



TÍTULO DEL TRABAJO

“Modelization of floating macrophytes (lemna sp.) ponds “

NOMBRE DE AUTORES

Jupsin H., Richard H., Vasel J.-L.^(*)

NOMBRE Y DIRECCIÓN DE LAS INSTITUCIONES

(*) Université de Liège, Unité “Assainissement et Environnement”. Avenue de Longwy, 185
6700 Arlon (Belgium).

NÚMERO DE TELÉFONO, FAX Y E-MAIL

Tel: +32.(0)63.230.849; Fax: +32.(0)63.230.840; E-mail: jl.vasel@ugl.ac.be

FIGURAS Y TABLAS

MODELIZATION OF FLOATING MACROPHYTES (LEMNA SP.) PONDS

Jupsin H., Richard H., Vasel J.-L.^(*)

(*) Université de Liège, Unité “Assainissement et Environnement”

ABSTRACT

The objective of the present study was to develop a methodology for the quantification of the growth rate of Lemnaceae biomass by digital image analysis. The effect of biomass surface coverage on the oxygen transfer coefficient (kl.a) was also quantified. Contribution of Lemnaceae to oxygen balance was evaluated by closed respirometry. Monod-like equations could be derived from growth rate coefficients in various experimental conditions. This opens the way to a deterministic model of Lemnaceae ponds where uptake of nitrogen and phosphorus (even heavy metals) can be calculated.

KEY WORDS: duckweeds, growth kinetics, oxygen balance, uptakes, mathematical model

RESUMEN

El objetivo de la presente investigación fue desarrollar una metodología para la cuantificación de la tasa de crecimiento de la biomasa Lemnaceae a través de un análisis de imagen digital. El efecto de la cobertura de la superficie de agua por la biomasa sobre el coeficiente de transferencia del oxígeno (kl.a) también fue cuantificado. La contribución del Lemnaceae al balance de oxígeno fue evaluado por medio de un analizador hermético de respirometría. Las ecuaciones del tipo Monod podrían ser derivadas a partir de los coeficientes de la tasa de crecimiento en varias condiciones experimentales. Ello nos conduce hacia un modelo determinista de lagunas de Lemnaceae donde la absorción del nitrógeno y del fósforo (incluso metales pesados) pueden ser calculadas.

PALABRAS CLAVE : lentejas de agua, cinética del crecimiento, balance de oxígeno, absorción, modelo matemático.

1. INTRODUCTION

Various types of floating macrophytes such as Lemnaceae, water lettuces (*Pistia stratiotes*) and water hyacinths (*Eichhornia crassipes*) have been used in constructed wetlands and ponds. The contribution of macrophytes to treatment plants has, however, mostly been regarded in the literature as a black or grey box model, as global first order kinetic coefficients are for example compared with similar coefficients for microphyte waste stabilization ponds. Yet the contribution of macrophytes to processes such as biochemical oxygen demand (BOD) and chemical oxygen demand (COD) removal, N (nitrogen) and P (phosphorus) uptake are directly related to the biomass production even if the precise driving mechanisms are still unknown (in the case of the effect of plants on BOD removal for example).

In this paper we describe how the growth rate of Lemnaceae biomass can be monitored by digital image analysis. The growth rate of the Lemnaceae biomass can then be modeled, enabling the evaluation of its influence on the pond system.

2. MATERIAL AND METHODS

Several methods have been used to quantify the lemna biomass in those systems but most are very tedious. Moreover they do not enable the evaluation of the percentage of surface covered by the biomass.

The authors therefore aimed to develop a method using digital image analysis.

2.1. Oxygen transfer coefficient

Oxygen transfer coefficients were measured by standard method (ASCE, 1984) in identical tanks with and without biomass. Oxygen transfer coefficients starting from zero dissolved oxygen concentration (after N₂ injection) to reach saturation have been quantified for various percentages of surface coverage by biomass.

