

# CREATING A CIRCULAR NITROGEN BIOECONOMY IN AGRICULTURAL SYSTEMS THROUGH NUTRIENT RECOVERY AND UPCYCLING BY MICROALGAE AND DUCKWEED: PAST EFFORTS AND FUTURE TRENDS



Pandara Valappil Femeena<sup>1</sup>, Gregory R. House<sup>1</sup>, Rachel A. Brennan<sup>1,\*</sup>

<sup>1</sup> Department of Civil and Environmental Engineering, The Pennsylvania State University, University Park, Pennsylvania, USA.

\* Correspondence: rab44@psu.edu

## HIGHLIGHTS

- Aquatic vegetation-based nutrient recovery offers an alternate approach for treating agricultural wastewater.
- Microalgae and duckweed can upcycle waste nutrients into valuable bio-based products.
- Producing feed, fertilizer, and fuel from manure-grown aquatic vegetation promotes a circular N-bioeconomy.

**ABSTRACT.** *The massive amounts of nutrients that are currently released into the environment as waste have the potential to be recovered and transformed from a liability into an asset through photosynthesis, industry insight, and ecologically informed engineering design aimed at circularity. Fast-growing aquatic plant-like vegetation such as microalgae and duckweed have the capacity to enable local communities to simultaneously treat their own polluted water and retain nutrients that underlie the productivity of modern agriculture. Not only are they highly effective at upcycling waste nutrients into protein-rich biomass, microalgae and duckweed also offer excellent opportunities to substitute or complement conventional synthetic fertilizers, feedstocks in biorefineries, and livestock feed while simultaneously reducing the energy consumption and greenhouse gas emissions that would otherwise be required for their production and transport to farms. Integrated systems growing microalgae or duckweed on manure or agricultural runoff, and subsequent reuse of the harvested biomass to produce animal feed, soil amendments, and biofuels, present a sustainable approach to advancing circularity in agricultural systems. This article provides a review of past efforts toward advancing the circular nitrogen bioeconomy using microalgae- and duckweed-based technologies to treat, recover, and upcycle nutrients from agricultural waste. The majority of the work with microalgae- and duckweed-based wastewater treatment has been concentrated on municipal and industrial effluents, with <50% of studies focusing on agricultural wastewater. In terms of scale, more than 91% of the microalgae-based studies and 58% of the duckweed-based studies were conducted at laboratory-scale. While the range of nutrient removals achieved using these technologies depends on various factors such as species, light, and media concentrations, 65% to 100% of total N, 82% to 100% of total P, 98% to 100% of  $\text{NO}_3^-$ , and 96% to 100% of  $\text{NH}_3/\text{NH}_4^+$  can be removed by treating wastewater with microalgae. For duckweed, removals of 75% to 98% total N, 81% to 93% total P, 72% to 98%  $\text{NH}_3/\text{NH}_4^+$ , and 57% to 92%  $\text{NO}_3^-$  have been reported. Operating conditions such as hydraulic retention time, pH, temperature, and the presence of toxic nutrient levels and competing species in the media should be given due consideration when designing these systems to yield optimum benefits. In addition to in-depth studies and scientific advancements, policies encouraging supply chain development, market penetration, and consumer acceptance of these technologies are vitally needed to overcome challenges and to yield substantial socio-economic and environmental benefits from microalgae- and duckweed-based agricultural wastewater treatment.*

**Keywords.** *Circular bioeconomy, Duckweed, Manure treatment, Microalgae, Nitrogen, Nutrient recycling, Wastewater treatment.*

**T**ransitioning the current agricultural sector from a linear to a circular system is required to effectively recycle valuable resources such as nitrogen (N). Considered one of the most important elements for plant growth, N also forms a key component of amino acids that make up the proteins required by humans and animals to meet their nutritional needs. Natural processes such as atmospheric deposition, N fixation, plant and animal N uptake,

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nitrification, and denitrification are all critical parts of the complex N cycle that affect the availability of N in the environment, in the forms of organic N, nitrate ( $\text{NO}_3^-$ ), nitrite ( $\text{NO}_2^-$ ), and ammonia ( $\text{NH}_3$ ), and its subsequent influence on air and water quality. In agricultural systems, the relatively recent changes in agricultural practices, such as extensive soil tillage and crop residue harvesting, and the increased use of chemical fertilizers have resulted in excessive N applications and subsequent N leaching through groundwater infiltration and surface runoff (Mazzoncini et al., 2011; Savci, 2012). Livestock farms that produce and release untreated manure are another major source of N pollution to surface waters (Kleinman et al., 2018; Ribaud, 2003). Excess nutrients can be carried down gradient in streams and rivers, resulting in the growth of harmful algal blooms that can cause eutrophication and hypoxia (oxygen depletion) in large water bodies such as the Gulf of Mexico, Chesapeake Bay, Lake Erie, Lake Victoria, and other regions around the world (Anderson et al., 2008; Kemp et al., 2005; Scavia et al., 2014). Agricultural wastewater thus often necessitates treatment or nutrient recovery techniques before being released for reuse; otherwise, long-lasting negative impacts on soil health, water quality, and biodiversity may result.

Although many N management strategies have been developed, full recovery of N from water sources is typically challenging without significant energy and financial investment. For instance, conventional N removal processes in wastewater treatment are known to cause serious environmental impacts by contributing to the release of nitrous oxide ( $\text{N}_2\text{O}$ ), a potent greenhouse gas (GHG) (D'Odorico et al., 2018; Sutton et al., 2011). Higher N removal from wastewater often requires higher energy and chemical demands, and in turn leads to increased operating costs and more GHG emissions (Hauck et al., 2016). Furthermore, most of the existing N removal technologies are focused on municipal and industrial wastewater treatment, with limited emphasis given to wastewater from agricultural sources. Typically, agricultural wastewaters (especially those from livestock farms that include manure, feedlot runoff, milking center washwater, etc.) are left untreated, spread on crop fields to increase soil fertility, or occasionally treated using constructed wetlands (Dordio and Carvalho, 2013). Untreated manure and agricultural soil mismanagement not only deteriorate streamwater quality but also increase  $\text{N}_2\text{O}$  emissions and overall N imbalances. Novel techniques and materials to remove and recover N from agricultural wastewater without deleterious climate change effects are therefore required to alleviate the environmental impacts from waste generation and improve soil, air, and water quality. One promising set of options are photosynthesis-based technologies that incorporate the use of aquatic vegetation to recover nutrients while simultaneously sequestering carbon dioxide ( $\text{CO}_2$ ) from the atmosphere and producing beneficial biomass. Evaluating the true impacts associated with these techniques requires a cradle-to-grave analysis, or life cycle assessment (LCA), of all processes and products generated within the wastewater treatment system. Most LCA studies in this area have focused on evaluating the environmental impacts of microalgae-based municipal wastewater treatment with concomitant biofuel production, with a few

studies concentrating on the benefits of growing microalgae on swine wastewater (Lopes et al., 2018; Maga, 2017; Wu et al., 2020). Although duckweed-based municipal wastewater treatment is gaining popularity, and laboratory- to full-scale experiments have been conducted to demonstrate the plant's nutrient recovery efficiency (Cheng and Stomp, 2009; Mohedano et al., 2012), LCA on this technique has only been done to a minimal extent (Roman and Brennan, 2021). Further, the concept of using microalgae and duckweed for treating agricultural runoff and manure is still evolving and requires additional research to holistically evaluate potential environmental impacts.

The transition to a resource recovery-focused approach for wastewater treatment over the past decade parallels the global trend toward a circular bioeconomy, which focuses on the conversion of biomass and other bio-waste into useful products in an effort to transition away from the overexploitation of fossil fuels (Ferreira et al., 2018; Nagarajan et al., 2020). A prime example is a biorefinery that uses biomass to produce bioethanol as an alternative to conventional petroleum refineries. Other examples include producing plant-based biodegradable plastics (Karan et al., 2019), pharmaceuticals (Kesik-Brodacka, 2018), and construction materials (Shanmugam et al., 2021). A circular N-bioeconomy specifically focuses on cycling N within the larger bioeconomy through efficient N recovery techniques such as using biofertilizers and compost, making plant-based biofuels, and producing animal feed from bio-waste. These techniques, when employed on a large scale, are not only environmentally sustainable but also more economically viable than traditional fossil fuel-based production processes (Awasthi et al., 2019; Nagarajan et al., 2020). Such a systems-level approach further provides opportunities to conduct LCAs on several interconnected N-bioeconomy processes and help address issues within the water-energy-food (WEF) nexus including, but not limited to: food insecurity, GHG emissions, water pollution, and eutrophication (Del Borghi et al., 2020; Ubando et al., 2020).

More than any other sector, agriculture has the largest impact on habitable land use (50%) and is the second largest contributor to GHG emissions (24%) after energy production (IPCC, 2014; Ritchie, 2019). Additionally, the farming stage of the food supply chain accounts for 25% of global terrestrial acidification and 74% of total freshwater and marine eutrophication (Poore and Nemecek, 2018). In agricultural systems, one of the ways to promote a circular N-bioeconomy is by producing beneficial byproducts from harvested or leftover biomass such as crop residues. For example, corn stover has been widely recognized as a good candidate for lignocellulose-based biofuel production (Kim et al., 2019; Qureshi et al., 2010), but corn stover-based biorefineries have not been yet been implemented on a large-scale, primarily due to the negative water quality impacts caused by the increased nutrient runoff that occurs with the removal of crop residues from agricultural fields (Battaglia et al., 2021; Cibin et al., 2012). Considering the tradeoffs between energy production and water quality deterioration, a futuristic pathway to advance the circular N-bioeconomy in agriculture is to employ nutrient recovery techniques that use fast-growing aquatic vegetation that naturally recover N

from agricultural runoff and enable subsequent reuse of the cultivated biomass for producing energy and other useful products such as soil amendments and animal feed. With technological advancements and process improvements, this practice could holistically tackle the issues within the larger WEF nexus, one such example being the use of wastewater-grown aquatic vegetation to sustainably produce proteins for animal consumption and to enhance food security.

