ABSTRACT

CHAIPRAPAT, SUMATE. Modeling Nutrient Uptake Process and Growth Kinetics of Duckweed *Spirodela punctata* 7776 for Nutrient Recovery from Swine Wastewater. (Under the direction of John Classen and Jiayang Cheng.)

Use of plants for swine waste management involves the removal of nutrients from the swine wastewater by the plants and the utilization of the plant biomass for other useful purposes such as feed supplement and soil amendment. Duckweed has gained much interest for this purpose in the past decades because of its high growth rate and high protein content. The goal of this research was to study the characteristics of duckweed growth and nutrient uptake from swine wastewater in order to improve the efficiency of duckweed nutrient recovery. In the first part of this research, nutrient distribution and transport in a quiescent duckweed-covered pond containing swine lagoon liquid were investigated and described mathematically. A superior duckweed strain for total protein production in swine wastewater *Spirodela punctata* 7776 was used as a subject of the study. Diffusive transport of ammonium was shown to be a limiting process in nitrogen removal by duckweed plants in static ponds. In addition, a pH profile developed along the depth of the pond, creating an additional barrier to ammonia volatilization from the pond covered with a duckweed mat. In the second part of the research, growth and nutrient uptake characteristics of *Spirodela punctata* 7776 in artificial swine medium were examined in sterile batch cultures. Growth of *Spirodela punctata* 7776 corresponded to the amount of nitrogen storage in its biomass rather than the nutrient concentration in the growth medium. The relationship followed Monod-like kinetics with a maximum specific growth rate of 0.2381 g/g/d. Reduction in the specific growth rate of *Spirodela punctata* 7776 was observed in the culture with higher crop density,

which signified the adverse effects of surface crowding. A mathematical expression to represent the effects of crop density (mass per unit area) on specific growth rate was developed, which can be used in optimization of crop density management in duckweed nutrient recovery systems.

MODELING NUTRIENT UPTAKE PROCESS AND GROWTH KINETICS OF DUCKWEED *SPIRODELA PUNCTATA* 7776 FOR NUTRIENT RECOVERY FROM SWINE WASTEWATER

by
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BIOGRAPHY

Sumate Chaiprapat was born in Nakhornratchasima province in the northeastern Thailand. He is the second of the three children in the family of Wirat and Kanit Chaiprapat. In his teenage years, he was rigorously trained and competed in several regional and national badminton tournaments. Graduating from high school with high mark in athletic credentials, he earned an eligibility to attend Kasetsart University in Bangkok. At Kasetsart, he played three years for its badminton varsity team and was a team's captain in his senior year. In 1994, he received his bachelor's degree in Mechanical Engineering and worked for a major construction company, Sino-Thai Engineering & Construction Ltd., in Rayong province prior to the departure from Thailand to pursue advanced studies in an environmental field in the United States.

In January of 1995, he arrived in the US, and then joined the Environmental Engineering program at Iowa State University in Ames, Iowa in the spring of 1996. His research involved industrial wastewater treatment using anaerobic sequencing batch reactor developed by Richard R. Dague and coworkers at Iowa State. After receiving his M.S. degree from Iowa State University in 1997, he accepted a position as a project engineer at the Economy Form Corporation in Des Monies, Iowa. In the following year, he decided to continue his graduate studies at Rutgers University in New Brunswick, New Jersey. The research then was dealing with anaerobic digestion of agricultural wastes as part of the Mission to Mars project. He transferred to North Carolina State University in the fall of 1999 to complete his doctoral studies in Biological Engineering.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

WASTE AND TREATMENT

In the past century, world population has experienced dramatic growth because of the advancements in science and medicine. Human society has become more organized; as such we have built towns and cities to accommodate better living conditions. Natural resources are rigorously explored and spent, and in so doing tremendous amount of wastes are generated. The liquid part, wastewater, is basically a water supply which was used by home, community, industry, and farm. If the wastewater is left untreated, malodorous gases will be produced, and pathogenic bacteria that survive in it will cause illnesses or even outbreak of disease when leaked into water supply systems. Public health issues have become a main concern to keep the community sustainable. Wastewater also contains minerals and nutrients. When discharged directly into surface waters, it can result in eutrophication or undesired aquatic plant bloom that can lead to conversion to marsh and eventually to dry land. While alive, this large amount of aquatic plants can cause a swing in dissolved oxygen levels and pH between day and night as a result of photosynthesis and respiration. Subsequent die-off of these aquatic weeds in a eutrophic water body will also cause oxygen demand in the sediment. In either case, the result is detrimental to aquatic life. Under some circumstances, especially in industrialized regions, wastes produced may contain toxic chemicals that are harmful to humans and can cause death of the organisms necessary for a balance of a natural habitat. The effects may not appear in the short term but will trickle through and eventually impinge on the community. With such great impacts and massive

amount currently generated, systematic, sound waste management practices, which include collection/storage, treatment and utilization/disposal, must be employed.

For municipal and typical industrial wastewaters, many systems are available in the market to serve different purposes to the different degrees of treatment desired. Although chemical treatment is applicable under certain circumstances, treatment by biological systems is dominant due mainly to the lower operating costs of the systems. Generally, solids are separated from the liquid stream and conveyed through stabilization processes such as digestion and drying. Meanwhile, the liquid stream will engage in aerobic or anaerobic regimes or a combination to remove carbonaceous compounds and other nutrients such as nitrogen and phosphorus. This process generates biosolids, which is primarily composed of the bacterial cells grown during the operation. Final effluent of the treatment systems must meet local and federal regulatory standards to be able to be discharged into the natural waterways. As for solids, incineration, landfilling, pelletizing for fertilizer blend, and direct land application are generally practiced.

With much success in municipal waste management, the scope of environmental awareness has expanded. Agricultural wastes have a great potential to become a major contributor to environmental pollution. In order to sustain our increasing population, food suppliers of today's society must keep pace and produce in a more efficient way. Agricultural industries have become larger and more intensive. Consequently, large amounts of waste are generated in a relatively small area. One of the issues that is of much concern is animal waste produced during livestock production. Since the operation must be efficient, animals are confined in housing units, thus creating a concentrated waste stream. Animal waste is rich in minerals and nutrients with a considerable amount of bacterial and viral

agents. Potential contamination of surface water by run-off and ground water by leaching can pose serious problems if sources are not identified and controlled. Emission of odorous compounds, ammonia and other gases from the livestock operations can cause effects ranging from merely a nuisance odor to nearby communities, to soil acidification in a larger area resulted from ammonia deposition, and to such a great extent as global warming by the release of greenhouse gases such as methane.

In 1973, the US Environmental Protection Agency (USEPA) mandated the National Pollutant Discharge Elimination System (NPDES) that considered concentrated animal feeding operations to be a point source and subject to NPDES permit. Since then manure management systems have been an integral part of farms in the United States. In North Carolina, the wastes typically are flushed from animal houses through pipe or gutters to anaerobic lagoons for treatment. However, a solid-liquid separation unit can be installed to reduce the amount of solid loading to the lagoons and, thus, extend the lagoon life. An example of the solid separation unit is shown in Figure 1. The unit intercepts flow of a solid-liquid mixture from animal houses to the lagoon. The liquid part flows into a treatment lagoon while the solids are left to sun dry for various end-uses. The lagoon is either open (facultative pond, Fig. 2) or enclosed (digester, Fig. 3). A certain degree of solid separation by sedimentation can be achieved in both types; however, the enclosed lagoon provides a means to collect biogas for energy. The supernatant in the lagoon can be used to re-flush animal houses or to irrigate onto cropland as an ultimate disposal.

Figure 1. Solid-liquid separation unit with two sides operated alternatively. While one side takes all the incoming wastes, the other is left to dry the solids.

Figure 2. Swine waste lagoon that receives liquid part from the solid-liquid separator.

Figure 3. Enclosed anaerobic lagoon that produces biogas for energy. The cover swells due to the gas production inside.

PLANTS AND WASTEWATER UTILIZATION

The goal of applying lagoon liquid to soil is to recover the nutrients as plant materials that can be useful for many purposes. The balance between plant requirements and the amount of nutrients applied must be considered in developing a land application plan. Over application causes nutrient overload leading to salt buildup in soil, which will harm the plants rather than enrich their growth. In addition, the over application can result in degradation of water quality for both surface water, by runoff with high concentration of nutrient, and ground water, by leaching from the saturated soil. However, the nutrient uptake efficiency of the plants is not generally high, plus nitrogen can be lost through volatilization and nitrification/denitrification. An application scheme must also take into account both nutrient requirements and nutrient losses.

Plant nutrients consist of macronutrients, such as nitrogen, phosphorus, and potassium, which are needed in large quantities, and micronutrients or trace elements that are

required in lesser amounts. Although nitrogen is one of the most abundant elements in the environment, it is usually the element that limits plant growth. This is due to the large quantity required by plants as a constituent of their cells. Besides, transformation of nitrogen species in the habitat occurs constantly, making it either less available or less accessible to the plant. Phosphorus is also a major contributor to plant growth because it is a component used in membrane and genetic material synthesis, as well as in nucleotides for energy metabolism. Available forms for plant uptake of nitrogen are NH_4^+ and NO_3^-, and of phosphorus are $H_2PO_4^-$ and HPO_4^{2-}. These two macronutrients are the focus of studies regarding plant growth and productivity in the field.

Often, a primary goal for an animal producer is to produce high quality animals while meeting a regulatory standard for waste management; as such, an incentive for crop production is usually not persuasive enough for investment in a sophisticated cropping system. Lagoon liquid is applied to soil mainly for disposal and hay grass is often a by-product. The most common delivery method is by spraying because it is much cheaper to operate and easier to maintain than the drip system and haul system. However, the spraying method is deemed unsuitable for leafy vegetation and grain crops due to the possible accumulation of pathogenic organisms on the plant that can damage the plant itself and be toxic to the animals feeding on it. The drip or trickle application system minimizes contact between wastewater and plant. It employs a distribution of piping network in the field with control valves to regulate the amount of irrigating water. Clogging and high cost of the system installation are the major drawbacks of this delivery method. The haul system uses trucking vehicles to move irrigation water to spread in the field. This method can be costly

and labor intensive. It is more applicable to the more concentrated materials such as the delivery of fertilizer or manure to soil.

Other alternative approaches of utilizing lagoon liquid are wetland systems and floating aquatic plant systems. The wetland system features a land area with shallow water depth, typically less than 2 feet, that supports growth of emergent plants such as cattail, bulrush, reeds, and sedges, as well as aquatic plants such as duckweed and water hyacinth. The vegetation provides attachment media for bacterial growth and at the same time takes up nutrients from the wastewater. No harvesting is necessary in this system, which means it acts strictly as a wastewater treatment system. Unlike the wetland, floating aquatic plant systems can accomplish both wastewater nutrient removal and production of valuable plant tissue by-products. The latter task relies on the selection of plants that possess the ability to produce biomass with high nutritional value. Although water hyacinths have been used effectively for many years in wastewater treatment, they are intolerant to low temperature and their tough fibers are of little nutritional value and limit the end-use as animal feed. Through observations and rigorous screenings by many researchers, duckweed has been identified as a promising choice in that regard (Bergmann et al. 2000a and b; Culley and Epps 1973; Fasakin 1999; Harvey and Fox 1973; Hillman and Culley 1978; Mbagwu and Adeniji 1988; Oron et al. 1987; Oron et al. 1986; Porath et al. 1979; Porath et al. 1985; Skillicorn et al. 1993).

DUCKWEED

Duckweed is a small free-floating aquatic plant that belongs to *Lemnacea* family. This family is subdivided into four genera ranging from biggest to the smallest as *Spirodela*,

Lemna, *Wolffiella*, and *Wolffia* (Fig. 4). The largest species is *Spirodela polyrhiza* (so-called "giant duckweed") with leaf length of 15 mm while the genus *Wolffia* can be smaller than 1 mm. *Lemnacea* exhibits reduction in physiological structure possessing only fronds that consist of a leaf-like structure and roots, although *Wolffiella*, and *Wolffia* generally do not have roots due to their small size. Duckweed can reproduce sexually by their flowers, but this means of reproduction rarely occurs in most species. Vegetative growth is the dominant mode of reproduction (Hillman and Culley 1978; Landolt and Kandeler 1987). In this mode, daughter fronds emerge from the mother frond and later separate to become a new plant. This type of growth to some extent resembles binary fission of microorganisms. Among other plants, duckweed is one of the fastest growing ones. In the laboratory, *Lemna aequinoctialis* and *Wolffia microscopica* were reportedly able to double in frond number within approximately 24 hours under optimal conditions (Landolt and Kandeler 1987). Many field data also indicate high growth rates, but there are considerable discrepancies

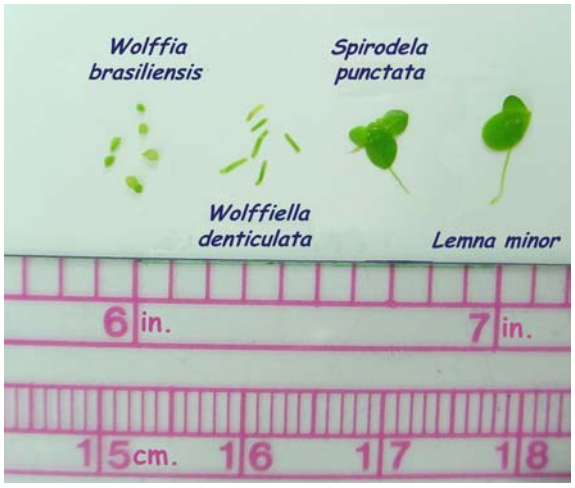

Figure 4. Different sizes of the four duckweed genera.

among the reported values even in the same duckweed species. Different climatic conditions, medium types, species, and more importantly the operating scheme of the duckweed pond can cause dramatic variation in growth.

Although fast growth often translates to higher rate of nutrient removal, it is not the only determining factor in plant selection. Potential for particular end-uses must be considered. In agriculture, attention has been drawn to duckweed because of its high nutritional value. Many researchers suggest suitability for animal (pig, chicken, cow, and fish) feed supplement and soil enhancement. Hillman and Culley (1978) showed a comparison of duckweed to soybean, cottonseed, peanuts, and alfalfa hay in terms of production rate and nutritional content. Crude protein content in duckweed was approximately 37 percent as opposed to the counterpart soybeans at 41.7 percent. However, in a given time and area, the total protein production by soybeans was only 10.2 percent of that of duckweed. This comparison is overwhelmingly in favor of duckweed because of its fast growing nature and the relatively much smaller area required for growth.

There are many other uses of duckweed besides for wastewater treatment and feedstock. Duckweed has long been used as an indicator plant for toxicity tests to monitor water quality (Landolt and Kandeler 1987; Pedersen and Petersen 1996; Wang 1990) due to the simple cultivation with small space requirement, and fast growth rate with genetically uniform culture that allows relatively quick responses to the toxic pollutants. Digestion of duckweed biomass for energy was proposed as an alternative for fossil fuel (Wolverton and McDonald 1981), but failed to gain popularity because of inexpensive energy from conventional sources. Duckweed is also a suitable host for genetic modification because it reproduces by cloning itself, thus loss of gene expression in the next generation is minimized.

This characteristic enables production of pharmaceutical compounds and enzymes by duckweed with the help of advanced biotechnology that involves insertion of desired genes into duckweed (Yamamoto et al. 2001). Other benefits of duckweed reported are reduction in water loss (10-30 percent) in arid regions (Oron 1990; Oron et al. 1985), and reduction in mosquito breeding (Culley and Epps 1973).

USE OF DUCKWEED FOR NUTRIENT RECOVERY

Duckweed has met many criteria as a suitable aquatic plant for reduction of nutrients in animal waste lagoons and utilization for feed supplement. It has a small size but is easy enough to harvest, and can grow in various climatic conditions. High nutritional value in its biomass and fast growing capability are the strongest characteristics that capture attention of researchers and agriculturists. There may be a misperception of duckweed for animal wastewater nutrient removal that the main purpose of growing duckweed is to treat the lagoon liquid. Rather, the use of duckweed in agricultural waste management emphasizes nutrient recycling in that duckweed takes up nutrients from animal wastes, and its biomass can be harvested and used as feed for animals (Fig.5). Unless a dedicated large land area is used to construct a series of duckweed ponds, a high degree of treatment would not be achieved. Some domestic wastewater treatment plants have used extensive duckweed systems mostly as a polishing unit and produce effluent that satisfactorily meets regulatory standards. However, waste management in farms can focus more on maximizing resource utilization, not cleaning up the wastewater, because land application does not require such clean irrigation water as that of the treated water to be discharged into the natural waterways.

Figure 5. Fast growing duckweed is harvested from a pond for animal feed.

A lot of work has been done on duckweed use with domestic and agricultural wastewaters, frequently to report the efficiency of the existing systems or to present the parameters that could help improve the system efficiencies. Although much detail is involved in the design of duckweed systems, most designs or proposed systems share some similar physical features. The most common ones are 1) the plug-flow hydraulic arrangement to provide incremental treatment of a wastewater stream with ease of probable recirculation when needed and to avoid short circuiting of the flow, and 2) the installation of floating grids to prevent the mat from shifting by wind (Alaerts et al. 1996; Bonomo et al. 1997; Skillicorn et al. 1993; Zirschky and Reed 1988). In many treatment systems where an effluent storage pond was used, the problem with excessive algal growth that degrades water quality of the effluent was solved by duckweed cover (Alaerts et al. 1996; Hammouda et al. 1995; van der Steen et al. 1998). With a dense cover of duckweed (Fig. 6), light penetration was reduced by 35 percent and 94 percent at surface mat density of 0.5 kg/m^2 and 3.9 kg/m^2, respectively (Zirschky and Reed 1988). The concept of integrated duckweed nutrient recycle

on dairy farm was illustrated by Hillman et al. (1978). This approach calls for an installation of a large duckweed system with harvesters, which could collect enough duckweed to supply about 60 percent of daily protein requirement for cattle in the farm. A pilot project was developed in Bangladesh to study the possibility of the duckweed farming for fish feed (Skillicorn et al. 1993), and the results were found extremely promising. Nevertheless, a decline in growth during the winter months and high requirement of land, especially in areas where land is expensive, have reportedly limited the acceptance of duckweed use (Bonomo et al. 1997).

Figure 6. Thick cover of floating duckweed in a pond limits light penetration and prevent algal growth. It can also reduce water evaporation, mosquito breeding, and gas/odor emission.

Greater success in using duckweed for nutrient recovery requires understanding of the plant and also its interactions with the environment created in our designed system. The first task would be to select for superior duckweed. Selection of suitable duckweed species, in our case for swine lagoon liquid, was challenging due to the great diversity within its family.

Many researchers have compared a few species that were easily accessible or native to their region (Chowdhury et al. 1999; Oron et al. 1986; Porath et al. 1979; Reddy and De Busk 1985; Vermaat and Hanif 1998); however, a recent systematic *in vitro* screening of a collection of the duckweed isolates from around the world revealed three promising genotypes based on the highest total protein production in swine lagoon liquid (Bergmann et al. 2000a), with the highest ranking being *Spirodela punctata* 7776 from Australia. Studies of growth and nutrient uptake characteristics of this strain could help enhance its use and application.

Light, temperature, pH of medium, crop density, and concentration of nutrients in the medium are known as key factors controlling growth and nutrient uptake of duckweed. However, light, temperature, and pH are not practically controllable in the field, while medium nutrient concentration may be adjusted simply by dilution and crop density can be managed by regular harvesting. Swine lagoon liquid contains a large amount of nutrients including nitrogen, phosphorus, and minerals (see Table B2 in APPENDIX B). Unlike other plants, duckweed preferentially takes up ammonium (Ingemarsson et al. 1984; Landolt and Kandeler 1987; Porath and Pollock 1982), which is the predominant form of nitrogen in swine lagoon liquid. This preference could explain why duckweed can tolerate and proliferate in such ammonium-concentrated medium as swine lagoon liquid, which is toxic to many other plants. One key to survival of duckweed in concentrated medium may be that the microenvironment around the duckweed mat is less concentrated. However, if the duckweed used were able to tolerate high concentration in the medium, reduced concentration around the mat would not give any advantage and may even allow starvation. In that situation, continuous exposure to concentrated medium should induce more growth and nutrient

uptake. Whether the nutrient levels near the water surface where duckweed is located become less than that in the other regions beneath, the parameters that control this phenomenon and the possible consequences deserve more attention.

Some researchers have observed that higher duckweed production rate was achieved in a more concentrated medium (Hammouda et al. 1995; van der Steen et al. 1998), but some reported decease in growth at higher medium concentration (Al-Nozaily et al. 2000; Bergmann et al. 2000b). This inconsistency is caused primarily by the different types of medium and duckweed used and also the difference in operational settings of the experiments. Concentration of nutrients in the growing medium was always thought to control the growth of the residing organisms. This perception may be inherited from the modeling practice of bacterial culture where the yield coefficient (biomass yield per COD or nutrients consumed) is assumed constant. It will undoubtedly hold true at steady state condition where influx of nutrient equals utilization rate. However, higher plants have a more complex cellular organization that provides flexibility for storing minerals and nutrients for later use. Modeling efforts for phytoplankton growth have linked this capability to its growth behavior (Collins 1980; Fong et al. 1994; Lehman et al. 1975). At stable environmental conditions, phytoplankton growth responded well with the internal nutrient content. Recent data from Cheng et al. (2002) indicates that there was an extended growth period of *Spirodela punctata* 7776 after nitrogen and phosphorus concentrations in the growth medium were exhausted. This growth pattern seems to show the effect of internal nutrient storage as in phytoplankton. Therefore, overall nutrient utilization of duckweed should be viewed as two coupled processes: the intake of nutrient for storage and the growth of biomass out of the storage pool.

Nevertheless, there is a major difference between phytoplankton and duckweed, in that growth and accumulation of duckweed are limited to the water surface. Thus, interference of surface crowding could play a significant role in controlling duckweed growth and nutrient uptake rates. Frequent harvesting of duckweed to maintain an optimal crop density should be performed. This will prevent the mat from being over crowded, which could limit growth and can introduce anaerobic degradation within the duckweed mat. On the other hand, over harvesting that leaves a thin duckweed mat will allow light penetration and thus algal growth. So far, only a few observations of density effects have been reported (Porath et al. 1979; Porath et al. 1985; Reddy and De Busk 1985; Said et al. 1979). Attempts to quantify the effects of density that could lead to optimization of this parameter are still lacking.

RESEARCH OBJECTIVES

The main objectives of this research were the following:

1) To investigate the nitrogen transport in a static pond covered with the selected duckweed strain *Spirodela punctata* 7776, and determine the parameters controlling the transport process,
2) To develop a mathematical model to describe the transport process,
3) To study the role of internal nutrient storage on growth and nutrient uptake of *Spirodela punctata* 7776, and
4) To identify the probable mathematical expression to represent the effects of crop density on the growth rate of duckweed.

REFERENCES

Alaerts, G. J., Mahbubar, M. R., and Kelderman, P. (1996). "Performance analysis of a full-scale duckweed-covered sewage lagoon." *Water Research*, 30(4), 843-852.

