ABSTRACT

LYERLY, COURTNEY NEIL. Swine Wastewater Treatment in an Integrated System of Anaerobic Digestion and Duckweed Nutrient Removal: Pilot Study. (Under the direction of Jiayang Cheng.)

Organics destruction and nutrient uptake in an integrated pilot system of anaerobic digestion and duckweed nutrient removal for swine wastewater treatment were monitored under field conditions. Raw swine wastewater of 100 gallons/day was first treated in a 1,000-gallon anaerobic digester with floating ballast rings. Organic compounds in the wastewater were digested to produce biogas. Many nutrients including nitrogen and phosphorus remain in the effluent of the anaerobic digester. Three duckweeds (*Lemna gibba* 8678, *Lemna minor* 8627, and *Spirodela, punctata* 7776) were grown in three 1,000-gallon tanks to recover nutrients from the anaerobic effluent. The duckweed was periodically harvested and can be used as animal, poultry, and fish feed. The Three species were compared for growth and nutrient removal characteristics. This research provides an initial understanding of the attached-growth anaerobic digester and the characteristics exhibited by duckweed in the treatment of swine wastewater under conditions similar to those found in North Carolina. Both the anaerobic digester and the duckweed tanks were run as completely mixed systems. The performance of the system was monitored by measuring chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN), ammonia nitrogen, total phosphorus (TP), ortho-phosphate-phosphorus, and pH in the influent and effluent of each treatment unit.

SWINE WASTEWATER TREATMENT IN AN INTEGRATED SYSTEM OF ANAEROBIG DIGESTION AND DUCKWEED NUTRIENT REMOVAL: PILOT STUDY

by
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A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the Degree of
Masters of Science

BIOLOGICAL AND AGRICULTURAL ENGINEERING

Raleigh

2004

APPROVED BY:

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BIOGRAPHY

Courtney Lyerly was born in Augusta, GA. He is the son of Linda and Neil Lyerly and has a brother, Daniel. He attended Clemson University in Clemson, SC, where he earned his bachelor’s degree in Biosystems Engineering in 2001. In May of 2002, he began his master's studies at North Carolina State University in Biological and Agricultural Engineering.

ACKNOWLEDGEMENTS

This work could not have been completed without the continual support of my advisor Dr. Cheng. I would like to thank Dr. Anne Stomp for her support in work with the duckweed plants, as well as Rachel Huie and the NCSU Environmental Analysis Laboratory for their hard work. Thanks to my other advisors Dr. Liehr and Dr. de los Reyes for their support. Finally thanks to my family and all the friends around Weaver Labs and N.C. State who supported me during work and play.

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Agricultural Wastewater Treatment

In North Carolina the rural landscape is dominated by agriculture particularly by hog farms in the eastern the part of the state. The state is also blessed with a very beautiful natural environment that includes many river basins, lowland creeks and wetlands. North Carolina is in the middle of a period of reevaluation of the dynamics between these two facets of life. The hog farms provide a large boost to the state's economy through production and also by providing jobs. The farms also produce large amounts of waste that must be prevented from reaching surrounding waters. The question being addressed is: what are the impacts on the environment from this industry? The concerns that surround hog waste are: the release of nutrients, organic matter, dissolved solids, pathogens, and odorous compounds to ground water, surface water, and atmosphere near the farms.

In the past 30 years there have been sweeping changes in the structure of the swine industry. The number of head in North Carolina has grown from around 2 million to near 10 million, while the number of farms has sharply declined from nearly 60,000 operations to only about 500 today. This large concentration of animals in farms has created concerns about the level of nutrients in waste that must be treated. The animals only consume about 50% of the nutrients in the feed. Therefore, half of the nutrients shipped to a farm to feed the animals go directly to the waste stream. This large influx of nitrogen and phosphorus can potentially disrupt the natural cycles in the surrounding environment.

In response to these concerns, the U.S. Environmental Protection Agency (EPA) has set up guidelines for Concentrated Animal Feeding Operations (CAFOs). These guidelines originate from the Clean Water Act (CWA), which regulates the discharge of pollutants from both point and non-point sources. Revisions of the CWA in 2001 required all farms with more than 1000 animal units (2,500 adult swine) to obtain a National Pollution Discharge Elimination System (NPDES) permit. North Carolina, like many other states has implemented its own, more restricted,

permitting system with the approval of the EPA to regulate CAFOs. In the late 1990's there were a few cases of spills and overflows from heavy rain events that grabbed the attention of the state. These incidents led to a government issued moratorium on the growth of the swine industry in North Carolina in 1997 (N.C. General Assembly 2003). This moratorium prohibited the expansion of existing operations, or the opening of new operations until "environmentally superior technologies" are implemented that reduce the possibilities of negative impacts on the environment. The research of these methods is ongoing, due in part to the Smithfield Agreement with North Carolina State University. Research has not yet produced a complete system proven to satisfy the conditions of the moratorium.

EPA studies have shown that approximately 18 percent of streams and lakes in the United States are negatively impacted by agriculture. Nearly half of all reported water quality problems in impaired rivers and streams is from agriculture. Of these rivers and streams, CAFOs are reported to affect about one fifth, or 24,616 miles of rivers and streams. The most common problem attributed to high levels of N and P is eutrophication. This occurs when algae and phytoplankton experience a large "bloom" in growth due to the abundance of nutrients. This new organic matter, along with any organic matter that may have been discharged to the surface water with the N and P, creates a much higher chemical oxygen demand (COD) in the water. As the algae die, the bacteria consuming them have the potential to remove most of the available oxygen in the environment. This results in fish kills and overall degradation of the quality of a body of water. There are also some toxic algal blooms, such as red tide or *Pfiesteria* that can directly harm the fish and cause kills. Such blooms have been seen off the North Carolina coast (Burkholder 1999). On the other extreme, in environments that have high oxygen availability there is the concern of nitrate being formed from the excess N. Higher levels of nitrate in drinking water can cause methemoglobinemia (Blue Baby Syndrome). There is also the general disapproval from populations living near CAFOs of the strong odor released from these operations. A large portion of that odor is from the ammonia volatilization from lagoons.

There is concern over the short life of NH_3 in the atmosphere. After volatilization from the animal houses and lagoons, 20 % to 40 % of the NH_3 will deposit near the source. With large-

scale operations, this addition of nitrogen to surrounding environment could alter the landscape of eastern North Carolina.

The traditional treatment system for hog waste is the flushing of the houses where the animals live and the collection of that wastewater in a lagoon. There are also methods used early in the treatment process such as screens or settling basins to remove larger suspended solids in the wastewater. The purpose of the lagoon is to provide treatment of the wastewater as well as storage until it can be removed. The treatment occurs by a large portion of the nutrients accumulating in the sludge layer on the bottom of the lagoon and also through volatilization of nitrogen to the atmosphere. The lagoon effluent is land applied to local fields for nutrient uptake by a crop selected to accept the applied nutrient load.

There have been problems with the method of lagoon and spray field application that have led to the concern over hog waste from CAFOs. Many of the older lagoons, and a few of the newer improperly lined lagoons have the potential for leaking pollutants to groundwater. In addition, high rainfall events such as the large hurricanes and tropical storms that reach the North Carolina coast can cause an overflow or breech of the lagoons. One method of treatment of the wastewater that could replace such large treatment lagoons is the process of anaerobic digestion.

Anaerobic Digestion

Anaerobic digestion is a common method used in the treatment of wastewater. Digesters can be found in many municipal wastewater treatment plants as well as in agricultural waste treatment. The process of anaerobic digestion is carried out by a host of different microorganisms and is efficient in the transformation of the biological, chemical and physical properties of a wastewater. The biological degradation of organics under anaerobic conditions is the main function of the digester. In this system the microbes use organic matter as a food source and produce the energy necessary to grow and reproduce, along with end products such as methane and carbon dioxide.

Swine waste is a high strength waste that contains a high amount of organics as well as large nutrient loads. A common COD concentration for flushed swine wastewater is between 3,000 mg/l and 9,000 mg/l. This is much larger than a municipal wastewater, which is in the

range of 500 mg/l. This high organics load presents the opportunity for biogas production using anaerobic digestion. There have been many sources which have looked at methane production using anaerobic digestion and contributed to our understanding of the process by defining the necessary conditions and parameters needed for successful operation (Lettinga et al. 1980; Durand et al. 1987; Boopathy and Sievers 1991; Hansen et al. 1997). The use of anaerobic digestion on hog waste may not replace the entire lagoon because a storage pond is needed to hold the digester effluent before it is land applied.

Operation

The process of anaerobic digestion is facilitated by a host of different microorganisms which each play a specific role in the transformation of organics into the methane that is desired from the system. As described in Grady et al. (1999), the basic process can be defined by three distinct functions: hydrolysis, acidogenesis, and methanogenesis. Hydrolysis is the solubilization and reduction in size of large organic particles to more basic components such as amino acids, simple sugars, and long chain fatty acids. These products are degraded through fermentation and anaerobic oxidation to acetic acid and hydrogen during acidogenesis. Also during this step, volatile fatty acids are formed as intermediaries. The methanogenesis can then occur via either of two pathways. The first is through acetoclastic methanogenesis, where the acetic acid is split into methane and carbon dioxide. The second is when hydrogen-oxidizing methanogens reduce carbon dioxide to methane.

The intricate ecosystem that supports these digestive microorganisms is complex, but the requirements necessary to keep these microorganisms viable and productive are rather simple. A constant temperature range is the first requirement. There are three ranges in which digestion can occur. The first is in the mesophilic range, which spans from 30°C to 40°C, and the microorganisms do best in this range around 35°C. There is also the thermophilic range which is above 50°C and the system is most efficient in this range around 60°C. The last, which is used less frequently, is psychrophilic or below 10°C. After setting a constant temperature range for the digester, the next decision is simply what to feed into the digester and at what rate. The

microorganisms needed for the digestion of the waste are latent within the waste, and under the appropriate conditions, will flourish. Therefore, as long as there is a constant manageable food source there will be gas production and organics reduction. However, the process can be inhibited by high loading rates or excessive levels of specific constituents in the waste.

The relationship of volatile fatty acids (VFA) and acetic acid concentration to digester performance and digester health is discussed in Hill and Holmberg (1987). In this study it is reaffirmed that levels of TVFA greater than 2,000 mg/l and acetic acid levels greater than 800 mg/L indicate failure of the digester. This study concludes that levels of long chain volatile fatty acids can indicate the health of the system prior to failure. This accumulation of acids within the digester is commonly referred to as the "souring" of the digester. When this occurs, there is no longer efficient methane conversion because acetoclastic methanogens are inhibited from degrading the acetic acid to methane.

The primary cause of inhibition of anaerobic digestion of swine waste has been determined to be from ammonia (Hansen et al. 1997). The highest load that has been treated without a loss in methane production is about 4 g-N/l (Hansen et al. 1997). Thermophilic digestion is more prone to ammonia inhibition due to the fact that as temperature increases the free ammonia concentration also increases (Hansen et al. 1998). There is also the possibility of inhibition due to Sulfide from the high nutrient waste that swine produce. Inhibition due to S is observed near 50 mg S_2-/L (Karhadkar et al. 1987).

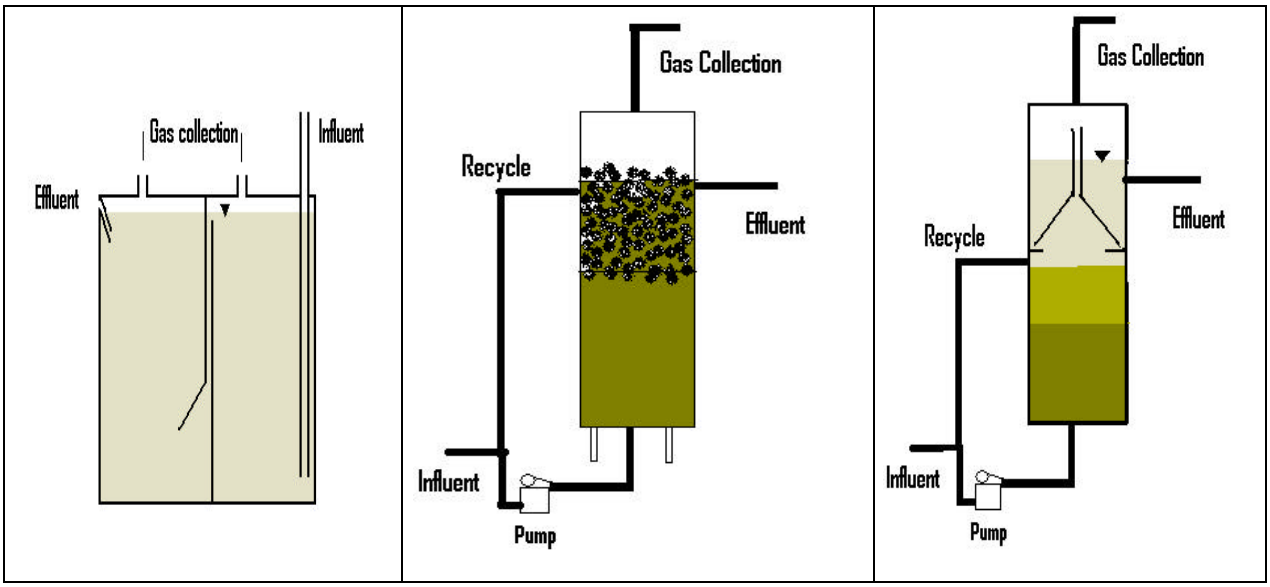
If this process is healthy then it can be expected that a biogas consisting of around 65%-70% methane and about 25%-30% carbon dioxide will be released during digestion. Standard anaerobic digestion is performed using a continuous stirred tank reactor where the sludge age and hydraulic retention time are equal. This means that the entire liquid volume in the reactor is well mixed and there is no solids separation. When dealing with such high solids loads and high COD, it is desirable for the solids retention time to be greater than the HRT. This is because many of the volatile solids in swine waste are contained in small particles (less than .21mm) (Boopathy and Sievers 1991), and lower SRTs will cause these particles to washout before they have been degraded. These smaller particles account for more than 50% of the available methane in swine

waste (Sievers et al. 1980). By increasing the SRT to HRT ratio, a larger volume of wastewater can be treated while providing for the necessary retention time for solids degradation and methane production.

Methods for Attaining High SRT to HRT Ratio

As mentioned above, there is a distinct advantage to having the solids remain in the digestive process for longer amounts of time. There are many different designs of digesters used to accomplish this result and most designs are based on one of three processes: biofilm growth on surfaces within the reactor, formation of settleable particles collected by sedimentation, retention of suspended solids (Grady et al. 1999).

The process of biofilm growth is the method used in an attached growth anaerobic digester. This type of anaerobic digestion is described in Grady et al. (1999). The packed-bed anaerobic digester (PBAD) and the fluidized-bed anaerobic digester (FBAD) are two attached-growth digesters that provide a stable surface on which microorganisms live and retain the solids that are to be degraded. The PBAD is described as simple to construct and operate, but clogs easily with high solids loads. The FBAD is very efficient in treating the swine waste, but has a high operating cost to provide the energy necessary to keep the bed fluidized. A solution provided by Cheng and Liu (2002) is to use floating ballast rings that have a large surface area and are less dense than water, so the upper layer of the digester volume is filled by the rings. The methane production in this system was found to be 0.22 and 0.24 m^3 CH_4 per kg COD removed at 10 and 5 day HRTs, respectively. The reactor configuration used in this research is shown in figure 1b and was adapted from Cheng and Liu (2002).



a. Baffled Reactor **b. Attached Growth** **c. UASB**

Figure 1.1 Configurations of Different Anaerobic Digesters Used in Treatment of Swine Waste

Boopathy (1998) used baffled reactors in anaerobic digestion. The design of the anaerobic baffled reactor is efficient in trapping solids in the lower portion of the cell and preventing them from quickly flowing through the system. Figure 1a. was adapted from Boopathy (1998) and shows an example of a double chambered baffled reactor. The research demonstrated that as the number of baffled chambers increased, the efficiency of the system increased as well. In addition, the methane yields were higher than many of the other methods of digestion. A five chamber anaerobic baffled reactor was reported to produce between 0.94 and 1.46 l/g VS added with a VS loading between 4 and 8 g/l/day. This was higher than conventional CSTR reactors which had a yield of 0.62 to 0.82 l/g VS added with a loading of 1.05 to 2.1 g VS/l/day (Kroeker et al. 1975). The advantage of the baffled reactor is that it can produce a high yield of biogas and therefore can handle a higher loading of organics. There were other reactors that handled high organics loading (Hashimoto 1983; Hasheider and Sievers 1984), but none were able to produce yields that compared to the work done by Boopathy (1998). Advantages of this system, beyond the high biogas yields, are that it avoids clogging and lessens the chance of sludge bed expansion. The smaller particles containing the higher levels of organics are trapped in the bottom half of the reactor and have the time necessary to be digested.

The use of the upflow anaerobic sludge blanket (UASB) reactors for animal waste treatment is a practice that has been documented in many studies. The advantage of this process is that, as in baffled reactors, the solids are retained near the bottom of the reactor by a sludge blanket that forms as the particles and microorganisms attach to each other (Ferreira et al. 2003). One unique action is the formation of granules that occurs when the particles conglomerate. Once formed, these granules fall to the bottom of the reactor (Lettinga et al. 1980). The UASB reactor illustrated in Figure 1.c was adapted from Chen (2000). Waste enters from the bottom of the reactor and is pumped up through the profile of the column. If the proper sludge blanket is established then the solids are intercepted and retained in the lower portion of the reactor. Once again this design provides for a longer SRT than HRT and provides the opportunity for more efficient digestion of organics and higher biogas yields.

In biogas production through anaerobic digestion, a higher SRT to HRT ratio is desired. This can be accomplished through different reactor configurations. Each of these designs has its own strengths and weaknesses. The stability of the microbial community that conducts biogas production is dependent upon a number of factors that are unrelated to the reactor configuration. The proper temperature range needs to be insured and the waste characteristics should be monitored to make sure that the waste is suitable for anaerobic digestion. Once a reactor is selected, then the main goal is to determine the proper HRT and loading rate so that the most efficient operation of the system is achieved.

One limitation to anaerobic digestion of swine waste that cannot be ignored is the high nutrient load that remains in the effluent after digestion. The process of dealing with the effluent from the digester still provides many areas for discussion. The common solution is land applying the effluent for nutrient recovery by some type of crop. There are also other technologies that have been examined for nutrient reduction. Reactors that promote advanced nitrification and denitrification are also used to treat the high nutrient load. One method similar to land application is the recovery of nutrients in plant biomass of aquatic plants that can grow on the wastewater.

Duckweed and Nutrient Removal

Duckweed, an aquatic plant, has shown to be effective in the treatment of many types of wastewaters (Culley and Epps 1973; Staves and Knaus 1985; Reddy and DeBusk 1987; Oron et al. 1988; van der Steen et al. 1998; House et al. 1999; Bergmann et al. 2000). While duckweed stabilization ponds may have a small impact on the degradation of organics in the wastewater (Körner et al. 1998), suspended solids (SS), biochemical oxygen demand (BOD) and pathogen removal in these ponds are often reported as similar to conventional wastewater treatment ponds (Bonomo et al. 1997). The main advantage of these plants is in the uptake of nutrients.

Duckweed is a small free-floating aquatic plant belonging to the family *Lemnaceae* which includes the smallest flowering plants in the world. The family *Lemnaceae* is composed of the five genera: *Lemna, Spirodela, Landolita, Wolffia, Wolfiella.* The plant is very hardy and can be found in almost any environment and location worldwide. Duckweed grows in many slow flowing waters as well as relatively polluted and eutrophic waters, and can even live in saline waters (Leng 1999). The typical pH range for these plants is 4.5-7.5, though growth is possible up to a pH of 10 (Bonomo et al. 1997). Extensive research of *Lemnaceae* has been conducted by Landolt and Kandeler (1987) and comparison has been performed for the different species located on all continents and ranging in size from as large as 15mm to s maller than 1mm (Figure 2, below).

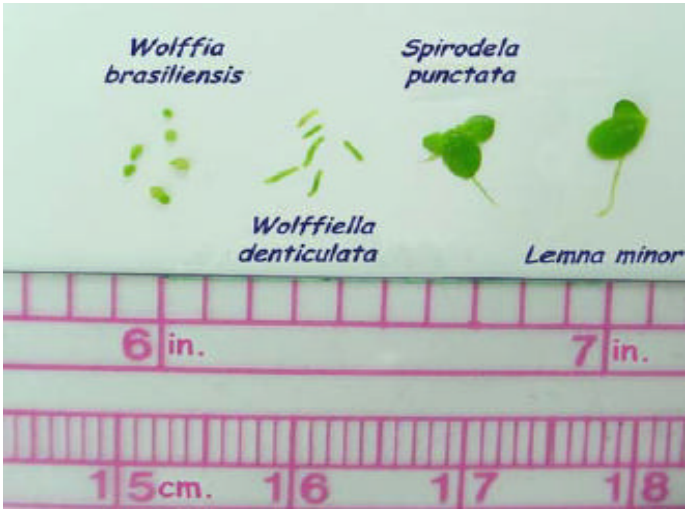


Figure 1.2 Sizes of different species of Duckweed

As seen in figure 1.3, the plants are completely vascular and composed primarily of a large buoyant frond surface. There are also one or two roots suspended in solution from the plants in *Lemna*, *Spirodela*, and *Landolita*. The common method of reproduction is through asexual vegetative reproduction, but the plants can also reproduce through flowering and seed production. The rate of reproduction is quite remarkable for these tiny plants. Duckweed prefers environments with large nutrient loads, and have been shown to be able to double their biomass in a matter of days. The rapid growth is facilitated by the asexual growth in which the plants bud, producing more and more biomass as long as water surface area is sufficient and the necessary nutrients are provided. The plants require macronutrients such as nitrogen, phosphorus and potassium as well as key micronutrients in order to grow and reproduce. As the properties of the duckweed are understood, the potential of the plant in wastewater treatment applications is better realized.

Figure 1.3 *Lemna gibba*

Research on nitrogen removal in duckweed-based treatment has listed the main mechanisms of removal as: plant uptake, ammonia volatilization, ammonia assimilation into algal biomass, and biological nitrification coupled with denitrification (Zimmo et al. 2000). How large of a role can duckweed play in nutrient removal? Körner and Vermaat (1998) claim that only a quarter of total nutrient losses were not directly or indirectly attributed to duckweed, while Vermaat and Hanif (1998) claim that denitrification is the major pathway for N removal and plant uptake is the major pathway for P removal. There is consensus that the plants can increase nutrient removal from a system.

Research concerning the growth rate and nutrient uptake capacity of duckweed has been central to the development of the plant as a viable process in wastewater treatment. Plants with high growth rates and high nutrient content should provide for good removal of N and P from the wastewater. As mentioned earlier, the plants are capable of very high biomass production. Some species have been shown to double their biomass in less than 24 hours (Landolt and Kandeler 1987). High growth rates have been reported by many different sources (Hillman 1961; Oron et al. 1984; Caicedo et al. 2000). The nutrient uptake is also very high as would be expected from a plant which grows so quickly. Certain species of duckweed are reported to be composed of crude protein between 30% and almost 50% of plant dry weight (Culley and Epps 1973; Mbagwu and Adeniji 1988). Protein, being composed of amino acids, is a good indication of the amount of nitrogen that is contained in the plant. Research by Bergmann et al. (2000b) has shown that nitrogen can constitute almost 6% of the plant's dry weight. While N uptake is the main goal of many treatment designs, the removal of P is also accomplished at a lower rate. Reddy and DeBusk (1987) has reported an annual mean N uptake rate of 350-1200 kgN ha^{-1} yr^{-1} compared with a lower rate for P of 116-400 kgP ha^{-1} yr^{-1}.

