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# Removal of nitrogen and phosphorus from municipal wastewater effluent using *Chlorella vulgaris* and its growth kinetics

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ABSTRACT

*Chlorella vulgaris* was used for the removal of residual ammonia/ammonium ion ($NH_3/NH_4^+$) and orthophosphate ion ($PO_4^{3-}$) from secondary wastewater effluent collected from a municipal wastewater treatment plant. The uptake rates for nitrogen and phosphorus were studied with different initial algal cell densities and the addition of $CO_2$ gas for pH control and supply of inorganic carbon. Our result showed that typical $NH_3/NH_4^+$ and $PO_4^{3-}$ concentrations could be readily removed within 48 h. It was found that the culture with an initial algal cell density of ~350 mg/L and $CO_2$ gas supply could significantly enhance both the rates of cell growth and nutrient uptake. The Monod equation well described the algal cell growth under substrate-limiting conditions, and could be used for the design and operation of photobioreactors for potential tertiary wastewater treatment.

*Keywords:* *Chlorella vulgaris*; Wastewater effluent; Nitrogen and phosphorus removal; Growth kinetics

## 1. Introduction

Microalgae-derived biodiesel production has recently received great attention due to their ability to produce triacylglycerols for biodiesel while using solar energy and carbon dioxide ($CO_2$). A previous life cycle assessment study [1,2] showed that most energy required for autotrophic algal cultivation is estimated to be used for the production of nutrients (for example, nitrogen and phosphorus) and the power consumption required to increase the mass transfer of $CO_2$ gas into the aqueous phase such as bubbling and mixing. The study recommended that ~50% of the total energy use associated with fertilizer production could be reduced by using nitrogen and phosphorous in wastewater. The use of wastewater for algae growth will reduce not only the cost associated with fertilizer, but also the residual nitrogen concentration present in the wastewater effluent stream, a major contributor to ecological eutrophication [3].

The removal of nutrients such as nitrogen and phosphorus for wastewater treatment was reported in previous studies [4–7] by using *Chlorella* sp. and *Phaeodactylum tricornutum*. Although the potential of microalgae for nutrients removal from wastewater was recognized, little information is available on the feasibility and growth kinetics using microalgae

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required for the design and operation of an algal pond. From a practical standpoint, a hydraulic retention time required for nutrient removal is likely to be one of the major constraints for the realization of tertiary-level wastewater treatment using microalgae [8,9]. *Chlorella vulgaris*, one of the fastest growing green microalgae, is used in this study. In this study, the feasibility of the removal of ammonia/ammonium ion ($NH_3/NH_4^+$) and orthophosphate ion ($PO_4^{3-}$) was studied for potential tertiary wastewater treatment with different initial cell densities. A growth model was also constructed for the design of an algal pond using wastewater.

## 2. Materials and methods

### 2.1. Cultivation medium and conditions

Secondary wastewater samples were collected from the Mill Creek plant located in Cincinnati, OH, USA, a major wastewater treatment facility, where ~70 and ~30% of the wastewater is typically collected from industrial and domestic sources, respectively. The wastewater was used as a culture medium. The capability of *C. vulgaris* to remove nutrients was examined using two initial algal cell densities. For a culture with a low initial algal cell density, a *C. vulgaris* sample suspended in shuisheng-4 medium [10] was added to a 3.7-L wastewater medium (W1) in a 4-L bottle (26 cm (height) × 15 cm (diameter)). The cell density of *C. vulgaris* in the wastewater medium was found to be 44.37 ± 0.60 mg/L after inoculation. During the culture in the wastewater medium, the bottle was closed in order to minimize the evaporation loss of dissolved $CO_2$ into air, and the media were mixed by using a magnetic stirrer at a speed of 350 rpm. Then, the concentrations of $NH_3/NH_4^+$, $PO_4^{3-}$, and total inorganic carbon ($TIC = [HCO_3^-]+[CO_3^{2-}]+[H_2CO_3]+[CO_2(aq)]$), cell density, and the pH of the medium were measured once daily. The experiment with a low initial algal cell density was performed in duplicate. For the culture with a high initial algal cell density, a *C. vulgaris* sample concentrated by centrifugation was added to a 3.7-L wastewater medium (W2) and the algal cell density was found to be 354.48 ± 2.14 mg/L after inoculation. Since the initial TIC concentration was low for the culture with a high algal cell density, $CO_2$ gas was bubbled through the medium every 12 h to supply TIC and control the pH at ~7.

