TÍTULO DEL TRABAJO

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

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FIGURAS Y TABLAS
MODELIZATION OF FLOATING MACROPHYTES (LEMNA SP.) PONDS


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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 (kLa) 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, uptake, mathematical model

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 modelized, 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\textsubscript{2} and CO\textsubscript{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\textsubscript{2} can be quantified to evaluate the fluxes between those phases.

![Figure 1a: Closed respirometer with known volumes of gas phase and liquid phase.](image1a)

![Figure 1b: Reactor for growth kinetic and gas transfer measurements.](image1b)

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.

![Figure 2a: Digital image analysis with calibrated object](image2a)

![Figure 2b: Processed black and white image (object count and surface coverage)](image2b)

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>
<thead>
<tr>
<th>Category</th>
<th>N</th>
<th>Area (mm²)</th>
<th>Perimeter (mm)</th>
<th>Major axis (mm)</th>
<th>Minor axis (mm)</th>
<th>Elongation</th>
<th>Compactness</th>
<th>Roundness</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 frond</td>
<td>50</td>
<td>Mean 3.67</td>
<td>8.67</td>
<td>2.79</td>
<td>1.77</td>
<td>1.63</td>
<td>0.76</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Std. Dev.</td>
<td>1.71</td>
<td>3.05</td>
<td>0.63</td>
<td>0.52</td>
<td>0.35</td>
<td>0.06</td>
</tr>
<tr>
<td>2 fronds</td>
<td>171</td>
<td>Mean 5.81</td>
<td>10.97</td>
<td>3.78</td>
<td>1.94</td>
<td>1.97</td>
<td>0.72</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Std. Dev.</td>
<td>1.62</td>
<td>1.98</td>
<td>0.68</td>
<td>0.31</td>
<td>0.31</td>
<td>0.05</td>
</tr>
<tr>
<td>3 fronds</td>
<td>125</td>
<td>Mean 8.28</td>
<td>14.71</td>
<td>4.58</td>
<td>2.62</td>
<td>1.8</td>
<td>0.71</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Std. Dev.</td>
<td>1.74</td>
<td>2.31</td>
<td>0.53</td>
<td>0.58</td>
<td>0.34</td>
<td>0.05</td>
</tr>
<tr>
<td>4 fronds</td>
<td>51</td>
<td>Mean 10.07</td>
<td>17.33</td>
<td>4.9</td>
<td>3.54</td>
<td>1.44</td>
<td>0.73</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Std. Dev.</td>
<td>2.05</td>
<td>2.8</td>
<td>0.66</td>
<td>0.77</td>
<td>0.3</td>
<td>0.05</td>
</tr>
<tr>
<td>5 fronds</td>
<td>6</td>
<td>Mean 11.4</td>
<td>19.26</td>
<td>5.37</td>
<td>3.9</td>
<td>0.73</td>
<td>0.71</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Std. Dev.</td>
<td>2.31</td>
<td>3.67</td>
<td>0.65</td>
<td>0.96</td>
<td>0.42</td>
<td>0.06</td>
</tr>
<tr>
<td>All objects</td>
<td>403</td>
<td>Mean 6.91</td>
<td>12.87</td>
<td>4.07</td>
<td>2.36</td>
<td>0.58</td>
<td>0.72</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Std. Dev.</td>
<td>2.73</td>
<td>3.82</td>
<td>0.96</td>
<td>0.78</td>
<td>0.38</td>
<td>0.06</td>
</tr>
</tbody>
</table>

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.

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![Figure 3: Relationship between dry weight (g.m⁻²) and percentage of area covered (%) by Lemma minor.](image)

![Figure 4: Example of growth kinetic measurements (Lemna minor).](image)
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>
<thead>
<tr>
<th>Authors</th>
<th>Species</th>
<th>Data</th>
<th>Photo irradiance</th>
</tr>
</thead>
<tbody>
<tr>
<td>This study</td>
<td><em>Lemna minor</em></td>
<td>RGR 0.05 g DW (gDW⁻¹ d⁻¹)</td>
<td>18.5 W/m²</td>
</tr>
<tr>
<td>Oron et al., 1996</td>
<td>Duckweed</td>
<td>RGR 0.10-0.35 g DW (gDW⁻¹ d⁻¹)</td>
<td>Outdoor</td>
</tr>
<tr>
<td></td>
<td>Duckweed</td>
<td>Doubling times 2.3-7.3 Days</td>
<td>Outdoor</td>
</tr>
<tr>
<td>Reddy and DeBusk, 1987</td>
<td>Duckweed</td>
<td>Annual productivity 6.0-26.0 tDW. ha⁻¹ y⁻¹</td>
<td>?</td>
</tr>
<tr>
<td></td>
<td>Duckweed</td>
<td>Growth rate 16-71 g DW m⁻² d⁻¹</td>
<td>?</td>
</tr>
<tr>
<td>Zimmo and al., 2002</td>
<td><em>Lemna minor</em></td>
<td>RGR 0.04-0.31 g DW (gDW⁻¹ d⁻¹)</td>
<td>68.5 W/m²</td>
</tr>
<tr>
<td></td>
<td><em>Lemna gibba</em></td>
<td>Production of duckweed 7.5-12.3 g DW m⁻² d⁻¹</td>
<td>Outdoor</td>
</tr>
</tbody>
</table>

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⁻¹ d⁻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 deducted.

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

\[
\frac{\frac{P}{K_{S,P}}}{\frac{N}{K_{I,N}}} \cdot \frac{1}{N + \frac{NH_{4}^{+}}{K_{I,N}}} \cdot \frac{1}{\frac{PO_{4}^{3-}}{K_{S,N}}} \cdot \frac{1}{f(I) \cdot f(T) \cdot ?} = \frac{P}{K_{S,P}} \cdot \frac{1}{f(I) \cdot f(T) \cdot ?} \cdot \frac{P}{K_{S,P}} \cdot \frac{1}{f(I) \cdot f(T) \cdot ?}
\]

(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). With \( f(T) = A \cdot \exp \frac{\frac{\frac{(T - Topt)^{2}}{2}}{Dti^{2}}}{2} \)

(2)

Where

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

3.4. Effect of biomass Activity on oxygen and CO$_2$ 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 CO$_2$ fluxes between the liquid phase and the
gas phase. From those results we concluded that for lemna minor less than 10% of the
oxygen (CO$_2$) 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 CO$_2$ 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 CO$_2$ 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
($k_{La}$) and decreased exponentially with surface coverage. In the case of Lemnaceae, the
contribution of macrophytes to O$_2$ and CO$_2$ balances can be quantified in a close respirometer.
Exchanges happen mostly directly within the gas phase. As growth rates are now easier to

\[ f(I) = \frac{I}{I_{M}} \exp \left( \frac{I}{I_{M}} \right) \]

(3)

$I_{M}$ = Optimal light intensity

$A_1$ = 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.
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


