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Modeling light and temperature influence on ammonium removal by *Scenedesmus* sp. under outdoor conditions

Short title: Modeling of light and temperature influence on ammonium removal by Scenedesmus

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ABSTRACT

The ammonium removal rate of the microalga *Scenedesmus* sp. was studied under outdoor conditions. Microalgae were grown in a 500 l flat-plate photobioreactor and fed with the effluent of a Submerged Anaerobic Membrane Bioreactor (SAnMBR). Temperature ranged between 9.5 ºC and 32.5 ºC and maximum light intensity was 1860 $\mu mol \cdot m^{-2} \cdot s^{-1}$. A maximum specific ammonium removal rate of 3.71 mg NH_4-N·g $TSS^{-1} \cdot h^{-1}$ was measured (at 22.6 ºC and with a light intensity of 1734 $\mu mol \cdot m^{-2} \cdot s^{-1}$). A mathematical model considering the influence of ammonium concentration, light and temperature was validated. The model successfully reproduced the observed values of ammonium removal rate obtained and it is thus presented as a useful tool for plant operation.

Keywords: Ammonium removal; light; microalgae; modeling; temperature; wastewater

INTRODUCTION

Microalgae are photosynthetic microorganisms whose ability to eliminate inorganic nutrients from different kind of wastewaters is well known (Wu et al., 2014). Their application for such purposes has also the advantages of atmospheric CO_2 fixation, less energy consumption than conventional wastewater treatment methods, and biomass generation, among others. Generated microalgal biomass can be used for biofuels obtention and as fertilizer (Brenan and Owende, 2010).

Predicting the behavior of microalgal cultures is a very complex task, especially when the cultivation takes place outdoors, under changing environmental conditions of light and temperature. Nutrient levels are also variable in applications which use real wastewaters as growth medium. Changing conditions, together with the microalgal responses to these external conditions (such as selfshading, photoacclimation or changes in pigments, metabolites and reserve compounds) and with the physical characteristics of the photobioreactor system (such as geometry and agitation, which influence mass and heat transfer) should all be taken into account in order to obtain the best reproduction of the processes taking place in the microalgal culture.

However, the higher the number of known phenomena taken into account, the higher the complexity of the models obtained. Thus, when a model is proposed in order to predict the microalgal behavior in a real wastewater treatment system, a compromise needs to be found between accuracy and ease of application and computation.

The aim of this work was to propose and validate a mathematical model which accounts for the effect of ammonium concentration, light and temperature on the microalgal ammonium removal rate under full scale changing outdoor conditions. This model would allow for real-time prediction of a photobioreactor system performance when

treating wastewater, which is of great help for plant control and operation. For this aim, the authors proposed a multiplicative combination of mathematical expressions which are able to accurately reproduce experimental data under stable laboratory conditions (Ruiz-Martinez et al., 2014; Ruiz-Martinez et al., 2015a; Ruiz-Martinez et al., 2015b). The suitability of these expressions to also reproduce the observed ammonium removal rates taking place in a bigger scale under outdoor conditions was therefore tested and validated, and the corresponding parameters were obtained, which allows for further application of the model in a photobioreactor-based wastewater treatment system.

MATERIALS AND METHODS

Microorganisms

Microalgae were isolated from the walls of the secondary clarifier in the "Cuenca del Carraixet" Wastewater Treatment Plant (Valencia, Spain) and maintained in the laboratory in a 7 L semicontinuous reactor (for details see Ruiz-Martinez et al., 2014), using as growth medium the effluent of a submerged anaerobic membrane bioreactor (SAnMBR) described in Giménez et al., 2011. The biomass formed a stable ecosystem where the dominant microalgae belonged to the Chloroccocal order, of which > 99% to the *Scenedesmus* genus. The photobioreactor (PBR) was seeded with this culture (10% of the PBR volume) and the effluent from the SAnMBR system (90% of the PBR volume). Microalgae were then allowed to grow in batch mode until a concentration of 600 mg $TSS \cdot L^{-1}$ was reached. The dominant microalgae genus was *Scenedesmus* for the whole duration of the experiment.

