Desalination and Water Treatment
www.deswater.com

doi: 10.5004/dwt.2017.0479

63 (2017) 327–342
February

The uses of duckweed in relation to water remediation

Paul Ziegler[a,*], Kandregula Sowjanya Sree[b], Klaus-Jürgen Appenroth[c]

[a]*Department of Plant Physiology, University of Bayreuth, Universitätsstr. 30, 95440 Bayreuth, Germany, Tel. +49-921-553037; email: paul.ziegler@uni-bayreuth.de*
[b]*Department of Environmental Science, Central University of Kerala, RSTC, Padannakad 671314, India, email: ksowsree@gmail.com*
[c]*Institute of General Botany and Plant Physiology, Friedrich-Schiller-University Jena, Dornburger Str. 159, 07743 Jena, Germany, Tel. +49-3641-949233; email: klaus.appenroth@uni-jena.de*

Received 29 April 2016; Accepted 31 July 2016

ABSTRACT

Duckweeds are small, simply structured floating plants that grow on surface waters. They grow rapidly and are easy to cultivate, harvest, process and analyze, which makes them useful in many ways. Duckweeds are of great value in illustrating the physiological effects of toxic water contaminants on plants and serving to indicate the presence and environmental risk of such toxins. The pronounced capacity of duckweeds to assimilate aqueous nutrients and to take up and mediate the removal of a variety of toxic substances from aqueous solution constitutes the potential of these organisms for wastewater remediation. The biomass yielded by duckweed growth – particularly on nutrient-rich wastewater – has a high nutritional value and is well suited for biofuel production, as well as being useful for fertilization, biosorption and soil and water amendment. Duckweeds thus have the potential for integrating a significant contribution to meeting food, feed and energy demands traditionally supplied by terrestrial crop plants and fossil fuels with the remediation of polluted waters. Duckweed growth can also be used to directly generate bioelectricity, and the success of genetically transforming duckweed plants opens the road to biomanufacturing with these organisms, both of which are in principle compatible with wastewater remediation. However, neither wastewater remediation by duckweeds nor the exploitation of the multiple potential benefits of wastewater-grown duckweed biomass has yet been widely implemented. The present review underlines the potential of duckweeds for combining resource management with water remediation, while examining the difficulties encountered in the realization of this potential.

Keywords: Duckweeds; Uses; Water remediation; Biomass production; Biomass utilization

1. Introduction

The current expansion of world population and industrialization gives rise to increasing production of wastewater. Municipal, agricultural and industrial wastewaters are often discharged untreated into the surroundings, and even when they are processed, in facilities ranging from simple septic tanks to large-scale sewage treatments plants, wastewater effluents are often not sufficiently cleared to the extent that they are of no hazard to the environment. In addition, leachates from solid wastes and fertilizer spread on fields and pesticides sprayed on crops can contaminate ground water and water reservoirs via rain and runoff. Duckweeds, small floating aquatic plants or macrophytes, can help to clear these waters of excessive nutrients, heavy metals and organic xenobiotics. Their potential for effecting the cleanup of wastewaters and polluted surface waters and some examples of their actual use in this regard have been described in a recent review [1]. Duckweed-based water remediation is the reference point of this review, and its essential features will be reiterated under consideration of recent relevant findings in the following.

* Corresponding author.

Presented at the 5th International Conference on Environmental Management, Engineering, Planning and Economics (CEMEPE), 14–18 June 2015, Mykonos, Greece.

Water remediation is not the only use to which duckweeds can be, and have been, put. Duckweeds have long been used as model organisms for investigating basic issues of plant biology, and they are also well-established as test organisms, especially for detecting and evaluating toxicity in aquatic environments. Duckweeds can serve as vehicles for recombinant protein and bioelectricity production, and have use in medicine. Duckweed biomass can be put to a great variety of uses, including animal and even human nutrition, bioenergy production, biosorption and soil improvement. A major aim of the present review is to emphasize that all of the uses for duckweeds are actually or potentially related to water remediation. Investigations utilizing the model investigative character of duckweeds point to the presence of pollutants present in wastewater and other surface waters and their deleterious physiological and environmental effects. The cultivation of duckweeds on contaminated waters to effect the removal of these pollutants can produce ample biomass with all its potential applications, and the duckweed growth could also be used to produce bioelectricity and recombinant proteins. Although they may at first sight appear to be very promising, neither wastewater remediation by duckweeds nor any of the potential benefits accruing from duckweed cultivation on wastewater have been successfully implemented to any significant extent. This review also discusses why this potential has not yet been realized.

The newsletter of the International Steering Committee on Duckweed Research and Applications (ISCDRA: [2]) is a valuable source of information on, among many other topics of duckweed research, current affairs in water remediation by duckweeds and the utilization of duckweed biomass. Some specific references will be made to articles in this newsletter, and the newsletter itself (see [2]) is recommended for keeping up to date on duckweed research and applications.

The versatile utilization potential of duckweeds is based on particular attributes of these organisms.

2. Duckweed attributes

Duckweeds are small aquatic plants or macrophytes that grow on the surface of bodies of still or slowly moving water. As summarized in a recent review [1], their architecture is simple, consisting principally of thallus-like vegetative bodies, or fronds, made up mostly of spongy mesophyll with large air spaces that confer buoyancy. The fronds are often flattened ovoids ranging from two to 15 mm across, but can also be globoid, cylindrical, ellipsoidal or narrow linear as little as 0.2 mm in width. Representing a fusion of leaves and stems, these simple forms constitute the extreme reduction of a vascular plant, which may or may not bear hairless roots on the underside. The duckweeds make up the family Lemnaceae that includes 5 genera encompassing 37 species (Fig. 1; [3]). The phylogenetic relationships between the duckweed species have been deduced on the basis of morphology, anatomy, biochemical data and DNA sequences from chloroplast and ribosomal genes [3–6]. Many duckweed species are widely distributed about the globe [7], and each species can encompass numerous clones, or strains or ecotypes, that have evolved in response to particular microenvironments. Since there are few morphological characters to differentiate between duckweeds, DNA bar-coding and amplified fragment length polymorphism analysis are modern approaches to identifying the species to which such ecotypes belong [8,9]. Although they are flowering plants, most duckweeds flower only infrequently under laboratory conditions (often as a response to stress [10]), and reproduce predominantly in the vegetative mode. Daughter fronds bud off from one or two pouches in mother fronds, while remaining attached for a time to form colonies. This vegetative reproduction can take place very rapidly.

Duckweeds are often touted as being the world's fastest growing higher plants (e.g., [11–13]). This rapid growth is on the one hand favored by the high proportion of photosynthetic tissue in the fronds and the good exposure to light ensured by the floating growth mode. In addition, duckweeds can proficiently take up nutrients (see [1]), and their vegetative propagation enables them to rapidly spread over the surface of the water on which they float (see, for example, [14]). Indeed, their ability to rapidly overgrow open, nutrient-rich water in conjunction with their vegetative reproduction and assimilatory prowess has led to duckweeds being compared with "Darwinian demons", highly reproductive and proliferative hypothetical organisms [15]. While this seemingly "demonic" potential can of course not be realized due to the presence of multiple real growth constraints (see [12]), duckweeds can indeed produce large amounts of biomass under favorable cultivation conditions.

The small size and rapid growth of duckweeds make these organisms amenable to cultivation and harvest on any scale, and their simple anatomy (e.g., lack of many common structural elements and lignin) makes their tissues easy to process. The clonal reproduction of the fronds is easily monitored, and it is conducive to stability in the genetic and physical makeup of the macrophytes over time. These properties all contribute to the various uses to which duckweeds have been put. However, the particular use that may be envisaged for duckweed must take into account the diversity shown by this family. This is particularly evident with regard to growth.

