributed to stress due to the high initial concentration of  $\text{NH}_4\text{-N}$  ( $130 \text{ mg/l}$ ) in the wastewater. High levels of  $\text{NH}_4\text{-N}$  ( $>200 \text{ mg/l}$ ) from feedlot runoff and methane digester effluent have been reported as a factor that may inhibit aquatic plant growth (Reddy et al., 1983). They are also often implicated in plant death in animal wastewater treatment wetlands (Cronk, 1996). Baldwin and Davenport (1994) reported that wastewater killed almost all the newly transplanted vegetation at a dairy in Maryland. Ammonium levels of  $188 \text{ mg/l}$  and organic N levels of  $114 \text{ mg/l}$  were considered high levels of N for constructed wetlands cells (Karpiscak et al., 1999).

Most of the reports in the literature indicate that the toxic effect is due to the un-ionized form of ammonia,  $\text{NH}_3$ , the concentration of which depends on the dissociation of  $\text{NH}_4^+$ , which is a function of pH (Vines and

Wedding, 1960; Azov and Goldman, 1982; Caicedo et al., 2000). However, in their study on the effect of total ammonia N and pH on duckweed (*Spirodela polyrrhiza*) growth, Caicedo et al. (2000) concluded that both forms of ammonium caused growth inhibition in duckweed. The growth inhibition by  $\text{NH}_4^+$  was attributed to saturation and depolarization of cell membranes, which inhibit anion transport.

However, in a study conducted by de Casabianca-Chassany et al. (1992) of water hyacinth grown on three ammonium-containing effluents (pectin production, carcass-treatment, and pig manure wastewaters), the authors found that optimal biomass production was obtained at  $\text{NH}_4\text{-N}$  concentrations of  $100\text{--}150 \text{ mg/l}$ . The conductivity of the effluents for this range of  $\text{NH}_4\text{-N}$  was  $760\text{--}2167 \mu\text{S/cm}$ . Inhibitory effects on growth were observed at  $\text{NH}_4\text{-N}$  concentrations of  $200 \text{ mg/l}$  (EC ranged from  $1025$  to  $2890 \mu\text{S/cm}$ ). The pH was 8.5 in all the effluents. de Casabianca-Chassany et al. (1992) indicated that sodium appeared to be one of the inhibitory elements affecting differences in productivity among the effluents. Growth inhibition occurred when the  $\text{Na}^+$  concentration was  $77 \text{ mg/l}$  for pectin production wastewater,  $63 \text{ mg/l}$  for carcass-treatment wastewater and  $108 \text{ mg/l}$  for pig manure wastewater. Conversely, Moorhead (1986) reported water hyacinth growth at  $\text{Na}^+$  concentrations of  $1100 \text{ mg/l}$ , where the plants were grown on the effluent of an anaerobic digester receiving water hyacinth as feedstock. The plant also survived in textile wastewater having  $\text{Na}^+$  concentrations of  $2550$  and  $2900 \text{ mg/l}$  (Trivedy and Gudekar, 1987).

The nature of the organic matter in the wastewater could provide another explanation for the growth inhibition of the plants. A wide range of soluble organic compounds, some from the anaerobic decomposition of cellulose and lignin, has been reported to be toxic to plants (Patrick et al., 1964). Barko and Smart (1983) found that aquatic sediments receiving refractory organic matter retained growth inhibiting properties for longer than those receiving labile organic matter. The presence of inhibitory soluble organic compounds could explain the failure of water hyacinth to thrive in undiluted primary dairy lagoon effluent encountered by DeBusk et al. (1995), despite an EC of  $1630 \mu\text{S/cm}$  and  $\text{NH}_4\text{-N}$  of  $53.8 \text{ mg/l}$ .

