DUCKWEED AND ITS BROAD-SPECTRUM APPLICATIONS

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Abstract:

World researchers being in constant search of novel and innovative alternatives to combat the changing dynamics of the earth agrees upon the utilization of small, floating, aquatic plants – duckweed, for solving most of the anthropogenic conditions and reverting nature to a healthy form. The global distribution of these aquatic plants and their high rate of reproduction makes them an ideal candidate for several scientific explorations. This morphologically simplest plant delivers a wide- range of applications and utilities, which becomes imperative for not only safeguarding the planet but also for providing a nutritive source for plants and animals. The environmental degradation due to the irrational release of wastewater from different sources and the heavy metal contamination of the ecosystem could be effectively neutralized by these floating plants. The energy and power crisis, occurring globally, makes duckweed far more important as they could be intensively utilized for production of biofuels, which is not only an economically feasible approach but also an eco-friendly, green, and practical alternative to pollution-causing power stations. The composting of duckweed enhances the fertility of the soil and improves the yield of the crops. The duckweed biomass, after processing, makes its significant place among the producers of bioactive compounds. The high protein and amino acid profile of the duckweed make them a promising candidate for their utilization as animal and human food. The duckweed food for human consumption has paved the way to alleviate several symptomatic and chronic diseases in humans. This paper discusses the broad-spectrum applications of duckweed and its role in human health.

Keywords:* *Duckweed, Phytoremediation, Animal feed, Human food, Antioxidants, Wastewater treatment.

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1 Introduction:

"Duckweed" holds an important spot among the vast category of aquatic floating plants, mostly observed as growing in thick, blanket-like mats over stagnant, nutrient-rich waters (which could either be freshwater or brackish water). These mats comprise a heterogenous mixture of varied genera and species, which could reach up to the thickness of 6 cm, creating anaerobic conditions in the water bodies, and encouraging denitrification and anaerobic digestion [1]. However, the deficiency of oxygen adversely impacts aquatic communities. Duckweed illustrates varying sizes ranging from less than 1mm to 20 mm.

They comprise five significant genera (*Spirodela, Landoltia, Lemna, Wolffiella,* and *Wolffia*), encompassing 36 species within the monocot order of Alismatales. [2]. All species of duckweed occasionally produce minuscule, almost invisible flowers, and seeds [3], however, the mechanism that triggers the flowering remains unknown.

Duckweed adapts readily and effectively to different environmental and climatic conditions, which makes their distribution worldwide and in extensive form. Except for deserts and enduringly frozen polar regions, duckweed makes their presence in tropical and temperate regions globally.

Duckweed has captured more attention worldwide, predominantly, due to its faster-growing ability, which occurs to be two times faster than any other vascular plant [1]. Under the optimum conditions, they could double their body mass in less than 48 hours [4]. The growth of the duckweed relies upon nutrient availability as well as density-dependent factors, such as water temperature, air temperature, humidity, carbon dioxide levels in the optimum atmosphere, and pH levels. Their optimum temperature lies between 20°C - 30°C, while below 17°C, some duckweed illustrates a reduction in their growth [5]. Furthermore, the effect of temperature on the growth occurs to be largely impacted by the light intensity, i.e., as the light increases the growth rate increases from 10°C to 30 °C. Their optimum pH lies in the range of 5.0 –7.0. Additionally, duckweed could successfully be cultivated in aquaponics, man-made ponds, greenhouses, and even in fish tanks, where all the growth conditions remain under control to efficiently produce these tiny aquatic plants.

They efficiently utilize (absorb), the water contaminants such as nitrogen, phosphorus, heavy metals, and carbon dioxide from the air for their growth and metabolism, making them a potent candidate for treating the contaminated water via different sources.

Out of the several advantages posed by the duckweed, the major ones include the following:

- Duckweed constitutes an ideal candidate for carrying out several experiments in the field of ecology, physiology, and toxicology, due to their ease of maintenance, rapid reproduction rate, and abundance [6].
- Suitable for the production of biofuel due to the rapid biomass production [7].
- Improves soil fertility when utilized as compost.
- Appropriate test organisms for ecotoxicological testing in the surface waters and waste waters due to their short lifespan, rapid vegetative propagation, and hyperaccumulation of heavy metals [8].
- Plays a vital role in phytoremediation, due to their unmatched capabilities of absorbing excess metals and minerals from the water bodies [9].
- Utilized as the protein source for animal and human consumption.

2 Morphological Characteristics

Duckweed (*Lemnoideae*) occur to be the smallest and the fastest growing, morphologically simplest flowering plants. The plant body remains as an organized structure referred to as a " thalloid," or "frond" [10]. An individual duckweed frond lacks a leaf, stem, or other specialized structure. The entire plant comprises flat and ovoid fronds [3] as depicted in figure 1. However, several species of duckweed may possess hair-like rootlets which provide structural stability to the plant. Different species of duckweed illustrate varying sizes of fronds. For example, species of the genus *Spirodela* consist of the largest fronds of around 20 mm across while those in *Wolffia* are observed to be 2mm or less in diameter [3]. Almost all the tissues of the duckweed could be potentially utilized as feed or food products and remain metabolically active. This forms a crucial characteristic of duckweed in contrast to other terrestrial crops, where a large amount of biomass remains unutilized after harvesting their useful parts.

