Kinetic Exploration Of The Microalgal Growth Rate Of Species Residing In A Waste Stabilization Pond

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ABSTRACT

Since the oxygen concentration is an important operational parameter of Waste Stabilization Ponds (WSP), accurate insight in the oxygen production kinetics of the microalgae living in such systems, is essential in view of system optimization. In this research the growth kinetics of the most dominant microalgal species, namely *Chlorella vulgaris* was investigated when different conditions of light intensity and temperature were imposed. This microalgal species was isolated from the WSP situated near Cuenca, Ecuador.

A combined respirometric and titrimetric set-up was used to assess the microalgal kinetics. The experimental results illustrated the interdependent relationship of light intensity and temperature, which had a significant influence on the microalgal growth rate. Consequently the growth kinetics, proposed in a previous developed model were extended with a mathematical function that describes this relationship. Additional combined respirometric and titrimetric data was used for model calibration and model validation. Two parameters were considered for model calibration, namely the maximum specific growth rate and the oxygen mass transfer coefficient. Based upon the Theil's Inequality Criterium, the model described the dissolved oxygen production and the related proton consumption rather good.

1. INTRODUCTION

Wastewater treatment by Waste Stabilization Ponds (WSP) is widely used in the world to treat different types of wastewater, ranging from domestic to industrial waste water. The most important advantage of this system is its simplicity in construction and operation. Moreover WSP exhibits a high reliability because its operation depends mainly on biological processes and does not depend on equipment performance that can fail (Von Sperling, 2007). In the treatment of domestic wastewater by WSP, the aerobic stabilization of organic compounds by bacteria and the oxygen production and nutrient removal by algal (photosynthetic) activity are the main occurring natural processes. The oxygen demand by bacteria for the assimilation of organic substrate is met by the oxygen produced through algal photosynthetic activity. The photosynthetic activity of algae depends on several environmental conditions such as light (Richmond, 2004), temperature (Bordel et al., 2009) and availability of nutrients (Yao et al., 2010; Broekhuizen et al., 2012). Further, Carvalho and Malcata (2003) demonstrated the interdependent relationship of light intensity and temperature. The basic assumption for this was, that for a given temperature, there is a direct relation between light intensity and activation energy and as such a light dependency of the activation energy (Carvalho and Malcata, 2003). Also, the light saturation level is influenced by the temperature. Indeed, augmentation of temperature shifts the light saturation point to higher light intensities and as such also the intensity at which photo-inhibition occurs (Sorokin and Krauss, 1962).

Alvarado (2012) stated that the oxygen production and consequently the amount of dissolved oxygen in the pond, is a fundamental operational parameter for both maintain a healthy aerobic biomass and to induce adverse conditions in the ponds for pathogen viability. Thus, it is essential to (accurately and frequently) quantify the influence of operational conditions over the oxygen production. Further in view of system optimization, good insight in the kinetics of the microalgal biomass is a prerequisite. For this, the combined respirometric and titrimetric methodology (Decostere et al., 2013) was used in this research to determine the growth kinetics of microalgal species in the facultative pond of the full scale installation at Ucubamba, Ecuador. This WSP is designed to treat the domestic effluent form the city of Cuenca (Ecuador). The combined respirometric and titrimetric methodology involves the measurement of the dissolved oxygen production and the proton consumption at constant pH by photosynthetic activity during a batch – wise conducted experiment. The main isolated microalgal species in the WSP was *Chlorella vulgaris* and therefore this species will be used during the experiments. In particular, the effect of light intensity and temperature was assessed. In addition a kinetic growth model, based upon the activated sludge models (ASM)

(Henze et al., 2000) was developed, calibration and validation was performed with the specific experimental data.

2. MATERIALS AND METHODS

2.1 Cultivation of the microalgae

Chlorella vulgaris was isolated from the biomass of the full – scale WSP installation located near Cuenca. Isolation was done by controlled growth on specific media. After isolation, the microalgal species was bred axenic in continuous stirred 3.0 L reactors with ideal inorganic carbon, nutrients and light availability. Also the reactors were periodically sparged with air to prevent settlement of the microalgae on the walls of the breeding reactors and stripping of oxygen produced by the microalgae. Further the microalgae were kept in exponential growth phase by bi-weekly refreshing of the breeding medium and harvesting of microalgae.

