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Review

Growing Duckweed to Recover Nutrients from Wastewaters and for Production of Fuel Ethanol and Animal Feed

Lemnaceae or duckweed is an aquatic plant that can be used to recover nutrients from wastewaters. The grown duckweed can be a good resource of proteins and starch, and utilized for the production of value-added products such as animal feed and fuel ethanol. In the last eleven years we have been working on growing duckweed on anaerobically treated swine wastewater and utilizing the duckweed for fuel ethanol production. Duckweed strains that grew well on the swine wastewater were screened in laboratory and greenhouse experiments. The selected duckweed strains were then tested for nutrient recovery under laboratory and field conditions. The rates of nitrogen and phosphorus uptake by the duckweed growing in the laboratory and field systems were determined in the study. The mechanisms of nutrient uptake by the duckweed and the growth of duckweed in a nutrient-limited environment have been studied. When there are nutrients (N and P) available in the wastewater, duckweed takes the nutrients from the wastewater to support its growth and to store the nutrients in its tissue. When the N and P are completely removed from the wastewater, duckweed can use its internally stored nutrients to keep its growth for a significant period of time. A modified Monod model has been developed to describe nitrogen transport in a duckweed-covered pond for nutrient recovery from anaerobically treated swine wastewater. Nutrient reserve in the duckweed biomass has been found the key to the kinetics of duckweed growth. Utilization of duckweed for value-added products has a good potential. Using duckweed to feed animals, poultry, and fish has been extensively studied with promising results. Duckweed is also an alternative starch source for fuel ethanol production. *Spirodela polyrrhiza* grown on anaerobically treated swine wastewater was found to have a starch content of 45.8% (dry weight). Enzymatic hydrolysis of the duckweed biomass with amylases yielded a hydrolysate with a reducing sugar content corresponding to 50.9% of the original dry duckweed biomass. Fermentation of the hydrolysate using yeast gave an ethanol yield of 25.8% of the original dry duckweed biomass. These results indicate that the duckweed biomass can produce significant quantities of starch that can be readily converted into ethanol.

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1 Introduction

The wastewater generated by livestock and municipalities, as well as some industries, e.g., food processing, fermentation and pharmaceutical, contains organic and inorganic nutrients that can be reused to support plant growth. It has been used to a limited extent as a source of nutrients for crops. However, its use is limited by safety concerns if the plant is directly destined for human consumption.

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For several decades researchers have been intrigued by the idea that plants of the *Lemnaceae*, commonly called duckweeds and water meals, could be developed as a major crop. Utilization of duckweed for municipal wastewater treatment through recovery of polluting nutrients by duckweed growth is not new [1]. These plants have been used for tertiary treatment of municipal wastewater for about two decades and limited commercialization in the US has occurred [2]. The impetus for the idea is based on the morphological and growth characteristics of the plants. All 37 *Lemnaceae* species are aquatic plants and therefore would require development of an aquatic agronomic production system. Wastewater from livestock production and municipalities would provide an excellent supply of the nutrients needed to produce massive quantities of duckweed biomass. Developing a duckweed cropping system based on wastewater could transform a ubiquitous and large source of pollution into a biomass production system of industrial scale.

In 1978, William Hillman and David Culley made a compelling case for the development of wild-type duckweed as an aquatic crop for protein production [3]. However, broad use of large-scale systems has not occurred because of a chicken-and-egg problem. Uses for the massive amounts of duckweed biomass that would be produced by large-scale wastewater systems do not exist. Therefore, the emphasis of engineering work focused on running these systems solely as wastewater treatment systems and not on developing them as duckweed cropping systems. As wastewater treatment systems, the problem of duckweed disposal is a disincentive for system use. Without a readily available supply of massive amounts of duckweed via cropping, there has not been an impetus to find products that could be made from duckweed.

However, the energy/climate change challenge and the role of plant biomass as a source of carbon compounds to supplant petroleum as an energy and chemical feedstock has altered the debate surrounding the idea of developing duckweed as a crop. For plant biomass to play a significant role as an energy and chemical feedstock, it would require massive increases in plant production. To avoid the potential collision between demands for energy with increasing demands for crop commodities such as grains and legumes, new crops and agricultural systems may be needed. In this article research focused on developing a wastewater treatment system that captures nutrients and produces large amounts of duckweed biomass will be discussed as well as the potential uses of duckweed biomass for two different products: ethanol as a sustainable biofuel and protein-rich animal feed.

For the purpose of this article, duckweed will be used to refer to the *Lemnaceae* plants, regardless of species. A substantial literature exists describing the ecology, systematics, life-cycle, metabolism, growth habit, reproductive biology, and development and anatomy of *Lemnaceae* species [4–6]. This article will focus on information that has relevance to the use of duckweed for wastewater treatment and the applications of duckweed biomass.

1.1 Why Duckweed?

Before describing the attributes of duckweed species, we consider the attributes for a hypothetical ideal plant and its cropping system that could utilize the nutrients in wastewater and that would provide the massive amounts of biomass needed for commercialization of biomass products. The ideal plant for capture of wastewater nutrients would be an aquatic plant because the aquatic growth habit provides several advantages. Aquatic plants, in contrast with soil-based crops, can directly absorb nutrients from wastewater. This obviates the need for land application with its potential for nutrient loss due to run-off and subsequent contamination of ground and surface waters. The cropping system would utilize ponds that could be built on land marginal or totally unsuitable for soil-grown crops. In that way an aquatic crop could add to the overall plant biomass global supply while not competing for the finite supply of arable crop land. Utilization of wastewater for the cropping system would provide the plant's water and nutrient needs, again preventing competition for irrigation water and fertilizers needed for soil-based crops. The ideal plant would be able to tolerate the variation of nutrient levels in wastewater and the various forms of nitrogen that can be present. Most notably, the plant should be able to tolerate and utilize relatively high concentrations of ammonia found in some wastewaters. Algal growth with its associated problems is a constant concern with wastewater. Therefore, the cropping system of the ideal plant should provide a strategy to minimize algal growth. The ideal aquatic plant would not be an invasive exotic species. By careful species selection the problems associated with accidental release into the environment can be minimized.

