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Duckweed: exploring its farm-to-fork potential for food production and biorefineries

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Duckweed is a promising and sustainable aquatic plant offering an eco-friendly alternative for synthesizing high-value bio-products and has potential across food industries, pharmaceuticals, and bioenergy production. This review explores duckweed cultivation, harvesting, and biorefining of duckweed into value-added products, with a focus on both traditional and innovative production methods. Advanced techniques, such as superhydrophobic coatings, bioreactor systems, and process waste management, are discussed to enhance biomass yield. Various impacts of abiotic factors that influences the cultivation practices are examined and effective management strategies (harvesting frequency, storage conditions, and appropriate pretreatment methods) are discussed. The biorefinery of duckweed biomass is extensively investigated for producing organic acids, biofuels, biochar, biofertilizer, enzymes, vitamins, and proteins. Current and future applications of duckweed in feed, wastewater treatment, pharmaceuticals, and functional foods are highlighted. Thus, duckweed biorefinery presents a versatile platform to meet the growing demand for sustainable resources. It also facilitates to capture the high value products with reduced environmental impacts by applying life cycle assessment (LCA) and techno-economic analysis. However, further research is essential to develop scalable and cost-effective solutions.

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Sustainability spotlight

In the face of an increasing global population, ensuring food security while mitigating environmental impact is imperative. Duckweed presents a versatile and sustainable resource with the potential to address growing global demands across various industries. Its environmentally friendly nature, coupled with innovations in cultivation and processing, make it a key player in the transition towards more sustainable bio-product production systems. This spotlight advocates for the adoption of duckweed as a sustainable and eco-friendly resource for the production of high-value bio-products. It emphasizes the potential of duckweed to replace conventional, resource-intensive methods across various sectors, such as food, pharmaceuticals, bioenergy, and wastewater treatment. By highlighting innovations in cultivation, advanced biorefinery technologies, and the applications of duckweed, the spotlight encourages further research and development to scale up duckweed-based biorefineries, ultimately promoting it as a key solution for meeting global sustainability goals. Aligning with the UN's Sustainable Goals, this work contributes notably to multiple goals. It address SDG 2 (Zero Hunger) and SDG 3 (Good Health and Well-being) through its potential as a nutritious food source and medicinal products. It also contributes to SDG 6 (Clean Water and Sanitation), SDG 7 (Affordable and Clean Energy), SDG 12 (Responsible Consumption and Production), and SDG 13 (Climate Action) by fostering sustainable resource use, renewable energy production, and environmental stewardship.

1. Introduction

Sustainability and the promotion of sustainable consumption and production patterns are central to the United Nations' strategies, particularly within the 12th Sustainable Development Goal (SDG), which focuses on reducing food waste.[1] The Farm-to-Fork (F2F) strategy further emphasizes the need for a sustainable food supply chain that operates within a circular and bio-based economy. In this context, the European Commission has identified the food industry's growing demand for new and alternative plant proteins, with duckweed emerging as a promising solution.[2] Duckweed is a small aquatic plant found in still or slow-flowing water bodies such as ponds, lakes, and marshes. It belongs to the Lemnaceae family, comprising at least 38 species across five genera: *Lemna*, *Spirodela*, *Wolffia*, *Landoltia*, and *Wolffiella*.[3] These species are distributed globally, except in the Antarctic and Arctic regions. Among them, *Lemna gibba* is native to Europe, Asia, and North America but invasive in parts of southern Africa. In contrast, *Wolffia arrhiza* and *Wolffia globosa* are consumed as food in the Asian region, and *Lemna minor* is increasingly recognized as a sustainable protein source.[4] *L. minor* is particularly noteworthy for its adaptability to diverse environmental conditions, rapid growth in favorable settings, and nutrient-rich composition, making it suitable for various applications. Recent studies have identified *L. minor* as a significant source of carotenoids and flavonoids,

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underscoring its potential in diverse applications.[5] In Ireland, *L. minuta* (tiny duckweed) an invasive species first discovered in Co. Cork in 1993, has spurred further research into duckweed's applications. This research aims to contribute to a circular bioeconomy by utilizing waste as a valuable resource.[6]

Duckweed cultivation offers significant benefits for both human nutrition and environmental sustainability. Rich in *C*-glycosyl-flavonoids, duckweed can enhance the human diet by providing a high-quality protein source.[7] It serves not only as a component of human nutrition but also as animal feed, an industrial raw material, and a resource for bioenergy production.[7–11] Duckweed's nutrient profile is impressive, containing approximately 20–35% protein, 4–10% starch, 4–7% fat, and 25% fiber by dry weight.[8] Additionally, studies have shown that duckweed consumption results in higher post-consumption blood concentrations of essential amino acids (12.4–184.4 μmol L^{-1}) and increased vitamin B12 levels (423 pg mL^{-1}) compared to other plant sources like green peas (31.3–179.3 μmol L^{-1}).[12] Importantly, toxicological examination of representative duckweed strains from five distinct genera has confirmed their safety, showing no adverse effects on human cell lines or in individuals consuming *L. minor*.[13,14] From an environmental perspective, duckweed plays a crucial role in water purification by absorbing and concentrating contaminants from its environment. It has been found to absorb heavy metals such as Cd, Cu, K, Mn, Na, Ni, and Zn more effectively than some macroalgae like *Chlorella sorokiniana*. However, it is worth noting that duckweed cultivated in contaminated water sources, such as thermal mineral waters, can accumulate potentially harmful levels of heavy metals like Pb, sometimes exceeding the maximum levels set by the European Union (EU) for food supplements. This emphasizes the need for careful monitoring of cultivation environments to ensure the safety of duckweed-based products.[15]

Duckweed is abundantly available and considered an economic resource. However, conventional cultivation methods in natural aquatic ecosystems are often inefficient, limiting its widespread adoption as a mainstream food source.[16] To improve production efficiency and sustainability, innovative cultivation strategies have been developed to unlock duckweed's potential as a valuable and underutilized food resource. These strategies include the use of bioreactor systems, waste stream utilization, and controlled environments, which offer greater control overgrowth conditions and resource use. By adopting these advanced methods, duckweed cultivation can be optimized to meet growing demands more sustainably and efficiently.[17–20]

While there are potential risks and safety considerations associated with duckweed consumption, numerous studies have extensively explored its benefits. The role of duckweed as a feed source, phytoremediator (a plant-based approach for removing pollutants), human food source, and biofuel feedstock has been widely discussed.[21–25] However, the risks mainly stem from its ability to absorb and concentrate environmental contaminants, which depend on the cultivation location and water quality. Therefore, suitable pre-treatment methods, such as chemical precipitation, heat treatment, or membrane filtration, are necessary to remove or neutralize contaminants in the water before it is used for duckweed cultivation. Additionally, further processing or alternative cultivation methods are required before incorporating duckweed into food products. Pretreatments can also improve the extraction process and increase the yield of high-value products from duckweed. Addressing these challenges is crucial to fully harnessing duckweed as a sustainable alternative source to meet the growing demand for proteins and other products. This review addresses the need for sustainable and renewable sources of plant-based proteins and other valuable products in response to global food demand, environmental challenges, and sustainability goals. Duckweed has emerged as a key resources due to its rapid growth, high nutrient content, and minimal resource requirements making it highly efficient for diverse applications. Its ability to purify water by absorbing excess nutrients also positions duckweed as an eco-friendly solution for food production, bioenergy generation, and environmental remediation. The objective of this review is to explore the full potential of duckweed, focusing on its cultivation, nutrient management, and its role in biorefinery processes that yield valuable by-products such as proteins, starch, and biofuels.

2. Cultivation of duckweed for biomass production

2.1. Diversity of duckweed species

The Lemnaceae family, comprising various duckweed species, presents a fascinating diversity with significant potential for multiple applications.[26] Duckweed plants can range in size from 1.5 cm (*Sp. polyrhiza*) to <1 mm (*Wo. angusta*) and consist of a frond—a leaf-stem structure. Some genera, like *Spirodela*, *Landoltia*, and *Lemna* possess roots, adding to their structural complexity.[27] The shape of the fronds varies among genera, from roundish to sickle-shaped. Different duckweed species, such as *Spirodela polyrhiza* (L.) Schleid and *Lemna minor* L., have clone-specific doubling times of approximately 2.3 and 1.7 day, respectively, highlighting their rapid growth potential.[26] Each duckweed species possesses unique characteristics that make them suitable for various applications. For instance, *Lemna aequinoctialis* has been noted for its effectiveness in wastewater remediation.[28] Meanwhile, species like *Spirodela* species, with their larger fronds, are ideal for biomass production and bioenergy feedstock due to their higher biomass yield. Conversely, smaller species such as *Wolffia* offer potential in pharmaceuticals and nutraceuticals, given their high protein content and rapid growth rates.[29–31] This diversity underscores the importance of selecting duckweed species based on specific traits and adaptability for targeted applications.

Duckweed's potential as a protein source has garnered considerable attention, with each species exhibiting distinct nutritional profiles influenced by growth conditions (Table 1). For example, *S. polyrhiza* contains high lipid concentrations rich in polyunsaturated fatty acids (PUFA). In contrast, *W. arrhiza* is characterized by high crude protein, low-fat, and abundant minerals and α-linolenic acid, known for its anti-

Table 1 Nutrient profiles of selected edible duckweed species (dry weight)

Species	Protein (%)	Ash (%)	Carbohydrate (%)	Lipid (%)	Starch (mg g^{-1})	References
L. minor	25.0–35.0	—	51.0–61.0	2.6–7.3	—	32
	27.0–31.0	—	52.3–60.0	4.7–10.0	—	33
	34.9–41.8	21.6–32.3	—	2.7–3.3	—	34
	7.5–10.2	11.8–14.0	54.0–58.1	2.0–2.6	—	35
	—	—	—	—	16.7–477.6	36
L. japonica	—	—	—	—	17.2–346.1	36
L. japonica	31.0–36.1	—	—	—	—	37
L. punctata	30.1	—	—	—	—	37
L. minuta	—	—	—	—	10.8–369.5	36
L. turionifera	—	—	—	—	25.2–323.9	36
W. arrhiza	50.9	11.7	31.3	6.1	—	38
	45.4–61.7	18.9–22.1	8.8–14.1	1.2–2.0	—	39
	20–30	—	—	0.5–5.3	—	40
	20.9–29.6	10.1–14.6	—	1.47–2.83	—	41
S. polyrhiza	24.1	1.8	57.9	1.7	—	42
	30.5–35.8	18.5–20.6	38.4–41.7	7.1–7.2	—	43
	16.7–29.3	—	18.0–33.1	—	—	44
	—	—	—	—	4.8–310.5	36

carcinogenic properties, thereby positioning duckweeds as sources of healthful fats.[31,43,45] These nutritional attributes position duckweeds as promising sources of both healthful fats and proteins, with significant potential in nutrition, health, and industrial applications.

2.2. Duckweed cultivation methods for sustainable biomass production

Confined initially to open pond systems, duckweed cultivation has evolved through the integration of laboratory techniques, sustainable practices, and advanced technologies.[46,47] This evolution was driven by the need for more efficient and sustainable biomass production. Table 2 presents an overview of various cultivation methods, highlighting their advantages, disadvantages, and innovative approaches and discussing them in detail in the following sections.

2.2.1. Conventional farming practices. Conventional duckweed cultivation typically involves growing the plant in open ponds or natural water bodies, leveraging its natural habitat. An optimal pond depth of less than 0.5 meters is recommended to ensure that duckweed has adequate access to essential nutrients. Economical and straightforward, open pond systems are commonly used for large-scale cultivation.[34] These ponds, whether shallow or deep, utilize solar energy for photosynthesis, offering benefits such as low setup and operating costs, ease of maintenance, and scalability.[58] However, this method faces several challenges due to environmental variability. Fluctuations in temperature, sunlight exposure, and nutrient concentrations can significantly affect growth rates and overall productivity, introducing unpredictability into the cultivation process.[59,60] Additionally, open pond systems are susceptible to contamination from external pollutants such as pesticides, heavy metals, and pathogens. This not only affects the quality and safety of duckweed biomass but also raises concerns for its use in human consumption and other downstream applications. Despite these challenges, conventional farming practices remain relevant for large-scale, cost-effective duckweed production, especially in regions where resource limitations make advanced methods less feasible.[61]

Table 2 Various cultivation methods of duckweed, advantages, disadvantages and innovative approaches

Cultivation method	Advantages	Disadvantages	Innovative approaches	References
Conventional farming practices	Low cost, accessibility, natural growth conditions	Environmental variability, contamination, lack of control	Influent pre-aeration	48 and 49
Laboratory cultivation	Precise control, high growth rates	Limited growth surface, medium limitations	Superhydrophobic coatings	50
Bioreactor systems	Controlled environment, scaling possibilities, reduced contamination risks	Operational complexity, cost considerations	Optimized flow dynamics, tray geometry modifications	9, 51 and 52
Waste streams	Sustainable resources, nutrient rich medium, adaptability in integrated biorefineries	Regulatory challenges, effluent composition impact, toxicity, emissions	pH adjustment, optimization of slurry dilution rates, nutrient supplementation, Ca: mg ratio optimization	53–56 and 57

Conventional farming practices, though prevalent, often lack precise control overgrowth conditions, highlighting the need for more innovative approaches. One promising method is influent pre-aeration, which has been shown to optimize duckweed growth in ponds. Studies indicate that the growth rate of duckweed in pre-aerated ponds (9.20 g per m^2 per day, dry weight basis) is significantly higher than in ponds without pre-aeration (6.88 g per m^2 per day).[48] However, the effectiveness of the pre-aeration may diminish over time, typically after about nine months. Despite this decline, pre-aeration has been found to enhance duckweed biomass production without affecting its nutrient quality. Further improvements have been observed when combining pre-aeration with an elastic carrier made of polypropylene fiber, which increases both growth rates and nutrient recovery compared to methods without the carrier.[49] Adopting pre-aeration represents a promising strategy to enhance duckweed cultivation in open ponds, effectively addressing the limitations of conventional farming practices. This approach not only boosts duckweed growth but also offers potential benefits for downstream applications. As demand for sustainable bioresources grows, such innovative strategies are crucial in developing more efficient duckweed cultivation practices.