The primary objective of this review is to identify past efforts toward advancing the circular N-bioeconomy in agricultural systems, with a specific focus on emerging sustainable methods for treating and recovering nutrients from agricultural wastewater, and to understand the limitations and future trends in this area. By reconciling the lessons learned from past studies, and through a comprehensive analysis of improved N recovery techniques, the environmental and economic benefits of adopting a circular N-bioeconomy approach in agricultural systems may be realized.

## **TOWARD A CIRCULAR N-BIOECONOMY IN AGRICULTURAL SYSTEMS**

Traditionally, manure from livestock farms is stored in deep pits or on-site lagoons and subsequently applied to crop fields, which helps enrich the soil with nutrients but can release  $\text{NH}_3$  into the atmosphere. Anaerobic digestion, a routine process used to treat manure prior to soil application, can reduce  $\text{CO}_2$  and methane ( $\text{CH}_4$ ) emissions from manure through useful biogas production; however, the remaining digestate, when applied on soil, still poses a risk of increased GHG emissions (Dietrich et al., 2020). Livestock farms in general have been reported to be the major source of non- $\text{CO}_2$  GHG emissions in the U.S. and China (Nagarajan et al., 2019). Although manure-fertilization of crop fields has been recommended as a way to encourage circularity in agricultural systems, runoff from these farms can cause pollution in adjacent water bodies if effective nutrient recovery techniques are not implemented. Using manure as a biorefinery feedstock has been studied as another pathway to promote the circular bioeconomy, but there are technical challenges associated with the conversion of manure to biofuel and other useful byproducts due to its heterogeneous composition (Chen et al., 2005).

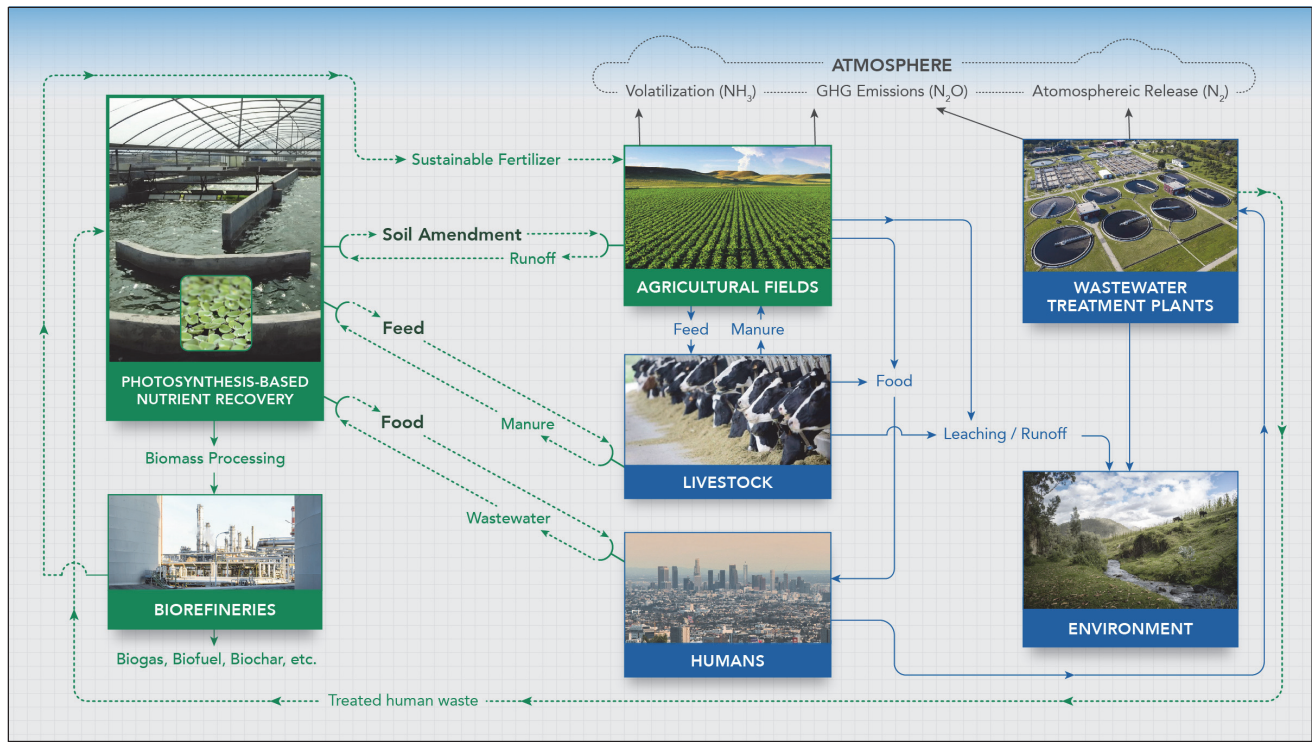
Cultivating protein-rich plant-like species including duckweed, azolla, seaweed, and microalgae on wastewater has gained popularity in recent years as a novel method to recover nutrients before they are released into the environment (Arumugam et al., 2018; Muradov et al., 2014; Nagarajan et al., 2020). Duckweed (of family Lemnaceae), azolla (of family Salviniaceae), seaweed (a form of macroalgae), and microalgae are all aquatic autotrophs with a wide-ranging diversity of species within each family. These species require a smaller areal footprint to produce equivalent biomass when compared to conventional land-grown crops and are promising sources of biomass feedstock and animal feed (Calicioglu et al., 2018; Hemalatha et al., 2019). In relation to conventional lignocellulosic biomass, both algae and duckweed have strong potential for use in large-scale systems for upcycling N into biomass due to their rapid growth

rates. Their high protein content (up to 50% by dry weight) and their ability to be pumped for transport are other benefits of using algae and duckweed for biomass, feed, and food production. An LCA on a duckweed-based ecological wastewater treatment facility indicated that without supplemental heating, such a facility can reduce energy consumption by a third and GHG emissions by half when compared to a conventional wastewater treatment system (Roman and Brennan, 2021). A sustainable farming system promoting the circular N-bioeconomy concept could involve growing these aquatic species on either diluted manure or bio-digester effluents and harvesting them for use in bioenergy production, as a fertilizer substitute, or as a protein supplement in animal feed. Figure 1 illustrates the existing linear N economy in agricultural systems along with the recommended pathways to transition toward a circular N-bioeconomy using aquatic vegetation for nutrient recovery.

The following section summarizes conventional farm nutrient management methods and reviews emerging microalgae- and duckweed-based nutrient recovery technologies, highlighting the benefits and challenges associated with each. Although a large share of published studies has been focused on using microalgae and duckweed for treating municipal wastewater, there is growing trend toward applying these technologies for treating agricultural runoff and manure. A circular N-bioeconomy can be realized in agricultural systems by applying these practices to integrated farming systems to generate value-added products.

### **PAST EFFORTS IN N RECOVERY METHODS BASED ON MICROALGAE AND DUCKWEED**

Typically, wastewater treatment plants providing dedicated N removal processes are normally used only to treat wastewater from domestic and industrial sources. Runoff from agricultural fields and livestock farms is often left untreated, leading to surface and groundwater contamination. In certain cases, manure and other organic waste from livestock farms are treated either using anaerobic digesters or waste stabilization ponds that promote sedimentation of waste solids and anaerobic decomposition to produce methane and other usable products such as biochar and compost. While anaerobic digesters have better treatment efficiency than settling ponds due to the added heating and mixing, they are a comparatively expensive treatment option. Settling ponds, on the other hand, while cost-effective, can contribute to high GHG and odor emissions (Craggs et al., 2014). Therefore, a cost-effective and environmentally friendly treatment method with high nutrient removal efficiency (e.g., using aquatic vegetation such as microalgae or duckweed) would offer a sorely needed alternative for treating and recovering N from farm wastewater. Existing practices to capture N from agricultural field runoff involve the use of constructed wetlands, buffer strips, denitrification bioreactors, etc. (Husk et al., 2017; Xia et al., 2020); there have been limited applications of using microalgae- and duckweed-based N recovery technologies to capture and treat runoff from crop fields due to the nonpoint-source nature of the runoff. However, manure generated on livestock farms is comparatively easier to collect and treat than runoff; therefore, much of the work conducted in the past on microalgae-



**Figure 1. Integrating wastewater-treatment and aquatic vegetation to promote a circular N-bioeconomy in agricultural systems. Blue lines refer to the existing linear economy and green dashed lines show pathways to promote a circular N-bioeconomy.**

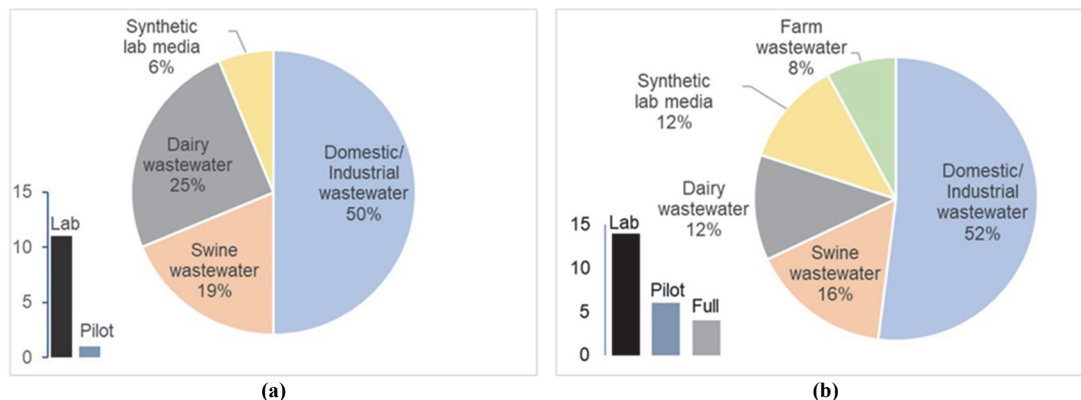
and duckweed-based N recovery from agricultural wastewater has been focused on manure from livestock farms. Theoretically, these recovery methods could be adopted to treat cropland runoff if an on-farm treatment system (such as a constructed wetland) is used to capture runoff from cropping areas.