Maximizing biomass yield and ease of handling, while minimizing production costs highlights other characteristics the ideal plant and its cropping system should possess. Production costs are associated with the amount of resources including area that are needed to produce a unit of plant biomass. The ideal plant should have a fast growth rate and the plant should be able to achieve this growth rate with minimal nutrient inputs. The plant would also provide for multiple crops per year. To maximize yield, the carbon component that is the product, e.g., protein, carbohydrate, lipids, specific chemicals, should be present in all the biomass, not just in a specific plant organ, such as a seed, root or stem. The cropping system would also maximize yield and minimize costs by simple harvesting methods of total plant biomass. In the traditional soil-based crops considerable biomass is lost through crop residues that are left in the field, e.g., roots, stems. The plant's characteristics should provide advantages for simple mechanization of harvesting and post-harvest handling. The target product component would need to be consistent in quality and quantity and be stable under biomass storage conditions. To minimize biomass post-harvest handling and transportation costs, the biomass should be readily amenable to fast drying, with minimal energy inputs. This attribute would be achieved with plant biomass having a high surface area to volume ratio, obviating the need for biomass milling or grinding, and one that is easily dried.

1.2 How does duckweed compare to the ideal plant?

Some of the duckweed species have morphological and growth characteristics that translate into the attributes of the ideal plant which makes them exceptional candidates for wastewater-based agriculture. The *Lemnaceae* is a monocotyledonous family of aquatic plants with four genera and a total of 37 species: *Spirodela*, *Lemna*, *Wolffia*, and *Wolfiella.* All members of the *Lemnaceae* are small, free-floating, fresh-water plants whose geographical ranges span the entire globe [5]. An example from each genus is shown in Fig. 1. The duckweeds inhabit freshwater ponds and pools, preferring those that are shallow and without flowing water. One species, *Spirodela polyrrhiza*, is the most widely distributed, and has been studied for its use for nutrient capture from wastewater [7]. Its wide geographical range means that a native strain of this species can be used to establish a wastewater treatment system at almost any global location thus avoiding the use of an invasive exotic species.

Members of the *Lemnaceae* are the most morphologically reduced species of higher plants (see Fig. 1). *Spirodela* and *Lemna* plants consist of disc-shaped fronds of varying sizes, shapes and thickness depending on species, a hair-like root or roots (the number is species specific) and, when present, one or two flowers. The morphologies of *Wolffia* species are further reduced, with plants consisting of tiny fronds resembling geometric solids (shape is species specific), no roots and rarely single flowers (see Fig. 1). *Wolfiella* species are the most varied in morphology. Regardless of species, the size of fronds of *Lemna* and *Spirodela* species vary from less than one to several millimeters in diameter, with roots elongating to no more than sev-

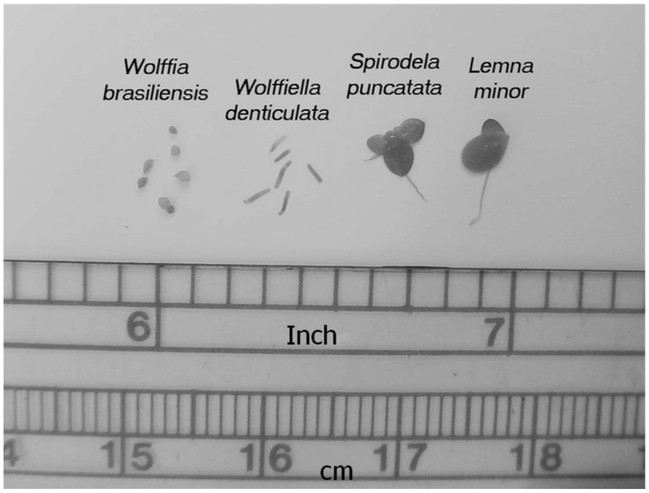

Figure 1. The size of duckweeds from four different genera.

eral centimeters in length; the fronds of *Wolffia* species are less than 2 mm in diameter. All *Lemnaceae* species proliferate primarily through vegetative budding of new fronds from parent fronds. Newly budded fronds remain attached to the parent frond to varying degrees. *Lemna* and *Spirodela* species are forming frond clusters of varying number and *Wolffia* species remaining solitary. Although parent fronds are limited in the number of progeny fronds that are produced before the parent frond dies, duckweed cultures achieve near exponential growth rates. Doubling times vary by species and environmental conditions and are as short as 20 to 24 hours and many species have doubling times of 2 to 3 days [8 – 10]. Intensive laboratory culture of duckweed has achieved high rates of biomass accumulation per unit time at culture densities of 1 – 2 kg/m^2 [6]. Greenhouse production levels of 1 kg fresh weight/m^{2}wk have been achieved (M. Edelman, personal communication). In our wastewater treatment research we have achieved a growth rate of 0.2 kg dry weight/m^{2}wk [11]. To achieve these growth rates, only low concentrations of nutrients are required. Oron and co-workers [1] achieved optimal growth rates at 20 ppm nitrogen utilizing municipal wastewater. Our research with wastewater indicates that high growth rates can be achieved at nitrogen levels less than 10 ppm [11 – 13].

