NUTRIENT REMOVAL FROM SWINE LAGOON LIQUID BY *LEMNA MINOR* 8627

J. Cheng, L. Landesman, B. A. Bergmann, J. J. Classen, J. W. Howard, Y. T. Yamamoto

ABSTRACT. *Nitrogen and phosphorus removal from swine lagoon liquid by growing* Lemna minor *8627, a promising duckweed identified in previous studies, was investigated under in vitro and field conditions. The rates of nitrogen and phosphorus uptake by the duckweed growing in the in vitro system were as high as 3.36 g m^{-2} day^{-1} and 0.20 g m^{-2} day^{-1}, respectively. The highest nitrogen and phosphorus removal rates in the field duckweed system were 2.11 g m^{-2} day^{-1} and 0.59 g m^{-2} day^{-1}, respectively. The highest observed duckweed growth rate was close to 29 g m^{-2} day^{-1} in both conditions.*

Wastewater concentrations and seasonal climate conditions had direct impacts on the duckweed growth and nutrient removal in outdoor tanks. The rate of duckweed production in diluted swine lagoon liquid increased as the dilution rate increased. Duckweed assimilation was the dominant mechanism for nitrogen and phosphorus removal from the swine lagoon liquid when the nutrient concentration in the wastewater was low, but became less important as nutrient concentration increased. Reasonably high light intensity and a longer period of warm temperature could result in a higher growth rate for the duckweed. Pre–acclimation of the duckweed with swine lagoon liquid could accelerate the start–up of a duckweed system to remove nutrients from the wastewater by preventing the lag phase of duckweed growth.

Keywords. *Ammonium, Duckweed,* Lemna minor*, Nitrogen, Nutrient removal, Phosphorus, Swine wastewater treatment.*

North Carolina has become the second–largest swine producer in the nation, as its swine industry has expanded rapidly in the last decade (USDA, 1999). Large amounts of hog feed are imported into the state, while most of the pork produced is exported out of the state. The swine manure generated in North Carolina contains large amounts of unused nutrients that need to be managed. Currently, most swine manure is treated and temporarily stored in anaerobic lagoons, and the lagoon effluent is irrigated onto the nearby cropland to utilize the nutrients in the effluent. In recent years, concerns have increased over potential environmental contamination from excess nutrients leaking from swine manure treatment lagoons, as well as leaching and runoff from land application fields. Production of aquatic plants to recover nutrients from the wastewater has promise as an alternative technology to convert the nutrients into potentially useful products and prevent nutrient pollution of the environment (Cheng et al., 2002; Classen et al., 2000).

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Duckweed is a small, free–floating aquatic plant belonging to the *Lemnaceae* family (Landolt, 1998). Various duckweed species have been used for the treatment of municipal and industrial wastewaters in many countries, including Bangladesh, Israel, and the U.S. (Alaerts et al., 1996; Culley et al., 1981; Oron, 1994; Oron et al., 1988; van der Steen et al., 1998). Alaerts et al. (1996) demonstrated that duckweed removed 74% of total Kjeldahl nitrogen (TKN) and 77% of total phosphorus (TP) in a Bangladesh sewage lagoon with a hydraulic retention time of 21 days during a local dry season. A final effluent with low values of 2.7 mg/L TKN and 0.4 mg/L TP was produced.

As a plant for wastewater treatment, duckweed has several advantages over other aquatic macrophytes, such as water hyacinth or *Salvinia*. First, duckweed has a high rate of nutrient uptake and preferentially takes up ammonium ions (Oron et al., 1988). Ammonium uptake is critically important for the treatment of swine wastewater, as ammonium is the primary form of nitrogen in this wastewater. Excess ammonium accelerates eutrophication in open ponds and results in nitrate formation if released into groundwater (Oron et al., 1988). Culley et al. (1981) indicated that a mixture of duckweed species could remove 1,378 kg of nitrogen (mostly as ammonium), 347 kg of phosphorus, and 441 kg of potassium from one hectare of water area in one year under the climatic conditions of Louisiana.

Along with efficient nutrient uptake ability, duckweed can tolerate high wastewater nutrient levels. For example, growth of *Spirodela polyrrhiza* in the presence of 1.0 g/L nitrogen and 1.5 g/L phosphorus has been reported (Landolt, 1986). High tolerance to nutrients is particularly valuable for treatment of swine wastewater because its nutrient levels are usually very high (200 to 800 mg/L nitrogen and 30 to 100 mg/L phosphorus). Another advantage of duckweed over other aquatic macrophytes is its ability to tolerate low

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temperatures. A number of duckweed geographic isolates that tolerate low temperatures have been collected from temperate areas (Landolt, 1998). In our previous study, *Lemna gibba* 8678 survived in outdoor wastewater treatment tanks at temperatures below freezing for several days, and resumed its growth when the temperature rose above freezing (Classen et al., 2000). Its cold tolerance allows duckweed to be used for year–round wastewater treatment in areas where tropical macrophytes, such as water hyacinths, can only grow in summer.

Duckweed multiplies vegetatively and accumulates biomass rapidly. Furthermore, duckweed biomass has a high protein content, ranging from 15% to 45% of dry weight (Landolt, 1986). Oron et al. (1988) showed that dry yield of duckweed grown in a wastewater treatment system was as high as 15 g m^{-2} day^{-1} with a protein content of approximately 30%. The high protein content of duckweed indicates a high assimilation capacity of nitrogen, a major pollutant from swine wastewater. Protein content is also an important characteristic for potential end uses of duckweed. Duckweed species have been fed to cattle, poultry, fish, and ducks as a protein–rich feed (Hassan and Edwards, 1992; Haustein et al., 1994; Hillman and Culley, 1978; Robinette et al., 1980; Skillicorn et al., 1993). Duckweed cell walls lack lignin and are easy to digest, making duckweed a good protein source for livestock (Leng et al., 1995).