Experimental setup and operation

Microalgae cultivation was performed during 30 days in a 500 L flat-plate PBR made of transparent methacrylate and placed outdoors, in the "Cuenca del Carraixet" WWTP. Its dimensions were 125 x 200 x 25 cm (height x length x width). The 125 x 200 cm surface (perpendicular to the ground) was facing south in order to improve solar irradiance. The PBR was continuously stirred by air sparging (0.06 vvm), which allowed homogenization of the culture and prevented wall fouling. pH was controlled at 7.5 by adding pure (99.9%) CO_2 through an automatic valve whenever the pH reached the maximum value established.

The PBR was fed with the effluent from the existing SAnMBR system described in Gimémez et al. (2011). This SAnMBR system is fed with the pre-treated urban wastewater (screening, degritter, and grease removal) of the "Cuenca del Carraixet" WWTP. Influent nutrient load was therefore variable (46.9 ± 4.3 mg NH_4-N·L^{-1} and 5.9 ± 1.3 mg PO_4-P·L^{-1}), depending on the influent to the WWTP and on the performance of the SAnMBR plant. Nitrite and nitrate concentration were negligible (~ 0 mg·L^{-1}), as expected from an anaerobic effluent.

The SAnMBR effluent was fed daily to the PBR in a total of 5 to 10 deliveries (depending on the cellular retention time (CRT)), which were evenly distributed during the light hours. The CRT at which the PBR was operated was varied during the operational period. It was established at 3 days during the first 5 days and at 5.5 days from day 6 until the end of the operational period. Temperature and solar irradiation varied freely at all times as a result of the changing environmental conditions.

A group of on-line sensors submerged in the reactor constantly monitored the culture. They consisted of the following: one pH-temperature transmitter (HachLange pHD-S

sc), one turbidity sensor to measure total suspended solids (TSS) (HachLange SOLITAX sc), one dissolved oxygen (DO) sensor (HachLange LDO) and one ammonium-nitrate (NH_4-N and NO_3-N) concentration sensor (HachLange AN-ISE sc). An irradiation sensor (HOBO® Smart Sensor, s-lia-m003), which measured only the photosynthetically active radiation (PAR), was located on the vertical surface of the PBR facing south. Data was continuously acquired and saved on a PC during the 30 days of operation, during which the PBR was fed around 200 times.

Analytical Methods

Phosphate level in the PBR was determined weekly according to Standard Methods (APHA 2005, 4500-P-F) in a Smartchem 200 automatic analyzer (Westco Scientific Instruments, Westco). Samples were analyzed in duplicate.

RESULTS AND DISCUSSION

Ammonium removal rates

Fig. 1 shows the evolution of light, temperature and ammonium concentration in the PBR on a sunny day when light intensity increased in the morning and decreased in the afternoon without important oscillations (dotted line). It was observed that temperature increase generally suffered a lag with respect to light intensity, so that maximum temperatures occurred during the last minutes of daylight (dashed line). Ammonium (filled line) started decreasing when light intensity increased (at sunrise), and continued to do so during the light hours, with the exceptions of the times when the SAnMBR effluent was added. At those points, ammonium concentration rapidly increased. Seven

of these rapid increases can be seen in fig. 1. For each day of the experiment, a temperature-light-ammonium profile was obtained. Since the PBR was placed outside, these profiles were different for each day.

The data taken by the ammonium sensor revealed the decrease of ammonium to be linear between two consecutive feed deliveries. Ammonium decrease was due to microalgae activity, who took it up from the medium, provided the light intensity was high enough. Microalgal ammonium uptake rate after every SAnMBR effluent injection was thus calculated -using Microsoft ® Excel 2007- as a linear regression of the ammonium concentration values represented versus time. Data provided by the suspended solids sensor allowed calculating the specific ammonium uptake rate. PAR intensity and temperature were averaged for each period of linear ammonium decrease between SAnMBR effluent additions from the information recorded by the respective sensors. When the light oscillation was too abrupt data were discarded since an average value would not be representative. Thus, 183 sets of data were obtained, each of them consisting of four values: the measured specific ammonium removal rate immediately after the feed injection and the corresponding averaged ammonium concentration, temperature and light intensity to which the culture was subject during the same period of linear ammonium decrease.