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

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

Bergmann, B. A., Cheng, J., Classen, J., and Stomp, A. M. (2000b). "Nutrient removal from swine lagoon effluent by duckweed." *Transactions of the ASAE*, 43(2), 263-269.

Bonomo, L., Pastorelli, G., and Zambon, N. (1997). "Advantages and limitations of duckweed-based wastewater treatment systems." *Water Science and Technology*, 35(5), 239-246.

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

Chowdhury, S. A., Sultana, N., Huque, K. S., and Huque, Q. M. E. (1999). "Manure based duckweed production in shallow sink: effect of genera on biomass and nutrient yield of duckweed under the same nutritional and management conditions." *Asian-Australasian Journal of Animal Sciences*, 13, 686-693.

Collins, C. D. (1980). "Formulation and validation of a mathematical model of phytoplankton growth." *Ecology*, 61(3), 639-649.

Culley, D. D. J., and Epps, E. A. (1973). "Use of duckweed for waste treatment and animal feed." *Journal Water Pollution Control Federation*, 45(2), 337-347.

Fasakin, E. A. (1999). "Nutrient quality of leaf protein concentrates produced from water fern (*Azolla africana* Desv) and duckweed (*Spirodela polyrrhiza* L. Schleiden)." *Bioresource Technology*, 69, 185-187.

Fong, P., Foin, T. C., and Zedler, J. B. (1994). "A simulation model of lagoon algae based on nitrogen competition and internal storage." *Ecological Monographs*, 64(2), 225-247.

Hammouda, O., Gaber, A., and Abdel-Hameed, M. S. (1995). "Assessment of the effectiveness of treatment of wastewater-contaminated aquatic systems with *Lemna gibba*." *Enzyme and Microbial Technology*, 17, 317-323.

Harvey, R. M., and Fox, J. L. (1973). "Nutrient removal using Lemna minor." *Journal Water Pollution Control Federation*, 45(9), 1928-1938.

Hillman, W. S., and Culley, D. D., Jr. (1978). "The uses of duckweed." *American Scientist*, 66(4), 442-451.

Ingemarsson, B., Johansson, L., and Larsson, C.-M. (1984). "Photosysthesis and nitrogen utilization in exponentially growing nitrogen-limited cultures of *Lemna gibba*." *Physiologia Plantarum*, 62, 363-369.

Landolt, E., and Kandeler, R. (1987). *The family of Lemnaceae, a monographic study*, Veröffentlichungen des Geobotanischen Institutes ETH.

Lehman, J. T., Botkin, D. B., and Likens, G. E. (1975). "The assumptions and rationales of a computer model of phytoplankton population dynamics." *Limnology and Ocenography*, 20(3), 343-364.

Mbagwu, I. G., and Adeniji, H. A. (1988). "The nutritional content of duckweed (*Lemna paucicostata* hegelm.) in the Kainji lake area, Nigeria." *Aquatic Botany*, 29, 357-366.

Oron, G. (1990). "Economic considerations in wastewater treatment with duckweed for effluent and nitrogen renovation." *Research Journal of the Water Pollution Control Federation*, 62(5), 692-696.

Oron, G., Porath, D., and Jansen, H. (1987). "Performance of the duckweed species *Lemna gibba* on municipal wastewater for effluent renovation and protein production." *Biotechnology and Bioengineering*, 29(2), 258-268.

Oron, G., Porath, D., and Wildschut, L. R. (1986). "Wastewater treatment and renovation by different duckweed species." *Journal of Environmental Engineering*, 112(2), 247-263.

Oron, G., Wildschut, L. R., and Porath, D. (1985). "Waste water recycling by duckweed for protein production and effluent renovation." *Water Science and Technology*, 17(4/5), 803-817.

Pedersen, F., and Petersen, G. I. (1996). "Variability of species sensitivity to complex mixtures." *Water Science and Technology*, 33(6), 109-119.

Porath, D., Hepher, B., and Koton, A. (1979). "Duckweed as an aquatic crop: evaluation of clones for aquaculture." *Aquatic Botany*, 7(3), 273-278.

Porath, D., Oron, G., and Granoth, G. (1985). "Duckweed as an aquatic crop; edible protein recovery, production and utilization." *Agricultural waste utilization and management : proceedings of the Fifth International Symposium on Agricultural Wastes*, Hyatt Regency Chicago, Illinois Center, Chicago, Illinois, 680-687.

Porath, D., and Pollock, J. (1982). "Ammonia stripping by duckweed and its feasibility in circulating aquaculture." *Aquatic Botany*, 13(2), 125-131.

Reddy, K. R., and De Busk, W. F. (1985). "Growth characteristics of aquatic macrophytes cultured in nutrient-enriched water: II. azolla, duckweed and salvinia." *Economic Botany*, 39(2), 200-208.

Said, M. Z. M., Culley, D. D., C., S. L., Epps, E. A., Myers, R. W., and Boney, S. A. (1979). "Effect of harvest rate, waste loading, and stocking density on the yield of duckweeds." *Proceedings of the World Mariculture Society*, 10, 769-780.

Skillicorn, P., Spira, W., and Journey, W. (1993). *Duckweed Aquaculture*, The International Bank for Reconstruction and Development/The World Bank, Washington, D.C.

van der Steen, P., Brenner, A., and Oron, G. (1998). "An integrated duckweed and algae pond system for nitrogen removal and renovation." *Water Science and Technology*, 38(1), 335-343.

Wolverton, B. C., and McDonald, R. C. (1981). "Energy from vascular plant wastewater treatment systems." *Economic Botany*, 35(2), 224-232.

Vermaat, J. E., and Hanif, M. K. (1998). "Performance of common duckweed species (*Lemnaceae*) and the waterfern *Azolla filiculoides* on different types of waste water." *Water Research*, 32(9), 2569-2576.

Wang, W. (1990). "Toxicity assessment of pretreated industrial wastewaters using higher plants." *Research Journal of the Water Pollution Control Federation*, 62(7), 853-860.

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

Zirschky, J., and Reed, S. C. (1988). "The use of duckweed for wastewater treatment." *Journal Water Pollution Control Federation*, 60(7), 1253-1258.

CHAPTER 2

MODELING NITROGEN TRANSPORT IN DUCKWEED POND FOR SECONDARY TREATMENT OF SWINE WASTEWATER

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ABSTRACT

A mathematical model was developed to describe nitrogen transport in duckweed-covered static ponds for nutrient recovery from swine lagoon water. A finite difference technique was used to solve the partial differential equations describing the ammonia transport and concentration in the pond. The key parameters in the model include the diffusion coefficient of ammonium in the medium (D) and kinetic constant of nitrogen uptake by duckweed (k). Using one order of magnitude parameter variations, the simulations showed that the model was clearly much more sensitive to D than to k, indicating the process of nitrogen removal in a static pond by duckweed is diffusion limited. Laboratory testing was conducted with *Spirodela punctata* 7776, a duckweed strain, to calibrate the model. The calibration of the model with experimental data yielded a new ammonium transport

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coefficient (T) that is 85 times of D value. Model results showed good agreement with depth-wise experimental ammonium concentration and the model also demonstrates that intermittent mixing every 3 hours can enhance ammonium uptake. Additionally, an apparent drop in pH near the duckweed mat at the surface was observed that may explain low rates of ammonia emission from duckweed ponds.

INTRODUCTION

With the large number of swine farms in North Carolina, a vast quantity of fecal waste is produced daily. Enormous amounts of various minerals, especially nitrogen and phosphorus, are contained in the waste. Treating swine waste by flushing the waste to traditional anaerobic lagoons has long been practiced. Lagoon effluent is often utilized by hay crops due to their high nitrogen uptake rates. However, the hay produced is often of low quality as a livestock feed, and of low value because of the excess amount available. Therefore the development of technologies to recover these nutrients as products with higher value than hay is perceived as a more desirable approach. Plant-based systems for nutrient sequestration that produce tissues suitable for animal feed and even human consumption are among the promising alternatives (Bergmann et al. 1997; Cheng et al. 2001; Harvey et al. 1996; Haustein et al. 1994; Oron et al. 1985; Rogers et al. 1996). Compared to other plants, duckweed has shown a great potential with many good traits compatible with nutrient recovery from domestic wastewater and animal waste lagoon liquid (Alaerts et al. 1996; Boniardi et al. 1994; Manimaran et al. 1997; Oron et al. 1988; Porath et al. 1985; Riggle 1998; Tripathi et al. 1991; Zirschky and Reed 1988).

Duckweed is a small free-floating aquatic macrophyte that belongs to the family *Lemnaceae*. The family consists of four genera (listed from largest to smallest): *Spirodela*, *Lemna*, *Wolfiella*, and *Wolffia*, with the largest species being *Spirodela polyrrhiza* at 1.5 cm long. Unlike water hyacinth that has an extensive root system and is high in fiber content making it difficult to harvest and process, duckweed's structure is highly reduced into only fronds (leaf-like) and roots, with very low concentrations of lignin and cellulose (Oron et al. 1985; Wolverton and McDonald 1981). Duckweed is reportedly able to produce a total protein content of 6.8 to as high as 45 percent (dry weight basis) depending on the growing conditions, and *Lemna aequinoctialis* and *Wolffia microscopica* can double in mass in only 20-24 hours (Landolt and Kandeler 1987). Because of the near neutral pH of swine lagoon liquid, ammonium (NH_4^+) is the predominant form of nitrogen, which provides a desirable setting for duckweed growth. Duckweed has the ability to thrive and proliferate in this type of wastewater because of its preferential uptake of ammonium ions (Ingemarsson et al. 1984; Landolt and Kandeler 1987; Porath and Pollock 1982) and the ability of some selected species to tolerate ammonium concentrations of up to 133 mgN/L (Bergmann et al. 2000) and 240 mgN/L (Cheng et al. 2002).

Studies on nutrient uptake and growth behavior of different duckweed species in various conditions are well documented (Alaerts et al. 1996; Bergmann et al. 2000; Caicedo et al. 2000; Cheng et al. 2002; Holst and Yopp 1979; Manimaran et al. 1997; Oron et al. 1987; Sutton and Ornes 1977; Vermaat and Hanif 1998; Wedge and Burris 1982). However, a common feature among these studies is that homogeneous nutrient concentration in the system was assumed or provided by regular mixing. Access to nutrients by duckweed is limited to the leaf's lower epidermis and root. Therefore, the evaluation of duckweed

nutrient recovery should account for the mechanism that conveys the nutrients to the root zone. This movement, which is presumably caused by diffusion, is particularly important in the static condition that occurs between mixing events. To date, only modest attention has been devoted to this aspect. Monselise and Kost (1993) observed a faster rate of ammonium-ion absorption in their stirred duckweed culture flasks compared to the unstirred. They suggested that depleting local NH_4^+ ions near the plant as a result of its nutrient uptake caused the reduced uptake. Al-Nozaily et al. (2000) reported that no statistically significant difference in total nitrogen (TKN) at different sampling depths was found in their 95 cm deep duckweed reactor but pointed out that mixing had a significant positive effect on the system. No further detailed description regarding the nutrient transport through the liquid column was presented. More elaborate analysis and experiments are still needed. The aim of the present study is to investigate the mechanism of the diffusion-uptake phenomenon which takes place in a static duckweed-covered pond, to develop a mathematical model to predict the ammonium-nitrogen recovery, and to examine the sensitivity of the nutrient transport and duckweed uptake kinetics in the system. The resulting simulation from the model with some adjustments from the experimental data will provide preliminary information that is regarded as the first step to design and manage duckweed-based nutrient recovery systems.

MODEL DEVELOPMENT

Governing Equation

Mass transport by diffusion follows Fick's law, which is expressed in terms of flux in one-dimensional coordinate system as

$$J = -D \frac{\partial c}{\partial x} \tag{1}$$

where J = mass flux of a chemical of interest in the system ($mg/m^2/hr$); c = concentration of the chemical (mg/m^3); x = coordinate direction in which mass diffuses (m); and D = diffusion coefficient of the chemical (m^2/hr). The negative sign indicates that mass moves from high to low concentration or in the direction of decreasing concentration. Diffusion coefficients of various chemicals are dependent on temperature, ionic concentration, and phases (e.g. air, water, soil) where diffusion is taking place. In this work, the chemical of interest is ammonium (NH_4^+). Eq. 1 is used in the mass balance on a differential volume to describe the diffusion process in the simulated duckweed-covered pond (Figure 1). The mass balance equation was developed by assuming no reaction in the differential volume and one-dimensional (vertical) transport of nutrients in the duckweed pond. The governing differential equation of the nutrient diffusion becomes

$$\frac{\partial c(x,t)}{\partial t} = D \frac{\partial^2 c(x,t)}{\partial x^2} \tag{2}$$

Complete derivation is available elsewhere (Choy and Reible 2000), and is also summarized in Appendix A.

Boundary Conditions

For one-dimensional transport, two boundary conditions must be set, one at each end of the control volume in the direction of diffusive mass flow. The first boundary condition (Eq. 3) in the static duckweed-covered pond is defined at the water surface (x = L) where duckweed grows (Figure 1).

$$-D \frac{\partial c(L,t)}{\partial x} = k\, c(L,t) \tag{3}$$

The nutrient flux (left hand side of Eq. 3) to the floating duckweed at the water surface must be equal to the nutrient mass taken up by duckweed (right hand side of Eq. 3), which is

assumed to follow first order kinetics. The rate constant k (m/hr) will be determined in a later section.

The second boundary condition (Eq. 4) is defined at the bottom of the pond (x = 0), where the mass flux is assumed to be zero, that is, there is no source of nutrient through the bottom of the system.

$$-D \frac{\partial c(0,t)}{\partial x} = 0 \quad (4)$$

Initial Condition

An initial condition is required to solve the governing equation. To observe the activity of mass transfer in the system from the start of the process, the initial condition was set as a uniform concentration, i.e. c_0, as shown in Eq. 5.

$$c(x,0) = c_0 \quad (5)$$

The change in concentration over time along the depth of the water column can be calculated, which will provide the information necessary to calculate nitrogen removed as a result of duckweed uptake. In a batch system with intermittent mixing, a lower homogeneous concentration would result at the end of each mix-cycle since duckweed has taken up a certain amount of nutrients from the liquid during the interval between two mixings. Thus, when intermittent mixing is employed, the homogeneous concentration at the end of each mixing must be calculated for use as an initial condition for the subsequent simulation cycle.

The two parameters of the model that must be determined are the diffusion coefficient of ammonium in water (D) and the rate constant (k) of nutrient uptake by duckweed.

Diffusion Coefficient (D)

The diffusion coefficient for ammonium ion in a dilute aqueous solution is 7.0452 x 10^{-6} m^2/hr (Lide 2000). In one liter of lagoon liquid, there are approximately 55.56 moles of water (a mole of water = 18 g) plus a small number of other ions. Various ions in one liter of lagoon liquid total approximately 64.3 x 10^{-3} moles (Bergmann et al. 2000) which is very low compared to 55.56 moles of water and can be neglected. In spite of this low ionic concentration, ionic charge concentration (normality, N) can play a significant role in diffusion transport of ions by imposing electromagnetic charges on the ionic components in the solution. Reid et al. (1987) showed that in many solutions, an increase in ionic concentration within the range of 0 to 2 N alters the diffusion coefficient in the solutions by less than 15 percent. The ionic charge concentration for lagoon liquid is estimated to be 81.0 x 10^{-3} N (Bergmann et al. 2000), which is also relatively low. Based on the relationship described by Reid et al. (1987), an increase of 81.0 x 10^{-3} N (from zero, of the dilute aqueous solution) of ionic charge in this case should lower the diffusion coefficient no more than approximately 10 percent. Moreover, our simulation took place in 1:1 dilution of the lagoon liquid that even further reduces the effect of the increase in both molarity (M) and normality (N) of ions in the liquid medium. Thus, the NH_4^+ diffusion coefficient of 7.0452 x 10^{-6} m^2/hr was used in this model.

First-Order Uptake Coefficient (k_1)

The uptake coefficient (rate constant) with first order kinetics was estimated by linear regression with the batch data from Cheng et al. (2002). In their experiment, duckweed *Spirodela punctata* 7776 was grown in 5.81 cm deep containers with 150 ml of swine artificial medium (SAM) at initial ammonium concentrations of 240, 125, and 63.1 mgN/L at

23°C under 40 μmol/m^2/s photon flux density. The mass balance of ammonium is described as

$$V \frac{d[NH_4^+]}{dt} = mass\ in - mass\ out + reaction \quad (6)$$

Mass neither entered nor left the system. The only reaction was ammonium uptake by duckweed at the surface. Note that the reaction term should take the form of an area-based expression due to the fact that the reaction takes place only at the surface of the liquid column. The area-based first order expression can be written as

$$V \frac{d[NH_4^+]}{dt} = -k_1 A_s [NH_4^+] \quad (7)$$

$$\frac{d[NH_4^+]}{dt} = -\frac{k_1}{L}[NH_4^+] \quad (8)$$

where $[NH_4^+]$ = ammonium concentration (mg/m^3); k_1 = area-based first order uptake coefficient (m/hr); A_s = surface area (m^2); and L = depth (m). Eq. 8 was rearranged and integrated to obtain the following:

$$\ln \frac{[NH_4^+]}{[NH_4^+]_{initial}} = -\frac{k_1}{L} t \quad (9)$$

Mathematically, this expression does not include the lag phase portion of the curve. This is not a concern because physiologically, duckweed in continuously operated systems should always be acclimated to the medium it inhabits. Thus, no lag phase was used in this model and data points in the lag phase were excluded in our parameter estimation process. Initial ammonium concentration in the simulation is the first data point after the lag period ended.

Evaluation of the Governing Equation on Parameter Sensitivity and Nitrogen Removal

Once the parameters k_1 and D were determined, the next step was to numerically solve the governing equation and determine the sensitivity of the model to variation in parameters of interest: diffusion coefficient (D) and duckweed uptake coefficient (k_1). Finite difference method utilizing the Crank-Nicolson scheme (Crank and Nicolson 1947) was implemented using Matlab (Appendix A).

The simulations were performed with the initial ammonium concentration of 64 mgN/L. This is also the actual concentration of the medium used in the experimental part of this study. Ammonium concentration profiles at every 24 hours were generated from the outputs of the Matlab computations. The sensitivity of the model to changes in the value of k_1 and D was tested. Values of k_1 and D were varied over two orders of magnitude and paired up to yield a total of five pairs, i.e. k_1-D, $0.1k_1$-D, $10k_1$-D, k_1-0.1D, and k_1-10D. All five pairs were simulated for a total simulation time of 144 hours (6 days) in 0.46 m (18 in) depth, and then compared. This depth was chosen because preliminary simulations showed no boundary effect in the ammonium concentration profiles. The output was then used to predict cumulative nitrogen removal (mgN/m^2) and nitrogen removal rate (NRR, $mgN/m^2/hr$) of each pair over time. Subsequently, the relative differences of nitrogen removal rate (RDNR, percent) with reference to the k_1-D pair over 144 hours were calculated according to the following:

$$RDNR_i = [(NRR_i - NRR_{k1-D}) / NRR_{k1-D}] \times 100\% \quad (10)$$

where NRR_i = nitrogen removal rate of pair i ($mgN/m^2/hr$); and $NRR_{k1\text{-}D}$ = nitrogen removal rate of the k_1-D pair ($mgN/m^2/hr$).

EXPERIMENTAL DESIGN

Simulation results were tested against data collected from laboratory-scale duckweed reactors. All experiments were carried out in a growth chamber which operated on continuous artificial lighting at an intensity of 25.3 μmol/m^2/s provided by 6 GE 48-inch 40W wide-spectrum fluorescent bulbs. Temperature was maintained at 26 ± 2°C. Measurements of the concentration profiles in the static duckweed-covered water column were taken from a total of nine cylindrical reactors of dimension 0.10 m (4 in) ID and 0.48 m (19 in) depth. The medium was prepared by allowing lagoon liquid to settle for 6 hours to separate solids and then diluting with tap water at 1:1 ratio. The initial ammonium concentration was 64 mgN/L with pH 7.43. The prepared medium filled the reactors to 0.46 m (18 in) deep. Duckweed *Spirodela punctata* 7776 in the amount of 2.85 g (wet weight) was placed in each reactor to completely cover the surface. Prior to the experiment, duckweed was preconditioned for two weeks in similar lighting and medium with daily medium replacement to minimize the lag phase that could potentially occur during the beginning of the experiment.

Samples from triplicate reactors were taken at 0, 24, 96, and 144 hours and total ammonia nitrogen (TAN) and pH of each sample were analyzed. Five 20-ml aliquots were collected from each reactor in sequence from sampling points located at distance 0.46 m (18 in, at surface), 0.41 m (16 in), 0.36 m (14 in), 0.20 m (8 in), and 0.01 m (0.5 in) measured from the bottom of the reactor. To minimize the disturbance of the ammonium ion diffusion, each reactor was discarded after one set of samplings at the five locations described. The results of the ammonium profiles were used to calibrate the model, which yielded optimal values of D and k_1 of the static system.

The second set of experiments was conducted to validate the model with another operational scenario, i.e. intermittent mixing. Two mixing intervals, 48 hours and 3 hours, were applied to 0.25 m (10 in) deep duckweed-covered water columns in 0.28 m (11 in) deep cylindrical reactors with a diameter of 0.28 m (11 in). The water depth of 0.25 m was chosen because a smaller volume will permit a larger change in medium concentration, thus, the difference between the two mixing cycles can be clearly observed in a reasonable time period. The setup consisted of nine reactors; four of which were operated with 3-hour cycle and the rest with 48-hour cycle. In each group, two reactors were seeded with 60 g (wet weight) of duckweed *Spriodela punctata* 7776 and two were covered with plastic flakes to imitate duckweed fronds. One reactor in the group of 48-hr mixing cycle was not seeded at all and left with a bare surface. Diluted lagoon liquid to tap water at 1:1 ratio was used. The initial ammonium concentration was 65.3 mgN/L. The 48-hour cycle reactors were agitated manually with a stirring paddle. Stir plates that were controlled by the ChronTrol® 4-circuit controller mixed the remaining reactors for 30 seconds every three hours. All agitation was done in such a way to ensure homogeneous nutrient concentration after each mixing and to not destroy the duckweed biomass. Samples were collected immediately after mixing at the same time every day for the 3-hour mixing cycle reactors and every other day for the set of 48-hour mixing cycle reactors. Samples were analyzed for TAN and pH.

RESULTS AND DISCUSSION

First Order Kinetics Evaluation

Analysis of the batch data of Cheng et al. (2002) gave a first order uptake coefficient, k_1, of 0.906 x 10^{-3} m/hr with R^2 of 0.96 for the initial ammonium concentrations of 63.1

mg/L. Figure 2 shows a curve fitting of the experimental ammonium concentration data using the first order kinetic model. Similar first order uptake coefficients were obtained from the other two data sets using different initial concentrations (240 and 125 mgN/L). Since the medium used in the experimental part of this study had an ammonium concentration of 64 mgN/L, this k_1 value of 0.906 x 10^{-3} m/hr from the data set where $[NH_4^+]_{initial}$ = 63.1 mgN/L was used for further analyses.