Open pond systems are inherently scalable due to their simple design and low cost, making them suitable for large-scale operations. However, scaling up pre-aeration techniques and incorporating elastic carriers can introduce additional complexity and infrastructure requirements. As the scale increases, maintaining consistent growth conditions and maintaining potential contamination risks become more challenging. While open pond systems offer a cost-effective solution for duckweed cultivation with minimal setup and operating expenses, integrating pre-aeration and elastic carriers could enhance productivity, potentially justifying the initial investment. The long-term economic feasibility of these advanced methods depends on balancing the improved growth rates with the costs of implementation and maintenance. In cases where the benefits of increased productivity outweigh these costs, these innovations could provide a viable pathway to more efficient and sustainable large-scale duckweed cultivation.

2.2.2. Laboratory cultivation techniques. Laboratory cultivation methods are widely used for growing duckweeds in controlled environments where light intensity, temperature, humidity, and nutrient concentrations can be precisely regulated.[53,62] Compared to natural environments, duckweed grown under laboratory conditions has been reported to contain higher concentrations of amino acids (lysine, arginine, valine, leucine, aspartic acid, glutamic acid, and alanine), non-fibrous carbohydrates, and minerals (calcium and sodium).[63] However, the selection of appropriate growth media is essential, as it provides the necessary nutrients for robust development.[58,64] Commonly used media include Schenk and Hildebrandt medium, Hutner medium, Murashige and Skoog medium, and Hoagland medium, with the latter being the most frequently reported in the literature for duckweed cultivation.[58] Studies have shown that *W. arrhiza* and *L. minor* demonstrate biomass productivity of 6.99–8.36 g and 1.31–1.76 g in Hoagland and Arnon (HA) medium, and 6.64–8.93 g and 1.40–1.67 g in Steinberg medium, respectively.[65] However, a key challenge arises when scaling up from laboratory settings to commercial production. In larger-scale operations, the cost and availability of high-quality growth media can become limiting factors, potentially affecting the economic feasibility of large-scale duckweed cultivation.

The protein content of duckweed can be significantly influenced by the growth medium. Under optimal conditions of 3.92 mM KH_2PO_4, 7.95 mM Ca $(NO_3)_2$, and a pH of 7.22, the protein content of duckweed increased markedly from 51.09% to 39.81%.[66] A modeled cultivation medium with inorganic salts and organic nutrients yielded even higher protein content (37.1–40.1% dry weight) compared to traditional HA and Steinberg media (18.2–32.5%). Additionally, supplementing mineral nutrition media with organic components like carbohydrates, vitamins, and amino acids significantly boosted biomass production (6.64–8.93 g) and protein content (22.1–32.5%) in *W. arrhiza*. Similarly, *L. gibba* exhibited approximately 1.4 times faster growth when cultivated in an organic nitrogen source medium compared to an inorganic nitrogen source, suggesting that enhancing the growth medium with organic nutrients can improve both yield and nutritional quality.[67]

Optimizing growth media can also target specific applications. For instance, cultivating duckweed in a diluted (1/6×) Hoagland medium with 800 mg L^{-1} uniconazole increased starch accumulation, making it more suitable for bioethanol fermentation. However, laboratory cultivation poses challenges, such as the stacking of duckweed due to limited growing surfaces, which can reduce light capture efficiency compared to natural environments.[68] To address this, superhydrophobic (SHP) coatings on acrylic sheets have been shown to significantly increase duckweed growth in laboratory settings.[50] In SHP environments, the relative growth rate was 2.17 times higher than in non-SHP conditions, with harvested duckweed exhibiting superior protein (15.6%), carbohydrate (9.47%), and lipid (0.71%) content. This indicates that SHP coatings not only provide more surface area for growth but also enhance biomass production while maintaining biochemical composition. Incorporating SHP coatings in cultivation platforms offers a promising strategy to optimize laboratory cultivation of duckweed. The application of optimized media and SHP coatings in laboratory settings shows promise for scalability, but translating these advancements to industrial-scale operations presents challenges. These include the need for consistent environmental control and the development of complex nutrient delivery systems. Economically, while advanced media and SHP coatings can significantly enhance biomass productivity and nutritional quality, they come with higher initial costs. However, their potential to boost yields could offset these expenses in the long run. The overall economic feasibility will ultimately depend on the costs of scaling up these methods and the market demand for high-protein, high-biomass duckweed products. This demand will play a crucial role in determining the cost-effectiveness of these advanced cultivation techniques for commercial applications.

2.2.3. Duckweed bioreactor systems. Cultivating duckweed in bioreactors offers numerous possibilities for producing novel, nutritious food products. Bioreactor systems have been utilized for both lab-scale duckweed biomass production.[46] A multi-tiered duckweed bioreactor system, as shown in Fig. 1, consists of four vertically stacked stainless-steel trays with a gravity-fed flow system that circulate the medium from a 500 L sump tank through the trays. This design also incorporates adjustable light emitting diode (LED) lighting to optimize growth conditions.[69] Optimal growth rates have been observed at specific flow conditions, such as a flow rate of 1.5 L min^{-1} with a 50 mm water depth, corresponding to a flow velocity of 0.0012 m s^{-1}.[70] Higher flow rates of up to 2.5 L min^{-1} with a 5 cm water depth have been found to minimize stagnant zones and surface channeling, enhancing nutrient distribution.[51]

Tray geometry also plays a crucial role in bioreactor performance. Modifying the number and positioning of inlet and outlet ports can improve hydrodynamic parameters and nutrient transfer efficiency, with configurations using three inlets and outlets proving effective.[51] The stocking density of the inoculant is another significant factor influencing duckweed growth. Bioreactors with an 80% inoculation density showed a low duckweed biomass yield (213.7 g m^{-2}), whereas bioreactors with a 20% inoculation density achieved a higher biomass yield (392.8 g m^{-2}) and a high starch yield rate of 1.20 g per m^2 per day. Conversely, the highest protein yield rate was observed at a moderate inoculation density of 60% (2.24 g per m^2 per day) compared to 1.90 g per m^2 per day at a 20% inoculation density. This suggests that lower densities favor higher biomass and starch yields, while moderate densities are optimal for protein production.[9] Additionally, lower inoculation densities (20% and 40%) enhanced the removal rates of nitrogen compounds (NO_3–N and NH_3–N) and phosphorus. The removal of sulfate (SO_4^{2-}) was also influenced by inoculation density, with higher removal rates (86.2%) observed in bioreactors with 20% inoculation density.[9] These findings highlight the potential of bioreactor systems for optimizing duckweed cultivation by fine-tuning environmental conditions, stocking density, and nutrient removal, making them a promising approach for sustainable biomass production.

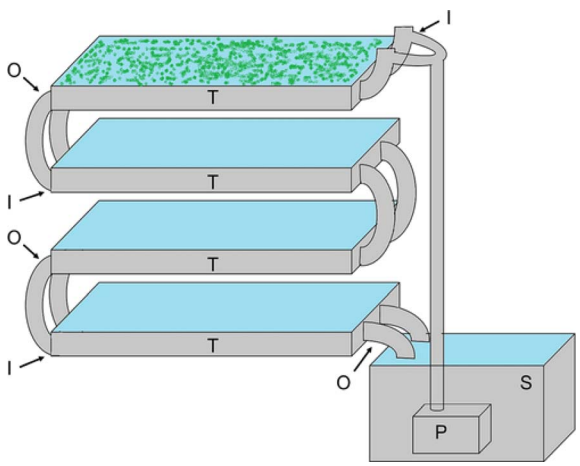

Fig. 1 Schematic overview of a multi-tiered duckweed bioreactor. S – sump; P – pump; t – duckweed trays; I – tray inlets; O – tray outlets.[69]

Transitioning from open-pond systems to bioreactor systems represents a shift towards more controlled and scalable duckweed cultivation methods. Bioreactors offer precise control over environmental conditions, such as nutrient levels, flow rates, and inoculation densities, making it feasible to scale up cultivation from laboratory to pilot and potentially industrial levels.[71] The ability to fine-tune these parameters is important for maintaining optimal growth conditions and maximizing productivity on a larger scale. However, this precision comes with higher construction costs compared to open-pond systems. While bioreactors can deliver better growth yields and efficiency, they also incur higher operational costs due to the energy required for maintaining flow rates and controlling environmental conditions.[72] Despite these higher operational costs, bioreactors offer advantages such as reduced contamination risks, improved nutrient utilization, and enhanced pollutant removal.[73] These benefits can lead to overall cost savings and increased profitability in the long term, particularly when considering the potential market value of high-quality duckweed products and the environmental gains from effective nutrient and pollutant management.

2.2.4. Duckweed cultivation with waste streams. Duckweed cultivation utilizing various waste streams has emerged as a promising strategy for sustainable biomass production and waste management (Fig. 2). This multifaceted approach involves harnessing the nutrient-rich composition of waste streams such as animal dung, food waste, and wastewater from agricultural and industrial processes to support duckweed growth.[52,54–57,74–76] Studies have shown that different duckweed species exhibit varying tolerance levels to these waste streams. For instance, *L. gibba* has been reported to tolerate swine lagoon effluent more effectively than *S. punctata*, *L. minor*, and *L. obscura*, maintaining healthier fronds and a higher protein content (16.9%).[52] Additionally, duckweed grown on swine manure has been found to produce protein-rich biomass with a protein content of 35%, and the levels of potentially harmful heavy metals were below the feed limits proposed by Directive 2002/32/EC.[54] By utilizing waste streams for cultivation, duckweed not only aids in nutrient recycling and waste management but also produces high-quality biomass that can serve as a sustainable protein source. This makes duckweed cultivation with waste streams a versatile approach that addresses both environmental and agricultural challenges.

Duckweeds can grow effectively, exhibiting a high protein content of 41.75% and ash content of 23.24% at slurry concentrations of 1.50%.[34] However, maintaining optimal environmental conditions, such as pH, total dissolved solids, electrical conductivity, salinity, and temperature is crucial for achieving maximum growth and productivity in pig slurry.[34] Duckweed cultivated in human urine has demonstrated a higher protein content (31.60%) compared to secondary treated wastewater (25.26%).[77] Additionally, cultivation in both human urine and wastewater has shown effective removal rates, with chemical oxygen demand (COD), Total Phosphorus (TP),

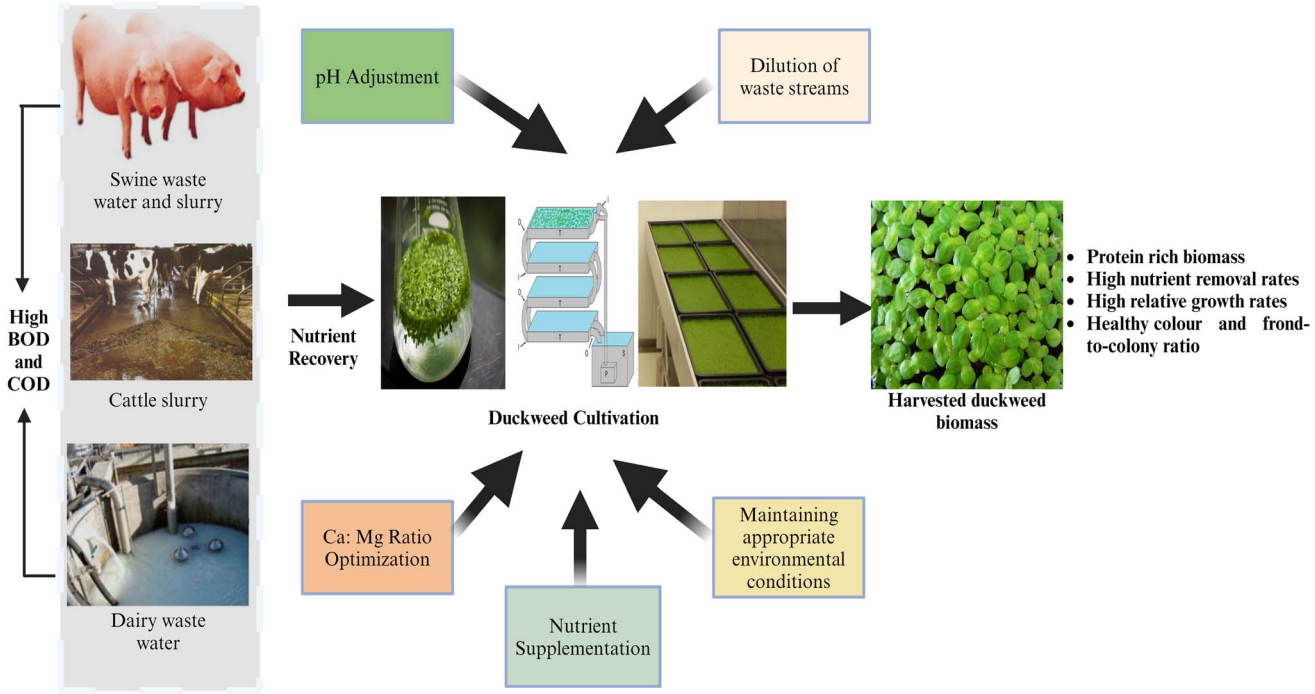

Fig. 2 Duckweed cultivation in waste streams.

and Total Nitrogen (TN) removal exceeding 80%, 90%, and 50%, respectively (Table 3).