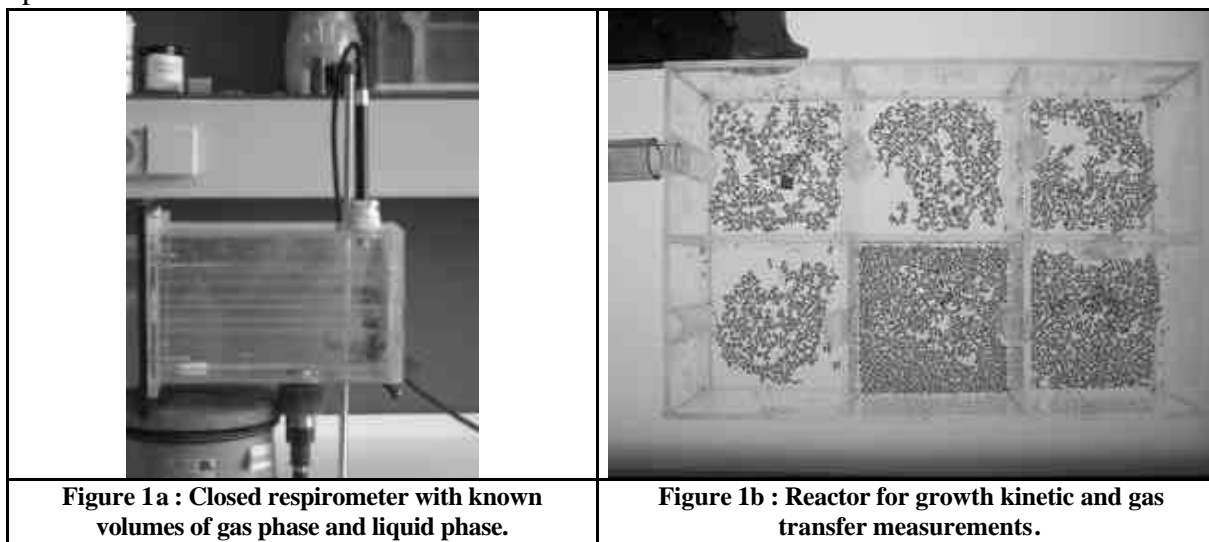
2.2. Digital image analysis

Camera: hp photosmart 715 (3.3 MP resolution : 2048*1536 pixels)

Software: UTHSCSA Image Tools (IT Version 2.0) software. More sophisticated softwares are available but the authors first wanted to check the feasibility of the procedure..

2.3. Closed respirometry

A closed respirometer has been designed to measure O_2 and CO_2 transfer rates between the gas phase and the liquid phase during an experiment. The “transparent” respirometer has known volumes of liquid and gas. Lemna can grow and cover the water surface while gas and liquid phases can be sampled without opening the respirometer. From those measurements mass balances on oxygen and CO_2 can be quantified to evaluate the fluxes between those phases.

