RESEARCH REVIEW

Multifaceted roles of duckweed in aquatic phytoremediation and bioproducts synthesis

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Abstract

Duckweed (Lemnaceae) is a fast-growing aquatic vascular plant. It has drawn an increasing attention worldwide due to its application in value-added nutritional products and in sewage disposal. In particular, duckweed is a promising feedstock for bioenergy production. In this review, we summarized applications of duckweed from the following four aspects. Firstly, duckweed could utilize nitrogen, phosphorus, and inorganic nutrition in wastewater and reduces water eutrophication efficiently. During these processes, microorganisms play an important role in promoting duckweed growth and improving its tolerance to stresses. We also introduced our pilot-scale test using duckweed for wastewater treatment and biomass production simultaneously. Secondly, its capability of fast accumulation of large amounts of starch makes duckweed a promising bioenergy feedstock, catering the currently increasing demand for bioethanol production. Pretreatment conditions prior to fermentation can be optimized to improve the conversion efficiency from starch to bioethanol. Furthermore, duckweed serves as an ideal source for food supply or animal feed because the composition of amino acids in duckweed is similar to that of whey protein, which is easily digested and assimilated by human and other animals. Finally, serving as a natural plant factory, duckweed has shown great potential in the production of pharmaceuticals and dietary supplements. With the surge of omics data and the development of Clustered Regularly Interspaced Short Palindromic Repeats technology, remodeling of the metabolic pathway in duckweed for synthetic biology study will be attainable in the future.

KEYWORDS
bioenergy, duckweed, eutrophication, omics, protein, synthetic biology

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1 | INTRODUCTION

Duckweed is one of the smallest, fastest growing, and simplest flowering plants in the world. They are classified into five genera (i.e., Spirodela, Landoltia, Lemna, Wolffsiella, and Wolffia) and 37 species based on their morphology, including root numbers and frond sizes (Banaszek & Musial, 2009; Les, Crawford, Landolt, Gabel, & Kimball, 2009; Sree & Appenroth, 2020). It is easy to distinguish them from each other albeit they are all very small. For instance, as the smallest flowering plants, Wolffia globosa is only 0.4–0.9 mm long. As the largest duckweed in North America, Spirodela polyrhiza is about 7–15 mm long, and is characterized with multiple roots and fronds with reddish-purple ventral surface.

Duckweed has an extremely wide adaptability to different environments (e.g., pH variation of 5–9, and temperature range of 6–33°C). In addition, duckweed is able to grow well even with limited nutrition (e.g., 60 mg/L of nitrogen and 1 mg/L of phosphorous; Leng, Stambolie, & Bell, 1995). Under suitable circumstances, duckweed has an overwhelming reproduction capability. The biomass of duckweed can be doubled every 48–96 hr depending on various species and culture conditions. However, the lifespan of an individual frond in the vegetative phase is relatively short, ranging from 3 to 10 weeks. Under unfavorable conditions, such as nutrient starvation, drought, or low temperature, the meristem can produce dormant fronds that are rich in starch. For example, S. polyrhiza forms dormant bodies, also called turions, and sink to the bottom of pond in winter. When the water temperature reaches 15°C or above in spring, new fronds can emerge from the dormant bodies by utilizing starch as the metabolism, and float on the surface of water again (Appenroth, Palharini, & Ziegler, 2013; Mejbel & Simons, 2018).

Among the five genera of duckweed, the sizes of fronds range from large to small, and root numbers vary from multiple to none. However, the genome sizes of different genera exhibit a large extent of variation ranging from 138Mb of Spirodela to 1.8Gb of Wolffia (Cao, Vu, et al., 2016; Hoang, Schubert, Meister, Fuchs, & Schubert, 2019; Van Hoeck et al., 2015; Wang et al., 2014; Wang, Kerstetter, & Michael, 2011). Comparative analysis of the chloroplast genome sequence is commonly employed as an effective approach to study the phylogenetic relationship of duckweed subfamilies from the evolutionary perspective. The chloroplast genomes of all five genera have been published thus far. The first completed chloroplast genome of Lemna minor was released in 2008. It has a circular structure and with of the genome size is 165,955 bp. Comparative genomics analysis of chloroplast genomes between L. minor with other monocots leads to a proposed model of rearrangements of the chloroplast genome (Mardanov et al., 2008). Taking the chloroplast genome of L. minor as the reference, the chloroplast genomes of S. polyrhiza, Wolffia australiana, and Wolffsiella lingulata were also assembled by filtering the whole DNA sequencing data using the SOLiD platform (Wang & Messing, 2011). Subsequently, the chloroplast genome with a total length of 171,013 bp was assembled for Landoltia punctata ZH0051 by integrating different bioinformatics tools (Ding, Fang, Jin, Zhao, & He, 2017). Taking advantage of the chloroplast genome data of the five genera, a reliable phylogenetic tree of duckweed is established (Wang et al., 2011). S. polyrhiza represents the first species with whole-genome sequence available in the duckweed family, and a high-confidence genetic map has been constructed based on the genomic data (Cao, Vu, et al., 2016). The subsequent research revealed no major structural rearrangements among seven S. polyrhiza clones (Hoang et al., 2018). Recently, the genomes of 68 genotypes representing the globally distributed of S. polyrhiza were sequenced using Illumina short-read sequencing with a 29x average coverage. The result showed that low genetic variation is associated with low mutation rate in S. polyrhiza (Xu et al., 2019). These high-quality genomic resources laid the foundation for molecular biology research in duckweed.