The literature review was performed using the Web of Science database (<https://www.webofknowledge.com>) by finding articles with keywords “duckweed”, “microalgae”, “bioeconomy”, “nutrient removal”, and “biomass production”. From the extensive list of studies, we shortlisted those in which microalgae and duckweed were used to treat agricultural, municipal, and industrial wastewater. Studies published between the years 1995 and 2020 are included in this review. Of the reviewed studies that focused on microalgae- and duckweed-based wastewater treatment, more than half

used wastewater from domestic and industrial sources, and the majority were conducted at laboratory-scale (fig. 2). For in-depth review, only studies focusing on agricultural wastewater treatment are summarized here (table 1). Tables A1 and A2 in the Appendix show the complete list of studies.

### **Microalgae-Based Wastewater Treatment**

Microalgae are unicellular photosynthetic microorganisms that can grow in marine and freshwater ecosystems and use sunlight, CO<sub>2</sub> or organic carbon, water, and nutrients to build biomass with high protein and lipid contents (40% and 30% by dry weight, respectively) (Acién Fernández et al., 2021; Su, 2021). Microalgae can double in mass in less than a day and produce biomass yields as high as 100 ton dry mass ha<sup>-1</sup> year<sup>-1</sup> (Acién Fernández et al., 2021). There are many strains of microalgae, with varying effectiveness in



**Figure 2. Sources of nutrients used in the reviewed articles that focused on (a) microalgae-based ( $n = 12$ ) and (b) duckweed-based ( $n = 24$ ) wastewater treatment. The experimental scales used in the studies (lab, pilot, or full-scale) are shown on the bottom left of each chart. Details of the studies reviewed are provided in tables A1 and A2 in the Appendix.**

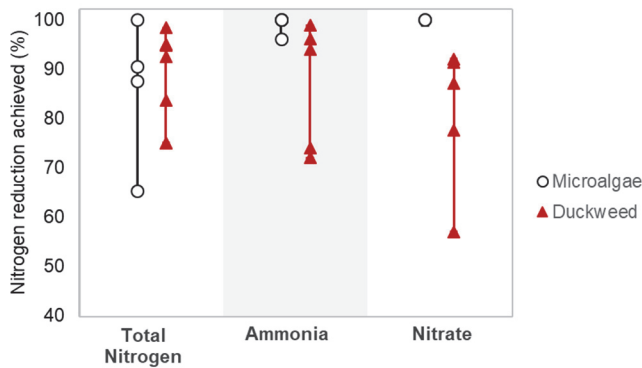
**Table 1. Summary of nitrogen removal and biomass production by microalgae and duckweed in selected agricultural wastewater treatment systems (HRT = hydraulic retention time, TN = total nitrogen, TKN = total Kjeldahl nitrogen, and TP = total phosphorus). Tables A1 and A2 in the Appendix provide a complete list of studies that include municipal and industrial wastewater treatment with microalgae and duckweed.**

Wastewater Type	Scale	Species Used	Experimental Conditions/Variables	Results	Reference
<b>Microalgae-Based Treatment</b>					
Poultry, swine, brewery, cattle, dairy, and urban wastewater	Lab	<i>Scenedesmus obliquus</i>	Pretreated cattle, dairy, and brewery wastewater	95% to 100% TN removal; 63% to 99% PO <sub>4</sub> <sup>3-</sup> removal; Biomass produced with 31% to 53% protein content, 12% to 26% sugars, and 8% to 23% lipids	Ferreira et al. (2018)
Dairy wastewater	Lab	<i>Acutodesmus dimorphus</i>	Untreated dairy wastewater; very low NO <sub>3</sub> <sup>-</sup> concentration	100% NO <sub>3</sub> <sup>-</sup> removal within 4 days; 100% NH <sub>3</sub> removal within 6 days; 1 kg biomass is theoretically calculated to produce up to 273 g of biofuels	Chokshi et al. (2016)
Dairy wastewater	Lab	Algal consortium: <i>Chlorella saccharophila</i> UTEX 2911, <i>Chlamydomonas pseudococcum</i> UTEX 214, <i>Scenedesmus</i> sp. UTEX 1589, and <i>Neochloris oleoabundans</i> UTEX 1185	Wastewater from collecting and holding tanks of dairy farm; three different CO <sub>2</sub> concentrations, irradiance of 80 mmol m <sup>-2</sup> s <sup>-1</sup> , 12 h daylength, for 10 days	98% TKN removal; 99% NH <sub>3</sub> removal; 86% NO <sub>3</sub> <sup>-</sup> removal	Hena et al. (2015)
Swine wastewater	Lab	<i>Chlorella vulgaris</i>	12 days	90.51% TN removal and 91.54% TP removal	Wen et al. (2017)
Swine wastewater	Lab and computer model	<i>Chlorella</i> sp.	Optimizing dilution rate and HRT	Modeled optimal biomass yield and N removal at 2.26-day HRT and 8-fold dilution rate; experiment removal rates of 38.4 mg L <sup>-1</sup> d <sup>-1</sup> of TN and 60.4 mg L <sup>-1</sup> d <sup>-1</sup> of NH <sub>3</sub>	Hu et al. (2013)
<b>Duckweed-Based Treatment</b>					
Swine wastewater	Lab	<i>Spirodela oligorrhiza</i>	Two-week harvest and 6% wastewater to 94% tap water	83.7% TN removal and 89.4% TP removal	Xu and Shen (2011)
Swine wastewater	Lab	<i>Lemna minor</i>	12 h light cycle, pretreated swine wastewater at 4% dilution	74% NH <sub>3</sub> removal; 0.14 g m <sup>-2</sup> d <sup>-1</sup> TN removal	Pena et al. (2017)
Diluted swine effluent	Lab	<i>Spirodela</i> spp.	Different N levels in growing media	Crude protein content increases from 15% at 1 to 4 mg N L <sup>-1</sup> to 37% at 10 to 15 mg N L <sup>-1</sup> ; toxic effect above 60 mg N L <sup>-1</sup>	Leng et al. (1995)
Effluent and digested slurry of biorefinery processing cattle slurry	Lab	<i>Lemna minuta</i>	Various concentrations of effluent from biorefinery and digested slurry	75% TN removal; 81% TP removal; higher concentrations had toxic levels of sodium and potassium	Soñta et al. (2020)
Mixture of domestic and agricultural wastewater	Pilot	<i>Lemna japonica</i> 0234	Comparative study with water hyacinth ( <i>Eichhornia crassipes</i> )	60% recovery of N over a year; 0.4 g m <sup>-2</sup> d <sup>-1</sup> TN removal	Zhao et al. (2014)
Mixture of domestic and agricultural wastewater	Pilot	<i>Lemna japonica</i> 0234	Combining duckweed and carrier biofilm	19.97% higher TN removal and 15.02% higher NH <sub>3</sub> removal with duckweed	Zhao et al. (2015)
Swine wastewater	Full	<i>Landoltia punctata</i>	One-year duration at 30-day HRT	98.3% TN removal; 98.8% NH <sub>3</sub> removal; 4.4 g m <sup>-2</sup> d <sup>-1</sup> TKN removal; 68 t ha <sup>-1</sup> year <sup>-1</sup> biomass yield	Mohedano et al. (2012)

removing nutrients and creating useful biomass; however, *Chlorella* and *Scenedesmus* are the most commonly used genera for wastewater treatment applications (Su, 2021). Up to 1 kg of microalgae can be produced per m<sup>3</sup> of human sewage; however, with the elevated concentrations of nutrients typically found in livestock manure, higher yields in the range of 10 to 100 kg m<sup>-3</sup> of effluent can be obtained (Acién Fernández et al., 2021), but this requires adequate dilution to avoid overloading the treatment system.

Microalgae exhibit a higher removal rate of NH<sub>4</sub><sup>+</sup> compared to NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> because the latter must be reduced to NH<sub>4</sub><sup>+</sup> (an energy-intensive process) before being used for building amino acids and then proteins in the cell (Cai et al., 2013; Maestrini et al., 1986). This is particularly important when treating livestock manure, which contains high levels

of NH<sub>4</sub><sup>+</sup>. The uptake of NO<sub>3</sub><sup>-</sup> by microalgae can be partially reduced in the presence of ambient NH<sub>4</sub><sup>+</sup>, an inhibitory effect that is further enhanced by factors such as limited light conditions and lower temperatures (Su, 2021). The phenomenon of NH<sub>3</sub> removal (but not recovery) is aided at elevated pH conditions because high pH causes NH<sub>4</sub><sup>+</sup> to convert to gaseous NH<sub>3</sub>, which is then released into the air (Ferreira et al., 2018; Zimmo et al., 2003). Microalgae can also remove N<sub>2</sub>O from wastewater (Qie et al., 2019). Using microalgae, 65% to 100% total N, 82% to 100% total P, 98% to 100% NO<sub>3</sub><sup>-</sup>, and 96% to 100% NH<sub>3</sub>/NH<sub>4</sub><sup>+</sup> removal has been achieved in treating farm, industrial, and municipal wastewaters (fig. 3; tables 1, A1, and A2). More studies concentrating on microalgal treatment of agricultural wastewater are required to fully understand the range of nutrient reductions that can



**Figure 3.** Ranges of nitrogen reductions achieved with microalgal- and duckweed-based wastewater treatment (summarized from 22 studies). Each symbol represents the results reported by an individual study.

potentially be achieved under different environmental conditions.

Some relatively new approaches, such as the addition of an organic carbon source to the growth medium, have been proposed to increase the growth rates of microalgae (Ma et al., 2016). Generally, higher growth rates are correlated with higher N removal efficiency (Ji et al., 2013). Due to its affinity for  $\text{NH}_3$  and the reduced metabolic cost to convert  $\text{NH}_4^+$  to organic matter compared to other nitrogen forms, microalgae tend to grow faster in water with high  $\text{NH}_3$  content. However, concentrations in excess of  $110 \text{ mg L}^{-1}$   $\text{NH}_3$  can be toxic and have detrimental effects on growth rate by disrupting the thylakoid transmembrane proton gradient, which is vital in supporting microalgal photosynthesis (Salbitani and Carfagna, 2021; Zheng et al., 2019); however, some strains of microalgae, such as *Chlorella vulgaris* and *Scenedesmus obliquus*, have been shown to grow in  $\text{NH}_3$  concentrations of up to  $360 \text{ mg L}^{-1}$  (Collos and Harrison, 2014; Morales-Amaral et al., 2015).