Ammonium Profile and Sensitivity Analysis

The governing equation with the specified boundary and initial conditions was numerically solved with values for D and k_1 of 7.0452 x 10^{-6} m^2/hr and 0.906 x 10^{-3} m/hr, respectively. The resulting ammonium ion concentration profile (Figure 3) exhibits a sharp drop near the duckweed-covered surface shortly after the initial time. This abrupt fall of concentration is primarily due to the uptake of ammonium ions by duckweed at the surface, which is much faster than the diffusion rate of the ions to the surface. The concentration difference (gradient) will be high near the surface and decrease with depth to the point below which the ion concentration is uniform, or in other words, the concentration gradient is zero.

In a two-step process, the slower step determines the speed of the entire process, which in this case is diffusion. The lower concentration near the duckweed surface indicates that the removal of ammonium ions from the liquid in this system is highly dependent on diffusion. It is clear that ammonium transport in a static pond is diffusion limited. Simulations of other k_1-D pairs also yielded similar concentration profiles, which support the conclusion of the diffusion-limited nature in this type of system. Nevertheless, the characteristics of the system could be altered by many factors in the field that cause changes in values of the parameters k_1 and D. Such factors include change in temperature (affecting

both parameters), different strains of duckweed, pH and light intensity (affecting k_1), and bacterial activities that cause internal agitation by gas production as well as the ionic concentration of the lagoon liquid (affecting D).

The model results were used to predict the cumulative nitrogen removal of the system over 144 hours (Figure 4). The nitrogen removal of the increased-D pair (k_1-10D) outperformed the other pairs. However, in the beginning increasing k_1 ($10k_1$-D pair) shows a slightly higher nitrogen removal until approximately hour 18. This implies that nitrogen removal in this system is initially more dependent on the uptake capacity or strains of duckweed grown in the pond, but improving the supply of ammonium ions to the surface through diffusion is far more influential to the performance of the system in the long run. In a full-scale pond, both an excellent duckweed strain and a suitable system operation must be exploited in order to obtain the highest possible nutrient recovery from the system. The preceding work by Bergmann et al. (2000) identified the superior duckweed strains for swine wastewater treatment, and this ensuing study is designed to investigate other ways of enhancing the efficiency of duckweed system through system operation.

Reduced k_1 and reduced D exhibited the same effect by lowering the overall nitrogen removal. Although the k_1-0.1D pair has a minimal advantage over $0.1k_1$-D pair for the entire simulation period of 144 hours, the difference in cumulative nitrogen removal narrowed over time. The diffusion coefficient D does not contribute as much to the system performance when duckweed uptake rate (k_1) is low because under such circumstances, although there are a lot of ammonium ions available at the duckweed mat as a result of diffusion, the removal is still regulated by a lower uptake coefficient ($0.1k_1$). Thus, increasing the diffusion under low duckweed uptake capacity will not cause much improvement in nitrogen removal. In

contrast, for normal duckweed (k_1), the higher D will promote greater nitrogen uptake by duckweed (compare k_1-0.1D, k_1-D, and k_1-10D in Figure 4), until the maximum uptake capacity of that k_1-duckweed is reached. Beyond that point, increasing D will no longer improve nitrogen removal. The nitrogen removal will become limited by the uptake capacity of the duckweed. This scenario is discussed in a later section.

Comparisons of the rate of nitrogen removal in relation to the k_1-D pair, according to Eq. 10, were performed as shown in Figure 5. The X-axis (at RDNR = 0) represents the k_1-D pair as a reference, and lines (a), (b), (c), and (d) represent the percent difference between nitrogen removal rate of each pair to nitrogen removal rate of k_1-D. RDNR of $10k_1$-D, (b), is high at the beginning as expected from the heightened uptake coefficient to $10k_1$. However, over time the k_1-10D RDNR, (d), increased and exceeded (b) at around the fourth hour. Increasing k_1 value only gave the advantage in removal speed at the beginning but would not sustain over a long period of time as (b) approaches zero (X-axis) in only a few hours. The decreasing k_1 value slows down nitrogen removal rate severely from the beginning, as shown by (a), although its RDNR seems to recover slightly over time. This suggests that healthy duckweed (with high k_1) would give a higher nitrogen removal rate in a short liquid retention static system. While maintaining duckweed health can sometimes be difficult due to the varying nutrient loading, seasonal change, and other environmental factors, increasing D by mixing would compensate for this drawback.

How much line (a), (b), (c), or (d) deviates from the X-axis indicates the degree of change (sensitivity) in the predicted nitrogen removal rate caused by the change in the parameter corresponding to that line, i.e. (a) and (b) for k_1, and (c) and (d) for D. In addition, the gaps between (a) and (b) and between (c) and (d) represent the sensitivity of the model to

parameters k_1 and D, respectively, at 2 orders of magnitude. Lines (c) and (d) are farther from the X-axis than either (a) or (b), and the (c)-(d) gap is obviously wider than that of (a)-(b). Nitrogen removal is more sensitive to parameter D than k_1 such that changing D would effectively alter the removal efficiency of the system. This leads to the use of the model to investigate the effect of the enhanced D value.

The maximum removal of ammonium nitrogen by duckweed in this type of system can be achieved when the concentration gradient is eliminated. Bringing the ions from the lower part of the pond to the surface to make the near-surface concentration higher will induce a higher duckweed uptake rate according to first order kinetics and therefore assures an increase in the nitrogen removal rate for a particular k_1 value. This can be done by, for example, introducing the influent to the pond through multiple inlets, such as in step feeding, to promote mixing, or simply by agitating the water column with a surface mixer or even by duckweed harvesting.

To investigate the effect of mixing on cumulative nitrogen removal, another simulation was conducted. Figure 6 shows the improvement in nitrogen removal of the 0.46 m (18 in) deep system by increasing D values with k_1 value held constant. The D value was repeatedly increased by a factor of two starting at 10D up to 640D and bounded by 1D and the completely mixed solutions. As shown, at high D values, the lines get close to each other and approach the completely mixed solution. The 640D line is in the region where further increases in D give only a small increase in nitrogen removal, i.e. less than 2.5 percent increase within 144 hours. On the other side, the removal with 1D was clearly lower, suggesting that if the observed nitrogen removal is higher than the model's prediction, an elevated ammonium diffusion coefficient could potentially be the cause. Figure 6 also shows

that less than 7.5 mg of nitrogen is removed per square meter in 6 days (144 hours). Actual field values of k_1 could be higher than our estimate due to the higher light intensity. Although that could improve the removal rate, it is still likely to be less than nitrification / denitrification systems. As a consequence, duckweed systems require a large land area for a high degree of nutrient removal. However, duckweed systems recover nutrients for utilization, unlike more space efficient nitrification / denitrification processes. The use of duckweed system as a polishing unit to produce agricultural irrigation water is the preferred application. As shown in Figure 6, the difference in removal is rather small at the beginning, i.e. 0 to 25 hours. Thus, even if a duckweed system is operated with the provision to enhance D values through mixing, the difference in removal efficiency may not be apparent at an early stage of operation.

The contribution of D to the increased removal is, however, limited. A separate series of simulations of ammonium profile development was carried out to determine the impact of an extreme D on increasing ammonium removal. The value of D was raised until the concentration gradient was decreased to zero; that is, the concentration was always homogeneous throughout the water depth. At approximately 1000D, the concentration profiles virtually became straight lines with depth. In this situation, nitrogen removal was not restricted by movement of the ions but by the duckweed ammonium uptake rate. Thus the system turned into an uptake-limiting type. The concentration values over time in the 1000D simulation were consistent with the first order kinetics model (Figure 2) that was derived from a completely mixed system.

Although mixing can enhance the removal rate of the system, under normal circumstances in the field it is more appropriate to provide only occasional or intermittent

mixing. Continuous mixing is undesirable primarily due to the energy costs. Moreover, duckweed prefers a stable water surface (Landolt and Kandeler 1987). Too much disturbance, such as produced by intense mixing, could cause slower growth leading to decreased removal efficiency of nitrogen and other nutrients (Al-Nozaily et al. 2000). A balance must be sought and maintained.

Experimental Results

Once the ammonium profile data were collected, they were compared to the model simulated profiles. Figure 7a shows the measured TAN and the model prediction at different depths using the original D and k_1 values. Because the pK_a of ammonia is 9.3 and the pH of the medium was 7.4, most of the ammonia was present in the ionic species, NH_4^+. Therefore, TAN can be used to represent NH_4^+ concentration reasonably well. The data indicate that a depth-wise ammonium profile developed as the model predicted. The concentration of ammonium ions was always lower near the surface over time confirming that the ammonium removal is diffusion-limited in this type of system. However, the simulation lines from k_1-D pair do not fit well with the data points. This poor agreement may be due to an inaccurate estimate of D that did not account for other transport mechanisms of ammonium besides molecular diffusion, such as the internal mixing caused by bacterial activities and possibly by the sampling procedure. These potential transport sources would increase the upward dispersion of the ammonium ions, thus increasing the D value. An experimentally determined transport coefficient should be used to include all transport mechanisms.

Best-fit values for D and k_1 were determined by minimizing the error sum of squares. The optimized D and k_1 values were 0.5989×10^{-3} m^2/hr and 0.8859×10^{-3} m/hr, respectively. When compared to the original values ($D = 7.0452 \times 10^{-6}$ m^2/hr, $k_1 = 0.906 \times$

10^{-3} m/hr), the ratio of the optimized value to the original value is 85.01 for D and 0.977 for k_1. These ratios suggest that the value of k_1 is acceptable but that D is a substantial underestimation of the overall transport process. The value of 85D was used to generate new ammonium profiles. The simulation results are clearly in better agreement with the experimental data (Figure 7b). This best fit diffusion coefficient could be regarded as the transport coefficient, T, of ammonium ions, which accounts for all ammonium transport processes. At this stage, the T value of 85D is only a preliminary estimate since there was no independent determination of the contribution from the other possible transport mechanisms. The model predicted lower concentrations than the data at 24 and 96 hours near the surface. This may be caused by the initial lag phase, despite a careful acclimation attempt, in duckweed nutrient uptake after duckweed was blot-dried for some time and weighed in preparation prior to transferring to the reactors. The predicted cumulative nitrogen removal using this T value of 85D is in the region where further increase of the D value would not substantially raise cumulative nitrogen removal (Figure 6). Therefore, improving D by additional mixing, which will increase costs of operation, may not improve nitrogen removal enough to be desirable.

Ammonia volatilization was assumed negligible in the model due to the shielding effect of duckweed. The pH also has an effect on ammonia volatilization. The pH values at different depths were measured over time at 0, 24, 48, and 144 hours (Figure 8). A zone of lower pH developed near the duckweed layer with the pH decreasing over time. This observation can be explained by the fact that duckweed plants possess a system of transport proteins that take NH_4^+ in exchange for H^+ (Ullrich 1987; Ullrich et al. 1984). Therefore, the pH will drop near the surface in association with NH_4^+ uptake. When the pH drops, the

volatile form of ammonia, NH_3, is converted to the ionic form, NH_4^+, which is no longer able to leave the liquid phase. This mechanism helps duckweed sequester nitrogen from its habitat. Nonetheless, extended exposure to the low pH environment may decrease the biochemical potential across the membrane necessary for efficient continuous cellular ion transport. Mixing restores the potential by dispersing the H^+ ions that build up. Duckweed provides not only a physical barrier by covering the pond but also the chemical barrier of reduced pH. Thus, a duckweed-covered pond reduces ammonia emissions to the atmosphere while carrying out nutrient recovery.

In the second set of experiments, duckweed was grown in reactors with 0.25 m (10 in) deep medium. Two mixing programs, every 3 and 48 hours, were applied to the reactors. Plastic simulated duckweed and bare surface reactors were used as controls. A loss of ammonium nitrogen was observed from reactors covered with plastic duckweed in both 48-hour and 3-hour mixing cycle systems (Figure 9). Bare surface reactors lost additional ammonium nitrogen due to the absence of cover effect (Figure 9a). The loss from the plastic simulated duckweed reactors was used to approximate the nitrogen loss through volatilization and/or bacterial activities from the duckweed reactors. This amount was added to the ammonium concentration from the duckweed reactors in order to estimate the concentration over time in the systems with duckweed uptake as the only ammonium loss mechanism (Figure 9a and 9b). This procedure yielded the adjusted 48-hour and 3-hour mixing cycle lines, which were then compared to the model prediction shown in Figure 10.

The model prediction in Figure 10 was generated with the ammonium transport coefficient T and uptake coefficient k_1. The 3-hour cycle was more efficient in nitrogen removal than the 48-hour cycle, as expected. Although the model predicted a smaller

difference of concentration over time between the two cycles, the calculated results are in good agreement with the data. The differences between the model predictions and the adjusted data are within 7.1 percent and 12.8 percent for 48-hour and 3-hour cycles, respectively. Overall, a better fit between the data and the model prediction was observed for the 48-hour cycle system than for the 3-hour cycle system. Compared to the 48-hour cycle system, the 3-hour system did not allow a reduced pH zone to build up because of the more frequent mixing. Ammonium uptake coefficient (k_1) of the duckweed could have been reduced in the 48-hour cycle as a result of the lower pH zone. This effect of the 48-hour mixing cycle is consistent with the data used to derive the k_1 value, which were collected from a system in which pH was adjusted only every 48 hours (Cheng et al. 2002). Thus, deviation from the model should be minimal for the 48-hour mixing cycle and the 3-hour cycle system would be expected to perform better than predicted.

Bacterial consumption of nitrogen is relatively small in anaerobic environments such as duckweed ponds and can be negligible. Thus, with known or estimable rate of ammonia loss through volatilization, the model developed in this study can reasonably predict the performance of the intermittently mixed duckweed system. Ammonia volatilization was not taken into account when the model was developed due to the lack of data or any existing model of ammonia emission from duckweed ponds. The volatilization data were used primarily to verify the uptake and transport components of the model. Ammonia volatilization from duckweed ponds varies under different conditions. Additional studies and experimental data will allow us to further improve the model.

CONCLUSIONS

Nitrogen removal by duckweed in a static pond depends on the combined action of ammonium transport, which conveys the ions to the surface, and duckweed ammonium uptake at the surface. Experimental results showed a lower ammonium ion concentration near the surface, indicating that ammonium transport is the limiting step in this process. A low concentration of nutrients near the surface is unfavorable to the nutrient recovery and removal. Design of duckweed ponds should be planned to avoid transport-limited conditions. In contrast, uptake-limited conditions will occur in well-mixed systems where the productivity of duckweed and the nutrient removal efficiency are at maximum, i.e., when the mixing intensity is below the adversity level that stresses the duckweed. In such a system with regular duckweed harvesting, the removal of nutrients is directly governed by the uptake and growth characteristics of the duckweed used. This nutrient removal process is species specific as extensively described by many researchers.

Molecular diffusion is not the only means for ammonium transport in lagoon liquid. The ammonium transport coefficient derived from experimental data was 85 times the ammonium diffusion coefficient (D). This new coefficient could be perceived as a lumped parameter that includes the effects of all transport mechanisms. Therefore, nutrient transport in unmixed duckweed systems can be much greater than molecular diffusion. Field-scale systems will have additional mixing mechanisms due to harvesting and wind action.

Another feature of duckweed ponds is the ion exchange between the intercellular hydrogen and ammonium ions in the liquid medium. Duckweed is deemed a suitable cover vegetation to minimize ammonia volatilization because of the localized pH drop near the surface and its small size that effectively covers the whole water surface. Providing mixing

will not only bring nutrients to the duckweed but also alleviate the exposure of duckweed to the low pH environment that occurs at the root zone.

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REFERENCES

Alaerts, G. J., Mahbubar, M. R., and Kelderman, P. (1996). "Performance analysis of a full-scale duckweed-covered sewage lagoon." *Water Research*, 30(4), 843-852.

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

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

Bergmann, B. A., Rubin, A. R., and Campbell, C. R. (1997). "Potential of *Paulownia elongata* trees for swine waste utilization." *Transactions of the ASAE*, 40(6), 1733-1738.

Boniardi, N., Vatta, G., Rota, R., Nano, G., and Carra, S. (1994). "Removal of water pollutants by *Lemna gibba*." *The Chemical Engineering Journal*, 54, B41-B48.

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

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

Cheng, J., Peet, M. M., Willits, D. H., and Pace, J. (2001). "Integrated farming for sustainable agriculture." *International Conference on Agricultural Science and Technology*, Beijing, China, 290-295.

Choy, B., and Reible, D. D. (2000). *Diffusion models of environmental transport*, Lewis Publishers, Boca Raton, Florida.

Crank, J., and Nicolson, P. (1947). "A practical method for numerical evaluation of solutions of partial differential equations of the heat-conduction type." *Proceedings of the Cambridge Philosophical Society*, 43, 50-67.

Harvey, R. W., Mueller, J. P., Barker, J. A., Poore, M. H., and Zublena, J. P. (1996). "Forage characteristics, steer performance, and water quality from bermudagrass pastures fertilized with two levels of nitrogen from swine lagoon effluent." *Journal of Animal Science*, 74(2), 457-464.

Haustein, A. T., Gilman, R. H., Skillicorn, P. W., Hannan, H., Diaz, F., Guevara, V., Vergara, V., Gastanaduy, A., and Gilman, J. B. (1994). "Performance of broiler chickens fed diets containing duckweed (*Lemna gibba*)." *Journal of Agricultural Science*, 122(2), 285-289.

Holst, R. W., and Yopp, J. H. (1979). "Comparative utilization of inorganic and organic compounds as sole nitrogen sources by the submergent duckweed, *Lemna trisulca* L." *Biologia Plantarum*, 21(4), 245-252.

Ingemarsson, B., Johansson, L., and Larsson, C.-M. (1984). "Photosysthesis and nitrogen utilization in exponentially growing nitrogen-limited cultures of *Lemna gibba*." *Physiologia Plantarum*, 62, 363-369.

Landolt, E., and Kandeler, R. (1987). *The family of Lemnaceae, a monographic study*, Veröffentlichungen des Geobotanischen Institutes ETH.

Lide, D. R. (2000). "CRC handbook of chemistry and physics." Chemical Rubber Company Press, Cleveland, Ohio, 2556.

Manimaran, B., Ramadhas, V., Santhanam, R., and Gopalakrishnan, P. e. (1997). "Cultivation of duckweed *Lemna minor* utilizing waste nutrients." *The National Seminar on Water Quality Issues in Aquaculture Systems*, Tamil Nadu Veterinary and Animal Sciences University, Tuticorin (India), 65-72.

Monselise, E. B.-I., and Kost, D. (1993). "Different ammonium-ion uptake, metabolism and detoxification efficiencies in two Lemnaceae." *Planta*, 189, 167-173.

Oron, G., De vegt, A., and Porath, D. (1988). "Nitrogen removal and conversion by duckweed grown on wastewater." *Water Research*, 22(2), 179-184.

Oron, G., Porath, D., and Jansen, H. (1987). "Performance of the duckweed species *Lemna gibba* on municipal wastewater for effluent renovation and protein production." *Biotechnology and Bioengineering*, 29(2), 258-268.

Oron, G., Wildschut, L. R., and Porath, D. (1985). "Waste water recycling by duckweed for protein production and effluent renovation." *Water Science and Technology*, 17(4/5), 803-817.

Porath, D., Oron, G., and Granoth, G. (1985). "Duckweed as an aquatic crop; edible protein recovery, production and utilization." *Agricultural waste utilization and management : proceedings of the Fifth International Symposium on Agricultural Wastes*, Hyatt Regency Chicago, Illinois Center, Chicago, Illinois, 680-687.

Porath, D., and Pollock, J. (1982). "Ammonia stripping by duckweed and its feasibility in circulating aquaculture." *Aquatic Botany*, 13(2), 125-131.

Reid, R. C., Prausnitz, J. M., and Poling, B. E. (1987). *The properties of gases and liquids*, McGraw-Hill, Inc.

Riggle, D. (1998). "Combining duckweed and high rate anaerobic treatments." *BioCycle*, 39, 64-65.

Rogers, J. R., Harvey, R. W., Poore, M. H., Mueller, J. P., and Barker, J. C. (1996). "Application of nitrogen from swine lagoon effluent to bermudagrass pastures: seasonal changes in forage nitrogenous constituents and effects of energy and escape protein supplementation on beef cattle performance." *Journal of Animal Science*, 74(5), 1126-1133.

Sutton, D. L., and Ornes, W. H. (1977). "Growth of *Spirodela polyrhiza* in static sewage effluent." *Aquatic Botany*, 3(3), 231-237.

Tripathi, B. D., Srivastava, J., and Misra, K. (1991). "Nitrogen and phosphorus removal-capacity of four chosen aquatic macrophytes in tropical freshwater ponds." *Environmental Conservation*, 18(2), 143-147.

Ullrich, W. R. (1987). "Nitrate and ammonium uptake in green algae and higher plants: mechanism and relationship with nitrate metabolism." Inorganic nitrogen metabolism, W. R. Ullrich, P. J. Aparicio, P. J. Syrett, and F. Castillo, eds., Springer-Verlag, Berlin, 35-36.

Ullrich, W. R., Larsson, M., Larsson, C.-M., Lesch, S., and Novacky, A. (1984). "Ammonium uptake in *Lemna gibba* G1, related membrane potential changes, and inhibition of anion uptake." *Physiologia Plantarum*, 61, 369-376.

Vermaat, J. E., and Hanif, M. K. (1998). "Performance of common duckweed species (*Lemnaceae*) and the waterfern *Azolla filiculoides* on different types of waste water." *Water Research*, 32(9), 2569-2576.

Wedge, R. M., and Burris, J. E. (1982). "Effects of light and temperature on duckweed photosynthesis." *Aquatic Botany*, 12, 133-140.

Wolverton, B. C., and McDonald, R. C. (1981). "Energy from vascular plant wastewater treatment systems." *Economic Botany*, 35(2), 224-232.

Zirschky, J., and Reed, S. C. (1988). "The use of duckweed for wastewater treatment." *Journal Water Pollution Control Federation*, 60(7), 1253-1258.