2 | DUCKWEED IN REMEDIATION OF WATER POLLUTION

It is estimated that more than 140,000 synthetics have been used in agricultural production since the Green Revolution in the 1950s. On the one hand, the applications of fertilizers and pesticides boost the grain yield and satisfy the human demand in food and energy. On the other hand, it inevitably imposes substantial threaten to the environment concurrently (Landrigan et al., 2018). Environmental pollution damages human health seriously. For example, water pollution can cause calculus, cancer, cardiovascular and digestive diseases, etc. Different physical, chemical, and biological approaches have been employed for water pollution restoration, among which, aquatic phytoremediation technology is regarded as an effective and environmentally friendly solution. As an aquatic plant, duckweed has various advantages in the phytoremediation of water pollution.

The substantially increased accumulation of nitrogen, phosphates, and other fertilizers in water results in dramatic water eutrophication, and aquatic plants blooming. It destroys the ecological balance of the water bodies, lowers the content of dissolved oxygen, deteriorates water quality, and even causes the death of aquatic creatures. Many studies had been shown that duckweed has been employed to treat agricultural, municipal, and even industrial wastewater streams into clean non-potable water (Ekperusi, Sikoki, & Nwachukwu, 2019; Yu et al., 2014). The advantages of using duckweed for the ecological restoration of eutrophic water are as follows: rapid growth and high biomass production, high photosynthesis efficiency, enormous nutrient
uptake capacity, wide adaptation to various aquatic ecosystems, and effortless harvesting. Duckweed can efficiently utilize nitrogen, phosphate, and other inorganic nutrients in water, and ameliorate the physicochemical properties and micro-environment of water. Generally, duckweed grows well at a concentration of nitrogen ranging from 7 to 84 mg/L. The optimum nitrogen concentration for prosperous duckweed growth is 28 mg/L, while nitrogen concentration exceeding 60 mg/L exerts substantial toxicity to water body (Priya, Avishek, & Pathak, 2012). Ammonium nitrogen (NH\textsubscript{4}\textsuperscript{+}) makes it suitable for remediation of nitrogen-enriched water body. Similarly, the phosphorous in water can be recycled by duckweed. A previous study showed that approximately 98.0% of nitrogen and 98.8% of phosphorous could be absorbed in duckweed-dominated pond, and the dissolved oxygen is increased from 0 to 3.0 mg/L in swine waste (Mohedano, Costa, Tavares, & Belli Filho, 2012). Apart from the concentrations of nutrient and dissolved oxygen, greenhouse gas emission is also an important index that should be considered in the wastewater treatment system by duckweed. It is estimated that carbon dioxide (CO\textsubscript{2}) emission rate ranges from 3,048 to 6,017 mg CO\textsubscript{2} m\textsuperscript{-2} day\textsuperscript{-1} and the CO\textsubscript{2} fixation rate ranges from 19,592 to 42,052 mg CO\textsubscript{2} m\textsuperscript{-2} day\textsuperscript{-1}. The amount of CO\textsubscript{2} fixed is almost three times higher than that of CO\textsubscript{2} released (Mohedano, Tonon, Costa, Pelissari, & Belli Filho, 2019). The capacity of sewage remediation reflects differences in adaptability to pollutants or stress in the environment, which varies among duckweed species. Yilmaz and Akbulut (2011) found that Lemna gibba removed four heavy metals (Pb, Ni, Mn, and Cu) in wastewater effluents as the ratio of 57%, 60%, 60%, and 62%, respectively. Interestingly, the best remediation effect is achieved through mixed cultivation of L. minor and L. gibba in the presence of wastewater with circulation. It is indicated that the national standards of biochemical oxygen demand 27–33 mg/L and chemical oxygen demand (COD) 62–78 mg/L were reached in wastewater effluents, respectively. In industrial wastewater, the total nitrogen concentration reached at 73 mg/L, the total phosphorus concentration was only 0.2 mg/L. Previous research suggested that the N removal rate was about 92%, accompanied by the P removal rate between 70%–75% for L. punctata and Lemna turionifera, by contrast, the N removal rate was 84% for Lemna aquinochialis and S. polyrhiza in industrial wastewater. Therefore, L. punctata and L. turionifera are more capable for the treatment of industrial wastewater with a high concentration of nitrogen and a low concentration of phosphorous (Zhou, Lin, et al., 2018).

