



Kinetic modelling of microalgae cultivation for wastewater treatment and carbon dioxide sequestration

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ABSTRACT

A simple and robust microalgae kinetic model has been developed for application in the prediction and control of algae cultivations in wastewater. The microalgae kinetic model was calibrated using experimental cultivation data from *Desmodesmus* sp. to determine specific microalgae growth rates (μ_{max} and μ_{maxNO_3}), microalgae death rates (μ_d), and the NH_4^+ to NO_3^- oxidation rate (μ_B). Model parameters obtained were: $\mu_{max} = 0.17 \text{ day}^{-1}$, $\mu_d = 0.004 \text{ day}^{-1}$, and $\mu_B = 0.14 \text{ day}^{-1}$. Microalgae specific growth rate based on NO_3^- alone ($\mu_{maxNO_3} = 0.1 \text{ day}^{-1}$) was lower than the overall growth rate (μ_{max}). The kinetic model was validated using additional experimental data for the *Desmodesmus* sp. and *Scenedesmus obliquus* cultivation in wastewater containing 0% and 7% landfill leachate, with accuracy above 98% in all cases. These results demonstrated the kinetic model was accurate in predicting microalgae growth, wastewater nutrient removal, and changes in the culture media pH. Biomass productivity of the algae culture was associated with an exponential increase in the media pH, which led to ammonia volatilisation and decreased carbon intake. Between 28.8 and 29.7% of the initial NH_4^+ was lost to ammonia volatilisation in wastewater containing 7% landfill leachate. Hence, loss of ammonium nitrogen contained in domestic wastewater must be avoided to ensure steady and efficient inorganic carbon utilisation which inherently maximises biomass production efficiency. The optimal pH for the microalgae culture was 8.1, at which point microalgae could achieve about 99% carbon fixation efficiency. To ensure constant pH in the microalgae growing system, immediate removal of the OH^- generated is needed, which could be facilitated by injections of 1.14 g CO_2 and 0.067 g OH^- per gram of produced algae when using NH_4^+ nutrient, and 1.54 g of CO_2 per gram of produced algae when using NO_3^- nutrient. This could be done in a wastewater pond by using an optical density-controlled smart CO_2 injection system.

1. Introduction

Disposal of wastewater without treatment often results in eutrophication, an excessive enrichment of the water body with nutrients which leads to algal blooms, and more long-term problems of heavy metals contamination [1,2]. Eutrophication has become a widespread and serious environmental concern since the mid-20th century, with about 48% of lakes and reservoirs in North America, 54% in Asia and the Pacific, 53% in Europe, 41% in South America, and 28% in Africa being affected by eutrophication [2]. In Mexico only 57% and 32% of the total municipal and industrial wastewater generated in the country is treated [3], therefore, eutrophication has had a great impact in receiving water bodies because of the discharges of untreated wastewater. These deleterious environmental consequences can be avoided through carbon-neutral wastewater treatment using microalgae. An integrated

microalgae-based process would lead to reductions in greenhouse gas emissions, production of usable microalgae biomass and low-cost water treatment [4]. However, the major challenge is an adequate understanding of the kinetics of microalgae growth in wastewater, to allow for optimal design and operation of ponds for wastewater treatment.

Microalgae cultivation is a promising approach for simultaneous CO_2 conversion and wastewater treatment. Microalgae autotrophic growth utilises inorganic carbon in the form of dissolved CO_2 in the bicarbonate-carbonate buffer system ($CO_2(aq) - HCO_3^- - CO_3^{2-}$) [5,6]. Previous studies have shown that CO_2 fixation efficiency, when injected continuously in microalgae culture, was in a range of 4–66% at input CO_2 concentration of 6–15% for *Scenedesmus* sp. [7–10], and 16–64% at an input CO_2 concentration of 1–15% for *Chlorella* sp. [8,11–13]. Within these CO_2 fixation yields, microalgae have reported growth rates between 0.19 and 1.24 d^{-1} . Although no specific value for

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CO₂ fixation efficiency was provided in the literature aforementioned, it was observed that the higher amount of CO₂ the lower CO₂ fixation efficiency due to the loss of CO₂ through outgassing. Therefore, controlled-dosing of CO₂ to match the inorganic carbon demand of the microalgae culture media may be required to minimise the outgassing of CO₂. It has been reported that CO₂ fixation efficiency increased from 64% when added continuously, to about 95% when intermittently injected into *Chlorella vulgaris* culture media [13]. Apart from inorganic carbon which could be obtained from CO₂ dissolved in water, the aquatic medium must contain sources of inorganic nitrogen, phosphorus, and iron for autotrophic microalgae growth [5,14]. Silica would also be required by many chrysophytes, such as golden algae (*Chrysophyceae*) and diatoms (*Bacillariophyceae*), as a nutrient for their cell walls [15,16]. From these, inorganic nitrogen and phosphorus are the two major nutrients required for microalgae cultivation [5,14]. The most important being nitrogen, as it constitutes about 1–10% weight of the algae biomass [5]. Microalgae require less phosphorus for their growth compared to inorganic carbon and nitrogen sources, with its content typically < 1% of the algae biomass [5,14]. Microalgae have been shown to effectively utilise the levels of nitrogen (2–1960 mg/L) and phosphorus (1–117 mg/L) found in different types of wastewater, sometimes even coupled with the biofixation of toxic metals [2,17,18]. Nitrogen sources commonly used are ammonium, nitrate, and urea. Ammonium is the most preferred because its assimilation requires less energy [17]. Ammonium exists in equilibrium with ammonia depending on the culture pH, with free ammonia concentration increasing with the pH [19,20]. High concentrations of free ammonia have been found toxic and reported to inhibit growth to most strains of microalgae, hence effluents with high ammonium nitrogen concentration (e.g. landfill leachate) need previous dilution [17,21]. Other shortcomings of the ammonia nitrogen source are volatilisation of ammonia, which has been reported in ammonium-rich aqueous media at pH above neutrality [19,22], and its reduction to nitrate by autotrophic bacteria [23].

Studies have shown that growth kinetics of microalgae are dependent on the availability of sources of inorganic carbon, nitrogen and phosphorus; plus light intensity and media temperature, [24–27]. For microalgae growth at constant light intensity, temperature, and homogenous mixing; usually substrate and nutrients availability determine the rate of the algae biomass accumulation. Microalgae growth kinetic models give an insight into the algae biomass production and the nutrient consumption rate, providing essential data for the design of ponds for high efficiency algae cultivation. A robust microalgae kinetic model is very crucial in prediction of nutrients removal, biomass growth, and optimisation of operating conditions for algae cultivation in wastewater. Among the kinetic models that have been used to study microalgae growth, the most favoured mathematical models are the Monod and Droop models, which are usually applied in studies of the specific growth rates of microalgae as a function of one of the three major substrates – inorganic carbon, nitrogen, or phosphorus [17,28–30]. Generally, the growth rates of organisms in both Monod and Droop models could be limited by the availability of any of the substrates as soon as its concentration becomes negligibly low. Hence, nutrient limitation can determine the maximum rate of microalgae growth, and this can be described using the Monod-type kinetics in Eq. (1), or Droop model [27,31], in Eq. (2). The maximum specific growth rate (μ_m , μ_D) in the Droop model equation is the specific growth rate of the algae at an infinite internal nutrient cell content (also called cell quota). The μ_m in the Monod equation is the maximum growth rate at infinite external nutrient concentration. Therefore, the Monod and Droop models can be related by Eq. (3).

$$\mu_m = \mu_{max} * \frac{[A]}{K_A + [A]} \quad (1)$$

$$\mu_D = \mu_{D,max} * \left(1 - \frac{Q_0}{q}\right) \quad (2)$$

$$\mu_{D,max} = \frac{\mu_m \rho_m}{\rho_m - \mu_m Q_0} \quad (3)$$

where:

μ_m : Monod specific growth rate (time⁻¹); μ_D : Droop specific growth rate (time⁻¹); μ_{max} : Monod maximum specific growth rate (time⁻¹); $\mu_{m,D}$: Droop maximum specific growth rate (time⁻¹); K_A : half-saturation constant for nutrient A (mg/L); $[A]$: concentration of the nutrient A (mg/L); Q_0 : minimum cell quota; q : cell quota; ρ_m : maximum nutrient uptake rate per cell.

