BENELUX
Young Water Professionals
Regional Conference

the international water association

Microalgae for wastewater treatment: measuring and modelling the autotrophic, heterotrophic and mixotrophic growth of *Chlorella vulgaris*

D. Manhaeghe[1*], T. Blomme[1], D.P.L. Rousseau[1], S.W.H. Van Hulle[1~]

[1] Laboratory for Industrial Water and Ecotechnology (LIWET), Department of Green Chemistry and Technology, Ghent University Campus Kortrijk, Graaf Karel de Goedelaan 5, B-8500 Kortrijk, Belgium
[*] Dave.Manhaeghe@UGent.be
[~] Stijn.VanHulle@UGent.be

Abstract: The use of microalgae for wastewater treatment shows great potential. However, optimization of this process is needed to make it economically more viable. A model-based approach is a cost-effective and efficient way to do this. Therefore, a microalgae model developed for the species *Chlorella vulgaris* is now further extended with the processes of heterotrophic and mixotrophic growth.

Keywords: microalgae; respirometry-titrimetry; modelling

Introduction

Microalgal based wastewater treatment is promising in view of making this treatment more sustainable. It omits the necessary aeration in the activated sludge process [1]. Moreover, microalgae are capable of storing N and P in their cells, which leads to nutrient recovery and avoids eutrophication of receiving water bodies [2]. The bottleneck, however, is that the microalgae harvesting process is still expensive. One of the reasons for this is the low biomass concentration [3]. This is inherent to the process since a highly concentrated microalgae suspension will lead to self-shading. A possible way to increase the biomass concentration is selecting microalgae that can grow both autotrophically and heterotrophically (i.e. mixotrophically), since the latter allows growth under dark conditions. As such, in this research, the growth of the microalgae species *Chlorella vulgaris* was measured for the first time under autotrophic, heterotrophic and mixotrophic conditions using combined respirometry-titrimetry. This data was then used to extend a previously developed microalgae model describing the autotrophic growth of microalgae [4].

Material and Methods

An inoculum of the microalgae strain *Chlorella vulgaris* was obtained from the Department of Biology from KU Leuven, Belgium. Microalgae cultivation was performed batch-wise in a 2 L bioreactor using a modified BG11 medium. To assess the autotrophic, heterotrophic and mixotrophic growth, a combined respirometric-titrimetric unit was used [4]. From the respirometric profile, the specific oxygen production/consumption rate could be derived (P_{O2}, g O_2.g $DW^{-1}.d^{-1}$). Analogously, from the titrimetric profile, the specific proton/hydroxide addition rate could be derived (P_{H+}, mole H^+. g $DW^{-1}.d^{-1}$/P_{OH-}, mole OH^-. g $DW^{-1}.d^{-1}$).

The parameters light, bicarbonate concentration (IC) and glucose concentration (OC) were varied according to table 1.1. The light intensity in all tests was 73 µmol.$m^{-2}.s^{-1}$, IC and OC were set at two levels (IC: 75 mg.L^{-1} and 190 mg.L^{-1}; OC: 75 mg.L^{-1} and 100 mg.L^{-1}). All 9 experiments were conducted in duplo with the carbon source as limiting nutrient. Based on a literature review, no tests were performed with organic carbon and inorganic carbon without light, and with inorganic carbon without light. Before and after each test, the nutrients (i.e. NO_3-N, NH_4-N, PO_4-P and COD) and dry weight concentration were determined according to standard methods [5].

Table 1.1: Experimental setup

	Light	IC	OC
Endogenous respiration			
Endogenous respiration + light	x		
Autotrophic growth	x	x	
Heterotrophic growth			x
Heterotrophic growth + light*	x		x
Mixotrophic growth	x	x	x

*only conducted with 75 mg glucose.L^{-1}

Results and Conclusions

The results indicated a net oxygen production in the heterotrophic test with the lights on (0.10 ± 0.02 g O_2.g $DW^{-1}.d^{-1}$). Since no IC was added during these tests, this indicates that the microalgae are capable of using the produced CO_2 during heterotrophic growth as an inorganic carbon source for autotrophic growth. Furthermore, the oxygen consumption was significantly larger during the heterotrophic tests than during the tests with only endogenous respiration. When comparing mixotrophic growth with autotrophic growth, no significant difference could be observed in both P_{O2} and P_{H+}. However, the increase in dry weight during the mixotrophic tests was higher than during the autotrophic tests. Moreover, the ratio of increase in dry weight during the mixotrophic tests to the sum of the increase in dry weight during the autotrophic tests and heterotrophic tests was 1.6 for the test with 75 mg.L^{-1} HCO_3^- and 75 mg.L^{-1} glucose, and 2.4 for the test with 190 mg.L^{-1} HCO_3^- and 100 mg.L^{-1} glucose. This indicates a synergistic effect during the mixotrophic growth which is attributed to the internal CO_2/O_2 recirculation [6]. This was supported by the titrimetric profiles. The first part of the mixotrophic profile was almost exactly the same as in the autotrophic test, which indicates an inhibition effect of HCO_3^- on the heterotrophic growth (figure 1.1, left). Furthermore, in the second part of the mixotrophic titrimetric profile, a reduced P_{OH-} was observed in comparison with the heterotrophic test, indicating synergistic gas exchange (figure 1.1, right).