Duckweed cultivation using dairy processing wastewater also presents a promising avenue for producing protein-rich biomass. However, the high organic nutrient loads of dairy wastewater can exceed the tolerable ranges for duckweed growth. For successful cultivation, dairy wastewater should be conditioned to reduce COD and biological oxygen demand (BOD) while retaining essential nitrogen and phosphorus.[78] Studies indicate that highly concentrated wastewater inhibits duckweed growth, necessitating treatment to prevent phytotoxic effects.[79] For example, duckweed grown on 10% diluted anaerobically digested dairy processing waste water (AD-DPW) showed higher nutrient removal rates (248.46 mg TN per md and 126.54 mg TP per md) compared to those on 5% AD-DPW (43.72 mg TN per md and 53.76 mg TP per md).[80] Notably, the relative growth rate of duckweed decreased as the concentration of AD-DPW increased, with plants grown on 100% AD-DPW displaying signs of stress, including fragmented colonies and death. Furthermore, studies have found that duckweed grows better on diluted nitrification-denitrification effluent (NDNE) from pig manure treatment than on undiluted medium.[81] High growth rates (0.23 per day) have also been reported at 24 °C using human urine with a 1 : 200 dilution factor.[67] While dilution effectively enhanced duckweed growth, exploring alternative conditioning methods such as chemical precipitation, aeration, membrane filtration, or pH adjustment could further improve wastewater characteristics and its suitability for duckweed cultivation.

Non-optimal concentrations of components like iron, manganese, sodium, chloride, and sulfate in synthetic dairy wastewater can negatively impact duckweed growth, resulting in poor growth rates.[55] The nutrient ratio is also critical for facilitating duckweed growth. For instance, an imbalance that favours magnesium over calcium can significantly reduce *L. minor* growth rate to as low as 0.05 per day. Conversely, adjusting this ratio to favor calcium through the addition of calcium sulfate can lead to a relative growth rate of 0.2–0.3 per day.[55] Research indicates that a calcium ratio of 1 : 1.6 or greater is necessary for optimal *L. minor* growth. Furthermore, maintaining a constant calcium concentration (0.12 nM) with a magnesium concentration of 0.2 nM has been shown to progressively increase *L. minor* growth. These findings suggest that monitoring and optimizing the Ca ratio in duckweed cultivation systems could maximize growth rates and enhance nutrient removal efficiency.[77] In addition to its role in wastewater treatment, duckweed grown on waste streams produces nutrient-rich biomass that can be utilized for various biorefinery products. For example, duckweed cultivated in sewage treatment plants has been used to produce biohydrogen,[82] while biogas has been generated from duckweed grown in food waste, alcohol wastewater, and cattle dung.[55,83,84] Furthermore, bio-oil has been derived from duckweed cultivated in rural domestic and agricultural wastewater.[85] Other applications, such as using duckweed for food production, should also be explored. However, safety concerns related to potential contamination may require additional pretreatment to ensure safe use.

Utilizing waste streams for duckweed cultivation is a scalable strategy, particularly when leveraging large volumes of available waste. However, scaling up requires effective waste conditioning and treatment to address issues like high organic loads and potential contamination. Implementing pre-treatment

Table 3 Growth rate, yield, and biomass production of different duckweed species under different cultivation medium[a]

Cultivated species	Cultivation medium	Findings	References
L. minor	Pig slurry	• Duckweed grows best on pig slurry, with optimal yields at concentrations of 1.50%, 1.00%, and 0.75%	34
L. minor	Diluted chicken manure	• Increased concentrations of chicken manure inhibited duckweed growth and protein production • A 1 : 8 dilution resulted in die-offs, while 1 : 16 and 1 : 12 dilutions supported acceptable growth and protein content	52
L. minor	Swine manure	• The average productivity was 4.5 g per m^2 per day • N and P were removed from the growth medium, resulting in the production of protein-rich biomass containing 35% of dry weight	74
L. minor	Waste water and Hoagland medium	• *L. minor* at 50% wastewater dilution exhibited the best performance in terms of weight, biomass, growth rates, and protein content	54
L. minor	Fresh cow manure, food waste and waste sorbents	• Bioreactors loaded with residual duckweed biomass (33.3%), inoculum (33.3%), and food waste (33.3%) provided the most significant biogas potential	55
L. minor	Swine lagoon and Schenk & Hildebrandt medium supplemented with 10 g L^{-1} sucrose	• Duckweed exhibits faster growth and higher biomass increase when grown in swine lagoon wastewater compared to Schenk & Hildebrandt medium supplemented with 10 g L^{-1} sucrose	75
Wolffia arrhiza	Outdoor cement pond-pig manure, chemical and hydroponic fertilizer	• Percentage yield in the range of 4.6–5.3% was obtained	56
Lemna sp. and *Spirodela* sp.	Treated sewage effluents from different wastewater treatment processes	• Biomass production rates and doubling times varied with the type of duckweed and effluent used • Primary treated sewage effluent resulted in higher and stable biomass production compared to conventional activated sludge effluent	57
L. punctata	Swine waste in duckweed ponds	• Ponds produced over 13 tons of biomass (68 t per ha per year of dry biomass), with 35% crude protein	76
L. minor	Anaerobically digested, dairy processing waste water (AD-DPW)	• Duckweed showed moderate growth across all concentrations of AD-DPW, with a maximum RGR of 0.13 per day • Increasing the concentration of AD-DPW resulted in a decrease in RGR overall	77

[a] AD-DPW = anaerobically digested, dairy processing waste water.

techniques and managing environmental variables are essential for successful large-scale operations. Using waste streams can also reduce input costs and offer the dual benefits of waste management and biomass production. The cost-effectiveness of this approach depends on the efficiency of waste treatment processes and the balance between waste handling expenses and the value of the produced biomass. Optimizing dilution and conditioning methods, while ensuring high nutrient removal efficiency can significantly enhance the economic viability of this sustainable cultivation strategy.

2.3. Effect of abiotic factors on duckweed growth, biomass production, nutrient removal rates, and biochemical composition

2.3.1. Temperature. In their natural habitat, duckweeds exhibit optimal growth within a temperature range of 6 to 33 °C, with the most favorable water temperatures being between 19 °C and 30 °C.[86,87] Optimal temperatures for maximum growth rates have been identified as 25 °C for *L. punctata* (4.2 g per m^2 per day) and 20 °C for *L. minor* (3.9 g per m^2 per day).[37] In mixed cultures of these two strains, growth rates align more closely with *L. punctata* at 20 °C, indicating the importance of tailoring cultivation systems to the specific temperature requirements of different duckweed species.

Temperature also significantly affects duckweed's protein production efficiency. Lower water temperatures ranging from 12 °C to 21 °C have been shown to result in higher crude protein content (36.16%) in duckweed biomass, compared to 25 °C to 31 °C, where protein content ranges between 19.75% and 22.25%.[37,88] Temperature also impacts starch accumulation in duckweed. As water temperature increases, starch content tends to decrease. For example, the lowest tested temperature of 5 °C led to the highest starch accumulation rate, reaching 26.6%.[89] Given the temperature-dependent nature of both protein and starch production in duckweed, careful management of water temperatures within the optimal range of 12 °C to 21 °C can help maximize these yields. Maintaining this temperature range consistently has been associated with higher crude protein content and can also support favorable starch accumulation rates, making it a key factor in optimizing duckweed cultivation for both nutritional and bioenergy purposes.

Duckweed also exhibits temperature-dependent efficiency in removing phosphorus from wastewater. At a low temperature of 8 °C, duckweed managed to remove over half of the phosphorus, and nearly complete phosphorus removal was achieved at 25 °C within four day.[90] Although growth is limited at 8 °C, duckweed continues to accumulate phosphorus internally, indicating its ability to remove this nutrient even under colder conditions. As the temperature increases from 18 °C to 25 °C, photosynthetic efficiency and nutrient absorption improve, which in turn stimulates duckweed growth. This enhanced growth potential may also help mitigate the adverse effects of cadmium toxicity.[91] These findings demonstrate a clear relationship between temperature fluctuations and the physiological responses of duckweed. Understanding this relationship is crucial for optimizing growth conditions and nutrient removal efficiency in duckweed cultivation, particularly in wastewater treatment applications.

2.3.2. Light intensity. Duckweed species exhibit distinct responses to varying light intensities (low, medium, and high intensities) and photoperiods.[92,93] Under light conditions of 105 μmol m^{-1} s^{-1} with a 12 hours light–dark cycle, duckweed achieved a protein content of 32%, with an average daily fresh weight yield of 0.9 kg.[19] The protein content and starch accumulation in duckweed are influenced not only by light intensity but also by light colour. For instance, exposure to red LED light significantly increased the protein content in rootless duckweed (*W. arrhiza*) to 41.6%, compared to approximately 10% in its natural environment.[94] Additionally, heightened light intensities (850 μmol ms^{-1}) were found to substantially increase protein content (% protein of fresh biomass).[95] Increased light intensity (150 μmol ms^{-1}) positively affected the relative growth rate and protein yield of duckweed while reducing chlorophyll content compared to lower intensities (50 and 100 μmol ms^{-1}).[96] Moreover, raising light intensity from 2000 to 5000 lux resulted in a 1.6- to 2.2-fold increase in starch content in *L. punctata* and *L. minor*.[94] These findings suggest that optimizing light intensity can enhance starch and protein synthesis, thereby improving the overall nutritional value of duckweed as a food or feed source. However, this optimization needs to be balanced with potential trade-offs. Excessively high light intensities can lead to light saturation and photoinhibition, causing a decrease in chlorophyll content and potential damage due to oxygen stress. Therefore, while elevated light intensity has a positive impact on duckweed growth and biochemical composition, careful management is required to avoid adverse effects.[94,97]

Nutrient removal rates, growth rate, and biomass production of duckweed are significantly influenced by light intensity. For *L. minor* grown on half-strength Hutner's medium, the total nitrogen removal rate increased with rising light intensity (10–850 μmol ms^{-1}).[95] The relative growth rate of *L. minor* also exhibited a proportional increase under these conditions, reaching 0.43 per day at 850 μmol ms^{-1}, while maintaining a steady rate of around 0.3 per day on synthetic wastewater. Similarly, *L. gibba* showed a 25% increase in growth rates when light intensity was increased from 100 to 700 μmol ms^{-1}.[97] It is important to note that the response of duckweed to light intensity is influenced by the species type, nutrient concentration, and temperature. For example, a combination of low light (7 μmol ms^{-1}) and high nutrient levels (100 mg N per L) was found to have interactive effects on the carbon-nitrogen balance in duckweed, resulting in reduced biomass production (0.01 g per day). This finding underscores the need to balance environmental variables when evaluating growth responses to light conditions in order to optimize biomass production.

2.3.3. Nutrient availability. Duckweed growth is closely linked to the nutrient content in its growth medium, with nitrogen and phosphorus playing key roles in its physiological processes.[37,79,98] Elevated levels of these nutrients are associated with the development of large, thin-fronded, dark green duckweed plants, while lower concentrations can result in smaller, thicker, and paler plants, indicating the sensitivity of duckweed morphology to nutrient availability.[92] When grown in a nutrient solution containing 30 ppm of both nitrogen (N) and phosphorus (P), duckweed exhibited a high biomass yield of 172 g per m^2 per day compared to 113.8 g per m^2 per day in the control group.[99] Additionally, increasing the combined application of N and P from 10 to 30 ppm notably enhanced protein content, raising it from 31.0% to 33.0%, whereas the control group had a lower protein content of 27.0. Further optimization was explored using macronutrients from Hoagland solution. Increasing concentrations of KH_2PO_4 up to 5 mM and $Ca(NO_3)_2$ up to 10 mM, with an adjusted pH of 9, resulted in a substantial

increase in protein content, reaching up to 54.24%. However, exceeding these nutrient and pH thresholds led to a decline in protein production, likely due to disrupted cellular activity at excessive nutrient concentrations.[66] These findings suggest that careful optimization of nutrient concentrations in the growth medium can significantly impact both the growth and protein content of duckweed. Balancing these nutrient levels is crucial for maximizing the nutritional value and productivity of duckweed in cultivation systems.

The growth of *L. puntata* was notably favoured by higher concentrations of N (345 ppm) and P (150 ppm). Growth rates decrease markedly when N levels drop below 34.5 mg L^{-1} and P levels fall below 15 mg L^{-1}. Conversely, as N and P concentrations decrease from 345 ppm N and 150 ppm P to 0 ppm, starch content increases substantially, with a 5.6-fold increase in *L. punctata* and a 9.9-fold increase in *L. minor*. Different N sources also have varied effects on the growth and biochemical composition of duckweed. For instance, using NH_4Cl as the N source resulted in a higher protein content (21.32%) in *L. minor* compared to $NaNO_3$ (16.84%) and $(NH_2)_2CO$ (17.52%). However, $NaNO_3$ promoted a higher growth rate (1.52%), while NH_4–N led to elevated carotene (144.667 mg/100 mL) and chlorophyll-a content (578.667 mg/100 mL).[100] These findings suggest that selecting the appropriate nitrogen source can be a strategic approach to tailoring the biochemical composition of duckweed. This strategy can be used to meet specific objectives, such as maximizing protein content for the food or feed industries or enhancing carotenoid content for pharmaceutical or nutraceutical applications.