Notwithstanding existing studies showing that the Droop model can accurately reproduce the dynamics of microalgae growth in a constant environment [31,32], this model is still not widely used by researchers compared to the Monod model [26,27]. The Monod model is commonly used because the external nutrient concentration in the culture media can be easily measured. Although microbial growth rates are more accurately determined by the internal cellular nutrient contents than on the concentration measured externally in the culture media [5], it is difficult to experimentally measure the cell quota of microalgae species, which limits the Droop model utilisation.

The Monod model in Eq. (1) is generally used to study microalgae growth that is limited by a single nutrient. The model does not take into considerations multiple-nutrient limited growth although existing studies have shown that two or more substrates could limit microbial growth rates [5,33]. This decreases the accuracy of specific growth rates predicted for microalgae limited by more than one substrate during cultivation. In such cases, the Monod model could be extended to explain dual nutrient limited growth of microalgae [25] as in Eq. (4), and also include carbon limitation as in Eq. (5).

$$\mu = \mu_{max} * \frac{[A]}{K_A + [A]} * \frac{[B]}{K_B + [B]} \quad (4)$$

$$\mu = \mu_{max} * \frac{[C]}{K_C + [C]} * \frac{[N]}{K_N + [N]} * \frac{[P]}{K_P + [P]} \quad (5)$$

In a complex culture media, like wastewater, microalgae growth could be dependent on the concentrations of main nutrients and carbon. Therefore, a Monod model as in Eq. (5), for the autotrophic growth of algae, considering inorganic carbon, nitrogen and phosphorus sources as limiting, could be used to explain microalgae growth kinetics [34,35].

The aim of this study is to develop a robust kinetic model that simulates microalgae growth in wastewater when limited by more than one substrate (inorganic carbon, nitrogen or phosphorous), incorporating the effects of NH₃ volatilisation, oxidations of the ammonium nitrogen to nitrate by autotrophic bacteria, and the inherent changes of pH in the culture media. With this, the model would predict CO₂ sequestration, and nutrient removal as a dual process of microalgae cultivation and wastewater treatment. The model will be calibrated and validated using experimental data from cultivations of microalgae in wastewater, in some cases, with added landfill leachate. This model development is an important step in predicting the performance of microalgae cultivation in wastewater ponds.

2. Materials and methods

2.1. Procedure for the microalgae cultivation in wastewater

Wastewater used for the microalgae cultivation was obtained from the Wastewater Treatment Plant (WWTP) at Cerro del Agua, located in Ciudad Universitaria (UNAM), México. The wastewater was collected in 20 L containers, filtered to remove suspended solids (Filter with 8 µm pore size), and stored at 4 °C. The wastewater had initial pH in the range of 8.0–8.5, 102.66 ± 1.68 mg/L ammonium nitrogen, 1.8 ± 1.3 mg/L nitrate, 25.60 ± 1.14 mg/L orthophosphate, and total inorganic carbon measured as alkalinity value (280 mg/L as CaCO₃).

Microalgae cultivation was conducted in polyethylene bottles (L:14 cm; W:12 cm; H: 25 cm), with a work volume of 4 L (1:1 microalgae culture – wastewater), under passive aeration conditions (manual shaking, 2 times a day), temperature of 23–24 °C, and illumination by 20 W white light LED lamps ($53 \mu\text{mol m}^{-2} \text{s}^{-1}$) with 12 h light and 12 dark cycles. A microalgae strain (*Desmodesmus* sp.) was cultivated, starting with wastewater media initially inoculated with 130 mg/L for *Desmodesmus* sp., and the biomass concentration (as total suspended solids, TSS) was used to monitor microalgae growth. Samples were taken from the microalgae culture vessels at various time intervals, filtered by glass microfiber filter with 1.6 μm pore size (Whatman, GF/A), and stored at -5°C for subsequent water quality analyses. The TSS and ammonium nitrogen contents were measured according to the APHA standard methods [36], and the orthophosphate and nitrate were determined by colorimetric methods using a HACH 3900 spectrophotometer. Total nitrogen was measured by a TOC-L_{CPH/CPN} analyzer (TOC-L_{CPH/CPN} Shimadzu). All the measurements were performed in duplicates. Experimental data obtained from the above procedure were used to calibrate the *Desmodesmus* sp. microalgae kinetic model. A second set of microalgae cultivations were conducted to generate data for the model validation, following the above procedure. In the experiments, two algae strains (*Desmodesmus* sp. and *Scenedesmus obliquus*) were cultivated in wastewater containing 0% and 7% landfill leachate, 44.3–167.5 mg/L ammonium nitrogen, 3.8–10.0 mg/L nitrate, 16.1–21.3 mg/L orthophosphate, and total inorganic carbon measured as alkalinity (340–715 mg/L as CaCO_3). All the cultures were conducted in batch mode.

2.2. Model development

The kinetic model for microalgae growth was developed using a Monod-type model for multi-nutrient limitations. The kinetic model discussed here applied all relevant reactions and dynamics of microalgae culture media. Components of the microalgae growth kinetic model include rate of utilisations of the inorganic carbon, ammonium nitrogen, nitrate, and the orthophosphate sources in the wastewater. Loss of ammonium nitrogen through ammonia volatilisation, and the oxidation to nitrate by autotrophic bacteria were also integrated into the model. Assumptions considered for the model are: fixed light intensity and temperature, no water evaporation from the culture, CO_2 taken from the environment and HCO_3^- from the wastewater, and no mass transfer limitation. Microalgae use inorganic carbon when the growth metabolism is autotrophic, organic carbon for heterotrophic growth, and a combination of organic and inorganic carbons for mixotrophic growth metabolism [37]. These microalgae growth metabolic processes are identifiable through the changes in the culture pH. There is an increase in culture pH during photoautotrophic microalgae growth due to removal of bicarbonate ions from the media, and a decrease in the pH because of utilisation of organic carbons when the microalgae growth is heterotrophic, whereas only negligible changes in the culture pH occurs for mixotrophic microalgae growth [37]. In this study, metabolism for the *Desmodesmus* sp. and *Scenedesmus obliquus* growth was predominantly photoautotrophic as evidenced by the sharp rise in the culture pH from 8.0–8.5 initially to about 10.5 during the microalgae cultivation. Therefore, the microalgae growth kinetics at the experimental conditions were modelled based on inorganic carbon, which is the sole carbon source for photoautotrophic metabolism in microalgae. The overall rate expressions for modelling of the microalgae growth kinetic are summarized in Eqs. (6)–(12).

$$\begin{aligned} \frac{d[M_b]}{dt} = & \mu_{\max} * [M_b] * \frac{[P]}{K_p + [P]} * \frac{[C]}{K_c + [C]} * \left(\frac{[NH_4^+]}{K_N + [NH_4^+]} \right) \\ & + \left(\frac{\mu_{\max} NO_3}{\mu_{\max}} \right) * \frac{[NO_3^-]}{K_N + [NO_3^-]} - \mu_d * [M_b] \end{aligned} \quad (6)$$

$$\begin{aligned} \frac{d[P]}{dt} = & -Y_p * \mu_{\max} * [M_b] * \frac{[P]}{K_p + [P]} * \frac{[C]}{K_c + [C]} * \left(\frac{[NH_4^+]}{K_N + [NH_4^+]} \right) \\ & + \left(\frac{\mu_{\max} NO_3}{\mu_{\max}} \right) * \frac{[NO_3^-]}{K_N + [NO_3^-]} - \mu_m * [P] \end{aligned} \quad (7)$$

$$\begin{aligned} \frac{d[C]}{dt} = & -Y_c * \mu_{\max} * [M_b] * \frac{[P]}{K_p + [P]} * \frac{[C]}{K_c + [C]} * \left(\frac{[NH_4^+]}{K_N + [NH_4^+]} \right) \\ & + \left(\frac{\mu_{\max} NO_3}{\mu_{\max}} \right) * \frac{[NO_3^-]}{K_N + [NO_3^-]} + K_a * \frac{P_0 * Y_0 * M_w}{K_H} \end{aligned} \quad (8)$$

