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Additional Information

Effect of temperature on ammonium removal in *Scenedesmus* sp.

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ABSTRACT

The effect of temperature on microalgal ammonium uptake was investigated by carrying out four batch experiments in which a mixed culture of microalgae, composed mainly of *Scenedesmus* sp., was cultivated under different temperatures within the usual temperature working range in Mediterranean climate (15-34 °C). Ammonium removal rates increased with temperature up to 26 °C and stabilized thereafter. Ratkowsky and Cardinal Temperatures models successfully reproduced the experimental data. Optimum (31.3 °C), minimum (8.8 °C) and maximum (46.1 °C) temperatures for ammonium removal by *Scenedesmus* sp. under the studied conditions were obtained as model parameters. These temperature-related parameters constitute very useful information for designing and operating wastewater treatment systems using these microalgae.

Keywords

Ammonium uptake; microalgae; modeling; temperature; wastewater.

HIGHLIGHTS

Ratkowsky's model accurately reproduced the temperature effect on ammonium removal

Cardinal temperatures model (CTM) obtained analogous results as Ratkowsky's equation

The CTM is preferred due to the biological significance of its parameters

The obtained theoretical optimum temperature for ammonium removal was 31 °C

1. INTRODUCTION

Interest in microalgae has risen in the last decades due to the combination of several factors, three of which are highlighted here for their somewhat bigger impact on research: a) microalgae can be used to obtain renewable fuels such as biodiesel, biohydrogen or biogas, therefore contributing to reduce fossil fuel consumption b) microalgae use CO₂ for their growth, and therefore contribute to reduce greenhouse gas emissions and c) pollutants such as phosphate, nitrate and ammonium can be successfully removed from wastewaters by microalgae, since they grow on inorganic nutrients which they take from the medium.

Several authors have indeed proved that the use of microalgae is a valid option for wastewater treatment, as reviewed by Wu et al. (2014). Many efforts have also been made to study their growth kinetics (Aslan and Kapdan 2006, Ruiz et al. 2013, Wu et al. 2013), or to predict their production of biomass or substances of interest, such as lipids or sugars (Adesanya et al. 2014, Tevatia et al. 2012). Temperature influence on microalgal growth has also been modeled in various ways. Béchet et al. (2013) and Ras et al. (2013) present reviews of this matter. The former recommends uncoupled models

(those which do not account for interdependence of light and temperature) and the latter insists on the important effect that high temperatures and microalgal adaptation to environmental conditions have on the functioning of outdoor production systems.

Essentially, the Arrhenius Law (eq. 1) has been widely used for describing temperature influence on microalgal growth (for instance by Geider et al.1998 or Ketheesan and Nirmalakhandan 2013), although it was originally proposed for chemical reaction rates and it does not describe properly the negative effect of high temperature on microbial growth.

$$\mu(T) = \mu(T_r) \cdot \theta^{(T-T_r)} \quad (1)$$

where T_r (°C) is the reference temperature and θ is the Arrhenius constant.

Ratkowsky et al. (1983) were the first to model the microbial growth rate reduction observed at temperatures above an optimum value (eq. 2).

$$\sqrt{\mu(T)} = b \cdot (T - T_{min}) \cdot \{1 - \exp[c \cdot (T - T_{max})]\} \quad (2)$$

where b and c are parameters with no biological meaning obtained by data fitting and T_{min} and T_{max} are the minimum and maximum temperatures, respectively, at which growth rate is zero.

Otherwise, Bernard and Rémond (2012) used the so-called cardinal temperature model with inflexion (CTMI) (eq. 3) to predict the effect of temperature on microalgal growth using parameters which all have a biological meaning:

$$\mu = \mu_{max} \cdot \frac{(T-T_{max}) \cdot (T-T_{min})^2}{(T_{opt}-T_{min}) \cdot [(T_{opt}-T_{min}) \cdot (T-T_{opt}) - (T_{opt}-T_{max}) \cdot (T_{opt}+T_{min}-2T)]} \quad (3)$$

Where T_{\min} (°C) is the temperature below which the growth is assumed to be zero, T_{\max} (°C) is the temperature above which there is no growth and at temperature T_{opt} (°C) maximal growth rate μ_{\max} (d^{-1}) occurs. To our knowledge, this expression has been previously used for microalgal growth only by this author.

The purpose of this study is to assess the direct influence of temperature on the ammonium uptake rate, due to the aforementioned interest on the microalgal ability to remove pollutants from wastewater, and to select a mathematical equation to model the observations. To this aim, four reactors seeded with a mixed culture of indigenous microalgae with clear predominance of *Scenedesmus* sp. were kept at different temperatures and the microalgal ammonium uptake rate was measured. The models previously detailed in this section were used to reproduce the observed data.

Temperature-related parameters for ammonium removal could thus be obtained.