Although laboratory and pilot-scale studies have been conducted to explore how algal ponds can be used to treat agricultural wastewater, limited studies have been conducted on its effectiveness for treating and removing nutrients at full scale. The varying concentrations of N and other elements in wastewater can largely affect the performance of microalgae-based wastewater treatment; therefore, supplementing with specific nutrients (C, N, P) may be required to achieve

optimal C/N and N/P ratios for enhanced N recovery (Su, 2021). For example, carbon in the form of  $\text{CO}_2$  is supplied to microalgal culture media to aid in the assimilation of inorganic N and P, an energy-intensive process that drives high operating costs (Mohsenpour et al., 2021). Maintaining the C/N and N/P ratios of the medium within an optimal range is specifically important to optimize biomass growth, which in turn affects nutrient removal and treatment efficiency. For example, cattle-slaughterhouse wastewater with a C/N ratio =  $49.6 \pm 9.4$  and an N/P ratio =  $6.7 \pm 3.8$  was found to be suitable for microalgal growth (Maroneze et al., 2014).

To make microalgal wastewater treatment techniques sustainable, it is necessary to maximize sunlight interception and to tailor the growth media specifically to the microalgae strain being cultivated (Acién Fernández et al., 2021). When comparing microalgae-based treatment to conventional wastewater treatment, there are lower energy demands, lower sludge production, reduced GHG emissions, and opportunities to convert biomass into useful products (table 2). Primary drawbacks of algal-based wastewater treatment include: high retention time (7 to 10 days); increased land use ( $10 \text{ m}^2$  of land per capita); the presence of competitive invasive species such as non-beneficial microalgae, parasites, and aquatic invertebrate predators; an inability to grow significant quantities of microalgae in highly turbid water due to low light penetration throughout the water column; and high harvesting costs (Nagarajan et al., 2020). Constructing algal ponds on marginal lands that are otherwise unsuitable for farming can mitigate the impacts arising from increased competition for arable land (Acién Fernández et al., 2021). Although synergistic relationships have been observed between microalgae and bacteria in wastewater, pilot studies have shown that the system can fail with excessive bacterial growth, resulting in competition for nutrients and a subsequent reduction in algal growth (Su, 2021). Microalgal wastewater treatment is considered an expensive option, mainly due to the harvesting operation, which accounts for a large share of the total production cost. This cost can be reduced with coagulation-flocculation and sedimentation systems to help settle microalgae (Matamoros et al., 2015). The high capital cost associated with the installation of microalgal-wastewater treatment systems can be mitigated in part by enhancing profits through measures that increase algal

**Table 2. Comparison of impacts from conventional, microalgae-based, and duckweed-based wastewater treatment systems.**

Criteria	Wastewater Treatment Impact		References
	Conventional	Microalgae- or Duckweed-Based	
N removal	Up to 99%	Up to 100% <sup>[m]</sup> ; up to 93% <sup>[d]</sup>	Henze, 1991 <sup>[c]</sup> ; McCarty, 2018 <sup>[c]</sup> ; Samori et al., 2013 <sup>[m]</sup> ; Li et al., 2019 <sup>[m]</sup> ; Costa et al., 2016 <sup>[d]</sup>
GHG emissions	High (0.005 to $0.8 \text{ kg CO}_2 \text{ eq. m}^{-3}$ )	Low ( $8.3$ to $14 \text{ g CO}_2 \text{ m}^{-3}$ ) <sup>[m]</sup> ; ( $1700$ to $3300 \text{ mg CO}_2 \text{ m}^2 \text{ d}^{-1}$ ) <sup>[d]</sup>	Gupta and Singh, 2012 <sup>[c]</sup> ; Monteith et al., 2005 <sup>[c]</sup> ; Alcántara et al., 2015 <sup>[m]</sup> ; Mohedano et al., 2019 <sup>[d]</sup> ; Sims et al., 2013 <sup>[d]</sup>
Land use	Low	High ( $5$ to $6.5 \text{ m}^2$ per capita) <sup>[m]</sup>	Acién Fernández et al., 2018 <sup>[m]</sup> ; Alcántara et al., 2015 <sup>[m]</sup>
Water demand	Low	High	Soñta et al., 2020 <sup>[d]</sup>
Energy demand	$0.3$ to $2.1 \text{ kWh m}^{-3}$	$0.02$ to $1 \text{ W m}^{-3}$ <sup>[m]</sup>	Capodaglio and Olsson, 2020 <sup>[c]</sup> ; Crawford and Sandino, 2010 <sup>[c]</sup> ; Pabi et al., 2013 <sup>[c]</sup> ; Alcántara et al., 2015 <sup>[m]</sup> ; Lopes et al., 2018 <sup>[m]</sup>
High-value products	Fertilizers, bioenergy	Fertilizers, animal feed, human food, biogas, biofuel feedstock	Cassidy, 1998 <sup>[c]</sup> ; Spolaore et al., 2006 <sup>[m]</sup> ; Cheng et al., 2019 <sup>[m]</sup> ; Leng 1999 <sup>[d]</sup> ; Calicioglu et al., 2019 <sup>[d]</sup>

<sup>[c]</sup> = Conventional wastewater treatment, <sup>[d]</sup> = duckweed-based wastewater treatment, and <sup>[m]</sup> = microalgae-based wastewater treatment.

biomass production, such as the use of greenhouses and wavelength filters (Kang et al., 2015).

### ***Duckweed-Based Wastewater Treatment***

Similar to microalgae, another sustainable technology to recycle N in the bioeconomy is to use duckweed to recover N from wastewater and subsequently use the harvested biomass to produce useful products. Duckweed is a free-floating aquatic plant in the Lemnaceae family with five genera and 36 known species (Bog et al., 2019). The macronutrient compositions of different duckweed species are similar, although the protein content can vary from 15% to 45% depending on the nutrient concentrations of the water in which the species are grown (Chantiratikul et al., 2010). When compared to microalgae, duckweed's enhanced effectiveness to treat wastewater is mainly attributable to its easy harvesting (Culley and Epps, 1973) and its ability to grow under a wide range of nutrient, temperature (5°C to 33°C), and pH (5.5 to 8.5) conditions (Ceschin et al., 2019). With a doubling rate of every 1 to 2 days, an initial duckweed mat covering an area of 10 cm<sup>2</sup> has the potential to cover up to 1 ha in less than 50 days (Leng, 1999). However, the rate at which duckweed grows and accumulates biomass can depend heavily on the pH, temperature, and nutrient concentrations in the growth media, as well as on the mat density, sunlight incidence, and day length.

Duckweed has been studied for removing N in swine, dairy, and municipal wastewaters, as well as dumpsite leachate and stormwater, among others (table 1). Like microalgae, which prefer NH<sub>4</sub><sup>+</sup> uptake over NO<sub>3</sub><sup>-</sup>, duckweed has an affinity for NH<sub>4</sub><sup>+</sup>, which is typically seen in high concentrations in agricultural wastewaters such as those coming from livestock farms (Nagarajan et al., 2019). Factors such as the state of N, temperature, pH, salts, metal concentrations, bacterial presence, and mixing of growth media can affect duckweed's nutrient removal rates (tables 1, A1, and A2). In treatment ponds, bacteria that become attached to duckweed fronds in the form of biofilm play a key role in increasing N removal through N fixation and aerobic degradation of complex compounds that make them easily available for plant uptake (Benjawan and Koottatep, 2007; Chen et al., 2019). Intermittent mixing of the growth media has been shown to promote nutrient removal, but excess mixing can deteriorate duckweed growth and nutrient uptake (Chaiprapat et al., 2003). Past studies demonstrated that 75% to 98% of total N, 81% to 92% of total P, 72% to 98% of NH<sub>3</sub>/NH<sub>4</sub><sup>+</sup>, and 57% to 92% of NO<sub>3</sub><sup>-</sup> can be removed from wastewater treated with duckweed (fig. 3). Although the maximum nutrient reductions were similar for microalgae and duckweed treatments, a wider range of removal rates was observed with duckweed, possibly due to the higher number of duckweed studies reviewed here. The differences in removal rates are also indicative of the wide range of growing conditions used in these studies, which can have a significant impact on overall nutrient uptake.

Duckweed has previously been shown to have resistance to high levels of macro- and micronutrients in its growth media; however, several studies have reported that high nutrient concentrations (in excess of 60 mg N L<sup>-1</sup>) can have negative impacts on duckweed growth (Iqbal and Baig, 2017; Soñta

et al., 2020). The optimum N concentration for supporting duckweed growth is around 60 mg L<sup>-1</sup>, which is within the concentration range of typical domestic wastewater sources, but far below many animal wastewaters (Ferreira et al., 2018). Although duckweed has better resistance to high nutrient concentrations compared to microalgae, both options require significant water demands for dilution, which increases the treatment costs (Soñta et al., 2020). For duckweed, N/P ratios of 4:1 to 5:1 have been found to be suitable for growth, but little work has been done to optimize the C/N and N/P ratios for maximum growth (Xu and Shen, 2011).

Through phytoremediation, duckweed can remove a wide range of contaminants, including agricultural chemicals (such as ammonium, nitrate, phosphate, 2,4-dichlorophenol, dimethomorph, and copper sulfate), nanomaterials (such as zinc oxide, alumina, and copper nanoparticles), and organic pollutants (such as petroleum hydrocarbons) (Ekperusi et al., 2020). Duckweed was able to remove up to 94% of BOD and COD, 63% to 87% of total suspended solids, 60% to 99% of total P, 35% to 87% of total dissolved solids, and 40% to 100% of heavy metals in studies across different scales (table 1). Duckweed's ability to effectively sequester up to three times more CO<sub>2</sub> than it emits (equaling 19,592 to 42,052 mg CO<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup>, as demonstrated in pilot-scale duckweed ponds) is particularly vital in addressing global warming (Mohedano et al., 2019). Studies are contradictory on whether duckweed-based wastewater treatment ponds are a source or a sink for CH<sub>4</sub> emissions due to the complex reactions that occur at the soil-water interface involving methane production by methanogens and oxidation by methanotrophs (Dai et al., 2015).