Maximum light intensity was 1860 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and temperature ranged between 9.5 ºC and 32.6 ºC. Suspended solids in the reactor were stable around 640 mg $TSS \cdot L^{-1}$ for the first 8 days and decreased afterwards to oscillate in the range of 320-480 mg $TSS \cdot L^{-1}$. Ammonium concentration ranged between 1.1 and 22.4 mg $NH_4\text{-}N \cdot L^{-1}$. The changes in ammonium and biomass concentration were not only influenced by microalgal metabolism and environmental conditions, but also by pilot plant operation (mainly the

138 modification in CRT). Phosphate levels in the PBR stayed relatively constant and above
139 1 mg PO_4-P·L^{-1}.

140 When the calculated ammonium removal rates are represented along a temperature axis,
141 a bell-shaped distribution can be observed (fig. 2). Maximum uptake rates for each
142 temperature increased with increasing temperature until reaching 22.6 ºC, where the
143 highest removal rate of the whole experimental period was obtained. Maximum
144 ammonium uptake rates for each temperature decreased thereafter. The maximum
145 ammonium uptake rate measured was 1.54 mg NH_4-N·L^{-1}·h^{-1}, which is similar to the
146 values reported by Wang and Lan (2011) (1.8 mg NH_4-N·L^{-1}·h^{-1} for *Neochloris*
147 *oleoabundans*) or Ackerstrom et al. (2014) (1.37-1.7 mg NH_4-N·L^{-1}·h^{-1} for *Chlorella*
148 sp.) and higher than the value reported by McGinn et al. (2012) (1 mg NH_4-N·L^{-1}·h^{-1} for
149 *Scenedesmus* sp.). The corresponding maximum specific ammonium uptake rate was
150 3.71 mg NH_4-N·$gTSS^{-1}$·h^{-1}. This ammonium uptake rate corresponded to averaged
151 ammonium concentration and light intensities of 7.7 mg NH_4-N·L^{-1} and 1734 µmol·m^{-2}·s^{-1}, respectively.

153 Since different removal rates were measured for the same temperature (data points
154 situated vertically above each other along the whole temperature range in figure 2), it is
155 clear that other factors, such as light intensity and ammonium concentration, also
156 affected the microalgal ammonium uptake rate. In order to partly account for this, in
157 figure 2 data was grouped by light intensity ranges. Analysis of this figure indicates that
158 lowest intensities (up to 400 µE·m^{-2}·s^{-1}) were normally associated to low temperatures,
159 and also ammonium uptake rates below 1 mgN·$gTSS^{-1}$·h^{-1} were achieved (fig. 2, black
160 dots). Between 400 and 1200 µE·m^{-2}·s^{-1} both associated temperatures and ammonium
161 uptake rates increase, although around the highest temperature range of 30 ºC no
162 ammonium removal rates above 0.5 mgN·$gTSS^{-1}$·h^{-1} were observed (fig. 2, dark grey

dots). The interval between 1200 and 1600 $\mu E \cdot m^{-2} \cdot s^{-1}$ confirms this observation: highest ammonium uptake rates under these conditions do not correspond to the highest temperatures achieved, but they rather appear between 10 and 25 ºC. (fig. 2, light grey dots). Data points presenting a temperature around 10 ºC and high light average (> 1100 $\mu mol \cdot m^{-2} \cdot s^{-1}$) represent times around noon, when light intensity has increased rapidly and temperature is still low. Finally, the highest ammonium removal rates were obtained, within the interval of 20-23 ºC, for the highest light intensities measured (fig. 2, empty dots).