2. MATERIALS AND METHODS

2.1. Microorganisms

Microalgae were isolated from the walls of the secondary clarifier in the WWTP “Cuenca del Carraixet” (Valencia, Spain) and maintained in the laboratory in a 7 l semicontinuous reactor, using as growth medium the effluent of a pilot-scale submerged anaerobic membrane bioreactor (SAnMBR) which operates in the WWTP (Giménez et al. 2011). The SAnMBR treats a small fraction of the WWTP incoming wastewater, and therefore its effluent displays variable nutrient content of 30-50 mg $\text{NH}_4\text{-N}\cdot\text{l}^{-1}$ and 4-7 mg $\text{PO}_4\text{-P}\cdot\text{l}^{-1}$. This effluent had previously proved to contain all necessary micronutrients to sustain algal growth (Ruiz-Martinez et al. 2012). The biomass formed a stable ecosystem where the dominant microalgae belonged to the Chlorococcal order, of which >99% to the *Scenedesmus* genus.

2.2. Experimental setup and operation

Four experiments were carried out in four cylindrical glass reactors with a total and working volume of 2 and 1.8 l, respectively. Around each reactor a coil of transparent silicon tube was installed for temperature control. Cooling/heating water temperatures were controlled by thermostatic baths (Lauda Alpha RA8). Reactors 1 to 4 were thus kept at 15 °C, 18 °C, 26 °C and 34 °C, respectively. Each reactor was equipped with electronic sensors in order to obtain on-line temperature and pH measurements. The probes were connected to a multiparametric analyzer (CONSORT C832, Belgium), which was in turn connected to a PC for data monitoring and storage. Data sampling was conducted every 60 s. A fine bubble diffuser was mounted at the bottom of each reactor in order to mix the algal culture by injecting compressed air. Pure (99.9%) CO₂ from a pressurized cylinder was injected into the compressed air flow whenever pH rose above 7.5. This pH control avoided phosphate precipitation and free ammonia stripping. The reactors stood in two confronted lines of two reactors each. From the front of the first line and from the back of the last line, a total of eight vertical fluorescent lamps (Sylvania GroLux, 18 W) constantly illuminated the reactors (four lamps in the front and four lamps in the back). Photosynthetically active radiation (PAR) of $180 \pm 21 \mu\text{E m}^{-2} \text{s}^{-1}$ was measured at the center of the empty setup.

At the beginning of the experiments, which were carried out in batch mode, 1300 ml of the SAnMBR effluent (containing 34 mg NH₄-N·l⁻¹ and 5.6 mg PO₄-P·l⁻¹) were transferred into each reactor. Phosphate (in the form of KH₂PO₄) and ammonium (in the form of (NH₄)₂SO₄) were added to each reactor in order to reach the concentrations of 14 mg PO₄-P·l⁻¹ and 42 mg NH₄-N·l⁻¹, respectively. These values were considered high enough for avoiding N or P deficiency during the whole experiment. When the

temperature setpoint was reached, 500 ml of biomass culture (taken from the semicontinuous reactor as explained in section 2.1) were added to each reactor. Total Suspended Solids (TSS) measured in this culture allowed us to calculate the initial biomass concentration resulting in each reactor, which was $115 \text{ mg TSS}\cdot\text{l}^{-1}$. The experiments lasted between 25 and 53 hours, during which ammonium and biomass concentrations were measured periodically.

2.3. Analytical Methods

Ammonium and phosphate were determined according to Standard Methods (APHA 2005) (4500-NH₃-G and 4500-P-F, respectively) in a Smartchem 200 automatic analyzer (Westco Scientific Instruments, Westco). TSS were determined at the beginning and the end of the experiment according to Standard Methods (APHA 2005), and calculated during the batch experiments using the equation which describes their linear relationship with absorbance. Absorbance was measured at 750 nm by a UV-VIS spectrophotometer (Merck Spectroquant® Pharo 300). All reported results were obtained from the previous analyses conducted in duplicate.

3. RESULTS AND DISCUSSION

3.1. Temperature influence on ammonium uptake

Initial ammonium concentration was measured in each reactor immediately after microalgae addition, and periodically during the rest of the experiment. Time evolution of ammonium concentrations in the four reactors is shown in figure 1. It is hypothesized that initial ammonium concentrations are not identical in all experiments due to the time lapse (for temperature stabilization) between nutrient addition in the reactors and microalgae addition (when the pH control system was switched on). Ammonium concentrations always decreased at a constant rate in all the reactors: four straight lines

can be observed in figure 1, one for each temperature tested. The slope of each line represents the ammonium removal rate of the corresponding reactor. The least-squares fitting method from Excel ® was applied to fit the obtained data and to calculate the slope of each trendline.

$$r_{\text{NH}_4-15^\circ\text{C}} = 4.3 \text{ mg N}\cdot\text{l}^{-1}\cdot\text{d}^{-1} \quad r_{\text{NH}_4-18^\circ\text{C}} = 6.7 \text{ mg N}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$$

$$r_{\text{NH}_4-26^\circ\text{C}} = 15.7 \text{ mg N}\cdot\text{l}^{-1}\cdot\text{d}^{-1} \quad r_{\text{NH}_4-34^\circ\text{C}} = 17 \text{ mg N}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$$

These values are in the same range or higher than those obtained by other authors. Park and Jin. (2010) reported an ammonium removal rate of 5-6 mg N·l⁻¹·d⁻¹ by *Scenedesmus* sp. from a piggery farm wastewater with no mention to temperature. Voltolina et al. (2005) reported an ammonium removal rate of 8-9 mg N·l⁻¹·d⁻¹ by *Scenedesmus obliquus* in artificial wastewater at 25.5 °C, which is a smaller value than the one obtained in this study for a similar temperature. Also at 25 °C, Kim et al. (2013) optimized nitrogen removal by *Scenedesmus* sp. by changing the wavelength of the used light, and reported a maximum removal rate of 15 mg N·l⁻¹·d⁻¹, which is similar to the value obtained in this study.

