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Comprehensive modeling of mat density effect on duckweed (*Lemna minor*) growth under controlled eutrophication

Monette Frédéric[a,*], *Lasfar Samir*[a], *Millette Louise*[b], *Azzouz Abdelkrim*[c]

[a]*Département de génie de la construction, École de technologie supérieure, 1100, rue Notre-Dame Ouest, Montréal, Qué., Canada H3C 1K3*
[b]*Département des génies civil, géologique et des mines, École Polytechnique de Montréal, C.P. 6079, succ. Centre-Ville, Montréal, Qué., Canada H3C 3A7*
[c]*Département de chimie, Université du Québec à Montréal, C.P. 8888, succ. Centre-Ville, Montréal, Qué., Canada H3C 3P8*

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ABSTRACT

The effect of mat density on duckweed (Lemna minor) growth was studied under controlled conditions: 12.5 h a day light exposure and 342 mol m^{-2} s^{-1} light intensity at 20 °C. The plant growth was carried out in Hoagland medium for 7 days without harvesting. The results revealed a maximal biomass growth rate of 88 g-dry m^{-2} (1470 g-wet m^{-2}) at an optimal initial mat density of 45 g-dry m^{-2} (750 g-wet m^{-2}), with removal rates for nitrogen (N) and phosphorus (P) of 483 mg-N m^{-2} d^{-1} and 128 mg-P m^{-2} d^{-1}, respectively. A mathematical model that takes into account the mat density was developed in order to simulate the growth of *Lemna minor* under controlled eutrophication. Based on experiments carried out, the model exhibits a reliability of 89% . The model remains to be validated at the full-scale level.

1. Introduction

Duckweed, also called duckmeat, belongs to the Lemnaceae family. It is composed of small-sized monocotyledon plants floating on the surface of stagnant or low water velocity pools, where water is rich in nutrients (USEPA, 1988; Oron and Willers, 1989; Haustein et al., 1990). The plant structure is relatively simple, devoid of distinct roots, stalks or leaves (Haustein et al., 1990). The worldwide spread of duckweed is due to its genetic adaptation leading to a wide variety of different species (Oron and Willers, 1989). The Lemnaceae family includes four main families, namely *Lemna*, *Spirodella*, *Wolffia* and *Wolfiella* (Cole and Voskuil, 1996). About 40 species have been inventoried worldwide in various aquatic environments (Haustein et al., 1990). Duckweed displays fast reproduction through gemmation and can absorb large amounts of nutrients such as nitrogen (N) and phosphorus (P) (Landolt, 1986; Oron and Willers, 1989; Vermaat and Hanif, 1998). For these reasons, duckweed has been more and more used in the treatment of household and agricultural wastewater, especially in the last two decades.

A series of biotic and abiotic factors, such as temperature, growth medium composition, light intensity and mat density, exert significant influence on duckweed growth. It appears that there exist optimal values of temperature (Zirschky and Reed, 1988; Oron and Willers, 1989; Boniardi et al., 1999; Iqbal 1999), pH (McLay, 1976), composition of the nourishing medium (Oron et al., 1987; Boniardi et al., 1999; Al-Nozaily

*Corresponding author. Tel.: +1 514 386 8498; fax: +1 514 396 8584.
E-mail address: frederic.monette@etsmtl.ca (M. Frédéric).
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et al., 2000) and light intensity (Filbin and Hough, 1985) beyond which the plant growth is slowed down and even stopped.

Under suitable conditions, duckweed can continuously develop, covering large water areas, unless the available water surface is limited. Indeed, on saturated water surfaces, the aquatic equilibrium is markedly modified since no light rays can pass through the dense plant mat. The growth of any given species is known to be governed by the size of its population; however, the specific effect of plant mat density has been scarcely studied.

So far, numerous mathematical models have been developed to describe plant growth, most of them being based on Michaelis–Menten kinetics. In these models, growth is regarded as being a first-order function of plant mat density. The specific growth rate is correlated to temperature, light intensity, some inhibiting compounds, biomass age, concentrations of nutrients N and P, as well as to the chemical oxygen demand (COD), as stated by Vatta et al. (1995), Takashi et al. (2000) and Boniardi et al. (1994). Plant mat density is either considered constant or devoid of any effect on plant growth. A slight improvement to these models occurs when some authors assume that, with periodical plant harvest (3–4 days), the effect of plant mat density can be neglected. On nearly saturated water surfaces, according to Krebs (1994), a species grows up to a certain asymptotic value of plant mat density, after which plant decay takes place.