A practical conclusion that can be drawn from figure 2 is that, under the studied conditions, high temperatures could be a bigger operational problem (in terms of low ammonium removal rates achieved) than high light intensities.

Model development

A mathematical model was proposed to describe the influence of ammonium concentration, light and temperature on the ammonium removal rate observed in the PBR. Influence of intracellular phosphorus content was not taken into account, since the level of phosphate in the medium stayed above 1 mg PO_4-$P \cdot L^{-1}$ during the whole duration of the study, and it was thus possible to assume that microalgae intracellular phosphorus concentration was relatively constant. This simplifies the effort and the time required to obtain the model input.

As previously proposed and validated for a laboratory scale microalgae culture (Ruiz-Martinez et al., 2014), the influence of ammonium concentration in the medium was represented using Monod kinetics (eq. 1), light influence was modeled by Steele's equation (eq. 2) and temperature was modeled using the Cardinal Temperatures Model

with inflexion (eq. 3) proposed by Bernard and Rémond (2012) for microalgae and previously used by the authors for modeling a laboratory scale microalgal system (Ruiz-Martinez et al., 2015b):

$$\frac{S_{NH4}}{k_S+S_{NH4}} \quad (1)$$

$$\frac{I}{k_i} \cdot exp\left(1-\frac{I}{k_i}\right) \quad (2)$$

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

where S_{NH4} (mg N·L^{-1}) represents ammonium concentration in the medium and k_S (mg N·L^{-1}) is the semisaturation constant for ammonium. I (µE·m^{-2}·s^{-1}) is light intensity and k_i (µE·m^{-2}·s^{-1}) is the optimal light intensity. T_{min} (ºC) is the temperature below which the growth is assumed to be zero, T_{max} (ºC) is the temperature above which there is no growth and at temperature T_{opt} (ºC) maximal growth rate occurs.

Thus, the expression used to predict microalgal specific ammonium removal rate was a combination of the above explained equations (eq. 4):

$$r_{spN} = r_{spNmax}\frac{S_{NH4}}{k_S+S_{NH4}}\frac{I}{k_i}\exp\left(1-\frac{I}{k_i}\right)\frac{(T-T_{max})\cdot(T-T_{min})^2}{(T_{opt}-T_{min})\cdot[(T_{opt}-T_{min})\cdot(T-T_{opt})-(T_{opt}-T_{max})\cdot(T_{opt}+T_{min}-2T)]} \quad (4)$$

where r_{spNmax} represents the maximum specific nitrogen uptake rate (mg N·h^{-1}·mg TSS^{-1}). I was calculated as an average light intensity, taking into account the reactor's geometry and Lambert-Beer's Law (eq. 5) for representing the selfshading effect of the biomass

$$I = I_0 \cdot \exp(-a \cdot TSS \cdot z) \quad (5)$$

where I_0 ($\mu E \cdot m^{-2} \cdot s^{-1}$) is incident light intensity, a ($m^2 \cdot gTSS^{-1}$) is the microalgal self-shading factor, and z (m) is the distance from the surface of the reactor. As in previous studies (Ruiz-Martinez et al., 2015a) a microalgal self-shading factor of 0.0758 $m^2 \cdot g\ TSS^{-1}$ was used.

Model calibration

The 183 sets of data obtained allowed calibration of the proposed model, 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. The initial values for the model parameters were selected based on previous results (Ruiz-Martinez et al., 2014) and on the obtained experimental data (fig. 2). The values obtained for the model parameters (Table 1) accurately reproduced the experimental data (fig. 3). Statistical analysis was carried out using SPSS 16.1, which showed a Pearson correlation coefficient of 0.876 (P-value < 0.01).