The structure of these rapidly growing plants is partially responsible for their success. Duckweed are void of any structural tissues that would require excess energy to create and maintain (Oron et al. 1984). This means that the entire plant is metabolically active. To provide the necessary nutrients quickly to the entire plant, all of the surface area of the plant absorbs nutrients and does not rely completely upon the central root system (Bonomo et al. 1997). Studies have shown that *Lemna minor* can use both the root and the frond for significant uptake of inorganic N (Cedergreen and Madsen 2002). The study and others have also shown that the roots do behave similarly to those of other higher plants and as nutrients become less available, the plant uses its energy to grow longer roots to help in the transport of nutrients to the plant.

What makes duckweed even more effective in a range of wastewater applications is that the plant preferentially removes N in the form of ammonia (Monselise et al. 1987). It has been shown that *L. gibba* will preferentially uptake NH_4^+ even at a ratio of 1:1000 with NO_3^- (Monselise and Kost 1993). This preferential uptake of ammonia results in the direct conversion of N to plant

protein, a more efficient rout than the assimilation and reduction needed to transform nitrate into plant protein (Oron et al. 1988). This is very important because ammonia is a large constituent of domestic wastewater at levels between 10 and 50 mg/l N. Ammonia levels can be as high as 200 mg/l N in industrial wastewater and domestic wastewater from arid areas (Konig et al. 1987). The goal for efficient nutrient removal is to provide the plant with the opportunity to remove as much ammonia N as possible. Chaiprapat et al. (2003) have shown that in static systems ammonium transport is the limiting factor for ammonium uptake, and plant growth is the limiting factor for uptake when the system is well mixed. If the plants are getting the proper exposure to ammonium then they can grow efficiently and remove N and P from the system. Maximum growth is also facilitated when the plant density is thin enough to allow space for growth, while at the same time dense enough to prevent algae from competing for nutrients.

While it has been shown that duckweed is advantageous because of its preferential uptake of the ammonium ion, there is the concern of growth inhibition at concentrations too high for the plant (Oron et al. 1984; Al-Nozaily et al. 2000). There is also concern of inhibition caused by ammonia only (Wang 1991). Ammonia and ammonium (un-ionized and ionized) concentrations are determined by the temperature and pH of a wastewater. The un-ionized form is the most toxic because it carries no charge. Therefore, it is lipid soluble and can more easily cross biological membranes than NH_4^+ (Körner et al. 2001). Knowing that either form can be detrimental to the plants, it is important to know the characteristics of the wastewater in order to know what loading is possible to attain proper growth. Based on pH, ammonia will be much higher as the pH rises away from neutral. While at neutral, ammonium will be the major form of nitrogen.

Duckweed is capable of removing other wastes such as heavy metals from water and surviving as long as the levels do not become toxic (Rodgers et al. 1978; Clark et al. 1981; Staves and Knaus 1985). Metals are common in the waste streams of industrial and textile operations, and have been found in effluent from municipal wastewater treatment facilities. It is also common that storm water runoff from urban areas and highways have high levels of metals such as Zn, Cd, Pb, Cd (Davis et al. 2001). Plants being used for metal removal would apply the same theory with metals as nutrients. If the duckweed are not harvested, then they will either die or depuration will

occur and release the metals back to the water (Clark et al. 1981). Duckweed have been subjected to metals such as Ag, B, Cd, Cr, Cu, Ni and Zn and have shown the ability to collect these metals within the plant at a much higher concentration than is present in the surrounding water (Staves and Knaus 1985). Duckweed has also shown the ability to survive in highly saline areas. Though duckweed does not concentrate the sodium ion in the plant, they can be found growing in 0.5 to 2.5% sodium chloride (Leng 1999).

Algae, as well as other aquatic plants, are used in wastewater treatment. A common plant mentioned for nutrient removal in natural treatment systems is water hyacinth (*Eichhornia crassipes*) (Reed et al. 1995). Algae and water hyacinths are both similar to duckweed in that they float in or on the surface of the wastewater and uptake nutrients as they grow. Algae are often not preferred in a system because they cannot be harvested easily like the larger plants. Once duckweed is able to cover the volume being treated, it prevents most algae growth. This leads to the reduction of TSS levels in the effluent due to algae (van der Steen et al. 1999). Duckweed has been shown to be competitive with the other plants for many reasons. Duckweed are able to grow at lower temperatures than the water hyacinth and therefore can extend the use of natural treatment systems for longer periods of time in colder climates (Culley and Epps 1973; Oron et al. 1984). Duckweed has been shown to contain twice the protein of hyacinths when grown on wastewater. However, even with the high protein concentration of duckweed, hyacinths have the capacity to remove more nutrients from a wastewater than duckweed under optimum growing conditions (Reddy and DeBusk 1985). Cooler climates need a process that can operate for longer periods of time. There are some challenges provided with large-scale duckweed systems. Some type of grid system is usually needed to prevent the plants from being blown around the surface of the water. In cooler climates, the system will not be able to run year round due to slow growth rates as temperatures drop.

It has been shown that duckweed ponds can achieve removal of 98% *Giardia*, 89% of *cryptosporidium*, 62% of fecal coliforms, and 40% of coliphages (Falabi et al. 2002). The research was done on the physical removal of these organisms and not their viability. It appears

that the removal of pathogens in duckweed ponds is based on size. The larger microorganisms such as *Giardia* and *Cryptosporidium* are removed more efficiently.

There are other important characteristics of duckweed treatment systems that can enhance the design and implementation of a system. Surface area coverage by duckweed has been shown to prevent mosquito reproduction, and there seem to be no serious pests which prevent plant growth (Culley and Epps 1973). The wide distribution of plant geographic isolates in many varied environments provides a broad selection of plants with genetic variability that can be used for very specific tasks (Landolt and Kandeler 1987; Skillicorn et al. 1993). There is also the potential of 30% greater water retention than in other wet processes due to the reduction of the evaporative effect (Oron et al. 1984).

A progressive method for addressing wastewater treatment is to look at it from a holistic approach that produces viable goods from the waste. When duckweed is used for wastewater treatment the main method of nutrient removal is harvesting the plant (Körner and Vermaat 1998). There is a large base of research conducted on the nutritional values of duckweed and many feel that duckweed could be used effectively as a dietary supplement for animals (Culley and Epps 1973; Mbagwu and Adeniji 1988; Skillicorn et al. 1993). This plant not only provides more protein than most other plants but the levels of the essential amino acids surpassed the FAO reference pattern, except for methionine which met 61.4% of the recommended value for dietary supplements for animals (Mbagwu and Adeniji 1988). Duckweed, *Lemna gibba* and *Lemna minor*, also have potential as plants that can be genetically engineered to produce an array of proteins (Yamamoto et al. 2001). This could increase the economic value of the plants that are harvested from a treatment system.

Much of the research into this natural treatment method has been conducted to identify a cost effective procedure for developing countries with water quality problems (Oron et al. 1984; Skillicorn et al. 1993; Caicedo et al. 2002). Usually the waste stream is very concentrated due to the lack of water for sanitary systems. In addition, funds are not available for the larger, more capital-intensive treatment systems. However, what these countries usually have and what is needed for natural treatment systems, is large amounts of land to properly treat the wastewater.

Duckweed and Swine Wastewater

Agricultural wastes are similar to high concentration domestic wastes, in that they have high organics and nutrient loads that must be removed. As mentioned above, there is room for improvement in the treatment of wastes from CAFOs. The traditional lagoon and field application, which is natural treatment systems, is seen to have many disadvantages in that it produces odor, leads to transformations in the nitrogen cycle in an environment, and changes the phosphorus conditions of a soil. If operated improperly, there could also be contamination of surrounding surface-water and ground-water.

With duckweed showing so much promise in applications of nutrient uptake, many researchers have made the extension of the science to the treatment of swine wastewater (Culley and Epps 1973; Bergmann et al. 2000; Cheng et al. 2002). Duckweed has a high potential for nutrient recovery from swine wastewater due to the fact that lagoons are near neutral pH, therefore the predominant form of nitrogen is ammonium (Chaiprapat et al. 2003). With duckweed, the nutrient uptake increases as the level of nutrients in the water increases (Culley and Epps 1973). This trend occurs until the level of N becomes too high and inhibition of plant growth and nutrient uptake occurs (Caicedo et al. 2000). Inhibition of growth by total ammonia ($NH_4^+ + NH_3$) is commonly attributed to the presence of NH_3 at higher levels. This would affect duckweed in the swine wastewater due to high nutrient loads or changes in temperature. There is also the argument that high NH_4^+ concentrations inhibit general anion transport in duckweed (Ingermarsson et al. 1987). There is no clear conclusion about whether ammonia or ammonium is the inhibiting factor in plant growth, but at similar pH and temperatures the level of total ammonia that is toxic to the plant can be determined. This nitrogen level should be the upper limit of how large of a loading can be applied to a duckweed treatment system. The level of nitrogen will have to be in balance with plant growth and other forms of removal in the system. The goal with swine waste then becomes to find the plant that can live in the highest nutrient concentrations and at the same time remove nutrients at a high rate.

Selection of geographic isolates for growth on swine waste

North Carolina State University houses Dr. Elias Landlot's worldwide germplasm collection of duckweed in the Environmental Biotechnology Laboratory. This collection contains approximately 1,000 isolates from 36 species of the four genera: *Lemna, Spirodela, Wolffia, Wolffiella*. Bergmann et al. (2000a) began the process of selecting a superior species and geographic isolate for growth on swine wastewater in North Carolina. This research tested 41 of the isolates including 12 species from the collection with the highest growth rates observed during culture maintenance. These 41 isolates were grown on a culture medium of swine artificial medium (SAM) in order to determine which isolates had a favorable ratio of quantity of biomass to protein produced. From this research there were 8 geographic isolates that were selected to be grown on swine lagoon effluent. These included the highest 6 ranked isolates along with 2 isolates of *Lemna minor* that can be genetically engineered. The results of this initial research listed *Lemna gibba* 8678 and *Spirodela punctata* 7776 as two isolates that could tolerate the lagoon effluent at full strength and showed slow growth. *Lemna minor* 8627 displayed about 50% survival and was the more favorable of the two isolates that are capable of being genetically engineered.

The next step in this research is reported by Bergmann et al. (2000b). The method of this research was to test the three selected isolates on an array of different lagoon effluent concentrations. *Lemna gibba* was placed on 67%, 50%, 33%, 25%, and 20% swine lagoon effluent, while *Lemna minor* and *Spirodela punctata* were each placed on 50% and 25% swine lagoon effluent. These treatments were grown in a greenhouse with water temperatures similar to average water temperatures in a lagoon in the summer in North Carolina (25 ± 2 °C). The results were that *Lemna gibba* could be grown on 50%, 33%, or 25% swine lagoon effluent for efficient treatment and healthy biomass production from the duckweed, while *Lemna minor* and *Spirodela punctata* should be grown at 50% swine lagoon effluent.

Now that these three duckweed geographic isolates have been selected and have been shown to do well on high concentrations of swine waste, the next step is to design a continuous flow treatment system that can handle the outdoor conditions to which a full scale system would

be subjected. This will require determining a nutrient-loading rate at which the plants can optimize growth and nutrient uptake. It is also important to determine during which portion of the year the plants will be able to actively grow. Cheng et al. (2002) tested the growth of *Lemna minor* 8627 under field conditions during two time periods (May to July and August to October) in Raleigh, North Carolina. This experiment was designed to test the growth of *Lemna minor* on four different initial lagoon waste concentrations (50%, 33%, 25%, 20%) under batch conditions. Results indicated that there was a significant difference in growth from spring to fall, with spring producing much more growth as well as nutrient uptake. This difference can be attributed to conditions such as time of exposure to daylight, warmer temperatures, and the different seasonal properties of the waste. The highest growth rates were reported at 29 g m^{-2} day^{-1} and highest N and P uptake were 3.36 g m^{-2} day^{-1} and 0.20 g m^{-2} day^{-1}, respectively. There also was significant COD and TOC reduction in the batch tests. Over 50% removal for both COD and TOC was accomplished in both the seasons.

The next step in this process is to design a pilot scale operation that can treat raw swine wastewater. One major concern is the treatment of the organics in the wastewater. It has been shown that COD reduction is possible, and is mainly attributed to bacteria which are present in the system (Körner and Vermaat 1998). The COD loading for a continuous treatment system would likely be too high for this reduction method. This is due to the lower oxygen concentrations found in duckweed treatment ponds (Reed et al. 1995). In order to release organically bound N and P, and to lower organics in the waste, pretreatment with an anaerobic digester has been suggested (Alaerts et al. 1996; van der Steen et al. 1999; Caicedo et al. 2002). The combination of anaerobic digestion along with duckweed growth has been used before on domestic waste by Caicedo et al. (2002). The combination of anaerobic digestion and duckweed based stabilization ponds to treat swine waste under field conditions in North Carolina has yet to be fully tested and understood.

Research Objectives: The purpose of this study was to evaluate the performance of swine wastewater treatment in an integrated system of anaerobic digestion and duckweed nutrient removal. The specific objectives were.

a. To define the fluctuations in the system operated year round in North Carolina.

b. To determine the waste loading rates possible for a healthy and effective system.

c. To observe the performance of anaerobic digestion of raw swine wastewater using an attached-growth reactor with floating plastic ballast rings.

d. To compare the biomass production and nutrient uptake of *Lemna gibba* 8678, *Lemna minor* 8627, and *Spirodela punctata* 7776 in treating effluent from anaerobic digestion.

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Chapter Two

Integrated System of Anaerobic Digestion and Duckweed Growth for Swine Wastewater Treatment: Pilot Study

Abstract

Organics destruction and nutrient uptake in an integrated pilot system of anaerobic digestion and duckweed nutrient removal for swine wastewater treatment were monitored under field conditions. Raw swine wastewater of 100 gallons/day was first treated in a 1,000-gallon anaerobic digester with floating ballast rings. Organic compounds in the wastewater were digested to produce biogas. Many nutrients including nitrogen and phosphorus remained in the effluent of the anaerobic digester. Duckweed (*Lemna gibba* 8678) was grown in three 1,250-gallon tanks to recover nutrients from the anaerobic effluent. The duckweed was periodically harvested and can be used as animal, poultry, and fish feed. This research provides an initial understanding of the attached-growth anaerobic digester and the characteristics exhibited by *Lemna gibba* in the treatment of swine wastewater under field conditions in North Carolina. Both the anaerobic digester and the duckweed tanks were run as completely mixed systems. The performance of the system was monitored by measuring chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN), ammonia nitrogen, total phosphorus (TP), ortho-phosphate-phosphorus, and pH in the influent and effluent of each treatment unit.

Introduction

For several years attention has been focused in North Carolina on the swine industry in the eastern part of the state and the wastes that are produced. Unlike the production facilities in Iowa, North Carolina does not have large amounts of arable land to use for application of the waste after the common treatment process of an anaerobic lagoon. Concerns have arisen over a number of possible problems such as: over application and possible runoff from the fields,

groundwater contamination from the seepage of the nutrients through the soils, and leaks in the liners of lagoons. The current operation of swine waste processes in the state use the crops, usually coastal Bermuda grass, for the purpose of nutrient uptake from land applied lagoon effluent. There is not a large market or use for the crop after the harvest, due to a low nutritional value. Considering that most nutrients fed to the animals are imported fromout of state, and animals consume only about 50% of the nutrients, this leads to a large influx of nutrients to the environment surrounding the concentrated swine industry. Accidents or improper operation from these facilities lead to the pollution of nutrients in surrounding waters, and problems such as eutrophication and high nitrate levels in the groundwater.

Swine wastewater is also a concern due to the high organics levels in the waste. A large COD in the waste represents these organics. Therefore it seems necessary to include more than one step in the treatment of the waste. This is already common as lagoons are followed by land application. There is the potential for the degradation of these organics using anaerobic digestion. This process would lower the high organics while at the same time producing a valuable biogas byproduct. This gas is approximately 70 % methane and could be combusted to produce energy and heat. The digester would provide for significant organics destruction, but the nutrients undergo little transformation during this process. It is necessary to include a nutrient removal step following the COD reduction.

One alternative process for the removal of nutrients from the wastewater is the cultivation of aquatic plants on the wastewater. It has been shown that the small macrophyte duckweed is a fast growing, high protein plant that also has the possibility of being used as a viable feed supplement after harvest from the wastewater. Duckweed grown on domestic wastewater has been used as a complete feed for fish production by PRISM in Bangladesh (Skillicorn et al. 1993; Alaerts et al. 1996). Duckweed is a sturdy plant that can grow in colder climates than other aquatic plants such as water hyacinth. Many species of duckweed can be found in North Carolina, usually in waters that have high nutrient levels. It has been shown that these plants are very efficient in the uptake of nutrients and that plants grown on highly concentrated wastes, such as

animal lagoon effluent, have a much higher nutrient level than others grown in nearby waters (Culley and Epps 1973).

Concern in agricultural operations has also focused on phosphorus levels. These are rising in the land applied soils due to the fact that most crops absorb the necessary nitrogen but do not use all of the land applied phosphorus. Duckweed has been shown to act as a phosphorus suppressor. It fosters bacterial removal of phosphorus and assimilates it during growth (Hammouda et al. 1995).

Research has been conducted leading up to the pilot scale treatment of swine wastewater to determine the proper plants to place in such a harsh environment (Bergmann et al. 2000b; Bergmann et al. 2000a). The focus of their research was to select the best performing plants from the worldwide collection at NCSU and grow them on a simulated swine waste (SAM) and determine which plants grew best. The plants were then taken to swine lagoon effluent and tested to see which plants could withstand the stress. Three geographic isolates were selected for further study: *Lemna minor* 8627, *Lemna gibba* 8678, and *Spirodela punctata* 7776. These plants were shown to survive the full strength lagoon effluent. After this result, further studies discovered that solid growth rates were obtained from these plants when grown on between 25% and 50% effluent concentrations. This research continues the observation of duckweed grown on swine waste, in particular including the plants in a pilot treatment system following an anaerobic digestion for organics destruction. The plants are grown outdoors to determine how well they react to the field conditions that a full-scale operation in North Carolina would require.

Methods

Pilot operation

Operation of a pilot system for the treatment of swine waste, incorporating anaerobic digestion and the growth of *Lemna gibba*, was monitored to determine organics destruction and nutrient uptake achieved by a continuous flow system under field conditions. The pilot system is located at the Lake Wheeler Road Field Laboratory of North Carolina State University in Raleigh, North Carolina. The system was constructed next to an existing settling basin and treatment lagoon for the experimental swine unit. The source of waste liquid for this research was the

screened effluent from the settling basin. The average properties of this wastewater influent for the duration of the study can be seen in Table 2.1. The pilot plant consisted of one 1,000-gallon Attached Growth Anaerobic Digester (AGAD), which feeds three 1,250-gallon concrete tanks used for duckweed growth (Figures 2.1 and 2.2).

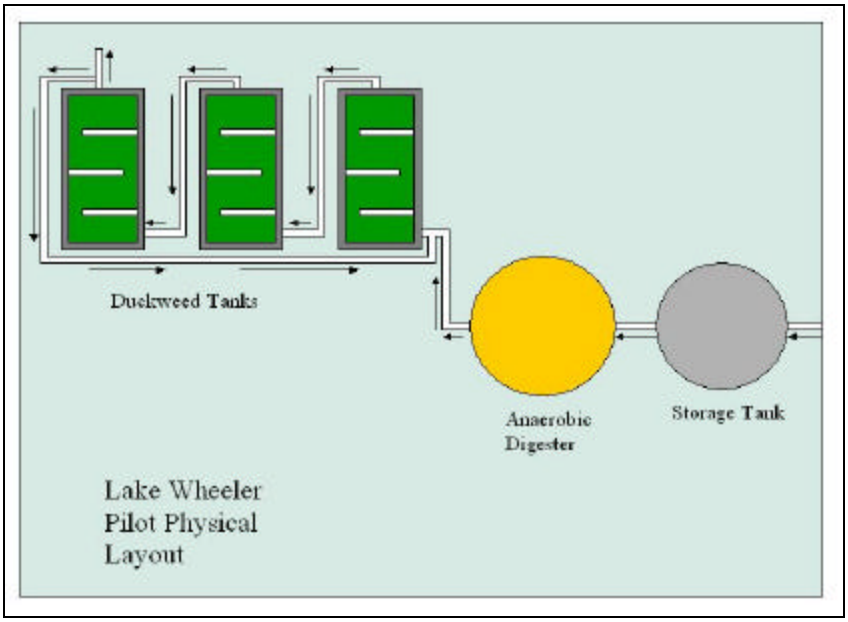


Figure 2.1 Diagram of pilot plant layout

Figure 2.2 AGAD and duckweed tanks

Table 2.1 Average raw swine wastewater properties including 13 samples during the pilot plant operation.

	TKN mg/l	NH3N mg/l	NO3N NO2N mg/l	TP mg/l	OP mg/l	COD mg/l	pH	%TS	%VS	ALKAL mg/l	TOC mg/l
AVG	350.4	175.5	0.0	144.3	99.5	5894.5	7.5	0.5	60.3	1336.9	553.2
StDev	70.3	27.0	0.0	38.7	27.1	6322.1	0.4	0.3	9.2	178.1	315.9
Max	463.0	236.0	0.0	218.0	149.0	24800.0	8.0	1.3	80.3	1600.0	1352.0
Min	238.0	138.0	0.0	67.1	64.6	1444.0	6.8	0.2	47.0	1050.0	283.0

Swine wastewater of 100 gallons per day was fed from a storage tank into the anaerobic digester. This feed was distributed among 20 periods every day in order to prevent solids from settling and clogging the lines. The volume of the anaerobic digester is 1,000 gallons and floating ballast rings provide a surface for attached growth of anaerobic bacteria within the digester. The ballast rings are 3 inch in diameter and 3 inch in height with a density of 0.98 g/cm3, a specific area of106 m2/m3 and a porosity in a packed reactor of 0.86. The hydraulic residence time of the reactor was 10 days and the temperature of the digester was kept near 35°C using water coils connected to a water heater. The digester was used for the conversion of organics to biogas, the composition of which is approximately 70% methane and 30% carbon

dioxide. The biogas production from the digester was measured using a wet test gas meter manufactured by Precision Scientific, Chicago, IL.

The effluent from the anaerobic digester was then taken to three 1,250-gallon concrete tanks in series where the *Lemna gibba* was grown. The dimensions of each tank were 3.2 feet deep, 10.5 feet long and 5 feet wide. The tanks were each divided into four sections to prevent the wind from moving the plants around. The tanks were initially filled with 75% tap water and 25% swine lagoon water, and circulation was provided in 30-minute periods 4 times per day to provide well-mixed conditions between all three tanks. *Lemna gibba* was present in the tanks initially and was removed and placed in small pools to remove older growth and establish a healthier group of plants. After a few weeks the duckweed was applied to the 75-25 mixed tanks at 6 lbs per tank. This amount provided full surface area coverage of the tanks by the plants. During the operation of the system the duckweed was harvested at a rate that would remove enough biomass to allow healthy growth, while not leaving any surface area exposed. Harvesting took place 2 to 3 times a week using screen harvesters that were 20% of the surface area of the tanks (Figure 2.3). The harvest was air dried for about 10 minutes in order to remove excess water. The weight of duckweed harvested was recorded to measure the growth of the plants. The effluent from the tanks was then wasted back into the treatment lagoon. The initial feed rate of 100 gal/day to the tanks resulted in a 37.5 day HRT, but later in the research the influent to the tanks was cut to 50 gals per day and the resulting HRT was 75 days.