Some authors reported the occurrence of an optimal mat density, but values greatly vary, e.g., 1600 g-wet m^{-2} (Alaerts et al., 1996), 1250 g-wet m^{-2} (Koles et al., 1987), to even smaller values such as 400–800 g-wet m^{-2} (Skillicorn et al., 1993). This observation is shared by other authors (DeBusk et al., 1981; Reddy and Tucker, 1983; Körner and Vermaat, 1998), who also state that an excessive increase of plant surface density should slow down duckweed growth.

Nonetheless, no model taking into account the real influence of mat density on plant growth is proposed by the literature. The main purpose of the present investigations is to develop a more comprehensive mathematical model taking into consideration the effect of plant mat density under controlled operating conditions. A better understanding of the growth mechanisms of *Lemna minor* would be of a great interest and would provide a new and improved approach to designing and managing duckweed-based wastewater treatment facilities.

2. Materials and methods

The duckweeds used in the present investigation originated from the Saint-Justin (Québec, Canada) wastewater treatment plant. Prior to laboratory incubation, the plants were repeatedly washed (≈5 times) with a 2% aqueous hypochlorite solution to eliminate bacteria, algae and other undesired organisms. Plant acclimatation required 2 weeks, using a growing Hoagland aqueous nourishing medium, as described by Cross (2002).

The duckweeds were cultivated indoors in six ($0.30 \times 0.30 \times 0.14\,m^3$) PET containers with opaque walls, impervious to light, to avoid algae proliferation. The containers were filled with Hoagland's growth medium and the water level was adjusted daily with distillated water to compensate for evaporation losses. All chemical analysis were evaluated according to the *Standard Methods for the Examination of Water and Wastewater* (APHA, 1995), except for nitrites and nitrates, which were analyzed according to Devarda's technique (Brière et al., 2000).

After the acclimatation period, the containers were placed in an insulated room with controlled light exposure (photoperiod) and temperature to prevent any external influence. Light exposure was provided using four 400 W light sources (Hydrofarm®, Super HPS LU1000B). Light intensity was measured using a Testo® 545 device, and kept at an average value of 342 mol $m^{-2}\,s^{-1}$. The exposure time was fixed at 12.5 h a day, for 7 days. The temperature was maintained at $20 \pm 1\,^{\circ}C$. No harvest took place for the duration of the experiments (7 days).

Fig. 1 provides a global view of the sampling methodology; N and P concentrations in filtrated and unfiltrated media were measured as well as the assessment of wet and dry biomass yield.

The excess water removal process was carried out in three steps: (1) the plants were sieved, (2) dried under vacuum for 5 min and (3) placed on absorbing paper for another 5 min. The initial dry biomass was estimated by extrapolation, using the dry/wet biomass ratio. The final dry biomass was directly assessed by dehydrating the entire biomass at 60 °C for 48 h.

The procedure developed was validated by using 49 mass balances, as described in Fig. 2.

The relative losses (E_c) in N and P concentrations were mainly due to the sampling procedure, the analysis and calculations of the different parameters, and was found to be less than 3.2%. The relative error ε ,which takes into account the total solute mass of the growth medium during the evaluation of duckweed dry weights, was expressed as follows:

$$\varepsilon = \frac{\text{Solute mass}}{\text{Dry mass} - \text{Solute mass}}$$

$$\varepsilon = \frac{\rho_S/(\rho_E) \times \left(1 - W_D/(W_H)\right)}{W_D/(W_H) - \rho_S/(\rho_E) \times \left(1 - W_D/(W_H)\right)} \leqslant \frac{1154/(10^6) \times (1 - 0.05)}{0.05 - 1154/(10^6) \times (1 - 0.05)} \approx 2.2\%,$$

where W_H and W_D are the wet and dry mass of duckweed, respectively, ρ_S is the total mass of the chemical compounds per liter of growth medium ($\rho_S = 1150\,mg\,L^{-1}$), ρ_E is the water density ($\rho_E = 1\,kg\,L^{-1}$) and W_D/W_H is the average value of duckweed dry-to-wet-weight ratio ($6 \pm 1\%$).

3. Results and discussion

The results indicate that the first-order growth rate (r) is dependent on the initial duckweed mat density and decreases when the density increases. The highest growth rate, 0.27 d^{-1}, was obtained with initial densities ranging between 3.4 and 9.6 g-dry m^{-2}. For higher densities, 86–128 g-dry m^{-2}, lower values of growth rates were observed (0.04–0.08 d^{-1}). Duckweed biomass production behaves differently and displays a maximum yield at an optimal initial mat density D_o. Fig. 3