Nevertheless, the growth rate and chlorophyll synthesis of duckweed are both inevitably inhibited when cultivated in wastewater, making it confronted with severe stress and become vulnerable during long-term cultivation (Caicedo, van Der Steen, Arce, & Gijzen, 2000; Liu, Dai, & Sun, 2017; Wang et al., 2016). Thus, it is necessary to search for aquatic microorganisms that can promote duckweed growth and/or increase its tolerance to stresses. Microbial communities living in symbiosis with duckweed include both plant growth-promoting bacteria (PGPB) and plant growth-inhibiting bacteria (Ishizawa, Kuroda, Morikawa, & Ike, 2017). Acinetobacter calcoaceticus P23, the first PGPB strain discovered in duckweed (L. minor), has been proved to effectively increase chlorophyll content and promote plant growth in both laboratory and natural waters (Suzuki, Sugawara, Miwa, & Morikawa, 2014; Toyama et al., 2017). Pseudomonas sp. Ps6, another PGPB strain, frequently adheres to and colonizes on the root of duckweed (L. minor). It enhances the growth of L. minor by 2- to 2.5-fold in 10 days, displaying a much higher activity than that of A. calcoaceticus P23 (Yamakawa, Jog, & Morikawa, 2018). The growth of duckweed rhizosphere-associated bacteria is dependent on the quality of water. A series of PGPB strains have been identified at different stages of rural wastewater treatment with duckweed. Rhodobacter, Bacteria vadin CA02, C9, and Flavobacterium play dominant roles at the initial stage, contributing to pollutant degradation and formation of biofilms. The percentages of Acinetobacter, Planctomycetes, and Methylibium are increased significantly at the stable stage, contributing to nutrient removal (Chen et al., 2019). Therefore, candidate strains should be screened from those rhizosphere-associated bacteria of duckweed according to the specific requirements of different sewage treatment, such as low pathogenicity, high phenol resistance, and the ability to utilize phenol as the sole carbon source. Five strains among 60 rhizosphere-associated bacteria were selected for bioremediation (Radulovic et al., 2018). The “recruiter duckweed-microbe co-cultivation” method has been established to isolate a wide variety of novel aquatic plant rhizosphere-associated microbes. Using this method, diverse microbes including two strains within the rarely cultivated phylum, Armatimonadetes, are effectively isolated (Tanaka et al., 2018). Inoculation of duckweed growth-promoting bacteria significantly enhances biomass yield and improves the removal efficiency of NH\textsubscript{4}\textsuperscript{+}-N and total nitrogen from eutrophic water (Ishizawa et al., 2017; Shen, Yin, Xia, Zhao, & Kang, 2019). Adding microbial carriers to rural wastewater improves the capacity of duckweed in sewage treatment, and the removal efficiency of nitrate nitrogen
and total nitrogen reaches 80.02% and 56.42%, respectively (Chen et al., 2019). The capability of duckweed and a bacterial to reduce the COD of metal-working fluid was evaluated. The result suggested that the reduction ratio of COD increased by about 41%, compared with the sole duckweed cultivation (Grijalbo, Becerril, Barrutia, Gutierrezmanero, & Garcia, 2016). Nevertheless, although the growth between duckweed and microorganisms is generally maintained synergistically, the requirements for some specific nutrients are diversified between plants and bacteria (O’Brien, Laurich, Lash, & Frederickson, 2018; Zhou, Chen, et al., 2018). From this perspective, the symbiosis system consisting of duckweed and the microbiome should be considered comprehensively in practical phytoremediation applications of the contaminated aquatic environment.