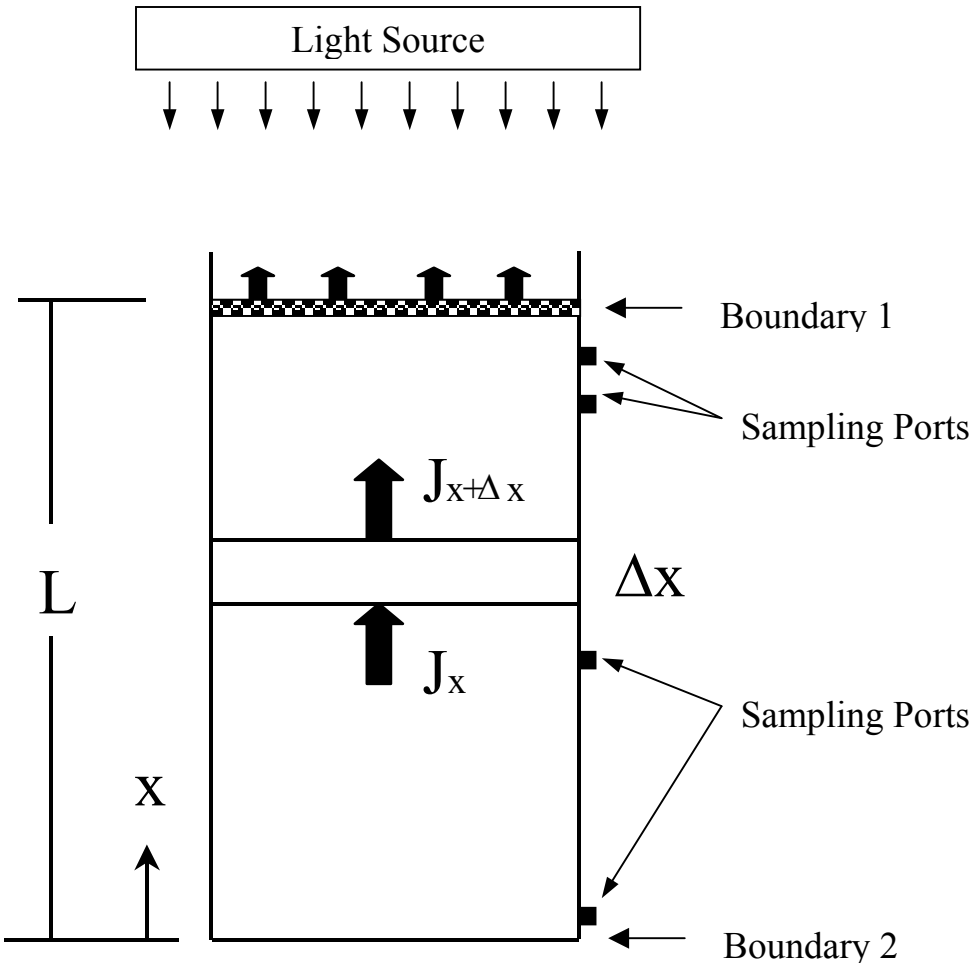


Figure 1. Schematic diagram of the simulated duckweed pond for nutrient removal from wastewater.

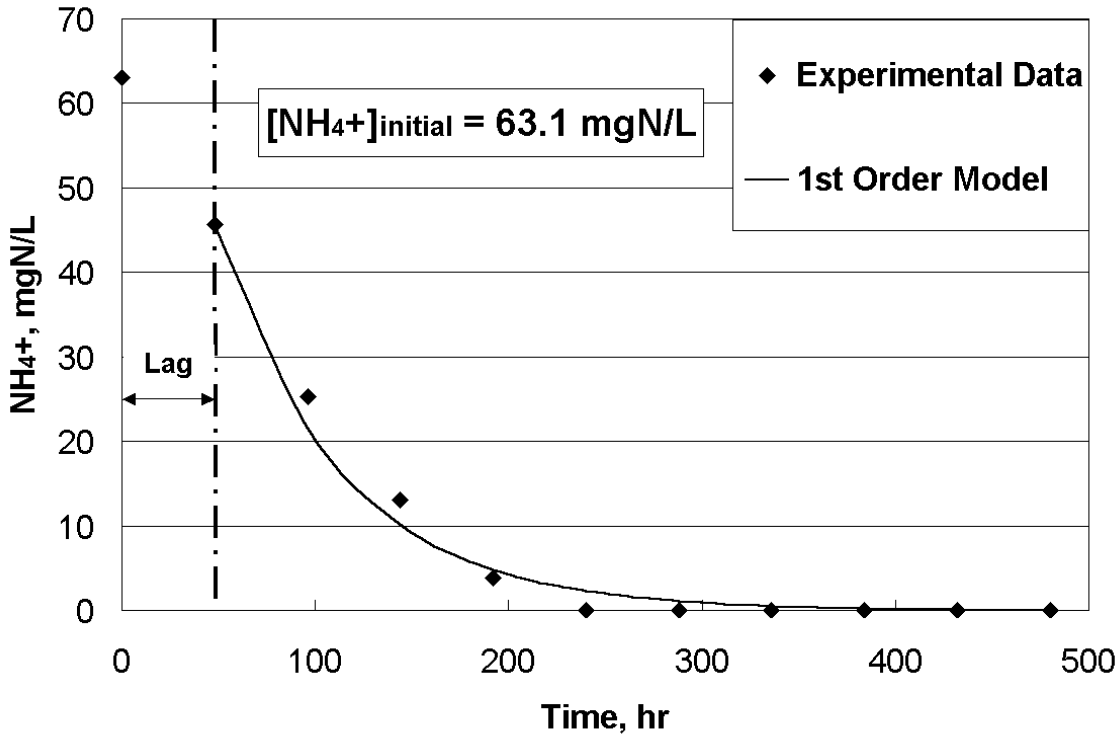


Figure 2. First order kinetic model fitted to the experimental data obtained from batch duckweed reactors with initial ammonium concentration of 63.1 mgN/L.

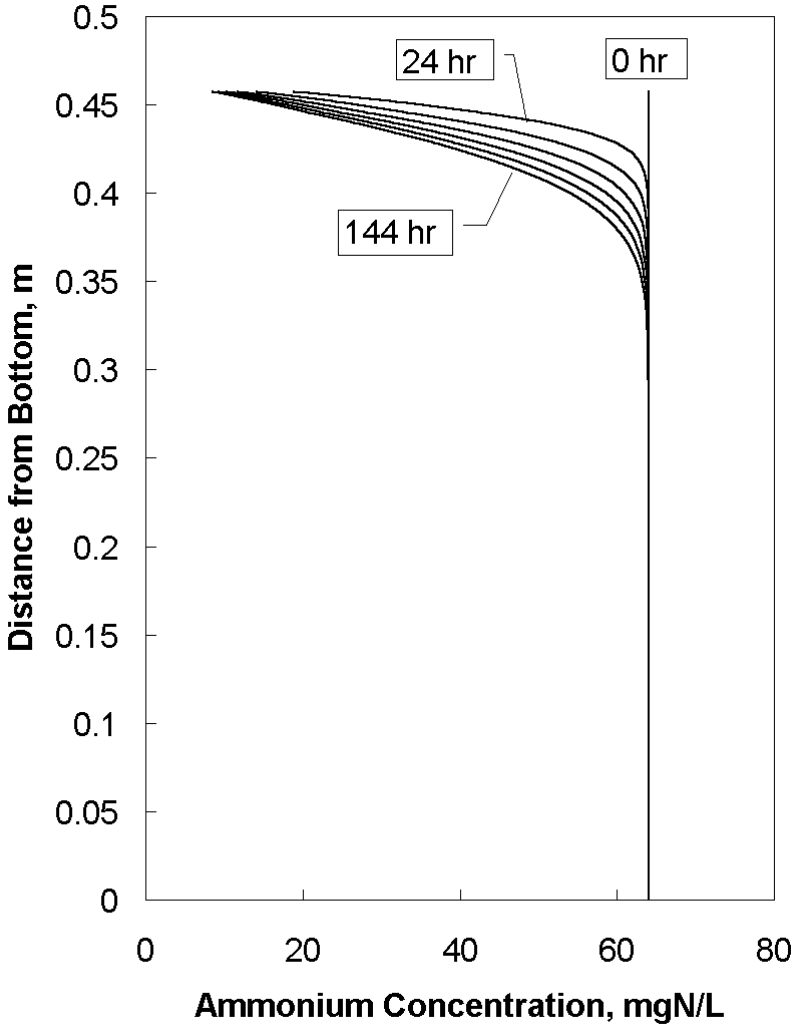


Figure 3. Ammonium ion concentration profiles in a simulated duckweed pond with initial concentration of 64 mgN/L, $k_1 = 0.906 \times 10^{-3}$ m/h, $D = 7.0452 \times 10^{-6}$ m^2/h at 24-hour increments.

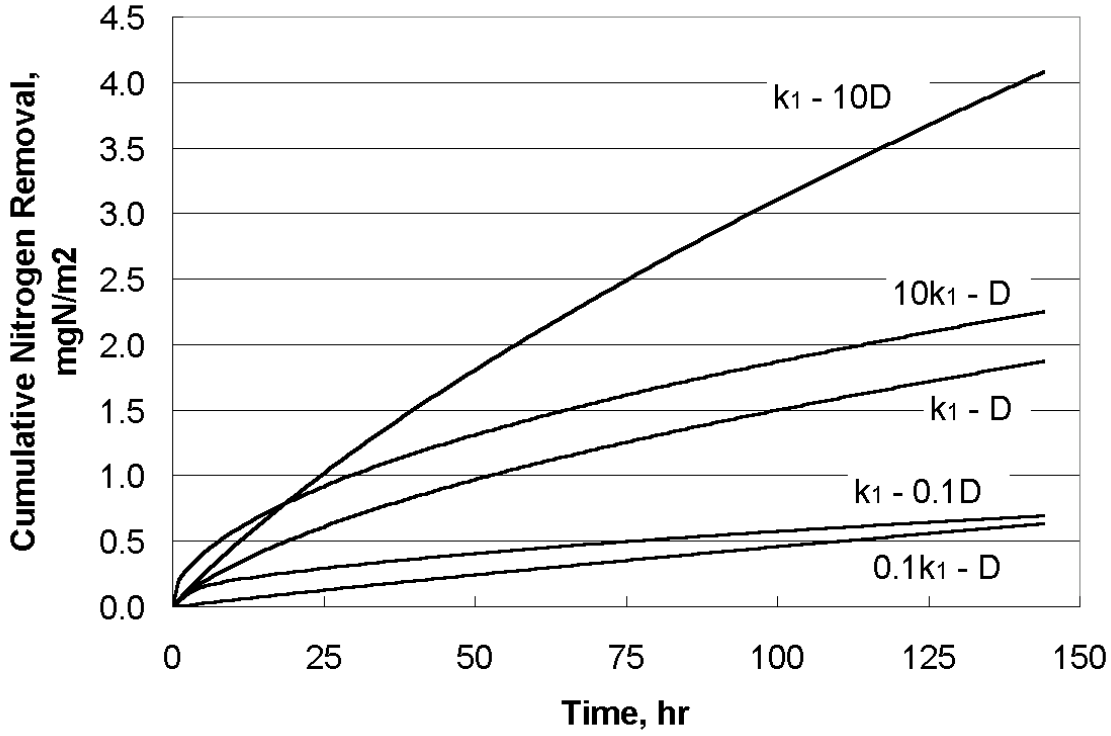


Figure 4. Responses of predicted cumulative nitrogen removal (mgN/m^2) to changes in ammonium diffusion coefficient (D) and duckweed ammonium uptake coefficient (k_1) in 0.46 m (18 in) deep static duckweed reactors.

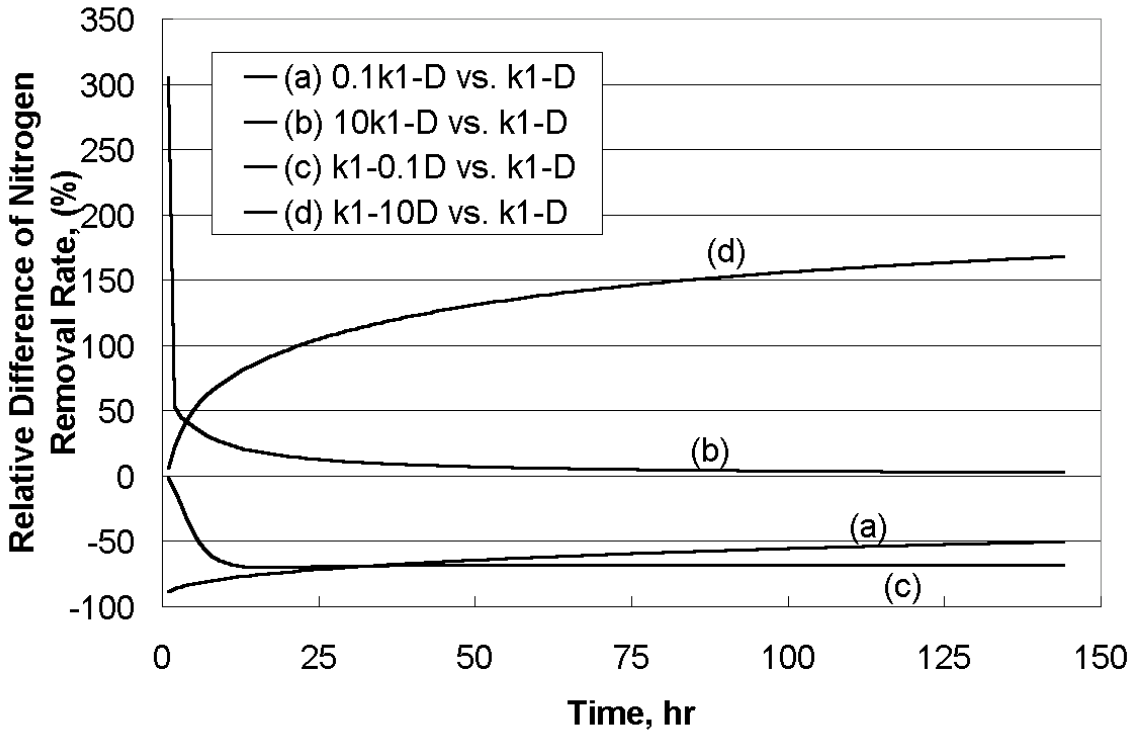


Figure 5. Effect of changes in ammonium diffusion coefficient (D) and duckweed ammonium uptake coefficient (k_1) on predicted nitrogen removal rate ($mgN/m^2/hr$) in 0.46 m (18 in) deep static duckweed reactors. The relative difference of nitrogen removal rates is calculated as a percent difference between predicted nitrogen removal rates using modified and unmodified coefficient values.

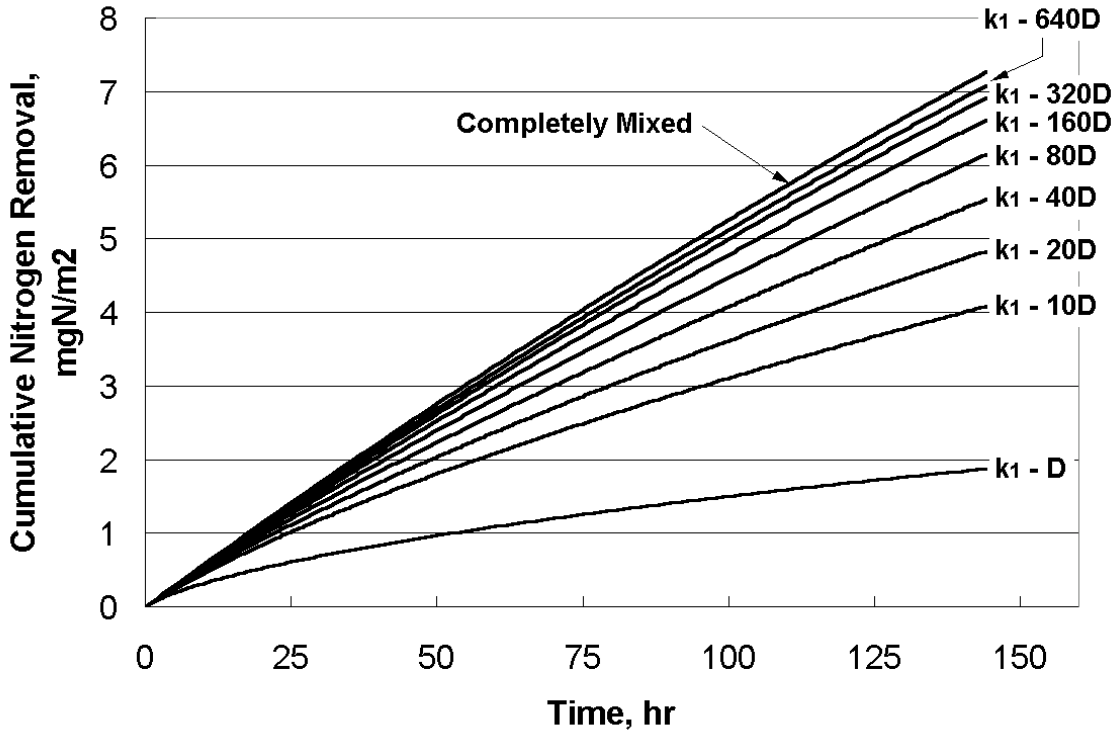


Figure 6. Predicted cumulative nitrogen removal (mgN/m^2) from 0.46 m (18 in) deep duckweed reactors using different values of ammonium diffusion coefficient (D).

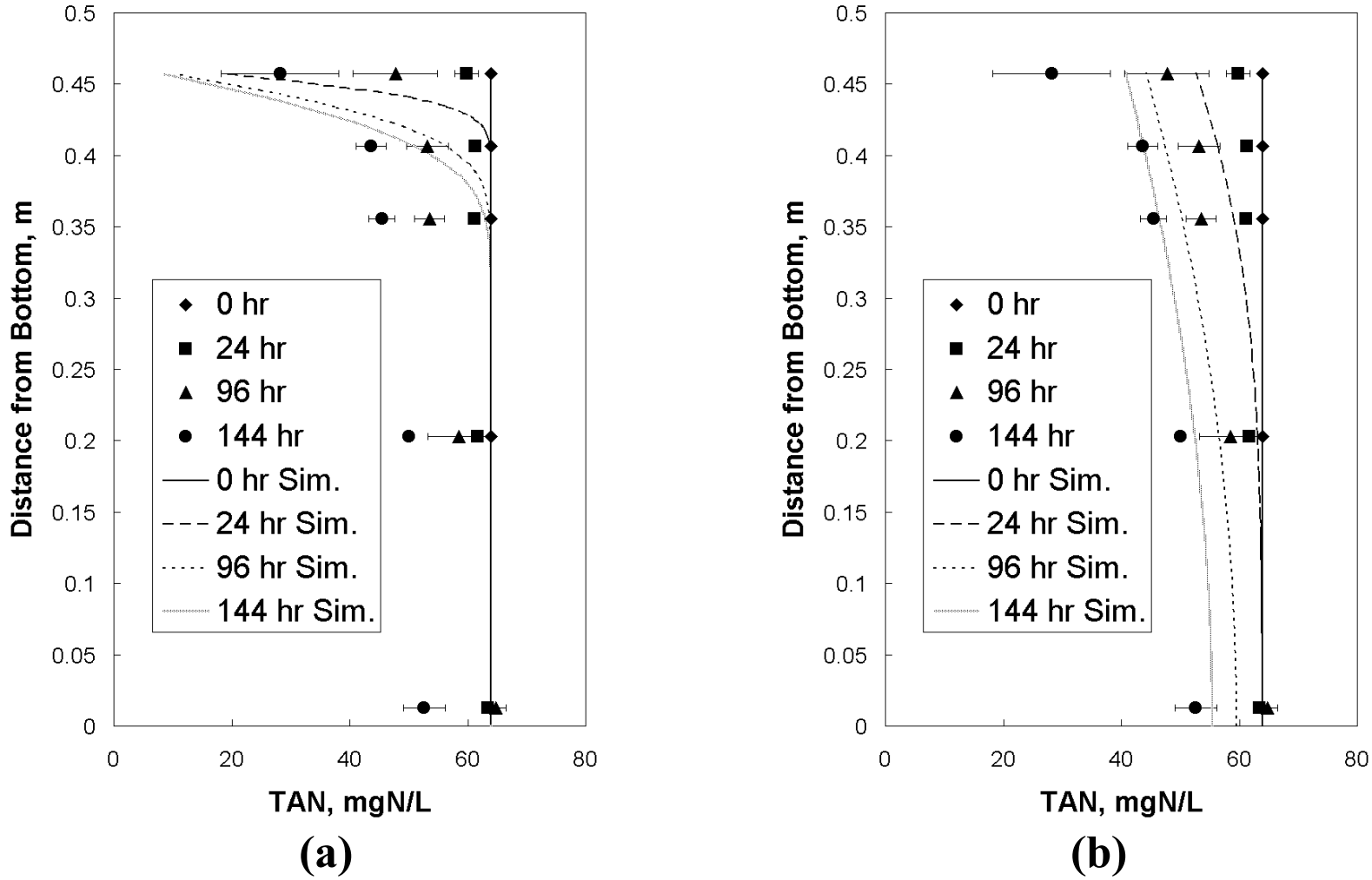


Figure 7. Comparison of simulated NH_4^+ profiles (lines) with experimental data (average ± standard deviation) at (a) k_1-D and (b) k_1-85D in 0.46 m (18 in) deep duckweed-covered reactors with swine lagoon liquid.