3 Distribution of Duckweed Globally

As mentioned earlier, duckweed inhabits tropical and temperate waters to a larger extent. They diversely occur in almost all climatic zones, except the dry and frozen areas. Duckweed occurs in abundance in various parts of Europe, North America, Asia, and Africa. Asia Pacific forms the dominant region in terms of production as well as utilization of duckweed for human and animal consumption. The utilization of duckweed has likewise increased substantially in Europe and America due to the increasing growth of wastewater treatment. Owing to its several applications (as discussed in the following sections), the duckweed market shall open the doors to different opportunities for development over the tenure of analysis, from 2020 to 2030.

Figure 1. Duckweed, the floating aquatic plants.

4 Types of Duckweed

The five major genera of duckweed are *Spirodela, Landoltia, Lemna, Wolffiella*, and *Wolffia*. *Spirodela* illustrates great tolerance against cold winters as compared to other genera through its dormant structure of turions in place of seeds. The presence of abundant starch within turions allows them to sink and escape the freezing surface waters [11]. The distinguishing features of *Landoltia* include reduced frond prophyllum, frond nerves (3 to 7), roots (up to 7), and root tracheids [12]. *Lemna* thalli comprise single roots as compared to multiple roots in *Spirodela* and *Landoltia* and no roots in *Wolffia* and *Wolffiella.* The nutritional profile of *Wolffia* remains most exceptional due to the absence of toxic substances such as oxalic acid [13]. As compared to other duckweed *Wolffiella* has low flowering frequency [14].

5 Duckweed as Animal Feed

Duckweed all over the world forms an appropriate animal feed due to its high nutritional value. Fresh duckweed fronds are estimated to contain around 92% to 94% of water [3], 20% to 35% of proteins, 4% to 7% fats, and starch from 4% to 10% per dry weight [15]. The estimated amino acid of *Lemna minor* accounts for 39.20% essential, 53.64% non-essential, and 7.13% of non-proteinogenic amino acids [16]. Fiber and ash content occurs to be higher in the duckweed colonies with slow growth. Likewise, cultures of duckweed contain a high concentration of trace minerals and pigments, predominantly beta carotene and xanthophyll, making the duckweed meal a valuable supplement for poultry, aquafeed, and other animal feed [3]. Duckweed consists of versatile carbohydrates such as starch, trace hemicellulose, and pectin. Additionally, they also form a rich source of minerals, vitamins, and phytochemicals. These plants contain high levels of necessary fatty acids such as (SCFA) such as C2 (11%), C3 (3.1%), C4 (1.4%), and C5 (0.4%) with a total short-chain fatty acid (SCFA) of 16.6% [17]. SCFA plays a vital role in controlling pathogens such as *Salmonella enteritidis* when utilized as the feed additive in poultry.

Duckweed under natural conditions creates a massive food source for ducks, swans, geese, pigs, sheep, cattle, and poultry [18]. Duckweed has been largely supplemented to animal feed for preventing incomplete nutrition obtained from traditional feeds and promoting animal growth, for example, research has been conducted to partially replace fish meal, soy meal, alfalfa leaf meal, and others with duckweeds in animal feed, providing fairly feasible results [19]. The species of *L. minor* and *S. polyrhiza* have been utilized as feed for fish, poultry, pigs, and others.

Several studies indicate the suitability of duckweed in feeding different species of livestock [20] [21], which also proves to be an economic alternative, to other costlier proteins utilized for animal feed. The nutritional value of duckweed as poultry feed has been established as early as 1954. In one of the feeding trials, the chicken diets containing 10% of duckweed, resulted in the same performance as chicken offered with the same amount of alfalfa meal-containing diet [22]. Dried *L. minor* could be used in broiler ration at 100 g/Kg diet, without affecting the weight gain of chickens and feeding efficiency [19]. The pigmentation in chicken occurs to increase when fed with duckweed. One of the studies concludes that the partial replacement of sesame oil cake with duckweed could increase the growth performance of broiler birds [23]. The high levels of carotene content of the duckweed have been illustrated to deepen the yellow colour of the broiler meat and skin. Some of the species of duckweed stimulate the birds to eat more due to their high palatability. However, it is important to note that though duckweed induces weight gain in the broiler chicken when replaced with around 6% of protein, the growth of the young broiler chickens may be retarded by increasing the levels of dehydrated duckweed in the meals [24]. This might be attributed to the incapability of the young chick to feed on the duckweed due to the bulkiness and low DM content [21]. This infers that the utilization of duckweed for young broiler chickens must be sparse.