During the culture, light intensity was also kept constant. Fluorescent lamps with 6,500-K color temperature similar to natural sunlight were used as a source of light, and the incoming light intensity to beakers was set to 6,000 lux (100.8 μmol $m^{-2}$ $s^{-1}$) by controlling the distance between the beaker and lamp. The light intensity was measured using a light intensity meter (HQRP digital lux meter, LX1010BS, Osprey-Talon Company), and a 16-h light and 8-h dark cycle was used for the culture.

### 2.2. Determination of cell density of C. vulgaris

The cell density of *C. vulgaris* was determined by measuring the optical density of a 15-mL sample at 682 nm [11] for every 24 h by using UV–Vis spectrophotometer (UV-1800, Shimadzu Scientific Instruments). The absorbance of UV–Vis spectrophotometer at 682 nm was calibrated by measuring the weight of *C. vulgaris* after harvesting and drying. Then, the weight of dried biomass was obtained from the prepared calibration curve.

### 2.3. Determination of total inorganic carbon (TIC) concentration

An acid–base titration method [12] was used to determine the concentrations of inorganic carbon species present in the aqueous phase. This titration method determines a TIC concentration in a 15-mL sample using 0.1 and 0.01 N HCl solutions for the titration of high and low carbon concentrations, respectively. The accuracy of this titration method was ensured by comparing a known amount of TIC dissolved from sodium bicarbonate or sodium carbonate with the amount of TIC determined by the acid–base titration method. All TIC concentrations were measured in duplicate.

### 2.4. Determination of $PO_4^{3-}$ and $(NH_3)/(NH_4^+)$ concentrations

Orthophosphate is measured using the Phosver 3 phosphate reagent available with the HACH Model PO-19 reactive phosphorus measurement kit. A 5-mL sample was taken from a culture medium and a Phosver 3 phosphate reagent was added to it. After 2 min, blue color appeared due to the presence of phosphorus, and the color intensity was proportional to the amount of an orthophosphate concentration in the solution. The color intensity was measured using a UV–Vis spectrophotometer (UV-1800, Shimadzu Scientific Instruments) at 890 nm.

The nitrogen concentrations in $NH_3$ and $NH_4^+$ were measured using an ammonia probe (Model: 9512HPBNWP Orion Thermo Scientific) [13]. All ammonium ions were converted into ammonia by raising the pH of the sample solution of the culture medium above 12, and the resultant ammonia

concentration was determined by the ammonia probe. A 15-mL sample was filtered out using a syringe filter (0.45 μm nominal pore with 24 mm diameter, Whatman filter) in order to avoid potential blockage of the membrane of the ammonia probe. All the measurements were carried out in duplicate.

## 3. Results and discussion

### 3.1. Growth and nutrients removal with low initial cell density

The characteristics of the wastewater samples used for this study are summarized in Table 1.

The growth of *C. vulgaris* and uptake of TIC were measured as shown in Fig. 1(a). The lag-phase period required for adaptation to the wastewater condition was found to be short (24 h), and the TIC concentration was not reduced during the period. During the growth phase, the cell density of *C. vulgaris* significantly increased, and the TIC concentration sharply decreased until 96 h, indicating active photosynthetic reaction. Microalgae can consume only dissolved $CO_2$(aq) and $HCO_3^-$, but, $CO_2$(aq) concentration is much lower than $HCO_3^-$ concentration and insignificant in the pH range between 7 and 10. The carbon content in microalgae was then estimated to be ~50% from a carbon mass balance between the inorganic carbon consumption ($36.28 \pm 1.68$ mg/L) and total biomass gain ($76.33 \pm 2.89$ mg/L) during the entire growth phase (96 h). The carbon content estimated from the culture of *C. vulgaris* is comparable to a carbon content (~50%) of *C. vulgaris* reported in a previous study [2].