When duckweed grows the individual fronds and frond clusters repel each other pushing the entire mat across the open water. Thus duckweed proliferation creates a floating photosynthetic surface that both maximizes capture of sunlight per unit area and shades out competing algal growth. Without continual harvesting proliferation creates dense mats of multiple frond layers that float at, or are slightly submerged relative to, the surface of the supporting fluid. To optimize production continuous harvesting of duckweed biomass is necessary. The floating particulate growth habit of duckweed facilitates harvesting and a variety of methods have been devised to corral and harvest duckweed biomass [14, 15]. Duckweed's small size gives the plant a large surface area to volume ratio. Duckweed also lacks a waxy cuticle, present on land plants to prevent water loss. Both of these characteristics mean that duckweed can be dried quickly with low energy inputs.

Duckweed growth can be optimized to produce high levels of protein or high levels of starch. The plant's dry weight accumulation varies by species and growth conditions and ranges from 6 to 20% of fresh weight [6, 16]. Protein content of a number of duckweed species grown under varying conditions has been reported to range from 15 to 45% dry weight [8, 17, 18]. These values place the protein content of dry duckweed biomass between alfalfa meal (20%) and soybean meal (41.7%) [4]. We routinely grow duckweed on dilute swine wastewater and get 30 to 35% protein of dry duckweed. Duckweed starch content is dependent on growth conditions, e. g., pH, phosphate concentration [19, 20] and developmental states controlled by the plant hormones, cytokinin [19, 51] and abscissic acid [6, 20]. Starch contents ranging from 3 to 75% have been reported [6, 21]. A duckweed starch content of 75% is comparable to corn, whose starch content ranges from 65 to 75% [22].

The possibility to manipulate growth to produce either high-protein or high-starch duckweed provides two opportunities to use duckweed biomass. The high-starch content suggests that duckweed could be used as an industrial feedstock for ethanol production for fuel. The high-protein content suggests that duckweed could be used as the protein component for animal feed. However, to commercialize either of these potential products requires a cropping system that can consistently produce stable duckweed biomass in massive quantities. For the past 10 years, we have been investigating the development of a coupled anaerobic digestion/duckweed system to recover energy and nutrients from swine wastewater and produce large quantities of either high-protein or high-starch duckweed. These studies have led to development of a duckweed cropping system and the applications of duckweed biomass.

2 Growing Duckweed for Nutrient Removal from Swine Wastewater

Swine wastewater treatment has been a major environmental concern in North Carolina in the last fifteen years because of a tremendous growth of swine production in the state in 1990s. A common swine wastewater treatment system widely used in the state is an anaerobic lagoon for organics destruction followed by a spray-field crop land irrigation for nutrient management. The system, especially the spray-field irrigation, has caused a serious environmental concern in potential nutrient contamination to surface and ground waters, and odor and greenhouse gas emissions. Growing duckweed on anaerobically pretreated swine wastewater to remove nutrients from the wastewater has been studied as an alternative to the spray-field irrigation.

2.1 Duckweed Selection

Duckweed geographic isolates that were promising in nutrient removal from swine wastewater were selected in two steps. In the first step, an in vitro protocol was used for the selection of promising duckweed strains with artificial swine wastewater that approximated anaerobically treated swine wastewater in terms of nutrient profile, total ionic strength, pH, and buffering capacity in a growing chamber [23]. As described in [23], the artificial swine wastewater had ammonium of 26.82 mM or 456 mg/L and phosphorus of 3.16 mM or 101 mg/L as well as minerals. According to the maintenance record of nearly a 1000 duckweed geographic isolates from the worldwide germplasm collection in Stomp's lab, forty-one strains were noted to be fast-growing duckweeds during routine collection maintenance (see Tab. 1). The 41 geographic isolates represented 12 species, at least one species from each of the four genera within *Lemnaceae*. Significant difference was observed on different duckweed

Table 1. Forty-one initial duckweed strains that were used for screening tests with artificial swine wastewater (Modified from [23]).

Genus	Species	Geographic isolates and origins
Lemna	*aequinoctialis*	7255 (Ghana), 7558 (USA), 8230 (Malaysia), 8654 (China), 8715 (Malaysia), 9049 (Zimbabwe), 9076 (Zimbabwe)
	gibba	G3 (unknown), 6861 (Italy), 7741 (Italy), 7784 (Ethiopia), 8405 (France), 8678 (India)
	minor	7501 (USA), 7968 (USA), 8626 (Denmark), 8627 (Denmark), 8731 (New Zealand), 8744 (Albania), 8745 (Canada)
	minuta	6600 (USA), 6747 (USA), 9008 (Columbia)
	obscura	7720 (USA)
	valdiviana	8821 (Argentina), 8829 (Argentina)
Spirodela	*intermedia*	7178 (Argentina)
	polyrrhiza	7401 (Poland), 7441 (Norway), 8197 (China), (USA), 8229 (Malaysia), 8240 (China), 8652 (China), 8683 (Kenya)
	punctata	7488 (USA), 7776 (Australia)
Wolffia	*australiana*	7267 (Australia)
Wolffiella	*lingulata*	8776 (USA)
	oblonga	7201 (Argentina), 8816 (Argentina), 9176 (Bolivia)

strains in terms of duckweed growth rate, nutrient removal from the wastewater, and protein or nitrogen accumulation in duckweed. The top eight duckweed candidates in terms of nitrogen removal from the wastewater and protein accumulation in duckweed were: *Spirodela punctata* 7776, *Lemna gibba* 8678, *Lemna minor* 7501, *S. punctata* 7488, *Lemna obscura* 7720, *Lemna aequinoctialis* 8715, *S. polyrrhiza* 8240, *L. minor* 8627. All the top eight duckweed geographic isolates with outstanding performance were from either *Spirodela* or *Lemna* species, the relatively big-size duckweeds. In the second step, the top eight duckweed strains with outstanding performance in nutrient removal from the artificial swine wastewater in in vitro tests were selected for a further screening test with real anaerobically treated swine wastewater in a greenhouse. The main characteristics of the wastewater include: chemical oxygen demand (COD) – 1287 mg/L, total Kjeldahl nitrogen (TKN) – 262 mg/L, and total phosphorus (TP) – 88 mg/L. The top three candidates that showed good tolerance to the swine wastewater and performed well in growth on the real wastewater in greenhouse were: *S. punctata* 7776, *L. gibba* 8678, and *L. minor* 8627. These top three candidates were selected for further testing on nutrient removal from and their growth on the anaerobically treated swine wastewater.