Previous studies of duckweed in wastewater treatment generally dealt with low nutrient concentrations, such as concentrations up to 100 mg/L TKN in domestic wastewater (Alaerts et al., 1996; Caicedo et al., 2000; Oron, 1994; Oron et al., 1988; van der Steen et al., 1998). In our previous studies, we investigated the use of duckweed for nutrient removal from anaerobically pretreated swine wastewater with high nitrogen and phosphorus contents (up to 345 mg/L TKN and 92 mg/L TP). We identified several duckweed geographic isolates that grew well in a synthetic medium (SAM) that approximated typical swine lagoon liquid in North Carolina (Bergmann et al., 2000a). These duckweed isolates were then tested in a greenhouse using swine lagoon liquid to determine promising duckweed candidates for the treatment of swine wastewater (Bergmann et al., 2000b). Three duckweed candidates, *Spirodela punctata* 7776, *Lemna gibba* 8678, and *Lemna minor* 8627, were superior to other isolates in biomass accumulation, nutrient recovery from the wastewater, and total protein production.

In addition to its great promise for wastewater treatment, duckweed, particularly some *Lemna* species, has an opportunity for future improvements through genetic engineering. Simple protocols for transferring foreign genes into both *L. gibba* and *L. minor* have been demonstrated (Yamamoto et al., 2001). This technology would allow manipulation of duckweed to remove more nutrients or other specific pollutants, or to generate products with a high commercial value.

The objective of the research presented here was to understand the dynamics of nutrient removal from swine lagoon liquid by *L. minor* 8627 under both in vitro and field conditions. The intrinsic nutrient uptake rate of the duckweed and its growth rate in relation to nutrient concentrations in the synthetic medium were determined under controlled conditions in the in vitro tests. The effects of light and temperature in different seasons on nutrient removal and duckweed growth in the duckweed system for swine lagoon liquid treatment were investigated under field conditions. This information will be useful in the design and operation of duckweed–based nutrient removal systems for swine wastewater treatment.

Materials and Methods

Duckweed

L. minor 8627 is a geographic isolate originally collected from Denmark in the worldwide duckweed germplasm collection (Landolt, 1998). Routine sterile cultures of *L. minor* 8627 were maintained in Schenk and Hildebrandt (SH) medium (Schenk and Hildebrandt, 1972) with 1% sucrose as a carbon source in a growth chamber at a constant temperature of 23° C. Lighting at a photosynthetic photon flux density of 40 μmol m^{-2} s^{-1} in a 16–hour photoperiod per day was provided by wide–spectrum fluorescent tubes (Bergmann et al., 2000a). The duckweed was cultured in the SH medium supplemented with 3% sucrose for two weeks prior to the transfer to the synthetic swine lagoon liquid (SAM) for in vitro tests. For field experiments, duckweed was first grown in the SH medium with 3% sucrose for two weeks, then in SAM containing 3% sucrose for two weeks, and bulked in a greenhouse using diluted (50%) swine lagoon liquid for six weeks.

In Vitro Tests

In order to determine the intrinsic dynamics of nutrient uptake from the synthetic medium (SAM) by the duckweed and its growth in relation to nutrient concentrations in the medium, other environmental parameters, such as initial duckweed biomass, temperature, and light intensity, were maintained constant. The synthetic medium (SAM) was formulated to closely resemble the nutrient profile of typical swine lagoon liquid in North Carolina (Bergmann et al., 2000a). Nutrient concentrations of SAM used as the medium in the in vitro tests are listed in table 1.

Batch tests on the rate of nutrient (N and P) uptake from SAM by *L. minor* 8627 were conducted using 300 mL (5.1 × 5.1 × 11.6 cm) polypropylene boxes (Magenta Corp.,

Table 1. Ion concentrations and pH of the buffered synthetic medium (SAM).

Ion (mg/L):	Buffered SAM[a]
NH_4–N	336.0
P	23.80
K	483.6
Ca	496
Mg	39.6
Cl	301.8
Fe	7.896
S	274.9
Na	175.5
B	0.693
Mn	8.8
Zn	3.074
Cu	1.207
Mo	0.02
Co	0.025
pH	7.0

[a] Buffering was accomplished by addition of 1.5 g/L citric acid.

Chicago, Ill.) in a growth chamber. Each box had a surface area of 25.8 cm^2 and contained 150 mL (5.8 cm deep in the box) of SAM. To prevent bacterial contamination, the medium was sterilized in an autoclave for 30 minutes at 121° C prior to use.

Four batch tests were conducted to test four dilutions of SAM (100%, 75%, 50%, and 25% strength). Each batch test consisted of 52 boxes containing a dilution of SAM. Of these boxes, 39 contained duckweed cultures, corresponding to triplicate samples for 13 time points, while 13 control boxes did not have any duckweed. Each duckweed culture was initiated with approximately the same amount of *L. minor* 8627 to cover the whole surface area with a single layer of duckweed. All boxes were then placed in the 23° C growth chamber with the same lighting condition as routine cultures. The medium and the duckweed in each box were mixed briefly every day to ensure uniform distribution. Because growing duckweed decreased the medium pH, it was necessary to adjust the pH to approximately 7.0 with drops of 10 M NaOH. During each test, three duckweed cultures and one control were removed for destructive sampling at every 48 hours to monitor the nutrient level and duckweed growth. Each batch test lasted for 22 to 24 days.

Field Experiments

In order to investigate nutrient removal from swine lagoon liquid by *L. minor* 8627 under natural climate conditions of North Carolina, field experiments were conducted using concrete outdoor tanks built adjacent to a swine waste treatment lagoon at the Lake Wheeler Road Field Laboratory of North Carolina State University in Raleigh, North Carolina. Seasonal effect on duckweed growth and nutrient removal was determined. The performance of the duckweed on the real swine lagoon liquid at different initial nutrient concentrations was compared with that in the in vitro tests. Two outdoor tanks were partitioned into 8 cells with PVC wallboard partitions. Each cell was 122 cm deep and had a surface area of 152 × 84 cm. The water depth in each cell during the experiments was maintained at 91 cm by adding tap water to compensate for evaporation and transpiration. The water volume and surface area of each cell were 1.16 m^3 and 1.28 m^2, respectively.