How well duckweeds actually can grow varies strongly with the individual duckweed species and strain (or clone/ecotype). Duckweed clones, or strains, show a wide range of growth rates under a particular set of conditions. In an investigation of the relative growth rates (RGRs), doubling times and relative yields of 39 clones representing 13 species from all five duckweed genera under favorable, standardized conditions on synthetic mineral salt medium [11], these growth parameters were found to vary strongly (e.g., RGRs ranging from 0.153 to 0.519 d^{-1}) with the individual ecotype, rather than with the genus or species. A similar examination of 25 clones representing all 11 species of the genus *Wolffia* revealed RGRs ranging from 0.155 to 0.559 d^{-1} [13]. The ability of duckweeds to grow under particular environmental conditions also varies strongly. E_rC_{50} values (concentrations at which 50% growth inhibition occurred) for 33 duckweed strains representing 13 species across all five genera ranged from 10 to 377 mM NaCl in standard mineral salt nutrient medium; 10 strains were considered to be suitable for cultivation in a six-fold dilution of sea water, and one *Spirodela polyrhiza* clone in an only three-fold dilution [16].

These data illustrate a general principle involved in choosing duckweed for a particular application: the necessity for extensive screening to identify a clone well suited to

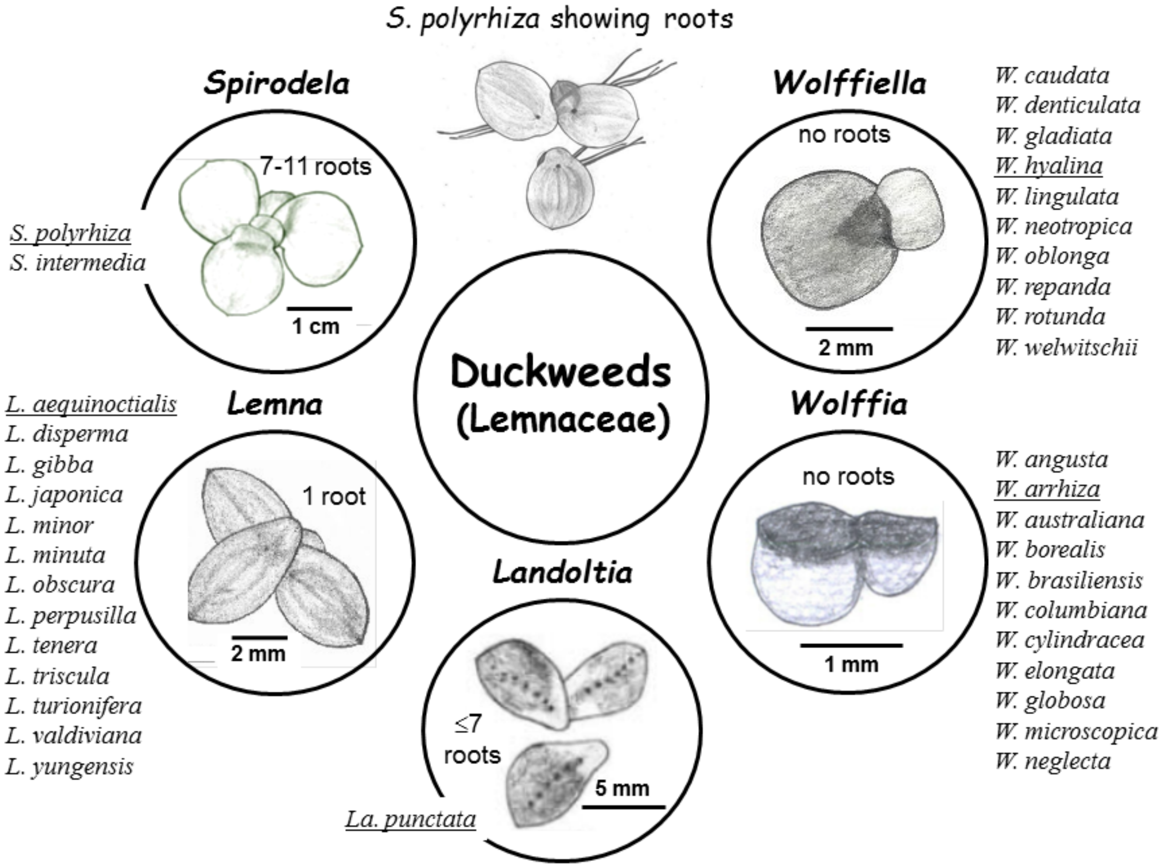

Fig. 1. Five genera and 37 species of the family of the Lemnaceae (duckweeds).
Note: Representative shapes, sizes of and groupings of fronds are shown for each genus (the actually depicted species is underlined), along with the number of roots that the fronds of the genus species exhibit. Drawings by Dr. K. Sowjanya Sree. The phylogenetical position of the family is shown in [4].

the purpose and the growth conditions at hand. If a particular attribute, for example, biomass itself (as the product of growth), or some constituent of the biomass, is the goal of a particular prospective duckweed cultivation project, screening for this attribute should be carried out under the conditions closely resembling those envisaged for the planned project-scale operation. Examples of experiments screening duckweeds for high biomass, protein, starch and fatty acid content will be mentioned in the following.

The ease with which duckweeds can be cultivated and analyzed has led to use of these macrophytes as model and test organisms. These roles are most important in determining the presence and the action of toxic water constituents that are targets of water remediation.

3. Duckweeds as model and test organisms

Many years ago, Hillman advocated using duckweeds as model organisms for studying plant biology [17], and these macrophytes have indeed long been employed for investigating plant physiology. A number of such studies are quoted in Table 1. The model character of duckweeds, in particular *Lemna minor* and *Lemna gibba*, has also long served to determine the deleterious effects of wastewater constituents on aquatic plants. Numerous examples of these investigations and the possibility of using duckweeds to establish biomarkers for toxic effects in aquatic plants have been discussed in a recent review [1]. The use of *L. minor* as a model organism for probing the use of ^{1}H NMR for identifying phytotoxin-elicited metabolic changes mentioned in Table 1 [27] is a further case in point. A recent report in this regard describes the biochemical changes and the oxidative stress alleviation activity elicited in *L. minor* by the pharmaceuticals diclofenac and paracetamol, which are often detected in aquatic systems [31].

Despite their value in elucidating plant physiology and wastewater toxicity, duckweeds have been largely eclipsed as model plants over the past 20 years by *Arabidopsis*, which initiated the era of plant genomics capable of rapidly tackling a multitude of important physiology questions, and by crop plants of great nutritional and commercial importance such as maize and soybean. The recent sequencing of the *S. polyrhiza* [32] and *L. minor* [33] genomes have now opened the way to all manner of genome-oriented investigations and manipulations with duckweeds, and interest in the potential of these macrophytes as model organisms for plant biology research is being renewed [34,35]. The use of *L. minor* cells transformed with bioluminescence markers for monitoring gene expression quoted in Table 1 [28] is a case in point. Efficient transformation and gene silencing techniques recently developed for *L. minor* [36] will facilitate the

Table 1
Use of duckweeds as model organisms to investigate plant biology

Investigation	Duckweed	Reference
Photoperiodic control of flowering	*Lemna perpusilla*	[18]
Biosynthesis of the branched-chain sugar apiose	*Lemna gibba*	[19]
Effect of light on the synthesis of ribulose-1,5-bisphosphate	*Lemna gibba*	[20]
Effect of light on the synthesis of light-harvesting chlorophyll proteins	*Lemna gibba*	[21]
Plant sulfur assimilation	*Lemna perpusilla*	[22]
Auxin biosynthesis	*Lemna gibba*	[23]
Dormant bud induction	*Spirodela polyrhiza*	[24,25]
Low-fluence phytochrome response in plant development	*Spirodela polyrhiza*	[26]
Use of ^{1}H NMR fingerprinting for studying metabolic changes due to phytotoxic substances	*Lemna minor*	[27]
Single-cell bioluminescence imaging for monitoring cellular plant gene expression	*Lemna minor*	[28]
Effects of heterogeneous nutrient dispersal on biomass accumulation	*Lemna minor*	[29]
Dietary transfer of metals/metal cycling in aquatic ecosystems	*Lemna minor*	[30]

use of duckweeds for elucidating physiology and improving biotechnology, as already illustrated by the development of a *L. minor* isoleucine auxotroph for recombinant protein expression [37]. Stable non-transgenic gene expression by means of genome duplication may lead to novel insights into metabolite production [38].