Figure 2.3 Harvest screens drying

Chemical analysis

Every day temperature, pH and DO (at 1 inch and at 1 foot) were measured in the duckweed tanks between the time period of 10 am and 2 pm. Grab samples were taken weekly from the influent to the digester, the effluent from the digester, and from the duckweed tanks and analyzed for TKN, NH_3 - N, NO_3 - N/ NO_2 - N, TP, OP, COD, pH, %TS, %VS, COND, ALKAL, TOC, K, Cu, Zn. Plant tissue was also sampled weekly and TKN, TP, %MC, K, Cu, Zn were measured. Analysis was performed by the Environmental Analysis Laboratory of the Biological and Agricultural Engineering Department at North Carolina State University using EPA methods (EPA 1983) and Standard Methods (APHA 1995). The pH, and temperature of the duckweed tanks were measured daily using an Orion model 1230 meter with a 9107 wp pH electrode. The dissolved oxygen (DO) was measured with the YSI 52 DO meter, Yellow Springs, OH.

Nitrogen mass balance

The following equation was used to conduct the mass balance of nitrogen in the duckweed tanks:

$$N_T = N_p + N_i - N_{dw} - N_o$$

where N_T is the amount of nitrogen in the duckweed tanks, N_p is the amount of nitrogen initially present in the tanks, N_i is the amount of influent nitrogen to the tanks, N_{dw} is the amount of nitrogen in the duckweed harvested from the tanks and N_o is the other forms of nitrogen loss including denitrification and ammonia volatilization. A similar formula was used to determine the phosphorus mass balance:

$$P_T = P_p + P_i - P_{dw} - P_o$$

Results and Discussion

Attached Growth Anaerobic Digestion

The use of anaerobic digestion is incorporated into this treatment process in order to reduce the large organics load that is present in swine waste. Figure 2.4 shows the reduction in COD achieved by the digester. The influent waste characteristics were quite variable, having an average COD of 8991.9 ± 7152.6 mg/l during the operation from 5/27/2002 until 12/16/2002. The COD in the effluent from the digester was effectively lowered to 846.6 ± 226.7 mg/l COD (91%) over this same time period. This is a much higher efficiency than the 66% removal reported by Cheng and Liu (2002) for a similar waste and a similar retention time using a 20 L bench scale test. One concern with the operation of the anaerobic digester was that the influent line became clogged easily as solids settled in the pipes from the pump. This possibly led to lower feed rates to the digester and a higher HRT in the digester. This high HRT would have led to greater removal of organics by the pilot scale digester. The AGAD displayed the ability to handle the large variations in the influent well. The standard deviation of the Influent COD was 79.5 %. The deviation in the effluent from the digester was 26.8% (Figure 2.4). There is also a large variation in the influent COD. This change could be caused by some sampling procedures. There was a large amount of solids in the storage tanks and there were some periods when the circulation pump

was not operating. This would allow for a high concentration of solids and organics in the effluent of the tank.

The TKN through the AGAD was reduced by 49% on average. This is a large reduction in TKN, but there was no significant change in the ammonia nitrogen through the digester. The loss of N in the AGAD should be due to the assimilation of ammonia into new biomass and the precipitation of struvite. There is also a large total phosphorus removal of near 60%. This removal is possibly due to both new biomass production, as well as the precipitation of struvite in the digester. The orthophosphate phosphorus is slightly reduced through digestion, and the pH is fairly stable, rising slightly in the effluent. The high alkalinity reported is common to swine waste and is beneficial for keeping the pH near neutral.

The above results are based on the operation leading up to December 16th 2002. The operation after that date was excluded in those calculations due to the "souring" of the digester. At some point during late December the feed line was clogged and there was no flow for a few days. This led to the continued heating of the digester above the mesophilic range, around 43ºC, that is optimal for the culture reducing the organics. This can be seen in the quick jump in effluent COD concentration in Figure 2.4. More proof lies in the rise from below 30% to over 60% VS in the effluent from the digester.

There were no tests done on the effluent gas composition from the digester. Common results would indicate that about 70% methane is produced while the other 30% of the biogas is carbon dioxide. The operation during this time produced about 425 l/day of biogas. This is an average of 0.138 m^3 of biogas produced for every kg of COD digested. Assuming that 70 % of the volume is methane then the methane productions would be 0.097 m^3 of CH_4 produced for every kg of COD digested. These numbers are lower than, 0.22 and 0.24 m^3 of CH_4 per kg of COD removed that was reported from the 20 L reactor by (Cheng and Liu 2002). Again, if the clogging of the lines led to a lower feed rate to the digester, there would have been less organics for destruction in the digester leading to lower biogas production.

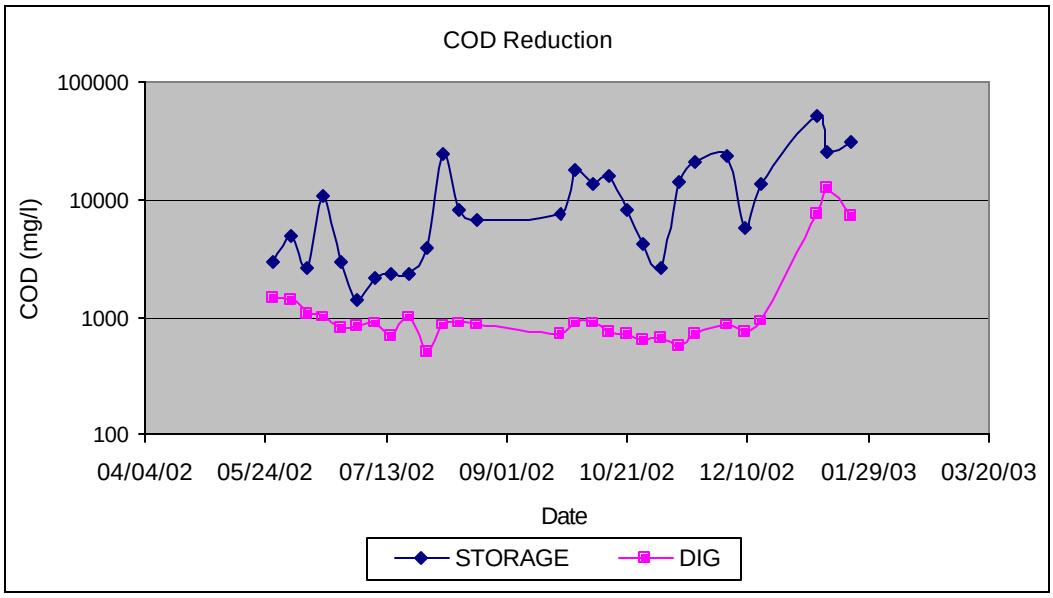


Figure 2.4 COD reduction by anaerobic digestion

Table 2.1 Performance of AGAD treating swine waste from 5/27/2002 to 12/16/2002 based on 27 samples.

	Unit	Influent			Digester Effluent			Removal Efficiency %
COD	mg/l	8991.96	±	7152.63	846.60	±	226.72	91
TOC	mg/l	610.66	±	331.93	269.63	±	106.85	56
TKN	mg/l	523.08	±	282.18	264.20	±	57.95	49
NH3N	mg/l	208.32	±	64.60	206.48	±	54.76	1
TP	mg/l	177.99	±	86.58	71.27	±	14.06	60
o-PO4-P	mg/l	74.74	±	38.62	71.27	±	14.06	5
PH		7.28	±	0.39	7.43	±	0.24	

Duckweed Tanks

There were two operation periods during the summer and fall of 2002. The first was from June 23rd until August 1st, while the 2nd period was from September 23rd until November 1st. This was necessary due to the inhibition of the growth of the plants. This will be discussed in detail later in the results. The tanks, while efficiently operating, provided a good environment for nitrogen and phosphorus removal from the wastewater.

There was a continuation in COD reduction as the effluent from the digester was then fed into the duckweed tanks (Figure 2.5). COD reduction in DW tanks is reported to be accomplished

by bacterial reduction from the organisms living in the habitat provided by the plants (Körner et al. 1998). The tanks held a constant effluent concentration of COD at approximately 38 % of the influent during the first operation period and 22% during the second period. The lower feed rate in the second operation would explain the lower concentration of COD in the effluent from the tanks.

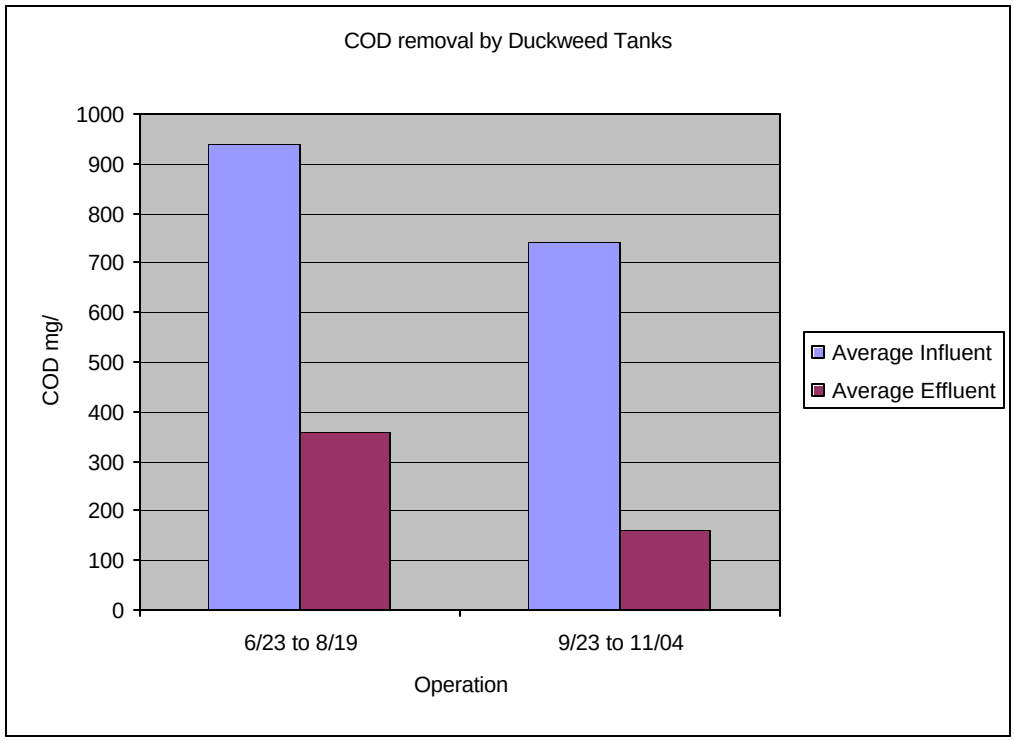
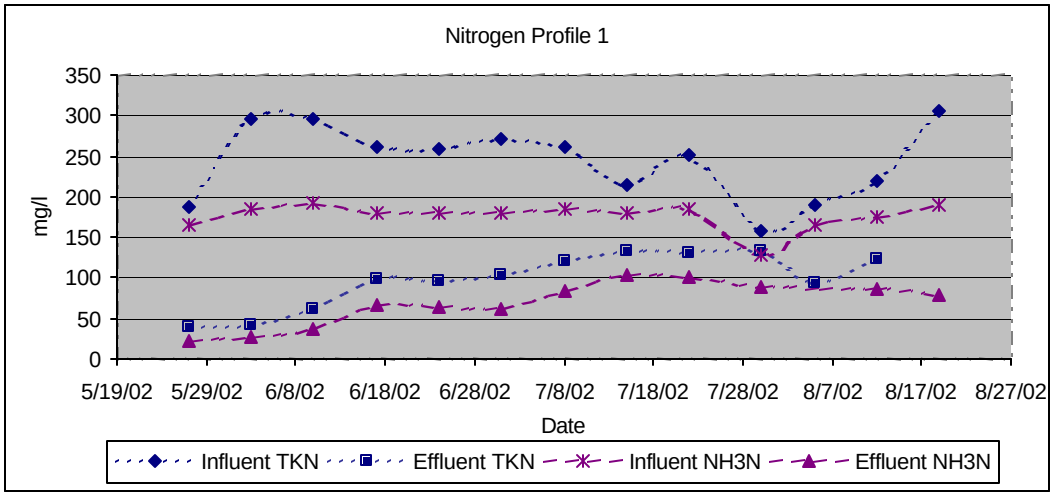


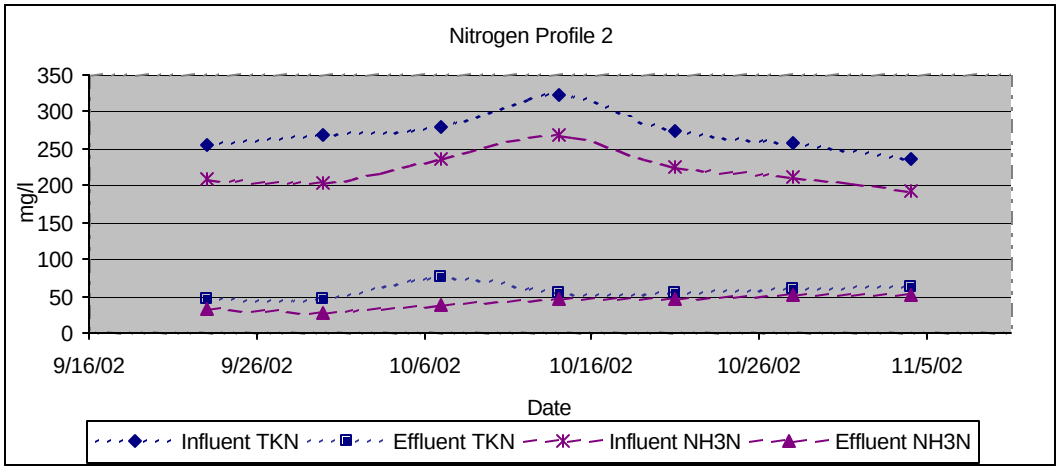
Figure 2.5 COD reduction in duckweed tanks

The nutrient removal from the tanks by duckweed growth is the main purpose for the incorporation of duckweed in the treatment of the digester effluent. Figure 2.6 shows the nutrient levels in the influent and effluent from the duckweed tanks. The operation of the system was initiated using a flow rate of 100 gal/day from the anaerobic digester through the duckweed tanks. The plants began growing well and became very dense on the surface of the wastewater ponds. The initial seeding was 0.55 kg (wet weight)/m^2 and provided just a thin complete surface coverage. After one week of growth the density of the plant coverage had grown to 1.733 kg/m^2. This was the minimum plant density for both periods of operation during the 2002 summer. The maximum density was similar for both operation periods at about 3.18 kg/m^2. These numbers

indicate the possibility for greater rates of harvesting to allow for less density and greater plant growth. This initial seeding was 0.1 kg/m^2 lower than that used by Zimmo et al. (2002) in a study on domestic waste. Zimmo et al. (2002) also returned the concentration to 0.4 kg/m^2 regularly. A higher yield could be attained from the growth, which would require more frequent harvesting, or harvesting of a greater area. This possibly would have allowed the system to function longer without the inhibition due to the accumulation of high nutrient levels.

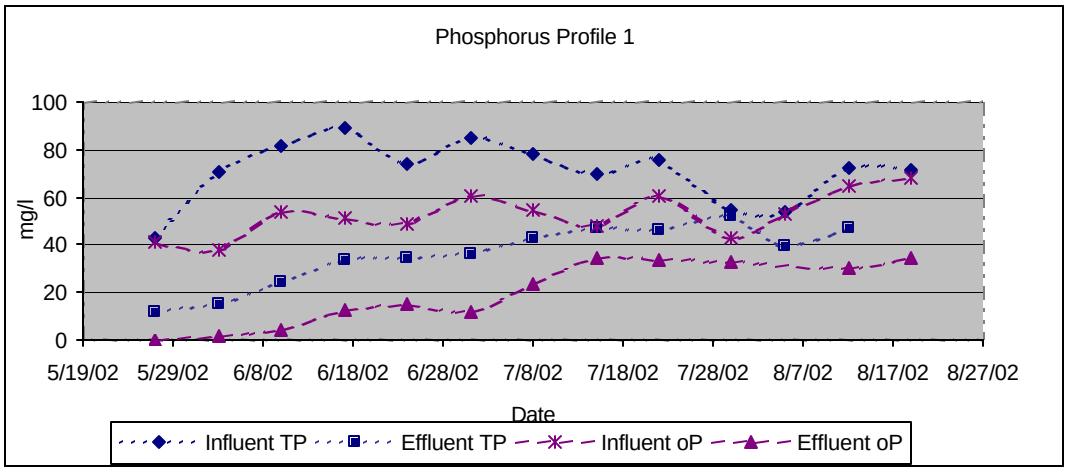


(a)

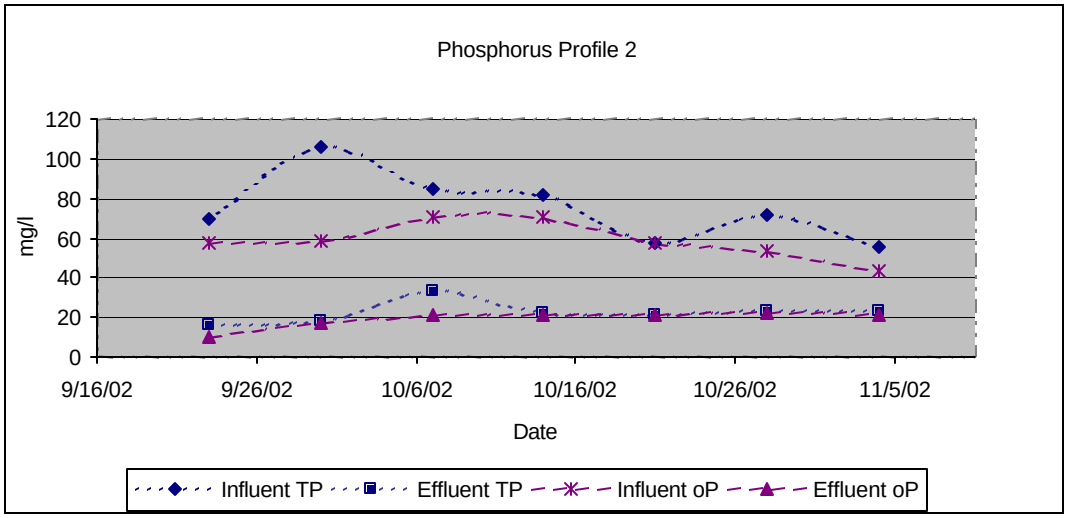


(b)

Figure 2.6 Nitrogen and Phosphorus concentrations in the influent and effluent of the duckweed tanks for the two operation periods of 5/27/02 - 8/22/02 and 9/23/02 - 10/23/02



(c)



(d)

Figure 2.6 Continued

The profiles of the nutrient for the operation indicate the trouble that was observed during the operation near the end of July. There was not enough nutrient removal from the tanks occurring to provide for healthy growth by the plants. The purpose of the initial operation of the pilot plant was to determine the parameters for successful treatment of swine waste and nutrient recovery using *Lemna gibba.* As the plants began to die in late July and early August it became evident that the loading rate of nitrogen had become too great for the mechanisms of removal to keep up and therefore resulted in an accumulation of nutrients in the tanks. The initial goal of this research was not to find inhibition levels for duckweed growth, but fortuitously, by killing the

plants in the first month, a very important upper range was determined for the operation of the duckweed system. The plants grew well in the time span leading up to their inhibition and eventual death, but there hit a point very quickly around the 21st of July when the plants began to deteriorate. This would lead to the conclusion that the ammonium level of around 100 mg NH_3-N/l caused inhibition of plant growth. Lab analysis of the swine waste reports NH_3 and NH_4^- as total ammonia nitrogen, but at the near neutral pH that is maintained by the high alkalinity, it is assumed that the predominant form of nitrogen will be ammonium. At this point the influent was halted to reestablish a healthy system. However, this never happened. A second seeding of the pond was then attempted using plants from a nearby pond. These plants suffered the same fate and were not able to survive on the highly concentrated waste.

The tanks were then purged and influent was reintroduced with a reduced flow rate from 100 gal/day to 50 gal/day around the middle of September. This flow rate was much more manageable and did not cause stress to the plants as the higher flow rate had done. This growth continued for a few months and slowed into the winter season. Again a very useful observation came from the pilot operation. The tanks were aboveground and therefore subject to larger variations in temperature. When the first freezing weather hit in late November, the plants were frozen in the top layer of the ponds. The 2002/2003 winter temperatures were below average and most of December saw the plants frozen and thawed many times. The coldest temperatures came in January and completely suspended all of the plants in a layer of ice that extended well past the root zone of the plants. The plants did not grow during these months, but as soon as the temperatures became milder and longer days afforded longer exposure to sunlight, the plants quickly regained full surface coverage of the tanks in mid March. This is a positive result for the implementation of a full-scale system, which would have the insulation of ground to make the winter water temperatures much less severe.

The reason for duckweed growth inhibition in high nutrient levels has been researched by many sources (Wang 1991; Körner et al. 2001). The point at which the plants began to show signs of ammonium inhibition was lower than the reports of inhibition at 133 mg/l ammonium by Bergmann (2000a). The possible differences could arise from the high temperatures, near 29ºC,

experienced by the plants as the ammonia/ammonium ratio is dictated by pH and temperature. Körner et al. (2001) suggested that ammonia inhibition would begin around 1 mg/l in pH region from 6.3 to 8.7 and that the maximum ammonia level that the plants could withstand would be around 8mg/l. Based on the equations below, and a pH of 7.5 at the time of death, ammonia would have been about 3% of the total ammonia. This would suggest that about 3 mg/l of un-ionized ammonia was present and around 97 mg/l of the ammonium ion. This value occurred in the range reported and suggests that both ammonia and ammonium were acting in the inhibition of growth. The TKN level at this point was near 130 mg/l. These values along with the nutrient removal rate, which are about to be covered, provide a strong framework for the establishment of a complete treatment system for nutrient recovery.

$$pK_a = \frac{0.09108 + 2729.92}{(273.2 + T)}$$

$$Un-ionized\ \ NH_3(\%) = \frac{100}{(1+10^{(pK_a - pH)})}$$

The plants grew at a rate of 8.46 and 7.17 g m^{-2} d^{-1} (dry weight) for periods 1 and 2 respectively. This growth rate is on the lower end of the 8 to 15 g m^{-2} d^{-1} scale for secondary wastewater treatment using duckweed (Oron et al. 1984). This growth rate is very low compared to the 28.5 g m^{-2} d^{-1} for *Lemna minor* by Cheng et al. (2002). The plant tissue analysis indicates that the plants were very nutrient rich and from this the assumption is made that the protein levels were very high as well. The percent nitrogen and percent phosphorus of the plant tissue by dry weight were around 6.5% and 2.25% respectively. This is a large increase from the values of 5.69% and 1.65% composition observed during greenhouse growth conducted by Bergmann et al. (2000b). It is possible that the growth of the duckweed outdoors provided an environment with uninhibited sunlight to promote a more active plant with healthier photosynthesis. This could have promoted the high nutrient composition of the plants. The high nutrient concentration in the

wastewater also promoted the high nutrient levels in the plants (Culley and Epps 1973). Table 2.3 shows the average treatment properties of the duckweed tanks during the first treatment period.

