Monitoring the influence of light intensity on the growth and mortality of duckweed (*Lemna minor*) through digital images processing

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Abstract
The growth of duckweed (*Lemna minor*), depending on biotic and abiotic factors (i.e. temperature, light intensity, photoperiod, pH, nutrients), is an important compartment of the treatment process in wastewater floating macrophytes ponds. Excess or shortage of this biomass might be responsible of the dysfunction of such ponds. Modeling these duckweed ponds through mass balances based on Petersen's matrix should help in an optimal management of such facilities. This article focused on (i) the influence of light intensity on the growth, and (ii) the mortality of *Lemna minor* under a constant temperature. Experiments were carried out in a growth chamber using a pilot consisting of transparent cubic tanks, with an initial fresh *Lemna minor* biomass. In order to monitor *Lemna minor* biomass, digital image processing was achieved in addition to fresh weight and dry weight measurements methods. The results showed that *Lemna minor* reached a maximum growth rate (0.19 d^{-1}) for light intensities ranging between 250 $\mu mol.m^{-2}.s^{-1}$ and 300 $\mu mol.m^{-2}.s^{-1}$. Light intensities from 300 $\mu mol.m^{-2}.s^{-1}$ to 400 $\mu mol.m^{-2}.s^{-1}$ induced a slight growth inhibition. This inhibition was strongly marked at 450 $\mu mol.m^{-2}.s^{-1}$. As for mortality, very low levels (< 0.05 d^{-1}) were determined. These results thus provide a contribution in the modeling of duckweed waste stabilization ponds.
Keywords: wastewater, *Lemna minor*, image processing, light intensity, growth, mortality

INTRODUCTION

Waste Stabilization Ponds are widely used worldwide in the treatment of domestic and industrial wastewaters. Numerous of organisms (bacteria, algae, protozoa, aquatic plants, etc) are involved in the treatment process. Among floating macrophytes, duckweed (*Lemna minor),* is most common species (Hilman, 1961; Cross, 2002). Duckweed are used because of their nitrogen and phosphorus assimilation potential and their performance in the elimination of carbon pollution (Debusk and Reedy, 1987; Vermaat and Hanif, 1998; Nozaily *et al*., 2000; Koné, 2002). However, their growth rate in optimal conditions (temperature, light intensity, nutrients, pH,…) can lead to a large biomass coverage percentage of ponds which might induce dysfunction of such ponds (Radoux and Kemp, 1992; Bonomo *et al.*, 1997; Korner and Vermaat; 1998; Monette *et al.,* 2006; Demirezen *et al.,* 2007; Lasfar *et al.,* 2007). In order to avoid an excess of biomass and any subsequent problem, mainly the death and the settling of duckweed creating a secondary pollution (Debusk *et al*., 1981; Reddy *et al*., 1983; Körneer and Vermaat, 1998; Jupsin *et al.,* 2004) or to valuate this biomass for animal feeding, a regularly harvesting should be achieved. On the other hand, an inappropriate harvesting could lead to a wrong purification and a development of microalgae.

For an appropriate use of this type of pond system it is necessary to characterize and quantify the duckweed life cycle (growth and mortality) and its dependence on environmental parameters (light intensity, temperature, pH, nitrogen, phosphorus, alkalinity, COD, …). On

the other hand, the growth of *Lemna* has also an effect on the bioreactor (N, P, O_2, CO_2) which should be quantified in order to optimize the system.
Although, the dry weight (DW) and fresh weight (FW) methods have been usually used for the monitoring of duckweed biomass (Edwards *and al*., 1992; Köner and Vermaat, 1998; Vermaat and Hanif, 1998; Rhamani and Sternberg, 1999; Caicédo *et al.*, 2000; Cedergreen and Madsen, 2002), they do not allow a continuous monitoring. In fact, the first method (DW) is reliable but destructive; and the second, non-destructive, is much less accurate and even difficult to duplicate. In this paper, digital image processing is used in addition to DW and FW methods for duckweed growth monitoring. Although, this method is non-destructive, it requires an appropriate methodology in taking pictures and image processing software (Jupsin *et al*., 2004).
Moreover modeling the duckweed ponds through mass balances, based on Petersen's matrix, should allow an optimal management of such facilities as for other treatment systems as Activated Sludge (Henze *et al.*, 1987; Jupsin *et al*., 2003). To reach a mathematical model of duckweed pond taking into account nitrogen and phosphorus removal , as well as CO_2 and oxygen fluxes requires to describe the growth kinetic and the stoechiometric parameters of the process.
A reactor has been designed to study the influence of various parameters (light, temperature, nitrogen and phosphorus concentration) on the growth rate and mortality of *Lemna*, using image processing. The impact of nutrients, mainly nitrogen and phosphorus, on the growth of *Lemna minor* was studied in our laboratories and the results of this study have been reported in Tangou *et al*. (2013). Thus, this study will focus on the influence of light intensity on (i) the growth and (ii) the mortality of *Lemna minor* under a constant temperature. The results of such a study will serve in the modeling of duckweed waste stabilization ponds.

MATERIAL AND METHODS

Duckweed used in this study were harvested in ponds of the Bertrix WWTP facility in Belgium. They were kept in plastic tray containing the original sewage water.
Experiments were carried out in a growth chamber using a pilot consisting of six open Plexiglas transparent cubic tanks (12 cm² x 12 cm²). Around 1 g of initial fresh plant biomass (*Lemna minor*) was placed in each tank of the pilot, in 500 ml volume containing various concentrations of nitrogen (NH_4Cl) and phosphorus ($Na_2HPO_4.2H_2O$) (**Table 1**). *In situ* pH was approximately neutral (between 6.5 and 7.5).Water losses caused by evapotranspiration were compensated by daily distilled water addition (Jupsin *et al*., 2004).

