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

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A B S T R A C T
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\text{max} and μ\text{maxNO}_3), microalgae death rates (μ\text{d}), and the NH₄⁺ to NO₃⁻ oxidation rate (μ\text{μ}). Model parameters obtained were: μ\text{max} = 0.17 day⁻¹, μ\text{d} = 0.004 day⁻¹, and μ\text{μ} = 0.14 day⁻¹. Microalgae specific growth rate based on NO₃⁻ alone (μ\text{maxNO}_3 = 0.1 day⁻¹) was lower than the overall growth rate (μ\text{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 NH₄⁺ 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 CO₂ and 0.067 g OH⁻ per gram of produced algae when using NH₄⁺ nutrient, and 1.54 g of CO₂ per gram of produced algae when using NO₃⁻ nutrient. This could be done in a wastewater pond by using an optical density-controlled smart CO₂ 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 CO₂ conversion and wastewater treatment. Microalgae autotrophic growth utilises inorganic carbon in the form of dissolved CO₂ in the bicarbonate-carbonate buffer system (CO₂(aq) → HCO₃⁻ → CO₃²⁻) [5,6]. Previous studies have shown that CO₂ fixation efficiency, when injected continuously in microalgae culture, was in a range of 4-66% at input CO₂ concentration of 6–15% for Scenedesmus sp. [7–10], and 16–64% at an input CO₂ concentration of 1–15% for Chlorella sp. [8,11–13]. Within these CO₂ fixation yields, microalgae have reported growth rates between 0.19 and 1.24 d⁻¹. Although no specific value for
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 phosphorous 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]. Ammonium sources commonly used are ammonium, nitrate, and urea. Ammonium is the most preferred because its assimilation requires less energy [17]. Ammonium exits 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 (μₘₐₓ, 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 μₘₐₓ 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).

\[ \mu_D = \frac{\mu_{\text{max},D} \left( 1 - \frac{Q_c}{q} \right)}{K_{Q_c} + \left( 1 - \frac{Q_c}{q} \right)} \]  

(2)

\[ \mu_{D,\text{max}} = \frac{\mu_m \rho_n}{K_m - \mu_m Q_Q} \]  

(3)

where:

- \( \mu_m \): Monod specific growth rate (time⁻¹);
- \( \mu_D \): Droop specific growth rate (time⁻¹);
- \( \mu_{D,\text{max}} \): Monod maximum specific growth rate (time⁻¹);
- \( \mu_{D,\text{max},D} \): Droop maximum specific growth rate (time⁻¹);
- \( K_A \): half-saturation constant for nitrogen A (mg/L/); \( [A] \): concentration of the nutrient A (mg/L); \( Q_Q \): minimum cell quota; \( q \): cell quota; \( \rho_{\text{max}} \): 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).

\[ \mu = \mu_{\text{max}} \frac{[A]}{K_A + [A]} \frac{[B]}{K_B + [B]} \]  

(4)

\[ \mu = \mu_{\text{max}} \frac{[C]}{K_C + [C]} \frac{[N]}{K_N + [N]} \frac{[P]}{K_P + [P]} \]  

(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).

\[ \mu_D = \frac{\mu_m \rho_n}{K_m - \mu_m Q_Q} \]  

(3)
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 μmol m⁻² 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-LCPH/CPN analyzer (TOC-LCPH/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 CaCO₃). 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 utilisation 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, CO₂ taken from the environment and HCO₃⁻ 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).

\[
\frac{d[M_c]}{dt} = \mu_{max} * [M_b] * \left( \frac{[P]}{K_P + [P]} + \frac{[C]}{K_C + [C]} \right) * \left( \frac{[NH_4^+]}{K_{NH_4} + [NH_4^+]} \right) + \left( \frac{\mu_{max}NO_3}{\mu_{max}} \right) * \left( \frac{[NO_3^-]}{K_N + [NO_3^-]} \right) - \mu_{d} * [M_b]
\]  