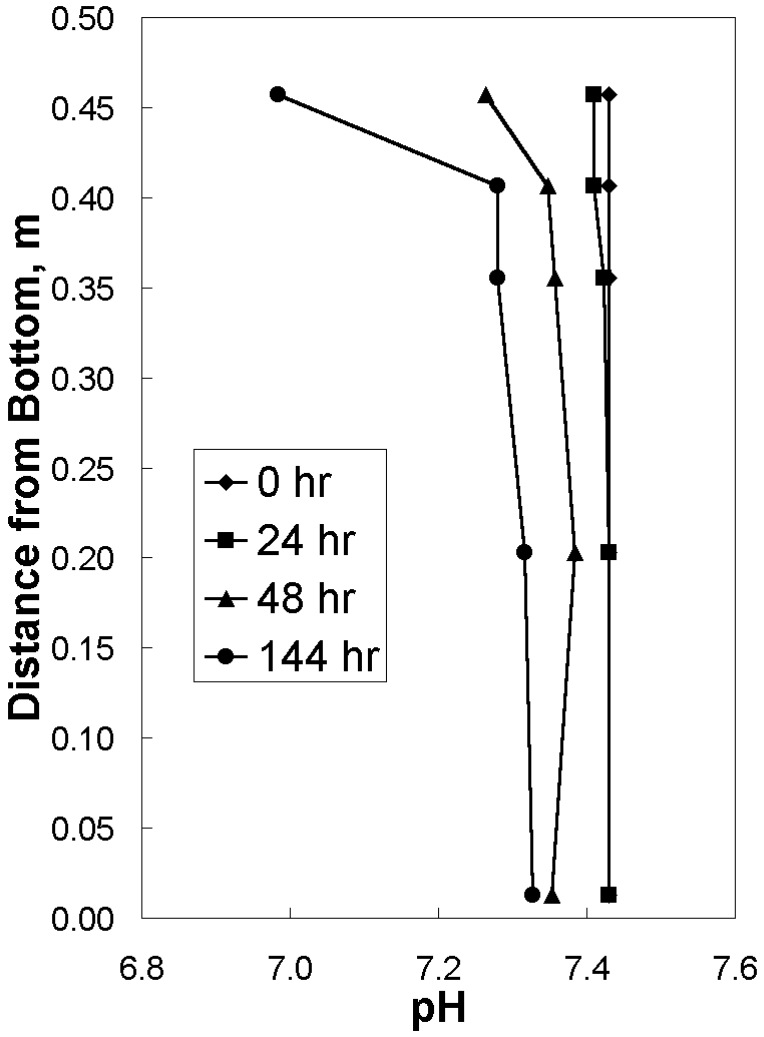
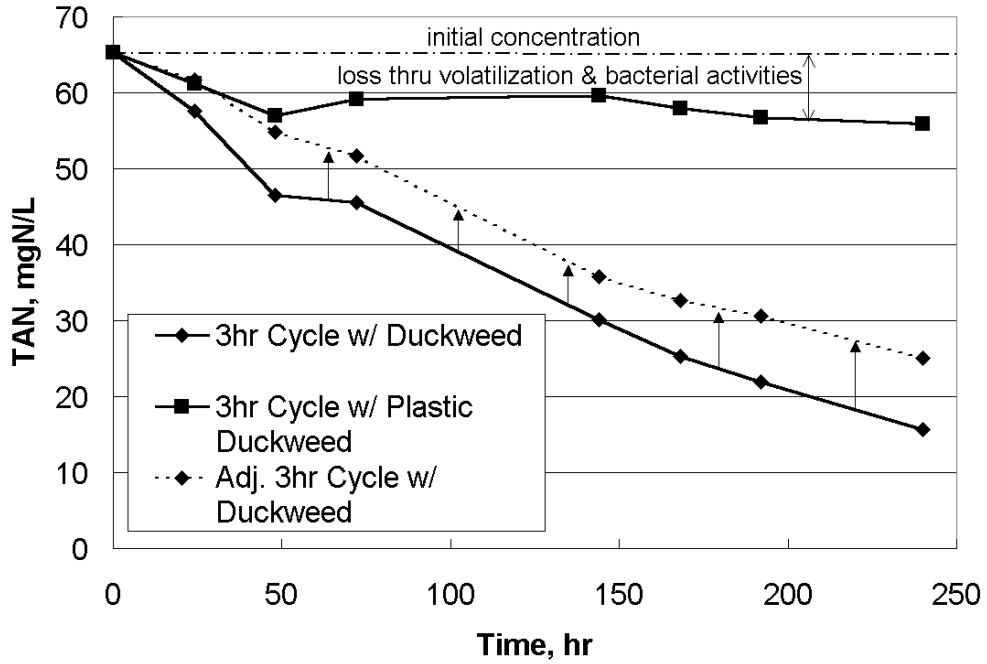
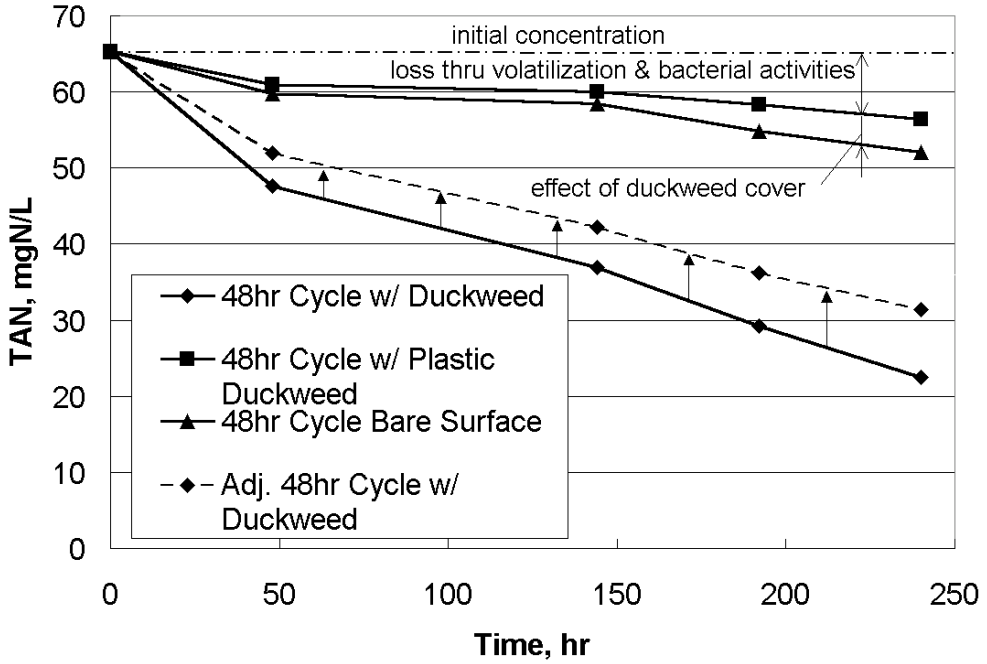


Figure 8. Measured pH profiles in 0.46 m (18 in) deep duckweed-covered reactors with swine lagoon liquid.



(a)



(b)

Figure 9. Influence of intermittent mixing at (a) 48-hour intervals and (b) 3-hour intervals on nitrogen loss from swine lagoon liquid in 0.25 m (10 in) deep reactors covered with plastic duckweed, live duckweed, and without cover (bare surface).

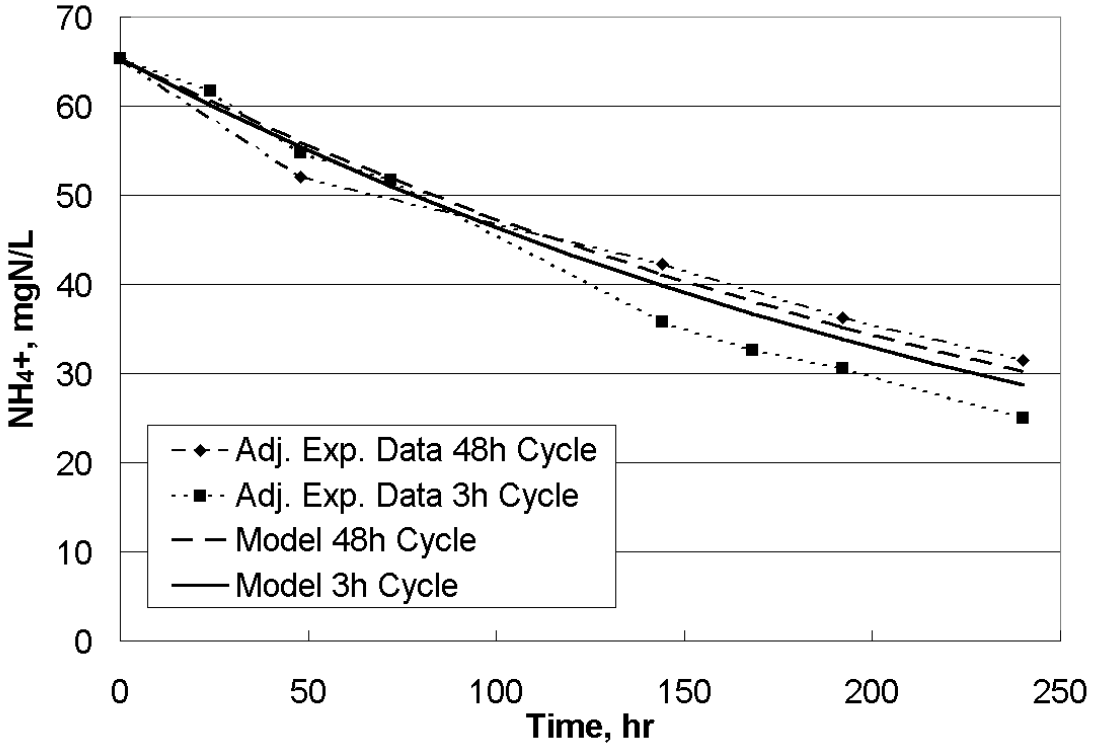


Figure 10. Comparison of predicted ammonium concentrations over time generated using optimized model parameters (ammonium transport coefficient T and first order ammonium uptake coefficient k_1) with experimental data in 0.25 m (10 in) deep duckweed reactors with swine lagoon liquid.

CHAPTER 3

ROLE OF INTERNAL NUTRIENT STORAGE IN DUCKWEED FOR SECONDARY TREATMENT OF SWINE WASTEWATER

By Sumate Chaiprapat[1], Jiayang Cheng[2], John J. Classen[3], and Sarah K. Liehr[4]

ABTRACT

The objective of this study was to investigate the relationship of duckweed growth in swine wastewater to nutrient content of duckweed biomass. Batch tests of *Spirodela punctata* 7776, the selected strain for highest total protein production, were conducted in an environment-controlled growth chamber operated at 24°C and 16-hour photoperiod. A prolonged growth period was observed after the nutrients in the medium were exhausted, indicating that duckweed could use its stored nutrients for growth. Prediction of growth using medium concentration as an independent variable was deemed unsuitable to describe this growth. Throughout the thirty-day growing period, nitrogen and phosphorus content in the biomass varied from 59.7 to 19.7 mg/g and from 14.8 to 6.8 mg/g (dry weight), respectively. The relationship between nitrogen content and specific growth rate of *Spirodela punctata* 7776 was found to follow Monod-type kinetics with μ_{max} of 0.2381 g/g/d and K_N of 28.8 mg/g. Reduced growth rate was observed in the duckweed culture with high

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duckweed density (mass per unit area). Effects of the duckweed density on growth rate and nutrient uptake were modeled and discussed.

INTRODUCTION

In conventional swine farms, animal wastes are flushed into anaerobic lagoons for partial treatment and the lagoon liquid is later applied to cropland as an ultimate disposal. However, in recent years, swine production has developed in such a way that larger-scale farming becomes necessary for survival in the industry. Associated with this trend is an increased concentration of animals in a given area, and cropland irrigation may no longer be sufficient to efficiently handle the excess nutrients contained in the waste. Moreover, periodic reductions occur in the amount of lagoon liquid that can be irrigated due to saturated soil during periods of frequent rainfall.

Storage of wastewater prior to land application can be more useful when nutrient recovery is implemented. Aquatic plant nutrient recovery systems could fill this niche as the plant takes up nutrients from the wastewater to produce value-added products, and at the same time reduces the amount of nutrients that has to be land-applied. Compared to other aquatic plants, duckweed has shown great potential with many good traits suitable for nutrient recovery from domestic wastewater and animal waste lagoon liquid. Many duckweed species have the ability to proliferate well in swine lagoon liquid (Bergmann et al. 2000b; Cheng et al. 2002), and the protein content can be as high as 45 percent dry weight basis in its tissue (Landolt and Kandeler 1987), which is a valuable property as the biomass can usually be used as dietary supplement for livestock and fish. The nitrogen levels in the healthy plant are comparable to those in commercial fertilizers; thus, the biomass could also

be utilized as a fertilizer supplement (Mbagwu and Adeniji 1988). Duckweed is a small free-floating aquatic plant, whose physical structure consists only of fronds (leaf-like structures) and roots. The size of a frond varies from 1.5 cm to less than 1 mm in length depending on species. Because of its small but seizable size, harvesting and processing are simple, as opposed to algae that is difficult to harvest, and water hyacinth that is hard to process due to its tough fibers and extensive root system. Additionally, duckweed preferentially takes up ammonium, while secreting hydrogen ions, thus creating a reduced pH environment at the water surface. This low pH environment plus the physical barrier of the fronds could help reduce ammonia emission to the atmosphere (Chaiprapat et al. *in review*).

In order to capitalize on the use of duckweed, a better understanding of its growth characteristics must be realized. There have been quite a few studies of duckweed growth in relation to nutrient acquirement and utilization in polluted waters. Since there are a variety of microorganisms in wastewater, association of bacteria with duckweed could have an effect on growth of a duckweed culture. Underwood et al. (1991) demonstrated that inoculation of a freshwater bacterium *Vibrio sp*. in the growth medium of *Lemna minor* caused significant increase in growth while inoculation with *Klebsiella sp*., *Enterobacter sp.* and *Serratia sp.* induced only moderate growth stimulation. In addition, presence of N_2-fixing heterotrophic bacteria and cyanobacteria was detected in duckweed mats sampled in Texas and Florida (Zuberer 1982). It was estimated that as much as 15 to 20 percent of nitrogen required for growth by duckweed could come from nitrogen fixation. Despite this possible benefit of bacteria, the majority of the nutrients used by duckweed are taken from the liquid medium.

Concentration of nutrients in its liquid habitat is one of the parameters believed to influence kinetics of growth and nutrient uptake of duckweed. Concentrations of various

forms of nitrogen have been used as the independent variable to predict duckweed growth and nutrient removal. Landesman et al. (1999) used total nitrogen concentration in their model to estimate the growth rate of *Lemna obscura* grown in diluted anaerobically digested cattle manure, while Caicedo et al. (2000) used separated concentrations of ammonia and ammonium to investigate growth of *Spirodela polyrrhiza*. More complex models were developed by Boniardi et al. (1994) and Vatta et al. (1995). Growth rate of *Lemna gibba* was described with a modified Monod model that contained concentrations of different nutrients including COD present in the wastewater medium. However, in recent batch test studies by Cheng et al. (2002), continued growth after a complete depletion of inorganic nitrogen and phosphorus in sterile growth medium was observed. This growth pattern implies that there could be other sources of nutrients available for growth besides those in the medium. Macronutrients nitrogen and phosphorus are required and are known to affect growth characteristics of plants. Plants are able to store nutrients internally in many forms, for instance, nitrogen as amino acid or proteins. This internal storage dictates the nutritional value of the plant tissues and could influence their growth rate as well. The objectives of this study were to verify the relationship of nutrient storage to the growth of the selected strain of duckweed *Spirodela punctata* 7776, to evaluate the behavior of growth and nutrient uptake in relation to the internal nutrient storage, and to determine other possible parameters affecting this relationship.

MATERIALS AND METHODS

Two batch tests were conducted for a period of 30 days to monitor the rate of biomass growth and nutrient uptake in swine artificial medium (SAM), which was formulated

(Appendix B) to resemble the chemical composition of typical North Carolina swine lagoon liquid (Bergmann et al. 2000a). The superior duckweed strain *Spirodela punctata* 7776, that yielded the highest protein production in *in vitro* screening of nearly 1000 geographic isolates by Bergmann et al. (2000a), was used in this study. The first batch test employed sterile full strength SAM while the second batch used sterile half strength SAM. Both experiments were carried out in a growth chamber operated on 16-hr photoperiod at a light intensity of 40 μmol/m^2/s provided by GE 48-inch 40W wide-spectrum fluorescent bulbs. Temperature was maintained at 24°C. In the full strength SAM experiment, a total of fifty-three boxes were used, which included forty-five duckweed-seeded boxes and eight control boxes that had no duckweed. Some duckweed-seeded boxes were discarded due to bacterial contamination. However, samples constituted at least duplicate duckweed-seeded boxes. The half strength SAM experiment also consisted of forty-five duckweed-seeded boxes and eight control boxes but with fifteen additional duckweed-seeded boxes as redundancies to ensure triplicate samples at each sample collection in case of contamination. An amount of 1 g fresh weight (± 5%) of *Spirodela punctata* 7776 was seeded to 6.35 cm × 6.35 cm × 7.62 cm (W×L×D) polypropylene boxes (Magenta, Chicago, IL) containing 100 ml of its respective full or half strength sterilized mediums. Sucrose (3% in full strength experiment) served as carbon source in SAM and the initial pH was 6.9-7.0. Prior to each experiment, duckweed was preconditioned in the growth chamber at least one week in full strength or half strength SAM with regular medium replacement. This process was performed in order to eliminate the lag phase that could occur at the beginning of the experiment. Due to a drop in pH during the experimental period, 10M NaOH was used to adjust the pH of all

duckweed-seeded boxes to 7.0 every 48 hours. This procedure was carried out under sterile conditions in a laminar airflow hood. All boxes were mixed daily.

Destructive sampling, in which boxes were destroyed during sampling, was used to collect liquid medium and duckweed biomass every 48 hours. To prevent sample contamination, duckweed biomass was sieved and rinsed with deionized water while liquid medium was filtered with a glass microfiber filter (20-25 μm particle retention, Whatman No. 41) to separate particulates and dead plant tissues. Every 96 hours, one control box was sampled. All liquid samples were analyzed for total ammonia nitrogen (TAN), phosphate (PO_4-P), total Kjeldahl nitrogen (TKN), and total phosphorus (TP) according to Standard Methods (APHA et al. 1995). After being rinsed, duckweed biomass was dried at 65°C over night and left to cool in a desiccator. Then, the tissue was analyzed for phosphorus content by nitric acid wet digestion followed by an inductively coupled plasma atomic emission spectrometer (Varian Inc., USA), and nitrogen content and carbon content by gas chromatography with NC 2100 Soil Analyzer (CE Elantech, Lakewood, NJ). Liquid sample analyses were done in the Environmental Analysis Laboratory of the Biological and Agricultural Engineering Department, and plant tissue analyses were done in the Forestry Nutrition Cooperative Laboratory of the Forestry Department at North Carolina State University.

RESULTS AND DISCUSSION

Biomass Growth

During the growth period of 30 days in batch culture, dry mass of duckweed was recorded over time (Fig. 1). *Spirodela punctata* 7776 was able to grow in full strength and

half strength SAM without a lag period at the early phase of growth. The linear regression line analysis for the full strength SAM, with an initial biomass of 0.1505 g, yielded an overall growth rate of 0.0754 g/d or 18.70 $g/m^2/d$. Although a high R-square value (0.984) was attained, a moderate exponential-like growth was noticed during the first 12 days (Fig. 1). During this period, the duckweed biomass in full strength SAM also exhibited slightly higher growth than that in the half strength SAM. A clear difference in biomass growth was, however, seen at approximately day 18 where the exhaustion of growth nutrients appeared in the half strength SAM experiment. The growth declined, came to stop at approximately 1.4 g, and the culture even lost some of its mass through decay. In full strength SAM, slight decrease in growth rate of duckweed was seen near the end of 30 days. If the experiment had been allowed to continue, the ultimate biomass production was expected to reach approximately 2.8 g because there was twice as much nutrient available in the full strength medium.

Nutrient Consumption

Ammonia nitrogen and orthophosphate phosphorus are the elements taken up in the largest quantities of all nutrients. Full strength SAM contained 343.0 mg/L of total ammonia nitrogen (TAN) and 135.0 mg/L of orthophosphate (PO_4-P) whereas half strength SAM contained approximately half at 174.0 mg/L TAN and 65.2 mg/L PO_4-P. Duckweed consumed virtually all of the TAN in 12 days and PO_4-P in 16 days in full strength SAM, and only 8 days for TAN and 10 days for PO_4-P in half strength SAM (Fig. 2). There was no change in nutrient concentration of the medium in all control boxes, which were without duckweed. Thus, no other nutrient removal activities in the medium were significant. Linear regression was performed on the straight portion of each curve to approximate the removal

rate. Total ammonia nitrogen and orthophosphate removal rates were 32.34 mg/L/d and 9.67 mg/L/d in full strength SAM, and 28.35 mg/L/d and 7.72 mg/L/d in half strength SAM, respectively. Both nutrients were removed faster in full strength SAM, by 12.3 percent for TAN and by 20.2 percent for PO_4-P even though the amount of biomass was approximately equal. In both cases, growth continued beyond these nutrient depletion points, implying that other sources of nutrients must have been available.

Nutrient Storage

Nutrient composition of duckweed can change in accordance with its habitat conditions (Hammouda et al. 1995; Landolt and Kandeler 1987; Oron et al. 1987; Oron et al. 1986; Sutton and Ornes 1975). Accumulation of various chemicals such as nitrogen, phosphorus, and some simple organics is desirable because the biomass could have higher nutritional value and could better serve the purpose of wastewater treatment. However, accumulation of toxic substances, especially heavy metals, is also possible (Chawla et al. 1991; Miranda and Ilangovan 1996; Rahmani and Sternberg 1999), which could make the biomass unsuitable for animal feed. Thus, sources of harvested duckweed must be evaluated prior to using it for feeding purposes. The highest nitrogen and phosphorus contents of *Spirodela punctata* reported were 7.2 percent (72 mg/g) and 2.4 percent (24 mg/g) on a dry mass basis, whereas the lowest nitrogen and phosphorus contents reported were 1.7 percent (17 mg/g) and 0.6 percent (6 mg/g), respectively (Landolt and Kandeler 1987). Therefore, 55 mg/g of nitrogen and 11 mg/g of phosphorus can be stored and used for growth. Excessive uptake or accumulation of nitrogen and phosphorus can occur whenever these elements are abundantly available in the habitat while there is capacity within the duckweed for storage. As seen in Figure 3, nitrogen and phosphorus contents rose during the first 4 days in both

experiments before starting to decline. Although full strength SAM is quite a concentrated medium, maximum values (72 mgN/g and 24 mgP/g) of nutrient contents were not reached in these experiments. Other factors besides the medium concentration, such as light, temperature and ratio of chemical composition of the medium, could also affect the storage capacity. Nitrogen and phosphorus contents of duckweed in the full strength SAM were higher than those in the half strength SAM (Fig. 3). However, the conclusion cannot be drawn that higher nutrient concentration produces duckweed with higher nutrient concentration in the biomass. This higher level of biomass nutrient content in full strength SAM is due primarily to the fact that the total amount of nutrient available for uptake in full strength SAM was twice as much, and so it lasted longer and at a higher concentration over time because the growth (before the nutrient depletion point) was fairly similar in both experiments. With such relatively higher nutrient concentration, continual nutrient uptake from the medium took place at a higher rate over time in the full strength SAM. Thus, delay in storage (nutrient contents) depletion occurred, which made nutrient content in biomass of the full strength SAM experiment higher over the course of the 30-day growth period. In order to determine the effect of medium concentration on the biomass nutrient content, comparison should be made in constant medium concentration or continuous culture experiment, such as that noted in Landolt and Kandeler (1987) that nitrogen and phosphorus concentrations in constant nutrient solution higher than about 4 mg/L did not raise nitrogen and phosphorus contents of duckweed further. Also shown in Figure 3 are the minimum nitrogen and phosphorus contents obtained from the half strength SAM experiment, which were 16.5 mg/g and 6.3 mg/g, respectively. These numbers are consistent with the reported values summarized in Landolt and Kandeler (1987).

Analysis of Growth Kinetics

Growth continued after complete depletion of nutrients in the medium and the presence of nutrient storage (the amount of nutrient content higher than the minimum level) lasted until the end of the growth phase. Therefore, nutrient content should be related to the rate of duckweed growth. Analysis of this relationship was carried out. Although the limiting nutrient could not be identified definitely at this stage, nitrogen was chosen for analysis because of its clear variation in the biomass (Fig. 3), corresponding to considerable variation in specific growth rates (μ, g/g/d) (Fig. 4a). Monod kinetics (Eq. 1) was used as a model to describe specific growth rate of *Spirodela punctata* 7776 in relation to nitrogen content as a limiting nutrient. Calculations of μ are shown in Equations 2 – 4 as follows.

$$\mu = \mu_{\max}\left(\frac{N_{resv}}{K_N + N_{resv}}\right) \quad (1)$$

where μ = specific growth rate (g/g/d),

μ_{max} = maximum specific growth rate (g/g/d),

N_{resv} = nitrogen reserve of the biomass (mg/g)

= (Nitrogen content) – (N_{min}),

N_{min} = minimum nitrogen content of the biomass (mg/g), and

K_N = half saturation constant (mg/g)

$$\frac{dX}{dt} = \mu X \quad (2)$$

$$\int_{X_i}^{X_{i+1}} \frac{dX}{X} = \int_{t_i}^{t_{i+1}} \mu \, dt \quad (3)$$

$$\mu = \frac{\ln\left(\frac{X_{i+1}}{X_i}\right)}{t_{i+1} - t_i} \quad (4)$$

where X = mass of the biomass (g), and

t = time (d)

In the half strength SAM experiment, curve fitting of the data yielded μ_{max} of 0.2381 d^{-1} with a half saturation constant (K_N) of 12.30 mg/g (Fig. 4a). This K_N value means that half of the μ_{max} would be reached at N_{resv} = 12.3 mg/g or at the nitrogen content of 28.8 mg/g. Note that duckweed growth would not take place at the nitrogen content below the minimum level, N_{min}, of 16.5 mg/g.