2.2 Nutrient Removal From Swine Wastewater by Growing Duckweed

Laboratory Testing

Nutrient removal from anaerobically treated swine wastewater by growing the duckweeds selected from the screening tests were performed under both laboratory and field conditions. To investigate the real nitrogen and phosphorus removal from the wastewater by the duckweed, batch laboratory tests were conducted with the same artificial swine wastewater as used in the in vitro screening tests. The artificial swine wastewater was sterilized to avoid any microbial activities during the tests. The batch tests were performed in a laboratory growth chamber with constant temperature (23°C), photoperiod (16 hours/day), and light intensity during the photoperiod (40 μmol/m^{2}s). Seed duckweed, that had been pre-cultured for two weeks on Schenk and Hildebrandt medium [24] supplemented with 3.0% sucrose, were used to initiate the tests. The performance of the three duckweed strains was very similar in terms of N and P removal and the duckweed growth rates. It took approximately four days for the duckweed to acclimate from the pre-culture to the artificial swine wastewater. After the acclimation period or lag phase, the duckweed grew at a linear rate with time with a growth rate of approximately 30 g/m^2/d (dry weight) [11, 13, 25]. During the linear growth period, the duckweed removed N and P from the wastewater in a rate of approximately 1.3 g/m^2/d and 0.18 g/m^2/d, respectively. An example of nutrient removal from the wastewater by growing *S. punctata* 7776 and the duckweed growth is shown in Fig. 2.

Field Testing

To investigate nutrient removal from anaerobically treated swine wastewater by growing the duckweeds selected from the previous screening tests under natural climate conditions, field testing was conducted in concrete outdoor tanks at the Lake Wheeler Road Field Laboratory of North Carolina State University in Raleigh, North Carolina, USA. Water temperature, light intensity, N and P removal from the wastewater, and duckweed growth were monitored during the testing. Seasonal effect on duckweed growth and nutrient removal was also observed. Again, the performance of different selected duckweeds was similar in terms of nutrient removal and duckweed growth in the same season. However, substantial difference was observed in different seasons. Generally, the duckweeds

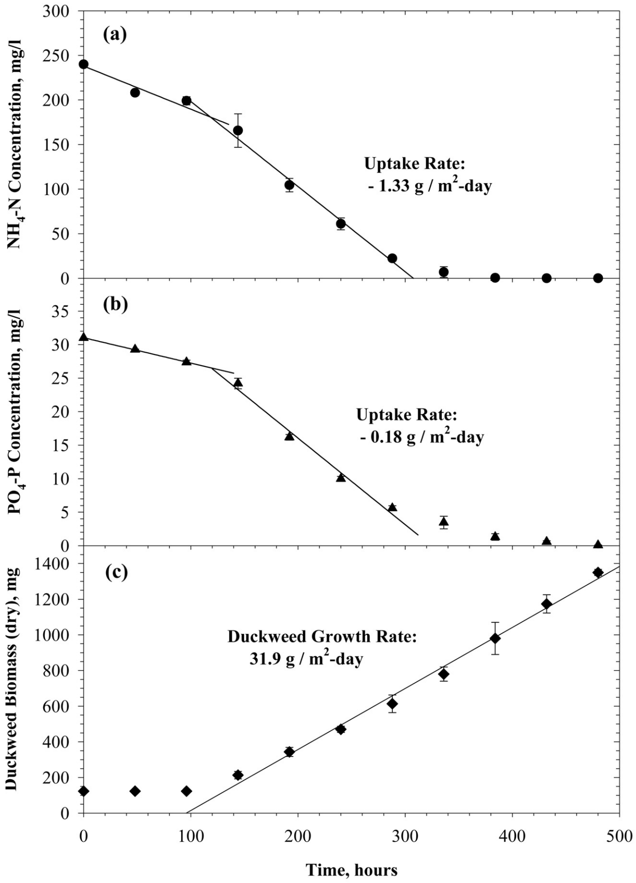

Figure 2. Nitrogen (a) and phosphorus (b) removal from artificial swine wastewater by growing *Spirodela punctata* 7776 and the duckweed growth (c) in a laboratory growth chamber (Modified from Cheng et al. 2002a).