Pilot-scale and full-scale studies have been used to assess how duckweed can be used for sustainable wastewater treatment while documenting the associated challenges. Like microalgae, the ideal HRT to effectively treat wastewater using duckweed is too high (15 to 20 days) to make it profitable at full scale; therefore, technological advancements are needed to increase removal rates in these systems (Acién Fernández et al., 2018; Shi et al., 2010). The toughest challenge in making duckweed an effective treatment solution is its land use and dilution water requirement. With full-scale treatment ponds and lagoons, there is an added challenge of adopting an appropriate harvesting regime for reliable biomass recovery and ensuring that duckweed is the dominant organism in the water. Table 3 lists the ideal operating conditions for microalgae- and duckweed-based wastewater treatment systems, summarized from the past studies reviewed in this section.

### ***Applications of Wastewater-Grown Microalgae Biomass***

Biofuel has been effectively generated from microalgal biomass grown in swine and municipal wastewater (Ma et al., 2014, 2016; Zhu et al., 2013). Besides producing biogas (CH<sub>4</sub> and CO<sub>2</sub>) through anaerobic digestion, digestate from microalgal biorefineries has the potential to be used as a soil amendment in place of synthetic fertilizers (Préat et al., 2020). When used as organic fertilizer, microalgae can prevent nutrient leaching by slow release of N and P, and can even result in higher crop yields (Coppens et al., 2016). Due

**Table 3. Comparison of ideal operating conditions and variables affecting nutrient removal in microalgae- and duckweed-based wastewater treatment systems.**

Variable	Wastewater Treatment System		References
	Microalgae-Based	Duckweed-Based	
Typical hydraulic retention time	7 to 10 days	15 to 20 days	Nagarajan et al. (2020); Shi et al. (2010)
Optimal temperature	15°C to 30°C	5°C to 33°C	van Esbroeck (2018)
Optimal pH	7 to 9	5.5 to 8.5	van Esbroeck (2018)
Biomass doubling rate	<1 day	1 to 2 days	Acien Fernández et al. (2021); Leng (1999)
Biomass yield	100 ton dry mass ha <sup>-1</sup> year <sup>-1</sup>	73 to 180 ton dry mass ha <sup>-1</sup> year <sup>-1</sup>	Acien Fernández et al. (2021); Leng (1999)
Optimal C/N ratio	49.6 ± 9.4	-	Maroneze et al. (2014)
Optimal N/P ratio	6.7 ± 3.8	4 to 5	Maroneze et al. (2014); Xu and Shen (2011)
Ammonia toxicity level	>110 mg NH <sub>4</sub> <sup>+</sup> -N L <sup>-1</sup>	>60 mg NH <sub>4</sub> <sup>+</sup> -N L <sup>-1</sup>	Salbitani and Carfagna (2021); Soñta et al. (2020)

to the high lipid content of some microalgae, it can be converted to biodiesel (Samori et al., 2013).

Microalgae have extensive applications in the food, feed, and health sectors. Within the last 50 years, the production of microalgae has increased due to its application in biochemicals, nutraceuticals, human nutrition, aquafeed, and biofertilizers (Spolaore et al., 2006). Microalgae have a high protein content and an essential amino acid composition similar to soybean and egg, making them suitable to feed humans, livestock, and fish (Bleakley and Hayes, 2017). They can be substituted for 5% to 10% of poultry feed and 33% of pig feed without causing any adverse health effects; replacing 1% to 5% of fish diet with microalgae is even shown to promote health and aid in early growth (Acien Fernández et al., 2021; Spolaore et al., 2006). Based on their high nutritional value and availability, microalgae can be used in diets for malnourished people around the world (Christaki et al., 2011). Major limitations to future research on microalgal applications include their high extraction cost and the lack of widespread public awareness on the health benefits of microalgae (Koyande et al., 2019).

Few studies have been completed on seaweed (a macroalgae) as an additional way to effectively complete the circular N-bioeconomy. Similar to microalgae and duckweed, seaweed can remove N from water and has a variety of applications in the food, energy, and agricultural sectors. Bioethanol, liquid fertilizers, and fish feed have been produced using seaweed biomass in pilot-scale and full-scale studies (Seghetta et al., 2016). Seaweed also has nutraceutical, food, and neuroactive agent applications (Barbosa et al., 2020). However, seaweed-based wastewater treatment projects are still in their preliminary stages and need additional studies to measure their feasibility and the biomass availability for large-scale use.

### ***Applications of Wastewater-Grown Duckweed Biomass***

Several valuable uses of duckweed biomass grown on wastewater have been explored in the past. The use of natural soil amendments that are produced by upcycling nutrient-rich duckweed provides an economical and sustainable alternative to existing synthetic inorganic fertilizers, which require costly and energy-intensive processes using atmospheric N (e.g., producing ammonia fertilizer using the Haber-Bosch process; Walsh et al., 2012). The potential effectiveness of duckweed as a replacement for conventional fertilizers is primarily attributed to its high N content and increased ability to retain that N in the soil (Kreider et al.,

2019; Ma et al., 2015). Along with N runoff into streams during rain events, NH<sub>3</sub> volatilization typically accounts for a significant portion of N loss in agriculture (Saggar et al., 2013); however, pairing duckweed with chemical fertilizer has been shown to significantly reduce NH<sub>3</sub> volatilization by 36% to 52% and added 10% to 11% overall economic benefit in rice fields compared to chemical fertilizer alone (Yao et al., 2017). The N and P bound within the duckweed biomass make it an ideal slow-release fertilizer and help retain the nutrients in the soil, effectively reducing nutrient runoff and pollution (Fernandez Pulido et al., 2021). In efforts to advance the circular bioeconomy, the use of other aquatic plants, such as seaweed, have been explored as soil amendments, especially for grain crops that have high N demands such as wheat, maize, and rice (Sadeghi et al., 2018).

Using duckweed grown on agricultural wastewater for bioenergy production is another approach to recycle otherwise untreated waste and close the N-bioeconomy cycle. Duckweed has potential for ethanol production due to its high starch content when grown on low-nutrient waters (Calicioglu et al., 2019; Cheng and Stomp, 2009). Using sequential fermentation and anaerobic digestion processes, an ethanol yield of 0.07 to 0.15 g ethanol and 328 to 390 mL CH<sub>4</sub> per gram of total solids was achieved with dried duckweed grown on treated wastewater, which was higher than lignocellulosic crops (such as straw) and within the range reported for starch crops (such as corn and potatoes) (Calicioglu and Brennan, 2018). After anaerobic digestion of duckweed to produce CH<sub>4</sub>, the resulting digestate can be used as an agricultural fertilizer (Calicioglu et al., 2019). A techno-economic analysis and LCA of a hypothetical integrated wastewater-derived duckweed biorefinery indicated that duckweed pond construction and operation account for the majority of capital and operating expenses, and that vertical farming options should be investigated to reduce the detrimental impacts of land use (Calicioglu et al., 2021). One of the most important applications of duckweed in agriculture is its use as feed for livestock and aquaculture. In addition to being a key protein source, duckweed can successfully accumulate microminerals such as potassium, calcium, magnesium, sodium, and iron, which are typically not present in adequate quantities in the livestock feed available to small-scale farmers (Leng et al., 1995). In Vietnam, duckweed farming has been practiced for many years, and duckweed grown on ponds with diluted manure and human waste is fed to ducks after mixing with cassava peelings (Leng, 1999). With overall protein production rates at 10.1 tons ac<sup>-1</sup> year<sup>-1</sup>,



duckweed can produce edible proteins 6 to 10 times faster than soybeans per area (Landesman et al., 2005; Roman and Brennan, 2019). Under optimal growing conditions, annual duckweed yield can range from 73 to 180 ton dry matter ha<sup>-1</sup> year<sup>-1</sup>; however, even less than optimal conditions can still provide an yield of 5 to 20 ton dry matter ha<sup>-1</sup> year<sup>-1</sup> (Leng, 1999). This is noticeably higher than the average yield for soybean (2.8 metric ton ha<sup>-1</sup>), which is conventionally used as a source of feed protein in livestock farms (Purdy and Langemeier, 2018), and on par or greater than the 2 to 100 ton dry matter ha<sup>-1</sup> year<sup>-1</sup> achievable with microalgae (Acién Fernández et al., 2021). In aquaponics, both fresh and dried duckweed have been shown to be effective feed in the production of fishes such as carp and tilapia (Skillicorn et al., 1993).

Although the potential for the use of duckweed in animal feed is high, some researchers have suggested adding only a small fraction of duckweed to existing feeds until further research is conducted on optimal inclusion rates so that any potential negative effects can be identified. A few feeding experiments conducted with duckweed on pigs, poultry, ruminants, and fish indicated that duckweed can be used as protein feed for these animals without any severe impact on health (Cheng and Stomp, 2009; Hamid et al., 1993). However, other studies reported decreased weight gain and low intake of feed when duckweed was added to animal diets (Soñta et al., 2019). This discrepancy in experimental outcomes can most likely be attributed to the fact that duckweed species and growth media composition can highly influence the nutritional quality of the resulting duckweed biomass (Roman et al., 2021). A study by Haustetn et al. (1990) on the potential of duckweed to replace soybean meal in poultry concluded that *Lemna* and *Wolffia* species are as good as soybean as a source of essential amino acids and have no effect on egg production. In ruminant animals, duckweed has a beneficial role in providing highly soluble and readily fermentable protein, with 80% to 94% rumen degradation observed with proteins in *Spirodela*, *Lemna*, and *Wolffia* species (Huque et al., 1996). A recent feeding trial conducted on mice demonstrated that replacing up to 25% of dietary casein protein with duckweed protein had no adverse effect on growth and organ development (Roman et al., 2021). Additional research focusing on the effect of a duckweed-supplemented diet on animal health and organ development, giving due importance to the type of duckweed used, is necessary to evaluate the feasibility of its large-scale application and to increase farmer confidence in using duckweed as animal feed.