Duckweeds have likewise been utilized for feeding the pigs. The pigs lack the natural ability to produce amino acids such as lysine and methionine, which could be readily made available by feeding the pigs with duckweed meal making it more efficient and economic as compared to other protein-based diets. Similarly, the presence of high content of minerals and vitamin A makes duckweed plants appropriate ingredients for pig feeds. One of the studies proves that Mong Cai piglets (indigenous pigs of Vietnam) could grow faster as compared to the Large White piglets, which could be attributed to a higher intake of protein by the Mong Cai piglets, implying a higher intake of duckweed plants in their diets [25]. The study also indicated the link between nutrition and genotype. The results of the studies concluded that apart from the factors of palatability and acceptability of feed by the piglets, their intake of duckweed, and thus their growth and development was influenced by their genetic make. Therefore, the levels of inclusion of duckweed in the pig diet also depend on determining their genotype, for desirable growth and development of piglets to the porker stage. Similarly, the study reported that piglets reflected a higher observable digestibility of N occurring in the diet when the duckweed was the major protein source as compared with cassava leaves owing to the difference in the cell wall content of both the plants, considering the small floating plants to have much less structural support [26] and thereby, cell wall content.

The duckweed plants have not only been used for the piglets but have been likewise utilized for the mature pigs. The diet of ensiled cassava root with 25% of the protein provided by the fresh duckweed was observed to totally replace the utilization of the rice by-products and protein meals in the diets of fattening pigs with no observable reduction in the growth rate [27]. This forms a vital factor for mitigating the effects of pigs competing with humans for the grain crops such as rice and maize. Additionally, the fresh duckweed plants are observed to replace all the soybean for Mong Cai pigs fed on ensiled cassava. roots or sugar cane, leading to improved performance. The improved performance of pigs fed with duckweed accounts for the high ideal digestibility of the organic matter, crude protein, and ether extract of the plant. The utilization of duckweed appears all the more beneficial in developing countries, as these plants possess higher biological value as compared to the meals prepare from the cassava leaves. However, some more research needs to be conducted for the utilization of duckweed in different classes of pigs such as board and pregnant sows.

Similarly, duckweed could be effectively utilized for feeding the ducks. Fresh duckweed could replace protein supplements in the diets of laying ducks without affecting their reproductive performance. However, it largely depends upon the duck breed. For example, in one of the studies, the replacement of soya bean and fishmeal by duckweeds in the diet of Cherry Valley laying ducks significantly reduced the laying rate and proportion of the fertile eggs, but the same amount of diets fed to the local breeding ducks increased the rates of laying and hatchability [28].

Duckweeds also hold a prime spot in their utilization as fish feed. The fish supplemented with duckweed in a polyculture system occurs to have a significantly high body weight of 20% [29]. Utilization of the duckweed (*Lemna minor*) powder in the polyculture resulted in higher production of fish such as rui (*Labeo rohita*), mrigal (*Cirrhinus cirrhosus*), and silver carp (*Hypophthalmicthys molitrix*) [30]. The utilization of the duckweed powder could prove quite beneficial while considering the economic aspect of polyculture. The adoption of this technique could greatly reduce the production cost of the fish culture.

The uncomplicatedness of the establishment of duckweed, its excellent growth habit, minimal carbon footprint, lower competition of land use with the food crops, and its nutritive value, make them a promising candidate for animal feed.

6 Duckweed as A Dietary Staple for Humans

Owing to the ever-increasing food insecurity globally and the deteriorating quality of crop production, the role of duckweed in the human diet, which uses no arable land for its cultivation, becomes quite crucial. The capability of producing rapidly and possessing high protein content makes the researchers keen for exploring the possibilities of utilizing duckweed for human consumption on large scale.

Currently, the major portion of the proteins in human diets get derived from animals, dairy, and plants, accounting for 46%, 16%, and 30% respectively [31]. The production of animal-derived proteins has several proven economic, health, and environmental disadvantages. Therefore, the obvious alternative to animal-derived seems to be plant protein. Promoting plant protein over animal proteins leads to a reduction in ecological disruption and several health issues. Therefore, to facilitate this, further research to bring duckweed into the mainstream of society must be conducted.

The consumption of duckweed as a source of protein has been already adopted in some parts of Southeast Asia, including Laos, Thailand, and Myanmar as a vegetable named 'Khai-Nam', where *Wolffia arrhiza* and *Wolffia globosa* are the predominating species used for human consumption [32]. The plant is eaten as chips, bread, and in soups or stir-fries in these regions. However, the lower acceptability of duckweed as a diet in other parts of the world may be due to the presence of oxalic acids in some species, causing a negative taste and difficulty in separating the pathogenic organisms such as worms, snails, protozoan, and bacteria from the plants [33]. However, duckweed occurs to be promising for human consumption globally.