3 | DUCKWEED IN THE PRODUCTION OF HIGH VALUE-ADDED PRODUCTS

3.1 | Bioenergy feedstock

According to the report, global energy consumption is growing with an average annual rate of 1.6% from 2015 to 2020. China’s energy consumption is predicted to peak at 3.75 billion tons of oil equivalent around 2035 (Campbell, 2013). Meanwhile, COx, SOx, hydrocarbons, and particulate matters released from fossil fuels have caused serious environmental pollution problems. Compared with fossil fuels, the consumption of bioenergy could substantially reduce emissions of exhaust gas and particulate matters. Therefore, the development and utilization of renewable energy especially bioenergy are of significant importance. Duckweed has been proposed to use as feedstock for the generation of bioenergy products including hydrogen, ethanol, butanol, and biogas. It has several outstanding characteristics as a promising bioenergy plant including higher productivity, no competition with agricultural crops for lands, higher proportion of carbohydrate, and relatively lower lignin content that guaranty higher saccharification efficiency (Table 1; Souto et al., 2019).

The characteristics of high starch accumulation capability of duckweed are a key determinant for its use in bioethanol production. The starch content of duckweed is dependent on the species and growing conditions. In fact, plants have evolved different adaptation mechanisms to optimize growth by distinctive light capture and utilization strategies (Valladares & Niinemets, 2008). For instance, L. minuta, an invasive aquatic plant, shows a relatively higher growth rate than L. minor when grown under medium or high light intensities. Further analysis showed that L. minuta has a higher Net Assimilation Rate, better photochemical quenching, and a higher quantum yield (Y(II)) than L. minor at high light intensities. In contrast, under low light intensities, L. minor has a marginally higher growth rate owing to a greater Leaf Area Ratio, and higher chlorophyll content than L. minuta. It is undoubtable that a higher growth rate is beneficial to the accumulation of starch in duckweed (Ceschin et al., 2019; Mariani, Di Giulio, Fattorini, & Ceschin, 2020; Paolacci, Harrison, & Jansen, 2018). In general, duckweed can either speed up photosynthate assimilation or slow down starch decomposition to increase starch accumulation. When duckweed is grown in a hermetic container at a CO2 concentration of 100,000 ppm for 7 days, the starch content is increased approximately 1.5-fold from 9.6% to 24.7%, and displays better performance for nitrate and phosphate removal, compared with the control group cultivated with 380 ppm CO2 concentration (Mohedano, Costa, & Belli Filho, 2016). To assure a high photosynthesis efficiency, both the light intensity and photoperiod regime are required to be optimized (Liu et al., 2018; Yin et al., 2015). However, it is usually difficult and costly to meet these requirements, especially in the outdoors cultivation system. By contrast, manipulations of growing conditions (i.e., temperature, nutrients, hormones, and inhibitory chemicals) to slow down starch decomposition are relatively feasible and

| Trait                                | Microalgae | Duckweed | Terrestrial plant |
|--------------------------------------|------------|----------|-------------------|
| Rapid growth                         | √          | √        |                   |
| Higher biomass per plant             |            |          |                   |
| No competition with crops for lands  | √          |          |                   |
| Lower water consumption              |            |          |                   |
| Using CO2 in the air                 | √          | √        |                   |
| Lower energy-consuming cultivation   | √          |          |                   |
| Effortless harvesting                | √          | √        |                   |
| Lower biorefinery cost              |            |          |                   |

Note: The check mark (√) represents that the species has a kind of advantage in bioenergy production.
less costly than stimulating photosynthate assimilation. *L. punctata* was cultivated under 16:8 or 24:0 hr light/dark photoperiod cycles in Hoagland’s solution with or without nutrient starvation for 21 days. The results showed that the maximum starch content reaches 60.03% and starch yield approaches 76.45 g/m² (dry weight) under 24:0 hr photoperiod and N starvation, which is higher than the other conditions (Liu et al., 2018). Hence, constant illumination combined with nutrient starvation represents an environmentally friendly and cost-effective method for starch accumulation. Integrated analysis of transcriptome and metabolites revealed that most of metabolic pathways are inhibited except for starch metabolism under the N starvation treatment. The fixed CO₂ fluxes into the starch synthesis pathways, leading to the rapid accumulation of starch-rich biomass (Yu et al., 2017). More importantly, the nitrogen use efficiency of duckweed reaches 61.3 ± 1.8 kg biomass/kg N, which is higher than most cereals and trees under N-sufficient conditions. Under N-insufficient conditions, the powerful and efficient autophagy and ubiquitin proteasome system activate the remobilization and recycling of endogenous N to support duckweed growth and starch accumulation (Guo et al., 2020). The effects of phytohormones on biomass production and starch accumulation have been systematically studied. Under the treatment of 1.0 μM 6-benzylaminopurine (6-BA), the biomass yield is increased by 37.41% in 7 days, which is much higher compared with the other plant hormone treatments (i.e., auxin, cytokinin, abscisic acid, gibberellins, and brassinosteroids). Moreover, starch accumulation in the Abscisic Acid (ABA) treatment is 3.3 times higher than the control. These results suggest that 6-BA and ABA play an important role in biomass production and starch accumulation in duckweed (Liu, Chen, et al., 2019).