2.3.4. pH and salinity. Duckweeds exhibit remarkable adaptability to a broad pH spectrum, with an optimal range between 6.5 and 8.[58] In most cases, duckweed can tolerate pH levels as low as 5.0 or even lower for short periods. Research has shown that while a pH range of 4.5 to 5.0 can support duckweed growth,[101] the highest growth rate (90 g per m^2 per day) was observed at pH 7. Conversely, the lowest growth rate (40 g per m^2 per day) occurred at pH 4.[99] This variation may result from differences in duckweed species or strains, each with specific pH preferences and physiological adaptations. Additionally, environmental factors such as temperature, light intensity, and nutrient availability may interact with pH levels, influencing duckweed growth rates.

pH levels also significantly affect the biochemical composition of duckweed, including protein, lipid, carbohydrate, and mineral content. Optimal nutrient levels are typically observed at pH 7 and 8, whereas both acidic (pH 4–5) and basic (pH 9–10) conditions negatively affect nutrient content.[100] For instance, protein content sharply declines at pH 4, peaking at 31 g/100 g dry weight at pH 7 and 8. In contrast, the lipid fraction increases at pH 9 and 10, and carbohydrate content reaches a maximum of 59.3 g/100 g dry weight.[99] These findings suggest that adjusting pH levels in duckweed cultivation systems can optimize nutrient content, thereby improving the efficiency and effectiveness of duckweed for various applications.

Duckweeds, known for their adaptability, can grow across a broad range of saline water conditions. Salinity measured in terms of electrical conductivity refers to the concentration of dissolved salts in water, including ions like sodium, chloride, sulfate, magnesium, and calcium.[101] The ideal conductivity range for duckweed cultivation in wastewater has been identified as 600–1400 μS cm^{-1}.[102] At this conductivity range, the wet weight relative growth rate peaked at 0.176 per day. Outside this range, duckweed growth and removal performance declined. Maximum removal rates within this range were 723.1 mg per m^2 per day for COD, 32.33 mg per m^2 per day for TKN (Total Kjeldahl Nitrogen), 77.99 mg per m^2 per day for fecal coliforms (100%), and 80.8% for turbidity. These findings suggest that duckweed, irrespective of species, may thrive within a conductivity range of 600–1400 μS cm^{-1}. However, the effect of salinity levels on the biochemical composition of duckweed has not been thoroughly explored, indicating a need for further research in this area. Overall, managing salinity levels within the recommended range is essential for achieving optimal duckweed performance in terms of growth and nutrient removal. Further studies are necessary to understand the specific impacts of salinity on the biochemical composition of different duckweed species and to validate the effectiveness of these salinity conditions across a broader range of applications.

3. Current state of duckweed research and industrial applications

In the past decade, duckweed has been extensively researched for its diverse applications, including the production of fuels, proteins, and chemicals (Fig. 3). Advances in genomics and metabolic pathway studies have optimized its use for these purposes.[103] Duckweed is increasingly recognized as a high-quality protein source, with species like *L. minor* and *W. arrhiza* containing up to 40% protein by dry weight, comparable to traditional plant-based proteins such as soybeans.[34,39] Its potential for wastewater treatment is being explored, offering an eco-friendly solution for treating agricultural and industrial

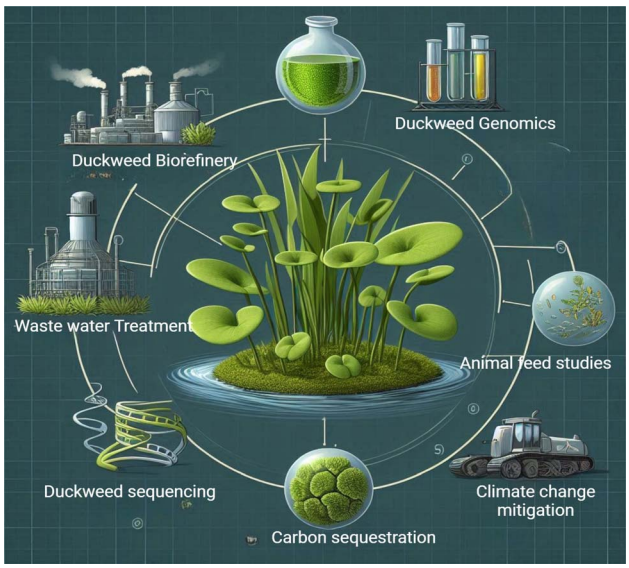

Fig. 3 Advances in duckweed research and application.

effluents while converting pollutants into valuable biomass for animal feed, biofertilizers, or biofuels. Additionally, duckweed's role in carbon sequestration is being studied, positioning it as a potential contributor to climate change mitigation efforts.

4. Post-harvest conditions for sustainable duckweed biomass management

4.1. Duckweed harvesting

The frequency and ratio of harvested duckweed biomass to the total available biomass play a key role in determining duckweed growth rate and biomass production. Harvesting duckweed twice per week resulted in higher biomass (533 g m^{-2} fresh weight (F.W.)) and growth rate (1.084 g per m^2 per day) compared to once per week, which produced lower biomass (402 g m^{-2} F.W.) and growth rate (0.803 g per m^2 per day).[104] Harvesting at 20 days intervals led to the highest average F.W. (1248.18 g m^{-2} F.W.) and dry weight (48.00 g m^{-2}) surpassing the yields from 5 days (657.95 g m^{-2} F.W.) and 10 days intervals (905.0 g m^{-2} F.W.).[105] Conversely, a shorter harvesting regime of 2, 4, and 6 days produced higher growth rates (6.60, 6.65, and 6.41 g dry mass per m^2 per day, respectively) than an 8 days regime (5.95 g dry mass per m^2 per day).[94] These variations may result from differences in duckweed species, genetic variability, and environmental conditions.[37]

The protein content of duckweed biomass is also influenced by harvesting frequency. A 20 days harvesting interval yielded the highest protein content (39.50%), compared to 5–15 days intervals, where protein content ranged from 32.77% to 35.91%.[99] An 8 days regime was found to be optimal for high protein content (36.30%) compared to 2 days (35.59%), 4 days (36.16%), and 6 days (35.29%) regimes.[94] These findings suggest that longer harvesting intervals may promote higher protein accumulation in duckweed.

4.2. Storage conditions for duckweed biomass

Proper storage conditions are critical for maintaining the quality and shelf life of duckweed biomass. Storing duckweed biomass at 25 °C led to rapid weight loss within 5 days, while storage at 4 °C and 10 °C extended the duration up to 5 and 4 days, respectively.[99] However, freshly harvested duckweed with high moisture content is prone to desiccation, resulting in water dullness, color darkening, and an abnormal odor. Effective moisture management during storage, including careful control of temperature and humidity, is crucial to prevent these undesirable effects and preserve the quality of the biomass.

4.3. Pretreatments for duckweed biomass

The pretreatment process for duckweed begins with harvesting mature and healthy biomass at the appropriate growth stage, followed by thorough cleaning to remove impurities such as debris and algae.[94] After cleaning, the harvested material is dried to reduce its higher moisture content, which enhances storage stability. Fresh duckweed typically contains about 95% moisture (wet basis), necessitating efficient drying techniques to extend its short shelf life.[106,107] Various drying methods, including solar drying, sun drying, microwave drying, shade drying, freeze drying, and vacuum-shelf drying, have been extensively explored as viable options for drying duckweed biomass.[8,41,107–109] Beyond simply removing moisture, drying extends shelf life and facilitates the extraction of valuable compounds, particularly proteins.[41] The choice of a specific drying method depends on the desired final moisture content and the properties of the target product, ensuring the preservation of biomass quality for efficient downstream processing.[109]

When dried under optimal conditions, duckweed becomes a rich source of nutritional components and bioactive compounds. Studies indicate that low-temperature drying at 50 °C and 60 °C for 4 and 6 hours yields higher concentrations of bioactive compounds,[39] while excessive heat and prolonged drying times can diminish these bioactive compounds. Microwave drying at higher power levels, such as 900 W, is particularly effective, producing shelf-stable duckweed with desirable quality characteristics while also reducing energy consumption and increasing energy efficiency.[107] Blanching at 100 °C for 3 min, especially when combined with sun drying, has been shown to make duckweed safe and nutritious for human consumption.[41] Blanching helps break down cell membranes, improving the accessibility of nutrients for extraction and enhancing the nutrient extraction efficiency during the drying process.[110,111] Depending on the intended use, the dried duckweed biomass may undergo size reduction processes, such as milling or grinding, to break down cellular structures and increase the surface area for extraction. This step improves the overall efficiency of the extraction procedure, maximizing the utility of the harvested material for various applications.

The composition of duckweed can be significantly altered depending on the pretreatment method used. Mechanical crushing has been reported to be more effective for extracting valuable bioactive compounds from duckweed compared to boiling and freeze-thawing.[112] In the study, mechanical crushing resulted in the highest crude protein content in the filtrate (42.02%) and a total phenolic content of 191.47 mg Gallic Acid Equivalent (GAE) per g in the residue, outperforming both boiling and freeze-thawing methods. This suggests that optimizing pretreatment methods can enhance protein extraction efficiency and the release of the antioxidant compound, paving the way for the development of functional ingredients or dietary supplements with potential health benefits. Further research is needed to explore additional pretreatment methods and their impact on the nutritional and functional properties of duckweed. Such studies could provide valuable insights for developing innovative processing technologies in the field of duckweed biorefinery.

5. Duckweed as a source of biomass and renewable feedstock

5.1. Duckweed as a source of biomass

Duckweed is recognized for its rapid growth, high nutrient uptake capabilities, and adaptability to various environments,

including wastewater and ponds. Species such as *Wolffia* and *Lemna* can double within days under favorable conditions, making duckweed a sustainable option for biomass production.[113] The growth rate and biomass yields vary significantly depending on the species and the cultivation media used (Table 3). Blending various duckweed species in cultivation has been proposed to enhance biomass yields.[37] In mixotrophic conditions, duckweed achieved its highest biomass production, reaching 340.09 g m^{-2} along with a notable protein yield of 40.59 g m^{-2}. However, starch content in mixotrophic duckweed is approximately 2.06 times lower compared to that grown under heterotrophic conditions.[114] Therefore, integrating and heterotrophic cultivation methods could optimize starch-enriched biomass production. Duckweed also exhibits superior growth rate and overall biomass when cultivated in mixed culture, particularly in environments like swine wastewater, compared to monocultures. Establishing an effective biomass production system requires careful selection of the most suitable duckweed strain from a diverse range of local varieties and optimization of nutrient levels. Additionally, choosing the appropriate culture media, such as wastewater, is fundamental for successful duckweed biomass cultivation.[115]

5.2. Duckweed as a renewable feedstock

Duckweed biomass is an excellent renewable feedstock due to its rich composition, which includes organic nitrogen in the form of proteins and free amino acids, alongside significant starch content.[84] These components contribute to its value as a highly fermentable feedstock with immense potential for various applications. The high protein and amino acid content makes duckweed a valuable source of bioavailable nitrogen, essential for numerous biological processes. This feature is particularly advantageous in applications where nitrogen is crucial, such as in developing microbial cultures or as a nutrient supplement in agricultural practices.[116] Duckweed is also an ideal biofuel feedstock due to its high cellulose and starch content and low lignin levels.[49] The starch in duckweed biomass enhances its suitability as a fermentable feedstock, facilitating various bioconversion processes, including anaerobic digestion for biogas production or fermentation for bioethanol production.[117,118] In the context of biorefinery, duckweed biomass emerges as a multifaceted solution capable of addressing both energy and nutritional demands. Whether used in biorefineries, bioenergy production, or as an ingredient in food or feed formulations, duckweed biomass stands out as a versatile renewable feedstock. Its potential to contribute to a more sustainable and resource-efficient future makes it a promising candidate for addressing global energy and nutritional challenges.

6. Biorefinery of duckweed biomass for producing value added products

Duckweed holds enormous potential for biorefinery applications (Fig. 4). Due to its rich and biochemical composition, duckweed is used in various industries, including food and feed, pharmaceuticals, wastewater treatment, and renewable energy. This positions duckweed as a key player in developing sustainable and innovative biotechnologies. Duckweed biorefineries offer several advantages over traditional biorefineries. They can target a wide range of products, from energy sources to bioactive compounds, using relatively simple reactor designs.

