In-situ UV-vis probe to monitor algal photobioreactors treating municipal wastewater

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Abstract: Algal cultivation has been proposed as a means to produce biofuels. However, the sustainability of algal biorefineries is compromised if microalgae are not cultivated on used water resources. Pending on the end product to be accumulated in algal biomass (e.g., lipids or pigments), cultivation conditions should be optimized. Nevertheless, online monitoring tools used in algal reactors are limited and mostly tested in systems using synthetic media. In this work we present a UV-Vis sensor, conventionally used to monitor municipal wastewater treatment plants, as a means to monitor algal cultivation. Different models have been successfully applied to estimate nitrate, total biomass and pigment concentration using the spectra collected by the sensor.

Keywords: sensor calibration; algal cultivation; pigments; municipal wastewater

INTRODUCTION

Green microalgae cultivation can be used for biogeochemical resource recovery from wastewater. Conventional control systems for photobioreactors (PBRs) rely on pH, temperature or turbidity measurements (Olivieri et al., 2014). However, resource recovery processes, e.g. fertigation or energy recovery, demand additional online measurements of several variables which can be related to nutrient or lipid content in the algae, such as soluble nutrients concentrations (e.g. nitrate) or algal pigmentation (Valverde-Pérez et al., 2016). UV-Vis sensors have been proposed to monitor multiple wastewater constituents (e.g. COD, TSS, nitrate, nitrite) using a single instrument, thus offering the potential to reduce monitoring complexity (Rieger et al., 2004, Mašić et al., 2015). Since algal pigments also absorb radiation in the UV-Vis range (Griffiths et al., 2011), we believe this type of sensor could also be used to monitor the metabolic state of the algae on the basis of their pigmentation.

The main objective of the study thus is to assess UV-Vis spectrophotometry to monitor constituents in microalgal PBRs including nitrate and total suspended solids concentrations (TSS). Additionally, the feasibility to quantify pigments is assessed.

MATERIALS AND METHODS

Microalgae cultivation

A monoculture of *Chlorella sp.* was cultivated for 85 days in 2 continuous-flow PBRs treating the effluent from an enhanced biological phosphorus removal system (operational conditions are described by Wágner (2016). Aeration was provided from the bottom of both PBRs through a diffuser for sufficient mixing and CO_2 supply, further used for pH control between 6.5 and 7.5.

Light was provided from the top of the reactor, with an average incident light intensity of 1100 µmol m^{-2} s^{-1}.

Analytical tools

Total nitrogen and phosphorus measurements in the suspension were done using commercial test kits (Hach-Lange©, USA), whilst ammonium, nitrate, nitrite and phosphate concentrations were measured using test kits by Merck© (USA) after sample filtration through 0.2 µm filter. TSS measurement was done based on the standard methods. Pigment extraction and analysis was executed as described in Wágner (2016). The analysis included the measurement of chlorophyll a and b, violaxanthin, lutein, and beta-carotene.

Triplicates of UV-VIS spectra were recorded between 220nm and 740nm using the Spectro::lyser™ (S::CAN Messtechnik, Vienna, Austria). Principal component analysis (PCA) was used to identify the main variables driving the variability of the collected spectra. Based on the identified principal components (PCs) a principal component regression (PCR) model was built to assess the prediction capacity of the sensor. A leave one out validation method was applied to identify the optimal number of PCs in each PCR model. Details and code demonstrating this procedure can be found elsewhere (Mašić et al., 2015).

RESULTS

Spectra collected during experiments are shown in Fig. 1. Three different regions can be observed, where absorbance is considerably higher than the background. At 220 nm the absorbance relates to the nitrate present in the system. The two peaks observed around 400 and 700 nm likely associate with the algal chlorophyll content (e.g., maximum light absorbance for chlorophyll a absorbance is at 428.6 nm and 664.9 nm). Finally, the high variability in the background may relate, among others, to the variability of the concentrations of the organic matter and suspended solids in the reactor – this is because organics and solids typically absorb light at all wavelengths. Additional backscattering effects may be expected from varying solids concentrations. When analysing the collected spectra via PCA it is shown that the first PC describes 77.6% of their variability (Fig. 2a). The second PC describes more than 20% of the spectra variability, which – based on the loading vector for this PC (Fig. 2b) – likely corresponds to the spectra variability due to nitrate concentration changes during the cultivation period. Finally, PC3 describes little variability (below 5%), which may however be related to variability of the chlorophyll concentration, as there is some significant contrast around 400 nm and 700 nm (Fig. 2b). Models built in a first stage are based on these 3 PCs.