Table 1: Experimental pilot involved in the monitoring of duckweed growth

Test (Medium)	*$[N\text{-}NH_4^+]_i$ ($mg.L^{-1}$)*	*$[P\text{-}PO_4^{3-}]_i$ ($mg.L^{-1}$)*	*Light intensity ($\mu mol.m^{-2}.s^{-1}$)*	*Temperature (°C)*	*Photoperiod (h/h)*
1	*5 - 15*	*1*	*200*	*20.6*	*12/12*
2	*5 - 15*	*1*	*250*	*20.9*	*12/12*
3	*5 - 15*	*1*	*300*	*21.2*	*12/12*
4	*5 - 15*	*1*	*350*	*21.5*	*12/12*
5	*5 - 15*	*1*	*400*	*21.7*	*12/12*
6	*5 - 15*	*1*	*450*	*21.9*	*12/12*

The growth of duckweed was monitored every week (five working days) during a period of six weeks. The light intensity in the growth chamber was provided by high pressure sodium lamps (400 watts), with a photoperiod of 12 h day and 12 h night. The air temperature was around 21°C in all series.
Biomass monitoring was realized through three methods: (i) the FW method, (ii) the DW method, and (iii) digital images processing. For the FW method, duckweeds are collected using a kitchen sieve and spread on a absorbent paper during five minutes, and finally weighted. For the DW method, duckweeds are dried in an oven at 105°C during 24 h. They were weighted before and after drying. Regarding the digital images processing method, images were taken using a Nikon® digital camera COOLPIX L120, 14.1 megapixels (picture size 4320 x 3240 pixels), with a focal length of 25-525 mm. The different steps are listed in **table 2**.

Table 2: Steps of Lemna minor camerawork

1. Duckweeds were placed in opened transparent Plexiglas cubic tanks in order to create a contrast between the container and the content (**Figure 1**).
2. To get a good image calibration, a floating colored (black or green) control (1 cm x 1 cm) was placed in each tank before taking the picture. This allows to easily switch from one value in pixels to the corresponding value in centimeter (**Figure 2**).
3. The biomass in the tank should not exceed 60 % of total coverage (Jupsin *et al*., 2004) throughout the experiment (4 days). A possible plants recovery could thereby be avoided, and image could be easily processed.
4. Natural light in the laboratory was used. The flash of the camera was blocked and the neon tubes in the room turned off to prevent any reflection on the water.
5. The camera was calibrated to the automatic option (denoted "auto") for a good resolution compensating the 'no zoom' effect.
6. The camera was maintained at 45 cm above the water by using a tripod (keep the same distance during the experiment).

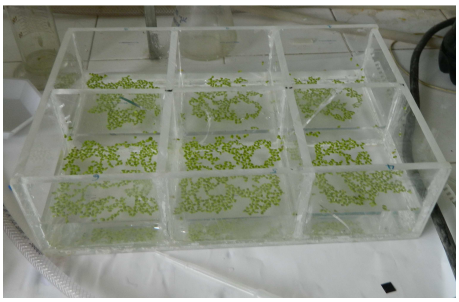

Figure 1. : Experimental cubic tanks

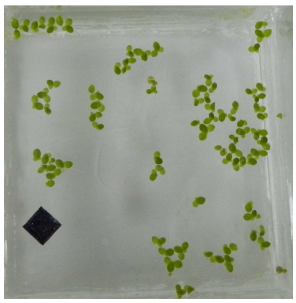

Figure 2. : Example of experimental cubic tank containing a control patch (in black)

Digital images were processed using two softwares: ACD-See® (pre-processing) and Image Pro-Plus® (processing). Pre-processing referred to crop the image to get only the area covered by plants. Whereas processing consisted in counting and determining the geometrical characteristics of *Lemna minor* (area, major axis, minor axis, perimeter). In this latter, manual mode involving the determination of duckweed color (i.e. green and white for living and dead duckweed, respectively) was used. Their automatic count was then achieved based on the corresponding color (**Figures 3 and 4**). Color intensities "Red-Green-Blue (RGB)" corresponding to the colors of living and dead duckweed were between 100-255 and 200-255, respectively. In addition, a synthetic table relating to the statistical characteristics of duckweeds was generated by the software (i.e. Image Pro-Plus®, **Tables 3 and 4**).

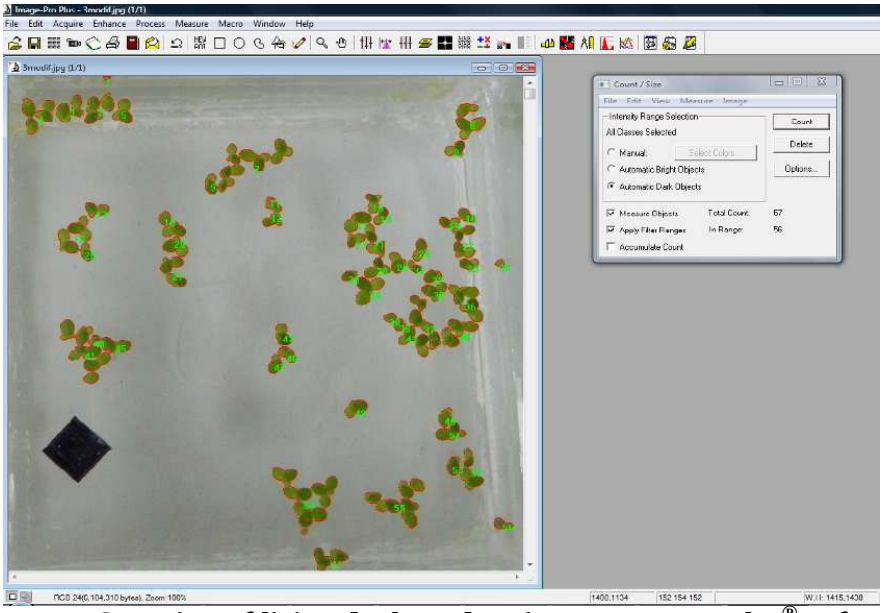

Figure 3: Counting of living duckweeds using Image Pro-Plus® software. Living duckweeds are green (red circles)