$$\begin{aligned} \frac{d[NH_4^+]}{dt} = & -\mu_B * e^{\mu_B * t} * [NH_4^+] - Y_{N,a} * \mu_{\max} * [M_b] * \frac{[P]}{K_p + [P]} * \frac{[C]}{K_c + [C]} \\ & * \frac{[NH_4^+]}{K_N + [NH_4^+]} - K_{des} * \frac{1}{1 + \frac{[H^+]}{K_b}} * [NH_4^+] \end{aligned} \quad (9)$$

$$\begin{aligned} \frac{d[NO_3^-]}{dt} = & \mu_B * e^{\mu_B * t} * [NH_4^+] - Y_{N,n} * \mu_{\max} NO_3 * [M_b] * \frac{[P]}{K_p + [P]} * \frac{[C]}{K_c + [C]} \\ & * \frac{[NO_3^-]}{K_N + [NO_3^-]} - \mu_m * [NO_3^-] \end{aligned} \quad (10)$$

$$\begin{aligned} \frac{d[NH_3]_{\text{gas}}}{dt} = & K_{des} * [NH_3]_{\text{aq}} = K_{des} * \frac{[NH_3]_{\text{aq}}}{[NH_3]_{\text{aq}} + [NH_4^+]_{\text{aq}}} * [NH_4^+]_0 \\ = & K_{des} * \frac{1}{1 + \frac{[H^+]}{K_b}} * [NH_4^+] \end{aligned} \quad (11)$$

$$[H^+] = \sqrt{\left(\left(-K_1 * \left(\frac{[C]}{R} - 1 \right) - \sqrt{\left(K_1 * \left(\frac{[C]}{R} - 1 \right) \right)^2 - 4 * K_1 * K_2 * \left(\frac{[C]}{R} \right)} \right) \div 2 * \left(\frac{[C]}{R} \right) \right)^2} \quad (12)$$

And, $R = [C]_0 + \left(K_a * \frac{P_0 * Y_0 * M_w}{K_H} \right)$

The μ_{\max} is the specific growth rate of the microalgae (time^{-1}) when using NH_4^+ as source of inorganic nitrogen, while $\mu_{\max} NO_3$ is the specific growth of the microalgae based on NO_3^- nitrogen. The ammonium nitrogen was being oxidised into NO_3^- by autotrophic bacteria in the wastewater. The rate of NH_4^+ oxidation was represented by μ_B . Ammonium nitrogen oxidation is an exponential function, probably due to exponential bacterial growth. It is expected that some of the microalgae would die during the cultivation, and this was represented by the specific death rate, μ_d (time^{-1}). μ_m is the rate of nutrient uptake for non-growth maintenance (time^{-1}), and $[M_b]$ is the initial microalgae concentration (mg/L). $[P]$ is the orthophosphate concentration (mg/L), $[C]$ is the inorganic carbon concentration (mg/L), $[\text{NH}_4^+]$ is the ammonium nitrogen concentration (mg/L), $[\text{NH}_3]$ is the ammonia concentration in equilibrium with ammonium (mg/L), and $[\text{NO}_3^-]$ is the nitrate concentration (mg/L).

The half-saturation constants for the inorganic carbon and nutrients were: K_N : for NH_4^+ and NO_3^- (mg/L), K_C for HCO_3^- (mg/L), and K_P for HPO_4^{2-} (mg/L). Half-saturation constants for ammonium nitrogen ($K_N = 31.5$ mg/L) and orthophosphate ($K_P = 10.5$ mg/L) were obtained from an existing study [38]. The half-saturation constant for the nitrate was assumed to be same as that of the ammonium nitrogen. A half-saturation constant ($K_C = 124.9$ mg/L) has been reported for inorganic carbon in a study using NaHCO_3 as a carbon source [24]. Removal coefficients for the inorganic carbon and the nutrients were represented as, $Y_{N,a}$ for NH_4^+ in gram NH_4^+ per gram algae, $Y_{N,n}$ for NO_3^- in gram NO_3^- per gram algae, Y_C for HCO_3^- in gram HCO_3^- per gram algae, and Y_P for the HPO_4^{2-} in gram HPO_4^{2-} per gram algae. In this model development, the yield coefficients for the inorganic carbon and the various nutrients, were obtained based on the reported molecular

weight of $C_{106}H_{263}O_{110}N_{16}P$ for microalgae [4]. The removal coefficients, in terms of the amount of nutrients required per algae biomass produced, were calculated, using the molar weights of the various reaction species: $HCO_3^- = 61$ g/mol; $NH_4^+ = 18$ g/mol; $NO_3^- = 62$ g/mol; $HPO_4^{2-} = 96$ g/mol; and microalgae = 3550 g/mol. The reaction stoichiometry in the microalgae formation equations [4] were also used to calculate the consumption coefficient for the HCO_3^- . For instance, the removal coefficient for the HPO_4^{2-} was $(1 * 96/3550) = 0.027$ g HPO_4^{2-} per gram of the microalgae.

K_{des} and K_a represent mass transfer coefficient of NH_3 gas and CO_2 in water, respectively; while K_H is the Henry's law constant for CO_2 gas. The pressure and mole fraction of CO_2 in the flue gas supplied to the microalgae culture were denoted by P_o and y_o . For our experiments, carbon supplied to microalgae was derived from carbonic acid (HCO_3^-) equilibrium. As such, carbonic acid formation could be originated from bicarbonate present in wastewater or CO_2 dissolved in equilibrium with water, see Eq. (8). CO_2 dissolved in equilibrium with water would be limited by Henry's constant, whether CO_2 is derived from air or the bacterial oxidation of organic carbon, at atmospheric conditions. Equilibrium constant K_b denoted the $NH_4^+ \leftrightarrow NH_3 + H^+$ process; K_1 the ionisation of carbonic acid, $H_2CO_{3(aq)} \leftrightarrow HCO_3^- + H^+$; and K_2 the bicarbonate dissociation in, $HCO_3^- \leftrightarrow CO_3^{2-} + H^+$. Molecular weight of CO_2 is shown as M_w .

The model considers the effects of NH_3 volatilisation and the oxidations of the ammonium nitrogen to nitrate by autotrophic bacteria. This was done through the addition of the parameter μ_B to account for the rate of oxidations of ammonium to nitrate, and Eq. (11) to account for ammonia volatilisation. The rate of NH_3 volatilisation (K_{des}) used in the model was obtained from the work of Solimeno et al. [23]. At the initial pH used in our experiments (8.0–8.5), which are also normally used in microalgae cultivation, the inorganic carbon was available to the algae as HCO_3^- . Bicarbonate concentration was taken to be proportional to carbonate alkalinity values. This is a valid assumption, as carbonate alkalinity mainly consists of HCO_3^- and CO_3^{2-} , which is dominated by HCO_3^- at pH between 4.5 and 8.3 [39]. HCO_3^- accounted for over 90% of the inorganic carbon at the initial pH range

Table 1

Kinetic parameters used in the microalgae model.

Parameters	Values	References
Rate of nutrient uptake for maintenance, μ_m	0.00385 h^{-1}	[40]
Initial microalgae concentration, M_b	130–150 mg/L	This study
NH_4^+ & NO_3^- half-saturation constant, K_N	31.5 mg/L	[38]
HPO_4^{2-} half-saturation constant, K_P	10.5 mg/L	[38]
HCO_3^- half-saturation constant, K_C	124.9 mg/L	[24]
NH_4^+ removal coefficient, $Y_{N,a}$	0.081 g/g. algae	This study
NO_3^- removal coefficient, $Y_{N,n}$	0.279 g/g. algae	This study
HPO_4^{2-} removal coefficient, Y_P	0.027 g/g. algae	This study
HCO_3^- consumption coefficient, Y_{IC}	1.821 g. HCO_3^- /g. algae	This study
Mass transfer coefficient for NH_3 gas, K_{des}	0.14 h^{-1}	[23]
Mass transfer coefficient for CO_2 gas, K_a	0.14 h^{-1}	[23]
Henry's law constant for CO_2 gas, K_H	29.4 bar/mol	[41]
Pressure of the CO_2 source, P_o	1 bar	This study
Mole fraction of CO_2 in the air, y_o	$3 * 10^{-4}$ mol/mol	This study
$NH_4^+ \leftrightarrow NH_3 + H^+$ dissociation constant, K_b	$4.95 * 10^{-10}$	[19,20]
$H_2CO_{3(aq)} \leftrightarrow HCO_3^- + H^+$ dissociation, K_1	$4.375 * 10^{-7}$	[42]
$HCO_3^- \leftrightarrow CO_3^{2-} + H^+$ dissociation, K_2	$1.358 * 10^{-10}$	[42]

used in the experiments, as shown in the Fig. 1. Therefore, change in the pH of the culture media was modelled based on the rates of the HCO_3^- utilisation by the microalgae, using Eq. (12).