L. minor has also found application as a test organism in the study of host immune responses to biofilm microbial infections [39] and as a high-throughput infection model for pathogenic bacteria [40]. In terms of wastewater science, however, especially *L. minor* and *L. gibba* have long been instrumental in detecting the presence of toxicity in water samples, in addition to elucidating the deleterious effects of water-borne toxins, as recently discussed in detail in [1]. As such, duckweeds are vital for pointing to the risks posed by wastewaters to aquatic plant life. In a related vein, *L. gibba* has been explored for its ability to determine the effect of diluted substances in a homeopathic context [41,42]. *L. minor* was also chosen as the model experimental plant to demonstrate the suitability of a disposable electrochemical sensor for monitoring heavy metal take-up from contaminated waters [43].

In addition to their usefulness in pointing to the presence and the deleterious effects of toxic substances in wastewater and surface waters, duckweeds are of great potential value in ridding these waters of such substances.

4. Wastewater remediation by duckweeds

Duckweeds are proficient and versatile in removing nutrients and other pollutants from water. If they do this efficiently enough, duckweed growth on wastewater and other contaminated surface waters can result in the release of an effluent harmless to the environment and suitable for irrigation.

4.1. Nutrient and pollutant take-up

The rapid growth of which duckweeds are capable shows that these organisms can take up and assimilate large amounts of macronutrients (NH_4^+, NO_3^- and PO_4^{2-}) from their supportive medium. The removal of especially NO_3^- from nutrient-rich waters may be largely due to the action of denitrifying bacteria associated with the duckweed rhizosphere [1]. The efficiency of total nitrogen and NH_4^+ removal from lake water in China by *Lemna japonica* was found to be improved by up to 20% by adding carrier biofilms to the wastewater that served as artificial roots and enhanced the growth of nitrifying and denitrifying bacteria [44].

Duckweeds can also tolerate and take up numerous water contaminants including many heavy metals and a variety of organic xenobiotics including pesticides and phenolics [1]. Some of the organic xenobiotics can also be degraded by the duckweeds. The quinolone aquaculture antibiotic flumequine can be removed from solution and degraded by *L. minor* [45], and *Lemna triscula* has been shown to efficiently remove anatoxin-a, a cyanobacterial neurotoxin, from culture medium and to subsequently degrade much of the toxin [46]. Bacteria associated with the duckweed rhizosphere are often involved in the removal of xenobiotics from solution and their breakdown [1]. Rhizosphere bacteria can be not only beneficial for duckweed growth and remediative activity but can also protect the macrophyte from the toxic effects of the substances that it takes up. The presence of the *L. minor* rhizobacterium *Exiguobacterium* sp. MH3 led to both better growth of and chromium uptake by the duckweed, while alleviating the toxic effects of the heavy metal [47]. However, not all of the bacteria that can utilize water contaminants as growth sources and can colonize duckweeds may actually be suited to removing these contaminants from polluted waters [48].

The rapid growth and xenobiotic uptake by duckweeds make these organisms obvious candidates for the bioremediation of wastewaters and polluted surface waters. Although many of the studies of nutrient and xenobiotic take-up by duckweeds have been carried out with spiked standard nutrient media, this does not mean that they are irrelevant for actual wastewaters. When the performance of *Lemna aequinoctialis* in removing nutrients from artificial culture medium and from sewage water was compared, NH_4^+ and total phosphorus were removed to an extent of more than 80% from each medium [49]. This indicates that experiments with artificial media can indeed give a good estimate of how efficient nutrient removal will be on actual wastewater.

Analogous to the variability in growth rates described above in Section 2, individual duckweed clones or ecotypes can vary considerably in their suitability for growing on and removing unwanted substances from a particular type of wastewater. This has been most convincingly shown in a study comparing the growth of 41 duckweed strains across 12 species and all five genera on synthetic medium formulated to closely resemble swine lagoon effluent that was particularly rich in NH_4^+ [50]. Increases in fresh weight and dry weight (DW) showed 11- and 3-fold ranges, respectively, among the strains, and there was a 28-fold difference between the most disparate strains with regard to total protein content, i.e., in the amount of NH_4^+ having been assimilated from the wastewater. This study led to the selection of a clone of each of *Landoltia punctata*, *L. gibba* and *L. minor* that were particularly well-suited for nutrient take-up from swine wastewater (see [1]).

4.2. Studies of wastewater remediation

A number of studies have demonstrated the potential of duckweeds for improving the quality of actual domestic, municipal, agricultural and industrial wastewaters. These encompass investigations in Ghana, Egypt, Israel (see [1]) and in several other countries as shown in Table 2. The duckweeds were shown to remove much of the organic and inorganic nitrogen and phosphorus of nutrient-rich wastewaters, in addition to reducing chemical and biological oxygen demand, removing suspended solids, mosquito larvae and coliform bacteria from the wastewaters and maintaining a neutral pH value. Two of the studies in Table 2 demonstrate that duckweeds can also facilitate the removal of heavy metals and unwanted chemical ions from industrial wastewaters. The finding that *L. minor* growing on subtropical paddy fields in China significantly (19%) reduced the overall greenhouse effect of CH_4 and N_2O release [63] may also be relevant for the remediation of detrimental effects of wastewaters.

Duckweeds may not, however, necessarily improve the remediation of polluted waters over that of more conventional wastewater treatment systems in some geographical or climatic contexts. Full technical scale "Lemna System" set-ups for domestic wastewater treatment consisting of primary aerated ponds serially connected with secondary duckweed ponds have been operated in Poland for several years. A recent study has shown, however, that reduction in the nutrient content and the chemical and biological oxygen demand of the water was independent of whether the duckweed (*L. minor*) was growing on the secondary pond or not [64].