Table 3: Statistical summary relating to living duckweeds assessed in Figure 3

Statistics	***Area (cm²)***	***Axis major (cm)***	***Axis minor (cm)***	***Perimeter (cm)***
Min	*0.0083*	*0.151*	*0.071*	*0.373*
(Object #)	*1*	*11*	*1*	*1*
Max	*0.674*	*1.462*	*0.886*	*5.560*
(Object #)	*54*	*7*	*54*	*7*
Range	*0.666*	*1.312*	*0.815*	*5.187*
Mean	*0.133*	*0.539*	*0.305*	*1.767*
Standard Deviation	*0.123*	*0.271*	*0.152*	*1.224*
Sum	***7.449***	***30.184***	***17.087***	***98.952***
Samples	*56*	*56*	*56*	*56*

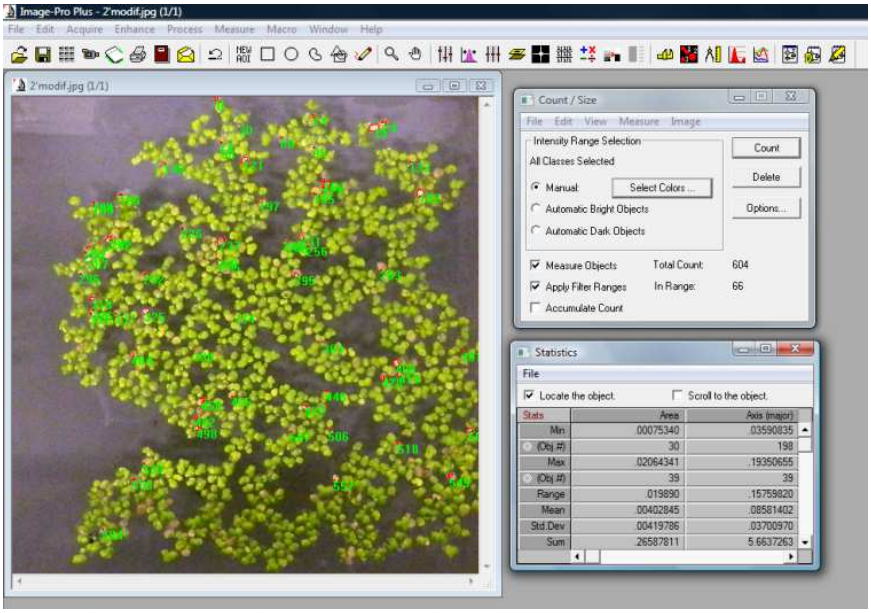

Figure 4: Counting of dead duckweeds using Image Pro-Plus® software. Dead duckweeds are white (red circles)

Table 4: Statistical summary relating to dead duckweeds assessed in Figure 4.

Statistics	*Area (cm²)*	*Axis major (cm)*	*Axis minor (cm)*	*Perimeter (cm)*
Min	*0.001*	*0.036*	*0.019*	*0.066*
(Object #)	*30*	*198*	*446*	*198*
Max	*0.021*	*0.194*	*0.137*	*0.647*
(Object #)	*39*	*39*	*39*	*237*
Range	*0.019*	*0.158*	*0.117*	*0.581*
Mean	*0.004*	*0.086*	*0.053*	*0.217*
Standard Deviation	*0.004*	*0.037*	*0.031*	*0.130*
Sum	***0.266***	***5.664***	***3.486***	***14.292***
Samples	*66*	*66*	*66*	*66*

After characterizing the duckweeds, a correlation between their area and their biomass was analyzed. To do this, the same steps of image capturing and processing (a described in Table 2) were performed on several duckweed biomasses (with known fresh weight) in order to get a standard. The relationship between the dry/fresh weight and the covering percentage was assessed as follows:

$FW\ (g.m^{-2}) = f(A)$ (1)

$DW\ (g.m^{-2}) = f(A)$ (2)

Where *A* (%) refers to the surface covered, and corresponds to the ratio between the real surface covered by duckweeds in a tank and the total area of an empty tank (144 cm²).

The experimental growth rate μ in the exponential growth part was calculated as follows:

$$\ln Xv = \mu t + c \quad (3)$$

where *Xv* (g) refers to the living biomass; μ designs the growth rate; *t* (day) refers to the time (d); and c is the intercept.

We assume that the exponential biomass growth obeys to a Monod kinetic equation (**Equation 4**) (Boniardi *et al.*, 1994; Vatta *et al.*, 1995), and the kinetic of inhibition by

substrate excess follows the Andrews kinetic equation (**Equation 5**) (Caicedo *et al*., 2000; Jupsin *et al*., 2004)

$$\mu = \mu_{\max} \times \left(\frac{S}{S + K_S} \right) \quad (4)$$

$$\mu = \mu_{\max} \times \left(\frac{1}{1 + \frac{K_S}{S} + \frac{S}{K_I}} \right) \quad (5)$$

Where μ_{max}, S, K_S and K_I respectively maximum growth rate (day^{-1}), concentration of substrate ($mg.L^{-1}$), half saturation constant ($mg.L^{-1}$) and inhibition constant ($mg.L^{-1}$).
If we assume that the mortality is proportional to the living biomass

$$\frac{dXm}{dt} = b.Xv \quad (6)$$

The mortality rate, b, can be calculated as follows: $$b = \frac{1}{Xv} . \frac{\Delta Xm}{\Delta t} \quad (7)$$

Where Xv refers to the living biomass and Xm refers to the dead biomass.
Therefore, $\mu_{tot} = \mu + b$ was also evaluated and have been reported on **figure 8**.

RESULTS AND DISCUSSIONS

The relationships between the covered area and dry or fresh weight are expressed in equations 8 and 9. The corresponding coefficient of determination (R^2) were high and close to 1. Thus, in our experimental conditions, digital images processing could be used as a satisfactory method for the determination of duckweed biomass (Tangou et al., 2013).