2.2 Experimental protocol

The combined respirometric and titrimetric unit (Fig. 1) was similar to Decostere et al. (2013). The 1.6 L reactor vessel was heat-jacketed to allow temperature control (Alpha R8, www.lauda.de) enabling the exploration of system behaviour at different temperatures. The light cage enclosing the reactor entirely consisted of 36 fluorescent lamps (Voltech,T5 8 W). Light intensity was measured using a lux light meter (FC 840020, Sper Scientific).

Dissolved oxygen (DO) and pH were measured online with an oxygen (Inpro 6870i, Mettler Toledo) and pH electrode (Inpro R 4260 i/SG/120, Mettler Toledo) and the data logged using a PCI-MIO-16XE-50 data acquisition card using LabView (www.ni.com). The pH was controlled online at a user defined set-point using a banded (+/- 0.1 pH) on-off feedback control algorithm implemented in LabView by dosing HCl or NaOH through two 3-way pinch solenoid valves (Cole Parmer). The rate and amount of 0.25 M HCl and 0.5 M NaOH dosed into the reactor vessel constitutes the titrimetric data.

For the respirometric tests microalgae from the breeding reactor were used, after centrifugation at 4000 rpm for 5 minutes (Heraeus Megafuge 8 Centrifuge, Thermo Scientific). The microalgae where then rinsed twice with demineralized water. Subsequently, the concentrated algae were diluted in 1.5 L of demineralized water. Next 100 ml of nutrient solution containing nitrogen and phosphorus was spiked into the solution. Then, a sample (200 ml) was taken to analyse the initial microalgal biomass and nutrient concentrations. Finally, 100 ml of bicarbonate solution was spiked before the start of each test. As such, it was strived

to keep the amount of nutrients and algal biomass for each separate test constant, namely 15 g NH_4^+ – N m^{-3}, 0.6 g NO_3^- – N m^{-3}, 1.5 g PO_4^{3-}P m^{-3} and 100 g HCO_3^- m^{-3}. The microalgal biomass concentration was 100 g DW m^{-3} . The pH was controlled at 7.5 ± 0.1 for each separate test.

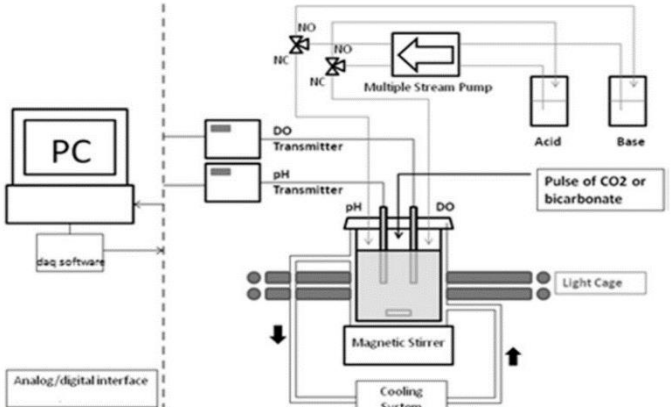

Fig 1. Schematic overview of the combined respirometric – titrimetric setup

In order to investigate the effect of light intensity and temperature and possible interaction between those environmental variables, a 2 level full factorial design (Box and Draper, 1987) was at first used. As such 7 experiments (Table 1) were performed. The maximal photosynthetic activity, expressed as g O_2 g $DW^{-1}d^{-1}$ was considered as response variable (y). This value was derived from the oxygen production rate (OPR) curve (Decostere et al., 2013), more specifically the maximum value of this curve. Next linear regression was used to determine the significant variables and possible interaction. The equation to describe this photosynthetic activity can be denoted as:

$$y = b_0 + b_1X_1 + b_{12}X_1X_2 + b_2X_2 \quad \text{(Eq.1)}$$

With b_0, b_1, b_{12} and b_3 the different coefficients and X_1, X_2 the variables influencing the photosynthetic activity, respectively light intensity (lux) and temperature (K). The interaction term in this equation is represented by $b_{12}X_1X_2$.

The statistical analysis was performed with SPSS.