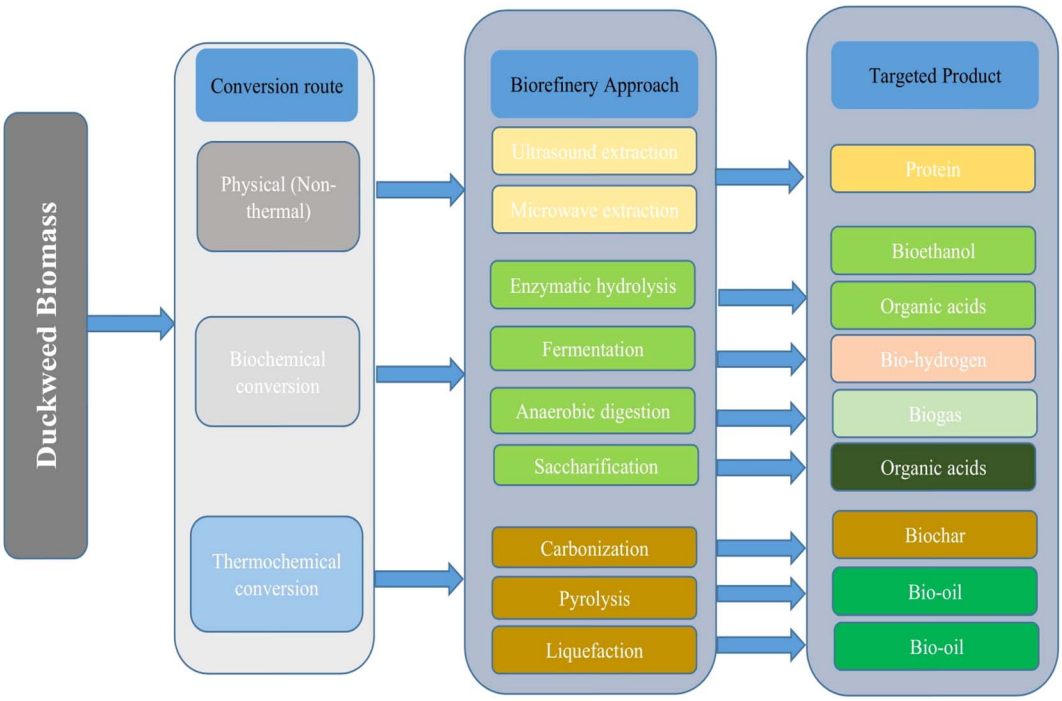

Fig. 4 . Duckweed biorefinery processes and integrated products.

Moreover, duckweed biorefineries harness natural resources like sunlight, air, and wastewater operating under ambient conditions. In contrast, traditional biorefineries based on lignocellulosic biomass or sugarcane often require complex reactor designs, energy-intensive pretreatment processes, and sterile conditions, all of which add complexity and cost. However, a key challenge in duckweed biorefineries is minimizing product yield loss during the separation of different components. The small size of duckweed cultures makes biomass recovery difficult, necessitating scalable, low-cost, and energy-efficient separation techniques to optimize the biorefinery process. Furthermore, duckweed biorefineries support a waste-free concept, as residues left after lipid or protein extraction can be repurposed for biofuel production or animal feed. However, a key challenge in duckweed biorefineries is minimizing product yield loss during the separation of different components. The following sections discuss some of the valuable products derived from duckweed biorefineries and the technologies employed to enhance yields and optimize production, as summarized in Table 4.

6.1. Bioethanol

Duckweed, especially *L. minor*, is rich in carbohydrates like starch and cellulose, making it a viable and environmentally sustainable feedstock for bioethanol production. By adjusting growth conditions, the starch content of duckweed can be enhanced, reaching levels up to six times higher than those in corn.[10] Duckweed shows significant promise for bioethanol production, particularly due to its soft biomass and high starch content, which facilitate easy saccharification into glucose, a crucial step in bioethanol production.[10,135,136] This multi-stage process involves pretreatment, enzymatic or acidic hydrolysis, fermentation, and distillation. Duckweed, especially *L. minor*, is rich in carbohydrates like starch and cellulose, making it a viable and environmentally sustainable feedstock for bioethanol production.[10,117] In the context of sustainable energy solutions, bioethanol serves as a cleaner alternative to conventional gasoline, helping to reduce the carbon footprint associated with transportation fuels. Efficient bioethanol production from duckweed requires the conversion of starch into simple sugars.[137] Duckweed starch consists of 35.7% amylose and 64.3% amylopectin,[138] which must undergo enzymatic saccharification to break down these polymers into fermentable sugars. The composition of starch, particularly the amylose-to-amylopectin ratio, varies across species and influences the efficiency of this conversion process.[49]

Achieving optimal saccharification of duckweed biomass involves careful exploration of factors such as enzyme concentration, pH, temperature, and incubation time.[139,140] Researchers have focused on optimizing saccharification in specific duckweed species, namely *L. punctata*, *L. aequinoctialis*, *S. polyrrhiza*, and *W. arrhiza*, to enhance bioethanol production. The saccharification process has been optimized by determining the ideal enzyme ratio for starch conversion, with the highest conversion achieved using a 2 : 1 (v/v) ratio of α-amylase to amyloglucosidase and a 24 hours incubation at 50 °C. Under these conditions, reported ethanol concentrations reached 0.19, 0.17, 0.19, and 0.16 g ethanol per g dry biomass for *L. punctata*, *L. aequinoctialis*, *S. polyrrhiza*, and *W. arrhiza*, respectively.[117]

Under conditions of nutrient deprivation or treatment with the plant hormone uniconazole, *L. punctata* has been shown to achieve a starch content ranging from 30% to 45%.[141,142] However, for duckweed biomass containing less than 45% starch, further enhancement is needed to make it a viable replacement for traditional starch feedstocks in commercial ethanol production.[10] One promising approach involves the application of abscisic acid, which has been demonstrated to promote biomass and starch accumulation in duckweed by regulating endogenous hormone levels and the activity of key starch metabolism enzymes.[143] In one study, this treatment resulted in duckweed biomass production reaching 59.70 and 63.93 g m^{-2} over six days, with starch content increasing from 2.29% to 46.18% after 14 days—a 2.6-fold increase compared to the control. The abscisic acid content in treated samples also reached 336.5 mg kg^{-1} (F.W.), 7.5-fold greater than in the control. Further enhancement of starch content has been achieved using a combination of nutrient deprivation, uniconazole treatment, and elevated CO_2 levels.[118] At the laboratory level, these conditions produced a peak growth rate of 9.4 g per m^2 per day and a starch content of 75.9%, resulting in an estimated starch yield of 30.8 t per ha per year. Remarkably, without any pretreatment, up to 88.4% of glucose from high-starch duckweed biomass was released, leading to an annual ethanol yield of 16 000 L ha^{-1}—approximately 2.6 times higher than the ethanol yield from maize.

Duckweed cultivation for bioethanol production is scalable and holds significant potential for large-scale biomass and starch. However, achieving and maintaining these optimal growth conditions requires careful management of light, temperature, and CO_2 levels and the application of abscisic acid and uniconazole. This method facilitates the rapid accumulation of high starch levels in duckweed but also demonstrates its potential as a competitive starch feedstock for sustainable bioethanol production.

6.2. Bio-oil

Bio-oil derived from duckweed is characterized by its diverse chemical composition, including long-chain hydrocarbons, esters, cyclic ketones, phenols, and a significant proportion of nitrogen-containing compounds (N-compounds).[120] These N-compounds, primarily originating from protein degradation, constitute a substantial fraction of the bio-oil, ranging from 41.8% to 55.4% of its total composition. The bio-oil exhibits notable physical properties, including a specific gravity of 1.009, which is higher than that of gasoline (0.68 to 0.74). With an American Petroleum Institute (API) gravity of 8.73 at 22 °C, it is classified as a very heavy oil. The viscosity of the bio-oil at 22 °C is 9.32 mm^2 s^{-1}, indicating a high viscosity and, its viscosity at 22 °C is 9.32 mm^2 s^{-1}, indicating a more concentrated nature compared to other biomass-derived oils.[119]

The chemical composition of duckweed bio-oil includes 19 carbon chain compounds (C8–C28), such as pristane and

Table 4 Emerging trends in duckweed biomass valorization and biorefinery potentials[a]

Targeted product	Biorefinery approach	Condition	Level of yield/findings	References
Bio-oil	Thermochemical conversion, fixed bed reactor (pyrolysis)	No catalyst, 30 °C starting temperature, 30 min heating, 26 °C cooling water	Bio-oil with a density of 1.076 g cm^{-3}, specific gravity of 1.009, viscosity of 9.32 mm^2 s^{-1}, API gravity of 8.73, and fixed carbon content of 86.56%	119
Bio-oil	Hydrothermal liquefaction using a stainless-steel autoclave, with product separation *via* filtration and solvent extraction	Varying temperatures 250 °C to 370 °C and 5.2 MPa to 21.1 MPa, with heating rates of 5 °C min^{-1} and reaction times of 15 to 60 minutes	Yield of 35.6% obtained at 370 °C with a 45 minutes residence time. Rich in N-heterocycles, cyclic ketones, esters, amides, long-chain hydrocarbons	85
Bio-oil	Hydrothermal liquefaction	Temperature range: 240 °C to 360 °C Reaction time: 60 minutes	Yield of 34.7% obtained at 360 °C containing nitrogen mainly as heterocycles and amides	120
Lactic acid	Substrate utilization and fermentation	50 mL substrate, using commercial yogurt as inoculum (2 : 1 ratio), at 37 °C, 120 rpm, pH 4.2, and anaerobic conditions for 5 days	Yield of 0.015 g/g dry biomass from the solid fraction and 0.0015 g/g dry biomass from the liquid fraction	121
Succinic acid	Enzymatic hydrolysis and fermentation	*A. succinogenes* GXAS137 grown in anaerobic conditions at 37 °C with glucose, yeast extract, corn steep liquor powder, $NaH_2PO_4 \cdot H_2O$, K_2HPO_4, and $NaHCO_3$	Separate saccharification and simultaneous fermentation process achieved a succinic acid yield of 85.26% with an SA concentration of 65.31 g L^{-1} and productivity of 1.36 g per L per h	122
Protein	Ultrasound extraction	Frequency of 120 kHz, power level 4, 15 m treatment at room temp, constant pH of 6.7	Protein content of 33.16% was obtained. Protein concentrate hydrolysate exhibited strong antimicrobial effects	123
Protein	Alkaline/ultrasound extraction (UAE)	Frequency of 20 kHz, 70% amplitude for 18 min; extraction at pH 8.5 followed by precipitation at pH 4.5	UAE significantly enhanced extraction yield and protein recovery. UAE had the highest protein recovery of 34.2%, followed by ultrasound/water assisted with 24.6%, and alkaline extraction with 17.1%	124
Protein	Ultrasound and enzymatic hydrolysis	Amplitude (50 and 100%) for 10 min	• UAE, especially in combination with enzymes improved protein extraction	123
Bio-hydrogen	Dark fermentation; integrated waste biomass feedstocks	*Lemna minor* and DKP as substrates with an inoculum from heated compost in a medium with 20 g TS per L, 100 mg L^{-1} L-cysteine HCl, and 20 mM $FeSO_4 \cdot 7H_2O$, grown at 37 °C for 16 hours, with 5% inoculation and argon-purged headspace	Optimal H_2 yield for LM-glucose experiments was 77.34 mL H_2/10 mL with 21 g L^{-1} glucose and 40 g L^{-1} LM, while for the LM-DKP experiments, the maximum H_2 yield was 125.35 mL H_2/10 mL with 60 g L^{-1} PERS and 40 g L^{-1} LM	125
Bio-hydrogen	Dark fermentation with hydrothermal pretreatment	Varying temperatures (35 °C, 50 °C, 55 °C), with operations over seven days	High hydrogen production rate was 0.97 mmol per day at 55 °C, with a maximum yield of 416 mL H_2 per g biomass. Substrate utilization was 57.27% at 55 °C, higher than 50.67% at 35 °C	82

Table 4 (*Contd.*)

Targeted product	Biorefinery approach	Condition	Level of yield/findings	References
Biochar	Carbonization	Carbonization at 800 °C, adsorption at 30–60 °C, pH adjustment, contact time 5–120 min	Carbonized duckweed achieved a maximum crystal violet dye removal efficiency of 96.94% at 40 °C with an adsorption capacity of 9.69 mg g^{-1}, an optimal pH of 6, and a dose of 0.5 g	126
Biogas	Anaerobic digestion	Bioreactor temperature maintained at 35 °C, with anaerobic digestion occurring over a period of 35 to 50 days using a discrete mode of operation	Bioreactor with a mixture of 33.3% residual duckweed biomass, 33.3% inoculum, and 33.3% food waste, achieved a high biogas yield of 0.16 L g^{-1} organic carbon and a methane concentration of 49.8% after 48 days	55
Biogas	Anaerobic digestion	Conducted at 38 °C with a 55 days hydraulic retention time, using duckweed (DW) cattle dung (CD) slurry mixtures in ratios of 75 : 25, 50 : 50, and 90 : 10	At a 55 days hydraulic retention time, biogas yields peaked at 20, 610, 580, and 560 mL per day for DW/CD ratios of 90 : 10, 75 : 25, 50 : 50, and CD 100 : 0, respectively, with the highest cumulative production of 12 070 mL and methane content of 64.3% in 100% cattle dung	84
Biogas	Anaerobic digestion	Duckweed and excess sludge at a 4 : 1 volatile solids ratio, NaOH pretreatment at 100 °C, substrate to inoculum ratios of 4 : 1, 2 : 1, 1 : 1, 1 : 2, and 1 : 2.5, an initial pH of 7.0, and a constant temperature of 37 °C	High biogas yield of 12 070 mL was achieved with a 50 : 50 duckweed to cattle dung ratio	83
Biogas	Anaerobic digestion	Duckweed and waste activated sludge co-digested with acclimatized anaerobic granular sludge in batch reactors, both with and without thermal pretreatment (autoclaving at 120 °C for 30 min)	Thermal pre-treatment significantly increased cumulative methane production by 13–24 times compared to non-pre-treated feedstock, with the best substrate-inoculum ratio for pre-treated substrates being 1 : 1.7	127
Biogas	Acidogenic fermentation, electrohydrogenesis, and methanogenesis	*Lemna minor* pretreated with 1% H_2SO_4 at 121 °C for 15 minutes, with processes conducted at pH 6 (acidogenic fermentation, electrohydrogenesis) and pH 7 (methanogenesis), at 37 °C, with 48- to 96 hours retention times	In two-stage processes, biogas yields ranged from 220 to 290 mL g^{-1} VS (volatile solids). Three-stage processes showed even higher efficiency, with yields up to 350 mL g^{-1} VS. The highest degradation efficiencies and energy recovery were observed in the three-stage processes	128
Biogas	Anaerobic digestion	Digesters placed at 38 °C for 30 days, using rumen fluid as inoculum	High biogas production observed with a 25% duckweed and 75% cattle manure mix, yielding 2000 mL per day of biogas	129
Bioethanol	Saccharification and fermentation	Sonication, enzymatically hydrolyzed at 100 °C for 1 hour, pH adjusted to 4.5,	Ethanol yield from high-starch duckweed after 24 hours of separate hydrolysis	130