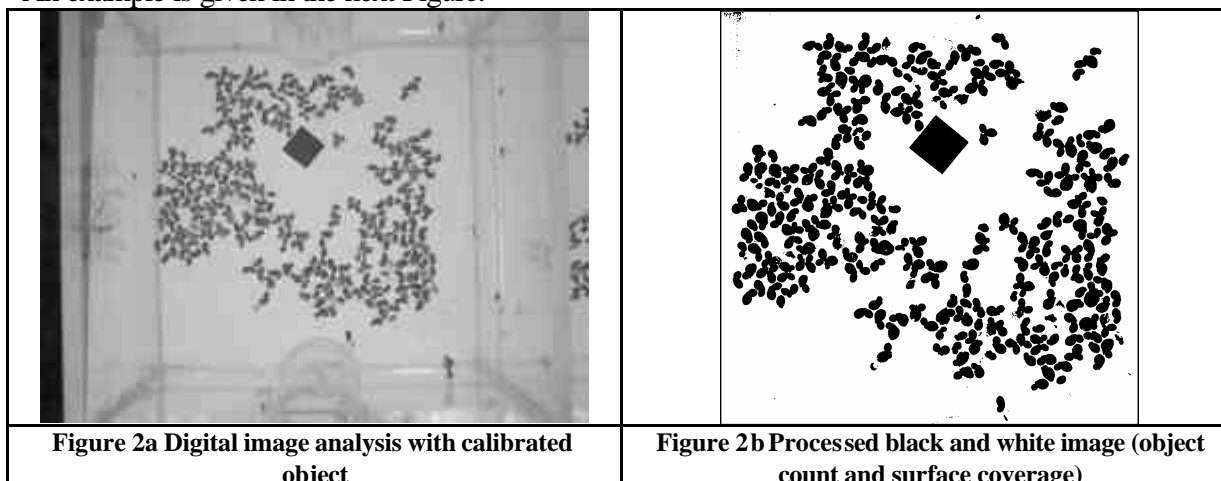


2.4. Biomass and growth rate measurements

Various steps are needed:

- The conversion of the image to a grey/black image,
- The definition of a threshold value to conserve only black and white pixels,
- Each object on the figure has to be numbered

An example is given in the next Figure.



Depending on the type of camera, various tests were made with a view to optimizing the results, such as changing the distance between the camera and the water surface, and trials with the zoom or artificial light.

An object of known dimensions was also placed on the water surface in order to facilitate the conversion of pixels into metric units.

From image analysis the geometric properties of the lemna “objects” could be characterized. If the minimum size could be clearly defined the maximum size is more difficult to characterize. We observed that for one individual the main parameters (surface, perimeter, large axis, small axis) have Gaussian distribution. We quantified the same properties for objects composed of more than one frond. Most of those properties (except the one in grey) provided in table 1 have gaussian distribution.

Table 1: Main size parameters of the lemna sp (average values and standard deviation).

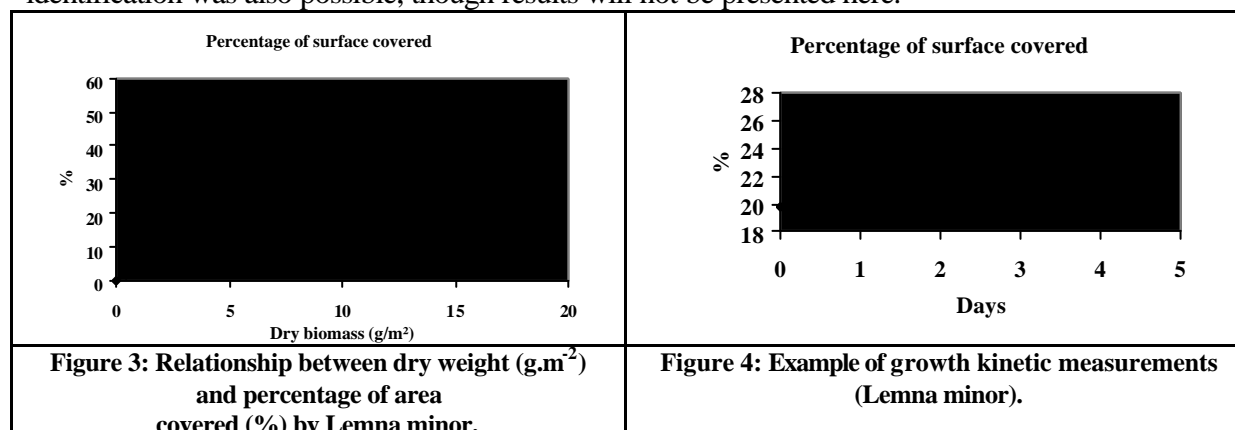
Category	N		Area (mm ²)	Perimeter (mm)	Major axis (mm)	Minus axis (mm)	Elongation	Compactness	Roundness
1 frond	50	Mean	3.67	8.67	2.79	1.77	1.63	0.76	0.65
		Std. Dev.	1.71	3.05	0.63	0.52	0.35	0.06	0.18
2 fronds	171	Mean	5.81	10.97	3.78	1.94	1.97	0.72	0.61
		Std. Dev.	1.62	1.98	0.68	0.31	0.31	0.05	0.09
3 fronds	125	Mean	8.28	14.71	4.58	2.62	1.8	0.71	0.49
		Std. Dev.	1.74	2.31	0.53	0.58	0.34	0.05	0.34
4 fronds	51	Mean	10.07	17.33	4.9	3.54	1.44	0.73	0.43
		Std. Dev.	2.05	2.8	0.66	0.77	0.3	0.05	0.08
5 fronds	6	Mean	11.4	19.26	5.37	3.9	0.73	0.71	0.4
		Std. Dev.	2.31	3.67	0.65	0.96	0.42	0.06	0.6
All objects	403	Mean	6.91	12.87	4.07	2.36	0.58	0.72	0.54
		Std. Dev.	2.73	3.82	0.96	0.78	0.38	0.06	0.13