Table 2.2 Performance of duckweed tanks treating digester effluent between 5/27/02 and 8/22/02 including 12 samples.

	Unit	Digester Effluent			Duckweed Tank Effluent			Removal Efficiency %
COD	mg/l	940.15	±	266.49	357.00	±	94.16	62%
TOC	mg/l	277.08	±	126.90	102.57	±	36.40	63%
TKN	mg/l	244.31	±	46.66	97.80	±	33.79	60%
NH3N	mg/l	176.15	±	17.09	68.43	±	27.23	61%
TP	mg/l	70.62	±	13.05	35.83	±	12.90	49%
o-PO4-P	mg/l	52.62	±	9.19	19.52	±	13.46	63%
pH		7.51	±	0.17	7.66	±	0.21	

As a treatment process the duckweed growth is not responsible for all of the nitrogen removal from the duckweed tanks. The accountable removal from the digester effluent in the duckweed tanks will be in these ways: assimilation into the biomass of the duckweed plants, nitrification/denitrification by organisms on and around the plant surface, and ammonia volatilization. The nutrient removal by *Lemna gibba* only represents a fraction of the total removal achieved by the duckweed tanks. During the first operation the TKN and TP removal by duckweed was 0.61 g m^{-2} d^{-1} and 0.18 g m^{-2} d^{-1} respectively. Similar values of 0.49 g m^{-2} d^{-1} and 0.20 g m^{-2} d^{-1} were observed during the second operation period. These values are on the lower range of reported uptake rates. In specific, the TKN removal rate is half of the 0.95 g m^{-2} d^{-1} reported for growth on 25% lagoon effluent under natural conditions (Cheng et al. 2002). The TP levels are also on the lower end of the results from this previous study. For the two periods of duckweed growth in the pilot system there was an average TKN loading of 6.41 g m^{-2} d^{-1} for the first period and 3.34 g m^{-2} d^{-1} for the reduced flow second period. The TP loading was 1.85 and 0.95 g m^{-2} d^{-1} for the first and second periods, respectively. The removal rates of the duckweed are clearly lower than the total removal rates and only contribute a fraction of the nutrient removal. The percentages of TKN removal achieved by duckweed were 14.6% and 19.0% for the two operation periods. The percentage TP removal were 15.9% and 33.0% for the two operations.

As mentioned earlier there is the need in future operations to harvest the plants at a greater rate to provide optimum growth and nutrient removal.

Complete System

The complete pilot system treatment characteristics are listed in tables 2.4 and 2.5 for the two operation periods. The two periods are quite different due to the variability of the influent properties and more importantly due to the reduction of feed rate from 100 to 50 gal/day. The nutrient removal levels are not as high as some batch systems that grow the plants on a wastewater until there is a limiting nutrient for plant growth (usually phosphorus) and most of the nitrogen is removed. When this pilot system is run at steady state the nutrient effluent will still be relatively high in order to provide maximum potential for nutrient uptake by the plants. The benefit from the system will be a lower concentrated wastewater and a smaller volume than is currently produced during common treatment practices.

Table 2.4 Performance of pilot system treating swine wastewater between 5/27/02 and 8/22/02 including 12 samples.

	Unit	Influent			Effluent			Removal Efficiency %
COD	mg/l	5894.54	±	6322.06	357.00	±	94.16	94%
TOC	mg/l	553.15	±	315.92	102.57	±	36.40	81%
TKN	mg/l	350.38	±	71.34	97.80	±	33.79	72%
NH3N	mg/l	175.46	±	27.00	68.43	±	27.23	61%
TP	mg/l	144.32	±	38.74	35.83	±	12.90	75%
o-PO4-P	mg/l	99.50	±	27.14	19.52	±	13.46	80%
pH		7.53	±	0.35	7.66	±	0.21	

Table 2.5 Performance of pilot system treating swine wastewater between 9/23/02 and 11/04/02 including 7 samples.

	Unit	Influent			Effluent			Removal Efficiency %
COD	mg/l	10067.14	±	5893.22	160.86	±	19.18	98%
TOC	mg/l	509.60	±	136.11	71.11	±	46.33	86%
TKN	mg/l	582.43	±	220.43	57.09	±	10.67	90%
NH3N	mg/l	213.43	±	59.90	41.97	±	9.76	80%
TP	mg/l	204.63	±	97.50	22.33	±	5.35	89%
o-PO4-P	mg/l	55.51	±	34.23	19.25	±	4.26	65%
pH		7.13	±	0.16	7.47	±	0.11	

Figure 2.7 compares the effluent from the pilot plant procedure to the effluent from the lagoon that lies just below the pilot plant and settling basin. It appears that the practical operating range for TKN is from about 50 mg/l to around 100 mg/l. If this is the effluent rate from the pilot plant, it will be well bellow the effluent level of the lagoon which was constantly between 150 and 300 mg/l. The final details for a comparison of these two treatment methods will be discussed in the following chapter of this thesis. The COD reduction is high for the entire process, but does not significantly differ from that of the treatment lagoon.

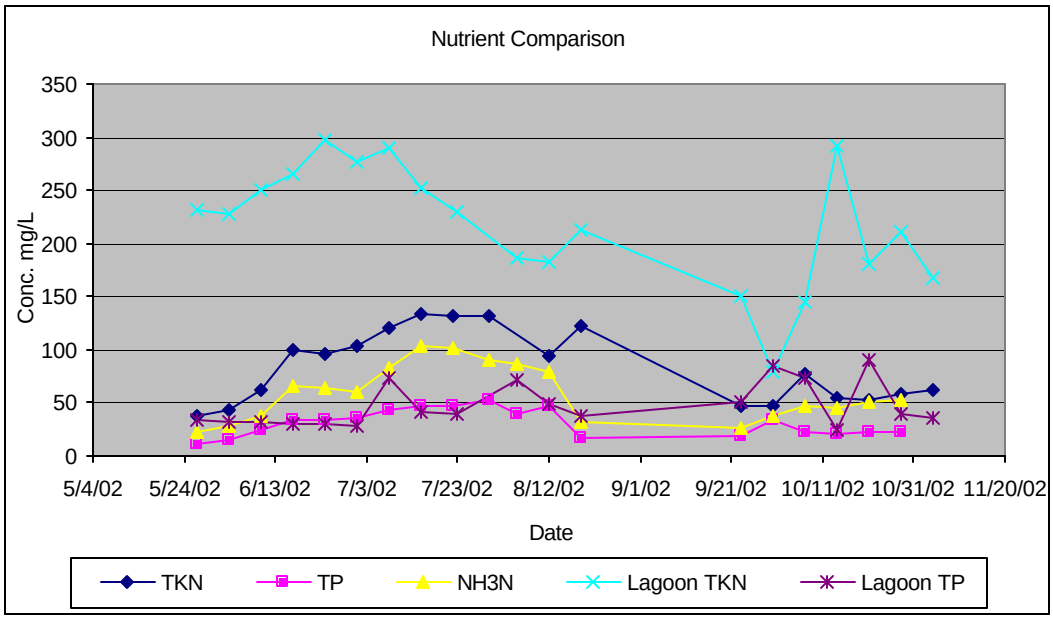
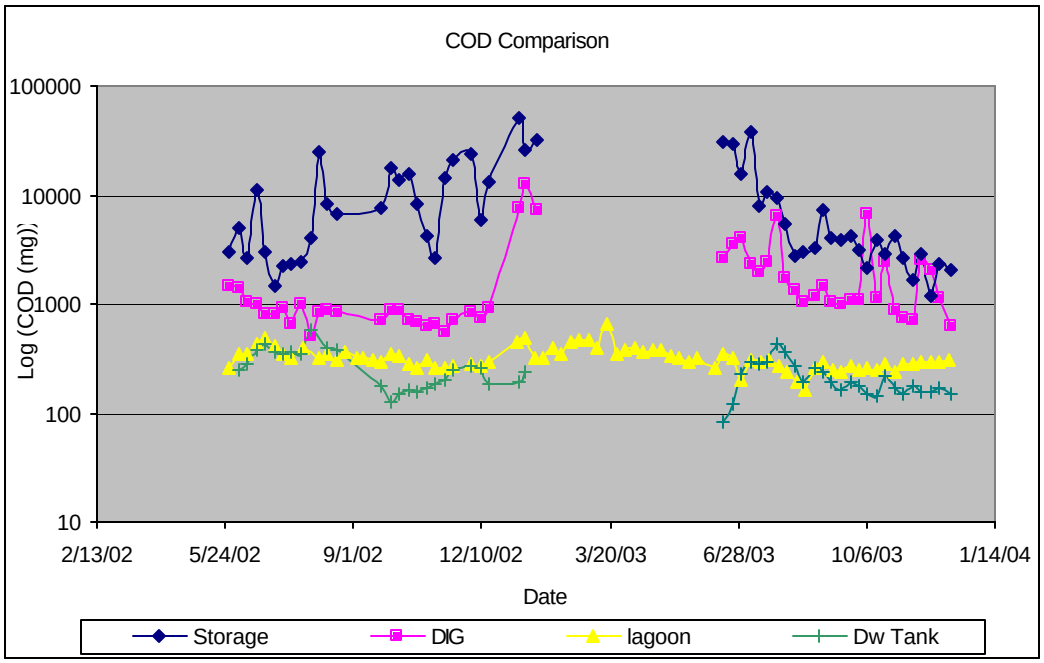


Figure 2.7 Comparison of effluent from lagoon and effluent from pilot plant (a) Nutrients (b)COD



(b)

Figure 2.7 Continued

Conclusion

From the operation of the pilot plant it appears that the feed rate at which this system would operate best would be in the range of 50 to 75 gal/day. This would be a loading of between 3 and 5 g TKN m^{-2} d^{-1} based on the current TKN concentration in the influent. Assuming that the plants were harvested at a high enough rate to encourage a larger TKN removal rate than the 0.61 g m^{-2} d^{-1} that was accomplished during this study the higher loading rates could be implemented. The digester operates efficiently treating 100 gallons per day. In order to run the duckweed system in line with the digestion, doubling the surface area would be required for duckweed growth to handle the nutrient load effectively. The point at which inhibition occurred was around 100 mg NH_3N /l. At this point it became impossible for the plant to grow and therefore the system regressed to properties similar to a lagoon. In the subsequent operation of the pilot plant, this 100 mg/l was used to prevent system failure from occurring again.

Three questions need be included in the planning and design of this system with respect to nutrients. The first is "what level of nutrient removal is desired by the duckweed system?"

The next is "what nutrient level is best suited for maximum plant growth?" Finally, "at what rate can the wastewater be treated?" If there is a profitable use for the duckweed then production of plant biomass will become the main concern of the treatment process. If there is very little land available for land application of the remaining effluent, then the design will be based on effluent properties. These are a few factors determining the design of a full scale system. It appears that complete treatment by the plants would produce a huge amo unt of duckweed and require that the harvested duckweed be utilized as a value added product. The practical application of this system is that it lowers the amount of nutrients that have to be land applied and produces a marketable byproduct.

Ackno wledgements

The authors would like to thank the Environmental Analysis Laboratory of the Biological and Agricultural Engineering Department at North Carolina State University, Dr. Yuri Yamamoto for providing the duckweed, and Jeremy Goodwin for assistance with the start up of the pilot plant.

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Chapter Three

Comparison of L. gibba 8678, S. punctata 7776, and L. minor 8627 for Nutrient Recovery from Swine Wastewater: Pilot Study

Abstract

Nutrient recovery from anaerobically pretreated swine wastewater by growing duckweed has been investigated under field conditions in Raleigh, North Carolina. Raw swine wastewater of 100 gallons/day was first treated in a 1,000-gallon anaerobic digester with floating ballast rings. Organic compounds in the wastewater were digested to produce biogas. Many nutrients including nitrogen and phosphorus remained in the effluent of the anaerobic digester. Three species of duckweed (*Lemna gibba* 8678, *Spirodela punctata* 7776 and *Lemna minor* 8627) were grown in 1,250-gallon tanks to recover nutrients from the anaerobic effluent. The duckweed was periodically harvested and can be used as animal, poultry, and fish feed. This research provides an initial understanding of the characteristics exhibited by these three duckweed species in the treatment of swine wastewater under field conditions in North Carolina. The duckweed tanks were run as completely mixed systems. The performance of the system was monitored by measuring chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN), ammonia nitrogen, total phosphorus (TP), ortho-phosphate-phosphorus, and pH in the influent and effluent of each duckweed tank.

Introduction

The operation of the pilot plant in 2002 provided an initial understanding of the performance of the system with the use of *Lemna gibba* 8678 for nutrient removal. This research is useful in attempting to produce more effective treatment methods for swine wastewater. One area of the current treatment system that this research seeks to improve is the value of byproducts from the treatments system. Anaerobic digestion is capable of reducing the organics load in the waste while also producing methane, but there is still a large amount of nutrients in the effluent that must be removed. Duckweed is a fast growing aquatic plant that can remove nutrients at high levels and has a large proportion of protein in the plant biomass. These high protein plants have the potential to be used as a valuable feed supplement. This would be more effective than the current Bermuda grass that is grown on the land applied effluent. Research has also shown the potential for genetically engineered duckweed use in the pharmaceutical industry for protein extraction(Yamamoto et al. 2001). The duckweed system is also more environmentally friendly because ammonia volatilization is reduced due to surface coverage by the plants and duckweed growth lowers the need for land application by removing nutrients.

In determining the most effective method to implement duckweed-based treatment systems, research was done to identify the geographic isolates that grew most effectively on swine wastewater. This research was initiated by selecting the plants from the worldwide germplasm collection housed at North Carolina State University that were observed to grow well during culture maintenance(Bergmann et al. 2000a). From this research the three plants that were selected for further study were *Lemna gibba* 8678, *Spirodela punctata* 7776, *and Lemna minor* 8627. *Lemna minor* 8627 was not in the top 3 for growth on swine effluent, but because it can be genetically engineered to produce certain proteins such as insulin, it was included in further research (Yamamoto et al. 2001). The research comparing these three geographic isolates also introduced the concentration of swine lagoon effluent and tap water as a variable. The plants did not grow well in Bergmann et al. (2000a) on pure lagoon effluent, and a suitable ratio was needed for further research. The results from the test

including the three isolates on the varying wastewater concentrations concluded that the plants should be grown on a wastewater in the range of 25% to 50% lagoon effluent. The plants selected by Bergmann et al. (2000b) for growth with the pilot system were *Lemna gibba* 8678 and *Lemna minor* 8627. This is interesting because the earlier research indicated that *Spirodela punctata* 7776 had the highest protein production during the in vitro studies.

The research detailed in this thesis examines the performance of these three isolates under field conditions to determine which plants should be used in the development of a full scale system. The pilot scale system in use is set up as continuous flow and hopefully through this research, parameters for effective nutrient removal can be determined for the three plants.

Methods

Pilot system operation

Operation of a pilot system for the treatment of swine wastewater, incorporating anaerobic digestion and the growth of *Lemna gibba, Spirodela punctata,* and *Lemna minor* was monitored to determine organics destruction and nutrient uptake achieved by a continuous flow system under field conditions. The pilot system is located at the Lake Wheeler Road Field Laboratory of North Carolina State University in Raleigh, North Carolina. The system was constructed next to an existing settling basin and treatment lagoon for the experimental swine unit. The source of waste liquid for this research was screened effluent from the settling basin. The average properties of this wastewater influent for the duration of the study can be seen in Table 3.1. The pilot plant consisted of one 1000-gallon Attached Growth Anaerobic Digester (AGAD), which feeds three 1250-gallon concrete tanks used for duckweed growth (Figures 3.1 and 3.2).

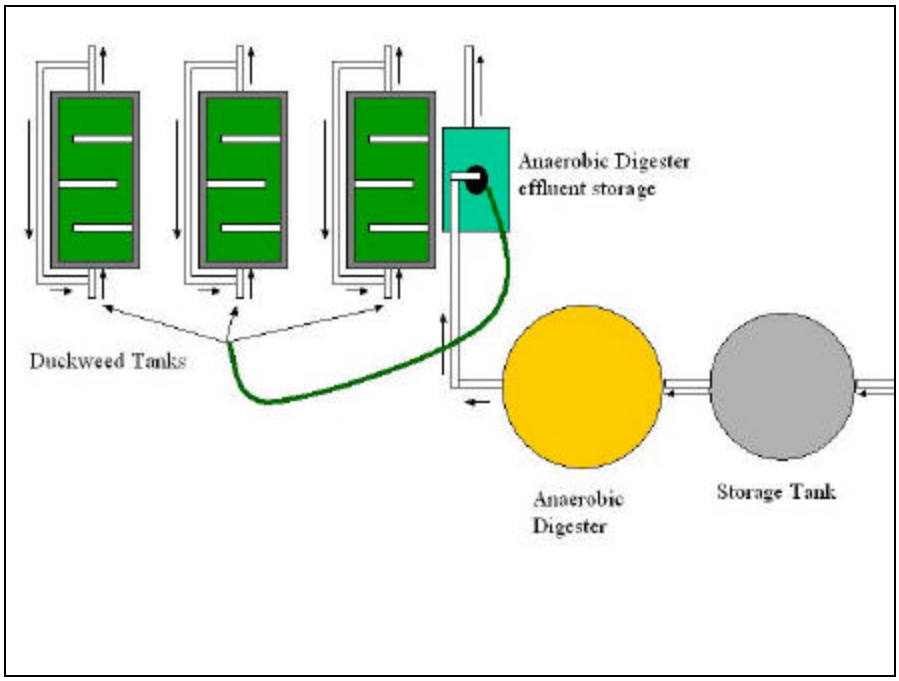


Figure 3.1 Diagram of pilot treatment process

Figure 3.2 AGAD and duckweed tanks

Table 3.1 Average raw swine wastewater properties including 26 samples during the pilot plant operation.

	TKN mg/l	NH3N mg/l	NO3N/NO2N mg/l	TP mg/l	OP mg/l	COD mg/l	pH	TS	VS	ALKAL mg/l	TOC mg/l
AVG	430.2	240.0	0.0	144.7	85.2	7898	7.20	0.53	62.0	1620	1417
StDev	185.3	91.4	0.0	86.6	57.6	9853	0.55	0.42	12.4	420	1488
Max	865.0	434.0	0.0	383.0	249.0	38200	8.68	1.56	80.3	2400	5090
Min	204.0	96.0	0.0	59.1	21.9	1200	6.02	0.37	17.0	900	19.8

The effluent from the anaerobic digester was taken to a 250 gallon holding tank. From the holding tank the digester effluent was manually distributed to the three 1250-gallon concrete tanks. The effluent was distributed to the duckweed tanks by placing large 50 gallon PVC containers on the edge of the tanks and filling them to the desired level. The containers were loaded daily in order to provide a 20 gal per day flow rate for each duckweed tank. The containers were then drained into the duckweed tanks. The excess digester effluent was then wasted to the lagoon. The dimensions of each duckweed tank are 3.2 feet deep, 10.5 feet long and 5 feet wide. The tanks were each divided into four sections to prevent the wind from moving the plants around. The tanks were initially filled with 75% tap water and 25% swine lagoon water and circulation was provided in 30-minute periods 4 times per day to provide well-mixed conditions in each of the tanks. Each tank contained a different geographic isolate of duckweed. Figure 3.3 shows the configuration of the tanks, and figure 3.4 depicts the three species growing in the tanks.

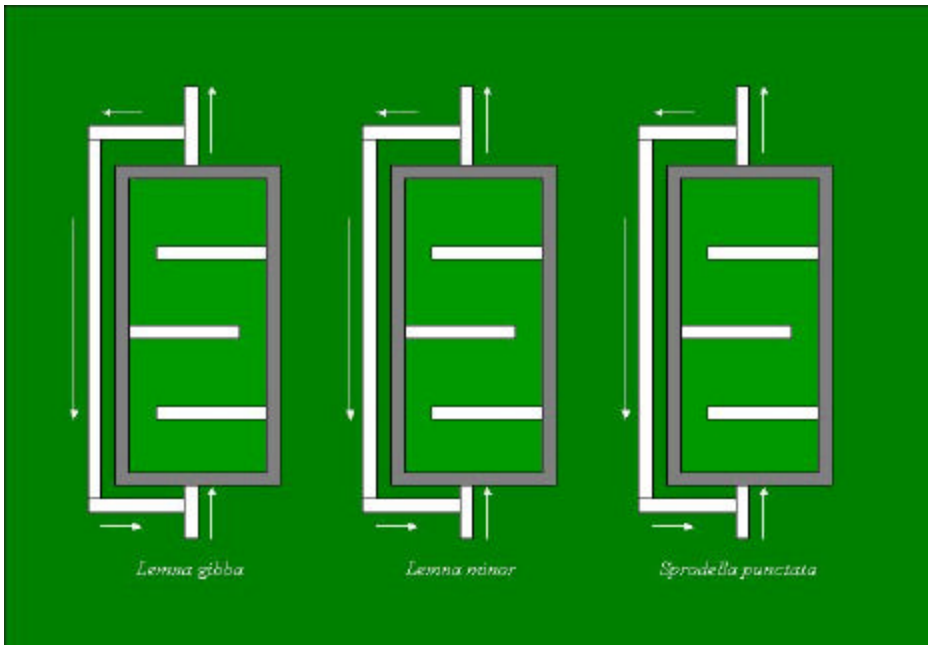
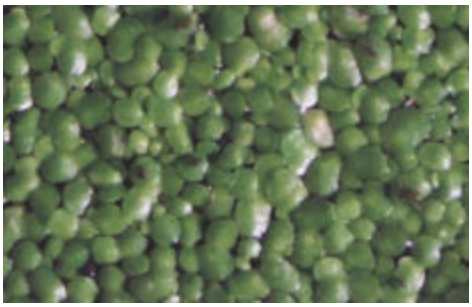
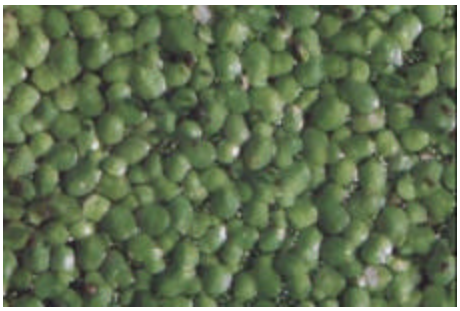


Figure 3.3 Configuration of duckweed tanks



(a)



(b)

(c)

Figure 3.4 (a) *Lemna minor* 8627 (b) *Lemna gibba* 8678 (c) *Spirodela punctata* 7776

The duckweed was initially located in the worldwide germplasm collection in the forestry department at NCSU. Small amounts of these three isolates were collected during routine culture maintenance and placed in baby food jars on 25 ml of SH media with 1% sucrose for growth (Schenk and Hilderbrandt 1972). The plants were then taken from the baby food jars once thick surface coverage was achieved, and separated into small boxes and grown on 40 ml of artificial swine medium (SAM3). The up scaling was done using a sterile hood. The SAM3 medium was produced using the following recipe:

For 1L of SAM3:

Add 10 ml each of stocks 1, 2, 3, and 4 to 950 ml H_2O

Along with:

30g Sucrose (3%)

1.15 g/l Citric acid

1.24 ml of concentrated NH_4OH/l of medium

226 mg/l $Ca(OH)_2$

Always add the NH_4OH last.