Our group in Qingdao Institute of Bioenergy and Process, Chinese Academy of Sciences, has established a stereoscopic duckweed cultivation system coupled with the livestock wastewater treatment with an area of 1,500 m². The anaerobic digestion with wastewater from a dairy farm (hosting 300 cows) reduces the COD. After solid–liquid separation, solid applies to organic fertilizers and liquid enters into duckweed wastewater treatment system. A three-layer rack is designed for duckweed cultivation in order to make full use of space and save the cost. More importantly, various light intensities ensure the optimal growth rate of duckweed for starch accumulation. Duckweed use nitrogen and phosphorus in wastewater to grow rapidly, and purified water is recycled into landscape ponds. The system accomplishes starch accumulation, sewage disposal, and recycling simultaneously (Figure 1). When firstly cultivated in the diluted pig effluent, then transferred into clean water for cultivation for 10 days, the starch content of *S. polyrhiza* is increased by 64.9% and the production rate reaches 9,420 kg ha⁻¹ year⁻¹.

**FIGURE 1** Multilayer duckweed cultivation and wastewater treatment system. (a) Wastewater and animal feces from a dairy farm (hosting 300 animals) was used for duckweed cultivation; (b) the tower was used for anaerobic fermentation of wastewater and animal feces; (c) solid–liquid separation device; (d) Duckweed grow in photovoltaic greenhouses; (e) duckweed pond for sewage disposal and starch accumulation; (f) stereoscopic and multilayer cultivation system.
Compared with the control group. After enzymatic hydrolysis and yeast fermentation, the theoretical ethanol yield reaches 6,420 L ha\(^{-1}\) year\(^{-1}\), which is 50% higher than the corn-based ethanol (Xu, Cui, Cheng, & Stomp, 2011). The catalytic efficiency of enzyme limits the conversion efficiency from starch to ethanol to some extent. A hydrolysate containing 50.9% reducing sugar of dry weight is produced by S. polyrhiza after saccharification with amylases and amyloglucosidase. The hydrolysate is further fermented with yeast and gives an ethanol yield of 25.8% of biomass (Cheng & Stomp, 2009). The molecular structure of starch influences the enzymatic saccharification efficiency as well, which is of significant importance to the production of glucose, a key precursor in ethanol production. The content of amylase with shorter chain length is negatively correlated with undigested starch content, while the amount of long-chain amylopectin is negatively correlated with the degradation rate efficiency (de Souza Moretti et al., 2019). This result provides new perspectives for the utilization of duckweed starch in biomass energy production. Furthermore, the gelatinized L. punctata can be directly fermented to produce butanol by Clostridium acetobutylicum (CICC 8012). A previous report demonstrated that 9.31 g/L butanol can be produced from total sugar at a concentration of 47.50 g/L. The ratio of butanol to total solvent is 68.46%, which is 14.11% higher than that of corn fermentation (Li, Jin, Gao, Zhang, & Zhao, 2012). Generally, methane is a more favorable product from duckweed than ethanol. Compared to the anaerobic digestion with dried biomass, fermentation of ethanol gives even higher (51.2%) methane yields, increasing the overall energy gain by 70.4% (Calicioglu & Brennan, 2018). Improvement of the pretreatment conditions is beneficial to bioenergy production from duckweed. Among various pretreatment methods, the hydrothermal process increases cellulose accessibility with minimal potentially inhibitory productions (Yu et al., 2010). The effect of hydrothermal pretreatment on bioethanol conversion is investigated in L. punctata. The optimum hydrothermal pretreatment condition is selected, and then subjected to saccharification and fermentation. It is estimated that the production of ethanol is increased by about 63%, compared to the untreated control (Souto et al., 2019). Acid hydrolysis with 1.0% H\(_2\)SO\(_4\) is another feasible strategy for pretreatment of duckweed biomass. The maximum production of hydrogen reaches 169.30 ml/g dry weight under a temperature of 35°C and an initial pH of 7.0 (Mu, Liu, Lin, Shukla, & Luo, 2020).