Table 4 (*Contd.*)

Targeted product	Biorefinery approach	Condition	Level of yield/findings	References
		further hydrolyzed at 60 °C for 2 hours, and autoclaved at 115 °C for 20 min, followed by ethanol fermentation with *Saccharomyces cerevisiae* at 30 °C for 24 hours	and fermentation was 4.98 g per flask, with a fermentation efficiency of 91.83%, achieving a potential bioethanol production of 8670 L per ha per year	
Bioethanol	Saccharification and fermentation	Enzymatically saccharified using α-amylase and amyloglucosidase enzymes at 50 °C for up to 36 hours, with subsequent fermentation carried out by *Saccharomyces cerevisiae* at 37 °C	*S. polyrrhiza* yields significant bioethanol production (0.19 g ethanol per g dry biomass) with 73% glucose-to-ethanol conversion efficiency across all duckweed species	117
Bioethanol	Saccharification and fermentation	Liquefaction with α-amylase at 90 °C for 45 min followed by saccharification with glucoamylase at 60 °C for 2 hours, with the resulting hydrolyzate fermented using *Saccharomyces cerevisiae* at 30 °C for 24 hours under anaerobic conditions	Ethanol yield from high-starch duckweed of 45.8 g L^{-1} with a conversion rate of 94.6% during low gravity fermentation and 106.3 g L^{-1} with a conversion rate of 95.2% during simultaneous saccharification and fermentation	131
Bioethanol	Heavy-ion irradiation mutagenesis of duckweed, enzymatic saccharification of the biomass, and fermentation	Enzymatic saccharification with α-amylase, amyloglucosidase, and pullulanase at 50 °C for 30 hours, followed by fermentation with yeast at 30 °C for 30 hours	Heavy-ion irradiation of *Lemna aequinoctialis* 6002 yields significantly higher ethanol yield of 0.232 g g^{-1} dry weight compared to the wild type (0.043 g g^{-1} dry weight)	132
Bioethanol	Hydrothermal pretreatment, simultaneous saccharification and fermentation	Pre-treatment at 130–210 °C for 10–40 min, followed by enzymatic hydrolysis with 50 FPU g^{-1} cellulase and fermentation with *Saccharomyces cerevisiae* at 25 °C for 144 hours	Hydrothermal pre-treatment resulted in the highest ethanol yield of 88.81% achieved at a severity factor of 3.9 (200 °C for 10 min), representing a 63% increase compared to untreated biomass	133
Bioethanol	Acid pretreatment and fermentation	Dilute acid pretreatment (0.1% H_2SO_4) at 120 °C for 20 min, followed by fermentation with *Saccharomyces cerevisiae* QG1 MK788210 at 30 °C for 48 hours	9.8% theoretical ethanol yield from after effective dilute acid pretreatment, with 0.01 g of ethanol per gram of volatile solids	134

[a] API = American Petroleum Institute; CD = Cattle Dung; DKP = *Diospyros kaki* Peels; LM = *L. Minor*; DW = Duckweed; UAE = Ultrasound/Alkaline Extraction; SA = Succinic acid.

phytane, with the most prevalent compound being n-C26, identified at a retention time of 16.926 min. The bio-oil also contains several polyaromatic hydrocarbons (PAHs) such as naphthalene, acenaphthene, fluorene, phenanthrene, and pyrene, each with distinct concentrations and retention times.[119] Research has identified optimal conditions for maximizing bio-oil yield as a temperature of 370 °C and a residence time of 45 min, resulting in a yield of 35.6%.[85] Duckweed-derived bio-oil can be fractionated into various fuel types based on the number of carbon atoms in its molecules. These fractions include gasoline (C6–C10), kerosene (C10–C16), diesel (C16–C20), lubricant oil (C20–C30), and heavy fuel oil.[119] This versatility suggests duckweed bio-oil has the potential to serve as an alternative energy source, offering a range of applications from transportation fuels to industrial lubricants.

The yield and quality of bio-oil derived from duckweed are significantly influenced by pretreatment, reaction temperature and residence time. Phosphoric acid pretreatment has been shown to enhance bio-oil quality by improving the yield and stability of valuable compounds like phenols and furans, while

reducing the formation of undesirable by-products.[144] This process facilitates the breakdown of complex biomolecules, resulting in a more refined and chemically stable bio-oil. Temperature and residence time are also critical factors in optimizing bio-oil yield.

Studies have demonstrated that increasing the temperature from 240 °C to 360 °C raises the bio-oil yield from 18.8 weight (wt%) to 34.7 wt%.[120] Additionally, extending the residence time from 30 to 60 min at 240 °C increases the yield by 1.9 wt%. An increase in yield from 19.9% to 33.9% has been observed as the temperature rises from 250 °C to 340 °C.[85] At higher temperatures, such as 370 °C, the yield increases from 33.6% to 35.6% with an extended residence time of up to 45 min, after which further improvement is minimal. These findings indicate that while lower temperatures benefit from longer residence times, higher temperatures require careful optimization to maximize yield. Duckweed-derived bio-oil holds strong potential for scalability due to its diverse chemical composition and high yield under optimized conditions. However, scaling up production necessitates precise control of temperature and residence time parameters to ensure consistent bio-oil quality and yield.

6.3. Biogas

Beyond bioethanol, duckweed-based bioenergy products include biogas, produced through the anaerobic digestion (AD) of organic waste materials.[145] Biogas production from duckweed has gained significant attention as a promising renewable fuel source. Duckweed's rapid growth in farm ponds and water bodies receiving agricultural runoff makes it an appealing biomass for integration into farm-scale anaerobic digesters.[146] Notably, its minimal lignin content, a common hindrance to microbial degradation, enhances its suitability for AD.[84]

Duckweed's suitability for biogas production is further demonstrated by its higher volatile solids content, approximately 20% more than cattle dung, facilitating enhanced biogas generation. Its lower ash content improves digestibility, while its favorable carbon-to-nitrogen (C/N) ratio (9.5) compared to cattle dung (22.7) positively influences methane production.[84] When comparing biogas yields among aquatic plants, *L. minor* produces 368 L kg^{-1}-volatile solids (VS), water hyacinth yields 410 L kg^{-1}-VS and *P. stratiotes* generates 269 L kg^{-1}-VS.[147] Researchers have explored various techniques to optimize biogas yields from duckweed, including solar drying at 35 °C and utilizing two-stage reactors.[148] Optimal operational temperatures around 35 °C have been identified for achieving the highest methane content in digesters, leading to increased biogas yields.[84,149] Duckweed is a viable candidate for large-scale biogas production. However, scaling up production requires efficient integration into farm-scale anaerobic digesters and consistent management of growth conditions. The use of solar drying and two-stage reactors can further enhance scalability by improving processing efficiency and biogas yield.

6.4. Bio-hydrogen

Bio-hydrogen is an emerging sustainable energy source with significant potential to replace fossil fuels. Duckweed offers a promising feedstock for bio-hydrogen production, particularly through dark fermentation. This method is preferred due to its ability to operate under ambient conditions, utilize a wide range of organic substrates, and require relatively simple and cost-effective reactor designs.[125,150] However, pretreatment is often crucial for enhancing hydrogen production during dark-fermentation.[151] Hydrothermal pretreatment of duckweed has proven effective in disrupting the plant's recalcitrant cell walls, making its biomass more accessible for microbial degradation during fermentation.[82] Achieving optimal hydrogen production from duckweed also involves balancing key nutrient ratios, particularly C/N and carbon-to-phosphorus (C/P) ratios. Duckweed's native composition is high in N and P, which necessitates modifications to achieve favorable ratios for H_2 production. A high yield of 77.34 mL H_2/10 mL was obtained by blending 40 g L^{-1} *L. minor* with 21 g L^{-1} glucose, providing optimal C/N and C/P ratios of 14.67 and 29.30, respectively. Beyond these concentrations, substrate inhibition was observed, indicating the need to carefully balance nutrient concentrations for effective hydrogen production.[125]

Waste biomass substrates have shown significant potential in improving hydrogen yield from duckweed. Blending duckweed with *Diospyros kaki* peels (DKP) significantly enhanced hydrogen yield and production rates compared to duckweed-glucose experiments.[125] The highest H_2 yield of 125.35 mL H_2/10 mL was achieved using 60 g L^{-1} DKP and 40 g L^{-1} *L. minor*. Temperature also plays a crucial role in the efficiency of H_2 production rates of 0.97 mmol per day at 55 °C, 0.84 mmol per day at 50 °C, and 0.38 mmol per day at 35 °C.[82] Additionally, COD utilization was higher under thermophilic conditions (57.27%) than mesophilic conditions (50.67%), leading to improved substrate degradation and hydrogen yield. The biomass conversion rate reached 416 mL H_2 per g at 55 °C, compared to 144 mL H_2 per g at 35 °C. Using duckweed for bio-hydrogen production not only offers a renewable energy source but also provides a sustainable solution for managing aquatic biomass in polluted water bodies. However, to optimize bio-hydrogen production from duckweed, it is important to balance nutrient management by adjusting the C/N and C/P ratios.

6.5. Organic acids

Duckweed biomass, particularly when rich in carbohydrates, is a valuable resource for the fermentative production of fine chemicals such as succinic acid and lactic acid.[152] Lactic acid is a versatile compound that can be converted into various useful chemicals, including pyruvic acid, lactate esters, 1,2-propanediol, and acrylic acid.[132] Duckweed has proven to be an effective substrate for lactic acid production, with lactic acid formation beginning on the first day of fermentation and peaking on the fifth day, reaching a concentration of 5110 mg L^{-1}.[121]

Duckweed is also an efficient substrate for succinic acid production. The optimal substrate concentration for maximum succinic acid production has been identified as 180 g L^{-1}.[122] The production process can be enhanced through enzymatic pretreatment, yielding up to 59.7 g L^{-1} of succinic acid, compared to 54.4 g L^{-1} obtained through acid hydrolysis.

Simultaneous saccharification and fermentation have been reported as the most effective method, producing 65.31 g L^{-1} of succinic acid with a productivity rate of 1.36 g per L per h within 48 hours. Compared to other biomass sources such as corn stover, sugarcane bagasse, and food waste, duckweed shows comparable efficiency in lactic acid production.[122] Additionally, duckweed has the added advantage of growing on wastewater, offering dual benefits of wastewater treatment and biomass production. This characteristic makes duckweed a more sustainable and environmentally friendly option than traditional crops used for organic acid production. For optimal production of succinic and lactic acid from duckweed, it is essential to ensure balanced N and P levels, maintain an appropriate C/N ratio, monitor pH to support efficient fermentation, and employ enzymatic pretreatment to improve substrate availability and enhance overall acid yield.

6.6. Biochar

Biochar can be produced from various biomass sources, including agricultural crops, wood, animal manure, and duckweed, using thermochemical processes such as pyrolysis, torrefaction, and hydrothermal carbonization.[153] Among these, catalytic fast pyrolysis of duckweed has proven efficient for generating aromatic hydrocarbons. Under optimal conditions of 736 °C and a catalyst-to-biomass ratio (CBR) of 16 : 1, total aromatic hydrocarbons can reach 27.2 mol%. At 750 °C and a CBR of 20 : 1, specific yields include benzene at 5.5 mol%, toluene at 8.0 mol%, and xylene at 6.2 mol%.[154] In water treatment applications, biochar derived from *L. minor*, produced at 400 °C (LM400) has demonstrated significant efficacy in reducing turbidity (92.33%) and NH_4^+–N (89.54%). However, its performance was less effective for removing Ni (37.22%) and PO_4^{3-}–P. Enhanced removal rates were achieved by combining LM400 biochar with $Ca(OH)_2$ precipitation. Optimal dosages of 5.0 g L^{-1} $Ca(OH)_2$ and 2.0–3.0 g L^{-1} LM400 improved Ni and PO_4^{3-}–P removal to 78.67% and 97.79%, respectively.[155] Additionally, biochar derived from duckweed has shown promise as an adsorbent for removing crystal violet from aqueous solutions, with an adsorption capacity of 18.5 mg g^{-1}.[126] To optimize the performance of duckweed-derived biochar, it is recommended to maintain balanced nitrogen and phosphorus levels in the feedstock, adjust the C/N ratio, and utilize pretreatment methods such as enzyme or acid treatments to enhance biomass properties. Ensuring thorough thermochemical processing is also crucial to achieving optimal yields of aromatic hydrocarbons and effective adsorption capacities for water treatment applications.