This study used a combination of experimental investigations and MATLAB numerical simulations of growth kinetic expressions for the microalgae, to develop a simple and robust predictive model for optimal operations of microalgae ponds, ensuring adequate pH control and efficient CO_2 dosing for microalgae ponds, and consequently healthy algae growth. The rate expressions in Eqs. (6)–(12), were numerically modelled in MATLAB 7.12.0 (R2011a) using ODE45 solver (Runge-Kutta method), and the model parameters calibrated by using

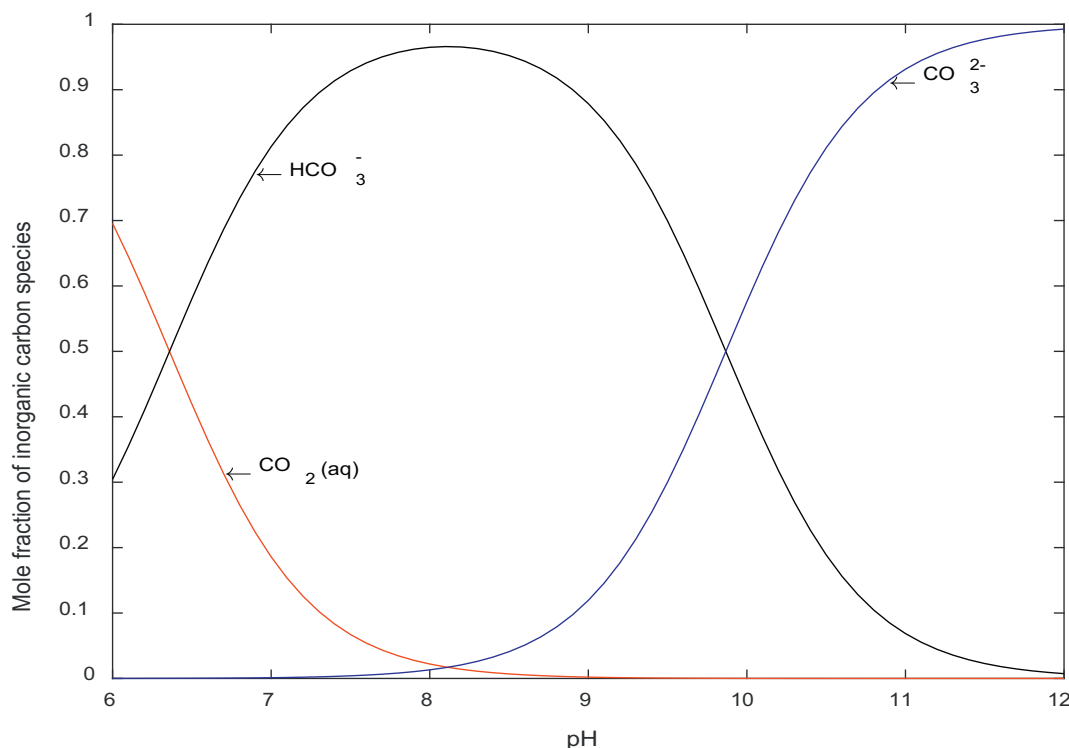


Fig. 1. Mole fractions of various inorganic carbon species at different media pH.

Table 2Microalgae growth and nutrient removal from wastewater using *Desmodesmus* sp. Data shown are the mean \pm SD of three independent measurements.

Duration (days)	<i>Desmodesmus</i> sp. biomass (mg/L)	NH ₄ ⁺ -N (mg/L)	HPO ₄ ²⁻ (mg/L)	NO ₃ ⁻ -N (mg/L)	Total Nitrogen (mg/L)
0	130	102.7 \pm 1.7	25.6 \pm 1.1	1.8 \pm 1.3	104.5 \pm 1.5
3	215 \pm 7.1	77.5 \pm 2.0	23.3 \pm 1.1	1.9 \pm 1.3	77.8 \pm 3.0
7	292.5 \pm 7.1	60.7 \pm 1.7	21.7 \pm 1.4	3.0 \pm 1.0	63.7 \pm 1.8
9	297.5 \pm 14.1	59.3 \pm 1.4	22.9 \pm 1.2	5.1 \pm 1.5	60.2 \pm 2.5
11	335.0 \pm 7.1	40.8 \pm 1.7	21.3 \pm 1.0	30.8 \pm 3.4	51.3 \pm 2.0
14	380 \pm 14.1	12.4 \pm 2.0	20.9 \pm 1.2	30.5 \pm 2.8	48.9 \pm 2.4
16	420 \pm 14.1	5.1 \pm 1.8	20.2 \pm 1.0	40.5 \pm 1.8	45.7 \pm 2.3
18	407.5 \pm 7.1	3.0 \pm 1.5	19.7 \pm 1.0	38.0 \pm 2.0	40.9 \pm 2.0
21	397.5 \pm 14.1	1.8 \pm 1.5	19.0 \pm 1.2	38.4 \pm 2.3	39.9 \pm 2.5
24	415 \pm 14.1	0.0 \pm 1.6	18.0 \pm 1.3	38.4 \pm 2.3	38.5 \pm 2.4
28	430.0 \pm 7.1	0.0 \pm 1.4	17.9 \pm 1.0	39.1 \pm 3.4	39.3 \pm 2.0

fminsearch function to minimise the sum squared errors (SSE < 0.05) between the predicted and experimental data. The developed kinetic model for microalgae cultivation was validated using the second set of experimental data. Some of the kinetic parameters used for the modelling are shown in Table 1.

3. Results and discussion

3.1. Wastewater treatment and microalgae growth kinetic model

Table 2 depicts experimental data from microalgae cultivation in wastewater. A nitrogen and phosphorus material balance showed that about 14% of the initial ammonium nitrogen was lost in the form of NH₃ volatilisation, while no loss in phosphorus occurred. This substantial loss in the ammonium nitrogen, justified the inclusion of ammonia volatilisation and pH prediction in the kinetic model.

The microalgae kinetic model developed in this study was applied to fit the above experimental data, as shown in the Fig. 2. From the model calibration, the following model parameters were determined for *Desmodesmus* sp. cultivation: specific microalgae growth rates based on ammonium nitrogen ($\mu_{max} = 0.17 \text{ day}^{-1}$) and based on NO₃⁻ ($\mu_{maxNO_3} = 0.1 \text{ day}^{-1}$), death rates ($\mu_d = 0.004 \text{ day}^{-1}$), and the NH₄⁺ to NO₃⁻ oxidation rate ($\mu_B = 0.14 \text{ day}^{-1}$). Growth kinetics for the

Desmodesmus sp. were found to be similar to that of *Scenedesmus obliquus* from the second phase of the experiments. The μ_{max} obtained in this study was consistent with $0.19\text{--}0.22 \text{ d}^{-1}$ when using flue gas containing 6 vol% CO₂, and $0.14\text{--}0.22 \text{ d}^{-1}$ for flue gas containing 12 vol% CO₂, in cultivation of *Scenedesmus obliquus* in a photobioreactor [7]. However, higher specific growth rates have been reported. For instance, specific growth rate obtained from our study is lower than $0.78\text{--}1.25 \text{ d}^{-1}$ reported for *Scenedesmus obliquus* cultivation using NaNO₃ and flue gas containing 10–20% CO₂ as the inorganic nitrogen and carbon sources, respectively [9]. Nutrient concentrations and the operating conditions for microalgae cultivation, are predominant factors in determining their specific growth and death rates.