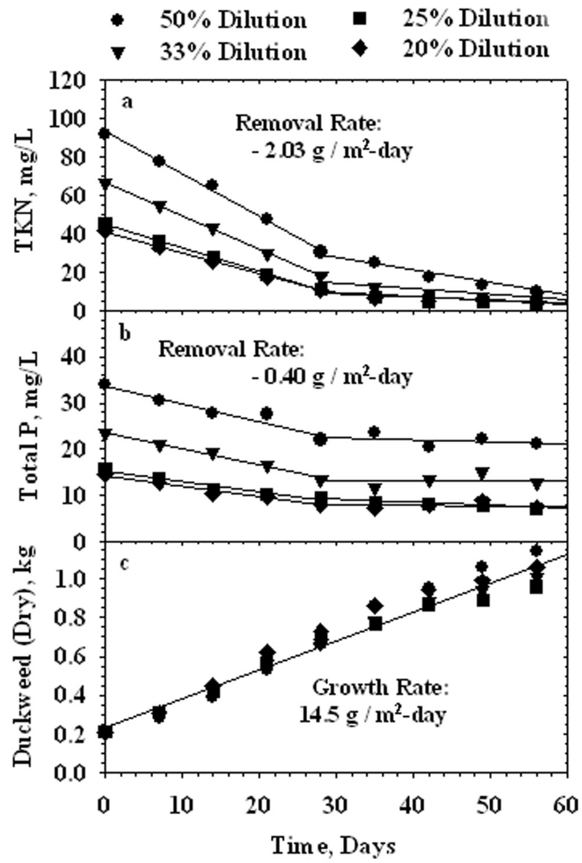

Figure 3. Nitrogen (a) and phosphorus (b) removal from diluted anaerobically treated swine wastewater by growing *Spirodela punctata* 7776 and the duckweed growth (c) under natural climate conditions of fall 2000 in Raleigh, North Carolina, USA.

grew well in the spring and fall, slightly slower in the summer, and hardly in the winter. The average temperature in the duckweed tanks was 15 to 25°C in the spring and fall, around 25°C in the summer, and 0 to 10°C in the winter. Figure 3 shows an example of N and P removal and the duckweed growth on an anaerobically treated swine wastewater with different dilutions in a fall test. In this test the daily average temperature was 12 to 24°C, and the light intensity 100–700 μmol/m²s with the average of 470 μmol/m²s. In the field tests, no lag phase in duckweed growth and nutrient removal was observed because the seed duckweed was acclimated to the nutritional environment of the same wastewater for six weeks during the duckweed culture preparation in a greenhouse, which is in agreement with [26] who also suggested that an acclimation period would help duckweed adjust to drastic changes within a system. It was noticed that both N and P removal rates in the field testing was substantially higher than that in the laboratory testing. This is because nutrient removal in the laboratory testing was solely through duckweed growth in a sterile environment, while in the field testing bacterial activities such as nitrification/denitrification could contribute to nutrient removal in addition to duckweed growth [27]. Substantial COD and TOC (total organic carbon) reduction, 62–76% and 52–73%, respectively, in the field duckweed tanks indicated that bacteria were quite active during the experiments. We have also noticed that the duckweed growth rate in the field testing was approximately half of that in the laboratory testing. This is most probably because of the constant temperature and light intensity and longer daily photoperiod during the laboratory testing. In addition, bacterial and algal activities in the field testing were competing again the duckweed for nutrients, which probably also contributed to the slower duckweed growth.

2.3 Mechanism and Kinetics of Nutrient Removal and Duckweed Growth

To understand the mechanism and kinetics of nutrient removal from the wastewater in a duckweed system, mathematic models have been developed to describe nitrogen transport and duckweed growth in the system [12, 28]. In the anaerobically treated swine wastewater, nitrogen is mainly present in the form of ammonium or NH_4^+. Duckweed is a floating aquatic plant and grows only at the water surface. Ammonium in the bulk wastewater needs to transfer to the duckweed or water surface before it is utilized by the duckweed. A diagram of ammonium transport from the bulk wastewater to the water surface where nutrient is utilized by the duckweed is shown in Fig. 4. The diffusion of ammonium in the wastewater can be mathematically described using the model developed by Choy and Reible [29] as the following equation:

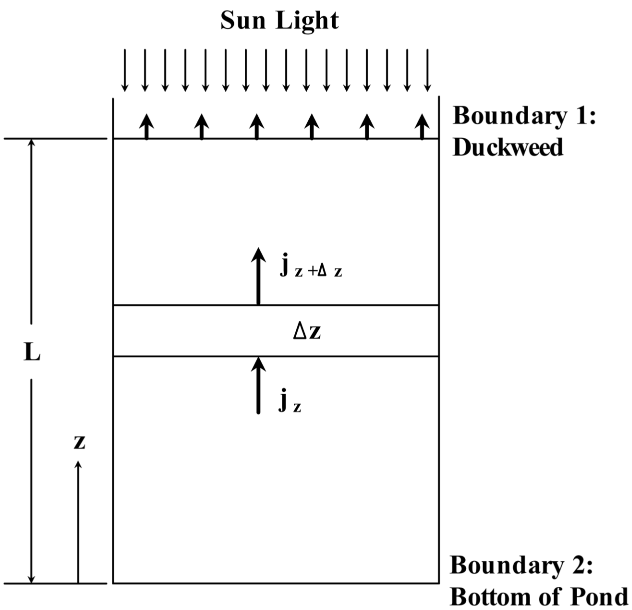

Figure 4. Nutrient transport from the bulk wastewater to the water surface where the nutrient is utilized by duckweed for its growth (Modified from [28]).

$$\frac{\partial c(z,t)}{\partial t} = D \cdot \frac{\partial^2 c(z,t)}{\partial z^2} \qquad (1)$$

where c is the concentration of the ammonium (mg/m^3), z the coordinate direction in which mass diffuses (m), D the diffusion coefficient of the ammonium in the wastewater (m^2/h), and t the time of diffusion (h).

Two boundaries are shown in Fig. 4 with the following boundary conditions:

At the first boundary at the water surface where duckweed grows ($z = L$), the ammonium flux to the floating duckweed must be equal to the rate of the ammonium uptake by the duckweed, or:

$$D \cdot \frac{\partial c}{\partial z}(z = L, t) = -r_{NH_4}(z = L, t) \qquad (2)$$

where r_{NH4} denotes the rate of ammonium accumulation at the water surface.

