Creating a Circular Nitrogen Bioeconomy in Agricultural Systems through Nutrient Recovery and Upcycling by Microalgae and Duckweed: Past Efforts and Future Trends

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 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 transportation 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 presents a sustainable approach to advancing circularity in agricultural systems. This article provides a review of past efforts made 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/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, 65-100% total N, 82-100% total P, 98-100% NO_3^-, and 96-100% NH_3/NH_4^+*

can be removed by treating wastewater with microalgae. For duckweed, removals of 75-98% of total N, 81-93% total P, 72-98% NH_3/ NH_4^+, and 57-92% 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 while 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; Microalgae; Wastewater treatment; Nitrogen; Nutrient recycling; Manure treatment*

INTRODUCTION

Transitioning 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 like atmospheric deposition, N-fixation, plant and animal N uptake, nitrification, and denitrification, are all critical elements of the complex N cycle that affects the availability of N in the environment (in the forms of organic N, nitrate (NO_3^-), nitrite (NO_2^-), and ammonia (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; Ribaudo, 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, or 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 (N_2O), 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 operational 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 wash water, etc.) are left untreated, spread on crop fields to increase soil fertility, or occasionally treated using constructed wetlands (Dordio & Carvalho, 2013). Untreated manure and agricultural soil mismanagement not only deteriorate stream water quality but also increase N_2O 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 (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 of the LCA studies in this area have focused on evaluating 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 & Stomp, 2009; Mohedano et al., 2012), LCA on this technique has only been done to a minimal extent (Roman & Brennan, 2021a). 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 utilizes 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 & 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 which utilize fast-growing aquatic vegetation that naturally recover N from agricultural runoff and enable the subsequent reuse 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 made toward advancing the circular N-bioeconomy in agricultural systems, with a specific focus on emerging sustainable methods of 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.

PROMOTING 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 NH_3 into the atmosphere. Anaerobic digestion, a routine process used to treat manure prior to soil application, can reduce CO_2 and methane (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-CO_2 GHG emissions in the United States 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 owing 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 the conventional lignocellulosic biomass, both algae and duckweed have strong potential to be used in large-scale systems for upcycling N into biomass due to their rapid growth rates. Their high protein content (of up to 50% by dry weight) and the ability to be pumped for transportation 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 & Brennan, 2021b). A sustainable farming system promoting the circular N-bioeconomy concept could involve growing these aquatic species on either diluted manure or bio-digestor effluents and harvesting them to be used for: 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 towards a circular N-bioeconomy using aquatic vegetation for nutrient recovery.

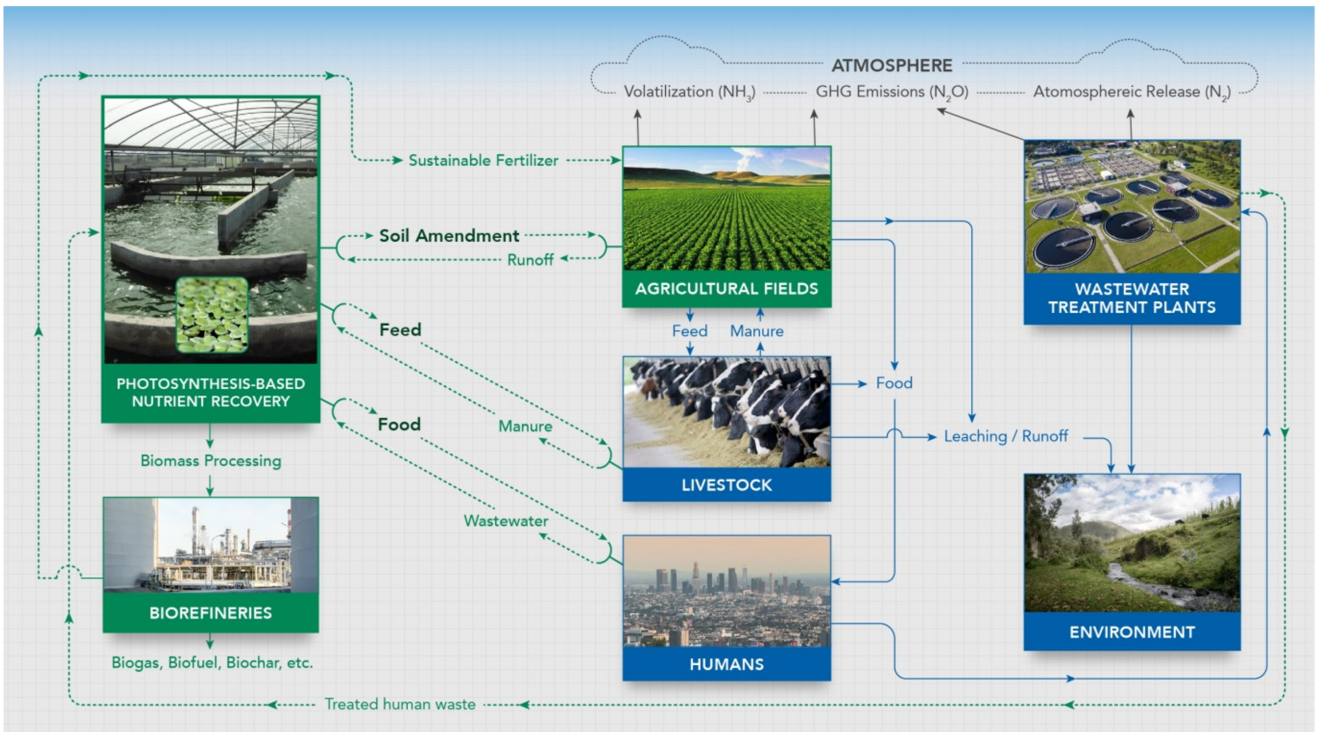

Figure 1. Integrating wastewater-treatment and aquatic vegetation to promote a circular N-bioeconomy in agricultural systems. Blue lines refer to the existing linear economy and green dashed lines show pathways to promote a circular N-bioeconomy.

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 MICROALGAE AND DUCKWEED-BASED NITROGEN RECOVERY METHODS

Typically, wastewater treatment plants providing dedicated N removal processes are normally only used to treat wastewater from domestic and industrial sources. Runoff from agricultural fields and livestock farms are 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 digestors 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 digestors have better treatment efficiency than settling ponds due to 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 non-point source nature of the runoff. Manure generated on livestock farms, however, is comparatively easier to collect and treat than runoff; therefore, much of the work conducted in the past on microalgae 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 utilized to capture runoff from cropping areas.

The literature review for this study was carried out using 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 papers found, we shortlisted those in which microalgae and duckweed were used to treat wastewater. Studies published between the years 1995-2020 are included in the review. **Table S-1** (Supplementary Information) shows the complete list of selected papers. 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 of them were conducted at laboratory scale (**Figure 2**). For in-depth review, only those papers focusing on agricultural wastewater treatment are summarized here (**Table 1**).

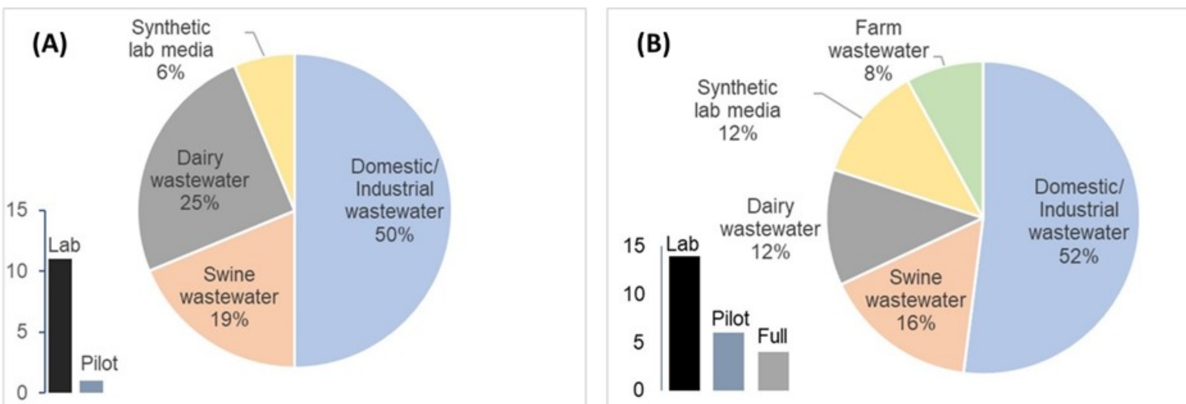

Figure 2. Source of nutrients used in the reviewed articles that focused on (A) microalgae-based (n=12) and (B) duckweed-based (n=24) wastewater treatment. The experimental scales used in the studies (lab, pilot, or full-scale) are shown on the bottom left of each chart. Details of the studies reviewed are provided in the Supplementary Information (Table S-1).

Microalgae-based Wastewater Treatment

Microalgae are unicellular photosynthetic microorganisms that can grow in marine and freshwater ecosystems, utilizing sunlight, CO_2 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/yr (Acién Fernández et al., 2021). There are many strains of microalgae, with varying effectiveness in 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^3 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^3 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_4^+ compared to NO_3^- and NO_2^- because the latter must be reduced to NH_4^+ (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 in the context of treating livestock manure since it contains high levels of NH_4^+. The uptake of NO_3^- by microalgae can be partially reduced in the presence of ambient NH_4^+, an inhibitory effect that is further enhanced by factors such as limited light conditions and lower temperatures (Su, 2021). The phenomenon of NH_3 removal (but not recovery) is aided at elevated pH conditions since high pH causes NH_4^+ to convert to gaseous NH_3, which is then released into the air (Ferreira et al., 2018; Zimmo et al., 2003). Microalgae can also remove N_2O from wastewater (Qie et al., 2019). Using microalgae, 65-100% total N, 82-100% total P, 98-100% NO_3^-, and 96-100% NH_3/NH_4^+ removal has been achieved in treating farm, industrial, and municipal wastewaters (**Figure 3, Table 1, Table S-1**). More studies concentrating on microalgal treatment of agricultural wastewater are required to fully understand the range of nutrient reductions that can potentially be achieved under different environmental conditions.