The kinetic model was accurate in predicting the growth of microalgae, and nutrient removals from wastewater. For instance, the predicted *Desmodesmus* sp. biomass were 294.8 mg/L at 9 days, 340.5 mg/L at 11 days, 414.5 at 28 days, which compares well with the experimental biomass concentrations of 297.5 mg/L at 9 days, 335.0 mg/L at 11 days and 430.0 mg/L at 28 days. However, the predicted algae biomass is slightly lower than the experimental values at 3 days and 7 days, probably due to microalgae growth rate modification by the rapid change in the culture media pH. Biomass growth was associated with removal of the inorganic nitrogen and phosphate from the culture. As shown in the Fig. 2, the experimental ammonium nitrogen decreased

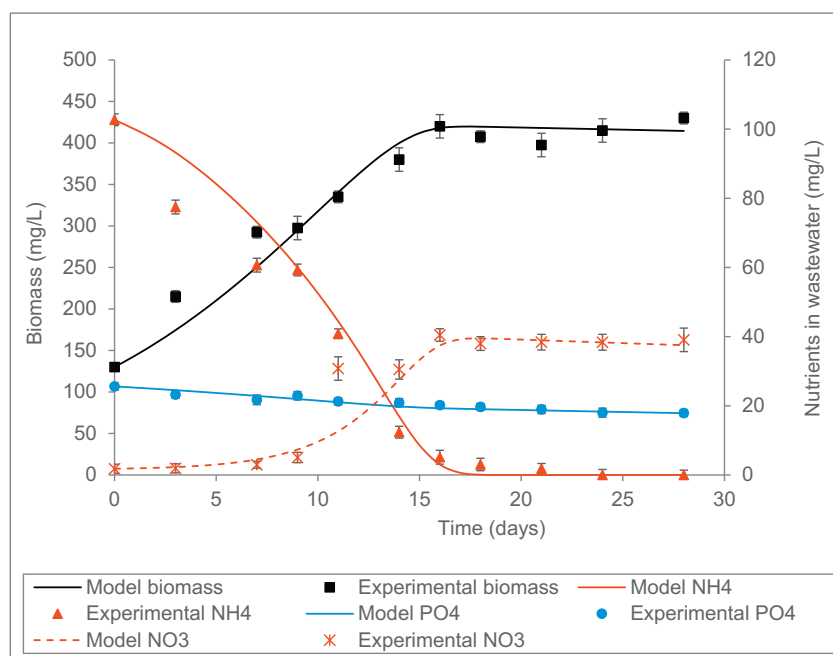


Fig. 2. Experimental and model predictions of biomass and nutrients during *Desmodesmus* sp. cultivation in wastewater. The experimental data shown are the mean \pm SD of three independent measurements.

rapidly from 102.7 mg/L initially, to 59.3 mg/L after 9 days, 5.2 mg/L after 16 days, and 0.0 mg/L after 24 days. The predicted NH_4^+ concentrations were 59.8 mg/L after 9 days, 3.0 mg/L after 16 days, and 0.0 mg/L after 24 days. The rapid decreases in ammonium concentration were followed by exponential rise in the NO_3^- content of the culture media, indicating an active oxidation of the ammonium nitrogen by bacterial nitrification. Measured nitrate concentrations agreed fairly well with model predictions. The experimental nitrate concentrations rose from 1.8 mg/L initially, to 40.5 mg/L after 16 days, and 39.1 mg/L after 28 days. There was a decrease in nitrate concentration after 16 days, likely due to removal by microalgae. Experimental phosphate content gradually decreased from the initial value of 25.6 mg/L, to 22.9 mg/L after 9 days, and 17.9 mg/L after 28 days, as compared the model predicted values of 22.0 mg/L after 9 days, and 17.9 mg/L after 28 days.

Nutrient removal efficiencies at 28 days, for the *Desmodesmus* sp. cultivation, were 30% for phosphorus and 62.6% for the nitrogen. However, the actual nitrogen fixation in the microalgae was 49%, as our findings showed that pH-driven NH_3 volatilisation occurred. The equivalent amount of CO_2 sequestered based on the algal biomass yield was 394 mg per litre of the microalgae culture (i.e., $106 \times 44 \times (430 - 130) / 3550 = 394$). The actual nitrogen removal efficiency (49%) in this study compares well with 50% that was reported after 10 days for *Chlorella vulgaris* cultivation in synthetic wastewater at fixed pH, using ammonium nitrogen concentrations in the ranges of 41.8–92.8 mg/L and 7.7 mg/L phosphate [38]. Aslan & Kapdan [38] noted that complete removal of the NH_4^+ nutrient was achieved at concentrations of 13.2–21.2 mg/L after 10 days; however, only 24% removal efficiency was obtained at NH_4^+ concentrations above 129 mg/L. The phosphate removal efficiency reported for the *Chlorella vulgaris* cultivation was 78% at 7.7 mg/L initial concentration, and < 30% removal efficiency at higher phosphate concentration [38]. Nutrients removal from our study are lower than 55% obtained for phosphate after 216 h, for batch cultivations of *Chlorella vulgaris* and *Scenedesmus dimorphus* in agro-industrial wastewater containing 111 mg/L of phosphate [43]. Nutrient removal efficiency of > 97% has also been reported for both nitrogen and phosphorus, in cultivations of *Scenedesmus obliquus* for 188 h at pH of 9.3, using wastewater containing 27.4 mg/L of NH_4^+ and 11.8 mg/L of phosphate [44]. Although removal efficiencies of nutrients in microalgae culture are dependent on initial nutrient concentrations, as well as cultivation conditions; nitrogen removal efficiency based on only NH_4^+ concentrations could be misleading, as substantial loss as NH_3 will occur at pH of 9 and above.

The kinetic model developed in this study clearly shows that pH of the culture media increased rapidly from an initial 8.3 to 9.6 after 10 days, and 10.2 after about 28 days (Fig. 3(a)). The experimental pH values were 8.0–8.5 initially and 10.5 after 28 days. The model shows that the rise in the culture media pH was responsible for the observed

ammonia volatilisation, which increased from a negligible value of 0.05 mg/L at the pH of 8.3, to 10.3 mg/L at pH of 9.6, and finally to 14.3 mg/L at pH of 10 and above (Fig. 3(a)). Ammonium-ammonia equilibrium plot in Fig. 3(b), using Eq. (11), shows that at pH above 10, over 90% of all the ammonium nitrogen in the microalgae culture existed as NH_3 , leading to massive loss of the NH_4^+ nitrogen due to NH_3 volatilisation.

Although there was still presence of substantial amounts of HPO_4^{2-} and NO_3^- in the culture media even after 28 days, the experimental data and the kinetic model demonstrate that the microalgae growth had reduced drastically after ammonium nitrogen in the culture medium had finished (Fig. 2). The uptake of these nutrients by microalgae substantially decreased. This observation can be attributed to main reasons: microalgae prefers ammonium nitrogen and utilises NO_3^- at slower rates [17,23], and the availability of the inorganic carbon in the form of CO_3^{2-} which is not utilisable by the microalgae [45]. The $\text{CO}_2(\text{aq}) - \text{HCO}_3^- - \text{CO}_3^{2-}$ equilibria in Fig. 1, clearly indicate that CO_2 and HCO_3^- required by photosynthetically active microalgae are abundant at less than pH 9.5. Above a pH of 10, the CO_3^{2-} species predominate in solution, and this form of inorganic carbon cannot be utilised by microalgae [23]. The rise in pH of the culture media reduced their potential production of biomass and nutrient removal from wastewater. To reduce the culture media pH and shift the inorganic carbon equilibrium towards CO_2 and HCO_3^- , extra addition of CO_2 or neutralisation with an acid was required. It has been proposed that injection of CO_2 , especially from flue gas ad biogas, are attractive strategies for pH control and to sustain healthy and steady biomass production in microalgae ponds [45,46].