### 3.2 | A suitable alternative of protein source

About 90% of sewage wastewater has been directly discharged into the natural environment without any treatments, resulting in poor water quality, eutrophication, and dead zones owing to high contents of nutrients and pollutes. Billions of people are suffering from protein malnutrition in the world due to scarcity of high-quality food. Waste of resources contrasts sharply with lacking of high-protein food for human. With the increasing of the population and rapid development of economy in developing countries, dairy and meat consumption is estimated to increase by 82% and 102%, respectively, in 2050 compared to that of 2000 (Boland et al., 2013). Meanwhile, the environmental and ecological impacts brought by the production of animal-derived proteins should not be neglected, which also hinders the further increase in animal protein production (Aiking, 2011; Van der Peet & Kamp, 2011). Therefore, searching for the alternative protein source for human food is in urgent need.

High-protein content and adequate essential amino acids and vitamins make duckweed an attractive plant alternative for protein production. Duckweed protein contains 2.7% of methionine and cysteine, 7.7% of phenylalanine and threonine, 4.8% of lysine, and is rich in leucine, threonine, valine, and isoleucine (Appenroth et al., 2017). Duckweed has been accepted as a quality protein source for farm animals and aquaculture, and possibly also for humans in the future (Ziegler, Adelmann, Zimmer, Schmidt, & Appenroth, 2015). The advantages of using duckweed as the alternative protein source are as follows:

- (a) Fast growth rate and wide stress tolerance enable it suitable for biological production;
- (b) the water used in duckweed cultivation can be recycled with a recovery rate above 95%. Floating on the surface of water can reduce the loss of water by evaporation; thus, the water amount needed in duckweed cultivation is relatively less compared with other crop;
- (c) duckweed can grow in water without the supplement of fertilizer and pesticide, and even can be cultivated in wastewater; therefore, it does not compete with arable land;
- (d) duckweed production has less CO\(_2\) emission. To obtain 1 kg of duckweed, only about 0.4 kg of CO\(_2\) equivalent is produced, while 37–85 kg of CO\(_2\) equivalent is produced for 1 kg of beef (Dumontier et al., 2012).

The protein content of duckweed can span from 15% to 45% of dry weight, depending on the species and the growth conditions (Appenroth et al., 2017; Chantiratikul et al., 2010). The protein content increases with increasing nitrogen uptake by duckweed, with a preference for ammonium (NH\(_4\)) over nitrate (NO\(_3\)) in synthesizing amino acids (Landesman, Parker, Fedler, & Konikoff, 2005). In another study, Lemma japonica/ minor and Wolffia columbiana were co-cultivated in wastewater. By contrast, rude protein contents do not increase with increasing nitrogen concentration in wastewater, but rather seem to be dependent on chemical composition of the water body and the composition of microbial communities (Roman & Brennan, 2019). Besides, trace amount of organic contaminant also has an effect on the content and quality of protein. Considering the above influencing factors, it is appropriate to grow duckweed for protein production at the end stage of wastewater treatment, which reduces the risks of contamination from pathogens and chemicals without sacrificing protein yield.
Currently, duckweed is widely used in animal feed and aquaculture production. Feeding with duckweed has been improved the productivity, fattening, and slaughter performance of the livestock and poultry as well as the quality of meat and eggs (Mwale & Gwaze, 2013; Rojas, Liu, & Stein, 2014; Sońta, Rekiel, & Batorska, 2019). Apart from animal feed, duckweed can also be potentially used for human food (Figure 2). The extracts from seven species (i.e., *S. polyrhiza*, *L. punctata*, *L. gibba*, *L. minor*, *Wolffiella hyalina*, *W. globosa*, and *Wolffia microscopica*) have been tested for cytotoxic effects on human cell lines (i.e., HUVEC, K-562, and HeLa) and for anti-proliferative activity. The results confirm that the extracts from duckweed do not possess any detectable anti-proliferative or cytotoxic effect (Sree et al., 2019). *W. globosa* (also called Mankai) is considered as a natural protein source or “vegetable meat ball” for human or animal feed. Its protein content reaches 65% dry weight and has high levels of micronutrients such as β-carotene, chlorophyll, folic acid, and lutein. Mankai contains nine essential amino acids, dietary fibers, iron, zinc, and vitamins A, E, and B12 (Kaplan et al., 2019; Natesh, Abbey, & Asiedu, 2017). Compared with spirulina and kale, the taste of Mankai is relatively milder. When processed into fine powder, it can be added to baked products, sport nutrition drinks, pasta, and snacks. Researchers at Ben-Gurion University of the Negev found that Manki milkshake has higher satiety compared with the same amount of yogurt. A randomized, controlled, and cross-over trial has shown that it helps to lower the blood glucose level after intake of carbohydrates. In addition, the iron derived from Mankai could maintain iron homeostasis in human, which is essential for vegetarianism (Yaskolka Meir et al., 2019). Hinoman, an Israeli company, produces pesticide- and herbicide-free Mankai products for human food by introducing a precision cultivation system in greenhouse. For *W. globosa* and *Wolffia arrhiza*, Hinoman and Green Onyx are able to comply with the generally recognized as safe (GRAS) requirement in the United States in 2015 and 2016, respectively. In March 2017, Ajinomoto, a condiment giant in Japan, agreed to invest $15 million in Hinoman in order to obtain Mankai’s exclusive seller rights in Japan. Hinoman took the lead in commercialization of duckweed product successfully. In 2017, Parabel launched a new product called Lentein Complete, which derives from lentil protein. This protein is highly digestible and rich in essential amino acids, Omega3, zinc, iron, vitamins A, E, B12, and minerals, and its nutritional value even exceeds the so-called superfood such as spirulina and chlorella. Currently, Parabel holds 95 patents on plant protein extraction and final application. The capacity of Parabel to produce lentils reaches 3,300 tons/year, and satisfies the growing demand for plant protein. The Food and Drug Administration firstly issued a letter of no objection to Parabel, agreeing to regard Lentein protein as GRAS. This sets a good example for the commercialization of duckweed-derived products.

