# DUCKWEED TECHNOLOGIES FOR INTEGRATE EFFLUENT TREATMENT AND PROTEIN FOOD PRODUCTION

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## ABSTRACT

Duckweeds are known by its great potential in biotechnology applications, mainly for wastewater treatment, food protein production and alternative biofuel. Thus, the present study aims to present the potential of duckweed technologies for protein biomass production obtained from wastewater treatment systems, with focus on operation. For this propose, bibliographic research was conducted systematizing information of design and operation of duckweed systems. The duckweed production facilities are commonly built as ponds in different size scales, but always shallow, below 1,0m depth. Regarding nutrient demands, duckweed ponds are dimensioned using superficial application load, assuming the maximum value to be around 50 kg.(ha.d)$^{-1}$ of ammonium nitrogen and 5 kg.(ha.d) $^{-1}$ of phosphorus. Population density is also an important operational parameter that should be between 400 and 600 g.m$^{-2}$ (fresh basis) are often used to avoid overcrowding problems. The crude protein content ranged from 18 to 42% depending on species and growth conditions, mainly the availability of nitrogen. In optimal conditions duckweed protein productivity could be 20 times higher than soybean (22 ton/ha.y$^{-1}$). For coliforms reduction in biomass was observed 3 log after drying at room temperature. Thus, reclaimed effluent can be sustainable source of nutrients and efficient option for protein production by duckweed.

**Keywords:** Water reuse. Nutrient recovery. Green economy.

## 1 INTRODUCTION

Duckweeds are widely known for their efficiency in wastewater treatment, biofuel production and animal feed[1]. In addition, other applications have been studied, such as in human food, as bio indicators, and in the pharmaceutical industry[1]. This is due to several reasons, such as their nutrient removal capacity, high growth rate, environmental resilience, and protein content[1].

In wastewater treatment, duckweeds are generally used in a polishing stage (secondary and tertiary treatment) focusing on nutrient removal (phosphorus and nitrogen)[2], but also on metals like zinc and copper ions[1]. It is important to highlight that both their growth rate and biochemical characteristics are influenced by environmental conditions such as nutrient availability, sunlight, and coverage density[3]. Additionally, the protein content in duckweeds can be favorably affected by sediment material in duckweed ponds[4].

In laboratory, duckweeds can be cultivated in synthetic culture media, with recommended compositions that may vary, such as the guidelines provided by the Organization for Economic Co-operation and Development (OECD)[5]. In contrast, industrial cultivation uses fertilizers, which can be either chemical or organic. In any case, the nutrients obtained from non-renewable sources result in a negative life cycle impact. Therefore, this review aims to guide stakeholders on the constructive and operational parameters of cultivating duckweeds in reclaimed water to utilize the biomass, aiming to enhance the sustainability of this bioprocess.

## 2 MATERIAL & METHODS

This systematic literature review was conducted using the portals Google Scholar, ScienceDirect, and SpringerLink, through the keywords "duckweed pond" and "reuse". In the following sequence, titles, abstracts, and full texts were analyzed to match this study. Due to the amount of results, Google Scholar results were examined until the frequency of relevant matches was considered low. Relevant studies on the topic of wastewater polishing using duckweeds in pilot or full-scale ponds, aimed at biomass utilization, were aggregated.

## 3 DESIGN

The cultivation units are commonly constructed as excavated ponds, sealed with geomembranes or masonry, where the inoculated duckweed population distributes horizontally. In the literature, duckweed ponds are found with depths ranging from 40 cm in pilot scale[6] to 90 cm in full scale[7]. The depth of the ponds is justified by duckweed being a floating macrophyte with roots that absorb nutrients present in the surface layers of the water. At greater depths, the nutrients become unavailable, and creates an anaerobic zone, which can lead to odor formation when using reclaimed water.

Similar to stabilization ponds (facultative and maturation)[8], macrophyte cultivation ponds can assume hydraulic models such as series, parallel, plug-flow, and dispersed flow. On the other hand, complete-mix models cannot be adopted due to the need to maintain the upper layer of duckweeds. In terms of nutrient absorption efficiency, plug-flow ponds (figure 1a) are the most suitable, followed by series ponds and dispersed flow ponds[8]. This is due to the path that nutrients take in contact with the duckweeds, which is longer in plug-flow ponds.

Regarding horizontal dimensions, to promote greater distribution of nutrients in the culture medium and reduce wind action on the surface, the width-to-length ratios of stabilization ponds can be applied[8]; from 1:2 to 1:4. In the literature, this ratio is 1:2 in pilot scale[2] and less than 1:3 in full scale[9]. As an alternative to reduce wind action, some authors suggest installing floating structures on the surface[5], which prevents duckweed drag by the wind without limiting liquid flow. It is also possible to find baffled ponds.