3.2. Experimental validations of the microalgae growth model

The microalgae kinetic model was validated using the experimental data for *Desmodesmus* sp. and *Scenedesmus obliquus* cultivated in wastewater containing 0% and 7% landfill leachate (Fig. 5). *Desmodesmus* sp. and *Scenedesmus obliquus* growth kinetics were found to be similar, hence, only the model versus predicted results for the *Desmodesmus* sp. are shown in Fig. 4. The results demonstrate that the kinetic model could be used to predict the microalgae growth, nutrient removals from wastewater, and changes in the culture media pH. The predicted biomass yields in Fig. 4(a), for the *Desmodesmus* sp. cultivation in wastewater containing 7% landfill leachate were 330 mg/L after 1 day, 541 mg/L after 9 days, 824 mg/L after 17 days, and 1251 mg/L after 28 days. These predicted biomass yields compare quite well with the experimental values at $R^2 > 99\%$. Initial nutrient concentrations were 167.4 mg/L for NH_4^+ , 10 mg/L for NO_3^- , and 21.3 mg/L for HPO_4^{2-} , while their experimentally measured values after 28 days were 30.4 mg/L for NH_4^+ , 6.9 mg/L for NO_3^- , and 12.2 mg/L for HPO_4^{2-} . These final nutrient concentrations compared to 26.7 mg/L for NH_4^+ ,

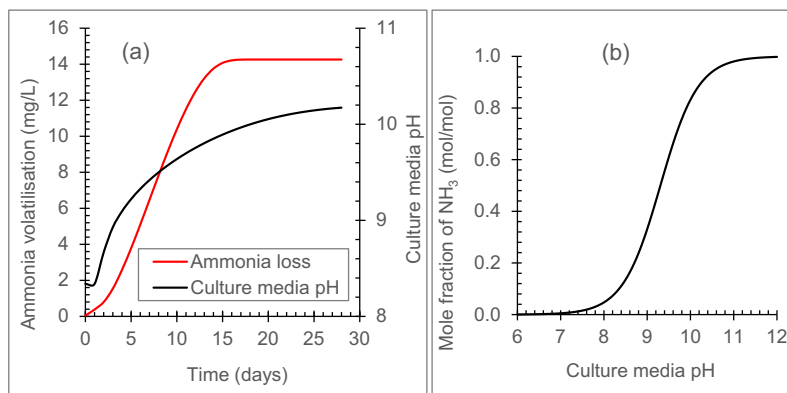


Fig. 3. Model predictions of ammonia volatilisation and changes in pH during the microalgae cultivation (a), and the dissociation of NH_4^+ into NH_3 with increase in pH (b). Wastewater was used as the culture media.

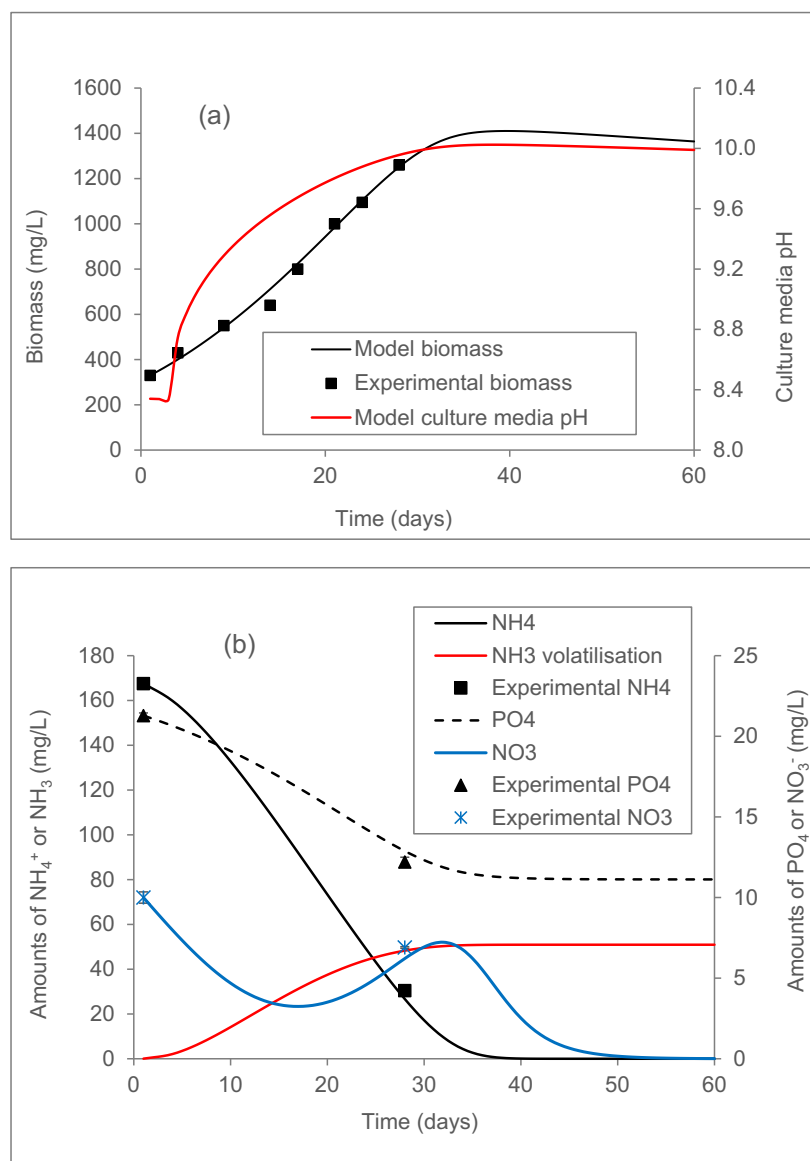


Fig. 4. Model validation for *Desmodesmus* sp. cultivation in wastewater containing 7% landfill leachate, (a) microalgae growth and culture media pH, (b) nutrients removals and NH_3 volatilisation. The wastewater/leachate mixture was used as the culture media. The experimental data shown are the mean \pm SD of three independent measurements.

6.2 mg/L for NO_3^- , and 12.9 mg/L for HPO_4^{2-} predicted by the model (Fig. 4(b)).

Fig. 4(a) shows that the pH of the culture media rose from 8.3 initially, to 9.3 after 10 days, and to 10.2 after about 28 days due to assimilation of HCO_3^- by the algae. The experimental pH values were 8.0–8.5 initially and 9.8 after 28 days. The rise in the culture pH was associated by increase in ammonia volatilisation, from about 0.05 mg/L at the pH of 8.3, to 48.3 mg/L (corresponding to about 28.8% loss in NH_4^+ nitrogen) at pH of 9.8 after 28 days. Fig. 4(a) also shows the model prediction of an achievable maximum biomass yield of 1403 mg/L after 40 days, with final nutrient concentration levels of 0.08 mg/L for NH_4^+ , 2.5 mg/L for NO_3^- , and 11.2 mg/L for HPO_4^{2-} . After 40 days of microalgae cultivation, the pH of the culture media had risen to 10, producing an overall ammonia volatilisation of 50.9 mg/L. It can be observed that the microalgae biomass yield from the period of 28 to 40 days (12 days) was only 152 mg/L. This was due to the reduced biomass production and nutrient uptake due to the increased pH. As shown in the Fig. 1, a rise in the culture media pH from 8.3 to about 9.5 could lead to approximately 40% loss in the inorganic carbon utilisation

efficiency due to CO_3^{2-} formation. In summary, the microalgae cultivation without supply of CO_2 -rich flue gas (Figs. 2 and 4) could be limited by availability of utilisable forms of inorganic carbon. Nitrogen limitation also occurred, even at high initial NH_4^+ concentration, as result of ammonia loss. Therefore, pH control is crucial in a microalgae culture system that uses ammonium nitrogen as nutrient.

Comparison of experimental data and model predictions, for microalgae cultivated in wastewater containing 0% and 7%, are shown in the Fig. 5. Accuracy of the prediction was above 98% for both the *Desmodesmus* sp. and the *Scenedesmus obliquus*. This clearly demonstrated that the kinetic model developed in the study can be effectively used to evaluate and predict microalgae cultivation. Addition of landfill leachate to the wastewater increased nutrient content, hence the higher biomass yields at 7% compared to the 0% leachate.