2.3 Analytical methods

Nitrate, phosphate and ammonium were measured according to the standard methods (APHA, 2005). The microalgal dry weight concentration was determined by turbidity measurements,

which was previously related to the microalgal dry weight by means of the linear equation shown below:

$$Y = 3.06\,X - 8.54 \quad \text{(Eq.2)}$$

With Y (g DW m^{-3}) the microalgal dry weight concentration and X (FTU) the feature of turbidity measurement.

2.4 Modelling software

For the simulations described in this work, the Flexible Modelling Environment (FME) package (Soetaert and Herman, 2009) was used. Although this open access package only uses a textual interface, recently it has been more intensively used in view of ecological modelling (Haario et al., 2009; Mannina et al., 2012)

Parameter estimation was performed by the minimization of an objective function by using an optimization algorithm. The objective function was defined as SSE between model prediction and measurements, which could be denoted as:

$$J\,(\theta) = \sum_j^z \sum_{i=1}^N (y_{i,j} - y_{i,j}(\theta))^2 \quad \text{(Eq.3)}$$

in which $J(\theta)$ represents the objective function based on N data points and $y_{i,j}$ and $y_{i,j}(\theta)$ represent the measured data and predicted value for the variable j respectively, with i the index of the data points ranging from 1 to N. θ includes the parameters to be estimated. It can be derived from this equation, that the objective function calculation was performed for the variables (here dissolved oxygen concentration and proton addition) simultaneously.

Minimization of the objective function was done by the Levenberg – Marquardt (Yu and Wilamowski, 2011) algorithm.

2.5 Goodness – of – fit

When model calibration and validation was performed, the goodness – of – fit between measured and calculated was quantified by calculating the Theil's inequality coefficient (TIC) (Theil, 1961) which can be denoted as follows:

$$TIC = \frac{\sqrt{\sum_i (y_i - y_{i,m})^2}}{\sqrt{\sum_i y_i^2} + \sqrt{\sum_i y_{i,m}^2}} \quad \text{(Eq. 4)}$$

in which y_i represents simulated data and $y_{i,m}$ represents measured data points. A TIC value lower than 0.3 (Coppens et al., 2014) thereby indicates a good agreement with measured data.

3. RESULTS AND DISCUSSION

3.1 Determination of significance of factors

To determine the significance of light intensity and temperature, seven separate experiments were run. The relative maximum photosynthetic activity of each experiment was considered as respons variable for the linear regression. This was derived from the respirometric profiles (Table 1). Results of the linear regression (95 % confidence level) showed the significance of the interdependency of light intensity and temperature (p – statistic = 0.024). The interrelationship between temperature and light intensity can be explained by the fact that there is a direct relation between light intensity and activation energy. Furthermore, the light saturation level is influenced as mentioned before by the temperature, next to the prevailing light intensity (Carvalho and Malcata, 2003). As such this aspect was taking into account for model development.

Table 1. Initial settings of light intensity and temperature and corresponding relative maximum photosynthetic activity for *C. vulgaris*

I (lux)	T (K)	$p_{O_{2,max}}$ (g O_2 g^{-1} DW d^{-1})
4810	283	0.08
4810	306	0.59
4810	290	0.13
10650	290	0.12
1000	290	0.26
10650	298	0.66
10650	303	1.00

3.2 Model development

In the previous section, experiments were performed to assess the effect of light intensity and temperature and the interdependent relationship between those variables. In this section, a model taking into account inorganic carbon, phosphorus and nitrogen kinetics (Decostere et al., 2016) was extended. This allows to describe several experiments conducted at different temperatures and light intensities by using one uniform combination of parameter values

The interrelationship between temperature and light intensity was described here as (Carvalho and Malcata, 2003):

$$f\ (I,T) = (\frac{K_1\ I}{K_2 T+I}) e^{-\beta(\frac{I}{T})} \quad \text{(Eq. 5)}$$

Where I (lux) and T (K) represent respectively the prevailing light intensity and temperature. ß (-) represents a constant related to the activation energy and ideal gas constant. K_1 (-) and K_2 (K^{-1}) are constants related to the light intensity and temperature.