The obtained maximum specific ammonium removal rate, r_{spNmax}= 4.7 mg N· g $TSS^{-1} \cdot h^{-1}$, is in accordance with the maximum ammonium uptake rate measured in the experiment (25% higher). A value of 2.5 mg $N \cdot L^{-1}$ for parameter k_S implies a high affinity of the microalgae for ammonium, which is reasonable for the given growth conditions. k_i presents a higher value than the parameters obtained in our previous laboratory scale studies (180 and 200 $\mu E \cdot m^2 \cdot s^{-1}$ according to Ruiz-Martinez et al., 2014 and Ruiz-Martinez et al., 2015b, respectively), possibly since in the outdoor pilot plant the microalgal culture is adapted to higher light intensities, and thus the optimal intensity for the present culture is higher than for the microalgae which have grown under lower light intensities in the laboratory experiments of the cited works. According

to Richmond (1986), species cultivated under outdoor conditions should be able to tolerate light variations and should have a high light saturation constant.

While the minimum temperature obtained in the present study is comparable to that one previously found in the laboratory (8.8 ºC in Ruiz-Martinez et al., 2015b), the optimum and maximum temperatures obtained in the present experiment are sensibly smaller. This discrepancy is possible due to the very different conditions in which microalgae are growing in the PBR outdoors and in the laboratory. Xin et al. (2011) actually reported an optimal temperature of 20 ºC for *Scenedesmus* sp. biomass production, which is in agreement with the result obtained in this study.

It can therefore be assumed that the mathematical expressions which reproduce data obtained in the laboratory can also be combined and used to predict the behavior of microalgae cultivated under outdoor conditions, which constitutes a useful tool for plant design and operation. It has been proved that the model proposed is easy to implement, since calculations are not complex and model input can be continuously obtained with the sensors that monitor the basic culture parameters.

CONCLUSIONS

The present work proposed a mathematical model which represents microalgal ammonium removal rate taking into account the ammonium concentration in the medium, light and temperature. Influences of these parameters were represented with functions which had previously been validated for laboratory scale cultures: a Monod kinetics term, the Steele function and the cardinal temperatures model, respectively. The combination of these terms successfully reproduced the experimental data, therefore validating its suitability for use at full scale and under changing outdoor conditions as

well. However, since the microalgal culture was adapted to different conditions, different model parameters were obtained.

ACKNOWLEDGEMENTS

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303

304 Figure 1: Experimental data: Ammonium, temperature and light intensity during day 14
305 of the experiment

306 Figure 2: Calculated ammonium removal rates distributed along a temperature axis and
307 grouped by light intensity ranges.

308 Figure 3: Parity plot: a comparison of model predictions against observed ammonium
309 uptake rates, using model parameters as indicated in table 1.