Therefore, despite the survival of the water hyacinth in the undiluted culture, the combined effect of initial

salt concentration ( $EC = 2510 \mu\text{S}/\text{cm}$ ), initial  $\text{Na}^+$  concentration ( $52.4 \text{ mg}/\text{l}$ ), initial  $\text{NH}_4\text{-N}$  concentration ( $130 \text{ mg}/\text{l}$ ), and the presence of uncharacterized soluble compounds likely stressed the plants and limited their ability to grow and develop as compared to the 1:1 diluted culture (with initial  $EC = 1450 \mu\text{S}/\text{cm}$ ,  $\text{Na}^+ = 30.8 \text{ mg}/\text{l}$  and  $\text{NH}_4\text{-N} = 69 \text{ mg}/\text{l}$ ).

#### 4.2. Biomass growth

While the varying initial biomass operational densities of the different aquatic macrophyte cultures (Table 2) may have influenced the extent of N and P removal, the average productivities of the plants suggest that growth of pennywort and water lettuce was limited in the diluted wastewater during this study. The productivity for pennywort was  $2.6 \text{ g dry wt. m}^{-2}$  per day, and for water lettuce it was only  $1.0 \text{ g dry wt. m}^{-2}$  per day. By contrast, the productivity for water hyacinth was  $24.7 \text{ g dry wt. m}^{-2}$  per day in the undiluted wastewater and  $51.9 \text{ g dry wt. m}^{-2}$  per day in the diluted wastewater. Reddy et al. (1983) reported productivity ranges for water hyacinth, pennywort, and water lettuce of 38–64, 5–20, and 19–40  $\text{g dry wt. m}^{-2}$  per day, respectively. DeBusk et al. (1995) measured the summertime productivity of water hyacinth in a 1:1 dilution of primary dairy lagoon effluent and found that it averaged  $33.2 \text{ g dry wt. m}^{-2}$  per day. Thus, the water hyacinth growth rates in the current study were similar to previous measurements, while growth rates for pennywort and water lettuce were lower.

#### 4.3. Wastewater treatment

Reduction of TKN (Fig. 1) in the plant cultures would mainly be due to plant uptake of  $\text{NH}_4\text{-N}$ , volatilization of  $\text{NH}_3$ , nitrification, entrapment of particulate matter (organic nitrogen) by the extensive root system, and settling. The detection of  $\text{NO}_3\text{-N}$  in the plant cultures indicates that nitrification occurred in these treatments, whereby ammonia was oxidized to  $\text{NO}_3\text{-N}$  by nitrifying bacteria. These nitrifiers form part of a consortium of microorganisms that may establish themselves on the aquatic plant roots, which provide a large surface area for microbial attachment. A major part of the degradation of pollutants (COD) in the wastewater is attributed to these microorgan-

isms, which may establish a symbiotic relationship with the plants. In this study, the support provided by the roots of the aquatic plants and the transport of oxygen to the roots may have provided more favorable conditions for nitrifying bacteria to establish in the plant cultures compared to the control/algal systems. Nitrate was likely taken up by the plants as levels of  $\text{NH}_4\text{-N}$  decreased. In the control/algal systems, nitrification probably did not occur due to the lack of support for the nitrifying biofilm to grow.

In addition to  $\text{NH}_4\text{-N}$  uptake for algal growth in the control/algal systems, volatilization would have been the likely mechanism by which  $\text{NH}_4\text{-N}$  was reduced; the process being enhanced by the increasing pH levels (7.9–9.6) in these two treatments, as shown in Fig. 2 for the diluted control/algal system. However, ammonia loss through volatilization is a concern because of its impact on atmospheric chemistry and acid deposition, conveying an advantage to floating aquatic macrophytes over algae-based nutrient removal systems.  $\text{NH}_4\text{-N}$  removal in the plant cultures may have been primarily due to plant uptake and nitrification, along with a lesser level of volatilization given that these systems had a less alkaline pH.