Two batch experiments were performed during consecutive two–month periods in 1999: one from late May through late July (referred to hereafter as the spring experiment), and the other from mid–August through mid–October (referred to hereafter as the fall experiment). Since our previous experimental results had indicated that full–strength swine lagoon liquid did not allow healthy growth of *L. minor* 8627 in a greenhouse (Bergmann et al., 2000a), four dilutions (approximately 50%, 33%, 25%, and 20%) of the swine lagoon liquid were prepared with tap water in duplicate cells. At the initiation of each test, 2 kg (wet weight) of seed duckweed, enough to cover the surface of a cell, were added to each cell. The wastewater and duckweed in each cell were gently stirred for about 5 minutes every day.

Wastewater and duckweed samples were taken every week after stirring to monitor the nutrient level in each cell. The biomass production was monitored by harvesting duckweed present on approximately 20% of the surface area of each cell every Monday, Wednesday, and Friday. Frequent harvesting also encouraged duckweed growth and helped maintain healthy culture. The wet weight of the harvested duckweed was determined with a field balance after excess water was removed with a paper towel. Wet duckweed samples were dried in an oven at 105° C for about 12 hours to determine the dry weight. Temperature in the wastewater was monitored with a StowAway TidbiT temperature recorder (Onset Computer Corporation, Bourne, Mass.), and the light intensity at the duckweed surface was recorded with a LICOR light meter (LICOR, Inc., Lincoln, Neb.). The temperature and light intensity data were recorded every 15 min with dataloggers.

Chemical Analysis

Samples from the media in both in vitro tests and field experiments were analyzed for TKN, ammonium nitrogen (NH_4–N), nitrate nitrogen (NO_3–N), TP, ortho–phosphate–phosphorus (o–PO_4–P), chemical oxygen demand (COD), total organic carbon (TOC), and pH. The moisture content of the duckweed was determined from the wet and dry weights. The dried duckweed biomass was analyzed for TKN and TP contents for the samples from the field experiments. All chemical analyses were conducted in the Environmental Analysis Laboratory of the Biological and Agricultural Engineering Department at North Carolina State University using EPA Methods (EPA, 1983) and APHA Standard Methods (APHA, 1995).

Results and Discussion

Performance of *L. Minor* 8627 in a Controlled Environment

Results from the in vitro tests demonstrated that the duckweed efficiently removed nitrogen and phosphorus from the synthetic medium (SAM) and incorporated them into its biomass. Figure 1 shows nitrogen and phosphorus removal from different dilutions (100%, 75%, 50%, and 25% strength) of SAM by growing *L. minor* 8627 and the duckweed growth during the in vitro tests. There were clearly two phases of nutrient uptake by the duckweed for both nitrogen and phosphorus in all the tests: an initial slow uptake, followed by a rapid uptake (figs. 1a and 1b). The removal of NH_4–N and o–PO_4–P from the media followed the same pattern as that of TKN and total–P, respectively (fig. 2). However, the duckweed growth experienced three phases: a lag phase, an exponential growth phase, and a linear growth phase (fig. 1c). The lag phase lasted for approximately 100 hours in all the tests, and the duckweed weight was almost constant in this phase. During the exponential growth phase, approximately from 100 to 336 hours, there were only small differences in duckweed growth rates in different dilutions of SAM. Beyond 336 hours, most nitrogen and phosphorus were removed from the media, and a linear duckweed growth was observed at about the same rate in all treatment conditions.

The values of the COD, TOC, TKN, NH_4–N, TP, and o–PO_4–P concentrations and pH in the control boxes without duckweed were almost constant during each in vitro test (data not shown). This indicates that biological activity other than duckweed growth can be neglected in these tests, and nitrogen and phosphorus removal from the media during the tests was solely a result of nutrient uptake by the duckweed.

The apparent lag phase during the first 100 hours was most likely due to the abrupt change in growth conditions for the

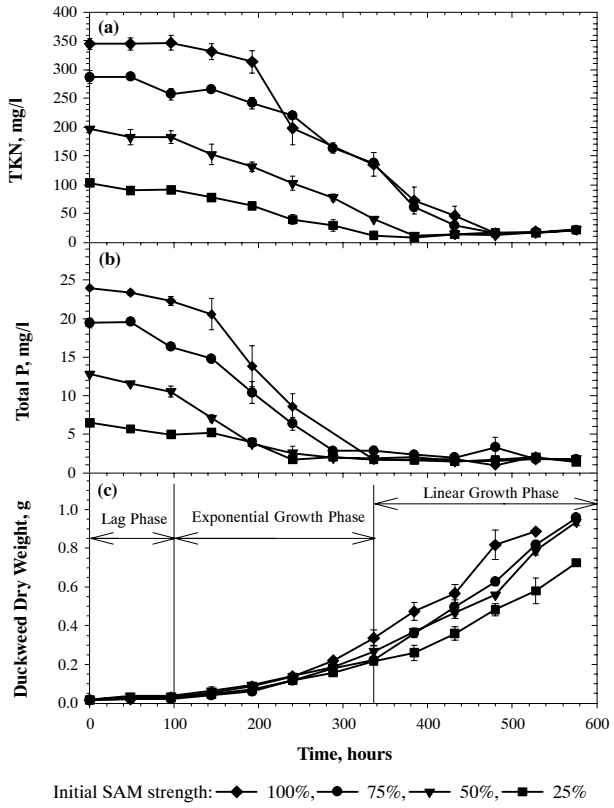

Figure 1. Removal of (a) total Kjeldahl N (TKN) and (b) total P from different dilutions of a synthetic swine lagoon liquid (SAM) by growing *Lemna minor* 8627, and (c) the duckweed growth in a growth chamber (temperature = 23° C; photon flux density = 40 µmol m^{-2} s^{-1}; photoperiod = 16 hours/day; medium volume = 150 mL; surface area = 25.8 cm^2).