### 3.3 | A chassis plant for synthetic biology

Synthetic biology technologies were used to design or redesign metabolic pathways to produce biological components and systems, which do not originally exist in plants, based on the engineering principle. Currently, the de novo synthesis of various natural products and their precursors has been achieved in microorganisms. Compared with animal and microorganism, plant synthetic biology is safer and more reliable because it is not easily contaminated by bacteria or virus, and costs on clearing pathogens can be reduced. Compared to other plants, the advantages of employing duckweed in synthetic biology are as follows: (a) high genetic stability: asexual propagation without interference from seeds and pollen makes it stable to inherit; (b) clear genetic background: *Spirodela* is regarded as a model plant for synthetic biology due to its small genome (158 M) and large fronds, which makes it convenient for molecular biology research and tissue culture operation in the laboratory (Wang et al., 2014). Using the third-generation...
PacBio single-molecule long-read sequencing, the assembled sequence of *Spirodela* genome has been improved by 44-fold contiguity with an N50 of 831 kb and filled 95.4% of the sequence loss area (An et al., 2019). Integrated transcriptome, metabolome, and proteome analyses have been performed for treatments of nutritional deficiency or heavy metal stress (Tao et al., 2017; Xu et al., 2018). Accumulative data in the omics database lay the foundation for synthetic biology studies; (c) well-established genetic transformation platform: The tissue culture and genetic transformation of duckweeds was firstly implemented successfully in the 1970s. To date, the genetic transformation of three genera (*Lemna*, *Spirodela*, and *Wolffia*) has been achieved successfully. The transformation efficiency varies, depending on the frond, callus, and hormone. The transform efficiency of *Lemna* reaches 10%–94% (Chhabra, Chaudhary, Sänger, & Jaiwal, 2011; Liu, Wang, et al., 2019; Yang, Fang, et al., 2018), while it is 5%–13% in *Spirodela* (Rival et al., 2008; Vunsh et al., 2007; Yang, Li, et al., 2018). By contrast, *Wolffia* has the lowest transform efficiency of about 0.14%–0.4% (Heenatigala et al., 2018; Pavel et al., 2018). Application of the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) technology to silence the phytoene gene (*LaPDS*) had been carried out in *L. aequinoctialis*. Fifteen biallelic mutants with the albino phenotypes are obtained (14.3% success rate). Successful implementation of the CRISPR technology in duckweed brings the synthetic biology into the field of gene editing (Cao, Wang, Le, & Vu, 2016; Liu, Wang, et al., 2019; Zhang, Pribil, Pulmgren, & Gao, 2020); (d) simple isolation and purification of products: Target proteins are secreted into the culture solution, which contains only inorganic salts and water; (e) low content of natural product precursors: The contents of lignin and high-value aromatic compounds are quite low, making it easy to synthesize and purify target products using duckweed (Figure 3).