(6)

\[
\frac{d[P]}{dt} = -\mu_P * \mu_{max} * [M_b] * \left( \frac{[P]}{K_P + [P]} \right) + \frac{\mu_{max}NO_3}{\mu_{max}} * \left( \frac{[NO_3^-]}{K_N + [NO_3^-]} \right) - \mu_{d} * [P]
\]  

\[
\frac{d[C]}{dt} = -\mu_C * \mu_{max} * [M_b] * \left( \frac{[C]}{K_C + [C]} \right) + \left( \frac{\mu_{max}NO_3}{\mu_{max}} \right) * \left( \frac{[NO_3^-]}{K_N + [NO_3^-]} \right) + \frac{K_{des} \cdot \frac{P_{max}}{K_I}}{K_I + \frac{P_{max}}{K_I}}
\]  

(7)

\[
\frac{d[NH_4^+]}{dt} = -\mu_{NH} * \mu_{max} * [M_b] * \left( \frac{[NH_4^+]}{K_{NH_4} + [NH_4^+]} \right) - K_{d,N} * \frac{[P]}{K_P + [P]} + \frac{[C]}{K_C + [C]} + \frac{[NO_3^-]}{K_N + [NO_3^-]} - \mu_{d} * [NH_4^+]
\]  

(8)

\[
\frac{d[NO_3^-]}{dt} = \mu_{NH} * \mu_{max} * [M_b] * \left( \frac{[NO_3^-]}{K_N + [NO_3^-]} \right) - K_{d,N} * \frac{[P]}{K_P + [P]} + \frac{[C]}{K_C + [C]} - \mu_{d} * [NO_3^-]
\]  

(9)

\[
\frac{d[NH_3]^i}{dt} = K_{d,N} * \left( [NH_3]^i \right) - K_{d,N} * \left( [NH_3]^i \right)
\]  

(10)

\[
[H^+] = \left( \left( -K_t * \frac{[C]}{R} - 1 \right) - \sqrt{K_t \left( \frac{[C]}{R} - 1 \right)^2 - 4 * K_t * K_s * \frac{[C]}{R}} \right) \left( \frac{C}{R} \right)^2
\]  

(12)

And, \( R = \frac{[C]}{K_{max}} \)

The \( \mu_{max} \) 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 \( \mu_{NH}. \) 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, \( \mu_{d} \) (time⁻¹). \( \mu_{d} \) 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), \( [NH_4^+] \) is the ammonium nitrogen concentration (mg/L), \( [NH_3]^i \) is the ammonia concentration in equilibrium with ammonia (mg/L), and \( [NO_3^-] \) is the nitrate concentration (mg/L).

The half-saturation constants for 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 \( (K_{max} = 31.5 \text{ mg/L}) \) and orthophosphate \( (K_P = 10.5 \text{ mg/L}) \) 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 \( (K_N = 124.9 \text{ mg/L}) \) has been reported for inorganic carbon in a study using NaHCO₃ as a carbon source [24]. Removal coefficients for the inorganic carbon and the nutrients were represented as, \( Y_{NH}, \) for \( NH_4^+ \) in gram \( NH_4^+ \) per gram algae, \( Y_{NP}, \) 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 \( HPO_4^{2-} \) 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 C106H263O110N16P 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: \( \text{HCO}_3^- = 61 \text{ g/mol} \); \( \text{NH}_4^+ = 18 \text{ g/mol} \); \( \text{NO}_3^- = 62 \text{ g/mol} \); \( \text{HPO}_4^{2-} = 96 \text{ g/mol} \); and microalgae = 3550 g/mol. The reaction stoichiometry in the microalgae formation equations [4] were also used to calculate the consumption coefficient for the \( \text{HCO}_3^- \). For instance, the removal coefficient for the \( \text{HPO}_4^{2-} \) was \( (1 * 96/3550) = 0.027 \text{ g HPO}_4^{2-} \text{ per gram of the microalgae.} \)