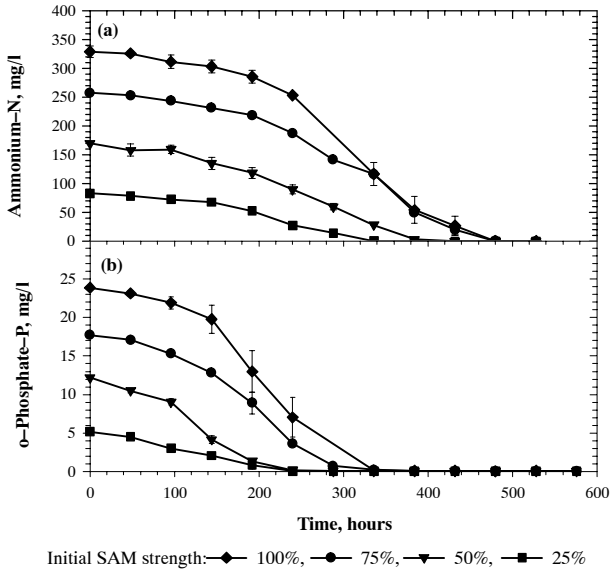

Figure 2. Removal of (a) ammonium–N and (b) ortho–phosphate–P from different dilutions of a synthetic swine lagoon liquid (SAM) by growing *Lemna minor* 8627 in a growth chamber (temperature = 23° C; photon flux density = 40 µmol m^{-2} s^{-1}; photoperiod = 16 hours/day; medium volume = 150 mL; surface area = 25.8 cm^2).

duckweed from the SH medium to SAM. For example, the majority of nitrogen in the SH medium was provided in the nitrate form, while SAM contained mostly ammonium nitrogen. The SH medium also contained organic components, such as myo–inositol and vitamins, that were not present in SAM. These differences probably necessitated physiological adjustments, and may have hindered duckweed growth. It is notable that there was a slight N and P reduction in the media during the lag period. This phenomenon indicates that the duckweed accumulated N and P in its cells without increasing its weight during the lag phase, resulting in a higher N and P contents of the duckweed. Landolt (1986) indicated that N and P contents of duckweed increase with the increase of N and P concentrations in the medium. Our observation agrees well with Landolt's conclusion.

After the duckweed was acclimated to the new media, it began to grow and rapidly remove nutrients from the media. Generally, higher rates of nitrogen and phosphorus uptake and duckweed growth occurred in the media with higher nutrient concentrations (fig. 1). The highest TKN uptake rate was observed in the 100% strength SAM at around 240 hours and was 0.014 mg cm^{-2} h^{-1} or 3.36 g m^{-2} day^{-1}. The highest TP uptake rate was observed in the 100% strength SAM at around 192 hours and was 8.2×10^{-4} mg cm^{-2} h^{-1} or 0.20 g m^{-2} day^{-1}. After the exponential growth, during which most N and P were removed from the media, duckweed growth rates in all different dilutions of SAM were similar (fig. 1c). The average duckweed growth rate during the linear phase was 1.19 g (dry) m^{-2} h^{-1} or 28.6 g m^{-2} day^{-1}.

Nutrient Removal from Swine Lagoon Liquid in Outdoor Tanks with Growing *L. Minor* 8627

Two batch experiments were conducted to study the nutrient dynamics of a duckweed–based swine wastewater renovation system in outdoor tanks under natural climate conditions of Raleigh, North Carolina, in the spring and fall of 1999. Different dilutions (50%, 33%, 25%, and 20%) of the swine lagoon liquid were used as media for growing duckweed, and the average N and P concentrations and other characteristics of each dilution are listed in table 2. Generally, the P:N ratio in the initial media in the fall experiment (0.47 to 0.62) was much higher than that in the spring experiment (0.30 to 0.34). Ortho–phosphate, in particular, was nearly non–existent in the spring wastewater, while it was relatively high in the fall lagoon liquid (table 2).

The pH in the testing cells of outdoor tanks with diluted swine lagoon liquid was fairly stable within a range of 7.1 to 8.1 during both experiments, indicating that the swine lagoon liquid had a strong buffering capacity. This is very different from SAM to which NaOH had to be added to maintain the neutral pH. The strong buffering capacity of the swine lagoon liquid is very important for maintaining duckweed growth because growth tends to lower the pH value of the media quickly without a buffer (from approximately 7.0 to approximately 5.0 within 24 hours).

Significant COD and TOC reduction was observed in both experiments. COD and TOC were reduced by 51% to 74% and 61% to 67%, respectively, in the spring experiment, and by 62% to 76% and 52% to 73%, respectively, in the fall experiment. This indicates that bacteria were quite active in the testing cells during the experiments.

Linear or close to linear rates of N and P removal and duckweed growth were observed in both field experiments (figs. 3 and 4), unlike in the controlled environment described in the preceding section. Considerable amounts of nutrients were still present in the more concentrated waste–

Table 2. Initial conditions of diluted swine lagoon liquid used as media for growing *L. minor* 8627 in batch field experiments in Raleigh, North Carolina, in 1999.