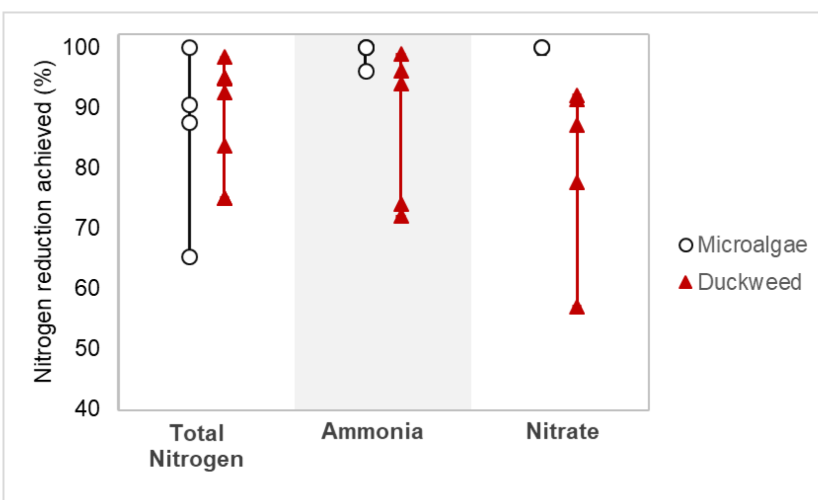

Figure 3. Ranges of nitrogen reductions achieved with microalgal- and duckweed-based wastewater treatment (summarized from 22 papers). Each symbol represents the results reported by an individual study.

Table 1. Summary of nitrogen removal and biomass production by microalgae and duckweed in selected agricultural wastewater treatment systems*.

Wastewater Type	Scale	Species used	Experimental Conditions/ Variables	Results	Citations
Microalgae-based					
Poultry, swine, brewery, cattle, dairy, and urban wastewater	Lab	*Scenedesmus obliquus*	Pre-treated cattle, dairy, and brewery wastewater	95-100% TN removal; 63-99% PO_4^{3-} removal; Biomass produced with 31-53% protein content, 12-26% sugars, and 8-23% lipids	Ferreira et al. (2018)
Dairy wastewater	Lab	*Acutodesmus dimorphus*	Untreated dairy wastewater; Very low NO_3^- concentration	100% NO_3^- removal within 4 days; 100% NH_3 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 *(Chlorella saccharophila* UTEX 2911, *Chlamydomonas pseudococcum* UTEX 214, *Scenedesmus sp* UTEX1589 and *Neochloris oleoabundans* UTEX 1185)	Wastewater from collecting and holding tanks of dairy farm; Three different CO_2 concentrations, irradiance of 80 mmol $m^{-2}s^{-1}$, 12 hr daylength, for 10 days	98% TKN removal; 99% NH_3 removal; 86% NO_3^- removal	Hena et al. (2015)
Swine wastewater	Lab	*Chlorella vulgaris*	12 days	90.51% TN removal and 91.54% TP removal	Wen et al. (2017)
Swine wastewater	Lab and computer model	*Chlorella* 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^{-1} d^{-1} of TN and 60.4 mg L^{-1} d^{-1} of NH_3	Hu et al. (2013)
Duckweed-based					
Swine wastewater	Lab	*Spirodela oligorrhiza*	Two-week harvest and 6% wastewater to 94% tap water	83.7% TN removal and 89.4% TP removal	Xu & Shen (2011)
Swine wastewater	Lab	*Lemna minor*	12 hr light cycle, pre-treated swine wastewater at 4% dilution	74% NH_3 removal; 0.14 g m-2 d-1 TN removal	Pena et al. (2017)
Diluted swine effluent	Lab	*Spirodela spp*	Different N levels in growing media	Crude protein content increases from 15% at 1 to 4 mg N L^{-1}, to 37% at 10 to 15 mg N L^{-1}; toxic effect above 60 mg N L^{-1}	Leng et al. (1995)
Effluent and digested slurry of biorefinery processing cattle slurry	Lab	*Lemna minuta*	Various concentrations of effluent from biorefinery and digested slurry	75% TN removal; 81% TP removal; higher concentrations had toxic levels of sodium and potassium	Sońta et al. (2020)
Mixture of domestic and agricultural wastewater	Pilot	*Lemna japonica 0234*	Comparative study with water hyacinth (*Eichhornia crassipes*)	60% recovery of N over a year; 0.4 g m^{-2} d^{-1} TN removal	Zhao et al. (2014)
Mixture of domestic and agricultural wastewater	Pilot	*Lemna japonica 0234*	Combining duckweed and carrier biofilm	19.97% higher TN removal and 15.02% higher NH_3 removal with duckweed	Zhao et al. (2015)
Swine wastewater	Full	*Landoltia punctata*	1 year duration at 30-day HRT	98.3% TN removal; 98.8% NH_3 removal; 4.4 g m-2 d^{-1} TKN removal; 68 t ha^{-1} yr^{-1} biomass yield	Mohedano et al. (2012)

HRT: Hydraulic Retention Time; TN: Total Nitrogen; TKN: Total Kjeldahl Nitrogen; TP: Total Phosphorus.

*A complete list of studies that include municipal and industrial wastewater treatment with microalgae and duckweed are provided in Table S-1 (Supporting Information).

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 NH_3 and the reduced metabolic cost to convert NH_4^+ to organic matter compared to other nitrogen forms, microalgae tend to grow faster in water with high NH_3 content. However, concentrations in excess of 110 mg L^{-1} 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 & Carfagna, 2021; Zheng et al., 2019); however, some strains of microalgae, such as *Chlorella vulgaris* and *Scenedesmus obliquus,* have been shown to grow in concentrations to up to 360 mg L^{-1} of NH_3 (Collos & 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 the full scale. The varying concentrations of N and other elements in wastewater can largely affect the performance of microalgae-based wastewater treatment and 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 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 ± 9.4 and an N/P ratio = 6.7 ± 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 growing 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 – 10 days); increased land-use (10 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 owing mainly due to the harvesting operation that 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 biomass production such as the utilization of greenhouses and wavelength filters (Kang et al., 2015).

Table 2. Comparison of beneficial and detrimental impacts from conventional wastewater treatment and microalgae/duckweed-based wastewater treatment systems.

Criteria	**Wastewater Treatment Impact**		**Sources**
	Conventional	Microalgae/Duckweed-based	
N removal	Up to 99%	Up to 100%[m] Up to 93%[d]	Henze, 1991[c]; McCarty, 2018[c]; Samorì et al., 2013[m]; Li et al., 2019[m]; Costa et al., 2016[d]
GHG Emissions	High (0.005 to 0.8 kg CO_2 eq./ m^3)	Low (1700 - 3300 mg CO_2 m^{-2} day^{-1})[d]; (8.3-14 g CO_2 m^{-3})[m]	Gupta & Singh, 2012[c]; Monteith et al., 2005[c]; Mohedano et al., 2019[d]; Sims et al., 2013[d]; Alcántara et al., 2015[m]
Land Use	Low	High (5-6.5 m^2 per capita)[m]	Acién Fernández et al., 2018[m]; Alcántara et al., 2015[m]
Water Demand	Low	High	Sońta et al., 2020[d]
Energy Demand	0.3-2.1 kWh m^{-3}	0.02- 1W m^{-3} [m]	Capodaglio & Olsson, 2020[c]; Crawford & Sandino, 2010[c]; Pabi et al., 2013[c]; Alcántara et al., 2015[m]; Lopes et al., 2018[m]
High value products	Fertilizers, bioenergy	Fertilizers, animal feed, human food, biogas, biofuel feedstock	Cassidy, 1998[c]; Spolaore et al., 2006[m]; Cheng et al., 2019[m]; Leng 1999[d]; Calicioglu et al., 2019[d]

[c]Conventional wastewater treatment; [m]Microalgae-based wastewater treatment; [d]Duckweed-based wastewater treatment.

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 macro-nutrient composition of different duckweed species are similar, though protein content can vary from 15-45% depending on the nutrient concentrations of the water in which they 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 & Epps, 1973) and ability to grow under a wide range of nutrient, temperature (5-33°C), and pH (5.5-8.5) conditions (Ceschin et al., 2019). With a doubling rate of every 1-2 days, an initial duckweed mat covering an area of 10 cm^2 has the potential to cover up to 1 hectare in under 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 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 storm water, among others (**Table 1**). Like microalgae, which prefer NH_4^+ uptake over NO_3^-, duckweed has an affinity for NH_4^+, 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 (**Table 1, Table S-1**). 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 & 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 demonstrate that 75-98% of total N, 81-92% total P, 72-98% NH_3/ NH_4^+, and 57-92% NO_3^- can be removed from wastewater treated with duckweed (**Figure 3**). Although maximum nutrient reductions were similar for microalgae and duckweed treatments, a wider range of removal rates were 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 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^{-1}) can have negative impacts on duckweed growth (Iqbal & Baig, 2017; Sońta et al., 2020). The optimum N concentration for supporting duckweed growth is around 60 mg L^{-1}, 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, increasing treatment costs (Sońta 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 & 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 sulphate), 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% BOD and COD, 63-87% total suspended solids, 60-99% total P, 35-87% total dissolved solids, and 40-100% heavy metals in studies across different scales (**Table 1**). Duckweed's ability to effectively sequester up to three times more CO_2 than it emits (equaling 19,592 to 42,052 mg CO_2 m^{-2} d^{-1} as demonstrated in pilot-scale duckweed ponds) is particularly vital in addressing global warming (Mohedano et al., 2019). Studies have contradicted on whether duckweed-based wastewater treatment ponds are a source or a sink for CH_4 emissions due to complex reactions occurring at the soil-water interface involving methane production by methanogens and oxidation by methanotrophs (Dai et al., 2015).