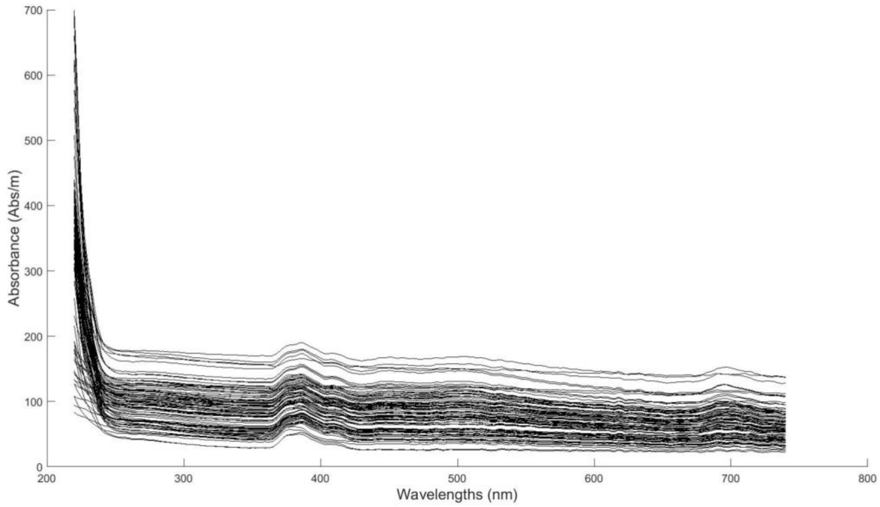

Figure 1. UV-Vis spectra collected during PBR operation.

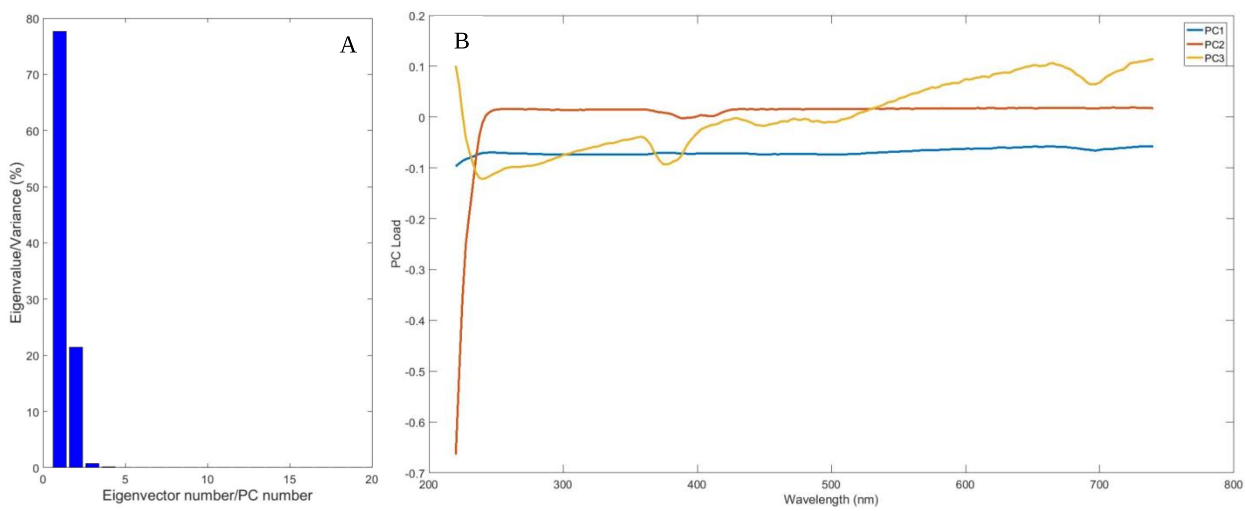

Figure 2. a) UV-Vis spectra collected during PBR operation; b) PC loadings showing the impact of the original variables (i.e. collected spectra) on the 3 first PC.