At the second boundary at the bottom of the duckweed pond (z = 0), the mass flux is assumed to be zero, i.e., there is no leaking of nutrient through the bottom of the pond, or:

$$\frac{\partial c}{\partial z}(z = 0, t) = 0 \qquad (3)$$

An initial condition is necessary to solve Eq. (1). At the beginning of the duckweed nutrient removal process, the ammonium concentration in the wastewater is known and should be uniform all over the pond. Therefore, we have the following initial condition:

$$c(z, t = 0) = c_0 \qquad (4)$$

where c_0 is the initial ammonium concentration in the wastewater.

To ultimately solve Eq. (1) and establish ammonium profile in the duckweed nutrient removal pond, we need to understand the kinetics of ammonium uptake by the duckweed, or the mathematic expression of $-r_{NH4}$. Boniardi et al. [30] and Vatta et al. [31] used a modified Monod model that contained concentrations of different nutrients including COD present in the wastewater medium to describe the growth rate of *L. gibba*. However, our experimental data on nutrient removal from anaerobically treated swine wastewater by growing duckweed have shown that duckweed continued to

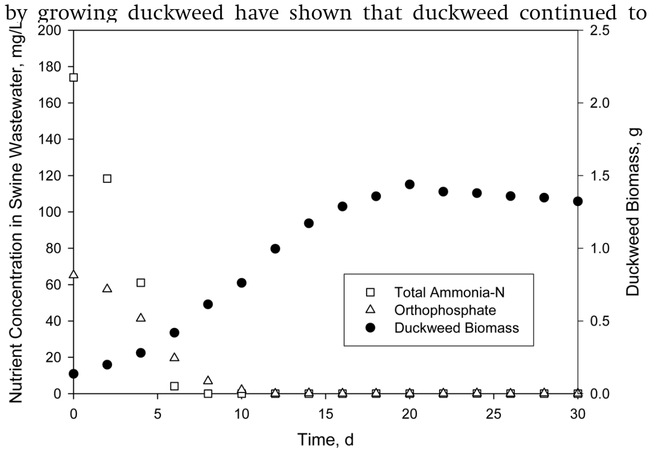

Figure 5. Nitrogen and phosphorus removal from anaerobically treated swine wastewater by growing duckweed (Modified from [12]).

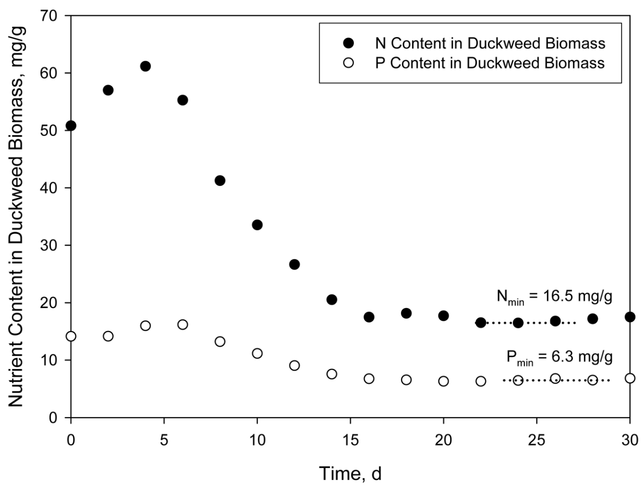

Figure 6. Nitrogen and phosphorus contents in duckweed (*Spirodela punctata*) biomass that grows on anaerobically treated swine wastewater (Modified from [12]).

grow for a quite a long period even after the inorganic nutrients (N and P) in the wastewater medium were completely removed (see Fig. 5) [12].

This phenomenon implies that there must be other sources of nutrients available to support the growth of the duckweed. Many plants are able to store nutrients such as N and P internally in many forms, e.g., nitrogen as amino acid or proteins. Some of the internally stored nutrients can be utilized to support the growth of the plant. In our duckweed nutrient removal experiment, we did find that nitrogen and phosphorus contents in the duckweed biomass changed during the tests. Figure 6 shows nitrogen and phosphorus contents in the duckweed biomass that are corresponding to the nutrient level in the swine wastewater and duckweed growth in Fig. 5. In the initial period when there were N and P present in the wastewater, both N and P contents in the duckweed biomass were quite high, as shown in Fig. 6. After all the N and P were removed from the wastewater, N and P contents in the duckweed biomass started to decrease while duckweed continued to grow (see Figs. 5 and 6). N

Table 2. Amino Acid Composition of Bulk Protein of *Lemnaceae* Species, Grains, Legumes, and Casein.

Amino Acids	*L. gibba*[a]	*S. punctata*[a]	*S. polyrrhiza*[a]	Green Grass[b]	Soybean Meal[b]	Peanut[b]	Rice[b]	Corn Gluten Meal[c]	Casein[b]	Recommended Levels of Essential Amino Acids for Chicken Feed[c]
	g/100 g protein									
Leu	7.15	6.88	6.85	10	8.0	6.7	8.2	15.3	10.0	7.5
Ile	3.87	3.76	3.75	5	6.0	4.6	5.2	4.9	7.5	5.0
Val	4.96	4.71	4.40	5	5.3	4.4	6.2	5.1	7.7	5.0
Met	0.83	1.07	0.83	2.5	1.7	1.0	3	2.35	3.5	2.0
Cys	NA[d]	NA	NA	2.0	1.9	1.6	1.3	1.65	0.4	3.6[e]
Phe	4.45	4.38	4.20	5–6	5.3	5.1	5.0	5.6	6.3	4.4
Tyr	2.91	3.14	3.05	5.0	4.0	4.4	5.7	2.3	6.4	6.4[f]
Lys	4.13	4.26	4.30	5.5	6.8	3.0	3.2	1.85	8.5	4.0
Thr	3.20	3.31	3.45	5.4	3.9	1.6	3.8	3.0	4.5	3.5
Trp	NA	NA	NA	2.2	1.4	1.0	1.3	0.5	1.3	1.0
His	1.89	1.90	2.15	2.0	2.9	2.1	1.7	2.1	3.2	1.9
Arg	4.29	4.86	5.25	7.0	7.3	11.3	7.2	3.25	4.2	5.0
Ser	2.61	2.83	2.80	5	4.2	NA	NA	NA	3.3	NA
Pro	2.93	2.95	3.28	NA	5.0	NA	NA	NA	13.1	NA
Gly	3.79	3.93	3.95	NA	NA	5.0	NA	NA	2.1	NA
Glu	7.60	7.69	8.00	11.5	18.4	17.7	NA	NA	23.0	NA
Asp	7.12	7.38	7.55	5.3	NA	NA	NA	NA	7.0	NA