Adjust Final pH to 7.1 using 0.1 M HCL or 0.1 M NaOH

Stock Preparation:

Stock 1

K_2SO_4	52.9 g/l	6.08 mM K and 3.04 mM SO_4 X 100
$MgSO_4\,7H_2O$	40.7 g/l	1.65 mM Mg and 1.65 mM SO_4 X 100
$ZnSO_4\,H_2O$	1.349 g/l	0.047 mM Zn and 0.047 mM SO_4 X 100
$MnSO_4\,H_2O$	0.27 g/l	0.016 mM Mn and 0.016 mM SO_4 X 100
Cu $SO_4\,5H_2O$	0.47 g/l	0.019 mM Cu and 0.019 mM SO_4 X 100
Na_2SO_4	52.19 g/l	7.35 mM Na and 3.675 mM SO_4 X 100

Stock 2

NH_4Cl	45.47 g/l	8.5 mM NH_4 and 8.5 mM Cl X 100
NH_4NO_3	0.12 g/l	0.015 mM NH_4 and 0.015 mM NO_3 X 100

Stock 3

K_2HPO_4	72.1 g/l	3.16 mM PO_4 and 6.32 mM K X 100
H_3BO_3	0.3895 g/l	0.063 mM B X 100
$CoCl_2\,6H_2O$	0.01 g/l	0.00042 mM Co and 0.00084 mM Cl X 100
Na_2MoO_4	0.00432 g/l	0.00021 mM Mo and 0.00042 mM Na X 100

Stock 4*

(A) $FeSO_4$ $7H_2O$	3.92 g/l	0.141 mM Fe and 0.141mM SO_4 X 100
(B)Na_2EDTA $2H_2O$	5.25 g/l	0.282 mM Na X 100

*Make solutions A and B separately in half the final volume, and then mix them together.

Once the media was made, the boxes or larger flasks used for growth were autoclaved to provide a sterile growth medium. The SAM3 medium was used in each subsequent upsizing until there was enough plant biomass to be moved to the greenhouses and grown on swine lagoon effluent. In the greenhouse, 100-liter baby pools were used to grow the plants. Each pool was initially filled with 25 % lagoon effluent and 75 % tap water for a total of near 80 liters. After the plants had developed the necessary biomass in the greenhouse, they were then transported to the pilot plant to seed the tanks. Each tank was seeded with six pounds by wet weight of one of the three different geographic isolates. This was enough to provide surface coverage.

During the operation of the system the duckweed was harvested at a rate that would remove enough biomass to allow healthy growth, while not leaving any surface area exposed. This was accomplished using screens that were 20% of the surface area of the tanks, and usually harvesting 3 times a week. The weight of duckweed harvested was recorded to measure the growth of the plants. The effluent from the tanks was then wasted into the lagoon. The initial feed rate of 60 gal/day to the tanks resulted in a 62.5 day HRT, but later in the research the influent to the tanks was cut to 30 gals per day and the resulting HRT was 125 days.

Chemical analysis

Every day temperature, pH and DO (at 1 inch and at 1 ft) were measured in the duckweed tanks between the time period of 10 am and 2 pm. Grab samples were taken weekly from the influent to the digester, the effluent from the digester, and from the duckweed tanks. It was then analyzed for TKN, NH_3 - N, NO_3 - N/ NO_2 - N, TP, OP, COD, pH, TS, VS, COND, ALKAL, TOC, K, Cu, Zn. Plant tissue was also sampled weekly and

TKN, TP, moisture content (MC), K, Cu, Zn were measured. Analysis was performed by the Environmental Analysis Laboratory of the Biological and Agricultural Engineering Department at North Carolina State University using EPA methods (EPA 1983) and Standard Methods (APHA 1995). The pH, dis solved oxygen (DO) and temperature of the duckweed tanks were measured daily on site using electrodes.

Nitrogen mass balance

The following equation was used to follow the mass balance of nitrogen in the duckweed tanks:

$$N_T = N_p + N_i - N_{dw} - N_o$$

N_T is the amount of nitrogen in the duckweed tanks, N_p is the amount of nitrogen initially present in the tanks, N_i is the amount of influent nitrogen to the tanks, N_{dw} is the amount of nitrogen in the duckweed harvested from the tanks and N_o is the other forms of nitrogen loss including denitrification and ammonia volatilization. A similar formula was used to determine the phosphorus mass balance:

$$P_T = P_p + P_i - P_{dw} - P_o$$

Statistical Analysis

The SAS System for Windows Version 8 was used for comparisons of the data collected from the duckweed growth. A multiple linear regression (MLR) was performed with the plant species as a class statement in order to determine if the plant species had an effect on the selected data. Tests with P values less than 0.05 were considered to be significantly different.

Results and Discussion

Anaerobic Digestion

The use of anaerobic digestion was incorporated into this treatment in the same manner as during the operation of 2002. There was a lower efficiency of COD removal during 2003. This resulted in a higher organics loading to the duckweed tanks despite the lower organics loading in the raw swine wastewater. The variability in the COD in the digester effluent in 2002 was around 27%, while the variability of the digester effluent in 2003 was near 73%. The change in properties most likely arises from operation problems including troubles with the thermometer and feed system. This led to constant fluctuations in the digester's temperature. While the digester was not operating at optimum conditions, there was still a considerable amount of organics destruction through the digestion process. COD levels were reduced by 74% and TOC was reduced 64%. Additional operation parameters of the digester during 2003 are available in the appendices.

Duckweed Seeding

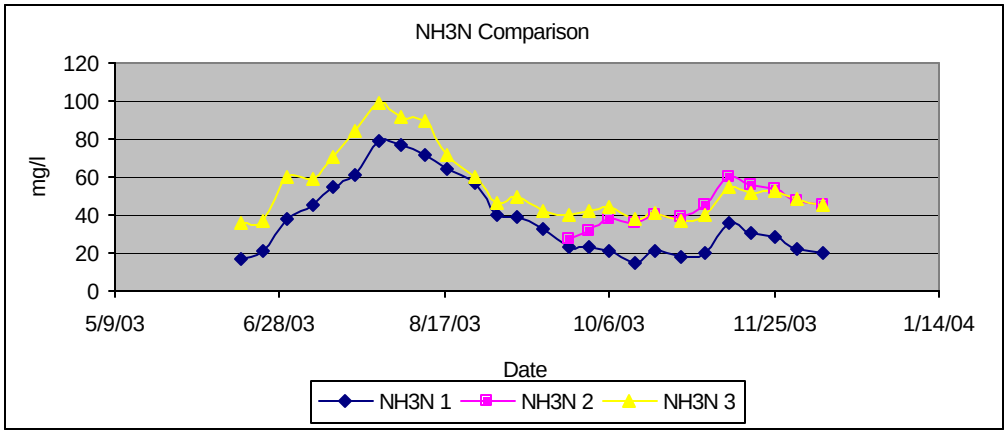
The three plants, *Lemna gibba* 8678, *Lemna minor* 8627, and *Spirodela punctata* 7776 used in this study are pictured above in Figure 3.4. A transition from the one species in the three tanks to the current setup of three species in individual tanks required upscaling of the duckweed from the germplasm collection to the mass needed for seeding. This was carried out during the winter and into the spring of 2003. Most of the details of this process are detailed in the Methods section of this chapter, but there were some challenges. Dr. Yuri Yamamoto provided the initial supply of plants needed to seed the baby pools, but there was some trouble with the greenhouse growth and the plants died in early January. Most likely the high temperature, near 45°C in the greenhouse, stressed the plants. Losing the plants setback the start of operation until late June. The next step was to work with Dr. Anne Stomp and produce the amount of plants necessary to move to the greenhouse again. This time the ambient temperatures were warmer, but there was a new paint on the houses to block light, lowering the internal temperatures. Cool water was added to keep the volume of water as large as possible and to lower the temperature in the pools.

The scaling up of the plants became difficult, as there was trouble with the *Lemna minor* 8627 being overcome by bacteria in the growth medium and not reproducing. This was possibly due to the location of the autoclave being located in an adjacent building. Carrying the freshly autoclaved boxes of media back might have allowed for contamination opportunities as the boxes cooled and pulled in air from the surrounding environment. The *Lemna minor* 8627 was then unable to compete with the contaminant, and did not grow efficiently until the plant was removed from the SAM3 and grown on a much more favorable medium. The other two plant species had less trouble with contamination and were able to quickly produce the wet weight needed to seed the pilot plant. This makes sense as the *Lemna gibba* and *Spirodela punctata* performed much better on the SAM than *Lemna minor 8627* in earlier studies (Bergmann et al. 2000a). The *Lemna minor 8627* could not be placed at the pilot plant until September due to the troubles with the lab growth.

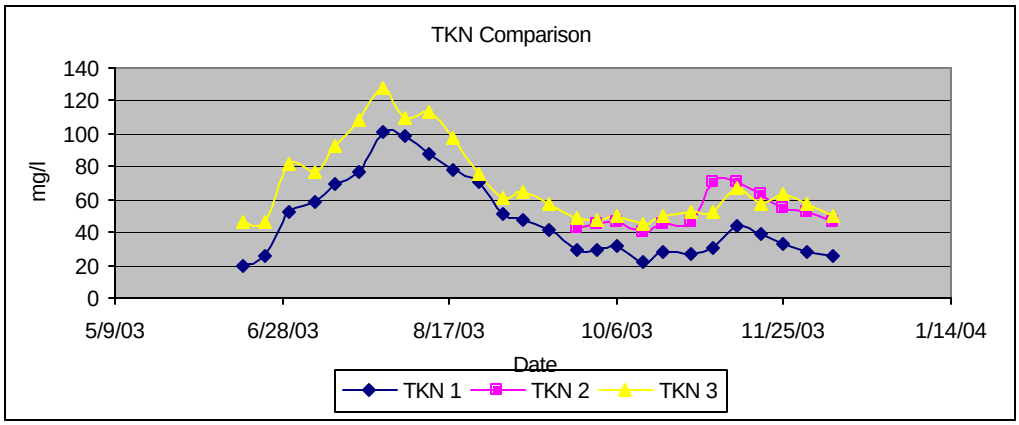
Duckweed Performance

The plants were initially grown on SAM3 in laboratory and then swine lagoon effluent was used to acclimate the plants to the wastewater in the greenhouse. This procedure was effective and as soon as the plants were placed in the tanks at the pilot plant, growth quickly began. The 6 lbs per tank of wet weight was just enough to cover the surface area. The plants quickly grew to around 30 lbs per tank by wet weight for both *Lemna gibba* and *Spirodela punctata* by the first harvest just a week later. The operation of the duckweed growth at the pilot plant went smoothly and the plants appeared healthy for the duration of the study with the exception of the winter months, when growth was stopped and the plants were dormant.

As plants were introduced, it became important to stabilize the operation of the tanks. Results of the 2002 pilot study supported a feed rate of about 60 gallons per day, or 20 gallons per tank. The data reporting the TKN and ammonia concentrations were used to determine the stability of the treatment process. The profiles of the TKN and ammonia for the 2003 operation are displayed in Figure 3.5.



(a)



(b)

Figure 3.5 Profile of **(a)** NH_3-N and **(b)** TKN in the three duckweed tanks.

As these figures indicate, there was a noticeable increase in the nitrogen present in the tanks from the start of the operation until the end of July. The TKN and NH_3N levels were approaching 120 mg/l and 100 mg/l respectively. The plants began to show the stress from the high nitrogen levels, and further increase in the tanks would have led to the death of the plants. The design flow rate of 20 gal per day to each tank was too high for the pilot plant in 2003. The problem stemmed from the fact that nitrogen was being fed into the tanks at a higher rate than the year before, due to the higher concentrations in the influent. The average feed rate per tank during stable operation in 2002 was 3.34 g TKN m^{-2} d^{-1} for the flow rate 50 gallons per day. The loading rate of nitrogen to the tanks in 2003 was an average of 6.05 g TKN m^{-2} d^{-1}. This number is higher due to the high average concentration of TKN

experienced during 2003. The flow rate of the duckweed tanks was cut to 10 gallons per tank per day in order to lower the nitrogen loading to the tanks. This reduction resulted in reduced nitrogen levels in the tanks and provided stable growing conditions.

It is clear from the two years of operation that the flow rate to the tanks must be based on the TKN and NH_3N concentrations in the influent. Basing the operation on flow rate would rely on a waste stream with little variability. In order to keep the effluent TKN concentrations near 100 mg/l, the loading rate to the duckweed tanks should be maintained around 3.5 g TKN m^{-2} d^{-1} to 4 g TKN m^{-2} d^{-1}. This is based on the dynamics in place at the Lake Wheeler Road Pilot Plant. The depth and volume of the growth tanks or pond will be a key role in the design of a duckweed treatment system. Research has highlighted the importance of allowing the plants access to the nutrients (Vermaat and Hanif 1998; Chaiprapat et al. 2003). If the water is too deep then there will be insufficient transport of ammonium to the plants, resulting in lower uptake and growth rates. Ideally a large surface area and low depth would allow the greatest growth and water treatment, but this is not possible due to land constraints. Lower percentages of nitrogen removal by the duckweed were recorded during this study than in other research (Körner and Vermaat 1998; Vermaat and Hanif 1998; Bergmann et al. 2000b). This is possible due to a lesser depth in other studies allowing for easier transport of the ammonia to the duckweed for growth. This prevents other methods such as nitrification and denitrification from playing as large of a role in the system, while allowing better transport of nutrients to the plants.

Response to temperature was another interest of this study. If the system is to be implemented in North Carolina, the plants must actively grow for most of the year. Figure 3.6 contains the temperature profile for the duration of the study. This figure illustrates a concern for growth at the pilot plant. The tanks are above ground concrete structures that provide little insulation against temperature change. This is evident by the high water temperatures in the tanks during the summer. This occurs because the temperature was measured during the warmest time period of the day, between 10 am and 2 pm. The same effect is observed during the colder weather. The tanks quickly froze at observed ambient temperatures of around -

2°C. As temperatures drop and daylight became less available the plants growth halted. By moving the pilot system to a ground-insulated pond, the plants would have the potential to grow year round. Right now the growth is possible from middle March until early December based on temperature fluctuations. Further discussion of the individual species reaction to the temperature is located in the next section.

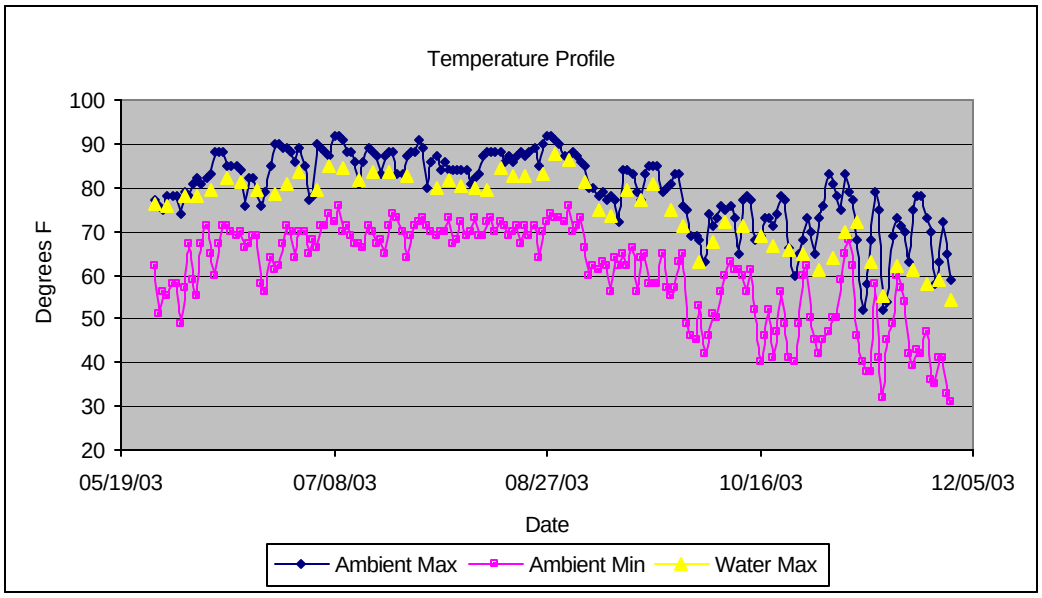


Figure 3.6 Temperature profile of ambient and water temperatures at the Lake Wheeler Road Pilot Plant

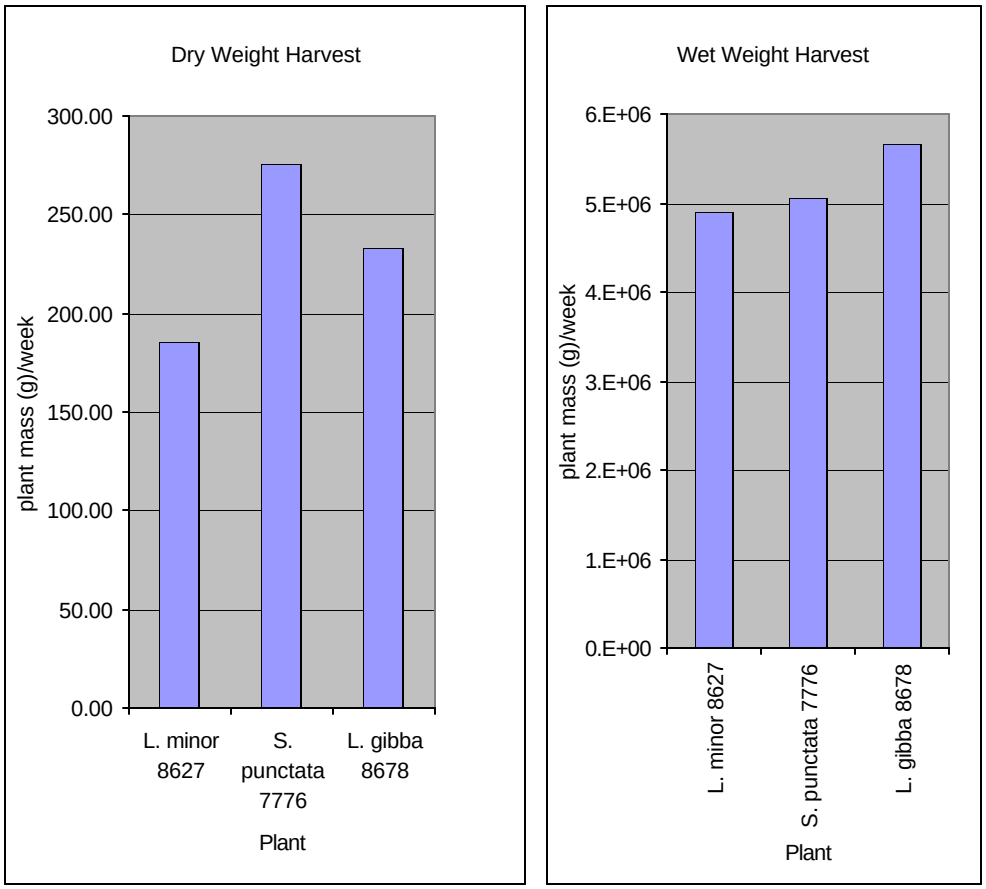
The other measurements associated with the tanks were pH and DO. The average pH of the three duckweed tanks during the study were all very similar at 7.55, 7.73 and 7.68 for tanks 1,2 and 3. These values ranged from 7.1 to 7.98 in the three tanks. pH is extremely stable in the tanks due to the high alkalinity in the swine waste. It is good for plant growth that the pH remains near neutral, because at higher pHs the toxicity due to ammonia increases and the ammonium concentration decreases. The DO in the system was recorded in the average range of 0.75 mg/l at 1 inch and 0.20 mg/l at 2 inches. These values were not significantly different between the three tanks. DO is important in the system for the nitrogen transformation. It is possible that higher DO levels near the surface could promote more nitrogen removal through nitrification (Caicedo et al. 2002).

Duckweed Species Comparison

The geographic isolates used in the operation of the pilot plant had all grown well on diluted swine waste. The research leading to the pilot study indicated that *Lemna gibba* 8678, and *Lemna minor* 8627 had higher biomass production than *Spirodela punctata* 7776 and suggested the use of the two *Lemna* geographic isolates in future system development (Bergmann et al. 2000b). The two *Lemna* isolates also had higher N and P concentrations during the study, indicating larger removal rates than *Spirodela punctata* 7776. This is in contrast with the results of Bergmann et al. (2000a) which found *Spirodela punctata* 7776 to be the highest protein producing plant of the three and therefore the plant which removed the highest levels of nitrogen from the wastewater. The difference arises in the media of the two studies. The punctata was most efficient when on the SAM media, but was not as effective and removed less nitrogen than the two *Lemna* isolates when grown on diluted lagoon effluent. Analyses of the data from the growth of these three isolates during 2003 allowed for further comparison in a pilot system simulating continuous flow treatment under field conditions. Due to the late incorporation of *Lemna minor* 8627, there were two analyses run for comparison of plant growth. The first includes *Lemna gibba* 8678 and *Spirodela punctata* 7776 for the duration of the study, while the second compares the three geographic isolates from the 24th of September until the end of the operation.

The first analysis concluded that there was no significant difference ($p = 0.93$) between the wet weight biomass production of *Lemna gibba* 8678 and *Spirodela punctata* 7776 during 2003. However there was a significant ($p = 0.011$) difference in the dry mass harvested during the 2003 operation of the pilot plant. This calculation of dry weight was based on the moisture content of the plants. The difference between the two isolates is that *Lemna gibba* 8678 had a moisture content of 96%, while *Spirodela punctata* 7776 had a moisture content of 95%. *Spirodela punctata* 7776 had the higher biomass production of the two over the duration of the research. *Lemna gibba* 8678 and *Spirodela punctata* 7776 had average dry weight harvests of 227 g and 311 g per week respectively. The ANOVA table results, harvest date and associated graphs are located in the accompanying appendices.

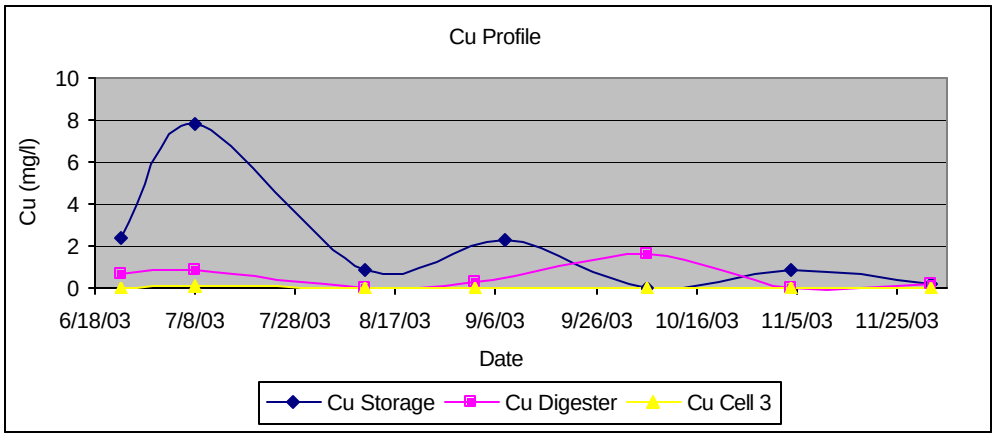
The comparison of the three different isolates between the end of September and beginning of December indicated that there was not a significant difference ($p = 0.53$) in the species wet weight harvested. There was, however, reasonable evidence of differences in the harvested dry mass of the three plants ($p = .012$). The average wet and dry weight production of the three plants are illustrated in figure 3.7. *Spirodela punctata* 7776 had the highest average dry weight production during this time period, with *Lemna gibba* 8678 producing around 42 g less per week. *Lemna minor* 8627 produces 48 g per week less than the *Lemna gibba* 8678. These results are similar to the first analysis and agree that though the wet harvest values are not significantly different, the actual biomass being produced is greater due to the specific moisture contents of the three plants. Over the course of the study the moisture content of the *Lemna minor* 8627 was 96%.