$DW\ (g.m^{-2}) = 0.5325 * A\ (\%).\ R^2 = 0.978$ (8)

$FW\ (g.m^{-2}) = 13.629 * A\ (\%).\ R^2 = 0.968$ (9)

Although these results are interesting in the determination of growth kinetics of duckweed in laboratory as well as in field experiment, the percentage of recovered *L. minor* biomass would not result in the same weight for the same area covered. The availability of nutrients in a given environment (controlled or natural) influence the growth and the final size of duckweeds (Hilman, 1961; Leng, 1999 ; Cedergreen et Madsen, 2002). Such empirical relationships are limited to the experimental conditions involved.
Furthermore, the accuracy of image processing based method decreases with an increasing covered duckweed biomass (difficulties in counting due to the recovering of duckweeds). Beyond 60% of recovering, the image processing method biomass should be either carried out in 2-3 cubic tanks (the final area determined through the sum) or in one large cubic tank (Jupsin *et al*., 2004).
Field experiment (i.e. on a lagoon) using this method should involve the use of suitable image processing software for discriminating the duckweeds and a rigorous and qualitative survey (for avoiding errors relating to quantitative harvesting of duckweeds in such conditions).
The growth and death rates of *L. minor* were determined in the different cubic tanks involved in our study. An example of different growth and death rates recorded is given in Appendix. Overall, our results showed that light intensities ranging between 200 $\mu mol.m^{-2}.s^{-1}$ and 250 $\mu mol.m^{-2}.s^{-1}$ were optimal for the growth of duckweed. For light intensities ranging from 250 $\mu mol.m^{-2}.s^{-1}$ to 400 $\mu mol.m^{-2}.s^{-1}$, a progressive inhibition of the growth was observed, with a decrease of the rate μ from 0.19 d^{-1} to 0.14 d^{-1}. And for light intensities greater than 450 $\mu mol.m^{-2}.s^{-1}$, the rate μ decreased and reached 0.07 d^{-1} (**Figure 5**). These results are in

accordance with literature (Bouarab *and al*., 2002). Indeed, the growth rate of aquatic plants relating to light intensity has two main phases: (i) a linear relationship between the growth and the light intensity to a maximum rate μ_{max}, which corresponds to a maximum light intensity, and (ii) a second phase characterized by the decrease of the rate μ (photo-inhibition).

Moreover, De la Noue and De Pauw (1988) noted that the efficiency of ponds systems based on aquatic plants was controlled by light and temperature when nutrients are no-limiting and with no physical turbulence. Therefore, light intensity plays an important role in the behavior of *Lemna minor*. Saturation range light intensities for duckweed growth were reported at 342 µmol.m^{-2}.s^{-1} and 440 µmol.m^{-2}.s^{-1} by Filbin and Hough (1985) and Lasfar *et al*. (2007). For intensities greater than this value, some adverse effects may occur (i.e. photo-inhibition). In our experiment, the light intensity of 450 µmol.m^{-2}.s^{-1} is beyond the optimal values for duckweed growth, leading to the photo-inhibition observed.

The evolution of the mortality rate according to the light intensity showed that this rate was constant (0.015 J^{-1}, **Figure 6**) for light intensities ranging between 200 µmole.m^{-2}.s^{-1} and 250 µmole.m^{-2}.s^{-1}. For light intensities greater than 250 µmole.m^{-2}.s^{-1}, *b* progressively decreased and reached 0.001 J^{-1} at 450 µmole.m^{-2}.s^{-1}. But for corresponding light intensities, the values of μ were greater than those of *b* (**Figure 7**).

The results of the influence of light on the duckweed growth are combined with the effect of nutrients (Tangou *et al*., 2013). The overall equation of the kinetics of *Lemna minor* growth can be written as follows:

$$\mu = \mu_{\max} \times f(I) \times f(T) \times \left(\frac{1}{1 + \frac{K_{S,N}}{[N - NH_4^+]} + \frac{[N - NH_4^+]}{K_{I,N}}} \right) \times \left(\frac{1}{1 + \frac{K_{S,P}}{[P - PO_4^{3-}]} + \frac{[P - PO43-]}{K_{I,P}}} \right)$$

with: $f(I) = A_I \frac{I}{I_M} \exp\left(1 - \frac{I}{I_M}\right)$

Where I is the average light intensity, I_M represents the optimal light intensity, and A_I is a parameter accounting for the differences between the solar and artificial wavelength spectra, which has been set equal to unity in our case (Vatta *et al*., 1995).

Thus, by iteration we get **I_M = 286.5 µmol.m^{-2}.s^{-1}** knowing that μ_{max} = 0.19 d^{-1}; T = 21°C, [N-NH_4^+] = 10 mg.L^{-1}; $K_{S,N}$ = 3.83 mg.L^{-1}; $K_{I,N}$ = 204.27 mg.L^{-1}; [P-PO_4^{3-}] = 1 mg.L^{-1}; $K_{S,P}$ = 1.26 mg.L^{-1} and $K_{I,P}$ = 13.33 mg.L^{-1}.

The experimental value obtained through our study (250 µmol.m^{-2}.s^{-1}) is quite close to the theoretical value (286.5 µmol.m^{-2}.s^{-1}). Under the conditions tested, the optimum intensity for the growth of *Lemna minor* was ranged between 250 and 300 µmol.m^{-2}.s^{-1}. Beyond 300 µmol.m^{-2}.s^{-1}, saturation was observed at 400 µmol.m^{-2}.s^{-1}.