The reduced growth of water hyacinth in the undiluted wastewater could explain the lower TP and SRP removal compared to the undiluted control/algal system (Table 3). However, in the diluted cultures, water hyacinth had the highest removal of TP and SRP (Table 3); it also had the highest growth rate (Table 2). It can be assumed that SRP reduction was principally due to algal uptake in the control/algal systems and due to plant uptake in the plant cultures. The complete coverage of the surface area in the water lettuce culture and the small change in reduction of SRP from day 15 onward (Fig. 1) indicates that either harvesting was necessary to improve SRP removal or else SRP concentration was low enough to limit removal in the later days of the experiment. Reduction in TP would have been mainly due to uptake of soluble P, filtration of particulate matter through the roots, and settling. Despite the relatively good performance of the control/algal system for nutrient removal in undiluted wastewater compared to water hyacinth, aquatic plants may have a practical advantage over algal systems in their ease of harvesting.

The relatively poor reduction in SCOD (Table 3) could be attributed to the refractory nature of the

soluble organic matter. Considering that the flushed dairy manure had undergone anaerobic digestion, most of the biodegradable compounds would have been degraded during that process, leaving a predominance of refractory components in the effluent. In particular, the phenolic residues that make up the largest portion of the wastewater SCOD are likely not very degradable and account for the persistence of SCOD after 31 days (Table 4).

The higher DO concentrations in the control/algal systems resulted from the increased photosynthetic oxygen production rate of algae compared to their respiration rate during the daytime. Lower DO concentrations in the plant cultures could be explained by reduced oxygen diffusion from the atmosphere into the water column due to the plant cover, higher root respiration rates, and oxygen uptake by microorganisms attached to the roots.

Differences in DO levels among the plant cultures were due to their different rate of exchange of oxygen from aerial tissue into the root zone. A study conducted by Moorhead and Reddy (1988) to evaluate oxygen transport into the root zone for several floating and emergent aquatic macrophytes showed that the highest transport rates were associated with plants having a small root mass. As root mass increased, the rate of oxygen transport decreased for the plants evaluated, suggesting that older plants and plants with extensive rooting systems probably consume more of the transported oxygen due to higher respiration rates. This phenomenon is clearly depicted in the present study with the highest DO concentration in the pennywort culture, which had the smallest root system compared to water hyacinth and water lettuce.

Electrical conductivity reduction in FAMTS has been reported mainly for water hyacinth. Moorhead (1986) found that for an initial EC of 2300  $\mu\text{S}/\text{cm}$ , a decrease of 1100  $\mu\text{S}/\text{cm}$  (48% reduction) was obtained in a 22-day hydraulic retention time plant culture grown on the effluent of an anaerobic digester receiving water hyacinth as feedstock. At a higher level of salinity (initial EC = 3750  $\mu\text{S}/\text{cm}$ ), Trivedy and Gudekar (1987) reported a decrease of 1725  $\mu\text{S}/\text{cm}$  (46% reduction) at a 4-day hydraulic retention time when the water hyacinth was grown in textile wastewater. These results compare to EC reductions of 52 and 89% for water hyacinth cultured in undiluted and diluted ADFDMW, respectively, in the present study.

A comparison of percentage reduction in EC with biomass yield (Tables 2 and 3) indicates that the reduction in EC may be related to root biomass rather than standing crop biomass, especially when considering the results for pennywort and water lettuce. The water lettuce has a longer and more extensive root system compared to pennywort, which therefore would explain the higher reduction in EC despite a lower biomass yield. The reduction in EC in relation to root biomass could be due to the ability of the roots to take up certain ions. Water hyacinth has the ability to absorb and accumulate or translocate metal, organic and inorganic substances through its roots (Pinto et al., 1987). The marked decrease in  $\text{Na}^+$  concentration in the diluted water hyacinth culture indicates that the uptake of sodium ion during plant growth was substantial (Table 4).

The high biomass yield of the diluted water hyacinth culture, which corresponded with high EC, nutrient and  $\text{Na}^+$  reductions, suggests that EC measurement might be a simple tool to monitor the performance of water hyacinth growth and nutrient reduction. To further examine this correspondence, the reduction rate constants for EC (Table 5) were compared using a Student's *t*-test to the corresponding reduction rate constants for TKN and SRP, and the differences between the rates were not significant for the diluted water hyacinth culture. However, the EC reduction rates for all other treatments (controls and plants) were found to be statistically different than their TKN and SRP reduction rates. This suggests that the use of EC measurements to monitor nutrient uptake may be valid only in FAMTS employing water hyacinths at high growth rates similar to that in the diluted water hyacinth culture.