One of the studies conducted on duckweed acceptability for human consumption in the Netherlands concluded that the provision of information about the nutritional and environmental benefits of duckweed led to a positive effect on duckweed acceptability as human food (as a fitting meal) in the Netherlands [34]. Certain duckweed species such as *L. minor, L. gibba, S. polyrhiza, L. punctata, Wo. globosa, Lemna sp.* forms a rich source of vital macro-nutrients, including proteins, polyunsaturated lipids, fibers, micronutrients, and several other bioactive compounds. Additionally, possessing a well-balanced amino acid profile, makes these plants a great candidate for formulating food for human consumption.

A partial substitution of wheat flour with duckweed flour could greatly enhance the nutritional profile of conventional cereal foods along with several health benefits such as the prevention of chronic diseases, owing to a better amino acid profile and higher content of vitamins, minerals, and antioxidants compared to wheat [18]. A protein concentrate could be effectively developed from the duckweed whole meal and utilized for enhancing the nutritional profile and food texture while producing several kinds of foods.

Duckweeds could very likely become a novel source of plant protein and shall eventually eliminate the issue of excessive arable land use for growing food crops. However, it must be noted that the extraction of proteins from duckweed could be more difficult than from any cereal or pulses, as predicted for any leaf proteins. Several economically feasible technologies should be developed and implemented for the extraction of duckweed proteins having desirable protein yield and purity.

Several bioactive compounds such as carotenoids, phytosterols, and other pigments synthesized by duckweed possess high antioxidant and anti-inflammatory properties, which have been widely used in several foods and nutraceuticals to protect from cancer, coronary heart disease, cataract, and free radicals [18]. The carotenoids and vitamin E (α-tocopherol) play a noteworthy role in opposing chronic inflammation. Likewise, zeaxanthin, lutein, and β-carotene can detoxify, reactive oxygen species (ROS) [35]. Carotenoids regulate the gene that functions in immune response or the balance of energy control. Some of the carotenoids including zeaxanthin and additional dietary nutraceuticals directly oppose obesity. The high content of lutein and zeaxanthin in these plants helps the prevention of age-related macular degeneration [36]. Additionally, *L. gibba* containing carotenoid [37], could be possibly used as a food colorant in dressing ice cream, pudding, chewing gums, and dairy products, owing to its taste and nutritional benefits. Certain duckweed species such as *L. minor, L. gibba, S. polyrhiza, L. punctata, Wo. globosa, Lemna sp*, may be utilized as a good option of space food for long-duration of space exploration. The high content of tocopherol adds up to the beneficial impact of the plant material.

Duckweeds having higher fiber content may also aid the regulation of blood sugar, which helps the energy balance levels and lowers insulin response, which in turn maintains the healthy body weight. Equally the high content of antioxidant in the duck weeds promotes healthy gut bacteria.

Though duckweed has great potential of being utilized as a nutritive and healthy human food and as well as a food ingredient, there may still be safety concerns associated with duckweed cultivation and harvesting, due to the accumulation of heavy metals, pathogens, toxicants, and pesticides in the duckweed. A report by FAO (1999), however, states the heavy of the heavy metals such as Cd, Cr, and others in the duckweed don't threaten human or animal health, but this largely depends upon the quality of the water, where duckweed plants are grown/cultivated [38]. Thereby, the safety concerns associated with heavy metals require thorough and continuous monitoring and risk assessment, and further evaluation.

Thus, duckweed could be a promising candidate for alleviating food insecurity globally, majorly in under-developing and developing countries, its potential must be explored further in much more depth, to replace the food crops and incorporate it into the mainstream diet. However, the techno-functional properties of the duckweed and protein extract still call for an exhaustive investigation, with the development of tasty and innovative food products from duckweeds for consumers.

7 Duckweeds and Wastewater Treatment

Duckweed species have illustrated an interesting potentiality of not only treating the wastewater but also being effective against nutrient recovery from the wastewater. Wastewater treatment by the utilization of the duckweed has several advantages over other aquatic macrophytes, which include a high rate of nutrient uptake, high tolerance against high wastewater nutrient levels, rapid vegetative propagation, and higher accumulation of biomass. A few more advantages of utilizing duckweed-based waste are depicted in figure 2. The duckweed wastewater treatment system has been studied widely for all kinds of wastewater. Most of the studies focus on nutrient removal efficiencies and removal rates [39]. The BOD_5 and COD removal rate has accounted for 50-95% of the duckweed-covered systems [40].

The duckweed wastewater treatment has been studied from dairy waste lagoons, raw diluted domestic sewage, secondary effluent, septage-loaded ponds, and fish culture. [39]. Several countries of the world such as Taiwan, China, Bangladesh, Belgium, and the USA already have a functional full-scale system of wastewater treatment with duckweeds. One of the studies concludes that the degradation of organic in a matter of BOD, as well as COD, occurs rapidly in the duckweed-covered wastewater treatment systems as compared to the uncovered systems [39]. The higher removal of the organic matter could be attributed to the higher surface area provided by the duckweed to the bacteria to carry on the process of decomposition of the organic matter present in the wastewater [39].