The model can accurately predict nitrate concentration below 20 mg-N/L (Fig. 3a). However, the signal gets saturated above this level, which is also demonstrated when analysing the residuals, which systematically deviate from zero and increase at higher concentrations (Fig. 3b). After removing samples beyond saturation the model prediction capacity considerably improves (R^2=0.84), thereby demonstrating that the UV-Vis probe can be a valuable tool to monitor nitrate in PBR treating wastewater. For the case of TSS (data not shown), 8 outliers (i.e., 8 samples) were removed to calibrate a model able to capture trends on biomass (R^2=0.78). Finally, for the case of chlorophyll (data not shown), after removing 7 outliers and data in the saturation region (i.e., beyond 7 mg-chlorophyll/L), the model was limited when describing chlorophyll trends (R^2=0.63). Models for violaxanthin, lutein and beta-carotene could not be set, likely due to the low concentration present during the experiments.

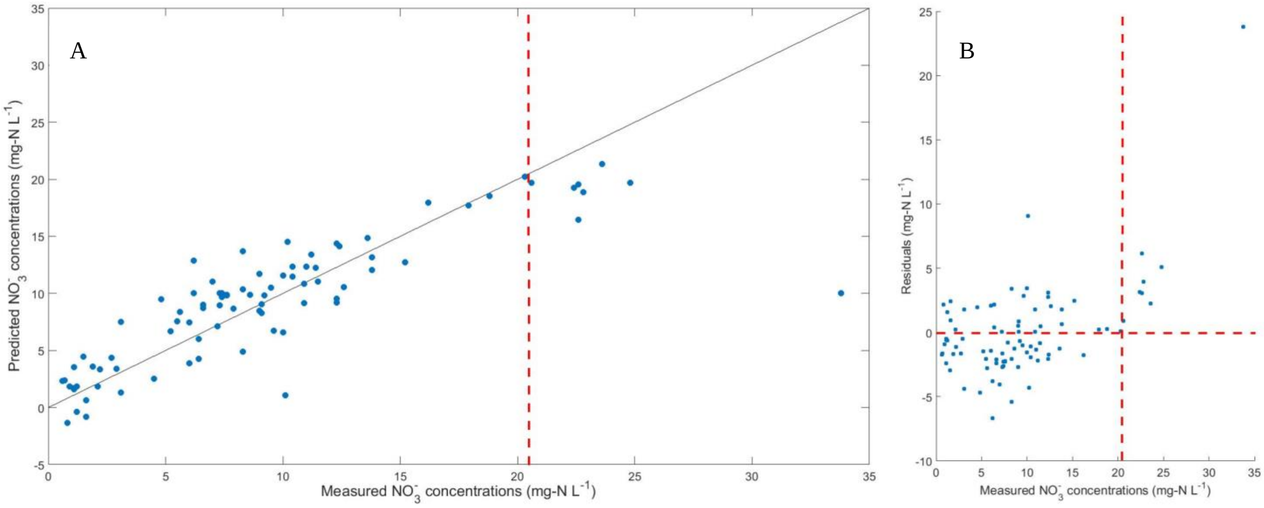

Figure 3. a) Predicted vs measured nitrate concentrations based on 3 PCs. The dashed line shows the starting concentration for signal saturation; b) Residuals. Dashed lines represent zero error (horizontal) and the concentration beyond residuals systematically increase, showing signal saturation (vertical).

Leave one out validation method was applied for the case of nitrate (Fig. 4a) and chlorophyll (Fig. 5a). Results suggest the optimal model for nitrate requires up to 14 PCs, which yields to a model that predicts well nitrate concentration (Fig. 4b; R^2=0.92). For the chlorophyll case, the optimal model requires 27 PCs (Fig. 5b; R^2=0.83), even though models with 15 PCs perform satisfactory below 7 mg-chlorophyll/L (data not shown).