		Nutrient Concentration (mg/L)[b]				COD[b]	TOC[b]	
Dilution of Swine Lagoon Liquid as Medium[a]		TKN	NH_4–N	TP	o–PO_4–P	(mg/L)	(mg/L)	pH[b]
Spring Experiment	50%	140	83	47	0.4	783	221	7.50
	33%	100	58	34	0.2	550	142	7.38
	25%	73	42	24	0.1	403	102	7.25
	20%	54	31	16	0.0	302	77	7.12
Fall Experiment	50%	196	112	92	43	1,058	434	7.93
	33%	86	51	45	27	507	149	8.05
	25%	48	40	26	21	215	57	8.00
	20%	34	26	21	18	178	46	7.83

[a] The actual initial nutrient concentrations may not be exactly the same as intended because of the deviations caused in the dilution operation in the field tanks.

[b] Each datum is an average of the results obtained from two replicate experimental cells.

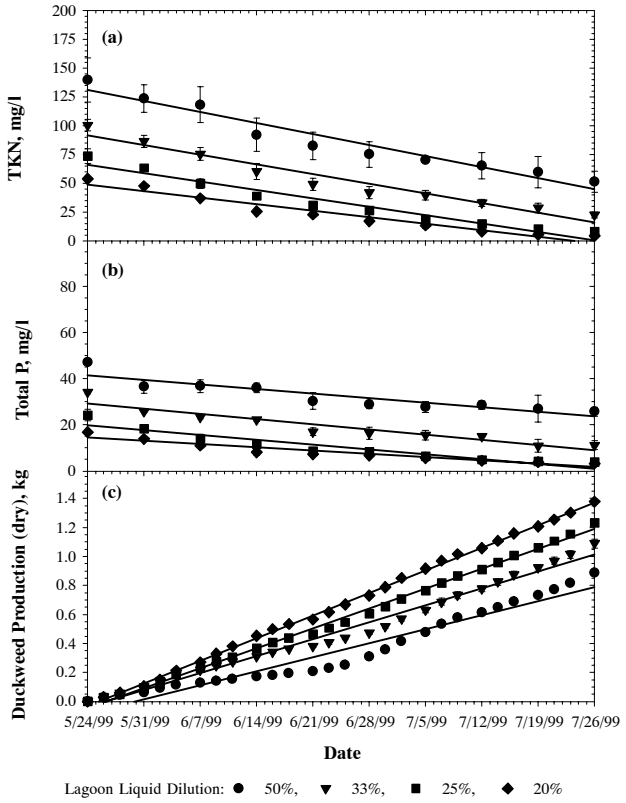

Figure 3. Removal of (a) total Kjeldahl N (TKN) and (b) total P from different dilutions of a swine lagoon liquid by growing *Lemna minor* 8627, and (c) the duckweed production under natural climate conditions in spring (May 24 through July 26) 1999 in Raleigh, North Carolina.

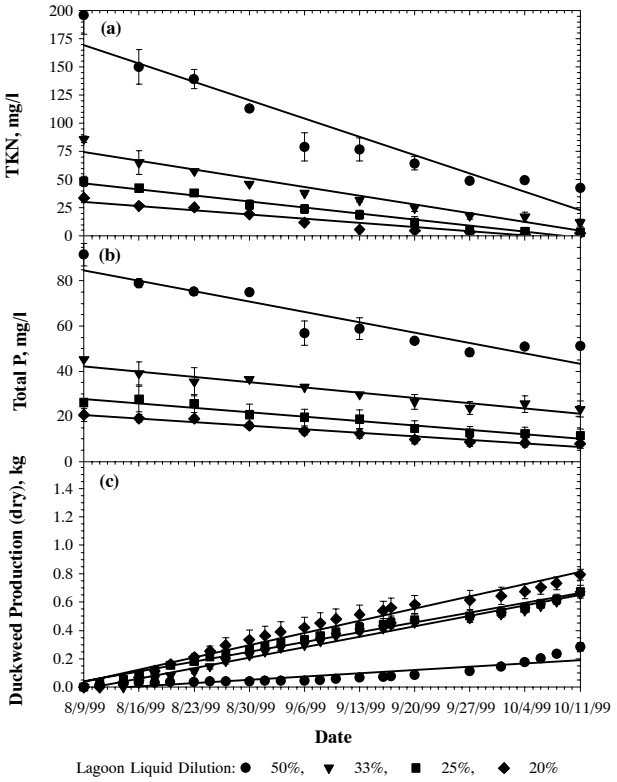

Figure 4. Removal of (a) total Kjeldahl N (TKN) and (b) total P from different dilutions of a swine lagoon liquid by growing *Lemna minor* 8627, and (c) the duckweed production under natural climate conditions in fall (August 9 through October 11) 1999 in Raleigh, North Carolina.

water at the end of the experiments. In the field tests, no lag phase in duckweed growth was observed, probably because the seed duckweed was acclimated to the nutritional environment of swine lagoon liquid during the greenhouse production period. Perniel et al. (1998) also suggested that an acclimation period would help duckweed adjust to drastic changes within a system. The field experiment results for N and P reduction and duckweed growth in various levels of diluted swine lagoon liquid show that the nutrient removal rates were generally higher in more concentrated wastewater, while the duckweed growth rate was higher in more diluted wastewater (table 3), which was different from the in vitro results.

The mass balance analysis presented in table 4 indicates that a large portion of removed nutrients was not taken up by the duckweed, especially in the 50% dilution of the fall experiment, which agrees with the growth data (table 3). Despite low rates of duckweed growth and nutrient uptake, the rate of overall nutrient removal was the highest in cells containing the most concentrated wastewater. This suggests that wastewater with higher nutrient concentrations may be more favorable to microbial growth than to duckweed growth. Some nitrogen was probably lost through ammonia volatilization, algal and microbial assimilation, and nitrification/denitrification (Al–Nozaily et al., 2000). Bonomo et al. (1997) reported that at pH values lower than 8.0, ammonia

Table 3. Nutrient (N and P) removal and duckweed growth rates in batch experiments with *L. minor* 8627 growing on diluted swine lagoon liquid under the natural climate condition of Raleigh, North Carolina, in 1999.