4.3. Is duckweed wastewater remediation viable?

Despite the obvious potential of duckweeds to improve the quality of wastewater and the demonstration of this potential in the treatment of actual wastewaters described above, the use of duckweeds in wastewater remediation

Table 2
Wastewater remediation with duckweeds

Wastewater	Location	Duckweed	Removal of	Reference
Settled residential wastewater	Near Thessaloniki, Greece	*Lemna minor*	Nutrients, BOD, TSS bacteria	[51]
Secondary treated university wastewater	Lesvos, Greece	*Lemna minor*	Nutrients, COD, antimicrobials	[52]
Mine gallery water	Keban, Turkey	*Lemna minor* *Lemna gibba*	Heavy metals	[53]
Settled municipal wastewater	Khirbet As-Samra, Central Valley, Jordan	*Lemna sp.*	Nutrients, BOD, bacteria	[54]
Drainage water	Gharbia, Nile Delta, Eygpt	*Lemna gibba*	Ammonia, COD	[55]
Activated sludge-treated domestic wastewater	Mahdia, Tunisia	*Lemna minor*	Nutrients, BOD, COD, coliform bacteria	[56]
Settled municipal wastewater	Taxila, Pakistan	Unspecified duckweed	Nutrients, BOD, COD	[57]
Untreated municipal and industrial wastewater effluents	Islamabad, Pakistan	*Lemna minor*	Heavy metals	[58]
Untreated domestic sewage	Jodhpur, India	*Lemna minor*	Nutrients, BOD, COD, TSS, TDS	[59]
Wetland with RBC-treated university wastewater effluent	Near Ranchi, India	*Lemna minor*	Phosphate, BOD	[60]
Oxygen-treated steel plant coking wastewater	Jamshedpur, India	*Lemna minor*	Chloride, sulfate, TDS	[61]
Constructed wetland with diluted raw dairy wastewater	Webberville, MI, USA	*Lemna minor*	Nutrients, COD, bacteria	[62]

Note: "Nutrients" refer to nitrogen-containing substances and phosphates, "BOD" and "COD" to biological and chemical oxygen demand, respectively, and "TSS" and "TDS" to total suspended and soluble solids, respectively.

has not become of large-scale importance. A good example of this is the recent report of the result of a national survey that duckweed ponds currently account for less than 1% of 108 natural treatment systems being used in India to treat a variety of wastewater effluents, in contrast to polishing and waste stabilization ponds which comprise the treatment system in nearly 98% of the facilities [65]. It is perhaps not to be expected that duckweeds will readily find a place in modern large-scale municipal sewage and industrial effluent treatment plants, which must be planned and constructed to ensure effective clearance of a large volume of wastewater in relatively restricted areas. As alluded to some time ago by Stomp [66], experience with wastewater remediation by duckweeds has apparently not provided economic arguments sufficient to procure research and development investments necessary to establish sophisticated wastewater remediation technology. Paul Skillicorn recently described the failure of an attempt to finance a duckweed wastewater treatment plant in Texas, citing unwillingness to challenge the status quo in implementing new technological endeavors in America [67]. But wastewater remediation by duckweeds is indeed now gaining importance in rural areas and less well-developed countries without extensive access to traditional water treatment technology (see again [67]), and is being integrated into comprehensive concepts being developed for wastewater treatment in such countries (e.g., [68]). This idea becomes attractive in view of efforts to implement the remediative potential of duckweeds by exploiting the biomass that the macrophytes growing on wastewater produce, i.e., by using wastewater remediation for the production of a crop plant. Before this is examined, however, a further potential benefit of wastewater remediation by duckweeds is indicated.

4.4. Generation of bioelectricity?

It has recently been demonstrated with *Lemna minuta* that duckweeds can produce bioelectricity while growing photoautotrophically in a newly designed direct photosynthetic plant fuel cell [69]. The duckweed could conceivably grow on wastewater in the fuel cell, and, if the fuel cell could be constructed on an appropriate scale, extensive wastewater remediation by duckweeds could be combined with significant electricity production. In this case, the bioelectricity generation could be a value-added product of water remediation lending incentive to implementation of the remediation logistics. Over 40% increases in soluble protein and reserve carbohydrate contents of *Lemna valdiviana* due to enhanced metabolic activity under the polarization conditions of the fuel cell [70] show that bioelectricity generation by duckweeds may also result in improved quality of the concomitantly produced biomass.

5. Wastewater remediation for biomass production

Duckweeds growing on nutrient-rich waters produce biomass that represents reclamation of the water-borne nutrients. The possibilities of exploiting nutrient-rich biomass derived from wastewater remediation was recognized long ago by Hillman and Culley [71], who proposed growing duckweed on lagoons fed by sludge from fermented dairy manure and using the harvested duckweed for fodder for the cattle. This seminal idea has been followed up in numerous studies and publications since then, and much attention has been given to the potential of using wastewater-derived duckweed biomass for animal feed and for biofuel production [72–75]. The most famous example is the use of duckweed to remediate hospital wastewater in Mirzapur, Bangladesh, and the use of the biomass for fish food in pisciculture ponds (see [72,73]).

However, no large-scale utilization of duckweed in any sense has been realized up to now. As discussed by Stomp in a review published in 2005 [66], the successful transformation of any wild plant (which a duckweeds is) into a crop plant is dependent on the identification of a valuable product that can be obtained from the plant, the development of processing methods to obtain specific products from the plant biomass, and the development of agronomic programs to scale up consistent production of the biomass. In addition, genetics programs should be developed to improve product quality, increase production and lower production costs. As discussed in the following Section 7, duckweed biomass does represent a valuable product in terms of nutrition, energy and remediation, and techniques required to process it to specific products are available. Large-scale wastewater remediation with duckweeds would immediately suggest itself as a vehicle for increasing biomass production, but it is subject to the economic and conceptual restrictions alluded to above. The large-scale realization of Hillman's remediation/utilization vision is only now really starting to come of age (see [34]); it is being fueled by the rising need for environmentally sustainable crops to complement traditional agriculture in meeting incipient global nutrition and energy demands. A facility to use wastewater-derived, starch-rich duckweed for biodegradable plastic production has now been established in Argentina [76] and other initiatives to exploit the nutritional value of wastewater-grown duckweed are in the planning or development stages in Holland [77] and the Philippines [78], as well as in other South American and Asian countries (see the newsletters of the ISCDRA [2]).

Whatever use is envisaged for duckweed biomass, as much of the biomass should be produced as possible under the conditions under which the duckweed can be cultivated. Duckweed biomass targeted for a particular usage should also have a high content of the appropriate constituent, for example, a high protein or starch content when nutrition or biofuel production is the goal, as will be discussed in the following. There are two basic strategies for optimizing the amount and/or desired attributes of the duckweed biomass to be produced. One is to identify a duckweed clone or ecotype that performs particularly well in the appropriate manner under the conditions under which it will be growing. The importance of this for biomass production *per se* is well illustrated in terms of the large variation in the growth rates (measured as biomass production!) of individual duckweed clones referred to in Section 2, and duckweeds particularly proficient in producing high protein- or high-starch biomass can also be identified by screening (see below). The other basic strategy is to optimize the conditions of cultivation for a particular duckweed strain. For example, a fuzzy-logic-based diagnosis system has been developed to optimize the pH and temperature conditions for *L. gibba* biomass production [79], and the light intensity and photoperiod required for maximum biomass and starch production with *L. aequinoctialis* have been evaluated [80]. Whereas these two studies were carried out with synthetic mineral growth media, the most favorable conditions of water depth, coverage rate and harvest regime for the production of high-protein *L. aequinoctialis* biomass on actual domestic sewage and industrial wastewater have been determined in China [81]. In addition, transforming duckweed with appropriate genes may be able to effect the enhancement or improvement of particular duckweed attributes.