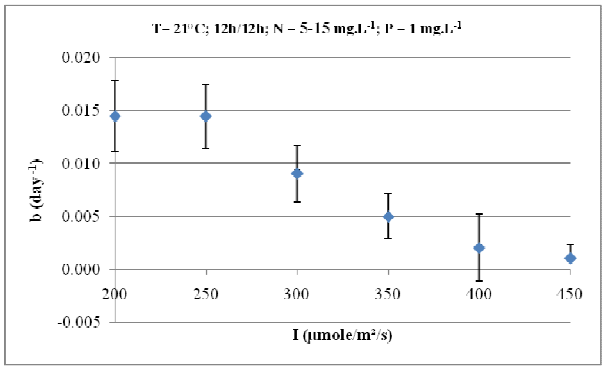

Figure 5: Evolution of Lemnar minor growth rate according to light intensity

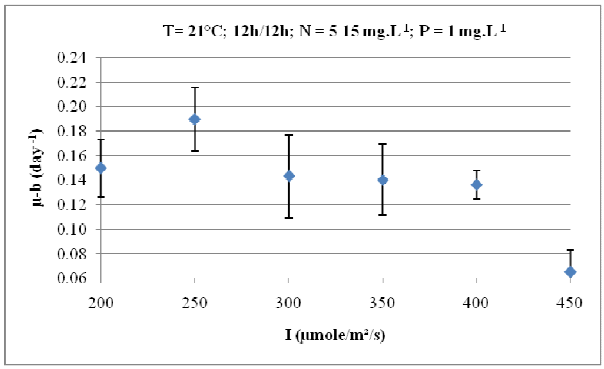

Figure 6: Evolution of Lemna minor mortality rate according to light intensity

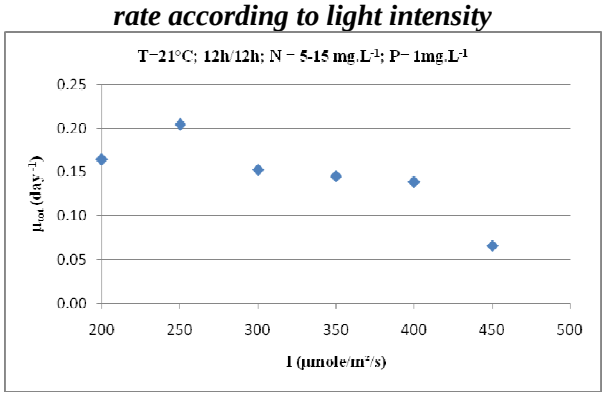

Figure 7: Evolution of Lemna minor growth and mortality rates according to light intensity

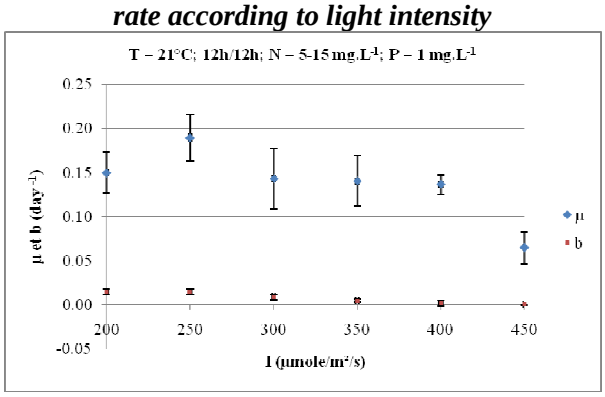

Figure 8: Evolution of Lemna minor total growth rate according to light intensity

CONCLUSION

This study sought to assess the influence of light intensity on the growth and mortality of *Lemna minor* in experimental laboratory conditions. Our results showed that the growth of *Lemna minor* was subdivided in two phases. A first phase characterized by a growth for optimal intensities ranging between 250 and 300-µmol.m^{-2}. s^{-1}. The maximum growth rate recorded was 0.19 d^{-1}. The second phase in turn, was characterized first by a progressive inhibition (with light intensities varying between 250 and 400 µmol.m^{-2}. s^{-1}), which became clear at 450 µmol.m^{-2}. s^{-1}. The corresponding growth rates decreased sharply from 0.15 d^{-1} to 0.07 d^{-1}. These results showed that the light intensity was a major factor that should be taken into account in the growth of *Lemna minor*.

A mortality rate was also determined at each light intensity involved. Overall, they were very low (< 0.05 d^{-1}). The experimental conditions were conducive to the growth of *Lemna minor* rather than their death. Moreover, the assumption of proportional relationship between living and dead biomass was tested according to the different light intensities. Despite the limits relating to empirical-based relationships, the image processing method involved in our study allowed a continuous and non destructive monitoring of duckweeds biomass. These different results are interesting as they could serve in the optimal management of *Lemna minor* and the modeling of biological reactors.

BIBLIOGRAPHY

Bonomo L., Pastorelli G. and Zambon N., 1997. Advantages and limitations of duckweed-based wastewater treatment systems. Water Science and Technology, **35**(5), 239-246.

Bouarab L., Loudiki M. and Dauta A. (2002). Croissance en autotrophie et mixotrophie de la microalgue *Micractinium pusillum* Fres. isolée d'un lagunage naturel : influence de la lumière et de la température (Autotrophic and mixotrophic growth of microalgae *Micractinium pusillum* Fres. isolated from a natural lagoon: influence of light and temperature).Revue Science de l'Eau, **15,** 73-86

Caicedo J. R., Van Der Steen P., Arce O. and Gijzen H., 2000. Effect of total ammonium nitrogen concentration and pH on growth rates of duckweed (*Spirodela polyrrhiza*). Water. Research, **34**(15), 3829-3835.

Cedergreen N. and Madsen T.V., 2002. Nitrogen uptake by the floating macrophyte *Lemna minor*. New Phytologist, **155,** 285-292.

Debusk T.A., Ryther J.H. et Hanisak M.D., 1981. Effects of seasonality and plant density on the productivity of some freshwater macrophytes. Aquatic Botany. **10**, 133-142.

Debusk T.A. and Reedy K.R., 1987. BOD removal in floating aquatic macrophytes based wastewater treatment. Water Science and Technology., **19**(12), 273-279.

Demirezen D., Aksoy A. and Uruç K., 2007. Effect of population density on growth, biomass and nickel accumulation capacity of *Lemna gibba* (Lemnaceae). Chemosphere **66**(3), 553-557.

De la Noue J. and De Pauw N. (1988). The potential of microalgae biotechnology: A review of production and uses of microalgae. Biotechnology Advances **6**(4): 725-770.

Edwards P., Hassan M.S., Chao C.H. and Pacharaprakiti C., 1992. Cultivation of Duckweeds in Spetage-Loades Earthen Ponds. Bioresource Technology **40**, 109-117.