Dilution of Swine Lagoon Liquid as Medium		Nutrient Removal Rate ($g\ m^{-2}\ day^{-1}$)[a]			Duckweed Growth Rate[a] ($g\ m^{-2}\ day^{-1}$)
		TKN	NH_4–N	TP	
Spring Experiment	50%	1.24	0.59	0.26	17.6
	33%	1.09	0.60	0.29	21.3
	25%	0.95	0.58	0.27	25.0
	20%	0.73	0.47	0.18	28.5
Fall Experiment	50%	2.11	1.29	0.59	4.3
	33%	1.00	0.59	0.30	13.5
	25%	0.70	0.61	0.25	12.7
	20%	0.48	0.40	0.20	15.7

[a] Each datum is an average of the results obtained from two replicate experimental cells.

volatilization in duckweed wastewater treatment systems was negligible, indicating that it could be neglected in our experiments. NH_4–N removal from the media followed the same pattern as TKN removal in both field experiments (figs. 5a and 6a).

At the beginning of the spring experiment, there was almost no o–PO_4–P in swine lagoon liquid (table 2), and its level fluctuated during the test (fig. 5b). This was probably due to bacterial degradation of organics in the wastewater and subsequent phosphorus uptake by the duckweed. During the fall experiment, o–PO_4–P also fluctuated but generally decreased during the experiment (fig. 6b). In addition to duckweed uptake, phosphate removal may have also occurred by microbial assimilation, precipitation with some minerals, and adsorption onto clay and organic matter (Al–Nozaily et al., 2000).

Overall, duckweed production rate was much lower during the fall experiment. This is likely due to a combination of climatic factors. Both light intensity and temperature were lower during the fall experiment (fig. 7) and day length was shorter, less favorable to duckweed growth. Differences in nutrient concentrations were probably not as important because the most diluted wastewater with the highest duckweed growth in the fall experiment did not support

Table 4. Mass balance of total N and P removal and the nutrient uptake by duckweed, *Lemna minor* 8627 from the diluted swine lagoon liquid in two batch field experiments under the natural climate condition of Raleigh, North Carolina, in 1999.

Dilution of Swine Lagoon Liquid as Medium		Total Nutrient Removal from the Media[a]				Nutrient Uptake by the Duckweed[a]			
		g		%		g		% of Total Removal	
		N	P	N	P	N	P	N	P
Spring Experiment	50%	102.4	24.9	63.1	45.7	50.3	11.9	49.1	47.8
	33%	90.5	26.8	78.0	68.0	63.0	16.5	69.6	61.6
	25%	76.0	23.5	89.7	84.4	66.4	18.6	87.4	79.1
	20%	57.4	15.8	91.6	85.1	51.6	14.0	90.0	88.6
Fall Experiment	50%	178.1	46.7	78.3	43.8	16.8	6.0	9.4	12.8
	33%	86.1	25.6	86.3	49.0	41.7	13.4	48.4	52.3
	25%	52.1	16.9	93.6	56.0	37.3	11.8	71.6	69.8
	20%	36.2	14.8	91.8	60.8	35.9	11.5	99.2	77.7

[a] Each datum is an average of the results obtained from two replicate experimental cells.

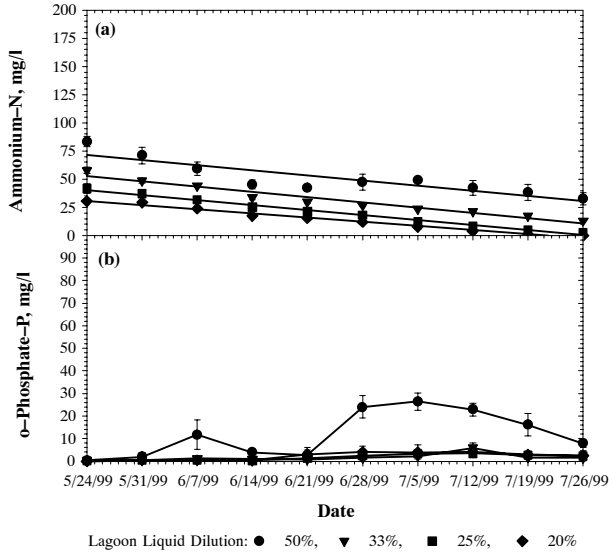

Figure 5. Concentrations of (a) ammonium–N and (b) ortho–phosphate–P in diluted swine lagoon liquid for growing *Lemna minor* 8627 under natural climate conditions in spring (May 24 through July 26) 1999 in Raleigh, North Carolina.

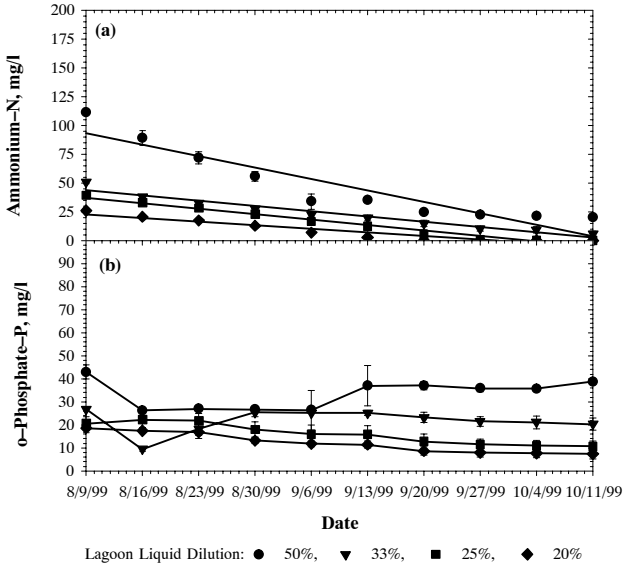

Figure 6. Concentrations of (a) ammonium–N and (b) ortho–phosphate–P in diluted swine lagoon liquid for growing *Lemna minor* 8627 under natural climate conditions in fall (August 9 through October 11) 1999 in Raleigh, North Carolina.

nearly as much duckweed growth as the most concentrated wastewater, the least productive environment in the spring experiment (figs. 3c and 4c). Significant short–term temperature fluctuations were observed in both spring and fall field experiments (fig. 7b). This is due to a relatively small amount of liquid in each tank. Normally, this kind of temperature fluctuations would not be expected in lagoons or ponds for animal wastewater treatment or storage.