In addition to protein, duckweed has high amounts of antioxidants that can be especially useful when incorporated into human diets (Soñta et al., 2020). Due to its ability to accrue micronutrients such as iodine, duckweed can be used in human diets to alleviate the problem of malnutrition in countries around the world (Vladimirova and Georgiyants, 2014). Duckweed's role in controlling mosquito populations has also been studied to some extent, with certain species such as *Lemna minor* being reported to release compounds that repelled female mosquito's oviposition and affected larval development in mosquitos (Eid et al., 1992; Marten et

al., 1996). This can have a widespread impact on public health in many regions around the world that are especially vulnerable to mosquito-borne diseases. Advances in duckweed genomics have resulted in three different genomes sequenced to date (*S. polyrhiza* 9509, *L. minor* 5500, and *W. australiana* 8730) (Acosta et al., 2021). Genomic studies open up a wide range of opportunities within the plant microbiology community by providing valuable information on species identification and traits present in these species. Moving forward, techniques such as gene editing and genetic transformations can be used to identify duckweed lines with superior traits that are most effective in nutrient recovery and useful in beneficial downstream applications such as combating malnutrition, controlling mosquito populations, and serving as a sustainable alternative to conventional fertilizers, feeds, and fuels.

## FUTURE TRENDS AND CHALLENGES

### *Localized Sustainable Production of Feed and Fertilizer*

The growing demand for animal-derived food products and the extensive use of conventional animal feed such as corn and soybean have caused the current livestock production system to become unsustainable. Alternate feed materials are therefore required to overcome this challenge and to transition from a linear to a circular system in the livestock industry. The potential of using algae and duckweed as animal feed has already been studied to some extent, as discussed in the preceding sections. Compared to the fish and soy sectors that produce 7000 kt year<sup>-1</sup> of fish-based feed and 200,000 kt year<sup>-1</sup> of soy-based feed (costing \$1.8 and \$0.6 per kg, respectively), microalgae production is still a small-scale industry, producing 100 kt year<sup>-1</sup> biomass, and is an expensive feed option, costing \$17 to \$30 per kg (Acién Fernández et al., 2021).

Importing feed products from off-site leads to increased expenses for farmers and greater GHG emissions compared to on-farm feed production (Sasu-Boakye et al., 2014). In agriculture, especially dairy farms, developing an integrated on-farm wastewater treatment and N recovery practice by growing protein-rich aquatic vegetation on diluted manure could result in sustainable localized feed production for the livestock. Another pathway to recycle the N contained in manure-grown algae or duckweed is to use them as fertilizer alternatives for crops or as amendment materials to improve soil fertility. This approach would be especially useful on large-scale farms consisting of mixed livestock and cropping systems if the algae- or duckweed-based fertilizers are processed on-site and applied to crop fields on the same farm. Such an on-site system would increase farm profits by decreasing feed and fertilizer imports and transportation requirements, and it would provide a more environmentally friendly option by reducing GHG emissions and overall carbon and water footprints (Sasu-Boakye et al., 2014). Considering the low nutrient content of microalgal biofertilizers (<5% N and <1% P), a better way to use microalgae may be as a fertilizer additive or biostimulant, which has been shown to reduce chemical fertilizer use by >10% at very low dosages of 2 L ha<sup>-1</sup> (Acién Fernández et al., 2021).

### ***Integrated Treatment and Biorefinery Systems for Farm Wastewater***

Research focusing on integrated models that combine microalgae- or duckweed-based domestic wastewater treatment and biorefinery systems has gained major attention in recent years, especially with the growing trend to transition from fossil fuels to renewable energy sources (Calicioglu et al., 2019; Nagarajan et al., 2019). However, this approach still needs to be studied in detail for biomass grown on agricultural wastes. In addition to offering a promising sustainable solution by upcycling farm wastewater nutrients into biomass, these approaches can help curb the long-term issue of food/feed versus fuel competition arising from the conventional use of corn for producing biofuel. Biorefineries based on wastewater-grown microalgae and duckweed are largely in their initial stages, with several processes and technologies still being developed.

### ***Sustainable Protein Sources for Humans***

Animal-derived protein currently accounts for approximately 45% of total human protein consumption, and this share is expected to increase significantly by 2050 (Boland et al., 2013). Human consumption of animal-based proteins is increasing at a high rate, aggravating global warming and creating a need for alternative plant-based protein substitutes. A report by the United Nations Food and Agricultural Organization (FAO) estimated that global livestock production releases 7.1 gigatonnes of CO<sub>2</sub> equivalent per year, accounting for 14.5% of anthropogenic GHG emissions in the form of CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O, and these emissions are expected to increase substantially in the coming years (Gerber et al., 2013). Animal-derived metabolic waste further contributes to other environmental impacts such as eutrophication, acidification, and GHG emissions (Wu et al., 2014). Livestock production also causes land use change impacts and subsequent soil erosion, with deforestation typically accounting for 85% of livestock-related GHG emissions (FAO, 2006). According to the FAO, 26% of the world's ice-free land is used for livestock grazing, and one-third of the arable land is used for cultivating livestock feed. A shift to a low-meat diet and plant-based proteins is recommended not only to alleviate the environmental impacts discussed above but is also beneficial for human health (Appenroth et al., 2018; Koyande et al., 2019). Edible versions of seaweed have long been consumed by people in the Asia-Pacific region but have recently gained popularity in other parts of the world, such as Europe. The global seaweed cultivation market is projected to be worth USD \$30.2 billion by 2025 (MarketsandMarkets, 2021). Duckweed and microalgae have been consumed in the past, predominantly by people in developing regions, but are now increasing in popularity as sustainable food sources in developed countries (Appenroth et al., 2018; Kusmayadi et al., 2021). Duckweed's ability to accumulate toxic heavy metals (such as cadmium, nickel, and lead) and carcinogens (such as arsenic) warrant careful monitoring and treatment technologies to curb excessive accumulation of these chemicals in the food chain (Khan et al., 2020). Similar to other vascular plants, duckweed has the potential to adsorb microplastics in its fronds and roots, which when consumed by humans can cause long-term

harmful health effects. Pretreatment methods such as density-driven separation, flocculation, and sedimentation, which can remove up to 88% of the microplastics in wastewaters, may be used in conjunction with duckweed-based wastewater treatment if high levels of microplastics are identified in the growth media (Vivekanand et al., 2021). Given that the nutritional composition and protein accumulation of algae and duckweed depend heavily on the growth media, the concept of growing them on wastewater merits further research to evaluate their nutritional value and safety for human consumption.

### ***Challenges in the Circular N-Bioeconomy***

The two biggest challenges in using wastewater-grown algae or duckweed to advance the circular N-bioeconomy are: (1) the production costs associated with cultivation and frequent harvesting, and (2) the sociological resistance to consuming vegetation grown on wastewater. The high production costs can be addressed to a great extent by implementing this approach on a large scale and producing a combination of valuable products, such as animal feeds, protein supplements, and crop fertilizers. Pond construction accounts for a major share of the production costs associated with duckweed-based biorefinery models (Calicioglu, 2018). Constructing the ponds on land inappropriate for agricultural purposes will avoid major competition for arable land (Kreider, 2015). Further, the emerging trend of vertical farming (using stacked trays of plants in growth media illuminated with LED lighting) can significantly reduce the land requirements for duckweed cultivation, which is anticipated to make the system more economical and sustainable compared to the typical pond-grown approach (Roman and Brennan, 2021).

The circular bioeconomy is heavily dependent on the availability of ample biomass to produce bio-based energy and products, especially for large-scale systems. For instance, in Belgium, the implementation of innovative conversion technologies to produce fertilizers and other valuable products from bio-based products was constrained by the lack of sufficient biomass (Maes and Van Passel, 2019). The logistical aspects related to the collection and transport of biomass products should be given high importance in a biorefinery system because they are a direct measure of the operating costs as well as the environmental impact in terms of carbon emissions (Ubando et al., 2020). Using pipes for pumping instead of ground transport for conveying biomass material (such as duckweed), and using natural sun-drying methods, are ways to encourage sustainability in this context. Studies in Vietnam have demonstrated that duckweed can be successfully used on small-scale farms, and that a major share of the costs derived from drying and transporting the duckweed can be mitigated by using inexpensive sun-drying methods (Leng, 1999).

### ***Supply Chain Model***

A robust supply chain must be designed for microalgae- and duckweed-based wastewater treatment systems to be both economical and sustainable (Mohseni and Pishvae, 2016). Considering that these systems have the potential to influence multiple sectors, such as energy, agriculture, and food processing, an efficient supply chain model is essential

to upscale locally developed practices to the national level and to eventually enter global markets. Inevitably, the end-use products of these systems should substitute for existing products (e.g., generating duckweed-based biofuel instead of petroleum-based fuel, substituting existing chemical fertilizers with duckweed-based soil amendments, supplementing livestock diets with duckweed-based proteins instead of soybeans, etc.). Systematically designing the supply chain to make the byproducts and end-use products available to consumers is equally important to making the system resilient. In addition, optimizing the processes and products in the entire value chain is required to develop a system that is cost-effective, beneficial to society, and has minimal environmental impacts. This provides increasing opportunities to use multi-scale modeling tools, optimization methods, and LCA to help policymakers and other stakeholders quantify the benefits and risks, and make decisions regarding the emerging practices within the circular N-bioeconomy.

### Policy Interventions and Socio-Economic Development

Effective policies have to be designed to encourage investments in technologies and products that advance the circular N-bioeconomy (Maes and Van Passel, 2019). Additionally, subsidizing the products and offering economic and social incentives for processing and/or using the products will make the production system more profitable. For instance, providing economic incentives for growing duckweed on manure waste and re-using it as feed or fertilizer would encourage more farmers to implement this technique, which would have a critical influence on the entire duckweed market. These incentives will help overcome the cultural resistance of farmers to cultivating duckweed instead of traditional crops and encourage farmers to develop the skills required to implement such integrated farming systems, which is usually a major constraint in establishing these practices. Additionally, supporting the development of

a local duckweed market, as in Vietnam, will be useful for promoting duckweed as a cash crop and encouraging farmers in rural communities to engage in duckweed farming (Leng, 1999). Creating more revenue streams through successful policy implementation will attract more private and public investments in the near-term and long-term. Environmental externalities (i.e., uncompensated environmental effects of production and consumption of a particular product) have to be incorporated into the true market pricing of the emerging alternative products to achieve reasonable profits and to run the system sustainably.