6.7. Enzymes

Duckweed exhibits great promise for cellulase production due to its favourable composition, which includes 30.4% cellulose, 23.6% hemicellulose, and a notably low lignin content of 2.4%.[156,157] The low lignin content is particularly advantageous, as it minimizes cellulase inactivation and promotes efficient enzyme production.[158] When tested at various concentrations (10 g L^{-1}, 30 g L^{-1}, 50 g L^{-1}, and 70 g L^{-1}), duckweed significantly enhanced cellulase production with the highest filter paper activity (FPA) of 6.5 FPU mL^{-1} observed at a concentration of 50 g L^{-1}.[159] Duckweed has also outperformed other common inducers in cellulase production. Cellulase production induced by duckweed was 195% higher than that induced by steam-exploded corn stalk, 55% higher than corn cob, and 32% higher than bagasse. These results suggest that duckweed duckweed is not only effective but also more efficient as a cellulase inducer, making it a promising candidate for scaling up to industrial enzyme production. Its cost-effectiveness and renewability further position it as a viable and sustainable resource for cellulase production. To optimize cellulase production using duckweed, it is recommended to maintain balanced N and P levels to support effective enzyme synthesis and ensure the biomass has low lignin content. Using duckweed at its optimal concentration is also crucial, as this significantly enhances enzyme production efficiency compared to other common inducers.

6.8. Protein

Duckweed serves as a valuable source of essential amino acids for human nutrition and can play a role in combating malnutrition and other health issues.[8] Proteins from duckweed are extracted using both conventional and novel technologies. Conventional extraction methods include alkaline and acid extraction processes (Fig. 3). In alkaline extraction, alkaline conditions facilitate protein solubilization by altering the protein's structure, and charge.[1,160] A wide range of extraction yields (37.8 to 60%) and protein purities (34.5 to 67.8%) have been achieved through this method by varying process conditions (Table 4). The effectiveness of the extraction process depends on several key variables, including extraction temperature, duckweed powder concentration, the pH of the alkaline solution, and the isoelectric point used for protein precipitation. Under optimal conditions of 80 °C and pH 11, duckweed protein solubilization yields of 72.0% to 77.8% were achieved.[160] By optimizing the extraction process, specifically focusing on temperature and pH conditions, it is possible to maximize the quantity of soluble proteins obtained from duckweed. This optimization can also enhance the functional and structural qualities of the protein extracts, making duckweed a more attractive and sustainable source of proteins for various industries.

The pH of precipitation significantly affects protein yield at concentrations of 2% and 4%, whereas concentration has a significant effect only at pH 4.[160] The optimal conditions for maximizing duckweed protein extraction yields through isoelectric point precipitation have been identified as an initial concentration of 2% or 4% with a precipitation pH of 4, resulting in maximum protein yields of 60.0% and 57.9%, respectively.[160] Protein yields ranging between 37.8% and 50.3% were obtained at concentrations of 6% and 8% with pH levels of 4.5–5.0 and 3.0–3.5. Specifically, precipitation at pH 5 and pH 4.5 with an initial concentration of 2% induced protein yields of 37.8% and 41.0%, respectively. A pH of 6 was shown to yield higher protein in the liquid fraction compared to pH 6.5.[161] A precipitation pH of 4.5 or 5.0 with a 2% initial powder concentration resulted in maximum protein purities of

approximately 60.1% and 61.1%, respectively, while other conditions (pH of 3.0–4.0 with powder concentrations of 4–8%) resulted in protein purities ranging from about 51.0% to 57.6%.[161] These findings highlight the need for further research on efficient fractionation procedures to attain higher protein purity due to the consistent presence of fiber in duckweed.

The acid extraction method has been investigated for the isolation of proteins from duckweed (*L. gibba*).[162] Decreasing the plant material-to-extractant volume ratios increased the extracted protein amount, reaching a maximum at a ratio of 1 : 20. Increasing the molarity of the HCl-solution from 0.1 M to 0.5 M HCl did not significantly enhance protein extraction, nor did substituting HCl with HNO_3. A re-extraction of the duckweed material resulted in an additional 3–5% increase in protein content compared to the initial extraction. A re-extraction of the duckweed material resulted in an additional 3–5% increase in protein content compared to the initial extraction. Optimized conditions for this process were identified as an extractant-to-sample volume ratio of 20 : 1, an extractant concentration of 0.1 M HCl, a 24 h extraction at room temperature, and an optional second extraction round. These optimized conditions can streamline the protein extraction processes from duckweed, improving overall efficiency.

Emerging technologies offer additional benefits in food processing, including protein extraction from duckweed. Ultrasound is one of the most common techniques applied to duckweed, recognized for its improved extraction efficiency. However, research on other emerging technologies, such as high-hydrostatic pressure, microwave-assisted extraction, and supercritical fluid extraction, remains limited. Ultrasound treatment has been reported to yield duckweed protein extracts and solutions with high solubilities across different pH levels.[123] Ultrasonication promotes both protein and non-protein extraction, while alkaline treatment is necessary to facilitate protein extraction.[124] Ultrasonic treatments, especially 80% and 100% have resulted in increased protein extraction compared to controls (water and buffer).[124] Although enzyme treatments alone did not significantly impact protein extraction yield, their combination with ultrasound increased the yield. The alkali extraction method showed minimal changes in protein extraction yield compared to controls, regardless of the duration, with the highest protein yield reported at approximately 65%.[163] To optimize protein extraction from duckweed, it is recommended to adjust pH and temperature during alkaline extraction to maximize protein solubilization and yield. Incorporating advanced techniques such as microwave or enzyme-assisted extraction can further improve efficiency and protein quality. Future research should focus on exploring and integrating these emerging technologies to enhance protein extraction processes, contributing to the advancement of sustainable food production practices.

6.9. Biofertilizers

Duckweed has been explored for composting and the formation of biodegradable value-added products like biofertilizers. Compared to organic fertilizers, biofertilizers made from duckweed offer the advantageous of maintaining soil fertility while reducing the need for irrigation water to enhance productivity.[164] Research indicates that composts enriched with duckweed can significantly improve soil nutrients content and crop yields. When incorporated into compost, duckweed increases essential soil nutrients, including total organic carbon (15.9–22.3%), total nitrogen (1.2–2.6%), available phosphorus (0.23–0.52%), total potassium (0.47–1.14%), and calcium (2.5–4.8%).[165] These improvements are particularly important for promoting plant growth in nutrients-depleted soils. Studies have shown that using duckweed-enriched compost as a biofertilizer can significantly boost crop yields. For example, when duckweed is used as a biofertilizer, bean plants exhibit a 20–30% increase in biomass and a 15–25% rise in seed yield compared to conventional fertilizers.[166] This increase suggests that duckweed not only contributes essential nutrients to the soil but also enhances the overall metabolic efficiency of plants. Duckweed also has the ability to accumulate selenium from selenium-impacted water.[167] When applied as a biofertilizer, it can increase soil selenium concentrations by 0.2–0.5 mg kg^{-1},[166] enhancing nutrient uptake in plants. Selenium, although required in trace amounts, plays a crucial role in plant metabolism and animal health. The increased bioavailability of selenium in duckweed-treated soils contributes to improved crop growth and the nutritional quality of the harvested products. These findings suggest that duckweed acts not only as a general nutrient provider but also as a vehicle for delivering specific micronutrients like selenium, which may be deficient in certain soils.

6.10. Vitamins

Duckweed is recognized as a promising sustainable source of essential nutrients, including vitamin B12. In a recent study, researchers utilized lyophilized duckweed samples, homogenizing them and employing the potassium cyanide-boiling method to convert various forms of vitamin B12 into cyanocobalamin.[168] The findings revealed that different duckweed species exhibit varying levels of vitamin B12, ranging from 0.31 to 3.43 μg/100 g dry weight, with certain species like *Spirodela polyrhiza* demonstrating significant amounts of both bioactive B12 and pseudo-B12. Additionally, cobalamin levels in *Lemna* species were found to be higher under mixotrophic conditions (1.83–2.76 μg B12/100 g dry weight (DW)) compared to phototrophic conditions (0.40–0.00 μg B12/100 g DW).[169] The investigation into the effects of tissue sterilization on vitamin B12 levels suggested that endophytic bacteria may contribute to the vitamin content in duckweed. This indicates a complex interaction between duckweed and its associated microbial community. Further research is needed to explore the mechanisms behind vitamin B12 production in duckweed and its potential applications in human nutrition, particularly in addressing vitamin B12 deficiencies in plant-based diets.

6.11. Current and prospective applications of duckweed other than as biofuels

In recent years, duckweed has gained attention for its potential in producing sustainable and renewable biofuels. However,

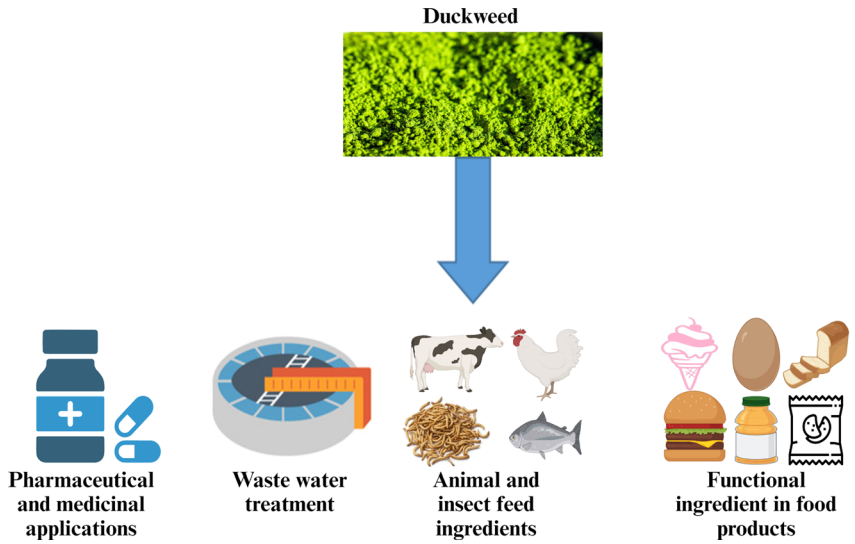

Fig. 5 Common applications of duckweed.

there are other more readily exploitable commercial opportunities for duckweed (Fig. 5). The following section discusses the current and potential commercial application of duckweed beyond biofuels.

6.11.1. Biomass/extracts duckweed for animal and insect feeding. The use of duckweed and its extracts in animal feed has attracted substantial interest due to the growing need for renewable and sustainable sources of animal protein, which alleviates pressure on land resources. Research has explored the benefits of incorporating fresh or dried duckweed into the diets of various animals and insects. In insect farming, duckweed alone has shown limited efficacy in promoting growth, as indicated by a low body weight of 0.04 g and a negative feed conversion ratio.[170] However, when combined with 75% semolina, yellow mealworms achieved a higher average body weight of 0.16 g, a survival rate of 90%, and an improved feed conversion ratio of 2.58.

In dairy cows, duckweed supplementation has been shown to significantly enhance several health indicators. Inclusion of duckweed improved red blood cell counts (4.78×10^3 mm^{-3} to 5.24×10^3 mm^{-3}), hemoglobin levels (9.5 g dl^{-1} to 12.8 g dl^{-1}), packed cell volume (3.87% to 37.46%), and total antioxidant status (9.96 nmol mg^{-1} to 13.31 nmol mg^{-1}).[171] For Horro rams, incorporating duckweed into commercial feed at levels up to 50% significantly enhanced growth parameters, including growth rate (48.60–77.07 g per day), daily dry matter intake (849.175–914.375 g per day), crude protein content (16.45–24.87%), and body weight gain (20.34–24.43 kg).[172]

In aquaculture, the inclusion of duckweed in fish diets has led to marked improvements in growth indices. For example, Nile tilapia fed with a 20% duckweed inclusion showed an increase in final weight (63.20–78.61 g), weight gain (47.6–63.2 g), and specific growth rate (3.19–3.57%), along with an increase in crude protein content from 16.42% to 17.36%.[173] Furthermore, incorporating blanched and sun-dried duckweed meal in *Oreochromis niloticus* diets resulted in significant improvements in growth performance, nutrient utilization, and economic profitability, with the highest growth and net profit observed in fish-fed diets containing 75% blanched duckweed meal.[174] Despite these benefits, duckweed is primarily utilized as a supplement rather than a complete feed source due to its essential amino acid profile being lower compared to traditional feed ingredients such as animal protein, soybean protein, fishmeal, and fish oil.