Clearly, duckweed yields were lower when grown in the presence of higher nutrient concentrations. This observation does not agree with the in vitro study, in which duckweed yields and nutrient concentrations showed a positive correlation up to 345 mg/L TKN and 24 mg/L TP in the 100% strength SAM. This could be due to a number of factors, such as the presence of undefined chemical components as well as other organisms such as microbes and insects in the field condition. Furthermore, duckweed grown in vitro depended mostly on sucrose in SAM for its energy source, while field–grown duckweed depended solely on photosynthesis, leading to very different physiological states. Nevertheless, the highest duckweed growth rate in the outdoor experiment (28.5 g m^{-2} day^{-1}) was very close to the duckweed growth rate in the lab test (28.6 g m^{-2} day^{-1}). These numbers are almost twice as high as a reported value of 15 g m^{-2} day^{-1} in municipal wastewater (Oron et al., 1988).

While both nitrogen and phosphorus uptake by the duckweed was the sole mechanism of the nutrient removal from SAM in the in vitro tests, it played only partial role in nitrogen and phosphorus removal from the swine lagoon liquid in the field experiments. Furthermore, relatively concentrated wastewater appears to inhibit growth and nutrient uptake by the duckweed under the field conditions. This effect is probably not due to the high nitrogen concentration because much higher nitrogen level in SAM is not inhibitory to duckweed growth or nutrient uptake. Reasons that cause lower duckweed growth rate in more concentrated swine lagoon liquid need to be further investigated. Effects of high phosphorus content or other components, such as other organisms, need to be determined in the future.

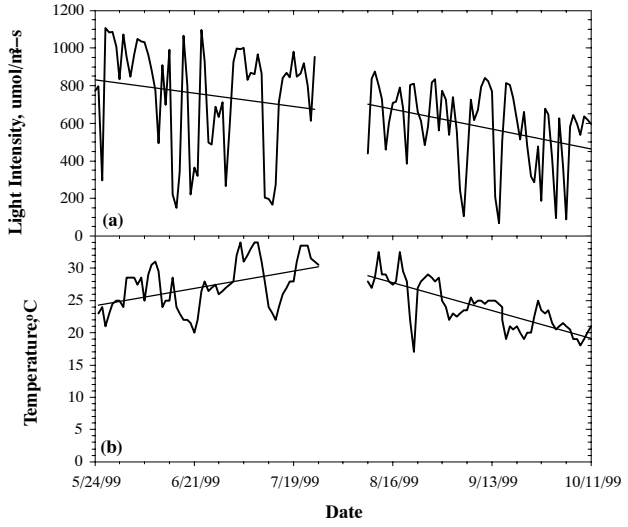

Figure 7. Light intensity (a) and water temperature (b) during the two field experiments on nutrient removal from a swine lagoon liquid by growing *Lemna minor* 8627 under natural climate conditions of spring (May 24 through July 26) and fall (August 9 through October 11) 1999 in Raleigh, North Carolina.

Data reported here indicate that wastewater should be diluted to at least below 100 mg/L TKN and 50 mg/L TP to maintain rapid growth and nutrient uptake by the duckweed. In the design and operation of a duckweed pond for secondary treatment of swine wastewater, these data would be very useful to the initiation of the duckweed system. To establish a duckweed mat in the pond, diluted swine lagoon liquid should be used for initial rapid growth of the duckweed. Once the duckweed mat is established, the duckweed would remove nutrients from the liquid and lower the nutrient concentrations in the liquid. When the nutrient concentrations are reduced to a desired level, the duckweed pond would be ready to take pretreated swine wastewater continuously.

The pretreated swine wastewater usually contains high nutrient concentrations, which are not favorable for rapid duckweed growth and nutrient uptake. To avoid nutrient shock loading on the duckweed, we recommend that some of the duckweed–treated liquid be recycled and mixed with the influent wastewater to lower the influent nutrient concentrations. In the normal operation of the duckweed pond, wastewater would flow into the pond continuously or semi–continuously, and the duckweed would be harvested periodically. The duckweed–treated liquid can be used for crop irrigation. The influent wastewater flow rate (or the size of the duckweed pond) and the amount of duckweed to be harvested should be determined by the duckweed growth and nutrient uptake rates.

Lemna minor 8627 has shown its capability of removing nitrogen and phosphorus from swine lagoon liquid under field conditions. Nevertheless, it is important to establish duckweed as a useful product to apply to in–field swine wastewater treatment systems. Although research reports have shown that duckweed is a good source of proteins for livestock, poultry, and fish feed, as mentioned earlier in this article, a market for utilizing duckweed has yet to be developed. Economic analysis of the whole duckweed wastewater polishing system is necessary to determine the cost–effectiveness of the system.

CONCLUSIONS

In summary, the following specific conclusions may be drawn from the work presented here:

- *Lemna minor* 8627 grew well on synthetic and actual swine lagoon liquid and effectively removed N and P from the wastewaters. The highest observed duckweed growth rate was close to 29 g m^{-2} day^{-1} in both systems. The rates of N and P uptake by the duckweed growing in the synthetic media were as high as 3.36 g m^{-2} day^{-1} and 0.20 g m^{-2} day^{-1}, respectively.
- Pre–acclimation of the duckweed with swine lagoon liquid could prevent the lag phase of duckweed growth, allowing efficient nutrient uptake throughout a treatment period.
- The relatively concentrated wastewater inhibited the duckweed growth and nutrient uptake from swine lagoon liquid.
- High light intensity and longer periods of warm temperature during the spring experiment resulted in a higher growth rate for the duckweed on diluted swine lagoon liquid.