Filbin G.J. and Hough A.R., 1985. Photosynthesis, photorespiration and productivity in *Lemna minor L.* Oceanography, **30**(2), 322-334.

Jupsin H., Praet E. and Vasel J.L., 2003. Dynamic mathematical model of high rate algal ponds. Water Science and Technology., **48**(2), 197-204.

Jupsin H., Richard H. and Vasel J-L., 2004. Contribution of floating macrophytes (lemna sp.) in pond modelization. 6th International Conference on Waste Stabilisation Ponds, September 28th - October 1st, 2004, Avignon, France, 387-393.

Henze M., Grady C.P.L., Gujer W., Marais G.V.R. and Matsuo T., 1987. Activated Sludge Model N°1. IAWQ, London, (ISSN 1025-0913, ISBN 1 900222 000).

Hillman W.S., 1961. The Lemnaceae, or Duckweed: a review of the descriptive and experimental literature. Botanical Review, **27**, 221-287.

Nozaily F., Alaerts G. and Veenstra S., 2000b. Performance of duckweed-covered sewage lagoons-II. Nitrogen and phosphorus balance and plant productivity. Water Research **34**(10), 2734-2741.

Koné D., 2002. Lagunage à microphytes et à macrophytes (Pistia stratiotes) en Afrique de l'Ouest et du Centre: Etat des lieux, performances épuratoires et critères de dimensionnement. Thèse de doctorat : Ecole Polytechnique Fédérale de Lausanne: Environnment naturel - Architectural and Construction Faculty (Suisse).

Körner 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., **32**(12), 3651-3661.

Lasfar S., Monette F., Millette L. and Azzouz A., 2007. Intrinsic growth rate: A new approach to evaluate the effects of temperature, photoperiod and phosphorus-nitrogen concentrations on duckweed growth under controlled eutrophication. Water Research, **41**(2007), 2333-2340.

Monette F., Lasfar S., Millette L. and Azzouz A., 2006. Comprehensive modeling of mat density effect on duckweed (*Lemna minor*) growth controlled eutrophication. Water Research, **40**(15), 2901-2910.

Reddy K.R. et Tucker J.C., 1983. Productivity and nutrient uptake of water hyacinth, Eichhornia crassipes 1 – Effect on nitrogen source. Economic Botany. **37**(2), 237-247.

Radoux M. and Kemp D., 1992. Rôle de la fréquence des prélèvements de la biomasse produite sur les capacités épuratrices de *Lemna minor L*. Revue Science de l'Eau, (5), 55-68.

Rahmani G. N. H. and Sternberg S. P. K., 1999. Bioremoval of lead from water using Lemna minor. Bioresource Technology, **70**: 225-230.

Tangou T.T., Baya D.T., Liady M.N.D., Musibono E.D. et Vasel J.L., 2013. Etude de la cinétique de croissance des lentilles d'eau (*Lemna minor*) par traitement d'images numériques : I. Cas de la Belgique. (Assessing the kinetics of duckweed (*Lemna minor*) growth through digital images processing: I. A case study of Belgium) ("Submitted")

Vatta G., Rota R., Boniardi N. and Nano G., 1995. Dynamic modelling of waste-water treatment plants based on *Lemna gibba*. The Chemical Engineering Journal., **57**:37-48.

Vermaat J. E. and Hanif M. K., 1998. Performance of common duckweed species (Lemnaceae) and waterfern Azolla filiculoides on different types of waste water. Water Research, **32**(9), 2569-2576.

Appendix : Monitoring of duckweeds **growth**

Table 1: Evolution of duckweeds biomass (covered area and dry weight) in our experimental conditions. Case of Test 2.

Test 2	Monday		Tuesday		Wednsday		Thursday		Friday		μ
	Day 1		Day 2		Day 3		Day 4		Day 5		$\ln Xv = \mu t + c$
	cm^2	mg DW	cm^2	mg DW	cm^2	mg DW	cm^2	mg DW	cm^2	mg DW	day^{-1}
Tank 1	18.349	**98.348**	22.848	**122.465**	28.845	**154.608**	35.501	**190.283**	39.646	**212.498**	0.198
Tank 2	20.630	**110.575**	23.935	**128.290**	26.566	**142.392**	31.292	**167.725**	36.211	**194.085**	0.139
Tank 3	20.235	**108.458**	22.949	**123.005**	29.686	**159.115**	32.238	**172.793**	38.893	**208.461**	0.164
Tank 4	18.106	**97.046**	21.535	**115.426**	28.663	**153.633**	34.294	**183.813**	43.564	**233.500**	0.222
Tank 5	18.257	**97.856**	22.527	**120.743**	29.563	**158.454**	37.561	**201.323**	42.456	**227.563**	0.219
Tank 6	19.797	**106.112**	22.548	**120.853**	30.251	**162.140**	38.412	**205.885**	40.715	**218.230**	0.197

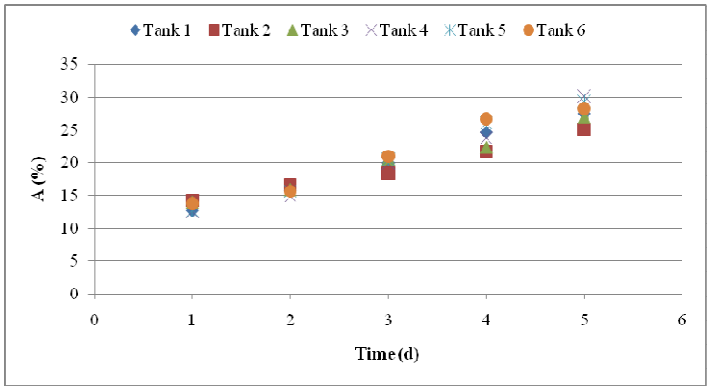

Figure 1: Evolution of the area covered by Lemna minor in the six cubic tanks of Test 2 experiment