One of the lab experiments conducted in a shallow, duckweed-covered semi-continuous batch system infers that duckweeds could possibly treat the wastewater containing very high total ammonia concentration till the pH levels don't exceed, 7.8. Thus, wastewater treatment using fat duckweed becomes almost impossible at a pH level approximately above 9.8. Thus, the efficiency of the wastewater treatment by the utilization of the duckweed could be possible by lowering the pH which could be effectively attained by the employment of anaerobic pre-treatment by the means of anaerobic ponds or sophisticated anaerobic reactors. Furthermore, the degradation of the organic matter also gets enhanced through both the additional oxygen supply and the additional surface area for bacterial growth [42]. The duckweed pond develops a thick floating mat of duckweed (*Lemna*), that uniformly covers the surface of the water and thereby, prevents the growth of algal blooms and water evaporation. This helps in maintaining the clear suspended solids effluent, pH, and dissolved oxygen condition, which in turn preserves the bacterial growth important for the organic degradation of the pollutants [41]. Over the course of, the excess duckweed could be effectively and readily harvested and with further processing utilized as different biological-active ingredients.

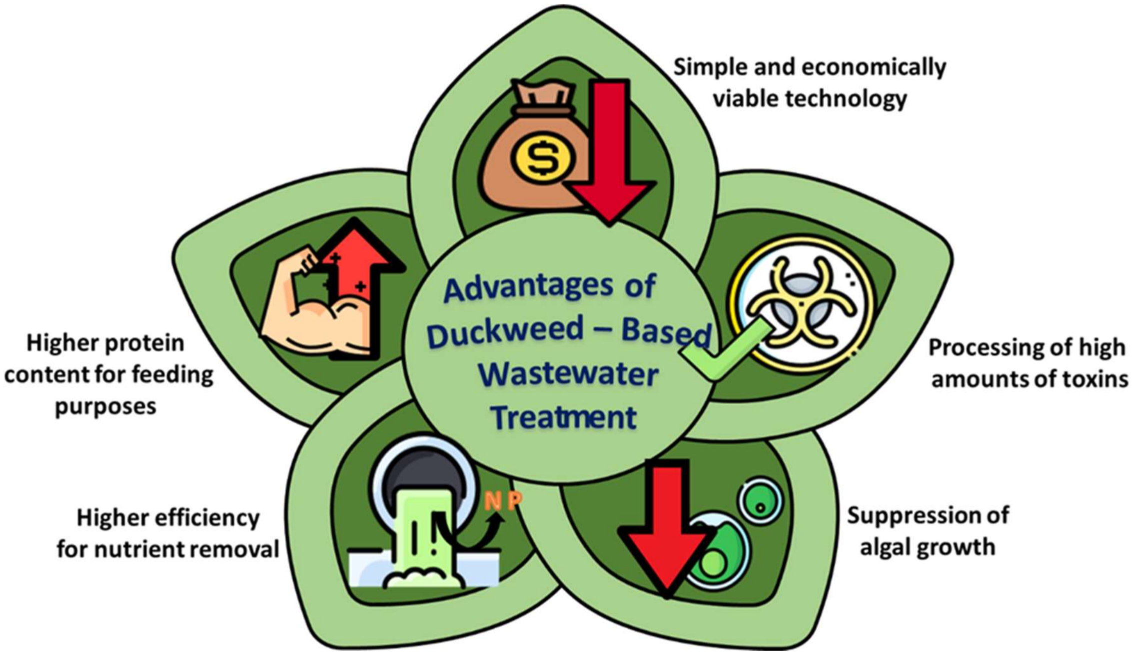

Figure 2. Advantages of Duckweed-based *wastewater treatment (illustration by author after [41].*

The role of duckweeds in the removal of organic matter from the wastewater still remains a topic of further research, as the capability of duckweed to remove the organic matter remains a little low then the water hyacinth, as the duckweed lacks the extensive root system that occurs to be the major determinant in providing the surface for the bacteria growth. However, fat duckweed was found to significantly enhance COD removal in the shallow batch systems. The bacteria present in the duckweed system may play a significant role in the degradation of the organic compounds occurring in the wastewater. The duckweed ponds generally serve as a secondary or tertiary treatment stage in the wastewater treatment system, where pretreatment is often recommended to allow the sedimentation of the suspended solids.

One of the experiments for treating the wastewater with duckweed [43] involved the following steps as indicated in figure 3.

- Introduction of the wastewater collected from the community into the storage tank, to mix the wastewater and remove the floating matter.
- Directing the wastewater to the cylindrical tank.
- The presence of a small cylindrical perforated plate in the center of the tank connected to the hopper, lying down of the sedimentation tank and utilized for sludge processing.