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REFERENCES

Alaerts, G. J., M. D. Rahman Mahbubar, and P. Kelderman. 1996. Performance analysis of a full–scale duckweed–covered sewage lagoon. *Water Res.* 30(4): 843–852.

Al–Nozaily, F., G. Alaerts, and S. Veenstra. 2000. Performance of duckweed–covered sewage lagoons: II. Nitrogen and phosphorus balance and plant productivity. *Water Res*. 34(10): 2734–2741.

APHA. 1995. *Standard Methods for the Examination of Water and Wastewater*, 19th ed. Washington, D.C.: American Public Health Association, American Water Works Association, and Water Environment Federation.

Bergmann, B. A., J. Cheng, J. J. Classen, and A.–M. Stomp. 2000a. *In vitro* selection of duckweed geographical isolates for potential use in swine lagoon effluent renovation. *Bioresource Technol.* 73(1): 13–20.

_____. 2000b. Nutrient removal from swine lagoon effluent by duckweed. *Trans. ASAE* 43(2): 263–269.

Bonomo, L., G. Pastorelli, and N. Zambon. 1997. Advantages and limitations of duckweed–based wastewater treatment systems. *Water Sci. Tech.* 35(5): 239–246.

Caicedo, J. R., N. P. van der Steen, O. Arce, and H. J. Gijzen. 2000. Effect of total ammonia nitrogen concentration and pH on growth rates of duckweed (*Spirodela polyrrhiza*). *Water Res.* 34(15): 3829–3835.

Cheng, J., B. A. Bergmann, J. J. Classen, A.–M. Stomp, and J. W. Howard. 2002. Nutrient recovery from swine lagoon water by *Spirodela punctata. Bioresource Technology* 81(1): 81–85.

Classen, J. J., J. Cheng, B. A. Bergmann, and A.–M. Stomp. 2000. *Lemna gibba* growth and nutrient uptake in response to different nutrient levels. In *Animal, Agricultural, and Food Processing Waste: Proc. Eighth International Symposium*, 40–45.Oct. 9–11. Des Moines, Iowa. St. Joseph, Mich.: ASAE.

Culley, D. D., Jr., E. Rejmankova, J. Kvet, and J. B. Frye. 1981. Production, chemical quality, and use of duckweed (*Lemnaceae*) in aquaculture, waste management, and animal feeds. *J. World Maric. Soc.* 12(2): 27–49.

EPA. 1983. *Methods for Chemical Analysis of Water and Waste.* Cincinnati, Ohio: U.S. Environmental Protection Agency.

Hassan, M. S., and P. Edwards. 1992. Evaluation of duckweed (*Lemna perpusilla* and *Spirodela polyrrhiza*) as feed for Nile tilapia (*Oreochromis niloticus*). *Aquaculture* 104(3–4): 315–326.

Haustein, A. T., R. H. Gilman, P. W. Skillicorn, H. Hannan, F. Diaz, V. Guevara, V. Vergara, A. Gastanaduy, and J. B. Gilman. 1994. Performance of broiler chickens fed diets containing duckweed (*Lemna gibba)*. *J. Agric. Sci. Cambridge* 122(part 2): 285–289.

Hillman, W. S., and D. D. Culley, Jr. 1978. The use of duckweed. *Amer. Scientist* 66(4): 442–451.

Landolt, E. 1986. Biosystematic investigation on the family of duckweeds: The family of *Lemnaceae.* A monograph study. Zurich, Switzerland: Geobotanischen Institute ETH.

_____. 1998. Anatomy of the *Lemnaceae* (duckweeds). In *Extreme Adaptations in Angiospermous Hydrophytes*, 1–116. Berlin, Germany: Gebrüder Borntraeger.

Leng, R. A., J. H. Stambolie, and R. Bell. 1995. Duckweed: A potential high–protein feed resource for domestic animals and fish. *Livestock Research for Rural Development* 7(1): 1–12. Available at: http://www.cipav.org.co/lrrd/lrrd7/1/3.htm.

Oron, G. 1994. Duckweed culture for wastewater renovation and biomass production. *Agric. Water Management* 26(1–2): 27–40.

Oron, G., A. de–Vegt, and D. Porath. 1988. Nitrogen removal and conversion by duckweed grown on wastewater. *Water Res.* 22(2): 179–184.

Perniel, M., R. Ruan, and B. Martinez. 1998. Nutrient removal from a stormwater detention pond using duckweed. *Applied Eng. in Agric.* 14(6): 605–609.

Robinette, H. R., M. W. Brunson, and E. J. Day. 1980. Use of duckweed in diets of channel catfish. *Proc. Annual Conf. S.E. Assoc. Fish Wildlife Agencies.* 34: 108–114.

Schenk, R. U., and A. C. Hildebrandt. 1972. Medium and techniques for induction and growth of monocotyledonous and dicotyledonous plant cell cultures. *Can. J. Bot.* 50(1): 199–204.

Skillicorn, P., W. Spira, and W. Journey. 1993. Duckweed aquaculture. Washington, D.C.: The International Bank for Reconstruction and Development/The World Bank.

USDA. 1999. National agricultural statistics. Washington, D.C.: USDA National Agricultural Statistics Service.

van der Steen, P., A. Brenner, and G. Oron. 1998. An integrated duckweed and algae pond system for nitrogen removal and renovation. *Water Sci. Tech.* 38(1): 335–343.

Yamamoto, Y. T., N. Rajbhandari, X. Lin, B. A. Bergmann, Y. Nishimura, and A.–M. Stomp. 2001. Genetic transformation of duckweed *Lemna gibba* and *Lemna minor*. *In Vitro Cellular and Developmental Biology – Plant.* 37(3): 349–353.