Pilot 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 (15-20 days) for making it profitable at the full-scale and 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.

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

Variable	Units	Wastewater Treatment System		Citations
		Microalgae-based	Duckweed-based	
Typical Hydraulic Retention Time	days	7 to 10	15 to 20	*Nagarajan et al., (2020); Shi et al. (2010)*
Optimum Temperature	ºC	15 to 30	5 to 33	*Esbroeck, (2018)*
Optimum pH	-	7 to 9	5.5 to 8.5	*Esbroeck, (2018)*
Biomass Doubling Rate	days	< 1	1 to 2	*Acién Fernández et al. (2021); Leng (1999)*
Biomass Yield	ton dry mass/ha/yr	100	73 to 180	*Acién Fernández et al. (2021); Leng (1999)*
Optimum C/N ratio	-	49.6 ± 9.4	-	*Maroneze et al. (2014)*
Optimum N/P ratio	-	6.7 ± 3.8	4 to 5	*Maroneze et al. (2014); Xu & Shen (2011)*
Ammonia Toxicity Level	mg NH_4^+-N /L	> 110	> 60	*Carfagna (2021); Sońta et al. (2020)*

Applications for Wastewater-grown Algae and Duckweed Biomass

Microalgae applications

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_4 and CO_2) through anaerobic digestion, digestate from microalgal biorefineries has the potential to be used as soil amendment in place of synthetic fertilizers (Préat et al., 2020). When used as organic fertilizers, 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 to the high lipid content of some microalgae, it can be converted to biodiesel (Samorì 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 & Hayes, 2017). They can be substituted in 5-10% of poultry feed and 33% of pig feed without causing any adverse health effects; replacing 1-5% of fish diet with microalgae is even shown to promote health and aid in early growth (Acién Fernández et al., 2021; Spolaore et al., 2006). Based on its high nutritional value and availability, microalgae can be used in diets of malnourished people around the world (Christaki et al., 2011). Major limitations to future research on microalgal applications include their high extraction cost and 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, seaweeds can remove N from water and have a variety of applications in food, energy, and agricultural sectors. Bioethanol, liquid fertilizers, and fish feed have been produced using seaweed biomass in pilot- 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 feasibility and biomass availability for large-scale use.

Duckweed applications

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 are produced in a 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_3 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_3 volatilization by 36-52% and added 10-11% overall economic benefit in rice fields compared to chemical fertilizer alone (Yao et al., 2017). The N and P bound within the duckweed biomass makes it an ideal slow-release fertilizer and helps retain the nutrients in soil for longer, 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 & 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_4 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 & Brennan, 2018). After anaerobic digestion of duckweed to produce CH_4, the resulting digestate can be utilized 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. Besides being a key protein source, duckweed can also successfully accumulate micro-minerals such as potassium, calcium, magnesium, sodium, and iron, which are typically not present in adequate quantities in the livestock feed available to small farm owners (Leng et al., 1995). In Vietnam, duckweed farming has been done for many years, where 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^{-1} yr^{-1}, duckweed can produce edible proteins 6-10 times faster than soybeans per area (Landesman et al., 2005; Roman & Brennan, 2019). Under optimum growing conditions, annual duckweed yield can range from 73 to 180 ton dry matter/ha/yr; however, even less than optimal conditions can still provide an yield of 5 to 20 ton dry matter/ha/yr (Leng, 1999). This is noticeably higher than the average yield for soybean (2.8 metric ton/ha) which is conventionally used as a source of feed protein in livestock farms (Purdy & Langemeier, 2018), and on par or greater than the 2 – 100 ton dry matter /ha/yr achievable with microalgae (Acién Fernández et al., 2021). In aquaponics, both fresh and dried duckweed have been shown as 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 suggest adding only a small fraction of duckweed to existing feeds until further research is conducted on optimum inclusion rates so that any potential negative effects can be identified. A few feeding experiments conducted with duckweed on pigs, poultry, ruminants, and fish indicate that duckweed can be given as protein feed to these animals without any severe impact on health (Cheng & 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 (Sońta et al., 2019). This discrepancy in experimental outcomes can most likely be attributed to the fact that duckweed species and growth medium 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 80-94% 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 the 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 (Sońta 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 & 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 like gene editing and genetic transformations can be used to identify duckweed lines with superior traits that are most effective in nutrient recovery and use 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 feed and fertilizer production

The growing demand for animal-derived food products and the extensive use of conventional animal feed such as corn and soybean has 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 sections above. Compared to fish and soy sectors that produce 7000 kt/year of fish-based feed and 200,000 kt/year of soy-based feed (costing $1.8 and $0.6 per kg, respectively), microalgal production is still a small-scale industry producing 100 kt/year biomass and offers an expensive feed option costing $17-$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 localized feed production on farms (Sasu-Boakye et al., 2014). In agriculture, especially in dairy farms, developing an integrated on-farm wastewater treatment-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 the manure-grown algae or duckweed is to use them as fertilizer alternatives for crops or as a useful soil amendment material 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 the crop fields within the same farm. Such an on-site system would not only increase farmer profit by decreasing feed and fertilizer imports and transportation requirements, but also be a more environmentally-friendly option by reducing 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 utilize microalgae may be as a fertilizer additive or biostimulant, which at very low dosages of 2 L/ha has been shown to reduce chemical fertilizer use by over 10% (Acién Fernández et al., 2021).

Integrated farm wastewater treatment-biorefinery systems

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. Besides offering a promising sustainable solution to upcycle 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 grains 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 calling for the need for alternative plant-based protein substitutes. A report by United Nations Food and Agricultural Organization (FAO) estimated that global livestock production releases 7.1 gigatonnes of CO_2 eq. per annum, accounting for 14.5% of anthropogenic GHG emissions in the form of CO_2, CH_4, and N_2O, 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 utilized 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 not only recommended to alleviate the environmental impacts discussed above, but also to benefit 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 some developing regions, but are now also rising 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 into the food chain (Khan et al., 2020). Similar to other vascular plants, duckweed has the potential to adsorb microplastics to their fronds and roots, which when consumed by humans can cause long-term harmful health effects. Pre-treatment methods such as density-driven separation, flocculation, and sedimentation that can remove up to 88% microplastics from wastewaters may be used in conjunction with duckweed-wastewater treatment if high levels of microplastics are identified in the growing media (Vivekanand et al., 2021). Given that the nutritional composition and protein accumulation of algae and duckweed depends heavily on the type of growth media, the concept of growing them on wastewater merits further research to evaluate their nutritional value and safety for human consumption.

Challenges within the circular N-bioeconomy

The two biggest challenges behind using wastewater-grown algae or duckweed to advance the circular N-bioeconomy concept are: (1) the production costs associated with cultivation and frequent harvesting; and (2) the sociological resistance to consuming vegetation grown on wastewater. The issue concerning high production cost can be addressed to a great extent by implementing this approach on a large scale and producing a combination of valuable products, for instance, animal feed, protein supplements, and crop fertilizer. Pond construction accounts for a major share of the production cost associated with duckweed-based biorefinery models (Calicioglu, 2019). 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 by LED lights) can be utilized to significantly reduce the land requirements for duckweed cultivation, which is anticipated to make the system more economical and sustainable compared to employing the typical pond-grown approach (Roman & Brennan, 2021a).

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 due to the lack of sufficient biomass (Maes & Van Passel, 2019). Logistical aspects related to the collection and transport of biomass products should be given high importance in a biorefinery system since it is a direct measure of operational costs as well as environmental impact in terms of carbon emissions (Ubando et al., 2020). Utilizing pipes for pumping instead of ground transport for conveying biomass material (such as duckweed), and using natural sun-drying techniques, are ways to encourage sustainability in this context. Studies in Vietnam have demonstrated that duckweed can successfully be used in 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- or duckweed-based wastewater treatment systems to be simultaneously economical and sustainable (Mohseni & Pishvaee, 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 the locally developed practices to the national level, and to eventually enter global markets. Inevitably, the end-use products of these systems should substitute the existing products (e.g. generating duckweed-based biofuel instead of petroleum-based fuel, substituting existing chemical fertilizers with duckweed-based soil amendments, supplementing livestock diet 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 also equally important to make the system resilient. In addition, optimizing the processes and products within the entire value chain is also required to develop a system that is cost-effective, beneficial to society, and has minimal environmental impacts. This provides growing opportunities to use multi-scale modeling tools, optimization methods, and LCA to help policy-makers and other stakeholders quantify 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 & Van Passel, 2019). Additionally, subsidizing these products and offering economic and social incentives for processing and/or using these 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 not only help overcome the cultural resistance of farmers in cultivating duckweed in place of traditional crops, but also encourage them 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 seen in the case of Vietnam, will be useful in 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 would attract more private and public investments in the near- 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 not only influence the current livestock and fertilizer market, but also expand the sustainable food, feed, and energy markets. The algae products market is projected to grow by 5.2% from 2016 to 2023; and 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 US $2000 million (Credence Research, 2017). The microalgae market in particular is currently valued at 50 million euros and predicted to be worth 70 million euros by 2025 (Acién Fernández et al., 2021). Emerging applications of microalgae, besides using them for 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 insight 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.