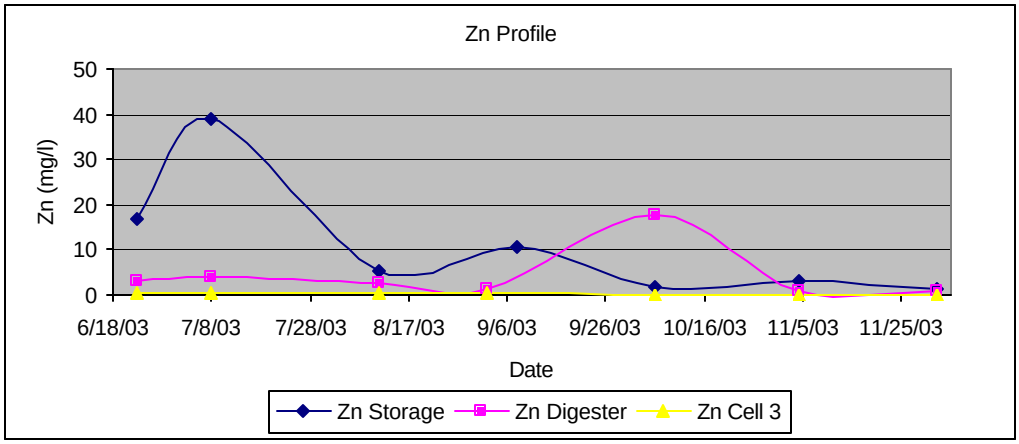
(a) (b)

Figure 3.7 Average (a) dry and (b) wet weight harvests of the three selected geographic isolates between 9/24/03 and 12/02/03.

Differences in the properties of the three plants such as nitrogen and phosphorus concentration in the plant tissue must be considered when evaluating the performance of the three isolates. Table 3.2 lists the average nutrient content of the plant tissue for the duration of the pilot study. *Lemna minor* 8627 had much lower Cu and Zn levels due to the late introduction of the plant to the system. The influent levels of Cu and Zn were much lower in the latter half of the operation (Figure 3.8) and *Lemna gibba* 8678, *Spirodela punctata* 7776 would have been exposed to higher levels of Cu and Zn and would concentrate the elements in plant tissue.



(a)



(b)

Figure 3.8 Profile of (a) Cu and (b) Zn in the three duckweed tanks

Table 3.2 Nutrient content for the geographic isolate during the pilot plant operation

Geographic Isolate	TKN (%)	TP (%)	K (%)	Cu ppm	Zn ppm
Lemna gibba 8678*	6.91 ± 0.74	1.96 ± 0.64	2.44 ± 0.50	42.7 ± 24.2	231.1 ± 113.7
Lemna minor 8627**	6.94 ± 0.50	1.89 ± 0.25	1.87 ± 0.53	12.5 ± 23.0	138.6 ± 115.7
Spirodela punctata 7776*	6.45 ± 0.48	1.62 ± 0.43	2.30 ± 0.26	31.3 ± 22.7	195.2 ± 117.8

** Averages based on 10 measurements
* Average based on 25 measurements

Analysis of nutrient removal by the three plants was performed in the same manner as the analysis of biomass production. One comparison for the entire operation was done to compare *Lemna gibba* 8678 and *Spirodela punctata* 7776. Then a second analysis of the data included *Lemna minor* 8627 along with the other two and used the data from September 24th until the beginning of December. Over the entire study there was a noticeable difference in the average TKN removed per week between *Lemna gibba* 8678 and *Spirodela punctata* 7776 ($p = 0.048$). The average removal of TKN by plant biomass harvest per week was 20 g for *Spirodela punctata* 7776 and 15.75 g for *Lemna gibba* 8678. Though the *Spirodela punctata* 7776 had a lower concentration of TKN in the plant tissue than *Lemna gibba*, greater biomass production resulted in higher nitrogen removal. This difference can be observed in the nitrogen concentrations that are present in the duckweed tanks. Figure 3.7 shows the concentration for *Spirodela punctata* 7776 as being much lower than the other two plants during stable operation. The results of the analysis of TP removal indicated that there was no difference in the two plants ($p = 0.44$). The phosphorus average removal per week was 5.1 g and 4.6 g for *Spirodela punctata* 7776 and *Lemna gibba* 8678, respectively.

The next analysis included *Lemna minor* 8627 in the comparison and only looked at the last few months of the operation. There was no significant difference in the three geographic isolates during the last two months of operation of the pilot plant with respect to TKN and TP removal by the plants ($p = 0.13$) and ($p = 0.3$). The weekly average TKN removal by plant biomass harvest was 17.4 g, 12.9 g, and 15.6 for *Lemna gibba* 8678, *Lemna minor* 8627 , and *Spirodela punctata* 7776, respectively. The average TP removal per week was 4.2 g, 3.5 g, and 4.5 g, respectively. Table 3.3 contains the uptake rates for the three species during the study.

Table 3.3 Uptake rates of the three geographic isolates during the 2003 pilot plant operation.

	Lemna gibba 8678 $g\ m^{-2}\ d^{-1}$	*Lemna minor* 8627 $g\ m^{-2}\ d^{-1}$	*Spirodela punctata*7776 $g\ m^{-2}\ d^{-1}$
TKN	0.44	0.40	0.58
TP	0.13	0.11	0.15

The primary reason for including the duckweed in the waste treatment process was for the removal of nutrients by harvesting plant biomass. A mass balance on the system indicated that the plant growth and harvest accounted for about 20% of TKN loss through the duckweed tanks. More frequent harvesting was suggested and carried out during 2003, and a slight increase in removal percentage was achieved than the previous year. In 2002 plant harvesting of *Lemna gibba* 8678 accounted for 17 % of TKN removal and 25 % of TP removal. The removal percentage by harvest of *Lemna gibba* 8678 during 2003 for TKN and TP were 20 % and 35 % respectively. The results for the three plants average percent removal of TKN and TP during 2003 are illustrated in figure 3.9.

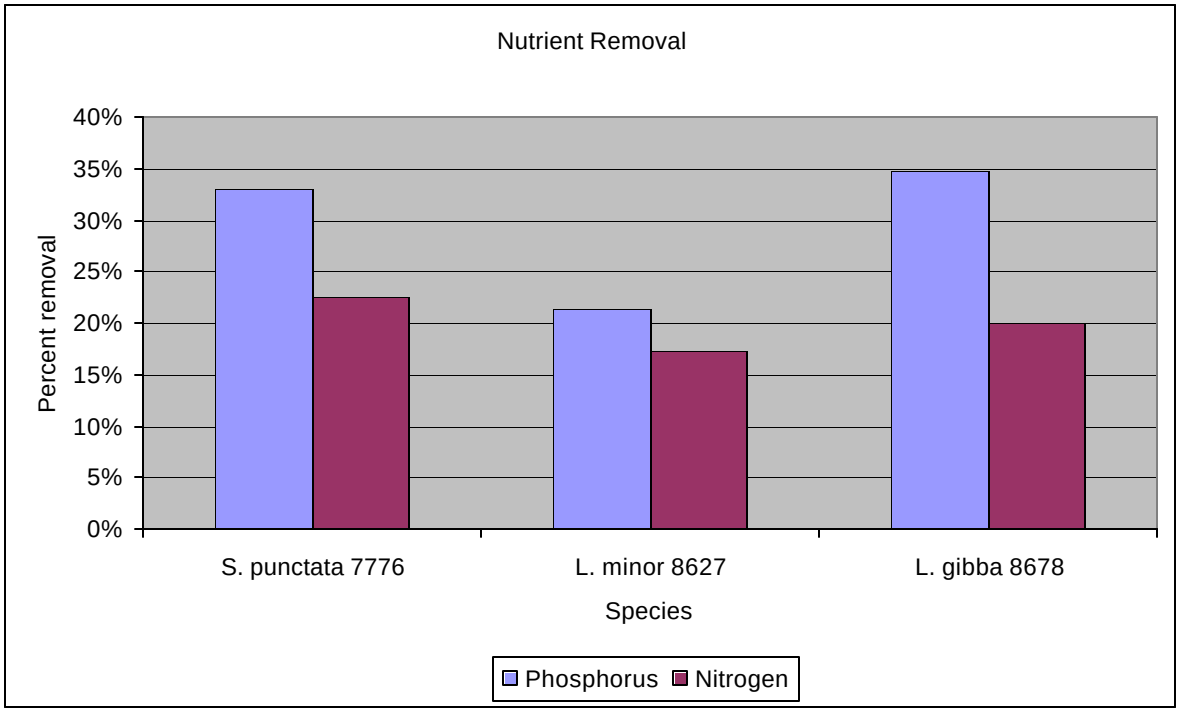


Figure 3.9 Percent of Nitrogen and Phosphorus removal from duckweed tanks accomplished through plant biomass harvest.

The loading rate of nutrients to the duckweed tanks was variable due to the constant change in influent properties of the wastewater. As mentioned earlier, the flow rate to the tanks was reduced from 60 gallons per day to 30 due to the high nitrogen concentrations. The First 35 days of operation the loading rate of nitrogen was 6.7 g TKN m^{-2} d^{-1}. The nitrogen loading level was reduced to 2.7 g m^{-2} d^{-1} for the remainder of the study. The TP loading rate was 0.6 g TP m^{-2} d^{-1} and 1.24 g TP m^{-2} d^{-1} for the two flow rates. Figure 3.5 illustrates the quick increase in TKN and NH_3N during the first 30 days and then the stabilization of the nutrient concentration in the effluent. The plants constitute a larger percentage of nitrogen removal when the loading rate is lowered. A balance must be created where a large enough volume of wastewater can be treated while collecting as much of the nutrients in the plant biomass as possible.

As mentioned earlier, there was concern about the lack of insulation for the tanks to prevent extreme temperature shifts. The *Lemna gibba* 8678 and *Lemna minor* 8627 both began to be stressed and stop growing the last week in November. The water temperatures in the tanks at that time were highest around 14° C during the day and probably dropped below 10°C during the night. The *Spirodela punctata* 7776 performed better as the temperatures were cooler and was still growing into the second week in December. The *Spirodela punctata* 7776 also had almost full surface area coverage before the first of many freezes that began in late January, while *Lemna gibba* 8678 and *Lemna minor* 8627 were much smaller and dormant at this time. *Lemna gibba* 8678 and *Lemna minor* 8627 were the only plants to return with the warmer weather and longer days in the spring. The pictures in figure 3.10 show the three tanks and the remnant plants from the winter. The growth of these plants was initiated during the second week of March and by the first week in April there was complete surface area coverage similar to figure 3.4 (a) and (b) by *Lemna minor* 8627 and *Lemna gibba* 8678. There were no signs of any live *Spirodela punctata* 7776 plants when the pictures were taken the 21st of March. Due to the similar physical properties of the two *Lemna* isolates, it would require genetic testing of plants from the two tanks to make certain that both plants returned in the spring. It is possible that the plants could have been transferred between tanks

in the open environment of the pilot plant. This is unlikely though, because nothing grew in the tank containing *Spirodela punctata* 7776.

(a)

(b)

(c)

Figure 3.10 Pictures of the tanks containing (a) *Lemna minor* 8627 (b) *Lemna gibba* 8678 and (c) *Spirodela punctata* 7776 the 21st of March

This simple observation of which plants were able to return after the winter is very important for a possible full-scale system. The initial seeding of a pond to attain full coverage by duckweed would require time and money and would preferably be a one-time procedure. These results indicate that *Lemna gibba* 8678 and *Lemna minor* 8627 would be preferable due to their ability to become dormant and survive the cold weather. While *Spirodela punctata* 7776 did not return after the winter, further studies with the plant might determine that if the plant were grown in a pond with sufficient insulation, the plant would not die over the winter. This is based on the observation of similar native isolates of *Spirodela punctata* 7776 that are found growing throughout the year in ditches and ponds around the state.

Conclusion

Comparing *Lemna minor* 8627, *Lemna gibba* 8678, and *Spirodela punctata* 7776, it appears that *Spirodela punctata* 7776 would be the geographic isolate to choose, based on nutrient removal from the duckweed tanks. The plant was significantly more effective at removing nitrogen than the other two over the course of the study. Unfortunately, *Spirodela punctata* 7776 was not able to survive the winter and therefore would be a poor choice if it could not naturally return in the spring. However further research could find suitable pond systems with less temperature variation that could support the year round growth of the plant. The *Lemna gibba* 8678 slightly outperformed the *Lemna minor* 8627 in biomass production, but was not significantly better at removing nitrogen and phosphorus from the tanks. One factor that might influence the use of *Lemna minor* 8627 in the future is its ability to be genetically engineered (Yamamoto et al. 2001).

The loading rate of nitrogen suggested for a future system would be 3.5 g TKN m^{-2} d^{-1}. Flow rate for a future system would have to take into close consideration of the TKN concentrations in the influent to achieve the desired loading rate. If the system were based on *Spirodela punctata* 7776 it is possible that a loading rate of over 4.0 g TKN m^{-2} d^{-1} could be implemented. While the *Lemna gibba* 8678 was stressed in the early operation of 2003, the *Spirodela punctata* 7776 ammonia levels were lower and the plant was never stressed in

respect to ammonia and ammonium concentration, therefore there is no suggestion as to what maximum nutrient levels the plant can handle from this research. Based on *Lemna gibba* 8678, concentrations should be kept below 125 mg/l TKN and 100 mg/l NH_3N. A system design would have to balance the trade offs of having a high flow rate system and a high nutrient effluent or a lower flow rate system and lower effluent levels. At the same time the volume of the ponds could allow for manipulation of treatment capacity. Designs based on this research should consider the parameters of a depth of around 3 feet and a steady mixing effect to help make the ammonium available to the plant for uptake.

Acknowledgments

The authors would like to thank Dr. Anne Stomp for her continual support in work with the duckweed plants, as well as Rachel Huie and the NCSU Environmental Analysis Laboratory.

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Appendix A

Schematics and Directions for Operation of the Pilot Plant at Lake Wheeler Road.

The following diagrams and information are provided to assist with the technical operation of the pilot plant in the future.

I

The section represented by diagram I is the pump that is located at the settling basin nest to the pilot plant. The submersible pump is attached to an underground pvc pipe that carries the wastewater from the settling basin to the storage tank. This pump functions best when rinsed regularly and placed just below the surface in-between the overflow and the screen that prevents solids from entering the overflow. Rope tied to the power supply post is sufficient to suspend the pump. The power cord is then plugged into the power supply. The power is controlled by the **STO** switch in the shed onsite. The switch is normally horizontal, and it flipped up to activate the pump. This process should take less than 30 minutes to fill up the entire storage tank. This is the most important process to be mindful about. If the pump is left on too long, large amounts of waste can be spilled onto the site.

II

In the storage tank there is a submersible pump that is run constantly to provide mixing and suspension of solids in the waste. This pump is dropped in through the manhole at the top of the tank and is powered by an extension cord that runs back to the shed. If this pump goes out then the feed to the digesters will be high in solids due to the settling of the solids in the storage tanks.

III

The pumps listed 2,4,6,7,8,9,AIR are all recirculation pumps used in the pilot system. Pumps 2, 4, and 6 are the recirculation pumps used to mix the three anaerobic digesters and are run continuously while the digesters are operating. Pumps 7,8, and 9 are used to recirculate the water in the duckweed tanks. They are on a timer system. The AIR pump is used with the aeration basin, separate from the duckweed tanks. All of the controls are in the shed for these pumps.

IV

Pumps 1, 3, and 5 are used to feed the digesters from the storage tank. These pumps control the flow rate of the system. They are controlled by timers mounted on the wall next to the control box in the shed. It is best to use these pumps for as small a runtime as possible for many times throughout the day. The reason for this is that solids settling in the pipes can clog the lines and inhibit the feed. When the pumps are run often this can be avoided. If the line does become clogged, then the outlet from the pump must be detached and the line can be backwashed.

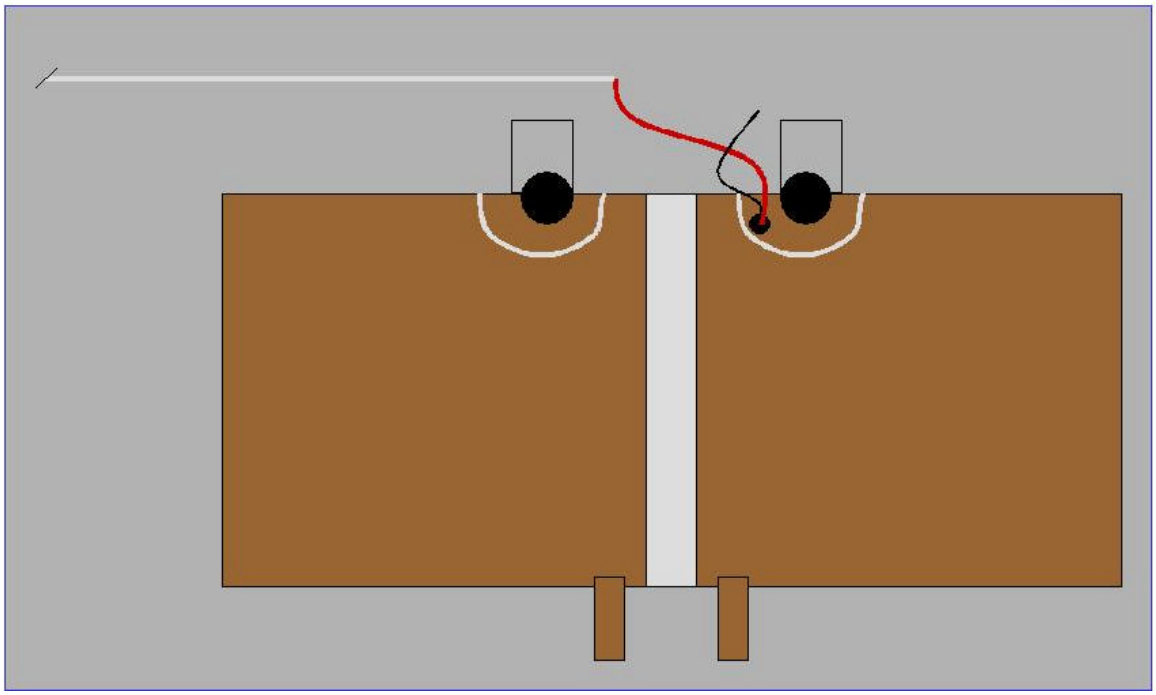
V

The operation of the water heater is used to control the temperature in the digesters. The system pumps water to and from the digesters. The water heater is similar to domestic heaters. The temperature control is located on the front outside of the tank and can be

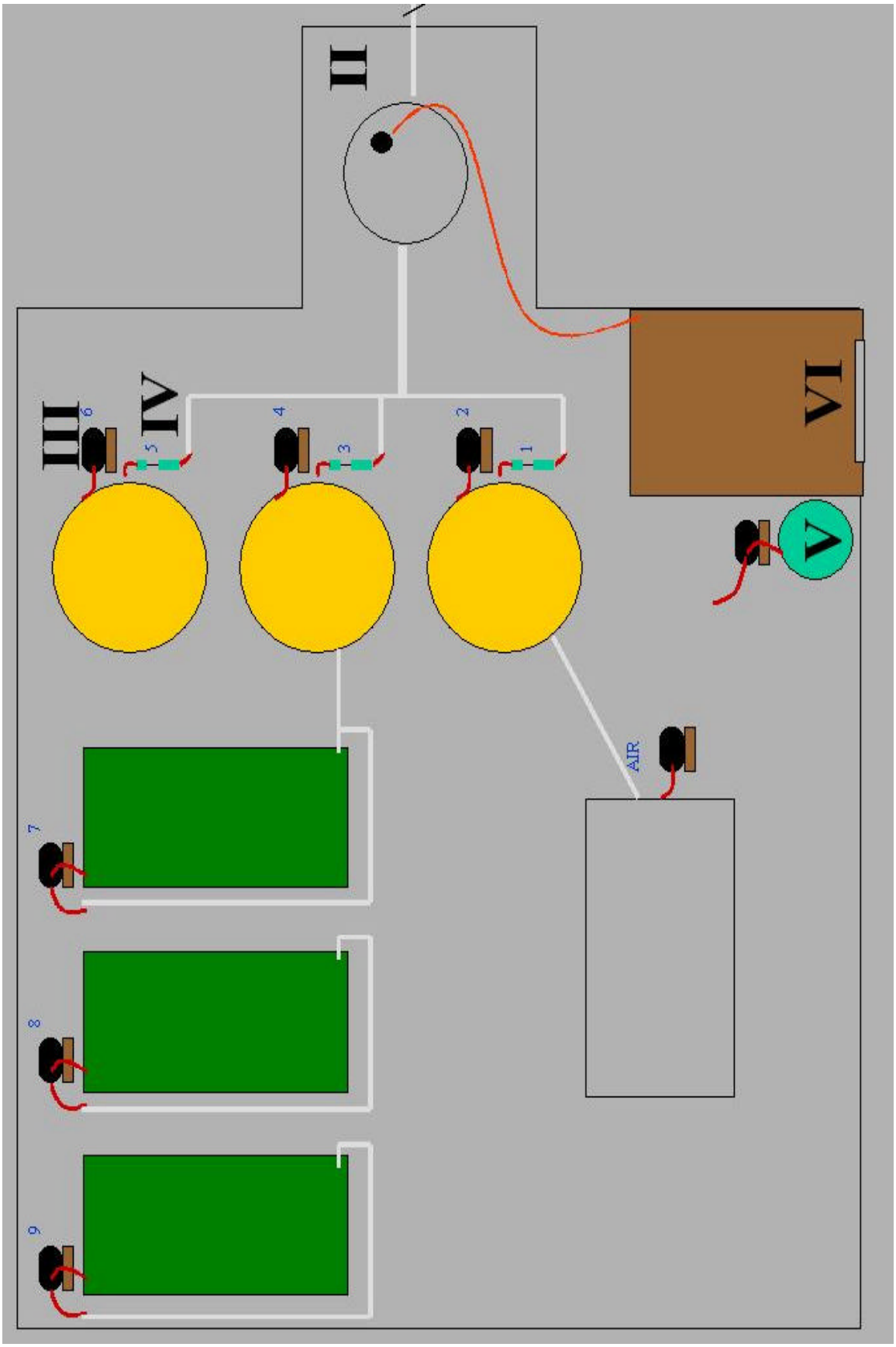
accessed using a flathead screwdriver. The heater can be adjusted to control the temp erature in the digesters. When starting up the heater, it is important to remove all of the air from the system. There is a release valve on the top of the heater that should be used every 15 minutes or so for the first hour of operation to purge the bubbles from the system. The recirculation pump is located next to the heater and should be run continuously when in operation.