Table 1
Characteristics of wastewater samples in Mill Creek plant

| Parameter | Wastewater 1 used for low initial algal cell density experiment (W1, mg/L) | Wastewater 2 used for high initial algal cell density experiment (W2, mg/L) |
|---|---|---|
| TIC | $50.18 \pm 0.83$ | $72.24 \pm 1.81$ |
| Nitrogen ($NH_4^+/NH_3$) | $8.05 \pm 0.16$ | $18.31 \pm 0.53$ |
| Phosphorus ($PO_4^{3-}$) | $1.85 \pm 0.10$ | $1.37 \pm 0.01$ |
| pH | $7.34 \pm 0.05$ | $7.88 \pm 0.07$ |

Microalgae produce hydroxyl ion ($OH^-$) when $HCO_3^-$ is consumed during photosynthesis within the algal cell by the following reaction: $HCO_3^- \rightarrow CO_2 + OH^-$ [14–16]. As a result, the pH of the wastewater medium continued to increase from the lag phase through the growth phase as shown in Fig. 1(b). Then the pH started to level off as the uptake rate of TIC discontinued after 96 h. The pH of the culture medium was roughly estimated from the carbon mass balance based on an assumption that only $HCO_3^-$ ion can be consumed by *C. vulgaris* and $OH^-$ ion can be generated as a result of $HCO_3^-$ consumption. The estimated final pH value overpredicted the measured pH values over the entire culture period as shown in Fig. 1(b) probably because some of TIC was released to the air as $CO_2$ gas during the culture and the lost fraction was not included in the calculation.

The removal of nitrogen in $NH_3/NH_4^+$ is shown in Fig. 2(a). Overall, the nitrogen removal showed almost the same pattern as the TIC uptake. As observed from the uptake of TIC, a small amount ($1.11 \pm 0.31$ mg/L) of nitrogen concentration was consumed during the lag phase, but then sharply decreased from $6.94 \pm 0.18$ to $0.56 \pm 0.04$ mg/L concomitantly with a decrease in TIC concentration during the rapid growth phase (i.e. 24–96 h). The total amount of nitrogen removed during 96 h was $7.48 \pm 0.20$ mg/L. Similar to the

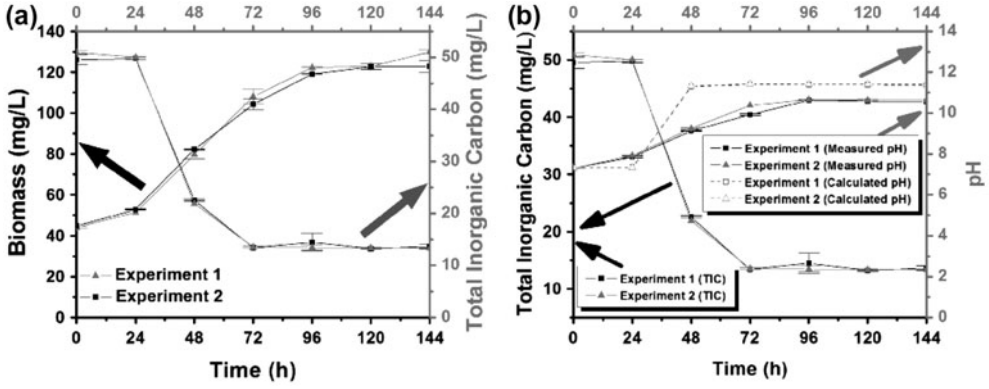


Fig. 1. (a) Growth of *C. vulgaris* and TIC uptake and (b) TIC uptake and pH increase with low initial algal cell density ($44.37 \pm 0.60$ mg/L).

carbon mass balance, the nitrogen content in *C. vulgaris* was estimated to be ~10% from a nitrogen mass balance between the total nitrogen removal (7.48 ± 0.20 mg/L) and total biomass gain (76.33 ± 2.89 mg/L) for 96 h. The removal of orthophosphate ($PO_4^{3-}$) is represented in Fig. 2(b). During the lag-phase period, a small amount (0.08 ± 0.02 mg/L) of phosphorus was consumed. During the growth-phase (i.e. 24–96 h) period, it significantly decreased from 0.47 ± 0.03 to 0.03 ± 0.01 mg/L. The phosphorus content of *C. vulgaris* was estimated to be ~0.7% from a phosphorus mass balance between the total phosphorus consumption (0.52 ± 0.04 mg/L) and total biomass gain (76.33 ± 2.89 mg/L) during 96 h.