310 Table 1: parameters obtained during model calibration.

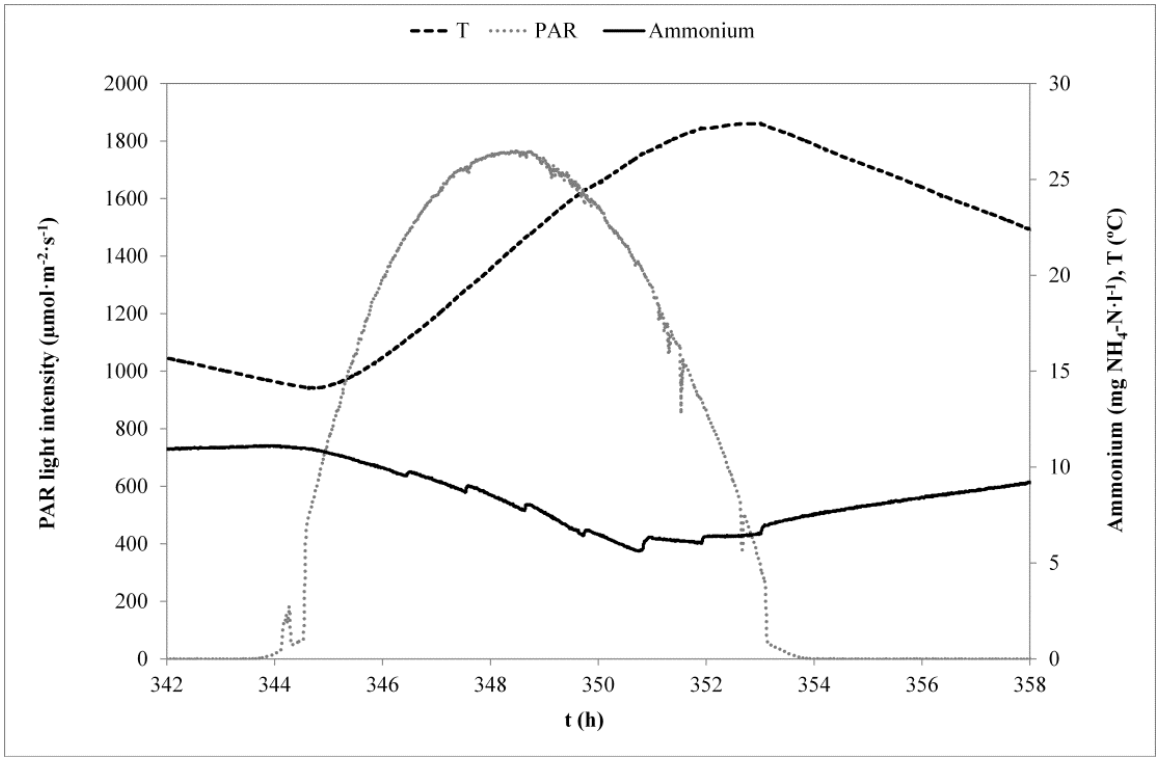
311

312 Table 1

Parameter	Units	Obtained value
r_{spNmax}	(mg N· g TSS^{-1}·h^{-1})	4.7
k_S	mg N·L^{-1}	2.5
k_I	$\mu E \cdot m^2 \cdot s^{-1}$	477
T_{min}	ºC	2
T_{max}	ºC	32
T_{opt}	ºC	20.5

313

314 Figure 1



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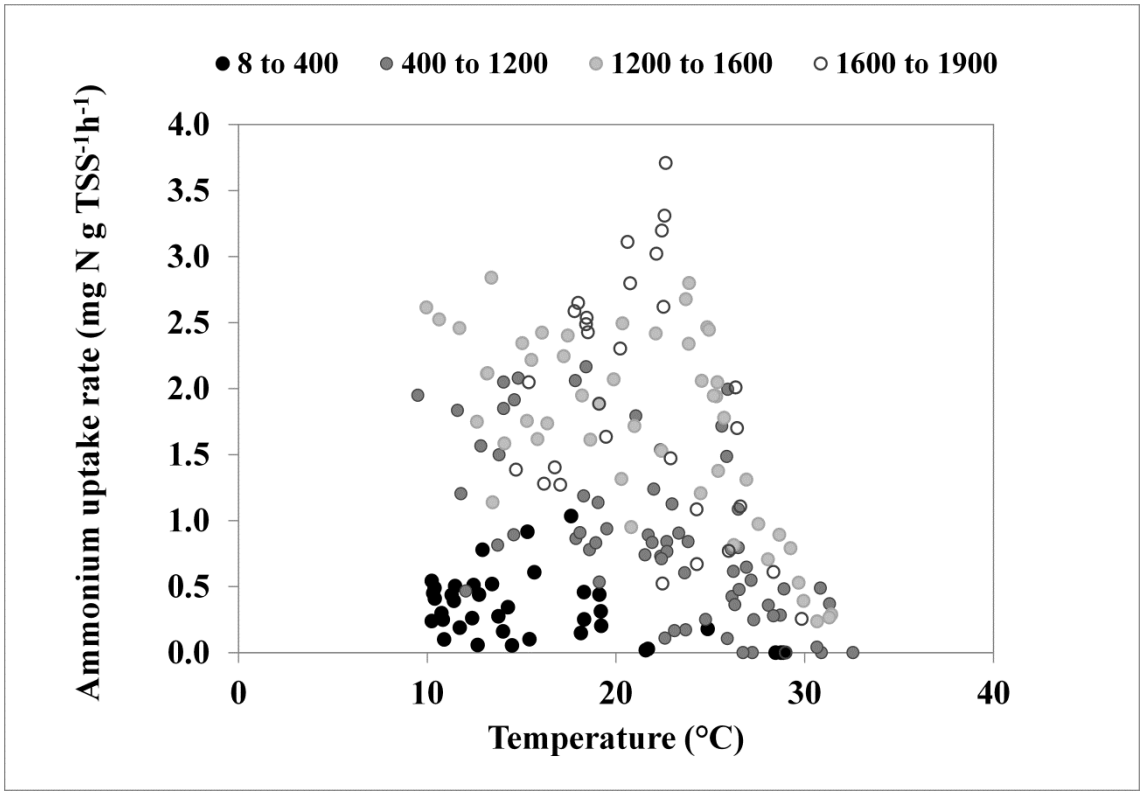
317 Figure 2