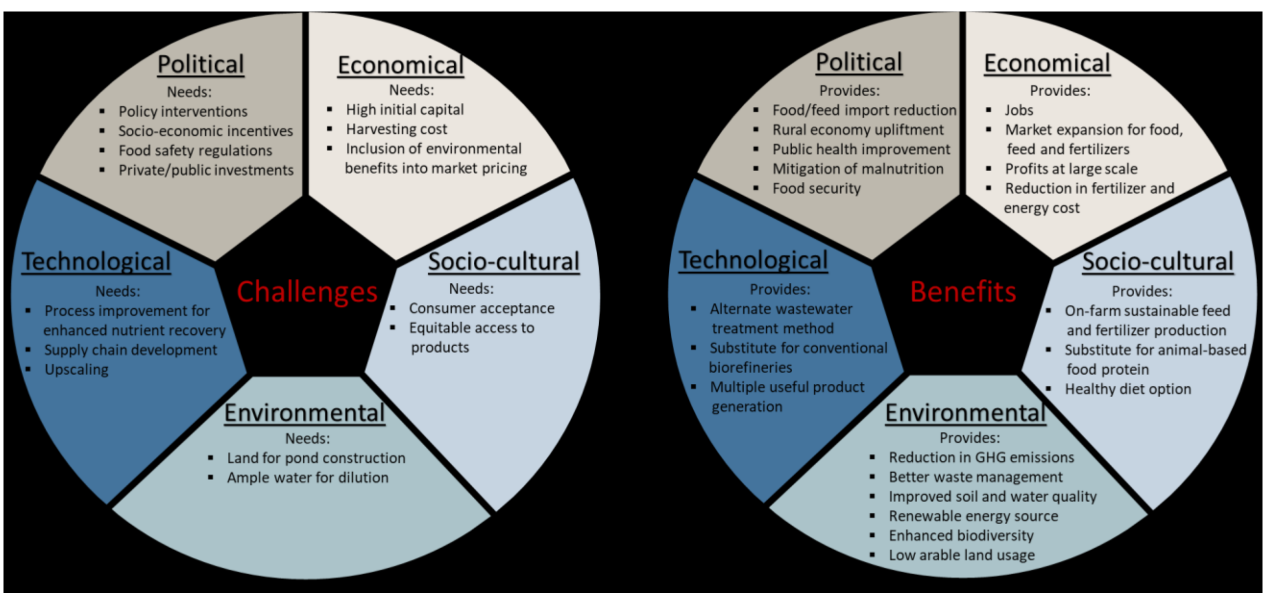

Figure 4. Challenges and benefits associated with using aquatic vegetation for wastewater treatment in the circular N-bioeconomy.

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 tested ability to grow under a wider range of environmental conditions gives duckweed some advantages over microalgae. Although over half of the reviewed studies utilized microalgae and duckweed for municipal/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 over 90% nitrate and ammonia removal, various applications of these aquatic organisms are being explored in the form of biofeedstock, fertilizers, animal feed, and human food as a way to transition from a linear to 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.

References

Acién Fernández, F. G., Gómez-Serrano, C., & Fernández-Sevilla, J. M. (2018). Recovery of Nutrients From Wastewaters Using Microalgae. *Frontiers in Sustainable Food Systems*, *2*(September), 1–13. https://doi.org/10.3389/fsufs.2018.00059

Acién Fernández, F. G., Reis, A., Wijffels, R. H., Barbosa, M., Verdelho, V., & Llamas, B. (2021). The role of microalgae in the bioeconomy. *New Biotechnology*, *61*, 99–107. https://doi.org/10.1016/j.nbt.2020.11.011

Acosta, K., Appenroth, K. J., Borisjuk, L., Edelman, M., Heinig, U., Jansen, M. A. K., Oyama, T., Pasaribu, B., Schubert, I., Sorrels, S., Sowjanya Sree, K., Xu, S., Michael, T. P., & Lam, E. (2021). Return of the Lemnaceae: duckweed as a model plant system in the genomics and postgenomics era. *The Plant Cell*, *33*(10), 3207–3234. https://doi.org/10.1093/PLCELL/KOAB189

Alcántara, C., Posadas, E., Guieysse, B., & Muñoz, R. (2015). Microalgae-based Wastewater Treatment. In *Handbook of Marine Microalgae: Biotechnology Advances* (pp. 439–455). Elsevier Inc. https://doi.org/10.1016/B978-0-12-800776-1.00029-7

Anderson, D. M., Burkholder, J. M., Cochlan, W. P., Glibert, P. M., Gobler, C. J., Heil, C. A., Kudela, R. M., Parsons, M. L., Rensel, J. E. J., Townsend, D. W., Trainer, V. L., & Vargo, G. A. (2008). Harmful algal blooms and eutrophication: Examining linkages from selected coastal regions of the United States. *Harmful Algae*, *8*(1), 39–53. https://doi.org/10.1016/j.hal.2008.08.017

Appenroth, K.-J., Sree, K. S., Bog, M., Ecker, J., Seeliger, C., Böhm, V., Lorkowski, S., Sommer, K., Vetter, W., Tolzin-Banasch, K., Kirmse, R., Leiterer, M., Dawczynski, C., Liebisch, G., & Jahreis, G. (2018). Nutritional Value of the Duckweed Species of the Genus Wolffia (Lemnaceae) as Human Food. *Frontiers in Chemistry*, *6*(OCT), 483. https://doi.org/10.3389/fchem.2018.00483

Arumugam, N., Chelliapan, S., Kamyab, H., Thirugnana, S., Othman, N., & Nasri, N. (2018). Treatment of Wastewater Using Seaweed: A Review. *International Journal of Environmental Research and Public Health*, *15*(12), 2851. https://doi.org/10.3390/ijerph15122851

Awasthi, M. K., Sarsaiya, S., Wainaina, S., Rajendran, K., Kumar, S., Quan, W., Duan, Y., Awasthi, S. K., Chen, H., Pandey, A., Zhang, Z., Jain, A., & Taherzadeh, M. J. (2019). A critical review of organic manure biorefinery models toward sustainable circular bioeconomy: Technological challenges, advancements, innovations, and future perspectives. *Renewable and Sustainable Energy Reviews*, *111*, 115–131. https://doi.org/10.1016/j.rser.2019.05.017

Barbosa, M., Valentão, P., Ferreres, F., Gil-Izquierdo, Á., & Andrade, P. B. (2020). In vitro multifunctionality of phlorotannin extracts from edible Fucus species on targets underpinning neurodegeneration. *Food Chemistry*, *333*(November 2019), 127456. https://doi.org/10.1016/j.foodchem.2020.127456

Battaglia, M., Thomason, W., Fike, J. H., Evanylo, G. K., von Cossel, M., Babur, E., Iqbal, Y., & Diatta, A. A. (2021). The broad impacts of corn stover and wheat straw removal for biofuel production on crop productivity, soil health and greenhouse gas emissions: A review. In *GCB Bioenergy* (Vol. 13, Issue 1, pp. 45–57). Blackwell Publishing Ltd. https://doi.org/10.1111/gcbb.12774

Benjawan, L., & Koottatep, T. (2007). Nitrogen removal in recirculated duckweed ponds system. *Water Science and Technology*, *55*(11), 103–110. https://doi.org/10.2166/wst.2007.360

Bleakley, S., & Hayes, M. (2017). Algal Proteins: Extraction, Application, and Challenges Concerning Production. *Foods*, *6*(5), 33. https://doi.org/10.3390/foods6050033

Bog, M., Appenroth, K. J., & Sree, K. S. (2019). Duckweed (Lemnaceae): Its Molecular Taxonomy. *Frontiers in Sustainable Food Systems*, *3*, 117. https://doi.org/10.3389/FSUFS.2019.00117/BIBTEX

Boland, M. J., Rae, A. N., Vereijken, J. M., Meuwissen, M. P. M., Fischer, A. R. H., van Boekel, M. A. J. S., Rutherfurd, S. M., Gruppen, H., Moughan, P. J., & Hendriks, W. H. (2013). The future supply of animal-derived protein for human consumption. In *Trends in Food Science and Technology* (Vol. 29, Issue 1, pp. 62–73). Elsevier. https://doi.org/10.1016/j.tifs.2012.07.002

Cai, T., Park, S. Y., & Li, Y. (2013). Nutrient recovery from wastewater streams by microalgae: Status and prospects. *Renewable and Sustainable Energy Reviews*, *19*, 360–369. https://doi.org/10.1016/j.rser.2012.11.030

Calicioglu, O. (2018). *Technical, economic, and environmental feasibility of wastewater-derived duckweed biorefineries*. The Pennsylvania State University.