### 3.2. Growth and nutrients removal with high initial cell density

The growth of *C. vulgaris* is shown in Fig. 3(a) when a high (354.48 ± 2.14 mg/L) initial algal cell density was used for faster nitrogen and phosphorus uptake. The lag-phase period was not observed, and $PO_4^{3-}$ was depleted within 12 h. The concentrations of $NH_3/NH_4^+$, $PO_4^{3-}$, and cells were measured every 3 h until $PO_4^{3-}$ was depleted. Almost all $NH_3/NH_4^+$ were also removed within 48 h. For this culture, the initial $NH_3/NH_4^+$ concentration in this sample (i.e. W2) was higher than that in the first W1 sample, and thus $PO_4^{3-}$ clearly became a limiting substrate for the growth.

### 3.3. Growth kinetics of C. vulgaris

The Monod equation is a well-known substrate-limiting growth model used to describe the growth of a micro-organism covering the growth and stationary phases as shown in Eq. (1):

$$\mu = \frac{\mu_{max} S}{K_s + S} \tag{1}$$

where $\mu_{max}$ is a maximum growth rate coefficient ($h^{-1}$), $\mu$ is a specific growth rate ($h^{-1}$), $K_s$ is the

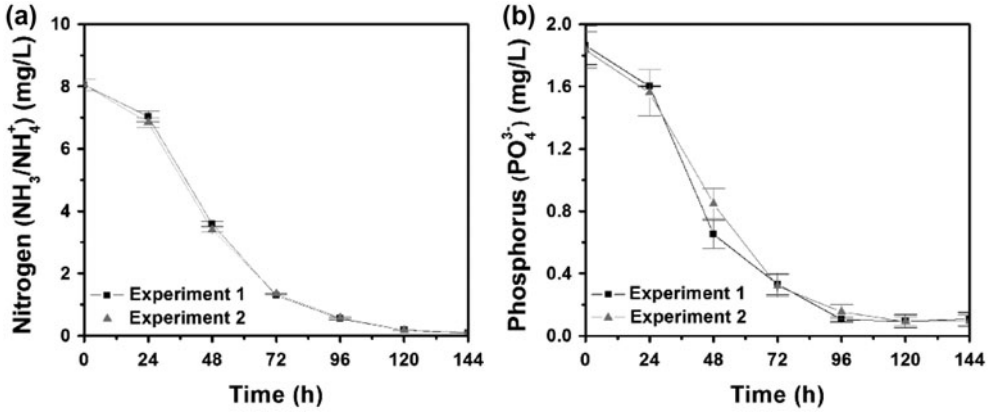


Fig. 2. Removal of (a) nitrogen in the form of $NH_3/NH_4^+$ and (b) phosphorus in the form of orthophosphate ($PO_4^{3-}$).

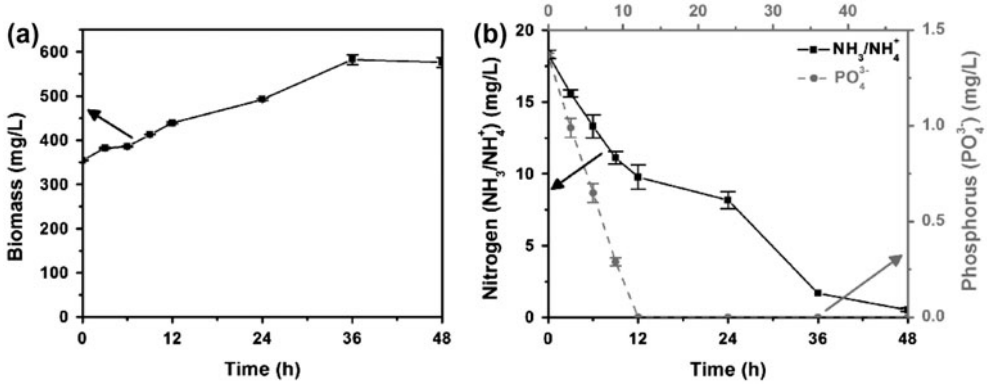


Fig. 3. (a) Growth of *C. vulgaris* and (b) removal of $NH_3/NH_4^+$ and $PO_4^{3-}$ with high initial algal cell density (354.48 ± 2.14 mg/L).