Analysis of the Effects of Surface Density on Growth

A similar procedure was applied to the data set from the full strength SAM experiment. There were two outliers of the specific growth rate, which are 0.3735 and 0.0429 d^{-1} at the nitrogen contents of 53.2 and 57.4 mg/g, respectively (Fig. 4a). These two outliers are caused by one peculiar data point of the duckweed biomass at day 2 (Fig. 1) that could be a mere random error in the data set. The parameters derived from them were displayed in later plots but neither was included in subsequent data analysis. Although curve fitting of the specific growth rate data was achievable, duckweed growth pattern in the full strength SAM culture did not show a good agreement with the Monod kinetics curve. There appeared to be decreases in specific growth rates, particularly at nitrogen content around 45 mg/g where a sharp drop occurred (Fig. 4a). Under such controlled environmental conditions as in this study, reduction in growth rate of duckweed in our culture that allowed accumulation of biomass could be caused by the limited light penetration and gas exchange

to the lower duckweed layers, and increased competition for space to grow. Each individual factor was not determined in this study, but they appeared to relate to the same source, the surface density (mass per unit area) of the duckweed culture. This was supported by reports by Said et al. (1979), Reddy and DeBusk (1985), and Porath et al. (1985) that maximum growth rate of *Lemna* and *Spirodela* were achieved at low plant density. Thus, surface density was used as a lumping parameter to represent all the effects mentioned.

Densities of duckweed at various nitrogen contents over the experimental period are shown in Figure 4b. The chronological order of density data is along the y-axis, from the least to the highest density. In the x-axis, the nitrogen content of duckweed increased at the beginning (least density) of each experiment as a result of the nutrient accumulation shown in Figure 3. The difference in specific growth rates was moderate at the beginning where nitrogen contents were higher at low density. At a nitrogen content of approximately 45 mg/g, where rapid decrease occurred in full strength SAM experiment, the biomass density was around 260 g/m^2. At this same density in the half strength SAM experiment, nitrogen content had already reached a low level at approximately 24 mg/g. Its specific growth rate was also low and falling into a steep portion of the Monod curve. Thus, density effects would not clearly be seen and not significantly affect the shape of the Monod curve in the half strength SAM data set. For the purpose of comparison and approximation of the form of the density effects function, we assumed minimal or no density effects on this set of data. The kinetic expression of the specific growth rate of duckweed in full strength SAM was then designated to resemble the Monod equation with an additional multiplying factor of density effects as follows:

$$\mu' = \mu\left(DF\right) \qquad (5)$$

$$\mu' = \mu_{\max}\left(\frac{N_{resv}}{K_N + N_{resv}}\right)(DF) \quad (6)$$

where μ' = specific growth rate with density effects in full strength SAM experiment (g/g/d),

μ = specific growth rate without density effects in half strength SAM experiment (g/g/d), and

DF = density function (value between 0 and 1)

Thus, the value of density function can be estimated as

$$DF = \frac{\mu'}{\mu} \quad (7)$$

In order to derive the data points of DF, μ' and μ in Eq. 7 must be at the same N_{resv}. Our data collection was time dependent and was controlled by N_{resv}. However, μ could be calculated by the Monod equation over any N_{resv} (N content – N_{min}) values (solid line in Fig. 4a), and the calculated μ at corresponding N_{resv} of μ' were used to derive DF. The resulting DF values were plotted against the density of biomass that corresponded to each μ' (Fig. 5). As a multiplying factor in the Monod expression, the maximum value of DF is unity, which obviously means that there is no reduction in growth as a result of density. Therefore, one data point at (0, 1) was added to the data set. In Figure 5, effects of density increased (DF declined) as density increased, first at a slower rate, and faster near the critical point where the highest rate of DF reduction occurs. After that, the reduction slows down and approaches saturation. At some point the increasing density would not reduce biomass growth any further.

This type of function may be represented by an appropriate mathematical expression. An enzyme kinetics expression with some modification is proposed. The Sigmoidal Hill's

equation (Eq. 8) has been used to describe various chemical reactions and biological systems (Fisher et al. 1996; Manso et al. 2001; Romero and Celis 1995) and was chosen initially in our study because of its characteristics that are comparable to the density factor data. Although linear and step functions may seem to fit to the data set to some extent, they were eliminated because of the lack of physical meaning. At indefinitely high surface density, there can still be growth of duckweed, which the linear model would, at some point, predict zero at X-intercept. Meanwhile, the step function would predict a sudden drop of the function at a certain value of independent variable, which usually does not reflect the changes in biological systems that are typically gradual and continuous. The features of the Sigmoidal Hill's equation are graphically shown in Figure 6. It has a minimum value at y_0 as the starting point for any increases of the function (y-y_0). The function reaches half of the maximum increase at $y_0+a/2$ where $x=K_x$. Slope of the mid part of the curve is controlled by a constant h ($h\geq1$), that is higher h value gives a steeper slope whereas the function turns into a Monod's curve at $h = 1$. However, what we sought was not exactly this function, but its inverse (y'), which can be achieved by subtracting the increase of the Sigmoidal Hill's function (y-y_0) from the saturation value of the function (y_0+a). The resulting equation (Eq. 9) is also illustrated in Figure 6.

$$y = y_0 + \frac{a\,x^h}{K_x^h + x^h} \tag{8}$$

$$y' = (y_0 + a) - (y - y_0) \tag{9}$$

Since this inverse function is a multiplying factor, the maximum is unity:

$$y_0 + a = 1 \tag{10}$$

Combining Eq. (8), (9), and (10) gives the following:

$$y' = 1 - \frac{a\, x^h}{K_x^h + x^h} \tag{11}$$

This equation can be rearranged to obtain an expression for y':

$$y' = \frac{K_x^h + y_0 x^h}{K_x^h + x^h} \tag{12}$$

Substitution of our parameters into Eq. 9 results in the following expression for DF:

$$DF = \frac{K_D^h + (DF_0)\, D^h}{K_D^h + D^h} \tag{13}$$

where DF = density factor,

DF_0 = minimum density factor,

K_D = half saturation constant (g/m^2),

D = density of duckweed (g/m^2), and

h = Hill's constant

Parameter estimation was carried out using optimization functions in Matlab. Two outliers, as indicated in Figure 5, were excluded from the data during this process. The resulting estimates were $DF_0 = 0.33$, $K_D = 201.25$ g/m^2, and $h = 2.02$. The plot of the DF function (Eq. 13) using these parameters is shown together with the data in Figure 7a. The DF function was used to predict the specific growth rate of duckweed in the full strength SAM using Eq. 6. The calculations utilized the kinetic parameters of the duckweed in the half strength SAM experiment: $\mu_{max} = 0.2381$ d^{-1} and $K_N = 12.3$ mg/g to calculate μ without density effects. Then DF was computed with Eq.13 and multiplied to μ at each corresponding N_{resv} in order to plot against the specific growth rates of duckweed in the full strength SAM. The predictions were able to reasonably represent the data of specific growth rate with density effects (Fig. 7b). Greater degree of fitness was hindered by the scattered

nature of the DF data with density shown in Figure 7a. Nevertheless, the analytical method presented could be used to mathematically quantify and assess the effects of crop density in a duckweed pond, which will be useful in optimization of harvesting schedule to enhance growth in a duckweed nutrient recovery system. It must also be recognized that effects of density will be of different magnitudes in duckweed grown in different environmental conditions. The assessment of density effects, thus, must be implemented on a site and species specific basis.

Nutrient Uptake

Analysis of nutrient uptake was also carried out. As previously discussed, nitrogen and phosphorus depletion rates in the medium were faster in the full strength SAM (Fig. 2). However, to compare the efficiency of the nutrient uptake of duckweed, nutrient consumption per unit biomass or the specific uptake rate was used. This specific uptake rate was derived from the increase of the total amount of nutrient in biomass rather than the decrease in nutrient concentration in the medium. Although comparable under most conditions, this method was more suitable, eliminating the possibility of sampling interference from dead cells or particulates in the medium that may have passed through the filter. Moreover, we could disregard the forms of nitrogen and phosphorus taken up because the nutrient content in biomass accounted for all forms of either nitrogen or phosphorus or other nutrients that were consumed.

The specific uptake rate (mg/g/d) of nitrogen and phosphorus were plotted against TKN and TP in the medium, respectively (Fig. 8). In the early stage at high TKN and TP concentrations in SAM, specific uptake rates of both nitrogen and phosphorus were high. The maximum rates obtained were 13.2 mgN/g/d and 3.5 mgP/g/d from the half strength

SAM experiment, and 11.6 mgN/g/d and 3.3 mgP/g/d from full strength SAM experiment. These rates occurred near the beginning of the experiment where nutrient accumulation took place (Fig. 3). This may indicate that starving duckweed could take up nutrients in a faster rate as to fill up its storage. When the storage is full, intake of nutrient is purely controlled by growth rate. However, specific uptake rate continued to drop over time. This was thought to be caused by the increasing density of the biomass. Thick culture limited access to nutrients by the duckweed located in the upper layers, and as discussed earlier, density negatively affected the growth rate of duckweed by limiting light, gas exchange, and space to grow, thus reducing the potential for nutrient uptake. It was clearly shown that the nitrogen (Fig. 8a) and phosphorus (Fig. 8b) specific uptake rates in the half strength SAM experiment (compared at the same medium nutrient concentrations in the full strength SAM experiment) were higher, indicating that the effects of density could have interrupted the uptake process in the full strength SAM culture that had higher crop density. Additionally, because nutrient uptake happens only at the water surface, diffusion of nutrients from within the liquid medium to the surface was reported as one of the important limiting factors for nutrient uptake of duckweed (Chaiprapat et al. *in review*). All of these factors make the nutrient removal process of duckweed cultures quite complicated, especially when interactions among them exist.

Despite all the obstructions to growth and nutrient uptake, higher surface density, however, offers some beneficial functions. A dense layer prevents light penetration to the liquid medium and thus inhibits algal growth. It could also help lower the ammonia emission to the atmosphere by first providing the physically denser duckweed blanket covering the surface, and second, creating the stratified lower pH layers near the water surface as a result

of nutrient uptake of duckweed (Chaiprapat et al. *in review*). Finally, the overall duckweed biomass production should be higher at high density even with a lowered specific growth rate. Higher total number of plants (in high density) to reproduce would at some point balance out and surpass the overall growth of the lower density culture with a higher specific growth rate. By considering all pieces together, optimization can be performed. However, it could be done only if quantification of each factor is justified. More research is still needed.

CONCLUSION

During the 30-day period in full strength SAM, *Spirodela punctata* 7776 exhibited a rather linear growth pattern at a rate of 18.70 $g/m^2/d$. However, moderate exponential growth pattern was observed in both full and half strength SAM cultures early in the experiments. In the half strength SAM experiment, biomass growth stopped at around 1.4 g dry weight basis with the nitrogen and phosphorus contents at minimum of 16.5 mgN/g and 6.3 mgP/g. In both experiments, growth of *Spirodela punctata* 7776 continued after the depletion of nutrient concentration in the medium indicating that duckweed *Spirodela punctata* 7776 utilized internal storage of nitrogen and phosphorus for growth. The relationship between nitrogen reserve and growth could be expressed in the form of Monod kinetics in the lower density culture of the half strength SAM experiment with maximum specific growth rate of 0.2381 d^{-1}. Reduction in growth of the high-density duckweed culture in the full strength SAM experiment was detected. A density factor (DF) was defined as a multiplying factor to the normal Monod kinetics to yield the specific growth rate with the effects of surface density. A system of mathematical equations was developed to describe

DF as a function of biomass density, and the proposed model was able to reasonably predict the specific growth rate with the effects of density.

With regard to nutrient removal efficiency, *Spirodela punctata* 7776 was able to remove at faster rates in the full strength SAM, at 32.34 mgNH_3-N/L/d and 9.67 mgPO_4-P/L/d, than in half strength SAM, at 28.35 mgNH_3-N/L/d and 7.72 mgPO_4-P/L/d. Another measure of the nutrient uptake as specific uptake rate (mg/g/d) was also considered. The maximum values of specific uptake rate obtained were 13.2 mgN/g/d and 3.5 mgP/g/d from the half strength SAM experiment, and 11.6 mgN/g/d and 3.3 mgP/g/d from full strength SAM experiment. These high values were found at the beginning of the experiment where accumulation of nutrient occurred and biomass density was low. Besides environmental and ecological factors, the level of internal nutrient storage and biomass density could significantly regulate the growth and nutrient uptake processes of duckweed, and hence the effectiveness of the duckweed nutrient recovery systems.

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REFERENCES

APHA, AWWA, and WWF. (1995). "Standard methods for the examination of water and wastewater." A. D. Eaton, L. S. Clesceri, A. E. Greenberg, and M. A. H. Franson, eds., United Book Press, Inc., Washington D.C.

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

Bergmann, B. A., Cheng, J., Classen, J., and Stomp, A. M. (2000b). "Nutrient removal from swine lagoon effluent by duckweed." *Transactions of the ASAE*, 43(2), 263-269.

Boniardi, N., Vatta, G., Rota, R., Nano, G., and Carra, S. (1994). "Removal of water pollutants by *Lemna gibba*." *The Chemical Engineering Journal*, 54, B41-B48.

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

Chaiprapat, S., Cheng, J., Classen, J. J., Ducoste, J. J., and Liehr, S. K. (*in review*). "Modeling nitrogen transport in duckweed pond for secondary treatment of swine wastewater."

Chawla, G., Singh, J., and Viswanathan, P. N. (1991). "Effect of pH and temperature on the uptake of cadmium by *Lemna monor* L." *Bulletin of Environmental Contamination and Toxicology*, 47, 84-90.

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

Fisher, P. R., Heins, R. D., and Lieth, J. H. (1996). "Quantifying the relationship between phases of stem elongation and flower initiation in poinsettia." *Journal of the American Society for Horticultural Science*, 121(4), 686-693.

Hammouda, O., Gaber, A., and Abdel-Hameed, M. S. (1995). "Assessment of the effectiveness of treatment of wastewater-contaminated aquatic systems with *Lemna gibba*." *Enzyme and Microbial Technology*, 17, 317-323.

Landesman, L., Patrick, B. P., Fedler, C. B., Konikoff, M., and Parker, N. C. (1999). "Effectiveness of duckweed to recycle nutrients from wastewater." *WEFTEC'99*, New Orleans, LA.

Landolt, E., and Kandeler, R. (1987). *The family of Lemnaceae, a monographic study*, Veröffentlichungen des Geobotanischen Institutes ETH.

Manso, M. C., Oliveira, F. A. R., Oliveira, J. C., and Frias, J. M. (2001). "Modelling ascorbic acid thermal degradation and browning in orange juice under aerobic conditions." *International Journal of Food Science and Technology*, 36(3), 303-312.

Mbagwu, I. G., and Adeniji, H. A. (1988). "The nutritional content of duckweed (*Lemna paucicostata* hegelm.) in the Kainji lake area, Nigeria." *Aquatic Botany*, 29, 357-366.

Miranda, M. G., and Ilangovan, K. (1996). "Uptake of lead by *Lemna gibba* L.: influence on specific growth rate and basic biochemical changes." *Bulletin of Environmental Contamination and Toxicology*, 56, 1000-7.

Oron, G., de-Vegt, A., and Porath, D. (1987). "The role of the operation regime in wastewater treatment with duckweed." *Water Science and Technology*, 19(1/2), 97-105.

Oron, G., Porath, D., and Wildschut, L. R. (1986). "Wastewater treatment and renovation by different duckweed species." *Journal of Environmental Engineering*, 112(2), 247-263.

Porath, D., Oron, G., and Granoth, G. (1985). "Duckweed as an aquatic crop; edible protein recovery, production and utilization." *Agricultural waste utilization and management : proceedings of the Fifth International Symposium on Agricultural Wastes*, Hyatt Regency Chicago, Illinois Center, Chicago, Illinois, 680-687.

Rahmani, G. N. H., and Sternberg, S. P. K. (1999). "Bioremoval of lead from water using *Lemna minor*." *Bioresource Technology*, 70, 225-230.

Reddy, K. R., and De Busk, W. F. (1985). "Growth characteristics of aquatic macrophytes cultured in nutrient-enriched water: II. azolla, duckweed and salvinia." *Economic Botany*, 39(2), 200-208.

Romero, I., and Celis, H. (1995). "Comparison of the hydrolysis of Zn-PPi-2- and the MgPPi-2- as substrates and the effect of free cations upon membrane-bound pyrophosphatase of Rhodospirillum rubrum." *Biochimie*, 77(12), 949-952.

Said, M. Z. M., Culley, D. D., C., S. L., Epps, E. A., Myers, R. W., and Boney, S. A. (1979). "Effect of harvest rate, waste loading, and stocking density on the yield of duckweeds." *Proceedings of the World Mariculture Society*, 10, 769-780.

Sutton, D. L., and Ornes, W. H. (1975). "Phosphorus removal from static sewage effluent using duckweed (*Lemna gibba*, *Lemna minor*)." *Journal of Environmental Quality*, 4(3), 367-370.

Underwood, G. J. C., and Baker, J. H. (1991). "The effect of various aquatic bacteria on the growth and senescence of duckweed (*Lemna minor*)." *Journal of Applied Bacteriology*, 70, 192-196.

Vatta, G., Rota, R., Boniardi, N., and Nano, G. (1995). "Dynamic modelling of waste-water treatment plants based on *Lemna gibba*." *The Chemical Engineering Journal*, 57(3), B37-B48.

Zuberer, D. A. (1982). "Nitrogen fixation (acetylene reduction) associated with duckweed (Lemnaceae) mats." *Applied and Environmental Microbiology*, 43(4), 823-828.

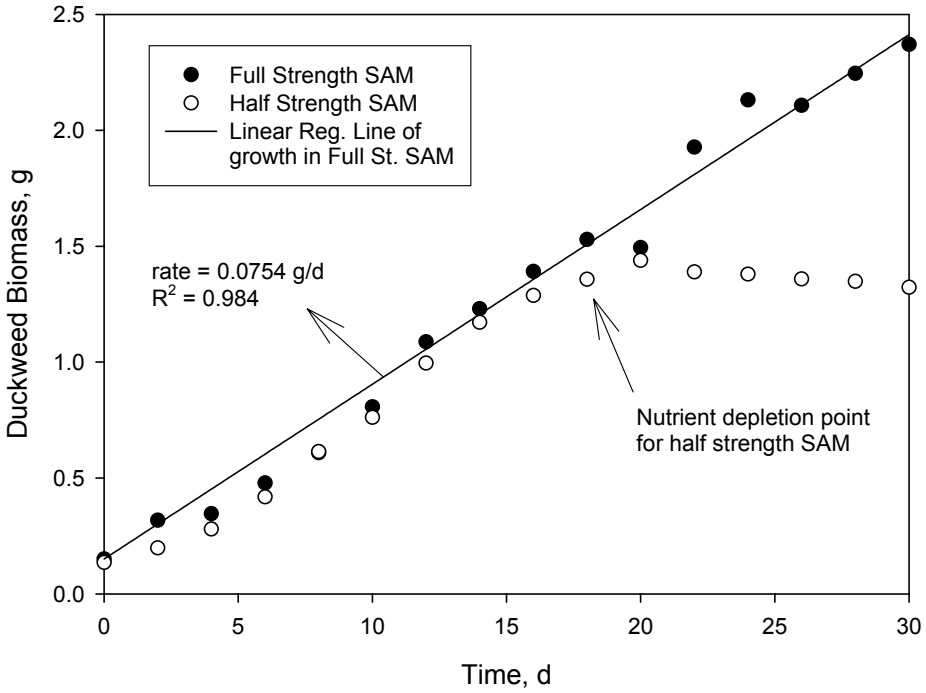
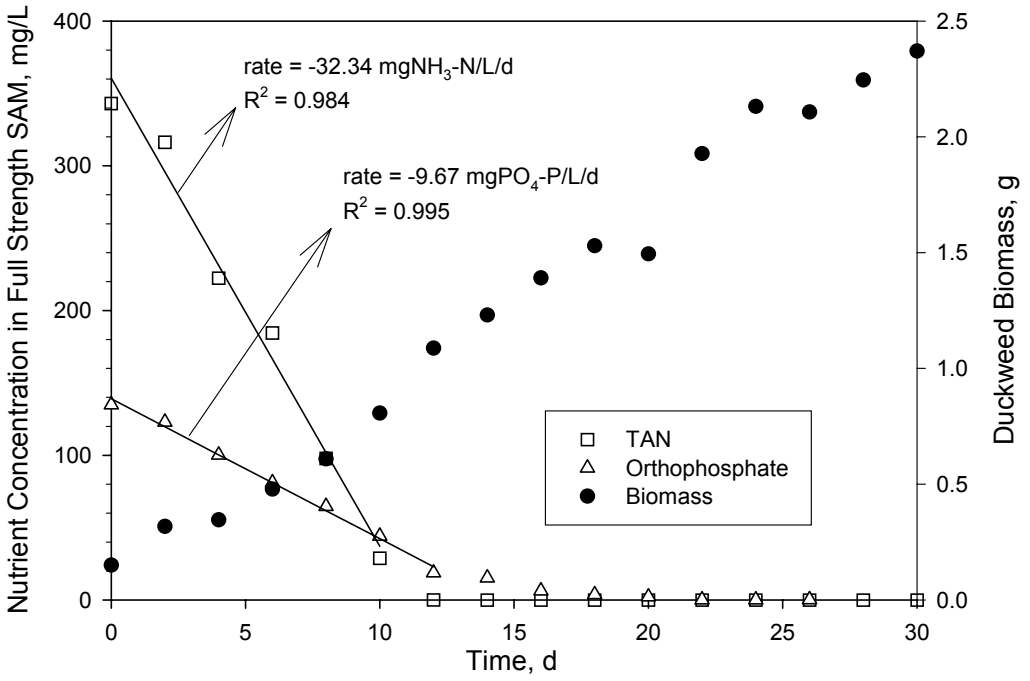
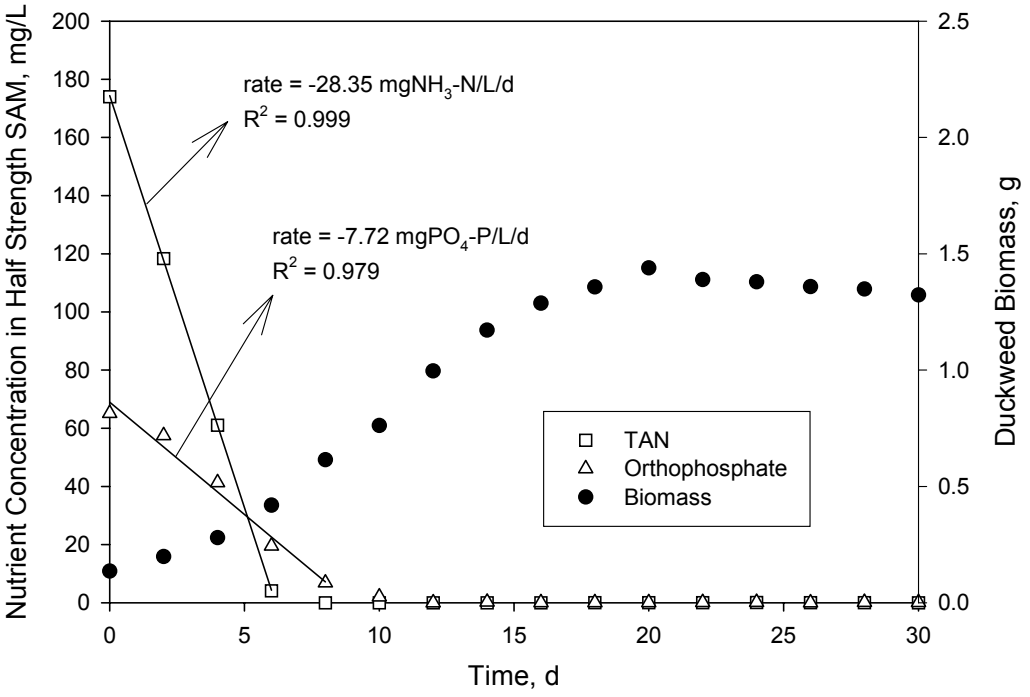


Figure 1. Average dry biomass of *Spirodela punctata* 7776 grown in batch test of full strength and half strength SAM with regular pH adjustment during experimental period of 30 days. Cultures were maintained in growth chamber under light intensity of 40 μmol/m^2/s 16-hr photoperiod at 24^{o}C.