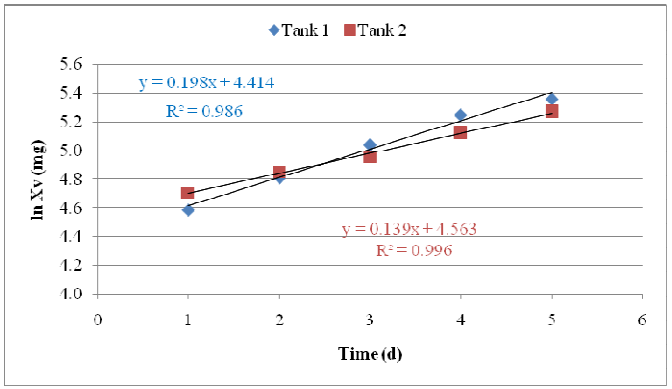

Figure 2: Determination of the growth rate μ in cubic tanks 1 and 2 (Test 2)

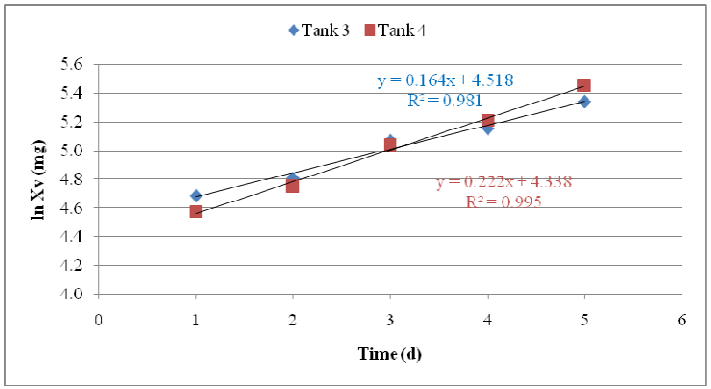

Figure 3: Determination of the growth rate μ in cubic tank 3 and 4 (Test 2)

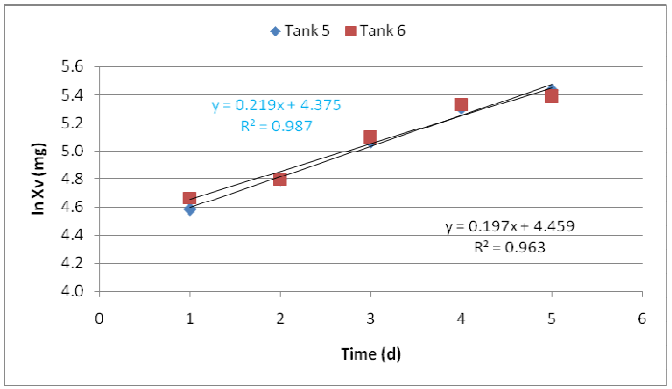

Figure 4: Determination of the growth rate μ in cubic tank 5 and 6 (Test 2)

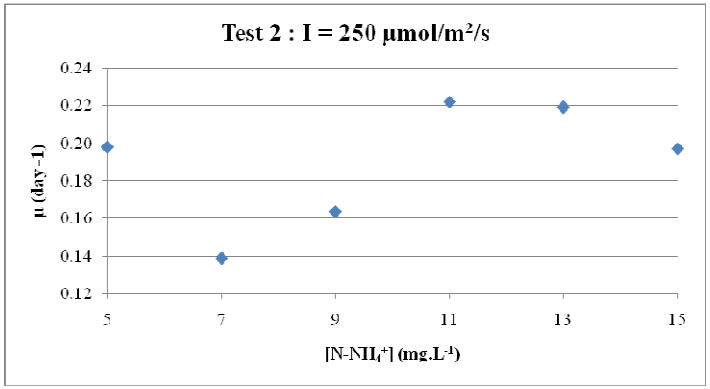

Figure 5: Evolution of Lemnar minor total growth rate according to the nitrogen concentration

Monitoring of duckweeds **decay in Test 2 experiment**

Table 2: Determination of duckweeds death rate in the cubic tank 1.

Tank 1	**Day 1**			**Day 2**			**Day 3**			**Day 4**			**Day 5**			**rate**
Process	**A(Cm²)**	**A(%)**	**DW(g/m²)**	**A(Cm²)**	**A(%)**	**DW(g/m²)**	**A(Cm²)**	**A(%)**	**DW(g/m²)**	A(Cm²)	A(%)	DW(g/m²)	A(Cm²)	A(%)	DW(g/m²)	**day $^{-1}$**
Growth	**18.349**	12.742	0.068	22.848	15.867	0.084	28.845	20.031	0.107	35.501	24.653	0.131	39.646	27.532	0.147	0.198
Decay	0.888	0.617	0.003	1.372	0.953	0.005	1.226	0.852	0.005	1.044	0.725	0.004	1.453	1.009	0.005	**0.004**
Total	19.237	13.359	0.071	24.221	16.820	0.090	30.071	20.883	0.111	36.545	25.378	0.135	41.099	28.541	0.152	0.202

Table 3: Determination of duckweeds death rate in the cubic tank 2.

Tank 2	**Day 1**			**Day 2**			**Day 3**			**Day 4**			**Day 5**			**rate**
Process	**A(Cm²)**	**A(%)**	**DW(g/m²)**	**A(Cm²)**	**A(%)**	**DW(g/m²)**	**A(Cm²)**	**A(%)**	**DW(g/m²)**	A(Cm²)	A(%)	DW(g/m²)	A(Cm²)	A(%)	DW(g/m²)	**day $^{-1}$**
Growth	**20.630**	14.326	0.076	23.935	16.622	0.089	26.566	18.449	0.098	31.292	21.731	0.116	36.211	25.146	0.134	0.139
Decay	0.783	0.544	0.003	1.550	1.076	0.006	1.099	0.763	0.004	0.928	0.645	0.003	1.013	0.703	0.004	**0.002**
Total	21.413	14.870	0.079	25.485	17.698	0.094	27.665	19.212	0.102	32.221	22.376	0.119	37.224	25.850	0.138	0.141

Table 4: Determination of duckweeds death rate in the cubic tank 3.