[a] Rusoff et al. [38];
[b] Block and Bollings [49];
[c] Scott et al. [50]; laying hen requirement;
[d] Not available;
[e] Sum of phenylalanine and tyrosine;
[f] Sum of phenylalanine and tyrosine.

and P contents in the duckweed biomass continued to decrease until they reached their minimum level, 16.5 and 6.3 mg/g, respectively, at which duckweed stopped growing. These results indicate that duckweed growth was directly related to the nutrient reserve in the duckweed biomass, instead of nutrient concentration in the wastewater. Based on the experimental findings, we have developed a modified Monod model to describe the duckweed growth on wastewater as follows:

$$\mu = \mu_{\max}\left(\frac{N_{resv}}{K_N + N_{resv}}\right) \tag{5}$$

where μ denotes the specific duckweed growth rate ($g_{biomass}/g_{biomass}$/day), μ_{max} the maximum specific duckweed growth rate ($g_{biomass}/g_{biomass}$/day), $N_{resv} = N$ reserve of the biomass ($mgN/g_{biomass}$) = N content of biomass − N_{min}, N_{min} the minimum nitrogen content of the biomass ($mgN/g_{biomass}$), and K_N is the half saturation constant ($mgN/g_{biomass}$).

This model has provided a realistic solution to describe duckweed growth on wastewater before and after the nutrients are removed from the wastewater. It worked well to predict N profiles and duckweed growth [12]. The rate of nutrient removal from the wastewater, e.g., $-r_{NH4}$, is proportional to the duckweed growth before the nutrients are completely removed from the wastewater.

3 Potential Products from Duckweed

As a by-product from wastewater treatment, duckweed can be utilized for different applications. Duckweed has a high protein content, from 15 to 45%, making it an excellent material for animal, poulty, and fish feed. Recent research has found that duckweed can be a good feedstock for starch production and eventually for fuel ethanol generation.

3.1 Duckweed Feed for Animal, Chicken, and Fish

The 1970s and 80s were the most productive decades for research concerning the use of duckweed as a source of proteins for animal feed. Three characteristics of *Lemnaceae* species prompted this interest. A protein content ranging from 15 to 45% dry weight can be achieved with duckweed depending on specific species and strain within species and on growing conditions [6]. This value compares favorably with soybeans, whose protein content can range from 33 to 49%, again depending on genotype and growth conditions [32] and is higher than the protein content of grains [33].

Protein quality is the second characteristic on interest when evaluating a protein source for animal feed. Protein quality or biological value is a reflection of how closely the amino acid balance of total protein matches that needed by the animal. Typically, plant proteins have lower biological value relative to those derived from animal sources. Of particular concern is the generality that legumes are deficient in sulfur containing amino acides, i.e., methionine and cystine, and grains are usually deficient in lysine. Table 2 is a compilation of amino acid contents of several duckweed species (*S. polyrrhiza, S. punctata*, and *L. gibba*) grown under differing conditions in comparison with protein from grains (rice and corn gluten meal), legumes (peanut and soybean meal) and milk (casein) with the amino acid requirement for laying hens. From Tab. 2 it is clear that soybean meal has an amino acid balance quite close to the dietary requirements of laying chickens, giving it a relatively high biologi-

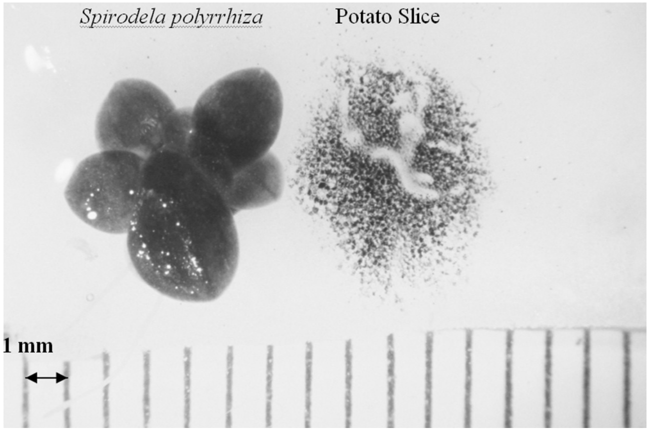

Figure 7. *Spirodela polyrrhiza* plants stained to visualize starch content. A slice of potato is also stained for comparison.

cal value. In contrast, the amino acid balance of *S. polyrrhiza* protein, one of the duckweed species utilized in wastewater systems, is notably low in leucine, isoleucine and valine, the sulfur-containing amino acids methionine and cysteine, and serine. The lysine content of duckweed protein is generally higher than that found in grains, which have low levels of this essential amino acid.