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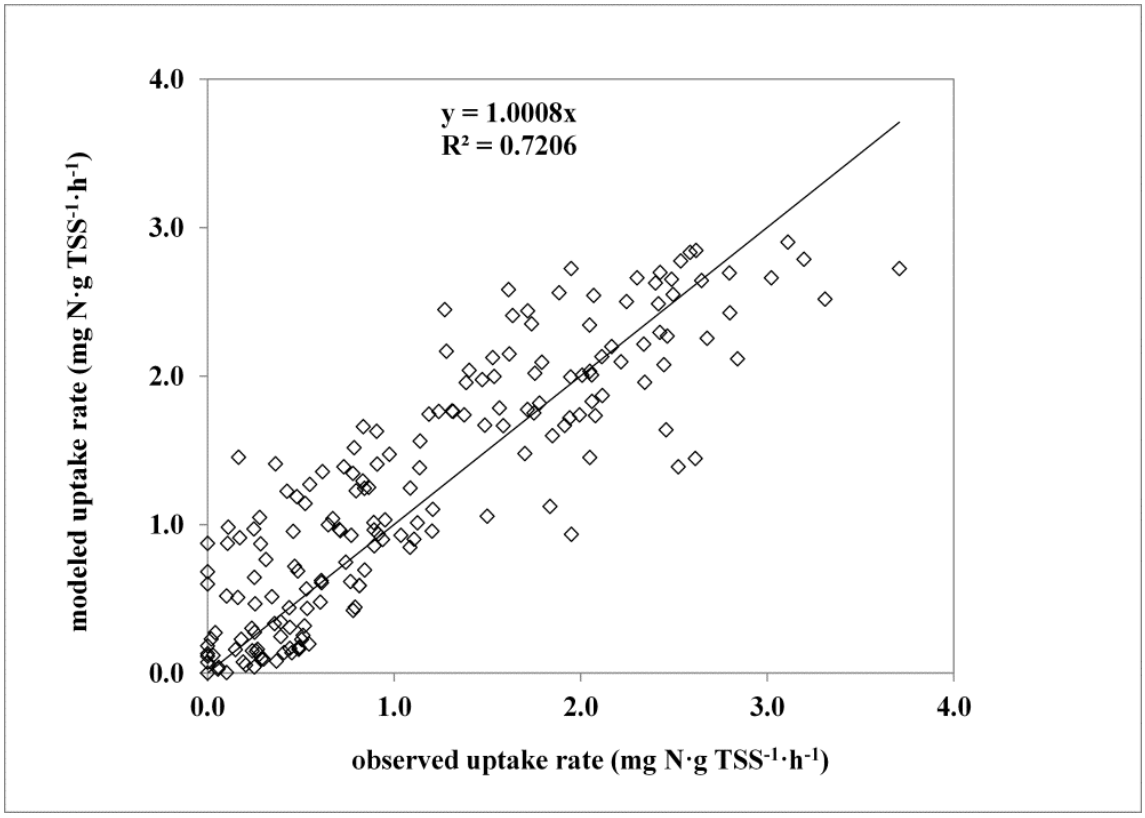
320 Figure 3



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