Calicioglu, O., & Brennan, R. A. (2018). Sequential ethanol fermentation and anaerobic digestion increases bioenergy yields from duckweed. *Bioresource Technology*, *257*(November 2017), 344–348. https://doi.org/10.1016/j.biortech.2018.02.053

Calicioglu, O., Femeena, P. V., Mutel, C. L., Sills, D. L., Richard, T. L., & Brennan, R. A. (2021). Techno-economic Analysis and Life Cycle Assessment of an Integrated Wastewater-Derived Duckweed Biorefinery. *ACS Sustainable Chemistry & Engineering*, *9*(28), 9395–9408. https://doi.org/10.1021/ACSSUSCHEMENG.1C02539

Calicioglu, O., Richard, T. L., & Brennan, R. A. (2019). Anaerobic bioprocessing of wastewater-derived duckweed: Maximizing product yields in a biorefinery value cascade. *Bioresource Technology*, *289*(May), 121716. https://doi.org/10.1016/j.biortech.2019.121716

Calicioglu, O., Shreve, M. J., Richard, T. L., & Brennan, R. A. (2018). Effect of pH and temperature on microbial community structure and carboxylic acid yield during the acidogenic digestion of duckweed. *Biotechnology for Biofuels*, *11*(1), 1–19. https://doi.org/10.1186/s13068-018-1278-6

Capodaglio, A. G., & Olsson, G. (2020). Energy issues in sustainable urban wastewater management: Use, demand reduction and recovery in the urban water cycle. *Sustainability (Switzerland)*, *12*(1). https://doi.org/10.3390/su12010266

Cassidy, S. (1998). Recovery of Valuable Products from Municipal Wastewater Sludge. In *Chemical Water and Wastewater Treatment V* (pp. 325–340). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-72279-0_26

Ceschin, S., Abati, S., Traversetti, L., Spani, F., Del Grosso, F., & Scalici, M. (2019). Effects of the invasive duckweed Lemna minuta on aquatic animals: evidence from an indoor experiment. *Plant Biosystems*, *153*(6), 749–755. https://doi.org/10.1080/11263504.2018.1549605

Chaiprapat, S., Cheng, J., Classen, J. J., Ducoste, J. J., & Liehr, S. K. (2003). Modeling Nitrogen Transport in Duckweed Pond for Secondary Treatment of Swine Wastewater. *Journal of Environmental Engineering*, *129*(8), 731–739. https://doi.org/10.1061/(asce)0733-9372(2003)129:8(731)

Chantiratikul, A., Chinrasri, O., Chantiratikul, P., Sangdee, A., Maneechote, U., & Bunchasak, C. (2010). Effect of replacement of protein from soybean meal with protein from wolffia meal

[Wolffia globosa (L). Wimm.] on performance and egg production in laying hens. *International Journal of Poultry Science*, *9*(3), 283–287. https://doi.org/10.3923/ijps.2010.283.287

Chen, G., Huang, J., Fang, Y., Zhao, Y., Tian, X., Jin, Y., & Zhao, H. (2019). Microbial community succession and pollutants removal of a novel carriers enhanced duckweed treatment system for rural wastewater in Dianchi Lake basin. *Bioresource Technology*, *276*(December 2018), 8–17. https://doi.org/10.1016/j.biortech.2018.12.102

Chen, S., Wen, Z., Liao, W., Liu, C., Kincaid, R. L., Harrison, J. H., Elliott, D. C., Brown, M. D., & Stevens, D. J. (2005). *Studies into Using Manure in a Biorefinery Concept*.

Cheng, D. L., Ngo, H. H., & Guo, W. S. (2019). Microalgae biomass from swine wastewater and its conversion to bioenergy. *Bioresource Technology*, *275*(December 2018), 109–122. https://doi.org/10.1016/j.biortech.2018.12.019

Cheng, J. J., & Stomp, A. M. (2009). Growing Duckweed to recover nutrients from wastewaters and for production of fuel ethanol and animal feed. *Clean - Soil, Air, Water*, *37*(1), 17–26. https://doi.org/10.1002/clen.200800210

Chokshi, K., Pancha, I., Ghosh, A., & Mishra, S. (2016). Microalgal biomass generation by phycoremediation of dairy industry wastewater : An integrated approach towards sustainable biofuel production. *Bioresource Technology*, *221*, 455–460. https://doi.org/10.1016/j.biortech.2016.09.070

Christaki, E., Florou-Paneri, P., & Bonos, E. (2011). Microalgae: A novel ingredient in nutrition. *International Journal of Food Sciences and Nutrition*, *62*(8), 794–799. https://doi.org/10.3109/09637486.2011.582460

Cibin, R., Chaubey, I., & Engel, B. (2012). Simulated watershed scale impacts of corn stover removal for biofuel on hydrology and water quality. *Hydrological Processes*, *26*(11), 1629–1641. https://doi.org/10.1002/hyp.8280

Collos, Y., & Harrison, P. J. (2014). Acclimation and toxicity of high ammonium concentrations to unicellular algae. *Marine Pollution Bulletin*, *80*(1–2), 8–23. https://doi.org/10.1016/j.marpolbul.2014.01.006

Coppens, J., Grunert, O., Van Den Hende, S., Vanhoutte, I., Boon, N., Haesaert, G., & De Gelder, L. (2016). The use of microalgae as a high-value organic slow-release fertilizer results in tomatoes with increased carotenoid and sugar levels. *Journal of Applied Phycology*, *28*(4), 2367–2377. https://doi.org/10.1007/S10811-015-0775-2/FIGURES/6

Costa, R. H. R., Tonon, G., Mohedano, R. A., Teles, C. C., Aguiar, S. R., Filho, P. B., Campus, / Ens, João, R., & Ferreira Lima, D. (2016). *Does Duckweed Ponds Used for Wastewater Treatment Emit or Sequester Greenhouse Gases (GHG)?*

Craggs, R., Park, J., Heubeck, S., & Sutherland, D. (2014). High rate algal pond systems for low-energy wastewater treatment, nutrient recovery and energy production. *New Zealand Journal of Botany*, *52*(1), 60–73. https://doi.org/10.1080/0028825X.2013.861855

Crawford, G., & Sandino, J. (2010). Energy Efficiency in Wastewater Treatment in North America: A Compendium of Best Practices and Case Studies of Novel Approaches: A Compendium of Best Practices and Case Studies of Novel Approaches. *Water Intelligence Online*, *9*. https://doi.org/10.2166/9781780403373

Credence Research. (2017). *Algae Products Market Size, Analysis, Trends, Growth And Forecast To 2027*. https://www.credenceresearch.com/report/algae-products-market

Culley, D. D., & Epps, E. A. (1973). Use of Duckweed for Waste Treatment and Animal Feed. In *Journal (Water Pollution Control Federation)* (Vol. 45, Issue 2).

D'Odorico, P., Davis, K. F., Rosa, L., Carr, J. A., Chiarelli, D., Dell'Angelo, J., Gephart, J., MacDonald, G. K., Seekell, D. A., Suweis, S., & Rulli, M. C. (2018). The Global Food-Energy-Water Nexus. *Reviews of Geophysics*, *56*(3), 456–531. https://doi.org/10.1029/2017RG000591

Dai, J., Zhang, C., Lin, C.-H., & Hu, Z. (2015). Emission of Carbon Dioxide and Methane from Duckweed Ponds for Stormwater Treatment. *Water Environment Research*, *87*(9), 805–812.

https://doi.org/10.2175/106143015x14362865226310

Del Borghi, A., Moreschi, L., & Gallo, M. (2020). Circular economy approach to reduce water–energy–food nexus. In *Current Opinion in Environmental Science and Health* (Vol. 13, pp. 23–28). Elsevier B.V. https://doi.org/10.1016/j.coesh.2019.10.002

Dietrich, M., Fongen, M., & Foereid, B. (2020). Greenhouse gas emissions from digestate in soil. *International Journal of Recycling Organic Waste in Agriculture*, *9*(1), 1–19. https://doi.org/10.30486/IJROWA.2020.1885341.1005

Dordio, A., & Carvalho, A. J. P. (2013). Constructed wetlands with light expanded clay aggregates for agricultural wastewater treatment. *Science of The Total Environment*, *463–464*, 454–461. https://doi.org/10.1016/J.SCITOTENV.2013.06.052

Eid, M. A. A., Kandil, M. A. E., Moursy, E. B., & Sayed, G. E. M. (1992). Bioassays of duck weed vegetation extracts. *International Journal of Tropical Insect Science*, *13*(05), 741–748. https://doi.org/10.1017/s1742758400007992

Ekperusi, A. O., Nwachukwu, E. O., & Sikoki, F. D. (2020). Assessing and Modelling the Efficacy of Lemna paucicostata for the Phytoremediation of Petroleum Hydrocarbons in Crude Oil-Contaminated Wetlands. *Scientific Reports*, *10*(1), 1–9. https://doi.org/10.1038/s41598-020-65389-z

Esbroeck, E. van. (2018). *Temperature control of microalgae cultivation under variable conditions* [Wageningen University & Research]. https://edepot.wur.nl/455088#:~:text=Every microalgae species has an,C microalgae growth will stop.

FAO. (2006). *Livestock's Long Shadow: Environmental Issues and Options*. http://www.fao.org/publications/card/en/c/9655af93-7f88-58fc-84e8-d70a9a4d8bec/

Fernandez Pulido, C. R., Caballero, J., Bruns, M. A., & Brennan, R. A. (2021). Recovery of waste nutrients by duckweed for reuse in sustainable agriculture: Second-year results of a field pilot study with sorghum. *Ecological Engineering*, *168*, 106273.

https://doi.org/10.1016/J.ECOLENG.2021.106273

Ferreira, A., Marques, P., Ribeiro, B., Assemany, P., de Mendonça, H. V., Barata, A., Oliveira, A. C., Reis, A., Pinheiro, H. M., & Gouveia, L. (2018). Combining biotechnology with circular bioeconomy: From poultry, swine, cattle, brewery, dairy and urban wastewaters to biohydrogen. *Environmental Research*, *164*(February), 32–38. https://doi.org/10.1016/j.envres.2018.02.007

Gerber, PJ, Steinfeld, Henderson, Mottet, Opio, Dijkman, Falcucci, & Tempio. (2013). *Tackling climate change through livestock: A global Assessment of emissions And mitigation opportunities*. www.fao.org/publications

Gupta, D., & Singh, S. K. (2012). Greenhouse Gas Emissions from Wastewater Treatment Plants: A Case Study of Noida. *Journal of Water Sustainability*, *1*(2), 131–140.