There is some question as to the tryptophan content of duckweed protein. Although the tryptophan content of bulk duckweed protein has been reported to be less than 1% [34, 38], these values may be underestimates of the actual tryptophan content. The tryptophan content is difficult to measure accurately [52, 53]. Given that duckweed is primarily green frond tissue, it would be expected to have the tryptophan content similar to grasses which ranges from 1 to 2% tryptophan [49]. The tryptophan content of green plant tissue is significantly biased towards the content of the two major leaf proteins, RuBP carboxylase and chlorophyll ab binding protein.

Our calculations from amino acid sequence data indicate that the tryptophan content by weight of these proteins is approximately 4%. Overall, the biological value of duckweed total proteins would be less than that of soybean meal. That said, numerous studies of duckweed species grown under differing growth conditions have reported significant variation in amino acid ratios of total proteins, with values for individual amino acids varying by 25 to 50% [6]. In a more recent study, Reid [34] utilized swine wastewater to grow *L. gibbba* for a goat feeding trial and found that using wastewater as growing media will give *L. gibba* bulk protein with an amino acid content that mirrors the average across several species. Finally, a third characteristic of interest is the protein yield per unit of growing area. Assuming average protein content for duckweed of 30% dry weight and 0.2 kg/m^{2}wk (10 000 kg/hectare yr) this wastewater system could produce approximately 3000 kg of proteins per hectare over a 12-month growing season. Bhanthumnavin and McGarry [35] reported *Wolffia globosa* protein production of approximately 2000 kg/hectare year in Thailand. For comparison the same authors reported protein production of approximately 300 kg /hectare/year for soybeans, 70 kg for rice and 180 kg for corn. Even taking the somewhat lower biological value of duckweed protein, *Lemnaceae* species yield much more protein per unit of cropping area, an important consideration as increasing human population continues to demand higher crop yields.

Culley and co-workers [36] published an excellent review of studies on feeding animals with duckweed species through 1980. Briefly, duckweed feeding studies have been done with dairy cows [37, 38], pigs [6], sheep [39], goats [34], poultry including ducks and chickens [40, 41], and fish [42, 43]. Because duckweed is a common and valuable natural protein source for fish, a number of investigations have focused on using duckweed as fish feed [44, 45]. Overall, these studies show that duckweed can supply a large proportion and in some cases all the protein required by the animals with no adverse effects. Animals supplied with a plant-based diet supplemented with duckweed typically had higher growth rates than those fed with the control diet alone. Some trials used duckweed as the sole animal feed and attained reasonable animal growth rates. One cautionary note is that species in the genera *Spirodela* and *Lemna* accumulate significant concentrations of oxalic acid both in soluble form and as crystals (raphids). Plants accumulate oxalic acid crystals as a feeding deterrent to herbivores. Oxalic acid at high concentrations can be toxic, although it should be noted that toxicity has not been noted in any of the animal feeding trials to date.

3.2 Fuel Ethanol

In recent years there has been a growing interest in renewable energy production worldwide because of the limited reserve of crude oil and natural gas and environmental concerns of using fossil fuels. Ethanol production from dedicated crops or agricultural residues is one form of renewable energy that addresses the critical need for sustainable transportation fuels. There is already a well-developed market for ethanol in the U.S.; over 1.5 billion gallons are already added to gasoline to improve emissions and boost octane. Currently, corn starch is the primary raw material for fuel ethanol production in the U.S. and accounts for approximately 92% of the total feedstocks in ethanol industry [46, 47]. However, it may not be practical to substantially increase ethanol production from corn because of the competition against food and feed production. Thus, there is a great interest in exploring alternative feedstock for ethanol production.

As discussed earlier in this article, duckweed starch content can be manipulated by adjusting growth conditions, e.g., pH, nutrient starvation, that affect frond proliferation. That duckweed plants can achieve starch contents comparable to corn provided the impetus for investigating the use of duckweed biomass as a source of fermentable carbohydrate for fuel ethanol production. In our laboratory study, *S. polyrrhiza,* a local duckweed strain, has shown a great potential for starch production. Figure 7 shows an example of the high starch content that we have been able to achieve with *S. polyrrhiza* through simple transfer of fresh duckweed fronds from a nutrient-rich solution to tap water for 5 days. The starch content in the duckweed was found to be 45.8% (dry based).

Enzymatic hydrolysis of the duckweed biomass with α-amylase (Sigma A3404), pullulanase (Sigma P2986), and amyloglucosidase (Sigma 10115), following the same protocol used for the saccharification of corn starch, yielded a hydrolysate of 509 mg reducing sugars per gram of dry duckweed. Fermentation of this solution using yeast gave an ethanol yield of 258 mg per gram of the dry duckweed biomass. These results indicate that duckweed biomass can produce starch in appreciable quantities that can be readily fermented into ethanol. In addition, duckweed biomass has several characteristics that provide duckweed biomass-to-ethanol process advantages and that could lower overall costs when compared to corn. Based on the preliminary results from our laboratory study, duckweed could produce starch in a rate of approximately 28 tons per hectare per year, compared to corn starch production of about 5.0 tons per hectare per year. Duckweed biomass would require little or no mechanical grinding because of the small size of the plants and because it is a green, hydrated biomass. The lack of a milling step to prepare biomass for fermentation translates into a substantial savings in energy, one of the major costs in the corn-to-ethanol process [48]. Duckweed has a protein content ranging between 15 and 45% dry weight [6] compared to 9% protein content for corn. This suggests that supplementation of the yeast fermentation mash with an N-source may not be necessary when using duckweed biomass. High protein content may also make "distilled grain", a by-product of the ethanol fermentation, from duckweed biomass a livestock feed supplement superior to that derived from corn.

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