\( K_{\text{des}} \) and \( K_{\text{m}} \) represent mass transfer coefficient of \( \text{NH}_3 \) gas and \( \text{CO}_2 \) in water, respectively; while \( K_{\text{hl}} \) is the Henry's law constant for \( \text{CO}_2 \) gas. The pressure and mole fraction of \( \text{CO}_2 \) 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 (\( \text{HCO}_3^- \)) equilibrium. As such, carbonic acid formation could be originated from biocarbonate present in wastewater or \( \text{CO}_2 \) dissolved in equilibrium with water, see Eq. (8). \( \text{CO}_2 \) dissolved in equilibrium with water would be limited by Henry's constant, whether \( \text{CO}_2 \) is derived from air or the bacterial oxidation of organic carbon, at atmospheric conditions. Equilibrium constant \( K_h \) denoted the \( \text{NH}_3^- \leftrightarrow \text{NH}_4^+ + \text{H}^+ \) process; \( K_1 \) the ionisation of carbonic acid, \( \text{H}_2\text{CO}_3(aq) \leftrightarrow \text{HCO}_3^{(aq)} + \text{H}^+(aq) \); and \( K_2 \) the bicarbonate dissociation in, \( \text{HCO}_3^{(aq)} \leftrightarrow \text{CO}_3^{2-} + \text{H}^+ \). Molecular weight of \( \text{CO}_2 \) is shown as \( M_w \).

The model considers the effects of \( \text{NH}_3 \) volatilisation and the oxidations of the ammonium nitrogen to nitrate by autotrophic bacteria. This was done through the addition of the parameter \( \mu_o \) to account for the rate of oxidations of ammonium to nitrate, and Eq. (11) to account for ammonia volatilisation. The rate of \( \text{NH}_3 \) volatilisation \( (K_{\text{des}}) \) used in the model was obtained from the work of Solimeno et al. [23]. At the initial \( \text{pH} \) 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 \( \text{HCO}_3^- \). Bicarbonate concentration was taken to be proportional to carbonate alkalinity values. This is a valid assumption, as carbonate alkalinity mainly consists of \( \text{HCO}_3^- \) and \( \text{CO}_3^{2-} \), which is dominated by \( \text{HCO}_3^- \) at pH between 4.5 and 8.3 [39]. \( \text{HCO}_3^- \) accounted for over 90% of the inorganic carbon at the initial pH range.

Table 1

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate of nutrient uptake for maintenance, ( \mu_o )</td>
<td>0.00385 h(^{-1})</td>
<td>[40]</td>
</tr>
<tr>
<td>Initial microalgae concentration, ( M_o )</td>
<td>130–150 mg/L</td>
<td>This study</td>
</tr>
<tr>
<td>( \text{NH}_4^+ ) &amp; ( \text{NO}_3^- ) half-saturation constant, ( K_o )</td>
<td>31.5 mg/L</td>
<td>[38]</td>
</tr>
<tr>
<td>( \text{HPO}_4^{2-} ) half-saturation constant, ( K_o )</td>
<td>10.5 mg/L</td>
<td>[38]</td>
</tr>
<tr>
<td>( \text{HCO}_3^- ) half-saturation constant, ( K_o )</td>
<td>124.9 mg/L</td>
<td>[24]</td>
</tr>
<tr>
<td>( \text{NH}<em>4^+ ) removal coefficient, ( Y</em>{\text{NH}_4} )</td>
<td>0.081 g/g. algae</td>
<td>This study</td>
</tr>
<tr>
<td>( \text{NO}<em>3^- ) removal coefficient, ( Y</em>{\text{NO}_3} )</td>
<td>0.279 g/g. algae</td>
<td>This study</td>
</tr>
<tr>
<td>( \text{HPO}<em>4^{2-} ) removal coefficient, ( Y</em>{\text{HPO}_4} )</td>
<td>0.027 g/g. algae</td>
<td>This study</td>
</tr>
<tr>
<td>( \text{HCO}<em>3^- ) consumption coefficient, ( Y</em>{\text{HCO}_3} )</td>
<td>1.821 g. ( \text{HCO}_3^- )/g. algae</td>
<td>This study</td>
</tr>
<tr>
<td>Mass transfer coefficient for ( \text{NH}<em>3 ) gas, ( K</em>{\text{m}} )</td>
<td>0.14 h(^{-1})</td>
<td>[23]</td>
</tr>
<tr>
<td>Mass transfer coefficient for ( \text{CO}<em>2 ) gas, ( K</em>{\text{m}} )</td>
<td>29.4 bar/mol</td>
<td>[41]</td>
</tr>
<tr>
<td>Henry's law constant for ( \text{CO}<em>2 ) gas, ( K</em>{\text{hl}} )</td>
<td>1 bar</td>
<td>This study</td>
</tr>
<tr>
<td>Mole fraction of ( \text{CO}_2 ) in the air, ( y_o )</td>
<td>3 ( \times 10^{-4} ) mol/mol</td>
<td>This study</td>
</tr>
<tr>
<td>Mass transfer coefficient for ( \text{NH}<em>3 ) gas, ( K</em>{\text{m}} )</td>
<td>4.95 ( \times 10^{-10} )</td>
<td>[19,20]</td>
</tr>
<tr>
<td>Pressure of the ( \text{CO}_2 ) source, ( P_o )</td>
<td>1 bar</td>
<td>This study</td>
</tr>
<tr>
<td>Mole fraction of ( \text{CO}_2 ) in the air, ( y_o )</td>
<td>3 ( \times 10^{-4} ) mol/mol</td>
<td>This study</td>
</tr>
<tr>
<td>( \text{NH}_4^+ \leftrightarrow \text{NH}_3 + \text{H}^+ ) dissociation constant, ( K_o )</td>
<td>4.375 ( \times 10^{-7} )</td>
<td>[42]</td>
</tr>
<tr>
<td>( \text{H}_2\text{CO}_3(aq) \leftrightarrow \text{HCO}_3^{(aq)} + \text{H}^+ ) dissociation, ( K_1 )</td>
<td>1.358 ( \times 10^{-10} )</td>
<td>[42]</td>
</tr>
<tr>
<td>( \text{HCO}_3^{(aq)} \leftrightarrow \text{CO}_3^{2-} + \text{H}^+ ) dissociation, ( K_2 )</td>
<td>4.375 ( \times 10^{-7} )</td>
<td>[42]</td>
</tr>
</tbody>
</table>