The growth rates proposed by Decostere et al. (2016) on different inorganic carbon sources and nutrients can subsequently be extended as follows:

$$\rho_{Alg(CO_2,NH_4^+,PO_4^{3-})} = \mu_{max} \left(\frac{S_{CO_2}}{K_{CO_2}+S_{CO_2}}\right)\left(\frac{S_{NH_4^+}}{K_{NH_4^+}+S_{NH_4^+}}\right)\left(\frac{S_{PO_4^{3-}}}{K_{PO_4^{3-}}+S_{PO_4^{3-}}}\right)\left(\frac{K_1\ I}{K_2\ T+I}\right) e^{(\frac{-\beta\ I}{T})}\ X_{Alg} \quad \text{(Eq.6)}$$

$$\rho_{Alg(CO_2,NO_3^-,PO_4^{3-})} = \mu_{max} \left(\frac{S_{CO_2}}{K_{CO_2}+S_{CO_2}}\right)\left(\frac{S_{NO_3^-}}{K_{NO_3^-}+S_{NO_3^-}}\right)\left(\frac{K_i NH_4^+}{K_i NH_4^+ + S_{NH_4^+}}\right)\left(\frac{S_{PO_4^{3-}}}{K_{PO_4^{3-}}+S_{PO_4^{3-}}}\right)\left(\frac{K_1\ I}{K_2\ T+I}\right) e^{(\frac{-\beta\ I}{T})} X_{Alg} \quad \text{(Eq.7)}$$

$$\rho_{Alg(HCO_3^-,NH_4^+,PO_4^{3-})} = \mu_{max} \left(\frac{S_{HCO_3^-}}{K_{HCO_3^-}+S_{HCO_3^-}}\right)\left(\frac{Ki_{CO_2}}{Ki_{CO_2}+S_{CO_2}}\right)\left(\frac{S_{NH_4^+}}{K_{NH_4^+}+S_{NH_4^+}}\right)\left(\frac{S_{PO_4^{3-}}}{K_{PO_4^{3-}}+S_{PO_4^{3-}}}\right)\left(\frac{K_1\ I}{K_2\ T+I}\right) e^{(\frac{-\beta\ I}{T})} X_{Alg} \quad \text{(Eq.8)}$$

$$\rho_{Alg(HCO_3^-,NO_3^-,PO_4^{3-})} = \mu_{max} \left(\frac{S_{HCO_3^-}}{K_{HCO_3^-}+S_{HCO_3^-}}\right)\left(\frac{Ki_{CO_2}}{Ki_{CO_2}+S_{CO_2}}\right)\left(\frac{K_i NH_4^+}{K_i NH_4^+ + S_{NH_4^+}}\right)\left(\frac{S_{NO_3^-}}{K_{NO_3^-}+S_{NO_3^-}}\right)\left(\frac{S_{PO_4^{3-}}}{K_{PO_4^{3-}}+S_{PO_4^{3-}}}\right)\left(\frac{K_1\ I}{K_2\ T+I}\right) e^{(\frac{-\beta\ I}{T})} X_{Alg}$$

(Eq. 9)

Considering the function of light intensity and temperature, parameters K_1, K_2 and β were fitted to the relative photosynthetic activity ($p_{O_{2,max}}$) values for the experiments described in section 3.1. After minimization of the SSE, values of K_1= 12.23 (-), K_2 = 21.75 (K^{-1}) and β = 0.013 (-) were obtained. Other biokinetic and physical – chemical parameters were taken from Decostere et al. (2016).

3.3 Model calibration

Two separate experiments were used for further model calibration. In particular, the maximum specific growth rate (μ_{max}) and the oxygen mass transfer coefficient ($K_L a$) were calibrated to the combined respirometric and titrimetric data. These parameters were considered for model calibration, because in Decostere et al. (2016), it was illustrated that these parameters are uniquely identifiable to the combined respirometric and titrimetric data. The first experiment was performed at a light intensity of I = 10650 lux. The temperature was controlled at 313 K and the initial microalgal biomass concentration equaled 91.75 g DW m^{-3}. The second calibration experiment was run at a light intensity of 4810 lux and a temperature of 308 K. The initial biomass concentration was 104 g DW m^{-3}. Like already mentioned before it was strived to use similar values of nitrate, phosphate and ammonium for each separate test, respectively 15 g NH_4^+- N m^{-3}, 0.6 g NO_3^- - N m^{-3} and 0.2 g PO_4^{3-} - P m^{-3} . In Figure 2, the predicted and experimental dissolved oxygen profiles and proton addition profiles are illustrated.