Designing new methods to reuse microalgae or duckweed grown on agricultural wastewater would influence the current livestock and fertilizer markets and expand the sustainable food, feed, and energy markets. The market for algae products is projected to grow by 5.2% from 2016 to 2023. With more use in cosmetics and natural colorants, the compounded annual growth rate of a single algal species (*Spirulina* spp.) is expected to be 10% by 2026, with a market value of USD \$2 billion (Credence Research, 2017). The microalgae market in particular is currently valued at 50 million euros and is predicted to be worth 70 million euros by 2025 (Acién Fernández et al., 2021). Emerging applications of microalgae, in addition to biofuel, include the production of biomaterials, biofertilizers, biostimulants, and biopesticides (Acién Fernández et al., 2021). Market expansion of wastewater treatment and biomass production technologies using aquatic vegetation would create more job opportunities and improve the rural economy, allowing further research into developing sustainable products and methods in agricultural systems. Socio-economic and techno-economic analyses would provide further insights into the long-term social and economic impacts triggered by these systems. Figure 4 summarizes the economical, socio-cultural, political, environmental, and technological challenges and benefits linked to using aquatic vegetation for fostering a circular N-bioeconomy in agricultural systems.

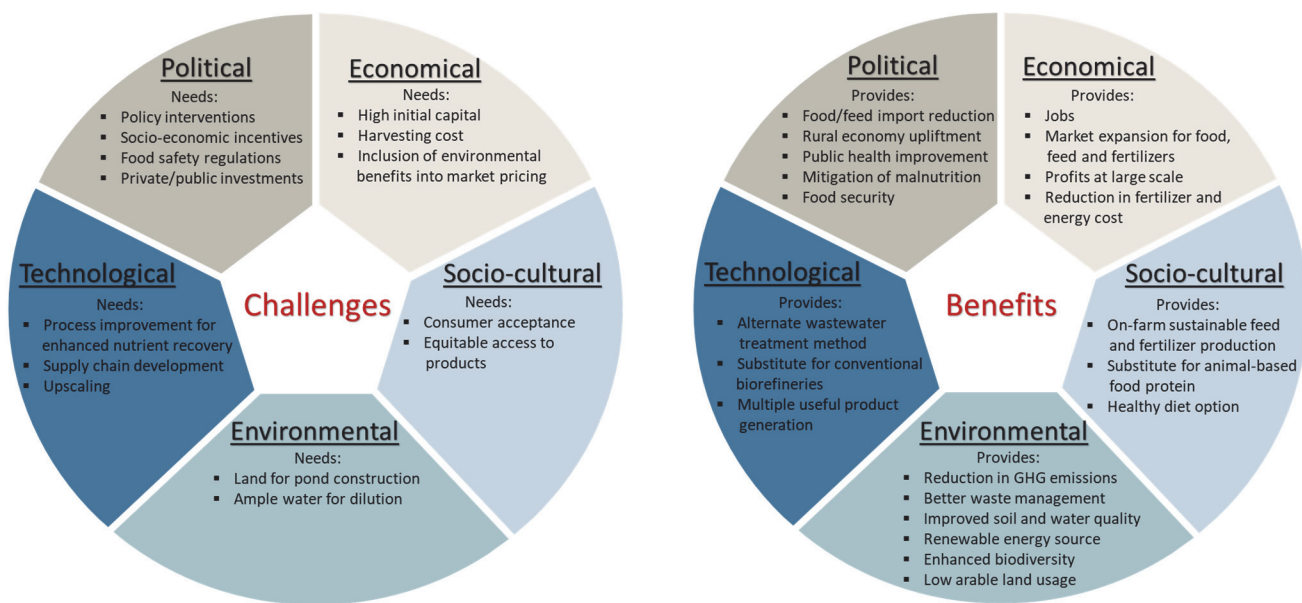


Figure 4. Challenges and benefits associated with using aquatic vegetation for wastewater treatment in the circular N-bioeconomy.

## CONCLUSIONS

Growing either microalgae or duckweed on manure and agricultural runoff and subsequently using the harvested plant biomass for the production of biofuels, animal feed, or soil amendments provides a promising opportunity to recycle N and promote a circular N-bioeconomy in agricultural systems. However, its ease of harvesting and its tested ability to grow under a wider range of environmental conditions give duckweed some advantages over microalgae. Although more than half of the reviewed studies used microalgae and duckweed for municipal or industrial wastewater treatment, there is a growing trend toward using this approach for capturing nutrients in livestock manure, which has promising potential. With a capacity of greater than 90% nitrate and ammonia removal, various applications of these aquatic organisms are being explored in the form of biofeedstocks, fertilizers, animal feed, and human food as a way to transition from a linear to a circular bioeconomy. Additional in-depth experimental trials are required to fully understand the nutrient interactions, uptake dynamics, and toxicity risks in microalgae- and duckweed-based wastewater treatment systems. LCA studies and techno-economic analyses specifically focusing on agricultural wastewater treatment are necessary to evaluate the environmental impacts and economic feasibility of using these technologies in the agricultural sector. With the help of effective policies and technological advancements, several of the political, socio-cultural, and infrastructural challenges that hinder large-scale implementation of these sustainable practices can be overcome.

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## APPENDIX

The literature review was performed using the Web of Science database (<https://www.webofknowledge.com>) by finding articles with keywords “duckweed”, “microalgae”, “bioeconomy”, “nutrient removal”, and “biomass production”. From the extensive list of studies, we shortlisted those in which microalgae and duckweed were used to treat

agricultural, municipal, and industrial wastewater. Studies published between 1995 and 2020 are included in the review. Tables A1 and A2 show the complete list of selected studies for microalgae and duckweed systems, respectively. For the in-depth review, only studies focusing on agricultural wastewater treatment were used (highlighted in tables A1 and A2 and listed in table 1).

**Table A1. Nutrient removal and biomass production by microalgae in wastewater treatment systems.**

Wastewater Type	Scale	Location	Species	Experimental Conditions/Variables	Results	Reference
Poultry, swine, brewery, cattle, dairy, and urban wastewater	Lab	Lisbon, Portugal	<i>Scenedesmus obliquus</i>	Pretreated cattle, dairy, and brewery wastewater	95% to 100% TN removal; 63% to 99% PO <sub>4</sub> <sup>3-</sup> removal; Biomass produced with 31% to 53% protein content, 12% to 26% sugars, and 8% to 23% lipids	Ferreira et al. (2018)
Effluent of wastewater reclamation facility	Lab	Emilia-Romagna Italy	<i>Desmodesmus communi</i> and algal consortium	Batch cultures in varying N/P ratios	Almost 100% removal of NH <sub>3</sub> and P	Samori et al. (2013)
Dairy wastewater	Lab	Bhavnagar, Gujarat, India	<i>Acutodesmus dimorphus</i>	Untreated dairy wastewater; very low NO <sub>3</sub> <sup>-</sup> concentration	100% NO <sub>3</sub> <sup>-</sup> removal within 4 days; 100% NH <sub>3</sub> removal within 6 days; 1 kg biomass is theoretically calculated to produce up to 273 g of biofuels	Chokshi et al. (2016)
Dairy wastewater	Lab	George Town, Penang, Malaysia	Algal consortium: <i>Chlorella saccharophila</i> UTEX 2911, <i>Chlamydomonas pseudococcum</i> UTEX 214, <i>Scenedesmus</i> sp. UTEX 1589, and <i>Neochloris oleoabundans</i> UTEX 1185	Wastewater from collecting and holding tanks of dairy farm; three different CO <sub>2</sub> concentrations, irradiance of 80 mmol m <sup>-2</sup> s <sup>-1</sup> , 12 h daylength, for 10 days	98% TKN removal; 99% NH <sub>3</sub> removal; 86% NO <sub>3</sub> <sup>-</sup> removal	Hena et al. (2015)
Activated sludge effluent	Lab	Shandong Province, China	<i>Chlorella pyrenoidosa</i>	Varying pH in different seasons	76% to 84% NH <sub>3</sub> removal at pH 5.7-6.5; 73% to 77% NH <sub>3</sub> removal at pH 6.8-7.3; 75% to 86% NH <sub>3</sub> removal at pH 7.6-8.1; 60% to 96% NH <sub>3</sub> removal at pH 8.3-8.8	Tan et al. (2016)
Swine wastewater	Lab	Fuzhou, China	<i>Chlorella vulgaris</i>	12 days	90.51% TN removal and 91.54% TP removal	Wen et al. (2017)
Simulated domestic wastewater	Lab	Zhejiang Province, China	<i>Chlorella vulgaris</i>	Artificial wastewater made using glucose and sodium acetate (NaAc); comparative study under photoautotrophic and mixotrophic conditions	63.5 and 55.2 mg L <sup>-1</sup> d <sup>-1</sup> biomass with glucose and NaAc, respectively; highest lipid content (17.35 mg L <sup>-1</sup> d <sup>-1</sup> ) with glucose; highest carbohydrate content (18.75 mg L <sup>-1</sup> d <sup>-1</sup> ) with NaAc	Peng et al. (2019)
Domestic, sewage, paper mill, and dairy wastewaters	Lab	Bhagwanpur, Uttarakhand, India	<i>Chlamydomonas debaryana</i> IITRIND3	Light intensity of 80 mmol m <sup>-2</sup> s <sup>-1</sup> , 16 h photoperiod, for 10 days	Maximum lipid productivity (87.5 ± 2.3 mg L <sup>-1</sup> d <sup>-1</sup> ) in dairy wastewater, with 87.56%, 82.17%, 78.57%, and 85.97% removal of TN, TP, COD, and total organic carbon, respectively	Arora et al. (2016)
Domestic wastewater	Lab	Busan, Korea	<i>Chlorella vulgaris</i>	Mixotrophic cultivation comparing carbon sources: glucose, glycerol, and acetate	Under optimal condition (5 g L <sup>-1</sup> glucose): 0.13 g L <sup>-1</sup> d <sup>-1</sup> biomass productivity with 19.29% total lipid, 41.4% carbohydrate, and 33.06% proteins; 96.9%, 65.3%, and 71.2% removal of COD, TN, and PO <sub>4</sub> <sup>3-</sup> , respectively	Gupta et al. (2016)
Swine wastewater	Lab and computer model	Waseca, Minnesota	<i>Chlorella</i> sp.	Optimizing dilution rate and HRT	Modeled optimal biomass yield and N removal at 2.26-day HRT and 8-fold dilution rate; experiment removal rates of 38.4 mg L <sup>-1</sup> d <sup>-1</sup> of TN and 60.4 mg L <sup>-1</sup> d <sup>-1</sup> of NH <sub>3</sub>	Hu et al. (2013)
Synthetic municipal wastewater	Lab	Texas	<i>Chlorella vulgaris</i>	Addition of crude glycerol	Lipid accumulation under alkaline conditions because triacylglycerols are derived from glycerol and fatty acids	Ma et al. (2016)
Urban wastewater	Pilot	Barcelona, Spain	<i>Stigeoclonium</i> sp., <i>Chlorella</i> sp., and <i>Monoraphidium</i> sp.	Examining effect of HRT and seasonality on removal efficiency of organic microcontaminants; two high-rate algal ponds at 4 d and 8 d HRT	Removal efficiencies range from 0% to 99%; Highest removal (>90%) in caffeine, acetaminophen, ibuprofen, methyl dihydrojasmonate, and hydrocinnamic acid	Matamoros et al. (2015)