Monod coefficient (mg/L), and $S$ is the concentration of a limiting nutrient (mg/L). The two parameters, $\mu_{max}$ and $K_s$, were determined from the growth and stationary phases using the Lineweaver–Bulk plot of $1/\mu$ vs. $1/S$ by taking a reciprocal for each term in the Monod equation as shown in Eq. (2).

$$\frac{1}{\mu} = \frac{K_s}{\mu_{max}}\frac{1}{S} + \frac{1}{\mu_{max}} \quad (2)$$

For the low initial cell density case, the remaining nitrogen and phosphorus concentrations after 144 h were both low at $1.10 \pm 0.01$ and $0.02 \pm 0.01$ mg/L, respectively, and it was difficult to determine a limiting substrate. Therefore, a comparison was also made for the $R^2$ values of the Lineweaver–Bulk plot when nitrogen and phosphorus were used as limiting nutrients. The average $R^2$ values (from Experiments 1 and 2) for nitrogen and phosphorus were 0.97 and 0.64, respectively. Therefore, nitrogen was determined to be a limiting substrate for the growth in the low initial algal cell density. Then, $\mu_{max}$ ($h^{-1}$) and $K_s$ (mg/L) were determined to be 0.01245 ($h^{-1}$) and 0.0696 (mg/L) for Experiment 1, and 0.01241 ($h^{-1}$) and 0.0864 (mg/L) for Experiment 2, respectively. The experimental data were re-plotted with the Monod equation for specific growth ($\mu$) vs. nitrogen concentration ($S$) in Fig. 4(b), and the both equations with the determined parameters well represent the growth.

For the culture with the high initial algal cell density, phosphorus was evidently a limiting substrate. Following the same procedure applied above, the two parameters ($\mu_{max}$ and $K_s$) were determined to be 32.85 ($h^{-1}$) and 0.99 (mg/L). When the Monod equation was compared for the two cases, the $\mu$ value for the culture with a high initial cell density and pH control using $CO_2$ gas was at least one order magnitude (e.g. ~40 times) greater than that for the culture with a low initial cell density and no pH control. The culture with a high initial cell density and pH control using $CO_2$ gas could accelerate simultaneous algal cell growth and residual nitrogen and phosphorus uptake. (Fig. 5(a) and (b)).

In this study, the Monod expression for nutrient removal has been obtained in a batch system. However, if an intrinsic kinetic expression is obtained, the expression can be incorporated into a reactor mass balance model taking into account, fluid flow and