- Introduction of the wastewater from the sedimentation tank to the duckweed tank covered with *Lemna gibba*, with a hydraulic retention time of 3 days.
- Sufficient harvesting of the duckweed from the duckweed tank, at a suitable rate, to remove enough biomass, without leaving any area exposed.
- Air drying/ oven drying of the harvested biomass with further processing, to obtain several valuable by-products.

The major portion of the TSS gets eliminated under the sedimentation tank. A minor fraction gets absorbed on the roots of the fronds, where microorganisms degrade a sufficient amount of the solids, and the remaining portion gets accumulated in the plants. Likewise, BOD gets aerobically digested by microorganisms attached to the duckweed fronds. Apart from the plant uptake, denitrification and volatilization of ammonia occur to be the most relevant processes for nitrogen removal in the duckweed systems. In the same line, phosphorus removal is associated with assimilation by plants as well as sedimentation in the duckweed system for treating wastewater. The removal efficiency of 34% to 99% for N% and 14 to 99% for P were reported for systems using fat duckweeds [42].

The removal of the pathogens largely occurs through the natural die-off mechanism owing to the long detention time. The lipophilic toxins get largely accumulated in the lipids of the cell membrane from where they get extracted to the interior side of the duckweed cell. Practically, the pond depth, the organic surface loading rate, sewage temperature, and hydraulic retention times appear to be inevitable while designing the duckweed ponds for wastewater treatment [42]. The indirect contribution of the duckweed to nutrient removal occurs due to its association with attached algae and bacteria biofilm, which promotes the process of nitrification–denitrification accounting for around 35 to 46% and 31 to 71% of the total N and P loss, respectively [42].

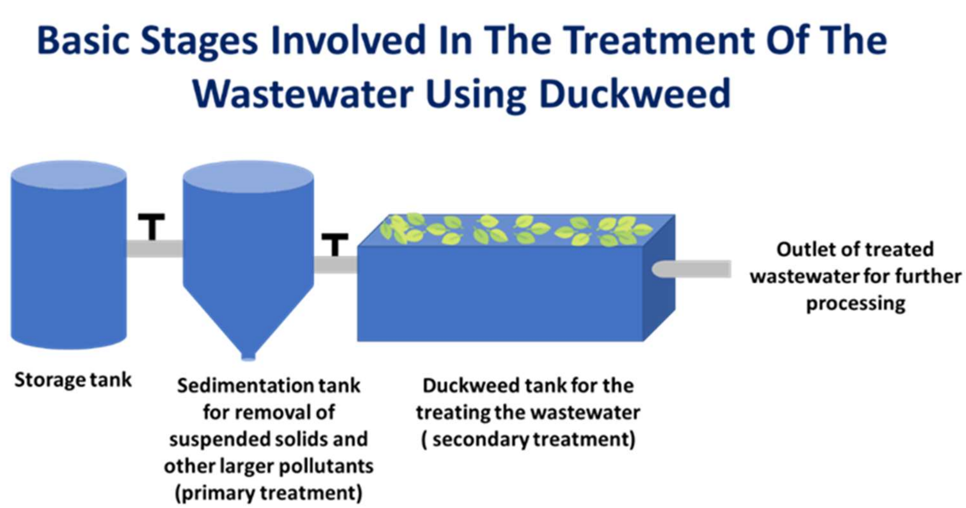

Figure 3. Schematic diagram illustrating the stages involved in the treatment of the wastewater by the utilization of the duckweed(designed by author after [43].

Some of the crucial factors to be considered while planning for the installation of the duckweed system for wastewater treatment involve the following:

- Installation of the duckweed wastewater treatment system near the natural habitat of duckweed, for making the system suitable for the rural areas and communities in the vicinity of duckweed ponds.
- Utilization of techniques to remove the pathogens from the duckweed prior to its further utilization, especially when the duckweed is cultivated on industrial wastewater.
- Investigation of possible health hazards associated with the wastewater-grown duckweed on poultry and other animals, when used as feed.
- Establishing the capability of duckweed to remove heavy metals from industrial wastewater.
- Establishing a well-studied comparison between the effectiveness of wastewater treatment by duckweed and algae in order to establish a better biological system to treat the wastewater.

Source: M. A. Al- Hashimi and R. A. Joda, "Treatment of Domestic Wastewater Using Duckweed Plant," Journal of King Saud University - Engineering Sciences, vol. 22, no. 1, pp. 11–18, Jan. 2010, doi: 10.1016/S1018-3639(18)30505-1.

8 Duckweed and Biofuel Production

Duckweed could be more effectively employed for production of biofuel as compared to other crop plants or agricultural waste, due to 1) its rapid growth rate and 2) its high ability to accumulate starch. The potential to produce a high-starch content provides an excellent opportunity to utilize duckweed biomass for production of ethanol.

The free-floating nature of the duckweed makes the task of harvesting an easy job and could be collected by fine net, vacuum, rake, or any other manual methods. The duckweed could be then dried and milled/ground for further processing.