Hamid, M. A., Chowdhury, S. D., Razzak, M. A., & Roy, C. R. (1993). Effects of feeding an aquatic weedLemna trisulaca as partial replacement of fish meal on the performance of growing ducklings. *Journal of the Science of Food and Agriculture*, *61*(1), 137–139. https://doi.org/10.1002/jsfa.2740610123

Hauck, M., Maalcke-Luesken, F. A., Jetten, M. S. M., & Huijbregts, M. A. J. (2016). Removing nitrogen from wastewater with side stream anammox: What are the trade-offs between environmental impacts? *Resources, Conservation and Recycling*, *107*, 212–219. https://doi.org/10.1016/j.resconrec.2015.11.019

Haustetn, A. T., Gilman, R. H., Skillicorn, P. W., Vergara, V., Guevara, V., & Gastanaduy, A. (1990). Duckweed, A Useful Strategy for Feeding Chickens: Performance of Layers Fed with Sewage-Grown Lemnacea Species. *Poultry Science*, *69*(11), 1835–1844. https://doi.org/10.3382/ps.0691835

Hemalatha, M., Sarkar, O., & Venkata Mohan, S. (2019). Self-sustainable azolla-biorefinery platform for valorization of biobased products with circular-cascading design. *Chemical Engineering Journal*, *373*, 1042–1053. https://doi.org/10.1016/j.cej.2019.04.013

Hena, S., Fatimah, S., & Tabassum, S. (2015). Cultivation of algae consortium in a dairy farm wastewater for biodiesel production. *Water Resources and Industry*, *10*, 1–14. https://doi.org/10.1016/j.wri.2015.02.002

Henze, M. (1991). Capabilities of biological nitrogen removal processes from wastewater. *Water Science and Technology*, *23*(4–6), 669–679. https://doi.org/10.2166/wst.1991.0517

Hu, B., Zhou, W., Min, M., Du, Z., Chen, P., Ma, X., Liu, Y., Lei, H., Shi, J., & Ruan, R. (2013). Development of an effective acidogenically digested swine manure-based algal system for improved wastewater treatment and biofuel and feed production. *Applied Energy*, *107*, 255–263. https://doi.org/10.1016/j.apenergy.2013.02.033

Huque, K. S., Chowdhury, S. A., & Kibria, S. S. (1996). Study on the potentiality of duckweeds as a feed for cattle. *Asian-Australasian Journal of Animal Sciences*, *9*(2), 133–137. https://doi.org/10.5713/ajas.1996.133

Husk, B. R., Anderson, B. C., Whalen, J. K., & Sanchez, J. S. (2017). Reducing nitrogen contamination from agricultural subsurface drainage with denitrification bioreactors and controlled drainage. *Biosystems Engineering*, *153*, 52–62. https://doi.org/10.1016/J.BIOSYSTEMSENG.2016.10.021

IPCC. (2014). *AR5 Climate Change 2014: Mitigation of Climate Change — IPCC*. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA. https://www.ipcc.ch/report/ar5/wg3/

Iqbal, J., & Baig, M. A. (2017). Nitrogen and phosphorous removal from leachate by duckweed (Lemna minor). *Environment Protection Engineering*, *43*(4), 123–134. https://doi.org/10.5277/epel70410

Ji, M. K., Kim, H. C., Sapireddy, V. R., Yun, H. S., Abou-Shanab, R. A. I., Choi, J., Lee, W., Timmes, T. C., Inamuddin, & Jeon, B. H. (2013). Simultaneous nutrient removal and lipid production from pretreated piggery wastewater by Chlorella vulgaris YSW-04. *Applied Microbiology and Biotechnology*, *97*(6), 2701–2710. https://doi.org/10.1007/s00253-012-4097-x

Kang, Z., Kim, B. H., Ramanan, R., Choi, J. E., Yang, J. W., Oh, H. M., & Kim, H. S. (2015). A cost analysis of microalgal biomass and biodiesel production in open raceways treating municipal wastewater and under optimum light wavelength. *Journal of Microbiology and Biotechnology*, *25*(1), 109–118. https://doi.org/10.4014/jmb.1409.09019

Karan, H., Funk, C., Grabert, M., Oey, M., & Hankamer, B. (2019). Green Bioplastics as Part of a Circular Bioeconomy. *Trends in Plant Science*, *24*(3), 237–249. https://doi.org/10.1016/J.TPLANTS.2018.11.010

Kemp, W. M., Boynton, W. R., Adolf, J. E., Boesch, D. F., Boicourt, W. C., Brush, G., Cornwell, J. C., Fisher, T. R., Glibert, P. M., Hagy, J. D., Harding, L. W., Houde, E. D., Kimmel, D. G., Miller, W. D., Newell, R. I. E., Roman, M. R., Smith, E. M., & Stevenson, J. C. (2005). Eutrophication of Chesapeake Bay: Historical trends and ecological interactions. In *Marine Ecology Progress Series* (Vol. 303, pp. 1–29). Inter-Research. https://doi.org/10.3354/meps303001

Kesik-Brodacka, M. (2018). Progress in biopharmaceutical development. *Biotechnology and Applied Biochemistry*, *65*(3), 306–322. https://doi.org/10.1002/BAB.1617

Khan, M. A., Wani, G. A., Majid, H., Farooq, F. U., Reshi, Z. A., Husaini, A. M., & Shah, M. A. (2020). Differential Bioaccumulation of Select Heavy Metals from Wastewater by Lemna minor. *Bulletin of Environmental Contamination and Toxicology*, *105*(5), 777–783. https://doi.org/10.1007/S00128-020-03016-3/TABLES/3

Kim, S., Dale, B. E., Jin, M., Thelen, K. D., Zhang, X., Meier, P., Reddy, A. D., Jones, C. D., Cesar Izaurralde, R., Balan, V., Runge, T., & Sharara, M. (2019). Integration in a depot-based decentralized biorefinery system: Corn stover-based cellulosic biofuel. *GCB Bioenergy*, *11*(7), 871–882. https://doi.org/10.1111/gcbb.12613

Kleinman, P. J. A., Buda, A. R., Sharpley, A. N., & Khosla, R. (2018). *Elements of Precision Manure Management* (pp. 165–192). https://doi.org/10.2134/agronmonogr59.c9

Koyande, A. K., Chew, K. W., Rambabu, K., Tao, Y., Chu, D. T., & Show, P. L. (2019). Microalgae: A potential alternative to health supplementation for humans. *Food Science and Human Wellness*, *8*(1), 16–24. https://doi.org/10.1016/j.fshw.2019.03.001

Kreider, A. N. (2015). *Behavior of duckweed as an agricultural amendment: Nitrogen mineralization, leaching, and sorghum uptake*. The Pennsylvania State University.

Kreider, A. N., Fernandez Pulido, C. R., Bruns, M. A., & Brennan, R. A. (2019). Duckweed as an Agricultural Amendment: Nitrogen Mineralization, Leaching, and Sorghum Uptake. *Journal of Environmental Quality*, *48*(2), 469–475. https://doi.org/10.2134/jeq2018.05.0207

Kusmayadi, A., Leong, Y. K., Yen, H.-W., Huang, C.-Y., & Chang, J.-S. (2021). Microalgae as sustainable food and feed sources for animals and humans – Biotechnological and environmental aspects. *Chemosphere*, *271*, 129800. https://doi.org/10.1016/j.chemosphere.2021.129800

Landesman, L., Parker, N. C., Fedler, C. B., Konikoff, M., & Village, E. S. K. (2005). *Modeling duckweed growth in wastewater treatment systems*. *17*(6), 1–8.

Leng, R. (1999). *Duckweed: a tiny aquatic plant with enormous potential for agriculture and environment*. http://www.fao.org/ag/AGAinfo/resources/documents/DW/Dw2.htm

Leng, R., Stambolie, J. H., & Bell, R. (1995). Duckweed - a potential high-protein feed resource for domestic animals and fish. *Livestock Research for Rural Development*, *7*(1).

Li, K., Liu, Q., Fang, F., Luo, R., Lu, Q., Zhou, W., Huo, S., Cheng, P., Liu, J., Addy, M., Chen, P., Chen, D., & Ruan, R. (2019). Microalgae-based wastewater treatment for nutrients recovery: A review. In *Bioresource Technology* (Vol. 291, p. 121934). Elsevier Ltd. https://doi.org/10.1016/j.biortech.2019.121934

Lopes, A., Valente, A., Iribarren, D., & González-Fernández, C. (2018). Energy balance and life cycle assessment of a microalgae-based wastewater treatment plant: A focus on alternative biogas uses. *Bioresource Technology*, *270*, 138–146. https://doi.org/10.1016/j.biortech.2018.09.005

Ma, Q., Wang, X., Li, H., Li, H., Zhang, F., Rengel, Z., & Shen, J. (2015). Comparing localized application of different N fertilizer species on maize grain yield and agronomic N-use efficiency on a calcareous soil. *Field Crops Research*, *180*(3), 72–79.

https://doi.org/10.1016/j.fcr.2015.05.011

Ma, X., Zheng, H., Addy, M., Anderson, E., Liu, Y., Chen, P., & Ruan, R. (2016). Cultivation of Chlorella vulgaris in wastewater with waste glycerol: Strategies for improving nutrients removal and enhancing lipid production. *Bioresource Technology*, *207*, 252–261. https://doi.org/10.1016/j.biortech.2016.02.013

Ma, X., Zhou, W., Fu, Z., Cheng, Y., Min, M., Liu, Y., Zhang, Y., Chen, P., & Ruan, R. (2014). Effect of wastewater-borne bacteria on algal growth and nutrients removal in wastewater-based algae cultivation system. *Bioresource Technology*, *167*, 8–13. https://doi.org/10.1016/j.biortech.2014.05.087

Maes, D., & Van Passel, S. (2019). Effective bioeconomy policies for the uptake of innovative technologies under resource constraints. *Biomass and Bioenergy*, *120*(July 2017), 91–106. https://doi.org/10.1016/j.biombioe.2018.11.008