Phosphate concentrations in the reactors were measured at the end of each experiment to rule out any possible effect of phosphorus limitation. Phosphorus limitation was discarded since phosphate levels in the reactors remained in all cases above 5 mg PO₄-P·l⁻¹.

Regarding the variation of ammonium removal rates with temperature, a bell-shaped curved is expected for describing the relationship between microalgal activity and temperature, although individual shapes are species dependent, and also influenced by environmental conditions (Ras et al. 2013). In this study, a fast (lineal) increase was

observed for the first three temperatures evaluated: ammonium removal rate at 26 °C was twice the value obtained at 18 °. In contrast, no substantial difference was observed between the values obtained at 26 °C and at 34 °C.

It is undeniable that temperature is an operation parameter which, being on the one hand relatively easy to control, defines on the other hand the refrigerating or heating costs of the process, which might amount to a high fraction of the total costs and affect process sustainability. Taking the influence of temperature on ammonium removal rate into account can help optimizing the compromise between operation costs and removal efficiency.

3.2. Mathematical models evaluation and calibration

Using the Solver program in Microsoft ® Excel 2007 software for minimizing the residual sum of squared errors between the experimental data and the model predictions, experimental data were accurately reproduced with Ratkowsky's expression (figure 2a), obtaining the following parameter values:

$$b = 0.36 \quad c = 0.04 \quad T_{\min} = 7.1 \text{ °C} \quad T_{\max} = 47.4 \text{ °C}$$

Statistical analysis was carried out using SPSS 16.1. Pearson correlation coefficient (P-value < 0.01) was 0.998.

The cardinal temperature model with inflexion (CTMI) adopted by Bernard and Rémond (2012) for microalgal growth could also accurately reproduce the experimental data of ammonium removal rates (figure 2b). Pearson coefficient (P-value<0.01) was 0.997 and the following parameter values were obtained:

$$r_{\text{NH}_4\text{max}}=17.5 \text{ mg N}\cdot\text{l}^{-1}\cdot\text{d}^{-1} \quad T_{\min} = 8.8 \text{ °C} \quad T_{\max} = 46.1 \text{ °C} \quad T_{\text{opt}} = 31.3 \text{ °C}$$

Maximum ammonium removal rate obtained is in agreement with our observations. Minimum and maximum temperatures are in accordance with the values obtained with Ratkowsky's model, as well as optimum temperature. Although Ratkowsky's model does not include a model parameter representing the optimum temperature as such, it does predict an optimum temperature around 30 °C (see figure 2a).

The shape of the removal rate curve versus temperature is in the case of the CTMI more asymmetrical, showing a faster decrease after the optimum temperature. The area under the curve is also smaller, since the minimum temperature is slightly higher than in the case of the Ratkowsky's equation, and the maximum temperature is slightly smaller. Nonetheless, the predictions obtained with both models are very similar, and both models accurately reproduced the experimental data. Under these circumstances, the CTMI expression was preferred for modeling the effect of the temperature on the ammonium removal rate of *Scenedesmus* sp. under the studied conditions, since the parameters have a biological significance whose meaning is straightforward to understand, thus making calibration easier (for instance the decision for the initial values of parameters). In contrast, parameters b and c in Ratkowsky's model have no direct biological interpretation, being mathematical parameters defined in order to reproduce the experimental data.

Hodaifa (2010) reported an optimum temperature for *Scenedesmus obliquus* growth rate of 29.6 °C, and Sanchez (2008) reported, for *Scenedesmus almeriensis*, optimum growth temperatures of 30-35 °C depending on light intensity and a maximum temperature of 48 °C. These parameters are in agreement with the ones obtained in this study.

4. CONCLUSIONS

This work describes the effect of temperature on ammonium removal rate of the microalgae *Scenedesmus* sp. The cardinal temperature model used by Bernard and Rédmond (2012) for microalgal growth was successfully used to fit the data and to obtain theoretical minimum, maximum and optimum temperatures for ammonium removal by *Scenedesmus* sp. This information is very useful for the operation of microalgae based wastewater treatment systems, since removal rates are affected by temperature, which is an easy to control parameter while at the same time responsible for a percentage of the operation costs.

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FIGURE CAPTIONS

Figure 1: Experimental data: Ammonium concentrations measured in each reactor during the experiment.

Figure 2: Data fitted using a) Ratkowsky's equation and b) the cardinal temperature model with inflexion