**Table A2. Nutrient removal and biomass production by duckweed in wastewater treatment systems.**

Wastewater Type	Scale	Location	Species	Experimental Conditions/Variables	Results	Reference
Dumpsite leachate	Lab	Rawalpindi, Pakistan	<i>Lemna minor</i>	10% leachate dilution	95% uptake of TN; 380 mg N m <sup>-2</sup> d <sup>-1</sup> removal rate; 6.4 g m <sup>-2</sup> d <sup>-1</sup> highest growth rate	Iqbal et al. (2019); Iqbal and Baig (2017)
Stormwater	Lab	Columbia, Missouri	<i>Lemna minor</i>	10-day HRT	94% NH <sub>3</sub> removal; 87% NO <sub>3</sub> <sup>-</sup>	Dai et al. (2015)
Swine wastewater	Lab	Shanghai, China	<i>Spirodela oligorrhiza</i>	Two-week harvest and 6% wastewater to 94% tap water	83.7% TN removal and 89.4% TP removal	Xu and Shen (2011)
Swine wastewater	Lab	Ribatejo, Portugal	<i>Lemna minor</i>	12 h light cycle, pretreated swine wastewater at 4% dilution	74% NH <sub>3</sub> removal; 0.14 g m <sup>-2</sup> d <sup>-1</sup> TN removal	Pena et al. (2017)
Diluted swine effluent	Lab	Armidale, Australia	<i>Spirodela</i> spp.	Different N levels in growing media	Crude protein content increases from 15% at 1 to 4 mg N L <sup>-1</sup> to 37% at 10 to 15 mg N L <sup>-1</sup> ; toxic effect above 60 mg N L <sup>-1</sup>	Leng et al. (1995)
Effluent and digested slurry of biorefinery processing cattle slurry	Lab	Hengelo, Netherlands	<i>Lemna minuta</i>	Various concentrations of effluent from biorefinery and digested slurry	75% TN removal; 81% TP removal; higher concentrations had toxic levels of sodium and potassium	Soñta et al. (2020)
Leachate		Rawalpindi, Pakistan	<i>Lemna gibba</i>	Leachate processed using solid waste collected from residential, commercial, and industrial areas	95% N uptake and 90% P uptake; 6.4 g m <sup>-2</sup> d <sup>-1</sup> peak growth rate	Iqbal an Baig (2017)
Tap water with metal loads	Lab	Milan, Italy	<i>Lemna gibba</i>	Varying metal concentrations, 24 h photoperiod	Growth performance not affected at high organic loading of iron (<20 mg L <sup>-1</sup> ), zinc (<20 mg L <sup>-1</sup> ), and aluminum (<30 mg L <sup>-1</sup> ); toxic levels of chromium at >0.1 mg L <sup>-1</sup> and copper at >0.1 mg L <sup>-1</sup>	Boniardi et al. (1999)
Hoagland solution with NaCl	Lab	Tianjin, China	<i>Lemna minor</i>	Varying NaCl concentrations from 0 to 100 mM cultured for 24 and 72 h	Withstand salt stress up to 75 mM NaCl; >100 mM NaCl cause release of nutrients and growth stops	Liu et al. (2017)
Lab-made concentrations of metals	Lab	Samsun, Turkey	<i>Lemna minor</i>	Varying metal concentrations	40% to 100% removal of lead, chromium, zinc, copper, and cadmium	Üçüncü et al. (2013); Üçüncü Tunca et al. (2017); Vaseem and Banerjee (2015)
Mixture of textile, distillery, and domestic wastewater	Lab	Ethiopia	<i>Lemna minor</i>	28-day batch system; comparative study with <i>Azolla filiculoides</i>	94.7%, 96.7%, 92.0%, 91.5%, and 78.0% removal of TN, TP, COD, BODs, and sulfate (SO <sub>4</sub> ), respectively; removal of Cd, Cr, Ni, and Cu below detection limit; removal of Co, Zn, Fe, and Mn by 72%, 91%, 80%, and 89%, respectively	Amare et al. (2018)
Paper mill wastewater	Lab	Rayagada District, Orissa, India	<i>Lemna minor</i>	Comparing duckweed to species of water weed, water primrose, water lettuce, water hyacinth, water chestnut	91.36%, 92.12%, 92.10%, 86.89%, 92.82% 92.25%, 93.91%, 91.94%, 91.32% 66.48%, and 71.42% removal of NO <sub>3</sub> <sup>-</sup> , phosphate (PO <sub>4</sub> <sup>3-</sup> ), conductivity, TSS, TDS, BOD, COD, SO <sub>4</sub> <sup>2-</sup> , K, Hg, and Cu, respectively; <i>Lemna minor</i> had highest removal rate in 8 of 11 categories	Mishra et al. (2013)
Upflow anaerobic sludge blanket reactor effluent from treating industrial wastewater sediment	Lab	Adiyaman, Turkey	<i>Lemna minor</i>	Diluted effluents to achieve COD of 1000 mg L <sup>-1</sup>	96%, 94%, 97%, 95%, 83%, and 88% removal of NH <sub>3</sub> , TKN, TP, PO <sub>4</sub> <sup>3-</sup> , BOD <sub>5</sub> , and COD, respectively; over 98% removal of Zn, Al, Cd, Co, Cu, Pb, and Ni; over 90% removal of As and Cr; 83% Hg removal	Tufaner (2020)
Domestic wastewater	Pilot	Ranchi, India	<i>Lemna minor</i>	Examining effect of pH on growth and nutrient removal	94.45% BOD removal, 79.39% orthophosphate removal; optimum pH range of 7 to 8	Priya et al. (2012)
Mixture of domestic and agricultural wastewater	Pilot	Kunming, China	<i>Lemna japonica</i> 0234	Comparative study with water hyacinth ( <i>Eichhornia crassipes</i> )	60% recovery of N over a year; 0.4 g m <sup>-2</sup> d <sup>-1</sup> TN removal	Zhao et al. (2014)
Municipal wastewater	Pilot	Khlong Nueng, Thailand	Mixture of <i>Lemna minor</i> and <i>Wolffia arrhiza</i>	Two different N loadings	At TN loading of 1.3 g <sup>-1</sup> m <sup>-2</sup> d <sup>-1</sup> , 75% TN, 89% TKN, and 92% NH <sub>3</sub> removal; at TN loading of 3.3 g <sup>-1</sup> m <sup>-2</sup> d <sup>-1</sup> , 73% TN, 74% TKN, and 76% NH <sub>3</sub> removal	Benjawan and Kootatep (2007)

**Table A2 (continued). Nutrient removal and biomass production by duckweed in wastewater treatment systems.**

Wastewater Type	Scale	Location	Species	Experimental Conditions/Variables	Results	Reference
Mixture of domestic and agricultural wastewater	Pilot	Kunming, China	<i>Lemna japonica</i> 0234	Combining duckweed and carrier biofilm	19.97% higher TN removal and 15.02% higher NH <sub>3</sub> removal with duckweed	Zhao et al. (2015)
Domestic wastewater	Pilot	Birzeit, West Bank Palestine	<i>Lemna gibba</i>	Comparative study with algae and duckweed; 28-day HRT	NH <sub>3</sub> volatilization: 7.2 to 37.4 mg N m <sup>-2</sup> d <sup>-1</sup> with algae and 6.4 to 31.5 mg N m <sup>-2</sup> d <sup>-1</sup> with duckweed	Zimmo et al., 2000
Septic tank wastewater	Full	Thessaloniki City, Greece	<i>Lemna minor</i>	Year-long study comparing pollutant removal efficiency during warm and cold seasons	Average of 72%, 94%, 63%, 99.65%, and 91.76% removal of NH <sub>3</sub> , BOD <sub>5</sub> , TSS, <i>E. coli</i> , and <i>Enterococci</i> , respectively	Papadopoulos and Tsihrintzis (2011)
Septic tank wastewater	Full	Thessaloniki City, Greece	<i>Lemna minor</i>	Comparing fecal bacteria removal during winter and summer conditions	Over 99.3% removal of <i>E. coli</i> and 88.9% removal of enterococcus	Papadopoulos et al. (2011)
Swine wastewater	Full	Santa Caterina, Brazil	<i>Landoltia punctata</i>	One-year duration at 30-day HRT	98.3% TN removal; 98.8% NH <sub>3</sub> removal; 4.4 g m <sup>-2</sup> d <sup>-1</sup> TKN removal; 68 t ha <sup>-1</sup> year <sup>-1</sup> biomass yield	Mohedano et al. (2012)
Municipal wastewater	Full	Islamabad, Pakistan	<i>Lemna minor</i>	Sequential treatment in ponds with different species: <i>Pistia stratiotes</i> (water lettuce), <i>Eichhornia crassipes</i> (water hyacinth), <i>hydrocotyle umbellata</i> (water pennywort), <i>Typha latifolia</i> (cattail), and <i>Scripus acutus</i> (hardstem bulrush)	77.6% NO <sub>3</sub> <sup>-</sup> removal; treatment reduced TDS, Cl <sup>-</sup> , HCO <sub>3</sub> <sup>-</sup> , Ca <sup>2+</sup> , and Mg <sup>2+</sup> by 35.5%, 61%, 29.2%, 45.7%, 32.3%, and 55.9%, respectively; sequential phytoremediation with different plants led to higher removal rates	Farid et al. (2014)