The percentage reduction of nutrients and physico-chemical parameters of the wastewater in the various treatments at the end of the study demonstrates the relative efficiency of the four plant cultures for use in wastewater remediation (Table 3). Among the plant cultures using the 1:1 dilution, the water hyacinth had the best performance in terms of nutrient removal, SS and VSS reduction as well as reduction in dissolved solids, as shown by the EC and SCOD values. However, in environments where colder weather could impact water hyacinth growth, the potential of alternate plants and polycultures is worthy of consideration.

## 5. Conclusion

This study evaluated the potential of water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*) and pennywort (*Hydrocotyle umbellata*) in improving the water quality of ADFDMW. The growth of water hyacinth was inhibited in undiluted ADFDMW and both pennywort and water lettuce failed to grow in this undiluted medium. In a 1:1 dilution of ADFDMW, all three floating aquatic macrophytes grew successfully. However, growth of pennywort and water lettuce was limited while water hyacinth growth was robust in the diluted medium. High salinity levels appear to be the principal reason for inhibited growth, along with the presence of uncharacterized soluble compounds making up a significant fraction of the wastewater SCOD.

In terms of nutrient, COD, solids and salinity reductions, water hyacinth performed better than water lettuce and pennywort in improving the water quality of diluted ADFDMW. The polyculture exhibited the next best performance compared to the other aquatic plants. The high biomass yield of the diluted water hyacinth culture corresponded with high EC and  $\text{Na}^+$  reductions, suggesting that EC measurement might be a simple tool to monitor performance of water hyacinth growth and nutrient reduction under high plant growth rate conditions.

Reduction in nutrients and COD followed first-order kinetics, with water hyacinth exhibiting the highest rates of removal among the plant cultures (1:1 dilution). For water hyacinth, TKN was reduced by 91.7%,  $\text{NH}_4\text{-N}$  by 99.6%, TP by 98.5%, and SRP by 96.5%. Nitrification occurred in most plant cultures, with  $\text{NO}_3\text{-N}$  being rapidly consumed at low  $\text{NH}_4\text{-N}$  levels. Dissolved oxygen was detected after the third day and ranged from 0.4 to 3.6 mg/l during the study. Oxidation–reduction potentials shifted from  $-285\text{ mV}$  to an average of  $100\text{ mV}$ . Maximum reduction in EC of 89% was obtained in the diluted water hyacinth culture.

The overall performance of the polyculture treatment indicates that such a system has potential in dairy wastewater treatment. Visual observation showed that water hyacinth positively influences growth of pennywort and the growth characteristic of the two plants in terms of their morphology (vertical growth when surface area is completely covered) makes them suitable for polyculture. In order to assess the potential

of such a system for year-round performance, further investigations need to be undertaken during the colder months.

The controls, in which algae flourished, exhibited the highest rate and level of  $\text{NH}_4\text{-N}$  reduction, and the undiluted control/algal culture exhibited higher levels of TP, SRP, SCOD, and alkalinity reduction than the undiluted water hyacinth culture. The high reduction of nutrients in the undiluted control/algal treatment would tend to make such a system more favorable than floating aquatic macrophytes. However, issues such as the environmental impact of ammonia volatilization and the ease of harvest for aquatic plants compared to algae justify consideration of floating aquatic plants for renovating dairy wastewater. While the dilution of ADFDMW required to achieve plant growth presents a challenge to the application of this technology, in practice, effluent from the aquatic plant treatment systems could be used for dilution water to mitigate the effects of high salinity. However, the role of uncharacterized soluble organics in plant growth inhibition requires further research in order to confirm the feasibility of this approach. In addition, year-round experiments should be conducted to evaluate variability of performance due to seasonal changes in light and temperature.

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