For the first calibration experiment a TIC = 0.06 and TIC = 0.05 was calculated for the respirometric and titrimetric profile, indicating good model performance (Coppens et al., 2014). Also good visual correspondence between experimental and predicted values was observed. Considering the second calibration experiment, a visual less good fit (respirometric profile) was observed. However still low values for TIC were calculated, namely TIC = 0.07 for the respirometric profile and TIC = 0.05 for the titrimetric profile. The optimized parameter values for the first experiment were μ_{max} = 0.56 ± 0.0008 d^{-1} and $K_L a$ = 10.02 ± 0.02 d^{-1}. For the second calibration experiment μ_{max} = 0.62 ± 0.0001 d^{-1} and $K_L a$ = 3.76 ± 0.003 d^{-1} were obtained. The optimal values for the maximum specific growth rate were akin for the two experiments.

3.4 Model validation

One additional experimental runs were used as validation experiment. The mean value of the optimized values for the maximum specific growth rate was used for the simulations. As such a value of μ_{max} = 0.59 d^{-1} was used. Considering the oxygen mass transfer coefficient, the mean of the two calibration was used. So a value of $K_L a$ = 6.43 d^{-1} was used. The validation experiment was run with a light intensity of I = 10650 lux and temperature T = 2

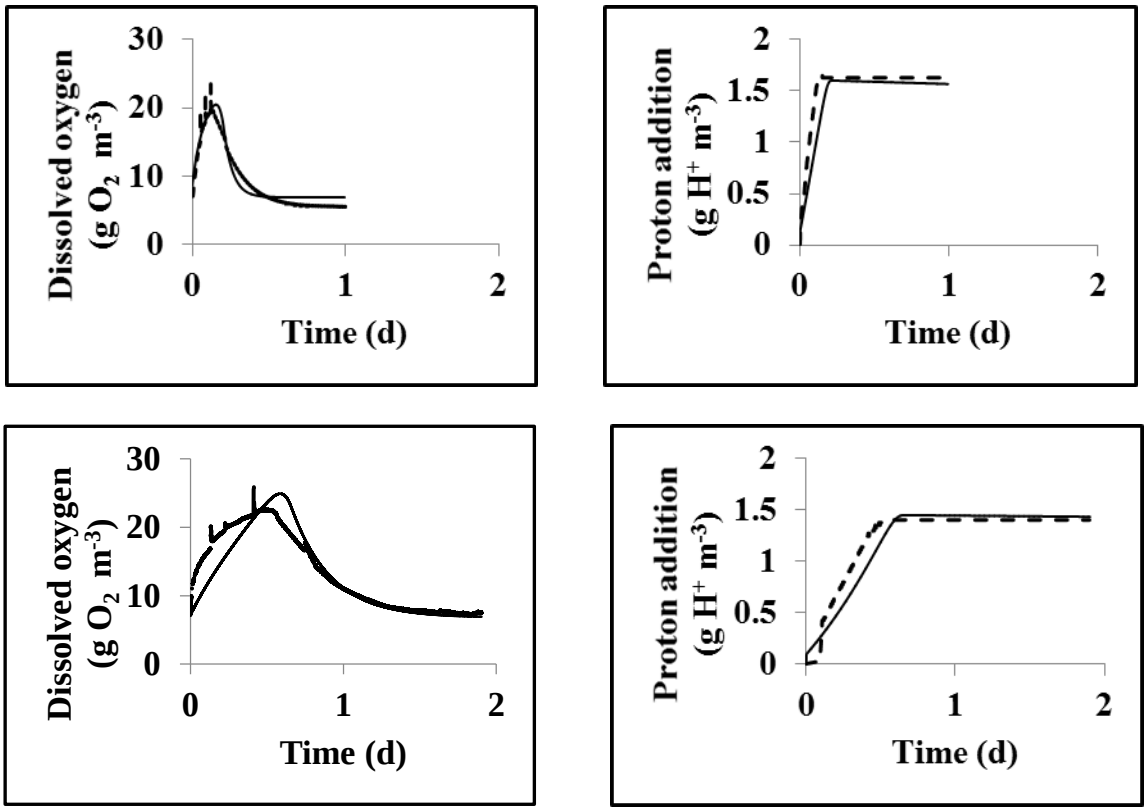

Fig 2. Experimental (dashed line) and predicted (full line) dissolved oxygen concentration (left hand) and proton addition (right hand) for the first (top) and second (bottom) calibration experiment with *Chlorella vulgaris.* The first experiment was performed at a light intensity of I = 10650 lux. The temperature was controlled at 313 K and the initial microalgal biomass concentration equaled 91.75 g DW m^{-3}. The second calibration experiment was run at a light intensity of 4810 lux and a temperature of 308 K. The initial biomass concentration was 104 g DW m^{-3}.