It is difficult to distinguish individual lemna when they are in contact. This means that although the total surface of lemna can be calculated, it is much harder to give the exact number of “individuals”.

We also checked that when we gathered the same number of individuals on a smaller surface we obtained the same total surface coverage (+/- 2%) which validated the method. Some results indicated that the physiological status of the biomass could also be obtained from (color) image analysis. Elements were numbered and finally the percentage of water area covered by macrophytes was calculated.

3. RESULTS

3.1. Correlation with biomass measurements. The following figure illustrates the correlation between biomass (dry weight) and surface area, demonstrating the feasibility of the method for the quantification of the Lemnaceae biomass. Conducting various experiments we found that the method was valid when the area coverage percentage was < 65%, otherwise overlapping individuals produced measurement errors (in this case a “dilution” of the surface covered is needed). More precise color image analysis was carried out, indicating that bud identification was also possible, though results will not be presented here.



As can be observed in Figure 3, there was a strong positive correlation between area coverage (< 60%) and dry biomass (and even with fresh biomass if the sampling procedure was carefully defined).

3.2. Growth kinetic measurement

Using the methodology developed to measure the biomass, the following experimental conditions were assayed: Light intensity: 18.5 W/m² (dark period = light period = 12 hours). Temperature = 25°C. Initial nutrients conditions: 0.031 mg P-PO₄/l and 2.42 mg N-NH₄/l, total alkalinity : 43 meq/l. The following table compares our results with literature.

Table 2: Growth kinetic measurements and bibliographic values .(RGR=relative growth rate)

Authors	Species	Datas			Photo irradiance
This study	<i>Lemna minor</i>	RGR	0.05	g DW (gDW) ⁻¹ d ⁻¹	18.5 W/m ²
Oron et al., 1996	Duckweed	RGR	0.10-0.35	g DW (gDW) ⁻¹ d ⁻¹	Outdoor
	Duckweed	Doubling times	2.3-7.3	Days	Outdoor
Reddy and DeBusk, 1987	Duckweed	Annual productivity	6.0-26.0	tDW. ha ⁻¹ .y ⁻¹	?
	Duckweed	Growth rate	16-71	g DW m ⁻² d ⁻¹	?
Cedergreen and al., 2002	<i>Lemna minor</i>	RGR	0.04-0.31	g DW (gDW) ⁻¹ d ⁻¹	68.5 W/m ²
Zimmo and al., 2002	<i>Lemna gibba</i>	Production of duckweed	7.5-12.3	g DW m ⁻² d ⁻¹	Outdoor

Mortality could also be evaluated thanks to the color modification of Lemnaceae (see figure 2a). The first growth kinetic experiments yielded a mortality coefficient of 0.0068 g dry g⁻¹d⁻¹.

Following this methodology therefore enabled the evaluation of the growth of biomass species such as Lemnaceae, as well as kinetic measurements in various conditions from which Monod kinetic coefficients could be deduced.

Based on those experiments and on data from literature it was possible to modelize the growth kinetics by the following formula:

$$f(I) \cdot f(T) = \frac{1}{1 + \frac{K_{S,N}}{N} + \frac{K_{I,N}}{N}} \cdot \frac{1}{1 + \frac{K_{S,P}}{P} + \frac{K_{I,P}}{P}} \quad (1)$$

With f(T) = Temperature effect function and f(I) = Light effect function.