Fig. 1. Mole fraction of various inorganic carbon species at different media pH.
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. The 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 $\mu_{max}$ obtained in this study was consistent with 0.19–0.22 d⁻¹ when using flue gas containing 6 vol% CO₂, and 0.14–0.22 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 0.78–1.25 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 ± SD of three independent measurements.](image-url)
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 $\text{NH}_4^+$ 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 concentrations were followed by exponential rise in the $\text{NO}_3^-$ 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/L 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 $\text{NH}_4^+$ nutrient was achieved at concentrations of 13.2–21.2 mg/L after 10 days; however, only 24% removal efficiency was obtained at $\text{NH}_4^+$ 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 $\text{NH}_4^+$ 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 $\text{NH}_4^+$ 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 $\text{NH}_4^+$ nitrogen due to NH$_3$ volatilisation.

Although there was still presence of substantial amounts of $\text{HPO}_4^{2-}$ and $\text{NO}_3^-$ 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 $\text{NO}_3^-$ at slower rates [17,23], and the availability of the inorganic carbon in the form of CO$_2^{2-}$ which is not utilisable by the microalgae [45]. The $\text{CO}_2(aq) - \text{HCO}_3^- - \text{CO}_3^{2-}$ 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$_2^{2-}$ 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 $\text{NH}_4^+$, 10 mg/L for $\text{NO}_3^-$, and 21.3 mg/L for $\text{HPO}_4^{2-}$, while their experimentally measured values after 28 days were 30.4 mg/L for $\text{NH}_4^+$, 6.9 mg/L for $\text{NO}_3^-$, and 12.2 mg/L for $\text{HPO}_4^{2-}$. These final nutrient concentrations compared to 26.7 mg/L for $\text{NH}_4^+$.