3.3. Microalgae substrate consumption from domestic wastewater

Wastewater is a useful source of inorganic carbon, nitrogen, and phosphate. Inorganic carbon in domestic wastewater is mainly in the

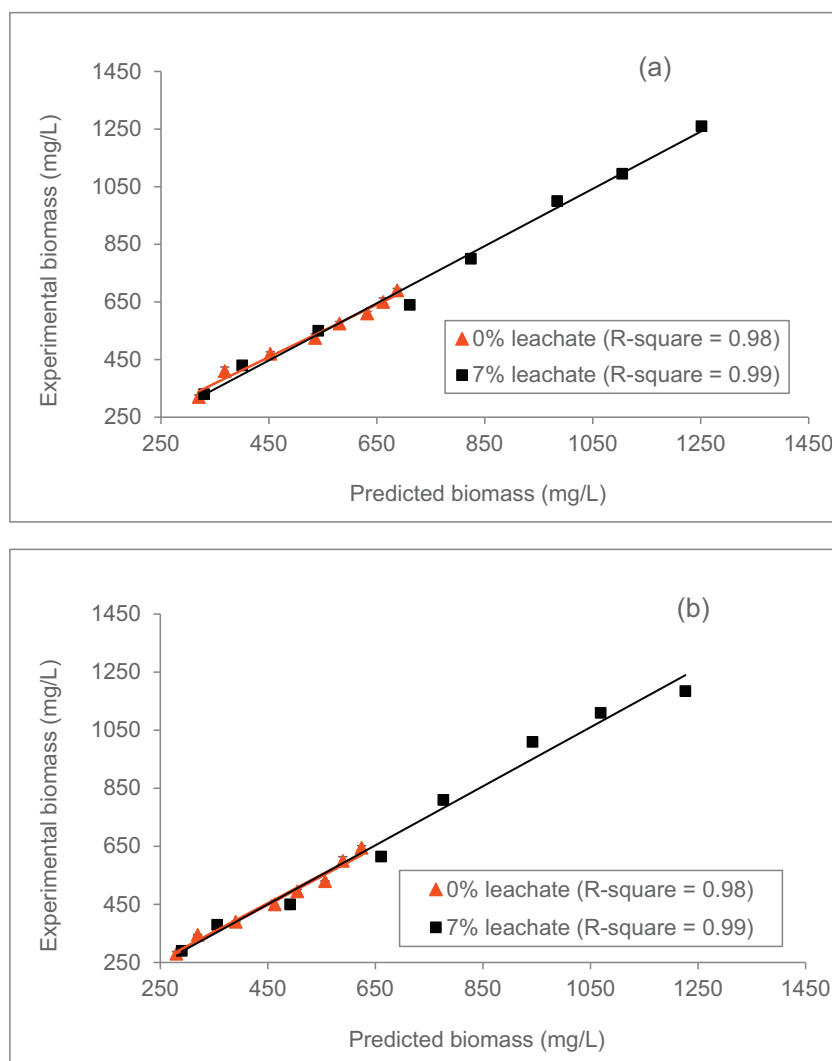


Fig. 5. Model validation using the biomass from microalgae cultivation in wastewater containing 0% and 7% landfill leachate, (a) *Desmodesmus* sp., and (b) *Scenedesmus obliquus*. The data shown are the mean \pm SD of three independent measurements.

forms of HCO_3^- and CO_3^{2-} , which comes from food wastes and cleaning. Hence dissolved CO_2 , HCO_3^- and CO_3^{2-} are major constituents dictating the alkalinity of domestic wastewater [39]. Domestic wastewater has been reported to have a carbon-to-nitrogen (C:N) ratio of approximately 3:1, which is considerably lower than the C:N ratios in the range of 5:1 to 10:1 required for microalgae cultivation [45]. The carbon to nitrogen ratio in wastewater has a serious implication in design of suitable ponds for microalgae cultivation, because of the carbon limitations which occur in absence of extra CO_2 supply. Carbon limitation during microalgae cultivation in domestic wastewater is evidenced by the rise in the culture media pH to values of up to 10 [45], also observed in our studies (Figs. 2 & 4). Therefore, extra CO_2 injection from inexpensive sources, such as flue gas, is recommended for carbonation of microalgae systems where there is limited inorganic supply [46]. Some carbonation strategies which have been proposed for algae cultivation are via the use of carbonation sump [10,47], and bubble column [6]. However, to ensure complete utilisation of the CO_2 injected into the culture media, an effective strategy for the CO_2 dosing, would be to link it with the amount of microalgae biomass present by monitoring optical density, or link it with the amount of ammonium or nitrate present.

Another option to avoid carbon limitation could be to use algae-bacteria symbiotic mechanism. Previous studies have shown that algae-bacteria symbiotic process could be used for wastewater treatment,

where the photosynthetic process in the algae generates O_2 , which is required by the bacteria for aerobic degradation of organic matter to produce CO_2 [48,49]. In this case, aerobic bacteria degradation of organic matter in the wastewater would produce CO_2 to stabilise the culture pH and sustain the microalgae growth. While the photosynthetic process in algae would generate O_2 required by the bacteria for oxidation of organic matter. However, the challenge of this mechanism would be to ensure controlled ratios of algae and bacteria cultures required to maintain the optimal pH for microalgae growth.

Fig. 6 presents results from combining the modelling of inorganic carbon equilibria and ammonia loss. At an initial experimental pH of 8.0–8.5, the microalgae culture has over 90% of the inorganic carbon source in the form of HCO_3^- , leading to very rapid biomass growth. This rapid biomass productivity is associated with exponential increase in the media pH, which must be controlled to avoid loss in biomass production efficiency. Considering the high ammonium nitrogen content of the domestic wastewater, control of the culture media pH serves dual functions – minimises ammonia volatilisation, and ensures steady and high efficiency inorganic carbon utilisation. Fig. 6 also demonstrates that pH in the range of 7.5 to 8.5, optimally at pH of 8.1, are required for high efficient utilisation of the inorganic carbon by microalgae.

For instance, about 97% of the inorganic carbon species are in the form of HCO_3^- and 2% as $\text{CO}_2(\text{aq})$ at pH of 8.1, showing that 99% of

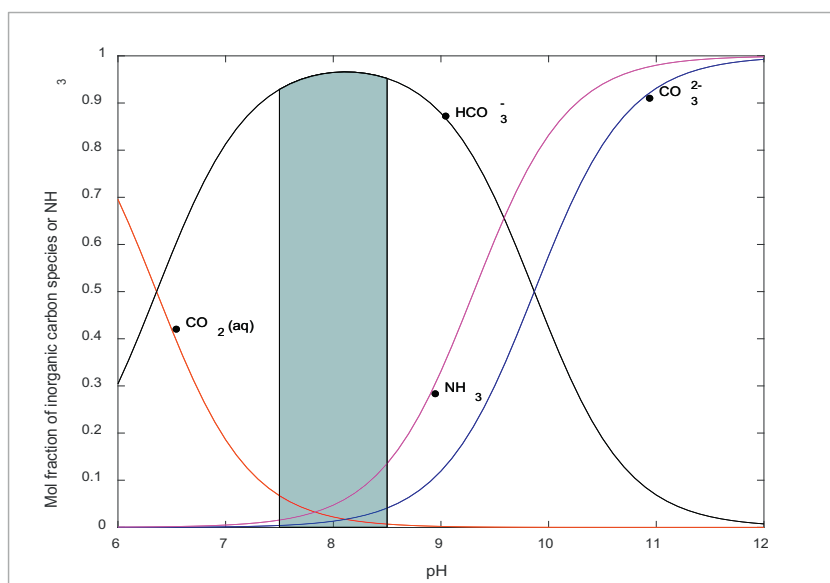
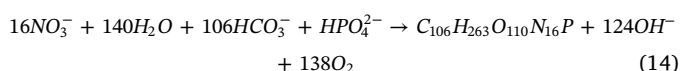
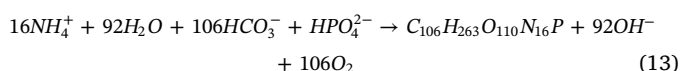


Fig. 6. Mole fractions of the inorganic carbon in the forms of $\text{CO}_2(\text{aq})$, HCO_3^- and CO_3^{2-} , and mole fractions of the NH_4^+ nitrogen dissociating into NH_3 , as a function of media pH.