As can be seen, the effect of nitrogen on growth rate includes an inhibition function (also observed by other authors (CAICEDO, 2000; ORON, 1986).

$$f(T) = A \cdot \exp\left(-\frac{(T - T_{opt})^2}{D_{ti}}\right) \quad (2)$$

Where

- A = Maximum activity at optimal temperature
- T = Temperature (°C)
- Topt = Optimal Temperature (°C)
- Dti = Temperature sensitivity (°C)

$$f(I) = A_I \cdot \frac{I}{I_M} \cdot \exp\left\{1 - \frac{I}{I_M}\right\} \quad (3)$$

I = Average light intensity

I_M = Optimal light intensity (for studied species)

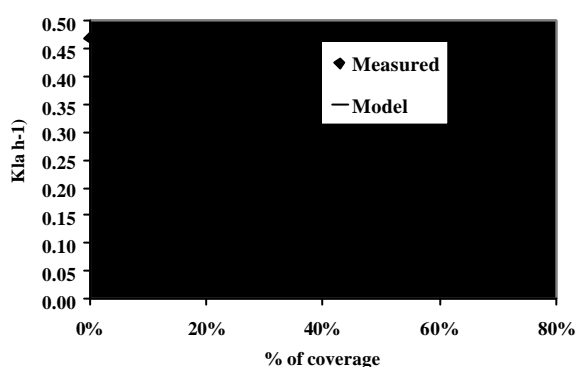
A_I = parameter accounting for the differences between the solar and artificial wavelength spectra (= 1 for artificial illumination)

Experiments to better fit those functions are still in progress

3.3. Effect of surface coverage on gas exchange with the atmosphere

Consequently the percentage of surface covered by the biomass indeed appears to have a drastic influence on factors such as extinction coefficients in the liquid phase under the surface or oxygen transfer coefficients, which were evaluated.

Figure 5: Effect of surface coverage on oxygen transfer coefficient.



$$\frac{Kla}{Kla_0} = \frac{Kla_{\gamma}}{Kla_{\gamma}} e^{-27.748\% \cdot \text{coverage}}$$

$$R^2 = 0.9869$$

$$Kla_0 = 0.47 \text{ h}^{-1}$$

$$Kla_8 = 0.119 \text{ h}^{-1}$$

3.4. Effect of biomass Activity on oxygen and CO₂ balances in the liquid phase

In an other part of the project a special closed respirometer was developed and installed in an experimental "phytotron" to measure oxygen and CO₂ fluxes between the liquid phase and the gas phase. From those results we concluded that for lemna minor less than 10 % of the oxygen (CO₂) produced is released (consumed) in the liquid phase. This means that this species will restrict the oxygen transfer with atmosphere and will not produce oxygen in the liquid phase. Similarly CO₂ is exchanged directly with the gas phase and the effect on alkalinity can be neglected in equation (1).

The resulting observations may be used to relate oxygen and CO₂ fluxes in the liquid phase to the growth rate of the aquatic biomass, thereby enabling to correlate growth rate and mass balance.

The quantification of these mechanisms offers new opportunities for the modelization of these systems.

This methodology can also probably be extended to other types of floating macrophytes.

4. CONCLUSIONS

Digital image analysis provides a promising method of quantification of growth kinetics (and population statistics) of floating macrophytes. Other characteristics such as rate of division or decaying should also be possible to obtain from further developments. The coverage of the water surface by floating macrophytes has a large effect on gas exchange rates with the atmosphere. In the case of oxygen the relationship between the oxygen transfer coefficient ($k_L a$) and decreased exponentially with surface coverage. In the case of Lemnaceae, the contribution of macrophytes to O₂ and CO₂ balances can be quantified in a close respirometer. Exchanges happen mostly directly within the gas phase. As growth rates are now easier to

measure, Monod-type growth kinetic models (such as equation 1) can be used to fit experimental data. The Lemnaceae model is now being combined with a microphyte pond model (Jupsin et al., 2003).

5. LITERATURE REFERENCES

ALAERTS G.J., RAHMAN MAHBURA M.D. and KELDERMAN P.; 1996, "Performance analysis of a full-scale duckweed-covered sewage lagoon", *Water Research*, Vol. 30, n°4, pp 843-852.