A number of studies illustrating high yields of biomass produced by duckweeds growing on agricultural and university wastewaters have been tabulated in a recent review [1], and some further examples of wastewater remediation to generate duckweed biomass, in some cases specifically targeted for animal feed and biofuel production, are shown in Table 3. Of course, duckweeds do not have to grow on actual wastewater to produce useful biomass, as shown by the example of protein-rich duckweed for broiler feed grown on irrigation pond water in Jordan [91]. Duckweeds are not the only macrophytes under consideration for producing useful biomass in the implementation of water remediation, and some other water plants may at first glance appear to be more promising than duckweeds in this regard. For instance, water hyacinth (*Eichhornia crassipes*) produced twice as much biomass as did *L. japonica* in a pilot-scale wastewater treatment system, while exhibiting rates of total nitrogen and phosphorus recovery from the wastewater similar to those shown by the duckweed. However, protein

Table 3
Recent examples of wastewater remediation targeted for duckweed biomass production

Growth medium	Location	Removal of	Yield	Duckweed	Reference
Secondary treated university wastewater	Lesvos, Greece	Nutrients, COD, antimicrobials	Protein- and starch-rich biomass	*Lemna minor*	[52]
Domestic wastewater septic tank effluent	Cairo, Egypt	Nutrients, BOD, COD, coliform bacteria	Protein-rich biomass for fish feed	*Lemna gibba*	[82]
Anaerobically digested swine lagoon wastewater	Melbourne, Australia	Nutrients	Biomass for bio-oil production	*Landoltia punctata*	[83]
Anaerobically treated domestic wastewater	Florianopolis, Brazil	Nutrients, COD, coliform bacteria	Protein-rich biomass for fish feed	*Lemna valdiviana*	[84]
Anaerobically digested swine wastewater	Raleigh, NC, USA	Nutrients	Protein-rich biomass for laying hens	Unspecified	[85]
Anaerobically digested swine wastewater	Zebulon, NC, USA	Nutrients	Biomass for bio-hydrogen production	*Spirodela polyrhiza*	[86]
Anaerobically treated swine wastewater	Shanghai, China	Nutrients	Biomass	*Spirodela oligorrhiza*[a]	[87]
Domestic sewage and agricultural runoff	Dianchi Lake near Kunming City, China	Nutrients	Protein-rich biomass	*Lemna aequinoctialis*	[81]
Domestic and agricultural wastewater	Dianchi Lake near Kunming City, China	Nutrients	Protein- and starch-rich biomass	*Lemna japonica*	[88]
Untreated swine lagoon wastewater	Leshan City, China	Nutrients	Protein-and starch-rich biomass	Several species	[89]
Sewage water	Quingdao, China	Nutrients, heavy metals	Starch-rich biomass for bio-ethanol production	*Lemna aequinoctialis*	[49]
Untreated and anaerobically digested swine wastewater	Chengdu, China	Nutrients	Biomass for biofuel production	*Spirodela polyrhiza*	[90]

Note: "Nutrients" in the third column refers mainly to nitrogen compounds (mostly NH_4^+) and phosphates, "BOD" and "COD" to biological and chemical oxygen demand, respectively.
[a]Currently accepted name: *Landoltia punctata* [3].

and starch contents were higher in the duckweed, which was considered to have converted wastewater nutrients more efficiently into biomass than did the water hyacinth [88].

Duckweeds growing on wastewater will produce biomass containing microbial organisms associated with growing duckweeds, as well as any pollutants that the macrophytes may have taken up, if these have not been degraded in the process. This especially holds true for incorporated or adsorbed heavy metals resistant to breakdown (see also [1]). As discussed in the following, the presence (or lack) of pathogens and/or toxic substances in the biomass will have consequences for the uses to which the biomass can be put.

If duckweeds are grown on wastewater only for purposes of remediation and/or there is no market for the generated biomass, the plant material must be disposed of in a safe manner. Pathogens can be killed by heating the harvested biomass, and the duckweed tissues may be safely used for landfill or compost if they do not contain significant amounts of toxicants. If they do contain heavy metals and/or toxic organic xenobiotics (which could leach out of landfill deposits), the only really safe option is incineration. Heavy metal removal from the intact biomass itself (e.g., [92]) would not be worthwhile, and incineration would eradicate any organic toxins. Biomass-derived heavy metals could be removed and reclaimed from the incinerator ash by chemical and biochemical leaching techniques (e.g., [93]) if they would compromise the disposal of the ash or if there was a market for them.

6. Uses for duckweed biomass

Duckweed biomass can be used as a foodstuff, for producing several types of biofuel production, for fertilizer and for water and soil amelioration. The biomass of accordingly genetically engineered duckweed can also be the source of recombinant proteins. All this also applies in principle to duckweed biomass derived from wastewater remediation (see Fig. 2), and the prospect of large-scale use of duckweed in water improvement implies potentially significant contributions to issues of nutrition and alternative energy supply in particular. However, the use of biomass from duckweeds grown on wastewater is in many cases dependent on the absence of unhealthy or toxic contaminants taken up or adsorbed from the water.

6.1. Nutrition

Nutrition was the first use envisaged for wastewater-grown duckweed biomass [71], and especially duckweed grown on nutrient-rich media is rich in protein content with an amino acid composition favorable for both animal and human nutrition [73,75,94,95]. Large-scale duckweed cultivation on nutrient-rich wastewater accordingly suggests itself for widespread utilization as animal feed and human food to complement or even partially replace the nutrition feedstocks traditionally supplied by soil-grown crops, especially when protein malnutrition is an issue.

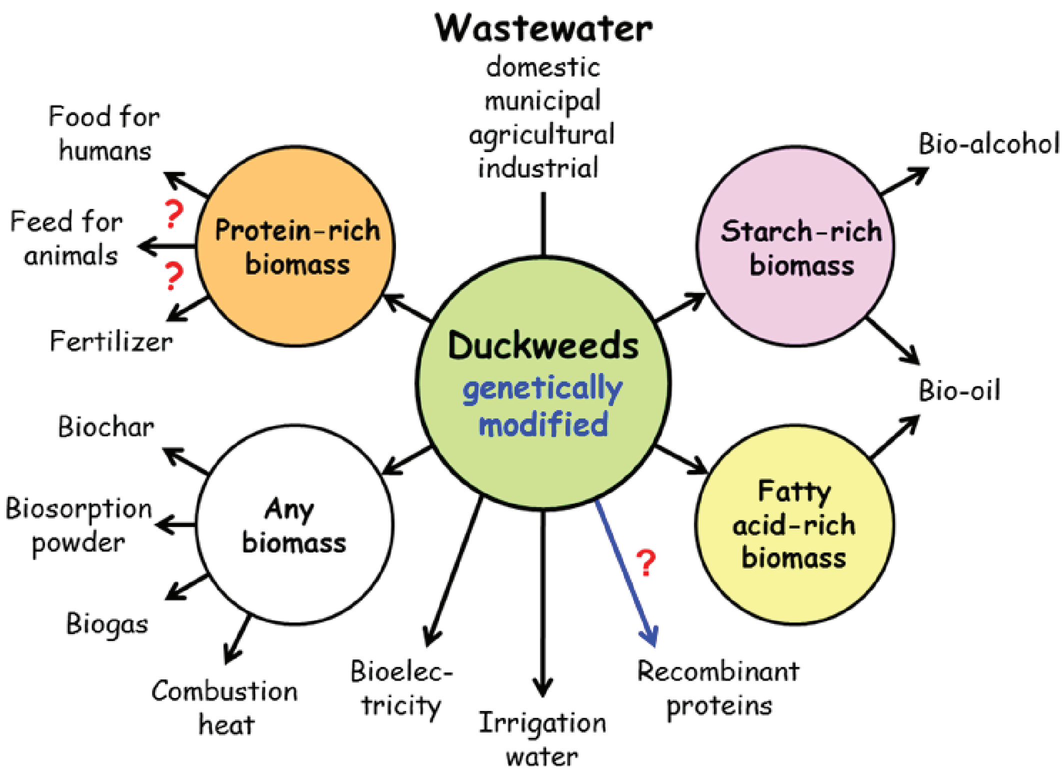

Fig. 2. Uses to which wastewater-grown duckweed biomass can be put.
Note: Successful remediation of the wastewater will result in a non-toxic effluent suitable for irrigation. Bioelectricity and recombinant proteins can be produced through the growth of duckweed. The question marks in red indicate that these uses are only acceptable in the absence of toxic substances and pathogens.