Maestrini, S. Y., Robert, J. M., Leftley, J. W., & Collos, Y. (1986). Ammonium thresholds for simultaneous uptake of ammonium and nitrate by oyster-pond algae. *Journal of Experimental Marine Biology and Ecology*, *102*(1), 75–98. https://doi.org/10.1016/0022-0981(86)90127-9

Maga, D. (2017). Life cycle assessment of biomethane produced from microalgae grown in municipal waste water. *Biomass Conversion and Biorefinery*, *7*(1), 1–10. https://doi.org/10.1007/s13399-016-0208-8

MarketsandMarkets. (2021). *Seaweed Cultivation Market Size, Share, Trends and Forecast to 2025*. https://www.marketsandmarkets.com/Market-Reports/commercial-seaweed-market-152763701.html

Maroneze, M. M., Barin, J. S., de Menezes, C. R., Queiroz, M. I., Zepka, L. Q., & Jacob-Lopes, E. (2014). Treatment of cattle-slaughterhouse wastewater and the reuse of sludge for biodiesel production by microalgal heterotrophic bioreactors. *Scientia Agricola*, *71*(6), 521–524. https://doi.org/10.1590/0103-9016-2014-0092

Marten, G., Suárez, M. F., Astaeza, R., & Erradicaci, S. N. de. (1996). An Ecological Survey of Anopheles albimanus Larval Habitats in Colombia. *Journal of Vector Ecology*, *21*(2), 122–131.

Matamoros, V., Gutiérrez, R., Ferrer, I., García, J., & Bayona, J. M. (2015). Capability of microalgae-based wastewater treatment systems to remove emerging organic contaminants: A pilot-scale study. *Journal of Hazardous Materials*, *288*, 34–42. https://doi.org/10.1016/j.jhazmat.2015.02.002

Mazzoncini, M., Sapkota, T. B., Bàrberi, P., Antichi, D., & Risaliti, R. (2011). Long-term effect of tillage, nitrogen fertilization and cover crops on soil organic carbon and total nitrogen content. *Soil and Tillage Research*, *114*(2), 165–174. https://doi.org/10.1016/j.still.2011.05.001

McCarty, P. L. (2018). What is the Best Biological Process for Nitrogen Removal: When and Why? *Environmental Science and Technology*, *52*(7), 3835–3841. https://doi.org/10.1021/acs.est.7b05832

Mohedano, R. A., Costa, R. H. R., Tavares, F. A., & Belli Filho, P. (2012). High nutrient removal rate from swine wastes and protein biomass production by full-scale duckweed ponds. *Bioresource Technology*, *112*, 98–104. https://doi.org/10.1016/j.biortech.2012.02.083

Mohedano, R. A., Tonon, G., Costa, R. H. R., Pelissari, C., & Belli Filho, P. (2019). Does duckweed ponds used for wastewater treatment emit or sequester greenhouse gases? *Science of the Total Environment*, *691*, 1043–1050. https://doi.org/10.1016/j.scitotenv.2019.07.169

Mohseni, S., & Pishvaee, M. S. (2016). A robust programming approach towards design and optimization of microalgae-based biofuel supply chain. *Computers and Industrial Engineering*, *100*, 58–71. https://doi.org/10.1016/j.cie.2016.08.003

Mohsenpour, S. F., Hennige, S., Willoughby, N., Adeloye, A., & Gutierrez, T. (2021). Integrating micro-algae into wastewater treatment: A review. *Science of The Total Environment*, *752*, 142168. https://doi.org/10.1016/J.SCITOTENV.2020.142168

Monteith, H. D., Sahely, H. R., MacLean, H. L., & Bagley, D. M. (2005). A Rational Procedure for Estimation of Greenhouse-Gas Emissions from Municipal Wastewater Treatment Plants. *Water*

Environment Research, *77*(4), 390–403. https://doi.org/10.1002/j.1554-7531.2005.tb00298.x

Morales-Amaral, M. del M., Gómez-Serrano, C., Acién, F. G., Fernández-Sevilla, J. M., & Molina-Grima, E. (2015). Outdoor production of Scenedesmus sp. in thin-layer and raceway reactors using centrate from anaerobic digestion as the sole nutrient source. *Algal Research*, *12*(November), 99–108. https://doi.org/10.1016/j.algal.2015.08.020

Muradov, N., Taha, M., Miranda, A. F., Kadali, K., Gujar, A., Rochfort, S., Stevenson, T., Ball, A. S., & Mouradov, A. (2014). Dual application of duckweed and azolla plants for wastewater treatment and renewable fuels and petrochemicals production. *Biotechnology for Biofuels*, *7*(1), 30. https://doi.org/10.1186/1754-6834-7-30

Nagarajan, D., Kusmayadi, A., Yen, H. W., Dong, C. Di, Lee, D. J., & Chang, J. S. (2019). Current advances in biological swine wastewater treatment using microalgae-based processes. *Bioresource Technology*, *289*(May), 121718. https://doi.org/10.1016/j.biortech.2019.121718

Nagarajan, D., Lee, D. J., Chen, C. Y., & Chang, J. S. (2020). Resource recovery from wastewaters using microalgae-based approaches: A circular bioeconomy perspective. *Bioresource Technology*, *302*(January), 122817. https://doi.org/10.1016/j.biortech.2020.122817

Pabi, S., Reekie, L., Amarnath, A., & Goldstein, R. (2013). *Electricity Use and Management in the Municipal Water Supply and Wastewater Industries*.

Pena, L., Oliveira, M., Fragoso, R., & Duarte, E. (2017). *Potential of Duckweed for Swine Wastewater Nutrient removal and Biomass Valorisation through Anaerobic Co-digestion*. 127–138.

Poore, J., & Nemecek, T. (2018). Reducing food's environmental impacts through producers and consumers. *Science*, *360*(6392), 987–992. https://doi.org/10.1126/science.aaq0216

Préat, N., Taelman, S. E., De Meester, S., Allais, F., & Dewulf, J. (2020). Identification of microalgae biorefinery scenarios and development of mass and energy balance flowsheets. *Algal Research*, *45*, 101737. https://doi.org/10.1016/j.algal.2019.101737

Purdy, R., & Langemeier, M. (2018). International Benchmarks for Soybean Production. In

International Benchmarks for Soybean Production. https://farmdocdaily.illinois.edu/2018/06/international-benchmarks-soybean-production.html

Qie, F., Zhu, J., Rong, J., & Zong, B. (2019). Biological removal of nitrogen oxides by microalgae, a promising strategy from nitrogen oxides to protein production. *Bioresource Technology*, *292*(June). https://doi.org/10.1016/j.biortech.2019.122037

Qureshi, N., Saha, B. C., Hector, R. E., Dien, B., Hughes, S., Liu, S., Iten, L., Bowman, M. J., Sarath, G., & Cotta, M. A. (2010). Production of butanol (a biofuel) from agricultural residues: Part II - Use of corn stover and switchgrass hydrolysates. *Biomass and Bioenergy*, *34*(4), 566–571. https://doi.org/10.1016/j.biombioe.2009.12.023

Ribaudo, M. (2003). *Managing Manure: New Clean Water Act Regulations Create Imperative for Livestock Producers*. U.S. Department of Agriculture Economic Research Service, Amber Waves, February. https://www.ers.usda.gov/amber-waves/2003/february/managing-manure/

Ritchie, H. (2019). *Half of the world's habitable land is used for agriculture*. Our World in Data. https://ourworldindata.org/global-land-for-agriculture

Roman, B., & Brennan, R. (2021a). Life cycle assessment of an Eco-Machine indicates beneficial environmental impacts for upcycling wastewater nutrients into protein-rich duckweed. *Journal of Environmental Management*, *in press*.

Roman, B., & Brennan, R. A. (2019). A beneficial by-product of ecological wastewater treatment: An evaluation of wastewater-grown duckweed as a protein supplement for sustainable agriculture. *Ecological Engineering: X*, *1*(April), 100004. https://doi.org/10.1016/j.ecoena.2019.100004

Roman, B., & Brennan, R. A. (2021b). Coupling ecological wastewater treatment with the production of livestock feed and irrigation water provides net benefits to human health and the environment: A life cycle assessment. *Journal of Environmental Management*, *288*, 112361. https://doi.org/10.1016/j.jenvman.2021.112361

Roman, B., Brennan, R. A. A., & Lambert, J. D. D. (2021). Duckweed protein supports the growth and organ development of mice : A feeding study comparison to conventional casein protein. *Journal of Food Science*, *86*(3), 1750-3841.15635. https://doi.org/10.1111/1750-3841.15635

Sadeghi, S. M., Noorhosseini, S. A., & Damalas, C. A. (2018). Environmental sustainability of corn (Zea mays L.) production on the basis of nitrogen fertilizer application: The case of Lahijan, Iran. *Renewable and Sustainable Energy Reviews*, *95*(July), 48–55. https://doi.org/10.1016/j.rser.2018.07.005

Saggar, S., Singh, J., Giltrap, D. L., Zaman, M., Luo, J., Rollo, M., Kim, D. G., Rys, G., & Der Weerden, T. J. va. (2013). Quantification of reductions in ammonia emissions from fertiliser urea and animal urine in grazed pastures with urease inhibitors for agriculture inventory: New Zealand as a case study. *Science of the Total Environment*, *465*, 136–146. https://doi.org/10.1016/j.scitotenv.2012.07.088

Salbitani, G., & Carfagna, S. (2021). Ammonium Utilization in Microalgae: A Sustainable Method for Wastewater Treatment. *Sustainability 2021, Vol. 13, Page 956*, *13*(2), 956. https://doi.org/10.3390/SU13020956