VI

The control board is located in the shed and is the most complicated part of the system. The board is briefly described in the attached figure. The power supply is located in the top left of the box. The wiring of the system is more than I properly understand. The fuses are located in the middle left system and can be shut off if any repair work needs to be done with the pumps. The switches are mounted on the right side of the box and are controlled by either switches on the outside panel or by the timers. Beyond this, if repairs need to be done help should be found in working with the electrical side of things. The instructions for the timers are located in the shed with the control system.



Settling Basin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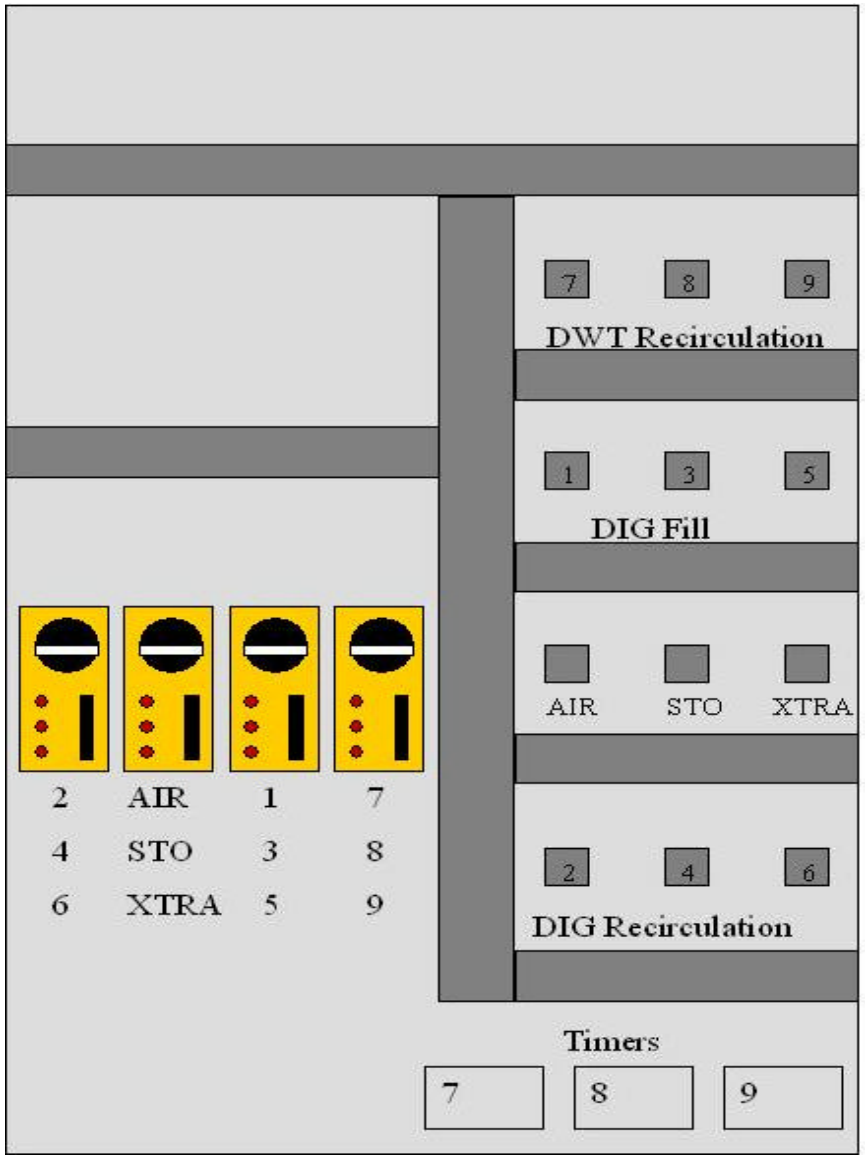
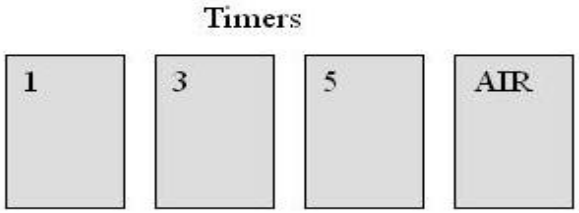
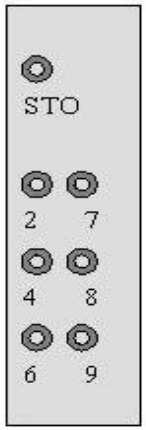
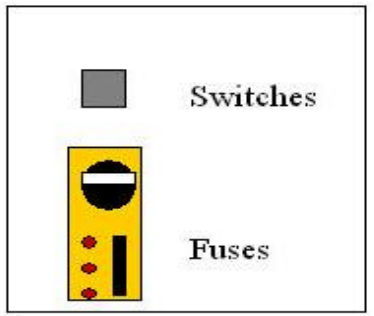
VI







Switches on side panel



Appendix B

Duckweed Harvest Data and Statistical Analysis.

The results of plant growth and plant tissue analysis for the duration of the study are included here along the analytical procedures mentioned in the text.

2002 Harvest Data

Wet weight

Lemna gibba

Date:	lb/harvest	mg/harvest	mg/week
5/31/2002	10.375	4706100	4706100
6/03/2002	11.6875	5301450	
6/05/2002	11.34375	5145525	
6/07/2002	13.3125	6038550	16485525
6/10/2002	17.5625	7966350	
6/12/2002	14.75	6690600	
6/14/2002	13.5625	6151950	20808900
6/17/2002	16.125	7314300	
6/19/2002	14.1875	6435450	
6/22/2002	13	5896800	19646550
6/25/2002	14.3175	6494418	
6/28/2002	12.755	5785668	12280086
7/02/2002	18.3125	8306550	
7/05/2002	20.4375	9270450	17577000
7/08/2002	20.8125	9440550	
7/11/2002	17.625	7994700	
7/14/2002	13.694	6213804	23649054
7/17/2002	13.63	6180300	6180300
Final Balance*	50.125	22736700	

* Balance performed by subtracting the initial seed amount from the final mass of duckweed in the pond to account for all duckweed produced.

Wet weight

Lemna gibba

Date:	lb/harvest	mg/harvest	mg/week

9/23/2002	16.38	7427700	
9/27/2002	13.25	6010200	13437900
10/1/2002	13.88	6293700	
10/4/2002	11.31	5131350	11425050
10/10/2002	17.38	7881300	7881300
10/18/2002	19.44	8816850	8816850
10/25/2002	16.27	7380072	7380072
10/31/2002	18.69	8476650	42695100
Final Balance*	75.4375	34218450	

* Balance performed by subtracting the initial seed amount from the final mass of duckweed in the pond to account for all duckweed produced.

Plant Tissue Analysis

Date	TKN ug/g	TP (ug/g)	%MC
5/27/02	31458	14121	94.4
6/3/02	64165	19527	94.4
6/10/02	66131	21295	94.6
6/17/02	57234	19358	94.9
6/24/02	69867	22332	95.6
7/1/02	69507	17842	95.98
7/8/02	65915	17309	94.28
7/15/02	63675	16634	95.6
7/22/02	70733	39520	95.7
7/30/02	58694	35023	95.01
8/12/02	70733	39520	95.86
8/19/02	58694	35023	96.71
9/23/02	66768	32661	94.98
9/30/02	67213	25977	95.6
10/7/02	64654	23142	95.99
10/14/02	69593	23406	94.7
10/21/02	65772	30601	89.93
10/28/02	69427	27108	95.28

Dry weight and mass balance

Date	Dry Weight Removed (g)	ug N/g Dryweight	N Removed (g)	Influent TKN (mg/l)	TKN (g)	Effluent TKN (mg/l)	Effluent TKN (g)	TKN Loss not from Duckweed	Total TKN Loss	% TKN Removed by Duckweed
5/27/02	263.54	31458	8.29	188	498.20	38.3	101.50	388.41	396.71	2.09%
6/3/02	923.19	64165	59.24	297	787.05	43	113.95	613.86	673.10	8.80%
6/10/02	1123.68	66131	74.31	297	787.05	61.4	162.71	550.03	624.34	11.90%
6/17/02	1001.97	57234	57.35	262	694.30	98.8	261.82	375.13	432.48	13.26%
6/24/02	540.32	69867	37.75	260	689.00	96.9	256.79	394.46	432.22	8.73%
7/1/02	703.08	69507	48.87	272	720.80	103	272.95	398.98	447.85	10.91%
7/8/02	1348.00	65915	88.85	262	694.30	120	318.00	287.45	376.30	23.61%

7/15/02	271.93	63675	81.02	214	567.10	133	352.45	133.63	214.65	37.74%

Date	Dry Weight Removed (g)	ug N/g Dryweight	N Removed (g)	Influent TKN (mg/l)	TKN (g)	Effluent TKN (mg/l)	Effluent TKN (g)	TKN Loss not from Duckweed	Total TKN Loss	%TKN Removed by Duckweed
9/23/02	674.58	66768	45.04	255	337.88	46.6	61.75	231.09	276.13	16.31%
9/30/02	502.70	67213	33.79	269	356.43	46.2	61.22	261.42	295.21	11.45%
10/7/02	316.04	64654	20.43	280	371.00	77	102.03	248.54	268.98	7.60%
10/14/02	467.29	69593	32.52	324	429.30	55	72.88	323.90	356.43	9.12%
10/21/02	743.17	65772	48.88	275	364.38	53	70.23	245.27	294.15	16.62%
10/28/02	2015.21	69427	139.91	259	343.18	58.9	78.04	125.22	265.13	52.77%

Date	Dry Weight Removed (g)	ug TP/g Dryweight	TP Removed (g)	Influent TP (mg/l)	TP (g)	Effluent TP (mg/l)	Effluent TP (g)	TP Loss not from Duckweed	Total TP Loss	%TP Removed by Duckweed
5/27/02	263.54	14121	3.72	43.1	114.22	11.6	30.74	79.75	83.48	4.46%
6/3/02	923.19	19527	18.03	70.6	187.09	15.4	40.81	128.25	146.28	12.32%
6/10/02	1123.68	21295	23.93	81.1	214.92	24.3	64.40	126.59	150.52	15.90%
6/17/02	1001.97	19358	19.40	89.3	236.65	33.6	89.04	128.21	147.61	13.14%
6/24/02	540.32	22332	12.07	74.2	196.63	34.1	90.37	94.20	106.27	11.36%
7/1/02	703.08	17842	12.54	84.5	223.93	36.4	96.46	114.92	127.47	9.84%
7/8/02	1348.00	17309	23.33	78.1	206.97	42.6	112.89	70.74	94.08	24.80%
7/15/02	271.93	16634	21.16	69.4	183.91	46.8	124.02	38.73	59.89	35.34%

Date	Dry Weight Removed (g)	ug TP/g Dryweight	TP Removed (g)	Influent TP (mg/l)	TP (g)	Effluent TP (mg/l)	Effluent TP (g)	TP Loss not from Duckweed	Total TP Loss	%TP Removed by Duckweed
9/23/02	674.58	32661	22.03	70	92.75	16.3	21.60	49.12	71.15	30.97%
9/30/02	502.70	25977	13.06	106	140.45	17.9	23.72	103.67	116.73	11.19%
10/7/02	316.04	23142	7.31	85	112.63	33	43.73	61.59	68.90	10.62%
10/14/02	467.29	23406	10.94	82	108.65	22.3	29.55	68.17	79.10	13.83%
10/21/02	743.17	30601	22.74	58	76.32	21.2	28.09	25.49	48.23	47.15%
10/28/02	2015.21	27108	54.63	71.7	95.00	22.9	30.34	10.03	64.66	84.49%

2003 Harvest Data *Lemna gibba* 8678

Wet weight

Lemna gibba

Date:	lb/harvest	mg/harvest	mg/week
24-Jun	4.00	1814400.00	4536000.00
29-Jun	6.00	2721600.00	
1-Jul	4.38	1984500.00	3628800.00
2-Jul	3.63	1644300.00	
8-Jul	5.50	2494800.00	7852950.00
11-Jul	5.13	2324700.00	

13-Jul	6.69	3033450.00	
15-Jul	5.88	2664900.00	
17-Jul	5.88	2664900.00	7342650.00
19-Jul	4.44	2012850.00	
21-Jul	4.19	1899450.00	3487050.00
26-Jul	3.50	1587600.00	
2-Aug	2.50	1134000.00	1134000.00
5-Aug	2.81	1275750.00	
7-Aug	4.31	1956150.00	5244750.00
10-Aug	4.44	2012850.00	
12-Aug	4.63	2097900.00	
14-Aug	5.69	2579850.00	7909650.00
17-Aug	7.13	3231900.00	
19-Aug	5.06	2296350.00	7966350.00
22-Aug	6.56	2976750.00	
24-Aug	5.94	2693250.00	
27-Aug	6.38	2891700.00	5584950.00
31-Aug	7.44	3373650.00	
3-Sep	6.06	2749950.00	5755050.00
6-Sep	6.63	3005100.00	
8-Sep	7.31	3316950.00	9383850.00
11-Sep	6.94	3146850.00	
15-Sep	6.44	2920050.00	
18-Sep	6.06	2749950.00	
20-Sep	5.75	2608200.00	8221500.00
22-Sep	6.31	2863350.00	
25-Sep	7.19	3260250.00	6180300.00
28-Sep	6.44	2920050.00	
30-Sep	6.31	2863350.00	8788500.00
2-Oct	6.25	2835000.00	
5-Oct	6.81	3090150.00	
10-Oct	6.38	2891700.00	
13-Oct	5.19	2353050.00	5244750.00
15-Oct	6.25	2835000.00	
17-Oct	5.38	2438100.00	7484400.00
19-Oct	4.88	2211300.00	
21-Oct	5.69	2579850.00	
24-Oct	4.19	1899450.00	6974100.00
27-Oct	5.50	2494800.00	
29-Oct	5.13	2324700.00	4337550.00
1-Nov	4.44	2012850.00	
4-Nov	5.25	2381400.00	6378750.00
6-Nov	4.38	1984500.00	
9-Nov	4.44	2012850.00	
12-Nov	4.69	2126250.00	3883950.00
15-Nov	3.88	1757700.00	
19-Nov	3.63	1644300.00	3373650.00
22-Nov	3.81	1729350.00	
25-Nov	2.44	1105650.00	1105650.00
Final Balance*	6.1875	2806650.00	

* Balance performed by subtracting the initial seed amount from the final mass of duckweed in the pond to account for all duckweed produced.

Plant Tissue Anlysis

Date	TKN ug/g	TP (ug/g)	%MC
6/16/2003	61534	11296	95.9
6/23/2003	75049	2651	96.43
6/30/2003	62784	38414	96.07
7/8/2003	68297	19212	94.9
7/14/2003	68297	19212	96.9
7/21/2003	70101	16395	96.37

7/28/2003	62986	17838	95.79
8/4/2003	79324	22917	96.52
8/11/2003	84488	20313	96.8
8/18/2003	59271	15699	96.67
8/26/2003	63425	18921	96.3
9/2/2003	70391	21750	96.34
9/8/2003	68635	22033	96.14
9/16/2003	64142	22231	96.1
9/24/2003	92190	32716	96.27
9/30/2003	60812	18819	95.78
10/7/2003	70803	23931	95.37
10/14/2003	66886	20033	96.43
10/20/2003	66377	18583	95.72
10/28/2003	69388	19106	96.04
11/4/2003	68319	17028	95.95
11/11/2003	65167	17089	95.82
11/18/2003	70556	18298	95.25
11/25/2003	70905	19359	95.81
12/2/2003	67811	17236	94.89

Dry Weight and Mass balance

Date	Dry Weight Removed (g)	ug N/g Dryweight	N Removed (g)	Influent TKN (mg/l)	TKN (g)	Effluent TKN (mg/l)	Effluent TKN (g)	TKN Loss not from Duckweed	Total TKN Loss	%TKN Removed by Duckweed
06/23/03	161.94	75049	12.15	318	168.54	46.43	24.61	131.78	143.93	8.44%
06/30/03	142.61	62784	8.95	426	225.78	81.21	43.04	173.78	182.74	4.90%
07/08/03	400.50	68297	27.35	382	202.46	77.01	40.82	134.29	161.64	16.92%
07/14/03	227.62	68297	15.55	373	197.69	92.43	48.99	133.16	148.70	10.45%
07/21/03	126.58	70101	8.87	418	221.54	108	57.24	155.43	164.30	5.40%
07/28/03	47.74	62986	3.01	307	81.36	128	33.92	44.43	47.44	6.34%
08/04/03	182.52	79324	14.48	384	101.76	109	28.89	58.40	72.88	19.87%
08/11/03	253.11	84488	21.38	385	102.03	114	30.13	50.51	71.89	29.74%
08/18/03	265.28	59271	15.72	365	96.73	97.11	25.73	55.27	70.99	22.15%
08/26/03	206.64	63425	13.11	326	86.39	75.1	19.90	53.38	66.49	19.71%
09/02/03	210.63	70391	14.83	325	86.13	60.71	16.09	55.21	70.04	21.17%
09/08/03	362.22	68635	24.86	297	78.71	64.29	17.04	36.81	61.67	40.31%
09/16/03	320.64	64142	20.57	329	87.19	56.85	15.07	51.55	72.12	28.52%
09/24/03	230.53	92190	21.25	288	76.32	49.19	13.04	42.03	63.28	33.58%
09/30/03	370.87	60812	22.55	262	69.43	47.95	12.71	34.17	56.72	39.76%
10/07/03	242.83	70803	17.19	436	115.54	50.03	13.26	85.09	102.28	16.81%
10/14/03	267.19	66886	17.87	276	73.14	45.51	12.06	43.21	61.08	29.26%
10/20/03	298.49	66377	19.81	315	83.48	50.48	13.38	50.28	70.10	28.26%
10/28/03	171.77	69388	11.92	327	86.66	52.5	13.91	60.82	72.74	16.38%
11/04/03	258.34	68319	17.65	441	116.87	52.19	13.83	85.39	103.03	17.13%
11/11/03	162.35	65167	10.58	376	99.64	66.55	17.64	71.42	82.00	12.90%
11/18/03	160.25	70556	11.31	432	114.48	57.05	15.12	88.06	99.36	11.38%

11/25/03	163.93	70905	11.62	293	77.65	63.39	16.80	49.22	60.85	19.10%

Date	Dry Weight Removed (g)	ug TP/g Dryweight	TP Removed (g)	Influent TP (mg/l)	TP (g)	Effluent TP (mg/l)	Effluent TP (g)	TP Loss not from Duckweed	Total TP Loss	%TP Removed by Duckweed
06/23/03	161.94	2651	0.43	81.94	43.43	15.21	8.06	34.94	35.37	1.21%
06/30/03	142.61	38414	5.48	82	43.46	21.47	11.38	26.60	32.08	17.08%
07/08/03	400.50	19212	7.69	52.67	27.92	20.5	10.87	9.36	17.05	45.13%
07/14/03	227.62	19212	4.37	42.56	22.56	21.74	11.52	6.66	11.03	39.63%
07/21/03	126.58	16395	2.08	48.22	25.56	21.78	11.54	11.94	14.01	14.81%
07/28/03	47.74	17838	0.85	57.91	15.35	24.17	6.41	8.09	8.94	9.52%
08/04/03	182.52	22917	4.18	54.6	14.47	22.8	6.04	4.24	8.43	49.64%
08/11/03	253.11	20313	5.14	55.38	14.68	21.45	5.68	3.85	8.99	57.18%
08/18/03	265.28	15699	4.16	74.6	19.77	19.94	5.28	10.32	14.48	28.75%
08/26/03	206.64	18921	3.91	46.22	12.25	18.04	4.78	3.56	7.47	52.36%
09/02/03	210.63	21750	4.58	59.06	15.65	16.44	4.36	6.71	11.29	40.56%
09/08/03	362.22	22033	7.98	44.33	11.75	16.88	4.47	-0.71	7.27	109.71%
09/16/03	320.64	22231	7.13	74.24	19.67	18.9	5.01	7.54	14.67	48.61%
09/24/03	230.53	32716	7.54	81.07	21.48	18.01	4.77	9.17	16.71	45.13%
09/30/03	370.87	18819	6.98	76.11	20.17	14.96	3.96	9.23	16.20	43.07%
10/07/03	242.83	23931	5.81	104	27.56	14.84	3.93	17.82	23.63	24.60%
10/14/03	267.19	20033	5.35	71.11	18.84	15.58	4.13	9.36	14.72	36.37%
10/20/03	298.49	18583	5.55	83.71	22.18	19.15	5.07	11.56	17.11	32.42%
10/28/03	171.77	19106	3.28	75.97	20.13	19.47	5.16	11.69	14.97	21.92%
11/04/03	258.34	17028	4.40	124	32.86	18.93	5.02	23.44	27.84	15.80%
11/11/03	162.35	17089	2.77	59.36	15.73	20.9	5.54	7.42	10.19	27.22%
11/18/03	160.25	18298	2.93	148	39.22	21.19	5.62	30.67	33.60	8.73%
11/25/03	163.93	19359	3.17	64.25	17.03	21.96	5.82	8.03	11.21	28.32%

2003 Harvest Data *Spirodela punctata* 7776

Wet weight

Spirodela punctata

Date:	lb/harvest	mg/harvest	mg/week
24-Jun	6.13	2778300.00	5641650.00
29-Jun	6.31	2863350.00	
1-Jul	8.56	3883950.00	7314300.00
2-Jul	7.56	3430350.00	
8-Jul	7.50	3402000.00	9950850.00
11-Jul	7.06	3203550.00	
13-Jul	7.38	3345300.00	
15-Jul	5.38	2438100.00	7344918.00
17-Jul	6.44	2920050.00	
19-Jul	4.38	1986768.00	
21-Jul	5.00	2268000.00	4110750.00
26-Jul	4.06	1842750.00	
2-Aug	4.50	2041200.00	2041200.00
5-Aug	5.88	2664900.00	
7-Aug	5.19	2353050.00	7881300.00
10-Aug	6.31	2863350.00	
12-Aug	6.75	3061800.00	8561700.00
14-Aug	6.38	2891700.00	
17-Aug	5.75	2608200.00	

19-Aug	5.19	2353050.00	7654500.00
22-Aug	5.25	2381400.00	
24-Aug	6.44	2920050.00	
27-Aug	6.13	2778300.00	
31-Aug	5.75	2608200.00	5386500.00
3-Sep	4.88	2211300.00	
6-Sep	6.69	3033450.00	5244750.00
8-Sep	5.25	2381400.00	
11-Sep	5.00	2268000.00	7030800.00
15-Sep	5.25	2381400.00	
18-Sep	4.81	2182950.00	
20-Sep	5.44	2466450.00	7314300.00
22-Sep	5.88	2664900.00	
25-Sep	4.94	2239650.00	4479300.00
28-Sep	4.94	2239650.00	
30-Sep	5.88	2664900.00	7796250.00
2-Oct	5.50	2494800.00	
5-Oct	5.81	2636550.00	
10-Oct	6.44	2920050.00	
13-Oct	4.25	1927800.00	4847850.00
15-Oct	5.13	2324700.00	
17-Oct	5.00	2268000.00	6435450.00
19-Oct	4.06	1842750.00	
21-Oct	4.81	2182950.00	
24-Oct	4.31	1956150.00	6265350.00
27-Oct	4.69	2126250.00	
29-Oct	4.31	1956150.00	3855600.00
1-Nov	4.19	1899450.00	
4-Nov	3.88	1757700.00	5755050.00
6-Nov	4.31	1956150.00	
9-Nov	4.50	2041200.00	
12-Nov	5.19	2353050.00	4592700.00
15-Nov	4.94	2239650.00	
19-Nov	4.19	1899450.00	3912300.00
22-Nov	4.44	2012850.00	
25-Nov	3.56	1615950.00	1615950.00
2-Dec	2.69	1219050.00	
7-Dec	2.88	1304100.00	3572100.00
10-Dec	2.31	1048950.00	
Final Balance*	5.5625	2523150.00	2523150.00

* Balance performed by subtracting the initial seed amount from the final mass of duckweed in the pond to account for all duckweed produced.