However, heavy metals, phenols, pesticides, dioxins, antibiotics, natural toxins and pathogens can be sequestered in the tissues of duckweeds growing on wastewaters, and duckweeds growing under open-environment conditions are associated with a microflora of bacteria, viruses, fungi, algae and minute invertebrates. In addition, *Spirodela*, *Landoltia* and *Lemna* species contain oxalic acid that impairs palatability [73,95,96]. Wastewater-grown duckweeds can thus pose serious health risks for consumption. Sporadic studies have shown such duckweeds targeted for animal feed to be harmless in terms of microbial content [97,98], but any duckweed biomass seriously being considered for animal feed and especially human consumption would have to be stringently tested for the presence of any health-threatening xenobiotics and pathogens that might have been present (and should be tested for) in the growth medium. This concern with health safety is most likely the reason that commercially available duckweed feed and food products (high-protein and high-fiber concentrates for both animal feed and human consumption from Parabel [99] and whole-plant human food preparations form Hinoman [100] and Green Onyx [101] derive from growth under strictly controlled, hygenic conditions.

6.1.1. Animal feed

Hillman and Culley first envisaged growing duckweed on dairy wastewater for cattle fodder in 1978 [71]. Although a number of monographs published in the 1990s [72–74] amount to handbooks for the cultivation of duckweed on agricultural and municipal wastewaters and the utilization of the duckweed biomass produced for feeding fish, poultry, pigs and ruminants, the use of duckweed as an animal feed supplement has not seriously developed further since then, due to concerns about toxic content and pathogen transfer ([96] and the abundance of cheap grain and soybean supplies and the lack of appropriate cropping systems [85]. Nevertheless, duckweeds growing on irrigation ponds are promising as a food source for broilers in Jordan, where feedstuffs are limited and expensive [91].

Recent studies illustrating benefits of using duckweeds used as a feed supplement may help to revive interest in wastewater-grown duckweed in animal nutrition. *L. minor* supplied in conjunction with lime to aquaculture ponds in Bangladesh resulted not only in a 60% increase in fish production but also in a 12-fold reduction in the euglenophyte content of the water [102]. In addition to enhancing the growth and production of the respective test animals, the inclusion of duckweed in animal feed has led to improved digestion in carp [103], shrimp [104] and lambs [105] and to a higher Omega 3 fatty acid content of hen eggs [85]. Duckweed in dog diets can improve stool consistency [106], and at least one entrepreneur is developing duckweed-based feed products specifically for pets [107].

6.1.2. Human consumption

Wolffia arrhiza has long been cultivated in South East Asia for human consumption [74, 108], and duckweed biomass, with its high content of protein with a favorable amino acid composition, would make duckweed an attractive human nutrition additive (see [74]). *W. arrhiza* has even been envisaged as an edible component of a controlled aquatic ecological life support system for use in a lunar or planetary base [109]! Although many publications tout its promise for relieving local and global nutrition concerns, duckweed has not become a widespread food source. The marketing of even duckweed grown on uncontaminated nutrient waters for food would be a challenge in terms of removing oxalic acid and offsetting the costs regarding cultivation, harvesting, drying, processing, packaging and distribution, and that grown on wastewater would be additionally subject to the health concerns mentioned above. Duckweed biomass stemming from wastewater remediation could thus only be considered fit for human consumption upon attestation of absolute harmlessness following cost-intensive extensive analysis of the water composition and of the attributes of the biomass itself, an unrealistic prospect at present.

6.2. Energy

Duckweed biomass can be used to produce a variety of energy-rich biofuel products: biogas and bio-alcohols, along with bio-oils and biochar. This usage is the topic of two comprehensive recent reviews [110,111]. Whereas biogas is produced by the anaerobic digestion of biomass and bio-alcohols by fermentation of hydrolyzed biomass starch, bio-oils and biochar derive from the hydrothermal liquefaction or pyrolysis of biomass. The health safety concerns due to heavy metal, organic xenobiotic and microorganism contamination of wastewater-grown duckweed biomass under consideration for use in nutrition, fertilization or biomanufacturing are of little relevance for generating energy products. The biomass contaminants will in most cases be destroyed by the processing conditions or sequestered from the formed biofuel products, and would not compromise the final products even if they were to be present.

6.2.1. Biogas

Biogas has long been produced by the anaerobic digestion of organic municipal, agricultural and industrial organic wastes to methane (see [110]), which is an energy-producing fuel through its combustion with oxygen. Duckweed biomass resulting from cultivation on wastewaters or even skimmed off untended ponds and lagoons receiving agricultural runoff can be profitably used in biogas production facilities if there is no other use for it. Studies have shown that duckweed biomass added to poultry, dairy and swine manure significantly improved biogas production in anaerobic digesters (see [110]). The mature technology and relatively low costs involved make biogas production a valuable option for disposing of "superfluous" duckweed.

Biogas generally contains carbon dioxide in addition to methane, diminishing the fuel value of the latter. The charcoal-like biochar that is a product of the pyrolysis of duckweed biomass [112] can, in addition to releasing energy when burned, be used for catalyzing the conversion of biogas to hydrogen- and carbon monoxide-containing syngas useful for synthesizing methanol and synthetic hydrocarbon fuels [113].

The clean and efficient fuel hydrogen gas can be produced from the fermentation of plant biomass. Up to 75 ml H_2 could be produced from 1 g DW of acid-pretreated, swine wastewater-grown *S. polyrhiza* by fermentation with anaerobic sludge microflora for 7 d [86]. The yield of H_2 (42% of the total biogas formed) was comparable with that obtained from the biomass of various important crop plants. H_2 production would be a good use for superfluous duckweed biomass; the H_2 will have to be separated from the rest of the biogas, if it is to be obtained in pure form.

6.2.2. Bioalcohol

The duckweed biomass component most readily suited to producing bioethanol is starch, which is hydrolyzed with amylolytic enzymes and the resultant glucose fermented with yeast to yield the alcohol. The saccharification of cell wall carbohydrates with cellulases and pectinases and fermentation of the released sugars can also contribute to bioalcohol production [110]. Duckweed biomass to be used for ethanol production should accordingly have a high starch content. Although it has long been evident that the starch contents of duckweeds grown on mineral medium vary enormously (ranging from 3% to about 50% of the DW: see [114]), screening studies aimed at identifying duckweed clones exhibiting particularly high starch contents in their biomass have not been systematically carried out. In their review, Cui and Cheng [110] have thoroughly described plant starch metabolism from the viewpoint of optimizing starch content for biofuel production. Since starch accumulation in plant tissues is the result of photosynthetic carbon assimilation taking place in excess of the utilization of starch in dissimilatory processes, the authors point out that starch content of duckweeds can be maximized by either increasing photosynthetic production without increasing dissimilation or inhibiting dissimilation without impairing photosynthetic processes.

Stimulating photosynthesis is dependent on increasing illumination and/or carbon dioxide concentration in the medium. These would be difficult and expensive to implement, especially outdoors [110], and thus appear impractical in terms of cultivating high-starch duckweeds on wastewater. Nevertheless, studies on improving the yield and starch content of duckweed biomass by optimizing growth conditions are being undertaken. A laboratory experiment with *L. aequinoctialis* growing on artificial mineral medium observed the highest biomass production (233 g m^{-2}) and the highest starch yield (99 g m^{-2}) at a light intensity of 110 μmol m^{-2} s^{-1} and a 24-h photoperiod over a period of 39 d [80]. These lighting data could possibly be profitably employed to optimize future industrial large-scale duckweed cultivation.