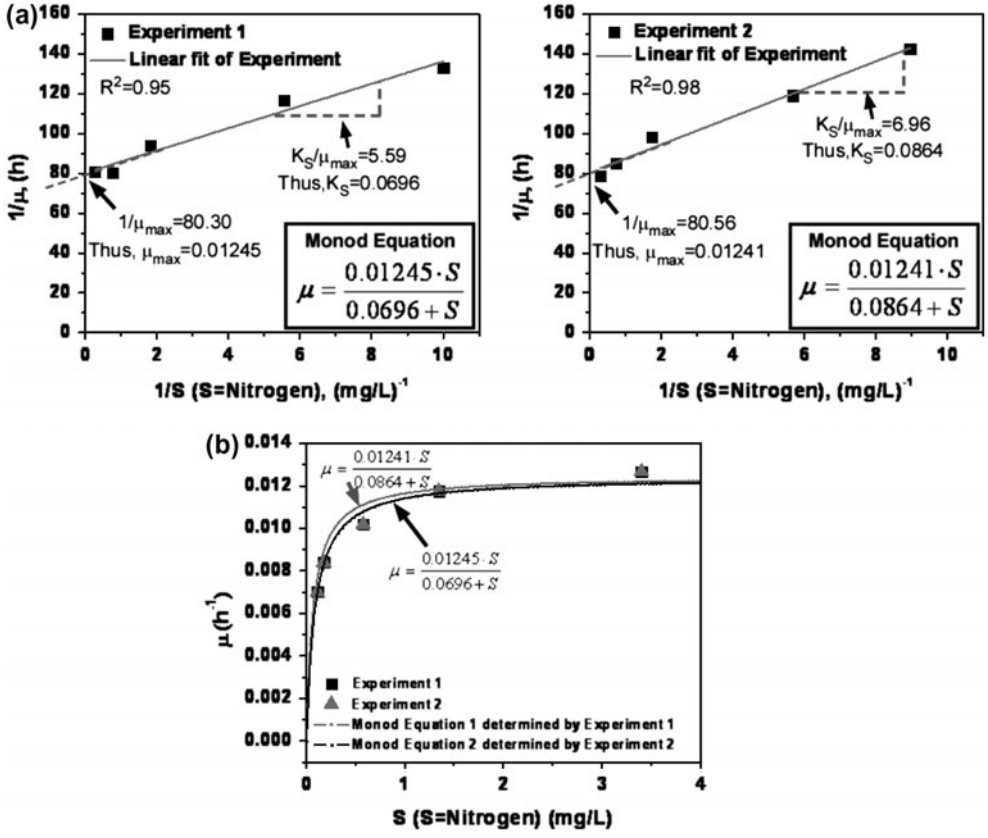


Fig. 4. (a) Determination of $\mu_{max}$ ($h^{-1}$) and $K_s$ (mg/L) of the Monod equation from the Lineweaver–Bulk plot of $1/\mu$ vs. $1/S$ and (b) Monod plot ($\mu$ vs. $S$) (substrate = nitrogen) for low initial algal cell density.

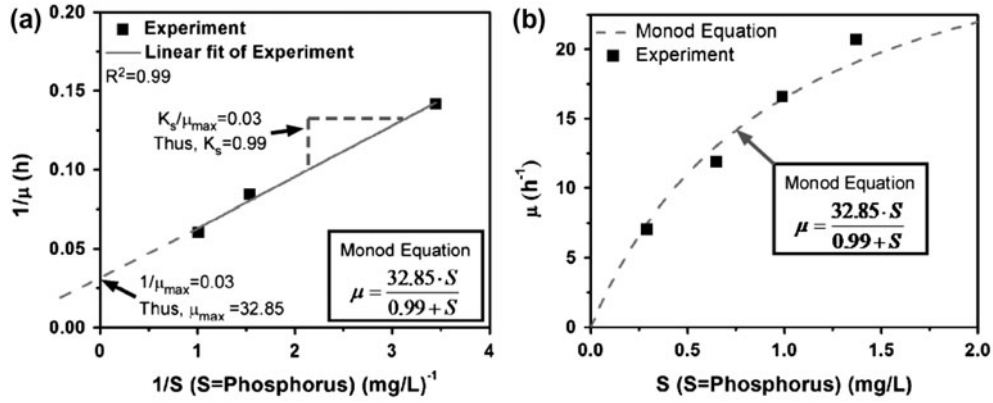


Fig. 5. (a) Determination of $\mu_{max}$ ($h^{-1}$) and $K_s$ (mg/L) of the Monod equation from the Lineweaver–Bulk plot of $1/\mu$ vs. $1/S$ and (b) Monod plot ($\mu$ vs. $S$) (substrate = orthophosphate) for high initial algal cell density.

mass transfer for the design of a continuous reactor. Although a possibility of nutrient uptake from wastewater is demonstrated, the harvest of microalgae from a voluminous wastewater effluent stream is a great challenge. Several options have been investigated, including chemical flocculation, autoflocculation, filtration, flotation, sedimentation or electrophoretic separation [17]. However, energy-efficient, effective, and reliable harvesting methodologies for large-scale wastewater treatment have not yet been realized and need to be developed. Total lipid content in a few strains including *Chlorella* sp. and *Scenedesmus* sp. grown in wastewaters was reported to range between 12 and 15% (wt.) in dry biomass [18].

## 4. Conclusions

The growth of *C. vulgaris* was studied for the uptake of residual nitrogen and phosphorus present in the secondary wastewater samples collected from a local wastewater treatment plant in Cincinnati, OH, USA. The following summary was found from this study:

(1) Between nitrogen and phosphorus present in the secondary municipal wastewater samples, either one could be a substrate limiting its growth depending on their initial concentrations.
(2) The residual nutrient removal rate can enhanced by high initial algal cell density and $CO_2$ gas supply, and almost all nitrogen and phosphorus could be removed within 48 h.
(3) The Monod equation could be used to express the algal cell growth for a limiting substrate. It would be applicable to the design and operation of any type of continuous photobioreactor by the incorporation into a reactor mass balance model taking into account fluid flow and mass transfer for the removal of nutrients from wastewater.

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