Plant Tissue Analysis

Date	TKN ug/g	TP (ug/g)	%MC
6/16/2003	69202	11182	85.42
6/23/2003	74073	19856	95.37
6/30/2003	68644	32839	95.35
7/8/2003	62489	12494	92.52
7/14/2003	62489	12494	95.57
7/21/2003	62835	12211	94.89
7/28/2003	60537	14173	94.33
8/4/2003	67591	15583	94.94
8/11/2003	64613	16150	95.16
8/18/2003	65010	16927	94.97
8/26/2003	68536	18093	95.47
9/2/2003	63849	15375	95.45
9/8/2003	64097	19724	94.58
9/16/2003	68317	18535	94.77

9/24/2003	71961	19364	95.26
9/30/2003	60598	15262	94.76
10/7/2003	63882	17950	94.43
10/14/2003	62346	15812	94.46
10/20/2003	60198	14443	94.63
10/28/2003	64005	13851	93.88
11/4/2003	65045	15486	94.02
11/11/2003	48441	11727	94.16
11/18/2003	65927	16387	92.96
11/25/2003	64573	14645	93.82
12/2/2003	62469	13274	96.28

Dry Weight and Mass balance

Date	Dry Weight Removed (g)	ug N/g Dryweight	N Removed (g)	Influent TKN (mg/l)	TKN (g)	Effluent TKN (mg/l)	Effluent TKN (g)	TKN Loss not from Duckweed	Total TKN Loss	%TKN Removed by Duckweed
6/23/03	261.21	74073	19.35	318	168.54	25.33	13.42	135.77	155.12	12.47%
6/30/03	340.11	68644	23.35	426	225.78	52.05	27.59	174.85	198.19	11.78%
7/8/03	744.32	62489	46.51	382	202.46	58.5	31.01	124.94	171.46	27.13%
7/14/03	325.38	62489	20.33	373	197.69	69.06	36.60	140.76	161.09	12.62%
7/21/03	210.06	62835	13.20	418	221.54	76.94	40.78	167.56	180.76	7.30%
7/28/03	115.74	60537	7.01	307	81.36	101	26.77	47.58	54.59	12.83%
8/4/03	398.79	67591	26.95	384	101.76	98.4	26.08	48.73	75.68	35.62%
8/11/03	414.39	64613	26.77	385	102.03	88.26	23.39	51.86	78.64	34.05%
8/18/03	385.02	65010	25.03	365	96.73	78.3	20.75	50.95	75.98	32.95%
8/26/03	244.01	68536	16.72	326	86.39	71.18	18.86	50.80	67.53	24.77%
9/2/03	238.64	63849	15.24	325	86.13	50.7	13.44	57.45	72.69	20.96%
9/8/03	381.07	64097	24.43	297	78.71	46.91	12.43	41.85	66.27	36.86%
9/16/03	382.54	68317	26.13	329	87.19	41.88	11.10	49.95	76.09	34.35%
9/24/03	212.32	71961	15.28	288	76.32	28.73	7.61	53.43	68.71	22.24%
9/30/03	408.52	60598	24.76	262	69.43	29.34	7.78	36.90	61.65	40.15%
10/7/03	270.03	63882	17.25	436	115.54	31.16	8.26	90.03	107.28	16.08%
10/14/03	356.52	62346	22.23	276	73.14	21.84	5.79	45.12	67.35	33.00%
10/20/03	336.45	60198	20.25	315	83.48	28.52	7.56	55.66	75.92	26.68%
10/28/03	235.96	64005	15.10	327	86.66	26.37	6.99	64.56	79.67	18.96%
11/4/03	344.15	65045	22.39	441	116.87	30.5	8.08	86.40	108.78	20.58%
11/11/03	268.21	48441	12.99	376	99.64	44.15	11.70	74.95	87.94	14.77%
11/18/03	275.43	65927	18.16	432	114.48	38.62	10.23	86.09	104.25	17.42%
11/25/03	99.87	64573	6.45	293	77.65	32.93	8.73	62.47	68.92	9.36%
12/2/03	226.74	62469	14.16	301	79.77	28.15	7.46	58.14	72.31	19.59%

Date	Dry Weight Removed (g)	ug TP/g Dryweight	TP Removed (g)	Influent TP (mg/l)	TP (g)	Effluent TP (mg/l)	Effluent TP (g)	TP Loss not from Duckweed	Total TP Loss	%TP Removed by Duckweed
6/23/03	261.21	19856	5.19	81.94	43.43	9.77	5.18	33.06	38.25	13.56%
6/30/03	340.11	32839	11.17	82	43.46	13.39	7.10	25.19	36.36	30.72%

7/8/03	744.32	12494	9.30	52.67	27.92	15	7.95	10.67	19.97	46.58%
7/14/03	325.38	12494	4.07	42.56	22.56	15.7	8.32	10.17	14.24	28.56%
7/21/03	210.06	12211	2.57	48.22	25.56	15.85	8.40	14.59	17.16	14.95%
7/28/03	115.74	14173	1.64	57.91	15.35	19.77	5.24	8.47	10.11	16.23%
8/4/03	398.79	15583	6.21	54.6	14.47	16.2	4.29	3.96	10.18	61.07%
8/11/03	414.39	16150	6.69	55.38	14.68	17	4.51	3.48	10.17	65.80%
8/18/03	385.02	16927	6.52	74.6	19.77	15.5	4.11	9.14	15.66	41.61%
8/26/03	244.01	18093	4.41	46.22	12.25	15.11	4.00	3.83	8.24	53.55%
9/2/03	238.64	15375	3.67	59.06	15.65	13.57	3.60	8.39	12.05	30.44%
9/8/03	381.07	19724	7.52	44.33	11.75	13.17	3.49	0.74	8.26	91.02%
9/16/03	382.54	18535	7.09	74.24	19.67	14.24	3.77	8.81	15.90	44.59%
9/24/03	212.32	19364	4.11	81.07	21.48	14.51	3.85	13.53	17.64	23.31%
9/30/03	408.52	15262	6.23	76.11	20.17	13.16	3.49	10.45	16.68	37.38%
10/7/03	270.03	17950	4.85	104	27.56	11.82	3.13	19.58	24.43	19.84%
10/14/03	356.52	15812	5.64	71.11	18.84	10.97	2.91	10.30	15.94	35.37%
10/20/03	336.45	14443	4.86	83.71	22.18	14.1	3.74	13.59	18.45	26.34%
10/28/03	235.96	13851	3.27	75.97	20.13	14.31	3.79	13.07	16.34	20.00%
11/4/03	344.15	15486	5.33	124	32.86	15.32	4.06	23.47	28.80	18.51%
11/11/03	268.21	11727	3.15	59.36	15.73	17.49	4.63	7.95	11.10	28.35%
11/18/03	275.43	16387	4.51	148	39.22	16.7	4.43	30.28	34.79	12.97%
11/25/03	99.87	14645	1.46	64.25	17.03	16.17	4.29	11.28	12.74	11.48%
12/2/03	226.74	13274	3.01	70.92	18.79	16.68	4.42	11.36	14.37	20.94%

2003 Harvest Data *Lemna minor* 8726

Wet weight

Lemna minor

Date:	lb/harvest	mg/harvest	mg/week
25-Sep	5.50	2494800.00	5358150.00
28-Sep	6.31	2863350.00	
30-Sep	4.13	1871100.00	6633900.00
2-Oct	4.69	2126250.00	
5-Oct	5.81	2636550.00	
10-Oct	5.25	2381400.00	4309200.00
13-Oct	4.25	1927800.00	
15-Oct	5.13	2324700.00	6690600.00
17-Oct	5.88	2664900.00	
19-Oct	3.75	1701000.00	
21-Oct	4.75	2154600.00	5840100.00
24-Oct	3.94	1786050.00	
27-Oct	4.19	1899450.00	
29-Oct	5.06	2296350.00	4025700.00
1-Nov	3.81	1729350.00	
4-Nov	4.44	2012850.00	5613300.00
6-Nov	4.06	1842750.00	
9-Nov	3.88	1757700.00	
12-Nov	5.38	2438100.00	4252500.00
15-Nov	4.00	1814400.00	
19-Nov	3.25	1474200.00	2806650.00
22-Nov	2.94	1332450.00	
25-Nov	2.25	1020600.00	1020600.00
Final Balance*	5.25	2381400.00	

* Balance performed by subtracting the initial seed amount from the final mass of duckweed in the pond to account for all duckweed produced.

Plant Tissue Analysis

Date	TKN ug/g	TP (ug/g)	%MC
9/30/2003	62305	19529	96.56
10/7/2003	66756	23376	96.53
10/14/2003	71105	18422	96.11
10/20/2003	71592	16076	95.97
10/28/2003	67950	16971	96.61
11/4/2003	72373	18796	96.1
11/11/2003	72117	20407	95.64
11/18/2003	79410	21958	95.37
11/25/2003	64280	16518	96.33
12/2/2003	65873	16624	96.28

Dry Weight and Mass balance

Date	Dry Weight Removed (g)	ug N/g Dryweight	N Removed (g)	Influent TKN (mg/l)	TKN (g)	Effluent TKN (mg/l)	Effluent TKN (g)	TKN Loss not from Duckweed	Total TKN Loss	%TKN Removed by Duckweed
9/30/03	228.21	62305	14.22	262	69.43	45.43	12.04	43.17	57.39	24.77%
10/7/03	149.53	66756	9.98	436	115.54	46.37	12.29	93.27	103.25	9.67%
10/14/03	260.26	71105	18.51	276	73.14	39.74	10.53	44.10	62.61	29.56%
10/20/03	235.36	71592	16.85	315	83.48	45.08	11.95	54.68	71.53	23.56%
10/28/03	136.47	67950	9.27	327	86.66	46.76	12.39	64.99	74.26	12.49%
11/4/03	218.92	72373	15.84	441	116.87	70.63	18.72	82.30	98.15	16.14%
11/11/03	185.41	72117	13.37	376	99.64	70.24	18.61	67.66	81.03	16.50%
11/18/03	129.95	79410	10.32	432	114.48	63.68	16.88	87.29	97.60	10.57%
11/25/03	124.85	64280	8.03	293	77.65	54.86	14.54	55.08	63.11	12.72%
9/30/03	228.21	62305	14.22	262	69.43	45.43	12.04	43.17	57.39	24.77%

Date	Dry Weight Removed (g)	ug TP/g Dryweight	TP Removed (g)	Influent TP (mg/l)	TP (g)	Effluent TP (mg/l)	Effluent TP (g)	TP Loss not from Duckweed	Total TP Loss	%TP Removed by Duckweed
9/30/03	228.21	19529	4.46	76.11	20.17	17.99	4.77	10.95	15.40	28.94%
10/7/03	149.53	23376	3.50	104	27.56	15.88	4.21	19.86	23.35	14.97%
10/14/03	260.26	18422	4.79	71.11	18.84	15.02	3.98	10.07	14.86	32.26%
10/20/03	235.36	16076	3.78	83.71	22.18	18.05	4.78	13.62	17.40	21.74%
10/28/03	136.47	16971	2.32	75.97	20.13	17.72	4.70	13.12	15.44	15.00%
11/4/03	218.92	18796	4.11	124	32.86	25.21	6.68	22.06	26.18	15.72%
11/11/03	185.41	20407	3.78	59.36	15.73	19.97	5.29	6.65	10.44	36.25%
11/18/03	129.95	21958	2.85	148	39.22	21.56	5.71	30.65	33.51	8.52%
11/25/03	124.85	16518	2.06	64.25	17.03	21.96	5.82	9.14	11.21	18.40%
9/30/03	228.21	19529	4.46	76.11	20.17	17.99	4.77	10.95	15.40	28.94%

Statistical Analysis

ANOVA table for the comparison of *Lemna gibba* and *Spirodela punctata* biomass production for the duration of the pilot plant operation:

The GLM Procedure

Class Level Information

Class	Levels	Values
Species	2	gibba punctata

Number of observations 109

NOTE: Due to missing values, only 47 observations can be used in this analysis.

MLR of Duckweed growth

The GLM Procedure

Dependent Variable: Harvest_dry Harvest dry

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	82649.7993	82649.7993	7.04	0.0110
Error	45	528255.8100	11739.0180		
Corrected Total	46	610905.6093			

R-Square	Coeff Var	Root MSE	Harvest_dry Mean
0.135291	40.06511	108.3467	270.4267

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Species	1	82649.79925	82649.79925	7.04	0.0110

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Species	1	82649.79925	82649.79925	7.04	0.0110

Parameter		Estimate	Standard Error	t Value	Pr > |t|
Intercept		311.4783126 B	22.11618751	14.08	<.0001
Species	gibba	-83.8880872 B	31.61515202	-2.65	0.0110
Species	punctata	0.0000000 B			.

NOTE: The X'X matrix has been found to be singular, and a generalized inverse was used to solve the normal
equations. Terms whose estimates are followed by the letter 'B' are not uniquely estimable.

ANOVA table for the comparison of *Lemna gibba* and *Spirodela punctata* wet weight production for the duration of the study:

The GLM Procedure

Class Level Information

Class	Levels	Values
Species	2	gibba punctata

Number of observations 109

NOTE: Due to missing values, only 47 observations can be used in this analysis.

MLR of Duckweed growth
The GLM Procedure

Dependent Variable: Harvest_wet Harvest wet

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	9162447206.4	9162447206.4	0.00	0.9630
Error	45	1.8946773E14	4.210394E12		
Corrected Total	46	1.8947689E14			

R-Square	Coeff Var	Root MSE	Harvest_wet Mean
0.000048	34.97585	2051924	5866689

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Species	1	9162447206	9162447206	0.00	0.9630

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Species	1	9162447206	9162447206	0.00	0.9630

Parameter	Estimate	Standard Error	t Value	Pr > |t|

```
                Intercept              5880357.000 B     418847.3267      14.04
<.0001
                Species   gibba         -27930.913 B     598743.4271      -0.05
0.9630
                Species   punctata           0.000 B                             .

NOTE: The X'X matrix has been found to be singular, and a generalized inverse was used to
solve the normal
      equations.  Terms whose estimates are followed by the letter 'B' are not uniquely

estimable.
```

ANOVA table for the comparison of *Lemna gibba, Lemna minor* and *Spirodela punctata* wet weight production from September 24th until December 1st:

```
                                    The GLM Procedure

                                  Class Level Information

                            Class         Levels    Values

                            species              3    gibba minor punctata

                                 Number of observations    31

NOTE: Due to missing values, only 30 observations can be used in this analysis.
                                      MLR of Duckweed growth

                                    The GLM Procedure

Dependent Variable: wet_weigt wet_weight

                                                 Sum of
            Source                       DF         Squares     Mean Square    F Value
Pr > F

            Model                         2    3.4506892E12    1.7253446E12       0.64
0.5331

            Error                        27    7.2337262E13    2.6791578E12

            Corrected Total              29    7.5787951E13

                             R-Square     Coeff Var      Root MSE       F2 Mean

                             0.045531      31.52098       1636813       5192775

            Source                       DF       Type I SS     Mean Square    F Value
Pr > F

            species                      2    3.4506892E12    1.7253446E12       0.64
0.5331
```

Source	DF	Type III SS	Mean Square	F Value	Pr > F
species	2	3.4506892E12	1.7253446E12	0.64	0.5331

Parameter		Estimate		Standard Error	t Value	Pr > |t|
Intercept		5059186.364 B		493517.7843	10.25	<.0001
species	gibba	596638.636 B		715175.2144	0.83	0.4115
species	minor	-217636.364 B		735692.8759	-0.30	0.7696
species	punctata	0.000 B				.

NOTE: The X'X matrix has been found to be singular, and a generalized inverse was used to solve the normal
equations. Terms whose estimates are followed by the letter 'B' are not uniquely

estimable.

ANOVA table for the comparison of *Lemna gibba, Lemna minor* and *Spirodela punctata* biomass production from September 24th until December 1st:

The GLM Procedure

Class Level Information

Class	Levels	Values
minor	3	gibba minor punctata

Number of observations 31

MLR of Duckweed growth

The GLM Procedure

Dependent Variable: dryweight dryweight

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	62678.7095	31339.3548	5.22	0.0118
Error	28	167992.8297	5999.7439		
Corrected Total	30	230671.5392			

R-Square	Coeff Var	Root MSE	F4 Mean
0.271723	34.15788	77.45801	226.7647

Source	DF	Type I SS	Mean Square	F Value	Pr > F
minor	2	62678.70954	31339.35477	5.22	0.0118

Source	DF	Type III SS	Mean Square	F Value	Pr > F
minor	2	62678.70954	31339.35477	5.22	0.0118

Parameter		Estimate	Standard Error	t Value	Pr > |t|
Intercept		275.8367373 B	23.35446992	11.81	<.0001
minor	gibba	-43.1821788 B	33.84384223	-1.28	0.2125
minor	minor	-108.9411378 B	33.84384223	-3.22	0.0032
minor	punctata	0.0000000 B			.

NOTE: The X'X matrix has been found to be singular, and a generalized inverse was used to solve the normal
equations. Terms whose estimates are followed by the letter 'B' are not uniquely estimable.

ANOVA table for the comparison of *Lemna gibba,* and *Spirodela punctata* nitrogen removal for the duration of the study:

MLR of nutrient removal
The GLM Procedure

Class Level Information

Class	Levels	Values
punctata	2	gibba punctata

Number of observations 46

MLR of nutrient
The GLM Procedure

Dependent Variable: N N

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	209.209151	209.209151	4.13	0.0482

```
Error                       44     2229.159648       50.662719

Corrected Total             45     2438.368800


                  R-Square     Coeff Var      Root MSE       F1 Mean

                  0.085799      39.76958      7.117775      17.89754


Source                      DF       Type I SS     Mean Square    F Value
Pr > F

punctata                     1     209.2091514     209.2091514       4.13
0.0482


Source                      DF     Type III SS     Mean Square    F Value
Pr > F

punctata                     1     209.2091514     209.2091514       4.13
0.0482


                                                      Standard
    Parameter                   Estimate             Error    t Value    Pr >
|t|

    Intercept               20.03014708 B      1.48415866      13.50
<.0001
    punctata  gibba         -4.26521982 B      2.09891730      -2.03
0.0482
    punctata  punctata       0.00000000 B                                .

NOTE: The X'X matrix has been found to be singular, and a generalized inverse was used to
solve the normal
      equations.  Terms whose estimates are followed by the letter 'B' are not uniquely
estimable.
```

ANOVA table for the comparison of *Lemna gibba,* and *Spirodela punctata* phosphorous removal for the duration of the study:

```
                                  MLR of nutrient removal
                                     The GLM Procedure

                                  Class Level Information

                            Class         Levels    Values

                            punctata           2    gibba punctata


                                 Number of observations    46
                                   MLR of nutrient removal
                                      The GLM Procedure

Dependent Variable: F3   F3

                                                Sum of
Source                      DF         Squares     Mean Square    F Value
Pr > F
```

Model	1	2.8694016	2.8694016	0.59	0.4468
Error	44	214.2330057	4.8689319		
Corrected Total	45	217.1024074			

R-Square	Coeff Var	Root MSE	F3 Mean
0.013217	45.50513	2.206566	4.849048

Source	DF	Type I SS	Mean Square	F Value	Pr > F
punctata	1	2.86940164	2.86940164	0.59	0.4468

Source	DF	Type III SS	Mean Square	F Value	Pr > F
punctata	1	2.86940164	2.86940164	0.59	0.4468

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	5.098804264 B	0.46010074	11.08	<.0001
punctata gibba	-0.499512949 B	0.65068071	-0.77	0.4468
punctata punctata	0.000000000 B			.

NOTE: The X'X matrix has been found to be singular, and a generalized inverse was used to solve the normal
equations. Terms whose estimates are followed by the letter 'B' are not uniquely estimable.

ANOVA table for the comparison of *Lemna gibba, Lemna minor* and *Spirodela punctata* nitrogen removal from September 24th until December 1st:

MLR of nutrient removal

The GLM Procedure

Class Level Information

Class	Levels	Values
Species	3	gibba minor punctata

Number of observations 31

NOTE: Due to missing values, only 28 observations can be used in this analysis.

MLR of nutrient removal 21:20 Monday, June 21, 2004 18

The GLM Procedure

Dependent Variable: N N

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	94.1692964	47.0846482	2.23	0.1280
Error	25	526.8438322	21.0737533		
Corrected Total	27	621.0131286			

R-Square	Coeff Var	Root MSE	N Mean
0.151638	29.84821	4.590616	15.37987

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Species	2	94.16929635	47.08464818	2.23	0.1280

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Species	2	94.16929635	47.08464818	2.23	0.1280

Parameter		Estimate	Standard Error	t Value	Pr > |t|
Intercept		17.37386346 B	1.45168017	11.97	<.0001
Species	gibba	-1.76177697 B	2.10924239	-0.84	0.4115
Species	minor	-4.44175608 B	2.10924239	-2.11	0.0454
Species	punctata	0.00000000 B			.

NOTE: The X'X matrix has been found to be singular, and a generalized inverse was used to solve the normal equations. Terms whose estimates are followed by the letter 'B' are not uniquely estimable.

ANOVA table for the comparison of *Lemna gibba, Lemna minor* and *Spirodela punctata* phosphorous removal from September 24th until December 1st:

MLR of nutrient removal
The GLM Procedure

Class Level Information

Class	Levels	Values
Species	3	gibba minor punctata

Number of observations 31

NOTE: Due to missing values, only 28 observations can be used in this analysis.

MLR of nutrient removal
The GLM Procedure

Dependent Variable: P P

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	4.45698241	2.22849120	1.25	0.3048
Error	25	44.70213074	1.78808523		
Corrected Total	27	49.15911314			

R-Square	Coeff Var	Root MSE	P Mean
0.090664	32.78038	1.337193	4.079248

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Species	2	4.45698241	2.22849120	1.25	0.3048

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Species	2	4.45698241	2.22849120	1.25	0.3048

Parameter		Estimate	Standard Error	t Value	Pr > |t|
Intercept		4.230745997 B	0.42285757	10.01	<.0001
Species	gibba	0.241595188 B	0.61439780	0.39	0.6975
Species	minor	-0.712921357 B	0.61439780	-1.16	0.2569
Species	punctata	0.000000000 B			.

NOTE: The X'X matrix has been found to be singular, and a generalized inverse was used to solve the normal

equations. Terms whose estimates are followed by the letter 'B' are not uniquely estimable.