Aprotinin is a basic small peptide substance with antibacterial activity. The coding sequence of Aprotinin gene is transformed into *Spirodela oligorrhiza*. The aprotinin content reaches 3.7% of soluble proteins in transgenic lines, and reaches 0.65 mg/L in the culture medium (Rival et al., 2008). M2 matrix protein (peptide M2e) is the extracellular domain of the avian influenza virus. M2e DNA sequence fused in-frame to the 3′-end of ricin toxin B chain is transformed into *L. minor*. ELISA results revealed that the fusion protein content ranges from 0.25 to 2.5 μg/g in transgenic lines (Aleksey et al., 2018). The BIOLEX company exploits the Lemna Expression System in *L. minor*. Using this system, 35 proteins have been successfully expressed including α-interferon (IFN), human growth hormone (hGH), Fab fragments, monoclonal antibodies (mAbs), recombinant human plasmin BLX-30 and BLX-155. The IFN product accounts for more than 30% of the total protein in the culture medium, while hGH, Fab fragments, and mAbs account for 0.81, 8.62, and 5.60 g/kg of the dry weight of duckweed, respectively (Cox et al., 2006; Gasdaska, Spencer, & Dickey, 2003). The French company LemnaGene has also transformed genes into *S. oligorrhiza*, including encoding genes for antigens, drugs, industrial enzymes, and vaccines. The target products account for 35%–50% of the duckweed dry weight, which can be made into capsules or tablets in the form of dry powder.

### 4 PROSPECTS

Great progress in fundamental research and application of duckweed has been made in recent years and duckweed has played multifaceted roles in wastewater treatment and bioenergy production. However, there are still some obstacles hampering wide application of duckweed. For example, duckweed normally floats on the surface of the water body;
Therefore, it needs a huge pond for large-scale cultivation and high biomass production. To overcome this problem, a stereoscopic and multilayer culture device for duckweed has been established by our group, which made full use of space and light energy and ensured the maximum duckweed biomass production per unit area. Furthermore, full-automatic control of duckweed harvest, salvage, and starch extraction were achieved in this system. It sets a good example for future cultivation and broad application of duckweed (Figure 1). More cultivation technologies should be developed to obtain sufficient duckweed biomass for cost-effective bioenergy production in future. Another problem is that the combustion efficiency of bioethanol is much lower than that of fossil fuels. Usually, generation of efficient alternative bioenergy sources such as methane and hydrogen rather than bioethanol is preferred. Pretreatment methods for duckweed biomass need to be modified for clean energy production. Volatile fatty acids acetate and butyrate can be used for the cultivation of *Chlorella acidophillus* or lipid production.

Besides bioenergy production, duckweed has also been employed for biotreatment of agricultural, municipal, and even industrial wastewater (Ekperusi et al., 2019; Yu et al., 2014). Based on previous studies, 37 species of the Lemnaceae family showed different nutrient absorption abilities in wastewater. Therefore, appropriate duckweed varieties should be screened to improve wastewater treatment efficiency. In addition, involvement of PGPB during duckweed-based wastewater treatment can improve the bioremediation efficiency by promoting duckweed growth and improving its tolerance to stresses. Further work should be done for suitable PGPB selection.

Furthermore, with the development of multi-omics and genetic transformation techniques in duckweed, using duckweed as a chassis cell to produce high-tech and high-value products is possible. Currently, bioproduction of specific medicines or vaccine is normally performed using microbial and animal cells. However, high cost, time-consuming, and susceptibility to contamination by pathogenic microorganisms are to be resolved to meet increasing demands globally. Development and utilization of plant-derived drugs are considered to be an effective and promising approach. Studies have shown that plant secondary metabolites, including phenols, flavones, triterpenes, alkaloids, and coumarin compounds, have the ability to fight against acquired immune deficiency syndrome and tuberculosis (Habibi, Soccol, Grossi-de-Sá, & Daniell, 2019). The capability of producing abundant secondary metabolites, fast growth rate, and anti-pathogen makes duckweed a good candidate for pharmaceutical production. Employment of natural products from duckweed could eliminate adverse drug reaction, such as drug interactions, toxicity, and multidrug resistance. Therefore, after molecular engineering, duckweed can be potentially applied for large-scale production of various effective recombinant proteins and peptides for treatment of diseases (Capell et al., 2020).

Nowadays, duckweed is widely applied in wastewater purification, animal feed, human nutrition, bioenergy resource and as plant-derived cell factories. In-depth understanding of these tiny and amazing plants and developing new technologies will help solve future challenges.

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**DATA AVAILABILITY STATEMENT**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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