AL-NOZAILY F., ALAERTS G. and VEENSTRA S.; 2000; "Performance of duckweed sewage lagoon", *Water Research*, Vol. 34 (10), pp 2734-2741.

ASCE, 1984 ASCE Standard, Measurement of oxygen transfer in clean water, Am. Soc. Civil Engineers, 39p.

BONIARDI N., VATTA G., ROTA R., NANO G. and CARRA S.; 1994, "Removal of water pollutant by *lemna gibba*", *The Chemical Engineering Journal*, Vol 54, pp B41-B48.

BONOMO L., PASTORELLI G. and ZAMBON N.; 1997, "Advantages and limitation of duckweed-based wastewater treatment systems", *Water Science Technology*, Vol. 35, n°5, pp 239-246.

CAICEDO, J.R., VAN DER STEEN N.P., ARCE O., GIJZEN H.J., 2000, Effect of total ammonia nitrogen concentration and pH on growth rates of duckweed (*spirodela polyrrhiza*), *Water Research*, Vol. 34, n°15, pp 3829-3835.

CEDERGREEN N., MADSEN T.V.; 2002, "Nitrogen uptake by the floating macrophyte *Lemna Minor*", *New Phytologist*, Vol 155, pp 285-292.

GORDON D. LEMON and USHER POSLUSZNY; 2000, "Comparative shoot development and evolution in the Lemnaceae", *Int. J. Plant Sci.*, 161(5), pp 733-748.

IWA Group, 2000, "Constructed wetlands for pollution control", Scientific and Technical Report , n°8,.

JUPSIN H., PRAET E. and VASEL, J.-L.; 2003, "Dynamic mathematical model of High Rate Algal Pond (HRAP)", *Water Science and Technology*, Vol 48(2), pp 197-204.

KORNER S., LYATUU G. and VERMAAT J.E.; 1998, "The influence of *lemna gibba* on the degradation of organic material in duckweed-covered domestic waste water", *Water Research*, Vol. 32, n°10, pp 3092-3098.

KORNER S. and VERMAAT J.E.; 1998, "The relative importance of *lemna gibba*, bacteria and algae for the nitrogen and phosphorus removal in duckweed-covered domestic wastewater", *Water Research*, Vol. 32, n°12, pp 3651-3661.

ORON G., WILDDSHUT L.R. and PORATH D.; 1985, "Waste water recycling by duckweed for protein production and effluent renovation", *Water Science Technology*, 17(4/5), pp 803-817.

ORON G., PORATH D., WILDSCHUT L.R., 1986, Wastewater Treatment and Renovation by different duckweed species, *Journal of Environmental Engineering*, Vol. 112, n°2.

REDDY K. and DEBUSK T.A.; 1987, "State of the art utilization of aquatic plants in water pollution control", *Water Science Technology*, 19(10), pp 61-79.

VALDERRAMA LUZ T., DEL CAMPO CLAUDIA M., RODRIGUEZ CLAUDIA M. and BASHAN YOAV; 2002, "Treatment of recalcitrant wastewater from ethanol and citric acid production using the microalga *Chlorella vulgaris* and the macrophyte *Lemna minuscula*", *Water Research*, 3906.

VERMATT JAN E. and HANIF KHALID M.; 1998, "Performance of common duckweed species and the waterfern *Azolla Filiculoides* on different types of waste water", *Water Research*, Vol.32, n°9, pp 2569-2576.

WAYNE HOWARD J., JIANG CHENG, BERGMANN BEN A. and CLASSEN JOHN J.; 2002, "Nutrient removal from anaerobically pretreated swine wastewater with growing duckweed: pilot study", Paper n° e21278a; IWA 3rd World Water Congress, April 7-12, 2002, Melbourne, Australia.

ZIMMO O.R.; 2003, Chapter 2: "Effect of dissolved oxygen and pH on nitrogen removal in batch incubations simulating algae and duckweed-based waste stabilization ponds, in Nitrogen transformations and removal mechanisms in algal and duckweed waste stabilisation ponds", dissertation for the degree of doctor, Delft, The Netherlands.