6.11.2. Waste water treatment. Duckweed has recently gained attention for its potential in removing organic matter and nutrients from effluents.[53,80] Studies have demonstrated its effectiveness in various settings. For example, duckweeds were shown to effectively remove 37.67% TKN, 83.33% of nitrate (N–NO_3^-), and 35.33% of nitrite (N–NO_2^-) from slaughterhouse waste water.[175] In palm oil mill effluent, duckweed achieved an 85% reduction in COD and an 80% reduction in ammonia nitrogen (NH_3–N) after 28 days of treatment.[176] Additionally, duckweed has demonstrated strong phytoremediation capabilities, reducing zinc concentrations from 0.24 mg L^{-1} to 0.04 mg L^{-1}. In agricultural wastewater treatment, duckweed has proven effective in several areas. It achieved a 30.09% removal rate for total dissolved solids (TDS), a 73.85% reduction in BOD, and a 90.26% decrease in nitrate concentrations. Furthermore, it demonstrated significant heavy metal removal efficiencies, reducing lead concentrations by 95.92% and completely eliminating cadmium with a 100% removal rate.[177] The performance of duckweed in wastewater treatment can be enhanced through the use of plant growth-promoting bacteria (PGPB). Co-cultivation studies have shown that these bacteria improve pollutant removal efficiency. For example, *Paracoccus marcusii* W7-16 achieved a 42.86% reduction in COD, 100% nitrate removal, and a 52.78% reduction in phosphate levels.[178] Similarly, *Acidovorax kalamii* W7-18 contributed to a 19.23% reduction in P levels and a 42.86% reduction in COD. These findings suggest the potential of integrating PGPB with duckweed in wastewater treatment processes. A key challenge in

integrating duckweed cultivation with wastewater treatment in biorefineries is balancing effective pollutant removal with economic feasibility, given the variability in treatment performance and the costs associated with bacterial inoculation and maintenance. Future research should focus on cost–benefit analyses of such integrated systems, particularly in the context of biorefineries, where wastewater can serve as a low-cost, abundant growth medium for biomass production and duckweed metabolites.

6.11.3. Duckweeds as pharmaceutics and medicinal products. Duckweeds have attracted considerable attention for their potential applications in pharmaceuticals and medicinal products offering significant health benefits. Hydrolyzed duckweed powder produces protein hydrolysates with strong ACE-inhibitory activity, indicating its potential in managing blood pressure.[179] *W. arrhiza* also exhibits exceptional antioxidant capacity, surpassing many vegetables and crops, and is rich in carotenoids like lutein and α-tocopherol, which are known for their role in mitigating oxidative stress.[38,45] Additionally, *L. minor* extract has been shown to reduce lung inflammation, fibrosis, and inflammatory cytokines in idiopathic fibrosis (IPF) models, suggesting its therapeutic potential for oxidative stress-related conditions.[180] Duckweeds also contain bioactive metabolites with pharmacological activities, particularly antimicrobial properties. *Spirodela* exhibits superior antibacterial effects compared to *Lemna*, while *L. minuta* ethanol extract is effective against various microorganisms though it shows limited antifungal activity.[181,182] Duckweed protein hydrolysates have been found to reduce bacterial and fungal populations.[123] In cancer research, duckweed-derived flavonoids have been shown to induce apoptosis in acute myeloid leukemia (AML) cells and inhibit tumor growth *in vivo*, highlighting their therapeutic potential.[131] In terms of metabolic health, *W. globosa* demonstrates antidiabetic effects by improving postprandial glucose control, resulting in lower glucose peaks and faster returns to baseline compared to yogurt shakes.[183] In a randomized crossover study involving 45 participants with stable type 2 diabetes (T2D), a post-dinner 300 mL Mankai drink significantly improved postprandial glycemic control by reducing glucose excursions and prolonging the time to peak glucose levels. Additionally, this intervention showed beneficial effects on key metabolic markers without causing adverse effects or affecting overall satiety.[184] Overall, duckweed holds great promise as a source of bioactive compounds with therapeutic potential across various medical fields. As ongoing research continues to reveal new bioactive compounds and mechanisms of action, duckweed is emerging as a potent source of health-promoting agents that could be harnessed for innovative medical and nutritional therapies.

6.11.4. Duckweed incorporation in foods with potential health benefits. The functional food sector has experienced rapid growth, driven by consumer demand for health-promoting foods. Duckweed has emerged as a promising ingredient due to its rich nutritional content and bioactive compounds (Table 5). As a valuable protein source, duckweed shows potential as a plant-based egg yolk replacement, offering good digestibility and bioaccessibility while mimicking the appearance of egg yolk.[188] Additionally, duckweed-derived RuBisCO solutions have been used to produce egg white analogs with comparable gelling and viscosity properties to egg whites, though improvements in color and texture are needed.[185] Duckweed protein's ability to provide air- and oil-

Table 5 Duckweed incorporation in different food products

Product	Duckweed incorporation	Addition (%)	Potential benefits	References
Snacks	*W. globosa*	20–40	Increased protein and fiber content, antioxidant properties and microbiological quality	185
Bread	Lemnaceae	—	Enhances the nutrient profile of bread, addressing protein distribution issues, and contributes positively to essential nutrients	186
Ice cream	*W. globosa* and *L. minor*	2	Increased protein, fiber and ash content, low fat, low total plate count, suitable for human nutritional needs	45
Plant based egg	*L. minor*	12.5	Duckweed-derived RuBisCO provides a heat-set protein, mimicking the cook ability and textural attributes of real eggs. It offers flexibility in emulsion design without compromising bioaccessibility	184
Beef burger	*L. minor*	0.1, 0.5, and 1	Enhances antioxidant activity, inhibits lipid oxidation, and contributes to an extended shelf life	187

binding properties suggests potential for various food applications, although further optimization is required. Research on incorporating duckweed into food products shows promising results. For instance, adding 2% duckweed powder to ice cream increases protein and fiber content by 8% and 13%, respectively.[45] Similarly, the inclusion of duckweed powder in snacks and bread has been shown to improve their nutritional profile, increasing crude protein by 51%, essential amino acids by 147%, and dietary fiber by 83%.[186,189] Despite these nutritional enhancements, challenges such as altered texture, color, and pH can affect consumer acceptance. Duckweed consumption has also been linked to increased vitamin B12 levels, offering a plant-based alternative to red meat.[187] Its antioxidant properties further position duckweed extracts as natural shelf-life extenders for meat products.[190] However, the green color of duckweed may influence consumer perceptions of taste and quality. Despite these challenges, the nutritional and health benefits of duckweed are significant. Future research should focus on refining formulations and expanding their applications in the food industry to improve consumer acceptance and product appeal.

7. Sustainability, economic potential, and technological challenges of duckweed biorefinery systems

7.1. Life cycle assessment and techno-economic aspects of duckweed biorefinery

Duckweed biomass is rich in high-value products and utilizes both natural and anthropogenic resources. Its potential for producing biofuels, food, feed, and pharmaceuticals has attracted the attention of various research groups, making duckweed a viable candidate for a biorefinery approach.[25,90] However, a comprehensive life-cycle analysis (LCA) is essential before advancing toward industrialization. Currently, LCA studies focused on duckweed biorefinery systems are limited. To date, only one study has reported the LCA for duckweed biorefinery, estimating the net global warming potential at 49 kg CO_2 equivalent per unit of wastewater treated and duckweed produced.[191] The environmental impacts of duckweed biorefinery products relative to substituted products such as gasoline, natural gas, and chemical fertilizers depend on the biorefinery size, with larger biorefineries potentially causing smaller detrimental environmental impacts. Substituting products such as gasoline and synthetic nitrogen fertilizer with those generated from the duckweed biorefinery resulted in significant benefits, including a reduction in global warming potential and human health damage. The techno-economic analysis of duckweed biorefinery systems further highlights the financial dynamics of these operations. A significant portion of capital expenses is attributed to pond construction (55.6%) and land costs (15.8%).[191] These findings suggest that while duckweed biorefineries require substantial upfront investment, they hold promise for reducing environmental impacts compared to conventional wastewater treatment methods and product substitutions.

7.2. Economic and environmental impact of duckweed

Duckweed offers substantial economic potential due to its versatility across various industries, including food, animal feed, bioenergy, and wastewater treatment. Its rapid growth rate and ability to thrive in nutrient-rich waters enable high biomass production at a low cost, making it economically viable for large-scale cultivation. Compared to traditional crops, duckweed requires significantly less land, water, and fertilizers, leading to reduce operational costs. Its application in biorefineries, where multiple high-value products such as proteins, starch, and biofuels are extracted, further enhances the economic viability of duckweed-based systems. Integration duckweed cultivation with waste management such as using wastewater as a growth medium not only lowers input costs but also generates revenue from bio-remediation and biomass production.

From an environmental perspective, duckweed aligns with global sustainability goals. It serves as a natural water purifier, efficiently absorbing excess nutrients like nitrogen and phosphorus, along with heavy metals from contaminated water sources.[192] This dual role in biomass production and environmental remediation makes duckweed an effective tool for mitigating water pollution, particularly in agricultural runoff areas. Furthermore, its capacity to sequester carbon and reduce greenhouse gas emissions makes duckweed an eco-friendly option for food and energy production.[193] By decreasing reliance on resource-intensive traditional agriculture, which contributes to deforestation and biodiversity loss, duckweed cultivation supports a more sustainable and circular bioeconomy.

7.3. Technological challenges and innovations

Duckweed biorefinery technologies face several challenges, but ongoing innovations are addressing these issues effectively. One significant challenge is inconsistent biomass yield. While duckweed grows rapidly, yields can vary significantly under suboptimal conditions. To tackle this, researchers are focusing on selective breeding and genetic engineering to develop high-yield varieties that can thrive in a range of environments, ultimately increasing biomass production.[194] Nutrient management presents another challenge, as duckweed's growth heavily depends on the nutrient availability, particularly nitrogen and phosphorus.[88] Imbalances nutrient levels can lead to poor growth or nutrient deficiencies. Innovations in this area include optimizing nutrient formulations and using wastewater as a growth medium[52,54–57,74–76] allowing duckweed to absorb excess nutrients from agricultural runoff or municipal waste. This dual-purpose approach enhances both biomass growth and environmental remediation.

Improving processing efficiency is another priority. Extracting valuable compounds such as proteins and starch from duckweed can be energy-intensive and costly. Advanced techniques, such as ultrasound-assisted extraction and enzymatic treatments, are being explored to maximize the yield of target compounds while minimizing degradation.[123,124,163] These innovations aim to make the extraction process more sustainable and economically viable.

Market acceptance of duckweed-based products remains uncertain in the mind of consumers and industries due to its

unfamiliarity and knowledge of its utilisation. Pilot projects are being implemented to showcase the versatility and benefits of duckweed products which can help raise awareness and drive adoption across various industries. Additionally, integrating multiple processing technologies into a cohesive biorefinery system can be complex. Modular biorefinery designs are emerging to facilitate the simultaneous processing of duckweed into multiple value-added products.[195] This approach enhances flexibility allowing various products to be derived from a single feedstock. Finally, water and resource management is critical, as duckweed requires nutrient-rich water for growth. Innovations such as closed-loop systems and recirculating aquaculture systems are being developed to improve water-use efficiency while minimizing environmental impact.[196]

8. Conclusion and future research directions

Duckweed is emerging as a valuable resource for biofuel production, protein, and other value-added products. Its rapid growth, favorable composition, adaptability to diverse environments, and minimal land requirements give it a distinct advantage over conventional terrestrial feedstocks. While the use of duckweed cultivated on waste streams shows promise, further research is needed to optimize treatment and cultivation processes to ensure sustainable and high-quality biomass production. Duckweed holds strong potential for producing bioethanol, bio-hydrogen, bio-oil, and biofertilizers. However, protein and organic acid production from duckweed remains underexplored and warrants further investigation. A duckweed-based biorefinery offers a sustainable approach to producing bioactive compounds, biofuels, and industrial chemicals. Incorporating duckweed into food and pharmaceutical products presents opportunities to enhance nutritional and health benefits. Nevertheless, the sensory impact of duckweed-based ingredients poses challenges for commercialization, necessitating efforts to refine pre-treatment, extraction technologies, and product formulations to address these issues. Specific research gaps in the current literature include the need for more comprehensive life cycle assessments to evaluate the environmental impacts of duckweed biorefineries, as well as studies focusing on the optimization of bioprocessing technologies. Investigating the efficiency of extraction methods for proteins and organic acids is particularly crucial, as this could enhance the economic viability of duckweed as a biomass source. Additionally, the long-term sustainability of using duckweed in various applications needs thorough investigation to ensure that ecological balances are maintained. Establishing clear guidelines for the cultivation and processing of duckweed, particularly when grown in wastewater, will be essential to address potential health and safety concerns. Policies that incentivize research and development in duckweed applications can accelerate its integration into the bioeconomy. As demand for enzymes, biofuels, and bioactive compounds grows, ensuring continuous biomass production, eco-friendly cultivation, and efficient extraction methods is essential. These insights contribute valuable knowledge to sustainable biomass utilization and functional food production, offering promising prospects for a greener future.

List of abbreviations

AD	Anaerobic Digestion
AD-	Diluted Anaerobically Digested Dairy Processing
DPW	Waste Water
AML	Acute Myeloid Leukemia
API	American Petroleum Institute
BOD	Biological Oxygen Demand
CBR	Catalyst-to-Biomass Ratio
COD	Chemical Oxygen Demand
DKP	*Diospyros kaki* Peels
DW	Dry Weight
F2F	Farm-to-Fork
FW	Fresh Weight
FPA	Filter Paper Activity
HA	Hoagland and Arnon
NDNE	Diluted Nitrification-Denitrification effluent
PGPB	Plant Growth-Promoting Bacteria
SHP	Superhydrophobic
T2D	Type 2 Diabetes
TN	Total Nitrogen
TP	Total Phosphorus
LCA	Life Cycle Analysis
LED	Light Emitting Diode

Data availability

No datasets were generated or analyzed during the current study.

Conflicts of interest

There are no conflicts to declare.

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