The initial microalgal biomass concentration was 60.94 g DW m^{-3}. In Figure 3 the predicted and experimental values of dissolved oxygen (left) and proton addition (right) are depicted. Good visual correspondence in the first part of the respirometric profile was observed. In the descending part a slight deviation between experimental and predicted values could be noted. This could be explained by the difference of experimental and optimized values of oxygen mass transfer coefficient. Indeed, the experimental value was determined without microalgal biomass present in the reactor vessel and this can have influence on the parameter (Pittoors et al., 2014). Considering the titrimetric profile also good correspondence in the first part was observed and minor deviation between experimental values and predicted values after the declination point. This can be explained by the fact that the amount of bicarbonate dosed to the reactor vessel slightly deviated from the foreseen amount. To overcome this drawback, an additional sensor to online measure the inorganic concentration in the liquid phase might be

suggested. Overall good model performance could be concluded since the calculated TIC values were 0.19 for the respirometric profile and 0.04 for the titrimetric profile.

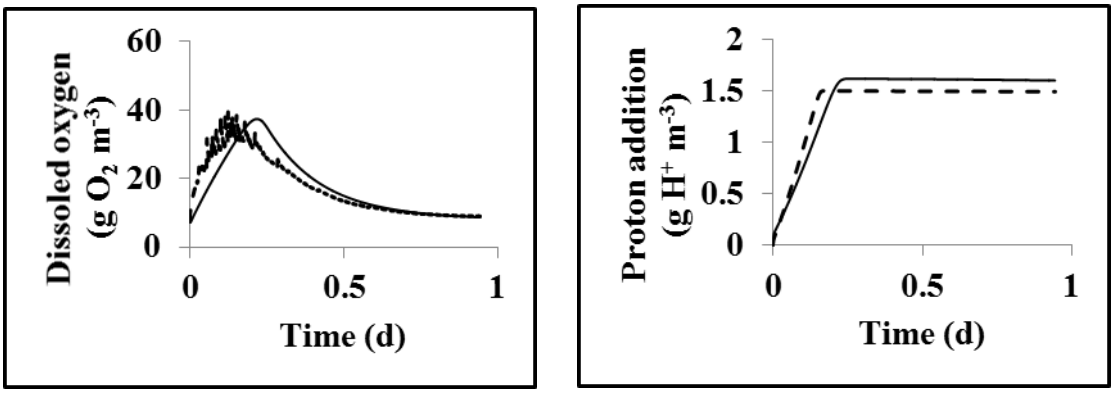

Fig 3. Experimental (dashed line) and predicted (full line) profiles for the validation experiments with *Chlorella vulgaris*. The experiment was run with I= 10650 lux and T= 298. Initial biomass concentration was 60.94 g DW m^{-3}.

4. CONCLUSIONS

In this research, the combined respirometric and titrimetric methodology was used to assess the microalgal growth kinetics of a microalgal species that was prior isolated from the biomass of a WSP. Results showed the interdependent effect of light intensity and temperature on the growth rate. As such a prior developed model (Decostere et al., 2016) was extended with a mathematical function which describes this interdependent relationship (Carvalho and Malcata, 2003). Good model performance with optimized parameter values of maximum specific growth rate and oxygen mass transfer coefficient was obtained. Further model validation with an additional experiment illustrated good model performance for both microalgal species. Next the optimized values for the maximum specific growth rates was μ_{max} = 0.590 d^{-1} for *Chlorella vulgaris* . This value is similar to growth rates reported in literature.

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