Tank 3	**Day 1**			**Day 2**			**Day 3**			**Day 4**			**Day 5**			**rate**
Process	**A(Cm²)**	**A(%)**	**DW(g/m²)**	**A(Cm²)**	**A(%)**	**DW(g/m²)**	A(Cm²)	A(%)	DW(g/m²)	A(Cm²)	A(%)	DW(g/m²)	A(Cm²)	A(%)	DW(g/m²)	**day $^{-1}$**
Growth	**20.235**	14.052	0.075	22.949	15.937	0.085	29.686	20.615	0.110	32.238	22.388	0.119	38.893	27.009	0.144	0.164
Decay	0.519	0.361	0.002	0.750	0.521	0.003	1.124	0.781	0.004	0.869	0.603	0.003	0.711	0.494	0.003	**0.001**
Total	20.754	14.413	0.077	23.699	16.457	0.088	30.810	21.396	0.114	33.107	22.991	0.122	39.603	27.502	0.146	0.165

Table 5: Determination of duckweeds death rate in the cubic tank 4.

Tank 4	**Day 1**			**Day 2**			**Day 3**			**Day 4**			**Day 5**			**rate**
Process	**A(Cm²)**	**A(%)**	**DW(g/m²)**	**A(Cm²)**	**A(%)**	**DW(g/m²)**	A(Cm²)	A(%)	DW(g/m²)	A(Cm²)	A(%)	DW(g/m²)	A(Cm²)	A(%)	DW(g/m²)	**day $^{-1}$**
Growth	**18.106**	12.574	0.067	21.535	14.955	0.080	28.663	19.905	0.106	34.294	23.815	0.127	43.564	30.253	0.161	0.222
Decay	0.643	0.447	0.002	1.216	0.844	0.004	1.193	0.829	0.004	0.930	0.646	0.003	0.756	0.525	0.003	**0.001**
Total	18.749	13.020	0.069	22.751	15.799	0.084	29.857	20.734	0.110	35.224	24.461	0.130	44.320	30.778	0.164	0.223

Table 6: Determination of duckweeds death rate in the cubic tank 5.

Tank 5	Day 1			Day 2			Day 3			Day 4			Day 5			rate
Process	**A(Cm²)**	**A(%)**	**DW(g/m²)**	**A(Cm²)**	**A(%)**	**DW(g/m²)**	A(Cm²)	A(%)	DW(g/m²)	A(Cm²)	A(%)	DW(g/m²)	A(Cm²)	A(%)	DW(g/m²)	**day $^{-1}$**
Growth	**18.257**	12.678	0.068	22.527	15.644	0.083	29.563	20.530	0.109	37.561	26.084	0.139	42.456	29.484	0.157	0.219
Decay	0.640	0.444	0.002	0.933	0.648	0.003	1.008	0.700	0.004	0.947	0.657	0.004	0.627	0.435	0.002	**0.000**
Total	18.897	13.123	0.070	23.460	16.292	0.087	30.571	21.230	0.113	38.507	26.741	0.142	43.083	29.919	0.159	0.219

Table 7: Determination of duckweeds death rate in the cubic tank 6.

Tank 6	Day 1			Day 2			Day 3			Day 4			Day 5			rate
Process	**A(Cm²)**	**A(%)**	**DW(g/m²)**	**A(Cm²)**	**A(%)**	**DW(g/m²)**	A(Cm²)	A(%)	DW(g/m²)	A(Cm²)	A(%)	DW(g/m²)	A(Cm²)	A(%)	DW(g/m²)	**day $^{-1}$**
Growth	**19.797**	13.748	0.073	22.548	15.658	0.083	30.251	21.007	0.112	38.412	26.675	0.142	40.715	28.274	0.151	0.197
Decay	0.600	0.417	0.060	0.941	0.654	0.003	1.158	0.804	0.004	0.922	0.640	0.003	0.484	0.336	0.002	**-0.001**
Total	20.398	14.165	2.048	23.489	16.312	0.087	31.409	21.812	0.116	39.334	27.315	0.145	41.200	28.611	0.152	0.196

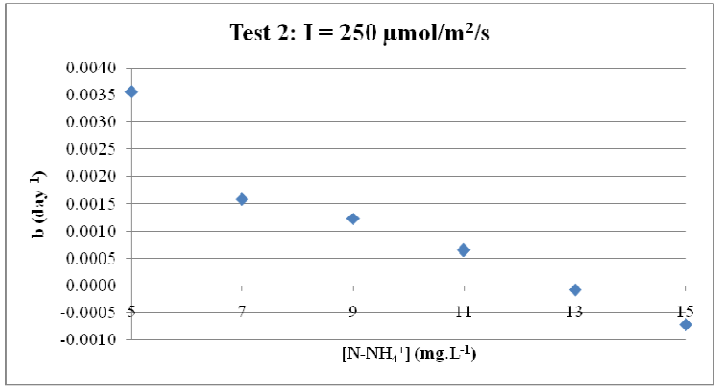

Figure 6: Evolution of Lemnar minor total growth rate according to the nitrogen concentration

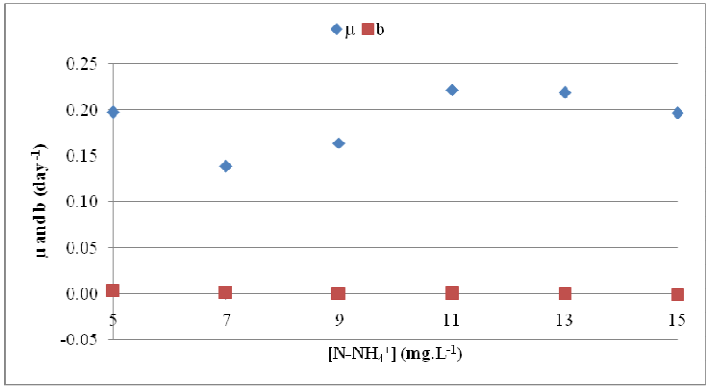

Figure 7: Evolution of Lemnar minor growth and mortality rates according to the nitrogen concentration