Samorì, G., Samorì, C., Guerrini, F., & Pistocchi, R. (2013). Growth and nitrogen removal capacity of Desmodesmus communis and of a natural microalgae consortium in a batch culture system in view of urban wastewater treatment: Part I. *Water Research*, *47*(2), 791–801. https://doi.org/10.1016/j.watres.2012.11.006

Sasu-Boakye, Y., Cederberg, C., & Wirsenius, S. (2014). Localising livestock protein feed production and the impact on land use and greenhouse gas emissions. *Animal*, *8*(8), 1339–1348. https://doi.org/10.1017/S1751731114001293

Savci, S. (2012). An Agricultural Pollutant: Chemical Fertilizer. *International Journal of Environmental Science and Development*, 73–80. https://doi.org/10.7763/ijesd.2012.v3.191

Scavia, D., David Allan, J., Arend, K. K., Bartell, S., Beletsky, D., Bosch, N. S., Brandt, S. B., Briland, R. D., Daloğlu, I., DePinto, J. V., Dolan, D. M., Evans, M. A., Farmer, T. M., Goto, D., Han, H., Höök, T. O., Knight, R., Ludsin, S. A., Mason, D., … Zhou, Y. (2014). Assessing and addressing the re-eutrophication of Lake Erie: Central basin hypoxia. In *Journal of Great Lakes Research* (Vol. 40, Issue 2, pp. 226–246). International Association of Great Lakes Research. https://doi.org/10.1016/j.jglr.2014.02.004

Seghetta, M., Hou, X., Bastianoni, S., Bjerre, A. B., & Thomsen, M. (2016). Life cycle assessment of macroalgal biorefinery for the production of ethanol, proteins and fertilizers – A step towards a regenerative bioeconomy. *Journal of Cleaner Production*, *137*, 1158–1169. https://doi.org/10.1016/j.jclepro.2016.07.195

Shanmugam, V., Mensah, R. A., Försth, M., Sas, G., Restás, Á., Addy, C., Xu, Q., Jiang, L., Neisiany, R. E., Singha, S., George, G., Jose E, T., Berto, F., Hedenqvist, M. S., Das, O., & Ramakrishna, S. (2021). Circular economy in biocomposite development: State-of-the-art, challenges and emerging trends. *Composites Part C: Open Access*, *5*, 100138. https://doi.org/10.1016/J.JCOMC.2021.100138

Shi, W., Wang, L., Rousseau, D. P. L., & Lens, P. N. L. (2010). Removal of estrone, 17α-ethinylestradiol, and 17ß-estradiol in algae and duckweed-based wastewater treatment systems. *Environmental Science and Pollution Research*, *17*(4), 824–833. https://doi.org/10.1007/s11356-010-0301-7

Sims, A., Gajaraj, S., & Hu, Z. (2013). Nutrient removal and greenhouse gas emissions in duckweed treatment ponds. *Water Research*, *47*(3), 1390–1398. https://doi.org/10.1016/j.watres.2012.12.009

Skillicorn, P., Spira, W., & Journey, W. (1993). *Duckweed Aquaculture: a new aquatic farming system for developing countries*. https://www.echocommunity.org/en/resources/771eac80-566c-48b3-8f12-9995143fe1f5

Sońta, M., Łozicki, A., Szymańska, M., Sosulski, T., Szara, E., Wąs, A., van Pruissen, G. W. P., & Cornelissen, R. L. (2020). Duckweed from a biorefinery system: Nutrient recovery efficiency and forage value. *Energies*, *13*(20). https://doi.org/10.3390/en13205261

Sońta, M., Rekiel, A., & Batorska, M. (2019). Use of duckweed (Lemna L.) in sustainable livestock production and aquaculture - A review. *Annals of Animal Science*, *19*(2), 257–271. https://doi.org/10.2478/aoas-2018-0048

Spolaore, P., Joannis-Cassan, C., Duran, E., & Isambert, A. (2006). Commercial applications of microalgae. *Journal of Bioscience and Bioengineering*, *101*(2), 87–96. https://doi.org/10.1263/jbb.101.87

Su, Y. (2021). Revisiting carbon, nitrogen, and phosphorus metabolisms in microalgae for wastewater treatment. In *Science of the Total Environment* (Vol. 762, p. 144590). Elsevier B.V. https://doi.org/10.1016/j.scitotenv.2020.144590

Sutton, M. A., Howard, C. M., Erisman, J. W., Bleeker, A., Billen, G., Grennfelt, P., Van Grinsven, H., & Grizzetti, B. (2011). The European Nitrogen Assessment: Sources, Effects and Policy Perspectives. In *The European Nitrogen Assessment*. Cambridge University Press: Cambridge, UK. https://doi.org/10.1017/cbo9780511976988

Ubando, A. T., Felix, C. B., & Chen, W. H. (2020). Biorefineries in circular bioeconomy: A comprehensive review. *Bioresource Technology*, *299*(December 2019), 122585. https://doi.org/10.1016/j.biortech.2019.122585

Vivekanand, A. C., Mohapatra, S., & Tyagi, V. K. (2021). Microplastics in aquatic environment: Challenges and perspectives. *Chemosphere*, *282*, 131151. https://doi.org/10.1016/J.CHEMOSPHERE.2021.131151

Vladimirova, I. N., & Georgiyants, V. A. (2014). Biologically Active Compounds from Lemna Minor S. F. Gray. *Pharmaceutical Chemistry Journal*, *47*(11), 599–601. https://doi.org/10.1007/s11094-014-1016-8

Walsh, J. J., Jones, D. L., Edwards-Jones, G., & Williams, A. P. (2012). Replacing inorganic fertilizer with anaerobic digestate may maintain agricultural productivity at less environmental cost. *Journal of Plant Nutrition and Soil Science*, *175*(6), 840–845. https://doi.org/10.1002/jpln.201200214

Wen, Y., He, Y., Ji, X., Li, S., Chen, L., Zhou, Y., Wang, M., & Chen, B. (2017). Isolation of an indigenous Chlorella vulgaris from swine wastewater and characterization of its nutrient removal ability in undiluted sewage. *Bioresource Technology*, *243*, 247–253. https://doi.org/10.1016/j.biortech.2017.06.094

Wu, G., Bazer, F. W., & Cross, H. R. (2014). Land-based production of animal protein: impacts, efficiency, and sustainability. *Annals of the New York Academy of Sciences*, *1328*(1), 18–28. https://doi.org/10.1111/nyas.12566

Wu, W., Cheng, L. C., & Chang, J. S. (2020). Environmental life cycle comparisons of pig farming integrated with anaerobic digestion and algae-based wastewater treatment. *Journal of Environmental Management*, *264*, 110512. https://doi.org/10.1016/j.jenvman.2020.110512

Xia, Y., Zhang, M., Tsang, D. C. W., Geng, N., Lu, D., Zhu, L., Igalavithana, A. D., Dissanayake, P. D., Rinklebe, J., Yang, X., & Ok, Y. S. (2020). Recent advances in control technologies for non-point source pollution with nitrogen and phosphorous from agricultural runoff: current practices and future prospects. *Applied Biological Chemistry*, *63*(1), 1–13. https://doi.org/10.1186/S13765-020-0493-6/METRICS

Xu, J., & Shen, G. (2011). Growing duckweed in swine wastewater for nutrient recovery and biomass production. *Bioresource Technology*, *102*(2), 848–853. https://doi.org/10.1016/j.biortech.2010.09.003

Yao, Y., Zhang, M., Tian, Y., Zhao, M., Zhang, B., Zhao, M., Zeng, K., & Yin, B. (2017). Duckweed (Spirodela polyrhiza) as green manure for increasing yield and reducing nitrogen loss in rice production. *Field Crops Research*, *214*(April), 273–282. https://doi.org/10.1016/j.fcr.2017.09.021

Zhao, Y., Fang, Y., Jin, Y., Huang, J., Bao, S., Fu, T., He, Z., Wang, F., Wang, M., & Zhao, H. (2015). Pilot-scale comparison of four duckweed strains from different genera for potential application in nutrient recovery from wastewater and valuable biomass production. *Plant Biology*, *17*(s1), 82–90. https://doi.org/10.1111/plb.12204

Zhao, Y., Fang, Y., Jin, Y., Huang, J., Bao, S., He, Z., Wang, F., & Zhao, H. (2014). Effects of operation parameters on nutrient removal from wastewater and high-protein biomass production in a duckweed-based (Lemma aequinoctialis) pilot-scale system. *Water Science and Technology*, *70*(7), 1195–1204. https://doi.org/10.2166/wst.2014.334

Zheng, H., Wu, X., Zou, G., Zhou, T., Liu, Y., & Ruan, R. (2019). Cultivation of Chlorella vulgaris in manure-free piggery wastewater with high-strength ammonium for nutrients removal and biomass production: Effect of ammonium concentration, carbon/nitrogen ratio and pH. *Bioresource Technology*, *273*(November 2018), 203–211. https://doi.org/10.1016/j.biortech.2018.11.019

Zhu, L., Wang, Z., Shu, Q., Takala, J., Hiltunen, E., Feng, P., & Yuan, Z. (2013). Nutrient removal and biodiesel production by integration of freshwater algae cultivation with piggery wastewater treatment. *Water Research*, *47*(13), 4294–4302. https://doi.org/10.1016/j.watres.2013.05.004

Zimmo, O. R., Van Der Steen, N. P., & Gijzen, H. J. (2003). Comparison of ammonia volatilisation rates in algae and duckweed-based waste stabilisation ponds treating domestic wastewater. *Water Research*, *37*(19), 4587–4594. https://doi.org/10.1016/j.watres.2003.08.013