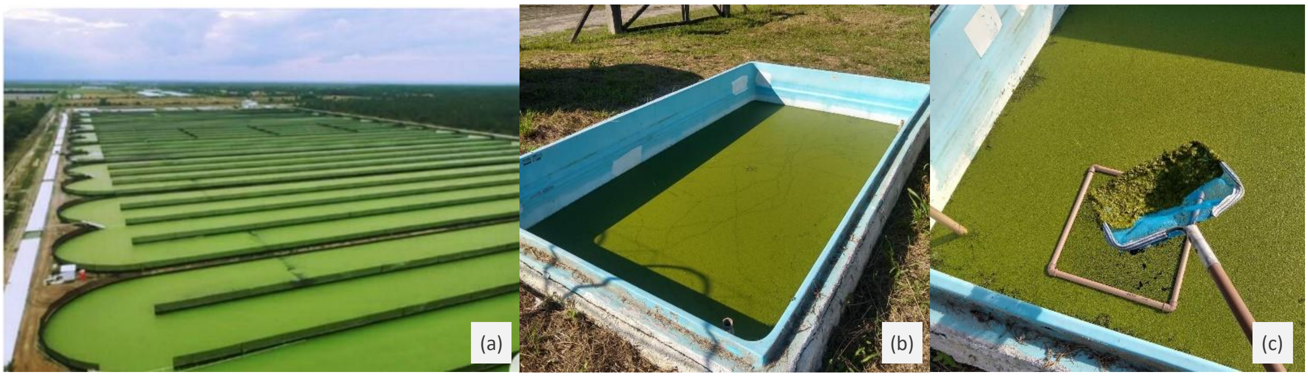


**Figure 1** Duckweed ponds: (a) plug-flow. (b) pilot scale. (c) harvesting.

## 4 NUTRIENT LOAD

In duckweed ponds, the nutrient load determines the flow rate to be applied, given a pond with a determined area, as duckweed ponds are delimited by this dimension. Thus, the flow rate (Q in $m^3.days^{-1}$) can be obtained by equation 1.

$$Q = \frac{V}{p} = \frac{\lambda s * A}{10 * C_0} \tag{1}$$

Where λs is the superficial application load in kg.(ha.d) $^{-1}$, *A* the surface area of the duckweed pond in m² and $C_0$ the nutrient concentration in mg.$L^{-1}$,.

In synthetic culture medium for toxicity tests, OECD Draft Guideline 221 recommends[5] nitrogen and phosphorus concentrations of 14 mg.$L^{-1}$ and 2.4 mg.$L^{-1}$, respectively, with the addition of concentrated solutions daily and the doubling of duckweed every two days. These values are lower than the nutrient discharge limits into water bodies established by the State Council for the Environment[10] of Santa Catarina (CONSEMA) and the National Council for the Environment[11] of Brazil (CONAMA), which are 20 mg.$L^{-1}$ and 4 mg.$L^{-1}$ for nitrogen and phosphorus, respectively.

In the literature[2], it is recommended the maximum surface application loads to be around 50 kg.(ha.d) $^{-1}$ and 5 kg.(ha.d) $^{-1}$, of nitrogen and phosphorus, respectively. However, the most commonly found values are about half of the mentioned[12, 13].

## 5 BIOMASS PRODUCTION AND PROTAIN

In optimal conditions, duckweed population could double its weight per day[5]. However, in studies considering real environmental conditions, this value tends to be lower. This is the case for those that used domestic effluent as a duckweed culture medium, where specific production rates ranging from 5 to 50 g.(m².d) $^{-1}$ [7, 12]. Meanwhile, in those using swine effluents for example, this value ranged from 80 to 170 g.(m².d) $^{-1}$ [(2, 9)]. Considering the cited growth rate, duckweed biomass can reach 42% of crude protein meaning 25 ton/ha.$y^{-1}$ of crude protein[9]. This protein yield could be 20 time higher than soybean protein production capacity.

Another parameter observed in duckweed production is density, as duckweeds require the pond surface to be completely covered to reduce light incidence below the surface and thus inhibit the growth of competitors such as microalgae. On the other hand, high-density values increase competition for resources among themselves. Thus, it was observed that densities between 400 and 600 g.$m^{-2}$ of duckweed fronds (fresh basis)[2, 4, 12] during pond operation are the most used. Maintaining an appropriate density value requires harvest management and daily growth monitoring.

## 6 HARVEST

In order to maintain duckweed density under control, it is necessary to implement proper biomass management, known as harvest. For this purpose, auxiliary structures for duckweeds removal can be adopted, taking into account a known area in order to remove excess biomass. The weight of duckweeds to be removed can be obtained using the equation 2.

$$W = A.(D_1 - D_0) \tag{2}$$

Where W is the weight of duckweeds to be removed in g, A the area of the pond in m²; $D_1$ and $D_0$ are the density of duckweeds before and after harvest, respectively, both in $g.m^{-2}$.

One of the techniques that may be adopted for measuring the current density[2] is the installation of a floating square (figure 1c) with a known area. Subsequently, the biomass inside this area is removed and weighed. Then, duckweeds are removed from the pond until the operational value of 400 to 600 $g.m^{-2}$ is reached, as mentioned.

## 7 PATHOGENS

The use of reclaimed water for duckweeds production raises concerns about the transfer of pathogenic microorganisms from the culture medium to the harvested biomass. One way to evaluate the presence of pathogenic agents may be through coliform counting.

As for coliforms, they are found in the biomass at levels of 7.5 log total coliforms and 7.0 log fecal coliforms[14]. Regardless of this, in dried biomass at room temperature[14], the concentrations are 4.0 log for both coliforms after 24 hours. However, there is observed the increase of these microorganisms by one log after 72 hours of drying at the same conditions[14].

Furthermore, it is expected that other drying alternatives, as well as processing stages of the biomass, may be more effective to the decay of pathogenic microorganisms.

## 8 CONCLUSION

Despite of informal and amateur usage of duckweed technologies, commonly observed, there are many operational conditions which should be observed to improve the results, i.e nutrient removal from effluents and protein biomass production. By this means, it is concluded that the use of reclaimed effluent for duckweed cultivation can be practicable under safety conditions generating a high quality protein biomass in small areas, with low costs. Finally, for future studies, it is recommended to analyze the various stages of biomass processing and other limiting factors besides pathogens.

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