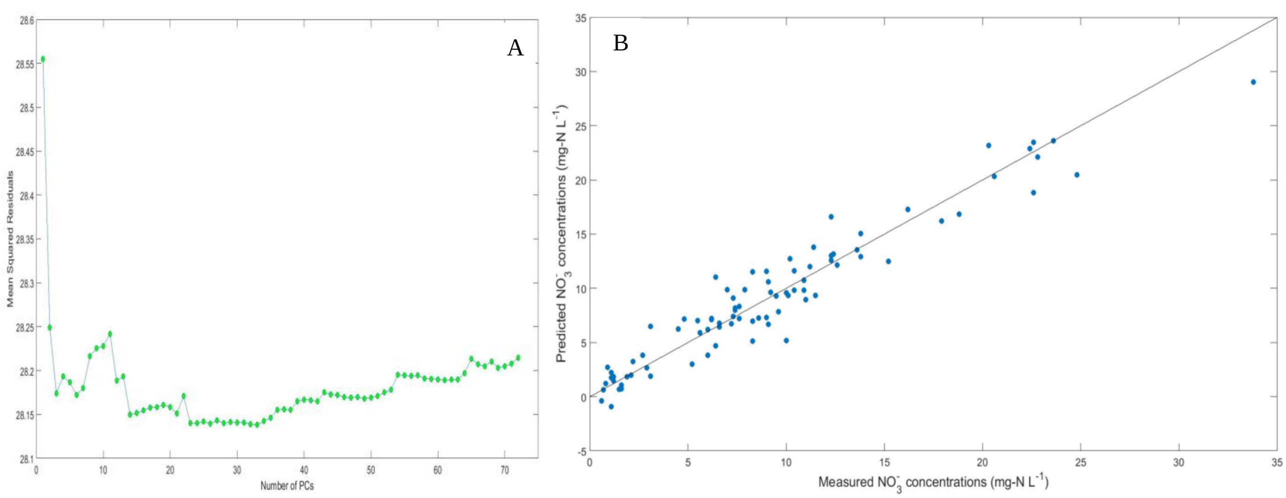

Figure 4. a) Mean squared residuals as function of the model dimensions for the leave one out validation for nitrate. b) Predicted vs measured nitrate concentrations based on 14 PCs.

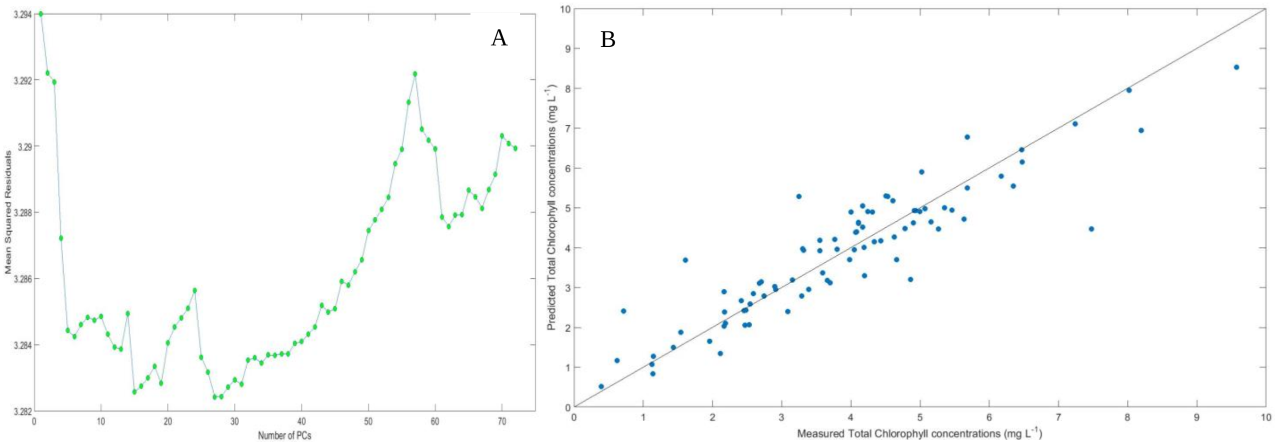

Figure 5. a) Mean squared residuals as function of the model dimensions for the leave one out validation for total chlorophyll. b) Predicted vs measured total chlorophyll concentrations based on 27 PCs.

OUTLOOK

In this work we demonstrate the capability of a UV-Vis probe to quantify nitrate, total chlorophyll and TSS in PBRs treating municipal wastewater for resource recovery. To our knowledge this is the first study demonstrating the use of UV-Vis spectrophotometry as an on-line tool to monitor TSS and chlorophyll in algal systems treating municipal wastewater (commercially available sensors for chlorophyll operate in the range of µg, making them unsuitable for wastewater applications – Havlik et al., 2013). The application of UV-Vis sensors opens new opportunities to develop novel control strategies for PBRs.

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