(a)



(b)

Figure 2. Nutrient concentration and duckweed biomass over time in batch culture of *Spirodela punctata* 7776 in (a) full strength SAM, and (b) half strength SAM.

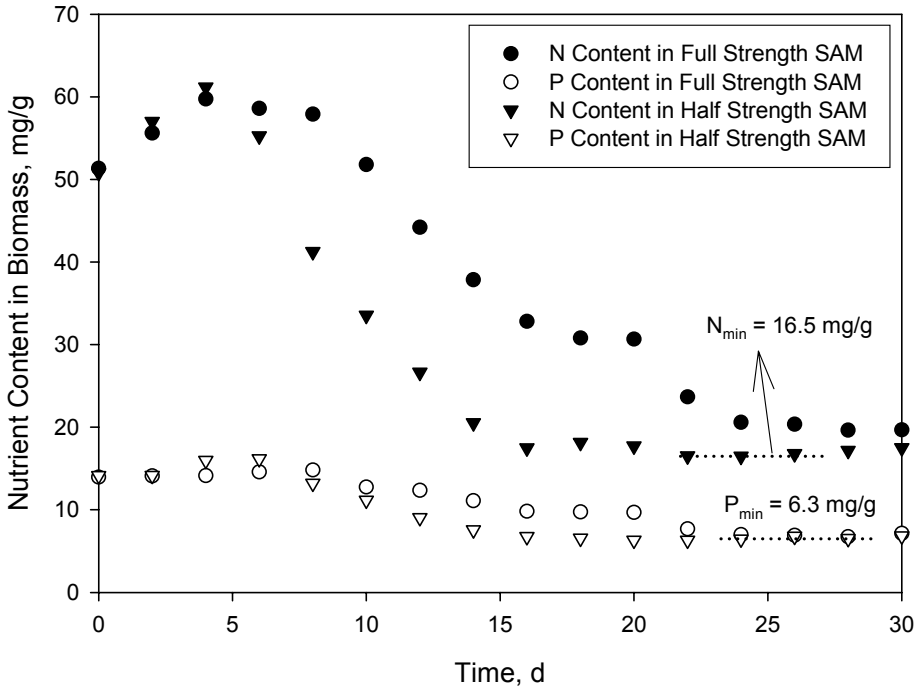
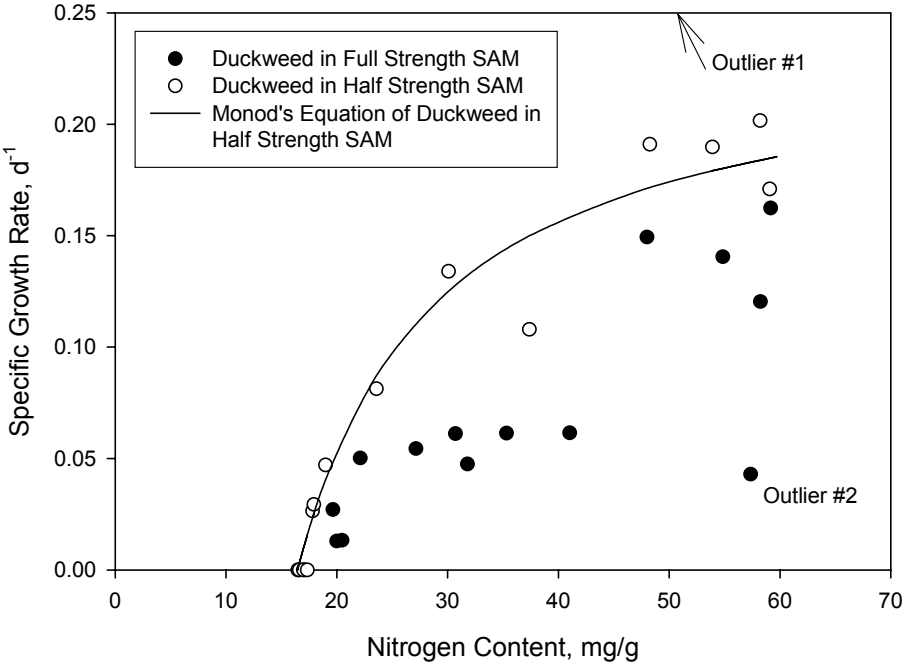
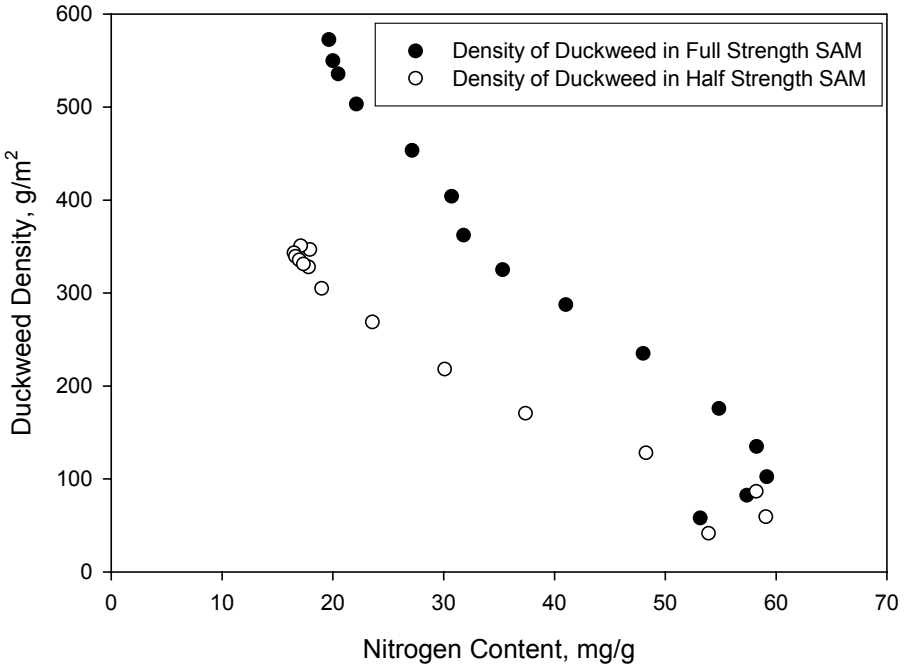


Figure 3. Nitrogen and phosphorus contents of duckweed *Spirodela punctata* grown for 30 days in batch culture of full strength and half strength SAM at light intensity of 40 μmol/m^2/s, 24°C.



(a)



(b)

Figure 4. (a) Specific growth rate (μ) of *Spirodela punctata* 7776 grown in half strength SAM, plotted with the fitted Monod's equation SAM, and in full strength SAM that was hypothetically reduced by the lumping parameter: surface density. There were 2 outliers in the data set of full strength SAM experiment. (b) Surface density of the cultures in both experiments in relation with nitrogen content of the biomass.

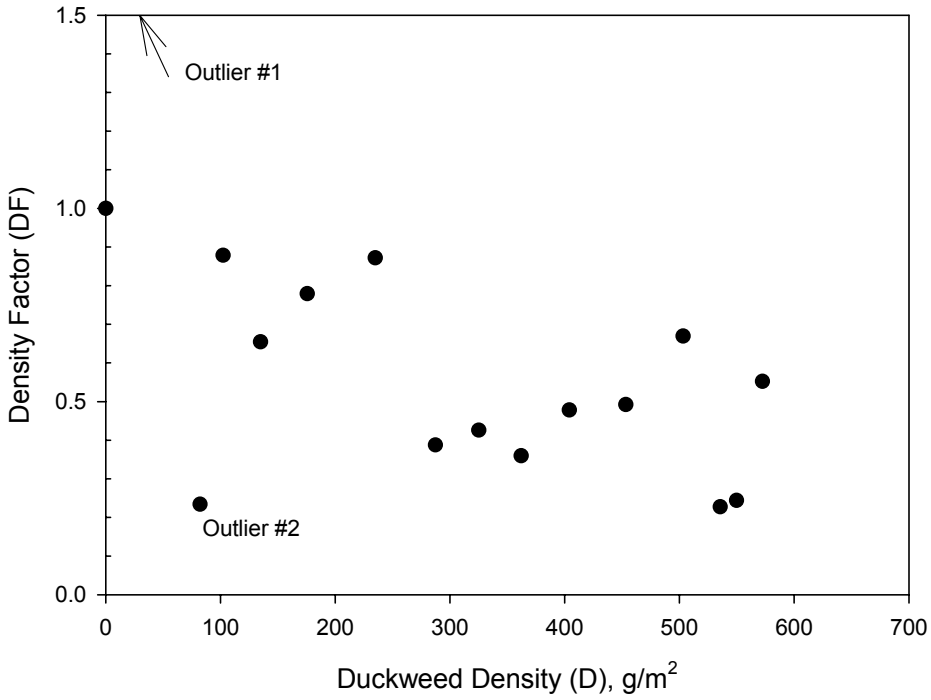


Figure 5. Plot of density factors that were derived from the ratios of specific growth rates of the full to half strength SAM data sets at different duckweed surface density.

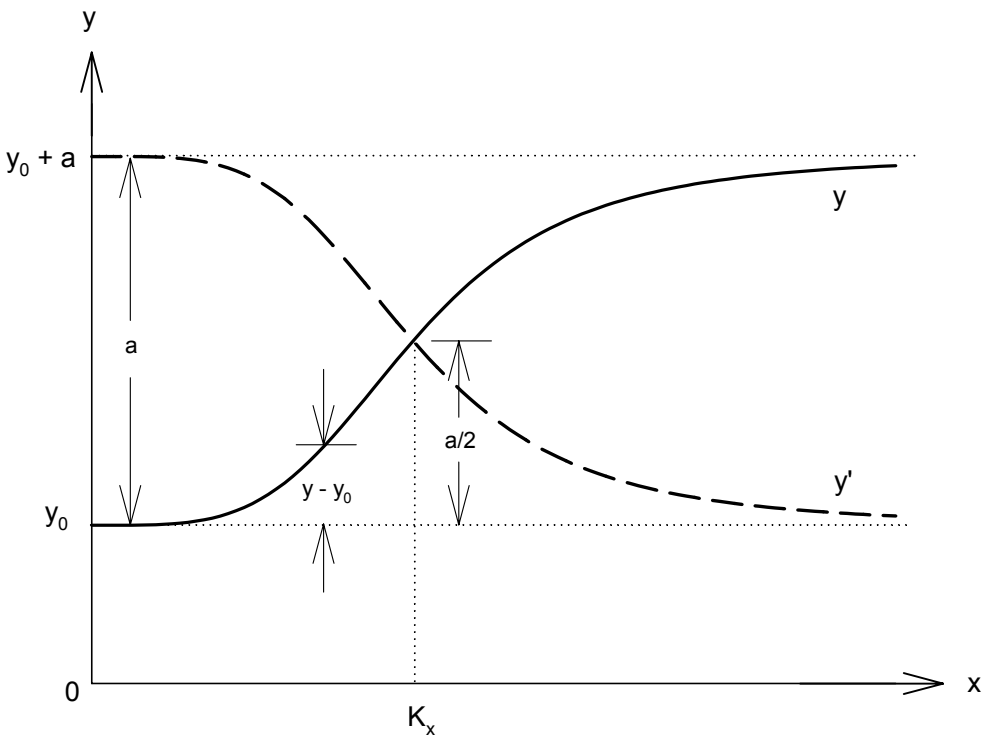
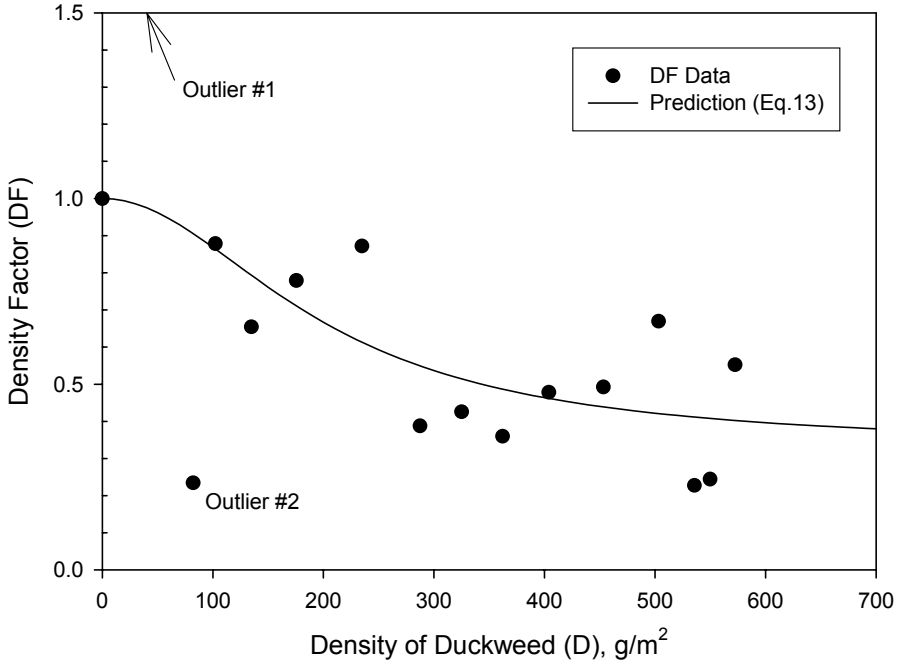
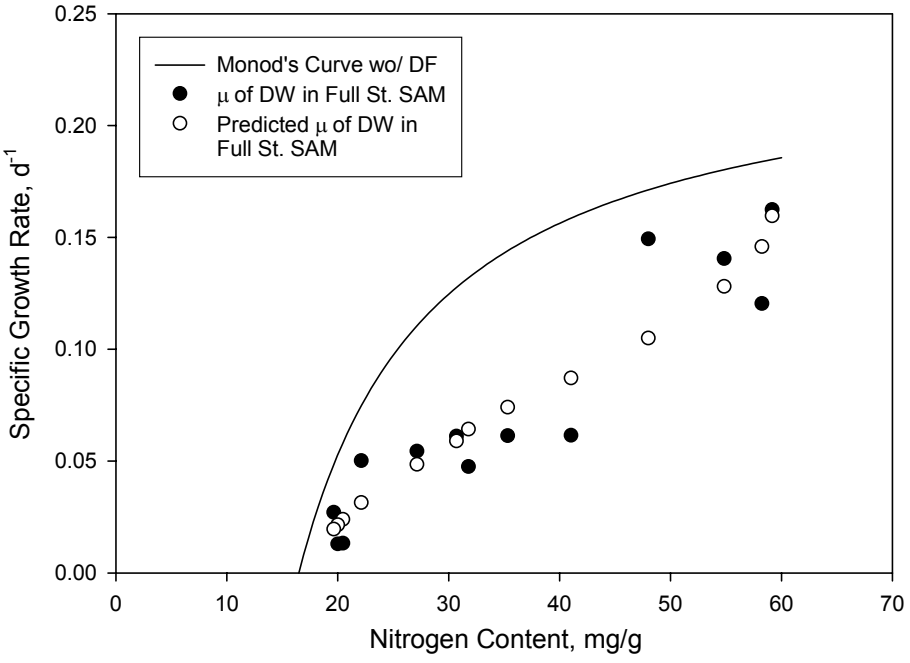


Figure 6. The Sigmoidal Hill's equation (solid line) and its inverse (dash line) that possesses the characteristics suitable to represent the density factors shown in Figure 5.

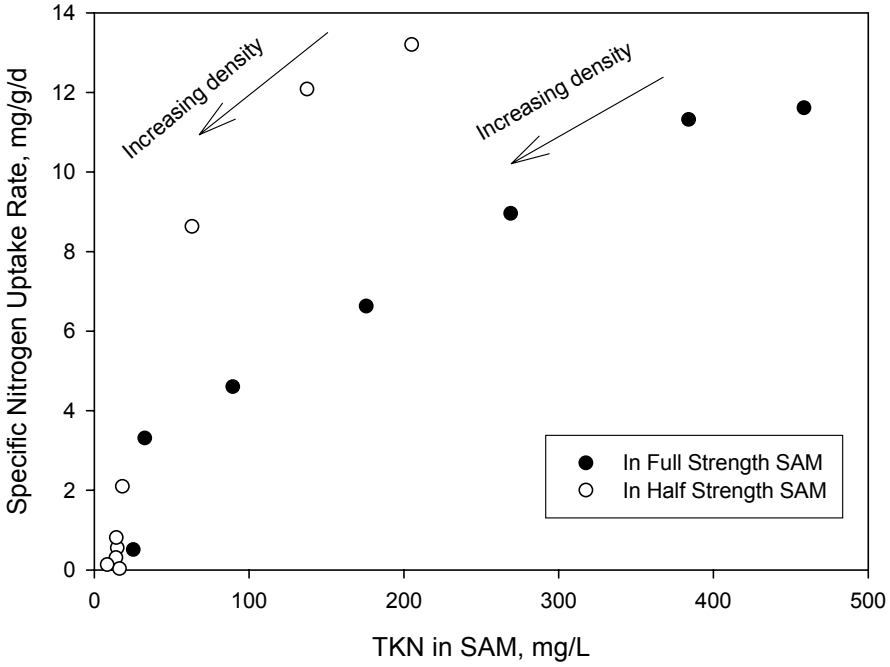


(a)

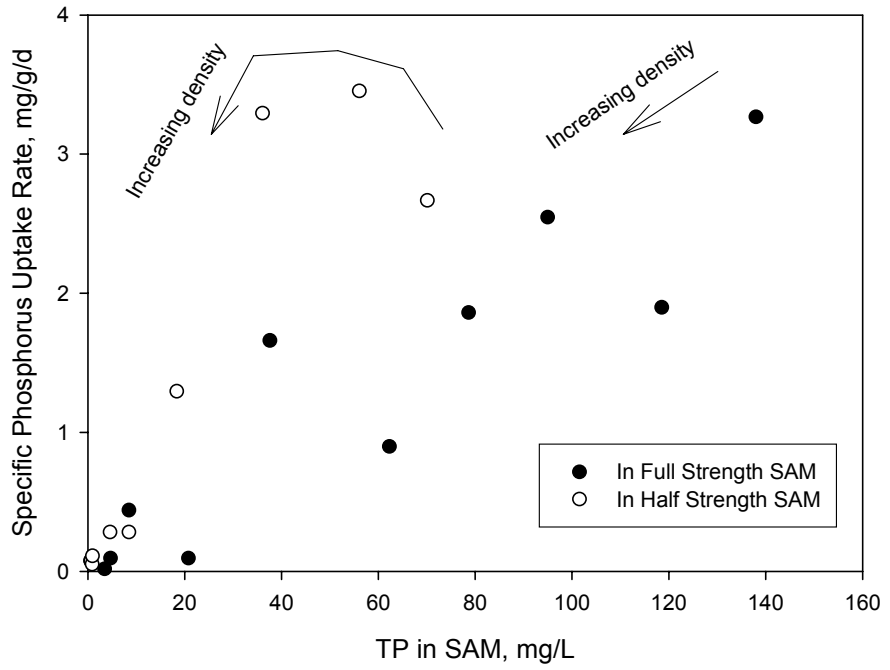


(b)

Figure 7. The model predictions of (a) DF by using the optimized parameters K_D = 201.25 g/m^2, DF_0 = 0.33, and h = 2.02, and (b) specific growth rate of the duckweed grown with density effects in full strength SAM obtained by multiplying the μ values without density effect (on Monod's curve) with the resulted DF function.



(a)



(b)

Figure 8. The specific uptake rate of (a) nitrogen and (b) phosphorus in batch tests of *Spirodela punctata* 7776 in full and half strength SAM.

RESEARCH SUMMARY

Nitrogen removal by duckweed in a static lagoon liquid is governed by, among other parameters, the transport of ammonium ions to the surface that is coupled with the ammonium uptake of the floating duckweed. Concentration gradients of ammonium ions were observed along the depth of the quiescent experimental lagoon water column indicating that the system was transport limited. In well-mixed systems where concentration is nearly homogenous over depth, nitrogen removal would be limited by the ammonium uptake and utilization capacity of the plant. Model calibration with the experimental data revealed an ammonium transport coefficient that was 85 times larger than the standard diffusion coefficient of ammonium. This transport coefficient included effects of other possible transport mechanisms besides diffusion at standard testing conditions. Therefore, mixing in the static duckweed pond could be applied at less frequency or totally omitted in the field where wind action and harvesting are also to take place at a regular basis. The mathematical model developed was also able to predict the concentration of ammonium over time in an intermittently mixed duckweed system with the exclusion of ammonia volatilization. Finally, local pH drop near the wastewater surface caused by ion exchange between duckweed and the lagoon liquid was observed. This lower pH layer could help minimize the ammonium volatilization in addition to the physical barrier that the duckweed mat already provides.

Although growth of duckweed is typically described as being related to the medium concentration, the examination of *Spirodela punctata* 7776 growth in batch cultures indicated that the rate of growth was more closely related to the internal nutrient storage in its biomass.