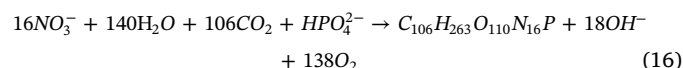
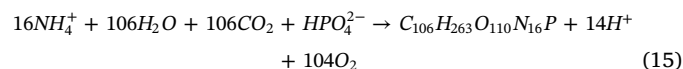
the inorganic carbon is available in suitable forms to the microalgae, such that 99% carbon fixation efficiency could be achieved. This pH region for more effective algae biomass production could be achieved through extra CO_2 supply, if needed. However, it is clear from Fig. 6 that continuous injection of excess CO_2 into the culture may lead to rapid decrease in pH, and consequently lower HCO_3^- and high $\text{CO}_2(\text{aq})$. An equilibrium towards higher dissolved CO_2 concentrations must be avoided, as this increases CO_2 outgassing and consequently the risk of environmental CO_2 emissions. Therefore, a pH-controlled smart CO_2 injection must be implemented to facilitate high efficiency microalgae culture system. This observation generally agrees with an existing study which demonstrated that about 95% CO_2 fixation was obtained in *Chlorella vulgaris* culture at pH in the range of 6.18–7.22, using intermittent injections of flue gas containing 15% CO_2 [13]. At pH of 7.22, 87% of the inorganic carbons exists in the form of HCO_3^- and 13% as $\text{CO}_2(\text{aq})$, therefore, 100% of the injected CO_2 are in the form suitable for the microalgae. However, a high dissolved CO_2 concentration would lead to outgassing and environmental CO_2 emissions, limiting the carbon fixation efficiency.

In view of the $\text{CO}_2(\text{aq})$ - HCO_3^- - CO_3^{2-} equilibrium dynamics (Fig. 6) and the trend in the culture media pH (Figs. 3b and 4a), HCO_3^- is the most utilisable form of inorganic carbon by the microalgae [23], and at pH of 8 and above, the amount of CO_2 in the culture media is negligible, hence, in this study microalgae growth was likely based on HCO_3^- . Our findings also showed that the active photosynthetic activity of the microalgae, which is represented by growth in the microalgae biomass, was associated with a rapid increase in the culture media pH. This observation clearly indicates that there is a reduction in the H^+ concentration, leading to increased culture media OH^- . Based on the observed pH trends, we have proposed Eqs. (13) and (14), as the reactions for microalgae biomass formation, based on reported molecular weight of microalgae [4].



Microalgae biomass formation reactions in Eqs. (13) and (14) expose a possible chemical kinetic approach which could be harnessed to

maximise biomass yield. Formations of OH^- during algae biomass production suggests that the desired pH of the culture media could be maintained by a proportionate removal of the OH^- from the system, just as soon as they are formed. The best strategy for the OH^- removal would be through reactions with CO_2 to generate bicarbonate ions ($\text{OH}^- + \text{CO}_2 = \text{HCO}_3^-$). Additions of CO_2 for pH control is more favourable to the consumption of nitrates due to positive stoichiometric OH^- imbalance (106HCO_3^- generates 124OH^-), ensuring that the bicarbonate ions regeneration continues. When NH_4^+ is used, the amount of OH^- generated was less than the stoichiometric HCO_3^- consumed, leading to gradual decline in total OH^- concentration in microalgae culture and slower rates of bicarbonate ions regeneration. An optimally-controlled microalgae cultivation in wastewater (pH ~ 8.10) should have regular or intermittent additions of CO_2 . According to Eq. (13) at rates of 5456 g CO_2 per 3550 g of produced algae biomass (1.14 g CO_2 per gram algae), when NH_4^+ is used as the nitrogen source. When NO_3^- is used as the nitrogen source (Eq. (14)), the CO_2 injection should be at the rate of 4048 g CO_2 per 3550 g of produced algae biomass (1.54 g CO_2 per gram algae). Also, considering the negative stoichiometric imbalance in OH^- generation for the utilisation of NH_4^+ , an input of OH^- is required at the rate of 238 g per 3550 g of produced algae biomass (0.067 g OH^- per gram algae). This strategy will prevent pH runaway, and ensure optimal and efficient biomass productivity in algae cultivation ponds. For microalgae cultivations at lower pH values ($6.5 < \text{pH}$) where CO_2 concentration dominates the inorganic carbon equilibria, we propose biomass formation in Eqs. (15) and (16).



These equations clearly demonstrate that CO_2 sequestration by microalgae when using NH_4^+ leads to negative OH^- imbalance (decrease in culture pH), while nitrate assimilation by the microalgae generates OH^- which cause the pH to rise, as previously reported [50,51]. Hence, selection of adequate strategies for pH control is an important step to ensure optimal productivity of microalgae biomass.

4. Conclusions

Microalgae growth kinetics in wastewater treatment and CO₂ sequestration have been investigated using both experimental and numerical modelling. A microalgae kinetic model was developed for predicting algae cultivations. The model was calibrated using experimental data from cultivation of *Desmodesmus* sp., to determine specific microalgae growth rates based on ammonium nitrogen (μ_{max}) and NO₃[−] (μ_{maxNO3}), microalgae death rates (μ_d), and the NH₄⁺ to NO₃[−] oxidation rate (μ_B). These parameters were: $\mu_{max} = 0.17 \text{ day}^{-1}$, $\mu_d = 0.004 \text{ day}^{-1}$, $\mu_B = 0.14 \text{ day}^{-1}$, and $\mu_{maxNO3} = 0.1 \text{ day}^{-1}$. The model was validated for the algal biomass from *Desmodesmus* sp. and *Scenedesmus obliquus* cultivation in wastewater that contained 0% and 7% landfill leachate, with accuracy of the prediction above 98% in all cases. The kinetic model also predicted other important parameters in microalgae cultivation such as wastewater nutrient removals, ammonia volatilisation, and changes in the culture pH. It was observed that microalgae growth in wastewater was associated with an exponential increase in the media pH, which must be controlled to avoid ammonia volatilisation and loss in biomass productivity. Between 28.8 and 29.7% of the initial NH₄⁺ was lost to ammonia volatilisation in wastewater containing 7% landfill leachate. The determined optimal pH for microalgae culture in this study was 8.1. At this pH, 99% of inorganic carbons are available in forms suitable to microalgae, at which 99% carbon fixation efficiency could be achieved, without CO₂ outgassing and emissions to the environment. Our findings showed that a combined model for the inorganic carbons equilibria and the ammonia loss, could be used to design an improved strategy for optimal biomass productivity in microalgae cultivation. The generation of OH[−] ions increases the culture pH during microalgae biomass formation, hence their removal from the system could be facilitated through reactions with proportionate amounts of CO₂ to regenerate the bicarbonate ions (OH[−] + CO₂ = HCO₃[−]). We propose that microalgae productions at a constant pH can be achieved through proportionate CO₂ injections based on the nitrogen source and rate of the algae biomass formation. This strategy will use optical density-controlled smart CO₂ injections to prevent pH runaway, ensure optimal and efficient biomass productivity in algae cultivation ponds.

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Author contributions

S.B.V.O. and V.C.E. conceptualized the study; A.H.G. conducted the experiments and acquisition of data; M.T.O.L. provided resources; V.C.E. and S.B.V.O. performed analysis and interpretation of data; V.C.E. wrote the original manuscript draft; S.B.V.O., V.C.E., M.T.O.L., I.M.R. and A.H.G. reviewed and edited the manuscript; S.B.V.O., M.T.O.L., and I.M.R. supervised the project.

Conflict of interest

All the authors declared that there are no conflicts of interest in publishing this article.

Statements regarding conflicts, consent, and human/animal rights

No conflicts, informed consent, human or animal rights applicable. All the authors agree in submitting this article in Algal Research.

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