The enzymatic hydrolysis of high-starch *S. polyrhiza* (45.8%) could be conducted by the utilization of enzymes such as α-amylase and pullulanase and amyloglucosidase. This could be further fermented by yeasts to produce a high yield of ethanol (potentially utilized as biofuel) from duckweed biomass [44]. Similarly, a continuously stirred tank reactor fermenter was utilized for the enzymatic hydrolysis and yeast fermentation of *S. polyrhiza,* which yield high amounts of ethanol almost 50% higher than that of maize-based ethanol [45]. An overall conversion of 94.7% has been achieved during the pilot-scale system, implying the high convertibility of duckweed to starch [46].

For the cultivation of high-starch-producing duckweed, it becomes imperative to provide the optimum growth conditions (depending upon the duckweed species). In order, the favor the accumulation of starch, a considerable excess of photosynthesis over respiration must exist. This is because green plants form starch to store the glucose generated through the process of photosynthesis and obtain energy from it via respiration, to synthesize new compounds and for their proliferation. Therefore, to have enough accumulation of starch, photosynthesis should over-power respiration. The process of photosynthesis could be possibly improved by increasing the light availability and CO_2 levels, whereas respiration could be reduced via nutrient starvation or by utilization of plant hormones such as abscisic acid, cytokinin, and other growth retarding chemicals. Nutrient starvation occurs to be the better and more economical option for enriching starch in the duckweed.

It occurs that nutrient deficiency could stimulate starch accumulation in the duckweed, by providing phosphorous [47], potassium [48], and nitrogen as the limiting nutrient source. The nutrients in a deficient level might lead to a reduction in the use of starch within the cell, resulting in its accumulation. Water containing low levels of nutrients appears to be a good medium for growing high-starch-containing duckweed. It was reported that *L. gibba* could accumulate a high amount of starch in a phosphate-deficient medium [49]. An experiment of simply transferring the fresh fronds of *Spirodela polyrhiza* from a nutrient-rich solution to tap water increased the starch content from about 20% to 45.8% (dry-based) after 5 days [44]. Additionally, the transfer of *Landoltia punctata* from a nutrient-rich solution to distilled water led activity of ADP glucose pyrophosphorylase – a key enzyme of starch synthesis, and starch content to increase readily under the nutrient starvation condition [50].

Cultivation of the duckweed as a waste-based crop for biofuel production creates an economical and environmental approach towards sustainability, which minimizes the dependence on crop-based ethanol production. Furthermore, this technique addresses the important concern over nutrient pollution from the unexercised release of wastewater into aquatic bodies. However, certain aspects shall be considered while developing commercial-scale duckweed systems. These aspects involve the selection of duckweed strains with a high starch-producing ability to enhance the ethanol yield, which could be conducted by utilizing DNA barcodes and near-infrared spectroscopy [46]. Likewise, the genetic modification shall play a vital role in manipulating the duckweed strains to accumulate higher content of starch. Even, the methods for conversion of starch to fermentable sugar could be optimized by pretreating the duckweed biomass to increase its digestibility and thereby, reduce the need for hydrolytic enzymes. Techniques involving rapid cultivation of the duckweeds, novel cultivation reactors as well as disease control methods must be developed, innovated, and implemented for the large scale of duckweeds for biofuel production.

9 Duckweed and Environmental Phytoremediation

"Phytoremediation" refers to the bioremediation of a polluted environment by the utilization of plants. Certain plants utilized in phytoremediation possess the capability of removing pollutants from the terrestrial as well as the aquatic environment.

Like several other macrophytes, duckweed could be possibly utilized for the bioremediation of the contaminated environment. *Lemna minor* remains the major species of duckweed involved in environmental phytoremediation. It illustrates a fairly high potential for the removal of lead as compared to any other aquatic plant from polluted water bodies. Likewise, *L. minor* represents a high removal potential for chromium, zinc, lead, and cadmium from textile wastewater [51]. One of the studies prominently proved the higher efficiency of nickel removal by *L. minor*, as compared to *E. crassipes* [52]. Similarly, the same species could accumulate uranium, boron, and arsenic from the toxic niches [53]. *L. trisulca,* possesses a high bioconcentration potential and thereby, is suitable for the formation of biosorbent for the removal of zinc from the wastewater. One of the studies indicates the utilization of *L. minor* with high efficiency to remove heavy metals such as copper, cadmium, lead, and zinc from wastewater of the oil refinery [54]. This species of duckweed likewise, get involved as a model in several toxicological studies and could bioaccumulate and bioremediate the iron from the mine effluent [55].