In both half and full strength North Carolina swine artificial mediums, *Spirodela punctata* 7776 grew well. Accumulation of nitrogen and phosphorus in duckweed tissues was observed at the beginning of the experiments when nutrient concentrations were high. This accumulation suggested the possibility of operating a *Spirodela punctata* 7776 system on full strength North Carolina lagoon liquid and still being able to produce a high nutritive biomass. However, implementation of the system will require additional testing especially for the interactions with microbial activities that were not present in our imitated medium. Growth of biomass in both half and full strength artificial swine medium experiments continued after all nutrients in the medium were exhausted. The pattern of growth in relation with internal nitrogen content in the half strength experiment could be expressed as Monod kinetics, which showed a specific growth rate of 0.2381 g/g/d. Meanwhile, high crop density in the full strength medium experiment reduced specific growth rate of the culture. The effects of crop density were lumped into the parameter Density Factor (DF) that was then defined mathematically. With the application of DF, the modified model was able to describe the growth of *Spirodela punctata* 7776 with density effects reasonably well. The analytical method presented could lead to the optimization of duckweed density that determines the duckweed-harvesting scheme.

Apparently, operations of duckweed systems in the field involve many environmental conditions that differ from our laboratory settings. The major differences that could significantly affect the performance of the system and model predictability include light intensity, photoperiod, temperature, biological agents in the wastewater, and chemical characteristics of the wastewater. These parameters can vary with geographical locations of the site, seasonal climates of the year, and operational schemes of each farm. In addition,

species of duckweed used will directly influence the overall growth and nutrient removal characteristics of the system. While this research has provided guidelines for analytical and mathematical assessments of the duckweed system, calibrations with field data, that could entail model modifications, are needed for practical use of the models. Future work should, therefore, emphasize year-round pilot testing of duckweed systems in the field to collect data for calibrating and/or modifying the models. This process can lead to optimization of the system in terms of nutrient removal and biomass production. Then, based on the subsequently adapted models, a following study could look at utilization of different species of duckweed in the system, one species at a time and mixed culture. This step followed by model recalibration and parameter adjustment would allow us to find the optimal operating regimes for systems with different types of duckweed, which are suitable or native to each site and geographical location of the application.

APPENDIX A

NUMERICAL METHOD FOR NITROGEN TRANSPORT MODELING

The numerical method in this section provides detailed mathematical concepts and derivations that were used but not presented in the analytical work of "MODELING NITROGEN TRANSPORT IN DUCKWEED POND FOR SECONDARY TREATMENT OF SWINE WASTEWATER." All parameters used in this appendix either were defined in the work earlier (Chapter 2) or are stated herein.

Derivation of the governing equation

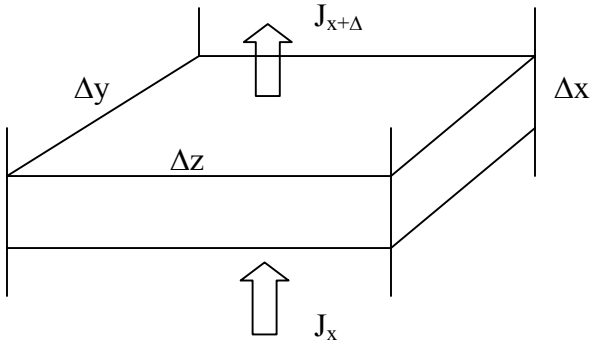


Figure 1 Differential volume of the mass diffusion

Mass flux equation for diffusion (Fick's First Law)

$$J = -D \cdot \frac{\partial c}{\partial x} \quad (1)$$

Mass balance of the differential volume

$$\begin{bmatrix} rate\ of\ accumulation \\ of\ nutrient\ per\ unit \\ volume \end{bmatrix} = \begin{bmatrix} rate\ of \\ nutrient \\ flow\ in \end{bmatrix} - \begin{bmatrix} rate\ of \\ nutrient \\ flow\ out \end{bmatrix} \pm \begin{bmatrix} nutrient \\ generation\ or \\ consumption \end{bmatrix} \quad (2)$$

For one dimensional transport

$$\frac{\partial c}{\partial t}\Delta x\Delta y\Delta z = \left[J_x - J_{x+\Delta x}\right]\Delta y\Delta z + \left[r\,x\,n\right]\Delta x\Delta y\Delta z \quad (3)$$

Divide through by Δx Δy Δz to yield

$$\frac{\partial c}{\partial t} = \left[\frac{J_x - J_{x+\Delta x}}{\Delta x}\right] + \left[r\,x\,n\right] \quad (4)$$

Set $\Delta x \rightarrow 0$ and it becomes

$$\frac{\partial c}{\partial t} = -\frac{\partial J}{\partial x} \pm \left[r\,x\,n\right] \quad (5)$$

Use the definition of the mass flux for diffusion in Eq. (1) and simplify the equation by assuming no reaction in the differential volume, the governing equation of the nutrient diffusion becomes

$$\frac{\partial c(x,t)}{\partial t} = D \cdot \frac{\partial^2 c(x,t)}{\partial x^2} \quad (6)$$

Implementation of the numerical calculations

After the parameters k_1 and D were estimated, the governing partial differential equation could be solved. First let's define all the components of the equation system in mathematical terms.

u = NH_4^+ concentration, i.e. C

U = numerical solution of u

u_t = first derivative of the NH_4^+ concentration with respect to time (t), i.e. $\frac{dC}{dt}$

u_{xx} = second derivative of the NH_4^+ concentration with respect to distance (x) from the bottom of the pond, i.e. $\frac{\partial^2 C}{\partial x^2}$

u_0 = initial NH_4^+ concentration at t = 0, i.e. C_0

i = distance index = 0 (bottom), 1, 2, … m (surface)

h = size of a grid in a space domain

n = time index

p = size of a time step

Finite difference approximation

$$u_t = \frac{u_x^{n+1} - u_x^n}{p} \tag{7}$$

$$u_{xx} = \frac{1}{h^2}\left[u_{x-h}^n - 2u_x^n + u_{x+h}^n\right] \tag{8}$$

Governing Equation

$$\frac{\partial C(x,t)}{\partial t} = D_A \cdot \frac{\partial^2 C(x,t)}{\partial x^2} \tag{9}$$

or

$$u_t = D\, u_{xx} \tag{10}$$

Boundary Condition #1 at x = L

$$-D \cdot \frac{\partial C(L,t)}{\partial x} = k_1 \cdot C(L,t) \tag{11}$$

or

$$u_x = \frac{D}{k_1} u \tag{12}$$

Boundary Condition #2 at x =0

$$\frac{\partial C(0,t)}{\partial x} = 0 \tag{13}$$

or $$u_x = 0 \tag{14}$$

Initial Condition at t = 0

$$C(x,0) = C_0 \tag{15}$$

or $$u = u_0 \tag{16}$$

Apply the finite difference approximation and using the Crank-Nicolson Scheme, the governing equation becomes

$$\frac{U_i^{n+1} - U_i^n}{k} = \frac{D}{2} \cdot \left[\frac{U_{i-1}^n - 2U_i^n + U_{i+1}^n}{h^2} + \frac{U_{i-1}^{n+1} - 2U_i^{n+1} + U_{i+1}^{n+1}}{h^2} \right] \tag{17}$$

Rearrange to yield

$$\left(-\frac{pD}{2h^2}\right)U_{i-1}^{n+1} + \left(1 + \frac{pD}{h^2}\right)U_i^{n+1} + \left(-\frac{pD}{2h^2}\right)U_{i+1}^{n+1} = \left(\frac{pD}{2h^2}\right)U_{i-1}^n + \left(1 - \frac{pD}{h^2}\right)U_i^n + \left(\frac{pD}{2h^2}\right)U_{i+1}^n \tag{18}$$

Apply the finite difference approximation to boundary condition #1

$$\frac{U_{m+1} - U_{m-1}}{2h} = -\frac{k_1}{D}U_m \tag{19}$$

$$U_{m+1} = -2h\frac{k_1}{D}U_m + U_{m-1} \tag{20}$$

Therefore, at i = m (at z = L), the numerical governing equation becomes

$$\left(-\frac{pD}{h^2}\right)U_{m-1}^{n+1} + \left(1 + \frac{pD}{h^2}\left(1 + \frac{k_1 h}{D}\right)\right)U_m^{n+1} = \left(\frac{pD}{h^2}\right)U_{m-1}^n + \left(1 - \frac{pD}{h^2}\left(1 + \frac{k_1 h}{D}\right)\right)U_m^n \tag{21}$$

Apply the finite difference approximation to boundary condition #2

$$\frac{U_1 - U_{-1}}{2h} = 0 \tag{22}$$

$$U_{-1} \quad = \quad U_1 \tag{23}$$

Therefore, at i = 0 (at z = 0), the numerical governing equation becomes

$$\left(1+\frac{pD}{h^2}\right)U_0^{n+1}+\left(-\frac{pD}{h^2}\right)U_1^{n+1} \quad = \quad \left(1-\frac{pD}{h^2}\right)U_0^{n}+\left(\frac{pD}{h^2}\right)U_1^{n} \tag{24}$$

Now we can arrange the matrix to calculate the concentration of the entire depth, i.e. at a time step t+p from the solutions at time step t. The matrix set-up is in the form of AU = b in which A is coefficient matrix, U is the matrix of solution at the next time step, and b is residual matrix of the previous time step. We, thus, can calculate U.

$$\begin{bmatrix} 1+\frac{pD}{h^2} & -\frac{pD}{h^2} & 0 & 0 & 0 & 0 \\ -\frac{pD}{2h^2} & 1+\frac{pD}{h^2} & -\frac{pD}{2h^2} & 0 & 0 & 0 \\ 0 & \ddots & \ddots & \ddots & \ddots & 0 \\ 0 & 0 & \ddots & & \ddots & -\frac{kD}{2h^2} \\ 0 & 0 & 0 & & -\frac{pD}{h^2} & 1+\frac{pD}{h^2}\left(1+\frac{k_1 h}{D}\right) \end{bmatrix} \begin{bmatrix} U_0^{n+1} \\ U_1^{n+1} \\ \vdots \\ U_{m-1}^{n+1} \\ U_m^{n+1} \end{bmatrix} = \begin{bmatrix} \left(1-\frac{pD}{h^2}\right)U_0^n+\left(\frac{pD}{h^2}\right)U_1^n \\ \left(\frac{pD}{2h^2}\right)U_0^n+\left(1-\frac{pD}{h^2}\right)U_1^n+\left(\frac{pD}{2h^2}\right)U_2^n \\ \left(\frac{pD}{2h^2}\right)U_1^n+\left(1-\frac{pD}{h^2}\right)U_2^n+\left(\frac{pD}{2h^2}\right)U_3^n \\ \vdots \quad \vdots \quad \vdots \\ \left(\frac{pD}{2h^2}\right)U_{m-2}^n+\left(1-\frac{pD}{h^2}\right)U_{m-1}^n+\left(\frac{pD}{2h^2}\right)U_m^n \\ \left(\frac{pD}{h^2}\right)U_{m-1}^n+\left(1-\frac{pD}{h^2}\left(1+\frac{k_1 h}{D}\right)\right)U_m^n \end{bmatrix} \tag{25}$$

Iteration was then performed time-wise (time step = p) until reaching the final time. The solutions at specific time steps, i.e. every 24 hours, are recorded in a separate file for further analytical work. Note that Matlab does not allow index 0, so the numeric index used in Matlab code is adjusted accordingly. Also, some parameters used in the code may not conform the nomenclature defined in the above numerical derivation but it is stated clearly in the code. The Matlab code is shown below.

```
%%%%%%%%%%%%  DUCKWEED MODELING  %%%%%%%%%%%%%%%%
%%%%%%%%%%%%  CRANK-NICOLSON METHOD  %%%%%%%%%%%%%

clear; close all

a = 0;
depth = input('Enter the water depth (m) :                ');
D = input('Enter diffusion coefficient D (m^2/h) :        ');
k = input('Enter uptake coefficient k1 (m/h) :            ');
W = input('Enter initial concentration C0 (mg/L) :        ');
tfinal = input('Enter final time (hours) :                ');
m = input('Enter grid points :                            ');
%suggested m = 80
pint = input('Enter data recording interval (hours) :             ');

h = (depth-a)/m;
p = h;                                                    %set time step equal grid size
h1=h*h;
n=round(tfinal/p);
t = 0;

%-------- Define the grid domain ---------
 for i=1:m+1,
   x(i) = a + (i-1)*h;
   u0(i) = W;
 end

%-------- Set-up the coefficient matrix  -----------

A = sparse(m+1,m+1);
for i=2:m,
  A(i,i) = 1+p*D/h1; A(i,i-1) = -0.5*p*D/h1; A(i,i+1)= -0.5*p*D/h1;
end
  A(1,1) = 1+p*D/h1; A(1,2) = -p*D/h1;
  A(m+1,m+1) = 1+p*D*(1+k*h/D)/h1; A(m+1,m) = -p*D/h1;

  b = zeros(m+1,1);

%--------------- Time Iteration --------------------------------
fid = fopen('output','w');                %write data in file named output, create if necessary

count=1;                    %set the index 'count' to calculate time step for extracting the data

 for j=1:n,

   for i=2:m
```

```
  b(i) = (.5*p*D/h1)*u0(i-1) + (1-p*D/h1)*u0(i) + (.5*p*D/h1)*u0(i+1);
end
  b(1) =  (1-p*D/h1)*u0(1) + (p*D/h1)*u0(2);                    % Neumann BC at x =a.
  b(m+1) = (p*D/h1)*u0(m) + (1-p*D*(1+k*h/D)/h1)*u0(m+1);   % Mixed BC at x =b.

u1 = A\b;

u0 = u1;

if j==round(count*pint/p);
        %Write the data when time equals count*pint/k. Note that data at time = 0 is not
        %recorded but it is known (C0)
  for ncount=1:m+1
        % set new index 'ncount' for position of data in solution vector
     fprintf(fid,'%1.10f ',u1(ncount));
       %write formatted data to file fid as 1 digit in front of decimal point and 10 after
       %at ncount
  end
  fprintf(fid,'\n');                          %write on the new line for each recording time
  count = count+1;                %get new j as an index where the data will be recorded
end

end

fclose(fid);                                                                %close file "output"

plot(x,u1); hold                                                    %plot/show final time profile

%%%%%%%%%%%%%%%%%%%%  END  %%%%%%%%%%%%%%%%%%%%
```

Once the ammonium profiles were measured from the reactors, they were compared to the profiles generated from the model. Parameter k_1 and D were varied by the Matlab built-in function (fminsearch) as to search for their optimized values that give the best fit to the data. The search procedure finds the values of k_1 and D which minimize the error sum of squares (ESS). The task was implemented with one main program (mainJ.m) and two subroutine programs (cp.m and kD.m). The codes with descriptions are listed below.

```
%%%%%%%%%%%%%%%%%%%% mainJ.m %%%%%%%%%%%%%%%%
clear all

global a W tfinal pint depth m h n N R p h1 s

a = 0;
W = input('Enter initial concentration C0 (mg/L) :          ');
tfinal = input('Enter final time (hours) :                  ');
depth = input('Enter the water depth (m) :                  ');
m = input('Enter grid points :                              ');
h = (depth-a)/m;  p = h; h1=h*h;
n=round(tfinal/p);
N=[4199 16798 25197];                %vector of time indexes for comparison to data points
R=[3 37 63 72 81];                   %vector of depth indexes for comparison to data points

x = fminsearch('cp',[0.0008861; 0.0005994])
%x is a vector composed of k1 and D that are being optimized. We supply the original
%value of k1 and D in the argument of function fminsearch to initiate the process. Vector
%x is then sent out to function cp.m. Function fminsearch will vary k1 and D, and
%eventually determine if ESS is minimum.

s                                    %show vector s which contains the optimal value of k1 and D

%%%%%%%%%%%%%%%%%%%%  END  %%%%%%%%%%%%%%%%%%%%
```

```
%%%%%%%%%%%%%%%%%%%  cp.m  %%%%%%%%%%%%%%%%%%%
function  s = cp(x)       %x = vector of k1 and D that the function cp receives from mainJ.m

global s

expt = [63.37          64.80          52.63;          %experimental data at 3 time steps
        61.73          58.43          50.03;          %(24h, 96h, and 144h) and 5
        61.13          53.53          45.43;          %depths (0", 2", 4", 10", and 17.5")
        61.3           53.133         43.633;
        59.8           47.73          28.20];

theo    = kD(x(1),x(2));        %Call function kD to run using k1 and D. Then receive the
                                %computed concentrations at specified times and depths
                                %from function kD thru parameter U.

s = 0;

for i = 1:size(expt,1)                              %calculate objective function or ESS
  for j = 1:size(expt,2)
    s    = s+(expt(i,j)-theo(i,j))^2;
  end
end

%%%%%%%%%%%%%%%%%%%  END  %%%%%%%%%%%%%%%%%%%
```

```
%%%%%%%%%%%%%%%%%%  kD.m  %%%%%%%%%%%%%%%%%%
function U = kD(k,D)

global a W tfinal pint depth m h n N R p h1

 t = 0;
 for i=1:m+1,
   x(i) = a + (i-1)*h;
   u0(i) = W;
 end

A = sparse(m+1,m+1);
for i=2:m,
  A(i,i) = 1+p*D/h1; A(i,i-1) = -0.5*p*D/h1; A(i,i+1)= -0.5*p*D/h1;
end
  A(1,1) = 1+p*D/h1; A(1,2) = -p*D/h1;
  A(m+1,m+1) = 1+p*D*(1+k*h/D)/h1; A(m+1,m) = -p*D/h1;

  b = zeros(m+1,1);

count=1;
kk = 0;
 for j=1:n,

   for i=2:m
     b(i) = (.5*p*D/h1)*u0(i-1) + (1-p*D/h1)*u0(i) + (.5*p*D/h1)*u0(i+1);
   end
     b(1) =  (1-p*D/h1)*u0(1) + (p*D/h1)*u0(2);
     b(m+1) = (p*D/h1)*u0(m) + (1-p*D*(1+k*h/D)/h1)*u0(m+1);

          u1 = A\b;

   if ismember(j,N)
      kk = kk+1;
      for ii=1:5
         U(ii,kk) = u1(R(ii));          %U is a matrix storing the computed concentrations at
                                        %specified depths and times which will be used to
                                        %calculate ESS in cp.m
      end
   end

   u0 = u1;
 end

%%%%%%%%%%%%%%%%%%  END  %%%%%%%%%%%%%%%%%%
```

In the mixing cycle simulation, new homogeneous concentration must be computed for use as an initial concentration of the subsequent cycle. Thus, Matlab code was modified to repeat the process of first computing the ammonium profile at the final time of each cycle and then from that information the initial homogeneous concentration for the next cycle. The modified code is shown below.

```
%%%%%%%%%%%%%%%%  MIXING CYCLE  %%%%%%%%%%%%%%%%%%

clear; close all

a = 0;
depth = input('Enter the water depth (m) :                  ');
D = input('Enter diffusion coefficient D (m^2/h) :          ');
k = input('Enter uptake coefficient k1 (m/h) :              ');
W = input('Enter initial concentration C0 (mg/L) :          ');
tfinal = input('Enter cycle duration (hours) :              ');
m = input('Enter grid points :                              ');
times = input('Enter number of cycles in the simulation :   ');

h = (depth-a)/m;
p = h;
h1=h*h;
n=round(tfinal/p);

for i=1:m+1,
   x(i) = a + (i-1)*h;
end

A = sparse(m+1,m+1);
for i=2:m,
  A(i,i) = 1+p*D/h1; A(i,i-1) = -0.5*p*D/h1; A(i,i+1)= -0.5*p*D/h1;
end
  A(1,1) = 1+p*D/h1; A(1,2) = -p*D/h1;
  A(m+1,m+1) = 1+p*D*(1+k*h/D)/h1; A(m+1,m) = -p*D/h1;

  b = zeros(m+1,1);

fid = fopen('output1','a');

for loop=1:times
u0(1:m+1) = W;
 for j=1:n,
   for i=2:m
     b(i) = (.5*p*D/h1)*u0(i-1) + (1-p*D/h1)*u0(i) + (.5*p*D/h1)*u0(i+1);
   end
     b(1) =  (1-p*D/h1)*u0(1) + (p*D/h1)*u0(2);
     b(m+1) = (p*D/h1)*u0(m) + (1-p*D*(1+k*h/D)/h1)*u0(m+1);
   u1 = A\b;
   u0 = u1;
 end

fprintf(fid,'%1.10f ',u1);
```

```
fprintf(fid,'\n');

area = 0;
for i=1:m
   area = area + (1/2*h*(u1(i)+u1(i+1)));
end
W = area/depth;
loop = loop+1;
end
fclose(fid);

 plot(x,u1); hold

 %%%%%%%%%%%%%%%%%%%  END  %%%%%%%%%%%%%%%%%%%
```

APPENDIX B

RECIPE FOR SWINE ARTIFICAIL MEDIUM

Table B1. The chemical compositions of stock solutions for making swine artificial medium. (Courtesy of the Forestry Department, North Carolina State University)

Stock Solution	Chemical composition	Amount required to make 1 L
No. 1	K_2SO_4	52.9
	$MgSO_4 \cdot 7H_2O$	40.7
	$ZnSO_4 \cdot 7H_2O$	1.349
	$MnSO_4 \cdot H_2O$	0.27
	$CuSO_4 \cdot 5H_2O$	0.47
	Na_2SO_4	52.19
No. 2	NH_4Cl	45.47
	NH_4NO_3	0.12
No. 3	K_2HPO_4	72.1
	H_3BO_3	0.3895
	$CoCl_2 \cdot 6H_2O$	0.01
	Na_2MoO_4	0.00432
No. 4*	(A) $FeSO_4 \cdot 7H_2O$	3.92
	(B) $Na_2EDTA \cdot 2H_2O$	5.25

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

To make 1 liter of sterile swine artificial medium (full strength), follow the procedure below;

1) Prepare 900 ml of DI water
2) Add 10 ml of each stock solution (1, 2, 3, and 4)
3) Mixing with magnetic stir bar should be provided throughout the procedure
4) Add 1.15 g of citric acid for buffer
5) Add a desired amount of sucrose (as a carbon source): 10 g for 1% or 30 g for 3%
6) Add 1.39 ml of NH_4OH
7) Add 0.226 g of $Ca(OH)_2$

8) Add DI water to make the final volume of 1 liter

9) Add enough citric acid to yield pH 7.1

10) Autoclave with liquid cycle for a proper duration to ensure sterilization

Table B2. Typical ionic concentration of swine lagoon liquid in North Carolina and the buffered swine artificial medium (SAM) formulated for use in the experiment (Courtesy of Bergmann et al. 2000).

Ion (mM)	Lagoon Liquid Ionic Concentration (mM)	Buffered SAM Ionic Concentration (mM)
NH_4	26.82	26.82
NO_3	0.015	0.015
P	3.16	3.16
K	12.40	12.40
Ca	3.05	12.40
Mg	1.65	1.65
Cl	8.50	8.50
Fe	0.141	0.141
S	1.06	8.59
Na	7.35	7.63
B	0.063	0.063
Mn	0.016	0.016
Zn	0.047	0.047
Cu	0.019	0.019
Mo	0.00021	0.00021
Co	0.00042	0.00042
Total	64.292	81.45
pH	7.0	7.0

APPENDIX C

SIMULATION RESULTS AND EXPERIMENTAL DATA

This section contains all the simulation results and experimental data that were used to construct all the figures in this dissertation. To view it, please click through this link.