![Fig. 3. Model predictions of ammonia volatilisation and changes in pH during the microalgae cultivation (a), and the dissociation of $\text{NH}_4^+$ into NH$_3$ with increase in pH (b). Wastewater was used as the culture media.](image-url)
6.2 mg/L for NO₃⁻, and 12.9 mg/L for HPO₄²⁻ 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 HCO₃⁻ 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 NH₄⁺ 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 NH₄⁺, 2.5 mg/L for NO₃⁻, and 11.2 mg/L for HPO₄²⁻. 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 CO₃²⁻ formation. In summary, the microalgae cultivation without supply of CO₂-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 NH₄⁺ 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
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 CO$_2$(aq) at pH of 8.1, showing that 99% of
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 CO₂ supply, if needed. However, it is clear from Fig. 6 that continuous injection of excess CO₂ into the culture may lead to rapid decrease in pH, and consequently lower HCO₃⁻ and high CO₂(aq). An equilibrium towards higher dissolved CO₂ concentrations must be avoided, as this increases CO₂ outgassing and consequently the risk of environmental CO₂ emissions. Therefore, a pH-controlled smart CO₂ injection must be implemented to facilitate high efficiency microalgae culture system. This observation generally agrees with an existing study which demonstrated that about 95% CO₂ 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% CO₂ [13]. At pH of 7.22, 87% of the inorganic carbons exits in the form of HCO₃⁻ and 13% as CO₂(aq), therefore, 100% of the injected CO₂ are in the form suitable for the microalgae. However, a high dissolved CO₂ concentration would lead to outgassing and environmental CO₂ emissions, limiting the carbon fixation efficiency.

In view of the CO₂(aq) · HCO₃⁻ · CO₃²⁻ equilibrium dynamics (Fig. 6) and the trend in the culture media pH (Figs. 3b and 4a), HCO₃⁻ is the most utilisable form of inorganic carbon by the microalgae [23], and at pH of 8 and above, the amount of CO₂ in the culture media is negligible, hence, in this study microalgae growth was likely based on HCO₃⁻. 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].

\[
16NH₄⁺ + 92H₂O + 106HCO₃⁻ + HPO₄²⁻ → C₁₀₀H₂₆₁O₁₁₀N₁₆P + 92OH⁻ + 106O₂ \tag{13}
\]

\[
16NO₃⁻ + 140H₂O + 106HCO₃⁻ + HPO₄²⁻ → C₁₀₆H₂₆₁O₁₁₀N₁₆P + 124OH⁻ + 138O₂ \tag{14}
\]

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 CO₂ to generate bicarbonate ions (OH⁻ + CO₂ = HCO₃⁻). Additions of CO₂ for pH control is more favourable to the consumption of nitrates due to positive stoichiometric OH⁻ imbalance (106HCO₃⁻ generates 124OH⁻), ensuring that the bicarbonate ions regeneration continues. When NH₄⁺ is used, the amount of OH⁻ generated was less than the stoichiometric HCO₃⁻ 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 CO₂. According to Eq. (13) at rates of 5456 g CO₂ per 3550 g of produced algae biomass (1.14 g CO₂ per gram algae), when NH₄⁺ is used as the nitrogen source. When NO₃⁻ is used as the nitrogen source (Eq. (14)), the CO₂ injection should be at the rate of 4048 g CO₂ per 3550 g of produced algae biomass (1.54 g CO₂ per gram algae). Also, considering the negative stoichiometric imbalance in OH⁻ generation for the utilisation of NH₄⁺, 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 < pH) where CO₂ concentration dominates the inorganic carbon equilibria, we propose biomass formation in Eqs. (15) and (16).

\[
16NH₄⁺ + 106H₂O + 106CO₂ + HPO₄²⁻ → C₁₀₀H₂₆₁O₁₁₀N₁₆P + 14H⁺ + 104O₂ \tag{15}
\]

\[
16NO₃⁻ + 140H₂O + 106CO₂ + HPO₄²⁻ → C₁₀₆H₂₆₁O₁₁₀N₁₆P + 18OH⁻ + 138O₂ \tag{16}
\]

These equations clearly demonstrate that CO₂ sequestration by microalgae when using NH₄⁺ 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 ($\mu_{\text{ammonia}}$) and NO3$^-$(NH3NO3), microalgae death rates ($\mu_d$), and the NH4$^+$ to NO3$^-$ oxidation rate ($\mu_p$). These parameters were: $\mu_{\text{ammonia}} = 0.17$ day$^{-1}$, $\mu_d = 0.004$ day$^{-1}$, $\mu_p = 0.14$ day$^{-1}$, and $\mu_{\text{maxNO3}} = 0.1$ 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 carbonates could be facilitated through reactions involving proportionate amounts of CO2 to regenerate the bicarbonate ions. 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.

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