Manipulating cultivation conditions to inhibit starch degradation is much easier than enhancing photosynthesis. Cui and Cheng [110] have described how nutrient starvation and exposure to cations, abscisic acid and other chemical inhibitors can repress starch breakdown in duckweeds by inhibiting growth, and Sree and Appenroth [115] emphasized that abiotic stresses leading to starch accumulation in *L. minor* must be suppressing growth more effectively than photosynthesis. The expression and activities of the key starch-synthesizing enzymes ADP-glucose pyrophosphorylase (ADPG-PP) and soluble starch synthase are enhanced, and those of the starch-degrading enzyme β-amylase are decreased, in *La. punctata* ("*La.*" denotes "*Landoltia*") upon nitrogen and phosphorus deficiency [116]. High-starch duckweed biomass can readily be produced by growing duckweeds on nutrient-rich medium to accumulate biomass and then transferring them to nutrient-poor water to effect the deposition of photosynthate as starch when growth is inhibited due to the lack of nutrients. This is a realistic strategy for producing starch-rich feedstock for bioethanol production, as municipal and agricultural wastewater support rapid growth, and transferring the harvested duckweeds for a few days to rain, tap or lake/river water would suffice to induce starch accumulation.

Methods to induce high starch accumulation in duckweed without any medium change would be convenient for producing high-starch biofuel feedstock. The presence of heavy metals, which are contaminants in many wastewaters, results in starch accumulation [110, 115], but heavy metals either present in the wastewater or added at the required concentrations would also impair long-term growth and thus overall starch accumulation. Several duckweed clones identified as being relatively tolerant of NaCl in a screen accumulated high amounts of starch upon exposure to the salt at concentrations (of about 100 mM) that only slightly inhibited growth (to at most 10%: [16]). This opens the prospect of growing duckweed on moderately salt-containing wastewater as a simple one-step procedure for producing high-starch biomass [16].

Recent experiments have demonstrated the even simpler possibility of enhancing duckweed tissue starch yield by simultaneously increasing photosynthetic activity and inhibiting starch degradation by the use of a growth retardant. *La. punctata* fronds sprayed with uniconazole had a higher chlorophyll content and photosynthetic activity than did control fronds, and accumulated 10% more DW and almost three times as much starch (48% of DW). This inducible starch accumulation could significantly enhance the value of duckweed biomass as a bioethanol feedstock. Both transcriptomic [117,118] and proteomic analysis [119] have indicated that uniconazole increases abscisic acid and cytokinin levels to promote chlorophyll biosynthesis and starch-synthesizing ADPG-PP activity, in addition to causing a decrease in the gibberellic acid level that may lead to an inactivation of starch-degrading α-amylase.

Many of the studies respective of high-starch biomass duckweed have used artificial mineral salt media for duckweed cultivation; this could lead to doubts as to relevance for growth on actual wastewater. The biomass production and biomass starch content of *L. aequinoctialis* grown on mineral salt culture medium and on sewage water were compared [49]. Although the growth of the duckweed was only half as fast on the sewage water, it was still considerable (4.3 g DW m^{-2} d^{-1}), and the starch content of the sewage water-derived biomass (34% of DW) and the ethanol yield derived from it (0.17 g g^{-1} DW) compared favorably with those of the duckweed grown on the artificial medium. This indicates that biomass, starch and ethanol yields produced in pilot experiments under standardized conditions can indeed indicate performance to be expected on actual wastewater media.

Even though the saccharification of duckweed starch and cellulose and the fermentation of the released sugars to ethanol have been well developed [110], efforts to improve the process continue to be made. *L. minor* biomass pre-treated by steam explosion (10 min at 210°C) and simultaneously saccharified with cellulose and β-glucosidase and fermented with yeast gave good yields of ethanol (70% of the theoretical yield from the tissue glucose) at the relatively high substrate density of 20% (w/v) [120].

Whereas high-starch duckweed biomass is usually thought of in terms of bioethanol production, even more succinate – a precursor to a number of chemicals and food and diet supplements – than ethanol can be produced from hydrolyzed *Wolffia globosa* starch when this is fermented with *Actinobacillus succiogenes* [121].

Duckweeds can also be used to produce biobutanol, which has a higher energy density than bioethanol. This has been achieved with *La. punctata* on the one hand by the acid hydrolysis of the plant material and fermentation of the released sugars with *Clostridium acetobutylicum* (see [110]). It has also been shown that 2-methyl-1-butanol, isobutanol and 3-methyl-1-butanol could be produced efficiently by fermentation of acid hydrolysates of *La. punctata* by bio-engineered *Cornebacterium crenatum* [122]. Butanol has also been formed at a high yield by the enzymatic hydrolysis of the starch and cellulose of *La. punctata* biomass and simultaneous fermentation with *Clostridium acetobutylicum* under control of the pH value [123].

6.2.3. Bio-oil

Oils for biofuel can be produced from duckweed according to two basic techniques [111]. Hydrothermal liquefaction, in which the freshly harvested plant material is heated at high temperatures and pressures in the presence of an inorganic catalyst, demonstrated the formation of predominantly heavy bio-oils (mainly resin and asphalt fractions) from unidentified duckweed collected from constructed wetlands [124]. Alternatively, bio-oils can stem from the pyrolysis of dried biomass at high temperatures in the absence of oxygen, as shown with *L. minor* [112]. Crude bio-oil derived from the hydrothermal liquefaction or pyrolysis of duckweed biomass can be upgraded to more valuable products in subcritical water by heating with gas and inorganic catalysts. Biocrude produced from *L. minor* underwent deoxygenation, desulfurization and denitrogenation when treated in this manner to form upgraded oils possessing similar properties to petroleum diesel [125]. Ongoing efforts to upscale the hydrothermal biomass liquefaction process [126] should give impetus to the utilization of duckweed biomass for bio-oil production.

Another source of bio-oil for fuel is the fatty acids present in free form in plant tissues or as triacylglycerides present in large amounts in the oil-bearing seeds of plants such as rape, soybean and peanut [127]. These fatty acids can be re-esterified with methanol to yield biodiesel. Duckweed fronds can have a total fatty acid content up to 15% of the tissue DW, but have – as do all plant leaves – a much lower triacylglyceride content (0.02%–0.15% of the tissue DW [128]) than do the oilseeds (up to over 50% by DW). However, tobacco leaves have been recently genetically engineered to accumulate more than 15% triacylglyceride by DW by expressing three genes involved in triacylglyceride production in *Arabidopsis thaliana* and sesame [129]. Given the success of transformation with duckweeds (see Section 3), similarly engineered duckweeds could become major producers of triacylglycerides for biofuel production.

6.3. Water and soil amendment

6.3.1. Fertilizer

Although duckweeds have a long history as an animal foodstuff, there are few references to it being used as a plant fertilizer. This is surprising, since duckweed biomass could improve the nutritive value and the texture of soil, and its use as fertilizer or compost would also be a productive means of disposing of superfluous duckweed. *L. minor* used as a complement to fertilizer nitrogen improved the growth and yield of rice plants and increased the nutrient contents of both the plant biomass and the soil in Bangladesh [130], and duckweed has been referred to as a fertilizer in Angola, China and Mexico [73]. More recently, fertilization with duckweeds in a consortium of lake weeds in Michigan significantly increased plant-available soil moisture, enhanced sod establishment and provided higher turf density, in addition to increasing turfgrass yield and quality [131]. However, plant-available N can be released excessively upon water weed application overload, and the presence of unwanted material ("trash") in the plant material may also discourage the use of this type of biomass as fertilizer [131]. Indeed, duckweed biomass contaminated with heavy metals and/or organic xenobiotics would not be suitable for use as fertilizer.