S. polyrhiza , could be effectively utilized for the Phyto-filtration of arsenic from contaminated soil and water [56]. It appears to accumulate arsenic through physicochemical adsorption and via the phosphate uptake pathway. The experiment conducted through nutrient enrichment proved to increase the tolerance of L.minor and *S. polyrhiza* to metals, making these species suitable for phytoremediation of even low levels of lead pollution [57]. One of the studies also proves the high efficiency of *S. polyrhiza* as a metal extractor from wastewater highly contaminated with chromium hexavalent [58]. One of the lab studies indicated that *S. polyrhiza* was capable of removing 40-53% and 42-52% of lead and cadmium respectively, from the media depending upon the initial cadmium and lead concentrations [59]. The same species likewise, illustrates a high efficiency for the removal of heavy metals such as lead, copper, zinc chromium, mercury, cobalt, manganese, and nickel from the contaminated wetlands [60].

Landoltia punctata, was found to extract copper quite easily from the copper oxide nanoparticles (CuO-NP) suspension, indicating its role in the removal of copper from the contaminated sites [52]. *Wolffia globosa (* rootless macrophyte*)* occurs to be a strong arsenic accumulator and an interesting plant model to study the As uptake and mechanism in plants [61]. *W. columbiana*, possesses a high capability of accumulating the highest concentration of Cd among the five species of Wolffia namely, *W. globosa, W. australiana, W. cylindracea, W. columbiana,* and *W. arrhiza* [62].

10 Criticality of the Duckweed-Based System

Though duckweed forms a promising alternative to most of the applications as already discussed, the system does account for several limitations as stated:

1. The development of a moderate amount of duckweed in natural/man-made ponds should not be a cause of concern. However, their extensive growth, as a thick mat on the surface of the nutrient-rich water, makes the sight unpleasant. Likewise, it creates a nuisance for aquatic wildlife, as they block the sunlight for the submerged plants. They also, prevent the exchange of gases with the air, creating anoxic conditions for aquatic animals. Thus, the extensive growth of duckweed adversely impacts the wildlife of the water bodies.

2. The warm temperatures, during the summers, account for the higher respiration and decomposition rate of oxygen-consuming animals, possibly resulting in the killing of fish due to further reduction of oxygen levels.

3. The outgrowth of duckweed in the ponds, limits the UV penetration in the ponds, causing a reduction in the killing of the pathogens, which in turn rise the growth of fecal coliforms.

4. The extensive growth of duckweed, for wastewater treatment, ceases in the winter months [63].

5. The nutrients takeup by the duckweed from the wastewater don't get broken down metabolically by the duckweeds and get released into the environment during the winter season when they die away. This causes a spike of nutrients in the water bodies during the winter season.

6. The acceptance of duckweed as the mainstream food appears to be minimum due to neophobia (fear of eating new or unfamiliar foods) [64], the “yuck” factor associated with the turbid ponds where the duckweed are grown, high content of crystallized oxalic acid (in some species of duckweed) resulting in a negative effect on the palatability, difficulty of removing pathogens from the duckweed and lack of nutritive knowledge in the large portion of the population.
7. As duckweed tends to rapidly absorb the nutrients and chemicals occurring in the wastewater system, the duckweeds must grow in pristine waters (which are available very little naturally), to process them as food. However, the plant could be easily cultivated in a hydroponics system.
8. During the wastewater treatment with the duckweed-based system, the duckweed could probably get released (escape) from the water lagoons into the receiving waterways, thereby, contaminating the treated effluent. A discharge pipe with a barrier skirt shall be utilized (an additional arrangement, attributing to the cost of the system), to prevent the duckweed from escaping from the pond.
9. The harvesting of the duckweed occurs to be quite laborious involving much of manual activities, such as netting or scooping.
10. For large-scale production of duckweed, outdoor cultivation forms a good technique, owing to its simplicity and cost-effectiveness. However, for commercial production of duckweed for human consumption and pharmaceutical products, indoor cultivation with highly monitored environmental parameters, with system optimization does the trick. The maintenance of the indoor system could be quite tactful [65].

11 Conclusion

Duckweeds being small, floating macrophytes possess immense potential in creating a sustainable approach for food, wastewater treatment, and environmental remediation. Being available largely all over the world, and having an extending high rate of reproduction, the accumulation of its biomass in most regions would be relatively straightforward. It could also be readily processed to form several useful bio-active compounds.

Because of its unique blend of physiological characteristics and food value, the global market for duckweed is expected to increase rapidly even in developed countries as a heat and drought-resilient alternative to traditional crops in the future, as the climate crisis worsens.

Typically, the high protein and amino acid content in the duckweed is of high value both as a meal alternative and potentially as a fundamental staple in future diets..

Another important reason for cultivating duckweed in the future involves its potential for treatment and remediation of untreated wastewater. Duckweed can effectively tolerate and absorb the heavy metals from the soil and water, thereby, improving their quality for further utilization.

Another future application for duckweed is as a biofuel source, as an eco-friendly alternative to petroleum and fossil fuels.

All applications for duckweed will require some degree of fine-tuning of the cultivation process, specific selection of which variants to use for which solution category, and mass production techniques. With that in mind and with appropriate scientific, agricultural processing, and commercial production technologies, duckweed is a promising option for further investigation and investment.

12 References

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