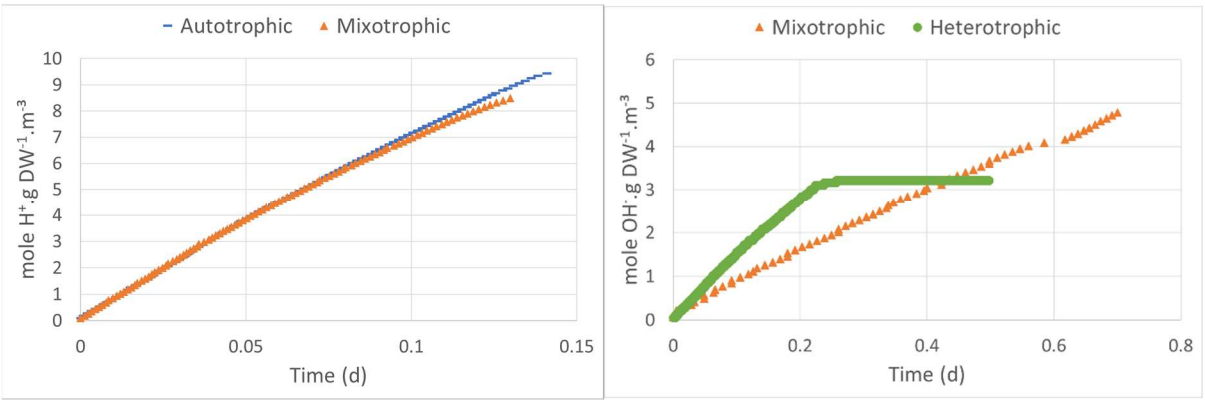

Figure 1.1: Titrimetric profile of the autotrophic tests (-) and first part of the mixotrophic tests (Δ) (left); Titrimetric profile of the heterotrophic tests (*) and second part of the mixotrophic tests (Δ) (right)

When modelling the heterotrophic growth, it was found that only 73% of the glucose was consumed. This indicates that a part of the glucose was stored inside the microalgae cell. As such, it was necessary to incorporate internal carbon storage in the model. In order to model the mixotrophic growth, an inhibition term for HCO_3^- was implemented and Monod kinetics were used in order to regulate the internal recirculation of CO_2 and O_2. The final microalgae model was able to predict all the observations, with Theil's Inequality Values below 0.3 [4].

References

[1] S. Van Den Hende, H. Vervaeren, H. Saveyn, G. Maes, and N. Boon, "Microalgal bacterial floc properties are improved by a balanced inorganic/organic carbon ratio," *Biotechnol. Bioeng.*, vol. 108, no. 3, pp. 549–558, 2011.

[2] I. T. D. Cabanelas *et al.*, "Comparing the use of different domestic wastewaters for coupling microalgal production and nutrient removal," *Bioresour. Technol.*, vol. 131, pp. 429–436, 2013.

[3] A. I. Barros, A. L. Gonçalves, M. Simões, and J. C. M. Pires, "Harvesting techniques applied to microalgae: A review," *Renew. Sustain. Energy Rev.*, vol. 41, pp. 1489–1500, 2015.

[4] D. Manhaeghe, S. Michels, D. P. L. Rousseau, and S. W. H. Van Hulle, "A semi-mechanistic model describing the influence of light and temperature on the respiration and photosynthetic growth of Chlorella vulgaris," *Bioresour. Technol.*, vol. 274, 2019.

[5] APHA, AWWA, and WEF, *Standard Methods for the Examination of Water and Wastewater*. 1999.

[6] R. T. Smith, K. Bangert, S. J. Wilkinson, and D. J. Gilmour, "Synergistic carbon metabolism in a fast growing mixotrophic freshwater microalgal species Micractinium inermum," *Biomass and Bioenergy*, vol. 82, pp. 73–86, 2015.