6.3.2. Biosorption

Not only live duckweeds but also non-living duckweed biomass can be used to remove heavy metals from solution on account of its adsorptive properties. Untreated dried *L. aequinoctialis* powder adsorbed water-soluble cadmium well [132], and finely divided dried *L. minor* was a good adsorbent for arsenic [133]. Dried, powdered *L. gibba* adsorbed the insecticide methyl parathion in addition to cadmium from nutrient solution spiked with these substances [134]. These findings indicate that dried duckweed can be a cost-effective biosorbent for the removal of toxins from polluted waters in place of the more expensive activated charcoal or other chemical sorbents traditionally used for this purpose. Heavy metals and/or organic xenobiotics having been taken up from wastewaters by duckweeds would not compromise the biosorptive action of the biomass, as the (adsorbed) contaminants would remain bound to the plant material.

6.3.3. Soil improvement

The biochar, or charcoal, co-product of the pyrolysis of plant biomass (see Section 6.2) can be used to improve the quality of soils. The application of charcoal can improve bioavailable water, build organic matter, enhance nutrient cycling and reduce leaching in soil, as well as sequestering carbon removed from the atmosphere [135]. The adsorptive properties of biochar make this substance also promising for the remediation of heavy metal-polluted soils (especially in conjunction with bioremediation [136]) and waters. Although large-scale biochar production has been envisaged only for harvestable forest and crop biomass [135], it could easily be complemented by the addition of otherwise superfluous duckweed biomass. Duckweed harvested from wastewater containing heavy metals or other toxic xenobiotics would pose no problem in this regard, as organic contaminants would be destroyed in the pyrolysis process, and any metal residues remaining in the biochar would be firmly adsorbed and not released into the environment.

6.4. Use in medicine

Duckweeds find employment as medicinal plants for treating a variety of illness, presumably due to the variety of biologically active compounds having been found in *L. minor* [137]. Duckweed biomass grown on wastewater containing heavy metals, (potentially) toxic organic xenobiotic contaminants, microflora or pathogens would certainly not be acceptable for this usage. The pectin polysaccharide lemnan from *L. minor* has been shown to be useful as an adjuvant for oral murine immunization [138] and as a cryoprotectant of human white blood cells, helping to preserve leucocyte membrane integrity and neutrophil phagocytic activity [139]. Duckweed grown on wastewater could conceivably serve as a feedstock for obtaining lemnan, as long as the procedure for purifying the polysaccharide is sufficiently selective.

6.5. Biomanufacturing

The stable transformation of some *Lemna* and *Spirodela* species has shown the potential of duckweeds for expressing recombinant proteins, polymers and small molecules [66,140]. Human monoclonal antibodies [141], a bacterial endoglucanase [142] and avian influenza antigens [37,143,144] have all been successfully expressed in *L. minor*, and the bovine serum inhibitor aprotinin in *Spirodela oligorrhiza* (correct modern name: *La. punctata*; see [3]) [145]. The recent stable transformation of *W. arrhiza* [146] shows the promise of this duckweed species for biomanufacturing as well.

Recombinant proteins are quintessential examples of valuable products that can be obtained from duckweeds via genetics programs and genetic engineering, and Stomp [66] has emphasized the suitability of duckweeds for biomanufacturing and analyzed the technological and economic problems of production, processing and safety and regulation relevant to the commercialization of recombinant duckweed proteins. In principle, recombinant proteins could be produced by and isolated from genetically engineered duckweed growing on wastewater. As with duckweed biomass targeted for human consumption, however, recombinant therapeutic proteins produced in this manner are not likely to be acceptable for health and safety reasons; marketable proteins would have to come from duckweeds grown under controlled, aseptic conditions. On the other hand, recombinant proteins such as industrial enzymes isolated from duckweeds grown on wastewater free of heavy metals and toxic organic xenobiotics could be commercially viable, as long as protein degradation stemming from duckweed-associated microorganisms could be circumvented. However, the obstacles encountered in serious efforts to establish a duckweed biotechnology firm producing recombinant therapeutic proteins [147] illustrate why it is so difficult to conduct any viable duckweed biomanufacturing at present.

7. Conclusions and outlook

The value of duckweeds for science, agriculture and industry is apparent from the various uses to which these small aquatic plants can be put, including acting as model organisms for investigating plant physiology and as test organisms in toxicology and medicine, partaking in wastewater remediation and producing protein- and starch-rich biomass for use in nutrition, biofuel production and soil and water amelioration, as well as acting as vehicles for biomanufacturing and bioelectricity production. It is intriguing that all of the uses which apply to duckweeds have an actual or potential relationship with the widespread environmental concern of water pollution. Duckweeds as investigative model organisms contribute heavily to elucidating the physiological effects of wastewater contaminants, and duckweeds as test organisms are important for the evaluation of the ecotoxicity of such contaminants. The generation of plant biomass for nutrition, fertilization, biosorption and as a feedstock for the production of biofuel and related products such as biochar can be a major asset of the remediative action of duckweeds on wastewater, and even recombinant protein expression and the generation of electricity can be effected by duckweeds growing on wastewater.

The (potential) usefulness of duckweeds in water toxicity testing will certainly increase with the genomic information and bioengineering techniques now available for these organisms, and the well-established associated role of duckweeds in environmental risk assessment can also only profit from these developments. However, the numerous investigations demonstrating the value of duckweeds for wastewater remediation have been mainly confined to laboratory investigations or pilot projects, and wastewater treatment by duckweeds has not been widely implemented and generally not been developed on a scale warranted by large volumes of wastewater or other contaminated surface waters in need of cleanup. It is to be hoped that the experimental results having been obtained and the experimental setups having been described will generate more interest in the potential of duckweeds for wastewater remediation than has been the case up to now, and that increased efforts will be devoted to confronting the technological and economic challenges posed by setting up duckweed water treatment systems. Although wastewater remediation systems established in larger communities will continue to rely on established technology, it should be possible to generate more interest in employing duckweed for final water cleanup in domestic, agricultural and industrial settings in which sophisticated wastewater treatment is not practicable.

It might be expected that awareness of the nutrient reclamation represented by the large amounts of biomass that can be produced by duckweeds growing on nutrient-rich wastewater and the numerous reports of the uses to which this biomass can be put would be an impetus for increasing the use of duckweeds for wastewater remediation. However, the prospect of using duckweeds for human consumption, which is actually attractive in terms of a high-value protein diet, is unlikely to be realized due to health and safety reasons when the plants are grown on wastewater. Health concerns and the degradative actions of wastewater contaminants on proteins similarly discourage the potential wastewater cultivation of genetically engineered duckweeds for recombinant protein expression. On the other hand, duckweeds grown on agricultural wastewaters that are rich in nutrients but contain negligible amounts of toxic xenobiotics indeed suggest themselves for large-scale animal feed and fertilizer usage. The multifaceted possibilities for exploiting duckweed biomass for biofuel and biochar production should not be compromised by the presence of contaminants present in wastewater which the duckweeds might be growing on, as these would not contaminate the processed fuel products themselves, and the generation of bioelectricity by duckweeds would be possible on any wastewater on which the plants would grow. Even if there is no other use for duckweed biomass, it can always produce heat by incineration. The technologies required for growing, harvesting and processing of duckweeds are all available, and especially process facilities for converting biomass to biofuel are ample due to the already established use of non-duckweed plant material. The at first glance so promising use of duckweeds for wastewater remediation and the exploitation of wastewater-derived duckweed biomass will be determined by the development and operational costs for growing, harvesting and processing the duckweeds and for marketing their products. It is to be hoped that financing can be obtained to meet these costs and thus help realize the potential of duckweeds for preserving the environment and alleviating food and energy shortages.

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